

Med Chem 535P – Diagnostic Medicinal Chemistry

Blood Proteins and Serum Enzymes

I. Blood Proteins

- A. Total Protein*
- B. Albumin*
- C. The Globulins
- D. Fibrinogen
- E. Serum Enzymes

II. Liver Function Tests

- A. Tests of Synthetic Function
 - 1. *Albumin**
 - 2. *Prothrombin time**/*INR**
- B. Tests of Bile Drainage (Cholestasis)
 - 1. *Alkaline phosphatase*
 - 2. *γ -Glutamyl transpeptidases*
 - 3. *Bilirubin**
- C. Tests for Liver Damage
 - 1. *Aspartate aminotransferase**
 - 2. *Alanine aminotransferase**
- D. Hepatic Encephalopathy

III. Tests for Cardiac Damage

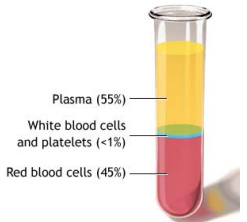
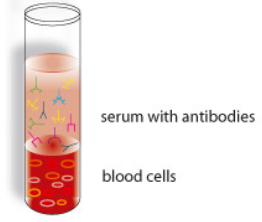
- A. Creatine phosphokinase
- B. Lactate dehydrogenase

IV. Pancreas

- A. Amylase

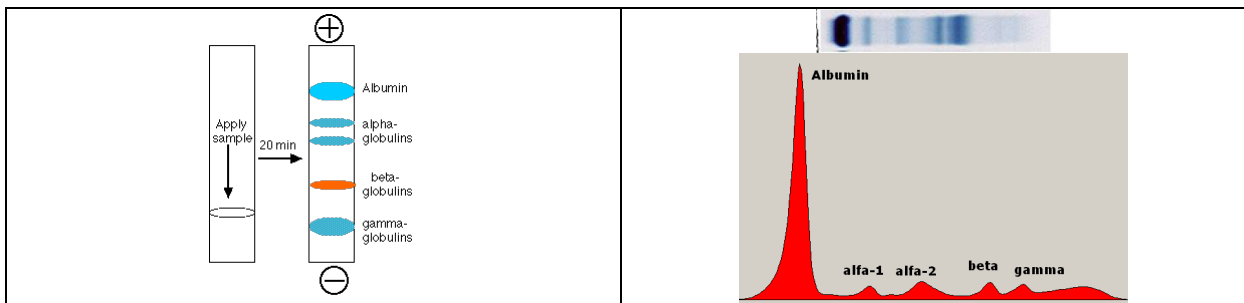
BLOOD CHEMISTRY ~ BLOOD PROTEINS AND SERUM ENZYMES

I. Blood Proteins. Remember: Blood -> Plasma -> Serum

<p>Blood ⇒</p> 	<p>⇒</p> 
<p>Plasma: 55% total volume 91% water 7% blood proteins (albumin, γ-globulins, fibrinogen) 2% nutrients (glucose, amino acids, hormones)</p> <p>Cells: Buffy coat (WBC, platelets) RBCs</p>	<p>Serum: Plasma minus clotting factors</p>

Proteins may be classified as blood proteins, plasma proteins, or serum proteins.

The proteins may be separated electrophoretically:



A. Total Protein (TP). Reference Range: 6 - 8 g/dL*.

This value increases with dehydration due to loss of fluids (vomiting, diarrhea, dialysis). TP can decrease as a result of kidney damage (loss of protein in the urine), with malnutrition (serum protein metabolism), and with the abuse of laxatives (malabsorption of nutrients).

Albumin, fibrinogen, and the globulins represent the major blood proteins.

