

**Liver – Jaundice Module**

**1. Objectives**

The student should be able to:

State the three main cell types in the liver and outline the important functions of each. Specifically for the hepatocyte, discuss it's function in protein synthesis, carbohydrate and lipid metabolism, detoxification and storage.

State the components of bile and discuss the regulation of biliary secretion.

Describe the formation and metabolism of bilirubin.

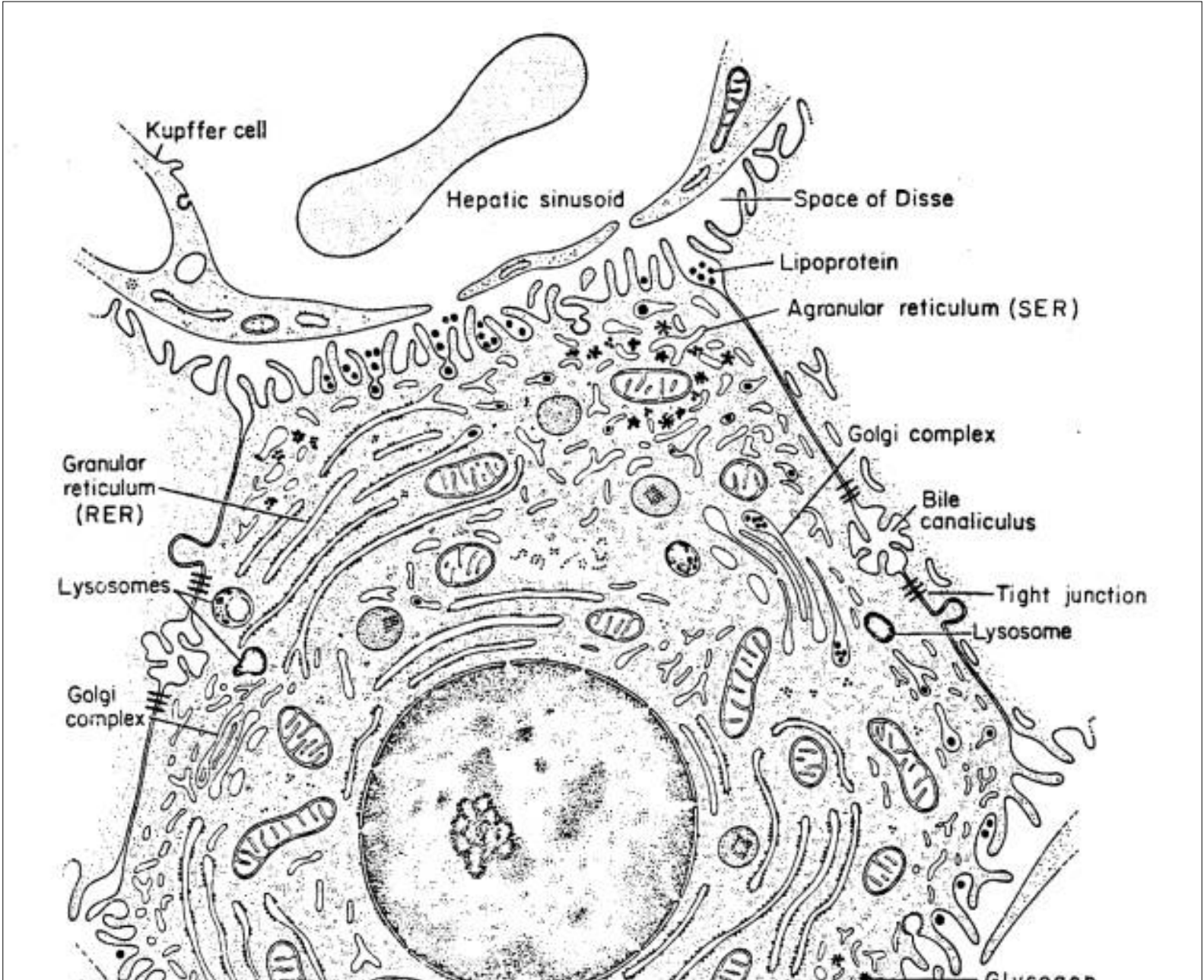
State the usual tests of liver function.

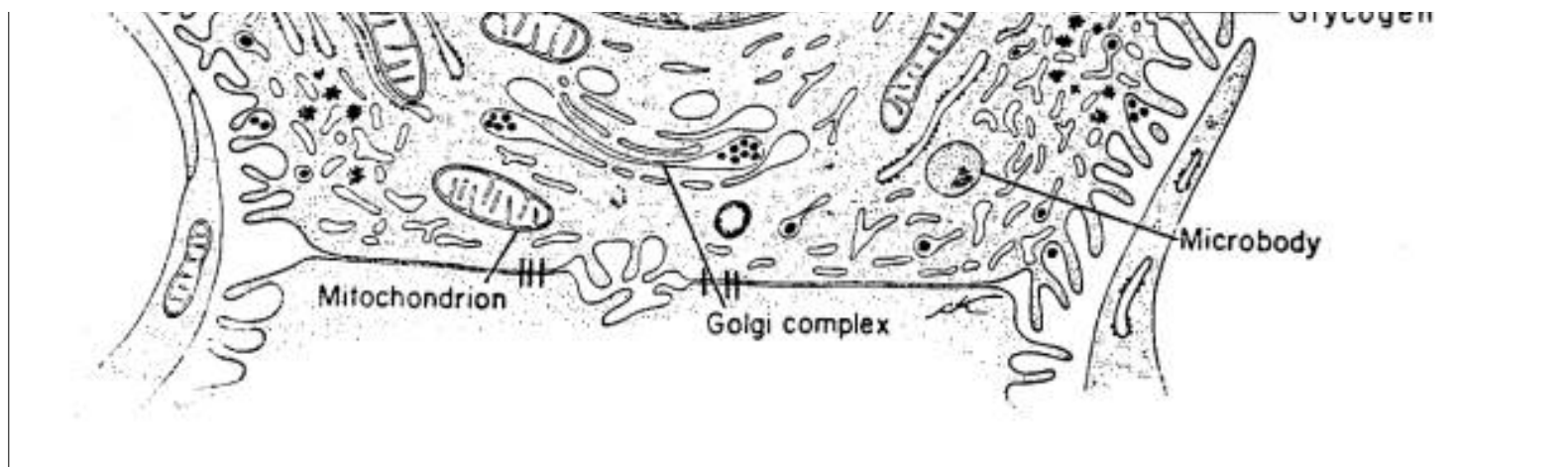
Understand the significance of positive test for bilirubin in the urine

