

BILIRUBIN METABOLISM & JAUNDICE

UNIVERSITY OF PNG

SCHOOL OF MEDICINE AND HEALTH SCIENCES

DISCIPLINE OF BIOCHEMISTRY & MOLECULAR BIOLOGY

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Brief description of Hemoglobin (Hb) structure

- Hemoglobin (Hb): Made up of 4-Subunits (Tetramer) held together by multiple non-covalent interactions;
- Each subunit consist of:
 - **Heme** (Ferro-Protoporphyrin),
 - **Globin** protein;
- **Heme**: Protoporphyrin IX and Ferrous ion (**Fe²⁺**);
- Globin protein folds around Heme group forming a protective Hydrophobic pocket;
- Heme is the site of Oxygen binding;

- There are different types of Hemoglobin, with different subunits:
- Foetal Hemoglobin (Hb F): $\alpha_2 \gamma_2$

Two types of Adult Hemoglobin (Hb A):

- **Hb A₁** represented as: $\alpha_2 \beta_2$
 - It is the major (98%) form of Hb in adults;
- **Hb A₂** represented as: $\alpha_2 \delta_2$
 - It is the minor (2%) form of Hb in adults;

What are the major sources of Heme in humans?

- RBC is major source 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,

What normally happens to RBC after 120 days?

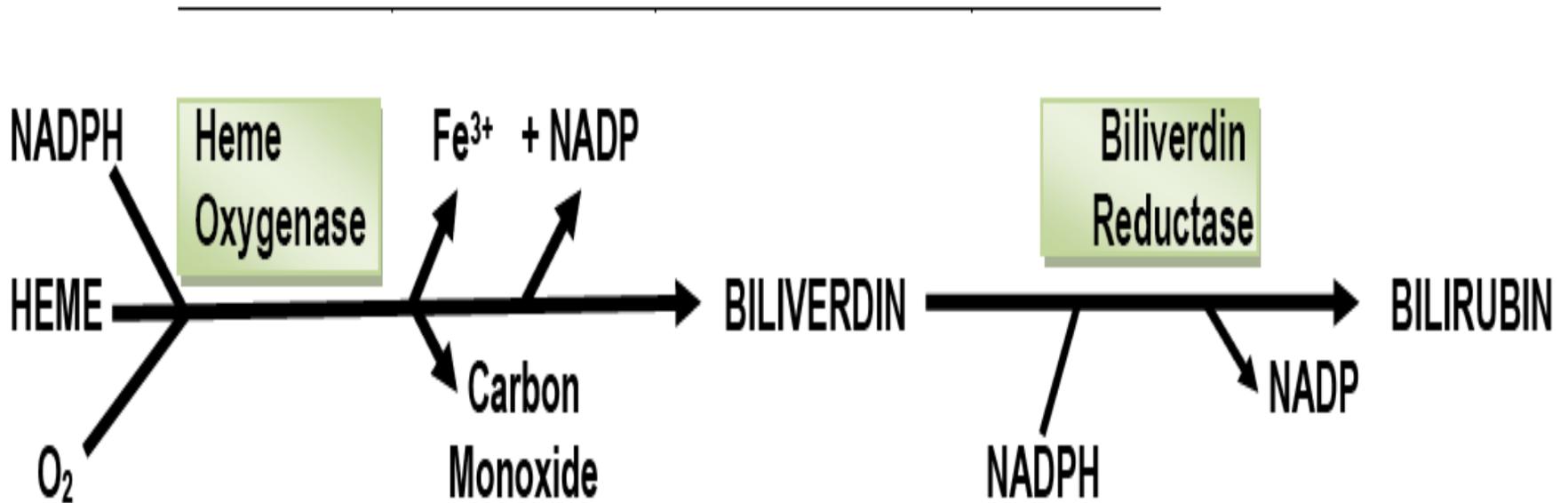
- RBC is destroyed mainly in Reticuloendothelial system (Extra-vascular system: Spleen and Liver);
- Daily turnover of Hb is about 6 g/day;
- Hb is broken down,
- Globin protein is hydrolyzed to amino acids,
- Protoporphyrin Ring in Heme is Hydrophobic, thus must be made soluble before it is excreted,
- Ferrous ion is removed and stored in Iron pool, for reuse,
- Protoporphyrin ring is metabolized to Bilirubin, mainly in Reticuloendothelial cells (spleen and bone marrow),

How is Heme metabolized to Bilirubin?

