SCHOOL OF MEDICINE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

PBL SEMINAR
BILIRUBIN METABOLISM AND JAUNDICE

Briefly outline the structure of Hemoglobin (Hb)

- Hemoglobin (Hb) consists of four (Tetramer) sub-units held together by multiple non-covalent interactions
- Each subunit consists of Heme (Ferro-Protoporphyrin) and Globin protein
- Globin protein folds around Heme group forming Hydrophobic pocket that protects the Heme, which is the site of Oxygen binding
- HbA_1 is major (98%) form in adults: represented as $\alpha_2\ \beta_2$
- HbA_2 is a minor (2%) form in adults: represented as $\alpha_2\ \delta_2$

What are the major sources of Heme in humans?

- RBC is largest repository of Heme in humans
  - Life span of RBC is about 120 days
- Other sources of Heme include:
  - Myoglobin (Mb): stores oxygen in muscle cells
  - Cytochromes: present in some enzymes
  - Catalase: an enzyme

- Daily turnover of Hb is about 6 g/day, which presents two problems
  - Protoporphyrin Ring in Heme is Hydrophobic and therefore must be made soluble before it is excreted
  - Iron must be conserved for new Heme synthesis

What normally happens to RBC after 120 days?

- Destruction of RBC occurs mainly in Reticuloendothelial system (Extra-vascular system: Spleen and Liver)
  - Hb is broken down
  - Globin protein is recycled or converted to amino acids for reuse, or catabolised as required
  - Iron (Fe) enters Iron pool, for reuse
  - Iron-free Protoporphyrin ring of Heme is degraded, mainly in Reticuloendothelial cells of liver, spleen and bone marrow

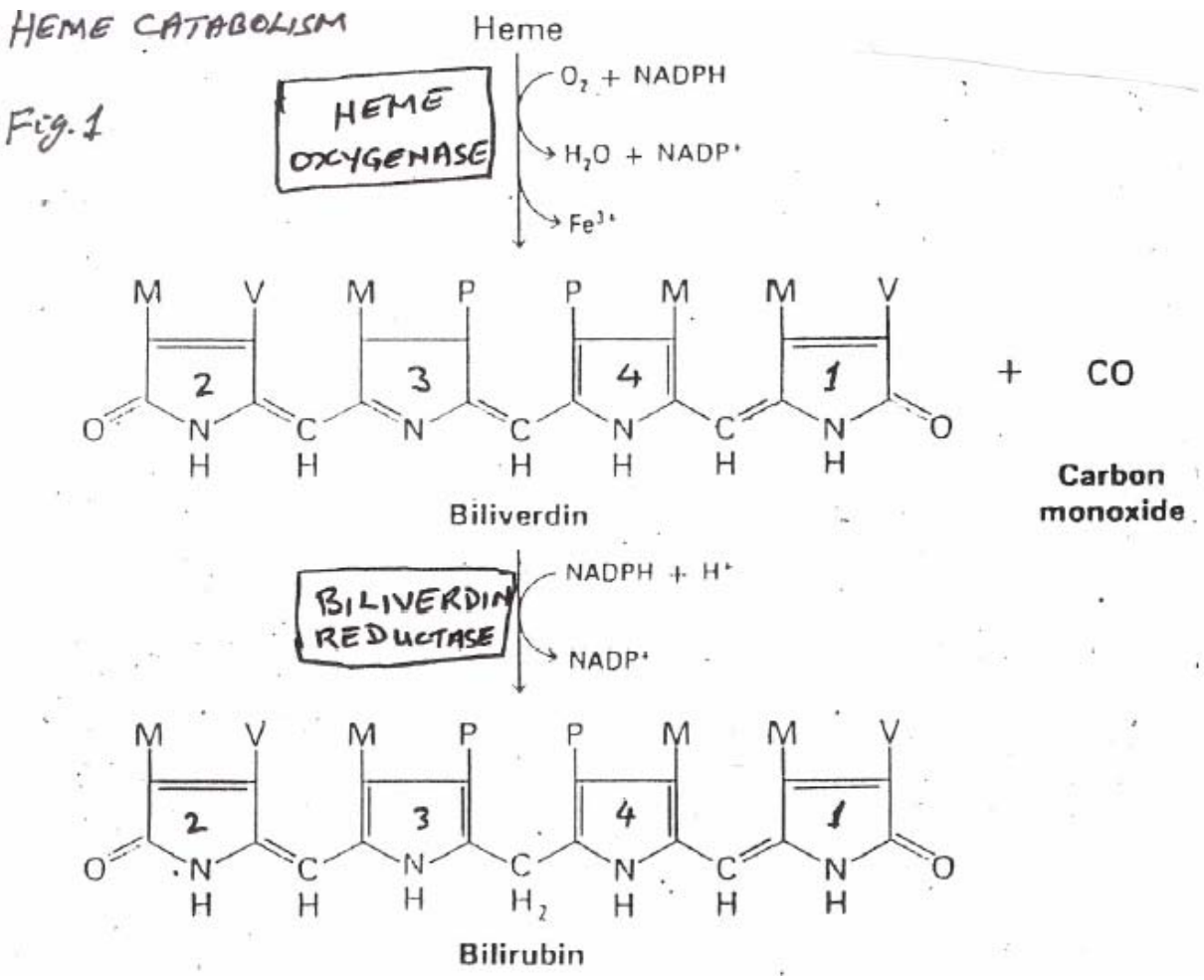
Explain formation of Bilirubin from Heme