Be able to classify disorders of the liver as either hepatocellular or cholestatic based on the liver enzyme profile

Order the appropriate diagnostic tests, in the correct order, for a patient with jaundice.

**2. Function of Components of the Liver**





**Figure 2.1**

There are three main cell types within the liver, each performing specific functions. The largest liver cell mass is that of the hepatocytes. The other cell types include the sinusoidal lining cells (Kupffer cells, endothelial cells, and stellate cells [lipocytes or Ito cells]), and the bile ducts.

## 2.1 Hepatocytes

The hepatocytes make up approximately 60-80% of the cytoplasmic mass within human liver tissue. These cells are essential for life. They function principally in the synthesis of proteins, storage and transformation of carbohydrates, synthesis of cholesterol, bile salts and phospholipids, and detoxification, modification and excretion of exogenous and endogenous substances (many of which have important biologic activities). In addition, the hepatocyte initiates the formation and secretion of bile.

### 2.1.1 Protein Synthesis:

*Sole site of some protein synthesis*

The hepatocyte is the only cell in the body that manufactures albumin, fibrinogen, and the prothrombin group of clotting factors. It is the main site for the synthesis of lipoproteins, ceruloplasmin, transferrin, and glycoproteins. In addition, the hepatocyte manufactures its own structural proteins and intracellular enzymes. The liver cells contain an extraordinarily well-developed system of organelles (e.g., mitochondria, lysosomes). Synthesis of proteins is undertaken by the rough endoplasmic reticulum, and both the rough and smooth endoplasmic reticulum are involved in secretion of the proteins formed. In addition, the endoplasmic reticulum is involved in conjugation of proteins to lipid and carbohydrate moieties synthesized by, or modified within, the hepatocytes.

### 2.1.2 Carbohydrate Metabolism:

*Glucose homeostasis*

The liver has accommodated our intermittent food intake by storing carbohydrate and releasing it upon demand. Following absorption by small intestine, galactose, mannose, fructose, and glucose are transported to the liver by the portal system. There, they are converted by enzymes in the cytosol of the hepatocyte to glucose or fructose phosphate, which may then be stored as a glucose polymer, glycogen. Upon physiological demand mediated by various hormones, this is depolymerized and released into the blood stream as glucose. The liver is about the only tissue source of blood glucose and has the major responsibility for supporting and maintaining a constantly adequate plasma concentration of it. Aside from conversion of glycogen to produce glucose (*glycogenolysis*), the hepatocytes may also derive glucose from amino acids (*gluconeogenesis*) and glycerol.

### 2.1.3 Lipid Metabolism:

*Bile is route of cholesterol excretion*

The liver forms fatty acids from carbohydrates and synthesizes triglycerides from fatty acids and glycerol. The hepatocytes also synthesize apoproteins with which they then assemble and export lipoproteins (VLDL, HDL). The liver receives many lipids from the systemic circulation and metabolizes chylomicron remnants. The liver synthesizes

cholesterol from acetate and then further synthesizes bile salts. The liver is the sole site of formation of bile salts. Lipoprotein, cholesterol, phospholipid, and bile acid metabolism are intimately related to the secretion of lipids into the bile and, in turn, to the biochemical alterations in bile associated with gallstone disease.

#### **2.1.4 Detoxification:**

*Liver metabolizes absorbed solutes carried in portal blood = "first pass metabolism"*

*Detoxification of NH<sub>3</sub> via urea cycle*

Hepatocytes have the ability to metabolize, detoxify, and inactivate exogenous compounds such as drugs and insecticides, and endogenous compounds such as steroids. Modifying these compounds will alter their physiological effect. Many such compounds (especially the steroid hormones) are converted to a relatively inactive form, whereas others are changed into a biologically even more active form (such as vitamin D). The drainage of the intestinal venous blood into the liver requires efficient detoxification of miscellaneous absorbed substances to maintain homeostasis and protect the body against ingested toxins. A number of substances are prepared for excretion via the bile by conjugation into a more water-soluble state (i.e., bilirubin and many drugs). The liver is the prime site for the metabolic inactivation of drugs, including many sedatives, steroids, ethanol, opiates, and certain antibiotics. This explains why patients with decreased hepatic function (i.e., cirrhosis) may have serious over-effects from relatively small doses of drugs. The liver is the main site for the detoxification of ammonia, one of the key noxious products of protein and amino acid catabolism. To carry out this responsibility a specific complex of interrelated enzyme systems exists in the hepatocyte through which ammonia is complexed with certain amino acids and finally transformed into urea. Urea is then delivered into the plasma for urinary and gastrointestinal excretion. This important biochemical operation is known as the urea or Krebs-Henseleit cycle. Reduced activity of its enzymes can result in toxic hyperammonemia. This may be caused by (a) liver disease or (b) a congenital absence of one or more of the enzymes.

