liver function tests

ANATOMY OF LIVER

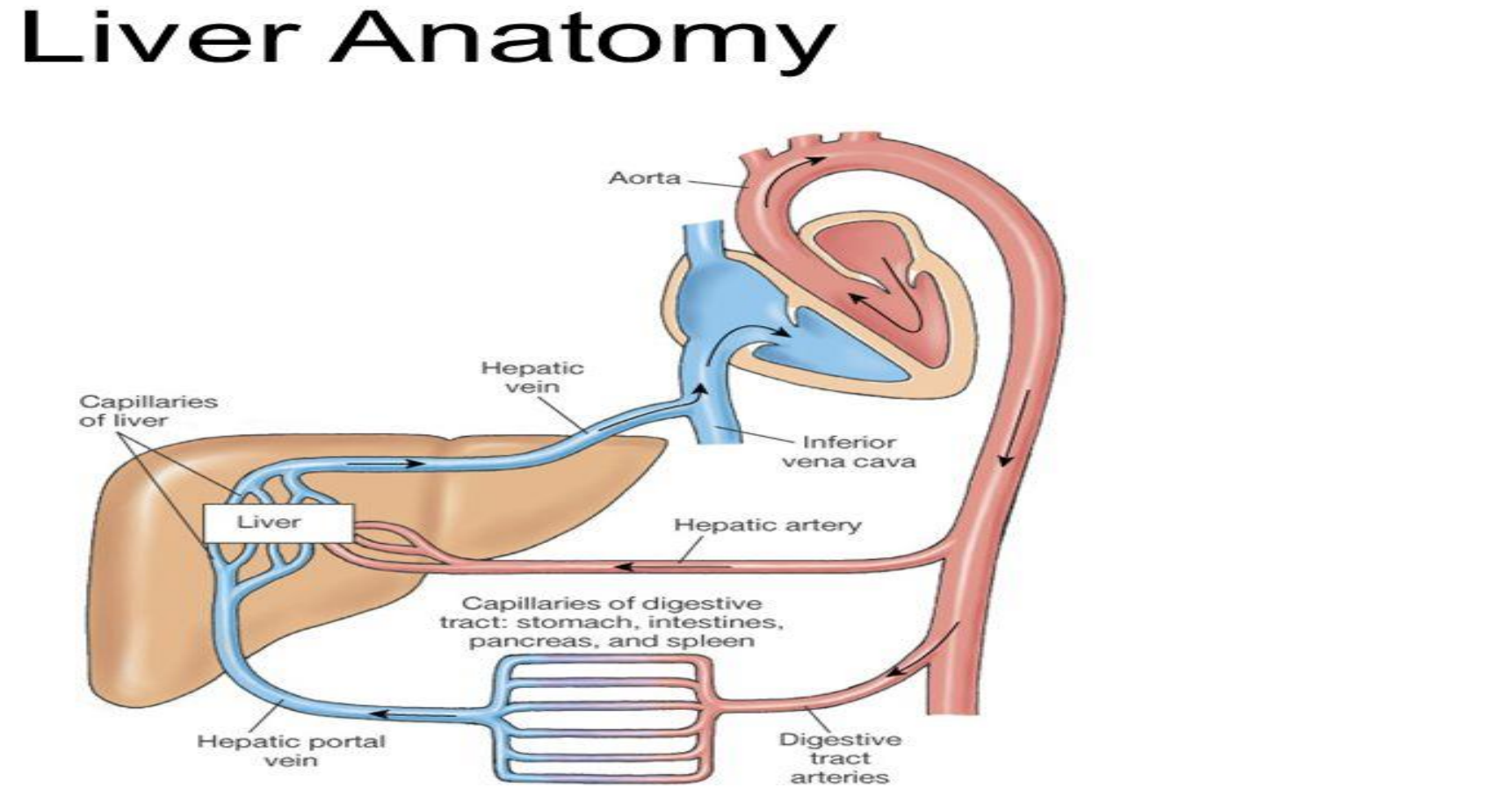
Liver is the largest organ in the human body (~1.5 kg in adults).

- **It is located in the** right upper quadrant of the abdomen and is attached by ligaments to the diaphragm.
- It has an abundant blood supply :

- **1. The portal vein**: delivers blood from the small intestine to the liver. This blood has the nutrients and other substances absorbed by the intestinal epithelial cells.
- **2. The hepatic artery**: delivers oxygenated blood from the heart to the liver.
- **3. The hepatic vein**: delivers deoxygenated blood from the liver to the heart.

- Liver has **reserve functional power** and can operate effectively when most of the hepatocytes are not working well. In addition, diseased hepatocytes can actually **regenerate** and return to normal function.
- The liver is unique in the sense that it is a relatively resilient organ that can regenerate cells that have been destroyed by some short-term injury or disease. However, if the liver is damaged repeatedly over a long period of time, it may undergo irreversible changes that permanently interfere with its essential functions.

MAJOR HEPATIC BLOOD VESSELS



FUNCTIONS OF LIVER

1) Metabolic Functions:

Carbohydrates (Glycogenesis, Glycogenolysis, Gluconeogenesis).

Lipids (beta-Oxidation, Ketogenesis, synthesis of TAG, VLDL, HDL and apolipoproteins).

Proteins (Deamination of AA, Urea cycle, synthesis of nonessential amino acids).

