Liver disease

Liver

- The liver is the largest, most versatile organ in the body
- It consists of two main lobes that, together, weigh from 1400-1600 g in the normal adult
- It has an abundant blood supply receiving about 15 ml/minute from two major vessels: the hepatic artery and the portal vein
- The hepatic artery a branch of the aorta, contributes 20% of the blood supply and provides most of the oxygen requirement
- The portal vein, which drains the gastrointestinal tract, transports the most recently absorbed material from the intestine to the liver

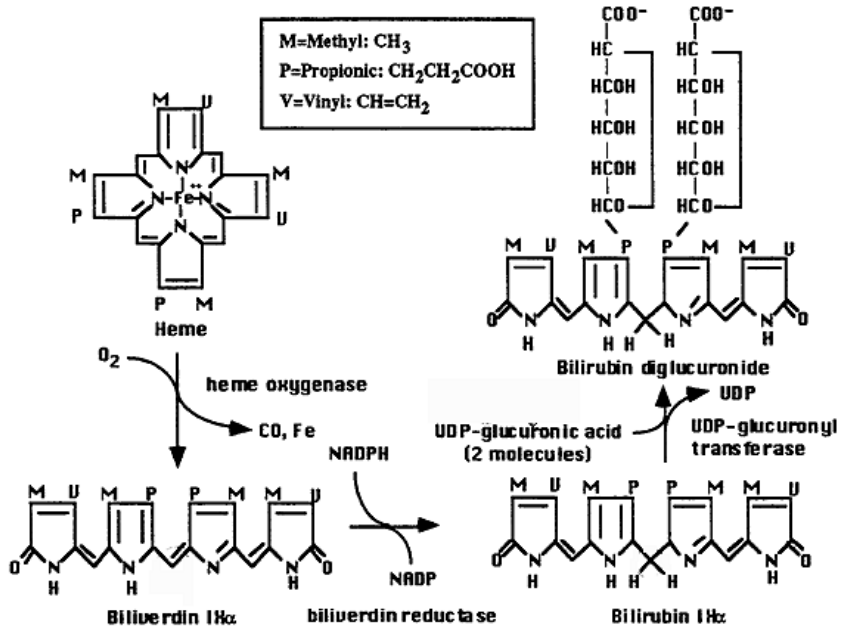
Liver function

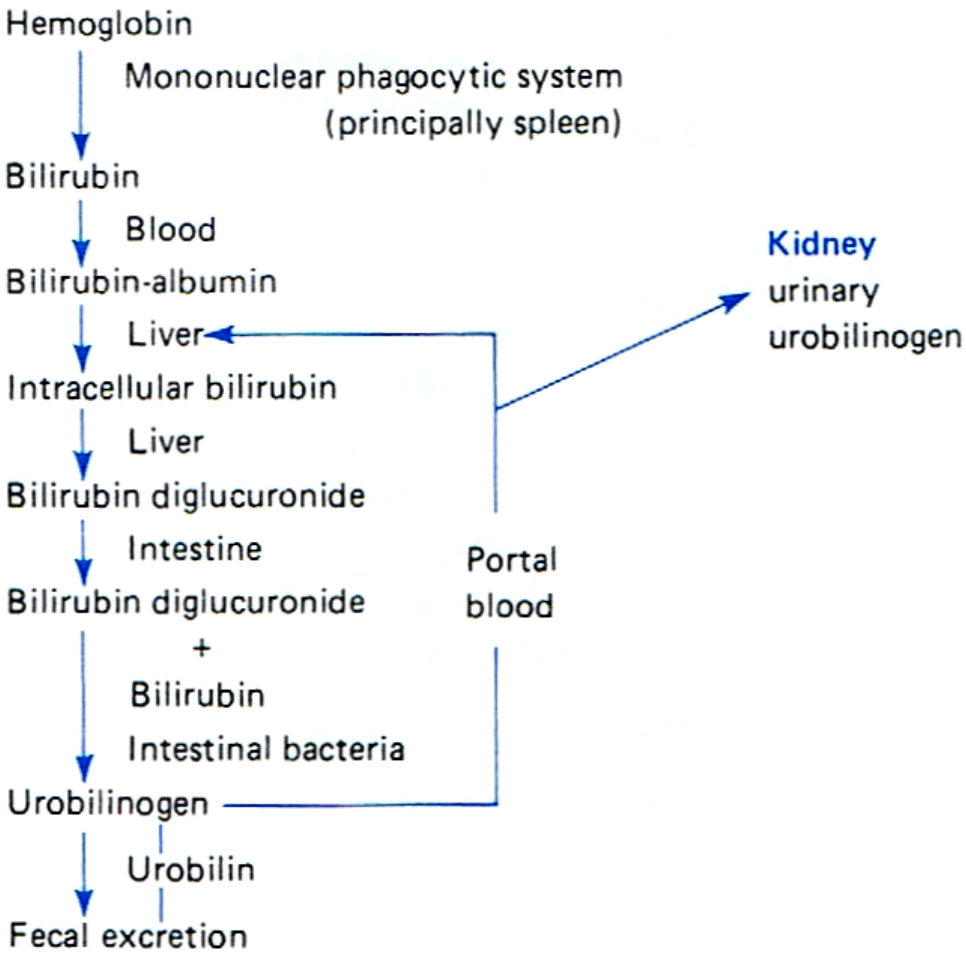
- **The excretion of bile**: Total bile production averages about 3 L per day, although only 1 L is excreted.
- The primary bile acids, cholic acid and chenodeoxycholic acid, are formed in the liver from cholesterol. The bile acids are conjugated with the amino acids glycine or taurine, forming bile salts.
- During fasting and between meals, bile acid pool is concentrated up to 10-fold in the gallbladder
- When the conjugated bile acids (salts) come into contact with bacteria in the terminal ileum and colon, dehydration to secondary bile acids occurs, and these secondary bile acids are subsequently absorbed
- The absorbed bile acids enter the portal circulation and return to the liver, where they are reconjugated and reexcreted. The enterohepatic circulation of bile occurs 2-5 times daily

Bilirubin

- The principal pigment in bile is derived from the breakdown of hemoglobin when aged red blood cells are phagocytized by the reticuloendothelial system, primarily in the spleen, liver, and bone marrow
