I. The Structure of the Liver

A. The liver is the largest organ in the body - It receives a tremendous systemic supply of oxygenated blood from the heart via the hepatic artery. Blood is carried back to the venous system by way of the hepatic vein.

1. The liver is unique in that it contains a second venous blood supply system.
    a. This second system involves the portal system. The portal vein brings blood from all parts of the gastrointestinal tract (the stomach, small intestines, large intestines, pancreas, and spleen) to the liver where it terminates in capillary-like vessels called sinusoids. Once blood passes through the sinusoids, it merges with the systemic system at the hepatic vein.
    b. The great physiological significance of the portal blood flow to the liver is that all nutrients that come from digestion of food in the GI tract pass first through the liver before entering the general (systemic) circulation for transmission to the rest of the body. (This is true except for lipids, which have already been discussed.)

2. The liver has approximately 30,000 different functions. If for some reason there was total loss of liver function, death would usually result in approximately 24 hours.

B. The anatomic structure of the liver is also unique - It has some 50,000 to 100,000 lobules, which is the functional unit of the liver. An artistic rendering of a single liver lobules is illustrated on the following page. In the diagram one can see that the sinusoids are lined with phagocytic cells known as Kupffer cells.

1. The purpose of these Kupffer cells is to remove antigenic material from the portal blood that the body does not recognize.
2. The section between the sinusoids is known as the hepatic plate - Each liver lobule has four hepatic plates.
    a. Moving into the hepatic plate from the cell membrane of the sinusoid there is a small interstitial space called the Space of Disse. There are small lymphatic ducts that drain this area and this is where most of the lymph originates (approximately one-third to one-half of all the lymph is generated here).
3. Beyond the Space of Disse is the hepatocyte. This is the liver cell that carries out most of the liver functions.
    a. Moving from the hepatocyte, bile that is formed in the hepatocyte is released into the bile canaliculus, which is a small canal. Many of these canaliculi come together to form the hepatic duct. The hepatic duct joins with the duct from the pancreas to form the common bile duct which empties into the intestine as illustrated in the second diagram below.

