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BCH 471 Experiment (9)

Estimation of Serum

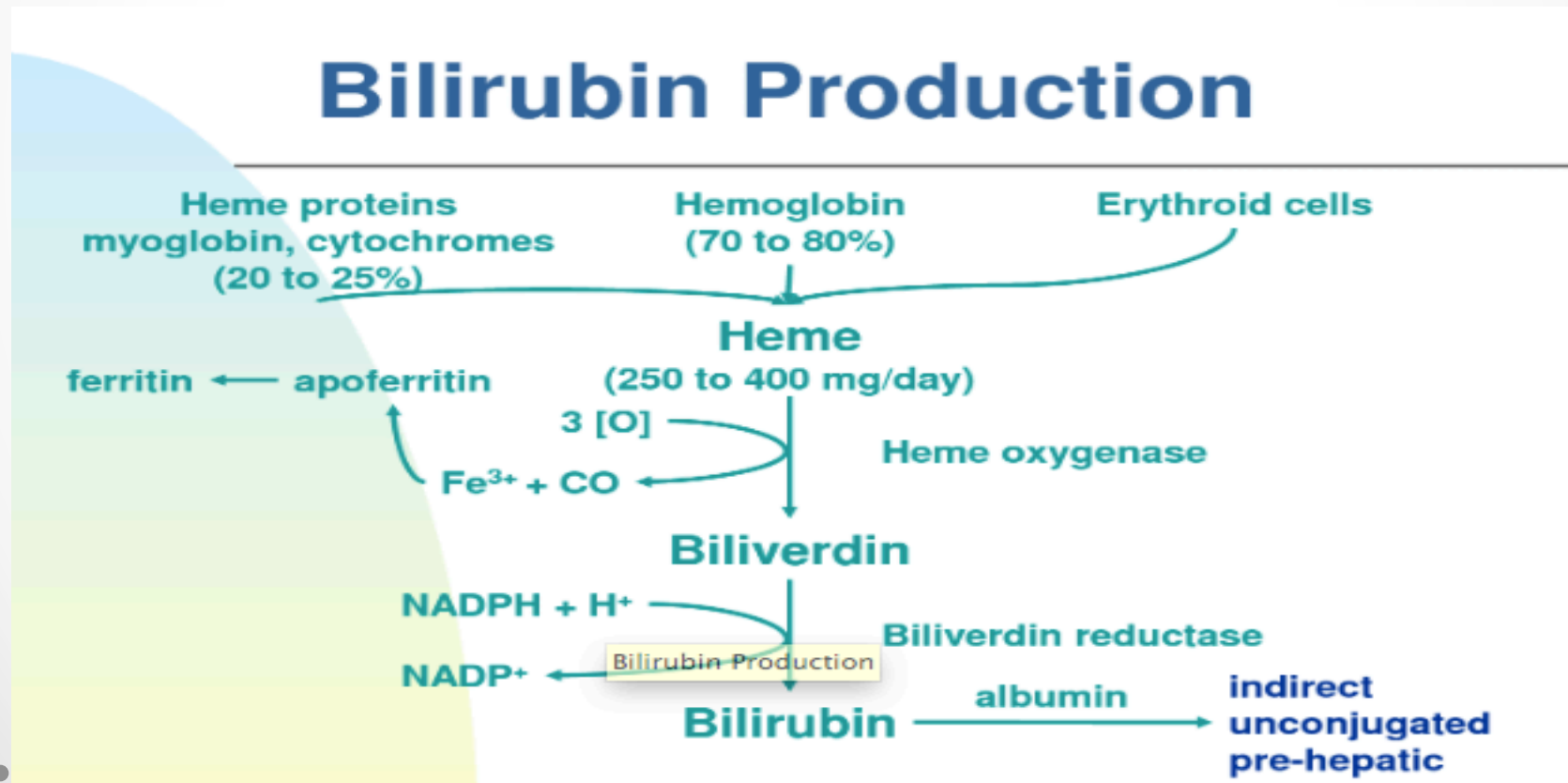
Bilirubin (Total & Direct)