- When hemoglobin is destroyed, the protein portion- globin-is reused by the body, The iron enters the body's iron stores and is also reused. The **porphyrin** is broken down as a waste product and excreted
- Bilirubin is transported to the liver in the bloodstream bound to albumin where it is conjugated with diglucuronide on it two carboxylic acids and excreted to the intestine
- The normal flora in the colon oxidize it further where it is reabsorbed, excreted to the stool (urobilin, reddish brown) or excreted in kidney (urobilinogen, colourless)

Bilirubin


M=Methyl: CH_3
P=Propionic: CH_2CH_2COOH
V=Vinyl: $CH=CH_2$
Heme
O_2
heme oxygenase
CO, Fe
Biliverdin IXα
NADPH
NADP
biliverdin reductase
Bilirubin IXα
UDP-glucuronic acid
(2 molecules)
UDP
UDP-glucuronyl
transferase
Bilirubin diglucuronide


Hemoglobin
Mononuclear phagocytic system
(principally spleen)
Bilirubin
Blood
Bilirubin-albumin
Liver
Intracellular bilirubin
Liver
Bilirubin diglucuronide
Intestine
Bilirubin diglucuronide
+
Bilirubin
Intestinal bacteria
Urobilinogen
Urobilin
Fecal excretion
Kidney
urinary
urobilinogen
Portal
blood

Bilirubin

- When the bilirubin concentration in the blood rises, the pigment begins to be deposited in the sclera of the eyes and in the skin. This yellowish pigmentation in the skin or sclera is known as jaundice, or icterus
- The cause can be:

  - **Prehepatic**: results when an excessive amount of bilirubin is presented to the liver for metabolism, such as in hemolytic anemia. This type of jaundice is characterized by unconjugated hyperbilirubinemia

  - The serum bilirubin levels rarely exceed 5 mg/dL because the normal liver is capable of handling most of the overload. bilirubin will not appear in the urine in this type of jaundice.