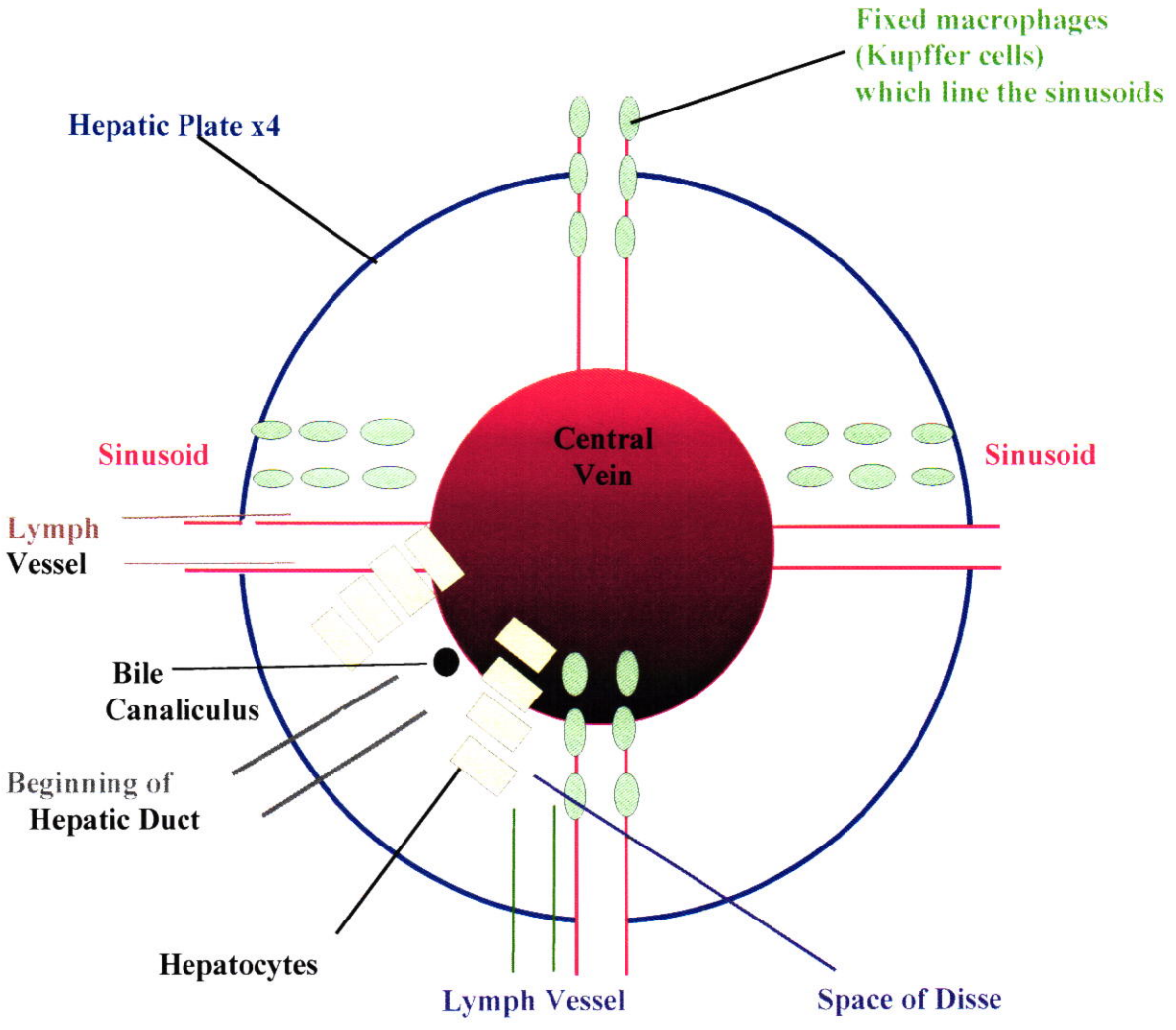
II. Specific Functions of the Liver

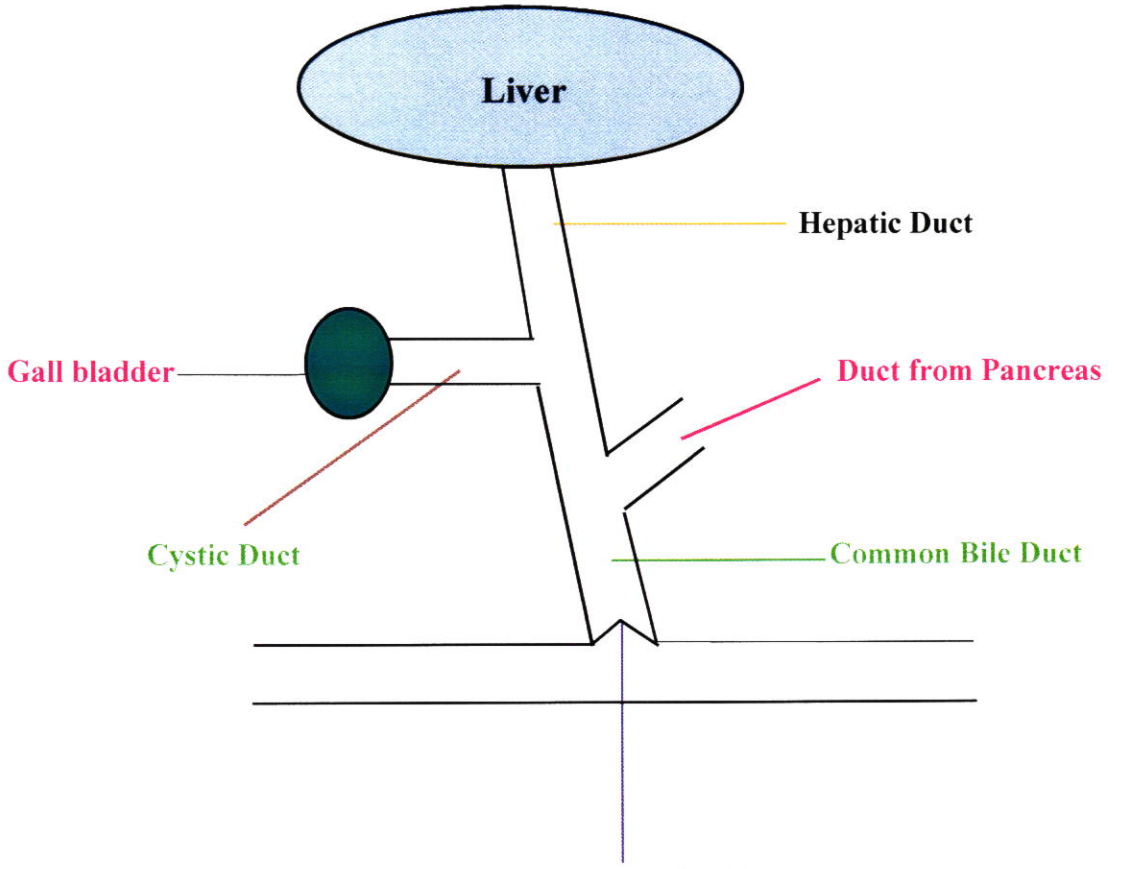
A. Metabolic Function of the Liver - One should recall from carbohydrate metabolism that glycogen is synthesized and stored in the liver during temporary periods of carbohydrate excess. Then, during periods of low plasma glucose, the stored glycogen is converted to glucose in an attempt to maintain constant blood glucose levels.

1. Also, amino acids and fatty acids may be converted into glucose by the liver during periods of great need and stored in the liver. Other hexoses are also converted into glucose by hepatic cells.

2. Almost all of the plasma proteins are synthesized in the liver. Two exceptions include immunoglobulins and hemoglobin. Lipoproteins are also synthesized in the liver.

Single Liver Lobule




Liver
Hepatic Duct
Gall bladder
Duct from Pancreas
Cystic Duct
Common Bile Duct
Sphincter of Oddi
(Located at the beginning of the first segment
of the small intestine; the duodenum)

3. In addition to lipoproteins, the endogenous lipids (cholesterol, phospholipids, and triglycerides) are synthesized here.
4. Also in the liver, cholesterol is degraded into bile acids and secreted into bile.
5. There are a number of other intermediary metabolic processes that occur in the liver.

B. Storage Function of the Liver

1. The liver is a primary storage site for glycogen, vitamin A, vitamin D, and vitamin B_{12}.
2. Significant amounts of iron are also stored in the liver.