2) Storage Functions: (Vit-A, Vit-D, Vit-B12, Glycogen and iron)

3) Synthetic Functions: (Albumin, clotting factors and all plasma proteins except Ig)

4) Excretory Functions: (Bilirubin, Cholesterol)

5) Detoxification (Drugs, Ammonia, Steroids, …. etc)

6) Hematological functions (Blood formation, Blood coagulation)

THE MAIN TYPES OF LIVER DISEASE INCLUDE:

- **1. Cirrhosis.**
- **2. Hepatitis.**
- **3. Liver failure.**
- **4. Liver Cancer.**
- **5. Wilson’s disease.**
- **6. Hemochromatosis.**

- Alcohol is a major cause of liver disease and accounts for 20% to 50% of the prevalence of cirrhosis of the liver.
- Liver is a common site of secondary metastases from a wide variety of primary tumors.
- Wilson’s disease: a disorder of copper metabolism that results in deposition of copper in body tissues including liver.
- Hemochromatosis: a disorder of iron metabolism that results in deposition of iron in body tissues including liver.

SYMPTOMS OF LIVER DISEASE:

- The main symptoms of liver disease include:

1- Jaundice.

2- Ascites.

3- Hypoglycemia.

4- Nausea, vomiting, fatigue, weigh loss…

ASSESSMENT OF LIVER FUNCTIONS - LIVER FUNCTION TESTS (LFT):

- LFTs are a group of blood tests used to assess liver injury rather than liver function.
- **- They do not assess quantitatively the capacity of liver to do its functions.**
- **-** They are measurements of blood components that provide a lead to the type of liver damage

1. METABOLIC FUNCTIONS:

- **1.a. Hepatic enzymes:** hepatic enzymes may elevate in serum in case of liver injury (recall that intracellular enzymes are released to blood at a higher rate in case of tissue injury due to cells necrosis).

- Hepatic enzymes:

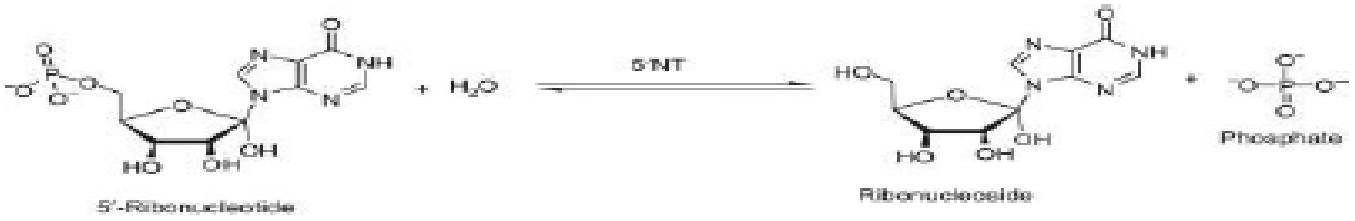
The most clinically useful include enzymes:

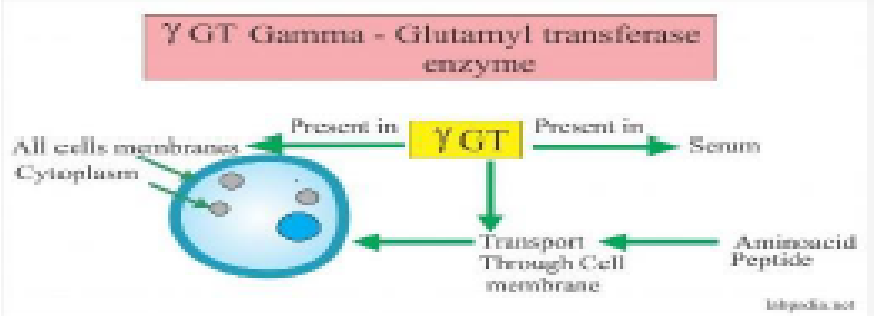
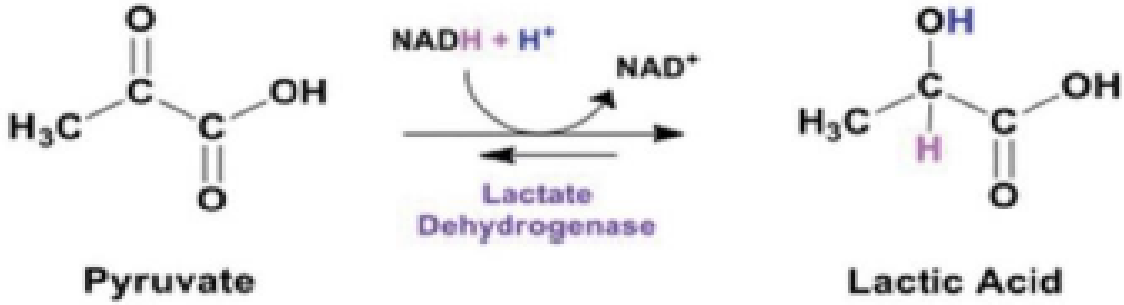
- **1.** Alanine aminotransferase (**ALT**) and Aspartate aminotransferase (**AST)**.
- **2.** Alkaline phosphatase (**ALP**).
- **3. 5-Neucleotidase (5'-NT).**
- **4.** γ-glutamyltransferase (**GGT**).
- **5.** Lactate dehydrogenase-5 (**LDH-5**).