#### **2.1.5 Storage:**

Hepatocytes are important depots for vitamin B12 and iron.

### **2.2 The Sinusoidal Lining Cells (Kupffer Cells, Endothelial Cells) and Stellate Cells**

#### **2.2.1 Endothelial Cells:**

Endothelial cells line the sinusoids and have fenestrae, which provide a graded barrier between the sinusoid and space of Disse. The size of the fenestrae determines the exchange of fluids and size of particular matter to and from the space of Disse and the hepatocyte. These cells are active in clearing macromolecules and small particles from the circulation. They also act as scavenger cells, removing harmful enzymes and pathogens.

#### **2.2.2 Kupffer Cells:**

*Liver is part of the RE system*

The Kupffer cells line the sinusoids of the liver and are attached to the endothelial cells. They are derived from blood monocytes and are the largest group of fixed macrophages in the body. They are highly mobile and are exceedingly active phagocytes. They are capable of removing particulate matter from the bloodstream, and phagocytose old cells, foreign particles, tumor cells, bacteria, yeast, viruses, and parasites. The large size of the liver and tremendous numbers of Kupffer cells make the sinusoids a very important location for clearance of particulate matter from the plasma. The liver's function as a filter can be appreciated when you remember that about one-third of the cardiac output flows through the liver.

#### **2.2.3 Stellate (Ito) Cells:**

The Stellate cells, also called lipocytes or Ito cells, are relatively small and morphologically bear some resemblance to fibroblasts. Their principal characteristic is the presence of numerous fat droplets within the cytoplasm. There is evidence that these cells store vitamin A. Stellate cells also have an important role in fibrogenesis, which is a significant component of chronic hepatitis and cirrhosis.

### **2.3 The Bile Duct Cells**

The bile duct cells form a tubular passage for the excretion of bile from the liver to the gut. It is strongly believed that,

secondary to neurohumoral stimulation, these cells make significant changes in the composition of bile as it flows past, particularly in its water and electrolyte components.

### **3. Biliary Secretion and its Control**

*Remember that bile contains bile salts and bilirubin*

Bile is an isotonic solution containing pigments, such as bilirubin conjugates, lipids (cholesterol), bile acids, and phospholipids: proteins and inorganic electrolytes. It is in part actively secreted by the hepatocytes into the biliary canaliculi. Indirect evidence indicates that this hepatic bile is modified by bidirectional movements of substances (chiefly water and electrolytes) as it passes through the intrahepatic biliary ductal system.

The knowledge about factors regulating hepatic bile secretion is incomplete, but important controls are (1) the supply of bile salts, (2) vagal stimuli and (3) gastrointestinal hormones. Bile salts are the most effective cholagogues (stimulators of bile flow). The production of hepatic bile depends upon the rate at which bile salts recirculate in the enterohepatic cycle. Secretin, gastrin, cholecystokinin and glucagon all cause an increase in bile volume with a rise in bicarbonate content; of these, secretin is the most potent.

Over a 24-hour period a normal adult human produces from 250-1100 ml of hepatic bile. The sphincter of Oddi holds the common bile duct relatively closed at the ampulla of Vater during the interdigestive period, diverting the hepatic bile into the gallbladder. Within the gallbladder the hepatic bile is converted into concentrated gallbladder bile. The gallbladder mucosa absorbs excess water and electrolytes and secretes hydrogen ions, concentrating and acidifying bile. This concentration may be up to 10-fold; however, isotonicity with plasma is maintained because the concentrated cations (largely sodium) become associated with the bile salt micellar aggregates, thus becoming osmotically inactive.

The passage of gallbladder bile into the duodenum requires a coordinated action involving the contraction of the gallbladder and relaxation of the sphincter of Oddi and is due to the release of CCK (cholecystokinin) from the mucosa of the proximal small intestine by products of intraluminal digestion. The concentrated bile solution is delivered to the duodenum where the bile salts aid in fat digestion and inhibit the further release of CCK. Then the gallbladder relaxes, sphincter tone returns and hepatic bile again flows into the gallbladder.