- Heme is degraded primarily in Reticuloendothelial cells by **Microsomal Enzyme system** that uses **O₂** and **NADPH**,
- **Heme Oxygenase** catalyzes break down of Heme to:
 - **Biliverdin**,
 - **Ferric iron (Fe³⁺)**, and
 - **Carbon Monoxide (CO)**;
- Iron is stored for reuse,
- CO is excreted via lungs and can be measured in breath,
 - CO content of expired air is a direct measure of activity of Heme Oxygenase and rate of Heme metabolism,

- **Biliverdin** is reduced to **Unconjugated Bilirubin** in reaction catalyzed by **Biliverdin Reductase** that requires NADPH, (**Fig. 1**)
- Unconjugated Bilirubin is Hydrophobic;
- It is transported in blood tightly bound to albumin,
- Presence of endogenous and exogenous binding competitors, like certain drugs, 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 cell membranes, including blood-brain barrier, leading to Neuro-toxicity,

Fig. 1: Metabolism of Heme to Bilirubin



IMPORTANT TO NOTE

- 1.0g of Hb yields about 35.0mg of Bilirubin,
- In healthy adults: 250 to 350 mg Bilirubin is formed daily;
 - Derived mainly from Hb, ineffective Erythropoiesis, Mb and Cytochromes P₄₅₀ ;
- Bilirubin in excess of 25 mg in plasma 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, especially in infants;

- 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 Hematoma, or Bruise, in which damaged tissue changes its color from:
 - Initial Dark Blue to Red-Yellow,
 - Finally to Yellow color before all the pigments are transported out of the affected tissue;

What are the stages of Bilirubin metabolism in liver?

- Metabolism of Bilirubin in liver is divided into three stages:
 - **Uptake** of Bilirubin by Hepatocytes,
 - **Conjugation** of Bilirubin in Smooth Endoplasmic Reticulum in Hepatocytes,
 - **Secretion** of Conjugated Bilirubin into Bile;
- **Let us take a brief look at each stage;**
- **Fig. 2 is schematic diagram of Bilirubin Metabolism;**

Briefly explain uptake of bilirubin by hepatocytes

- Bilirubin-albumin complex in plasma reaches the liver,
- Bilirubin enters Hepatocytes, and binds to **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,
- Bilirubin is removed from albumin and taken up at the Sinusoidal surface of Hepatocytes by special transport system,
- Net uptake of bilirubin depends on removal of bilirubin by subsequent metabolic pathways in the liver,

How is Bilirubin conjugated in the liver?

- Conjugation: conversion of insoluble bilirubin to soluble bilirubin, which is readily soluble in bile,
 - It involves addition of **2 Glucuronic Acids** to Bilirubin,
 - **Bilirubin Diglucuronide** is called **Conjugated Bilirubin**;
- **Reaction is catalyzed by UDP-Glucuronyl Transferase** located in the Smooth Endoplasmic Reticulum,

UDP-Glucuronyl Transferase reaction:



- Bilirubin excreted in bile is **Conjugated Bilirubin**,

How is Conjugated Bilirubin secreted into Bile?

- Secretion of conjugated bilirubin into bile occurs via Active Transport mechanism, which is rate-limiting for the process of hepatic bilirubin metabolism,
- Under normal physiological conditions, all Bilirubin secreted into bile is Conjugated,
- After Phototherapy, large amount of Unconjugated Bilirubin can be found in bile (**Why?**)
- Because Phototherapy converts unconjugated bilirubin to **Lumirubin** which is soluble in aqueous medium;

- De-conjugation of conjugated bilirubin by **beta-Glucuronidase** located in brush border, can occur in Proximal Small Intestine,
- Unconjugated bilirubin formed can be reabsorbed into circulation, increasing total plasma unconjugated bilirubin level,
 - Cycle of bilirubin Uptake, Conjugation, Excretion, De-conjugation, and Reabsorption is termed: **Enterohepatic Circulation of Bilirubin;**
- It occurs mainly in neonates;

How is Conjugated Bilirubin metabolized in Intestine?