- Biochemical assessment of ALT and AST is widely used for the evaluation of hepatic functions. ALT is more specific for liver functions, whereas AST can be elevated in conditions other than hepatic disorders including myocardial infarction, pancreatitis, muscle injury, renal failure, anemia… Thus ALT/ AST ratio is useful in differential diagnosis of hepatic against these diseases. **Normal ALT/AST ratio ≈ 1.15**.
- Remember that ALP has two major isozymes; one in liver and the other in bones. Thus, total ALP can be elevated in case of both bone and liver disease.

- Remember that ALT is more specific to liver than AST and is more *affected* by deterioration in liver functions compared to AST, whereas AST can be elevated in conditions other than liver disease.

HEPATIC ENZYMES, THEIR FUNCTIONS AND CATALYSED REACTIONS

Enzyme	Function	Catalyzed reaction
ALT (SGPT)	transfer of an amino group from alanine to α-ketoglutarate reversibly	
AST (SGOT)	transfer of an α-amino group between aspartate and glutamate reversibly	
ALP	Dephosphorylation	
5'-NT	hydrolysis of a nucleotide into a nucleoside and a phosphate. (A nucleotide + H_2O = a nucleoside + phosphate.)	

Enzyme	Function	Catalyzed reaction
GGT	transfer of gamma-glutamyl functional groups from molecules such as glutathione to an acceptor that may be an amino acid, a peptide or water (forming glutamate).	(5-L-glutamyl)-peptide + an amino acid ⇌ peptide + 5-L-glutamyl amino acid 
LDH	oxidation of lactate to pyruvate and the reversible reduction of lactate to pyruvate using NAD+/NADH respectively.	

2. SYNTHETIC FUNCTIONS:

- A healthy functioning liver is required for the synthesis of serum proteins (except the immunoglobulins). The measurement of serum proteins, therefore, can be used to assess the synthetic ability of the liver. Although these tests are not sensitive to minimal liver damage, they may be useful in quantitating the severity of hepatic dysfunction.
- **1. Albumin**
- **2. globulin**
- **3. Prothrombin**.

ALBUMIN AND GLOBULINS

- Globulins are a group of proteins in your blood. They are made in your liver by your immune system. Globulins play an important role in liver function, such as fighting infection. There are four main types of globulins
- liver synthesizes albumin which is the major plasma protein responsible for maintaining a balanced oncotic pressure. Impaired liver functions result in decreased albumin synthesis which results in edema. Liver disease results in a special type of edema where body fluids accumulate in the abdominal cavity; a condition termed "**ascites**":
- Impaired liver functions → ↓ albumin synthesis → Hypoalbunemia → disturbance in oncotic pressure → edema (Ascites).

- **Prothrombin** is a major clotting protein that is synthesized by the liver. Impaired liver functions can lead to low prothrombin production which increases the susceptibility to bleeding.
- **Alfa-fetoprotein (AFP)** is a fetal protein that is produced by the fetus liver during development. In adults, the level of this protein is very low under normal conditions. However, under hepatic malignant conditions this protein is elevated indicating liver cancer.

3. DETOXIFICATION:

- The liver plays a major role in detoxifying toxic substances either natural or foreign (xenobiotics). One of the most dangerous natural metabolites that should be detoxified is ammonia. Liver is responsible for ammonia detoxification via converting it to urea which finally excreted by the kidney in urine. In liver failure, ammonia and other toxins increase in the bloodstream and may ultimately cause hepatic coma (**Hepatic encephalopathy**)
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LIVER FUNCTION TESTS

The biochemical investigations can assist in differentiating the following:

Blood Tests	Clinical implication of abnormality
Bilirubin	(Excretory function) Cholestasis or biliary obstruction
Alkaline phosphatase (ALP)	
γ-glutamyl transferase (GGT)	
Alanine Transaminase (ALT)	Hepatocellular damage
Aspartate Transaminase (AST)	
Albumin	Synthetic function
Prothrombin time (PT)	

BILIRUBIN

- **Bilirubin** is a yellow compound that results from the breakdown of heme. Bilirubin is an open ring derived from the larger heme porphyrin ring. Bilirubin is a lipid - soluble compound. bilirubin is excreted in feces and urine, and elevated levels in blood, feces or urine may indicate diseases of liver, gallbladder or blood.
- Hyperbilirubinemia results in jaundice, a yellowish pigmentation of the skin and whites of the eyes due to high bilirubin levels. In newborns, bilirubin may penetrate blood -brain barrier causing damage to brain cells.