B. Albumin. Reference Range: 3.5 – 5 g/dL*.

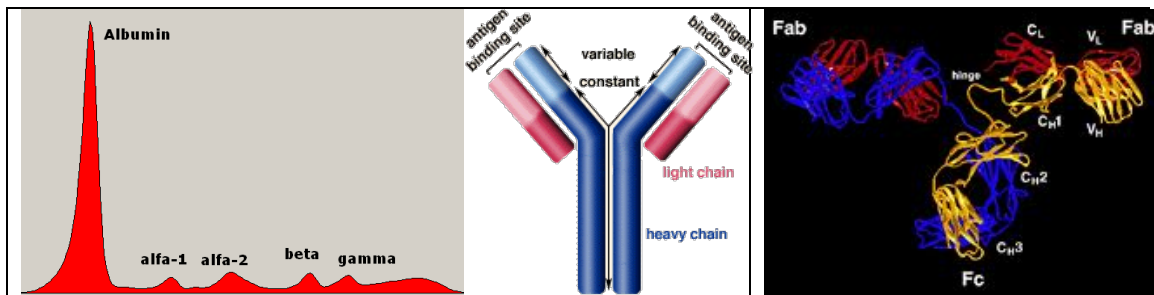
This is a small 67 kDa protein that makes up ~ 55% of the total serum proteins. It is responsible for maintaining osmotic pressure in the blood. It also binds many acidic compounds and thus plays a fundamental role in the transport of fatty acids, drugs, metabolites, and endogenous molecules.

Albumin is synthesized in the liver and is part of the liver function panel (LFT).

TP and albumin (after removal of the globulins by precipitation) can be assayed spectrally with dyes that bind to protein and change their color (Biuret, bromocresol green), by ELISA (albumin), and by electrophoresis.

C. Globulins. Reference Range: 2 – 3 g/dL.

The *globulins* make up the second largest fraction of the blood proteins (~ 38%), of which the most prominent are the circulating antibodies (a.k.a. immunoglobulins and γ -globulins).

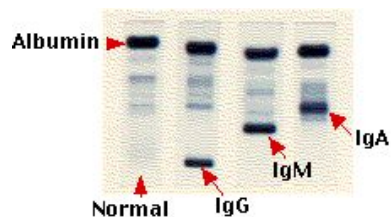


Antibodies (*Ab*, the γ -globulins) bind with high specificity and high avidity to *antigens* (*Ag*). Antigens can be proteins, peptides, DNA, carbohydrates, lipids, and even small molecules (haptens).

The specific region on the target molecule recognized by the *Ab* is the *epitope*. A large molecule (e.g., a protein) can have several epitopes. Thus, several different antibodies can react with a single protein antigen, each recognizing a specific epitope (*polyclonal* antibodies).

Monoclonal Abs now exist for a variety of specific epitopes.

There are several γ -globulins that can be measured by electrophoresis.



Most important is the **A/G ratio** (albumin/globin), which is *normally* ~ 1.2 – 1.5; this can decrease with chronic infections, liver disease and with neoplasms.

D. Fibrinogen (a major clotting factor) makes up the third largest component of the plasma proteins (~ 7%). *Removal by clotting affords serum.*

Fibrinogen can be assayed using a clotting assay (initiated with thrombin) and by ELISA.

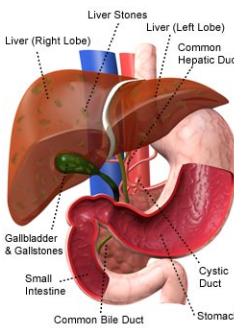
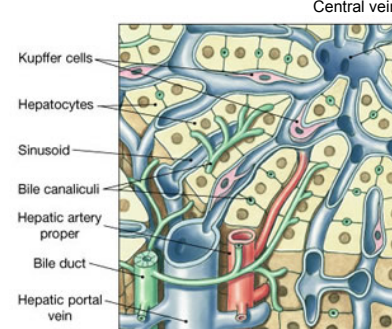
E. Serum Enzymes

Elevations in several enzymes in the serum that are normally present at only low concentrations indicate their leakage from damaged tissues, organs, and/or cells.

Usually, catalytic activities of the enzymes are measured and reported in units of activity. A common unit is the International Unit (IU) where one IU = the amount of enzyme that catalyzes the transformation of one μmole of a standard substrate per minute under defined reaction conditions. This is often recorded as U/L of serum.

II. **Liver Function Tests**

The liver is responsible for the synthesis of a variety of proteins (albumin, clotting factors, lipoproteins), for the chemical modification of endogenous molecules and xenobiotics (toxins, drugs), for the metabolism of amino acids, for carbohydrate metabolism (gluconeogenesis, glycogenesis, glycogenolysis), and for the production of bile (emulsification of lipids).