- Heme is degraded primarily in Reticuloendothelial cells by **Microsomal Enzyme system** that requires O_2 and NADPH
- **Heme Oxygenase** catalyzes break down of Heme to:
  - **Biliverdin, Ferric iron (Fe^{3+}), and Carbon Monoxide (CO)**

- Iron is conserved for reuse, Carbon Monoxide is excreted through the lungs and can be measured in the breath
- CO content of expired air is a direct measure of activity of Heme Oxygenase and the rate of Heme metabolism

- **Biliverdin** is reduced to **Unconjugated Bilirubin** in a reaction catalyzed by **Biliverdin Reductase** that requires NADPH (**Fig. 1**)

- Unconjugated Bilirubin is Hydrophobic, thus it is insoluble in water
- Unconjugated bilirubin is transported in plasma tightly bound to albumin
- Unconjugated Bilirubin also binds to other proteins and to RBC
- Presence of endogenous and exogenous binding competitors, such as certain drugs, also decreases the binding affinity of albumin for bilirubin
- Small fraction of unconjugated bilirubin in plasma is not bound to albumin
- Free unconjugated bilirubin can cross membranes, including blood-brain barrier, leading to Neuro-toxicity



TAKE NOTE OF THE FOLLOWING

- 1.0g of Hb yields about 35.0mg of Bilirubin
- In healthy adult, between 250 to 350 mg Bilirubin is formed daily;
  - Derived mainly from Hb, but also from ineffective erythropoiesis and from various other Heme proteins such as, Mb and Cytochromes P_{450}
- About 100 ml of plasma can dissolve approximately 25 mg of Bilirubin that is tightly bound to albumin,
- Bilirubin in excess of 25 mg can be bound only loosely to albumin, thus it can easily be displaced from albumin and diffuse into tissues
- Antibiotics and other drugs compete with bilirubin for high-affinity binding sites on albumin
  - These compounds can displace bilirubin from albumin and therefore have significant clinical effects
- Color of Biliverdin is Blue-green, color of Bilirubin is Yellow-red
- Change in color as Biliverdin is converted to Bilirubin is partly responsible for progressive changes in color of a Hematoma, or Bruise, in which damaged tissue changes its color from:
  - Initial Dark Blue to Red-Yellow and finally to Yellow color before all the pigments are transported out of the affected tissue

What are the stages of Bilirubin metabolized in the liver?

- Metabolism of Bilirubin in liver is divided into three stages:
  - Uptake of Bilirubin by Liver cells (Hepatocytes),
  - Conjugation of Bilirubin in Smooth Endoplasmic Reticulum in Hepatocytes,
  - Secretion of Conjugated Bilirubin into Bile

Explain the uptake of Bilirubin by Liver cells

- Bilirubin-albumin complex reaches the liver
  - Bilirubin is transported into Hepatocytes, where it partially binds to the protein **Ligandin**
  - Uptake of bilirubin into Hepatocytes increases with increasing Ligandin concentration
  - Ligandin concentration is low at birth, but increases rapidly over the first few weeks of life
- In the liver, bilirubin is removed from albumin and taken up at the Sinusoidal surface of Hepatocytes by special transport system
- Net uptake of bilirubin in liver depends upon the removal of bilirubin by subsequent metabolic pathways in the liver

How is Bilirubin conjugated in the liver?