Bilirubin

- **Hepatic**: Impaired cellular uptake, defective conjugation, or abnormal secretion of bilirubin by the liver cell are the main causes of this kind of jaundice

- **Posthepatic**: results from the impaired excretion of bilirubin caused by mechanical obstruction of the flow of bile into the intestine. This may be due to gallstones or a tumor

- When bile ceases to flow into the intestine, there is a rise in the serum level of conjugated bilirubin and the stool loses its source of normal pigmentation and becomes clay-colored

- Conjugated bilirubin appears in the urine, and urine urobilinogen levels decrease

Major Synthetic Activity

- The liver plays an important role in **plasma protein production**, synthesizing albumin and the majority of the α and β-globulins. All the blood-clotting factors (except VIII) are synthesized in the liver
- The deamination of glutamate in the liver is the primary source of ammonia, which is then converted to **urea**
- Glycogenesis and gluconeogenesis, lipogenesis, metabolism of cholesterol into bile acids, Very-low-density lipoproteins (transport TG into the tissues), High-density lipoproteins, phospholipids are all made in the liver
- The formation of **ketone bodies**
- The liver is the storage site for all fat-soluble vitamins **(A, D, E, and K)** and several water-soluble vitamins, such as **B12** and is responsible for the conversion of carotene into **vitamin A**

Synthesis of liver enzymes

- Many enzymes are synthesized by liver cells, but not all of them have been found useful in the diagnosis of hepatobiliary disorders, this includes:
  - **Aspartate aminotransferase (AST)** and **alanine aminotransferase (ALT)** which escape into the plasma from **damaged liver** cells
  - **Alkaline phosphatase (ALP)** and **5'-nucleotidase (5NT):** induced or released when the canalicular membrane is damaged and **biliary obstruction** occurs
  - **γ-glutamyltransferase (GGT):** increased in both **hepatocellular** and **obstructive disorders**

Detoxification and Drug Metabolism

- The liver protects the body from **potentially injurious substances** absorbed from the intestinal tract and toxic by-products of metabolism.

- The most important mechanism in this detoxification activity is the **microsomal drug-metabolizing system** of the liver. It is responsible for many detoxification mechanisms, including oxidation, reduction, hydrolysis, hydroxylation, carboxylation, and demethylation that convert many insoluble compounds into other forms that are less toxic or more water-soluble and, so excretable by the kidney.

- Conjugation with moieties, such as glycine, glucuronic acid, sulfuric acid, glutamine, acetate, cysteine, and glutathione, occurs mainly in the cytosol or smooth ER. This mechanism is the mode of bilirubin and bile acid excretion.

Disorders of the liver
jaundice

- Jaundice, or icterus: is the yellowish discoloration of the skin and sclerae resulting from hyperbilirubinemia
- Although the upper limit of normal for total serum bilirubin is 1 mg/dL, jaundice is not clinically apparent until the bilirubin level exceeds 2-3 mg/dL
- In African American or Asian patients, yellowing of the sclerae may be the only clinical evidence of jaundice
- Except in infants, hyperbilirubinemia is generally well tolerated.
- In infants, hyperbilirubinemia (>15-20 mg/dL) may be associated with kernicterus (serious disorder of the CNS resulting from increased bilirubin levels) it only occurs in infants because the immature CNS does not have a well-developed blood-brain barrier

Jaundice

- Although all cases of jaundice result from hyperbilirubinemia, not all are caused by hepatic dysfunction.
- hyperbilirubinemia may also result from **erythrocyte destruction**, or hemolysis in patients with normal liver function
- Hypercarotenemia (excessive ingestion of vitamin A) may produce skin discoloration indistinguishable from that of hyperbilirubinemia. In hypercarotenemia, the sclerae are usually not discolored.

Cirrhosis

- Cirrhosis refers to the irreversible scarring process by which normal liver architecture is transformed into abnormal nodular architecture
- One way to classify cirrhosis is by the appearance of the liver (by the size of the nodules). These conditions are referred to as **macronodular** and **micronodular** cirrhosis, although **mixed** forms occur
- In the USA, Canada, and Western Europe, the leading cause of cirrhosis is alcohol abuse, which leads to a micronodular type of cirrhosis
- Other causes of cirrhosis include hemochromatosis, postnecrotic cirrhosis (occurs as a late consequence of hepatitis), and primary biliary cirrhosis (an autoimmune disorder).