<p>The hepatic artery supplies oxygenated blood to the liver; the portal veins shunt blood from the intestines to the liver (first-pass).</p> <p>Blood flows through the sinusoids, into the central veins and ultimately exit the liver via the hepatic veins.</p> <p>The liver is composed of thousands of lobules; hepatocytes secrete bile into the bile canaliculi, which drains into the bile ducts and then into the gall bladder (for storage) and ultimately the duodenum.</p>		
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Liver damage may result from alteration of four broad processes, which is reflected in alteration of specific lab values:

Process	Lab Test
<i>Protein Synthesis</i>	Albumin* PT/INR*
<i>Cholestasis (bile drainage)</i>	Alkaline phosphatase γ -Glutamyl transpeptidase Bilirubin*
<i>Hepatocellular Injury</i>	Aspartate aminotransferase* Alanine aminotransferase*
<i>Detoxification</i>	Ammonia (NH_4^+)

Liver dysfunction can be classified as *cholestatic* ~ impairment of excretory function, or *hepatocellular* ~ primary inflammation/damage to the hepatocytes.

A. Tests of Liver Synthetic Function. The liver has an enormous synthetic capacity so modest dysfunction may not be detectable in early stages of dysfunction.

Synthetic capacity may decrease in cirrhosis secondary to alcoholism, viral hepatitis, and chemical toxicity.

1. *Albumin*. Reference Range: 3.5 – 5 g/dL*

The albumin half-life is ~ 20 days so alteration is not always seen in acute hepatitis.

2. *Prothrombin time/INR*. Reference PT*: 10 – 13 seconds (variable)
Reference INR*: 0.9 – 1.1

PT and INR measure the time required to generate a fibrin clot in the patient's serum. This test assesses deficiencies in the *extrinsic* and *common* coagulation pathways, which reflects the concentration and activity of clotting factors V, VII, X, prothrombin (II) and fibrinogen (I).

The majority of the clotting factors are synthesized in the liver and PT/INR will become prolonged in the presence of extensive hepatic impairment. The clotting factors have short half-lives and PT/INR changes occur more rapidly than those observed with albumin (24 hours); however, significant hepatic impairment is still necessary to see an increase in PT/INR.

PT/INR will also increase with a deficiency in Vitamin K.

A fibrinogen assay is also based on clotting time, initiated with thrombin.

B. Tests of Bile Drainage (Cholestasis)

1. *Alkaline phosphatase (alk phos, ALP)*. Reference Range: 20 – 120 U/L
(age dependent)

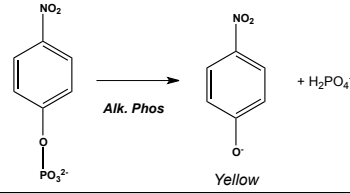
ALPs are a group of enzymes synthesized in a variety of tissues but those observed in the serum come primarily from the liver (**AP-1**) and bone (**AP-2**); *isoenzymes*.

Increases in ALP are observed in hepatobiliary diseases (*cholestasis*) and in some bone diseases (osteomalacia, osteogenic sarcoma).

Modest increases are observed during periods of bone growth in children, after bone fractures, and during the 3rd trimester of pregnancy (released from placenta)

Decreases can occur with retarded bone growth or too much fluoride intake.

Colorimetric Assay: Hydrolysis of para-nitro phenol (PNP) at pH 8.



Note: Acid phosphatase is found mostly in the prostate gland; increases can be observed with metastatic prostate cancer. The assay is the same as for ALP but is performed at pH 4.

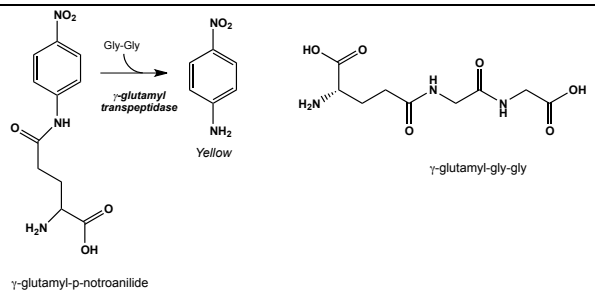
Prostate cancer is the #1 cancer incidence in men and the #2 in mortality. An increase in acid phosphatase is observed in only ~ 20% of individuals with prostate cancer and the *Prostate Specific Antigen (PSA)* test has been used to screen for and monitor the progression of prostate cancer; desirable PSA < 2.5 ng/mL serum.

2. γ -Glutamyl transpeptidases (GGTP, GGT). Reference Range: 1 – 95 U/L

GGT transfers a γ -glutamate from one peptide to another. The enzyme plays major roles in (i) the γ -glutamyl cycle, a pathway for the synthesis and (ii) the degradation of glutathione and xenobiotic detoxification.

