

**CDC 4T051N**

**Medical Laboratory  
Journeyman –  
Administration and  
Chemistry**

**Volume 2. Clinical Chemistry**



**Air Force Institute for Advanced Distributed Learning  
The Air University  
Air Education and Training Command**

**Authors:** MSgt Karla L. Puterbaugh and MSgt Christopher Lange  
82d Training Squadron  
882d Training Group (AETC)  
382 TRS/XYAC  
917 Missile Road, Suite 3  
Sheppard Air Force Base, Texas 76311-2263  
DSN: 736-4089  
E-mail address: karla.puterbaugh@sheppard.af.mil or  
chris.lange@sheppard.af.mil

**Instructional Systems**

**Specialist:** Timothy B. Bush

**Editor:** Geri W. Lang

Air Force Institute for Advanced Distributed Learning  
The Air University (AETC)  
Maxwell Air Force Base, Gunter Annex, Alabama 36118-5643

In this volume, you will build upon basic chemistry principles that were discussed in the previous volume. You'll learn about specific analytes, their individual testing methods, and their diagnostic importance.

A glossary of terms, abbreviations and acronyms is located at the back of the volume. Use them as the text directs.

Code numbers on figures are for preparing agency identification only.

The use of a name of any specific manufacturer, commercial product, commodity, or service in this publication does not imply endorsement by the Air Force.

To get a response to your questions concerning subject matter in this course, or to point out technical errors in the text, unit review exercises, or course examination, call or write the author using the contact information on the inside front cover of this volume.

**NOTE:** Do not use the IDEA Program to submit corrections for printing or typographical errors.

Consult your education officer, training officer, or NCOIC if you have questions on course enrollment or administration, *Your Key to a Successful Course*, and irregularities (possible scoring errors, printing errors, etc.) on the unit review exercises and course examination. Send questions these people cannot answer to AFIADL/ ACSA, 50 South Turner Blvd, Maxwell AFB, Gunter Annex AL 36118-5643, on our Form 17, Student Request for Assistance. You may choose to complete Form 17 on the Internet at this site: [http://www.maxwell.af.mil/au/afiadl/registrar/download\\_fr.htm](http://www.maxwell.af.mil/au/afiadl/registrar/download_fr.htm).

**NOTE:** When you complete this course, please complete the student survey on the Internet at this site: [http://www.maxwell.af.mil/au/afiadl/operation/survey\\_fr.htm](http://www.maxwell.af.mil/au/afiadl/operation/survey_fr.htm).

This volume is valued at 18 hours and 6 points.

## Acknowledgements

The author would like to take a few moments to acknowledge those who assisted with the development of this volume. Their combined knowledge, experience and unique talents have helped to bring an informative, yet user-friendly, product to life.

Many thanks goes to Capt Halupa and Capt Morris and the rest of the Medical Laboratory Apprentice training course team for their assistance and innovation towards developing this course.

### NOTE:

In this volume, the subject matter is divided into self-contained units. A unit menu begins each unit, identifying the lesson headings and numbers. After reading the unit menu page and unit introduction, study the section, answer the self-test questions, and compare your answers with those given at the end of the unit. Then do the unit review exercises.

	<i>Page</i>
<b>Unit 1. Electrolytes, Blood Gases and pH, and Other Inorganic Metabolites.....</b>	<b>1–1</b>
1–1. Electrolytes.....	1–1
1–2. Blood Gases and pH .....	1–14
1–3. Other Inorganic Metabolites .....	1–23
<b>Unit 2. Hepatic Function, Carbohydrates, Enzymes, and Cardiac Markers .....</b>	<b>2–1</b>
2–1. Hepatic Function .....	2–1
2–2. Carbohydrates .....	2–12
2–3. Enzymes .....	2–24
2–4. Cardiac Markers.....	2–47
<b>Unit 3. Proteins, Lipids, Hormones, Therapeutic Drug Monitoring, Toxicology, and Tumor Markers.....</b>	<b>3–1</b>
3–1. Proteins.....	3–1
3–2. Lipids .....	3–11
3–3. Hormones .....	3–20
3–4. Therapeutic Drug Monitoring .....	3–31
3–5. Toxicology .....	3–36
3–6. Tumor Markers.....	3–46
<b><i>Glossary</i>.....</b>	<b><i>G–1</i></b>

**Please read the menu page for Unit 1 and begin ➔**

# Unit 1. Electrolytes, Blood Gases and pH, and Other Inorganic Metabolites

<b>1-1. Electrolytes.....</b>	<b>1-1</b>
201. Terms.....	1-1
202. Analytes evaluated as a single unit.....	1-5
<b>1-2. Blood Gases and pH.....</b>	<b>1-14</b>
203. Blood gases.....	1-14
204. pH function in the body .....	1-19
<b>1-3. Other Inorganic Metabolites .....</b>	<b>1-23</b>
205. Calcium and phosphorus.....	1-23
206. Magnesium and lithium .....	1-26
207. Iron studies .....	1-28

**A**CCORDING to the 29<sup>th</sup> edition of *Dorland's Illustrated Medical Dictionary*, an analyte is a substance undergoing analysis. As a laboratory technician, your job involves determining the concentrations of analytes. These analytes may include vitamins, minerals, proteins, and enzymes; they may be combinations of chemicals, metabolites, or by-products of bodily functions. Regardless of the substance, each has its own story to tell about the overall health of the patient. Your laboratory tests allow the provider to form the best diagnosis possible so we can provide the patient with the best quality healthcare available. Isn't that what we all want?

## 1-1. Electrolytes

Anyone who has watched an emergency room drama on television has heard the doctor call for a list of tests to be performed on the patient, including, "...lytes, STAT!" The term *lytes* refers to electrolytes, primarily sodium, potassium, chloride, and bicarbonate. These electrolytes are almost always performed together because a change in one is usually accompanied by a change in the others. As a laboratory technician, you'll perform countless sets of electrolytes because these tests are vital to diagnosing the patient's immediate status and their reaction to treatment.

### 201. Terms

One of the many keys to learning is having an understanding of the fundamentals of a subject. Acquiring a basic comprehension of the concepts and terms relating to electrolytes helps you apply meaning to the tests you perform. You'll also have a better understanding of the important role the laboratory plays in quality patient care and accurate laboratory testing.

### Body water

An electrolyte can be defined as a substance that conducts electricity through a solution. Water is the major component of the solutions found in the human body. In order for the body to regulate itself, a number of things must be correct; things that water and electrolytes must work hand-in-hand to accomplish. As you will learn later in this volume, the body must maintain a delicate balance of water, osmolality, electrolytes, pH, and blood gases. The ability of a patient to recover from a traumatic event or illness may depend on the ability to regulate body water and electrolytes. Water is found in spaces both inside and outside the cells of the body.

### Intracellular water

The term intracellular water describes water found within the cells.

### ***Extracellular water***

Extracellular water describes the water found outside of the cells. About two-thirds of the total body water is intracellular, while about one third is extracellular.

**Average Total Body Water of An Adult**

Male		Female*	
<b>Obese</b>	55%	<b>Obese</b>	45%
<b>Thin</b>	65%	<b>Thin</b>	55%
*Adult women usually average a 10 percent less overall than men.			

### ***Balance***

The intracellular and extracellular compartments of water must remain at a constant volume, a concept implying that the rate of water loss must equal the rate of water intake. This concept is known as *water balance*. The average adult takes in about 2,500 ml of water daily in the form of drink and food, and another 200 to 300 ml per day is formed in the body by tissue metabolism. The same amount of water gained daily by the body is released daily in the form of urine, feces, and sweat. Water balance is regulated by such factors as the body's thirst mechanism, the anti-diuretic hormone produced by the posterior pituitary gland, and the excretion or reabsorption of water by the kidneys.

### ***Imbalance***

Fluid imbalances can result from a simple illness. Think about when you have a cold. With a sore throat, you really don't want to drink or eat anything because it is hard for you to swallow. The body can compensate for this lack of fluid intake briefly, but soon an imbalance occurs. Vomiting, excessive urination, sweating, diarrhea, bleeding, or the oozing of fluids (called exudation) through burns or other skin injuries can also cause fluid imbalances.

### ***Nature of electrolytes***

In chemistry, the term *electrolytes* traditionally refer only to sodium, potassium, chloride, and bicarbonate. These analytes are the major ions in the blood. Remember that electrolytes conduct electricity in a solution and when they are placed in a solution they dissociate to produce ions—charged particles—in the solution. There are other electrolytes, to include calcium, magnesium, sulfate, and phosphate. Some of which are discussed separately later in this volume.

### ***Sodium chloride***

An example of an electrolyte is the compound sodium chloride, most commonly known as table salt. In water, it breaks down to form sodium ions ( $\text{Na}^+$ ) and chloride ions ( $\text{Cl}^-$ ). The sodium ion is a *cation* because it is positively charged, and the chloride ion is an *anion* because it is negatively charged. The cations most frequently measured in the laboratory are sodium and potassium. The anions most frequently measured are chloride and bicarbonate.

### ***Function***

Electrolytes function in many ways to sustain life processes. Their importance is such that almost every metabolic process in the body is dependent on or affected by electrolytes. Among the functions regulated by electrolytes are water distribution between the intracellular and extracellular fluids, osmotic pressure, and maintaining blood pH within the narrow range of 7.35 and 7.45. In addition, electrolytes influence the regulation of the heart and other muscles, the body's oxidation-reduction (electron transfer) reactions, cell permeability, and nerve impulse conduction. Due to their importance in body processes, electrolyte analysis is often performed as an emergency procedure. For many patients, an electrolyte imbalance cannot be tolerated very long and irreversible damage or death can result.

### ***Analysis***

When interpreting electrolyte results, the provider must consider two major aspects of the patient's



profile beyond the individual analysis of each electrolyte. First is the relationship between a particular electrolyte and the proper functioning of an organ or body function. For example, chloride levels have a direct relationship on the proper functioning of the stomach and pancreas. Second, each electrolyte must be evaluated in relationship to other electrolytes. For example, sodium and chloride concentrations have a direct effect on each other. This is why electrolytes are normally ordered and performed as a set. As a laboratory technician, you should have knowledge of the electrolyte's relationships to each other. This helps you evaluate the quality of your work and provide the provider with the best results. Never let your basic knowledge of these relationships influence the results you report; never *tweak* a result so it arrives at a logical balance with other results. What is logical to the laboratory may not be logical to the provider who is familiar with the patient's clinical condition.

### **Distribution of electrolytes**

Electrolytes are not equally distributed throughout the body. Their concentration depends on whether they are intracellular or extracellular in nature and the type of body tissue involved. Sodium ions are the principal cations of the extracellular fluid. Potassium ions make up the majority of the cations found in intracellular fluid. Chloride and bicarbonate anions are found predominately in extracellular fluid.

### **Osmosis**

As you know, osmosis is defined as the movement of solvent from a lower concentrated solution through a semipermeable membrane in order to achieve solute concentration balance. In most cases, it is the cell membrane that serves as the semipermeable membrane for osmosis in the body. One of the most significant solvents involved in osmosis is water. Water passes from an area of lower ion concentration to one of higher ion concentration. The physical force exerted from this process is called *osmotic pressure*. Keep in mind that osmotic pressure is due to the many ions both inside and outside the cells. Most clinical chemistry analysis is performed on serum or plasma because it is more practical to measure extracellular ions than intracellular ions. Evaluations of extracellular ion levels indicate the functional integrity of cells.

### **Ion concentrations**

In addition to serum or plasma analysis, valuable information can be obtained by analyzing ion concentrations in urine, spinal fluid, and sweat. The kidneys normally conserve water and electrolytes, which can otherwise be lost or excreted. If water loss is left uncompensated, the body can suffer from dehydration, which could have a profound effect on ionic concentrations and bodily functions. Dehydration can be brought on by a variety of conditions, including sustained fever, diarrhea, or inadequate fluid intake. Untreated water loss and ionic imbalance can lead to shock or even death.

At first, dehydration affects the extracellular fluid volume; this can cause the patient's blood pressure to fall. This is due to loss of extracellular water and osmotic pressure differences formed between the extracellular water in the plasma and the intracellular water within the cells. The extracellular water naturally transfers into the cells when the concentration of ions within the cells exceeds the ion concentration of the extracellular water. If the patient continues to lose water, the extracellular fluid ion concentration may eventually exceed that of the interior of the cells; water then leaves the cells to achieve an ionic balance. You can see where the timely and accurate assessment of a patient's electrolyte balance during dehydration is critical to the proper treatment of the patient. This is just one condition where electrolyte analysis is useful to the provider.

You know that the electrolyte concentration by itself neither fully evaluates the patient's fluid volume nor completely reveals accurate information about total electrolyte gain or loss. For example, sodium and potassium can be lost from the body, and as long as there is a proportional loss of water, there is very little visible effect on electrolyte concentration. This is because concentration depends on the amount of solute and the volume of solvent; if lost proportionally, detection is difficult. The provider can typically diagnose the patient using both the laboratory results and the clinical picture of the patient.

**Anion gap**

Electrolyte analysis usually includes the measurement of sodium, potassium, chloride, and bicarbonate. Automated chemistry analyzers can perform this set of tests quickly. In addition to the individual analyte results, analyzers can provide supplemental data information calculated from the electrolyte results. This data can be both clinically significant and useful to the laboratory technician. One example of this is the *anion gap*.

**Calculation**

The anion gap is computed mathematically by subtracting the total chloride and bicarbonate anions from the total sodium cations. The resulting difference between the anions and cations is the anion gap.

**Formula**

The anion gap formula looks like this:

$$\text{Anion gap} = (\text{Na}^+) - (\text{Cl}^- + \text{HCO}_3^-)$$

**Use**

To further illustrate this, let's use the formula. Given the following information, calculate the patient's anion gap: sodium = 135 mmol/l, chloride = 100 mmol/l, and bicarbonate = 25 mmol/l. If you came up with an answer of 10 mmol/l, you were correct. By plugging in the numbers provided, your solution equation should have looked like this:

$$\text{Anion gap} = (135 \text{ mmol/l}) - (100 \text{ mmol/l} + 25 \text{ mmol/l})$$

$$\text{Anion gap} = 135 \text{ mmol/l} - 125 \text{ mmol/l}$$

$$\text{Anion gap} = 10 \text{ mmol/l}$$

**Meaning**

Now that you know how to calculate the anion gap, what does it mean? First, the very term anion gap is a misnomer. If the body fluids are to remain balanced, the total positive charges (positive ions,  $\text{Na}^+$ ,  $\text{K}^+$ , and other cations) MUST equal the total negative charges (negative ions,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ , and other anions.) At no time is there a deficiency of anions in relation to cations. The term *anion gap*, in fact, refers to an analytical gap showing the difference between the unmeasured anions and the unmeasured cations.

**Disagreement**

There's some disagreement about what is considered a normal anion gap. Although most current literature determines the anion gap using the formula explained above, there are some sources that use sodium plus potassium rather than sodium alone for the cation number. Normal values can also differ because they are based on different patient populations. Most reference books calculate the anion gap using *only* the sodium measurement. A common reference range used is 8 to 16 mmol/l with a mean of 12 mmol/l. Be sure any statements you make concerning the anion gap values are based on the testing methods you are using and patient reference ranges established for your facility.

**Normal balance**

In normal patients, there is a balance between the cations and anions. An increased production or retention of organic acids, as well as other abnormal physiological factors can disturb this balance. Increases in the anion gap are seen whenever there is a decrease in the unmeasured cations or an increase in the unmeasured anions. Some of the physiological reasons for an increased or decreased anion gap are listed in the following table.

Increased Anion Gap	Cause
Decreased unmeasured cations.	Hypocalcemia, hypomagnesemia, hypokalemia.
Increased unmeasured anions ( $\beta$ -hydroxybutyrate, acetoacetate).	Ketoacidosis caused by diabetes, alcoholism, or starvation.
Increased unmeasured anions (Lactate).	Lactic acidosis.
Increased unmeasured anions (Hippurate, glycolate, oxalate).	Poisoning caused by aspirin, methanol, ethanol, or ethylene glycol.
Increased unmeasured anions (Sulfate, phosphate).	Retained due to renal failure.
Decreased Anion Gap	Cause
Decreased unmeasured anions.	Hypoalbuminemia.
Increased unmeasured cations.	Hypercalcemia, hypermagnesemia, hyperkalemia, or lithium toxicity.

### ***Abnormal balance***

Anytime an anion gap value is outside the normal range, you must investigate your findings. Begin by rechecking your calculations, and then retest the sample. If possible, use a different method or instrument to perform the repeat testing. In itself, an initial abnormal anion gap value is not diagnostically significant. But once it has been determined the testing and retesting are valid, the significance of the anion gap value increases. The provider combines the anion gap values with other data, such as patient history, other laboratory tests, and physical examination, to determine its diagnostic significance. Anion gap values of 15–20 mmol/l are commonly seen in patients undergoing renal failure. Values of >25 mmol/l are diagnostic of diabetic patients, with >300 mg/dl plasma glucose levels, experiencing metabolic ketoacidosis.

### ***Quality control***

Calculation of the anion gap is also a valuable tool in the quality control of electrolyte testing. If you start to see a trend of low or high anion gaps in patient samples, this indicates there may be a consistent error in at least one of your analytes. Realize the anion gap lies within given reference ranges; results shifting away from that range indicate a need to investigate your calculations and procedures. The possibility of a testing error is why you always review your calculations and procedures *before* reporting out what may be an abnormal anion gap. Remember the anion gap is a calculation that can indicate a problem with your equipment or an abnormality in the patient. It's your responsibility to determine which applies.

## **202. Analytes evaluated as a single unit**

As you have learned from the previous lesson, electrolytes are normally ordered as a test battery. The following analytes are generally ordered and evaluated as a single unit—they are sodium, potassium chloride, and bicarbonate. In this lesson, you'll look at each analyte and learn how it contributes to the overall assessment of the patient.

### **Sodium**

Sodium is the major cation of extracellular fluid. In fact, sodium makes up more than 90 percent of all positively charged ions in the plasma. The average sized adult has about 80g of sodium in their body. Of this total amount, roughly 35g of sodium can be found in the extracellular fluids. The daily dietary intake of sodium is usually quite substantial, averaging about 3g daily. Even though the amount of sodium consumed varies with the amount and types of food eaten, the amount of sodium in the body remains fairly constant. Most sodium not required by the body is excreted in the urine, and to a lesser extent, through perspiration. When sodium salt is ingested and absorbed by the body, this action causes a temporary increase in the extracellular fluid volume. This is because the absorbed sodium ions, and the water that carries them, equilibrate between the plasma and the interstitial fluid. Interstitial fluid is an extracellular fluid that is found between the cells of the body. A small

temporary exchange of sodium and potassium occurs inside the cells. Keep in mind the sodium content of the fluid outside the cell is about 10 times that of the sodium inside the cell. Since the cell membrane is semipermeable, the sodium ions are pumped out of the cell and the potassium ions are pumped into the cell. The kidneys are the ultimate regulator of the sodium levels in the body. The kidneys reabsorb the sodium amounts required to maintain balance, while excesses are excreted. Hormones also play a part in maintaining sodium concentration in the body. Aldosterone, a hormone secreted by the adrenal cortex, influences the kidneys to reabsorb sodium. Aldosterone also accelerates the exchange of sodium and potassium ions across cell walls. This exchange helps the body retain sodium and excrete potassium.

### ***Clinical significance***

Sodium levels are more commonly decreased than increased.

#### ***Hyponatremia***

Decreased plasma concentration of sodium is known as hyponatremia. Predictably, when a patient is not feeling well, they may not be eating properly and not ingesting enough sodium. Add this to the fact the patient may be losing sodium due to diarrhea, prolonged vomiting, or sweating due to fever. Sodium may also be lost to a variety of other factors, some of which are:

- Sodium levels can decrease due to metabolic acidosis caused by diabetes mellitus. Just before a patient goes into a diabetic coma, large amounts of sodium and potassium are excreted into the urine as salts of ketoacids.
- Renal disease can diminish the tubular reabsorption of sodium by the kidneys. A patient's inappropriate choice, dose, or use of diuretics can also cause this diminished activity.
- Addison's disease is a condition in which there is a decreased secretion of aldosterone affecting sodium levels. Aldosterone is a hormone that regulates the salt and water balance in the body. Decreased levels of the hormone lead to decreased salt levels.

#### ***Hypernatremia***

Increased plasma concentration of sodium is known as hypernatremia. This can be caused by any of the following conditions:

- Severe dehydration.
- Cushing's Syndrome – A condition that results from the overproduction of adrenal steroids causing an excessive reabsorption of sodium in the renal tubules.
- Following the insulin treatment of comatose diabetic patients, the sodium in the patient's cells is replaced by potassium.
- Inappropriate therapy with saline solution or feedings of high-sodium content foods without sufficient fluid intake.

### ***Laboratory procedures and limitations***

Sodium testing is performed by atomic absorption spectrophotometry, flame emission photometry, or electrochemically using a sodium ion-selective electrode (ISE). Of these methods, two are commonly used in the laboratory—ISE and flame emission photometry. You should note that flame emission photometry is rapidly being replaced by ISE methods. When analyzing sodium by ISE, the measurement corresponds to the activity of the ion in the water-volume fraction in which it is dissolved. Sodium electrodes have glass membranes that selectively exchange sodium ions. As the sodium ion interacts with the membrane of the ISE, a potential-measuring circuit between the measuring electrode and the reference electrode determines a change in the electromotive force. This change is proportional to the sodium concentration in the sample. Normally, sodium analysis is

performed directly on whole blood or serum, provided the standards used are similar in ionic strength to that of the samples.

#### *Categories of error*

There are two categories of error commonly observed when performing ISE sodium analysis. The first category includes lack of selectivity of the ISE, protein coating of the ion-sensitive membrane, and contamination of the membrane by competing ions—all of which can alter the final result. The second applies to sampling methods. For example, when samples are diluted prior to testing, it is called an *indirect method* of testing. Indirect methods are affected by samples with high lipid or protein content. If the volume of the sample tested has a disproportionate amount of lipid or protein in it, the lipids or proteins displace fluid that would normally contain sodium ions and considerably affect test results.

#### *Collection*

Serum, heparinized plasma, whole blood, sweat, urine, feces, and gastrointestinal fluids can be analyzed for sodium. Timed collections of urine, feces, or gastrointestinal fluids are required to allow comparison of sodium values to a reference standard or for the calculation of rates of electrolyte loss. Urine, plasma, and serum can be stored at 2 to 4°C or frozen if testing is delayed. Hemolyzed samples normally do not affect sodium analysis because red blood cells contain approximately one-tenth of the sodium found in the serum or plasma, except when the hemolysis is severe, in which case a dilutional effect could occur.

#### **Potassium**

Potassium is primarily an intracellular cation. Only a small amount, roughly 2 percent of the total body potassium, is extracellular. Most adults consume about 2 to 3g of potassium in their diet daily. That same amount is excreted daily in the form of salts. Potassium is absorbed rapidly in the intestinal lumen but has little effect on the plasma levels. After ingestion, the increase is slight and very brief. Once the needs of the body tissues are met, the kidney excretes the excess potassium. Potassium levels, like sodium levels, are influenced by adrenocortical hormones.

#### *Clinical significance*

Potassium plays an important part in the muscle activity of the body. Elevated concentrations of potassium can inhibit the irritability of the muscles. Remember the heart is a muscle. By inhibiting the irritability of the heart, the heart becomes paralyzed and ceases to beat. Low potassium levels are also cause for concern. Low potassium levels increase the irritability of the heart to the point the heartbeat ceases during systole contraction. Low potassium levels are usually corrected by intravenous fluid therapy. Changes in heart irritability caused by increased or decreased potassium levels can be detected by altered electrocardiographic patterns.

#### *Hyperkalemia*

Increased plasma levels of potassium are known as hyperkalemia. This condition occurs when potassium leaves the cells at a rate faster than the kidneys can excrete it. This overload happens in conditions of anoxia and metabolic or renal tubular acidosis. In fact, one of the purposes of kidney dialysis is to remove the accumulated plasma potassium. Hyperkalemia can also be caused by shock or circulatory failure, dehydration, decreased aldosterone production (Addison's disease), or severe red blood cell destruction as in a transfusion reaction. Regulating the potassium level of a patient in renal failure is a major concern for the provider.

#### *Hypokalemia*

Decreased plasma levels of potassium are known as hypokalemia. This condition occurs as a result of low potassium intake over a period of time or an increased loss of potassium through vomiting, diarrhea, gastrointestinal problems, or long-term use of diuretics. The fluids in the gastrointestinal tract have high potassium content, and their removal or loss can have serious implications for the

patient. In addition, increased aldosterone production causes increased potassium excretion by the kidneys. Low potassium levels are frequently found in chronic starvation and post-operative patients. The potassium level of a post-operative patient may be decreased because of receiving large amounts of fluid low in potassium. This is one of the reasons why you often see post-operative potassium testing requested. Low potassium levels can result in cell damage, particularly to the cells of the kidney tubules.

### ***Laboratory procedures and limitations***

Potassium testing is performed by atomic absorption spectrophotometry, flame emission photometry, or electrochemically using a potassium ISE. The ISE method is most commonly used. The ISE method for potassium is very similar to the one described for sodium, except the potassium ions react with a liquid ion-exchange membrane that incorporates valinomycin. Since most of the body's potassium is intracellular, hemolysis in a sample can greatly affect the potassium level of a sample. Take great care not to hemolyze samples for potassium analysis. Should your testing method use whole blood, you may want to centrifuge a small portion of the sample to visually check for hemolysis.



### **Chloride**

Chloride is the major extracellular anion, and probably the most important anion. Its purpose is to counterbalance sodium in extracellular fluids and maintain the required electrical neutrality of the body fluids. Electrical neutrality simply means the total charges of the cations must equal the total charges of the anions. The concentration of chloride can vary at times due to any number of conditions. Chloride levels vary inversely with bicarbonate levels due to the electrochemical neutrality just mentioned. In metabolic acidosis, chloride levels rise as bicarbonate levels decrease. In metabolic alkalosis, the reverse is true; chloride levels decrease while bicarbonate levels increase.

Chloride is found in the serum, plasma, cerebrospinal fluid, tissue fluid, and urine. With the exception of red blood cells, chloride ions do not enter the cells and are found only in extracellular spaces. In the blood, about two-thirds of the chloride is found in the plasma. The remaining one-third is found in the red blood cells. About 2.5g of chloride is consumed daily by the average adult. Chloride is normally consumed in the form of a salt, such as sodium chloride, potassium chloride, calcium chloride, or magnesium chloride. Chloride is readily absorbed in the intestines, and excesses are removed in urine or perspiration.

Chloride has two primary functions. First, chloride, along with sodium, represents the majority of the osmotically active components of the plasma. They have significant involvement in maintaining the osmotic pressure, water distribution, and anion-cation balance in the extracellular fluids. Second, chloride is important in maintaining the body's acid-base balance by playing a role in the exchange of oxygen and carbon dioxide in the red blood cells. When blood is oxygenated, chloride travels from the red blood cells to the plasma. At the same time, bicarbonate leaves the plasma and enters the red blood cell. During carbon dioxide transport, bicarbonate exits in the red blood cells and is replaced by an equivalent amount of chloride anions.

### ***Hypochloremia***

Decreased plasma levels of chloride are called hypochloremia. Hypochloremia occurs in some forms of metabolic acidosis when there is an increased formation or decreased excretion of organic acids, such as diabetic ketoacidosis or renal failure. Gastric secretions contain a high concentration of  $H^+$  and  $Cl^-$ . When there is prolonged vomiting or intestinal blockage, chloride levels decrease. Low chloride values are seen in Addison's disease and in metabolic alkalosis when plasma chloride levels tend to decrease while bicarbonate levels increase.

### ***Hyperchloremia***

Increased plasma chloride levels are called hyperchloremia. These high concentrations usually occur in cases of dehydration, renal tubular acidosis, acute renal failure, and during metabolic acidosis associated with prolonged diarrhea and loss of sodium bicarbonate. Slight increases in chloride levels can occur during respiratory alkalosis. Hyperchloremia can result when extremely large amounts of salt are consumed, or a patient is over-treated with saline solutions.

### ***Laboratory procedures and limitations***

Chloride is commonly assayed in serum, plasma, urine, or sweat. Since two-thirds of the chloride in the blood is found in the plasma, plasma or serum is most often requested for chloride analysis. Sampling methods should eliminate any chances of chloride contamination and testing error. Tap water can contain high levels of chlorine, so ensure glassware or pipettes are free of any residual chlorine. Do *not* use sodium fluoride tubes for chloride analysis because fluoride is a halogen, just like chloride, and they both react the same way in an assay. Remove serum or plasma from the red blood cells as soon as possible and limit exposure to room air to prevent a chloride shift. A chloride shift occurs when gaseous carbon dioxide is lost from the red blood cells altering the distribution of chloride ions between the cells and plasma. Moderate hemolysis in the serum or plasma does not significantly affect the concentration of chloride in serum or plasma.

### ***Analysis***

The most common method for chloride analysis in serum, plasma, or whole blood is ISE. The membrane for the chloride ISE is a composite of silver sulfide ( $\text{Ag}_2\text{S}$ ) and silver chloride ( $\text{AgCl}$ ). The membrane selectively admits all halogen ions (fluoride, bromide, chloride, and iodide). Patients taking medication that contain bromides have altered serum chloride results. All serum chloride methods fail to distinguish chloride from bromide. Chloride can also be determined by colorimetric methods found in many automated analyzers. Chloride ions in the sample react with undissociated mercuric thiocyanate to form undissociated mercuric chloride and free thiocyanate ions. The released thiocyanate reacts with a ferric nitrate reagent to form a red color. This red color is read colorimetrically. This procedure is temperature sensitive, so ensure your analyzer is within the proper temperature range.

### ***Cerebrospinal fluid***

The chloride concentration of cerebrospinal fluid (CSF) is higher than that of serum because CSF has a lower protein concentration and thus fewer protein anions. The chloride level of CSF decreases in cases of bacterial meningitis because the bacteria increase the protein concentration in the fluid. CSF chloride analysis is normally performed in the same manner as serum.

### ***Urinary chloride***

Urinary chloride levels vary greatly depending on dietary intake. Patients on low-salt diets excrete very little chloride. Urine tests for chloride should only be run on accurately timed collections. The pH of the urine sample should be adjusted to a pH of 3 with dilute nitric acid. Urine chloride analysis is performed using the same methods as serum chloride testing. Sample dilution may be required prior to testing when the chloride levels are extremely high.

### ***Sweat chloride***

Sweat contains significant amounts of chloride. In the disease cystic fibrosis, the concentration of chloride ions in sweat is elevated because of a defect in the gene and corresponding protein known as cystic fibrosis transmembranous conductance regulator. Sweat chloride tests are usually requested on children as a cystic fibrosis screening test. An adequate sweat sample, usually about 50 mg, is collected from the arm of the child. The thigh may be used if the child is very small. It's very dangerous to induce total body sweating so usually sweating is induced in only a small area. Sweating is usually induced by using the drug Pilocarpine and equipment that is commercially available just for this purpose. Once the sweat is collected, it is analyzed using coulometric-amperometric methods

(discussed under chemistry analyzer principles later in this volume). Although chloride can be detected in sweat by ISE methods, the Cystic Fibrosis Foundation does not accept testing by this method.

### **Bicarbonate**

The bicarbonate ( $\text{HCO}_3^-$ ) ion is also a major extracellular anion. In many textbooks and reference manuals, bicarbonate is also called total carbon dioxide or just carbon dioxide. Although interchanging these terms is acceptable in many cases, it can be confusing. Be sure to use the proper context of the term when determining its meaning. Bicarbonate in plasma can consist of carbon dioxide in physical solution, carbon dioxide bound to amine groups in proteins, as bicarbonate ions ( $\text{HCO}_3^-$ ), carbonate ions ( $\text{CO}_3^{2-}$ ), or as carbonic acid ( $\text{H}_2\text{CO}_3$ ). When the blood passes through the lungs, carbon dioxide and water are formed to be expelled during exhalation, normally leaving only small amounts of carbon dioxide in the plasma. During metabolic processes, carbonic acid dissociates, and forms bicarbonate. The kidneys filter bicarbonate, with all of it being reabsorbed in the proximal and distal tubules. Very little, if any, bicarbonate is normally found in the urine. Bicarbonate is measured together with all other forms of carbon dioxide and expressed as total  $\text{CO}_2$ . Since about 90 percent of all the carbon dioxide in the blood is in the form of bicarbonate, this combined form closely approximates the actual bicarbonate amount.

### ***Clinical significance***

Changes in the bicarbonate and carbon dioxide content of the plasma are characteristic of acid-base imbalances. The nature of the imbalance cannot be determined based on the bicarbonate value alone. It must be viewed in relation to the other electrolytes.

#### ***Increased***

The bicarbonate content of plasma and serum is *increased* in metabolic alkalosis, compensated respiratory acidosis (a sudden stop in breathing—can be caused by drugs, a blockage, or a long-term disease, such as pulmonary disease), or alkalosis accompanying a large potassium deficiency.

#### ***Decreased***

The bicarbonate level of plasma and serum is *decreased* in metabolic acidosis and compensated respiratory alkalosis (hyperventilation—deep, rapid breathing).

### ***Laboratory procedures and limitations***

Bicarbonate testing can be performed on either serum or heparinized plasma. Avoid using other anticoagulants as they can upset the balance between the red blood cells and plasma  $\text{CO}_2$ . Venous blood is normally collected in an evacuated tube, although capillary blood collected in microcontainers or capillary tubes may be used. The bicarbonate concentration is most accurate if it is determined shortly after collection and immediately after the tube is opened. Ambient (room) air contains far less  $\text{CO}_2$  than plasma does. If the sample is left open and exposed to room air, the  $\text{CO}_2$  in the sample escapes into the room air causing falsely decreased results. Bicarbonate analysis can be performed by enzymatic, colorimetric, and ISE methods.

#### ***Methods***

In the automated enzymatic method, all forms of  $\text{CO}_2$  present in the sample are converted to bicarbonate by the addition of a base. The bicarbonate is then converted to oxaloacetic acid by adding the enzyme phosphoenolpyruvate carboxylase. The amount of oxaloacetic acid formed can be monitored spectrophotometrically by measuring its conversion to malate by malate dehydrogenase with a corresponding consumption of an ultraviolet light chromogen.

In automated colorimetric methods, the  $\text{CO}_2$  in the sample (regardless of its form—bicarbonate, carbonic acid, bound to protein amine groups) is released by the addition of an acid. The gaseous  $\text{CO}_2$  is passed through a silicone-rubber, gas-dialysis membrane into a buffered solution of cresol red at a



pH of 9.2. As the CO<sub>2</sub> passes through the membrane, it lowers the pH of the buffered cresol red solution. The decrease in color intensity is proportional to the CO<sub>2</sub> content and is measured spectrophotometrically. An ISE method, commonly used by other instruments, utilizes the *p*CO<sub>2</sub> electrode to measure the CO<sub>2</sub> released by addition of acid to the serum sample.

---

### Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

#### 201. Terms

1. What are the body processes that water and electrolytes work hand-in-hand to accomplish?
2. What are intracellular and extracellular water?
3. What is the concept of water balance?
4. List the factors that regulate water balance?
5. What are the six causes of fluid imbalances?
6. In chemistry, the term *electrolytes* refer to what analytes?
7. What two electrolytes are components of table salt?
8. What are some bodily functions regulated by electrolytes?
9. Why is electrolyte analysis often performed as an emergency procedure?
10. In what fluids do you find sodium, potassium, chloride, and bicarbonate?
11. What is osmotic pressure?

12. Why is most clinical chemistry analysis performed on serum or plasma?
13. In what fluids do you find sodium, potassium, chloride, and bicarbonate?
14. What conditions bring on dehydration?
15. How do you calculate an anion gap?
16. Given the following patient electrolyte values—sodium=130 mmol/l, chloride = 100 mmol/l, and bicarbonate = 25 mmol/l—what is the anion gap?
17. What are two conditions that cause an increased anion gap?
18. When does an abnormal anion gap become clinically significant?
19. In addition to clinical diagnostic applications, how else does a laboratory technician use anion gap results?

**202. Analytes evaluated as a single unit**

1. What is the major cation of extracellular fluid?
2. What organ regulates sodium levels?
3. What hormone helps to regulate sodium levels?
4. What is hyponatremia and what causes it?
5. What is hypernatremia and what causes it?

6. How does the sodium ISE work?
7. How are indirect methods of sodium analysis affected by samples with high lipid or protein content?
8. Potassium is primarily what type of cation?
9. How is potassium absorbed and excreted?
10. How do potassium levels affect the heart?
11. What is hyperkalemia and what causes it?
12. What is hypokalemia and what causes it?
13. How does hemolysis affect potassium analysis?
14. Chloride is an anion found in what type of fluid?
15. Chloride is *only* found inside of what type of cell?
16. What two primary functions does chloride serve?
17. What is hypochloremia and what causes it?
18. What is hyperchloremia and what causes it?
19. Chloride contamination, prior to analysis, can result from what sources?

20. What is the chloride ISE membrane made of and what medications can affect this test method?
21. Why is bicarbonate sometimes referred to as total carbon dioxide?
22. What conditions cause increased bicarbonate levels?
23. What conditions cause decreased bicarbonate levels?
24. What are sources of error affecting samples for bicarbonate analysis?
25. What are three methods commonly used for bicarbonate analysis?

## 1-2. Blood Gases and pH

The measures taken to preserve life in patients with cardiopulmonary problems have increased greatly with the development of instruments that can accurately measure oxygen and carbon dioxide in blood and expired air. These patients can now be treated with a mixture of gases tailored to their needs based in part on our laboratory findings. Today's blood gas instruments are extremely complex but very easy to operate. In many facilities, the responsibility for performing blood gas analysis is removed from the laboratory and given to special teams from respiratory therapy or a special cardiopulmonary laboratory. Regardless of who performs the blood gases, you still need to understand the significance of the analysis and its impact on patient care.

### 203. Blood gases

Blood gas analysis is the study of arterial or venous blood for the purpose of determining oxygen and carbon dioxide levels in the bloodstream, and to also measure the hydrogen ion concentration (pH) of the blood. Determination of these parameters is very important to the diagnosis and treatment of many diseases and is essential to determining patient progress in many emergency room settings. In order to understand blood gases, you first need to understand a few terms.

#### Terms

At first glance, the various abbreviations for all of the blood gas analytes can seem pretty overwhelming and almost a new language by themselves. After learning what each abbreviation means and linking them together with the analytes you will be testing for, it becomes simple. The following table shows some of the more commonly used prefixes and abbreviations used in blood gas terminology.

**Prefixes and Abbreviations Used for Blood Gas Terminology**

General Prefix	What it Means	How it is Used
<i>p</i>	Partial pressure or tension.	$pO_2$ , $pCO_2$
<i>s</i>	Saturation fraction	$sO_2$

Specimen Origin (lower case letters)		Specimen Type (upper case letters)	
a	Arterial	B	Whole blood
v	Venous	P	Plasma
c	Capillary		

## Forms

Now that you have seen some of the preferred abbreviations, let's begin to link them together with the forms you will be testing for.

Form	Explanation
$p\text{CO}_2$	The measure of the partial pressure of carbon dioxide in the blood. The word partial is used because it refers only to the amount of pressure exerted by a particular gas in a mixture of gases. $p\text{CO}_2$ is sometimes referred to as the respiratory component in acid-base determinations because the lungs primarily control this value. As the $\text{CO}_2$ level increases, the blood pH decreases ( $\text{CO}_2$ and blood pH are inversely proportional).
$p\text{O}_2$	Refers to the partial pressure of oxygen dissolved in the blood plasma.
$s\text{O}_2$	The $s\text{O}_2$ describes the percentage of available hemoglobin that is saturated with oxygen. Body tissues are adequately provided with oxygen when 95 to 100 percent of the hemoglobin carries oxygen. Since the $p\text{O}_2$ and the $s\text{O}_2$ are concerned with oxygen transport, we must look at them both when determining the adequacy of oxygenation.
$\text{HCO}_3$	Indicates the metabolic (renal) component of the acid-base equilibrium. As bicarbonate levels increase, so does the blood pH. The relationship of bicarbonate to pH is directly proportional.
pH	An expression of the extent to which the blood is acidic or alkaline. The pH is used to measure the acid-base balance of the body. A normal pH is essential to enzyme function and other biochemical processes in the body.

## Physiology

Blood gases are used to evaluate the patient's ability to transport oxygen. In addition, they are diagnostic in cases where there is a disturbance of the fluid or electrolyte balance. Because the lungs and kidneys act as regulators of the acid-base balance, a patient with a disorder affecting these organs is probably evaluated with blood gas analyses.

### External respiration

Lets look at the process of respiration. Respiration is responsible for supplying the oxygen needed for cellular metabolism and for removing the carbon dioxide waste product formed by those processes. The exchange of oxygen and carbon dioxide in the lungs is called *external respiration*. When a person inhales, air is taken into the lungs. The lungs are made up of successively smaller passages ending at the alveoli. Alveoli are small, sac-like chambers with very thin walls that are close to pulmonary capillaries. Gases are exchanged across the alveolar and capillary membranes. Oxygen travels from the lungs into the bloodstream and carbon dioxide travels from the bloodstream into the lungs. As a person exhales, carbon dioxide is expelled from the body. Breathing is simply these processes occurring on a continuous basis.

### Dalton's law

In order to understand external respiration a little better, let's look at the entire process in more detail. The concept of partial pressure is summed up by Dalton's law that states, "Each gas in a mixture exerts pressure on the outside walls of a container." If you were to add up the pressures of all the gases in the mixture, you would get the total pressure inside the container. For our purposes, the containers are our lungs, and the pulmonary capillaries and the gases are oxygen and carbon dioxide.

### Henry's law

Another law, Henry's law, states, "The amount of gas dissolved in a liquid, at a constant temperature, is directly proportional to the partial pressure of the gas and how soluble it is." So what does all this mean in plain English? Remembering back to the lesson regarding the concept of osmosis, you'll

recall that substances in an area of higher concentration diffuse to an area of lower concentration. This is what happens in the lungs. The partial pressure of oxygen in the inhaled air (in the lungs) is higher than the partial pressure of oxygen in the pulmonary capillaries so oxygen diffuses from the lungs into the bloodstream. The partial pressure of carbon dioxide is higher in the bloodstream than that of the inhaled air so it diffuses outward into the lungs and out of the body. Partial pressure is measured in millimeters of mercury (mm Hg). See figure 1-1 to help visualize how partial pressure affects gas exchange.

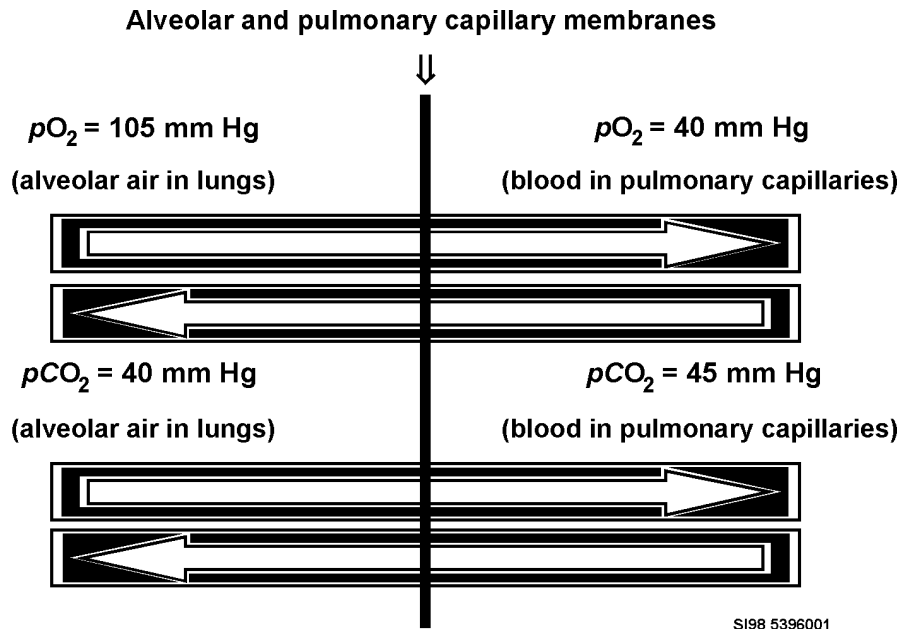


Figure 1-1. Gas exchange across alveolar and pulmonary membranes.

### ***Internal respiration***

The oxygen added to the blood from the lungs is of no use to tissue cells until they receive it. Carbon dioxide released by the cells also needs to be transported to the lungs so it can be expelled. The gas exchange of oxygen and carbon dioxide that takes place between the plasma in the capillaries around the tissue cells and the tissue cells themselves is called *internal respiration*.

### ***Oxygen in blood***

The blood obviously plays a crucial role in internal and external respiration. The amount of oxygen found in the blood plasma is very small, about 2 percent. The remaining 98 percent is bound to the hemoglobin in the red blood cells.

### ***Oxyhemoglobin***

Hemoglobin bound to oxygen is called oxyhemoglobin.

### ***Carbaminohemoglobin***

Hemoglobin with bound carbon dioxide is called carbaminohemoglobin. Arterial blood carries oxygenated blood to the tissues while venous blood carries carbon dioxide-laden blood from the tissues back to the lungs.

### ***Chemoreceptors***

The gas exchange that takes place between the blood and the tissue cells of the body is similar to the gas exchange in the lungs. The partial pressure of oxygen in arterial blood is about 90 mm Hg, and the partial pressure of carbon dioxide is about 40 mm Hg. At the surface of the tissue cells, the partial pressure of oxygen is only about 20 mm Hg and carbon dioxide is about 60 mm Hg. Once again, the

gradient for oxygen moves inward into the tissue cells and the carbon dioxide moves outward into the bloodstream. Breathing is controlled by respiratory control centers located in the brain and is based on the needs of the body. Chemoreceptors (special cells adapted for excitation by chemical substances) are sensitive to the levels of partial pressure of oxygen and carbon dioxide, sending impulses to the brain to speed up or decrease breathing as needed.

### **Clinical significance**

Blood gas analysis provides valuable information for use in assessing and managing a patient's respiratory and metabolic problems. When patients are treated with oxygen-enriched air, their  $pO_2$  is greatly increased. Exercise in both healthy individuals and cardiac patients results in a temporary increase in oxygen levels. Deficient oxygenation of the blood, called hypoxemia, is usually a medical emergency. One or more of the following conditions can cause hypoxemia:

- Decreased  $pO_2$  due to high altitudes or entry into areas low in oxygen (such as a room filled with carbon monoxide).
- Hypoventilation due to suffocation, submersion, trauma, or drug-induced respiratory failure (overdoes of barbiturates or morphine).
- Premature mixing of venous and arterial blood in cases such as congenital heart disease, pneumonia, pulmonary edema, or shock.
- Inability to oxygenate arterial blood due to diffusion difficulties such as pneumonia, respiratory distress syndrome in newborns or adults, and lung cancer.

### ***Hypercapnia***

Increased blood  $pCO_2$ , hypercapnia, causes respiratory acidosis. Increases in  $pCO_2$  occur when there is decreased alveolar ventilation due to lung disease, airway blockage, or by breathing  $CO_2$ -enriched air. Drug usage, which depresses the rate and depth of breathing, can also lead to increased levels of retained  $CO_2$ . In metabolic alkalosis, the lungs attempt to compensate by retaining  $CO_2$  (thus increasing  $pCO_2$ ) so that pH is lowered.

### ***Hypocapnia***

Decreased  $pCO_2$  is known as hypocapnia. This condition can be caused by increased breathing rates. Breathing rates can be increased artificially in cases such as mechanical ventilators, or naturally as in cases of hyperventilation due to stress, pain, or emotions. More information on the interaction of  $pCO_2$  and  $pO_2$  in respiratory and metabolic conditions is discussed in the pH lesson.

### **Laboratory procedures and limitations**

The correct collection of blood gas specimens is critical to accurate analysis. Factors, such as time, temperature, and exposure to ambient air, must be taken into account.

### ***Sample collection***

Blood is generally used to perform blood gas analysis but there are facilities that have equipment to analyze exhaled air. Arterial blood is the specimen of choice for blood gas analysis but venous blood can also be used. Partial pressure of oxygen ( $pO_2$ ) analysis can only be performed on an arterial blood sample. Venous samples are adequate when the provider is only requesting a pH and partial pressure of carbon dioxide ( $pCO_2$ ) analysis. Collect venous blood without stasis (no tourniquet) and without the patient clenching their fist. Prolonged application of a tourniquet or muscular activity from fist clenching decreases venous  $pO_2$  and allows acidic metabolites to accumulate. Be aware that collecting samples for blood gas analysis is extremely difficult, thus making the sample a very precious commodity, so handle it with care. This is especially true when the sample is from a neonate and the amount of sample has to be as small as possible. Laboratory technicians rarely have anything to do with the actual collection of the sample. Because of the medical risks involved with collecting blood gas samples, most hospitals only allow providers or specially trained personnel to perform

collections. Under no circumstances should you attempt to collect a blood gas sample without receiving specialized training or certification.

### ***Factors affecting sample collection***

Blood gas samples are best collected anaerobically using liquid heparin as the anti-coagulant. Many times, glass syringes are used because plastic syringes can allow gas exchange through the barrel wall. In most cases, prepackaged disposable sets are used. The syringes in these sets normally range from 1 to 5 ml and contain an appropriate amount of anti-coagulant. The sets have a rubber plug or block for the needle to be imbedded into following collection. This is done so the syringe can be transported safely, without having to remove the needle, and to minimize gas exchange between the sample and the room air.

Knowing the proper specimen-handling methods is important to your safety and the accurate analysis of the sample. Take care not to expose the sample to room air for any longer than is absolutely necessary. Exposing a sample to room air normally increases the  $pO_2$  in the sample because room air has a higher concentration of oxygen than arterial blood. One exception is a sample from a patient undergoing oxygen therapy. These patients have arterial blood concentrations higher than room air, so exposure of the sample may falsely decrease their  $pO_2$  result. Exposure to room air decreases the  $pCO_2$  in the sample because the concentration of carbon dioxide in the sample is much higher than that in room air. As the sample loses carbon dioxide, an increase in the pH is observed. Immediately expelling any air bubbles present in the syringe following collection minimizes errors. This may be out of your control. If a sample is received with air bubbles, make sure you annotate the condition it was in when it arrived in the laboratory on the final laboratory report. Another factor critical to the sample is temperature. Blood gas samples should be placed on wet ice immediately following collection, and transported to and processed by the laboratory as soon as possible. If all conditions are ideal, a properly collected and preserved sample is stable for up to 1 hour. Many providers will not accept results from samples that exceed 30 minutes. The key is knowing the directives of your facility and following them to the letter!

### ***Sample analysis***

The analyzer performs all blood gas measurements simultaneously. Some of the analytes are directly measured, while the analyzer calculates others. Most analyzers use ISE methodologies for analysis. The analyzer's electrodes are encased in a fluid or metal heating block. This heating block maintains the electrodes at 37°C. Microprocessors control all of the functions of the analyzer to include periodic calibrations and washings. This allows the analyzer to be ready at all times to accept a sample.

#### ***$pO_2$***

The  $pO_2$  electrode has a membrane made of Teflon or polyethylene, and is permeable to oxygen but not to most other blood constituents. The  $pO_2$  electrode consists of a platinum cathode and a silver-silver chloride anode. A negative charge is sent through the platinum electrode and the oxygen in the sample is reduced. This reduction causes a change in the ionic current and the change is then amplified and measured. The  $pO_2$  is then calculated by comparing the measuring electrode to one with a known concentration of  $pO_2$ .

#### ***$pCO_2$***

The  $pCO_2$  electrode consists of a glass electrode surrounded by a weak bicarbonate solution enclosed in a silicone membrane. This membrane is permeable to  $CO_2$  gas. As  $CO_2$  diffuses through the membrane, it lowers the pH of the bicarbonate solution, and this change is proportional to the  $CO_2$  content of the sample. The measurement signal is compared to a silver-silver chloride reference electrode and the difference between the two is then converted to provide the  $CO_2$  result.



## pH

The analyzer also measures the pH of the sample, but how that is performed is discussed in the next lesson. After measuring the pH and  $p\text{CO}_2$ , the  $\text{HCO}_3^-$  (bicarbonate) and total  $\text{CO}_2$  content can be calculated. A mathematical correlation exists between these analytes. The final readout from the analyzer displays all analytes requested.

### *Noninvasive methods*

There are non-invasive methods available for the monitoring of blood gases. They are not functions of laboratory personnel and are performed by the nursing staff at the patient's bedside. One of the most common methods is the pulse oximetry. Pulse oximetry is useful in only *monitoring* a patient. However, abnormal findings or cases of acid-base imbalances must be confirmed using a blood gas analyzer.

## 204. pH function in the body

Earlier in this course, you learned about hydrogen ions and how they relate to solutions. Acids are substances that donate hydrogen ions ( $\text{H}^+$ ) to a water solution, and bases are substances that donate hydroxide ions ( $\text{OH}^-$ ). In this lesson, you'll expand your knowledge about the function of pH in the body.

### **Physiology**

The term pH comes from the French expression, "puissance hydrogen," that roughly translated means strength or power of hydrogen. The strength and importance of pH in the blood is critical. A pH outside of the range of 6.8 to 7.8 is incompatible with life. In order to maintain this delicate balance, the body regulates its pH by using a series of chemical buffers and the actions of the lungs and the kidneys. The pH range in a normal adult is from 7.35 to 7.45.

### *Chemical buffers*

A buffer is a substance that can bind acids (hydrogen ions) or bases (hydroxide ions), thus maintaining the pH within a relatively narrow range. Our body's major buffering systems are the bicarbonate buffer system and the protein buffer system. Bicarbonate is probably the most important buffer in the body because it's present in large amounts, and the kidneys and the lungs can control its level in the body. Carbon dioxide is the product of cellular metabolism. After it is formed in the cell, it is released into the bloodstream. In the bloodstream, carbon dioxide diffuses into the red blood cells where it binds with water to form carbonic acid ( $\text{CO}_2 + \text{H}_2\text{O} = \text{H}_2\text{CO}_3$ ). Carbonic acid breaks down easily to form hydrogen ions ( $\text{H}^+$ ) and bicarbonate ions ( $\text{HCO}_3^-$ ). Eventually, only a small amount of carbon dioxide and carbonic acid remains in the plasma—about 5 percent of the original amount. The majority of the original carbon dioxide, about 75 percent, is carried in the blood as bicarbonate. The remainder, about 20 percent, is bound with hemoglobin and other plasma proteins as part of the protein buffer system. Proteins take hydrogen ions out of the solution and bind them to their structure, thus reducing the hydrogen ions concentration and minimizing pH changes.

### *Lungs*

Changing breathing rate and breathing pattern also regulates pH. Remember, external respiration also helps to control the carbon dioxide concentration in the blood. The more carbon dioxide in the blood, the more carbonic acid and hydrogen ions are formed. Free hydrogen ions join with bicarbonate ions to form carbonic acid. As the blood containing carbonic acid circulates through the lungs, the lungs convert the carbonic acid into carbon dioxide and water, which are expelled during exhalation. This ongoing process can handle most of the normal levels of hydrogen ions. When an excess amount is formed, the kidneys assist in maintaining proper pH.

### *Kidneys*

The kidneys play a major role in getting rid of excess hydrogen ions. They either directly excrete the hydrogen ions into the urine, or take the hydrogen ions and expel them in the form of water ( $\text{H}_2\text{O}$ ),

sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ ), or ammonium chloride ( $\text{NH}_4\text{Cl}$ ). The kidneys also form bicarbonate, as needed, that buffers the excess hydrogen ions in the plasma.

### Clinical significance

The normal pH of blood ranges from 7.35 to 7.45. When it falls below 7.35, *acidosis* exists. When it increases above 7.45, *alkalosis* exists. Both acidosis and alkalosis are further categorized based on the cause of the problem. For example, if the acidosis is caused by a respiratory problem, the term used is respiratory acidosis. If the cause is a metabolic problem, then the term metabolic acidosis is used. The same holds true for alkalosis; respiratory alkalosis is for respiratory problems and metabolic alkalosis for metabolic problems. The following table shows some of the more common causes of pH disturbances and body compensation methods.