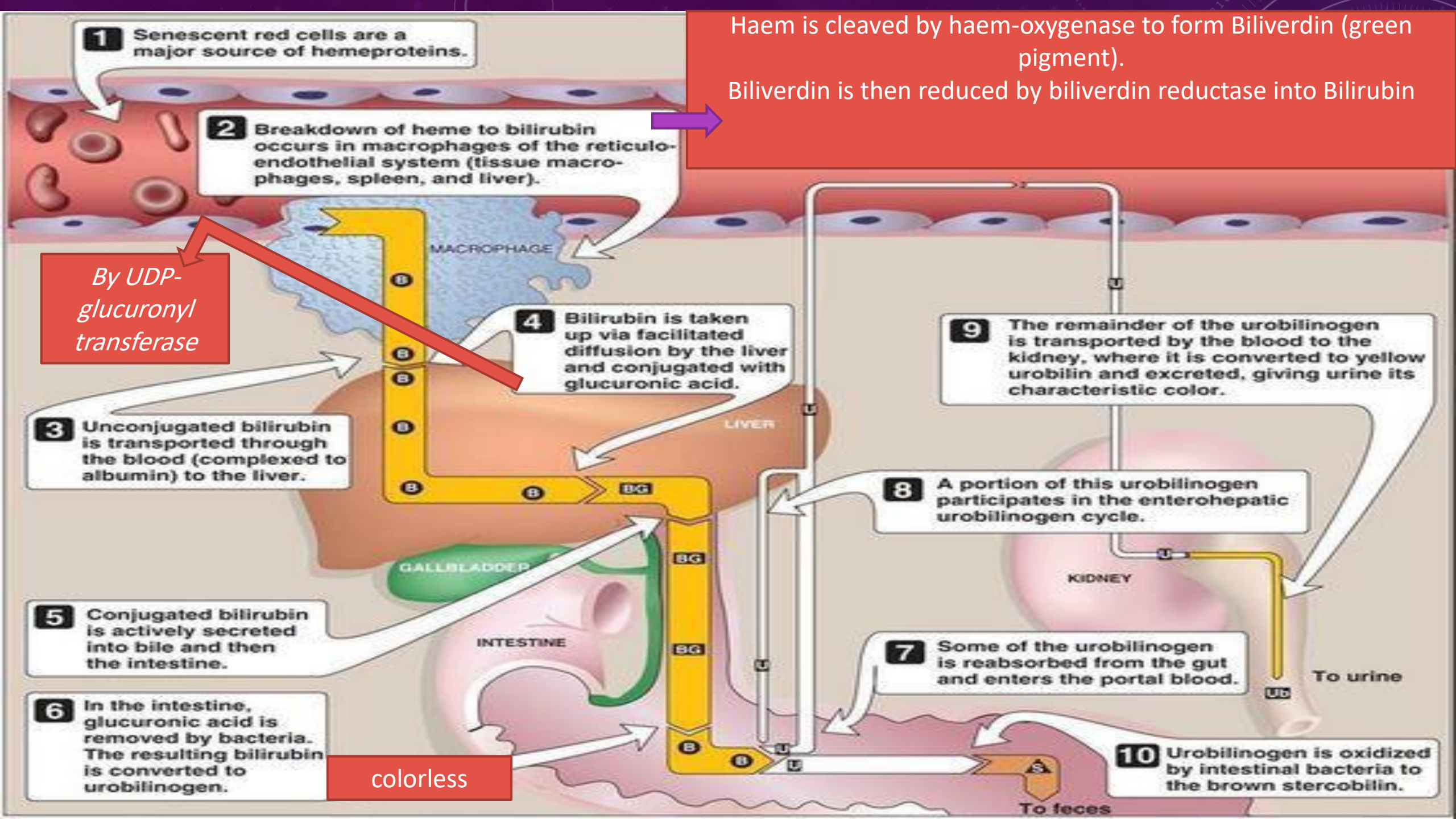
- Bilirubin is derived from Haem, mainly found in Haemoglobin of RBCs
- **- The average life span of red blood cells is 120 days.**
- - At the end of this time, they are removed from circulation by reticulo-endothelial cells in liver, spleen and bone marrow where they are haemolysed and haemoglobin released.
- **- Globin molecule is hydrolysed into free amino acids.**
- **- Haem gives iron and bilirubin as follows**

- 1) Haem is cleaved by haem-oxygenase to form Biliverdin (green pigment) and iron is removed for re-use.
- 2) Biliverdin is then reduced by biliverdin reductase into Bilirubin.
- 3) Transport of bilirubin in the plasma:
- Bilirubin is non-polar, insoluble in plasma; therefore it binds by non-covalent bonds to albumin to form unconjugated or indirect bilirubin.
- 4) Uptake of bilirubin by the liver:
- Bilirubin dissociates from the albumin molecule and enters hepatocytes. Bilirubin is conjugated with one or two molecules of glucuronic acid to form conjugated bilirubin (Direct bilirubin) by *UDP-glucuronyl transferase* (25% bilirubin mono-glucuronoid and 75% bilirubin di-glucuronoid).

- 5) Secretion of bilirubin into bile:
- Conjugated bilirubin is transported into bile canaliculi then into the bile.
- 6) Formation of urobilin in the intestine:
- a) Removal of glucuronides.
- b) Reduction of bilirubin to colourless compounds called urobilinogens.

- Intestinal bacteria act on conjugated bilirubin leading to:
- 7) Excretion of urobilinogens in stool and urine:

- Most of urobilinogens are oxidized to coloured stercobilin which excreted in stool giving its characteristic brown colour.
- Part of urobilinogens are reabsorbed to the liver then to blood to be excreted by the kidney in urine and converted to urobilin.
- Urobilin, gives the characteristic yellow colour of urine.



Haem is cleaved by haem-oxygenase to form Biliverdin (green pigment).