### **4. Laboratory Tests of Hepatobiliary Function**

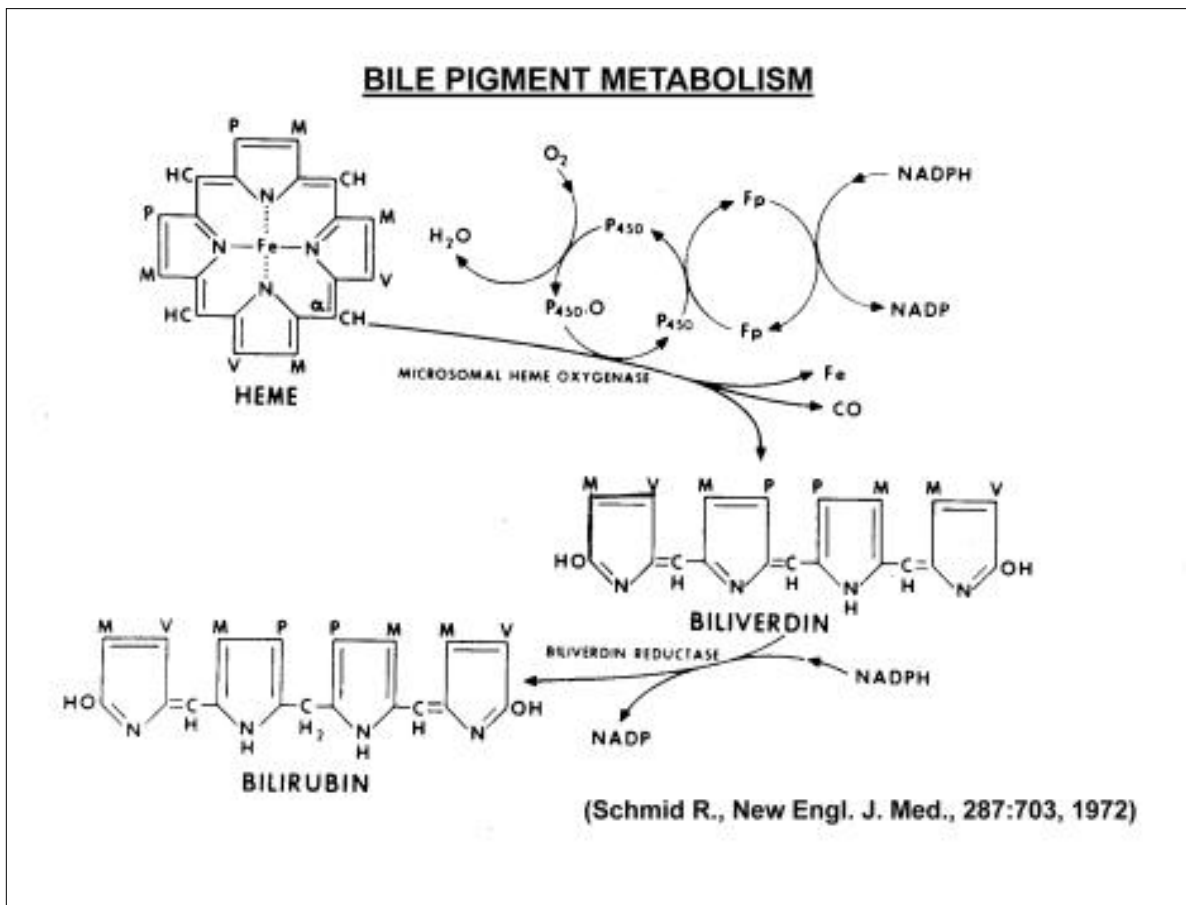
#### **Types of Laboratory Tests**

No single laboratory test or battery of tests is sufficient to provide a complete estimate of the function of the liver in every clinical situation. Biochemical tests of hepatobiliary disturbances are grouped according to which of the general functions of the liver they assess. These include (a) tests that measure the efficiency of the liver to transport organic compounds, (b) tests that reflect hepatobiliary injury (degree of leakage of hepatocyte intracellular enzymes into the plasma), (c) tests that measure hepatic synthetic function (synthesis rates of substances made only by the hepatocyte),

#### **4.1 Tests Measuring Efficiency of Hepatobiliary Transport**

Many organic compounds (e.g., bilirubin, bile salts, and drugs) are normally removed rapidly from the plasma by the hepatocyte and excreted in the bile. An overall hepatic clearance assessment reflects the combination of (1) delivery to the hepatocyte (hepatic blood flow), (2) uptake by the hepatocyte, (3) transport within the hepatocyte, (4) molecular alterations by intracellular enzymes (endoplasmic reticulum, cytosol), (5) transport across the hepatocyte membrane into the biliary canalicula, and (6) passage down the bile ducts into the duodenum.