C. Excretory Function of the Liver

1. The liver is excretory in that many pigments and waste products are passed into the small intestine for excretion or removal from the body.
   a. Many of the materials that are excreted by the liver are waste products. If these were not removed from the body by the liver, their concentrations would increase to a point that this material would become toxic.

D. Secretory Function of the Liver

1. The liver is secretory in that it produces bile acids from cholesterol that pass into the small intestine for the digestion of fats and for the absorption of fat-soluble vitamins.

E. Protective Function of the Liver

1. The liver helps protect the body from various foreign materials or dangerous materials by two primary mechanisms:
   a. phagocytic action
   b. detoxification reactions
2. The liver contains a large number of phagocytic Kupffer cells.
   a. These cells line the sinusoids and actively remove foreign materials from the blood.
   b. Kupffer cells are a part of the reticuloendothelial system.
3. In regard to the detoxification reactions - many noxious or relatively insoluble compounds are converted to forms that are either less toxic or more water soluble. Which ever the case, these resulting products can then be more readily excreted by the kidneys in most cases.
   a. The conversion to a less toxic form may involve conjugation, esterification, methylation, oxidation, reduction, or other changes.
      1) One example of this type of mechanism is illustrated with ammonia. Ammonia is a very toxic substance that comes from the large intestine through bacterial action upon amino acids. Ammonia enters the liver by the portal vein, and, when in the liver, it is converted to urea by the hepatocytes.
      2) Esterification with glucuronic acid is another mechanism of detoxification. This a common mechanism for converting lipid soluble materials into water soluble compounds. The advantage of this step is water soluble compounds/products are more easily excreted by the kidney. An example of this type of mechanism is seen with bilirubin, which is a lipid soluble pigment which will be discussed below.

F. Circulatory Function of the Liver - There are essentially three ways the liver functions in this capacity:

1. The liver plays a role in immunologic defense by the presence of the Kupffer cells which remove foreign materials from the blood stream.
2. The liver helps regulate the blood volume by serving as a blood storage area.
3. The liver serves as a means for mixing portal blood with the rest of the systemic circulation.

G. Blood Coagulation Function of the Liver - There are some proteins that are essential for coagulation that are synthesized only by the liver. These include:

1. fibrinogen
2. prothrombin
3. factors 5, 7, 9, and 10
    a. Factors 5, 7, 9, and 10 have very short half lives and therefore can be depleted rapidly in cases of severe hepatic dysfunction.

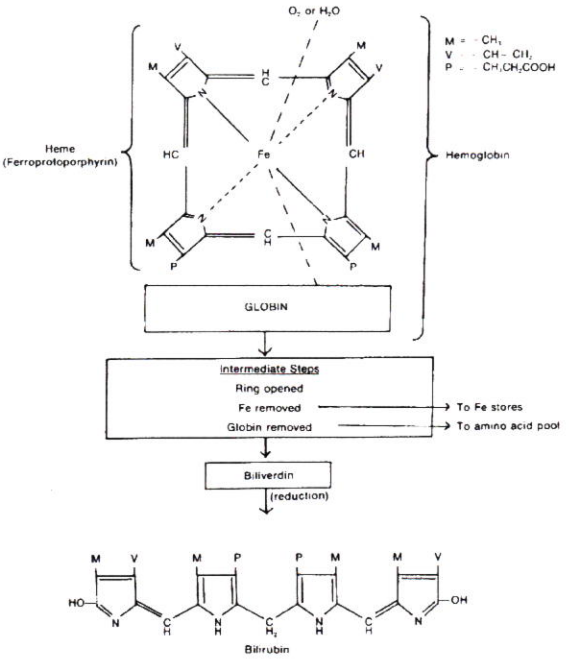
III. Laboratory Tests Useful in Evaluating Liver Function

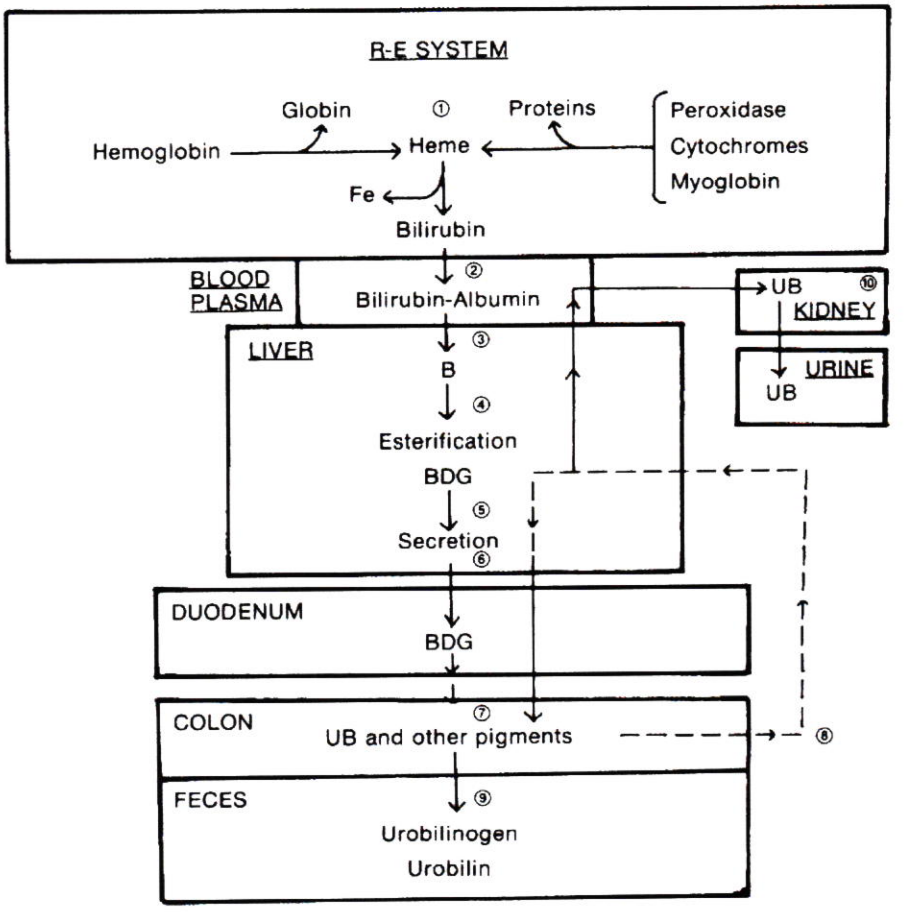
A. Laboratory tests are very important in evaluating liver function. - This is because it is difficult to study the liver by physical examination or X-rays. Also, since the liver has so many lobules carrying out the same function, most liver diseases do not initially result in altering liver function.