Objective

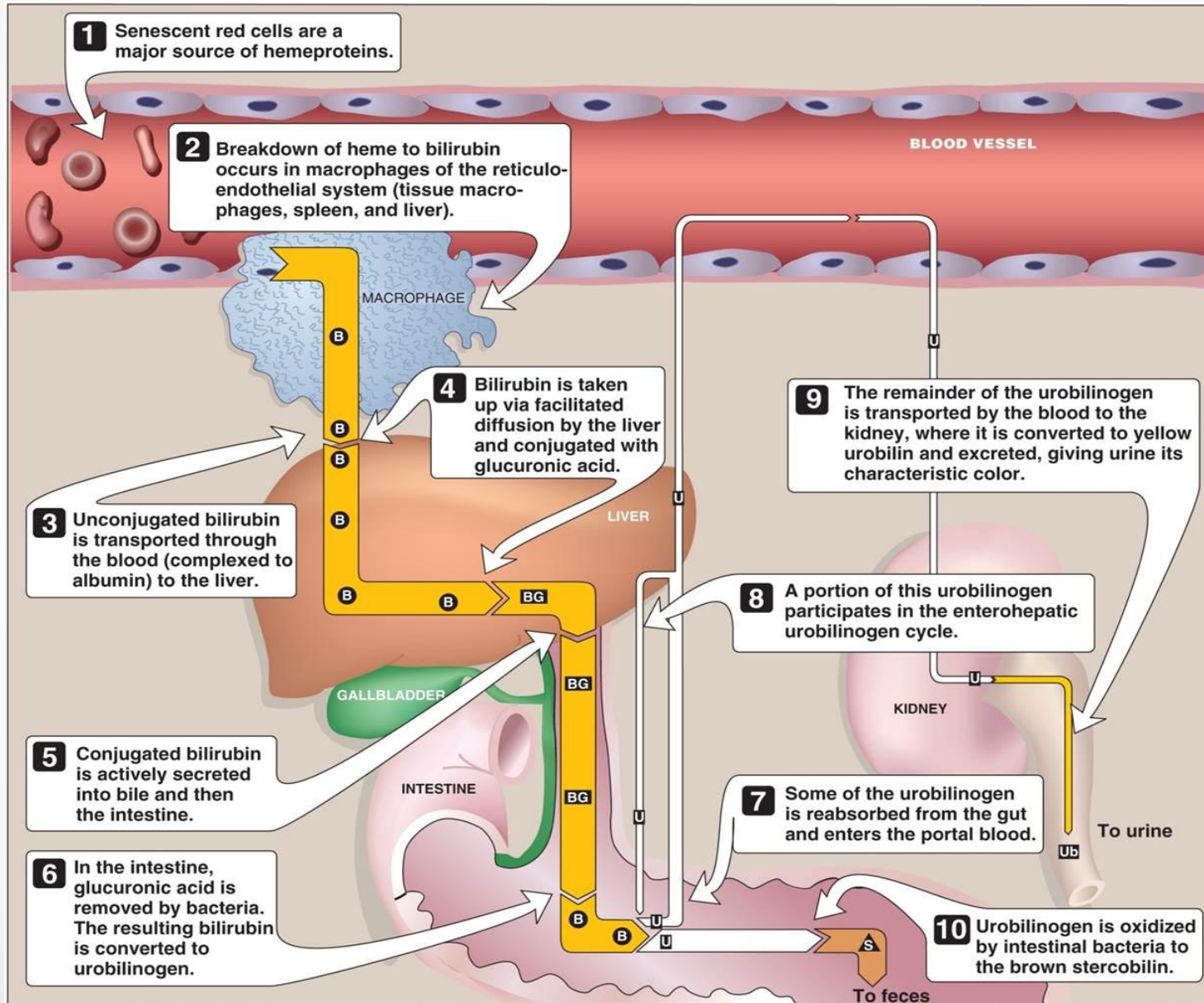
To estimate the amount of bilirubin in serum.

Bilirubin

- Bilirubin is a by-product of the breakdown of hemoglobin.
- It is the yellowish pigment responsible for jaundice.



Bilirubin Metabolism



Types of Bilirubin

- **Direct bilirubin:** Conjugated with glucuronic acid by means of *Bilirubin-UDP Glucuronosyl Transferase*.
- **Indirect bilirubin:** unconjugated, insoluble in water
- **Total bilirubin:** sum of the direct and indirect of bilirubin.
- **Note:** About **200 mg** per day of unconjugated bilirubin are transported to the liver and disturbances in the powers of conjugated and/or excretion of the liver of this yellow compound will lead to raised levels in serum. Above about 2 mg/dl in the blood, leads to disease called **Jaundice**.