##### **4.1.1 Bilirubin:**

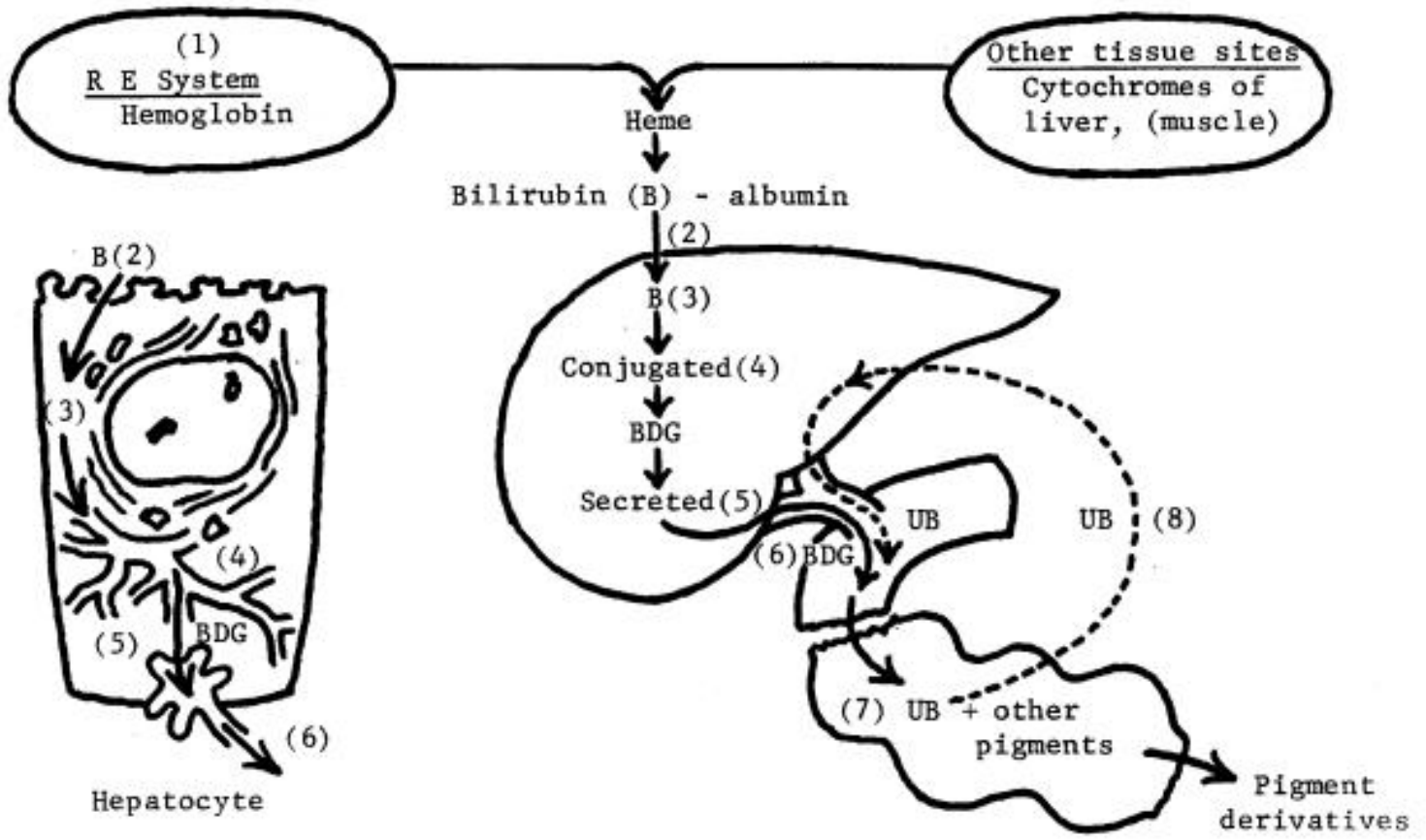


**Figure 4.1**

Bilirubin is an endogenous organic anion derived mainly from the degradation of hemoglobin. It is structurally a tetrapyrrole derived from the protoporphyrin heme (Figure 4.1)

The continuous production of bilirubin within the body (300 mg/day in a normal male adult) provides a built-in "load test" of the efficiency of the hepatobiliary excretory pathways. Normal hepatic functional reserve may allow clearance of two or three times this amount without plasma accumulation of bilirubin. The normal pathways of bilirubin metabolism are shown in Figure 4.2. An abnormality at any of the points (1) through (6), or in combinations causes plasma accumulation of the pigment (Normal persons have up to 17  $\mu$  mol/L of unconjugated plus conjugated bilirubin ("total" bilirubin). When plasma levels of bilirubin and its conjugates total more than 35  $\mu$  mol/L, the yellow color may be seen in the eyes and skin – a condition termed **jaundice**.

## PATHWAYS OF NORMAL BILIRUBIN METABOLISM



**Figure 4.2 Pathways of Normal Bilirubin Metabolism**

- (1) In the reticuloendothelial cells, the heme portion of hemoglobin is degraded to bilirubin (Fig. 4.1). The bilirubin passes into the plasma where it binds to albumin as a complex. Hemoglobin breakdown accounts for more than 80% of the bilirubin formed.
- (2) Bilirubin (B) is then transported from the sinusoidal plasma across the hepatocyte membrane into the hepatic cytoplasm. In this process of hepatic cell "uptake" it is released from the albumin attachment.
- (3) Within the hepatocyte, bilirubin is transported to the site of conjugation – the endoplasmic reticulum (ER). Presumably this transport step involves attachment to one or more specific cytoplasmic proteins.
- (4) Within the hepatocyte, bilirubin is conjugated (esterified) sequentially with glucuronide into bilirubin monoglucuronide (BMG) and then bilirubin diglucuronide. The latter occurs in or adjacent to the bile capillary membrane. This conjugation renders the compound water-soluble and suitable for biliary secretion.
- (5) Most of the conjugated bilirubin (BDG) is transported across the hepatic-biliary membrane into the bile canaliculus, an energy-dependent, rate-limited enzymatic process.
- (6) As a constituent of bile, BDG is excreted into the duodenum.
- (7) Bacteria of the colon hydrolyze and reduce the BDG to colorless urobilinogen (UB) and other derivatives. The usual brown pigment of the feces is presumably due to degradation products of the various bile pigments.
- (8) A small amount of urobilinogen is absorbed from the colon into the portal venous circulation. Most if this is cleared by the liver into the bile, and then into the intestine (an enterohepatic circulation). From the plasma, a small amount is excreted into the urine (about 1% of the colonic content).

**4.1.2 Bile Salts:**

Bile acids and their salts are the major organic anions that are synthesized from cholesterol in the liver. They are conjugated to glycine or taurine and excreted by the liver into the bile. Detection of bile acids/salts in the plasma provides a useful index of impaired liver function, and more specifically reflects hepatic excretory function. Sensitive assays are becoming available for measuring plasma levels of endogenous bile salts, as well as the plasma clearance of radiolabeled bile salts. As these assays become more generally available, they will be sensitive indicators of liver dysfunction.