Acid-Base Disturbances and Compensation Methods			
Disturbance type	pH	Common causes	Compensation method
Respiratory acidosis	Decreased	Respiratory depression (drugs or central nervous system trauma), pulmonary disease, pneumonia, or bronchitis.	Kidneys retain greater amounts of bicarbonate to increase pH.
Respiratory alkalosis	Increased	Hyperventilation (emotions, pain, or respirator over-ventilation).	Kidneys excrete increased amounts of bicarbonate to lower pH.
Metabolic acidosis	Decreased	Diabetes mellitus, shock, renal failure, or intestinal disturbances.	Lungs blow off $\text{CO}_2$ to raise pH.
Metabolic alkalosis	Increased	Prolonged vomiting or sodium bicarbonate overdose.	Lungs retain $\text{CO}_2$ to lower pH.

### Laboratory procedures and limitations

Blood pH is normally determined as part of blood gas analysis on a blood gas analyzer. ISE technology is used for the determination of pH. In the pH electrode, a glass membrane is used and the electrode is filled with a potassium chloride solution. As the sample comes into contact with the electrode, a difference in hydrogen ion activity across the glass membrane causes a change in the potential difference between the measuring and reference electrodes. The potential difference is measured by a voltmeter and converted into a pH result.

### Buffers

Blood gas analyzer pH meters are calibrated using two buffers. These buffers are standard solutions of differing pH levels that fall within the ranges compatible with life. Although the pH meters in blood gas analyzers are very similar to the free-standing pH meters found in most laboratories, the blood gas pH meter is much more sensitive because it must be calibrated to measure such a narrow range.

### Methods

Sample handling methods are the same as those for blood gas samples. Once again, use of a tourniquet during sample collection can cause a decrease of venous  $p\text{O}_2$  due to stagnant flow and this can affect pH readings. Results can also be affected by a buildup of protein on the electrode or by bacterial contamination of the reagents. Be sure to maintain cleanliness of the sample probe and electrodes on your analyzer.

### Temperature-sensitive

Since pH is a temperature-sensitive procedure, it is important to maintain the analyzer's temperature bath or block at  $37 \pm 0.05^\circ\text{C}$ . At room temperature, plasma pH decreases about 0.015 pH units every 30 minutes. In some hospitals, the blood pH may be corrected based on the patient's body temperature, since blood pH varies inversely with temperature. A sample from a patient with a high

fever needs a negative correction to pH. A sample from a patient suffering from hypothermia, decreased body temperature, needs to have a positive pH correction. There is a disagreement among medical professionals as to the pros and cons of calculating pH changes based on patient body temperatures. If your facility provides corrected pH readings, use the established criteria and correction formulas for your analyzers. Always report out corrected results with the original pH results measured at 37°C and ensure each is annotated accordingly for proper identification.

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 203. Blood gases

1. Match the item in column B with the statement it relates to in column A by placing the letter of the item in column B beside the statement in column A. Each item in column B may be used only once.

#### Column A

- \_\_\_\_\_ (1) The measure of the partial pressure of carbon dioxide in the blood.
- \_\_\_\_\_ (2) The partial pressure of oxygen dissolved in blood plasma.
- \_\_\_\_\_ (3) The percentage of available hemoglobin that is saturated with oxygen.
- \_\_\_\_\_ (4) The ion that measures the metabolic component of the acid-base equilibrium.
- \_\_\_\_\_ (5) Expresses the extent to which the blood is acidic or alkaline.

#### Column B

- a. pH
- b.  $\text{HCO}_3^-$
- c.  $p\text{CO}_2$
- d.  $s\text{O}_2$
- e.  $p\text{O}_2$

2. Blood gases are used to evaluate what functions?
3. What is *external respiration*?
4. What concept allows oxygen and carbon dioxide to move across the alveolar and capillary membranes?
5. What is *internal respiration*?
6. What are oxyhemoglobin and carbaminohemoglobin and how are they transported?
7. What are chemoreceptors and what function do they play in breathing?
8. What is hypoxemia and what can cause this condition?

9. What is the difference between hypercapnia and hypocapnia?
10. When specimens are collected for venous blood gas analysis, why should a tourniquet not be used or the fist clenched?
11. Why are plastic syringes avoided when blood gas samples are collected?
12. Why do blood gas samples have their exposure to room air minimized and air bubbles expressed out immediately following collection?

#### **204. pH**

1. Why is blood pH important and how does the body regulate it?
2. What is the body's most important buffer and why?
3. In what forms is carbon dioxide, produced by cellular metabolism, carried in the bloodstream?
4. How do the lungs help expel carbon dioxide?
5. How do the kidneys help regulate pH?
6. What are acidosis and alkalosis, and how are they further categorized?
7. How does the pH meter in a blood gas analyzer differ from the typical pH meter found in a laboratory?
8. What are some sources of error for blood pH testing?

### 1-3. Other Inorganic Metabolites

Previous lessons in this unit have focused on the primary electrolytes of the body, their functions, their abnormalities, and their diagnostic significance to providers. We furthered our discussion on electrolytes by exploring blood gases. We need to complete our lesson by looking at some of the other major anions and cations of the body. Our lesson is limited to information about calcium, phosphorus, magnesium, and lithium.

#### 205. Calcium and phosphorus

Calcium and phosphorus are two inorganic ions frequently ordered to evaluate metabolic disorders. As you will soon learn, their functions and metabolism are closely linked.

##### Calcium

Of all the minerals in the body, calcium is the one present in the largest amount. Calcium is found in three main body compartments—the skeleton, soft tissues, and extracellular fluids. The skeleton contains about 99 percent of the body's calcium; the remainder is in the soft tissues and extracellular fluids. In the blood, about all the calcium is found in the plasma and very little is found in red blood cells. The small amount of calcium found intracellularly is mostly bound to proteins in the cell membranes, mitochondria, and nucleus. Serum calcium plays a role in nerve and muscle tissue function, the coagulation process, and more.

Calcium is the predominant cation in the body. In the blood, calcium can be found in one of three states. One state is the free or ionized state of calcium; it makes up 50 percent of the calcium in the blood. Another is the calcium bound to plasma proteins, primarily albumin, and makes up about 40 percent of the calcium in the blood. The remaining 10 percent of blood calcium is complexed to diffusible anions, such as phosphate, bicarbonate, lactate, and citrate. Because calcium binds to negatively charged sites on albumin, the rate of binding is pH-dependent. Alkalosis leads to an increase in the binding of calcium and a decrease in the amount of free calcium in the blood. The reverse is true for acidosis; calcium binding is less likely to occur so there is more free calcium in the blood. Calcium is a very active cation and functions as an intracellular messenger by binding or being released from specific intracellular proteins. When these proteins bind or release calcium, their structure and functions change. The calcium messenger system is responsible for the contraction of muscle fibers, the secretion of hormones and fluids, the transfer of ions across cell membranes, and mitosis, just to name a few. Extracellular calcium is the source of calcium for the intracellular functions. In addition, extracellular calcium provides calcium ions for bone mineralization, the coagulation process, and maintains cell membrane potential.

Most people maintain a good balance of calcium in their body through continued ingestion and excretion. The average adult ingests about 500 to 1,000 mg of calcium daily and excretes about the same amount in the urine and feces. Dairy products, such as milk and cheese, are the best sources of dietary calcium.

##### *Regulated*

Plasma concentration is regulated by two substances, the parathyroid hormone (PTH) and 1,25-dihydroxy vitamin D<sub>3</sub>, the active form of vitamin D. The 1,25-dihydroxy vitamin D<sub>3</sub> can increase the intestinal absorption of dietary calcium as needed. The parathyroid secretes PTH in response to decreases in plasma calcium concentrations. PTH responds to decreased plasma calcium by retrieving calcium and phosphate from the bone, increasing its reabsorption by the kidneys, and increasing the absorption of calcium in the intestines.

##### *Clinical significance*

A decreased level of calcium is known as hypocalcemia. Critically decreased levels of calcium are normally associated with tetany, a hyperexcitability of nerves and muscles. Mild forms of tetany can occur during and after pregnancy, especially during lactation, due to the increased demands for

calcium brought on by these conditions. Additional factors that can cause low calcium levels include hypoparathyroidism (decreased PTH production), vitamin D deficiency, gastrointestinal problems that block the absorption of calcium or vitamin D, chronic renal failure, and magnesium deficiencies (magnesium deficiency impairs PTH production).

An increased calcium level is known as hypercalcemia. Hypercalcemia is found fairly commonly in clinical practice as either a chemical abnormality in an asymptomatic patient or in association with a severe illness. Prolonged elevation of calcium levels can lead to deposits of insoluble calcium salts in soft tissues and the formation of renal calculi. Primary hyperparathyroidism is the most common cause of hypercalcemia in outpatients and malignancy is the most common cause in inpatients. Other causes of hypercalcemia include vitamin D overdose, ingestion of large amounts of dairy products, excessive ingestion of antacids, Paget's disease, and chronic renal disease. Chronic renal problems can cause both hypercalcemia and hypocalcemia.

### ***Laboratory procedures and limitations***

Calcium analysis consists of measuring the free calcium or the total calcium (free, protein-bound, and complexed-combined). Free calcium analysis provides a better indication of the calcium status because it is biologically active and tightly regulated by the calcium-regulating hormones. Total calcium, in part, is bound to serum proteins and is affected by protein concentrations, especially albumin. Even though free calcium is more useful in clinical determinations, most analyzers perform total calcium determination methods. Instrument manufacturers are only beginning to include ISE methods on their instruments so free calcium levels may be determined.

#### ***Free calcium***

Only the free form of calcium acts as an intracellular messenger. This is why free calcium provides a better clinical picture than total calcium. Measurement of free calcium requires more careful handling of the sample and instrument maintenance than total calcium methods. The free calcium ISE consists of a calcium-selective membrane enclosing an inner reference solution of calcium chloride and an internal reference electrode. The external electrode comes in contact with the specimen and the potential difference across the cell is related to the activity of free calcium in the sample. The electrodes currently in use have a higher selectivity for calcium than sodium, potassium, magnesium, lithium, and hydrogen ions. At normal concentrations these other cations have minimal effect on the accuracy of free calcium results. High levels of magnesium and lithium in the sample may influence free calcium results. Free calcium analysis can be performed on serum, plasma, or whole blood.

Anticoagulants such as citrates, oxalates, or EDTA should not be used for sample collection because these anticoagulants bind with free calcium and decrease its concentration in the sample. Heparin is the only anticoagulant acceptable for free calcium determinations.

Other factors affecting free calcium testing include temperature and pH. Temperature affects electrode response and the binding response of calcium to proteins and anions. Most analyzers maintain samples at 37°C to reduce errors caused by this factor. The binding of calcium is also affected by pH. The majority of the protein-bound calcium in the blood is bound to albumin. By increasing the pH of a sample, both the ionization and negative charge of albumin is increased. This causes more calcium to bind with the albumin and lowers free calcium values. Decreasing the pH of the sample has the opposite effect; it decreases the negative charge of albumin, which decreases the amount of calcium bound to albumin, and increases the free calcium values. When exposed to air, samples lose carbon dioxide and become more alkaline. Therefore, anaerobic conditions should be maintained.

#### ***Total calcium***

Total serum calcium is most often determined by spectrophotometric methods using chelating agents. Chelating agents are defined as chemical bonding compounds that remove substances. The chelating agent produces a red color when bound to the calcium. The calcium chelator, o-cresolphthalein

complexone, is one of the most widely used. This method is precise and easily automated. In an alkaline solution, o-cresolphthalein complexone dye binds tightly with calcium in the sample. The reaction mixture also contains the following ingredients to improve performance: 8-hydroxyquinoline to bind magnesium and prevent its interference, urea to decrease the turbidity of a lipemic sample and to increase the color intensity of the calcium-dye complex, and ethanol to decrease the absorbance of the blank. Serum is the sample of choice although heparinized plasma is acceptable.

Specimens collected in anti-coagulants, such as citrate, oxalate, or EDTA, should not be used because of the actions of the anti-coagulants. Serum calcium levels are stable for several days at 4°C and for several months when frozen, provided care is taken to avoid evaporation of the sample. Hemolyzed, icteric, or lipemic samples can interfere with total calcium testing methods.

### **Phosphorus**

Most of the body's phosphorus, or phosphate, is combined with calcium within the skeleton. The remainder is found mostly in soft tissues in the form of organic phosphate esters. Both inorganic and organic phosphate ions are present within the cells, with the vast majority in the organic form. Within the cells, the organic phosphate ions are incorporated into nucleic acids, phospholipids, phosphoproteins (part of the enzyme system that affects the oxygen carrying capacity of hemoglobin), and high-energy compounds involved in cellular integrity and metabolism. The majority of the inorganic phosphate ions are found in extracellular fluid serving as part of the body's buffer system.

#### ***Dietary phosphorus***

Phosphorus is found in almost everything you eat, so obtaining enough phosphorus from the diet is usually not a problem. Dietary phosphorus is absorbed in the small intestine and the principal route for excretion of absorbed phosphorus is through the urine. The control of plasma phosphate concentration is closely linked to that of calcium because they are deposited together in the bone as hydrated calcium phosphate.

#### ***Phosphorus regulators***

The regulators of calcium (PTH and active vitamin D) affect phosphate concentrations. PTH stimulates the kidneys to excrete phosphate while retaining calcium. This action causes an inverse relationship between the levels of calcium and phosphate in the blood. A decrease in one, results in an increase in the other. Conversely, an increase in one causes a decrease in the other.

#### ***Clinical significance***

A decreased level of phosphate is called hypophosphatemia. Chronic hypophosphatemia is most often caused by a decrease in renal absorption of phosphate. This decreased absorption is usually the result of hyperparathyroidism, rickets (vitamin D deficiency), and renal diseases where tubular reabsorption of phosphate is impaired. Other causes include the prolonged ingestion of antacids, chronic starvation, and malabsorption conditions (especially if combined with vomiting or diarrhea). Recent carbohydrate ingestion, including IV glucose solutions, can cause a temporary decrease in serum phosphate because phosphate enters the cells with glucose. Severe decreases in phosphate levels can result in neuromuscular disturbances, hemolytic anemia, and decreased release of oxygen from the hemoglobin, impaired white blood cell and platelet function, and profound muscle weakness. The more severe cases of hypophosphatemia are usually found in patients recovering from diabetic ketoacidosis, severe respiratory alkalosis, acute alcoholism, and severe burns.

An abnormally increased level of phosphate is called hyperphosphatemia. This condition can be caused by chronic or acute renal failure, hypoparathyroidism, vitamin D intoxication, or hypersecretion of growth hormone. In infants and children, high phosphate levels are associated with normal increased levels of growth hormone.

***Laboratory procedures and limitations***

All of the common methods for phosphate analysis are based on the reaction of phosphate ions with ammonium molybdate. When joined, these compounds form a phosphomolybdate complex. This complex is colorless and can be read directly by ultraviolet absorption. Variations of this method reduce the phosphomolybdate compound to form an intensely colored, blue phosphomolybdate complex that is then read at 600 to 700 nm depending on the method. Absorbance readings are proportional to the serum phosphate concentration. In order for the complexes to be formed, an acidic pH is required. The pH level must be tightly controlled because a less acidic pH can result in spontaneous reduction of the molybdate complex. About three-fourths of all laboratory facilities use the direct UV procedure over reduction methods because of the simplicity, speed, and stability of the direct method.

Serum is the preferred sample for phosphate analysis. Do not use anti-coagulants, such as citrate, oxalate, or EDTA, because these anti-coagulants interfere with the formation of the phosphomolybdate complex. Do not use hemolyzed samples because red blood cells contain high concentrations of organic phosphate esters. These esters may be hydrolyzed to inorganic phosphate during storage. In a hemolyzed sample, inorganic phosphate levels increase 4 to 5 mg/dl each day when stored at 4°C and even more if stored at room temperature. It is important to separate the serum from the red blood cells promptly because phosphate levels increase when serum is stored on the red cells at room temperature.

Interference in testing has also been reported in samples that are lipemic or icteric. Ensure any glassware used in testing has been properly cleaned and rinsed because phosphate is a common component of many detergents. When properly separated from the clot, samples for phosphate testing are stable for several days if stored at 4°C and several months frozen.

**206. Magnesium and lithium**

The last two inorganic ions we'll study are magnesium and lithium. Magnesium is associated with calcium and phosphate processes and plays a role in muscle contraction. Lithium is used to treat mental health disorders.

**Magnesium**

Magnesium is the fourth most abundant cation in the body and most of it is found in the bones along with calcium and phosphate. Within the cells, magnesium is a cation second only to potassium. In extracellular fluids, the levels of magnesium are low with about 30 percent of the magnesium bound to albumin, 15 percent complexed with phosphate, citrate and other anions, and the rest existing as ionized or free magnesium. The main dietary sources of magnesium are meat and green vegetables. Magnesium is absorbed in the upper intestines and does not require vitamin D for absorption. The kidney is responsible for controlling magnesium balance. It can conserve magnesium when intake is low and excrete excesses when intake is high.

The exact control mechanism for magnesium is unknown but it is believed the hormone aldosterone plays a role. Aldosterone promotes the excretion of magnesium and potassium, and promotes the retention of sodium. Magnesium serves as an activator for more than 300 different enzymes. Therefore, magnesium's intracellular function assists in phosphorylation, protein synthesis, glycolysis, cell replication, and nucleotide metabolism. Magnesium also affects neuromuscular excitability. Extracellular magnesium serves as the source for maintaining intracellular magnesium levels. Decreased plasma magnesium levels cause tetany, and increased levels depress the central nervous system and alter the conducting mechanism of the heart.

***Clinical significance***

A decreased level of magnesium is called hypomagnesemia. This condition is very common and it is estimated that as many as 68 percent of all patients in intensive care units may be hypomagnesemic. Loss of magnesium from either the gastrointestinal tract or the kidney is the most common cause. The



loss may be a result from prolonged diarrhea, prolonged use of diuretics, or fluid therapy without magnesium supplements. Clinical causes such as renal problems, diabetes mellitus, and acute alcoholism can also contribute to hypomagnesemia. Magnesium deficiency is usually secondary to some other disease process or therapeutic cause. The features of the primary disease often mask or complicate the magnesium deficiency. As you know, low magnesium levels can cause tetany. However, tetany can also be caused by hypocalcemia.

Magnesium deficiency is one of the most common causes of hypocalcemia. It impairs PTH secretion and makes the kidneys and bones resistant to the effects of PTH. One of the more serious complications of magnesium deficiency is the effect on the cardiovascular system. Low magnesium can cause tachycardia (abnormally fast heart beat) and fibrillation (abnormal muscle contraction).

An increased level of magnesium, hypermagnesemia, is not common. Cases of increased magnesium levels are usually due to excessive intake of magnesium salts usually taken for constipation. Most of these patients have some type of renal problem that limits the kidney's ability to excrete the excess magnesium. In addition to renal problems, increased magnesium levels can be observed in patients suffering from severe dehydration and aldosterone deficiencies. Magnesium is also the standard form of therapy for pre-eclampsia and eclampsia in pregnant women. Both mothers and neonates can have increased magnesium levels during and following this therapy. Neuromuscular symptoms are the most common sign of magnesium intoxication. Deep tendon reflexes disappear and breathing rates may slow or stop due to muscle paralysis. High magnesium levels can cause cardiac arrest. Hypermagnesemia also causes decreased plasma calcium levels because high plasma magnesium levels also decrease PTH secretion.

#### ***Procedures and limitations***

Serum is the sample of choice for magnesium analysis, although heparinized plasma may be used. Avoid anti-coagulants, such as citrates, oxalates, and EDTA, because they form complexes with magnesium affecting test results. Red blood cells contain high levels of magnesium. It is recommended that hemolyzed samples not be used, and you separate the serum from the cells as soon as possible. Icteric or lipemic samples can cause interference depending on the methods of analysis used. Most clinical laboratories determine serum magnesium levels spectrophotometrically. Measurement by atomic absorption spectrometry is very accurate, but too time consuming for most laboratories to use.



#### **Lithium**

Lithium levels may be requested from the laboratory as a tracking mechanism for lithium carbonate treatments. Lithium carbonate is used to treat the manic phase of affective disorders like mania and manic-depressive illness.

#### ***Clinical significance***

Lithium carbonate has a calming effect on the manic stage of the manic-depressive patient and is frequently given as a therapeutic measure to prevent attacks. Lithium acts by enhancing the reuptake of neurotransmitters. The neurotransmitters, by being reabsorbed, have a lower concentration in the neuron junctions. This produces a sedating effect on the central nervous system. Plasma concentrations of lithium are toxic at levels of about 2 mmol/l or greater. Serum levels for patients on lithium are usually monitored weekly during the early phase of treatment. Early signs of lithium intoxication include apathy, sluggishness, drowsiness, speech difficulties, and twitching. Muscle rigidity, hyperactive deep tendon reflexes, and epileptic seizures characterize severe intoxication, levels exceeding 2.5 mmol/l. A serum level of 5 mmol/l can be lethal to the patient.

***Procedures and limitations***

Lithium analysis can be performed by flame emission photometry, ISE methods, or atomic absorption spectrometry. Most facilities that test for lithium do so by flame emission photometry. Because development of the lithium ISE is recent, many facilities are just beginning to explore this method. The principle of testing for lithium by flame emission photometry is not covered in depth here because it was discussed previously. The sample is aspirated into a flame along with an internal standard, cesium or potassium, and the lithium atoms are forced into an activated state by thermal collisions. As the atoms return to their ground state, they give off light of a characteristic wavelength. The emitted light of the lithium is compared to that of the internal standard and the lithium value is determined.

**207. Iron studies**

Iron is essential to the body for a multitude of functions, to include the production of red blood cells, enzymes, and oxygen transport. Iron studies encompass a variety of tests to reveal underlying conditions and disease processes. The focus of the study of iron will regard testing for serum iron, total iron binding capacity (TIBC), ferritin, transferrin, and hemosiderin, as well as iron deficiency and overload.

**Iron and iron components utilization and distribution**

The majority of iron is found in hemoglobin at rates of 65-70 percent. A small amount of iron is found in myoglobin. The remaining iron is distributed in various forms, such as ferritin, transferrin, hemosiderin, enzymatic, and organic. Iron levels must be regulated for homeostasis, because, unlike most other elements that are regulated by excretion, the intestines absorb the majority of the body's iron, with additional absorption to a lesser extent in other organs and the bloodstream. The intestines must control iron absorption so iron levels do not become toxic in the tissues. This absorption process is so well refined there is minimal daily loss of iron. In the bloodstream and other organs, iron reabsorption relates to the breakdown of the erythrocyte and hemoglobin, and the subsequent reclamation of iron from this process, which will be covered in more detail in 4T051P. The diet found in the western world normally provides iron replenishment. Consumption of foods containing vitamin C will increase iron uptake. The body stores iron in the forms of ferritin and hemosiderin that remain in a stand-by capacity to preserve homeostasis. Daily losses for adult males is approximately 1mg while losses for adult females is slightly higher at approximately 1.3 mg. Female losses are greater due to normal menstruation, as well as the burdens associated with pregnancy.

***Serum iron***

There are numerous methods to test for iron, and they all measure the amount of iron carried by transferrin, not including iron in the serum as free hemoglobin. In this state, iron is bound to the transferrin and must be separated for measurement. Following separation, the iron is allowed to react with a chromogen to create an iron-chromogen complex, the absorbance of which is directly proportional to the iron concentration. Because of the wide-ranging differences found between commercial methods, each laboratory must establish the local reference ranges.

Because iron is widely distributed in the environment, great care must be taken to avoid contamination of testing equipment or reagents.

***TIBC***

Typically, about one third of transferrin iron binding sites are occupied by iron, resulting in a reserve iron binding capacity that can be measured. TIBC measures the maximum amount of iron the blood could carry if all transferrin was saturated. To perform the test, iron is added to the sample in an amount sufficient to saturate the transferrin iron binding sites. The excess unbound iron will then be removed and measured revealing the TIBC, which can provide an indirect measurement of transferrin by using the following calculation:

$$0.7 \times \text{TIBC (mcg/dL)} = \text{Transferrin (mg/dL)}$$

The direct measurement of transferrin is preferred because the calculation described above can provide results that fluctuate greatly from actual values.

Additionally, once the TIBC is obtained, the transferrin saturation can be calculated multiplying the serum iron by 100 and dividing that result by the TIBC.

$$\% \text{ Transferrin} = \frac{\text{Serum Iron (mcg/dL)} \times 100}{\text{TIBC (mcg/dL)}}$$

### ***Ferritin***

Iron is stored mainly as ferritin and is found in almost all cells of the body. Ferritin remains in a stand-by capacity to form hemoglobin or other heme proteins as needed. Ferritin will remain in equilibrium with iron in the body. Normal ferritin iron levels vary through early development until adulthood is reached. The newborn will have levels that mirror adult concentrations, but, within the first month to 60 days, the level skyrockets from one to two months of age to nearly double adult levels. From two months to five months of age, the levels again mirror that of an adult, and then reduce even more during the subsequent toddler, pre-teen, and early adolescent years. In the middle adolescent years, adult levels of iron are achieved.

In iron deficiency, ferritin levels will be decreased prior to changes in hemoglobin concentration, and this result is considered a reliable indicator as a precursor to iron deficiency. Ferritin is increased in a variety of conditions to include chronic infections, rheumatoid arthritis, renal disease, heart disease, and several malignancies. When these conditions are present with concurrent iron deficiency, the ferritin levels may be within normal limits, but would still be considered as increased because of the other underlying condition(s). These potential sources of error must be kept in mind. Many methods exist for ferritin determinations to include RIA, EIA, ELISA and chemiluminescence assays. All of these methods are commercially available.

### ***Transferrin***

The liver produces transferrin and it is also known as siderophillin. Transferrin experiences diurnal variation with higher levels in the morning. Transferrin can bind two oxygen molecules. Transferrin is increased in periods of low iron stores in the body. When iron levels are high, transferrin levels are reduced and vice-versa. Many laboratories elect to measure the transferrin saturation in conjunction with the TIBC.

### ***Hemosiderin***

Hemosiderin is another storage form of iron accounting for approximately 20 – 30 percent of iron stores. It is associated with the iron overload conditions hemosiderosis and hemochromatosis.

### ***Iron overload***

Iron overload has two distinct presentations, hemosiderosis and hemochromatosis.

#### ***Hemosiderosis***

This term is used to indicate iron overload without tissue injury.

#### ***Hemochromatosis***

Hemochromatosis can be present as hereditary, acquired (secondary), or, rarely, neonatal. The hereditary disorder is a recessive genetic disorder of iron metabolism usually affecting Caucasians of northern European origin. The hereditary form of the disease will manifest in men (usually striking in their 40's) about 20 years earlier than in women because of the protection from menstruation. Women are typically affected post-menopausal. Alcoholism can cause the effects of this condition to occur more rapidly. Left untreated, hemochromatosis can adversely affect multiple organs, such as the liver, heart, pancreas, skin, joints, and gonads.

The acquired or secondary form of this disease can be caused by a variety of conditions. These include those caused as a secondary effect from another disease process, excessive dietary intake, and as a result of multiple blood transfusions.

The neonatal form of the disease will appear *in utero* or within the first few weeks of life, and cause large deposits of iron in many organ tissues. It is a rare condition and is fatal usually within four months.

If hemochromatosis is diagnosed before organ damage occurs and therapy promptly begins, the prognosis for the patient is good. If organ damage has occurred, the effects are often irreversible. Treatment is by periodic therapeutic phlebotomy.

### Iron deficiency

Iron deficiency may be caused by a number of factors to include improper diet, major trauma, occult bleeding, heavy menstrual periods, hookworm infestation, and blood donations. Patients with iron deficiency may exhibit or complain of fatigue, tachycardia, and paleness of the skin. Diagnosis is typically through a general examination and blood tests associated with iron studies, with hemoglobin and ferritin being the most commonly used indicators. In some cases, a bone marrow biopsy will be required for diagnosis. Treatment is through diet and/or iron supplements.

Guidelines for Diagnosing Iron Deficiency		
Analyte	♂	♀
Serum Ferritin	≤20 ng/mL	≤15 ng/mL
Hemoglobin	≤11.9 g/dL	≤11.9 g/dL

In addition to these guidelines from the Centers for Disease Control and Prevention (CDC), serum iron will be decreased, and transferrin and TIBC will be increased in iron deficiency anemia. An above-normal TIBC is specific for iron deficiency. Iron deficient anemias will be explored further in 4T051C.

---

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 205. Calcium and phosphorus

1. In what three states can calcium be found in the blood?
2. How do alkalosis and acidosis affect the amount of free calcium in the bloodstream?
3. How does calcium function as an intracellular messenger?
4. How are plasma concentrations of calcium regulated?
5. What is hypocalcemia and how is it caused?

6. What is hypercalcemia and how is it caused?
7. Why are anti-coagulants, such as citrate, oxalate, and EDTA, not used for collecting samples for calcium analysis?
8. How does pH affect the binding of calcium in the blood?
9. How are organic and inorganic phosphate found in the body?
10. How does the body obtain phosphate and how are its levels regulated?
11. What is hypophosphatemia and what kind of clinical problems might be observed with this condition?
12. What is hyperphosphatemia and how is it caused?
13. Why is pH important during phosphate analysis?
14. Why do you avoid using anti-coagulated samples for phosphate analysis?
15. Why do you avoid using hemolyzed samples for phosphate analysis?

**206. Magnesium and lithium**

1. What are the functions of magnesium in the body?
2. What is hypomagnesemia and what are some of the causes and clinical indications of this condition?

3. What is hypermagnesemia and what are some of the causes and clinical indications of this condition?
4. Why is hemolysis avoided in magnesium samples?
5. Why is lithium analysis usually requested?
6. What are the signs of lithium intoxication?
7. When performing flame emission photometry for lithium analysis, what two substances are used as the internal standards?

#### **207. Iron Studies**

1. What process does the body use to regulate iron and why is this unique?
2. Why do adult females typically suffer greater losses of iron than do adult males?
3. Why must laboratories establish local reference ranges for iron?
4. Name a potential source of error to consider when conducting iron studies.
5. In the total iron binding capacity (TIBC) test, why is iron added to the sample?
6. What are potential sources of error in ferritin assessments?
7. Why are the effects of hereditary hemochromatosis manifested in men often twenty years or more before the same effects are seen in women?

8. What is the treatment regimen for hemochromatosis?

---

## Answers to Self-Test Questions

### 201

1. Water balance, osmolality, electrolyte balance, pH balance, and blood gas exchange.
2. Intracellular water is found within the cells. Extracellular water is water found outside of the cells.
3. Water balance is keeping the intracellular and extracellular compartments of water at a constant volume. This is done by ensuring that the rate of water loss is equal to the rate of water intake.
4. It's regulated by the body's thirst mechanism, anti-diuretic hormone produced by the posterior pituitary gland, and excretion or reabsorption of water by the kidneys.
5. (1) Vomiting.  
(2) Excessive urination.  
(3) Sweating.
- (4) (4) Diarrhea.  
(5) Bleeding.  
(6) Exudation.
6. Sodium, potassium, chloride, and bicarbonate.
7. Sodium ions (cations) and chloride ions (anions).
8. Electrolytes regulate water distribution (intracellular and extracellular fluids), osmotic pressure, maintain blood pH, regulate heart and other muscles, play a part in the body's oxidation-reduction reactions, cell permeability, and nerve impulse conduction.
9. Because an imbalance cannot be tolerated by a patient for very long and irreversible damage or death can result.
10. Sodium ions are the principal cations of extracellular fluid. Potassium ions make up the majority of the cations found in the intracellular fluid. Chloride and bicarbonate anions are found predominately in extracellular fluid.
11. The physical force that is exerted when water passes from an area of lower ion concentration to one of higher ion concentration.
12. Because it's more practical to measure the extracellular ions than to measure intracellular ions.
13. Urine, spinal fluid, and sweat.
14. Sustained fever, diarrhea, inadequate fluid intake, and other clinical problems.
15. By subtracting the total chloride and bicarbonate anions from the total sodium cations.
16. Anion gap =  $(130 \text{ mmol/l}) - (100 \text{ mmol/l} + 25 \text{ mmol/l})$   
Anion gap =  $130 \text{ mmol/l} - 125 \text{ mmol/l}$   
Anion gap =  $5 \text{ mmol/l}$
17. A decrease in the unmeasured cations or an increase in the unmeasured anions.
18. Once it has been determined that the testing and retesting was valid.
19. As a quality control measure.

### 202

1. Sodium.
2. The kidneys.
3. Aldosterone.
4. Hyponatremia is a decreased plasma concentration of sodium that can be caused by the patient not ingesting enough sodium or sodium loss due to diarrhea, prolonged vomiting, or sweating brought on by fever.

Decreased sodium levels can also be caused by metabolic acidosis (due to diabetes mellitus), renal disease, diuretics, and Addison's disease.

5. Hyponatremia is an increased plasma concentration of sodium that can be caused by severe dehydration, Cushing's syndrome, insulin treatment of diabetic coma patients, and inappropriate forms of saline treatment or high sodium feedings.
6. The sodium ISE has a glass membrane that selectively exchanges sodium ions. As the sodium ions react with the membrane, a potential-measuring circuit between the measuring electrode and reference electrode determines a change in the electromotive force. This change is proportional to the sodium concentration.
7. Indirect samples for sodium are diluted before testing. Samples high in lipids or proteins can have these (lipids and proteins) displace some of the fluid that would normally be tested for sodium content.
8. An intracellular cation.
9. Potassium is absorbed rapidly in the intestinal lumen and excesses are excreted by the kidneys.
10. Too much potassium inhibits the irritability of the heart and paralyzes it so that it ceases to beat. Not enough potassium increases the irritability of the heart to the point where it ceases during a contraction.
11. Hyperkalemia is an increased plasma level of potassium. It occurs when potassium leaves the cells at a rate faster than the kidneys can excrete it. This overload occurs in conditions of anoxia, metabolic or renal acidosis, shock or circulatory failure, Addison's disease, dehydration, or severe red blood cell lysis.
12. Hypokalemia is a decreased plasma level of potassium. This condition occurs as a result of low potassium intake over a period of time, increased loss of potassium through vomiting, diarrhea, gastrointestinal problems, or long-term use of diuretics. In addition, low potassium levels are seen in cases of increased aldosterone production, causing increased excretion of potassium by the kidneys, in chronic starvation patients and post-operative patients.
13. Since most of the body's potassium is intracellular, hemolysis of a sample greatly affects the potassium level of the sample.
14. Extracellular.
15. Red blood cells.
16. Maintaining osmotic pressure and acid-base balance.
17. Decreased plasma chloride levels caused by uncontrolled diabetes (overproduction of ketoacids), renal disease (chloride lost as salts not reabsorbed), prolonged vomiting, intestinal blockage, and Addison's disease.
18. Increased plasma chloride levels caused by dehydration, prolonged diarrhea, renal tubular acidosis, respiratory alkalosis, heavy salt ingestion, and over-treatment with saline solutions.
19. Tap water and the use of sodium fluoride as an anti-coagulant.
20. A composite of silver sulfide and silver chloride. Medications containing bromides.
21. Bicarbonate is a term often interchanged with others used to describe carbon dioxide levels. About 90 percent of all carbon dioxide in the bloodstream is in the form of bicarbonate.
22. Metabolic alkalosis, compensated respiratory acidosis (sudden stop in breathing—can be caused by drugs, blockage, or long-term pulmonary disease), or alkalosis accompanying a large potassium deficiency
23. Metabolic acidosis and compensated respiratory alkalosis (hyperventilation—deep, rapid breathing).
24. Blood collected in anti-coagulants other than heparin can upset the balance between the red blood cells and the plasma  $\text{CO}_2$ . Allowing the sample to become exposed to room air also affects sample levels as  $\text{CO}_2$  in the sample escapes into the room air (room air has a lower  $\text{CO}_2$  concentration than blood).
25. Enzymatic, colorimetric, and ISE.

## 203

1. (1) c.  
(2) e.  
(3) d.  
(4) b.  
(5) a.



2. A patient's ability to transport oxygen, to assess disturbances in the fluid or electrolyte balance, and to monitor problems with the lungs or kidneys (since these organs regulate the body's acid-base balance).
3. The exchange of oxygen and carbon dioxide that takes place in the lungs as a person inhales and exhales.
4. Partial pressure helps oxygen and carbon dioxide move across the alveolar and capillary membranes through the process of osmosis.
5. It is the exchange of gases (oxygen and carbon dioxide) between the plasma in the capillaries and the tissue cells.
6. Oxyhemoglobin is hemoglobin that has bound with oxygen. Carbaminohemoglobin is hemoglobin that has bound with carbon dioxide. Arterial blood carries the oxygenated blood to the tissue cells. Venous blood carries the carbon dioxide laden blood from the tissues back to the lungs.
7. They are special cells adapted to excitation by chemical substances. They are sensitive to the levels of  $pO_2$  and  $pCO_2$  in the body, sending impulses to the brain to speed up or decrease breathing as needed.
8. Deficient oxygenation of the blood. It can be caused by breathing air low in oxygen, hypoventilation (from suffocation, submersion, trauma, obesity, or drug-induced respiratory failure), premature mixing of venous and arterial blood, or inability to oxygenate blood due to diffusion difficulties.
9. Hypercapnia is increased  $pCO_2$  in the blood (caused by decreased alveolar ventilation), while hypocapnia is a decreased  $pCO_2$  level (caused by increased ventilation rates).
10. Because it decreases the venous  $pO_2$  and allow acid metabolites to accumulate.
11. Because plastic syringes can allow gases to be exchanged between the blood sample and the outside air through the barrel wall.
12. Exposure to room air affects the blood gas levels in a sample. Exposure to room air normally increases the  $pO_2$  in the sample because the oxygen in the room air is of a higher concentration than that in the blood. Exposure to room air decreases the  $pCO_2$  because the concentration in the sample is much higher than that in room air. Air bubbles in the sample must be expressed out so that any gases in the bubbles are not allowed to affect the sample's blood gas levels.

## 204

1. Blood pH is important because levels outside the range of 6.8 to 7.8 are incompatible with life. The body uses a series of chemical buffers, the lungs and the kidneys to regulate pH.
2. Bicarbonate is probably the most important buffer in the body because it is present in large amounts and can be controlled by the kidneys and the lungs.
3. A small amount of carbon dioxide is carried in the bloodstream as carbon dioxide and carbonic acid (about 5 percent), the majority is carried as bicarbonate (about 75 percent), and the remainder (about 20 percent) is bound to hemoglobin and other plasma proteins.
4. As blood containing carbonic acid circulates through the lungs, the lungs convert the carbonic acid into carbon dioxide and water, which are expelled during exhalation.
5. They play a major role in getting rid of excess hydrogen ions. They will either directly excrete them into the urine or take them and expel them in the form of water, sodium dihydrogen phosphate, or ammonium chloride. The kidneys also form bicarbonate, as needed, which buffers the excess hydrogen ions in the plasma.
6. Acidosis is when the pH of the blood falls below 7.35 and alkalosis is when the pH of the blood rises above 7.45. The terms are categorized further based on what they are caused by. Respiratory acidosis and respiratory alkalosis are caused by respiratory problems while metabolic acidosis and metabolic alkalosis are caused by metabolic problems.
7. The pH meter in a blood gas analyzer is calibrated using two buffers that fall within the ranges compatible with life. The pH meter in a blood gas analyzer is also much more sensitive because it has to be calibrated to measure within such a narrow range.
8. Some blood pH testing sources of error include improper sample handling (same as for blood gas testing), use of tourniquet during sample collection, protein buildup on electrodes, bacterial contamination of the sample probe, and improper instrument temperature.

## 205

1. In the first state, calcium is free or ionized. In the second state, calcium is bound to plasma proteins (primarily albumin). In the third, calcium is bound to diffusible anions such as phosphate, bicarbonate, lactate, and citrate.
2. Because calcium binds to the negatively charged sites on albumin, the rate of binding is pH dependent. Alkalosis leads to an increase in the binding of calcium and a decrease in the free calcium in the blood. Acidosis causes less calcium to bind with albumin, so there is more free calcium found in the blood.
3. By binding or being released from specific intracellular proteins. When proteins bind or release calcium their structure and function change. The calcium messenger system is responsible for things such as the contraction of muscles, secretion of fluids and hormones, mitosis, and the transfer of ions across cell membranes.
4. By two substances, parathyroid hormone (PTH) and the active form of vitamin D (1,25-dihydroxy vitamin D<sub>3</sub>). The 1,25-dihydroxy vitamin D<sub>3</sub> can increase the intestinal absorption of dietary calcium. PTH is secreted in response to low levels of calcium in the blood. The body responds by retrieving calcium and phosphate from the bones, increasing the reabsorption of calcium by the kidneys and increasing the absorption of calcium in the intestines from foods eaten.
5. Hypocalcemia is a decreased level of calcium. Critically decreased levels of calcium are associated with tetany. Low calcium levels can be caused by hypoparathyroidism, vitamin D deficiency, gastrointestinal problems that block the absorption of calcium or vitamin D, chronic renal failure, magnesium deficiencies and by increased demand due to pregnancy or lactation.
6. Hypercalcemia is an increased level of calcium. Primary hyperparathyroidism is the most common cause of hypercalcemia in outpatients, and malignancy is the most common cause in inpatients. It can also be caused by vitamin D overdose, ingestion of large amounts of dairy products, excessive ingestion of antacids, Paget's disease, and chronic renal disease.
7. Because they form complexes with the calcium in the sample and affect test results.
8. When the sample pH is raised, the ionization and negative charge of the albumin is also raised, causing more calcium to bind with the albumin (thus lowering the free calcium values). Decreasing the pH has the reverse effect, decreasing the calcium bound to the albumin and increasing the free calcium values.
9. Organic phosphate is found in soft tissues in the form of organic phosphate esters. Both organic and inorganic phosphate ions are present within the cell, but most of the phosphates are found in the cells are of the organic form. Within the cells the organic phosphate ions are incorporated into nucleic acids, phospholipids, and high-energy compounds involved in cellular integrity and metabolism. Inorganic phosphate is found in extracellular fluids and serves as part of the body's buffer system.
10. Phosphorus is in almost everything you eat. It is absorbed by the body in the small bowel. Phosphorus is primarily excreted through the urine. The control of phosphate is closely linked to that of calcium. The regulators of calcium (PTH and active vitamin D) affect phosphate concentration. PTH stimulates the kidneys to excrete phosphate while retaining calcium. This causes an inverse relationship between calcium and phosphorus (as one goes up, the other goes down and vice versa).
11. Hypophosphatemia is a decreased level of phosphate. Severe decreases in phosphate can result in neuromuscular disturbances, hemolytic anemia, decreased oxygen released from hemoglobin, and profound muscle weakness. These conditions are normally found in patients recovering from diabetic ketoacidosis, severe respiratory alkalosis, acute alcoholism, and severe burns.
12. Hyperphosphatemia is a increased level of phosphate. This condition is caused by chronic or acute renal failure, hypoparathyroidism, vitamin D intoxication, or hypersecretion of growth hormone. In infants and children high phosphate levels are associated with normal increased levels of growth hormone.
13. All the common testing methods for phosphate are based on the reaction of phosphate ions with ammonium molybdate. In order for the complex to be formed, an acidic pH is required. The pH level must be carefully controlled because a less acidic pH can result in spontaneous reduction of the molybdate complex.
14. Because the anti-coagulants interfere with the formation of the phosphomolybdate complex in the testing procedure.
15. Because red blood cells contain high concentrations of organic phosphate esters. When the cells lyse, these esters are released and can be hydrolyzed into inorganic phosphates during storage (raising phosphate levels).

**206**

1. Magnesium is an activator for more than 300 enzymes in the body. Its intracellular roles include phosphorylation, protein synthesis, glycolysis, cell replication, and nucleotide metabolism, and it affects neuromuscular excitability.
2. Hypomagnesemia is a decreased level of magnesium in the body. Low magnesium levels can cause tetany, impair PTH secretion, and tachycardia (abnormally fast heartbeat) and fibrillation (abnormal heart muscle contractions).
3. Hypermagnesemia is an increased level of magnesium. This condition is usually caused by an excessive intake of magnesium salts (used to relieve constipation), renal problems, severe dehydration, aldosterone deficiencies, and during therapy for pre-eclampsia and eclampsia in pregnant women. The most common signs of magnesium intoxication are neuromuscular symptoms. Deep tendon reflexes disappear and breathing rates can slow or stop. High magnesium levels can also cause cardiac arrest.
4. Red blood cells contain high levels of magnesium.
5. As a tracking mechanism for lithium carbonate treatments. Lithium carbonate is used to treat the manic phase of affective disorders, mania, and manic-depressive illness.
6. Early signs of lithium intoxication (about 2 mmol/l) include apathy, sluggishness, drowsiness, speech difficulties, and twitching. Severe intoxication (about 2.5 mmol/l) is characterized by muscle rigidity, hyperactive deep tendon reflexes, and epileptic seizures.
7. Cesium or potassium.

**207**

1. Intestinal absorption (and to a lesser degree, the bloodstream), which is unique because most other elements are regulated by excretion.
2. Female losses are greater due to normal menstruation as well as the burdens associated with pregnancy.
3. Because of the wide-ranging differences found between commercial methods.
4. Because iron is widely distributed in the environment great care must be taken to avoid contamination of testing equipment or reagents.
5. To saturate the transferrin iron binding sites.
6. In conditions such as chronic infections, rheumatoid arthritis, renal disease, heart disease and several types of malignancies, that are present with concurrent iron deficiency, the ferritin levels may be within normal limits, but would still be considered as increased because of the other underlying condition(s).
7. Because of the protection from menstruation. Women are typically affected post-menopausal.
8. Periodic therapeutic phlebotomy.

**Do the unit review exercises before going to the next unit.**

## Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter. When you have completed all unit review exercises, transfer your answers to ECI (AFIADL) Form 34, Field Scoring Answer Sheet.

**Do not return your answer sheet to AFIADL.**

1. (201) The term that *best* describes the water found within cells is
  - a. water balance.
  - b. total body water.
  - c. intracellular water.
  - d. extracellular water.
2. (201) An example of calculated supplemental data is
  - a. the anion gap.
  - b. a cholesterol level.
  - c. an electrolyte panel.
  - d. the serum glucose level.
3. (201) A trend in high or low anion gaps indicates that
  - a. the patient sample is normal.
  - b. your quality control fluid has expired.
  - c. there may be severe hemolysis in one of the reagents.
  - d. there may be a consistent error in at least one of the analytes.
4. (202) The ultimate regulators of sodium levels in the body are the
  - a. insulin and follicle stimulating hormone.
  - b. adrenal glands through enzymatic action.
  - c. lungs through cardiopulmonary exchange.
  - d. kidneys through reabsorption and excretion.
5. (202) Hemolyzed samples for potassium analysis should be avoided because
  - a. the released hemoglobin will react with sodium.
  - b. intracellular potassium will be released, affecting results.
  - c. of interference with ion-selective electrode (ISE) methods.
  - d. color interference will affect flame emission photometry results.
6. (202) In the disease cystic fibrosis, the concentration of chloride ions in sweat is
  - a. decreased due to insulin.
  - b. elevated due to hormones.
  - c. decreased due to evaporation.
  - d. elevated due to a genetic defect.
7. (202) If you discover that a sample for bicarbonate analysis has been left open in a test tube rack, the result could be
  - a. decreased due to red cell hemolysis.
  - b. increased due to CO<sub>2</sub> absorption from the air.
  - c. falsely decreased due to CO<sub>2</sub> escaping into the air.
  - d. falsely increased due to evaporation of the sample.

- 
- 
8. (203) Because the lungs and kidneys regulate acid-base balance, a patient with disorders affecting these organs will be followed using
    - a. an EKG.
    - b. a hepatic panel.
    - c. blood gas analysis.
    - d. treadmill and blood enzyme analysis.
  9. (203) What happens during internal respiration when the partial pressure of oxygen is 90 mm Hg and carbon dioxide is about 40 mm Hg?
    - a. Carboxyhemoglobin is formed.
    - b. Patient begins to hyperventilate.
    - c. Oxygen moves out of the cell and carbon dioxide moves into the cell.
    - d. Oxygen moves into the cell and carbon dioxide moves out of the cell.
  10. (203) Blood gas samples should be collected only by
    - a. technicians on ward rounds.
    - b. nurses and cardiopulmonary personnel.
    - c. providers or specially trained personnel.
    - d. seasoned lab technicians that know how.
  11. (203) What do you do if you receive a blood gas sample with an air bubble in it?
    - a. Report everything but the  $p\text{CO}_2$ .
    - b. Discard the  $p\text{O}_2$  because it will be elevated.
    - c. Dispose of the original sample and call the ward for a new sample.
    - d. Annotate the final report with the condition the sample was in when it arrived in the laboratory.
  12. (204) The importance of pH in the blood is critical because
    - a. all enzyme reactions occur at a pH of 7.0.
    - b. a pH less than 6.5 causes severe dehydration.
    - c. a pH outside of 6.8 to 7.8 is incompatible with life.
    - d. a pH greater than 8.0 can cause respiratory acidosis.
  13. (204) Which one of the following substances is a buffer found in large amounts and has its concentrations within the body controlled by the lungs and the kidneys?
    - a. Zinc.
    - b. Sodium.
    - c. Ammonia.
    - d. Bicarbonate.
  14. (204) The lungs help maintain body pH by converting carbonic acid into carbon dioxide and water, which
    - a. form bicarbonate.
    - b. form a lung surfactant.
    - c. are then reabsorbed by the kidneys.
    - d. are then expelled during exhalation.
  15. (204) Respiratory acidosis means the blood pH is
    - a. increased due to a metabolic problem.
    - b. decreased due to a metabolic problem.
    - c. increased due to a respiratory problem.
    - d. decreased due to a respiratory problem.

16. (204) A tourniquet, used during venous blood collection, affects pH readings by causing
  - a. the pH result to not be detectable.
  - b. severe hemolysis, resulting in a decreased pH level.
  - c. formation of carbonic acid, resulting in a decreased pH.
  - d. the blood to stagnate, resulting in a decrease of venous  $pO_2$ .
17. (205) Decreased levels of calcium are often associated with tetany,
  - a. a loss of fluids.
  - b. an abnormal heartbeat.
  - c. a hyperexcitability of nerves and muscles.
  - d. the increased secretion of the enzyme pepsin.
18. (205) Increased calcium levels are known as
  - a. hypocalcemia.
  - b. hypercalcemia.
  - c. hypocalcitonin.
  - d. hyperneocalcium.
19. (205) Hemolyzed samples should *not* be used for phosphate analysis because
  - a. released phosphate will bind with free calcium.
  - b. hemoglobin from red blood cells will bind with free phosphate.
  - c. color from the hemoglobin will interfere with colorimetric methods.
  - d. red blood cells contain high concentrations of organic phosphate esters.
20. (206) The *main* dietary sources of magnesium are
  - a. animal fat.
  - b. soybean products.
  - c. meat and green vegetables.
  - d. fruits, such as apples and oranges.
21. (206) One of the *more serious* complications of magnesium deficiency is the effect on the
  - a. renal system.
  - b. hepatic system.
  - c. cardiovascular system.
  - d. gastrointestinal system.
22. (206) Analysis of which one of the following analytes is often requested to track patients with manic-depressive illnesses?
  - a. Sodium.
  - b. Lithium.
  - c. Palladium.
  - d. Magnesium.
23. (207) *All* test methods for serum iron measure
  - a. iron carried by transferrin.
  - b. iron bound to ferritin.
  - c. hemosiderin.
  - d. free iron.
24. (207) Iron levels in the body are regulated by
  - a. dietary intake.
  - b. vitamin C.
  - c. absorption.
  - d. excretion.

25. (207) Total iron binding capacity (TIBC) measures
- a. transferrin-bound iron.
  - b. unsaturated transferrin.
  - c. reserve iron binding sites.
  - d. maximum amount of iron-carrying capacity of transferrin.
26. (207) Iron overload may be accelerated by
- a. menstruation.
  - b. a vegetarian diet.
  - c. chronic alcoholism.
  - d. environmental factors.

## **Student Notes**



## Unit 2. Hepatic Function, Carbohydrates, Enzymes, and Cardiac Markers

<b>2–1. Hepatic Function .....</b>	<b>2–1</b>
208. Liver anatomy and physiology .....	2–1
209. Direct and indirect bilirubin tests.....	2–6
<b>2–2. Carbohydrates .....</b>	<b>2–12</b>
210. Important energy source .....	2–12
211. Glucose .....	2–14
<b>2–3. Enzymes.....</b>	<b>2–24</b>
212. Diagnosing with enzymes.....	2–24
213. Amylase .....	2–27
214. Alkaline phosphatase .....	2–28
215. Alanine aminotransferase and aspartate aminotransferase .....	2–30
216. Gamma glutamyltransferase .....	2–31
217. Lactate dehydrogenase.....	2–32
218. Creatine kinase .....	2–34
219. Acid phosphatase and prostate specific antigen.....	2–37
220. Lipase .....	2–38
<b>2–4. Cardiac Markers .....</b>	<b>2–47</b>
221. Cardiac markers .....	2–47

**T**HIS unit covers the largest organ of the body, the liver, as well as what the body uses for fuel and the catalysts for bodily chemical reactions. Although the procedures you’ll learn about are grouped under various headings, do not think they *only* apply to that given organ or subject. As you will learn, many bodily processes are woven together, and laboratory procedures often serve more than one purpose when diagnosing a patient’s clinical picture.

### 2–1. Hepatic Function

The functions of the human liver are quite numerous and complex. In addition to metabolizing compounds, the liver synthesizes various proteins required by the body. The liver also plays an essential role in the detoxification of poisons ingested or formed by the body. As a clinical laboratory technician, you’ll be called upon to perform series of tests to check liver functions. To fully understand the importance of your work, you should have a basic understanding of the liver.

#### 208. Liver anatomy and physiology

The liver is the largest organ in the human body. The average human liver weighs between 1.2 to 1.5 kg (roughly 2.5 to 3.5 pounds) and is located in the upper right quadrant of the abdomen.

##### Anatomy and physiology

The lungs and diaphragm overlay the upper portion of the liver. The lower portion of the liver overlaps the stomach and intestines. The liver is covered by a collagenous (supportive protein connective tissue) capsule that extends along the structures that enter into the liver. The liver appears to be divided into left and right halves, but closer examination shows it is actually divided into four lobes—left, right, quadrate, and caudate.

### **Blood supply**

The liver receives its blood supply from two sources—the portal vein and the hepatic artery. The portal vein carries blood from the capillary bed of the digestive system to the liver and provides about three-fourths of the blood supplied to the liver. The hepatic artery carries well-oxygenated blood to the liver. The right and left hepatic veins carry blood leaving the liver.

### **Liver function overview**

Among the liver's various functions is the ability to store glycogen for energy. The basic fuel for the body is the simple sugar known as *glucose*. The liver converts glucose (which goes to the liver following digestion) into glycogen for storage. It then reconverts glycogen into glucose, as needed, to keep sugar levels in the blood stable. Although this is usually a slow and continuous process, the liver can respond to epinephrine, which is released into the blood during emergencies, by releasing large amounts of sugar quickly for use by the muscles. The liver also has the ability to store certain vitamins until other organs in the body need them, and it makes many of the essential proteins used in bodily processes. In its role as a clearinghouse, the liver disposes of worn-out red blood cells by breaking them down into different components. Some of these components are stored for future use, while others are sent to the kidneys for excretion in the urine. The liver also filters and destroys bacteria, as well as neutralizes poisons.