Types of Jaundice

- *Jaundice* is the discoloration of skin and sclera of the eye.
- It occurs because RBCs are being broken down too fast for the liver to process, because of a disease in the liver, or because of a bile duct blockage.
- *The causes of jaundice may be classified as:*
 - ❖ Pre-Hepatic Jaundice
 - ✓ *haemolytic disease*
 - ❖ Hepatic Jaundice
 - ✓ *Cirrhosis of the liver*
 - ✓ *Infective Hepatitis*
 - ✓ *Neonatal Jaundice*
 - ❖ Post-Hepatic Jaundice
 - ✓ *Cholecystitis.*

Pre-Hepatic Jaundice

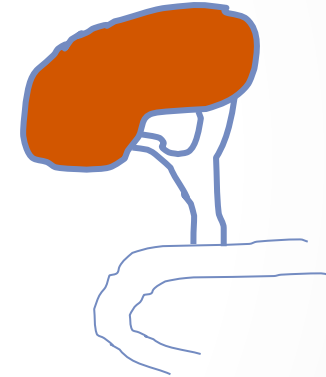
Haemolytic disease

- The production of un-conjugated bilirubin may exceed the conjugating capacity of the liver and hence the serum levels of **indirect (and of total) bilirubin will be raised** and that of direct in the upper normal range or just a little elevated.
- The other liver function tests will usually give normal results

A. Hemolytic anemia



excess
hemolysis



↑ unconjugated bilirubin
(in blood)

upper normal range conjugated
bilirubin (released to bile duct)

Hepatic Jaundice

❖ *Cirrhosis (in the absence of infection)*

Destruction of liver cells will lead to a reduced conjugating capacity with a:

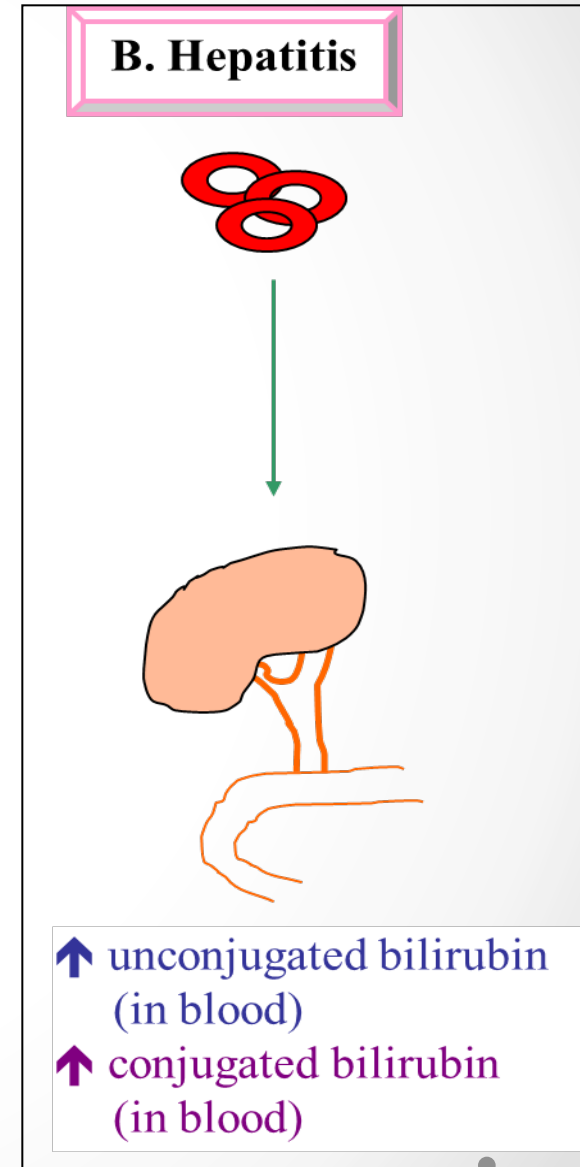
- Raised serum level of indirect (and of total) bilirubin, with a low level of direct bilirubin
- and an abnormally high release, into the blood, of the enzymes: AST, ALT and ALP.
- Synthesizing power of liver will be diminished and hence low levels of total protein, albumin and cholesterol

Hepatic Jaundice

• *Hepatitis*

The conjugative capacity of the liver is approximately normal, but there is the inability to transport the conjugated bilirubin from the liver cells to the biliary system, and it will be regurgitated back into the blood. Hence:

- The serum level of unconjugated bilirubin will be normal, and that of conjugated (and total) bilirubin will be raised.
- Synthesizing power is diminished leading to low serum levels of proteins but the raising of antibodies to infection usually leads to raised total proteins level.