1. Therefore, the laboratory enzymes that detect liver cell damage or obstruction are extremely important in the study of liver disease.
2. In addition to monitoring enzyme activities, another very common way to assess liver function is to evaluate the level of bilirubin and its metabolites in the body.

B. **Bilirubin** - Bilirubin is derived from the breakdown of hemoglobin when erythrocytes are phagocytized. This breakdown process is illustrated on the following page.

1. Erythrocytes are loaded with hemoglobin, which is a complex molecule containing four heme groups attached to a protein. (Note that the following diagram shows only one heme group, ferroprotoporphyrin.)
2. Erythrocyte degradation - Erythrocytes survive for approximately 120 days and then they are engulfed or digested by phagocytic cells. The following diagrams show schematically the degradation of hemoglobin.
    a. About 80 percent of the bilirubin formed daily is derived from the breakdown of old erythrocytes.
    b. The remainder comes from the degradation in the bone marrow of immature erythrocytes and from the destruction of other heme containing proteins, such as myoglobin and catalase.
    c. To put this on a weight basis, approximately 6 to 6.5 g of hemoglobin is broken down daily in a normal adult to form approximately 220 mg of bilirubin.
    d. 50 to 60 mg of bilirubin originates from other sources.


O_2 or H_2O
M = $-CH_3$
V = $-CH=CH_2$
P = $-CH_2CH_2COOH$
Heme
(Ferroprotoporphyrin)
Hemoglobin
GLOBIN
Intermediate Steps
Ring opened
Fe removed
To Fe stores
Globin removed
To amino acid pool
Biliverdin
(reduction)
Bilirubin


R-E SYSTEM
Globin
①
Proteins
Peroxidase
Cytochromes
Myoglobin
Hemoglobin
Heme
Fe
Bilirubin
BLOOD PLASMA
②
Bilirubin-Albumin
UB
⑩
KIDNEY
URINE
UB
LIVER
③
B
④
Esterification
BDG
⑤
Secretion
⑥
DUODENUM
BDG
COLON
⑦
UB and other pigments
⑧
FECES
⑨
Urobilinogen
Urobilin

3. Breakdown of Hemoglobin

a. Step 1 - Hemoglobin breakdown begins with the splitting off of a protein globin, which may be reused or it may be hydrolyzed to amino acids.
   1) Also, the porphyrin ring of the heme molecule is broke open by a microsomal heme oxygenase, with loss of one of the methine groups connecting the four pyrrole rings.
   2) As a result of the opening of this chain, it losses ferric iron which returns to plasma transferrin which may in turn distribute to ferritin for tissue storage. This is where the iron stays until it is reutilized again for the synthesis of new heme compounds in the bone marrow.
   3) The resulting product, biliverdin, is then reduced to bilirubin by biliverdin reductase. Bilirubin is a reddish-yellow waste product that must be excreted.

b. Step 2 - Bilirubin leaves the reticuloendothelial cell and is solubilized in plasma by firmly binding to albumin. If it were not bound, then it would not be soluble in the aqueous system.
   1) It can be seen from the structure of bilirubin that it is a non-polar material. This bilirubin is known as unconjugated (or indirect) bilirubin because it has not been conjugated by metabolism at this point.

c. Step 3 - Upon reaching the liver sinusoids, the bilirubin is transferred to the hepatocyte by an active process. The bilirubin is transported inside the liver cell to the microsomes of the rough endoplasmic reticulum.

d. Step 4 - Esterification (or conjugation) of bilirubin takes place in the endoplasmic reticulum.
   1) The enzyme, uridyldiphosphate glucuronyl transferase (UDPG) transfers a glucuronic acid molecule to each of the two propionic acid side chains in bilirubin, converting bilirubin into the diglucuronide ester.
   2) Bilirubin diglucuronide (abbreviated BDG in the preceding diagram) is frequently referred to as conjugated (or direct) bilirubin.

e. Step 5 - The conjugated form of bilirubin is now water soluble. It is secreted from the hepatic cell by a transport system into the bile canaliculi.