Males produce a certain level of female hormones and females produce a certain level of male hormones; it is the liver that regulates these hormone amounts. When an excess of the opposite sex hormone is produced, the liver takes up this excess and disposes of it. Other components of the hepatic system include the bile ducts and the gall bladder. The liver manufactures bile—a clear, golden-brown to greenish-yellow liquid, that is used in the small intestine for digestion. Bile salts emulsify fats, breaking large fat globules into smaller ones that can then be acted on by fat-splitting enzymes of the intestines and pancreas. Bile flows from the liver into the gallbladder where it is concentrated and stored. The function of the three bile ducts are defined in the table below. Also, refer to figure 2-1 to help you visualize the process.

Hepatic duct	The hepatic duct drains bile from the liver.
Cystic duct	The cystic duct drains bile from the gallbladder.
Common duct	The common duct, into which the hepatic and cystic ducts empty, carries bile to a point where it merges with the pancreatic duct, known as the Ampulla of Vater, and into the small intestines.

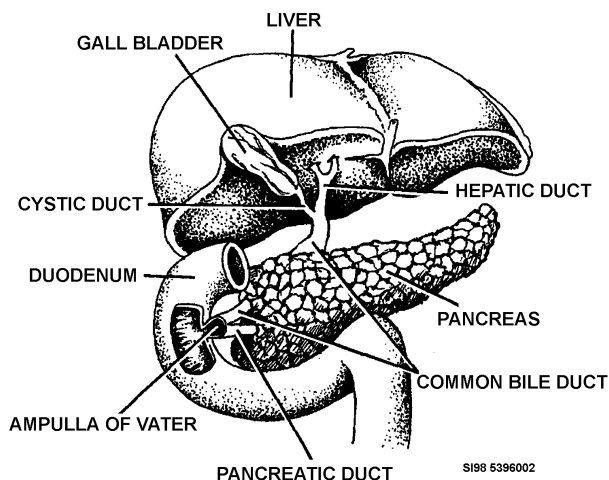


Figure 2-1. The hepatic system.

**Liver functions**

The liver is an organ that carries on a multitude of processes, to include metabolic, storage, excretory, protective, circulatory, and blood coagulation functions. Let's take a look at some of these functions in closer detail.

***Metabolic***

As mentioned previously, the liver plays a key role in the metabolism of carbohydrates. The liver converts glucose to glycogen through the process known as *glycogenesis*. When energy is needed, the liver uses enzymes to convert glycogen back to glucose for use by the body. This process is known as *glycogenolysis*. The liver also converts the lactate formed by working muscles back into glucose. The liver, based on the neural and hormonal signals it receives, maintains glucose levels in the serum. With the exception of gamma globulins, all of the plasma proteins are synthesized in the liver. Some of the plasma proteins the liver makes are albumin, apolipoproteins, complement C<sub>3</sub>, C-reactive protein, and haptoglobin. The liver also produces a variety of proteins needed for blood coagulation. Among these are proteins, such as prothrombin and fibrinogen, and factors, such as factor V, VII, X (all from the extrinsic clotting system), and factor IX (from the intrinsic clotting system). The liver is active in both the anabolism and catabolism of lipids. The liver synthesizes triglycerides, cholesterol, and phospholipids, in addition to the lipoproteins required to carry these lipids to the tissues. Hepatocytes, or hepatic cells, also break down lipids into other compounds used to produce energy. Hepatic cells break down cholesterol to form bile acids. These bile acids act as emulsifiers in the intestine, breaking down ingested fats so they can be digested and absorbed.

***Storage***

You have learned the liver can convert glucose into glycogen for storage. It can also store up to 20 percent of its weight in glycogen and is the primary storage site for this compound. It also temporarily stores small amounts of protein and lipid. Other items stored in the liver include vitamins such as A, D, and B<sub>12</sub>. Iron is also stored in the liver in significant amounts in the hepatocytes.

***Excretory***

You know the liver produces and excretes bile, and that bile is stored in the gallbladder. Bile is a viscous fluid made up of bilirubin esters, bile acid conjugates, cholesterol, phospholipids, and other materials. The bile is stored in the gallbladder until mealtime. After a meal, the intestines produce a hormone that stimulates the gallbladder to contract and eject bile into the small intestine. Bile in the intestines aids in the digestion and absorption of all types of lipids from the foods ingested.

***Protective***

The liver helps protect the body by destroying foreign objects and detoxifying poisons. The liver contains phagocytic cells known as Kupffer cells. These cells remove foreign materials, such as bacteria, from the blood. The Kupffer cells are scavengers and are part of the mononuclear phagocytic system (MPS). Another protective ability of the liver is to take toxic chemicals and convert them to less toxic forms that are water-soluble and can be excreted by the kidneys. One example of this conversion is that of ammonia. Ammonia is produced by bacterial action in the intestines upon amino acids. The ammonia is then carried by the portal vein to the liver, where it is converted to urea by the hepatocytes.

***Circulatory***

The liver serves as a blood storage area and helps to regulate blood volume. The liver also serves as a means for mixing blood from the portal system with the blood circulating throughout the rest of the body.

***Blood coagulation***

As you know, the liver synthesizes protein-clotting factors. Many of these factors have a short life span and need to be replaced continuously. Certain factors (prothrombin, factors VII, IX, and X) also require vitamin K, a fat-soluble vitamin, for their synthesis.

***Liver dysfunction***

There are a wide variety of reasons for decreased liver function performance. The patient may feel pain or illness in some cases but not necessarily in all cases. Causes of liver dysfunction include viral hepatitis, alcoholic liver disease, hepatic toxicity, and blockage.

***Viral Hepatitis***

The term hepatitis indicates an inflammation of the liver. If a person has viral hepatitis, it is a viral agent that is causing the inflammation of the liver. Viral hepatitis is a disease found worldwide. Because there are several causative agents of viral hepatitis, each infection is a little different in the way it's contracted, its severity, its incubation period, and its long-term effects on the patient.

***Hepatitis A virus***

Hepatitis A virus (HAV) is transmitted almost exclusively through the fecal-oral route. Outbreaks are common in areas where there is overcrowding and poor sanitation. The disease is spread rapidly because the feces of those infected are heavily laden with the virus in the early stages of the illness. Transmission can also occur through close contact with an infected person if personal hygiene is minimal. Day-care centers and other institutional settings are common places for outbreaks. The incidences of HAV infections are increasing among homosexuals and people with human immunodeficiency virus (HIV) infections. The incubation period for the HAV is 15 to 45 days, its severity is mild, and most patients recover completely. Hepatitis A has no chronic carrier state.

***Hepatitis B virus***

The hepatitis B virus (HBV) is a blood-borne virus; it can be transmitted through the parenteral route (e.g. needle sharing among drug users, transfusion with contaminated blood products, or accidental puncture with a needle containing infectious blood), sexually, or by the maternal-neonatal path. This type of hepatitis is also increasing among HIV-positive people and accounts for 5 to 10 percent of posttransfusion cases in the United States. Once a person is infected, all of their body fluids contain the HBV. The incubation time can range from 1 to 6 months. Most patients recover completely. The recovery rate worsens with age and debility of the patient. Occasionally, some HBV patients will become chronic carriers of the live HBV.

***Hepatitis C virus***

It is thought that prior to the virus-specific kits that came out around 1990, 90 percent of the transfusion-related cases of viral hepatitis were attributed to the hepatitis C virus (HCV). The transmission of hepatitis by transfusion should decrease as donors are now tested for both HBV and HCV. For many years the detection of HCV was dependent on the identification of circulating antibodies. This method of testing posed a problem for the donor centers. It may take up to 3 months for the HCV antibodies to reach detectable levels. This delay in sufficient antibody levels for testing meant that routine donor testing possibly did not identify all HCV-infected units. Testing for HCV is now performed by nucleic acid amplification (NAT). The NAT technology detects viral nucleic acids using gene amplification procedures, such as polymerase chain reaction (PCR). The use of the NAT technology will greatly reduce the risk of transfusion-acquired HCV infections. Today, HCV still account for about 20 percent of all viral hepatitis as well as most posttransfusion cases. HCV is blood-borne and has an incubation time of 15 to 160 days. As with HBV, HCV is transmitted by the parenteral route. The severity of the disease is moderate and 10 to 50 percent of patients with HCV infections will become chronic carriers.

### *Hepatitis D virus*

In the United States hepatitis D virus (HDV) infections are generally confined to people frequently exposed to blood and blood products, such as I.V. drug users and hemophiliacs. HDV occurs as a simultaneous infection with hepatitis B (coinfection) or as a superimposed infection in someone with chronic hepatitis B (superinfection). A coinfection usually runs the same course as the hepatitis B and resolves spontaneously as the hepatitis B resolves. A superinfection often results in a severe course and should be suspected in a chronically infected HBV patient who worsens. HDV is transmitted through parenteral routes, and its incubation time is 14 to 64 days.

### *Hepatitis E virus*

Hepatitis E virus (HEV) infection closely resembles HAV. HEV is transmitted primarily by fecal-oral route, has an incubation time of 14 to 60 days, and has no chronic or carrier state. HEV is rare in the United States and Europe. This type of viral hepatitis mainly occurs in people who have visited an endemic area, such as India, Africa, Asia, or Central America.

The following chart summarizes the key characteristics of each type of viral hepatitis.

Feature	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis D	Hepatitis E
Incubation	15 to 45 days	30 to 180 days	15 to 160 days	14 to 64 days	14 to 60 days
Onset	Acute	Insidious	Insidious	Acute and Chronic	Acute
Age-group most affected	Children, young adults	Any age	More common in adults	Any age	Ages 20 to 40
Transmission	Fecal-oral, sexual (especially oral-anal contact), non-percutaneous (sexual, maternal, neonatal), percutaneous (rare)	Blood-born; parenteral route, sexual, maternal-neonatal; virus is shed in all body fluids	Blood-borne; parenteral route	Parenteral route; most people infected with hepatitis D are also infected with hepatitis B	Primary fecal-oral
Severity	Mild	Often severe	Moderate	Can be severe and lead to fulminant hepatitis	Highly virulent with common progression to fulminant hepatitis and hepatic failure, especially in pregnant patients
Prognosis	Generally good	Worsens with age and debility	Moderate	Fair; worsens in chronic cases, can lead to chronic hepatitis D and chronic liver disease	Good unless pregnant
Progression to chronicity	None	Occasional	10% to 50% of cases	Occasional	None

### *Alcoholic liver disease*

Long-term consumption of excessive amounts of alcohol can have a detrimental effect on liver functions. When ethanol is catabolized, acetaldehyde is formed. This compound is toxic to hepatic

cells when overindulgence leads to high acetaldehyde concentrations. Injury begins in the liver with the accumulation of fats, and then progresses to fibrosis, which is an increase in the formation of fibrous connective tissue. This fibrous connective tissue replaces normal tissue in the liver. If allowed to continue, the condition can advance to liver cirrhosis and eventually death. The fibrosis and cirrhosis caused by excessive alcohol intake is irreversible.

### ***Hepatic toxicity due to drugs***

One of the prime functions of the liver is to break down compounds so they can be solubilized and excreted or rendered inactive. Some of the drugs, or their metabolites, transported to the liver are toxic to hepatic cells and can cause damage to these cells. Injury to the hepatic cells from some drugs can be predicted and is directly related to the dose of the drug ingested. Other drugs are less predictable in their toxic effects on the liver cells, and must be monitored closely. Drugs that commonly cause liver damage are acetaminophen overdoses, isoniazid (used to treat tuberculosis), tetracycline, chlorpromazine (a tranquilizer), and thiazides (diuretics).

### ***Blockage***

Whenever there is a blockage or obstruction in the flow of bile, a backup in the excretion of bilirubin occurs. This may be caused by either mechanical obstruction to the flow of the bile, due to stones in the bile duct of the gallbladder, tumors around the common bile duct, or by hepatocellular inflammation or damage due to viral hepatitis or drug toxicity. This condition is called cholestasis.

## **209. Direct and indirect bilirubin tests**

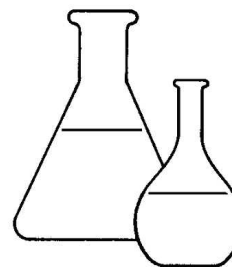
The liver plays an essential role in many of the bodily processes needed for life. When damage or injury occurs to the liver, one or more of the functions of the liver will be affected to some degree. Damage to the hepatic cells usually results in a release of the cells' fluids and enzymes into the blood. These chemicals are what you, the laboratory technician, will test for so the provider can diagnose, track, and confirm recovery of the liver due to disease or trauma. One of the most common tests used to determine the condition of the liver is bilirubin.

### ***Physiology***

Bilirubin is the principal pigment in bile. It is mainly produced when old red blood cells are phagocytized and the hemoglobin released is broken down. Red blood cells normally live in the body for about 120 days. The scavenger cells that destroy the old red blood cells are found in the spleen, liver, and bone marrow.

### ***Hemoglobin breakdown***

The exact process of hemoglobin breakdown is not fully understood. When hemoglobin is broken down, the iron in the hemoglobin is stored for later use and bilirubin, an orange-yellow waste product, is produced. In order for the bilirubin to be excreted, it is bound to albumin as it leaves the MPS. In the liver, the bilirubin-albumin complex attaches to hepatocyte membranes. The bilirubin is then detached from the albumin carrier and transported into the hepatic cells.



### ***Bilirubin***

Inside the hepatic cells, bilirubin becomes conjugated (joined) with glucuronic acid. This bound compound is referred to as *conjugated bilirubin* or *direct bilirubin*. Serum bilirubin that has yet to be conjugated is called *unconjugated bilirubin* or *indirect bilirubin*. The conjugated bilirubin is secreted by the hepatic cells and used in the formation of bile. Bile, as you may recall, is transported through bile ducts into the intestines. As bile approaches the large intestine, bacteria begin to break down the conjugated bilirubin (the glucuronic acid is detached in addition to other reductions). The product of these reductions is known as *urobilinogen*.

### ***Urobilinogen***

A small portion of urobilinogen is absorbed into the portal blood where it is transported back to the liver. Here, it is re-excreted into the bile. Any urobilinogen that escapes excretion by the hepatocytes will be circulated in the peripheral bloodstream and eventually excreted by the kidneys into the urine. Urobilinogen not absorbed in the colon becomes partially oxidized to form the bile pigments urobilin, stercobilin, and mesobilin, which are orange-brown and are the major pigments of the stool. Almost all bilirubin is eliminated in the feces in the form of these pigments. A small amount of urobilinogen is eliminated in the urine, and a very small amount of bilirubin can be found in normal plasma.

### **Clinical significance**

A disturbance in the flow of bile will result in the condition known as jaundice. The deposit of a yellowish pigment in the eyes and skin characterizes jaundice, and when this condition is detected visibly, the bilirubin level is well above normal. Because there are several non-hepatic disorders that cause jaundice and almost all liver diseases cause jaundice, it becomes very important to differentiate the cause of jaundice. Serum bilirubin can be increased by one of several conditions—excess bilirubin in the liver, Gilbert's syndrome, impaired conjugation, enzyme deficiency, and excretion disturbances.

### ***Excess bilirubin in the liver***

In hemolytic diseases or conditions in which too many red blood cells are being destroyed, there is an excess of bilirubin in the liver. The elevation of total serum bilirubin (conjugated and unconjugated bilirubin values combined) in patients with *chronic* hemolytic anemia is usually very mild. The liver has the ability to conjugate and excrete higher levels of bilirubin than it does on a normal basis. So, during chronic states only a slight increase in total bilirubin will be observed. The unconjugated bilirubin will normally be increased, while the conjugated bilirubin will remain at a normal level. When there are increased amounts of bilirubin conjugated by the liver and excreted into the gut, a correspondingly larger amount of urobilinogen is also formed. As a result, fecal urobilinogen and fecal pigments are greatly increased and urine urobilinogen is moderately increased.

### ***Gilbert's syndrome***

Gilbert's syndrome is a congenital defect in the transport system that carries bilirubin through the hepatic cell membrane into the cell microsomes. This defect usually produces a harmless, mild jaundice that is detected during routine bilirubin testing.

### ***Impaired conjugation***

In newborns, the transferase enzyme system is not fully developed. This enzyme system is responsible for converting bilirubin into a glucuronide ester. In healthy, full-term infants, it can take several days for the enzyme to be produced in sufficient amounts to convert the bilirubin present. The serum bilirubin can rise to levels as high as 8 mg/dl by the third to sixth day of life before dropping off to normal levels. This condition is known as *physiologic jaundice of the newborn*. In premature infants, this condition is aggravated because there is a longer waiting time for production of sufficient levels of the esterifying (conjugating) enzymes. The serum bilirubin of premature infants can climb as high as 16 mg/dl, based on this condition alone, or higher, if other problems are also present.

### ***Isoimmune hemolytic disease***

Impaired conjugation of bilirubin can also be caused by isoimmune hemolytic disease (IHD). IHD is caused by incompatibilities with ABO, Rh, or other blood group systems. In adults, IHD will most commonly be seen in the form of a hemolytic transfusion reaction (e.g., when an Rh negative person is transfused with Rh positive blood).

### ***Hemolytic disease of the newborn***

In infants, IHD is called *hemolytic disease of the newborn* (HDN), and is a result of a blood group incompatibility between the mother and the fetus. If the mother has been sensitized previously, by

either transfusion or another pregnancy, IgG antibodies from the mother cross the placenta and bind to their specific red blood cell antigen of the fetus. Then, activated complement binds to the attached IgG antibody and destruction of the infant's red blood cell membranes occurs. This hemolysis produces large amounts of bilirubin, which the immature infant's liver is unable to completely conjugate. Bilirubin binds to plasma albumin until the entire albumin volume becomes saturated. At that point, the bilirubin begins to enter other sites, such as the brain. When bilirubin enters the brain cells, irreversible damage occurs. Left untreated, mental retardation will usually occur if the infant survives. Exchange transfusions can lower the bilirubin concentrations, preventing any serious outcome.

### ***Enzyme deficiency***

Another congenital defect is that of a transferase enzyme that leads to a severe jaundice similar to the type seen in patients with HDN. This condition is known as Crigler-Najjar syndrome. Although this condition is rare, it is extremely severe. More than half of the patients having this condition die within 1 year of birth, and many of the survivors suffer from irreversible brain damage.

### ***Excretion disturbances***

The majority of patients with jaundice fall into this category. In this disorder, the increased total serum bilirubin includes an increase of both conjugated and unconjugated forms of bilirubin. The bilirubin is still allowed into the liver to be conjugated. Because of obstruction or impairment of the excretion of bile into the intestines, the concentration of bilirubin builds up in the hepatocytes and is regurgitated into the bloodstream. In addition, because there is already an increased amount of bilirubin in the hepatocytes, the transport system that normally delivers bilirubin into the liver cells slows down. This slowdown results in a further increase in serum bilirubin levels (hyperbilirubinemia, of which about 50 to 70 percent of the total is conjugated bilirubin). With the decreased amount of bile reaching the intestines, fewer pigments are produced. Feces will vary in color from pale brown or yellow to clay-colored. When the flow of bile is completely stopped due to obstruction, there is a failure in the ability to digest and absorb ingested fats due to the lack of bile salts to act as emulsifiers. The disorders that can cause excretion limitations can be divided into two types: those that originate within the liver (intrahepatic) and those that originate beyond the liver (posthepatic).

#### ***Intrahepatic***

Disorders taking place within the liver are most commonly caused by damage or destruction of the liver cells. Causes can include viral hepatitis, hepatitis caused by toxins (such as drugs and chemicals), or intrahepatic cholestasis. Intrahepatic conditions must be treated by medically managing the patient rather than by surgical means.

#### ***Posthepatic***

These disorders encompass all those that cause the obstruction of the flow of bile into the intestine. Obstructions can be caused by the presence of stones in the gallbladder or bile ducts. Other causes include tumors that obstruct the common bile duct or strictures of the common bile duct. Surgical methods are used to alleviate these conditions and return bilirubin levels to normal. Because surgery can be used to correct posthepatic problems, but cannot be used for intrahepatic conditions, it is important that a correct diagnosis for the cause of jaundice is made.

### ***Bilirubin concentration***

Increased total bilirubin will be mild in chronic hemolytic disease (less than 5 mg/dl), and moderately to severely increased in hepatocellular disease (10 to 30 mg/dl). In cases involving an obstruction, the bilirubin is markedly increased (10 to 60 mg/dl). Conjugated bilirubin is increased in both hepatocellular diseases and obstructive conditions. Unconjugated bilirubin is increased in hemolytic conditions, such as HDN. Almost all of the bilirubin increase caused by hemolytic conditions will be in the form of unconjugated bilirubin. In cases of hepatocellular diseases or obstruction, the majority



of the increased bilirubin will be in the conjugated form. A decreased concentration of bilirubin is of no clinical significance.

### **Laboratory procedures and limitations**

As you know, the liver performs many different functions. Numerous tests are available to evaluate the many functions of the liver. Unfortunately, most of the tests are non-specific because so many diseases cause similar changes in the liver. Usually, a panel of tests is used to determine liver performance. The purpose of the panel is to help determine where in the hepatic system the problem exists, to estimate the amount of damage to the liver that has occurred, and to track the patient's treatment. Assays include serum and urine bilirubin analysis, serum protein analysis, and serum enzyme analysis. Since the focus of this lesson is on bilirubin, only that aspect of liver testing is discussed here. The other procedures analyzing blood are covered later in this volume and urine testing is covered later.

### ***Diazo reaction***

The procedures most widely used for bilirubin analysis are based on the diazo reaction, which was first described in 1883. In this reaction, diazotized sulfanilic acid (the diazo reagent) reacts with bilirubin to form a reddish-purple color at a neutral pH, and a blue color at high or low pH ranges. In 1916, researchers discovered alcohol would speed up the reaction of the diazo reagent and the unconjugated bilirubin. They described the reaction that took place without alcohol as the direct bilirubin fraction. They would then add alcohol to the mixture to achieve a total bilirubin result. By subtracting the direct result from the total, they were able to calculate the indirect fraction.

### ***Modified Jendrassik-Grof method***

Over the years, there have been several modifications to the original methods used to determine bilirubin concentrations. Currently the *modified Jendrassik-Grof* method is used. In order to determine total bilirubin, serum is mixed with a slightly acidic solution of caffeine and sodium benzoate. These chemicals accelerate the diazotization of the unconjugated bilirubin. Diazotized sulfanilic acid reacts with both forms of bilirubin (conjugated and unconjugated) to form an azo dye. When alkaline tartrate (with a pH of 13) is added to the mixture, the azobilirubin is converted to a blue color, which is measured at 600 nm. The total bilirubin concentration is proportional to the amount of color produced. Testing for the conjugated bilirubin begins with mixing the serum sample with diazo reagent *without* the accelerators. Only conjugated bilirubin will react under these conditions within the first 5 minutes. After the 5-minute mark, ascorbic acid is added to stop the reaction by decomposing the excess diazo salt. The solution is then made alkaline and read at 600 nm. The concentration of conjugated bilirubin is proportional to the amount of color produced. To determine unconjugated bilirubin, the conjugated bilirubin result is subtracted from the total bilirubin result.

### ***Sample collection***

Serum or plasma can be used for bilirubin testing. A morning fasting specimen is recommended so lipemia in the sample is avoided. Avoid samples that have been hemolyzed because hemolysis can cause falsely low values with diazo methods. Both conjugated and unconjugated bilirubin are light-sensitive and will break down when exposed to either artificial or natural light. Protect samples from direct exposure to light as soon as possible after being drawn. The sensitivity of bilirubin to light is also affected by temperature. For best results, store samples in the dark at normal refrigerator temperatures. Bilirubin samples are stable for up to 3 days if stored in a dark refrigerator and up to 3 months if stored frozen at  $-70^{\circ}\text{C}$  (in the dark).

### ***Normal values***

In adults, serum bilirubin levels are barely detectable because it's normally excreted in the bile. Total serum bilirubin concentration range approximately from 0.2 to 1.0 mg/dl. Serum bilirubin concentrations for most full-term infants will rise 3 to 5 days after delivery and then begin to fall as

the infant's liver matures and bilirubin-conjugating enzymes are produced. Infants usually reach adult ranges of bilirubin by the time they are 1 month old. In premature infants, bilirubin concentrations will not normally exceed 16 mg/dl, providing no other complications exist.

---

### **Self-Test Questions**

**After you complete these questions, you may check your answers at the end of the unit.**

#### **208. Liver anatomy and physiology**

1. Describe the location and appearance of the liver.
  
2. What are the two sources of blood to the liver?
  
3. What are the liver's functions for providing energy to the body?
  
4. What are some of the clearinghouse functions of the liver?
  
5. Where is bile formed, what route does it take, and what is its function?
  
6. What are some of the metabolism functions that take place in the liver?
  
7. What other substances are stored in the liver besides glycogen?
  
8. Where is bile stored?
  
9. How does the liver help protect the body?
  
10. How does the liver help with blood circulation and the coagulation process?

11. Match the item in column B with the statement it relates to in column A by placing the letter of the item in column B beside the statement in column A. Each item in column B may be used once or more than once.

*Column A**Column B*

- |  |                                   |
|--|-----------------------------------|
| ____ (1) Liver inflammation caused by a viral agent.                               | a. Blockage or obstruction.       |
| ____ (2) Hepatitis that is transmitted most commonly through the fecal oral route. | b. Hepatic toxicity due to drugs. |
| ____ (3) Hepatitis that is transmitted through the parenteral route.               | c. Alcoholic liver disease.       |
| ____ (4) It may take up to 3 months for its antibodies to reach detectable levels. | d. Hepatitis D virus.             |
| ____ (5) Rare in the U.S. and Europe.  | e. Hepatitis E virus.             |
| ____ (6) Occurs in coinfection and superinfection forms.                           | f. Hepatitis C virus.             |
| ____ (7) Caused by overindulgence of ethanol.                                      | g. Viral hepatitis.               |
| ____ (8) Damage to the hepatic cells can be unpredictable.                         | h. Hepatitis A virus.             |
| ____ (9) Creates a backup in the excretion of bilirubin.                           | i. Hepatitis B virus.             |

**209. Direct and indirect bilirubin tests**

- From what is bilirubin derived and how does it get into the hepatic cells?
- Define conjugated bilirubin and unconjugated bilirubin.
- What are the bile pigments that are the major pigments of the stool?
- What is jaundice and how is it characterized?
- Match the item in column B with the statement it relates to in column A by placing the letter of the item in column B beside the statement in column A. Each item in column B may be used only once.

*Column A**Column B*

- |   |                                   |
|---|-----------------------------------|
| ____ (1) This condition is caused when too many red blood cells are being destroyed.                              | a. Enzyme deficiency.             |
| ____ (2) A congenital defect in the transport system that carries bilirubin to the hepatic cells.                 | b. Impaired conjugation.          |
| ____ (3) Known as Crigler-Najjar syndrome, more than half of the patients die within 1 year of birth due to this. | c. Excess bilirubin in the liver. |
| ____ (4) In newborns, this condition is caused because the transferase enzyme system is not fully developed.      | d. Gilbert's syndrome.            |

6. What is the cause of hemolytic disease of the newborn?
7. How do excretion disturbances increase serum bilirubin levels?
8. What are intrahepatic and posthepatic excretion disturbances?
9. Hemolytic conditions cause an increase in what type of bilirubin?
10. What is the principle of the modified Jendrassik-Grof method of total bilirubin analysis?
11. What are the principles of testing for conjugated bilirubin and how the unconjugated bilirubin is determined?
12. Why should fasting samples be used for bilirubin testing and hemolyzed samples be avoided?
13. What special handling do bilirubin samples require and why?
14. In healthy newborns, when are adult bilirubin levels reached?

## **2-2. Carbohydrates**

Regardless of your laboratory size, one of the most common procedures performed is glucose analysis. Many laboratories also test for other sugars and starches, all of which fall under the category of carbohydrates. In this section, you'll learn about the function and metabolism of carbohydrates and the many types of carbohydrate analysis.

### **210. Important energy source**

Carbohydrates are an important source of energy for the body and a major component of the human diet. Glucose is the principal carbohydrate found in the blood. Under normal conditions, all body tissues use glucose for energy. During fasting conditions, certain tissues rely on glucose exclusively. These tissues are the brain, erythrocytes, platelets, leukocytes, and the kidney medulla. Other tissues have the ability to oxidize fatty acids and ketones for their energy purposes. Carbohydrates are present in most foods in small quantities, but are primarily found in sugars and starches. The carbohydrates from food serve as an immediate source of energy.

## Structure

Carbohydrates are compounds made up of carbon, hydrogen, and oxygen. The very term *carbohydrate* refers to hydrates (water) of carbon. When looking at the chemical formulas for carbohydrates, you'll see these compounds contain approximately one molecule of water for every carbon atom (e.g., glucose is  $C_6H_{12}O_6$  and lactose is  $C_{12}H_{22}O_{11}$ ).

## Classification

Carbohydrates are normally classified according to the number of sugars they can be broken down into during hydrolysis. The simple sugars (monosaccharides) include glucose and fructose. Double sugars (disaccharides) include sucrose, which is found in sugar cane, and lactose, which is sometimes referred to as milk sugar. All ripe fruits and many vegetables contain these and possibly other natural sugars. The more complex carbohydrates (polysaccharides) are commonly found as starches and cellulose in foods such as rice, wheat, and potatoes. The following table further defines the different classifications of carbohydrates.

Carbohydrate	Explanation
Monosaccharides	Are the simplest of the carbohydrates and cannot be hydrolyzed to simpler structures.
Disaccharides	Will yield two monosaccharides when hydrolyzed.
Polysaccharides	Carbohydrates with more than two saccharide groups.

## Storage and use

Normally, the body converts carbohydrates into glycogen for storage and future use. When excessive amounts of carbohydrates are eaten, the body will change some of the excess into fats and store them in that form.

## Digestion and absorption of carbohydrates

The digestion process of carbohydrates begins in the mouth, while you are chewing. Starches and glycogen are partially digested by salivary amylase to form maltose. Maltose, along with any ingested lactose or sucrose, is further hydrolyzed by enzymes in the digestive tract to form the monosaccharides glucose, galactose, and fructose. Monosaccharides are absorbed into the blood from the small intestines at a fairly constant rate; however, different monosaccharides are absorbed at different rates, and this process depends on the selectivity of the intestinal mucosa. For example, glucose has an absorption rate several times higher than that of xylose. After absorption, the monosaccharides are transported to the liver by the portal vein, to be metabolized according to the needs of the body.

## Carbohydrate metabolism

The metabolism of monosaccharides is dependent on the body's needs. They are metabolized to perform one of these processes: (1) to be used for energy production by conversion to carbon dioxide and water, (2) to be converted to glycogen for storage in the liver or converted to triglycerides for storage in the adipose tissue, or (3) to be converted into other products such as keto acids, amino acids, or proteins. The steps in the metabolism of glycogen are rather complex. A specific enzyme catalyzes each step in the process. Some steps require two different enzymes, depending in which direction the reaction is moving. That is, one enzyme to form a product and another to help break that same product back down into its original form.

Remember that carbohydrate metabolism is not limited to glycogenolysis, the breakdown of glycogen into glucose, in the liver. Glycogenolysis can also take place in the muscle. Glucose can then be broken down further, into lactate or pyruvate, releasing energy for the body in the process. This process is called *glycolysis*. The function of glycolysis is to supply energy to the body tissues. Carbohydrate metabolism disorders can cause a wide variety of conditions in your patients, some of

which are very severe. These conditions can be the result of hormonal imbalances, such as diabetes, or are hereditary or other enzymatic defects, such as galactosemia and glycogen storage disease. These metabolism disorders will be covered in more depth as you explore specific carbohydrates in later lessons.

## **211. Glucose**

Because of its importance as an energy source to the cells, glucose analysis is one of the most commonly requested tests. If glucose levels are impaired, bodily functions can quickly be altered. Performance of glucose analysis may be used to either diagnose new disorders or follow-up the treatment for an existing problem.

### **Physiology**

Glucose,  $C_6H_{12}O_6$ , is an end product of carbohydrate metabolism. It can also be found in food items, such as fruits. Shortly after digestion, other monosaccharides (such as fructose and galactose) are converted into glucose. Glucose is the chief source of energy for living organisms and is the only monosaccharide normally found in bodily fluids in significant amounts.

### **Concentration**

The concentration of glucose in the blood is regulated by a complex series of pathways and the interactions of numerous hormones. Glycogenolysis is the breakdown of stored glycogen into glucose for release into the bloodstream. This is done on a continuous basis, so the glucose levels are maintained at a fairly steady level. Glucose can also be formed in the body from non-carbohydrate sources, such as amino acids, glycerol, or lactate. This type of glucose production is known as *gluconeogenesis*. Gluconeogenesis plays a significant role in maintaining steady blood glucose levels during periods of prolonged fasting.

### **Demand**

The demand for glucose constantly fluctuates in the body. Your body requires more glucose if you are active—running the Air Force marathon—then it does if you are sedentary—reading your CDCs! The concentration of blood glucose is narrowly maintained by hormones that control the movement of glucose into and out of the blood. Insulin is the only hormone that has the ability to decrease the blood glucose levels, while the hormones glucagon, cortisol, epinephrine, and the growth hormone (GH) all increase blood glucose levels.

### **Insulin**

Insulin is a hormone secreted by the pancreas. It is the only hormone that lowers the blood glucose levels. It stimulates the uptake of glucose into fat and muscle cells, it promotes the conversion of glucose to glycogen or fat for storage, inhibits glucose production by the liver, promotes the synthesis of amino acids, inhibits protein breakdown, and increases glucose utilization by the body. A deficiency in, or lack of, insulin is the cause of diabetes mellitus.

### **Counter-regulatory hormones**

Counter-regulatory hormones are those that increase hepatic glucose production. This is accomplished initially by enhancing the breakdown of glycogen into glucose and then, if needed, stimulating the synthesis of glucose through gluconeogenesis. The following table defines the specific functions of the counter-regulatory hormones.

Hormone	Function
Glucagon	Glucagon is a polypeptide secreted by the pancreas. It stimulates the production of glucose in the liver and the oxidation of fatty acids. Glucagon levels are regulated by the concentrations of glucose in the plasma. The effects of glucagon are confined to the liver.
Cortisol	Cortisol is secreted by the adrenal cortex in response to adrenocorticotropic hormone (ACTH). Cortisol stimulates gluconeogenesis and increases the catabolism of protein and fat.
Epinephrine	Epinephrine is a catecholamine secreted by the adrenal medulla. Epinephrine stimulates glucose production (glycogenolysis). It also stimulates the secretion of glucagon and inhibits the secretion of insulin by the pancreas. Physical or emotional stress increases epinephrine production, which, in turn, increases glucose levels for energy.
GH	GH is a polypeptide secreted by the anterior pituitary gland. This hormone stimulates gluconeogenesis, enhances lipolysis, and antagonizes insulin-stimulated glucose uptake.

### ***Other glucose-influencing hormones***

Other hormones that can have an impact on glucose levels include thyroxine and somatostatin.

#### ***Thyroxine***

Thyroxine ( $T_4$ ) is secreted by the thyroid gland. Its effect on maintaining blood glucose levels is of stimulating glycogenolysis, and increasing the rate of gastric emptying and intestinal glucose absorption.

#### ***Somatostatin***

Somatostatin is a polypeptide found in several areas of the body, but it is primarily found in the hypothalamus and the delta cells of the pancreatic islets. This hormone does not seem to have a direct effect on carbohydrate metabolism, but it does inhibit the release of growth hormone. Somatostatin also inhibits the secretion of glucagon and insulin, producing a reciprocal relationship between these two hormones.

### **Clinical significance**

The disorders relating to faulty carbohydrate metabolism are usually grouped into categories based primarily on the laboratory findings. The conditions that can occur and warrant study through laboratory testing include the conditions shown in the following table.

Condition	Explanation
Hyperglycemia	An increased plasma glucose concentration.
Hypoglycemia	A decreased plasma glucose concentration.

### **Hyperglycemia**

Hyperglycemia is a higher than normal level of glucose in the blood. Most often, you'll see patients with this condition if they are suffering from one of the many types of diabetes. Also, there are a few other hormonal disorders that can result in elevated glucose levels in the patient.

### ***Diabetes mellitus***

Diabetes mellitus is a group of diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Patients with chronic hyperglycemia as a result of diabetes mellitus can suffer long-term damage, dysfunction, or failure of many different organs. The disease will particularly affect the eyes, kidneys, nerves, heart, and blood vessels.

**Symptoms**

Symptoms of hyperglycemia include polyuria, weight loss sometimes with polyphasia, and blurred vision. Stunted growth and susceptibility to infections may also occur in patients with chronic hyperglycemia. The occurrence of acute life-threatening ketoacidosis is of major concern to these patients.

**Long-term effects**

The long-term effects of diabetes mellitus include permanent damage to the eyes (with the potential loss of vision), the kidneys (leading to renal failure), the legs (including foot ulcers and possible amputations). These patients generally have an increased incidence of gastrointestinal, genitourinary, cardiovascular, sexual dysfunction, and cerebrovascular problems. Hypertension, lipoprotein metabolism disorders, and periodontal disease can often be found in these patients. There is also the emotional and social impact of being diabetic and complying with the constant therapy—it places a great stress on both the patient and their families.

**Classification**

For many years, diabetes was categorized according to the therapy required to treat the condition and not by the cause of the condition itself. The two major categories we placed the diabetic patient into were insulin dependent diabetes mellitus (IDDM) or noninsulin dependent diabetes mellitus (NIDDM).

**Old**

The *old way* of categorizing the diabetic patient has been retired and replaced by a method that categorizes the patient by the cause of their disease rather than the treatment of it. This method went into effect in January of 1999 as proposed by the American Diabetics Association (ADA). The simple truth is the treatments and therapies for individual diabetic patients may change through the course of their disease, but the cause of the disorder will not. This approach simplifies the classification system, but is not absolute. As with the old system, the category the patient is placed into often depends on the circumstances present when they are diagnosed, and many diabetics do not easily fit into a single class. For example, a person diagnosed with gestational diabetes mellitus may continue to be hyperglycemic after delivery and may, in fact, be another class of diabetic. Another example are individuals that acquire diabetes because of taking large doses of steroids to treat another condition, but once the steroid therapy is over the patient's glucose levels return to normal. Overall, providers are finding it less important to label the patient as having a particular type of diabetes than to understand the cause of the hyperglycemia and treat the patient effectively. The laboratory plays a big role in the categorizing and treatment of these patients; we must work hand-in-hand with the providers to allow the best course of action for these patients.

**New**

The new classification system consists of 4 categories, which are Type 1 diabetics, Type 2 diabetics, *other specific types*, and *Gestational diabetes mellitus (GDM)*. The majority of patients fall into the first two categories that encompass the two most common causes of diabetes mellitus. The first category, Type 1 diabetics, includes patients with conditions that cause an absolute deficiency of insulin secretion. Individuals at an increased risk of developing Type 1 diabetes can often be identified serologically by identifying autoimmune conditions of the pancreas or genetic markers. With Type 2 diabetics, the cause of the diabetes is a combination of resistance to insulin action by the body and/or an inadequate response by the body to compensate for the lack of insulin function. There are more Type 2 diabetic patients than Type 1 patients. Type 2 patients may have a level of hyperglycemia that causes pathological and functional changes to their tissues and organs, but remain clinically asymptomatic for long periods of time. Their diabetes may go undetected until the cellular destruction and symptoms finally present themselves.



### *Type 1 diabetes*

Type 1 diabetes is generally defined as an immune mediated diabetes and usually leads to an absolute insulin deficiency. Type 1 diabetes was previously called IDDM, Type I diabetes, or juvenile on-set diabetes. Most Type 1 diabetes results from cellular-mediated autoimmune destruction of the insulin producing beta cells of the pancreas. The patient produces autoantibodies that destroy their insulin-producing cells. Autoantibodies against insulin itself have also been identified in some Type 1 patients. Type 1 diabetes is usually diagnosed during childhood or adolescence, but some patients are not diagnosed until they are in their 80's or 90's. The rate of beta cell destruction seems to be dependent of the patient's age. The destruction tends to be more rapid in infants and children, and slower in adults. All patients with Type 1 diabetes are at risk of ketoacidosis; the risk increases as the cellular destruction increases. As the amount of cellular destruction increases, so does the need for insulin therapy. Many adult on-set Type 1 patients will only start insulin therapy when the risk of ketoacidosis increases. Some Type 1 patients can convert from moderate hyperglycemic states into severe hyperglycemic states because of infection or stress. Type 1 diabetic patients are also prone to other autoimmune disorders such as Grave's disease, Addison's disease, and pernicious anemia. Unlike Type 2 patients, Type 1 diabetics are rarely obese when they are diagnosed. A very small percentage of Type 1 diabetics are defined as having *idiopathic diabetes*. The term idiopathic means the nature of the condition is unknown and self-originated. These patients have a permanent insulinopenia, lack of insulin, and are prone to ketoacidosis like the other Type 1 diabetics, but possess no autoantibodies. This form of diabetes usually occurs in patients of African or Asian origin and is strongly inherited. The patient requirement for insulin therapy comes and goes.

### *Type 2 diabetes*

Type 2 diabetes was previously referred to as NIDDM, Type II diabetes mellitus, or adult-onset diabetes. The conditions Type 2 diabetics have, which cause their hyperglycemia, range from disorders that cause insulin resistance along with decreased insulin production to problems with insulin secretion from the pancreas combined with insulin resistance. Patients with insulin resistance produce insulin in sufficient amounts, but do not respond to its effects. Type 2 diabetes can go undiagnosed for many years because the hyperglycemia gradually develops, allowing them to remain asymptomatic until the increased glucose levels start to cause systemic problems. The cause of this type of diabetes is unknown; patients are placed in this classification because they do not fit the criteria for the other three. As the identification of disease processes and genetic defects improve, these patients will probably be reclassified. Type 2 patients generally do not require constant insulin therapy. Most of the patients with this form of diabetes are obese. Obesity can cause insulin resistance, or have an increased percentage of body fat distributed predominantly in the abdominal region. Treatment for these patients includes weight reduction and possible hyperglycemic drug therapy. Type 2 patients seldom experience ketoacidosis as compared to Type 1 patients. Type 2 diabetes occurs more frequently in women with prior GDM, individuals with hypertension or lipid metabolism disorders, or a family history of diabetes. Some ethnic groups, such as African-Americans, Hispanic-Americans and Native-Americans, have an increased incidence of Type 2 diabetes.

### *Other specific types*

This category of diabetes includes a long list of conditions and disorders that result in chronic hyperglycemia. The treatment of these patients is based on the existing conditions and the extent of the hyperglycemia. This category includes, but is not limited to, patients with genetic defects of the beta cells or insulin receptor sites, diseases of the pancreas, hormonal disorders (such as GH, glucagons, or cortisol), or drug, chemical, or infectious agent destruction of the beta cells.

### *GDM*

GDM is defined as any degree of glucose intolerance initially recognized during pregnancy. The treatment for GDM usually involves diet modification and possible insulin therapy. GDM may or may not persist after the delivery of the baby. Approximately 4 percent of all pregnant women

screened in the U.S. are diagnosed as having GDM. Diagnosis of GDM is very important because of the severe problems that can result in both the mother and the newborn if left untreated. GDM is associated with increased intrauterine deaths, hypoglycemic and hypocalcemic newborns, and with increased frequency of maternal hypertensive disorders and the need for cesarean delivery. Generally, babies of GDM mothers have a higher-than-average birth weight. Once diagnosed with GDM, the mother is monitored closely and, after delivery, reassessed to determine the need for continued therapy. The GDM screening test consists of the patient ingesting a 50g oral glucose load (either glucose solution or meal) and, 1 hour after ingesting the glucose, having a plasma glucose level collected. The patient does not fast prior to this test. A result of >140 mg/dl indicates the need for a full-diagnostic, 100g, 3-hour, oral glucose tolerance test (OGTT) be performed in a fasting state. Screening for GDM should be performed between the 24<sup>th</sup> and 28<sup>th</sup> week of gestation in women meeting one or more of the following criteria.

- 25 years of age or older.
- Less than 25-years-old and obese, or greater than 20 percent over desired body weight.
- Family history of diabetes.
- Member of an ethnic group with a high prevalence of diabetes.

### ***Hypoglycemia***

Hypoglycemia is a condition in which the patient's plasma glucose level falls below the fasting limits. Because all of the bodily functions, especially the brain, require adequate amounts of glucose for energy, this condition can have severe consequences. Symptoms may include fainting, weakness, dizziness, tremors, hunger, heart palpitations, confusion, and motor skills impairment. When plasma glucose levels fall below 40 mg/dl in an adult, the patient can lose consciousness; how fast the glucose level drops is a factor in how quickly they lose consciousness. Convulsions are not uncommon either just before or shortly after unconsciousness. Newborn infants are not as sensitive to decreased plasma glucose level and will not normally go into convulsions unless the level drops below 25 to 30 mg/dl. In either case, the lower the plasma glucose level falls, the deeper the coma.

### ***Cortical centers***

In the brain, the cortical centers are the first to be affected. They require the most energy. The lower centers of the brain will also be affected over time. If hypoglycemia has been allowed for too long, irreversible brain damage and/or death can occur. If the coma has lasted for less than 20 minutes and an intravenous solution of glucose is started, consciousness is usually restored immediately and no permanent brain damage occurs. When you are resulting patient samples and come across a hypoglycemic level, contact the provider at once. Hypoglycemia is mainly caused by either hormonal or hepatic disorders.

### ***Hormonal***

An overabundance of insulin can cause hypoglycemia. Several things can cause this to occur, such as too much insulin is injected during therapy, the patient fails to eat after their usual insulin dosage, or there may be an excessive production of insulin by the pancreas. Other hormonal causes include a deficiency of glucagon, a deficiency of cortisol due to Addison's disease, or an anterior pituitary deficiency.

### ***Hepatic***

The liver can cause hypoglycemia. Low glucose levels can be directly attributed to lowered glycogen stores resulting from prolonged fasting, hepatocellular damage, or an acute drug toxicity. Genetic defects in the liver can also be the cause of low glucose levels. A deficiency of glucose-6-phosphatase (due to von Gierke's disease) affects the liver's ability to release liver glycogen.

### **Laboratory procedures and limitations**

The diagnosis of hyperglycemia and hypoglycemia is usually made based on presenting symptoms of the patient (such as hunger, thirst, frequent urination, weight loss, and abnormal glucose test results). The ADA recommends using the following criteria to diagnosis diabetes mellitus patients.

- Symptoms of diabetes plus a casual glucose concentration (CGC) greater than or equal to 200 mg/dl. CGC is defined as a sample taken at any time during the day without regard to the time of the last meal. CGC values meeting this criteria should be evaluated on two subsequent days and confirmed by criteria 3 testing.
- Fasting plasma glucose (FPG) of greater than or equal to 126 mg/dl. FPG samples are collected after the patient has had no caloric intake for at least 8 hours. Patients meeting this criteria have an impaired fasting glucose (IFG). IFG values meeting this criteria should be evaluated on two subsequent days and confirmed by criteria 3 testing
- 2 hour postload glucose (2-h PG) of greater than or equal to 200 mg/dl during an OGTT. The glucose load given to perform the OGTT should contain 75 g of glucose. Patients meeting this criteria have an impaired glucose tolerance (IGT).

### ***Evaluating risk factors***

Even if the patient has met the recommended criteria, the final diagnosis of diabetes mellitus will include evaluating the patient's risk factors, family history of diabetes, and serological testing. Risk factors evaluated include lipoprotein analysis, hypertension, and cardiovascular assessment.

### **Laboratory evaluations**

Laboratory evaluation for diabetes mellitus and other forms of diabetes includes fasting plasma glucose testing and the OGTT.

### ***Fasting plasma glucose***

A fasting glucose concentration of 109 mg/dl has now been chosen by the ADA as the upper limit of "normal." Normoglycemia is defined as plasma glucose levels <110 mg/dl in the FPG and a 2-h postload value of <140 mg/dl in the OGTT. FPG levels greater than or equal to 126 mg/dl are abnormal and are defined as an IFG. Patients are requested to fast (no caloric intake) for 8 hours prior to the collection of the sample.

### ***OGTT***

The OGTT requires the patient be placed on a diet containing at least 150g of carbohydrates per day for 3 days before the scheduled test. Drugs that can affect test results are withheld for the 3 days before the test. Please note it is the provider who should instruct the patient which drugs to take or not take. Some of the drugs that can affect testing results include steroids, estrogen, propranolol (a cardiac drug), phenytoin, and thiazides. The test procedure begins with a blood sample collected from the patient following an 8-hour fast. After the fasting sample has been collected, the patient is asked to ingest a liquid solution containing 75g of glucose. Most facilities purchase these solutions commercially in a variety of flavors in order to make the solution more palatable to the patient (YUM!). The patient ingests the solution within a 5-minute period. Blood samples are collected from the patient 1, 2, and maybe 3 hours after the solution is ingested. The collection times are dependent on the provider's orders. The glucose values of the samples are evaluated using the criteria stated earlier in this lesson.

### ***Glucose testing in CSF***

CSF and other body fluid glucose analysis is performed using the same methods as blood glucose analysis. The concentration of glucose in CSF is about 60 to 75 percent of that in plasma. Changes in the patient's CSF glucose level occur more slowly than that of the blood; it normally takes about 2 hours for a corresponding change to occur in CSF after a change in the blood levels. Blood glucose

levels should be tested in conjunction with CSF glucose levels for this reason. CSF glucose analysis is useful in helping to diagnose meningitis. The bacteria and leukocytes present in the CSF during meningitis will utilize the glucose quickly and lower its concentration. CSF glucose levels, in normal patients, are between 45 to 70 mg/dl, but in patients with bacterial meningitis, this level usually drops to below 30 mg/dl. Viral meningitis usually does not affect CSF glucose levels.

### ***Samples for glucose testing***

Most clinical laboratories use venous blood samples, either serum or plasma, for glucose testing. Analyzers use whole blood samples for self-monitoring or point-of-care testing. (**NOTE:** Plasma or serum glucose levels are 10 to 15 percent higher than whole blood glucose levels). The whole blood glucose levels are lower because of the difference in the water content; the glucose is distributed evenly between the cell and the plasma water. Keep this in mind when comparing samples and methodologies. Serum glucose samples should not be allowed to sit for extended periods of time on the red blood cells. Glycolysis can decrease the serum glucose level by as much as 5 to 10 mg/dl for every hour the sample is left uncentrifuged at room temperature. This rate is increased even more if the sample is contaminated by bacteria or if the patient has an increased number of leukocytes. Using the anti-coagulant sodium fluoride inhibits glycolysis in the sample and stabilizes the glucose level for as long as three days at room temperature. Fluoride ions prevent glycolysis by inhibiting the enzyme enolase. The use of sodium fluoride is not required for samples, as long as the serum and cells are separated within 60 minutes of collection. If a patient is known to have a high leukocyte count, it is required that the sample be collected using a glycolysis inhibitor. Levels with and without inhibitors have been found to vary up to 65 mg/dl after only 1 to 2 hours of contact with red blood cells. CSF glucose analysis should be performed immediately. A delay in testing could allow the bacteria or other cellular contamination in the CSF to utilize the glucose in the sample, decreasing the results. If a delay in testing is unavoidable, the sample should be centrifuged and stored at 4°C or -20°C.

### ***Testing methods***

Enzymatic procedures have become the standard for glucose analysis. They are highly specific, have quick turnaround times, are easily automated, and use 10 µl or less of sample. There are two enzymatic procedures commonly used in clinical laboratories—the hexokinase method and the glucose oxidase method.

#### ***Hexokinase***

Glucose is phosphorylated by adenosine triphosphate (ATP) in the presence of hexokinase and magnesium ions resulting in the formation of glucose-6-phosphate and adenosine diphosphate (ADP). The glucose-6-phosphate is then converted by a second enzyme, glucose-6-phosphate dehydrogenase (G-6-PD), in the presence of nicotinamide-adenine dinucleotide phosphate (NADP) to form 6-phosphogluconate and nicotinamide-adenine dinucleotide phosphate hydrogen (NADPH). The NADPH produced is directly proportional to the amount of glucose present in the sample when read at 340 nm.

- **Advantage.** One advantage of the hexokinase method over the glucose oxidase method is fewer substances—medications or other plasma constituents—will react and interfere with this method.
- **Disadvantage.** A drawback is this method also phosphorylates mannose and fructose; these sugars are not normally present in levels high enough to be of consequence.

#### ***Glucose oxidase***

The glucose oxidase method is specific for the β-isomer of glucose. In the presence of oxygen, glucose oxidase converts the β-isomer of glucose into gluconic acid and hydrogen peroxide. The glucose level in the sample is proportional to the oxygen consumed in the reaction or to the amount of hydrogen peroxide produced. Some instruments measure the rate of oxygen consumption through the

use of an oxygen electrode and convert this reading to one of glucose concentration. When hydrogen peroxide measurement is used for glucose analysis, the reaction uses a chemical such as o-tolidine or o-dianisidine to donate hydrogen to the reaction. These chemicals will turn an intense color when they give up two hydrogen atoms to the oxygen coming from the decomposition of the hydrogen peroxide. The color in the reaction is proportional to the glucose level in the sample. The specificity of glucose measurement, the different isomers, can be increased by using different peroxide indicators for the different isomers. The glucose oxidase method is suitable for the CSF glucose analysis—urine samples should *not* be tested using this method. Urine samples contain high concentrations of substances, such as uric acid, that can falsely decrease the results.

---

### Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

#### 210. Important energy source

1. During fasting conditions, what tissues rely on glucose exclusively for energy?
2. Carbohydrates are made up of what three elements and what is unique about their chemical formulas?
3. How are carbohydrates classified?
4. What are monosaccharides, disaccharides, and polysaccharides?
5. What is the function of salivary amylase?
6. After carbohydrates are digested, where are monosaccharides absorbed and at what rate?
7. Monosaccharides are metabolized to perform what three processes?
8. Glycolysis, in the muscle tissue, serves what purpose?

#### 211. Glucose

1. Other than glucose, which monosaccharides are normally found in bodily fluids in significant amounts?

2. Define glycogenolysis and gluconeogenesis.
3. Match the item in column B with the statement it relates to in column A by placing the letter of the item in column B beside the statement in column A. Each item in column B may be used once or more than once.

*Column A*

- \_\_\_\_\_ (1) This hormone is secreted in the pancreas and stimulates the uptake of glucose into muscle.
- \_\_\_\_\_ (2) This hormone promotes the conversion of glucose into glycogen or fat for storage.
- \_\_\_\_\_ (3) This hormone has its effects regulated by plasma glucose levels and its effects are confined to the liver.
- \_\_\_\_\_ (4) This hormone stimulates gluconeogenesis and increases the catabolism of protein and fat.
- \_\_\_\_\_ (5) Physical or emotional stress increases the production of this hormone that in turn increases glucose levels.
- \_\_\_\_\_ (6) This hormone stimulates gluconeogenesis, enhances lypolysis, and antagonizes insulin-stimulated glucose uptake.
- \_\_\_\_\_ (7) This hormone affects glucose levels by increasing the rate of gastric emptying and intestinal glucose absorption.
- \_\_\_\_\_ (8) This hormone inhibits the release of growth hormone as well as the secretion of glucagon and insulin.

*Column B*

- a. Glucagon.
- b. Epinephrine.
- c. Cortisol.
- d. Insulin.
- e. Somatostatin.
- f. Thyroxine.
- g. Growth hormone.

3. Define hyperglycemia and hypoglycemia.
4. What are the long-term effects of diabetes mellitus?
5. By what criteria is diabetes now categorized?
7. What are the four categories of diabetes?
8. What causes *Type 1 diabetes*?
9. What causes *Type 2 diabetic* patients to have hyperglycemia?
10. The *other specific types* diabetes category includes patients with what conditions?

11. Define *gestational diabetes mellitus* (GDM).
12. What are the criteria used when screening for GDM?
13. A patient can loss consciousness when their plasma glucose levels fall below what level?
14. How do hormonal and hepatic problems cause hypoglycemia?
15. Briefly describe the three criteria used to diagnose diabetes mellitus.
16. Define normoglycemia, and impaired fasting glucose (IFG).
17. When performing an oral glucose tolerance test (OGTT), how much glucose is given to the patient after the fasting sample has been collected?
18. How do cerebrospinal fluids (CSF) glucose levels correlate to blood glucose levels and what is CSF glucose analysis used to diagnose?
19. Why are serum samples for glucose analysis centrifuged and separated shortly after they have clotted?
20. What is one advantage of the hexokinase method over the glucose oxidase method for glucose analysis?
21. In the glucose oxidase method, what two products can be measured to determine the concentration of glucose in the sample?
22. Why should you NOT use the glucose oxidase method for urine glucose analysis?