It is a membrane protein that is found in highest concentrations in kidney tissue; however, most *serum* GGT originates from the bile duct ~ cholestasis results in release of GGT from the canalicular membrane by the detergent action of the bile salts.

Assay: Dye labeled substrate



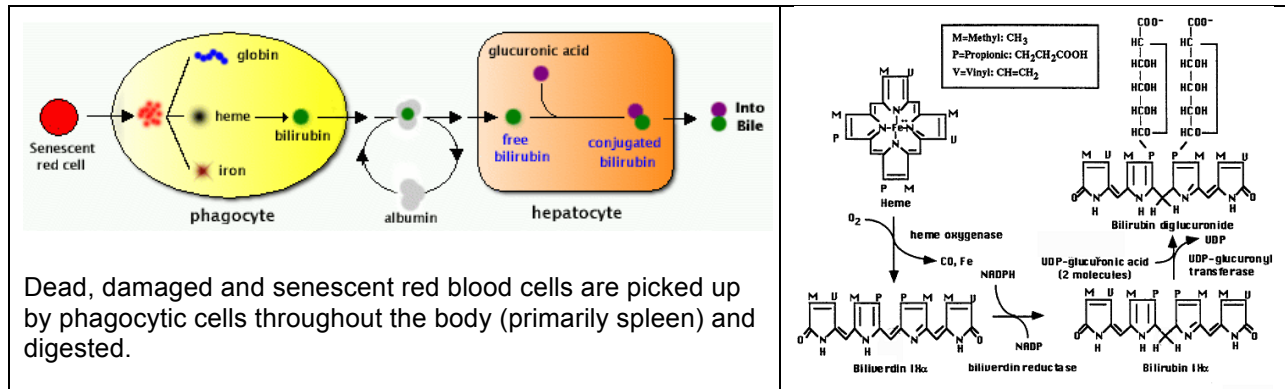
ALP and GGT levels generally increase in parallel in cholestatic disease, but NOT in bone disorders. It is therefore useful in combination with ALP to identify hepatic disorders.

An increase in GGT much greater than that of ALP (ratio > 2.5) may indicate alcohol abuse.

Many drugs that are inducers of microsomal enzymes can also increase GGT (carbamazepine, phenytoin, barbituates, rifampicin, alcohol).

3. **Bilirubin. Reference Range:** Total (TBili): 0.3 – 1.0 mg/dL*
 Direct (DBili): 0.1 – 0.3 mg/dL (conjugated)
 Indirect = Total – Direct (unconjugated)
 T/D ratio ~ 3*

Bilirubin is a breakdown product of heme. It is conjugated with glucuronic acid in the liver and secreted into the bile.



Dead, damaged and senescent red blood cells are picked up by phagocytic cells throughout the body (primarily spleen) and digested.

The globin chains are degraded and the amino acids recycled.

The enzyme *heme oxygenase* degrades the heme to iron (transferrin/ferritin) and biliverdin, which is then reduced to bilirubin by *biliverdin reductase*.

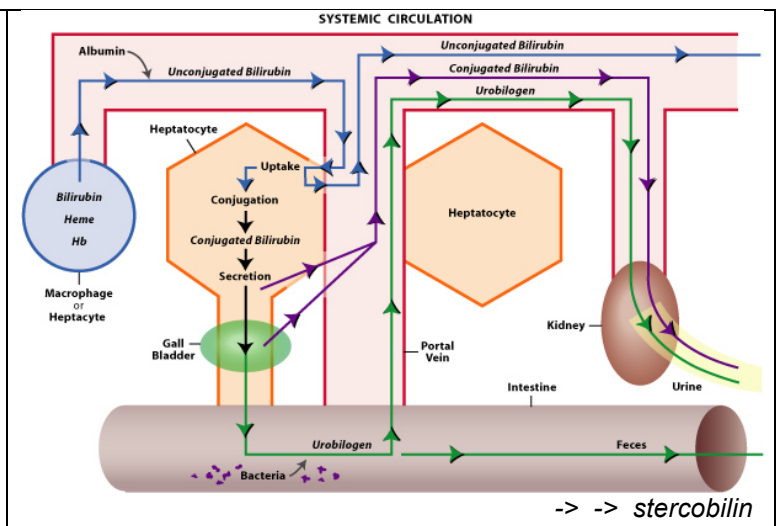
Bilirubin is secreted into the bloodstream; unconjugated bilirubin is hydrophobic and is *transported by albumin* to the liver. Here the enzyme *glucuronyl transferase* conjugates bilirubin with two glucuronide molecules.