**4.1.3 Drugs:**

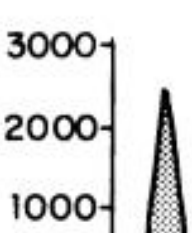
Clearance by the liver is especially dependent upon the efficiency of hepatocyte drug-metabolizing enzymes. These enzymes convert the parent compound into more polar, water-soluble derivatives, which are then usually excreted into bile.

**4.2 Tests Reflecting Hepatobiliary Injury**

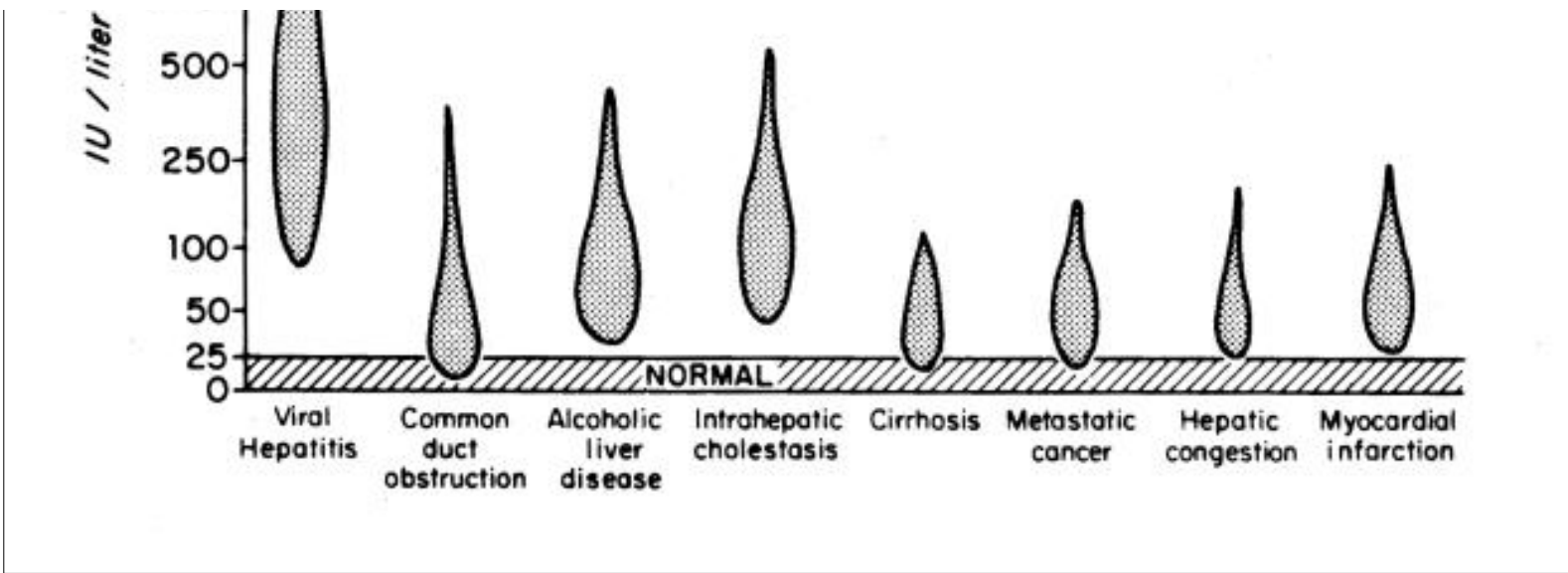
Following hepatocyte injury, the plasma concentration of hepatocyte intracellular enzymes rises. These enzymes vary in their degree of specificity for liver injury as compared with injury to other body organs, in their sensitivity of reflecting hepatocellular injury, and in the time and degree of their elevation following hepatocellular injury (Fig. 4.3).

Intracellular enzymes  
Tissue distribution, showing relative specificity for liver.  
(Note: Enzyme activities are given as % relative to that of liver (100%).)

|             | GGT    | AST  | ALT  |
|-------------|--------|------|------|
| Liver       | 100%   | 100% | 100% |
| Kidney      | 1000   | 63   | 42   |
| Heart       | ?      | 110  | 16   |
| Skeletal M. | -      | 70   | 11   |
| Pancreas    | 150    | 20   | 5    |
| Lung        | -      | 7    | 2    |
| GI tract    | Little | -    | -    |
| Prostate    | 300    | -    | -    |



RANGES, AST



**Figure 4.3**

#### 4.2.1 Aminotransferases:

*Serum transaminases reflect hepatocyte injury*

The serum aminotransferases – aspartate aminotransferase (AST) and alanine aminotransferase (ALT) – are the most commonly utilized indicators of hepatic injury and represent hepatocellular necrosis. Serum levels of AST and ALT are elevated to some extent in almost all liver diseases. Following liver cell injury, their plasma concentration rises – with highest levels seen in severe viral hepatitis, drug- or toxin-induced hepatitis, and ischemic hepatitis. Although levels may reflect the extent of hepatic necrosis, they do not correlate with eventual outcome.