## 2-3. Enzymes

The use of enzyme analysis as a diagnostic tool has expanded greatly over the course of my time in the laboratory. It has been estimated that enzyme determinations now account for as much as 20 to 25 percent of the laboratory's total workload. In this section, we'll first discuss general information about enzymes and their characteristics, and then, to further our knowledge about enzymes, we will take a closer look at individual enzymes—focusing on their functions in the body and their diagnostic significance. Enzymes are useful in the diagnosis of a heart attack/myocardial infarction, with some being more specific than others. This lesson will provide information regarding some of the enzymes useful in this diagnosis but that lack the specificity for a myocardial infarction (MI) found in the enzymes that will be discussed in Lesson 2-4.

### 212. Diagnosing with enzymes

The study of enzyme activity in the clinical laboratory is not a new concept; in fact, the measurement of digestive enzymes dates back to the early 1900s. The use of enzymes as a diagnostic tool has increased dramatically in recent decades. This is due primarily to our better understanding of bodily processes and technological advances.

#### **The nature of enzymes**

Enzymes are proteins capable of acting as catalysts for specific compounds in specific reactions. Many chemical reactions involving the transformation of organic compounds normally require extremely high temperatures to occur. Since living cells cannot be subjected to these high temperatures, a catalyst must be used to allow the reaction to occur at a safe temperature. The biological catalysts that cause these reactions to take place are called enzymes. Enzymes are present in every cell of the body and while most enzymes target a specific reaction, other enzymes have a lesser degree of specificity and can react with a group of compounds. An example of this latter type is the enzyme hexokinase. Hexokinase phosphorylates glucose, but it also catalyzes phosphorylation of fructose, mannose, and other isomers of glucose. Enzymes catalyze all essential reactions in the body that supply energy and/or chemical changes (such as oxidation, reduction, synthesis, and hydrolysis). Some of the bodily functions that rely on enzymes include breathing, digestion, growth, reproduction, muscle contractions, nerve impulses, and body temperature. Enzymes are defined as biological catalysts. A catalyst is a substance that increases the speed of a chemical reaction or process and is not consumed during the reaction or process. Enzymes are proteins and are synthesized under the control of specific genes. Deficiencies in enzyme proteins or enzyme activity are sometimes traced back to genetic defects.

#### **Enzyme nomenclature**

As the number of identified enzymes increased, it became apparent there was a need to create a systematic method to name the enzymes. Initially, common or trivial names were given to enzymes regardless of the type of reaction they were involved in or the substance they acted upon. Later, enzymes were named by adding the suffix *ase* to the name of the compound being acted on by the enzyme. For example, the enzyme that acted on urea became urease and the enzymes that acted on phosphate esters became phosphatases. Although this was an improvement over previous methods, there was still concern about how to name enzymes that acted on the same substance. In 1955, the International Union of Biochemistry (IUB) established the Enzyme Commission (EC) to develop a system for classifying and naming enzymes.

#### **Names**

Under the system developed by the EC, enzymes are given two names. The first, a systematic name, describes the nature of the reaction catalyzed and is associated with a unique numerical code. The second, a trivial name, is one that has been simplified for everyday use. Although not formally approved by the commission, it has become common practice in the laboratory to use capital letter abbreviations for the names of certain enzymes (such as ALT for alanine aminotransferase).



### Classes

The system the EC established created six enzyme classes. The classes are (1) oxidoreductases, (2) transferases, (3) hydrolases, (4) lyases, (5) isomerases, and (6) ligases. Each enzyme was also given a four-number EC classification number, with each number sequence separated by a period. The first number sequence designates the class to which the enzyme belongs. The next two number sequences indicate the subclasses the enzyme belongs to, and the final number sequence is a unique serial number for that specific enzyme in its subclass. The following table should help you visualize this concept.

EC number	Systematic name	Trivial name	Abbreviation
1.1.1.27	L-Lactate: NAD <sup>+</sup> oxidoreductase	Lactate dehydrogenase	LDH, LD
2.3.2.2	(5-Glutamyl)-peptide: amino-acid 5-glutamyltransferase	$\gamma$ -Glutamyltransferase	GGT
2.6.1.1	L-Aspartate: 2-oxoglutarate aminotransferase	Aspartate aminotransferase	AST
2.6.1.2	L-Alanine: 2-oxoglutarate aminotransferase	Alanine aminotransferase	ALT
2.7.3.2	ATP: creatine N-phosphotransferase	Creatine kinase	CK

### Enzyme action

The exact properties, methods of action, and specific reactions of enzymes are very complex. In this lesson, we will only do a basic overview about the actions of enzymes.

#### Enzyme-substrate complex

The first step in the action of an enzyme is the formation of the enzyme-substrate complex. This is the binding of the substrate molecule to the enzyme. Some enzymes require the presence of a coenzyme, a non-protein substance, before they form the complex with the substrate. Most coenzymes are small, heat-stable, non-protein compounds that are usually derivatives of vitamin B.

#### Active sites

Enzymes possess *active sites*. It is at the site, on the enzyme, that the substrate bonds to the enzyme. When the substrate is bound to the active site, the bonds in the substrate are strained and ruptured, converting it, the substrate, into the new product. The active site has a specific three-dimensional structure, depending on the amino acid sequence of the enzyme itself. Either part or all of the substrate fits into the active site—just as a key fits into a lock—making this process highly specific. Some enzymes also require the presence of an *activator* to induce the correct configuration of the active site so proper binding to the substrate can occur. The most common activators are the inorganic ions—calcium, magnesium, and zinc.

#### After action

After the enzymatic action, the strain and rupturing of the bonds in the substrate, the new substrate product is no longer specific for the enzyme's active site and is released from the enzyme. The free enzyme can now bind to another substrate and start the process all over again.

### Enzyme inhibition

The enzyme's catalytic action depends on its specific protein configuration. Inhibitors can decrease enzyme activity by either changing the enzyme's configuration, such as when drugs, charged molecules, or heavy metals bind to the enzyme, or by the attachment of substances that closely resemble the enzyme's intended substrate not allowing the true substrate to attach. Enzyme inhibition can be caused by either competitive or non-competitive inhibitors.

### *Competitive inhibitors*

Some inhibitors resemble the intended substrate so closely, in shape and charge, they can actually bind to the active sites on the enzyme. These molecules are called competitive inhibitors, because the substrate and inhibitor both compete for the binding sites. When competitive inhibitors are present and more substrate is added to the reaction, the simple increase in mass of the substrate in the reaction will displace the inhibitors from the enzyme's active sites allowing the substrate to attach and, consequently, increase the activity of the enzyme. Competitive inhibitors that result from common metabolic reaction are called antimetabolites.

### *Non-competitive inhibitors*

Non-competitive inhibitors act by altering the configuration of the enzyme. This reduces or eliminates the substrates access to the active site, which results in a decrease in enzyme activity. The addition of more substrate to the reaction has no effect on this type of enzyme inhibitor. The structural alteration that has occurred cannot be reversed. Examples of non-competitive inhibitors include specific drugs or heavy metals.

### ***Factors affecting enzyme activity***

As with any reaction, various factors can affect the final outcome of enzyme activity. These factors include concentration of the enzyme, concentration of the substrate, temperature, pH, inhibitors, and electrolyte environment.

### *Concentration of the enzyme*

If the concentration of the substrate is high and constant during an enzyme reaction, the speed of the reaction is directly proportional to the enzyme concentration. Reactions proceed faster when more enzyme molecules are present to bind with the abundant amount of substrate; this is a *mass action effect*.

### *Concentration of the substrate*

The concentration of an enzyme determines the enzyme's rate of activity. Within certain ranges, enzyme activity is linear; that is, as you add more enzyme, the rate of the reaction increases when a suitable amount of substrate is present. This statement is true until a certain point where the reaction rate plateaus. At this plateau, there are few enzyme active sites available for the substrate to bind to and the reaction rate has reached its fullest potential. The point where the concentration of enzymes no longer affect the rate of the reaction is known as the *zero order reaction*. At the zero order reaction point, there is an excess of substrate and the substrate concentration no longer influences the reaction. Most enzyme tests are designed to be performed under zero order reaction conditions so there is an excess of substrate and the only limiting factor is the amount of enzyme present in the sample. By taking multiple absorbance readings over a period of time while the reaction is at zero order, the concentration of the enzyme can be calculated.

### *Temperature*

Most chemical reactions increase in rate with an increase in temperature, and reaction rates approximately double with each 10°C increase. Most of our bodies' enzymes have an optimum temperature range of 37 to 38°C. Enzymes, like all other proteins, will quickly denature and become insoluble when subjected to temperatures of 60°C or higher. Once denatured, the enzyme activity ceases. Low temperatures will decrease the enzyme activity, but this inactivity is reversible. Samples for enzyme testing can be preserved for months by freezing at -20 to -70°C. Agreement on what temperature to perform enzyme analysis at is not universal, so temperature remains one of the factors responsible for the variation of enzyme results from laboratory to laboratory, because even a slight variance in temperature during analysis can greatly alter the final results. Most analyzers control their temperature to within  $\pm 0.1^\circ\text{C}$  of the optimal range.

### *pH*

As proteins, an enzyme's molecules carry a net charge that is sensitive to changes in pH. Changes in pH can affect the enzyme binding sites that, in turn, affect the attachment of the substrate. The optimum pH range for the effectiveness of an enzyme is very narrow, and the activity of the enzyme usually falls off very quickly once the optimum range has been surpassed. Some enzymes are even characterized based on the pH range they are most active at, such as acid and alkaline phosphatase.

### *Inhibitors*

Remember competitive or noncompetitive inhibitors can cause the enzyme's inhibition. Another example of enzyme inhibition is when the product of the reactant itself inhibits further enzyme activity. For example, a reaction product formed during the hydrolysis of organic phosphate esters by alkaline phosphatase is an actual competitive inhibitor of the enzyme alkaline phosphatase—strange or what!

### *Electrolyte environment*

Many enzymes are sensitive to ions included in the testing media. As we already know, some enzymes require *activators*, usually cations, for their full activation. The correct type and proper ionic strength of required activators should be included in the reaction media.

## **213. Amylase**

An elevated amylase level is usually associated with pancreatitis. But, its diagnostic significance is not limited only to pancreatitis. Amylase originates from both the pancreas and salivary glands. Amylase aids with the break down of carbohydrates into simple sugars.

### **Physiology**

In plants, carbohydrates are stored as starch. In animals, carbohydrates are stored as glycogen. Both starch and glycogen are polymers of glucose molecules broken down into more simple structures by amylase. Both the salivary and pancreatic glands secrete amylase; most of the amylase ends up in the gastrointestinal tract. Pancreatic amylase is the primary source of amylase responsible for carbohydrate break down. Salivary amylase is deactivated by the acidic conditions found in the stomach. Although amylase is predominately found in the pancreas and salivary glands, it is also found in other parts of the body. Amylase activity has been detected in fluids, such as semen, milk, and tears, and in the tissues of the testes, ovaries, fallopian tubes, striated muscles, and lungs. Both salivary and pancreatic amylases are made up of several isoenzymes. Not all of the isoenzymes are present in the serum of a normal individual. Amylase isoenzyme analysis is performed by electrophoresis. The most prominent electrophoretic peaks found in a normal person will include the P<sub>2</sub> peak (the intermediate migrating pancreas band) and the S<sub>1</sub> peak (the slowest migrating salivary band). The P<sub>3</sub> peak frequently occurs in patients suffering from acute or chronic pancreatitis or renal transplants.

### **Clinical significance**

Let's take a look at the possible clinical significance of increased or decreased amylase levels.

#### ***Increased activity***

Increased amylase levels will be observed when there is a blockage in the secretion of amylase from either the pancreas or the salivary glands. Most often the increase is attributed to a pancreatic problem. Serum amylase levels will considerably increase when the pancreatic ducts are blocked or during acute pancreatitis. Serum amylase levels are mildly elevated when there is a blockage of the salivary glands. When there is a blockage of the pancreatic ducts, the increase in serum amylase levels is rapid but temporary. Blockages are caused by stones, inflammation, or compression of the common bile duct by cancerous growths. An acute pancreatitis can be caused by a blockage of the pancreatic ducts, direct injury to the pancreatic tissues by trauma, toxins or inflammation, or impaired

blood flow to the pancreas. These conditions cause the serum amylase level to peak after about 24 hours and return to normal in 2 to 3 days.

Increased serum amylase levels due to pancreatic conditions can reach 6 to 10 times the upper normal limit. The increase in serum amylase levels due to salivary gland conditions will usually be less than four times the upper normal limit. Salivary gland blockage can be the result of an infection, stones in the parotid duct, surgery, or the mumps. Because serum amylase is cleared quickly by the kidneys, urine amylase testing is often ordered in conjunction with serum testing to assist in the diagnosis of acute pancreatitis. Patients with acute pancreatitis excrete large amounts of amylase in their urine. The increased urinary excretion of amylase persists longer than the increased serum levels and generally not returning to normal until 7 to 10 days after the attack. Providers find it useful to compare the serum and urine amylase levels when diagnosing acute pancreatitis.

Amylase levels can also be increased during lung or ovarian tumors, acute appendicitis, ruptured ectopic pregnancy, and acute alcoholic intoxication. Some forms of renal damage can mildly increase serum amylase levels due to impaired urinary amylase excretion from acute or chronic pancreatitis or renal transplants.

### ***Decreased activity***

Acute or chronic hepatocellular damage can cause decreased serum amylase concentration. Amylase measurements are not a sensitive liver function test and should not be used to diagnose liver damage.

### **Laboratory procedures and limitations**

Serum is the specimen of choice for performing amylase analysis. Heparinized plasma is also acceptable; all other common anti-coagulants inhibit amylase activity by as much as 15 percent. For this reason, anti-coagulated samples should be avoided. Amylase activity in serum is very stable; the amount of activity lost after 1 week at room temperature or 2 months refrigerated is negligible. There are thought to be over 200 methods to measure amylase activity. Of these methods, the majority fall into three main categories—amylolytic, saccharogenic, and chromogenic. These three testing categories are known as *starch-based* methods. These test methods differ in regards to how the action of amylase on the starch substrate is measured.

### ***Amylolytic assays***

The amylolytic assays measure the disappearance of the starch substrate. The amount of starch hydrolyzed in a fixed time period is measured photometrically (a blue starch-iodine color compound is used) and the readings are compared to a starch-iodine calibration curve. Results are calculated based on the calibration curve. This manual method is rarely used any more.

### ***Saccharogenic assays***

In saccharogenic assays, the amylase activity is determined by measuring the reducing sugars, glucose, and maltose produced as a result of enzymatic action. Many chemistry analyzer manufacturers use variations of this principle.

### ***Chromogenic assays***

Chromogenic assays use dye-labeled amylase substrates. Linking amylose, amylopectin, or oligosaccharide to a dye makes these substrates. These substrates are insoluble. As amylase in the serum sample breaks down the bonds of the substrate, small bits containing dye are released and the dye becomes soluble. The released, and now soluble, dye can be measured photometrically. Again, variations of this method have been developed and used by several analyzer manufacturers.

## **214. Alkaline phosphatase**

Alkaline phosphatase (ALP) is an enzyme found in many body tissues. It is called *alkaline* because its function is increased in an alkaline environment. Detection of this enzyme is important in diagnosing liver and bone disorders.

**Physiology**

ALP is an enzyme that splits off the phosphate group from organic phosphate esters in an alkaline solution. The optimum activity for this enzyme usually occurs around a pH of 10, although this number can vary depending on the particular substrate and isoenzyme of ALP involved. ALP is found in many tissues, including the intestinal mucosa and placenta, but its highest concentrations is found in the liver and bone. In normal people, the majority of serum ALP will be derived from liver and bone. Although ALP acts on a number of natural and synthetic substrates, the exact substrates affected within the body are unknown. It appears the primary function of ALP is to help with lipid transport in the intestines, and with the calcification process in the bone.

**Clinical significance**

ALP analysis is used mainly to diagnose liver and bone disorders. In addition to patient clinical symptoms, ALP isoenzymes analysis is used to distinguish between the two types of disorders.

***Increased activity***

During biliary tree obstructions, the liver increases its production of ALP. In turn, the serum ALP levels increase. The serum ALP level tends to be markedly high during extrahepatic obstructions, such as stones or cancer in the head of the pancreas. Serum levels may reach 10 to 12 times the upper normal limit and quickly return to normal following surgical removal of the obstruction.

***Intrahepatic obstruction***

Intrahepatic obstruction of bile flow will also increase serum ALP levels; the increase is usually only 2 to 5 times the upper normal limit. Invading cancer cells or certain drugs can affect the biliary tree causing intrahepatic obstructions. Liver diseases, such as infectious hepatitis, can also cause increased ALP levels.

***Paget's disease***

The highest ALP serum levels associated with bone disease is from Paget's disease. In these patients, increased serum ALP levels are the result of osteoblastic cells (the cells responsible for the formation of bone) trying to rebuild bone that has been reabsorbed by the uncontrolled activity of osteoclasts (cells that break down bone formations). Values 10 to 25 times the upper normal limit are not uncommon in Paget's disease.

***Other bone disorders***

Other bone disorders that can increase serum ALP levels include rickets and bone cancer. Temporary increases are often seen in patients recovering from fractures. Normal bone growth in children will also produce elevated ALP results. Growing children will normally have levels 1.5 to 2.5 times higher than that of normal adults.

***Pregnancy***

An increase in ALP may be observed in pregnant women during their third trimester. The increase is usually about 2 to 3 times the normal limit. The placenta produces the additional enzyme. Upward or downward trends in ALP levels can indicate pregnancy complications. But, because of the wide variations that normally occur in placental ALP levels, a single test result is of little diagnostic value.

***Decreased activity***

Low levels of ALP are found in *hypophosphatasia*, a rare congenital defect found in dwarfs as a result of decreased osteoblastic activity. Deficiencies of thyroid hormones in hypothyroidism and vitamin B<sub>12</sub> in pernicious anemia are also known to cause decreased levels of ALP. Patients with these conditions show premature tooth loss and rickets-like bone lesions. Dentists often identify these conditions during childhood checkups.

### Laboratory procedures and limitations

ALP analysis can be performed on either serum or heparinized plasma. The sample should be free of hemolysis. Avoid other anti-coagulants because they can inactivate the enzyme. For optimum results, keep samples at room temperature and test no later than 4 hours after collection. If testing is delayed, freeze the sample to temporarily deactivate the enzyme action; the action will be restored upon thawing. Keep thawed samples at room temperature for 18 to 24 hours before testing to ensure full enzyme reactivation. The method most commonly used for ALP analysis involves the following steps: (1) ALP catalyzes the hydrolysis of p-nitrophenylphosphate into p-nitrophenylate ion and phosphate. The substrate is colorless prior to the reaction, but the products of the reaction are yellow at the reaction pH. (2) The amount of ions produced during the reaction equals the amount of ALP in the sample. The reaction is continuously monitored at 405 nm. ALP from different tissues can display only slightly different properties, making them hard to identify. Distinguishing between some of these multiple forms of ALP and its isoenzymes can require using several techniques. While there are several methods for examining isoenzymes, two of the most common methods for examining ALP isoenzymes are electrophoretic separation and heat-inactivation analysis. The following chart gives a brief explanation of each method.

Methods	Explanation
Electrophoretic separation	Separates serum samples containing the various ALP isoenzymes into recognizable patterns. One concern of using only the electrophoretic separation method for determination is that some of the isoenzyme zones overlap each other.
Heat-inactivation analysis	Some ALP isoenzymes are differentiated based on their stability when exposed to heat. For example, placental ALP has a pronounced stability when exposed to heat. Placental ALP is still stable after being heated to 65°C for 30 minutes. Because other isoenzymes will be deactivated by heat, heating can determine the presence of this particular isoenzyme.

### 215. Alanine aminotransferase and aspartate aminotransferase

The two aminotransferases of clinical importance are alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The term *transaminase* is an acceptable alternative to the term aminotransferase. These two analytes are routinely requested because of their immediate value in diagnosing liver disorders.

#### Physiology

The aminotransferase enzymes are widely distributed throughout the body. Both ALT and AST are found in human plasma, bile, CSF, and saliva. These enzymes are only found in the urine if a patient is suffering from kidney lesions. These enzymes catalyze the reversible transfer of an amino group from an amino acid to a keto acid. This transfer results in the formation of both a new amino acid and keto acid.

#### *Alanine aminotransferase*

The concentration of ALT in body tissues is not as great as that of AST. ALT is found in moderately high concentrations in the liver, but has low concentrations in skeletal and cardiac muscle and other tissues. In clinical applications, ALT is primarily used to diagnose intracellular hepatic diseases. Of the aminotransferases, ALT is the more specific for liver functions. Measuring ALT can be helpful in discriminating hepatocellular jaundice from obstructive jaundice because hepatocellular jaundice will have marked increases of ALT. Obstructive jaundice will only exhibit mild elevations in ALT levels.

#### *Aspartate aminotransferase*

AST is found in practically every body tissue, including red blood cells. The concentration of AST is particularly high in cardiac muscle and liver. Moderate levels of AST are found in skeletal muscle

and the kidneys. The concentration of AST in all other tissues is very low. In addition to helping assess liver damage, AST is used to determine myocardial injury. Although AST is an indicator of cardiac muscle injury, it does not have a high specificity, so its use for this purpose is limited.

### **Clinical significance**

During viral hepatitis, or other liver diseases that cause hepatic necrosis, ALT and AST levels increase even before patients exhibit any clinical signs of disease. In liver disease, AST and ALT levels will most often be 20 to 50 times higher than normal. Values will peak between the 7<sup>th</sup> and 12<sup>th</sup> day of the condition and then gradually decrease. Normal levels will be reached in about 3 to 5 weeks, if the recovery is uneventful. AST and ALT levels will also increase during toxic hepatitis, mononucleosis, and liver cancers. After an MI, the AST levels will increase as the AST concentrated in the heart muscle is released from the damaged tissue. On the average, AST levels do not reach abnormal levels until 6 to 8 hours after the onset of chest pain. The AST values will peak after 18 to 24 hours following the MI. Levels will return to normal by the 4<sup>th</sup> or 5<sup>th</sup> day, providing no other attacks occur. The peak value of AST will be roughly proportional to the extent of tissue damage. During post MI, the ALT levels will remain at or near normal because the levels of ALT in the heart muscle are only a fraction of those of AST. AST activity is also increased in conditions affecting the skeletal muscles, such as during progressive muscular dystrophy; AST levels can increase up to 8 times that of normal. Elevations 2 to 5 times that of normal have also been observed during acute pancreatitis, crushed muscle injury, gangrene, and hemolytic diseases.

### **Laboratory procedures and limitations**

Serum is the specimen of choice for both ALT and AST analysis; use non-hemolyzed specimens. ALT activity in red blood cells is 7 times that of the serum, and the AST levels are 15 times that of the serum. There is some disagreement over the stability of ALT and AST activity in serum samples. It is recommended that samples be stored at 4°C if testing is performed within 1 to 3 days, or frozen if testing is not done within 3 days. If the sample's enzyme activity is beyond the linearity of the analyzer, the serum sample should be diluted and rerun. Saline may be used as the diluent, but an albumin solution is preferred. Consult your operating instruction to determine which diluent to use. Many of the automated analyzers use the following principles for analyzing ALT and AST levels.

#### ***Alanine aminotransferase***

ALT catalyzes transfer of an amino group from alanine to  $\alpha$ -ketoglutarate, forming pyruvate and glutamate. The rate of formation of pyruvate is determined by coupling this reaction with lactate dehydrogenase. In the second part of the reaction, the pyruvate is converted to lactate and the reaction is read at 340 nm. The change in absorbance indicates the amount of ALT present in the sample.

#### ***Aspartate aminotransferase***

AST catalyzes transfer of an amino group from aspartate to  $\alpha$ -ketoglutarate to form oxaloacetate. Oxaloacetate and NADH are then coupled with malate dehydrogenase. This reduces oxaloacetate to malate and oxidizes NADH to  $\text{NAD}^+$ . The decrease in absorbance, read at 340 nm, reflects the amount of AST present in the sample. The reactions listed above are abbreviated versions of the reactions that occur during ALT and AST analysis, giving you only a basic understanding of the process.

## **216. Gamma glutamyltransferase**

The enzyme gamma glutamyltransferase (GGT) helps in the transfer of amino acids and peptides across cellular membranes, and also participates in glutathione metabolism. GGT analysis is performed to detect liver cell dysfunction.

### **Physiology**

GGT catalyzes the transfer of a gamma-glutamyl group to a peptide, an amino acid, or even water. GGT was first noted in kidney tissue, but because of its function in transporting amino acids across

cell membranes, it is now known to be found in all cells, except those in muscle. It is found in highest concentrations in the kidney, liver, pancreas, and prostate. The majority of GGT in the serum seems to originate from the hepatobiliary system.

### **Clinical significance**

GGT is one of the most sensitive enzymatic indicators of hepatobiliary disease. While GGT is elevated in all forms of liver disease, it is highest in cases of intrahepatic or post-hepatic biliary obstruction. GGT levels in obstruction cases may reach 5 to 30 times normal levels. GGT is considered a better indicator of obstructive jaundice than AST, ALT, or ALP because it increases earlier and persists longer than the other enzymes.

GGT levels also increase during infectious hepatitis, liver cancer, acute and chronic pancreatitis, and some pancreatic malignancies. Normal GGT values are rarely observed during liver disease. GGT values cannot be used to differentiate between the various kinds of liver disease, but its specificity surpasses other enzymes in diagnosing hepatic disorders.

An important clinical characteristic of GGT is it can be used to detect alcohol ingestion. GGT values will increase quickly even after a small intake of alcohol. Elevated GGT levels will be observed in the majority of heavy drinkers and patients suffering from alcoholic cirrhosis. GGT levels are also affected in patients whose bodies are attempting to detoxify certain drugs. The release of GGT into the serum reflects the toxic effects of the alcohol or drugs on the microsomal structures of the liver cells. GGT levels correlate well with the duration of the drug action.

GGT levels help determine whether an elevated ALP is due to a bone or liver condition. GGT levels are normal during skeletal disease while ALP levels are elevated. This implies the problem is in the bones. If both the ALP and GGT levels were increased, this would imply the problem is hepatic in nature. The same would be true during muscular diseases; GGT levels are not elevated but AST levels are. Because there are high levels of GGT present in the prostate, normal male GGT values are higher than female values. Increased serum GGT levels can be observed in patients with prostatic cancer. When dealing with malignant diseases in general, an elevated GGT level can arouse suspicion that the disease has metastasized to the liver.

### **Laboratory procedures and limitations**

Serum free of hemolysis is the specimen of choice for GGT analysis. Anti-coagulated samples can cause turbidity in the reaction mixture or depress the activity of the enzyme. GGT activity is stable for 1 week refrigerated and up to 2 months frozen. Although there are several modifications and substitutions to the substrate used for GGT analysis, most procedures are based on the use of  $\gamma$ -glutamyl-p-nitroanilide (GGPNA) as the substrate and glycylglycine as the acceptor. In the presence of GGT and a buffer,  $\gamma$ -glutamylglycylglycine and p-nitroaniline are produced. An increase in absorbance due to the formation of p-nitroaniline in the reaction is measured at 405 nm and the amount of GGT in the sample is calculated.

## **217. Lactate dehydrogenase**

Lactate dehydrogenase (LD) is an enzyme found in the cytoplasm of all cells. LD is primarily used to assess MI and liver disease. It can also be used to diagnose muscle trauma, renal infarction, and hemolytic diseases.

### **Physiology**

LD catalyzes the reversible conversion of lactate to pyruvate. The direction the reaction goes is pH-dependent. The conversion of lactate to pyruvate requires an alkaline pH, while the conversion of pyruvate to lactate occurs at a neutral pH. LD is found in all body tissues, but its concentration is highest in the heart, liver, kidneys, skeletal muscle, brain, red blood cells, and lungs. Since LD is so widely distributed in the body, the diagnostic value of an increased total LD concentration is questionable. Some researchers have compared the clinical usefulness of an increase in total LD



concentration to that of an increased sedimentation rate. Both indicate an ongoing disease process, but neither shed much light on the specific origin of the problem.

### **Individual isoenzymes**

By analyzing the individual LD isoenzymes, we can pinpoint the cause of the increased LD activity.

### **Five LD isoenzymes**

The five LD isoenzymes commonly analyzed, because of their clinical diagnostic value, are LD<sub>1</sub>, LD<sub>2</sub>, LD<sub>3</sub>, LD<sub>4</sub>, and LD<sub>5</sub>. Other LD isoenzymes exist, but their analysis is not of clinical value yet. Each of the five isoenzymes is made up of four peptide chains. These peptide chains are classified into two types: M-type typical of skeletal muscle and H-type from heart muscle. The following table shows the makeup of the various isoenzymes and where they are primarily found in the body.

Isoenzyme	Chain Structure	Prevalent in
LD <sub>1</sub>	HHHH (H <sub>4</sub> )	Heart muscle, red blood cells, and kidneys.
LD <sub>2</sub>	HHHM (H <sub>3</sub> M)	Heart muscle, red blood cells, and MPS.
LD <sub>3</sub>	HHMM (H <sub>2</sub> M <sub>2</sub> )	Lung, kidney, and brain.
LD <sub>4</sub>	HMMM (HM <sub>3</sub> )	Kidney, placenta, and pancreas.
LD <sub>5</sub>	MMMM (M <sub>4</sub> )	Liver and striated muscle.

### **Clinical significance**

LD activity is present in all of the cells of the body. LD levels in the tissues are about 500 times that of serum LD levels. As such, even a small amount of tissue damage can cause a significant increase in serum LD levels. Total LD levels are commonly ordered as a *screening test* when a particular condition is suspected. If the total LD is increased, a request for LD isoenzymes will follow. Each specific isoenzyme, either by itself or in conjunction with other LD isoenzymes, can help the provider accurately diagnose the patient's disorder.

### **Myocardial infarction**

One of the most common uses of LD analysis is to determine whether or not a patient has suffered an MI. The serum LD levels increase after an MI, but the increase occurs later than that of the enzyme creatine kinase. This delayed increase allows LD analysis to be very useful in the delayed diagnosis of an MI, such as when patients finally presents themselves after having severe chest pains 3 or 4 days ago. In a normal LD isoenzyme pattern, the LD<sub>2</sub> isoenzyme level is greater than that of the LD<sub>1</sub> isoenzyme level. When a patient has suffered an MI, the isoenzyme pattern is said to be *flipped*; that is, the LD<sub>1</sub> isoenzyme level is greater than the LD<sub>2</sub> level.

### **Hemolytic conditions**

Any condition that causes severe hemolysis can also cause an increase in serum LD activity. Hemolytic conditions usually result in an LD isoenzyme pattern similar to that of an MI. Research suggests, however, that this pattern (due to hemolysis) depends on an increased reticulocyte level of 10 percent or greater. Megaloblastic anemias resulting from a deficiency of folate or vitamin B<sub>12</sub> can cause red blood cell precursors to break down while in the bone marrow. As these cells break down, they release large quantities of LD<sub>1</sub> and LD<sub>2</sub>. After appropriate treatment, the patient's LD levels quickly return to normal.

### **Other conditions**

Increased LD activity can be observed during liver diseases, but the aminotransferase (ALT and AST) activity is much more increased. LD levels can increase ten-fold during toxic hepatitis, and slightly lower LD values are seen in viral hepatitis and infectious mononucleosis. In cirrhosis or obstructive jaundice, LD levels can range from normal to only twice the normal level. Increased serum LD levels are observed in about one-third of the patients suffering from renal disease, especially those with tubular necrosis or pyelonephritis. The LD isoenzyme patterns in renal disease are very similar to

normal serum patterns. In renal infarction, the LD isoenzyme pattern also resembles that of an MI. In muscle disease, such as progressive muscular dystrophy, the total LD levels can be moderately increased. During the early to middle stages of the disease, the LD increase is confined to the LD<sub>5</sub> isoenzyme. In the later stages of the disease, most of the muscle mass containing LD<sub>5</sub> has been lost, so LD levels will decline. The decline in some patients has gone as far as falling to normal LD levels.

Patients with malignant diseases, such as Hodgkin's or abdominal or lung cancer, often show an increased serum LD activity. As a general rule, LD levels in cancer patients are too erratic to be a useful diagnostic tool, although LD can be helpful in monitoring the effectiveness of chemotherapy against the tumor.

### ***CSF activity***

LD activity in normal spinal fluid is much lower than that of the serum, and the levels of isoenzymes LD<sub>4</sub> and LD<sub>5</sub> are practically undetectable. When the spinal fluid is contaminated due to hemorrhage or by disease to the blood-brain barrier, the LD level will increase. The increase is caused by LD isoenzymes released from the cells that have entered the spinal fluid. During bacterial meningitis, a granulocytosis occurs, causing an increase in LD<sub>4</sub> and LD<sub>5</sub>, while viral meningitis generates a lymphocytosis causing an increase in LD<sub>1</sub>, LD<sub>2</sub>, and LD<sub>3</sub>. In neonates, an increased level of spinal fluid LD is associated with intracranial hemorrhage and hydrocephalus.

### **Laboratory procedures and limitations**

Serum or heparinized plasma can be used for LD analysis, but must be separated from the cells as soon as possible. Hemolyzed samples cannot be used, as red blood cells have an LD level 150 times that of serum, and ruptured cells will falsely elevate results.

### ***Sample storage***

Serum samples may be stored at room temperature for 2 or 3 days without any loss of LD activity. The different isoenzymes vary in their sensitivity to cold. LD<sub>4</sub> and LD<sub>5</sub> quickly lose their activity if refrigerated and will lose all activity if frozen. Some experts feel that, if samples must be stored for longer than 3 days, nicotinamide-adenine dinucleotide (NAD<sup>+</sup>) or glutathione must be added to preserve the activity of LD<sub>4</sub> and LD<sub>5</sub>, while others feel that storage at 4°C alone is adequate. Consult your laboratory operating instructions to determine specimen storage policies.

### ***Automated analyzers***

Automated analyzers either use the forward reaction, lactate to pyruvate, or the reverse reaction, pyruvate to lactate, to measure LD activity. In the forward reaction, LD converts lactate to pyruvate while reducing nicotinamide-adenine dinucleotide (NAD<sup>+</sup>) to nicotinamide-adenine dinucleotide reduced (NADH). The rate of increase in the absorbance of NADH, at 340 nm, is proportional to the LD activity in the sample. In the reverse reaction, pyruvate to lactate, the decrease in absorbance is measured. Here, the NADH is oxidized back to NAD<sup>+</sup>.

### ***Isoenzyme analysis***

LD isoenzymes analysis is performed by electrophoresis. The separation of LD isoenzymes is quite similar to that of serum proteins. After the isoenzymes have been separated by electrophoresis, a reaction mixture is layered over the separation medium. Depending on the manufacturer and method, the final product can be interpreted by fluorescence, long-wave ultraviolet light, or densitometry.

## **218. Creatine kinase**

Creatine kinase (CK) belongs to a group of enzymes known as kinases; these enzymes require magnesium ions to be present as activators to their reactions.

## Physiology

CK takes part in a reversible reaction. In the forward reaction, CK and magnesium ions catalyze the transfer of a phosphate from ATP to creatine, forming creatine phosphate and ADP. This reaction is reversible; creatine phosphate and ADP, in the presence of CK and magnesium ions, will yield creatine and ATP. The direction of the reaction is dependent on the pH of the reaction media. An alkaline pH favors the forward reaction (forming creatine phosphate) and a neutral pH favors a reverse reaction (formation of ATP).

## Clinical significance

CK is found predominately in skeletal muscle, heart muscle, and the brain. Increase in serum CK levels are normally attributed to damage of the striated muscle (either skeletal or cardiac) and, in rare cases, to the brain. Differentiation between these conditions can sometimes be made based on present clinical evidence, but often the use of CK isoenzymes is required for accurate differentiation.

## Isoenzyme structure

CK isoenzymes are broken down into two polypeptide subunits classified as B for brain and M for muscle. There are three primary CK isoenzymes: CK-BB (CK<sub>1</sub>), made up of two B chains; CK-MB (CK<sub>2</sub>), made up of one M and one B chain; and CK-MM (CK<sub>3</sub>), made up of two M chains. The following table shows the isoenzyme structures and where they are predominately found.

Isoenzyme Name	Isoenzyme Structure	Isoenzyme Distribution
CK-BB (CK <sub>1</sub> )	BB: two B chains	Brain.
CK-MB (CK <sub>2</sub> )	MB: one B chain, one M chain	Cardiac muscle.
CK-MM (CK <sub>3</sub> )	MM: two M chains	Skeletal muscle.

## CK-MB activity

Serum CK activity is most commonly used in assessing MI patients. CK activity will begin to increase within 3 to 6 hours after an MI and will usually reach its maximum level about 24 hours after onset. In most cases, the increase in CK levels will be about 5 to 8 times the upper normal limit. The higher the increase in CK activity, the worse the prognosis. Severe cases of MI can increase levels as high as 10 to 20 times that of normal. As long as no further MIs occur, CK levels will return to normal in 2 to 3 days. CK-MB determinations are the single best test for the early diagnosis of an acute MI. Other cardiac conditions or procedures, such as cardiac trauma or cardiac surgery, have been known to cause increased CK and CK-MB levels sufficient to mask a subsequent MI. Marathon running and heavy/endurance exercising have been shown to cause elevated CK-MB levels.

## CK-MM activity

In normal serum, most of the circulating CK is of the isoenzyme CK-MM. When total CK level increases are due to an increase in CK-MM, the cause is usually some kind of stress or injury to skeletal muscle. Vigorous exercise, multiple intramuscular injections, electroconvulsive therapy, and surgery can increase CK-MM levels. In muscular diseases, such as progressive muscular dystrophy, CK-MM levels can reach as high as 50 times the upper normal limit, and these levels can be reached long before the patient exhibits any outward clinical symptoms. Increased CK-MM levels in infants and children are especially indicative of muscular disease.

## CK-BB activity

Because CK-BB is found predominately in the brain, patients suffering from head trauma will normally show increased CK-BB activity. The degree of CK-BB elevation will usually correlate to the extent of brain damage and the prognosis of the patient. In some cases of brain damage, an increase in CK-MB levels is observed. This could be due to myocardial damage following the cerebral accident. In children suffering from Reye's syndrome (a childhood disorder where there is

acute brain swelling), the total CK activity can increase as much as 70 times the normal limit, and only CK-BB will be present in the serum.

### **Laboratory procedures and limitations**

Serum is the sample of choice for CK analysis. Plasma samples containing heparin, EDTA, citrate, or fluoride have been known to yield unpredictable reaction rates. The activity of CK in serum is unstable, and is lost quickly during storage. Different isoenzymes lose activity at different rates. CK-MM is only stable for 1 day at room temperature, 1 week at 4°C, and 1 month at -20°C. CK-MB is stable at room temperature for 12 hours, 3 days at 4°C, and 1 month at -20°C. The stability of CK-BB is even less stable than that of CK-MB. CK activity is decreased by exposure to bright light and increased pH. The pH increase can result from sample exposure to carbon dioxide in room air. Store samples for CK testing in the dark and in tightly sealed tubes. Although a non-hemolyzed sample is best, a slight degree of hemolysis can be tolerated, as red blood cells do not contain any CK activity. Do not use moderately or severely hemolyzed samples because chemicals regulating cellular functions will be released and may affect some of the side reactions taking place during the CK assay.

### **Methods**

There are numerous photometric, fluorimetric, and coupled enzyme methods available for CK analysis. These methods all utilize either the forward creatine-to-creatine phosphate, or reverse creatine phosphate-to-creatine reaction. If reaction conditions are optimized, the reverse reaction proceeds six times faster than the forward reaction. For this reason, most analyzers use the reverse reaction methods to determine CK levels. In the reverse reaction, creatine phosphate and ADP in the presence of CK will yield creatine and ATP. The ATP produced is then added to glucose in the presence of hexokinase. This reaction yields glucose-6-phosphate (G-6-P) and ADP. The G-6-P that results is then added to NADP<sup>+</sup> in the presence of glucose-6-phosphate dehydrogenase (G-6-PD), resulting in 6-phosphogluconate, NADPH, and H<sup>+</sup>. The rate of formation of NADPH is a measure of the concentration of CK activity in the sample.

### **Isoenzyme analysis**

The three most commonly used methods for CK isoenzymes analysis are electrophoresis, ion-exchange chromatography, and immunological.

#### *Electrophoresis*

In electrophoresis, the CK isoenzymes are separated on agar, agarose, or cellulose acetate. The isoenzyme bands are then treated with a mixture prompting the reverse reaction. The NADPH formed by this reverse reaction can then be visualized under fluorescent or visible light, and quantified by scanning on a densitometer or fluorometer.

#### *Ion-exchange*

Ion-exchange methods used to separate CK isoenzymes use either batch adsorption or column chromatography using various media. The basic process involves the CK isoenzymes adsorbing onto the medium, usually a gel. The gel is then washed and the CK isoenzymes are then eluted using TRIS buffers with varying sodium chloride concentrations. A major drawback to this method is the CK isoenzymes may be greatly diluted during the elution process, requiring the fractions to be concentrated after collection or some other method used to reduce the dilution effect.

#### *Immunological methods*

Immunological methods require the use of specific antisera against either the M or B subunits in order to measure the CK isoenzymes. When measuring CK-BB, an anti-CK-M serum inhibits any M subunits so only CK-BB activity is measured. When measuring CK-MM, an anti-CK-B sera inhibits any B subunit activity so only CK-MM activity is measured. Determining the activity of CK-



MB presents a unique challenge. In order to determine CK-MB activity, a sandwich technique is often used. In this technique, two antibodies having different affinities for different parts of the CK-MB molecules are used sequentially. This method provides an estimate of CK-MB activity because neither CK-MM nor CK-BB will react with both antibodies.

As advances are made in technology, new methods will affect the sensitivity and specificity for isoenzyme testing.

## **219. Acid phosphatase and prostate specific antigen**

Cancer of the prostate is a disease that primarily attacks elderly men, and its early diagnosis is often difficult due to its lack of clinical signs and symptoms. Two of the tests commonly performed by the clinical laboratory to diagnose and track the treatment and progress of prostate cancer are acid phosphatase (ACP) and prostate-specific antigen (PSA).

### **Physiology**

The prostate is a gland rich in ACP; it has a higher concentration of this enzyme than any other tissue in the human body. ACP is found, to some degree, in the liver, spleen, erythrocytes, platelets, and bone marrow. In healthy males, the prostate only contributes a very small portion of the ACP found in the serum. The majority of the ACP found in normal serum comes from the osteoclasts. Prostate ACP reacts best at a pH of 5 to 6 to split off a phosphate group from various organic esters. PSA is a glycoprotein, part carbohydrate and part protein, found only in the cytoplasm of prostatic epithelial cells. Like ACP, PSA is used to investigate and monitor prostatic cancer.

### **Clinical significance and limitations**

Laboratory analysis to detect and monitor the treatment of prostatic cancer includes ACP and PSA testing.

### **ACP**

Serum ACP analysis is almost always performed to detect or monitor cancer of the prostate. This form of cancer is the third leading cause of cancer-related deaths in men after lung and colon cancers. One of the problems associated with using ACP to detect prostatic cancer is ACP levels often remain normal until the cancer metastasizes. Only after the carcinoma metastasizes, or invades blood capillaries, lymph channels, and other tissues, will the ACP activity increase dramatically. If the prostatic tumor is successfully treated by surgery, ACP levels will return to normal within several days. If the tumor is treated by estrogen therapy, the enzyme levels will return to normal within several weeks. Should the levels of ACP continue to increase after therapy, the prognosis for the patient is usually poor and indicates the cancer has spread to other parts of the body. Abnormal ACP activity is not limited to males. Increased ACP activity is also observed in Paget's disease (a bone disease) and the malignant invasion of bones by cancers, such as breast cancer. Because ACP is also found in relatively high concentrations in seminal fluid, ACP testing is sometimes performed on vaginal secretions. This testing may occur during investigations of rape or similar offenses.

### **PSA**

PSA is an antigen that can be detected in all males; however, the levels of this antigen are greatly increased in patients who have prostatic cancer. The higher the antigen level, the greater the burden of the tumor. Because PSA is only produced by the prostate, this makes this glycoprotein an excellent tumor marker. Cancerous prostatic tissue produces ten times more PSA than normal prostatic tissue. Like ACP, levels will decline if treatment has been successful. If levels fail to decline, then patient prognosis is poor. PSA is replacing the use of ACP as a screening tool for the early detection of prostatic cancer. This is due to the lack of sensitivity of ACP in the early stages of the disease. However, ACP is still quite useful in determining the staging of prostatic cancer because an elevated level of ACP is usually associated with metastatic disease.

**Laboratory procedures and limitations**

Serum is the sample of choice for both ACP and PSA testing. For ACP testing, separate the serum from the red blood cells immediately and stabilize the specimen. You can stabilize the samples by adding commercially available disodium citrate monohydrate tablets or acetic acid to the serum until it reaches a pH of 5.4. At this pH, the sample is stable for several hours if left at room temperature and up to a week if refrigerated. Although the substrate used in ACP testing is fairly insensitive to the ACP found in red blood cells, avoid moderately to highly hemolyzed samples. Also avoid lipemic samples because the turbidity can interfere with measurements. The ACP from non-prostatic sources will hydrolyze certain substrates at a rate much lower than prostatic ACP. Since the object of ACP testing is almost always to determine prostatic ACP, substrates preferred by this enzyme are used in assay systems.

***Thymolphthalein monophosphate***

In continuous-monitoring methods, one of the most commonly used substrates is thymolphthalein monophosphate. Thymolphthalein monophosphate is hydrolyzed by prostatic ACP at a pH of 5.4. The reaction is stopped after 30 minutes by the addition of a sodium hydroxide-sodium carbonate solution. This develops an alkaline color with liberated thymolphthalein. The reaction is read at 595 nm, and the absorbance of the solution corresponds to the ACP activity.

***Immunological methods***

The ACP activity can also be measured using immunological methods. These methods determine their specificity for the source of the ACP based on the specificity of their antiserum. In most of these methods, the total activity of ACP is first measured, and then, a specific antiserum is used to capture the prostatic ACP. The difference between the two measurements determines the prostatic enzyme's activity. PSA is also measured immunologically. Several methods are commercially available using radioactive, enzyme, and fluorescent labels.

**220. Lipase**

The pancreas is the primary source of lipase production. In the presence of the cofactor colipase (required), lipase breaks down lipids into fatty acids and glycerol, which is then able to permeate the linings of the stomach and the small intestines for use by the body. The rate of lipase action is dependent upon the amount of surface area of the substrate available; the more available, the faster the rate of lipolysis. Surface area is made available through the action of bile salts (by breaking down the larger molecules), keeping the substrate free of other proteins, and in conjunction with the actions of colipase, allowing lipase activity to progress. Calcium is required for lipase activity, but, at higher concentrations, it will act as an inhibitor to lipase activity.

**Clinical significance**

Lipase is the enzyme of choice in conjunction with amylase to assess and diagnose pancreatitis. Lipase is typically measured to determine pancreatitis, but can be used for assessing other pancreatic disorders. Pancreatitis can be acute (sudden painful onset) or chronic (develops gradually over time). In chronic pancreatitis, the pancreatic secretions are blocked from reaching the duodenum and begin to digest the pancreas itself—a condition called autodigestion. Pancreatitis occurs in a number of presentations:

Type of Pancreatitis	Presentation
Gallstone	Gallstones lodge in the duct shared by the pancreas and gallbladder; more common in ♀.
Alcoholic	Occurring in individuals with long-term abuse; more common in ♂.
Drug-induced	A multitude of drugs can cause pancreatitis.
Post-ECRP	Endoscopic Retrograde Cholangiopancreatography; surgical procedure that involves dye injection into bile & pancreatic ducts – affects 3-5% of patients having this procedure.
Hereditary	Number of causes; example = familial hypertriglyceridemia (triglyceride $\geq 1000$ mg/dl).
Idiopathic	No underlying cause; 30% of all cases.
Other	Infections, mumps, abdominal trauma.

Lipase increases rapidly following an attack of acute pancreatitis, ranging from 2 to 50 times the upper limit of the normal range. Lipase activity in this instance will rise within 4 to 8 hours, peak around 24 hours, and remain at elevated levels for 8 to 14 days, which is a longer period than amylase. Lipase activity is not proportional to the severity of an attack nor is it specific for diagnosing pancreatitis exclusively, although it is a good indicator in the case of *acute* pancreatitis. Lipase can be elevated by a variety of other conditions to include mumps, alcoholism, carcinoma, renal diseases, abdominal trauma, and other abdominal disorders, blockages, or diseases. Because the pancreas is sensitive to proximal abdominal disorders (such as liver trauma, intestinal problems, etc), the specificity of the lipase test for pancreatitis is reduced. Lipase testing for chronic pancreatitis may be somewhat useful; however, it appears that determining the ratio of pancreatic amylase to lipase is a useful indicator for gauging the stage of the disease.

### Laboratory procedures and limitations

Lipase can be widely dispersed, allowing for testing on serum, acitic, and pleural fluids. Serum is most commonly used and is where our study will be focused. Serum specimens are stable for one week at room temperature and increased stability is achieved via refrigeration or freezing. The optimal testing pH range is 7.0 – 9.0 and the optimal reaction temperature is 40° C. Icterus, lipemia, and hemolysis have not demonstrated interference with turbidimetric methods. In addition to turbidimetric methods, immunoassay, titrimetric, turbidimetric, spectrophotometric, fluorometric, and agarose gel methods are available. Other diagnostic procedures outside the realm of laboratory testing, such as X-rays, fine needle aspiration, and computerized tomography (CT) scans can aid in determining the root cause of pancreatitis.

#### *Titrimetric methods*

This is the classic method developed by Cherry and Crandall in 1932 and was modified by Tietz and Fiereck in the 1970s. Olive oil acts as the substrate in this procedure. In this method, lipase activity was determined by the amount of free fatty acids released during the incubation period and estimated by titration to neutrality with an alkali. In the original procedure, the incubation time was 16-18 hours making the test too slow to be diagnostically useful. The Tietz modification reduced the time required for testing whereby it could be completed on the same day.

#### *Turbidimetric methods*

Turbidimetric procedures are based on the emulsion of fats in water-creating micelles (an aggregation of molecules) that make the solution milky in appearance, because these micelles absorb and scatter light that can be measured. Lipase will hydrolyze the triglycerides in the micelle causing disintegration with resultant decreases in turbidity and light scattering. However, these decreases are not necessarily proportional to lipase activity. Therefore, all turbidimetric methods employed must be calibrated against a titrimetric method. Turbidimetric procedures are fraught with difficulty since they are subject to analytical imprecision due to unstable substrates, as well as false elevations due to interference from rheumatoid factor.

**Automated methods**

Further advances in methodology, such as the Johnson & Johnson Vitros and DuPont ACA, have rapid turnaround and are available on a random access basis allowing results to be available within hours or minutes in some cases.

---

**Self-Test Questions**

After you complete these questions, you may check your answers at the end of the unit.

**212. Diagnosing with enzymes**

1. Why are enzymes defined as biological catalysts?
2. How may genetic defects affect enzyme activity?
3. Under the system established by the Enzyme Commission (EC), what are the enzyme's systematic name and trivial name?
4. What is the significance of the four-number code used by the EC in its classification system?
5. Match the item in column B with the statement it relates to in column A by placing the letter of the item in column B beside the statement in column A. Each item in column B may be used only once.

*Column A*

- \_\_\_ (1) The speed of the reaction is directly proportional to this.
- \_\_\_ (2) At this point in a reaction, the substrate has reached an excess, so it no longer influences the reaction.
- \_\_\_ (3) Enzyme activity can be irreversibly ceased by raising this factor too high.
- \_\_\_ (4) Some enzymes are characterized based on this factor when describing their activity.
- \_\_\_ (5) These items cause a decrease in the amount of enzyme activity by resembling the enzyme's substrate.
- \_\_\_ (6) These items cause a decrease in the amount of enzyme activity by altering the configuration of the enzyme.
- \_\_\_ (7) Reactions proceed faster when more enzyme molecules are present to bind with the abundant amount of substrate.

*Column B*

- a. Competitive inhibitors.
- b. pH.
- c. Non-competitive inhibitors.
- d. Concentration of the enzyme.
- e. Mass action effect.
- f. Zero order reaction.
- g. Temperature.

**213. Amylase.**

1. What is the function of amylase and what organs secrete it?
2. Why is pancreatic amylase the primary source of amylase in the intestines?



3. In what *other* tissues and fluids is amylase found?
4. What is the significance of the P<sub>3</sub> amylase isoenzyme?
5. When the pancreatic ducts or salivary glands are blocked, how does this effect serum amylase level?
6. When serum amylase levels are increased due to pancreatic problems, for how long will peak levels occur and when will the levels return to normal?
7. Following an acute pancreatitis, how do urine and serum amylase levels compare?
8. How does liver damage affect serum amylase levels?
9. What is the specimen of choice for amylase analysis and how stable is it?
10. What are the three starch-based methods used for amylase analysis, and what is the principle of each method?

#### **214. Alkaline phosphatase**

1. What does the *alkaline* signify in the name alkaline phosphatase (ALP), and what function does ALP serve?
2. Where are the highest concentrations of ALP found?
3. ALP analysis detects disorders in what areas of the body?
4. In extrahepatic conditions, such as biliary tree obstructions, what changes occur in serum ALP levels?
5. How high will ALP levels increase during intrahepatic obstruction of the bile flow?

6. How does Paget's disease effect serum ALP levels?
7. How does the patient's age effect normal ALP ranges?
8. How does pregnancy effect ALP levels?
9. What conditions cause decreased serum ALP levels?
10. If a sample for ALP testing has been frozen, how soon after thawing can it be analyzed and why?
11. What are the two methods used for ALP isoenzyme analysis, and what is the principle of each method?

#### **215. Alanine aminotransferase and aspartate aminotransferase**

1. Why are alanine aminotransferase (ALT) and aspartate aminotransferase (AST) analysis routinely requested?
2. When are ALT and AST observed in the urine?
3. Of the two enzymes, ALT and AST, which is primarily used to diagnose intracellular hepatic diseases? Why?
4. Where, in the body, is AST found?
5. What is AST analysis primarily used to diagnose?
6. What happens to ALT and AST levels in patients that are suffering from a condition that causes hepatic necrosis?
7. What happens to ALT and AST levels following a myocardial infarction?

8. How do hemolyzed samples alter ALT and AST results?
9. What course of action is taken when samples for ALT or AST testing exceed analyzer linearity?
10. What are the reactions that take place during ALT analysis?
11. What are the reactions that take place during AST analysis?

**216. Gamma glutamyltransferase**

1. What is the function of gamma glutamyltransferase (GGT) and where, in the body, is it found?
2. What is the clinical significance of GGT in regard to hepatobiliary disease?
3. How does the consumption of drugs or alcohol affect GGT levels?
4. How do GGT levels help distinguish between liver and bone disease?
5. How do GGT levels help distinguish liver disease from muscle or hemolytic conditions from muscle disease?
6. Why are GGT values higher in males than in females?
7. What do you suspect when there are increased GGT levels in patients with malignancies?
8. Explain the principle used for GGT analysis.