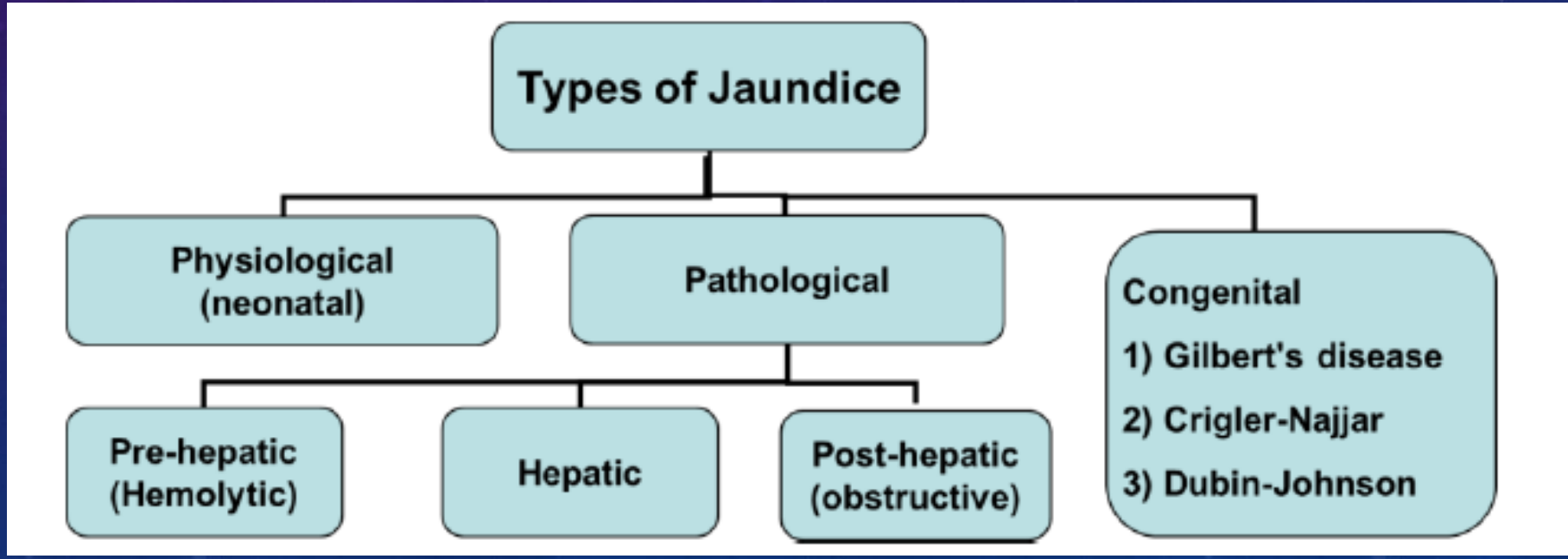
Biliverdin is then reduced by biliverdin reductase into Bilirubin

Bilirubin Is End Product Of Haem Catabolism And Present In 2 Forms Unconjugated & Conjugated.

Direct (Conjugated)	Indirect (Unconjugated)
Present normally in bile	Present normally in plasma
Has small M.Wt. and if present in plasma can be filtered by kidney	Has high M.Wt. can not be filtered through the kidney
Conjugated to glucuronic acid	Attached non-covalently to albumin
Polar, soluble in plasma & can not cross blood brain barrier	Non-polar, insoluble in plasma & can cross blood brain barrier in infants causing brain damage (Kernicterus)
Give Direct Van den Bergh reaction	Give Indirect Van den Bergh reaction

JAUNDICE (ICTERUS)

- - It is a yellow discoloration of skin and sclera.
- - It is due to increase plasma bilirubin above 3.0 mg% (50 µmol/L)
- - Normal is < 1 mg% (17µmol/L).



PHYSIOLOGICAL JAUNDICE

It is transient condition occurs in some new-borns especially if they are premature.

- **-** Causes:
- 1) At birth, the liver contains very little UDP-glucuronyl transferase enzyme, which is responsible for conjugation of bilirubin
- 2) Accelerated hemolysis of RBCs
- 3) Presence of fetal hemoglobin
- **- Effects:** - This condition leads to increase the unconjugated (Indirect) bilirubin.
- - If unconjugated bilirubin exceeds the concentration which can be tightly bound to plasma albumin (20 - 25 mg/dl), free bilirubin can pass BBB causing **Kernicterus** (toxic encephalopathy) which can cause mental retardation and death.

- Treatment:
- - Phenobarbital (liver enzyme inducers) and oral glucose (converted into glucuronic acid) to aid UDP-glucuronyl transferase
- - If the concentration > 20 mg%, phototherapy should be used to break down bilirubin. Babies with neonatal jaundice are placed under blue fluorescent (wavelength around 450 nm) light (NOT UV). This results in transformation of bilirubin to more water-soluble isomers can be excreted into bile without need for conjugation.
- **- If the concentration >** 25 mg%, blood transfusion is necessary.

PATHOLOGICAL JAUNDICE

- 1) Pre-hepatic jaundice (Haemolytic jaundice):
- - It is characterized by increased unconjugated bilirubin. It occurs in all types of haemolytic anaemia (excessive destruction of RBCs inside blood vessels).
- - The increase of unconjugated bilirubin is more than the capacity of the liver.

- Causes :
- 1- hemolytic disorder: abnormal haemoglobin (sickle cell anaemia). RBC membrane defects (hereditary spherocytosis).
- 2- ineffective erythropoiesis: megablastic anaemia
- 3- Blood group incompatibilities : Rh factor or ABO systems commonly responsible.
- 4-Drugs : salicylates and sulphonamides leads to displace bilirubin from plasma albumin . Novobiocin inhibit UDP-glucuronyl transferase.