#### 4.2.2 Alkaline Phosphatases:

*Elevated alk phos suggests cholestasis*

Alkaline phosphatase is a family of multimolecular forms (isoenzymes) that are found in the liver, small intestine, kidney, placenta, bone, and kidneys. Probably all of these tissues contribute to the normal plasma level. The enzyme is found in bile. In hepatic injury and biliary obstruction, plasma concentrations usually rise. These elevations are especially high in injuries and obstructions of the biliary channels (Fig. 4.4). Evidence indicates that this is due to induction of alkaline phosphatase synthesis rather than inability to secrete the enzyme into the bile.

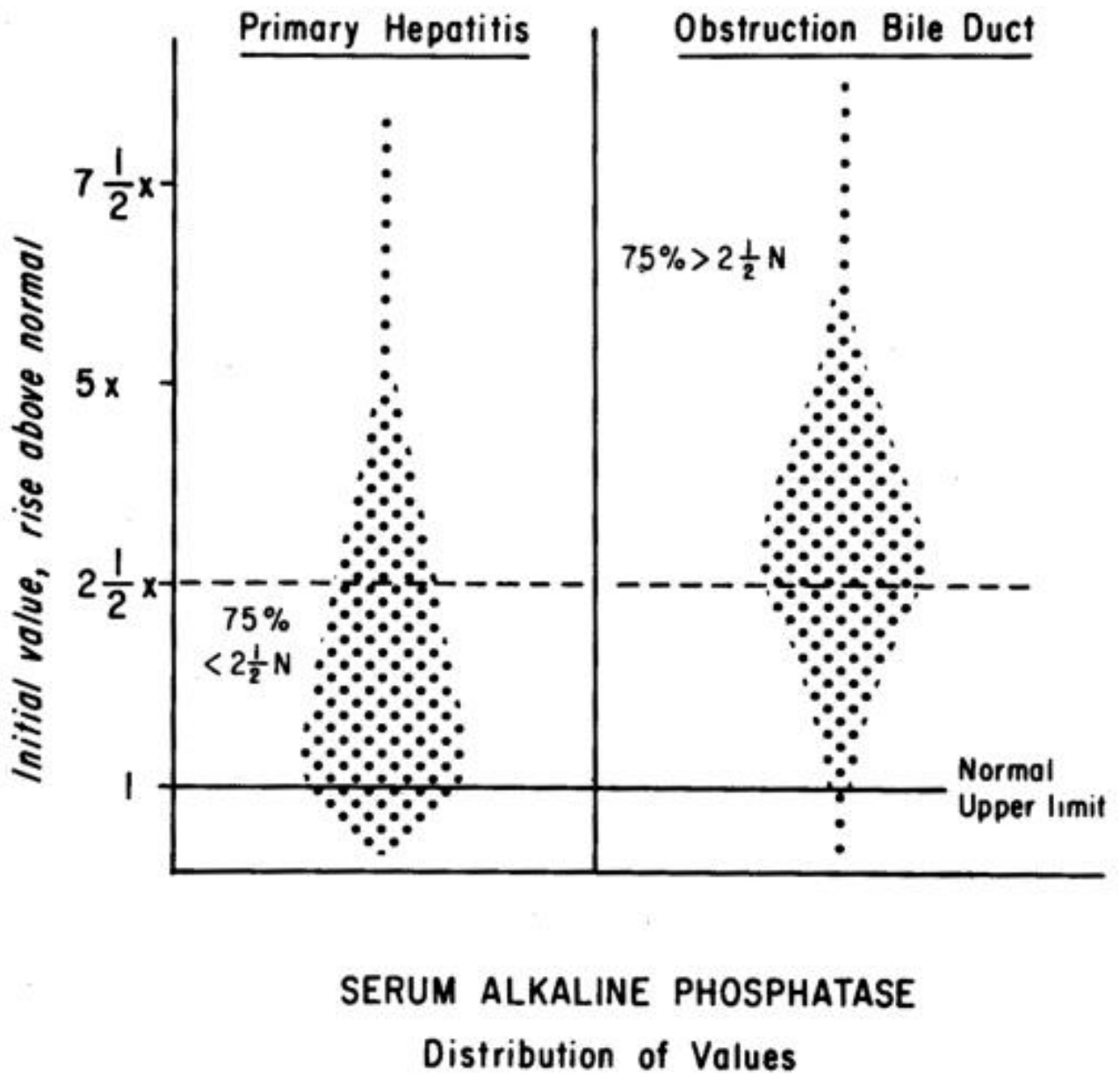


Figure 4.4

**4.2.3 Transpeptidases:**

$\gamma$ -glutamyl transpeptidase (GGT) has been localized to the entire hepatobiliary tree. It is a sensitive test for detecting hepatobiliary disease and is the most sensitive indicator of biliary tract disease. Its primary usefulness is limited to the exclusion of bone disease as the source of an elevated serum alkaline phosphatase level (GGT is not found in bone).

**4.3 Tests Measuring Hepatic Synthetic Function**

The liver is the only source of many proteins found in the body (including coagulation factors and albumin). The concentration of certain plasma proteins may be taken as an indirect measure of their synthesis rate.

**4.3.1 INR:**

*t*-1/2 of factor 7 is ~12 hours

Only the hepatocyte synthesizes coagulation factors 1 (fibrinogen), 2 (prothrombin), 5, 7, 9, and 10. The INR measures blood clotting as regulated by these factors and is prolonged when factors 1, 2, 5, 7, and 10 are deficient (either singly or in combination). All of these factors have very short half-lives and turn over in the plasma very rapidly. Acute or chronic liver injury may be reflected in a prolonged INR due to failure of hepatic synthesis. Other causes of prolonged INR include congenital deficiencies of coagulation factors, consumptive coagulopathies, drugs, and vitamin K deficiency. Vitamin K is essential for the synthesis of factors 2, 7, 9, and 10. Being a lipid, vitamin K requires the presence of bile salts in the upper intestine for efficient absorption. Vitamin K deficiency may complicate chronic liver disease either from decreased bile output or dietary intake.