**217. Lactate dehydrogenase**

1. Why is the diagnostic value of a total lactate dehydrogenase (LD) level questioned as opposed to LD isoenzyme levels?

2. What are the two types of LD isoenzyme peptide chains and what do they represent?
3. A patient had a myocardial infarction (MI) and exhibits an increased LD<sub>1</sub> isoenzyme level. What is the peptide chain structure for this isoenzyme?
4. A patient with progressive muscular dystrophy exhibits an increased LD<sub>5</sub> isoenzyme level. What is the peptide chain structure for this isoenzyme?
5. How does pH affect the LD reactions?
6. Why are LD levels especially useful in a delayed MI diagnosis?
7. What is meant by *flipped* LD level?
8. How do deficiencies of folate or vitamin B<sub>12</sub> affect LD levels?
9. How do liver and renal diseases effect LD levels?
10. How do malignant diseases effect LD levels?
11. Why will LD<sub>5</sub> levels decrease in the later stages of progressive muscular dystrophy?
12. How does spinal fluid LD isoenzyme analysis in patients with bacterial meningitis or viral meningitis compare?
13. Why avoid hemolyzed samples for LD testing, and why don't you freeze LD samples?
14. What are the forward and reverse reactions used during LD analysis?

15. What method is used for LD isoenzymes analysis?

**218. Creatine kinase**

1. What are the forward and reverse reactions of creatine kinase (CK), and what part does pH play in controlling these reactions?
2. What do M and B subunits signify?
3. Where are the CK isoenzymes predominately found in the body?
4. How is CK analysis used to assess MI patients?
5. Most of the circulating CK in normal serum is CK-MM. What conditions cause an increase in this isoenzyme?
6. What types of conditions can cause an increase in CK-BB activity?
7. You have just received a non-hemolyzed serum sample collected 2 hours ago. Can you use this sample for CK analysis?
8. Why is the reverse CK reaction most commonly used for CK analysis?
9. Describe the reverse CK reaction for CK analysis.
10. Describe the electrophoretic method for CK isoenzymes analysis.
11. Describe the ion-exchange method for CK isoenzyme analysis.
12. Describe the immunological methods for CK isoenzyme analysis.

**219. Acid phosphatase and prostate specific antigen**

1. Why are acid phosphatase (ACP) and prostate specific antigen (PSA) analysis used to diagnose and monitor prostatic cancer?
2. What is the problem associated with using ACP for the detection of prostate phosphate?
3. The majority of the ACP found in normal serum comes from what cell?
4. What conditions cause increased ACP levels in women?
5. Why is PSA such a good tumor marker, and why is it replacing ACP as a screening procedure for the detection of prostatic cancer?
6. How are samples for ACP testing stabilized?
7. How is ACP activity determined when using thymolphthalein monophosphate as a substrate?
6. What is the immunological method that sandwiches ACP in order to determine its level?

**220. Lipase**

1. What controls the rate of lipase action?
2. Why is the specificity of lipase to diagnose pancreatitis reduced?
3. If left untreated, what is likely to occur when pancreatic secretions are blocked from reaching the duodenum?
4. What can cause difficulty with turbidimetric methods for lipase?

## 2-4. Cardiac Markers

According to the CDC, the principle components of cardiovascular disease—heart disease and stroke—are the first and third leading causes of death in the United States, respectively. These conditions account for nearly 40 percent of all deaths or about 950,000 deaths per year in the United States. The need for diagnostic and specific laboratory tests for cardiovascular diseases is always growing. These tests can assist the provider in delivering rapid, life-saving care to the patient. As you recall from previous lessons, there are several enzymes, such as LD, CK, and AST, that provide some indication of a heart attack or the synonymous MI. In this lesson, you will learn about some of the more specific tests that have been developed for MI detection and diagnosis; many of these are used in conjunction with the enzymes previously presented in other lessons.

### 221. Cardiac markers

#### Physiology and clinical significance

Confirmation of an MI (heart attack) can only be made several hours after the event through the detection of elevated cardiac enzymes and cardiac (protein) markers in the blood. Cardiac enzymes are muscle proteins released into the bloodstream by damaged and dying heart muscle cells when their surrounding membranes dissolve; these enzymes include LD, AST, CK, and isoenzymes of CK. Many of these tests (namely AST, LD, CK) are falling into disfavor and obsolescence due to the advent of troponin tests with specificity. Troponin and myoglobin will be the primary focus of study in this lesson on cardiac markers. Cardiac markers are diagnostic of an MI since so many other factors can mimic an MI.

#### Laboratory procedures and limitations

##### *Myoglobin*

Myoglobin is a low molecular weight heme protein, much like hemoglobin, that transports oxygen within muscle cells during periods of low oxygen tension and is the first analyte to rise above normal levels following an MI. Myoglobin rises earlier than creatine kinase. Initially within the first hour following an MI, myoglobin will rise dramatically and peak between four to twelve hours following the infarction and return to normal levels within 18 hours. Due to myoglobin's presence in all types of muscle (smooth, skeletal, and cardiac), its specificity as an absolute determinant of MI is somewhat diminished, although it has been reported that the level of myoglobin released parallels the infarction size. The best use of myoglobin measurement is to rule out an MI. If measurements of myoglobin taken after the onset of chest pain remain constant and within the reference range three to six hours after the initial complaint, it can be concluded that no muscle injury has occurred. If muscle injury is great enough, myoglobin can be detected in the urine.

Myoglobin Detection		
Condition	Serum	Urine
Acute MI	Released within 4 –6 hrs; peaks by 12 hrs post-MI.	Following MI, appears in urine within 48 hrs.
Skeletal Muscle	Trauma (crush injury), surgery, severe burns, overexertion, rhabdomyolysis, and so forth.	Trauma (crush injury), surgery, severe burns, overexertion, rhabdomyolysis, and so forth.
Viral	Usually Influenza.	Usually Influenza.

##### *Troponin*

Troponin is a complex of three proteins that play a part in muscle contraction. The three cardiac proteins can be classified by their function as follows:

Cardiac Troponin (cTn) Abbreviation	Function
cTnC	Binds calcium at initiation of the contraction.
CTnI	An inhibitor that blocks contractions in the absence of $\text{Ca}^{++}$ .
cTnT	Causes muscle contraction.

*cTnI*

cTnI is specific for cardiac muscle. Elevation following an MI occurs within 4-6 hours, peaking within 8-20 hours, and remains elevated for seven days. There are no reports of cTnI exhibiting interference from other substances

*cTnT*

cTnT does not have the specificity for cardiac muscle that is seen in cTnI, as there are some known interfering substances. During an MI, elevation of cTnT will occur within 3-4 hours, peaking between 10-24 hours after onset, and will remain elevated 10 to 14 days. Interference is seen in renal disease and inflamed muscular conditions, such as myositis and dystrophies.

Skeletal and cardiac troponins have differing amino acid sequences allowing for laboratory test methods that have cardiac specificity. Troponin will be elevated within six hours of an MI. Troponin will rise to higher levels and will remain elevated longer (9-10 days) than other associated analytes and enzymes used to measure MI. Troponin is not normally found in the serum, as reflected by the narrow reference range of 0 – 0.1 ng/mL. Following an MI, troponin begins to rise much on the same curve as CK-MB, except troponin will rise much more (upwards of 80 times the upper limit of normal) than CK-MB. Also, troponin will remain elevated for approximately 10 days after an MI, longer than any other analyte currently used to assess and diagnose MI.

Unlike CK-MB, troponin is not affected by heavy exercise (i.e., marathon runners) and is only seen after myocardial damage. The specificity and reliability of this test can be considered high, as the only reported cause of false positives for cTnT is found in approximately 40 percent of renal patients receiving dialysis or suffering from end-stage renal disease. As of this writing, several international scientific organizations are closely studying troponin and, due to its specificity, it is anticipated it will replace CK-MB as the “gold standard” for the diagnosis of MI. Individual laboratories must decide what troponin assay or assays to provide.

---

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 221. Cardiac markers

1. Where is myoglobin found in the body?
2. What is the function of myoglobin?
3. Describe the actions of myoglobin following an MI.
4. Why does cardiac troponin have specificity?



- Following an MI, what happens to troponin levels?

---

## Answers to Self-Test Questions

### 208

- It is located in the upper right quadrant of the abdomen. The upper portion of the liver is overlaid by the lungs and diaphragm. The lower portion of the liver overlaps the stomach and intestines. The liver is covered by a collagenous capsule, and although it appears to be divided into a right and left half, it is actually divided into four lobes.
- The portal vein and the hepatic artery. The portal vein carries blood from the capillary bed of the digestive system to the liver and the hepatic artery carries well-oxygenated blood to the liver.
- The liver converts glucose into glycogen for storage. When energy is needed, the liver converts the glycogen back into glucose for release into the body. The liver maintains the release of glucose as a steady process, but in emergencies can release massive amounts of glucose in response to epinephrine.
- The liver disposes of worn out red blood cells by breaking them down into different components, some of which are stored for future use, and others, which are excreted by the kidneys. The liver also filters and destroys bacteria, neutralizes poisons, and regulates sex hormone levels.
- Bile is formed in the liver and flows into the gallbladder where it is concentrated and stored. Bile is released from the gallbladder into the intestines. In the intestines, bile (in the form of bile salts) breaks down large fat globules into smaller ones that can be acted on by fat-splitting enzymes.
- The liver plays a key role in the metabolism of carbohydrates. The liver is also responsible for synthesizing all plasma proteins except for gamma globulins. Other proteins formed in the liver are proteins for blood coagulation. The liver is also active in the anabolism and catabolism of lipids.
- The liver stores vitamins A, D, and B<sub>12</sub>. It stores iron in significant amounts. And, it temporarily stores small amounts of proteins and lipids.
- In the gallbladder.
- By destroying foreign material (such as bacteria). Kupffer cells in the liver are phagocytic cells that remove foreign material from the blood. The liver also detoxifies toxic chemicals by converting them to less toxic forms or by making them water-soluble so they can be excreted by the kidneys.
- It serves as a storage area for blood and helps regulate blood volume. It also serves to mix blood from the portal system with blood in circulation to the rest of the body. It continuously synthesizes protein-clotting factors, as many of these factors have a short life span and need to be replaced regularly.
- g.
  - e, h.
  - d, f, i.
  - f.
  - e.
  - d.
  - c.
  - b.
  - a.

### 209

- It is derived when old red blood cells are phagocytized and the hemoglobin released is broken down. Bilirubin is bound to albumin as it leaves the MPS, and this complex attaches to the hepatocyte membranes. The bilirubin then detaches from the albumin carrier and is transported into the hepatic cells.
- Conjugated bilirubin is bilirubin that has been joined with glucuronic acid inside of the hepatocytes. Bilirubin that has not been bound to glucuronic acid is called unconjugated bilirubin.

3. Urobilin, stercobilin, and mesobilin.
4. Jaundice is a disturbance in the flow of bile. It is characterized by the deposit of a yellowish pigment in the eyes and skin. When this condition is detectable visually, the bilirubin levels are well above normal.
5. (1) c.  
(2) d.  
(3) a.  
(4) b.
6. It is caused by a blood group incompatibility between the mother and the fetus. If the mother has been sensitized previously, the IgG antibodies from the mother cross the placenta and cause the destruction of the infant's red blood cell membranes.
7. Obstruction or impairment of the excretion of bile into the intestines will cause the concentration of bilirubin to build up in the hepatocytes and be regurgitated into the bloodstream. This condition is further worsened because the increased levels of bilirubin in the hepatocytes also cause a slow down in the bilirubin transport system, further increasing the bilirubin levels in the bloodstream.
8. Intrahepatic disturbances are disorders that take place within the liver, caused by damage or destruction of the liver cells due to viral hepatitis, toxins, or liver cirrhosis. Posthepatic disturbances are those that cause the obstruction of the flow of bile into the intestines, caused by stones in the gallbladder or bile ducts as well as tumors or carcinomas.
9. Unconjugated bilirubin.
10. The serum sample is mixed with a slightly acidic solution of caffeine and sodium benzoate. These chemicals accelerate the diazotization of the unconjugated bilirubin. Diazotized sulfanilic acid reacts with both forms of bilirubin to form an azo dye. When alkaline tartrate (with a pH of 13) is added, the azobilirubin is converted to a blue color, which is measured at 600 nm. The total bilirubin concentration is proportional to the amount of color produced.
11. To test for conjugated bilirubin, a serum sample is mixed with diazo reagent without accelerators. Only conjugated bilirubin will react under these conditions within the first 5 minutes. At the 5-minute mark, ascorbic acid is added to decompose the excess diazo salt and halt the reaction. The solution is then read at 600 nm and the color produced will be proportional to the amount of conjugated bilirubin in the sample. To determine the unconjugated bilirubin, the conjugated bilirubin result is subtracted from the total bilirubin result.
12. To avoid lipemia in the sample. Hemolyzed samples should not be used because hemolysis can cause falsely low values with diazo methods.
13. Because bilirubin is light sensitive and will break down if exposed to either artificial or natural light, protect samples from direct exposure to light as soon as possible after collection. The sensitivity of bilirubin to light is also affected by temperature. Samples are stable for up to 3 days if refrigerated at low temperatures and kept in the dark. Samples kept in the dark and frozen at  $-70^{\circ}\text{C}$  will be stable for up to 3 months.
14. By the time the infant is 1 month old.

**210**

1. The brain, erythrocytes, platelets, leukocytes, and kidney medulla.
2. Carbohydrates are made up of carbon, hydrogen and oxygen. Carbohydrates approximately contain one molecule of water for every carbon atom.
3. Carbohydrates are classified based on the number of simple sugars that can be broken down into during the process of hydrolysis.
4. Monosaccharides are the simplest of the sugars and cannot be hydrolyzed into simpler structures. Disaccharides are sugars that will yield two monosaccharides when hydrolyzed. Polysaccharides are sugars with more than two saccharide groups.
5. Starches and glycogen are partially digested by salivary amylase to form maltose.
6. They are absorbed primarily in the small intestines. The rate of absorption is determined by the selectivity of the intestinal mucosa (different monosaccharides absorb at different rates).
7. (1) To be used for energy production by conversion to carbon dioxide and water.

- (2) To be converted to glycogen for storage in the liver or converted to triglycerides for storage in the adipose tissue.
- (3) To be converted into other products such as keto acids, amino acids, or proteins.
- 8. To supply energy to the muscle tissues.

## 211

1. None.
2. Glycogenolysis is the breakdown of stored glycogen into glucose for release into the blood. Gluconeogenesis is the formation of glucose in the body from non-carbohydrate sources such as amino acids, glycerol, or lactate.
3. (1) d.  
(2) d.  
(3) a.  
(4) c.  
(5) b.  
(6) g.  
(7) f.  
(8) e.
4. Hyperglycemia is an increased plasma glucose while hypoglycemia is a decreased plasma glucose.
5. They include permanent damage to the eyes with the potential loss of vision, the kidneys leading to renal failure, the leg including foot ulcers and possible amputations.
6. The patient is categorized by the cause of their disease rather than the treatment of it.
7. Type 1 diabetes, Type 2 diabetes, Other specific types, and Gestational diabetes mellitus.
8. It is an immune mediated diabetes, the patient produces autoantibodies that destroy their own insulin producing cells.
9. Their hyperglycemia range from disorders that cause insulin resistance along with decreased insulin production to problems with insulin secretion from the pancreas combined with insulin resistance.
10. Genetic defects of the beta cells or insulin receptor sites, diseases of the pancreas, hormonal disorders, and drug, chemical or infectious agent destruction of the beta cells.
11. Any degree of glucose intolerance that is initially recognized during pregnancy.
12. Performed between the 24<sup>th</sup> and 28<sup>th</sup> week of gestation. Patient should meet one or more of the following criteria: 25 years of age or older, less than 25 years of age and obese or greater than 20 percent over their desired body weight, family history of diabetes, and member of an ethnic group with a high prevalence of diabetes.
13. When levels drop below 40 mg/dl in an adult, the patient may lose consciousness.
14. Hormonal reasons include an overabundance of insulin caused by an overdose of injected insulin, failure of the patient to eat after an insulin dose, or an increased production of insulin by the pancreas. Other hormonal causes include a deficiency of glucagon or cortisol. Hepatic causes include decreased stores of glycogen due to prolonged fasting, hepatocellular damage, acute drug toxicity, or genetic defects in the liver, such as von Gierke's disease.
15. Symptoms of diabetes plus a casual glucose concentration (CGC) greater than or equal to 200 mg/dl, fasting plasma glucose (FPG) of greater than or equal to 126 mg/dl, or 2 hour postload glucose (2-h PG) of greater than or equal to 200 mg/dl during an oral glucose tolerance test (OGTT).
16. Normoglycemia is defined as plasma glucose levels <110 mg/dl in a FPG and a 2-h postload value of <140 mg/dl in the OGTT. FPG levels greater than or equal to 126 mg/dl are abnormal and are defined as an impaired fasting glucose (IFG).
17. 75g.
18. The concentration of glucose in CSF is about 60 to 75 percent of that in a patient's plasma. CSF glucose analysis is useful in helping to diagnose bacterial meningitis.

19. Due to glycolysis in the sample. Glycolysis can decrease the glucose in a sample by as much as 5 to 10 mg/dl for every hour the sample is left uncentrifuged at room temperature.
20. It is less likely to be affected by medications or other constituents in the plasma.
21. The glucose level in the sample is proportional to the oxygen consumed in the reaction or the amount of hydrogen peroxide produced.
22. Because urine contains high concentrations of substances (such as uric acid), which produce falsely, low results with the glucose oxidase method.

**212**

1. Because they cause reactions to take place in the body.
2. Enzymes are proteins and are synthesized under the control of specific genes. Deficiencies in enzyme proteins or enzyme activity are sometimes tracked back to genetic defects.
3. The systematic name is used to describe the nature of the reaction catalyzed by the enzyme. The trivial name is the one that has been simplified for everyday use.
4. The first number sequence designates the class that the enzyme belongs to. The next two number sequences indicate the subclasses of the enzyme. The final number in the sequence is a unique serial number for that specific enzyme in its subclass.
5. (1) d.  
(2) f.  
(3) g.  
(4) b.  
(5) a.  
(6) c.  
(7) e.

**213**

1. Aids in the breaking down of carbohydrates into simpler sugars; pancreas and salivary glands.
2. Because salivary amylase is deactivated by stomach acids as it travels through the stomach.
3. Amylase is found in semen, milk, tears, fallopian tubes, testes, ovaries, striated muscle, and lungs.
4. The P<sub>3</sub> isoenzyme peak frequently occurs in patients suffering from acute or chronic pancreatitis or renal transplants.
5. Increased serum amylase level will be observed when there is a blockage in the secretion of amylase from either source.
6. Following acute pancreatitis, serum amylase levels will peak after about 24 hours and will return to normal in 2 to 3 days.
7. Increased urinary excretion of amylase persists longer than the increased serum levels, generally not returning to normal until 7 to 10 days after the attack.
8. Acute or chronic hepatocellular damage can cause decrease levels. Note that amylase is not a sensitive liver function test.
9. Serum is the specimen of choice. Anticoagulants, except for heparin, can inhibit amylase activity by as much as 15 percent. Samples are stable for up to 1 week at room temperature and up to 2 months if refrigerated.
10. The amylolytic assays measure the disappearance of the starch substrate. The amount of starch hydrolyzed is read photometrically and compared to a starch-iodine calibration curve. Saccharogenic assays determine amylase activity by measuring the reducing sugars produced as a result of enzymatic action. Chromogenic assays determine amylase activity by using a dye-labeled amylase substrate. These substrates are insoluble and, when acted upon by the enzyme, release dye that then becomes soluble. The released dye is then measured photometrically.

**214**

1. *Alkaline* signifies that this enzyme's activity is increased in an alkaline environment; to help with lipid transport in the intestines and with the calcification process in the bone.

2. In the liver and bones.
3. Liver and bone. Isoenzymes of ALP are used to distinguish these conditions.
4. ALP levels will increase as much as 10 to 12 times the upper normal limit. Levels will return to normal shortly after surgical removal of the obstruction.
5. ALP levels can increase up to 2 to 5 times the upper normal limit.
6. Values as high as 10 to 25 times the upper normal limit are common in Paget's disease.
7. Normal bone growth in children will also produce elevated ALP results. Growing children will normally have levels 1.5 to 2.5 time higher than that of normal adults.
8. During the third trimester of pregnancy, an increase in ALP levels may be observed. This increase is usually 2 to 3 times the normal upper limit with the additional enzyme being produced by the placenta. Upward or downward trends can indicate pregnancy complications.
9. During hypophosphatasia and deficiencies of thyroid hormones and vitamin B<sub>12</sub>.
10. Thaw and then stored at room temperature for 18 to 24 hours before testing. This allows for full enzyme reactivation. During freezing, enzyme activity is temporarily inactivated.
11. Electrophoretic methods are used to separate serum samples into recognizable patterns to detect the isoenzymes. Heat-inactivation methods differentiate the isoenzymes based on their stability when exposed to heat.

## 215

1. Because of their immediate value in diagnosing liver disorders.
2. When a patient is suffering from kidney lesions.
3. ALT, because it is the more specific of the two (ALT and AST) for liver functions.
4. AST is found in practically every body tissue, including red blood cells. The concentration is particularly high in cardiac muscle and the liver. Moderate levels are found in skeletal muscle and the kidneys. The concentration of AST in all other tissues is very low.
5. Liver damage and in determining the extent of injury to cardiac muscle.
6. Their levels will increase even before the patient shows any clinical signs of the disease. Values will peak between the 7<sup>th</sup> and 12<sup>th</sup> day of illness and levels will run 20 to 50 times that of normal levels. If recovery is uneventful, levels will return to normal after 3 to 5 weeks.
7. AST levels will increase. AST levels do not reach an abnormal level until 6 to 8 hours after the onset of chest pain. AST levels will peak after 18 to 24 hours following the infarction. Levels will return to normal by the fourth or fifth day, providing no other attacks occur. ALT levels will remain at or near normal because the levels of ALT in the heart muscle are only a fraction of those of AST.
8. They would be falsely elevated. ALT activity in red blood cells is 7 times that of serum and AST levels are 15 times that of serum.
9. They should be diluted and rerun. Saline may be used, but an albumin solution is preferred.
10. ALT catalyzes transfers of an amino group from alanine to  $\alpha$ -ketoglutarate, forming pyruvate and glutamate. The rate of formation of pyruvate is determined by coupling this reaction with lactate dehydrogenase. In the second part of the reaction, the pyruvate is converted to lactate and the reaction is read at 340 nm. The change in absorbance indicates the amount of ALT present in the sample.
11. AST catalyzes transfers of an amino group from aspartate to  $\alpha$ -ketoglutarate to form oxaloacetate. Oxaloacetate and NADH are then coupled with malate dehydrogenase. This reduces oxaloacetate to malate, oxidizes NADH to NAD<sup>+</sup>. The decrease in absorbance, read at 340 nm, reflect the amount of AST present in the sample.

## 216

1. Catalyzes the transfer of a gamma-glutamyl group to a peptide, amino acid, or even water. It is found in all cells except those in the muscle.
2. GGT is one of the most sensitive enzymatic indicators of hepatobiliary disease. While GGT is elevated in all forms of liver disease, it is highest in cases of intrahepatic or post-hepatic biliary obstruction, often reaching 5 to 30 times that of normal levels. GGT is considered a better indicator of obstructive jaundice than AST, ALT, or ALP because it increases earlier and persists longer than other enzymes.

3. Values will increase quickly even after a small intake of alcohol. Its levels are also affected in patients whose bodies are attempting to detoxify certain drugs.
4. GGT levels are normal during skeletal disease, while ALP levels are elevated. This implies that the problem is in the bones.
5. GGT levels are not elevated during muscular disease, while AST levels are elevated.
6. Because there are high levels of GGT in the prostate; normal male GGT values are higher than female values.
7. That the disease has metastasized to the liver.
8. In the presence of GGT and a buffer,  $\gamma$ -glutamylglycylglycine and p-nitroaniline are produced. An increase in absorbance due to the formation of p-nitroaniline in the reaction is measured spectrophotometrically at 405 nm and the amount of GGT in the sample is calculated.

## 217

1. Because it is so widely distributed in the body, the diagnostic value of an increase in total LD concentration is questionable. It indicates an ongoing disease process but offers little specifics on the problem's origin. By analyzing LD isoenzymes we can pinpoint the cause of the increased LD activity.
2. The M-type is typical of skeletal muscle while the H-type is from cardiac muscle.
3. The peptide chain structure of LD<sub>1</sub> is HHHH (H<sub>4</sub>).
4. The peptide chain structure for LD<sub>5</sub> is MMMM (M<sub>4</sub>).
5. The direction of LD enzymatic reactions is dependent on pH. In an alkaline pH, LD converts lactate to pyruvate. In a neutral pH environment, LD converts pyruvate back to lactate.
6. LD levels will rise later than that of the enzyme creatine kinase. This later rise is very useful in the diagnosis of patients who complain of chest pains that occurred 3 or 4 days ago.
7. Normal LD isoenzyme pattern is: LD<sub>2</sub> levels are greater than those of LD<sub>1</sub>. A *flipped* LD level has LD<sub>1</sub> levels greater than those of LD<sub>2</sub>.
8. In megaloblastic anemias resulting from a deficiency of folate or vitamin B<sub>12</sub> can cause red blood cell precursors in the bone marrow break down. As these cells break down, they release large quantities of LD<sub>1</sub> and LD<sub>2</sub> isoenzymes. After appropriate treatment, LD levels quickly return to normal.
9. Increased LD activity can be observed during liver disease, but the aminotransferase (ALT and AST) activity is much more increased. In renal diseases, the increased LD levels do not correlate very well with the other parameters of the disease, and isoenzyme patterns can resemble normal serum patterns.
10. Patients with malignant diseases often show an increase in serum LD activity; however, the LD activity is too erratic to be useful as a diagnostic tool. LD activity is useful, though, in monitoring the effectiveness of chemotherapy against the tumor.
11. Most of a patient's muscle mass containing LD<sub>5</sub> has been lost, so LD levels will decline.
12. Patients with bacterial meningitis will have granulocytosis and their spinal fluid will show an increase in the LD<sub>4</sub> and LD<sub>5</sub> isoenzymes. Patients with viral meningitis will have lymphocytosis and an increase in the LD<sub>1</sub> and LD<sub>3</sub> isoenzymes.
13. Red blood cells have a LD level 150 times that of serum, and ruptured cells will cause falsely elevated results. Freezing samples should be avoided because some of the LD isoenzymes are significantly affected by low temperatures. LD<sub>4</sub> and LD<sub>5</sub> lose all activity if frozen.
14. In the forward LD reaction, the enzyme LD converts lactate to pyruvate while reducing NAD<sup>+</sup> to NADH. The rate of increase in the absorbance of NADH at 340 nm is proportional to the LD activity of the sample. In the reverse reaction, pyruvate is converted back to lactate. Here, the NADH is oxidized back to NAD<sup>+</sup> and the decrease in absorbance is measured.
15. Electrophoresis.

## 218

1. The forward reaction of creatine kinase (CK) and magnesium ions catalyze the transfer of a phosphate from adenosine triphosphate (ATP) to creatine, forming creatine phosphate and adenosine diphosphate (ADP). In the reverse reaction, takes creatine phosphate and ADP, in the presence of CK and magnesium ions, will yield creatine and ATP. The direction of the reaction is dependent on the pH of the reaction media. An alkaline pH favors the forward reaction, while a neutral pH favors the reverse reaction.

2. The M signifies a polypeptide subunit for muscle and the B signifies a polypeptide subunit for brain.
3. CK-BB is found in the brain, CK-MB is found in the cardiac muscle, and CK-MM is found in the skeletal muscle.
4. In a patient with a MI, CK activity will begin to increase 3 to 6 hours after a MI and will usually reach its maximum level about 24 hours after onset. As long as no further MI's occur, CK levels will normally return to normal within 2 to 3 days. CK-MB determinations are the single best test for early diagnosis of a MI.
5. The cause is usually some kind of stress or injury to skeletal muscle. Vigorous exercise, multiple intramuscular injections, electroconvulsive therapy, and surgery can cause a rise in CK-MM activity. Muscular diseases, such as progressive muscular dystrophy, can also cause a dramatic rise in CK-MM levels.
6. Patients suffering from head trauma will normally show increased activity. Diseases that cause swelling to the brain, such as Reye's syndrome in children, can also cause increased CK-BB levels.
7. This sample is suitable for CK analysis.
8. Because this reaction proceeds at a much faster rate than the forward reaction.
9. In the reverse reaction, creatine phosphate and adenosine diphosphate (ADP) in the presence of CK will yield creatine and adenosine triphosphate (ATP). The ATP produced is then added to glucose in the presence of hexokinase. This reaction yields glucose-6-phosphate (G-6-P) and ADP. The G-6-P that results is then added to nicotinamide-adenine dinucleotide phosphate ( $\text{NADP}^+$ ) in the presence of glucose-6-phosphate dehydrogenase (G6PD), resulting in 6-phosphogluconate, NADPH, and  $\text{H}^+$ . The rate of formation of NADPH is a measure of the concentration of CK activity in the sample.
10. In electrophoresis, the CK isoenzymes are separated on agar, agarose, or cellulose acetate. The isoenzyme bands are then treated with a mixture, prompting the reverse reaction. The NADPH formed by this reverse reaction can then be visualized under fluorescent or visible light and quantified by scanning on a densitometer or fluorometer.
11. Ion-exchange methods have been used to separate CK isoenzymes using either batch adsorption or column chromatography using various mediums. The basic process involves the CK isoenzymes adsorbing onto the medium, usually a gel. The gel is then washed, and the CK isoenzymes are then eluted, using TRIS buffers with varying sodium chloride concentrations. A major drawback to this method is that the CK isoenzymes can be greatly diluted during the elution process, requiring the fractions to be concentrated after collection or some other method used to reduce the dilution effect.
12. Immunological methods require the use of specific antisera against either the M or B subunits in order to measure the CK isoenzymes. When measuring CK-BB, an anti-CK-M sera inhibits any M subunits so that only CK-BB activity is measured. When measuring CK-MM, an anti-CK-B sera inhibits any B subunit activity so only CK-MM activity is measured. Determining the activity of CK-MB presents a unique challenge. In order to determine CK-MB activity, a sandwich technique is often used. In this technique, two antibodies having different affinities for different parts of the CK-MB molecules are used sequentially. This method provides an estimate of CK-MB activity because neither CK-MM nor CK-BB will react with both antibodies.

## 219

1. Because they are very specific for the prostate. Acid phosphatase (ACP) is an enzyme that has its highest concentrations in the prostate. Prostate specific antigen (PSA) is a glycoprotein only found in prostatic epithelial cells.
2. ACP levels often remain normal until the cancer metastasizes. Only after the cancer spreads to blood capillaries, lymph channels, or other tissues will ACP activity rise dramatically.
3. From the osteoclasts.
4. When they are suffering from bone diseases such as Paget's disease or the malignant invasion of the bones by cancers such as breast cancer.
5. PSA is an antigen that can be detected in all males. The levels of this antigen are greatly increased in patients who have prostatic cancer. The higher the antigen level the greater the tumor burden. Because PSA is only produced by the prostate, this makes this glycoprotein an excellent tumor marker. PSA is replacing ACP as an early screen for prostate cancer because it is much more sensitive in determining the early stages of the disease.

6. By the addition of commercially available disodium citrate monohydrate tablets or by adding acetic acid to the serum until the pH reaches a level of 5.4, the level at which the enzyme is stable. At this pH, the sample is stable for several hours if left at room temperature and up to a week if refrigerated.
7. Thymolphthalein monophosphate is hydrolyzed by prostatic ACP at a pH of 5.4. The reaction is stopped after 30 minutes by adding a sodium hydroxide-sodium carbonate solution. This develops an alkaline color with liberated thymolphthalein. The reaction is read at 595 nm, and the absorbance of the solution corresponds to the ACP activity.
8. These methods determine their specificity for the source of the ACP based on the specificity of their antiserum. In most of these methods, the total ACP activity is first measured, and then, a specific antiserum is used to capture the prostatic ACP. The difference between the two measurements determines the prostatic enzyme's activity.

## 220

1. The rate of lipase action is dependent upon the amount of surface area available, with the more surface area available the faster the rate of lipolysis
2. Lipase can be elevated by a variety of other conditions to include mumps, alcoholism, carcinoma, renal diseases, abdominal trauma and other abdominal disorders, blockages, or diseases. Because the pancreas is sensitive to proximal abdominal disorders the specificity of the lipase test for pancreatitis is reduced.
3. In chronic pancreatitis the pancreatic secretions are blocked and from reaching the duodenum and begin to digest the pancreas itself, a condition called autodigestion.
4. They are subject to analytical imprecision due to unstable substrates as well as false elevations due to interference from rheumatoid factor.

## 221

1. Myoglobin is found in all muscle types (smooth, skeletal, and cardiac).
2. Myoglobin's function is to transport oxygen within muscle cells.
3. The first analyte to rise above normal levels following a MI is myoglobin. Myoglobin rises earlier than creatine kinase. Initially within the first hour following an infarct it will rise dramatically and peak between four to twelve hours following the infarct and return to normal levels within 18 hours.
4. Specificity for cardiac troponin exists because it has a differing amino acid sequence from skeletal troponin.
5. Troponin levels rise rapidly within the first few hours (<6) following a MI and remain elevated longer than all other associated analytes and enzymes used to measure MI.

**Do the unit review exercises before going to the next unit.**



## Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter.

27. (208) Among some of the liver's functions is the ability to
  - a. control the endocrine system.
  - b. produce digestive enzymes such as pepsin.
  - c. produce gallstones, which help to grind up ingested food.
  - d. convert glucose to glycogen and glycogen back to glucose, as needed.
28. (208) The liver is the *primary* storage site for
  - a. glycogen.
  - b. phosphate.
  - c. hemoglobin.
  - d. epinephrine.
29. (208) Hepatitis A virus is *primarily* transmitted through
  - a. tick bites.
  - b. sexual intercourse.
  - c. the parenteral route.
  - d. the fecal oral route.
30. (208) Due to the amount of time it takes for antibodies to reach detectable levels and *prior* to the use of nucleic acid amplification testing (NAT), which type of hepatitis could not be eliminated completely by blood donor screening?
  - a. Hepatitis A virus.
  - b. Hepatitis B virus.
  - c. Hepatitis C virus.
  - d. Hepatitis D virus.
31. (208) What hepatitis virus occurs as a simultaneous infection with the Hepatitis B (coinfection) or as a superimposed infection in someone with chronic Hepatitis B (superinfection)?
  - a. Hepatitis A.
  - b. Hepatitis B.
  - c. Hepatitis C.
  - d. Hepatitis D.
32. (209) Bilirubin that has become joined with glucuronic acid is called
  - a. indirect bilirubin.
  - b. conjugated bilirubin.
  - c. unconjugated bilirubin.
  - d. conjugated urobilinogen.
33. (209) When jaundice, a condition characterized by the deposit of a yellowish pigment in the skin and eyes, can be detected visually, the bilirubin level is
  - a. decreasing.
  - b. slightly increased.
  - c. well above normal.
  - d. well below normal.

34. (209) In newborns, bilirubin levels can increase for the first few days of life before returning to normal because
- a. of immature immuno-cells.
  - b. of hemolytic disease of the newborn.
  - c. maternal red blood cells are being destroyed.
  - d. the transferase enzyme system is not fully developed.
35. (209) Bilirubin levels increase due to the impairment or obstruction of the excretion of bile into the intestines, because bilirubin builds up in the hepatocytes, and is
- a. regurgitated into the blood.
  - b. reabsorbed into the intestines.
  - c. the cause of primary hepatic necrosis.
  - d. indirectly converted into urobilinogen.
36. (209) Conditions such as hepatitis, which damage or destroy liver cells, are known as
- a. hepatic necrosis.
  - b. prehepatic disorders.
  - c. posthepatic disorders.
  - d. intrahepatic disorders.
37. (209) Posthepatic disorders differ from intrahepatic disorders in that posthepatic disorders
- a. are caused by alcoholism.
  - b. are caused by drug overdoses.
  - c. can be treated by enzymatic methods.
  - d. can be corrected by surgical methods.
38. (209) Samples for bilirubin analysis should be protected from light because
- a. light hemolyzes red blood cells.
  - b. light minimizes lipemia in the sample.
  - c. both conjugated and unconjugated bilirubin are light sensitive.
  - d. both conjugated and unconjugated values will increase if exposed to light.
39. (210) Carbohydrate classes, such as monosaccharides, disaccharides, and polysaccharides, are determined based on
- a. their starch complexes.
  - b. how they react with enzymes.
  - c. how many sugar molecules are produced during gluconeogenesis.
  - d. the number of sugars a carbohydrate can be broken down into during hydrolysis.
40. (210) When excess carbohydrates are eaten, the body will change some of the excess into
- a. fats and store them in that form.
  - b. water for excretion from the body.
  - c. bile salts to aid in the digestive process.
  - d. carbon dioxide so it can be expelled through the lungs.
41. (210) The breakdown of glycogen into glucose is called
- a. glycolysis.
  - b. fractionation.
  - c. glycogenolysis.
  - d. gluconeogenesis.

- 
- 
42. (211) The *chief* source of energy and the *only* monosaccharide found in significant amounts in the body fluids of living organisms is
- lactose.
  - sucrose.
  - glucose.
  - cellulose.
43. (211) When epinephrine is released due to physical or emotional stress, it causes
- decreased glycogenesis.
  - decreased plasma glucose levels.
  - increased glucose levels for energy.
  - increased glucagon release to all cells of the body.
44. (211) The cause of Type 2 diabetes is
- unknown.
  - insulin receptor site damage.
  - genetic defect of the beta cells.
  - chemical destruction of the beta cells.
45. (211) If an unconscious patient's glucose result has been verified and it's 35 mg/dl,
- don't be alarmed, the patient is normal.
  - contact a supervisor for further guidance.
  - notify the attending physician at once, because the patient is hypoglycemic.
  - request a new sample because anti-coagulants may have caused interference.
46. (211) When diagnosing diabetes mellitus, once the patient has met all required criteria, what other evaluations must be performed?
- Family history of hypoglycemia.
  - Lipoprotein and enzyme analysis.
  - Serological and electrolyte testing.
  - Hypertension and cardiovascular assessment.
47. (211) Which one of the following glucose tests requires patient preparation for 3 days prior to the testing date?
- Fasting glucose.
  - Postprandial glucose.
  - Gestational screening.
  - Oral glucose tolerance.
48. (211) Cerebrospinal fluid (CSF) glucose levels in bacterial meningitis patients are usually
- lowered due to CSF leakage.
  - increased due to dehydration.
  - increased because bacteria produce glucose.
  - lowered because glucose is utilized by the bacteria.
49. (211) Serum for glucose testing should *not* be allowed to sit on the red blood cells for extended periods of time because
- glycolysis can increase the glucose level.
  - glycolysis can decrease the glucose level.
  - hemolysis will release free glucose into the sample.
  - putrefaction can cause increased amounts of glucose.

50. (211) One advantage of the hexokinase method over the glucose oxidase method, for glucose analysis, is
- hexokinase is more linear.
  - hexokinase is more stable than glucose oxidase.
  - fewer substances will interfere with the hexokinase method.
  - the hexokinase method is better adapted to automated methods.
51. (212) The active site of an enzyme is where the substrate bonds are strained, ruptured, and the
- enzymes are chemically changed.
  - enzymes produces heat for metabolism.
  - substrate is converted into a new substance.
  - substrate is released to bind to an identical enzyme molecule.
52. (212) Substances that decrease enzyme activity, and are either competitive or non-competitive, are called
- activators.
  - inhibitors.
  - coenzymes.
  - glycoproteins.
53. (212) The point where the concentration of enzyme no longer effects the rate of the reaction is known as the
- saturation.
  - progression point.
  - zero order reaction.
  - enzymatic solubility.
54. (212) Enzyme activators are usually
- anions.
  - cations.
  - other enzymes.
  - conjugated proteins.
55. (213) Amylase is secreted by the
- thyroid and pancreas.
  - lymph nodes and liver.
  - adrenal gland and kidney.
  - salivary and pancreatic glands.
56. (213) What source of amylase is the *primary* source of amylase responsible for carbohydrate break down?
- Lung.
  - Salivary.
  - Testicular.
  - Pancreatic.
57. (213) All of the following are starch-based methods used to measure amylase activity, *except*
- amyloclastic.
  - chromogenic.
  - saccharogenic.
  - flame photometric.

- 
- 
58. (214) The enzyme that splits off the phosphate group from organic phosphate esters in an alkaline solution is
- acid esterase.
  - alkaline esterase.
  - acid phosphatase.
  - alkaline phosphatase.
59. (214) Elevated amounts of alkaline phosphatase is normal in children because
- of normal bone growth.
  - the growth hormone produces the excess.
  - they have better diets than do most adults.
  - they are smaller, thus the enzyme is more concentrated.
60. (215) Because alanine aminotransferase is found in moderately high concentrations in the liver and has low concentrations in skeletal and cardiac muscle, it is *primarily* used to
- verify cardiac arrest.
  - check for Paget's disease.
  - diagnose intracellular hepatic diseases.
  - check for kidney lesions that affect sodium reabsorption.
61. (215) Alanine aminotransferase (ALT) will increase, even before the patient shows any clinical signs, in cases of
- cerebral hemorrhage.
  - myocardial infarction (MI).
  - complete renal failure where the patient requires dialysis.
  - viral hepatitis or other liver diseases, which cause hepatic necrosis.
62. (215) After a myocardial infarction (MI), aspartate aminotransferase (AST) levels
- and alanine aminotransferase (ALT) will increase significantly.
  - remain normal, ALT levels will remain near normal.
  - increase and ALT levels will remain near normal.
  - decrease and ALT levels will increase.
63. (215) You have just run a panel and the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are elevated beyond the linear range of your analyzer. Although you can dilute the sample with saline, the *preferred* dilutant is
- methanol.
  - buffered saline.
  - deionized water.
  - albumin solution.
64. (216) The function of gamma glutamyltransferase (GGT) is to
- oxidize peptide bonds.
  - activate transferase reduction.
  - metabolize carbohydrate molecules.
  - transport amino acids across cell membranes.
65. (216) Gamma glutamyltransferase (GGT) is considered a better indicator of obstructive jaundice than aspartate aminotransferase (AST), alanine aminotransferase (ALT), or alkaline phosphatase (ALP) because
- it's easier to test for than the other enzymes.
  - it's cheaper to test for than the other enzymes.
  - it's the only enzyme to increase in cases of obstructive jaundice.
  - its levels increase earlier and persist longer than the other enzymes.

66. (216) In addition to liver disease, the release of gamma glutamyltransferase (GGT) into the serum reflects
- irreversible liver damage.
  - immune system response to the liver damage.
  - the toxic effects of alcohol or drugs on the liver cells.
  - complications involving other organs such as lungs and heart.
67. (216) Normal reference ranges for gamma glutamyltransferase (GGT) run *higher* in males than in females because
- GGT binds with testosterone.
  - GGT is controlled by estrogen.
  - males are more prone to liver disease.
  - there are high levels of GGT in the prostate.
68. (217) The peptide chains that make up lactic dehydrogenase (LD) isoenzymes are classified into two types—
- B for bone and BB for brain.
  - M for skeletal muscle and H for heart muscle.
  - L for hepatic enzyme and LL for lung enzyme.
  - D for digestive enzymes and N for neurotransmitters.
69. (217) Lactate dehydrogenase (LD) testing is often useful in making a delayed diagnosis of a myocardial infarction (MI) because
- LD activity will not change.
  - the LD isoenzyme M<sub>4</sub> will flip.
  - the increase in LD occurs later than the increase in creatine kinase (CK).
  - LD levels will always be three times that of alanine aminotransferase (ALT).
70. (217) In a normal lactate dehydrogenase (LD) isoenzyme pattern, the LD<sub>2</sub> isoenzyme level is greater than the LD<sub>1</sub> isoenzyme level. In a myocardial infarction (MI), the patient's isoenzyme pattern will
- be flipped, with LD<sub>1</sub> greater than LD<sub>2</sub>.
  - show increases in the MMMM (M<sub>4</sub>) isoenzyme.
  - essentially remain normal, but all peaks will be larger.
  - show increased activity in the brain due to increased stress.
71. (217) Lactate dehydrogenase (LD) isoenzymes analysis is performed by
- electrophoresis.
  - atomic absorption.
  - flame emission photometry.
  - fluorescent immunoassay polarization.
72. (218) Creatine kinase (CK) is an enzyme that takes part in a reversible reaction—the direction of the reaction is dependent on the
- pH of the reaction media.
  - amount of enzyme in the reaction media.
  - amount of substrate present in the media.
  - enzyme activators present in the solution.
73. (218) Although creatine kinase (CK) is found predominately in the heart, skeletal muscle, and brain, accurate differentiation for conditions involving CK is accomplished using
- CK isoenzymes.
  - hepatic enzyme panels.
  - lactate dehydrogenase (LD) isoenzymes.
  - aspartate aminotransferase (AST) and LD as indicators.

- 
- 
74. (218) Creatine kinase (CK) activity is *most* commonly used to assess patients with a myocardial infarction (MI) because it
- decreases with MI severity.
  - increases 2 to 5 days after the MI.
  - appears flipped when compared to LD.
  - begins to increase 3 to 6 hours after the MI.
75. (218) What creatine kinase (CK) isoenzyme makes up *most* of the normal circulating CK, but can be increased with exercise?
- BBBB.
  - MMMM.
  - CK-BB (CK<sub>1</sub>).
  - CK-MM (CK<sub>3</sub>).
76. (218) Most analyzer use the reverse reaction for creatine kinase (CK) analysis because
- it is a less complex reaction.
  - fewer reagents are needed for testing.
  - the reverse reaction is six times faster than the forward reaction.
  - the pH of the reverse reaction is easier to control than the forward reaction.
77. (219) In addition to acid phosphatase (ACP), which of the following helps to investigate and monitor prostatic cancer?
- HB<sub>s</sub>Ag.
  - Amylase.
  - Prostate-specific antigen (PSA).
  - Alkaline phosphatase (ALP) and aspartate aminotransferase (AST).
78. (219) Forensic testing for acid phosphatase (ACP) is sometimes done during rape investigations because
- only males have the ACP enzyme.
  - ACP can be tracked like a fingerprint.
  - seminal fluid contains high levels of ACP.
  - genetic patterns can be derived from ACP.
79. (219) Prostate-specific antigen (PSA) can be detected in all males; however,
- placenta formation causes increase in females.
  - females with prostatic cancer will show a slight increase.
  - levels are greatly increased in males with prostatic cancer.
  - levels are greatly increased in males suffering from liver disease.
80. (219) Prostate-specific antigen (PSA) is replacing acid phosphatase (ACP) as a screening tool for early detection of prostatic cancer due to
- test procedure costs.
  - amount of sample needed for testing.
  - lack of sensitivity of ACP in early disease stages.
  - sensitivity of PSA to interfering substances like hemoglobin.
81. (220) Calcium is required for lipase activity, but at high concentrations will serve as a(n)
- catalyst.
  - inhibitor.
  - neutralizer.
  - accelerator.

82. (221) The best use of a myoglobin determinations is
- a. to rule out a myocardial infarction.
  - b. to confirm a myocardial infarction.
  - c. to assess severity of deep muscle tissue injury.
  - d. to monitor the long-term regeneration of cardiac tissue.
83. (221) Interference with troponin cTnT studies can be caused by
- a. LDH<sub>2</sub>.
  - b. myoglobin.
  - c. renal dialysis.
  - d. marathon running and endurance exercising.
84. (221) It is anticipated that the gold standard for the diagnosis of myocardial infarction (MI) will be
- a. CK-MB.
  - b. troponin.
  - c. myoglobin.
  - d. cardiac isozymes.



## Unit 3. Proteins, Lipids, Hormones, Therapeutic Drug Monitoring, Toxicology, and Tumor Markers

<b>3-1. Proteins.....</b>	<b>3-1</b>
222. Proteins .....	3-1
223. Serum proteins .....	3-3
224. Protein in cerebrospinal fluid.....	3-7
<b>3-2. Lipids .....</b>	<b>3-11</b>
225. Lipids .....	3-11
226. Cholesterol.....	3-13
227. Triglycerides .....	3-16
<b>3-3. Hormones .....</b>	<b>3-20</b>
228. Hormones .....	3-20
229. Thyroid hormones.....	3-22
230. Other medically important hormones .....	3-25
<b>3-4. Therapeutic Drug Monitoring.....</b>	<b>3-31</b>
231. Tracking drug concentrations .....	3-32
232. Medically important drugs.....	3-33
<b>3-5. Toxicology .....</b>	<b>3-36</b>
233. Alcohol .....	3-37
234. Acetaminophen and salicylate .....	3-39
235. Other toxic substances .....	3-40
<b>3-6. Tumor Markers .....</b>	<b>3-46</b>
236. Tumor markers .....	3-46

**T**HERE are still a few more aspects of clinical chemistry for you to investigate. This unit will introduce you to the study of some other areas of human biochemistry, such as proteins, lipids, and hormones. Also, part of a laboratory technician's time may be spent performing therapeutic drug monitoring or toxicology studies. These are more specialized areas of the chemistry department we all need to have knowledge of.

### 3-1. Proteins

The functional need for proteins and their interactions in your body is endless; they make-up the structural elements of all cells, and they serve as enzymes, hormones and immunoglobulins, just to name a few. They are also involved in the transport of many substances in your body and serve as a source of energy when needed. Understanding the functions of proteins and the clinical significance of their testing is basic to all laboratory technicians. In this section, we will explore the general characteristics and functions of proteins and their analysis in the laboratory.

#### 222. Proteins

Proteins are large molecules composed of chains of amino acids. The amino acids are linked together by peptide bonds formed between the terminal amino group of one amino acid and the carboxyl group of another amino acid. The synthesis of proteins takes place on the cell ribosomes with the blueprint for their construction coded genetically in DNA. Some researchers have estimated there are 30,000 to 50,000 genetic codes for the structure of proteins, while other researchers put their estimates at twice this number. Regardless of the number of genetic codes, it has been estimated the number of distinct proteins within a single cell is between 3,000 and 5,000.

**Characteristics of amino acids**

The sequencing and individual characteristics of amino acids help to determine the nature of the proteins they form. Keep in mind, protein molecules have a three-dimensional structure and the ways the molecules can be geometrically arranged is almost infinite. The exact arrangement of the molecule plays a significant part in determining the exact function of that particular protein.

***Simple proteins***

Proteins containing only amino acids or their derivatives are classified as *simple proteins*. Albumin and globulin are examples of simple proteins.

***Conjugated proteins***

Proteins containing an additional non-protein component, called a *prosthetic group*, are referred to as *conjugated proteins*. Conjugated proteins are classified according to the nature of their prosthetic group. Examples of conjugated proteins include nucleoproteins, mucoproteins, lipoproteins, chromoproteins, and phosphoproteins. Due to the large size of most protein molecules, they behave as colloids in water. Colloids are particles suspended in a medium and do not settle out readily. When applying this characteristic, it explains why proteins suspend themselves in body fluids without settling out. The proteins are held in solution due to an attraction between the water molecules and the protein molecules. Proteins have the interesting ability of being able to change their electrical charge (positive, negative, or neutral) as conditions vary. The electrical charge of a protein molecule is determined by the pH of the solution it is in.

***Plasma proteins***

In a previous lesson (Analytical chemistry and biochemistry), you learned about some of the functions of proteins. Of particular interest to clinical chemistry personnel are the proteins found in body fluids, especially plasma and serum. Most of the plasma proteins, with the exception of protein hormones, immunoglobulins, and a few coagulation factors, are made in the liver. We'll be focusing most of our lesson on total serum protein levels—specifically albumin and globulin function—and their analysis. The proteins are made by the hepatocytes and are then released into the blood. Plasma proteins circulate in the blood and between the extracellular tissue spaces and the blood. Differences in the size and makeup of the various types of proteins determine the transport mechanisms for each different type. These characteristics account for the type and concentrations of proteins in the different tissues and fluids. Diseases often affect the type and amount of specific proteins in body fluids, and these changes are what you, the laboratory technician, will detect during specimen analysis. Our basic understanding of the structure and function of proteins is growing. But, before we move on and take a more thorough look at serum protein analysis, let's look at some other forms of proteins of diagnostic importance to the healthcare provider.

**C-reactive protein**

C-reactive protein (CRP) belongs to a group of proteins called *acute phase reactants*. These reactants are proteins that are useful as markers for tissue damage and inflammation. The CRP is one of the most sensitive of the acute phase reactants. The concentration of CRP can increase several hundredfold in cases of inflammation. When combined with fever, white blood cell count, and increased sedimentation rate, CRP can be helpful in diagnosing an overt infection. CRP is synthesized by the liver and binds to polysaccharides found in many bacteria, fungi, and parasites. Like antibodies, CRP can help initiate phagocytosis and the lysis of invading cells. One of the main roles of CRP is to recognize and bind with endotoxins released from damaged tissue, and then detoxify or eliminate these toxins from the blood. CRP can also be used to determine other disease processes and conditions. It can be used for assessing inflammatory diseases, such as rheumatoid arthritis, infections associated with systemic lupus erythematosus, and leukemia. CRP levels are being used to aid in the screening of individuals at a high risk for strokes. Healthy people with high CRP levels have been found to have more strokes than those with normal levels. CRP analysis is useful in assessing the possibility of neonatal septicemia and meningitis when collecting enough blood for culture and

bacterial investigation is not practical. CRP is most often determined by rate nephelometry, enzyme immunoassay, or radial immunodiffusion.

### Complement proteins

Serum complement is a group of proteins that migrate with globulins in electrophoresis, and act as enzymes. These proteins facilitate the body's immunologic and inflammatory responses. Complement proteins increase vascular permeability, catalyzing delivery of antibodies and white blood cells to the area of inflammation. Complement proteins also act to increase chemotaxis (the attraction of white blood cells to the area of infection), phagocytosis of the invading substances, and the response of antibody to antigens. Serum complement levels are important in the detection of autoimmune diseases, such as lupus erythematosus and serum sickness. You'll learn more detailed information about complement proteins and their roles in the 4T051C CDC.

### Immunoglobulins

There are five classes of immunoglobulins, and they are designated as IgG, IgA, IgM, IgD, and IgE. Immunoglobulins have two functions—(1) to recognize antigens and (2) to initiate a response for the destruction or neutralization of the antigens. While most plasma proteins are synthesized in the liver, immunoglobulins are synthesized and secreted by plasma cells.

Class	Explanation
IgG	Found in the highest concentration in serum and most circulating antibodies fall into this class.
IgA	Protect mucous membrane surfaces from bacterial and viral attack.
IgM	Is the largest in molecular size, and is the most primitive and least specialized of the immunoglobulins. It is the only immunoglobulin synthesized by neonates.
IgG, IgA, and IgM	Are usually found in normal patient serum.
IgD and IgE	Are not found in any great amount in normal patient serum and were first discovered in the serum of patients with multiple myeloma. IgD's primary function is still unknown, and IgE is firmly bound to basophilic granulocytes and their tissue equivalent mast cells.

These immunoglobulins are also discussed in further detail in the 4T051C CDC.

## 223. Serum proteins

As you recall, proteins play many key roles in the body. Serum proteins serve many unique functions, such as affecting the distribution of fluids between the cells; serving as carriers for bilirubin, hormones, lipids; circulating as antibodies, clotting factors, enzymes, and hormones; and providing the body with a source of energy when needed.

### Total protein

Albumin and globulin make up most of the protein within the blood and are measured together as *total protein*. Albumin and globulin levels can also be determined independent of each other.

### Albumin

Albumin is a protein formed in the liver and makes up about 60 percent of the total protein in the serum or plasma. The major purpose of albumin within the blood is to maintain colloidal osmotic pressure; albumin also transports important blood constituents such as drugs, hormones, and lipids. Because albumin is synthesized in the liver, it can be used as a measure of hepatic function. When disease affects liver cells, they are not able to produce albumin in normal levels.

**Globulins**

Globulins are the key building blocks of antibodies, and some are synthesized in the liver, while others are made in the mononuclear phagocytic system. Globulins' role in maintaining osmotic pressure is much less than that of albumin. Serum albumin and globulin are also used as measures of nutrition. Malnourished patients will often show decreased levels of these serum proteins.