f. Step 6 - Conjugated bilirubin, along with the rest of the bile, passes through the bile duct.
   1) During periods of food digestion, bile moves directly into the intestine.
   2) When there is no digestion taking place, bile is stored in the gall bladder where it is concentrated by the absorption of water.

g. Step 7 - Once conjugated bilirubin is in the intestine, it moves through the small intestine, and, as it approaches the colon of the large intestine, it is exposed to bacteria.
   1) Bacterial enzymes cleave off the glucuronic acid groups and reduce (or hydrogenate) the bilirubin molecule.
   2) The reduction product is called urobilinogen (also known as stercobilinogen).

h. Step 8 - A portion of this urobilinogen is absorbed from the colon into the portal circulation. This in turn passes to the liver. A healthy liver removes most all of the urobilinogen as it passes through and re-excretes it. The small portion of urobilinogen that is not removed by the liver enters the

systemic circulation. This urobilinogen is removed by the kidney and is excreted into the urine.

i. Step 9 - The urobilinogen that is not absorbed from the colon becomes partially oxidized to urobilin (also known as stercobilin) and other brownish pigments that are excreted in the feces.

C. Delta Bilirubin - Delta bilirubin is an additional subfraction of bilirubin that is bound to albumin. It was first described by researchers in 1966 when they separated bilirubin fractions by column chromatography using methodology that did not precipitate proteins. In addition to the expected unconjugated and conjugated bilirubin fractions, they found a fraction that was tightly bound to albumin. This fraction is not synonymous with the previously discussed unconjugated bilirubin that is loosely bound to albumin. This new bilirubin fraction was named delta bilirubin.

1. Delta bilirubin is thought to arise by a nonenzymatic, but as yet incompletely defined, reaction between conjugated bilirubin and albumin that occurs in plasma. It does not appear in patients with unconjugated (or indirect) hyperbilirubinemia. Since it is bound to albumin, it is not filtered by the kidneys in patients with normal renal function, and therefore does not appear in the urine. Also, its half-life is the same as that of albumin, which is 12 to 14 days. Finally, one of the most important aspects of delta bilirubin is that many automated analyzers read this form of bilirubin as direct bilirubin. There are a few other automated assays, in particular dry chemistry assays, that measure only actual (true) conjugated bilirubin and do not detect this protein-bound bilirubin. Delta bilirubin can be measured most accurately by high performance liquid chromatography.

   The clinical significance of delta bilirubin and its measurement will be considered below.

IV. Laboratory Tests Used to Evaluate Hepatic Function

We have previously considered the utilization of enzymes in the evaluation of hepatic function. This discussion will be limited to the utilization of bilirubin and its derivatives in the evaluation of hepatic function.

A. Specimen Considerations

1. The specimen for bilirubin determination should be free of lipemia.
2. A hemolyzed sample should also be avoided. Hemoglobin has an absorbance maximum very close to bilirubin. Therefore, it can very easily interfere.
3. Serum samples for bilirubin analysis should be kept in the dark prior to analysis. Direct exposure to sunlight may cause up to a 50 percent decrease in bilirubin within one hour. This is especially true if the blood is kept in a capillary tube.
4. Bilirubin analyses should be carried out as soon as possible, and no later than two to three hours after the blood has clotted.

B. Determination of Bilirubin

1. There are two classic methods for bilirubin determination:
   a. Jendrassik-Grof Method
   b. Malloy and Evelyn Method(some refer to this as the Evelyn and Malloy Method)

2. Jendrassik-Grof Total Bilirubin Assay - Either serum or plasma may be used for this method. For the determination of total bilirubin, the specimen is added to a solution of the following:
   a. sodium acetate - buffers the pH of the diazotization reaction
   b. caffeine-sodium benzoate - mixture is known as the accelerator where it accelerates the coupling of bilirubin with the diazo reagent. (Actually, this liberates the unconjugated bilirubin from albumin so it can react.)
   c. diazotized sulfanilic acid - known as "diazo reagent" - The diazo reagent is the diazonium salt of sulfanilic acid and has to be prepared fresh prior to use because of its reactive nature.
      1) The diazo reagent is the main active ingredient. Diazo reagent functions by splitting both bilirubin and bilirubin diglucuronide in the middle to form two dipyrroles. Each dipyrrole forms an "azo-dye" by coupling with the diazonium salt. These resulting materials are known as azobilirubins, which have the same UV/Visible spectral characteristics.

   d. There are several variations of this reaction which may, or may not, be used as part of the reaction system. These include

      1) This reaction may be terminated by the addition of ascorbic acid. The ascorbic acid functions to destroy the excess diazo reagent. Some procedures will use cysteine hydrochloride to destroy the diazo reagent.
      2) The reaction mixture may be made strongly alkaline with alkaline tartrate. Alkaline tartrate converts the purple azobilirubin to blue azobilirubin, which can be analyzed at 600 nm. The absorbance is proportional to the concentration of bilirubin. By changing the λ_{max} to 600 nm, the wavelength has been moved away from where the effect of non-bilirubin pigments interfere. (Ex. yellow, red, and brown pigments.)

   e. The normal reference value for total bilirubin is 0.1 - 1.0 mg/dL.