- Bile containing conjugated bilirubin is released in GIT,
- Conjugated bilirubin may be De-conjugated by bacteria, resulting in Enterohepatic circulation of bilirubin,
- Fecal flora converts some conjugated bilirubin into Urobilinogens,
- Some urobilinogen is reabsorbed and re-excreted via liver to constitute **Intra-hepatic Urobilinogen cycle**,
- Some urobilinogen is excreted in the urine,
- Urobilinogen is excreted in feces and oxidized to Urobilin,
- Darkening of feces in air is due to oxidation of residual Urobilinogen to Urobilin,

HYPERBILIRUBINEMIA

What is Hyperbilirubinemia?

- Hyperbilirubinemia:
 - Accumulation of Bilirubin in blood,
 - Bilirubin level exceeds **1.0mg/dL** (17.1 $\mu\text{mol/L}$),

List some causes of Hyperbilirubinemia

- Production of more bilirubin than normal liver can excrete,
- Failure of damaged liver to excrete bilirubin produced in normal amounts,
- Obstruction to excretory ducts of liver preventing excretion of bilirubin,
- **Unconjugated Hyperbilirubinemia:**
 - Accumulation of Unconjugated Bilirubin in blood;
- **Conjugated Hyperbilirubinemia:**
 - Accumulation of Conjugated Bilirubin in blood;

What are the 3 major causes of Hyperbilirubinemia?

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

How is Hyperbilirubinemia classified?

- Depending on the type of bilirubin (Conjugated bilirubin or Unconjugated bilirubin) present in the plasma, Hyperbilirubinemia may be classified as:
- **Retention Hyperbilirubinemia:**
 - Due to overproduction of bilirubin,
 - Accumulation of Unconjugated Bilirubin in blood;
- **Regurgitation Hyperbilirubinemia:**
 - Due to reflux of bilirubin into the blood stream because of biliary obstruction,
 - Accumulation of Conjugated and Unconjugated Bilirubin in blood;

What is Jaundice?

- **Jaundice (Icterus):** yellowish discoloration of Skin and Sclera due to deposit of Bilirubin,
 - When bilirubin in blood is about **2 – 2.5 mg/dl**,
(34.2 – 42.8 μ mol/L)
- Hyperbilirubinemia can occur without jaundice, but jaundice cannot occur without Hyperbilirubinemia,

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** (Obstruction of common bile duct);

Other causes of Jaundice

Unconjugated Hyperbilirubinemia:

- Inherited disorders of Bilirubin metabolism leading to decreased clearance of bilirubin: Examples:
 - **Crigler-Najjar syndrome:**
 - Severe Unconjugated Hyperbilirubinemia due to Low activity of UDP-GT;
 - **Gilbert syndrome:**
 - Unconjugated Hyperbilirubinemia due to decreased expression of conjugating enzyme (UDP-GT)

IMPORTANT TO NOTE

- Encephalopathy due to Hyperbilirubinemia (Kernicterus) occurs in patients with unconjugated hyperbilirubinemia, as in Retention Hyperbilirubinemia;
- Conjugated bilirubin is soluble in aqueous medium, thus only Conjugated bilirubin can appear in urine,
- **Choluric Jaundice:** Choluria = presence of biliary pigment in urine, occurs in Regurgitation Hyperbilirubinemia (**Why?**)
- **Acholuric Jaundice:** No bile pigment in urine, occurs in Retention Hyperbilirubinemia, (**Why?**)

What lab tests are used for diagnosis of jaundice?

- **Liver Function Tests (LFT)** are the major lab tests for diagnosis of Jaundice:
 - Total Bilirubin,
 - Conjugated Bilirubin,
 - Transaminases (ALT & AST),
 - Alkaline Phosphatase (ALP),
 - Gamma Glutamyl Transpeptidase (GGTP),
 - Total Protein & Albumin,
- Other tests may include:
 - Urinary Urobilinogen and Bilirubin,
 - Inspection of color of Stool samples,

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