Cirrhosis

- Cirrhosis is a serious disorder and one of the ten leading causes of death in the United States. It causes many complications:
  - **Portal hypertension** results when blood flow through the portal vein is obstructed by the cirrhotic liver. This may result in splenomegaly and esophageal varices (may rupture and lead to fatal hemorrhage)
  - The synthetic ability of the liver is reduced, causing **hypoalbuminemia** and deficiency of the clotting factors, which may lead to hemorrhage
  - **Ascitic fluid** may accumulate in the abdomen
  - Although some patients with cirrhosis are capable of prolonged survival, generally this diagnosis is an ominous one

Tumors

- On a worldwide basis, primary malignant tumors of the liver, known as hepatocellular carcinoma are an important cause of cancer mortality
- In the United States, these tumors are relatively uncommon. Most cases of hepatocellular carcinoma can be related to previous infection with a hepatitis virus.
- Liver is frequently involved secondarily by tumors arising in other organs. Metastatic tumors to the liver from primary sites, such as the lung, pancreas, gastrointestinal tract, or ovary, are common. Benign tumors of the liver are relatively uncommon
- Whether primary or secondary any malignant tumor in the liver is a serious finding with a poor prognosis
- The only hope For cure relies on surgical resection, which is usually impossible. Patients with malignancies of the liver usually have a survival measured in months

Reye's Syndrome

- Reye's syndrome is a disorder of unknown cause, involving the liver and arising primarily in children,
- It is a form of hepatic destruction that usually occurs following recovery from a viral infection, such as varicella (chickenpox) or influenza.
- It has been related to aspirin therapy. Shortly after the infection, the patient develops neurologic abnormalities, which may include seizures or coma
- Liver functions are always abnormal, but the bilirubin level is not usually elevated
- Without treatment, rapid clinical deterioration, leading to death, may occur

Drug- and Alcohol-Related Disorders

- Many drugs and chemicals are toxic to the liver. This toxicity may take the form of overwhelming hepatic necrosis, leading to coma and death, or it may be subclinical and pass entirely unnoticed

- In small amounts, **alcohol** may cause mild, inapparent injury. Heavier consumption leads to more serious damage, and prolonged, heavy use may lead to cirrhosis (exact amount is unknown)

- Certain **drugs**, including tranquilizers (phenothiazines), certain antibiotics, antineoplastic agents, and anti-inflammatory drugs, may cause liver injury

- Usually this is mild and manifested only by elevation of liver function tests, which return to normal when the drug is discontinued. This may lead to massive hepatic failure or cirrhosis

- The most common drug associated with serious hepatic injury is **acetaminophen**. When taken in massive overdose, it produces fatal hepatic necrosis unless rapid treatment is initiated

Assessment Of Liver Function
Analysis of Bilirubin (Method Selection)

- Unfortunately, no single method for the determination of bilirubin will meet all the requirements of the clinical laboratory
  - For the evaluation of jaundice in newborns (no lipochromes), the direct spectrophotometric method is satisfactory
  - The sources of error in this technique are turbidity, hemolysis, and yellow lipochrome pigments
  - Hemolysis and turbidity can be blanked out by measuring a second wavelength, but the yellow lipochromes cannot be blanked out.
  - In patients older than 1 month, a diazo-colorimetric procedure is necessary

jendrassik-Grof Method for Total and Conjugated Billirubin Determination

- Serum or plasma is added to a solution of sodium acetate and caffeine-sodium benzoate, which is then added to diazotized sulfanilic acid to form **purple azobilirubin**
- The **sodium acetate** buffers the pH of the diazotization reaction, whereas the **caffeine-sodium benzoate** accelerates the coupling of bilirubin with diazotized sulfanilic acid
- This reaction is terminated by the addition of **ascorbic acid**, which destroys the excess diazo reagent
- A **strongly alkaline tartrate** solution is then added to convert the purple azobilirubin to blue azo-bilirubin, and the intensity of the color is read at 600 nm
- A **fasting** serum specimen, which is **neither hemolyzed** nor **lipemic**, is preferred.
- The specimen should be **kept in dark** after collection, analyzed within 3hrs, kept for 1 week in the refrigarator or 3 months in -20