About 30% of the unconjugated bilirubin is conjugated in each pass through a healthy liver.

In the intestine, conjugated bilirubin may be (i) metabolized by colonic bacteria, (ii) eliminated, or (iii) reabsorbed.

Bilirubin is metabolized into urobilinogen (yellow), which may be reabsorbed and ultimately excreted in the urine.

Urobilinogen is metabolized to stercobilin (brown); white or clay colored stool is an indicator for a blockage in bilirubin processing and thus potential liver dysfunction or cholestasis.



Increased bilirubin levels are observed liver dysfunction, but also with hemolysis and with congenital disorders (Gilbert's Syndrome).

Clinical Assay ~ The Erlich Test. Reaction of bilirubin with Erlich's reagent ("diazotized" sulfanilic acid) affords a blue-colored conjugate.

The conjugated glucuronide is more water-soluble and reacts much faster than unconjugated bilirubin. So,

Direct Bilirubin read at 1 minute (*Direct = conjugated, soluble*)

Total Bilirubin read at 10 minutes and/or with the addition of "accelerators"

Indirect Bilirubin = Total – Direct (*Indirect (calculated) = unconjugated*)

Strong reducing agents such as ascorbic acid can reduce the colored ion and lead to falsely low test results.

Some drugs can induce glucuronyl transferase enzymes (e.g., anticonvulsants). This can lead to increased conjugation and T/D < 3.

Some drugs can displace bilirubin from protein binding sites (e.g., acidic drugs in large doses ~ PCN, cephalosporins)

Babies are often born with yellow skin and eyes due to immature glucuronidation capacity. A bilirubin level of more than 5 mg/dL manifests as clinical jaundice in neonates. Bilirubin can get into the brain, which can result in mental retardation.



The 4Z, 15Z *trans* isomer is the normal isomer; it is less soluble than the *cis*-form. Blue light catalyzes the transition from the *trans* to the *cis* isomer, which is excreted in the bile.

Disease State	Drugs	Major Serum Form	Mechanism
Hemolysis, Hemorrhage (<i>Pre-Hepatic</i>)	-	Unconjugated T/D > 3	Overproduction of pigment or inadequate uptake
Hepatitis, Cirrhosis, Hepatic Necrosis	acetaminophen, EtOH, isoniazid	Both T/D ~ 3	Liver cell destruction
Cholestasis; obstruction (<i>Post-Hepatic</i>)	anabolic steroids, estrogens, sulfonamides	Conjugated T/D < 3	Cholestasis, defective excretion of bile
Neonatal Jaundice*	-	Unconjugated T/D > 3	Conjugation defect (glucuronyl transferase)

The diagnosis is narrowed down further by looking at the levels of direct bilirubin.

If direct (i.e. conjugated) bilirubin is normal, then the problem is an excess of unconjugated bilirubin (indirect bilirubin), and the location of the problem is upstream of bilirubin conjugation in the liver. Hemolysis, viral hepatitis, or cirrhosis can be suspected.

If direct bilirubin is elevated, then the liver is conjugating bilirubin normally, but is not able to excrete it. Bile duct obstruction by gallstones or cancer should be suspected.

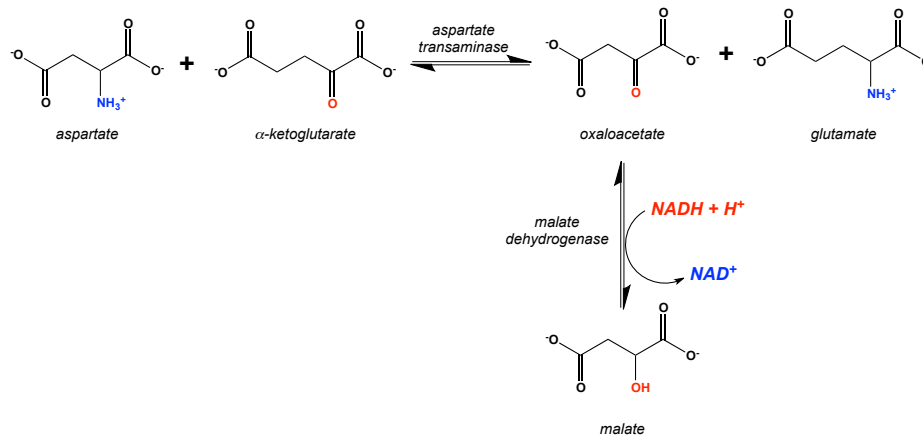
C. Tests for Liver Damage

1. *Aspartate aminotransferase (AST): Reference Range < 35 U/L **

2. *Alanine aminotransferase (ALT): Reference Range < 35 U/L **

These are intracellular pyridoxal (vitamin B6) requiring enzymes involved in amino acid metabolism (transamination reactions).