**Protein levels**

In some diseases, albumin is diminished and the globulin levels are increased to maintain a normal total protein level. For example, in lupus erythematosus, capillary permeability is increased. This causes albumin (which is a smaller molecule than globulin) to be selectively lost in the extravascular space. Another disease characterized by low albumin, high globulin, and normal total protein levels is chronic liver disease. In this condition, the patient's liver cannot produce albumin, but the mononuclear phagocytic system makes up the difference by producing greater amounts of globulin. In each of these examples, the total protein level will be normal because of the increased globulin level.

**Physiology**

Dietary sources for proteins are both animal and vegetable in nature. The most common sources are grains, beans, meat, fish, poultry, dairy products, and eggs. These foods are digested initially in the stomach by pepsin. Large protein molecules are partially digested by pepsin, in an acidic medium, into polypeptides and amino acids. Complete digestion of proteins takes place in the intestines under the action of enzymes such as trypsin, chymotrypsin, and peptidases.

**Basic building blocks**

When protein digestion is complete, they are finally broken down to their basic building blocks—amino acids. These amino acids are then absorbed into the blood. From the blood, the amino acids are carried to the liver and other tissues where they are assembled to form the specific proteins needed for body functions. Amino acids not used to form proteins can be stored for future use or eliminated from the body.

**Essential amino acids**

There are 20 different recognized amino acids, which the body combines to form all required proteins. Our bodies can synthesize 11 of the 20 amino acids. The other nine must be provided by our diet and are defined as the *essential amino acids*.

**Metabolism**

Proteins in the body are constantly being broken down and reformed. Although not all of these processes are completely understood, protein metabolism is an ongoing dynamic process. Proteins can be used for energy in cases of malnutrition or metabolic dysfunctions. In starvation, muscle tissue wastes away because the proteins in the muscles are used to produce energy.

**Albumin**

Serum albumin consists of a single type of protein and is the most prominent band observed during serum protein electrophoresis. As mentioned previously, albumin makes up about 60 percent of the total serum protein level, and it plays a major role in osmotic pressure and transport. Its level in the plasma determines the rate at which it is produced by the liver. Albumin is an anion with a pH of 7.4 and has over 200 negative charges per molecule. Because of all these charges, albumin has a vast capacity for binding with other substances and transporting them throughout the body.

**Globulins**

When total serum protein is broken down into its fractions by electrophoresis, five fractions are observed. The first is albumin; the remaining four fractions are globulins. Globulin molecules are larger than albumin molecules. Globulin molecules are broken down into three main groups: alpha

globulins ( $\alpha$ -globulins), beta globulins ( $\beta$ -globulins), and gamma globulins ( $\gamma$ -globulins). The alpha globulins are further broken down into two types.

Group	Explanation	
<i>Alpha globulins (<math>\alpha</math>-globulins)</i>	There are two types of $\alpha$ -globulins:	
	$\alpha_1$ -globulin	The second fastest band to be seen on electrophoresis (it is next to albumin, but closer to the cathode). Many of the proteins in this band are combined with carbohydrates and are called glycoproteins, while others are combined with lipids and are called $\alpha_1$ -lipoproteins. In general, the $\alpha_1$ -globulins increase from non-specific causes, such as infection, trauma, and neoplasms (abnormal growths of tissue). The $\alpha_1$ -globulins are formed in the liver.
	$\alpha_2$ -globulin	The third band in protein electrophoresis is the $\alpha_2$ -globulin band. This fraction also contains some $\alpha_2$ -lipoproteins. A few of the well-known proteins in this fraction include haptoglobin (a hemoglobin-binding protein) and ceruloplasmin (a copper-binding protein). Proteins in the $\alpha_2$ -globulin band increase in inflammatory conditions.
<i>Beta globulins (<math>\beta</math>-globulins)</i>	The fourth band contains $\beta$ -globulins, which are proteins, such as the $\beta$ -lipoproteins, fibrinogen, transferrin (the iron-transporting protein), and others. Any condition that causes an increase in $\beta$ -lipoproteins will cause this band to become more prominent because lipoproteins are predominant in this fraction.	
<i>Gamma globulins (<math>\gamma</math>-globulins)</i>	The slowest band to migrate in serum electrophoresis is the fifth and final band, the $\gamma$ -globulins. This band contains immunoglobulins, circulating antibodies needed for the body's defenses against foreign proteins. The $\gamma$ -globulins are produced by the cells of the mononuclear phagocytic system—plasma cells and lymphocytes.	

### Clinical significance

Let's next take a look at the diagnostic value of protein analysis.

#### Total serum protein

Usually, the first step in assessing a patient's overall serum or plasma protein composition is to perform a total serum protein analysis. There are generally two conditions that cause changes in total serum protein levels. The first is a change in the volume of water in the plasma and the second is a change in the concentration of one or more of the specific proteins in the plasma. A decrease in the volume of water in the plasma, known as hemoconcentration, can cause *hyperproteinemia*, an increase in total serum protein levels. This condition can be due to inadequate water intake or severe water loss from severe vomiting, diarrhea, Addison's disease, or diabetic acidosis.

#### Hemodilution

A reverse condition, in which there is too much water, is *hemodilution*, causing hypoproteinemia. Hemodilution can occur when there is overindulgence in the intake of water (water intoxication), in salt retention syndromes, and during massive intravenous transfusions.

#### Hypoproteinemia

*Hypoproteinemia* is a decrease in the total serum protein level. Because albumin makes up the majority of the total serum protein, a decrease in the level of albumin can cause a marked hypoproteinemia. When levels of proteins normally found in low concentrations are increased, the overall total protein can also increase, causing a mild hyperproteinemia. Marked hyperproteinemia

can be the result of high levels of immunoglobulins produced in multiple myeloma or other malignant conditions.

### ***Albumin***

Plasma levels of albumin are frequently used to examine a patient's nutritional status because protein levels depend on dietary intake. *Hyperalbuminemia*, an increased serum albumin level, is of little diagnostic significance with the exception of dehydration. *Hypoalbuminemia*, a decrease in serum albumin levels, is another story. Hypoalbuminemia is very common in many illnesses and can result from one or more of the following factors:

- Impaired protein synthesis as a result of liver disease or decreased protein intake.
- Increased catabolism as a result of inflammation or tissue damage.
- Malabsorption syndromes or malnutrition resulting in the reduced absorption of amino acids.
- Protein loss due to conditions such as renal problems, diabetes, or severe burns.

### ***Globulins***

Globulins are often decreased in cases of malnutrition or immunologic deficiencies and increases are usually caused by immunologic malignancies such as multiple myeloma. The following table gives a further breakdown based on the different globulin fractions:

<b>Globulin Type</b>	<b>Clinical Significance</b>
<i><math>\alpha_1</math>-globulins</i>	Often increased when serum albumin levels fall, particularly in infections and inflammatory diseases. Lowered concentrations are observed in acute hepatitis.
<i><math>\alpha_2</math>-globulins</i>	Greatly increased in nephrotic syndrome. Increased in inflammatory conditions, such as rheumatoid arthritis and lupus erythematosus, or after an MI. Lowered concentrations seen in acute hepatocellular disease.
<i><math>\beta</math>-globulins</i>	Usually increased in various types of hyperlipemias.
<i><math>\gamma</math>-globulins</i>	Increases often seen in a monoclonal disease, such as multiple myeloma. Increased in viral hepatitis, chronic infections, and some leukemias and lymphomas. Decreased levels occur when synthesis of these proteins is decreased due to impaired lymphocytes.

### **Laboratory procedures and limitations**

When testing for total protein levels in the blood, either serum or plasma can be used, but serum is preferred. A fasting specimen is not required, but you may wish to consider this in order to decrease lipemia. Avoid hemolyzed samples for analysis. Tightly capped samples are stable for 1 week at room temperature and up to 2 months if refrigerated at 2 to 4°C. Should you use a frozen sample for total protein, be sure the sample is thawed and well mixed before testing.

### ***Determination of total serum protein***

As the name implies, total serum protein is the sum of all of the independent proteins. When performing electrophoresis, the total protein is determined first, and then each protein fraction percentage is multiplied times this value to quantify each band. Aside from this reason, total serum protein levels offer little significant information on the individual protein fractions except for their relationship to changes in plasma or fluid volume. Two methods are most commonly used to determine total serum protein concentrations—the biuret and refractive index.

### ***Biuret method***

In the biuret method, copper ions react in an alkaline solution with the peptide linkages of proteins to form a violet-colored complex. The intensity of the color produced is proportional to the amount of protein present. Because this method is colorimetric, lipemia and hemolysis may cause interference.

*Refractive index method*

Refractometry is a quick and reasonably accurate alternative method to chemical analysis for total serum protein determinations. Many laboratories use this method of analysis to measure total serum protein levels prior to serum protein electrophoresis. Many refractometers have a calibrated scale that gives a direct readout of the total serum protein concentration. These instruments measure the total solids dissolved in the serum sample just as they do when they measure the specific gravity in a urine sample. Go into urinalysis and see if there are two scales on your refractometer!

*Determination of albumin*

The specimen of choice for albumin testing is serum. Grossly lipemic samples should be avoided. When collecting the sample, avoid venostasis (that is, leaving the tourniquet on too long). This condition can cause hemoconcentration leading to an increase in the concentration of albumin and other plasma proteins. Albumin analysis can be performed by either electrophoresis or, more commonly, by its ability to bind with anionic dyes. The dye binding methods, bromocresol green or bromocresol purple, are widely used. These dyes have a higher affinity for albumin than for the other protein fractions, so they bind, at a specific pH, primarily with albumin in the sample. The absorption of the dye-albumin complex is determined spectrophotometrically at 628 nm for bromocresol green and at 603 nm for bromocresol purple.

*Determination of globulins*

Electrophoresis is the method used to fractionate and quantify the various types of globulins. Because you have already studied the principles of electrophoretic separation, the lesson does not go into detail. Procedural details vary from manufacturer to manufacturer, so review the instructions that apply to the equipment in your facility (if you perform electrophoresis).

*Albumin/globulin ratio*

Changes in a patient's total serum protein level may result from abnormal levels of albumin, globulin, or both. The provider may need to know which fraction of the total serum protein has been affected. The most accurate method for determining serum protein fractions is electrophoresis. However, not all laboratories have the expensive equipment needed to perform this test. At smaller health care facilities, providers can request an albumin/globulin ratio (A/G ratio) to diagnose their patients and determine if more extensive testing is required. An A/G ratio is simple to calculate. First, the patient's total serum protein and albumin levels are determined. Then the albumin level is subtracted from the total serum protein value to determine an estimate of the total globulin concentration. The albumin level is then compared to the globulin level and expressed as a ratio. The normal A/G ratio is >1.0:1 (albumin to globulin). A/G ratios are not always calculated on patients with abnormal total serum protein levels. As an example, patients with an acute phase response, increased globulin levels, and decreased albumin levels, may still exhibit normal total serum protein levels.

**224. Protein in cerebrospinal fluid**

CSF is a clear, colorless liquid that circulates in the brain over the cerebral hemispheres and down over the spinal cord. Normally, only small amounts of protein are found in CSF because protein molecules are large and do not cross the blood-brain barrier. However, some disease processes alter the permeability of this protective membrane and allow proteins to leak in.

**Physiology**

CSF is secreted by the choroid plexuses, around the cerebral vessels, and along the walls of the ventricles of the brain. The turnover of CSF is rapid; it is exchanged about four times each day. Most of the protein present in the CSF (about 80 percent) comes from plasma that is ultrafiltered through the walls of the capillaries into the meninges and choroid plexuses. As CSF works its way down the spinal cord, the level of protein increases, with the highest levels being found in the lumbar spine. The lumbar spine is the area from which CSF specimens are usually collected.

**Clinical significance**

The protein content of CSF is increased in patients who have infections, such as bacterial meningitis, or inflammatory processes, such as encephalitis or poliomyelitis. Trauma and tumors can also cause increased levels of protein to be detected in CSF. When tumors occur in the spinal cord and obstruct the flow of CSF, the fluid in the lumbar spine area becomes stagnant. Proteins will equilibrate across the walls of the meningeal capillaries into the stagnant CSF raising proteins levels. CSF protein levels can also become increased when lesions inflame the meninges, when the patient suffers from a cerebral hemorrhage, or in cases of neurosyphilis. In patients with multiple sclerosis or other demyelinating diseases (conditions where the destruction or removal of the *myelin* covering nerve fibers results in their impaired function), an increased CSF protein is seen. This is caused by an increased synthesis of IgG within the central nervous system. Decreased levels of CSF protein are of minimal clinical significance.

**Laboratory procedures and limitations**

Because it is difficult to obtain a CSF sample and several laboratory sections must share its limited volume, every effort must be made to conserve the sample during testing. Only a small amount of CSF can be removed from the patient without causing a severe headache. Normally, only 5 to 10 ml of sample can be collected at a time. Spinal punctures are extremely painful for the patient and repeated punctures should be avoided because total protein levels can be falsely increased due to the trauma of previous punctures. Total protein content, even in a clear supernatant of a bloody specimen (often called a bloody tap), will reflect the contamination of blood proteins. Testing of CSF specimens will normally include glucose and protein in the chemistry department, cell counts in hematology, and cultures in microbiology. Any sample remaining after testing can be stored in the refrigerator for up to 2 weeks in the event other testing is required. The collection and handling of CSF samples are covered in the 4T051B CDC.

Testing of CSF for total protein levels is usually accomplished using the same methods described for total serum protein. Because these methods have been covered previously, they are not discussed here. CSF protein can also be determined by a turbidimetric method in which CSF proteins are precipitated by a combination of sulfosalicylic acid and sodium sulfate. The dye-binding methods are preferred because they require as little as .025 ml of sample, whereas the turbidimetric methods require up to 0.5 ml of sample.

In turbidimetric methods, a sulfosalicylic acid and sodium sulfate reagent produces a fine suspension of protein when added to CSF. The protein concentration is proportional to the turbidity produced. This turbidity is measured as a decrease in light transmittance at 620 nm. Interference from hemoglobin or drugs that precipitate protein can affect results. CSF proteins can also be fractionated by electrophoresis to evaluate the  $\gamma$ -globulin fractions. Patients with multiple sclerosis or other demyelinating diseases may or may not show an obvious elevation in CSF protein, but these conditions are more easily detected using electrophoresis. When the total protein of a CSF sample is normal, the specimen must be concentrated at least 100-fold in order for electrophoresis techniques to be successful. In normal CSF samples, the  $\gamma$ -globulin is less than 11 percent of the total protein, but in multiple sclerosis its level increases to more than 18 percent.

---

**Self-Test Questions**

After you complete these questions, you may check your answers at the end of the unit.

**222. Proteins**

1. What determines the nature of proteins?



2. What is the difference between simple and conjugated proteins? Give examples of each.
3. What is meant by the expression proteins behave as colloids?
4. What determines the electrical charge of proteins?
5. What are acute phase reactants? Give an example.
6. Where is C-reactive protein (CRP) synthesized and what are some of its functions?
7. What are some of the roles of serum complement?
8. What are two functions of immunoglobulins?
9. Which of the immunoglobulins are found in normal patient serum and which additional ones are found in patients with multiple myeloma?

**223. Serum proteins**

1. What percentage of total protein is made up of albumin, where is albumin formed, and what are some of the functions of albumin?
2. Where are globulins synthesized and what are their functions?
3. Why might a patient with chronic liver disease have a decreased albumin but increased globulin level?
4. Proteins are broken down into amino acids by the action of what intestinal enzymes?
5. Why does albumin have such a vast capacity for binding and transporting substances?

6. When total protein is broken down by electrophoresis, how many globulins will become evident, and what are they called?
7. What are some of the proteins that fall into the various globulin fractions?
8. What two conditions generally cause a change in a patient's total serum protein level?
9. Define hyperproteinemia, hemodilution, and hypoproteinemia.
10. What is the diagnostic significance of hyperalbuminemia?
11. What conditions can produce hypoalbuminemia?
12. What are the causes of increased or decreased serum globulin levels?
13. If frozen serum is used for total protein analysis, how should the sample be handled?
14. Explain the principle of the biuret method of total serum protein analysis.
15. Explain how refractometers measure total serum protein.
16. Explain the principles of the dye-binding methods for albumin analysis.
17. How is an albumin/globulin (A/G) ratio determined and what is the normal range?

**224. Protein in cerebrospinal fluid**

1. Why are protein levels in CSF lower than that of serum, and why do some diseases allow proteins to leak into the CSF?

2. Where does most of the protein found in CSF come from, and what can be said about its concentration in the spinal cord?
3. Why do CSF protein levels increase when there is a tumor obstructing the spinal cord?
4. What other conditions can cause increased CSF protein?
5. Why should repeated spinal punctures and bloody tap specimens be avoided?
6. Once testing is completed, what is done with the leftover CSF sample?
7. Why are dye-binding methods for CSF protein analysis preferred over turbidimetric methods?
8. Explain the principle of turbidimetric testing for CSF protein and list sources of test interference.
9. Why are CSF specimens, from patients with multiple sclerosis or demyelinating diseases, fractionated by electrophoresis?

## 3-2. Lipids

Lipids are essential to many life processes, such as aiding digestion, providing energy and energy storage, functioning and acting as structural components of membranes, serving as hormones or hormone precursors, and acting as insulators of nerves. In recent years, the emphasis on *lipid* testing has mainly come to be associated with the monitoring of cardiovascular disease states or the assessment of the patient's cardiovascular risk factors. Let's start our lesson by looking at the different types of lipids our body requires and how we use them.

### 225. Lipids

The term *lipid* refers to a class of compounds that are soluble in organic solvents and almost insoluble in water. Their chemical structure involves either compounds that yield fatty acids when hydrolyzed or complex alcohols that combine with fatty acids to form esters. Lipids are classified based on their chemical structure and complexity. The classes of lipids most important to laboratory technicians are fatty acids, triglycerides, phospholipids, plant sterols, sphingolipids, and cholesterol. All of these forms will be covered in this section. Triglycerides and cholesterol will be discussed in further detail later in this unit.

**Fatty acids**

Fatty acids are one of the simpler forms of lipids when looking at molecular structure. Fatty acids are often identified based on the number of carbon atoms and the number of double bonds in their structure. Fatty acids are either saturated (no double bonds) or unsaturated (one or more double bonds). In general, the fatty acids derived from plant seeds or fish oils are typically unsaturated. These fatty acids are among the essential fatty acids that humans require but cannot synthesize. Most of the fats in the human body are derived from dietary sources. Long-chain fatty acids are oxidized in the mitochondria of cells for the production of energy. Unit for unit, the amount of energy produced by metabolizing a fatty acid is twice that of metabolizing a carbohydrate such as glucose. Storage of fatty acids is also more efficient than that of carbohydrates, and when in the form of triglycerides, provide excellent insulation.

**Triglycerides**

Most of the fatty acids in the body are components of triglycerides and are stored in the adipose tissues as fat. In order for cells to convert fatty acids into triglycerides, glucose must be present in the cells. When glucose is absent due to starvation, fasting, or uncontrolled diabetes mellitus, triglycerides from fat deposits are broken down by hydrolysis to release fatty acids. Fatty acids then appear in blood plasma as free fatty acids bound to albumin (acting as a carrier). Various tissues in the body, but particularly the muscles, use fatty acids for energy purposes. Triglycerides make up about 90 to 95% of the stored fats in the human body.

**Phospholipids**

Phospholipids are essential components of cell membranes. They have the ability to align themselves between water and lipid phases of the cell membrane, providing the lipid portion of the membrane. Phospholipids play a role in mitochondrial metabolism, blood coagulation, and lipid transport, and are important in the structure of cellular membranes. In spite of all of these important roles, there is very little clinical information gained by attempting to measure the concentration of plasma phospholipids.

**Plant sterols**

Plant sterols are steroids made up of long, open (aliphatic) chains, which form a 4-ring structure that have at least one hydroxyl group. Plant sterols closely resemble cholesterol from animal sources. The only difference between plant sterols and animal cholesterol are the small variations in the chain structure. Despite their close similarity to animal cholesterol, plant sterols are not absorbed very well. When plant sterols are ingested, they help to inhibit the absorption of cholesterol. Although the exact reason for this inhibition is not completely understood, plant sterols are used to treat patients with elevated levels of plasma cholesterol.

**Sphingolipids**

Sphingolipids are essential components of cell membranes, particularly red blood cells and nervous system cells. Some of the membrane sphingolipids also play a role in cell recognition and blood typing.

**Cholesterol**

Cholesterol is found primarily in animals and humans, it is the primary body sterol, and its chemical makeup is that of a complex alcohol. Every cell and virtually all body fluids contain cholesterol. In the cells, cholesterol serves as a structural component and as a precursor of steroid hormones. Although some cholesterol is derived from the diet, the liver synthesizes the majority of cholesterol. Dietary cholesterol is obtained from foods such as meat, eggs, and whole-fat dairy products. Most of the dietary cholesterol is absorbed in the small intestines.

## 226. Cholesterol

Cholesterol is the primary lipid associated with an increased risk of cardiovascular disease. Even though high levels of cholesterol are associated with heart disease, it is still required, in small amounts, for the production of steroid hormones, bile acids, and cellular membranes.

### Physiology

Even though all cells have the ability to synthesize cholesterol, the liver synthesizes 90 percent of the cholesterol produced in your body. Your body has the ability to produce all of the cholesterol it needs, so dietary intake is not required. But, when dietary cholesterol is introduced, the liver will stop producing cholesterol. This feedback system is the body's attempt to keep your cholesterol levels within normal limits. Once synthesized or ingested, cholesterol is bound in the liver to a lipoprotein and then released into the circulation for transport to other cells. The following table lists lipoproteins and their functions.

Lipoprotein Name	Lipoprotein Origin	Function
<i>Chylomicrons</i>	The largest and least dense of all the lipoproteins, they are synthesized in the intestines.	Transport ingested triglycerides to adipose tissue and muscle cells.
<i>Very low-density lipoprotein (VLDL)</i>	VLDL is made in the liver.	Transports triglycerides synthesized by the liver to muscle and adipose cells.
<i>Low-density lipoprotein (LDL)</i>	LDL is rich in cholesterol and is created in the plasma from other lipoproteins.	LDL is responsible for the transport of cholesterol to extrahepatic tissues for the making of new cell membranes. It's also used as a precursor for of steroid hormones, or for the storage of cholesterol esters.
<i>High-density lipoprotein (HDL)</i>	Both the liver and intestinal mucosa cells make HDL particles. HDL has a complicated life cycle and experiences many changes after its formation.	HDL's life cycle is not completely understood, but HDL is thought to play a part in the outward movement of cholesterol from the tissues. Because of its role in reverse cholesterol transport, HDL is thought to protect against cardiovascular disease.

### Clinical significance

Since the liver plays a major role in the metabolism of ingested cholesterol and cholesterol synthesis, below normal cholesterol levels can be indicative of severe liver disease. Malnutrition has also been associated with low cholesterol levels.

### Cholesterol transport

When cholesterol is transported in the blood, 75 percent of it is bound to LDL and 25 percent is bound to HDL.

#### *Low-density lipoprotein*

LDL carries cholesterol to the cells where specific LDL receptor molecules line the cell surface. After the LDL is bound to these receptor sites, it is taken inside the cell where enzymes break it down, liberating the cholesterol from cholesterol esters. The cell then uses the cholesterol. In some cases, however, there are not enough LDL receptors. When this occurs, the serum cholesterol levels will increase, which increases the risk of cardiovascular disease in the patient. A high LDL level means there is also a high cholesterol level in the plasma since the LDL cannot get into the cell to be metabolized. So, both increased serum cholesterol levels and increased LDL levels will increase the patient's risk of cardiovascular disease.

### *High-density lipoprotein*

HDL transports cholesterol from the cells to the liver. In the liver, the cholesterol is used in the formation of bile. This action essentially decreases circulating cholesterol. Several studies have suggested that HDL levels have an inverse relationship with the risk of cardiovascular disease. That is, the higher the HDL, the lower the risk of cardiovascular disease. HDL is sometimes referred to as the *good* cholesterol and LDL has been called the *bad* cholesterol!

### ***Cholesterol increase***

Cholesterol levels can increase during pregnancy, uncontrolled diabetes mellitus, hypothyroidism, and biliary cirrhosis. Increased levels are observed in patients with abnormal levels of hormones such as epinephrine or adrenocorticotrophic hormone. The most common cause of increased levels is the most obvious — a high-cholesterol diet.

### ***Cholesterol decrease***

Cholesterol levels can be decreased in cases of malabsorption or malnutrition, cholesterol-lowering medications, liver disease, anemia, and hyperthyroidism. Drugs that can decrease cholesterol levels include bile-salt binding agents, androgens, erythromycin and neomycin, niacin, and nitrates. Also, a patient's serum cholesterol level can decrease as much as 10 percent when they change from a standing (outpatient) position to a reclining (inpatient) position.

### **Laboratory procedures and limitations**

Cholesterol analysis is most commonly requested as part of a lipid panel (cholesterol, triglyceride, HDL, and LDL).

### ***Specimens for analysis***

Serum cholesterol levels are not significantly affected by non-fasting samples; however, triglyceride analysis is, so samples for cholesterol are normally collected after a patient has fasted. Serum is the specimen of choice for cholesterol analysis. EDTA plasma can be used but the plasma value is converted to a serum value by multiplying the plasma value by 1.03. Specimens should be analyzed the day of collection but may be stored at 4°C for up to 3 or 4 days before analysis. Samples stored at -20°C will remain stable for several months; those stored at -70°C will remain stable for several years. Once the specimen is thawed, it should be brought back to room temperature and mixed well before analysis.

### ***Methods***

Most laboratories analyze total cholesterol by enzymatic methods. These methods are simpler, faster, and use less corrosive reagents than previous chemical methods. During testing, the cholesterol esters in the sample are hydrolyzed into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, free cholesterol is then oxidized by cholesterol oxidase to form cholest-4-ene-3-one and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The H<sub>2</sub>O<sub>2</sub> reacts with phenol and 4-aminophenazone in the presence of peroxidase to form o-quinoneimine dye. The intensity of the color formed is proportional to the cholesterol concentration in the sample. The final reaction is measured photometrically between 480 and 520 nm. When performing enzymatic methods, keep in mind the method listed above is linear up to 500 mg/dl. If a sample exceeds this amount, it must be retested after a 1:1 dilution with normal saline. Of course, the result must be multiplied by 2 prior to reporting the final result. It is recommended that two quality control samples be run, one at a 175 to 200 mg/dl range and the other at a 240 to 260 mg/dl range. This fully monitors analytical performance. Whichever method you use to perform total cholesterol analysis, also use it to measure HDL cholesterol. This increases the validity of your LDL calculations.

### ***Determination of HDL cholesterol***

Using serum cholesterol levels *only* is of limited value when determining the patient's risk of cardiovascular disease. Two lipoproteins, HDL, and LDL transport cholesterol; the measurement of

all three components will yield a more complete diagnostic picture. HDL carries cholesterol from tissues to the liver for conversion to bile salts, while LDL carries cholesterol to the tissues for deposit (to include deposit in the blood vessels). HDL competes with LDL for binding sites on the tissue receptors and, in doing so, reduces the cholesterol accumulation in the blood vessels, which reduces cardiovascular risk. This is why HDL is called the *good cholesterol*.

#### *Specimens for analysis*

Either serum or plasma samples may be used for analysis. Collect plasma samples in dry disodium-EDTA. Samples collected in liquid EDTA can be falsely decreased due to dilution with the liquid anti-coagulant. Again, if plasma is used, the results must be multiplied by a factor of 1.03 to make values equivalent to serum results. Serum values are used to determine normal ranges and evaluate the patient. Store the sample at 4°C before testing. HDL is stable for up to 4 days at 4°C, but significant changes occur by the seventh day following collection. Frozen samples kept at -20°C will show some decrease in HDL, but it is not clinically significant when tested as late as 7 to 14 days after collection. If possible, perform the HDL separation procedure on the day of collection.

#### *HDL analysis*

HDL analysis is performed as follows: first, the major classes of lipoproteins (chylomicrons, HDL, LDL, and VLDL) are isolated by forming an insoluble lipoprotein-polyanion-divalent cation complex. After the addition of magnesium and dextran sulfate to the sample, the larger, lipid-rich chylomicrons, VLDL, and LDL are precipitated selectively, leaving the HDL in solution. The precipitated lipoproteins are then removed by centrifugation, and the remaining clear supernatant is tested for HDL using the same enzymatic methods utilized for cholesterol testing. Controls for HDL cholesterol testing should have a target value between 45 and 60 mg/dl.

#### ***Determination of LDL cholesterol***

It is very difficult to measure the concentration of LDL cholesterol directly, so it is usually calculated from the measurements of total cholesterol, HDL cholesterol, and triglycerides. The risk for coronary artery disease increases with an increase of LDL, but decreases if there is an increased amount of HDL. For this reason, many laboratories calculate a ratio of LDL cholesterol to HDL cholesterol (LDL to HDL) to estimate the degree of risk. A ratio of less than 3:1 is considered desirable. The formula for calculating LDL is:

$$\text{LDL} = \text{Total cholesterol} - [\text{HDL cholesterol} + (\text{Triglycerides} \div 5)]$$

#### *Calculations*

To ensure you understand the formula, use the following results to determine the patient's LDL: total cholesterol level of 200 mg/dl, HDL cholesterol of 50 mg/dl, and triglyceride level of 200 mg/dl. Your first step is to solve the portion inside the parenthesis (Triglycerides  $\div$  5). In the example, this is  $200 \div 5$ , which equals 40. Next, solve for the equation within the brackets, [HDL cholesterol + 40]. In the example, this is  $50 + 40$ , which equals 90. Finally, solve the rest of the equation (Total cholesterol - 90). Again, based on the example, the figures are  $200 - 90$ , which equals 110. You have just calculated that the patient's LDL is 110 mg/dl. Without peeking at the answer, try your hand at one more example to ensure you fully understand the concept of calculating LDLs. In this example, your patient results are: total cholesterol is 330 mg/dl, HDL cholesterol is 30 mg/dl, and triglyceride is 360 mg/dl. Your calculations to determine this patient's LDL should look like this.

$$(360 \div 5) = 72$$

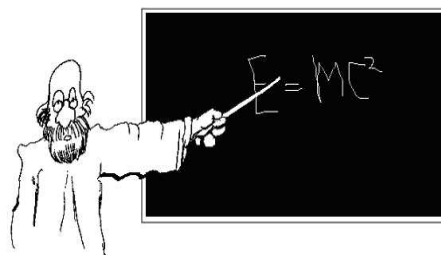
$$[30 + 72] = 102$$

$$330 - 102 = 228$$

$$\text{LDL} = 228 \text{ mg/dl}$$

### *Order of operations*

People tend to forget the order of operations for mathematical equations; that is, you always do *multiplication* and *division* before you do *addition* and *subtraction*. Remember the order of operation!



### *Other concerns*

There are a few other items of concern when you are determining LDL levels. Remember that because LDL is based on a calculation, if any of the three other values (total cholesterol, HDL cholesterol, or triglycerides) are in error, your LDL value will be, too. In addition, the formula for calculating LDL levels is not accurate if the triglycerides value is above 400 mg/dl.

## **227. Triglycerides**

Let's finish our unit on lipids by discussing the roles of triglycerides and the importance of triglyceride testing.

### **Physiology**

Triglycerides act as an energy storage source for the body. Triglycerides are stored in the adipose tissue. They are composed of 3 molecules of fatty acids bound to 1 molecule of glycerol. The adipose tissue and liver cells convert fatty acids into triglycerides by esterification with glycerol-3-phosphate. Glycerol-3-phosphate is a product of glucose metabolism. For a cell to have the ability to form triglycerides, it *must* contain glucose.

### ***Digestion of triglycerides***

Triglycerides are digested through the actions of bile acids and lipases in the small intestine. This action hydrolyzes the triglycerides into fatty acids and glycerol, and these substances are absorbed into the blood. After absorption, the triglycerides are resynthesized in the epithelial cells and combined with cholesterol and other substances to form chylomicrons. Not all of the fatty acids are converted back into triglyceride; some are used immediately by the tissues for energy (especially the muscle tissue). Only the excess fatty acids are used for resynthesizing triglycerides. Excess ingested carbohydrates are stored as triglycerides. Insulin promotes the formation of triglycerides by the adipose tissues, whereas a deficiency of insulin increases triglyceride hydrolysis. As mentioned before, triglycerides are a stored form of energy. When tissues use triglycerides for energy, the energy produced is twice that of an equal amount of protein or carbohydrate. The high energy released per unit of triglyceride burned, coupled with its ready availability in the adipose tissue, makes triglyceride an excellent source of energy.

### ***Transport of triglycerides***

Triglycerides are transported in the body primarily by chylomicrons and VLDLs. Chylomicrons transport digested triglycerides to the adipose tissues and muscles. Have you ever noticed the milky serum of a non-fasting patient? It is most likely due to the presence of chylomicrons in the patient's sample. Chylomicrons are metabolized quickly and will normally be absent from the serum within 10 hours after eating. Another triglyceride-rich lipoprotein is VLDL. VLDL transports triglycerides that have been synthesized in the liver to the muscles and adipose cells.

### **Clinical significance**

Like cholesterol, triglycerides analysis is primarily used as an indicator of a patient's risk of cardiovascular disease. A variety of conditions and diseases can affect triglyceride levels.



***Increased triglyceride levels***

Increased triglyceride levels have been found in conditions such as glycogen storage disease, hyperlipidemias, hypothyroidism, and high carbohydrate diets. Poorly controlled diabetes, nephrotic syndrome, alcoholic cirrhosis, and even pregnancy have been found to also cause increased triglyceride levels. Drugs that can cause increased triglyceride levels include oral contraceptives, alcohol, estrogens, and cholestyramine (used to prevent the reabsorption of bile salts in the intestine).

***Decreased triglyceride levels***

Malabsorption syndromes, malnutrition, and hyperthyroidism can cause decreased triglyceride levels. Drugs that decrease triglyceride levels include ascorbic acid, asparaginase (used to help treat leukemia), and clofibrate and colestipol (both used to treat hyperlipoproteinemia).

**Laboratory procedures and limitations**

Triglyceride analysis is most commonly performed by enzymatic methods. Techniques such as fluorometry or colorimetry can require purification or extraction steps and are labor intensive. Enzymatic methods eliminate these extra steps and are easily automated.

***Specimens for analysis***

Patients *must* fast at least 12 hours prior to sample collected for triglyceride analysis. Unlike cholesterol, triglyceride levels are quickly affected by food in-take. For example, a meal containing 50 g of fat (such as a hamburger and an order of fries) can raise levels as high as 260 mg/dl! Peak triglyceride levels occur about 5 hours after eating. Serum is the specimen of choice for analysis, although EDTA plasma can be used. If using EDTA plasma, results must be converted to serum values by multiplying by 1.03. Store specimens at 4°C before testing. When refrigerated, samples are stable up to 3 days. If stored at -20°C, samples are stable for several weeks, and if frozen at -70°C, for even longer.

***Triglyceride analysis***

During enzymatic triglyceride testing, triglycerides are hydrolyzed by microbial lipase to produce glycerol and free fatty acids. The glycerol is then put through several other coupled enzymatic reactions to finally form red quinoneimine dye. The absorbency of the dye is read at 510 nm and is proportional to the triglyceride concentration. Sources of error for triglyceride testing include the use of collection tubes with stoppers that have been lubricated with glycerol. Tubes for pediatric use may have glycerol as a lubricant. Also be sure to monitor the stability of your reagents as some reagents have very limited stability after reconstitution.

---

**Self-Test Questions**

After you complete these questions, you may check your answers at the end of the unit.

**225. Lipids**

1. What are the classes of lipids most important to laboratory technicians?
2. What is unique about the unsaturated fatty acids derived from plant seed or fish oils?
3. How does the energy produced from fatty acid metabolism compare to the energy produced from the metabolism of carbohydrates?

4. Triglycerides make up what percentage of the stored body fats?
5. Why are phospholipids essential components of cell membranes?
6. Why are sterols derived from plant sources used for patients with increased cholesterol levels?
7. Sphingolipids are essential parts of which cellular membranes?
8. Cholesterol is acquired from what sources?

**226. Cholesterol**

1. LDL and HDL serve what functions in the transport of cholesterol?
2. What are the conditions that can cause increased cholesterol levels?
3. An outpatient's cholesterol level was 200 mg/dl 2 weeks ago, and it is now 180 mg/dl. Aside from medications or dietary changes, what could account for this difference?
4. If an EDTA plasma sample is used for cholesterol analysis, what correction must be made to the results?
5.  $\text{H}_2\text{O}_2$  serves what function during enzymatic cholesterol analysis?
6. To what upper limits are results linear during enzymatic cholesterol analysis? And, what must be done to the sample prior to retesting?
7. Why is it important to perform HDL cholesterol testing as soon as possible after specimen collection?
8. Why is HDL is sometimes called the *good* cholesterol?

9. Magnesium and dextran sulfate serve what function during HDL analysis?
10. What is a desirable LDL:HDL ratio?
11. What formula is used to calculate LDL cholesterol?
12. Using the formula from the above question, calculate the LDL level for the patient with the following results: total cholesterol = 180 mg/dl, HDL cholesterol = 40 mg/dl, and triglyceride = 250 mg/dl.
13. What is the importance of having accurate total cholesterol, HDL cholesterol, and triglyceride values when calculating LDL levels?
14. When would LDL concentrations not be calculated?

**227. Triglycerides**

1. What is the primary purpose of triglycerides and of what are they made of?
2. Why must cells that synthesize triglycerides contain glucose?
3. What is insulin's effect on triglycerides levels?
4. How do the roles of chylomicrons and VLDL differ in the transport of triglycerides?
5. Triglyceride testing is primarily used to diagnose what condition?
6. Why are enzymatic methods of triglyceride testing more commonly used?
7. Why are patients instructed to fast for at least 12 hours prior to sample collection for triglyceride testing?

8. Describe the principle of enzymatic triglyceride testing.
9. What are the sources of error in enzymatic triglyceride testing?

### **3-3. Hormones**

The study of hormones is known as endocrinology. Hormones are defined as chemical substances produced by specialized organs and tissues, and transported by the blood to other areas of the body where they have a specific regulatory effect. Hormones act as chemical messengers inside the body; they stimulate some processes and retard others. Growth, reproduction, sexual attributes, blood sugar levels, and even mental conditions are just some of the many processes dependent on hormones.

#### **228. Hormones**

In this unit, we'll study the functions of hormones and how they're secreted and regulated. We will also take an in-depth look at the thyroid gland, its regulatory functions, and hormone production. Finally, we will cover other medically important hormones.

#### **Functions of hormones**

The functions of hormones are quite complex and diverse. Their functions are grouped into three general categories: regulatory function, morphogenesis, and integrative action.

##### ***Regulatory functions***

Regulatory functions are concerned with maintaining the chemical composition of extracellular and intracellular fluids. This process is accomplished through the closely regulated metabolism of substances, such as salts, water, fats, carbohydrates, and proteins, by appropriate hormones. For example, if the concentration of plasma glucose increases, the pancreas secretes the hormone insulin to increase the usage of glucose. The pancreas will continue to secrete insulin until the plasma glucose levels are back to normal. In addition to regulating normal bodily functions, hormones are used by the body to respond to emergency demands, such as starvation, infection, trauma, and psychological stress. Hormones also regulate reproductive functions such as sexual maturation and sexual rhythms.

##### ***Morphogenesis***

Hormones play an important role in your growth and development, which is what morphogenesis is. The development of male and female sex characteristics under the influence of the sex hormones testosterone and estradiol, respectively, is one of the best examples of morphogenesis.

##### ***Integrative action***

The integrative action of hormones is a complex concept. Each hormone has a specific function and each individual hormone regulates a specific action. Many times two or more hormones must work together to achieve the desired outcome. Let's look at the regulation of glucose again to help understand this concept. We know plasma glucose levels are affected by the action of insulin. But, we also learned in Unit 2 of this CDC that the action of insulin alone is not adequate to maintain the balance of carbohydrate metabolism. Other hormones, such as glucagon, GH, thyroxine, and epinephrine, work in conjunction with insulin to maintain this balance.

#### **Hormone secretion and regulation**

The production of hormones is not continuous and uniform. The synthesis of hormones is a dynamic process based on circulating levels of each hormone and the body's need for them. As you learn about

hormones and the organs that produce and secrete them, you may want to refer to figure 3-1 to familiarize yourself with the organs of the endocrine system and their placement in the body.

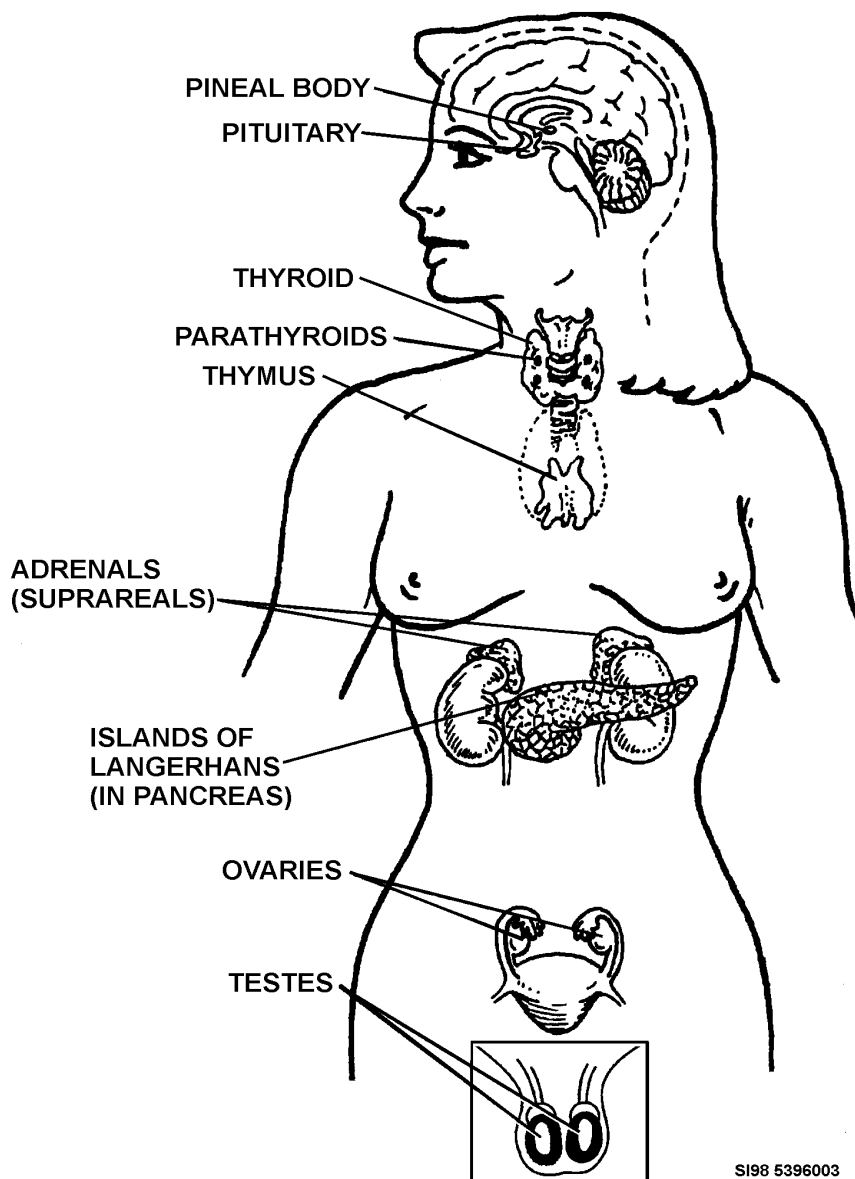


Figure 3-1. The endocrine system.

### ***Production and secretion of hormones***

There are several signals that can initiate the production and secretion of hormones. For example, the cerebral cortex or neural center can be stimulated by thoughts, emotions, stress, and circadian (periodic) rhythms that are daily, monthly, or seasonal. These stimuli, in addition to chemical transmitters at nerve junctions, stimulate the release of hypothalamic or adrenal medullary hormones. Changes in plasma concentration of specific ions or compounds can cause the secretion of hormones in order to maintain normal plasma concentrations. For example, parathyroid hormone is secreted when plasma concentrations of calcium are low, insulin is secreted when plasma glucose levels are high, and the posterior pituitary gland releases anti-diuretic hormone when blood osmolality is increased. Hormones are also released when food is detected in the gastrointestinal tract. For example, food in the stomach causes the secretion of gastrin by the stomach.

### ***Regulation of hormone secretions***

The secretion of hormones by the endocrine glands is carefully regulated by the interaction between the endocrine system and the nervous system. Neural centers can trigger or suppress the release of hormones from the endocrine glands. An example of this action is when you experience a sudden feeling of fear or anxiety. The brain transmits a message by the sympathetic nervous system to the adrenal medulla that results in the secretion of epinephrine. Anxiety and stress can also cause disturbances affecting the regularity of the menstrual cycle. The reverse is also true of the relationship between the endocrine and nervous systems. Hormone imbalances can affect moods and emotions. The biological response of a targeted cell or organ to a particular hormone is due to the binding of the hormone at specific receptor sites on the cell or organ. The term *target* is used to describe this receptor site for any hormone. For example, the thyroid gland is the target site for the thyroid-stimulating hormone (TSH), which is produced in the anterior pituitary gland. The receptor site provides the target cell with a means of recognizing the hormone. Once recognized, the hormone-receptor complex begins the chain of events that eventually produces the effect of the hormone. Hormone-receptor complexes have several characteristics.

1. They are highly specific. This specificity allows the target cell to recognize the active hormone from all the other substances the cell is exposed to.
2. The complexes have a saturation point. There are a set number of receptor sites on the target cells. This means the cells have a maximum hormone-binding capacity. The degree of hormonal activity of the target cell is directly proportional to the number of receptor sites that have been activated. Once all sites have been filled, the maximum hormonal response has been attained.
3. They have a high affinity. In order for hormone-receptor complexes to form in the presence of very low levels of circulating hormones, the affinity of the targets cells and hormones must be very high for one another.

At times, the receptor sites may not function properly. The receptor sites are made up of proteins and are not static parts of the cells. They can vary in both quality and quantity. The variations can be in response to abnormal stimuli or physiological changes in the patient, such as disease states. For example, obese patients often have chronically elevated levels of insulin. This chronic increase in the level of the circulating insulin will causes the cells' decreased sensitivity and response to it. As a general rule, hormones control their own receptor site activity and will only have affinities for their own receptor site. But some hormones have a very strong affinity for their own receptor sites and *some* affinity for the receptor sites of another hormone. This effect is known as *specificity spillover*. This crossover effect normally occurs between hormones with similar structures. The exact hormones involved and their circulating levels in the patient will determine if this occurrence will or will not cause any harm to the patient. Receptor site dysfunctions occur due to a variety of acquired or congenital endocrine diseases. These disorders can be due to a lack of receptor sites, an interference with receptor binding (such as the presence of an anti-receptor antibody), a structural abnormality in the receptor site, or a defect in the chemical processes that occur after the receptor binding takes place.

### **229. Thyroid hormones**

The thyroid gland is located in the front and sides of the neck, just below the larynx. The thyroid gland produces three different hormones. Two of them,  $T_3$  and  $T_4$ , are vital in maintaining normal growth and metabolism and the third, calcitonin, helps regulate plasma calcium levels. Our thyroid discussion is limited to the clinical significance of  $T_3$  and  $T_4$ .

#### **Physiology**

The element iodine is important in the synthesis of thyroid hormones. Iodine is normally obtained from foods and iodized salt. The iodine, in the form of iodides, is transported to the follicular cells of

the thyroid gland where the iodides are concentrated. Iodide is then oxidized in the thyroid gland to the more reactive form, iodine. It is then bound to the tyrosine molecules attached to a thyroidal protein called *thyroglobulin*.

### **Formation of $T_3$ and $T_4$**

Thyroglobulin is stored in the thyroid follicles until it's needed. Once a signal is received for thyroglobulin release, the thyroglobulin is acted upon by the enzyme tyrosine iodine, forming free monoiodotyrosine (MIT) and diiodotyrosine (DIT). Two DIT molecules will condense to form *tetraiodothyronine* ( $T_4$ ), commonly called *thyroxine*, and one MIT molecule plus one DIT molecule will condense to form *triiodothyronine* ( $T_3$ ).

### **Thyroid stimulating hormone**

Each step in the synthesis of the thyroid hormones is regulated by a pituitary hormone, the TSH, which stimulates the concentration of iodides, thyroglobulin synthesis, and the synthesis of  $T_3$  and  $T_4$  by follicular cells. TSH also controls the breakdown rate of thyroglobulin to release  $T_3$  and  $T_4$ . Prolonged TSH stimulation leads to increased vascularity of the thyroid gland, causing the gland to physically increase in size. The plasma concentration of thyroid hormones, in a healthy person, is kept constant by negative feedback control. This feedback control utilizes plasma free  $T_4$  levels to control the pituitary gland's release of TSH. Once released,  $T_3$  and  $T_4$  rapidly diffuse into the blood where they are bound to circulating plasma proteins.  $T_4$  is the hormone secreted in the larger amount.  $T_3$ , while secreted at less than half the amount of  $T_4$ , is 4 to 5 times more potent. Most of the total metabolic effect of the thyroid hormones is attributed to  $T_3$ .

### **Clinical significance**

Free  $T_3$  and  $T_4$  are the *only* forms of the thyroid hormones that attach to cell surfaces and affect cellular metabolism. While the exact mechanics are not fully understood, it is known they act as metabolic accelerators, increasing oxygen consumption, heat production, growth, and so forth as their concentration increases. Together,  $T_3$  and  $T_4$  act as catalysts, stimulating specific organs, tissues, and cells. They affect the growth, development, and sexual maturation of individuals. Their other actions include the stimulation of heart rate and heart contraction, the stimulation of protein synthesis and carbohydrate metabolism, and increases in the synthesis and degradation of cholesterol and triglycerides. Excessive thyroid activity increases metabolism, causing nervousness, heart palpitations, restlessness, and insomnia. This condition is called *hyperthyroidism*. An enlargement of the thyroid gland is called a *goiter*, and this condition usually accompanies hyperthyroidism. Decreased thyroid activity produces drowsiness, fatigue, and lethargy. A marked decrease in thyroid activity can cause weight gain, coarsened features, and thick, scaly skin (myxedema). A decrease in thyroid activity is called *hypothyroidism*.

### **Hypothyroidism**

Hypothyroidism is classified as either primary, secondary, or congenital.

#### *Primary hypothyroidism*

Primary hypothyroidism is caused by a defect in the thyroid gland itself; it has somehow become damaged and is not able to produce sufficient amounts of  $T_3$  and  $T_4$ . Because the thyroid gland is not producing enough  $T_3$  and  $T_4$ , increasing amounts of TSH are generally produced in an attempt to stimulate thyroid hormone production. A two-fold drop in free  $T_4$  levels will cause a 160-fold increase in TSH levels. TSH measurement becomes a powerful tool in the early diagnosis of thyroid failure. In mild or early hypothyroidism, the  $T_4$  levels may still be within the lower normal limits, but the TSH levels will be elevated.

#### *Secondary hypothyroidism*

Secondary hypothyroidism results from a defect of the anterior pituitary gland or the hypothalamus. This defect causes a deficiency of TSH, *thyrotropin releasing hormone* (TRH), or both. The TRH is

produced by the hypothalamus. TRH acts on the anterior pituitary gland, causing the synthesis and release of TSH. This class of hypothyroidism is more difficult to diagnose than primary hypothyroidism, because there are two possible sources of the problem. Patients with TSH deficiencies-only are rare; most patients with secondary hypothyroidism will have other pituitary hormone deficiencies. Patients with secondary hypothyroidism will have decreased serum thyroid hormone levels, but the TSH level can be low, within normal levels, or slightly elevated. When  $T_4$  and TSH levels are both low, a TRH test may be ordered. This test involves administering TRH to the patient and, at timed intervals following the administration, checking the patient's TSH levels. If the secondary hypothyroidism is caused by a defect in the pituitary gland, the TSH level will not be effected. If the secondary hypothyroidism is caused by a defect in the hypothalamus gland, the TSH levels will temporarily return to normal.

#### *Congenital hypothyroidism*

Congenital hypothyroidism may be due to the absence of the thyroid gland or a defect in the synthesis of the thyroid hormones. This condition occurs in approximately one out of every 5,000 live births. Early identification and treatment of this condition is critical. If left untreated, severe mental retardation will occur. TSH and  $T_4$  screening tests have been developed that utilize whole blood samples collected on special filter paper. This form of testing has greatly reduced the incidence of undetected newborn hypothyroidism, and minimized its devastating effects.

#### *Hyperthyroidism*

Hyperthyroidism is generally easier to diagnose by looking at the patient's clinical picture than hypothyroidism, and the symptoms of hyperthyroidism tend to be easier to recognize. In almost all cases of hyperthyroidism, the TSH levels will be decreased. Establishing a low TSH level and an elevated free  $T_4$  value is usually enough to diagnose a patient with hyperthyroidism. Hyperthyroidism is most commonly caused by a problem with the thyroid gland, rather than non-thyroidal conditions. The most common form of hyperthyroidism is Graves' disease, which is also called diffuse toxic goiter. Patients with Graves' disease have produced thyroid-stimulating immunoglobulins, which have bound to the TSH receptor sites on the thyroid. This binding causes the thyroid gland to produce and secrete excessive amounts of thyroid hormones.

#### **Laboratory procedures and limitations**

The procedures we will cover in this CDC to evaluate thyroid function are total  $T_3$ , total  $T_4$ , and TSH. These are not the only procedures available to evaluate thyroid function. Other tests you may see requested include free  $T_3$  and free  $T_4$ ,  $T_3$  uptake and free thyroxine index, reverse  $T_3$ , and thyroid hormone binding ratio. For a number of years, thyroid testing relied on the use of radioimmunoassay procedures. As such, these facilities required special licenses, special waste disposal methods, and specially trained personnel. In response to all of these concerns, procedures were developed using non-isotopic methods. These methods have been gaining in popularity and are now routinely seen in non-reference laboratories. The non-isotopic methods reviewed in the lesson are enzymatic in nature. Enzyme assay methods are easily automated, which also adds to their popularity.

#### ***$T_3$ testing***

The preferred specimen for  $T_3$  testing is serum. EDTA or heparinized plasma can also be used. When using plasma, remember that these samples tend to form fibrin clots after freezing and thawing, and that fibrin clots can interfere with automated testing systems.  $T_3$  is stable at room temperature and can be stored up to 1 week with little loss in activity. Samples are best stored at 4°C if they will not be tested within 24 hours of collection. If testing is not accomplished within 1 week, freeze the sample. Frozen specimens are stable for up to 1 month. Avoid grossly hemolyzed samples, as the hemolysis can lower results due to a dilution factor.



*Infant samples*

When collecting samples from infants for testing, either capillary tubes or filter paper may be used. Dried blood samples are stable and easily transported. Neonate testing is performed to screen for congenital hypothyroidism. The sample for this screening procedure should be collected 3 to 7 days after birth. Avoid touching the collection area of the filter paper or exposing it to extreme heat or light.

*Analysis*

Many non-isotopic immunoassay methods are available, using a wide variety of labels. These include using enzymes, such as peroxidase and alkaline phosphatase, to label  $T_3$  antigens or  $T_3$  antibodies. The enzyme activity is then determined using either sensitive, photometric fluorescent or chemiluminescent substrates. Most methods for  $T_3$  analysis have very little cross-reactivity with  $T_4$ , allowing direct measurement of  $T_3$  in the samples. Some  $T_3$  autoantibodies can cause interference with the assays and results may be higher or lower, depending on the particular assay method.

 *$T_4$  testing*

The specimen requirements for  $T_4$  testing are the same as those for  $T_3$ . The non-isotopic methods used for  $T_4$  analysis are also based on immunoassay principles. Enzymes, such as horseradish peroxidase, alkaline phosphatase, and  $\beta$ -D-galactosidase, are the most widely used. Enzyme immunoassays require the physical separation of free and bound  $T_4$ . Most enzyme methods use labeled  $T_4$  as an antigen, and photometric fluorescent or luminescent substrates are used to monitor enzyme activity. Some enzymatic methods use plastic beads to immobilize the  $T_4$  antibody. The bound and free hormones are then separated by decanting and washing the beads. Other methods use ferromagnetic particles, and the free and bound  $T_4$  are separated magnetically.