#### **4.3.2 Serum Albumin:**

Albumin is quantitatively the most important protein in the plasma synthesized by the liver. It is a useful indicator of hepatic function. Its concentration is the composite result of hepatic synthesis rate, catabolism (including external loss), and equilibration with extravascular fluids.

Albumin is synthesized only by hepatocytes and has a relatively long half-life – about 17 days. Therefore, a decreased synthetic rate will not be detected by a change in plasma (serum) albumin concentration for 2-3 weeks. Thus, serum albumin tends to be normal in acute liver disease and decreased in chronic liver disease. The serum concentration in normal individuals ranges 35-45 g/L. In the setting of severe stress, sepsis, or multiple organ failure, however, various cytokines may affect albumin metabolism and a striking decrease in the serum concentration of albumin may be noted over a several day period (e.g. acute alcoholic hepatitis).

#### **4.4 Selection of Liver Enzyme and Function Tests**

Most physicians refer to all liver laboratory tests as "liver function tests." In truth, only the albumin and INR are actually tests of liver "function." The remainder are markers of hepatobiliary damage. Most tests are relatively insensitive for assessing the degree of hepatic injury or amount of hepatic functional reserve. As little as 1/4 to 1/3 of completely normal functioning livers may yield normal values for the usual laboratory tests. Although hundreds of different tests have been devised, a physician does best if he/she employs only a few. The clinical situations wherein tests of liver enzymes and function are most helpful are the: (a) differential diagnosis of jaundice; (b) detection of hepatic dysfunction in the non-jaundiced patient; (c) rough measure of the degree of hepatic dysfunction in a patient with known liver disease; and (d) assessment of progress of known hepatic illness under serial observation.

### **5. Igor's Approach to the Differential Diagnosis of Jaundice**

The object here is to identify the nature of the disturbance of bilirubin metabolism – such as unconjugated vs conjugated hyperbilirubinemia, and for the latter whether it is hepatocellular or cholestatic. The approach can be summarized by a series of questions and the appropriate tests to answer the question.

#### **Question 1: Is the jaundice a result of unconjugated or conjugated hyperbilirubinemia?**

##### **Test 1: Urine dipstick for bilirubin**

*Only conjugated bilirubin in urine!*

In hemolysis, the increased rate of production of bilirubin overwhelms the ability of the hepatocytes to take it up from plasma. Plasma retention, therefore, is chiefly of the unconjugated form of bilirubin. Since only conjugated bilirubin can appear in urine, the urine remains free of bilirubin. Specific tests for hemolysis can be done, such as reticulocyte count, plasma free hemoglobin, plasma methemalbumin, urinary urobilinogen, and hemosiderin.

Defects in bilirubin conjugation will give a similar picture. These include severe congenital defects, as well as the common and mild Gilbert's Syndrome.

An increase in conjugated (direct) bilirubin in plasma is a regular feature of both hepatitis and cholestasis. Since renal tubular reabsorption of conjugated bilirubin is so limited, conjugated bilirubin appears in the urine.

#### **Question 2: If conjugated, is the problem hepatocellular or cholestatic?**

##### **Test 2: Liver enzyme profile**

Laboratory differentiation between hepatitis and cholestasis depends upon determining if other hepatocyte functions are significantly disturbed, such as protein synthesis, or leakage rate of intracellular enzymes into the plasma.

Measurement of plasma alkaline phosphatase is also useful, because values rise statistically higher in conditions of biliary tract injury and obstruction. Diseases of the intrahepatic biliary ducts give increases similar to those found in extrahepatic bile duct obstructions.

Characteristically, conditions of non-alcoholic acute hepatocellular injury causing jaundice show:

- Plasma elevations of both conjugated and unconjugated bilirubin.
- A vigorous leak of hepatocyte intracellular enzymes into plasma, plasma transaminases usually more than ten times the upper limit of normal.
- A mild to moderate increase in plasma alkaline phosphatase, usually less than 2-3 times normal upper limits.
- If severe, prolongation of INR due to decreased concentration of clotting factors. If prolonged, INR is unresponsive to supplements of vitamin K.

In comparison, cholestatic jaundice shows:

- Plasma elevations of both conjugated and unconjugated bilirubin.
- Normal or only moderately increased plasma transaminase activity (up to 5-6 times upper limit of normal).
- Plasma alkaline phosphatase activity greater than 2-3 times normal.
- Normal INR. If prolonged, synthesis is rapidly restored by injection of vitamin K (correcting for its malabsorption in the absence of bile salts).

**Question #3: If cholestatic, is it intrahepatic or obstructive?**

### **Test #3: Ultrasound**

Pain on clinical presentation is often a clue to biliary obstruction due to choledocolithiasis (stones within the common bile duct). Ultrasound will assess whether there is dilation of the biliary system due to obstruction. This could be due to stone, tumour or stricture. Endoscopic retrograde cholangiopancreatography (ERCP) is a method of injecting contrast material into the duct for more specific assessment of the anatomy and can be used for intervention.