- Process of converting non-polar bilirubin molecules to polar bilirubin molecules, which are readily soluble in bile, is called Conjugation
- Conjugation occurs in Hepatocytes
- Conjugation involves addition of Glucuronic Acid molecules to Bilirubin
- Bilirubin is converted to Bilirubin Diglucuronide (conjugated bilirubin)

    - **Reaction is catalyzed by UDP-Glucuronyl Transferase** located in the Smooth Endoplasmic Reticulum
- Bilirubin excreted in the bile is Conjugated Bilirubin

UDP-Glucuronyl Transferase

2 UDP-Glucuronic Acid + Bilirubin =====➔ Bilirubin Diglucuronide

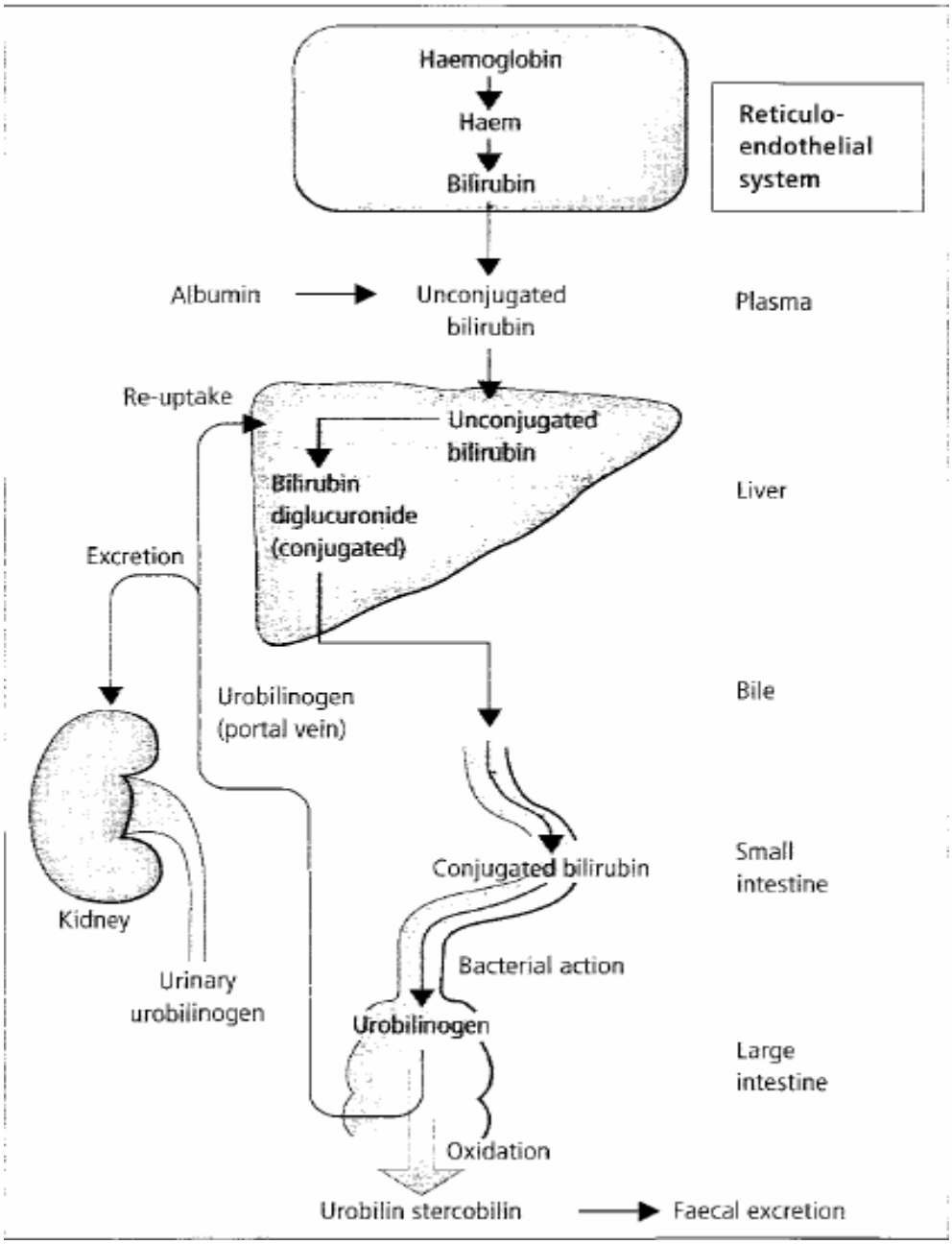
How is Conjugated Bilirubin secreted into Bile?