- 2) Hepatic (Hepatocellular jaundice):
- - It is due liver cell damage by cirrhosis, infective hepatitis, or toxins (Carbon tetrachloride, Paracetamol poisoning).
- - It is characterized by increased both direct and indirect bilirubin. Also liver enzymes (ALT and AST) are elevated

- 3) Post-hepatic (Cholestatic or obstructive jaundice):
- **-** Cholestasis (stoppage of bile flow) may be due to mechanical obstruction by gall stone in common bile duct or cancer head of pancreas (which exerts pressure on biliary tract). It is mostly of conjugated type.
- **- If the blockage is complete, both bilirubin and ALP are raised**

- If the blockage is partial, only ALP may by high while bilirubin is normal as the functioning part of the liver is sufficient to process and excrete the bilirubin
- **-** In obstructive jaundice bilirubin and bile salts are returns to blood. Increased bile salts in blood lead to Itching, because bile salts are irritant to sensory nerve. Bradycardia, because bile salts are toxic to cardiac muscles.

Differential Diagnosis of Jaundice

Type	Hemolytic (Pre-)	Hepatocellular (Hepatic)	Obstructive (Post-)
Total Bilirubin	↑	↑	↑
Conjugated Bilirubin	Normal	↑	↑
Unconjugated Bilirubin	↑	↑	Normal
ALP	Normal	Normal / ↑ later	↑
AST and ALT	Normal	↑	Normal
Urine color	Normal	Dark	Dark
Stool color	Normal	Normal/Pale	Pale

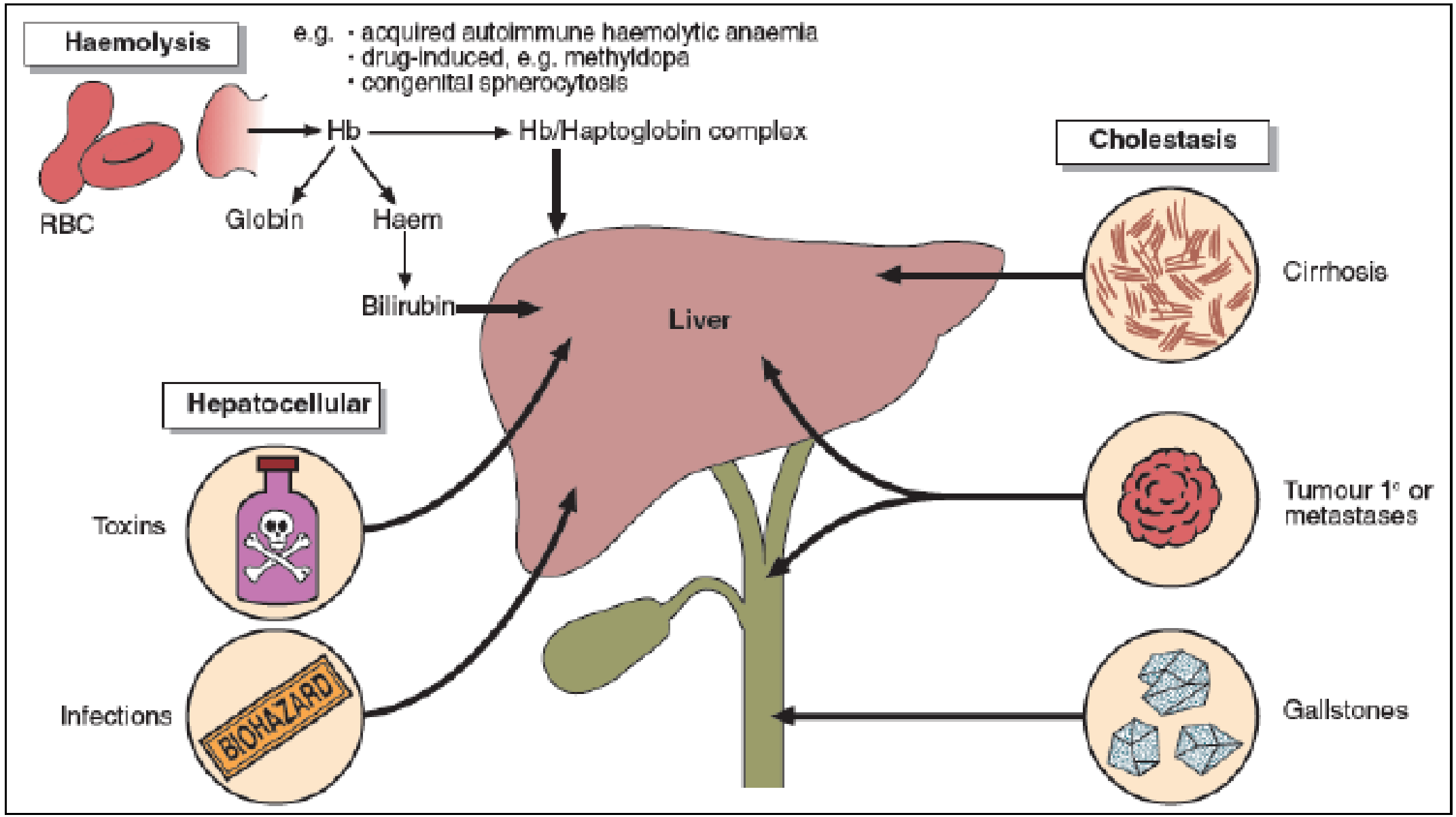


Figure 36: Types pf pathological jaundice

CONGENITAL JAUNDICE

- 1) Gilbert's Syndrome: It is asymptomatic mild unconjugated hyperbilirubinemia due to defective hepatic uptake of bilirubin. It is harmless and doesn't require treatment , however made worse by viral infections.
- Less than 3 mg%
- Caused by decreased expression of UDPGT (insertion of TA)

- 2) Crigler-Najjar syndrome: It is severe unconjugated hyperbilirubinemia due to absence of UDP-glucuronyl transferase enzyme (usually bilirubin concentration exceeds 20 mg/dl). It occurs in neonates leading to Kernicterus and often early death.