*TSH testing*

The specimen of choice for TSH testing is serum, although plasma can be used (remember the  $T_3$  frozen plasma warning). Specimens are stable for up to 5 days if refrigerated at 4°C and up to 1 month frozen. In addition to the safety and other considerations regarding radioimmunoassays, enzymatic immunometric assay methods are gaining in popularity because they are more sensitive and faster than the radioactive methods. As with  $T_3$  and  $T_4$  methods, TSH immunoassay methods use enzymes, such as peroxidase and alkaline phosphatase, to label the detection antibody, and then use a photometric fluorescent, or chemiluminescent substrate to measure enzymatic activity.

**230. Other medically important hormones**

There are many other medically important hormones, other than the ones the thyroid produces, that your laboratory will be requested to analyze. We will complete our lesson by providing an overview of information about many of the most commonly requested hormone assays. This lesson will mainly focus on the functions of the hormones and how their analysis assists the provider in making a proper diagnosis, not on the variety of methods used to test for them. These other medically important hormones are usually tested at reference laboratories or the larger medical centers with specialized equipment. For the majority of Air Force laboratory technicians, these procedures will be ship-outs.

**Pituitary hormones**

The pituitary gland is composed of two parts—the anterior lobe and the posterior lobe. As such, the pituitary hormones are classified based on the portion of the gland they are secreted from.

*Anterior pituitary hormones*

The anterior pituitary hormones include GH, prolactin, adrenocorticotropin, gonadotropins, and TSH. The functions of TSH were discussed in the previous lesson.

### *GH*

GH is the hormone produced in the largest quantity by the anterior pituitary. The GH affects many metabolic processes to include promoting skeletal growth and protein synthesis in young mammals. A deficiency of GH in young children can cause dwarfism or subnormal growth. The prolonged excess secretions of this hormone, prior to the closure of the long bones at puberty, can cause excessive growth or gigantism in children. Hyper-secretion of GH by a pituitary tumor in adults causes a condition called acromegaly, in which the person is large and has gross features.

### *Prolactin*

Prolactin, along with gonadal steroids, participates in breast growth during pregnancy. After childbirth, prolactin stimulates milk secretion. Elevated levels of prolactin can cause menstrual irregularities, infertility, and galactorrhea (an inappropriate production of breast milk). Prolactinomas (prolactin-secreting tumors) are the most common type of secretory pituitary tumors. Prolactin levels can be increased slightly by conditions such as exercise, fasting, stress, or a breast exam during a physical examination.

### *Adrenocorticotropin*

Adrenocorticotropin (ACTH) is a hormone that binds to the cells of the adrenal cortex and influences their activities in secreting cortisol and adrenal androgens. High levels of ACTH can be found in three conditions—primary adrenal cortical deficiency, Cushing's disease (hyperactivity of the adrenal cortex), and ectopic tumors that produce ACTH.

### *Gonadotropins*

The gonadotropins include follicle stimulating hormone (FSH) and luteinizing hormone (LH). These hormones are required for the proper maturation and function of the gonads in both men and women. These hormones induce the growth of the gonads and the secretion of gonadal hormones. They are required for the reproductive process—development of ova in females and sperm in males. The gonadotropins are present in the serum of both sexes at all ages. In ovulating females, there is a sharp rise in LH and FSH just before ovulation, and then a fall afterward. After menopause, the LH and FSH levels in women will rise and stay increased for the remainder of their lives. Gonadotropin analysis is useful when diagnosing menstrual and fertility disorders.

### ***Posterior pituitary hormones***

The posterior lobe of the pituitary gland is connected to the hypothalamus by a stalk through which a nerve tract and blood vessels pass. The hypothalamus produces both anti-diuretic hormone (ADH) and oxytocin. The posterior pituitary stores and secretes ADH and oxytocin. The primary function of ADH is to increase the reabsorption of water by the renal tubules of the kidney. This hormone also affects blood pressure. An ADH deficiency occurs in patients with diabetes insipidus. Oxytocin is a potent stimulant for smooth muscle contractions. Providers often use this hormone to induce labor by promoting uterine contractions. Oxytocin also stimulates the initial release of milk from the mammary glands.

### **Adrenocortical hormones**

These hormones are produced in the adrenal glands, located above each kidney. The adrenal glands produce steroid hormones from cholesterol through a series of enzymatic steps. The main hormone produced by the adrenal glands in both males and females is glucocorticoid cortisol. Cortisol alters various metabolic processes. It accelerates the enzymatic breakdown of muscle proteins and converts their amino acids into glucose in the liver. Cortisol stimulates the breakdown of fat in the adipose tissues for energy, and inhibits the uptake of glucose by muscle, acting as an insulin antagonist. Cortisol also reduces cellular reactions to inflammatory agents and lessens immune responses by inhibiting antibody formation. Overproduction of cortisol produces a variety of symptoms, which are collectively known as Cushing's syndrome. The cause of overproduction can be primary to the

adrenal glands, or secondary to an over-production of ACTH. An under secretion of cortisol can be caused by autoimmune disease or infection, or can be secondary to ACTH deficiency.

### **Adrenomedullary hormones**

The adrenal medulla is part of the adrenal gland, and its cells originate in a developing fetus from cells of the sympathetic nervous system. This close relationship is reflected later in life by the hormones the adrenal medulla produces.

### ***Norepinephrine and epinephrine***

One such hormone, norepinephrine (noradrenaline), is a neurotransmitter produced in the brain and nerve synapses. Epinephrine (adrenaline) is the main medullary hormone produced. This hormone produces the effects that occur when the sympathetic nervous system is stimulated by fear, anger, or aggression, such as increased heart rate and blood pressure. Both of these hormones assist with the generation of energy for the body. They can increase plasma glucose levels by promoting liver glycogenolysis and increase the amount of free fatty acids by promoting lipolysis in the adipose tissues. The hormones are so potent physiologically, that only small amounts are needed to obtain the desired effects, yet their action is transitory because they are inactivated rapidly.

### ***Catecholamines***

*Catecholamines* is a term used to collectively describe products of the adrenal medulla, to include epinephrine, norepinephrine, and dopamine. Analysis of urinary catecholamines or their metabolites is used to diagnose two distinct clinical conditions—adrenal tumors as a cause of high blood pressure and neuroblastoma, a usually fatal malignant condition in children in which cancer of the nervous system causes an excess production of norepinephrine.

### **PTH**

The parathyroid glands are usually four small glands found bilaterally at the base of the thyroid gland. The PTH regulates the homeostasis of both calcium and phosphorus by acting on the bones and the kidneys. PTH increases plasma calcium levels directly by pulling calcium out of the bone into the plasma and decreasing renal excretion of calcium by promoting tubular reabsorption of calcium. Its indirect control of calcium levels can be accomplished by enhancing the formation of the active form of vitamin D, which increases the intestinal absorption of calcium. The PTH's influence on plasma phosphorus levels involves the hormone's ability to promote the excretion of phosphorus from the kidneys. PTH levels are elevated in hyperparathyroidism, and can be normal or undetected in hypercalcemia and hypoparathyroidism.

### **Pancreatic hormones**

The pancreas produces and secretes insulin and glucagon—two of the hormones responsible for regulating plasma glucose levels.

### ***Insulin***

Insulin is the only hormone with the ability to lower plasma glucose concentrations. It does so by (1) stimulating the uptake of glucose by muscle and fat cells, (2) promoting glycogen storage in the liver, (3) inhibiting lipolysis and enhancing triglyceride formation in adipose tissue, (4) increasing protein formation from amino acids, and (5) increasing glucose utilization. A deficiency in, or total lack of, insulin can be the cause of diabetes mellitus. Plasma insulin analysis aids in the diagnosis of pancreatic tumors; it is of no value in the diagnosis of diabetes mellitus.

### ***Glucagon***

Glucagon is a hormone produced to counter the effects of insulin. The pancreas secretes glucagon in response to a fall in plasma glucose levels, whereas insulin is secreted in response to increased plasma glucose levels. Its action is to promote both the rapid breakdown of glycogen stored in the liver and the formation of glucose from amino acids, resulting in increased plasma glucose levels.

### Hormones of the gastrointestinal tract

The gastrointestinal tract secretes several hormones that regulate the entire digestive process. Generally food in the gut stimulates the release of the gastrointestinal hormones. The following table shows the sources and actions of some of the gastrointestinal hormones.

Hormone	Source	Action
Gastrin	Stomach	Increases secretion of gastric hydrochloric acid.
Secretin	Pancreas	Increases secretion of pancreatic fluid.
Cholecystokinin-pancreozymin	Gallbladder, pancreas	Gallbladder contraction; increases pancreatic secretions.
Vasointestinal peptide (VIP)	Gastrointestinal tract	Increases secretions; relaxes gut muscles.

### Female sex hormones

The female gonads, the ovaries, not only serve as the site of production and maturation of the ova, but also serve to produce and secrete the female sex hormones. The reproductive system of females is much more complicated than that of males because of the cyclical events that take place during the menstrual cycle and the changes that occur during pregnancy. The ovaries in non-pregnant women produce two different types of steroid hormones. During pregnancy, these hormones are still produced, but they are produced in different proportions. Also during pregnancy, the placenta produces hormones needed for the maintenance of the pregnancy.

#### *Estrogens*

The first group of female sex hormones you'll study are the estrogens. The estrogens originate in the ovarian follicles and, during pregnancy, the placenta. Estrogens play a role in the menstrual cycle and are essential for the development and maintenance of the reproductive organs and secondary sex characteristics.

#### *Progesterone*

The second group of female sex hormones includes progesterone and its metabolites. Progesterone is formed in the corpus luteum, a mass in the ovary formed when an ovarian follicle has matured and discharged its ovum. Progesterone stimulates the uterus to undergo changes in preparation for implantation of a fertilized ovum. If pregnancy occurs, progesterone produced by the corpus luteum, and later the placenta, suppresses the menstrual cycle for the duration of the pregnancy.

#### *Human chorionic gonadotropin*

As the placenta begins to develop during pregnancy, it begins to secrete human chorionic gonadotropin (hCG). This secretion begins shortly after the implantation of the fertilized egg. The concentration of hCG rises steadily from the first few days after conception until the 10<sup>th</sup> or 12<sup>th</sup> week of pregnancy. Detection of this hormone in urine or serum allows confirmation of pregnancy. Greatly increased levels of hCG can also be seen in women suffering from choriocarcinoma (a malignant condition resulting from retained products of conception) and in men suffering from testicular carcinoma.

### Male sex hormones

The male gonads are the testes, which serve a dual purpose. The testes secrete the male hormone testosterone and produce spermatozoa. In men, elevated testosterone levels can be due to testicular cancers or some abnormality of pituitary gonadotropin. Decreased levels of testosterone can be caused by a variety of conditions that directly affect the testes, by pituitary failure, or by chromosomal abnormalities. In women, testosterone levels can be increased as a result of adrenal or ovarian tumors.

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 228. Hormones

1. Briefly define the three categories of hormone functions.
2. On what is the synthesis of hormones based?
3. How is the cerebral cortex, or neural center, stimulated to initiate the production and secretion of hormones?
4. Give an example showing how a substance's change in plasma concentration can cause a hormonal response.
5. When you develop a feeling of sudden fear or anxiety, how may your neural centers react?
6. Define *target* in relationship to hormone responses.
7. What are the three characteristics of the hormone-receptor complexes?
8. Why do many obese patients have increased insulin levels?
9. What is *specificity spillover*?
10. What are the causes of receptor site dysfunction?

### 229. Thyroid hormones

1. How does iodine become bound to the thyroidal protein, thyroglobulin?
2. How are  $T_3$  and  $T_4$  formed?

3. What are the roles TSH plays in synthesis of thyroid hormones?
4. Which of the thyroid hormones is secreted in the largest amount and which is more potent?
5. Which forms of the thyroid hormones are capable of attaching to cell surfaces?
6. What are the characteristics of hyperthyroidism and hypothyroidism?
7. What causes primary hypothyroidism and how does it effect TSH levels?
8. What causes secondary hypothyroidism and how does it effect TSH levels?
9. What is the cause of congenital hypothyroidism?
10. What effect does hyperthyroidism have on TSH and  $T_4$  levels?
11. Why are non-isotopic immunologic methods for thyroid hormone testing so popular?
12. What special considerations must be taken when using plasma for thyroid hormone analysis?
13. Define specimen collection requirements for neonate  $T_3$  analysis.

14. What are the enzyme labels and substrates used to determine enzyme activity while performing  $T_3$  testing?
15.  $T_4$  testing methods require the separation of free and bound  $T_4$ . Briefly explain two methods of separation.
16. For TSH analysis, why are enzymatic immunometric methods gaining in popularity?

### 230. Other medically important hormones

1. Match the item in column B with the statement it relates to in column A by placing the letter of the item in column B beside the statement in column A. Each item in column B may be used once, more than once, or not at all.

<i>Column A</i>	<i>Column B</i>
____ (1) These hormones include growth hormone, prolactin, and TSH.	a. Testosterone.
____ (2) This hormone can cause dwarfism and giantism.	b. Human chorionic gonadotropin.
____ (3) This hormone stimulates initial release of milk from the mammary glands.	c. Estrogens.
____ (4) These hormones induce the growth of gonads and the secretion of gonadal hormones.	d. Parathyroid hormone.
____ (5) Anti-diuretic hormone and oxytocin fall into this class of hormones.	e. Adrenocortical hormones.
____ (6) These hormones are formed by glands located above each kidney.	f. Progesterone.
____ (7) Catecholamines is the term used to collectively describe these hormones.	g. Hormones of the gastrointestinal tract.
____ (8) This hormone regulates both calcium and phosphorus homeostasis.	h. Adrenomedullary hormones.
____ (9) These hormones include insulin and glucagon.	i. Pancreatic hormones.
____ (10) Food in the gut stimulates the releases of these hormones.	j. Posterior pituitary hormones.
____ (11) This hormone prepares the uterus for implantation of a fertilized ovum.	k. Oxytocin.
____ (12) This hormone is greatly increased in women with a choriocarcinoma.	l. Growth hormone.
____ (13) Found in increased amounts in men suffering from testicular cancer.	m. Gonadotropins.
____ (14) Male sex hormone and also seen in women with adrenal or ovarian tumors.	n. Anterior pituitary hormones.

## 3-4. Therapeutic Drug Monitoring

Up until now, you have explored some of the various chemical reactions that occur within the body and are regulated *by* the body. In this section, you are going to explore chemical reactions that occur

when our bodies are acted upon by influences from *outside* the body—namely, the use of therapeutic drugs.

### 231. Tracking drug concentrations

At times, part of going to the doctor means being prescribed a medication to help make you well, or at least help you survive the symptoms of an illness. While many medications are used in the short-term to treat a passing condition, there are others that must be used over extended periods of time. For these latter medications, many times the provider must often monitor the concentration of the drug in the patient in order to effectively adjust the dosage for optimum treatment.

#### Therapeutic drug monitoring

The tracking of a patient's drug concentration is known as *therapeutic drug monitoring* (TDM). The use of TDM has been increasing in the clinical laboratory because of the different medications becoming available on the market, and the fact that providers are getting away from standard or fixed dosing and are moving toward individualizing each patient's therapy. The purpose of any drug therapy is to supply the patient with the full benefit of the drug, as quickly as possible, while avoiding any drug-induced side effects. This fine line between the two is usually determined by monitoring the drug level (or the drug's metabolites) in the patient's serum. Many factors must be taken into account when monitoring a drug's level in the blood, to include:

- Absorption rate of the drug into the blood.
- Distribution of the drug within the body tissues.
- Metabolism of the drug by the body tissues.
- Excretion of the drug.

Add to these factors those of the individual patient, their height, weight, sex, and age. While there are numerous factors involved in how well the patient will respond to drug therapy, the key is still to ensure the well being of the patient. There is little benefit gained from curing one problem with a drug and giving a patient another problem due to the drug's toxic side effects. You'll explore toxicology in a later lesson.

#### Absorption

There are three common routes used to administer drugs: oral, intravenous, and rectal. For drugs administered orally or rectally, the absorption rate is dependent on the ability of the drug to dissolve and then diffuse across membrane barriers into the blood. The amount of a drug that eventually makes it into systemic circulation is referred to as the drug's *bioavailability*. Some drugs that are absorbed have a low bioavailability because they are carried by the portal blood system to the liver and are metabolized before they get out into systemic circulation. This phenomenon is known as the *first-pass effect*. One advantage of intravenous administration of drugs is this method allows an increased bioavailability. This is because the drug is being delivered directly to the bloodstream and circumnavigates any of the liver's first-pass effects.

#### Distribution

Distribution is the process of getting the drug from the blood into the cells of the targeted organ. Once the drug is in the blood, it can interact with many components in the blood. One such component is plasma protein, mainly albumin. Some of the drug will be bound to protein and some of the drug will remain in a free state. Only the free drug has the ability to cross over cellular membranes and interact with the receptor sites, and produces a biological response. Anything that alters the concentration of free drug in the plasma ultimately alters the ability of the drug to enter the tissues. Different factors can change the affinity of a drug and the way it binds with plasma proteins. Other chemicals that have a higher affinity for the protein can displace drugs that are already bound, or a disease state can



decrease the amount of plasma protein available. All these factors can alter the ultimate effect of the drug.

### **Metabolism**

Metabolism, or the biotransformation, of a drug primarily occurs in the liver. The role of the liver is to transform the drug into a water-soluble substance. As the water-solubility of a drug increases, so does its excretion by the kidneys. For this reason, some drugs are given in a precursor form and do not become active until the liver metabolizes them. Other drugs must be administered in large doses in order to counteract the first-pass effect. Metabolism of drugs can also be affected by various disease states. For example, renal disease can change the protein-binding capacity and the amount of drug eliminated by the body—both factors can increase free drug levels. Liver impairment can affect the amount of drug converted into the water-soluble state. Changes in the patient can also affect drug metabolism rates. As children enter into puberty, their body patterns change from childhood to adult metabolism. Pregnant women experience a whole new metabolism pattern. Metabolism patterns can slow down considerably in geriatric patients; any drugs they may have taken for years, with no ill effects, may need to be reduced in dosage, as they get older.

### **Excretion**

Accumulation of a drug in the blood will occur as the amount of drug intake exceeds the rate of drug elimination from the body. The amount of time it takes for the body to reduce the blood concentration level of a drug by one-half is referred to as the drug's *half-life*. It takes one half-life to eliminate 50% of a drug and five half-lives to essentially eliminate all of a drug from a person's system. Excretion of drugs occurs by biliary, intestinal, pulmonary, or renal routes. The primary route of drug excretion is through the renal route. As such, any alteration in the functions of the kidneys will have a major effect on the clearance and half-life of therapeutic drugs.

### **Sample collection**

In addition to performing TDM analysis, ensuring proper specimen collection prior to testing can be a responsibility of the laboratory. For many drugs, there is an optimal time for collection. You have probably already drawn samples for peak and trough levels on various patients. The *trough* level occurs just before a drug dose is administered. The *peak* level occurs when the drug reaches its maximum absorption or concentration. The *steady state* of the drug occurs when there is an average constant level between the peaks and troughs. Steady state is fully defined as the point of dosage when the amount entering the circulation (governed by dosage) equals the amount eliminated (governed by the elimination rate).

#### ***Administered orally***

When the drug is administered orally, peak sample collections will range from 1 to 5 hours after ingestion, depending on variables. Variables include the type of drug (some are digested and absorbed faster than others), the drug carrier (liquid, tablet, or capsule), and the state of the patient (fasting, impaired gastrointestinal tract, etc.).

#### ***Intravenous or intramuscular***

When receiving drugs through intravenous or intramuscular injection, the peak time frames for sample collection are much shorter. Following intravenous administration, blood is collected 30 minutes after the infusion has stopped. For intramuscular injection, collection is about 60 minutes after the injection. Understanding these basic concepts of the TDM process will help you realize the importance of accurate therapeutic drug monitoring. Let's next look at some commonly monitored drugs.

### **232. Medically important drugs**

The number of medications available for use today is incredible. With all the advances in technology and the growing knowledge of the human body, new drugs are added all the time. We, as laboratory

technicians, must continue to look for new ways to assist the providers in monitoring these new drugs, so the patients gain all the benefits possible from them.

### **Digoxin**

Digoxin is one of a group of drugs normally used to treat congestive heart failure and cardiac arrhythmias. Digoxin's specific action is restoring the force of cardiac contractions. The absorption rate of digoxin varies somewhat and depends on the manufacturer. Once in the plasma, about 25% of the digoxin is bound to blood proteins and the kidneys excrete the remainder of the drug. Because the kidneys excrete the bulk of digoxin, patients who have a decrease in renal functions will have an increase in digoxin levels. Digoxin accumulates in the tissues, and cardiac tissue levels are usually about 15 to 30 times that of plasma levels. After an oral dose of digoxin, plasma levels will peak after about 2 to 3 hours, but tissue levels peak after 6 to 10 hours. When determining peak concentrations, the measurement should not be plasma peak but rather the tissue peak. In order to determine this level, sufficient time should be allowed so the plasma and tissue levels equilibrate. The equilibration normally occurs about 8 hours after the last dose (this time frame is contrary to what you learned in the TDM lesson). Results drawn before the 8-hour mark are misleading and do not correlate well to tissue concentrations of digoxin. Enzyme immunoassay and fluorescence polarization immunoassay methods are used to determine digoxin levels.

### **Phenytoin**

Phenytoin's trade name is Dilantin. Phenytoin falls into a group of drugs known as anticonvulsants and is used primarily to treat epilepsy. The action of this drug is to reduce synaptic transmissions between nerves and to aid in the control of abnormal neuron excitability. Phenytoin is not very soluble in aqueous solutions. It is slow to be absorbed when given by intramuscular injection. Absorption of oral phenytoin is also slow and sometimes incomplete. Once the drug is absorbed, 90 to 95% of the drug binds with plasma proteins, leaving very little of the drug in the free state to cross cellular membranes. The liver metabolizes phenytoin; patients with conditions such as chronic liver disease or neonates with immature livers may have increased serum or plasma levels. Drugs or diseases that affect plasma protein levels will also affect the bioavailability of phenytoin. The time to collect a sample for phenytoin analysis depends on the reason for the testing. If the sample has been requested to check for toxicity, then a peak blood sample should be drawn. The peak sample time for phenytoin is 4 to 5 hours after the dose is administered. If the request for the sampling is to monitor the adequacy of therapy, then a trough blood sample should be collected. Phenytoin levels are measured using enzyme immunoassay and fluorescence polarization immunoassay methods.

### **Gentamicin**

Gentamicin is an antibiotic used to treat severe infections. This drug belongs to a group of antibiotics known as aminoglycosides. It destroys the bacterial organisms by binding to a subunit of its ribosomes and inhibiting protein synthesis in the organism. Gentamicin levels are closely monitored because of the adverse effects the drug can have on a patient's renal system and hearing. Gentamicin is poorly absorbed in the intestinal tract, so it is commonly delivered by intravenous or intramuscular methods. When administered directly into the blood, gentamicin rapidly diffuses into extracellular fluids, but it does not cross cell membranes or bond with plasma proteins. The half-life of gentamicin is very short—about 3 to 4 hours. Gentamicin is primarily excreted by the kidneys, but the drug is nephrotoxic. Any condition that affects the patient's kidneys will affect the clearance rate of gentamicin. Dose corrections must be made for patients with compromised renal functions. Peak plasma levels for gentamicin should be drawn about 30 minutes after the completion of the dose by intravenous methods. Both enzyme immunoassay and fluorescence polarization immunoassay methods are used to determine gentamicin levels.

### **Theophylline**

Theophylline is a bronchodilator used for the treatment of asthma. The action of the drug, although not fully understood, seems to relax the bronchial smooth muscles, relieving or preventing asthmatic

attacks. Theophylline is normally given by oral or intravenous routes and is readily absorbed. If the drug is taken orally without food, the blood concentration peaks after about 2 hours. If taken with food or in a slow-release form, the blood peak level will be reached after about 3 to 5 hours. About 50 percent of the drug is bound to proteins. The elimination half-life of the drug is 3 to 4 hours in smokers and children, and about 9 hours in non-smoking adults. The half-life in neonates and adults with congestive heart failure can be prolonged to as much as 20 to 30 hours, depending on the degree of liver immaturity or loss of liver function.

When theophylline reaches toxic levels, the effects are very serious. Cardiac arrhythmias and seizures are usually associated with serum concentrations over 30  $\mu\text{g/ml}$ . Once seizure activity begins, the prognosis for the patient is poor, with morbidity being reported in nearly all patients and mortality rates as high as 50 percent. Theophylline levels are normally measured using enzyme immunoassay and fluorescence polarization immunoassay methods. In the past few years, new bronchodilating drugs, such as Albuteral, have been introduced. Some of these can be used without monitoring blood concentrations because of their relatively mild toxicity, and they are effective over a broad concentration range. As the usage of these drugs increases, the number of specimens submitted for theophylline levels should decrease.

### **Antipsychotic drugs**

Drugs used in psychiatric care that are commonly monitored include lithium, antidepressants, and neuroleptics. Lithium is often used to treat mood disorders, such as mania and manic-depression. Antidepressants are used to treat endogenous depression—a condition where no organic or societal cause of the behavior change seems to exist. Neuroleptic drugs are used to treat patients who are psychotic—those who are detached from reality and have impaired perceptions. Lithium acts by enhancing neurotransmitters, thereby reducing their concentration in the neuronal junction. This has a sedating effect on the central nervous system. Absorption of lithium in the intestinal tract is complete and reaches its peak 2 to 4 hours after a normal dose. Lithium does not bind with protein. Lithium is primarily excreted by the kidneys, and its excretion rate parallels that of sodium. In conditions where the patient is vulnerable to dehydration, such as fever or vomiting, the potential for lithium intoxication is increased. Reduced renal function causes prolonged clearance times. Flame emission photometry, atomic absorption spectrometry, or ion-selective electrodes determine lithium levels.

---

## **Self-Test Questions**

**After you complete these questions, you may check your answers at the end of the unit.**

### **231. Tracking drug concentrations**

1. Define therapeutic drug monitoring (TDM).
2. What are some of the factors that must be taken into account when monitoring a drug's level in the blood?
3. Define bioavailability and first-pass effect.
4. What is drug distribution?

5. Drug metabolism, or biotransformation, occurs in what organ and what happens to the drug during this process?
6. What happens to a geriatric patient's ability to metabolize drugs?
7. Define drug half-life.
8. Define peak and trough.
9. Define steady state.
10. When are peak samples collected for oral, intramuscular, and intravenous drugs?

**232. Medically important drugs**

1. Match the item in column B with the statement it relates to in column A by placing the letter of the item in column B beside the statement in column A. Each item in column B may be used more than once.

<i>Column A</i>	<i>Column B</i>
____ (1) This drug restores the force of cardiac contractions.	a. Gentamicin.
____ (2) This drug accumulates in cardiac tissues at levels 15 to 30 times that of plasma.	b. Theophylline.
____ (3) Peak concentrations for this drug should be drawn after equilibration between tissue and plasma levels.	c. Phenytoin.
____ (4) This anticonvulsant is used to treat epilepsy.	d. Lithium.
____ (5) For this drug, peak samples are drawn to check for toxicity and trough samples are collected to check for therapy adequacy.	e. Digoxin.
____ (6) This drug is an antibiotic used to treat severe infections.	
____ (7) This drug is closely monitored because it adversely effects the patient's kidneys and hearing.	
____ (8) Its primary use is for the treatment of asthma.	
____ (9) If allowed to reach toxic levels, this drug can cause death rates as high as 50%.	
____ (10) This drug acts on neurotransmitters and has a sedating effect on the central nervous system.	
____ (11) Ion-selective electrode methods are used to measure levels of this drug.	

**3-5. Toxicology**

Toxicology is defined as the "The study of poisons, their actions, their detection, and the treatment of the conditions produced by them." As you already know, even drugs designed to be helpful can, in

fact, become poisonous if not taken correctly. This is true when a patient, who feels great taking one pill, may justify taking two because it will make them feel even better, or the occurrences of accidental poisoning, such as little children taking medicines thinking that it is candy. In this lesson, we will view toxicology by learning about alcohol, acetaminophen and salicylates, carbon monoxide and heavy metal poisoning, and drug abuse. Your job, as a laboratory technician, is to provide the provider with the “*their detection*” part of our definition so they can successfully treat the patient.

### **233. Alcohol**

One of the most commonly abused chemical substances is ethanol, or alcohol. Of all the various toxicology procedures performed in the laboratory, ethanol analysis is at the top of the list. Individuals may also consume other forms of alcohol, such as methanol or isopropanol. Regardless if this consumption was accidental or intentional, the patients may present themselves as if they had consumed ethanol. Testing for other forms of alcohol should be available because the route of treatment for each substance vastly differs.

#### **Types of alcohol**

The three types of alcohol are ethanol, methanol, and isopropanol.

##### ***Ethanol***

The principal action of ethanol is depression of the central nervous system. The affects on the patient’s central nervous system will vary according to the concentration of alcohol in their body and the tolerance limits of the individual. Someone that consumes large amounts of alcohol regularly will have a greater tolerance than a non-drinker. Generally, at lower concentrations (< 50 mg/dl), the patient will have a feeling of euphoria and decreased inhibitions. At slightly higher levels (100–300 mg/dl), the patient will have increased disorientation and lack of coordination. At extremely high levels (> 400 mg/dl), the patient could slip into a coma or even die. Ethanol is principally metabolized by the liver. First, it is metabolized by the action of alcohol dehydrogenase into acetaldehyde; it is further oxidized by aldehyde dehydrogenase to form acetic acid. Although the rate of elimination varies from person to person, it averages about 15 to 18 mg/dl per hour (about one drink containing 1 ounce of ethanol per hour). The elimination rate is also influenced by the drinking practices of the patient. Alcoholics eliminate alcohol at a rate of about 30 mg/dl per hour.

##### ***Methanol***

Methanol is found in products such as cleaning solutions, antifreeze, and canned fuels. It is sometimes consumed intentionally by alcoholics as an ethanol substitute or accidentally by children. The effects of methanol on the central nervous system are less severe than that of ethanol. Methanol is metabolized in the liver at a rate about one-tenth that of ethanol. When metabolized, its intermediate product is formaldehyde. Formaldehyde is rapidly oxidized by aldehyde dehydrogenase to form formic acid. Formic acid build-up can cause a serious form of metabolic acidosis. It can also affect the optic nerves, possibly causing blindness, or even cause death.

##### ***Isopropanol***

Isopropanol, or rubbing alcohol, is not quite as toxic as methanol, but it has twice the central nervous system depressant action as ethanol. Isopropanol is also metabolized in the liver, to a final end product of acetone. The acetone is primarily eliminated from the body through the lungs and urine. Severe isopropanol intoxication, like ethanol, can result in coma or death.

#### **Medical and legal considerations**

Ethanol analysis can initially be requested for either medical or legal reasons. At times, what was at first a medical concern may become a legal matter, or a legally directed test may turn into a medical emergency. We, as laboratory technicians, must be familiar with local directives and laboratory operating instructions that cover the specimen collection, handling, and processing of ethanol samples. It is not uncommon for a commander to refer an individual to the medical treatment facility

for a sobriety determination. The term *sobriety* refers to a clinical opinion of the person's state of intoxication. While a legal blood alcohol test is part of the sobriety exam, only the provider is professionally qualified to determine the state of intoxication. Laboratory technicians may sometimes find themselves testifying about what they witnessed (the defendant's manner, speech, and so on), but more importantly, they will be defending the manner in which the alcohol analysis was accomplished. Any deviations you make away from the operating instructions could put your results and you in jeopardy. To avoid any chance of this happening, *never* deviate from the operating instructions or local policies. Plus, never hesitate to call a supervisor if you don't feel comfortable with the procedure, or if someone is rushing you. Medical alcohol testing can be requested for a variety of reasons. For example, when a patient is found unconscious, a medical alcohol level can at least rule-in or rule-out ethanol as the cause of the patient's condition, allowing the provider to make a proper diagnosis. There are other conditions that can cause unconsciousness, but present themselves similar to alcohol intoxication.

### **Sample collection**

Some special precautions should be taken when collecting blood from an intoxicated individual. They may refuse to let you collect their blood or become hostile. Never attempt to collect blood from someone who has not consented to the procedure *unless told to do so by a competent authority*. In most cases, the competent authority will be the base commander. They will direct the collection of the sample after being advised by the Staff Judge Advocate. Blood alcohol testing can be performed on serum, plasma, or whole blood, depending on the test method and instrument used. Verify the specimen requirements unique to your facility. When you collect a sample for alcohol testing, remember that you do *not* use alcohol as a skin-cleansing agent. In most cases, you'll use an aqueous iodine solution or Merthiolate before performing the venipuncture.

### **Chain of custody**

In most legal cases, the key element is the *chain of custody*. The chain of custody is the tracking of who had access to the sample from the time it was collected to the time the result was released to the proper authority. Most bases have a locally approved form used to document the chain of custody. Be sure this form is completed according to local policy and the specimen has not been tampered with *before* you sign for the specimen. For legal blood alcohols, the specimen must be labeled and secured in such a way that it cannot be tampered with or confused with another specimen. Laboratories may have a special lock-box in a refrigerator just for this purpose. Remember, access to this box must be controlled. Do not give out the combination or have someone else put the sample away for you.

### **Laboratory procedures and limitations**

Alcohol analysis can be accomplished by gas chromatography or by enzymatic methods. Enzymatic methods are used in most laboratories because they are simple and quickly performed, compared to gas chromatography methods. The principle of enzymatic alcohol analysis is as follows: (1) ethanol in the presence of nicotinamide-adenine dinucleotide ( $\text{NAD}^+$ ) is oxidized to acetaldehyde by alcohol dehydrogenase; (2) as this reaction occurs, the  $\text{NAD}^+$  is reduced to NADH plus hydrogen ( $\text{H}^+$ ); and (3) the NADH that is formed is read at 340 nm, and is proportional to the amount of ethanol in the sample. Enzymatic kits using the above reactions are *reasonably* specific for ethanol—meaning a small amount of cross reactivity with other alcohols may occur during testing. This fact must be clearly communicated to health care providers who are treating acutely intoxicated patients. Patients who have ingested methanol or isopropanol will have very low results, and this might be misinterpreted as a low alcohol result. If it is suspected that a patient has swallowed an alcohol other than ethanol, the specimen must be tested by gas chromatography to identify what they have consumed. Because of the volatile nature of all alcohols, specimens for ethanol analysis must remain tightly sealed prior to testing to avoid evaporative loss of the ethanol. In other words, exposing the sample to room air will decrease the ethanol level in the sample, causing inaccurate results.

### 234. Acetaminophen and salicylate

Acetaminophen (Tylenol) and salicylate (aspirin) are common over-the-counter medications. In spite of the use of child safety caps, these drugs are still ingested, in large quantities, by unsupervised children. Occasionally, adults take them during suicide attempts. An overdose of either one of these medications may result in fatal consequences.

#### Acetaminophen

This medication is present in many over-the-counter medications, in addition to being sold as a singular drug. It is a common analgesic drug used to relieve pain or reduce fever. When taken in massive amounts, severe liver damage or even death can result. The initial clinical findings of an acetaminophen overdose are relatively mild and non-specific, and are not predictive of the degree of possible liver damage the patient will have developed. The full extent of liver damage will only become evident 3 to 5 days following ingestion. For this reason, fast and accurate serum acetaminophen analysis is extremely important for determining the level of overdose and for making proper treatment decisions. Blood samples for acetaminophen determinations should not be drawn any earlier than 4 hours after ingestion. Drawing a sample at this time ensures absorption of the drug is complete. If the time of ingestion is unknown, samples should be taken at 2 to 3 hour intervals to see if levels are climbing or declining. Damage to the liver begins when the acetaminophen half-life is greater than 4 hours and hepatic coma is probable when the half-life is greater than 12 hours.

The antidote for an acetaminophen overdose is N-acetylcysteine. This antidote helps detoxify the acetaminophen metabolites. The sooner the antidote is administered, the more efficient it will be. Maximum results occur when it is administered within 8 hours of ingestion; detoxification results decline sharply if given between 18 and 24 hours after ingestion. If testing is not available locally for acetaminophen (within 8 hours), the antidote treatment should be started immediately. The antidote treatments can always be stopped if results show they are not required. Serum is the preferred sample for acetaminophen analysis. Testing for acetaminophen can be accomplished using photometric methods, although these methods are subject to a number of interfering substances. Immunoassay methods are most commonly used—they are rapid, easily performed, and very accurate. Chromatography methods can also be used, and because of their accuracy, they are normally reserved as the reference method.

#### Salicylate

Salicylate, like acetaminophen, is an analgesic found in many over-the-counter medications, particularly in cold remedies and pain relievers. Salicylate is commonly known as aspirin (acetylsalicylic acid). Once aspirin is ingested, it is hydrolyzed into its active form—salicylate. The hydrolysis starts in the gastrointestinal tract and is completed in the tissues. To determine toxic drug levels when an aspirin overdose is suspected, you test for its metabolite—salicylate—not acetylsalicylic acid itself. Under normal therapeutic conditions, aspirin will interfere with platelet aggregation and cause prolong bleeding times. Aspirin is often taken in low doses for its platelet inhibition effect by patients who are at risk for thromboembolic disease. There has been some association between aspirin ingestion in children or adolescents and Reye's syndrome, so aspirin should not be given to children or adolescents. Once ingested, the half-life of aspirin is about 15 minutes, at which time it is converted to salicylate. At high therapeutic or toxic levels, the half-life of salicylate is prolonged 15 to 30 hours versus 2 to 3 hours for low doses.

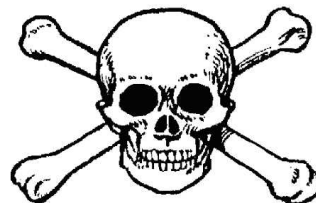
Overdoses of salicylate initially cause hyperventilation because the respiratory center of the brain is stimulated. Prolonged hyperventilation can first lead to respiratory alkalosis because of low  $\text{CO}_2$  concentration. After 2 to 5 hours of ingestion, the clinical picture changes to that of metabolic acidosis because of organic acid accumulation. In severe cases, coma and seizures can result. The measurement of serum salicylate is important for assessing the severity of the intoxication. Because some brands of aspirin are coated to give them a slow-release characteristic, consideration must be given to when peak absorption has taken place. If sample collection begins before 6 hours post-

ingestion, sample collection should continue every 2 to 3 hours to ensure absorption is complete. Testing beyond this point is done to assess the effectiveness of therapy.

Treatment for salicylate intoxication aims to prevent further absorption of the drug. Syrup of ipecac can be given to induce vomiting (expelling the salicylate), or activated charcoal can be given to bind with the salicylate and prevent further absorption. Sodium bicarbonate is given to the patient to prevent metabolic acidosis. Serum is the specimen of choice for salicylate testing, although plasma collected in EDTA, heparin, or oxalate is acceptable. Salicylate levels are determined by photometric methods, fluorescence polarization immunoassay, and chromatographic methods. Gas and liquid chromatography methods, although the most specific, are not practical for emergency use.

### 235. Other toxic substances

As you have already learned from previous lessons, even commonly used substances, such as Tylenol and aspirin, can be harmful in the wrong hands or taken in large amounts. Their healing effects can turn into that of a *poison*. True poisons are defined as any substance, which when relatively small amounts are ingested, inhaled, absorbed, applied to, injected into, or developed within the body, has a chemical action that may cause damage of structure, or disturbance of function-producing symptoms, illness, or death. There are an infinite amount of poisons around all of us every day. The effects of poisons are local or remote. A *local effect* means there is a direct action to the point where the poison is applied. An example of this action would be dropping a corrosive on your skin. A *remote effect* is caused when the action of the poison takes place at a site away from the site of application. Overdosing on Tylenol and experiencing your liver shutting down is an example of this effect.



#### Poisoning

Poisoning is either acute or chronic.

##### *Acute poisoning*

Acute poisoning is brought on by a single dose of the poison. A child accidentally eating the contents of an entire bottle of Tylenol is an example of acute poisoning.

##### *Chronic poisoning*

*Chronic poisoning* is brought on by repeated exposure over a period of time. Industrial workers, such as fuel handlers and painters, can become exposed to chemicals whose effects build up over a long period of time. Individuals such as these would be at risk for chronic poisoning. At times, the laboratory will be involved in assisting the provider in determining if patients have poisoned themselves. Many times, this will only involve the collecting and shipping of samples to a reference laboratory for toxicology studies, and assisting the provider with the continuous monitoring of the patient. In this lesson, we will only cover information on a couple of commonly encountered poisonous substances. More information can be gained from any good toxicology text.

#### Carbon monoxide

Carbon monoxide is a colorless, odorless, tasteless gas that is a by-product of burning fossil fuels. Common sources of carbon monoxide are cigarettes, gasoline engines, and improperly ventilated heating systems. When inhaled, carbon monoxide combines tightly with hemoglobin to form carboxyhemoglobin. The binding affinity of hemoglobin for carbon monoxide is about 250 times greater than that of oxygen. Not only does carbon monoxide decrease the oxygen content of the blood, but it also decreases the availability of oxygen to the tissues.



***Hypoxia***

The primary effect of carbon monoxide poisoning is hypoxia. Organs with a high demand for oxygen, such as the brain and heart, are the most sensitive to hypoxia. The longer the length of exposure, the more severe the effects. Initial exposure to carbon monoxide can result in headache, irritability, nausea, and vomiting. As exposure is continued, vertigo, lethargy, and eventually collapse and coma can follow. If the person is not removed from the carbon monoxide-contaminated environment and treated, death will result.

***Treatment***

Treatment for carbon monoxide poisoning involves removing the individual from the contaminated area and administering oxygen. The half-life of carboxyhemoglobin is 5 to 6 hours if the patient is breathing room air, but it is only 1½ hours if breathing pure oxygen. Carbon monoxide levels can be measured using gas chromatography or spectrophotometry methods.

***Metals***

Many metals have been recognized as toxins for years. Arsenic poisoning was the preferred method of getting rid of royalty during the Middle Ages and the Renaissance Age. Mercury, used in the making of felt from beaver pelts, was commonly the cause of behavioral changes in hat makers in the 18<sup>th</sup> century (that is where the phrase “mad as a hatter” comes from). Metal toxicity can also be called heavy metal poisoning. Even though we are more aware of the effects of heavy metal poisoning today, it still occurs. Still a common heavy metal exposure is that of infants and young children eating lead-based paint chips. This occurs primarily in older homes built before laws made the use of lead-based paints illegal. Generally, exposure to heavy metals is unintentional and usually traced back to an unknown exposure in the workplace or home. An example is household insecticides that contain heavy metals. Improper use of these products can lead to significant exposure (read the label!). Heavy metal testing is normally accomplished through the use of atomic absorption spectrophotometry or mass spectrometry methods.

***Drugs of abuse***

Illegal drug use and abuse is unfortunately widespread in our society despite public awareness and the government’s efforts to curb their use. More and more, testing for the illegal use of drugs has become a part of the workplace. In the laboratory, the need for drug screening is also on the increase. When unconscious patients are brought into the hospital and the reason for their condition is unknown, drug screening can immediately assist the provider with the proper diagnosis.

***Medical testing***

Testing for medical purposes and testing to identify the illegal use of drugs are quite similar. It usually involves the collection and processing of a single urine specimen. Urine drug testing cannot determine the degree of impairment, amount of drug taken, or exact time of use; it can only determine what type of drug was recently used. For the provider, this information is enough to provide treatment for the patient. Although there are a variety of rapid screens for drug abuse appearing on the market, definitive testing is accomplished using chromatography or mass spectrophotometry methods.

***Demand reduction program***

Drug testing in the Air Force, for non-medical reasons, is of great importance. The Air Force maintains a drug urinalysis-testing program—the Demand Reduction Program. It is used as a screening tool and a deterrent against illegal drug use. Many jobs in the Air Force have the potential of causing a great number of casualties if not performed correctly. You would not want to be flying on an aircraft that had an engine repaired by someone impaired, nor would you want to receive a transfusion in a hospital where the laboratory technician was high as a kite.

The Demand Reduction Program is designed to screen out people before problems of this nature can occur. The program also has a deterrent effect in that if a person never knows when they might be

tested, they are less likely to try illegal drugs. At many Air Force bases, the hospital laboratory plays a role in the collection and shipment of specimens in support of the Demand Reduction Program. The base commander or equivalent and/or delegate usually dictate the amount of involvement the laboratory has in the program. The legal requirement of the drug urine testing program is quite similar to those concerning legal blood alcohol. Chain of custody and adherence to exact operating instructions is extremely important to the success and application (prosecution) of the program. Of particular note for technicians such as yourself – either on the cusp of, or already fully engaged in laboratory middle management, – is that in recent years, the Demand Reduction program has migrated fully under the umbrella of Mental Health Service specialty after being a laboratory function in the years preceding this change.

### ***Drug abuse***

There are a variety of commonly abused drugs. Some are legally obtained and some are not. Taking a drug prescribed for someone else constitutes drug abuse, as well as taking a drug that was otherwise illegally obtained. The following drugs are representative of those normally encountered in drug screens.

### ***Amphetamines and methamphetamines***

Amphetamines and methamphetamines are central nervous system stimulants often used to treat narcolepsy, obesity, and attention-deficit disorder. These drugs however, produce an initial feeling of euphoria, so they have a high potential for abuse. In addition to euphoria, amphetamines and methamphetamines produce a feeling of well-being and self-esteem with heightened mental and physical capacity. Overdoses of amphetamines and methamphetamines can cause dizziness, tremors, irritability, and cardiac arrhythmias. If the overdose is severe, convulsions, coma, and cerebral hemorrhage can occur.

### ***Barbiturates***

Barbiturates suppress the central nervous system and thus have a sedating and hypnotic effect on the person. Barbiturates are often prescribed as an anticonvulsant drug and to treat intracranial pressure due to head trauma or injury. The primary actions of barbiturates are depression of the nervous and cardiovascular systems, and decreases of the respiratory system. Overdoses of barbiturates can result in coma and cardiorespiratory arrest.

### ***Cannabinoids***

Cannabinoids are compounds found in the plant species *Cannabis Sativa*. These drugs derive their effect from the chemical tetrahydrocannabinol (THC). THC is most often obtained by smoking marijuana—a mixture of crushed leaves, flowers, and stems of the cannabis plant. Hashish, a dried resin secretion of the plant, is also smoked. The primary effect of this drug is euphoria and a sense of relaxation and well-being. This effect begins minutes after smoking marijuana and can persist for as long as 2 to 4 hours after inhalation. Loss of short-term memory and impaired intellectual performance (recall and problem-solving) are associated with continued use of this drug. While under the influence of the drug, psychomotor skills are impaired, and the operation of equipment is negatively affected. Release of THC from tissues is slow, and urine samples are positive for up to 5 days following the last exposure to the drug. Heavy marijuana smokers can test positive for up to 2 months after their last encounter with the drug.

### ***Cocaine***

Cocaine is an alkaloid present in the leaves of the coca plant—not to be confused with the cocoa plant used to make chocolate! Cocaine is a potent stimulant of the central nervous system that results in an increased state of alertness and euphoria. When illegally used, cocaine is normally administered by snorting—that is, nasal inhalation. Cocaine is more volatile when converted from the salt form to free-base form so it can be inhaled by smoking. This free-base form is known as *crack*. The effects of cocaine include increased blood pressure and heart rate, as well as increased body temperature.

Overdoses of cocaine produce seizures, arrhythmias, and MIs. Sudden death due to cardiotoxicity can occur following cocaine use. In occasional users, cocaine can be detected in the urine 8 to 12 hours after use. In chronic users, it can be detected up to 4 or 5 days.

### ***Opiates***

Opiates are naturally occurring analgesic alkaloids derived from opium—a dried, milky juice from the unripe seeds of the poppy plant. Some opiates are semisynthetic, also being derived from opium. Opiates are clinically used for their analgesic properties, and they can cause sedation, euphoria, and respiratory depression. Nausea, vomiting, coma, pulmonary edema, and death can result from overdose. It is because of their analgesic and euphoric properties that opiates have a high abuse rate. The opiates class includes drugs such as morphine, heroin, and codeine. Heroin is the preferred form of the drug by opiate abusers because of the rapid onset of its effects. It is generally administered by intravenous injection. Heroin itself is not active, but it is quickly metabolized into morphine by the body. Codeine is not quite as potent as the other opiate drugs and can be found in some cough medicines. The consumption of poppy seeds in baked goods can lead to urinary excretion of morphine and codeine. However, these levels are normally much lower than those that would be detected in someone engaging in illegal drug usage. Threshold levels for determination of a positive level in drug testing programs are normally set accordingly.

### ***Phencyclidine***

Phencyclidine (PCP) was originally used as a surgical anesthetic, but was withdrawn from use because of its adverse side effects. The euphoric and hallucinogenic properties of this drug make it popular for illegal use. The effects of PCP vary and are unpredictable. It can cause delusions of grandeur, anxiety, agitation, hostility, paranoia, stupor, and coma. PCP is commonly administered by smoking drug-laced tobacco, marijuana, or parsley leaves.

---

## **Self-Test Questions**

**After you complete these questions, you may check your answers at the end of the unit.**

### **233. Alcohol**

1. How do non-drinkers and heavy drinkers differ regarding their tolerance and metabolism of ethanol?
2. In what products do you find methanol, and what problems can it cause if ingested?
3. Compare isopropanol to methanol and ethanol.
4. What is the meaning of the term *sobriety*?
5. If you are required to testify in court, what will you, as the laboratory technician, be defending?

6. You are preparing to collect a legal blood alcohol specimen, and the patient is combative and refuses to consent. Under what conditions can you collect their blood despite their objections?
7. Briefly explain any special conditions used to clean the phlebotomy site before collection of a sample for alcohol testing.
8. Describe one way to secure a blood alcohol sample and explain what is meant by *chain of custody*.
9. What happens when you expose an ethanol sample to room air?
10. An emergency room provider has ordered a blood alcohol test on a child who has swallowed a bottle of rubbing alcohol, and your lab only performs the enzymatic test for ethanol. What type of results can you expect and what should you recommend to the provider?

**234. Acetaminophen and salicylate**

1. What are two common, over-the-counter drugs frequently ingested accidentally by children or taken by adults in suicide attempts?
2. What could be the results of taking massive amounts of acetaminophen and what are the initial clinical findings of a person who has?
3. When is the full extent of hepatic damage evident following an acetaminophen overdose?
4. When are blood samples for a suspected acetaminophen overdose collected if the time of ingestion is known? What if the time of ingestion is unknown?
5. What is the effectiveness of the antidote (N-acetylcysteine) for acetaminophen overdose?
6. Why are immunoassay methods normally used for acetaminophen analysis rather than chromatography methods?

7. What analyte is tested for in an aspirin overdose?
8. Briefly discuss the half-life of aspirin and its metabolite salicylate.
9. What happens to the patient during an aspirin overdose?
10. How do slow-release forms of aspirin affect salicylate analysis, and what is done to counteract this effect?
11. What purpose does the treatment for salicylate intoxication serve and what forms of treatment are used?

**235. Other toxic substances**

1. What is the difference between local and remote effects of poisons?
2. Define acute poisoning and chronic poisoning.
3. How is carbon monoxide produced and describe its interaction with hemoglobin?
4. What are the primary effects of carbon monoxide exposure and which organs are most sensitive to the effects?
5. Describe the treatment for carbon monoxide poisoning and its half-life under room air and pure oxygen conditions.
6. How could people be exposed to heavy metals in their homes?
7. What methods are used to perform heavy metal analysis?

8. Briefly explain why the Air Force maintains a drug urinalysis testing program and what involvement the laboratory has with the program.
9. Why do amphetamines have a high potential for abuse?
10. What effect do barbiturates have on the patient and for what are they prescribed?
11. How is tetrahydrocannabinol (THC) most often administered?
12. What is the difference between the coca plant and the cocoa plant?
13. What are some effects of cocaine use and what can occur in an overdose?
14. What opiate is preferred by illegal drug users and how is it administered?
15. How can someone who has not taken opiates still have morphine or codeine in their urine?
16. What was the original use of phencyclidine and why is it popular for illegal use?

### **3-6. Tumor Markers**

Cancer still remains a major health problem in the United States and, unfortunately, seems to touch all of us at least once in our lives. It was estimated in 2003 that approximately 1.3 million people in the US would be diagnosed with cancer, and that more than 500,000 people would die of cancer in the US in the same year. It is our job as laboratory technicians to carefully evaluate the test protocol and reagents used to perform the screening procedures and confirmatory tests used to detect cancer.

#### **236. Tumor markers**

This unit will give you a brief overview of the cancerous disease process, define what tumor markers are, describe what qualities makes a “good” marker, and give you some examples of the most commonly used markers available to you today. In order for you to learn how to identify, select, and use tumor markers for the maximum diagnosis of cancer and the management of your patients, you need to first be familiar with the fundamentals of the neoplasm processes.

**Clonal Theory**

The clonal theory of carcinogenesis (the production of a cancer or carcinoma) states that cancers derive from an original, transformed cell. This transformed cell, or clone, is a normal cell whose genetic material has been altered. This alteration causes this cell and its progeny (offspring) to lose their normal regulatory functions that direct cell replication and cell death. Then, by an evolutionary, multi-step process, cells resulting from the initially modified cell begin to replicate, uncontrolled by their normal inhibitory systems and often invading other parts of the body. This last phase, called metastases, is usually the cause of the death of the patient.

**Insights to development**

Various clues have led scientists to believe that one or more of the genes within the cell must be altered before a malignant cell is formed. These clues include:

1. The relationship between cancer and the age of the patient is an empirical one; that is, as a person ages, the likelihood of them getting cancer increases. It is thought that time is needed to allow the buildup of genetic damage, which will lead to the transformed state of the cells.
2. Cancer cells can be shown to have multiple genetic lesions (flaws).
3. Cancer is more likely to occur in cells that proliferate. For example, cancer of the heart is very rare, but cancers of the white blood cells are very common. This is because white blood cells proliferate, allowing them a far greater potential expression of their genetic lesions and thus allowing for the process of cell division to become unregulated.

**Cancer: the cause of the disease**

What has caused the patient's original healthy cells to transform? Again, it has been theorized that the cause of cancer is a multi-stage genetic process. The three stages are:

1. The initial DNA damages to the cell.
2. The chromosome breakdown and rearrangement, and then gene replication.
3. The selection of successful growing mutant cells.

The initial changes in cellular DNA can be caused by a variety of carcinogens, such as radiation, chemicals, viruses, and unknown agents. The damage to the cells leads to flawed cellular growth control and loss of chromosomal stability. The chromosome breakage and rearrangement occurs in several continuous phases after the initial cell division. This later manifests itself in terms of abnormal chromosomal transpositions, which lead to the rearrangement of the cell's genes.

The changes in DNA and chromosomes within the cells result in a new pattern of gene expression for the cell, creating a new cell phenotype in which previously inactive genes are now being expressed, previously expressed genes are now inactive, or there is an over-expression of certain key genes for that cell. It is now believed the earliest change in gene expression, which can lead to a transformed cell, occur in genes that normally regulate cell growth and cell death. These newly expressed or suppressed genes are known as *oncogenes*. Assays that detect oncogenes in human cancer tissues are rapidly becoming available. These types of assays may be potentially useful for the prediction of the development of oncogene-associated cancers in high-risk groups.

Only transformed cells have the potential for developing into cancer cells. The expression of the cancer phenotype requires cell division, which can either be induced by additional genetic damage or can result from a natural initiation of cell division. As the cancer cells multiply, there may be additional phenotype changes in the now-unstable genetic material. As a result, a process of natural selection allows the most "successful" cancer cells to proliferate the most and to control the cancer mass. And, as the environment surrounding the cancer changes, such as that from therapy, the selection process continues. Now that we have a general idea of what happens to the patient's cells during the cancerous process, let's take a look at how to identify the cancerous cells.