Assay: coupled enzyme assay that ultimately measures NADH oxidation spectrophotometrically.



The presence of AST/ALT in the serum is a sensitive indicator of even mild liver damage. Unfortunately, they are somewhat non-specific.

While the AST/ALT serum concentrations increase slightly with cholestatic disease, it is much less prominent than ALP, GGT, and bilirubin.

Their serum half-life is short so an increase is associated with active disease.

ALT is found almost exclusively in the liver, while AST is found in the liver and also in heart muscle.

An increase in serum AST is also observed with myocardial infarction.

D. Hepatic Encephalopathy refers to a metabolic dysfunction of the brain that occurs with acute or chronic liver failure. Symptoms range from mild alteration of personality to coma and death. While controversial, ammonia appears to play a role.

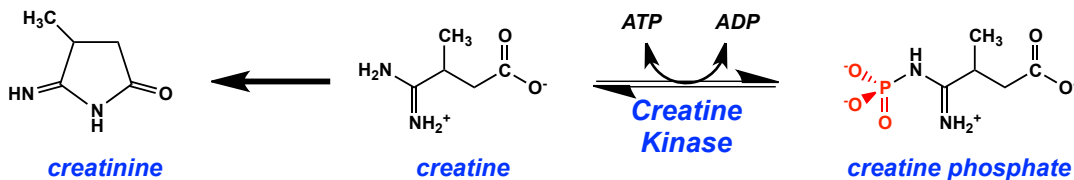
Serum Ammonia (NH₃). Reference Range: 10 – 80 µg/dL

The majority of NH₃ enters the body from the intestines where it is formed by bacterial protein catabolism in the gut. Normally, > 90% is detoxified by the liver in the first-pass. Elevated NH₃ correlates better with acute vs. chronic hepatic encephalopathy.

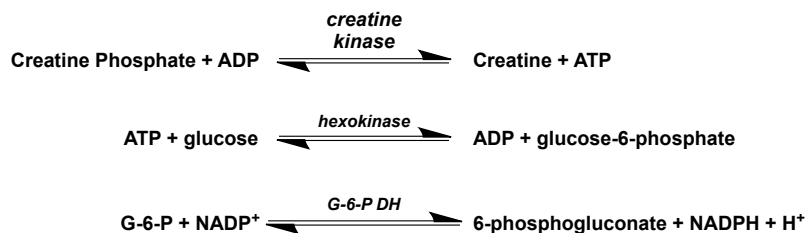
III. Tests for Cardiac Damage

A. Creatine Kinase (CK, CPK). *Reference Range: 55 – 170 U/L (male)
30 – 135 U/L (female)*

This enzyme reversibly transfers a phosphate group from ATP to creatine; phosphocreatine serves as a “reservoir” for ATP in cells that consume ATP rapidly, such as skeletal muscle, myocardium and the brain.



Assay: Coupled enzyme assay; measure NADPH formation spectrophotometrically.



The isoforms can be fractionated by electrophoresis and quantified by fluorometric analysis. ELISA assays using specific monoclonal antibodies are also available.

CK is a dimer that can be assembled from B monomers and M monomers. Homo and hetero dimers (isoenzymes) are found in specific tissues:

CK1 = CK-MM: skeletal and cardiac muscle

CK2 = CK-MB: predominantly heart muscle; lesser in skeletal muscle

CK3 = CK-BB: brain (increased in seizures), lungs, intestine

Serum CK is directly related to muscle mass. Concentrations rise rapidly (hours) in MI (5 – 10X). They are also elevated in muscular dystrophy (50 – 100X), striated muscle damage (crush injury), and even IM injections.

CK2 is most useful in the diagnosis of an MI. This can be measured as enzyme activity or as the ratio of CK-MB:Total (*Reference Range: 3% - 6%*)

A variety of drugs can also increase CK. In particular, statin drugs can cause muscle damage and should be monitored annually.