- 3) Dubin-Johnson syndrome: It is conjugated hyperbilirubinemia without elevation of ALT or AST which occurs during adult life due to defect in hepatic secretion of conjugated bilirubin into bile.

ALKALINE PHOSPHATASE (ALP)

- - ALP is found in cells lining biliary ducts.
- **- It is also found in bone, placenta, small intestine and kidneys.**
- **- In normal blood, the ALP activity is derived mainly from liver and bone**
- - Cholestasis, even for short duration, results in an increased to at least twice the upper limit of the reference interval.
- - Osteomalacia, rickets, children and pregnancy also show high level of ALP. However, an elevated GGT would suggest that the liver is the source of the increased ALP.

GAMMA-GLUTAMYL TRANSFERASE (GGT)

- **- GGT is found in bile ducts and kidneys.**
- - GGT level is raised in cholestasis. It's utilized as a supplementary test uses to confirm that elevated ALP comes from bile tract
- - Alcoholism results in an increased GGT serum level.

AMINOTRANSFERASES

- - These enzymes are located in liver cells and leak out into blood stream when liver cells are damaged.
- **1) Aspartate transaminase (AST):** is found normally in liver, heart, RBCs and muscle. AST elevates in diseases of liver, heart as myocardial infarction, and muscle as muscle trauma.
- **2) Alanine transaminase (ALT):** is found primarily in hepatocyte thus more specific for liver.

Serum levels of ALT and AST	Indicates
10 times the the upper limit of normal	Acute liver diseases
3-5 times the the upper limit of normal	Chronic liver diseases
Normal ALT level with high AST	Myocardial infarction

PLASMA ALBUMIN

- - Albumin is synthesized in the liver and its concentration in the plasma is in part a reflection of the functional capacity of the organ.
- - Plasma albumin concentration tends to decrease in chronic liver disease, but is usually normal in the early stages of acute hepatitis owing to its long half-life (approximately 20 days).
- - **Ascites:** an accumulation of fluid in the peritoneal cavity due to decrease in the plasma osmolality

PROTHROMBIN TIME

- - Liver is responsible for synthesis of prothrombin and other vitamin-K-dependent clotting factors.
- - In liver disease, there is a diminished synthesis, thus PT becomes longer (Normal PT: 10-13 seconds).
- - To decrease variability for lab to lab, PT obtained is compared to a normal patient's blood. This ratio is called International Normalized Ratio (INR).
- - INR of more than 1.2 is often an early feature of acute liver disease or vitamin-K deficiency.

LIVER DISEASES

- **▪ Acute liver disease**
- **-** Acute liver disease is usually caused by:
- 1) Viral infection (particularly with hepatitis viruses A, B, C, D and E but also Epstein–Barr virus and cytomegalovirus)
- 2) Toxins (e.g. alcohol, carbon tetrachloride, paracetamol).
- 3) Inadequate perfusion.
- **-** Patients may present with jaundice (icteric) but there is often without jaundice (pre-icteric) stage with relatively non-specific symptoms such as anorexia and malaise.

BIOCHEMICAL CHANGES DURING ACUTE HEPATITIS:

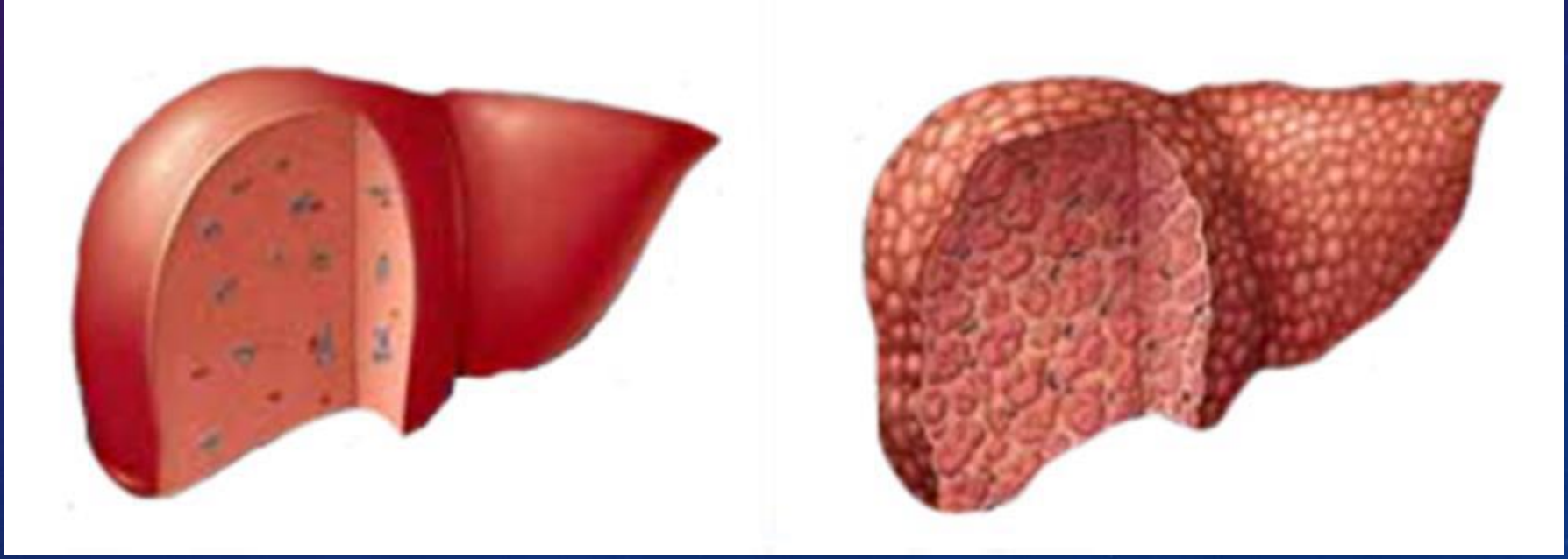
	Pre-icteric	icteric
Serum Bilirubin	Normal	↑
Aminotransferases	↑↑↑	↑
Serum ALP	Normal	↑
Urinary Bilirubin	↑	↑