Hepatic Jaundice

❖ *Neonatal Jaundice*

Conjugating enzymes in the liver are often absent at birth. Hence:

- Raised serum level of indirect (and total) bilirubin is to be expected
- Low level of direct bilirubin.
- The other liver functions are normal.

The indirect bilirubin level will rise for the first few days after birth until the conjugating enzymes begin to synthesize. If the latter process is delayed and the serum level of indirect bilirubin rises towards 20 mg/dl, an ultraviolet therapy or an exchange blood transfusion should be carried out owing to the danger of deposition of the insoluble unconjugated bilirubin in the basal ganglia of the brain leading permanent **Brain Damage**.

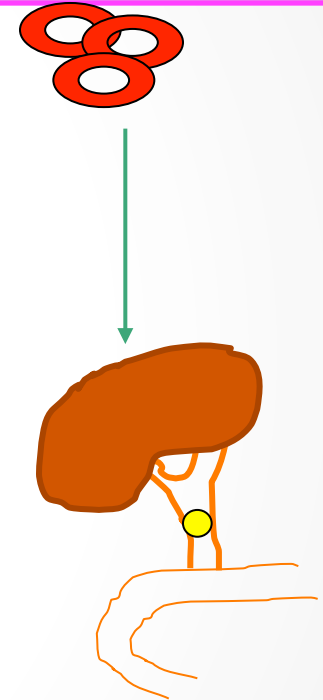
Post-Hepatic Jaundice

Cholecystitis

The bile duct is blocked.

- The indirect bilirubin level is normal but conjugated bilirubin is regurgitated into the blood and excreted into the urine (raised conjugated and total bilirubin).
- Enzymes will be regurgitated into the blood giving raised levels.
- The other liver function tests are normal

C. Biliary duct stone



Normal unconjugated bilirubin (in blood)

↑ conjugated bilirubin (in blood)

Principle

- Bilirubin in serum is coupled with *diazotized Sulphanilic acid* to form azobilirubin .
 - The water soluble conjugated bilirubin (*direct bilirubin*) reacts easily with reagents such as *diazotized sulphanic acid*.
 - while the water insoluble unconjugated bilirubin (*indirect bilirubin*) requires a solubilizing reagent, such as *Caffeine*, in order to react with the diazotized sulphanic acid.
- In this experiment, the direct bilirubin is estimated in the absence of the solubilizing agent and then further bilirubin estimation in the presence of the solubilizing agent will give the total bilirubin level.
- The indirect or unconjugated bilirubin is then found by difference.

Method

Label 4 tubes as TT (total test), TC (total control), DT (direct test), DC (direct control).

| | TT | TB | DT | DB |
|---|-----------|---------|-----------|---------|
| Solution 1 | 0.20 ml | 0.20 ml | 0.20 ml | 0.20 ml |
| Solution 2 Sodium nitrate | (0.05 ml) | – | (0.05 ml) | – |
| Solution 3/ NaCl solution 0.9% | 1.00 ml | 1.00 ml | 2.00 ml | 2.00 ml |
| Sample | 0.20 ml | 0.20 ml | 0.20 ml | 0.20 ml |
| Mix, let stand for 5 min. at 20-25°C. Read absorbance of test against blank (A_{DB}) for direct only at 546 nm. FOR total stand for 30 min at 20-25°C. | | | | |
| For total bilirubin add solution 4 | 1.00 ml | 1.00 ml | - | - |
| Mix and let stand for 15 min and read the absorbance at 546 nm against blank (A_{TB}). | | | | |

Calculation

- Concentration of direct bilirubin in mg/ml serum
 - = (abs. DT - abs. DB) X 14.4 = mg /dl
 - Normal range Up to: 0.25 mg/dl

- Concentration of total bilirubin in mg/ml serum
 - = (abs. TT - abs. TB)X 10.8 = mg /dl
 - Normal range Up to 1 mg/dl

- Concentration of indirect bilirubin in mg/ml serum
 - = Conc of total bilirubin – Conc of direct bilirubin= mg /dl