Reference range

- Normal blood contains no conjugated bilirubin.
- Some conjugated bilirubin is reported as normal because current available methodology picks up some of the total bilirubin as a false positive
- For **adults**
  - Conjugated: 0-0.2 mg/dL (0-3 µmoI/L)
  - Unconjugated: 0.2-0.8 mg/dL (3-14 µmol/L)
  - Total: 0.2-1.0 mg/dL (3-17 µmol/L)
- For **infants**

INFANTS	PREMATURE, TOTAL	FULL TERM, TOTAL
24 hours	1–6 mg/dL	2–6 mg/dL
48 hours	6–8 mg/dL	6–7 mg/dL
3–5 days	10–12 mg/dL	4–6 mg/dL

Direct Spectrophotometric Method for Determination of Total Bilirubin in Serum

- The absorbance of bilirubin in serum at 455 nm is proportional to its concentration
- The serum of newborns does not contain lipochromes, such as carotene, that would increase the absorbance at **455 nm**. The absorbance of hemoglobin at 455 nm is corrected by subtracting the absorbance at **575 nm**.
- Error will be introduced if the buffer is turbid. Because the method depends on the extinction coefficient of bilirubin, **all volumes must be accurate** and **cuvettes must be flat-surfaced**, with a path length of exactly 1 cm
- This method is relatively insensitive to hemolysis, which is often present in specimens obtained from infants, due to difficulty in skin puncture technique
- it is significantly affected by the presence of lipochromes and so cannot be used in infants older than a few months of age

Urobilinogen in Urine and Feces

- Urobilinogin is a colorless end product of bilirubin metabolism that is oxidized by intestinal bacteria to the brown pigment urobilin
- In the normal individual, part of the urobilinogen is excreted in the **feces**, and the remainder is reabsorbed into the portal blood and returned to the **liver.** A small portion that is not taken up by the hepatocytes is excreted by the **kidney** as urobilinogen
- Increased levels of urinary urobilinogen are found in hemolytic disease and in defective liver-cell function, such as hepatitis
- **Absence of urobilinogen** from the urine and stool is most often seen with complete **biliary obstruction.** Fecal urobilinogen is also decreased in biliary obstruction and in hepatocellular disease

Urobilinogen in Urine and Feces

- Most quantitative methods for urobilinogen are based on the reaction of this substance with **p-dimethylaminobenzaldehyde** to form a red color.
- Major improvements were made by using **alkaline ferrous hydroxide** to reduce urobilin to urobilinogen and added **sodium acetate** to eliminate interference from such compounds as indole
- The use of **petroleum ether** rather than diethyl ether for the extraction of urobilinogen was introduced to help in the removal of other interfering substances

Determination of Urine Urobilinogen (Semiquantitative)

- Principle.
  - Urobilinogen reacts with p-dimethyl aminobenzaldehyde (Ehrlich's reagent) to form a red color, which is then measured spectrophotometrically.
  - Ascorbic acid is added as a reducing agent to maintain urobilinogen in the reduced state.
  - The use of saturated sodium acetate stops the reaction and minimizes the combination of other chromogens with the Ehrlich's reagent.

- Specimen
  - A fresh 2-hour urine is collected which should be kept cool and protected from light.

Sources of Error

- The results of this test are reported in **Ehrlich units** rather than in milligrams of urobilinogen because of interfering substances

- Compounds, other than urobilinogen, that may be present in the urine and react with Ehrlich's reagent include porphobilinogen, sulfonamides, procaine, and 5-hydroxyindoleacetic acid. Bilirubin will form a green color and, therefore, must be removed, as previously described

- Fresh urine is necessary and the test must be performed without delay to prevent oxidation of urobilinogen to urobilin. Similarly the spectrophotometric readings should be made within 5 minutes after color production because the urobilinogen-aldehyde color slowly decreases in intensity.