CHRONIC LIVER DISEASE

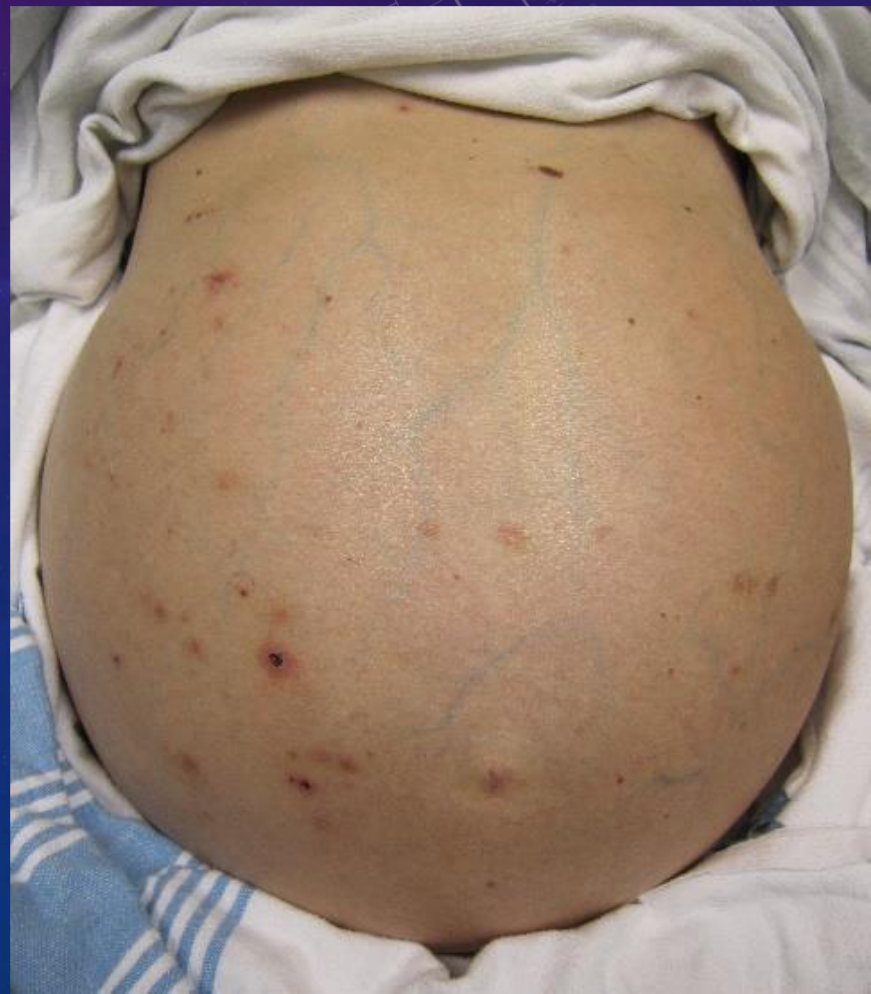
- - Chronic liver disease is defined as hepatic inflammation persisting for more than six months.
- **- There are many causes:**
- 1) Autoimmune hepatitis
- 2) Chronic infection with hepatitis B or C
- 3) Alcoholic fatty liver
- - Plasma aminotransferase activities are usually elevated in chronic hepatitis, but other liver function tests are often normal unless cirrhosis develops.
- - Chronic liver disease may progress to cirrhosis.

LIVER CIRRHOSIS

- **- Cirrhosis is the end stage of chronic hepatitis and characterized by extensive liver fibrosis.**
- **-** It is **IRREVERSIBLE**.
- **-** It is characterized by shrinking of the liver with disorganization of its architecture.
- **-** Fibrosis is the formation of a scar tissues resulting in the disorganization of liver architecture and its shrinkage.



ASCITES



BIOCHEMICAL INVESTIGATION

- - Due to the great functional capacity of the liver, metabolic and clinical abnormalities may not become apparent until late in the course of the disease; until this time, the cirrhosis is said to be ‘compensated’.
- - There are no reliable, simple biochemical tests to diagnose subclinical disease. Biopsy has been considered to be the definitive technique for this purpose but is invasive.
- - There has been considerable interest in the development of non-invasive methods of detecting hepatic fibrosis in patients at risk of cirrhosis (e.g. patients with hepatitis C, or alcoholic or non-alcoholic fatty liver disease), with a view to instituting treatment to slow or prevent disease progression.

- The end