### What is a tumor marker?

The first tumor marker identified in the laboratory was Bence Jones protein. Bence Jones protein is a light chain immunoglobulin produced in excess by about half of the patients with multiple myeloma, and it is associated with the presence of monoclonal immunoglobulins in the blood. By using immunoassay methods to test for this protein, we have learned the amount of Bence Jones protein found in the urine, or the amount of myeloma immunoglobulin found in the serum, can be used to follow up on the effectiveness of the patient's therapy. It was also found that the urine concentrations of these proteins closely reflects the size of the myeloma tumor mass. Bence Jones protein, therefore, was the first tumor marker that taught us about these novel ways of monitoring cancer patients during treatment, and about the *sensitivity* and *specificity* (terms which will be defined later in this lesson) of tumor markers.



There are a large number of tumor markers present in blood. Because the blood level of serum tumor markers usually reflects the changes in the tumor's size and activity, the measurement of serological tumor markers has become a common means for the detection and diagnosis of neoplastic diseases, as well as an aid in monitoring the course of treatment for the patient. The ease of blood sampling and the sensitivity of these assays make serological testing far superior to other clinical examinations that were based on physical methods only.

In general, tumor markers are molecules produced by the tumor, or by the body in response to the tumor. Tumor markers are not only found in the blood, they can be found in all body fluids, including urine, CSF, and effusions (exudates or transudates). Tumor markers are represented in small and large molecules, such as peptides, proteins, glycoproteins, enzymes, hormones, immunoglobulins, and mucins. Most tumor markers are incidentally involved in the production of the tumor (tumorigenesis) and are the byproducts of the malignant transformation of the involved cells.

### Roles of laboratory tests

There are four roles that our laboratory testing can serve the cancer patient and their providers. These roles are the detection or screening of the cancer, the confirmation of the condition, the classification or staging of the condition, and the monitoring of the condition.

#### Detection (screening)

Many times, the laboratory will be involved with the patient's initial cancer screening. The following lists several screening tests commonly used for the early detection of cancer:

Site of Cancer	Screening Test
Bladder	Cytological analysis of urine.
Breast	Mammography, physical examination, and self-examination.
Cervix	Papanicolaou smear and pelvic examination.
Colon and rectum	Testing stool for occult blood.
Lung	X-ray and cytological analysis of sputum.
Stomach	Saline wash and cytological examination of gastric contents, and examination of stool for occult blood.

The quality of each cancer-screening test is usually based on its clinical diagnostic *sensitivity* and *specificity*. The *diagnostic sensitivity* of a test is defined as the probability of obtaining a positive result for a patient with a given disease; that is, the percentage of individuals with the disease who will test positive. For example, carcinoembryonic antigen (CEA) is elevated in only 10% of patients with early colorectal cancer, but its values are increased in 60-70 percent of patients with the metastatic (advanced) disease. This makes the diagnostic sensitivity for CEA very low for the screening of patients with early stages of colon cancer. In contrast, the *diagnostic specificity* of a test



defines the probability of obtaining a negative result for a patient without the disease; that is, the percentage of people without the disease who test negative. Using these definitions, the observations of the screening tests are divided into negative and positive results. The patients screened are then classified as either a diseased or non-diseased person. It is thought this very rigid classification of screening tests into only positive and negative results may at times be too simplistic, whereas, results of screening tests can usually be defined as being very negative or very positive. This approach allows for a more sophisticated test interpretation of the screening procedures. For example patients whose results are not negative but are also not alarming enough to justify immediate diagnostic action can be scheduled for earlier repeat screening. Another example is a using “stepwise” screening policy in which only patients with positive results at the first screening are later put through more diagnostic testing.

Finally, sometimes more than one screening tests may be used. The combined results of two tests may yield better screening results. Generally, it is more efficient screening to combine two tests that are complimentary (that is, are directed at different anatomical or biochemical features of the tumor) than to combine tests directed at the same type of features. An example of complimentary testing would be the combined use of a sputum cytological examination and a chest X-ray examination for the screening of lung cancer.

### ***Confirmation***

Additional tests may have to be used to confirm the cancer diagnosis. Examples of tests used to confirm the presence of a cancer would include a bone marrow examination for leukemia and alpha-fetoprotein testing for testicular cancer. Confirmatory test results must be above a certain “decision level.” This means that for a laboratory test to be confirmatory, it should have 100 percent diagnostic specificity – that there should be *no* false-positive results.

### ***Classification and staging***

The classification of the tumor is used to describe the degree of tumor *differentiation*. Tumors are classified as well differentiated, moderately well differentiated, and poorly differentiated. Poorly differentiated tumors are generally more aggressive and have a poorer prognosis. Surgical pathologists have developed various *staging* approaches based on the size and the extent of invasion of surrounding tissues by the tumor, the number of cancer cell-positive lymph nodes, and the presence or absence of metastasis. Metastasis is the process of the cancer cells spreading to other organs, forming growing colonies, and invading the organs. This is called the TNM (tumor, node, metastasis) system. There is a threefold purpose for this staging system. It gives us a reasonable estimate of (1) the patient prognosis (that is, recurrence of the cancer), (2) the patient’s possible response to therapy, and (3) the most likely course their disease will take.

### ***Monitoring***

Probably the most important function of laboratory testing, to the cancer patient, is the monitoring of the course of their disease and its response to therapy. To best monitor patients, the medical community has established various monitoring strategies. For example, patients with either established colon or prostate cancers have been monitored on the basis of their CEA or PSA values, respectively. An increased CEA or PSA value is a signal to the provider to explore the patient surgically to remove additional cancer cells or to change the course of their chemotherapy. Regardless, once the cancer has been identified (screened and confirmed) and initially treated, the laboratory will still play an important role in the treatment of the patient.

### **What are the characteristics of an ideal tumor marker?**

If we lived in the perfect world, the *ideal* tumor marker would only be produced by the malignant tissue itself or would only be elevated in tissue prone to becoming malignant. And then, such a tumor marker would be elevated in the blood of all patients with that particular cancer. On the other side of the coin, all patients without a particular cancer would have very low or no detectable levels of the

tumor marker in their blood. However, in our world no such tumor marker exists with those properties! So how can we define what a good tumor marker is and use this to our advantage? We have already looked at the definitions of *diagnostic sensitivity* and *specificity*, and their relationships to dictating if a tumor marker is effective or not. So what other criteria need be considered when picking out the best tumor markers for our patients?

One source suggested the *ideal* tumor marker should meet the following criteria:

- Be easy and inexpensive to measure in readily available body fluids.
- Be specific to the tumor studied and commonly associated with it.
- Have a direct relation between the fluid level (i.e. plasma) of the marker and the size of the tumor mass.
- Have an abnormal plasma level, urine level, or both in the presence of micrometastases; that is, have detectable levels at a stage at which no clinical or presently available diagnostic methods reveal their presence.
- Have plasma levels, urine levels, or both that are stable and not subject to wild fluctuations.
- If present in the plasma of healthy individuals, exists at a much lower concentration than that found in association with any and all stages of the cancer.

Obviously we have a lot of research to do before we can satisfy all of the above criteria. So, until the ideal tumor markers have been discovered, we need to evaluate the tests we have available to us by these three reasonable benchmarks:

1. The marker should predict a higher or lower risk for eventual development of recurrence.
2. The marker should change as the current status of the tumor changes over time.
3. The marker should precede and predict recurrence before it is clinically detectable.

The following chart lists some of the tumor markers currently available for our use. This list will only grow as researchers learn more about the neoplastic process and then find better ways to identify and treat the cancers.

Tumor Marker	Clinical Potential
Alpha fetoprotein (AFP)	Elevate levels of AFP are seen in hepatocellular carcinoma, and acute and chronic hepatitis. Abnormally elevated levels of AFP and $\beta$ hCG are diagnostic for germ cell tumors.
Beta-2-microglobulins	A low molecular weight molecule increased in B-cell lymphoma, chronic lymphocytic leukemia, Waldenstrom's disease, and multiple myeloma.
Calcitonin	A hormone elaborated by the thyroid; its levels rise during medullary thyroid carcinoma
CEA	A fetal antigen whose association with GI-tract cancer was described in the mid-1960s. CEA is elevated in liver and lung cancer.
CA-125	Elevated levels have been found in approximately 80 percent of all patients with epithelial ovarian cancer.
CA15-3 (BR27.29)	A high molecular weight mucin elevated in metastatic breast cancer. Its decreasing levels are associated with successful treatment.
CA19-9	A high molecular weight mucin associated with pancreatic and colon cancer.
5-Hydroxyindoleacetic Acid (5-HIAA)	5-HIAA is the catabolic end product of the neurotransmitter, serotonin. 5-HIAA is elevated in the urine of patients with carcinoid tumors of the GI tract.

Homovanillic Acid (HVA)	Urinary HVA is the end metabolite of catecholamines, dopamine, and urinary vanillylmandelic acid (VMA). HVA is useful in diagnosing and monitoring therapy in patients with neuroblastoma.
$\beta$ -Human Chorionic Gonadotropin ( $\beta$ hCG)	A placenta antigen long known for its association with choriocarcinoma. Also important in combination with AFP in the diagnosis of germ cell tumors.
Immunoglobulins (paraproteins)	Elevated in plasma cell dyscrasia, such as multiple myeloma and Waldenstrom's macroglobulinemia
Lactic Acid Dehydrogenase Isoenzymes	LD isoenzyme 5 occurs in leptomeningeal carcinomatosis.
Neuron-Specific Endolase (NSE)	NSE is a glycolytic enzyme elevated in patients with neuroblastoma, pancreatic islet cell carcinoma, or small-cell lung disease.
PSA	Presently, PSA is the most sensitive non-invasive test for prostate cancer. PSA and digital rectal examination are recommended as a screening test for prostate cancer.
Prostatic Acid Phosphatase (PAP)	An enzyme classically associated with prostate cancer that has metastasized to the bone.
Terminal deoxynucleotidyl transferase (Tdt)	Tdt is a DNA polymerase found to be elevated in patients with acute lymphocytic leukemia.
Thyroglobulin	Increased levels are seen in inflammatory thyroid disease, thyroid hyperfunction, or differentiated thyroid cancer. Important in identifying patients with residual metastatic cancer.
Urokinase Plasminogen Activator ( $\mu$ PA)	A protease implicated in experimental metastasis. Its elevated concentrations have been seen in breast cancer tissues and is associated with poor prognosis.
VMA	Urinary VMA is elevated in both neuroblastoma and pheochromocytoma.

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 236. Tumor marker

1. The clonal theory of carcinogenesis (the production of a cancer or carcinoma) states that cancers derive from what type of cell?
2. List the clues that have led scientists to believe that one or more genes must be altered before a malignant cell is formed.
3. List the stages of the genetic process that causes a patient's cells to go from healthy to that of cancerous.
4. In stage one of the genetic process, what causes the initial DNA damage to the cell?
5. What are oncogenes?

6. Name the first tumor marker and the disease it is associated with.
7. Why has the measurement of serological tumor markers become a common means for the detection and diagnosis of neoplastic disease?
8. Where are tumor markers found and what type of substances are they?
9. What four roles do laboratory tests serve the cancer patient and their providers?
10. Define diagnostic sensitivity.
11. Define diagnostic specificity.
12. When a “stepwise” screening policy for cancer is used, which patients receive further testing?
13. What is the general rule to use to increase the efficiency of cancer screening when combining two screening tests.
14. What is meant by the requirement that confirmatory tests for cancer diagnosis must be above a certain “decision level?”
15. Tumor classification is used to describe the degree of tumor differentiation. List the terms used to describe tumor differentiation.
16. What is the threefold purpose of the staging system for tumors?
17. When monitoring a patient with an established prostate cancer, what would an increased PSA value signal the provider to do?
18. What suggested criteria should the *ideal* tumor marker possess?

19. What three benchmarks can be used to evaluate the tumor markers we have available to us now?

---

## Answers to Self-Test Questions

---

### 222

1. The sequence and individual characteristics of amino acids.
2. Simple proteins are those that contain only amino acids or their derivatives. Conjugated proteins are those containing an additional non-protein component, called a prosthetic group. Albumin and globulin are examples of simple proteins. Nucleoproteins, mucoproteins, chromoproteins, phosphoproteins, and lipoproteins are examples of conjugated proteins.
3. Colloids are particles that stay suspended in a medium and do not settle out. Proteins are held in solution due to an attraction between the water molecules and the protein molecules, making them behave as colloids.
4. The pH of the solution that the protein is in.
5. They are proteins that are useful as markers for tissue damage and inflammation. CRP is one of the most sensitive acute phase reactants.
6. CRP is synthesized by the liver and bonds to the polysaccharides found in many bacteria, fungi, and parasites. CRP can help initiate phagocytosis and the lysis of invading cells. CRP recognizes and binds with endotoxins released by bacteria and detoxifies or eliminates these toxins from the blood.
7. These proteins facilitate the body's immunologic and inflammatory responses. It increases vascular permeability, allowing antibodies and white blood cells to be delivered to the area of inflammation. It also acts to increase chemotaxis, phagocytosis of invading substances, and the response of antibody to antigens.
8. To recognize antigens and initiate a response for the destruction or neutralization of the antigens.
9. IgG, IgA, and IgM are found in normal patient serum. IgD and IgE are found in the serum of patients with multiple myeloma.

### 223

1. About 60 percent of total serum protein is made up of albumin. Albumin is formed in the liver and is responsible for maintaining colloidal osmotic pressure and transporting blood constituents such as drugs, hormones, and lipids.
2. Globulins are either synthesized in the liver or the mononuclear phagocytic system. Globulins are the key building blocks of antibodies, and they play a lesser role in osmotic pressure and transport.
3. The patient's liver cannot produce proper amounts of albumin. So their mononuclear phagocytic system produces greater amounts of globulins to maintain a normal total serum protein level.
4. Trypsin, chymotrypsin, and peptidases.
5. Albumin is an anion at a pH of 7.4 and has over 200 negative charges per molecule. Because of all these charges, albumin has a vast capacity for binding with other substances and transporting them throughout the body.
6. There will be five fractions, four of which are globulin bands. The four globulin bands are: alpha one globulin, alpha two globulin, beta globulin, and gamma globulin.
7. Glycoproteins and lipoproteins are seen in the  $\alpha_1$ -globulin fraction. The  $\alpha_2$ -globulin fraction contains  $\alpha_2$ -lipoproteins, haptoglobin and ceruloplasmin. The  $\beta$ -globulin fraction contains the  $\beta$ -lipoproteins, fibrinogen, and transferrin. The  $\gamma$ -globulin fraction contains the immunoglobulins.
8. Either from a change in the plasma water volume level or a change in the concentration of one or more of plasma proteins.
9. Hyperproteinemia: an increased protein level caused by decreased plasma water volume. Hemodilution: too much plasma water. Hypoproteinemia: decrease in the total serum protein level.
10. It is of no diagnostic significance with the exception of dehydration.

11. Impaired protein synthesis as a result of liver disease or decreased protein intake. Increased catabolism as a result of inflammation or tissue damage. Malabsorption syndromes or malnutrition resulting in the reduced absorption of amino acids. Protein loss due to conditions such as renal problems, diabetes, or severe burns.
12. Globulins are usually decreased in cases of malnutrition or immunologic deficiencies and increased by immunologic malignancies such as multiple myeloma.
13. The sample should be thawed and well mixed prior to testing.
14. Copper ions react in an alkaline solution with the peptide linkages of proteins to form a violet-colored complex. The intensity of the color produced is proportional to the amount of protein present.
15. Many refractometers have a calibrated scale that gives a direct readout of total serum protein concentration. This instrument measures the total solids dissolved in the serum.
16. In the dye binding methods, bromcresol green or bromcresol purple are widely used. These dyes have a higher affinity for albumin than for the other protein fractions so they bind primarily with albumin in the sample. The absorption of the dye-albumin complex is determined spectrophotometrically at 628 nm for bromcresol green and at 603 nm for bromcresol purple.
17. The albumin level is subtracted from the total serum protein value to determine an estimate of the total globulin concentration. The albumin level is then compared to the globulin level and expressed as a ratio. The normal A/G ratio is  $>1.0:1$ .

## 224

1. Because protein molecules are large and do not cross the blood-brain barrier. Some disease processes alter the permeability of this protective membrane and allow proteins to leak in.
2. From plasma that is ultrafiltered through the walls of the meninges and choroid plexuses. Protein levels increase as CSF works its way down the spinal cord, with the highest levels found in the lumbar spine.
3. The tumors impede the flow of CSF and the CSF in the lumbar spine becomes stagnant. Proteins equilibrate across the walls of the meningeal capillaries into the CSF raising protein levels.
4. When lesions inflame the meninges, when there is a cerebral hemorrhage, or in cases of neurosyphilis. Also occurs in patients with multiple sclerosis or other demyelinating diseases.
5. Repeated punctures should be avoided because total protein levels may be increased due to the trauma of the previous punctures and not be truly indicative of the patient's condition. Total protein content, even in a clear supernatant of a bloody specimen, will reflect the contamination of blood proteins.
6. They should be stored refrigerated for up to 2 weeks in the event other testing may be required.
7. Because they require as little as .025 ml of sample, while turbidimetric methods can require as much as 0.5 ml of sample.
8. Sulfosalicylic acid and sodium sulfate reagent produces a fine suspension of protein when added to CSF. The protein concentration is proportional to the turbidity produced. Turbidity is measured as a decrease in light transmittance at 620 nm. Interference from hemoglobin or drugs that precipitate protein can affect results.
9. Patients with these diseases may or may not show an obvious elevation in CSF protein; these conditions are more easily detected using electrophoresis. In normal CSF samples, the  $\gamma$ -globulin is less than 11% of the total protein, but in multiple sclerosis its level increases to more than 18%.

## 225

1. They are fatty acids, triglycerides, phospholipids, plant sterols, sphingolipids, and cholesterol.
2. They contain many of the essential fatty acids that humans require but cannot synthesize.
3. Unit for unit, the amount of energy produced by metabolizing a fatty acid is twice that of metabolizing a carbohydrate.
4. About 90 to 95%.
5. Because of their ability to align themselves between water and lipid phases. Providing the lipid portion of the cell membrane.
6. When ingested, they help to inhibit the absorption of cholesterol. Plant sterols are used to treat patients with elevated levels of plasma cholesterol.
7. Particularly those of red blood cells and central nervous system cells.

8. Some from the diet, but the majority is synthesized by the liver.

**226**

1. LDL transports cholesterol to the tissues, and HDL transports cholesterol away from tissues to the liver.
2. Increases occur during pregnancy, uncontrolled diabetes mellitus, hypothyroidism, and biliary cirrhosis.
3. It could be due to inpatient status; they are now in a reclining position. Cholesterol levels can decrease by as much as 10 percent when a patient goes from an erect to reclining position.
4. EDTA samples must be corrected so that results are converted to serum values. This is done by multiplying the EDTA value by a factor of 1.03.
5. To react with phenol and 4-aminophenazone in the presence of peroxidase to form o-quinoneimine dye. The intensity of the color that is formed is proportional to the cholesterol concentration in the sample.
6. Upper limits are usually about 500 mg/dl. The sample must be diluted 1:1 with normal saline before retesting.
7. While HDL is stable for up to 4 days at 4°C, significant changes in the levels will occur by the 7<sup>th</sup> day following collection.
8. HDL carries cholesterol from tissues to the liver for conversion to bile salts, while LDL carries cholesterol to the tissues for deposit (this includes the deposit in the blood vessels). HDL competes with LDL for binding sites on the tissue receptors and, in doing so, reduces the cholesterol accumulation in the blood vessels, which reduces cardiovascular risk.
9. When magnesium and dextran sulfate are added to a sample, the larger, lipid-rich chylomicrons, VLDL and LDL, are precipitated selectively, leaving behind the HDL.
10. A ratio of less than 3:1 (LDL:HDL).
11.  $\text{LDL} = \text{Total cholesterol} - [\text{HDL cholesterol} + (\text{Triglycerides} \div 5)]$
12.  $(250 \div 5) = 50$   
 $[40 + 50] = 90$   
 $180 - 90 = 90$   
 $\text{LDL} = 90 \text{ mg/dl}$
13. LDL determinations are based on a calculation involving total cholesterol, HDL cholesterol, and triglyceride. An error in any of these values would also create an error in determining the LDL concentration.
14. If the patient's triglyceride level is above 400 mg/dl. The formula is not accurate if triglycerides are above this level.

**227**

1. To act as a storage source for energy in the body. They are made up of 3 molecules of fatty acids bound to 1 molecule of glycerol.
2. The cells that synthesize triglycerides do so by converting fatty acids into triglycerides by esterification with glycerol-3-phosphate, a compound that is a product of glucose metabolism. Because of this required product of glucose metabolism, cells must contain glucose in order to have the ability to form triglycerides.
3. Insulin promotes the formation of triglycerides by the adipose tissues and a decreased amount of insulin increases the hydrolysis of triglycerides.
4. Chylomicrons transport triglycerides from dietary sources in the intestines to the tissues, and VLDL transports the triglycerides that have been synthesized in the liver to the tissues.
5. Cardiovascular risk.
6. They do not require purification or extraction steps and are easily automated.
7. Triglyceride levels are quickly affected by food in-take. If the patient has not been fasting for at least 12 hours before collection, the triglyceride results will be falsely elevated.
8. The triglycerides are hydrolyzed by microbial lipase to produce glycerol and free fatty acids. The glycerol is then put through several other coupled enzymatic reactions with the last product formed being a red quinoneimine dye. The absorbance of the dye is read at 510 nm and is proportional to the triglyceride concentration.

9. Sources of error include the use of stoppers that have been lubricated with glycerol and not monitoring the stability of the reagents.

**228**

1. (1) Regulatory function is concerned with maintaining the chemical composition of extracellular and intracellular fluids.  
(2) Hormones play an important role in your growth and development, which is what morphogenesis is.  
(3) Integrative action is when two or more hormones work together to achieve the desired outcome.
2. Based on the circulating levels of each hormone and the body's need for them.
3. By thoughts, emotions, stress, and circadian rhythms.
4. For example, parathyroid hormone is secreted when plasma concentrations of calcium are low or insulin is secreted when plasma glucose levels are high.
5. The neural centers in the brain transmit a message by the sympathetic nervous system to the adrenal medulla that results in the secretion of epinephrine.
6. The term *target* is used to describe a receptor site for any hormone.
7. They are highly specific. The complexes have a saturation point. They have a high affinity.
8. The target cell receptor response in an obese patient can be decreased. Obese patients often have a chronically elevated level of the hormone insulin. This increased level causes the cells' decreased sensitivity and response to insulin.
9. Some hormones have a very strong affinity for their own receptor sites and some affinity for the receptor sites for another hormone. This effect is known as *specificity spillover*. This effect normally occurs between hormones with similar structures.
10. Receptor site dysfunctions may occur due to a variety of acquired or congenital endocrine diseases. These disorders may be due to a lack in the number of receptor sites, an interference with the receptor binding (such as the presence of an anti-receptor antibody), a structural abnormality in the receptor site, or a defect in the chemical processes that occur after the receptor binding takes place.

**229**

1. The iodine from food, in the form of iodides, is transported to the follicular cells of the thyroid gland where the iodides are concentrated. Iodide is oxidized in the thyroid gland and bound to the tyrosine molecules in a thyroidal protein called *thyroglobulin*.
2. Once the signal is received for thyroglobulin to be released, the thyroglobulin is acted upon by the enzyme tyrosine iodinase, forming free moniodotyrosine (MIT) and diiodotyrosine (DIT). Two DIT molecules will condense to form tetraiodothyronine ( $T_4$ ), and one MIT molecule plus one DIT molecule will condense to form triiodothyronine ( $T_3$ ).
3. Each step in the synthesis of thyroid hormones is regulated by the pituitary hormone TSH (thyroid stimulating hormone). This hormone stimulates the concentration of iodides, thyroglobulin synthesis, and the synthesis of  $T_3$  and  $T_4$  by follicular cells. The breakdown rate of thyroglobulin to release  $T_3$  and  $T_4$  is also controlled by TSH.
4.  $T_4$  is the hormone secreted in the larger amount.  $T_3$ , although in an amount less than half that of  $T_4$ , is 4–5 times more potent. Most of the total metabolic effect of thyroid hormones is attributed to  $T_3$ .
5. Free  $T_3$  and  $T_4$ .
6. Hyperthyroidism is an increased activity of the thyroid gland. It is characterized by nervousness, heart palpitations, restlessness, and insomnia. Hyperthyroidism can be accompanied by an enlarged thyroid (called a goiter). Hypothyroidism is a decrease in thyroid activity and is characterized by drowsiness, fatigue, and lethargy. Marked hypothyroidism can cause weight gain, coarsened features, and thick, scaly skin.
7. It is caused by a defect in the thyroid gland itself. TSH levels are increased.
8. It is caused by a defect in the anterior pituitary gland or the hypothalamus. TSH level can be low, within normal limits or slightly elevated.
9. It is due to the absence of the thyroid gland itself or a defect in the synthesis of the thyroid hormones.
10. TSH levels are low and free  $T_4$  levels are elevated.



11. Because they are safer (no radioactive waste) and do not require the special licenses or training that radioimmunoassay methods require. In addition, non-isotopic methods are easily automated.
12. The sample can develop fibrin clots after freezing and thawing, and the fibrin clots can interfere with automated testing.
13. Either capillary tubes or filter paper can be used. Dried blood samples are stable and easily transported.  $T_3$  neonate testing is performed to screen for congenital hypothyroidism, and the sample should be collected 3 to 7 days after birth. You should avoid touching the filter paper (collection area) or exposing it to extreme heat or light.
14. Enzymes used in  $T_3$  assays may include peroxidase and alkaline phosphatase. The substrates used may be either fluorescent or chemiluminescent to determine activity.
15. Some enzymatic methods use plastic beads to immobilize the  $T_4$  antibody. The bound and free analyte are then separated by decanting and washing the beads. Other methods use ferromagnetic particles and the free and bound  $T_4$  are separated magnetically.
16. In addition to the safety and other considerations regarding radioimmunoassays, enzymatic immunometric assay methods are gaining in popularity because they are more sensitive and faster than the radioactive methods.

**230**

1. (1) n.
- (2) l.
- (3) k.
- (4) m.
- (5) j.
- (6) e, h.
- (7) h.
- (8) d.
- (9) i.
- (10) g.
- (11) f.
- (12) b.
- (13) b.
- (14) a.

**231**

1. The tracking of a patient's drug concentration.
2. Absorption rate of the drug into the blood, distribution of the drug within the body tissues, metabolism of the drug by body tissues, and excretion of the drug. Additional factors include the patient's height, weight, sex, and age.
3. Bioavailability is the amount of drug that eventually makes its way into systemic circulation. The first-pass effect describes the effect of the liver on drugs that have been absorbed and metabolized, thus decreasing their bioavailability.
4. Drug distribution is the process of getting the drug from the blood into the cells of the targeted organ.
5. The liver transforms drugs into water-soluble substances.
6. The metabolism pattern can slow down considerably, and the drug that they have taken for years, with no ill effects, may have to be reduced in dosage.
7. A half-life is the amount of time it takes for the body to reduce the blood concentration level of a drug by 50 percent.
8. A peak level is drawn when the drug reaches its maximum absorption or concentration. A trough level is drawn just before a dose is administered.
9. The steady state of a drug occurs when there is an average constant level between the peaks and troughs.

- 10 Orally, will range from 1 to 5 hours after ingestion; intramuscular, collection should occur about 60 minutes after the injection; and intravenous, 30 minutes after the infusion has stopped.

**232**

- (1) (1) e.
- (2) e.
- (3) e.
- (4) c.
- (5) c.
- (6) a.
- (7) a.
- (8) b.
- (9) b.
- (10) d.
- (11) d.

**233**

- 1. A heavy drinker will have a greater tolerance for the effects of the alcohol. A non-drinker will metabolize about 15 to 18 mg/dl per hour while a heavy drinker will metabolize about 30 mg/dl per hour.
- 2. It is found in cleaning solutions, antifreeze, and canned fuels. It is sometimes consumed intentionally by alcoholics as an ethanol substitute or accidentally by children. If ingested, methanol can cause blindness or death.
- 3. Isopropanol is not quite as toxic as methanol, but it has twice the central nervous system depressant action as ethanol.
- 4. Sobriety is the term used to describe the clinical opinion of an individual's state of intoxication.
- 5. The manner in which the testing was accomplished.
- 6. You should never attempt to draw the blood of someone who has not consented to the procedure *unless told to do so by a competent authority*. In most cases, the competent authority will be the base commander. This person will direct the collection of the sample after being advised by the Staff Judge Advocate.
- 7. Do *not* use alcohol as a skin-cleansing agent. Use an aqueous iodine solution or Merthiolate before performing the venipuncture.
- 8. One way is to use a special locked box in the refrigerator. The chain of custody is the tracking system used to determine who had access to the sample from the time it was collected to the time the result was released to the proper authority.
- 9. It will decrease the ethanol level of the sample and cause inaccurate results.
- 10. The results will appear very low. You should recommend testing the specimen by gas chromatography because this methods can distinguish between the various types of alcohols.

**234**

- 1. Acetaminophen (Tylenol) and salicylate (aspirin).
- 2. It can cause severe liver damage or even death. The initial clinical findings are not a very good indication of the degree of hepatic damage because the findings are so mild.
- 3. It will become evident 3 to 5 days after the ingestion of acetaminophen.
- 4. The samples should not be drawn any earlier than 4 hours after ingestion. If the time of ingestion is unknown, then samples should be taken at 2- to 3-hour intervals to see if levels are climbing or declining.
- 5. The sooner the antidote is administered, the more efficient it is. Maximum efficiency is seen if the antidote is administered within 8 hours of ingestion, but it declines sharply if given between 18 and 24 hours after ingestion.
- 6. Because they are rapid, easily performed, and very accurate.
- 7. Salicylate.

8. Once ingested, the half-life of aspirin is about 15 minutes, at which time it is converted to salicylate. At high therapeutic or toxic levels, the half-life of salicylate is prolonged: 15 to 30 hours at high levels versus 2 to 3 hours for low doses.
9. Overdoses of salicylate initially cause hyperventilation because the respiratory center of the brain is stimulated. Prolonged hyperventilation can first lead to respiratory alkalosis, this later changes to a metabolic acidosis. In severe cases coma and seizures can result.
10. Consideration must be given to when peak absorption has taken place. Slow release formulas can delay the peak absorption of the drug. If sample collection begins before 6 hours post-ingestion, sample collection should continue every 2 to 3 hours to ensure absorption is complete.
11. Treatment is aimed at preventing further absorption of the drug. Syrup of ipecac or activated charcoal can be used. Sodium bicarbonate is given to the patient to prevent metabolic acidosis.

## 235

1. A local effect means that there is a direct action at the point where the poison is applied. A remote effect is caused when the action of the poison takes place at a site away from the site of application.
2. Acute poisoning is a condition brought on by a single dose of the poison. Chronic poisoning is a condition brought on by repeated exposure over a period of time.
3. It is a by-product of burning fossil fuels. Common sources of carbon monoxide are cigarettes, gasoline engines, and improperly ventilated heating system. When inhaled, carbon monoxide combines tightly with hemoglobin to form carboxyhemoglobin. This action displaces any oxygen that may bind with hemoglobin because carbon monoxide has an affinity for hemoglobin that is about 250 times greater than that of oxygen.
4. The primary effect is hypoxia. Organs with a high demand for oxygen, such as the brain and the heart, are the most sensitive to hypoxia.
5. Treatment for carbon monoxide poisoning involves removing the individual from the contaminated area and administering oxygen. The half life of carbon monoxide is 5 to 6 hours if the patient is breathing room air, but it is only 1 1/2 hours if breathing pure oxygen.
6. The ingestion of lead-based paint chips and improper use of insecticides containing heavy metals.
7. Atomic absorption spectrophotometry and mass spectrophotometry.
8. It is used as a screening tool and a deterrent to illegal drug use. The laboratory may play a role in the collection and shipment of specimens. Our amount of involvement is usually dictated by the base commander.
9. Because they produce a feeling of euphoria, a feeling of well-being and self-esteem, and heightened mental and physical capacity.
10. They suppress the central nervous system and have a sedating and hypnotic effect on the individual. They are often prescribed as an anticonvulsant drug and are also to treat intracranial pressure due to head trauma or injury.
11. THC is most often administered by smoking marijuana or hashish.
12. The coca plant is used to make the drug cocaine. The cocoa plant is used to make chocolate.
13. The effects include increased blood pressure and heart rate, as well as increased body temperature. Overdoses produce seizures, arrhythmias, and myocardial infarction. Sudden death due to cardiotoxicity can occur.
14. Heroin is the preferred opiate by illegal drug users. It is generally administered by intravenous injection.
15. The consumption of poppy seeds in baked goods, such as cakes, muffins, and bread rolls, can lead to urinary excretion of morphine and codeine.
16. As a surgical anesthetic. The euphoric and hallucinogenic properties of this drug make it popular for illegal use.

## 236

1. A transformed cell.
2. First, the relationship between cancer and the age of the patient is empirical, second, cancer cells can be shown to have multiple genetic lesions, and thirdly, cancer is more likely to occur in cells that proliferate.

3. Stage One – the initial DNA damage to the cell.  
Stage Two – the chromosome breakdown and rearrangement, and then gene replication.  
Stage Three – the selection of successful growing mutant cells.
4. The DNA damage can be caused by a variety of carcinogens, such as radiation, chemicals, viruses, or unknown agents.
5. They are newly expressed, or suppressed, genes that have been created as a result of the DNA changes to the cell.
6. Bence Jones protein and multiple myeloma.
7. Because the blood levels of serum tumor markers usually reflect the changes in the tumor's size and activity. They also aid in monitoring the patients course of treatment.
8. Tumor markers are not only found in the blood, they can be found in all body fluids including urine, CSF, and effusions (exudates and transudates). Tumor markers are represented in small and large molecules, such as peptides, proteins, glycoproteins, enzymes, hormones, immunoglobulins, and mucins.
9. The roles are the detection or screening of the cancer, the confirmation of the condition, the classification or staging of the condition, and the monitoring of the condition.
10. The diagnostic sensitivity of a test is defined as the probability of obtaining a positive result for a patient with a given disease, that is, the percentage of individuals with a disease who will be positive.
11. The diagnostic specificity of a test defines the probability of obtaining a negative result for a patient without the disease, that is, the percentage of people without the disease who test negative.
12. When using a stepwise screening policy, only the patients with positive results at the first screening are later put through more diagnostic testing.
13. Generally, it is more efficient screening to combine two tests that are complimentary (that is, are directed at different anatomical or biochemical features of the tumor) than to combine tests that are directed at the same type of features. An example of complimentary testing would be the combined use of a sputum cytological examination and a chest X-ray examination for the screening of lung cancer.
14. Laboratory tests for the confirmation of a cancer diagnosis should have a 100% diagnostic specificity – there should be *no* false positive results.
15. Tumors are classified as: well differentiated, moderately well differentiated, or poorly differentiated. Poorly differentiated tumors are generally more aggressive and have a poorer prognosis.
16. It gives a reasonable estimate of: (1) the patient prognosis (that is, recurrence of the cancer), (2) the patients possible response to therapy, and (3) the most likely course their disease will take.
17. It would signal the provider to explore the patient surgically again to remove the additional cancer cells or to change the course of their chemotherapy.
18. It should: be easy and inexpensive to measure in readily available body fluids, be specific to the tumor studied and commonly associated with it, have a direct relation between the fluid level (i.e. plasma) of the marker and the size of the tumor mass, have an abnormal plasma level, urine level or both in the presence of micrometastases, have plasma levels, urine levels, or both, that are stable and not subject to wild fluctuations, and if present in the plasma of healthy individuals, exists at a much lower concentration than that found in association with any and all stages of the cancer.
19. The three benchmarks are: (1) that the marker should predict a higher or lower risk for eventual development of recurrence, (2) that the marker should change as the current status of the tumor changes over time, and (3) that the marker should precede and predict recurrence before it is clinically detectable.

## Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter. When you have completed all unit review exercises, transfer your answers to ECI Form 34, Field Scoring Answer Sheet.

**Do not return your answer sheet to AFIADL.**

85. (222) Serum complement is a group of proteins, which act as enzymes, that facilitate the
- a. acid-base buffering systems.
  - b. exchange of carbon dioxide in the lungs.
  - c. formation of hormones such as  $T_3$ ,  $T_4$ , and TSH.
  - d. body's immunologic and inflammatory responses.
86. (222) The functions of immunoglobulins are to recognize antigens and
- a. bond with foreign substances.
  - b. phagocytize invading viruses and bacteria.
  - c. bond with fetal and maternal hemoglobin.
  - d. initiate a response for destruction or neutralization of the antigen.
87. (222) The IgM class of immunoglobulins
- a. has an unknown function.
  - b. is firmly bound to basophilic granulocytes.
  - c. is the largest in molecular size and are the most primitive.
  - d. is the antibodies in the highest concentration in the serum.
88. (223) Albumin and globulin make up most of the protein in the body and can be measured together as
- a. total protein.
  - b. alpha protein.
  - c. ionized protein.
  - d. colloidal osmotic protein.
89. (223) When serum proteins are separated by electrophoresis, the most prominent band of protein seen is
- a. globulin.
  - b. albumin.
  - c. hemoglobin.
  - d. acute phase reactants.
90. (223) Total protein is most commonly determined by
- a. electrophoresis.
  - b. manual spectrophotometry.
  - c. atomic absorption photometry.
  - d. either biuret or refractive index.
91. (224) If a *bloody tap* cerebrospinal fluid (CSF) sample is sent to the laboratory, the results will reflect
- a. bacterial contaminants.
  - b. increased glucose levels.
  - c. increased phosphate levels.
  - d. contamination of blood proteins.

92. (224) Dye-binding methods for testing cerebrospinal fluid (CSF) protein are preferred over turbidimetric methods because
- a. it's quicker.
  - b. it's more economical.
  - c. it's a more accurate method.
  - d. a smaller amount of sample is needed.
93. (225) In order for cells to covert fatty acids into triglycerides, what other substance *must* be present?
- a. Glucose.
  - b. Albumin.
  - c. Magnesium ions.
  - d. Alkaline phosphatase.
94. (225) Which of the following substances make up 90 to 95 percent of the stored fats in the body?
- a. Fatty acids.
  - b. Cholesterol.
  - c. Triglycerides.
  - d. Phospholipids.
95. (226) Cholesterol levels decrease in *all* of the following conditions, *except*
- a. liver disease.
  - b. malnutrition.
  - c. malabsorption.
  - d. high animal fat diets.
96. (226) Cholesterol samples are normally drawn fasting even though cholesterol levels are
- a. derived from insulin increase.
  - b. the cause of a patient's hypoglycemia.
  - c. *not* significantly affected by non-fasting samples as triglycerides are.
  - d. somewhat raised due to the body processing fats during the fasting state.
97. (226) When possible, perform high-density lipoprotein (HDL) separation
- a. on the day of collection.
  - b. 36 hours after collection.
  - c. whenever it is convenient.
  - d. no later than three days after collection.
98. (227) Specimens for triglyceride testing should not be drawn *unless* the patient has been fasting for at least 12 hours because triglyceride levels
- a. drop as insulin is produced.
  - b. stabilize 11 hours into the fast.
  - c. are quickly affected by food in-take.
  - d. flip their ratio to cholesterol after eating.
99. (227) A potential source of error for triglyceride testing includes using
- a. a fasting serum sample.
  - b. in-dated reagents and controls.
  - c. a recently calibrated instrument.
  - d. tubes with stoppers that have been lubricated with glycerol.

- 
- 
100. (228) The three general categories for the functions of hormones are
- adrenal, medullary, and parathyroidal.
  - regulatory, morphogenesis, and integrative action.
  - metabolism, steroid regulation, and integrative synthesis.
  - regulatory cyclic, slow-producing, and epinephrine-induced.
101. (228) The specificity of hormone-receptor complexes allow the target cell to
- begin replication.
  - begin membrane repair.
  - initiate hormone production and secretion.
  - recognize the hormone from all other substances.
102. (229) Each step in the synthesis of thyroid hormones is regulated by the pituitary hormone
- triiodothyronine.
  - tetraiodothyronine.
  - thyroid releasing hormone.
  - thyroid stimulating hormone.
103. (229) When collecting samples for thyroid testing, remember that although plasma samples may be used, these samples
- are only stable for 24 hours.
  - must* be separated immediately.
  - tend to form fibrin clots after freezing and thawing.
  - will* have a tendency to increase all chemical reaction times.
104. (230) Pituitary hormones are classified based on
- the ability to produce insulin.
  - the activity in the presence of steroids.
  - their interaction with other hormones.
  - the portion of the gland they are secreted from.
105. (230) In addition to promoting protein synthesis in young mammals, growth hormone (GH) also promotes
- fat storage.
  - skeletal growth.
  - glucose metabolism.
  - immunoglobulin production.
106. (230) An adrenomedullary hormone that facilitates the generation of energy in response to fear, anger, or aggression is
- epinephrine.
  - gonadotropin.
  - adrenocorticotropin.
  - prolactin corticotropin.
107. (231) The tracking of a patient's drug concentration is known as
- pharmacology.
  - collecting a peak.
  - steady state monitoring.
  - therapeutic drug monitoring.

108. (231) The phenomenon that occurs when the liver metabolizes a drug before it gets into the systemic circulation is known as the
- drug half-life.
  - drug digression.
  - first-pass effect.
  - hepatic inversion.
109. (231) The difference between a *peak* and a *trough* sample is the
- trough is drawn while fasting.
  - trough is drawn just after a dose is given.
  - peak is drawn just before a dose is given.
  - peak is drawn at a drug's maximum absorption.
110. (232) When monitoring antipsychotic drugs, one of the *most* commonly observed requests is for
- sodium.
  - lithium.
  - bicarbonate.
  - phenobarbital.
111. (233) When ingested, the *principle* action of ethanol is
- to lower blood glucose.
  - to cause anxiety and paranoia.
  - the depression of the central nervous system.
  - the heightening of reflexes and mental processes.
112. (233) Which one of the following statements *best* illustrates a reason for a legal blood alcohol case to be dismissed?
- Physician determined the state of intoxication.
  - Sample was collected using a non-alcoholic skin cleanser.
  - Technician did not follow laboratory operating instructions.
  - Equipment was recently calibrated and all quality control results verified.
113. (233) When an intoxicated patient refuses to have his or her blood drawn for an alcohol test, who can authorize the sample collection anyway?
- Laboratory technician.
  - Attending health care provider.
  - Any member of the security forces squadron.
  - Competent authority, usually with the advise of the Staff Judge Advocate.
114. (233) What can cause a low ethanol result in a patient who is obviously intoxicated?
- Reagent pack was just opened.
  - The patient had mixed his or her drinks.
  - Patient drank isopropanol and you performed an enzymatic ethanol test.
  - Patient has had so much alcohol to drink that the analyzer cannot run effectively.
115. (234) The problem with initial clinical findings in patients who have overdosed on acetaminophen is the
- initial findings require stomach-pumping.
  - patients will dehydrate, making sample collection difficult.
  - patients usually vomit digested tablets, which cannot be counted.
  - initial findings are mild and do not indication the degree of hepatic damage.



- 
- 
116. (234) When testing for an overdose of aspirin, you are actually
- establishing a baseline level.
  - performing reverse comparison testing.
  - testing for the metabolite, salicylate, and not the original drug.
  - determining whether or not the patient will require an antidote.
117. (234) Treatment for salicylate intoxication is aimed at preventing further absorption of the drug. This is accomplished by
- the administration of an antidote.
  - drinking a mild base to offset the acid.
  - drinking vegetable oil to line the stomach.
  - inducing vomiting or by giving activated charcoal.
118. (235) The effects of poisons are either
- local or remote.
  - acute or chronic.
  - local or systemic.
  - intracellular or extracellular.
119. (235) Improper use of insecticides can lead to significant exposure to
- nitrogen.
  - heavy metals.
  - carbon monoxide.
  - oxygenated compounds.
120. (235) The threshold levels for *positive* codeine and morphine, in urine drug testing programs, are usually adjusted to take into account the
- possibility of a prescription.
  - natural secretion of these items by the body.
  - consumption of poppy seeds in breads, cakes, and muffins.
  - exposure of an individual to marijuana smoke at a rock concert.
121. (236) It has been theorized that cancer is a multi-stage genetic process. Which of the following represents the stages in the correct sequence?
- Selection of successful growing mutant cells, chromosome breakdown, DNA damage.
  - DNA damage, chromosome breakdown, selection of successful growing mutant cells.
  - Selection of successful growing mutant cells, DNA damage, chromosome breakdown.
  - DNA damage, selection of successful growing mutant cells, chromosome breakdown.
122. (236) Which of the following carcinogens could cause the initial change to the cellular DNA during the cancerous process?
- Viruses.
  - Radiation.
  - Chemicals.
  - All the above.
123. (236) Which of the following types of tumor are generally more aggressive and have a poorer prognosis?
- Well differentiated.
  - Poorly differentiated.
  - Moderately differentiated.
  - Moderately well differentiated.

124. (236) Which of the following is *not* a suggested criterion for the *ideal* tumor marker?
- a. Be specific to the tumor studied and commonly associated with it.
  - b. Be easy and inexpensive to measure in readily available body fluid.
  - c. Be non-specific to the tumor studied and commonly associated with it.
  - d. Have stable plasma levels, urine levels, or both, not subject to wild fluctuation.

**When you complete this course, please complete the student survey on the Internet at this URL:**  
**[http://www.maxwell.af.mil/au/afiadl/operation/survey\\_fr.htm](http://www.maxwell.af.mil/au/afiadl/operation/survey_fr.htm).**

## Glossary

### Terms

**Acidosis** –Conditions where blood pH falls below 7.35.

**Alkalosis**–The condition where pH of the blood rises above 7.45.

**Anabolism**–The constructive phase of metabolism where molecules are joined to form new compounds.

**Anion**–Negatively charged ion.

**Anoxia**–A total lack of oxygen.

**Arrhythmia**–An abnormal heartbeat rhythm.

**Buffer**–A substance that can bind acid (hydrogen ions) or base (hydroxide ions) thus maintaining the pH in a relatively narrow range.

**Carbaminohemoglobin**–Hemoglobin that has bonded with carbon dioxide.

**Catabolism**–The destructive phase of metabolism where larger molecules are broken down into simpler compounds.

**Cation**–Positively charged ion.

**Chronicity**–The quality of becoming chronic.

**Electrophoresis**–A separation technique using electrical current.

**Enzymes**–Biological catalyst.

**External respiration**–The exchange of oxygen and carbon dioxide in the lungs.

**Extracellular water**–Water that is found outside of the cells.

**Fulminant**–To occur suddenly or with great intensity.

**Gluconeogenesis**–The production of glucose from noncarbohydrate sources.

**Glycogenesis**–The formation of glycogen from glucose.

**Glycogenolysis**–The breakdown of glycogen into glucose.

**Glycolysis**–The breakdown of glucose into lactate or pyruvate releasing energy for the body in the process.

**Half-life**–The amount of time it takes for the body to reduce the blood concentration level of a drug by one-half.

**Hydrolysis**–The splitting of a compound into fragments by the addition of water.

**Hyperalbuminemia**–An increased serum albumin level.

**Hypercalcemia**–An increased calcium level.

**Hypercapnia**–Increased blood  $p\text{CO}_2$  that causes respiratory acidosis.

**Hyperchloremia**–An increased plasma chloride level.

**Hyperglycemia**–An increased plasma glucose concentration

**Hyperkalemia**–Increased level of potassium.

**Hypermagnesemia**–Increased levels of magnesium.

**Hypernatremia**—An increased sodium level.

**Hyperphosphatemia**—An increased level of phosphate.

**Hyperproteinemia**—An increase in total serum protein level.

**Hyperthyroidism**—Excessive thyroid activity.

**Hypoalbuminemia**—A decreased serum albumin level.

**Hypocalcemia**—Decreased levels of calcium.

**Hypocapnia**—Decreased  $p\text{CO}_2$  that can be caused by increased breathing rates.

**Hypochloremia**—Decreased levels of chloride.

**Hypoglycemia**—A decreased plasma glucose concentration

**Hypokalemia**—Decreased level of potassium.

**Hypomagnesemia**—Decreased level of magnesium.

**Hyponatremia**—Decreased level of sodium.

**Hypophosphatemia**—Decreased levels of phosphate.

**Hypoproteinemia**—A decrease in total serum protein levels.

**Hypothyroidism**—A decrease in thyroid activity.

**Hypoxemia**—Deficient oxygenation of the blood; hypoxia.

**Insidious**—Coming on in a stealthy manner; of gradual and subtle development.

**Internal respiration**—The gas exchange (oxygen and carbon dioxide) that takes place between the plasma in the system capillaries and the tissue cells.

**Intracellular water**—Water that is found within the cells.

**Lipolysis**—The decomposition or splitting up of fat.

**Lypolysis**—The splitting-up of fat, or fat breakdown.

**Metabolism**—The combined physical and chemical processes that take place in the body. There are two types: anabolism and catabolism.

**Neoplasm**—Any new and abnormal growth; specifically a new growth of tissue in which the growth is uncontrolled and progressive.

**Neoplastic**—Pertaining to or like a neoplasm.

**Osmotic pressure**—The physical force that is exerted when water passes from an area of lower ion concentration to one of higher ion concentration.

**Oxyhemoglobin**—Hemoglobin bound to oxygen.

**Peak**—Blood levels of a drug when the drug reaches its maximum absorption or concentration in the patient.

**Phenotype**—The expression of a single gene or gene pair.

**Polydipsia**—Chronic excessive in-take of water.

**Polyphagia**—Excessive eating, gluttony.

**Proliferate**—To spread excessively or rapidly.

**Rhabdomyolysis**—Disintegration or dissolution of muscle; evidence of which is typically the detection of myoglobin in the urine. Can be caused by heavy physical exertion as well.

**Steady state**—Blood levels of a drug which occurs when there is an average constant level between the peak and the trough.

**Specific gravity**—A term used to express the concentration of particles in a solution compared to that of pure water.

**Tachycardia**—Excessive rapidity in the action of the heart, usually  $\geq 100$  beats per minute

**Transposition**—The movement of genetic information from one locus to another. A locus is the position of a gene on a chromosome.

**Trough**—Blood levels of a drug just before a drug dose is administered.

## Abbreviations, Acronyms and Symbols



<b>2-h PG</b>	2 hour postload glucose.
<b>5-HIAA</b>	5-hydroxyindoleacetic acid.
<b>A/G ratio</b>	Albumin/globulin ratio.
<b>ACP</b>	Acid phosphatase.
<b>ACTH</b>	Adrenocorticotrophic hormone.
<b>ADA</b>	American Diabetics Association.
<b>ADH</b>	Antidiuretic hormone.
<b>ADP</b>	adenosine diphosphate.
<b>AFP</b>	alpha fetoprotein.
<b>ALP</b>	alkaline phosphatase.
<b>ALT</b>	Alanine aminotransferase.
<b>AST</b>	Aspartate aminotransferase.
<b>ATP</b>	adenosine triphosphate.
<b>CDC</b>	Career Development Course/Center for Disease Control and Prevention.
<b>CEA</b>	carcinoembryonic antigen.
<b>CFETP</b>	Career Field Education and Training Plan
<b>CGC</b>	casual glucose concentration.
<b>CK</b>	Creatine kinase.
<b>CRP</b>	C-reactive protein.
<b>CSF</b>	Cerebrospinal fluid.
<b>CT</b>	computerized tomography.
<b>cTn</b>	cardiac troponin.
<b>DIT</b>	diiodotyrosine.
<b>DNA</b>	Deoxyribonucleic acid.
<b>EC</b>	Enzyme Commission.
<b>EIA</b>	Enzyme immunoassay.

<b>ELISA</b>	Enzyme Linked Immunoassay
<b>FPG</b>	Fasting plasma glucose.
<b>FSH</b>	follicle-stimulating hormone.
<b>G-6-PD</b>	Glucose-6-phosphate dehydrogenase.
<b>GC</b>	Gas chromatography.
<b>GDM</b>	Gestational diabetes mellitus.
<b>GGT</b>	Gamma glutamyltransferase.
<b>GH</b>	Growth hormone.
<b>H<sub>2</sub>CO<sub>3</sub></b>	Carbonic acid.
<b>HAV</b>	Hepatitis A virus.
<b>HBV</b>	Hepatitis B virus.
<b>hCG</b>	Human chorionic gonadotropin.
<b>HCO<sub>3</sub><sup>-</sup></b>	Bicarbonate.
<b>HCV</b>	Hepatitis C virus.
<b>HDL</b>	High-density lipoprotein.
<b>HDN</b>	Hemolytic disease of the newborn.
<b>HDV</b>	Delta hepatitis virus.
<b>HEV</b>	Hepatitis E virus.
<b>HVA</b>	Homovanillic acid.
<b>IDDM</b>	insulin-dependent diabetes mellitus.
<b>IFG</b>	Impaired fasting glucose.
<b>IGT</b>	Impaired glucose tolerance.
<b>IHD</b>	Isoimmune hemolytic disease.
<b>ISE</b>	Ion-selective electrode(s).
<b>IUB</b>	International Union of Biochemistry.
<b>LD</b>	Lactate dehydrogenase.
<b>LDL</b>	Low-density lipoprotein.
<b>LH</b>	lutinizing hormone.
<b>MI</b>	myocardial infarction.
<b>MIT</b>	moniodotyrosine.
<b>MPS</b>	Mononuclear phagocytic system.
<b>NAD<sup>+</sup></b>	nicotinamide-adenine dinucleotide plus.
<b>NADH</b>	nicotinamide-adenine dinucleotide reduced.
<b>NADP</b>	nicotinamide-adenine dinucleotide phosphate.
<b>NADPH</b>	nicotinamide-adenine dinucleotide phosphate hydrogen.
<b>NAT</b>	nucleic acid amplification testing.

---



---

<b>NIDDM</b>	non-insulin dependent diabetes mellitus.
<b>NSE</b>	neuron-specific endolase.
<b>OGTT</b>	Oral glucose tolerance test.
<b>PAP</b>	prostatic acid phosphatase.
<b><math>p\text{CO}_2</math></b>	Partial pressure of carbon dioxide in the blood.
<b>PCP</b>	phencyclidine.
<b>PCR</b>	polymerase chain reaction.
<b>PG</b>	postload glucose.
<b>pH</b>	Used to measure the acid-base balance of the body.
<b><math>p\text{O}_2</math></b>	Partial pressure of oxygen in the blood.
<b>PSA</b>	Prostate specific antigen.
<b>PTH</b>	Parathyroid hormone.
<b>QC</b>	Quality control.
<b>RIA</b>	Radioimmunoassay.
<b>RNA</b>	Ribonucleic acid.
<b><math>s\text{O}_2</math></b>	Hemoglobin that is saturated with oxygen in the blood.
<b><math>\text{T}_3</math></b>	Triiodothyronine.
<b><math>\text{T}_4</math></b>	Thyroxine, also known as tetraiodothyronine.
<b>TDM</b>	Therapeutic drug monitoring.
<b>tDt</b>	terminal deoxynucleotidyl transferase.
<b>THC</b>	tetrahydrocannabinol.
<b>TIBC</b>	total iron binding capacity.
<b>TLC</b>	Thin-layer chromatography.
<b>TNM</b>	tumor, node, metastasis.
<b>TRH</b>	Thyrotropin releasing hormone.
<b>TSH</b>	Thyroid stimulating hormone.
<b>VIP</b>	Vasointestinal peptide.
<b>VLDL</b>	Very low-density lipoprotein.
<b>VMA</b>	vanillylmandelic acid.
	Symbol representing the male of any species.
	Symbol representing the female of any species.
<b>&lt; or ≤</b>	Symbols representing either less than or less than or equal to.
<b>&gt; or ≥</b>	. Symbols representing either greater than or greater than or equal to.

## **Student Notes**



## **Student Notes**

**AFSC 4T051**  
**4T051N 02 0401**  
**Edit Code 03**