Reference Range: Urine urobilinogen, 0.1-1.0 Ehrlich units/2 hr or 0.5-4.0 Ehrlich units/day (0.8 - 6.8 mmol/day); 1 Ehrlich unit is equivalent to approximately 1 mg of urobilinogen

Fecal Urobilinogen

- Visual inspection of the feces usually suffices to detect decreased urobilinogen.
- The semiquantitative determination of fecal urobilinogen is available It is carried out in an aqueous extract of fresh feces, and any urobilin present is reduced to urobilinogen by treatment with alkaline ferrous hydroxide before Ehrlich's reagent is added
- A range of 75-275 Ehrlich units/100 g of fresh feces or 75-400 Ehrlich units per 24-hour specimen is considered a normal reference range.

Measurement of Serum Bile Acids

- Unfortunately; complex methods are required for the analysis of bile acids in serum

- they involve extraction with organic solvents, partition chromatography; gas chromatography-mass spectroscopy spectrophotometry ultraviolet light absorption, fluorescence, radioimmunoassay and enzyme immunoassay methods

- Although serum bile acid levels are elevated in liver disease, the total concentration is extremely variable and adds no diagnostic value to other tests of liver function.

Enzyme Tests in Liver Disease

- Any injury to the liver that results in cytolysis and necrosis causes the liberation of various enzymes.
- The most common enzymes assayed in hepatobiliary disease include ALP and the aminotransferases.
- Used less often are γ-glutamyltransferase, lactate dehydrogenase (LD) and its isoenzymes, 5'-nucleotidase, ornithine carbamoyltransferase, and leucine aminopeptidase

Enzyme Tests in Liver Disease

Alkaline Phosphatase: in the clinical diagnosis of **bone** and **liver** disease.

- The most striking elevations occur in extrahepatic biliary obstruction, such as a stone in the common bile duct, or in intrahepatic cholestasis, such as drug cholestasis or primary biliary cirrhosis. This enzyme is almost always increased in metastatic liver disease and may be the only abnormality on routine liver function tests.
- The enzyme is found in **placenta**, and **pregnant** women also have elevated levels

Aminotransferases (Transaminases)

- AST and ALT are two enzymes widely used to assess hepatocellular damage. AST is found in all tissue, especially heart, liver, and skeletal muscle.
- ALT is more “liver specific”

Enzyme Tests in Liver Disease

5'-Nucleotidase: is another phosphatase in the liver and used clinically to determine whether an ALP elevation is caused by liver or bone disease

- This enzyme is much more sensitive to metastatic liver disease than is ALP because, unlike ALP, its level is **not** significantly elevated in other conditions, such as **pregnancy** or **childhood**

- Some increase in its activity may occur after **abdominal surgery**

Enzyme Tests in Liver Disease

- **γ-Glutamyltransferase (GGT)**: high in kidney and the liver and is elevated in the serum of almost all patients with hepatobiliary disorders
  - It is not specific for any type of liver disease but is frequently the first abnormal liver function test demonstrated in the serum of persons who consume large amounts of alcohol
  - The highest levels are seen in biliary obstruction
  - Sensitive test for alcoholic liver disease
  - Measurement of this enzyme is also useful if jaundice is absent for the confirmation of hepatic neoplasms and to confirm hepatic disease in patients with elevated ALP

- **Leucine Aminopeptidase**: widely distributed in human tissue, is found in the pancreas, gastric mucosa, liver, spleen, brain, large and small intestine, and kidney.
  - The serum activity of leucine aminopeptidase cannot be used to differentiate hepatocellular from obstructive jaundice.
  - The measurement of this enzyme does not provide any useful information

Enzyme Tests in Liver Disease

- **Lactate Dehydrogenase**: Measurement of total serum LD is usually not helpful diagnostically because LD is present in all organs and released into the serum from various tissue injuries
- Fractionation of LD into its five tissue-specific isoenzymes may give useful information about the site of origin of the LD elevation
- LD-5 is mostly present in liver and skeletal muscle. elevated LD-5 is noted in a patient with jaundice
- Moderate elevations of total serum LD levels are common in acute viral hepatitis and in cirrhosis, whereas biliary tract disease may produce only slight elevations
- High serum levels may be found in metastatic carcinoma of the liver.