- Secretion of conjugated bilirubin into bile occurs via an Active Transport mechanism, which is rate-limiting for the entire process of hepatic bilirubin metabolism
- Under normal physiological conditions, all the bilirubin secreted into bile is conjugated
- After phototherapy, significant quantities of unconjugated bilirubin can be found in bile (**Why?**)
    - Phototherapy converts unconjugated bilirubin to Lumirubin which is hydrophilic
- De-conjugation of conjugated bilirubin by **β-Glucuronidase** located in brush border, can occur in proximal small intestine
    - Unconjugated bilirubin formed can be reabsorbed into the circulation, increasing the total plasma unconjugated bilirubin pool
- Cycle of bilirubin Uptake, Conjugation, Excretion, De-conjugation, and Reabsorption are termed: **Enterohepatic Circulation of Bilirubin**

How is Conjugated Bilirubin metabolized in the Intestine?

- Conjugated bilirubin in bile reaches Terminal Ileum and Large Intestine,
    - Conjugated bilirubin may be De-conjugated by bacterial enzymes, resulting in Enterohepatic circulation of bilirubin
- Fecal flora converts remaining conjugated bilirubin into Urobilinogens
- Some Urobilinogen is reabsorbed and re-excreted via the liver to constitute **Intra-hepatic Urobilinogen cycle (Fig. 3)**
- Some Urobilinogen is excreted in the urine
- Urobilinogens in Colon are excreted in feces and oxidized to Urobilins
- Darkening of feces upon standing in air is due to oxidation of residual Urobilinogens to Urobilins

Fig 3: Metabolism of Bilirubin showing Intra-hepatic Urobilinogen cycle



HYPERBILIRUBINEMIA

What is Hyperbilirubinemia?

- Hyperbilirubinemia is accumulation of Bilirubin in blood
- Hyperbilirubinemia: Bilirubin level in blood exceeds 1.0 mg/dL (17.1 μmol/L)

List some causes of Hyperbilirubinemia

- Production of more bilirubin than the normal liver can excrete, or
- Failure of a damaged liver to excrete bilirubin produced in normal amounts
- Obstruction to excretory ducts of the liver (preventing excretion of bilirubin)
- **Unconjugated Hyperbilirubinemia**: due to accumulation of unconjugated bilirubin in blood
- **Conjugated Hyperbilirubinemia**: due to accumulation of conjugated bilirubin in blood

List three major reasons for Hyperbilirubinemia

- Hemolysis:
  - Increased Hb breakdown produces Bilirubin, which overloads the conjugating mechanism in the liver
- Failure of conjugating mechanism within Hepatocytes,
- Obstruction in Biliary system

How can Hyperbilirubinemia be classified?

- Depending on the type of bilirubin (conjugated bilirubin or unconjugated bilirubin) present in the plasma, Hyperbilirubinemia may be classified as follows:
  - **Retention Hyperbilirubinemia**: Due to overproduction of bilirubin
  - **Regurgitation Hyperbilirubinemia**: Due to reflux into the blood stream because of biliary obstruction