3. Jendrassik-Grof Direct Bilirubin Assay - Either serum or plasma may be used for this method as well.The direct bilirubin assay is very similar to the total bilirubin assay.
   a. In the direct method, the specimen is first added to a dilute acid solution (such as a 0.05 M HC1 solution). This is then followed by the addition of the diazo reagent.

b. The dilute acid is used to solubilize only the conjugated bilirubin. Since no accelerator is used here, the unconjugated (albumin-bound) bilirubin does not react; only the direct bilirubin will react with the diazonium salt of sulfanilic acid.

c. This reaction may also be terminated by the addition of ascorbic acid or cysteine HCl, and, the reaction mixture may be made alkaline with alkaline tartrate in order to form the blue azobilirubin.

d. Normal reference values for direct bilirubin os 0 - 0.2 mg/dL.

4. Malloy and Evelyn Method for Total and Direct Bilirubin - The Malloy Evelyn method is almost identical to the Jendrassik-Grof method. The primary difference in the two methods is the Malloy and Evelyn procedure uses methanol as the accelerator.
5. Calculation of the indirect bilirubin - Once the total and direct bilirubin are obtained, the indirect bilirubin level can be found by simply subtracting the direct bilirubin level from the total bilirubin level.
   a. Normal reference values for indirect bilirubin are 0.1 - 0.8 mg/dL.

6. The typical approach in the laboratory analysis of bilirubin is to measure total bilirubin first. If total bilirubin is elevated, then direct bilirubin may be measured and indirect bilirubin calculated. Bilirubin is not typically fractionated unless the total is elevated.

C. Urine Bilirubin Determinations

Bilirubin is not detectable by conventional methods in the urine of a normal health individual.

1. When bilirubin is found, it is usually indicative of hepatocellular disease or obstructive jaundice.
   a. One should also keep in mind that the form of bilirubin seen here is conjugated.
      1) Once conjugated in the liver, this water soluble form of bilirubin may enter the blood stream,(it is more or less "regurgitated" by the liver since it cannot be excreted as usual in the feces) especially in disease. Conjugated bilirubin is water soluble and freely dissolved in the blood stream, requiring no protein binding. As blood passes through the kidney, conjugated bilirubin is freely filtered at the glomerulus and excreted in the urine.

2. In the lab, qualitative tests are primarily used for the detection of urinary bilirubin because the main interest is whether or not it is present, rather than its actual concentration. The two most common tests for urine bilirubin levels that were considered in urinalysis are:
   a. Fouchet's Test
   b. Ictotest
3. Normally, we do not expect to find any bilirubin in the urine so any positive results are considered abnormal and are clinical significant.

D. Urine Urobilinogen - The mechanism by which urobilinogen enters the urine has been described above. Based on that discussion, some urobilinogen is expected in the urine.

1. Normal values for urine urobilinogen are 1-4 mg/24 hours.
2. Methods of analysis of urine urobilinogen by dipstick use para-dimethylaminobenzaldehyde as the active ingredient.

V. Classification of Jaundice

A. When the bilirubin levels in the blood stream begin to rise, bilirubin will begin to be deposited in the sclera (white-coverings) of the eyes and in the skin.

1. This becomes evident to an experienced observer when the serum bilirubin reaches a concentration of about 2.5 mg/dL or greater. This yellowish pigmentation of the skin or sclera is known as jaundice, or icterus.
2. Most liver diseases and some non-liver diseases are accompanied by jaundice. Therefore, the differential diagnosis of jaundice plays an important role in diagnosis and treatment of the liver problem.

3. The table on the following page divides hyperbilirubinemia (or jaundice) into two categories:

    a. Unconjugated Hyperbilirubinemia
    b. Conjugated Hyperbilirubinemia

B. Unconjugated Hyperbilirubinemia - The conjugated category of hyperbilirubinemia, as a whole, includes the forms of jaundice in which at least 80 percent of the serum bilirubin is indirect.

1. Hemolytic jaundice - The prehepatic type of unconjugated hyperbilirubinemia is commonly referred to as hemolytic jaundice.
    a. This occurs because of excessively rapid destruction of erythrocytes. This results in the production of bilirubin at a rate that exceeds the ability of the liver to conjugate and excrete it. (Note that there is nothing wrong with the liver conjugation system.) This will result from any of the genetic or acquired types of hemolytic disease.
    b. Considering the laboratory data, hyperbilirubinemia will be primarily of the unconjugated (or indirect) type. With the increased amount of bilirubin reaching the liver and the liver metabolizing bilirubin at its maximal capacity, an increased fecal urobilinogen would be expected. Along with an increased fecal urobilinogen would go an increased urine urobilinogen. In cases of hemolytic jaundice, the urine levels of bilirubin itself are usually not elevated. This is because the elevated form of bilirubin is unconjugated which is present in the blood stream bound to albumin and cannot be filtered.