Tests Measuring Hepatic Synthetic Ability

- The measurement of the end products of hepatic synthetic activity can be used to assess liver disease. Although these tests are not sensitive to minimal liver damage, they are useful in quantitating the severity of hepatic dysfunction

- Most serum proteins are produced by the liver. A decreased serum **albumin** may be a result of decreased liver protein synthesis. The albumin level correlates well with the severity of functional impairment and is found more often in chronic rather than acute liver disease. The serum **α-globulins** (α1-antitrypsin) tend to decrease with chronic liver disease

- Serum **γ-globulin** levels are transiently increased in acute liver disease and remain **elevated in chronic liver disease**. The highest elevations are found in **chronic active hepatitis** and postnecrotic cirrhosis.

- lgG and IgM levels are more consistently elevated in chronic active hepatitis, IgM in primary biliary cirrhosis, and IgA in alcoholic cirrhosis.

Tests Measuring Hepatic Synthetic Ability

- **Prothrombin time** is commonly increased in liver disease because the liver is unable to manufacture adequate amounts of clotting factor or because the disruption of bile flow results in inadequate absorption of vitamin K from the intestine

- Response of the prothrombin time to the administration of vitamin K is of some value in differentiating intrahepatic disease with decreased synthesizing capacity from extrahepatic obstruction with decreased absorption of fat-soluble vitamins.

- A marked prolongation of the prothrombin time indicates severe diffuse liver disease and a poor prognosis

Tests Measuring Nitrogen Metabolism

- The liver plays a major role in removing ammonia from the bloodstream and converting it to urea so in liver failure ammonia will increase leading to coma

- In brain, glutamate react with ammonia to give glutamine which increases in CSF to cause encephalopathy

Hepatitis

- inflammation of the liver, may be caused by viruses, bacteria, parasites, radiation, drugs, chemicals, or toxins.
- Among the viruses causing hepatitis are hepatitis types A, B, C, D (or delta), and E, **cytomegalovirus**, **Epstein-Barr** virus, and probably several others.
- Hepatitis A is usually transmitted by the **fecal/oral** route and causes a mild or inapparent infection with no tendency to chronic disease.
- Hepatitis B and C are primarily transmitted **parenterally**. Hepatitis B causes a serious illness in a minority of patients, however, in many patients, the infection is mild or even inapparent

Hepatitis

- Acute infection with hepatitis C is usually mild to inapparent
- Hepatitis B has a slight tendency to chronic disease, while most patients with hepatitis C infection develop chronic infection.
- Delta hepatitis is a unique satellite virus that causes a superinfection in patients already infected with hepatitis B.
- Hepatitis E is primarily transmitted by the **fecal/oral** route and causes serious disease **only** in **pregnant** women
- Chronic hepatitis is a major cause of morbidity and mortality worldwide
- Chronic hepatitis is a major risk factor for the development of hepatocellular carcinoma

Case study

CASE STUDY 22-1

The following laboratory test results were obtained in a patient with severe jaundice, right upper quadrant abdominal pain, fever, and chills (Case Study Table 22-1.1).

Question

1. What is the most likely cause of jaundice in this patient?

CASE STUDY TABLE 22-1.1. LABORATORY RESULTS

Serum alkaline phosphatase	4 times normal
Serum cholesterol	Increased
AST (SGOT)	Normal or slightly increased
5′-Nucleotidase	Increased
Total serum bilirubin	25 mg/dL
Conjugated bilirubin	19 mg/dL
Prothrombin time	Prolonged but improves with a vitamin K injection

Case study

CASE STUDY 22-2

The following laboratory test results were found in a patient with mild weight loss and nausea and vomiting, who later developed jaundice and an enlarged liver (Case Study Table 22-2.1).

Question

1. What disease process is most likely in this patient?

CASE STUDY TABLE 22-2.1. LABORATORY RESULTS

Total serum bilirubin	20 mg/dL
Conjugated bilirubin	10 mg/dL
Alkaline phosphatase	Mildly elevated
AST (SGOT)	Significantly elevated
ALT (SGPT)	Moderately elevated
Albumin	Decreased
γ-Globulin	Increased