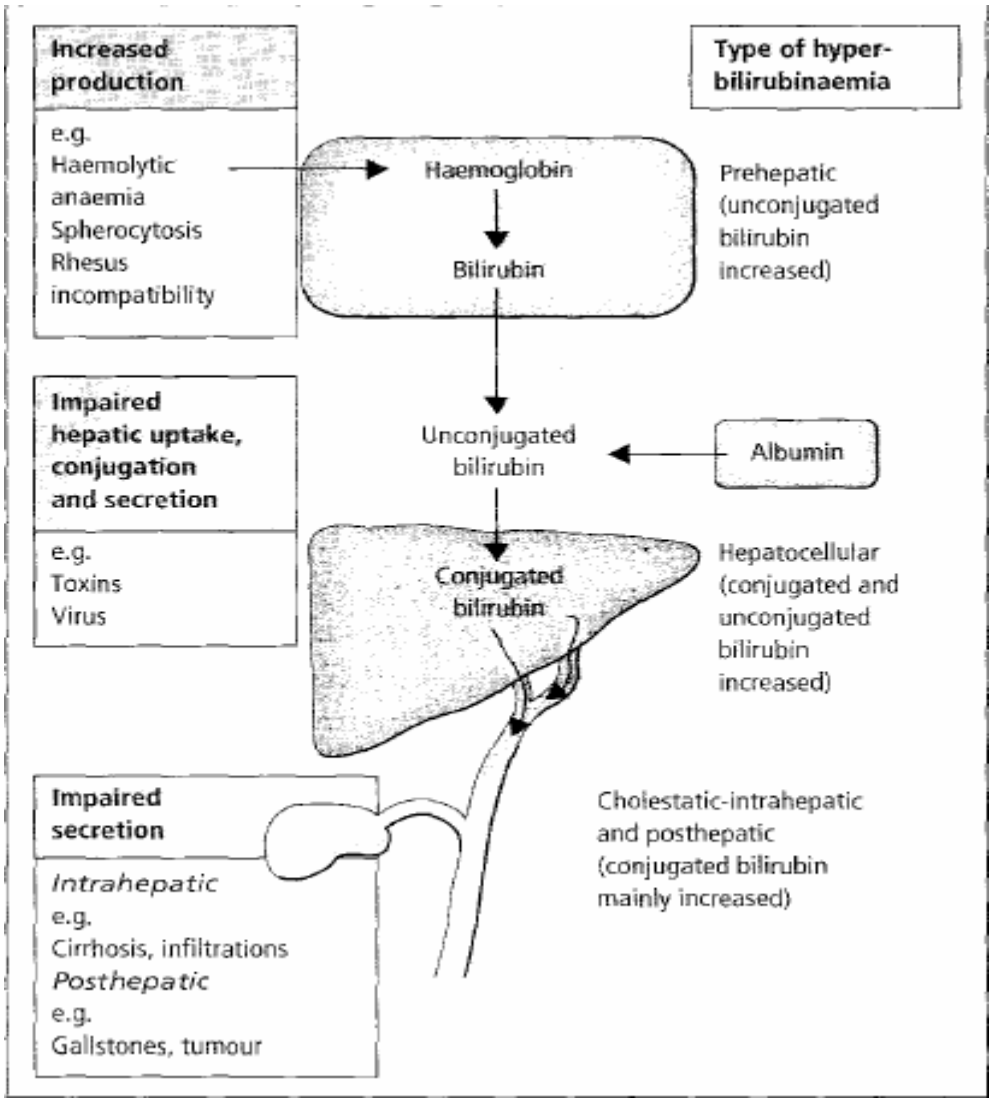
What is Jaundice?

- Jaundice (Icterus) is yellowish discoloration of Skin and Sclera due to deposit of Bilirubin
  - Occurs when Bilirubin in blood is about 2 – 2.5 mg/dl,
- Hyperbilirubinemia can occur without jaundice, but jaundice cannot occur without Hyperbilirubinemia

Give a simple classification of the causes of jaundice

- Causes of jaundice can be classified as follows:
  - Pre-hepatic jaundice (e.g., Hemolytic anemia),
  - Hepatic jaundice (e.g., Hepatitis),
  - Post-hepatic jaundice (e.g., Obstruction of common bile duct)

Types of Hyperbilirubinemia and Jaundice



TAKE NOTE:

- Encephalopathy due to Hyperbilirubinemia (Kernicterus) can occur only in connection with unconjugated hyperbilirubinemia, as found in Retention Hyperbilirubinemia
- Conjugated bilirubin is soluble in water, thus only Conjugated bilirubin can appear in the urine
- **Choluric Jaundice** (Choluria = presence of biliary derivatives in urine) occurs only in Regurgitation Hyperbilirubinemia (**Why?**)
- **Acholuric Jaundice** occurs only in the presence of excess unconjugated bilirubin (Retention Hyperbilirubinemia).

What laboratory tests are used to distinguish between the various types of jaundice?

- Measurements of:
  - Plasma Conjugated Bilirubin
  - Total Bilirubin,
  - Urinary Urobilinogen and Bilirubin,
  - Certain plasma enzymes
  - Inspection of Stool samples