B. Lactate dehydrogenase (LDH): *Reference Range: 100 - 200 U/L*

This is an intracellular enzyme that catalyzes the conversion of lactate to pyruvate. It is found in a variety of tissues and increases in serum levels correlate with MI, but are not specific. *Intravascular hemolysis is also associated with an increase in serum LDH.*

Assay: spectrophotometric assay that measures NADH formation



The enzyme is a tetramer of two subunits ~ H (heart) and M (muscle) and there are five isozymes; LDH1 (H₄) is found predominantly in cardiac muscle and erythrocytes. The isoenzymes may be separated electrophoretically, but this is not routinely done.

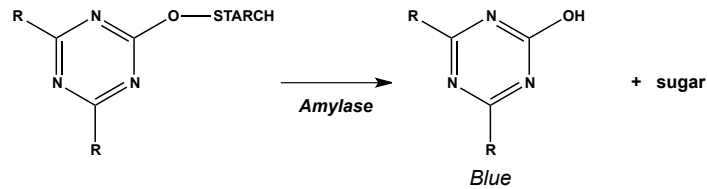
LDH1 activity can be assayed by first precipitating M-containing subunits with a monoclonal antibody.

III. Pancreas

A. Amylase. Reference Range: 35 – 120 U/L

This enzyme is found in the pancreas and salivary glands. It hydrolyzes starches (oligosaccharides) to simple sugars.

Assay: dye labeled substrate (amylochrome)



Increases are observed in pancreatitis associated with alcoholism, gallstones, biliary tract disease or damage, and cystic fibrosis. (*serum lipase* parallels amyase in pancreatitis).

Mumps and other trauma to the salivary glands, such as can occur in seizures, can also increase serum amylase.

Drugs that can cause pancreatitis: steroids, sulfomamides, thiazides, furosemide, cimetidine.

Blood Proteins and Serum Enzymes Study Guide

Terms You Need to Know

Blood, Plasma, Serum

International unit

γ -globulins

Polyclonal vs. monoclonal antibodies

Be prepared to discuss the relationship between blood, plasma, and serum. What components are found in each fraction and how are they derived from blood. You should be prepared to provide the three major classes of blood proteins.

Be prepared to discuss the conditions that can lead to increased and decreased total plasma protein concentration.

Be prepared to discuss the biological role of albumin and the conditions that can lead to hyper- and hypoalbuminemia.

You should be prepared to discuss the significance of an altered A/G ratio.

You should be prepared to discuss the significance of cellular enzymes in the blood and the type of test used to identify their presence. Be prepared to describe how an Enzyme Coupled Assay is performed. Why is enzyme coupling necessary?

You should be prepared to describe the essential features of a liver lobule and how liver damage relates to alteration of protein synthesis, bile flow, and enzyme leakage from the hepatocyte.

Be prepared to discuss the lab tests used to probe for liver function and to identify synthetic vs. cholestatic vs. hepatocyte injury liver issues based on LFTs. Which of the LFTs are associated with other pathologies (bone, cardiac, seizures, etc.)? How can ALP and GGT be used to probe for alcoholic cirrhosis?

You should be prepared to compare and contrast alkaline and acid phosphatase?

You should be prepared to discuss the fate of an "old" red blood cell and what happens to the hemoglobin within the cell when it is destroyed. Be prepared to discuss the relationship between total, direct, and indirect bilirubin? Where in the body are they formed and how are they transported? What are their fates?

Be prepared to describe how total, direct, and indirect bilirubin are measured (i.e., the basic nature of the test). Be prepared to discuss how they change with hemolysis, hepatocyte damage, and cholestasis.

Be prepared to discuss neonatal jaundice, why it occurs, and the treatment for the condition. Be prepared to describe how phototherapy works in a basic sense so that your patient can understand.

You should be prepared to discuss the lab tests used to probe for myocardial infarction. What factors can lead to an increase in serum creatine kinase activity. Which CK isoenzyme is most closely associated with an MI. What class of drugs is associated with muscle damage and an increase in CK?

Be prepared to use a combination of lab values to differentiate between bone issues, myocardial infarction, seizures, and cholestatic vs. hepatocellular injury.

You should be prepared to discuss the organ distribution of amylase and the significance of increased concentrations of this enzyme in the blood. What factors that can cause an increase in serum amylase?

Direct bilirubin (conjugated bilirubin)