Classification of Jaundice

Tests of Bilirubin Metabolism

Classifications of Hyperbilirubinemia	Physiologic Defect	Examples of Etiology	Serum Bilirubin Direct: total in %	Urine bilirubin	Urobilinogen	Fecal Urobilinogen
Unconjugated						
Prehepatic	Excessive production of bilirubin	Hemolytic states Extensive hematoma	<20	-	(↑)	↑
Hepatic	Defective transport of bilirubin from sinusoidal blood into hepatocyte	Gilbert syndrome Some toxins	<20	-	N	N
	Inability to conjugate	Crigler-Najjar syndrome Neonatal jaundice Some drugs	<20	-	N	N
Conjugated						
Hepatic						
Hepatocellular	Hepatocyte injury	viral or toxic hepatitis, cirrhosis, alcoholic hepatitis, other cuases of hepatocyte injury	>40	0	N, ↑, or ↓	↓
Hepatocanalicular	Intrahepatic cholestasis owing to defective transport of bilirubin into canaliculus	some drugs (chlorpromazine, anabolic steroids), viral hepatitis, primary biliary cirrhosis, some forms of familial jaundice	>40	0	N, ↑, or ↓	↓
Post Hepatic						
Obstructive Jaundice	Mechanical onstruction of biliary tree	Carcinome of pancreas or common bile duct Choledocholithiasis Other anatomic destruction	>40	+	↓	↓

2. Hepatic jaundice - The hepatic type of unconjugated hyperbilirubinemia includes
    a. The Gilbert syndrome - impaired uptake by the hepatocytes. A somewhat mild condition that appears to result form a genetic defect in the transport of bilirubin from the sinusoidal blood into the hepatocyte. This results from impaired uptake by the hepatocytes.
        1) Frequently a partial deficiency of conjugating enzyme - glucuronsyltransferase.
        2) Small increase in indirect bilirubin.
        3) Excellent prognosis
    b. The Crigler-Najjar syndrome - a severe disease that results from a genetic deficiency of the microsomal enzyme UDPG-transferase. This enzyme is needed for the conjugation of bilirubin.
        1) Type I - Rare, complete abscence of enzyme.
            a) most infants die from neurological complications before age 1.
        2) Type II - partial deficiency; neurological symptoms are rare.
        3) Defects in the conjugation reaction
    c. Considering the laboratory data, hyperbilirubinemia will be primarily of the unconjugated (or indirect) type. With the defect being associated with either transport or enzyme activity, the amount of bilirubin conjugated will be decreased.
        1) As a result, the amount of urobilinogen in the feces and urine will be decreased. As with hemolytic jaundice, the urine levels of bilirubin itself are usually not elevated. This is because the elevated form of bilirubin is unconjugated which is present in the blood stream bound to albumin and cannot be filtered.

C. Conjugated Hyperbilirubinemia (Reduced Excretion)

1. **Post-hepatic jaundice** - has a more common name which is obstructive jaundice. This is usually the result of obstruction of the common bile duct or hepatic duct by a variety of problems. Some of the most common are:
    a. carcinoma of the pancreas
    b. carcinoma of the common bile duct
    c. pancreatitis
2. Obstruction of the biliary tree, whatever the cause, results in jaundice due to the blockage preventing bilirubin from entering the duodenum. (Note that this bilirubin has already been conjugated.) As a result of this blockage, the bilirubin is "regurgitated" into the blood stream.
3. Hereditary disorders of biliary excretion - Dubin Johnson, Rotor syndromes
    a. increased levels of direct bilirubin
    b. Routine liver function tests are normal
    c. Good prognosis.

4. Considering the laboratory data, hyperbilirubinemia will be primarily of the conjugated (or direct) type. Since the bilirubin that has been metabolized cannot easily reach the intestine, fecal urobilinogen will be decreased. Likewise, urine urobilinogen will be decreased. Finally, urine bilirubin will be increased. The conjugated form of bilirubin that enters the blood stream is very water soluble and will pass to the kidney for removal and excretion.

D. Hepatic conjugated hyperbilirubinemia - subdivided into two categories.

1. Hepatocellular hyperbilirubinemia - types of hepatic hyperbilirubinemia result from injury to the parenchyma (the essential functional elements of an organ - specifically the hepatocyte). This is seen in diseases such as

a. viral hepatitis, toxic hepatitis, and cirrhosis to name a few.

b. Theoretically hepatic damage might be expected to produce an unconjugated hyperbilirubinemia due to impairment of the conjugation process. This is indeed the case in the late stages of convalescent hepatitis (i.e., the majority of the bilirubin is in the unconjugated form).

c. During the more deeply jaundiced phases of hepatitis, however, there are laboratory features that are similar to those seen in post-hepatic jaundice.

1) Therefore, in viral hepatitis for example, there is a distinct elevation in the direct bilirubin fraction of the blood.

2) there will be bilirubin found in the urine.

d. In hepatocellular types of hepatic jaundice, more of the bilirubin is allowed into the small intestine than is seen with post-hepatic jaundice. Therefore, the stools are only somewhat lighter than normal.

1) The urobilinogen levels of the stool is decreased, but not to the extent seen with post-hepatic jaundice.

2) Even though there are lesser amounts of bilirubin entering the duodenum than normal, the hepatic damage prevents the adequate clearing of the urobilinogen that is absorbed from the intestine (i.e. urobilinogen that passes back into portal circulation is not cleared by hepatocytes because of damage and therefore leads to an increase in urobilinogen in the urine).

e. conjugated bilirubin is seen in the blood stream, which is believed to occur through regurgitation.

1) This regurgitation is believed to occur through necrotic cells or through increased canalicular permeability.

f. Indicators of Hepatocellular Injury -

1) Hyperbilirubinemia with bilirubinuria
2) Elevated AST/ALT activity
3) Acute phase reactant responses - iron/ferritin elevations
4) Reduced synthetic function - prolonged prothrombin time, low albumin and cholesterol

2. **Hepatocanalicular** hyperbilirubinemia - very similar to post-hepatic jaundice.
    a. A more common name used for this type of hyperbilirubinemia is intrahepatic cholestasis, or just cholestasis.
        1) This term is used because it describes the fact that bile flow into the duodenum is inhibited or significantly decreased by the intrahepatic disease. The problem is believed to be based on the fact that bile acids are necessary for the transport of conjugated bilirubin from the hepatocyte into the canaliculus. With some problem in bile acid synthesis, this block in transport may lead to regurgitation.

    b. This type of jaundice is seen most commonly with certain drug reactions such as chlorpramazine, organic arsenicals, and methyltestosterone. This type of hyperbilirubinemia is thought to occur occasionally in association with viral hepatitis, or it may be idiopathic. It is very difficult to distinguish between the two types of hepatic conjugated hyperbilirubinemia. Therefore, other laboratory tests are needed to assist in the distinction. These indicators of summarized below. ALT and AST help in this assessment in addition to bilirubin and ALP. If bilirubin and ALP activity are elevated proportionally more than AST and ALT activities, the pattern is called predominately cholestatic. Confirmation of the liver as the source of ALP is generally not necessary when bilirubin is elevated. If bilirubin is normal, the hepatic origin of the increased ALP activity must be confirmed. This can be done with ALP isoenzymes or by finding elevated γGT or 5'N activity.

    c. Indicators of Cholestasis
        1) Hyperbilirubinemia with bilirubinuria
        2) Elevated ALP activity (total)
        3) Elevated γGT or 5'N activity - or - elevated hepatic ALP activityHypercholesterolemia

Recap - five mechanisms that lead to hyperbilirubemia and jaundice

1. Overproduction
2. Impaired uptake by hepatocytes
3. Defects in the conjugation reaction
4. Reduced excretion into bile
5. Obstruction to the flow of bile

<u>Returning to Delta Bilirubin</u>

When a disease process causing hyperbilirubinemia abates or is treated successfully, as in the removal of common bile duct stone, the level of conjugated bilirubin drops at a faster rate than does the delta bilirubin. Therein lies the clinical importance of delta bilirubin. In the recovery phase of diseases that cause direct hyperbilirubinemia, the delta bilirubin as a percentage of the total bilirubin increases markedly, sometimes representing as much as 90% of the total. Therefore, one can envision a situation in which the conjugated bilirubin has dropped to near normal levels, but the "direct" bilirubin may still

be elevated because the delta bilirubin is read as direct bilirubin in most analyzers.

VI. Bilirubin in the Newborn Infant

A. Physiologic Jaundice of the Newborn - As previously described , the UDPG-transferase (uridyldiphosphate glucuronyl transferase) enzyme system is responsible for converting bilirubin into the diglucuronide form. At birth, this enzyme system is usually not fully developed. In the full-term infant, it takes several days before the enzyme is produced in sufficient quantity to conjugate bilirubin.

1. The serum bilirubin may rise as high as 12 mg/dL in the normal full-term infant by the third to fifth day of life as a result of the enzyme immaturity. Usually, after this time interval, the enzyme system becomes fully functional and the bilirubin levels fall to normal adult levels. (Hepatocytes mature rapidly and jaundice resolves spontaneously - usually in 7-10 days.)
   a. This process is aggravated in the premature infant because they may have to wait a longer time for the enzyme system to become functional. In these premature infants, the serum bilirubin concentration may climb as high as 15-16 mg/dL in the absence of any disease process.
2. Normal bilirubin levels for newborns are as follows (for comparison purposes only - do not memorize):

	Premature (mg/dL)	Full-Term(mg/dL)
Cord	<2.0	<2.0
0 - 1days	<8.0	<6.0
1 - 2days	<12.0	<8.0
3 - 5days	<16.0	<12.0
Thereafter	<2.0	<0.2-1.0

B. Hemolytic Disease of the Newborn (HDN) - HDN is caused by either an Rh or ABO system incompatibility. As one would expect, this would intensify the jaundice usually encountered in the newborn. This is because there will be increased amounts of bilirubin produced from the phagocytized cells. Since the immature liver cannot conjugate the bilirubin, the concentration of bilirubin rises in the serum. It is important to remember that this conjugation is a necessary step for the excretion of bilirubin.

1. At this point, there are high levels of unconjugated bilirubin in the plasma. Albumin will bind to this bilirubin in order to transport it around. The only problem is plasma albumin has a limited binding capacity for bilirubin. When these primary sites on albumin are saturated, bilirubin then binds to secondary sites. Bilirubin is less tightly bound to these secondary sites and when the blood circulates to the brain, the loosely bound bilirubin is partitioned between the lipid covering of the brain cells and the plasma. The bilirubin then enters the brain cells and causes irreversible damage to the basal ganglia. This type of condition is known as kernicterus. Many infants who survive kernicterus suffer from mental retardation.

2. The critical concentration of serum bilirubin for possible brain damage in newborns is around 20 mg/dL. This may vary depending upon the concentration of serum albumin, the administration of certain drugs, and other factors.

3. An exchange transfusion is usually performed in order to lower the concentration of circulating bilirubin when bilirubin gets to a level around 20 mg/dL. In some cases, this has to be repeated several times. In situations of significant hyperbilirubinemia, the clinician depends solely on the laboratory results in making the final decision to perform an exchange transfusion.