Experiment

Estimation of Serum Bilirubin (Total & Direct)

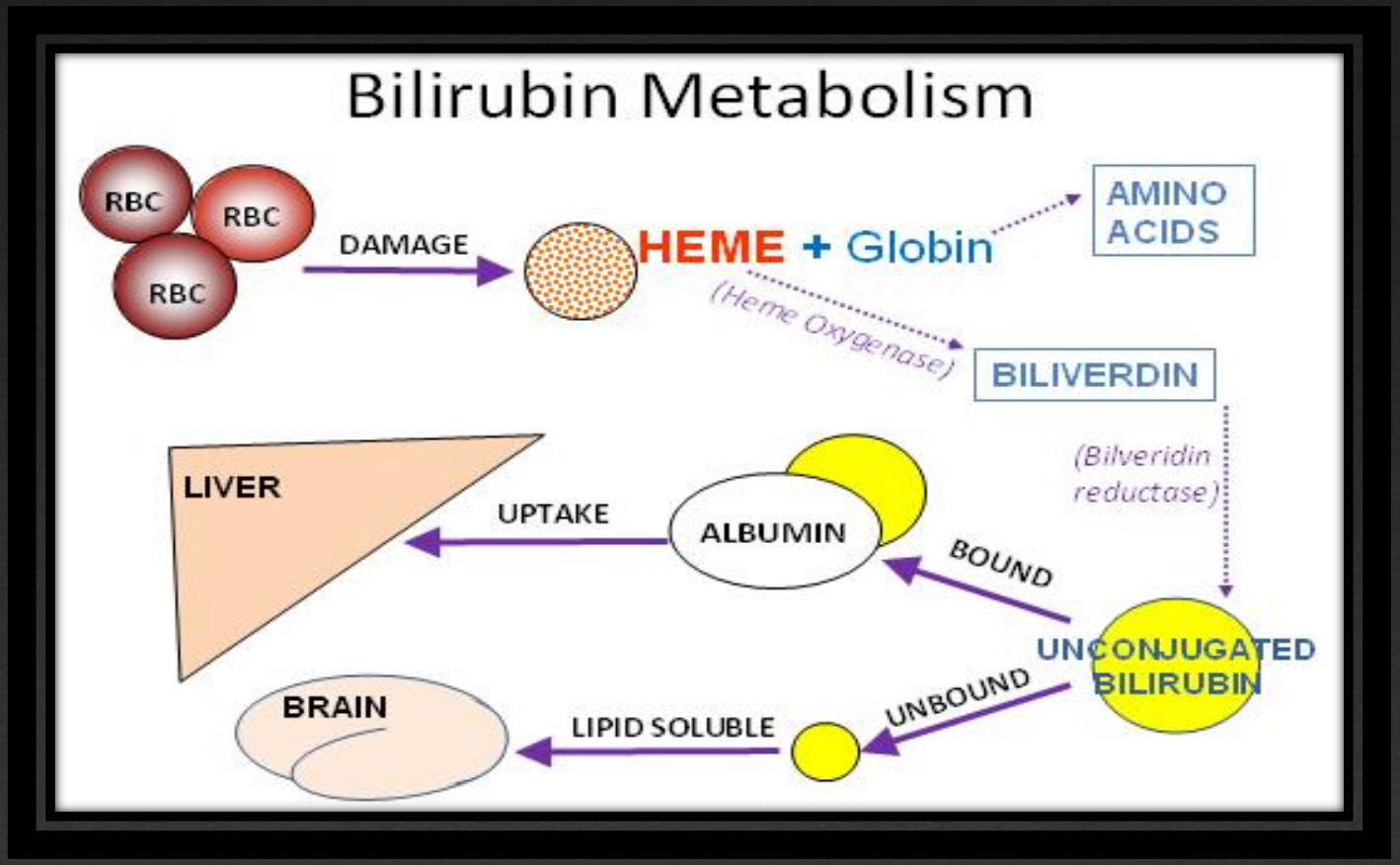
Objective

To estimate the amount of bilirubin in serum.

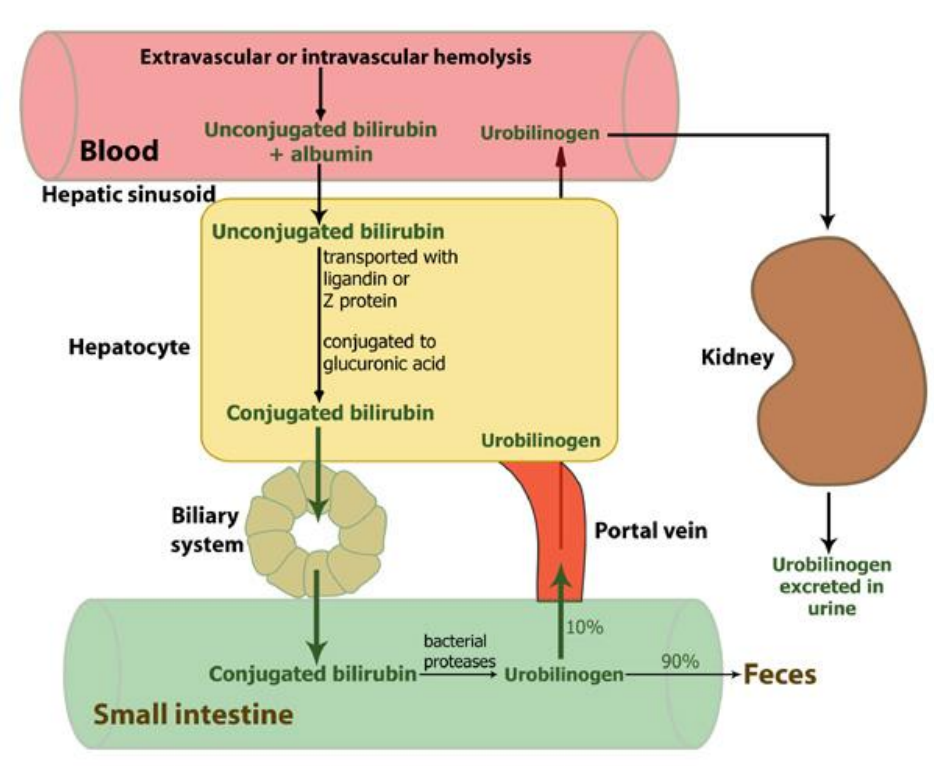
Introduction
Bilirubin

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

Bilirubin Metabolism



Bilirubin Metabolism



Bilirubin-UDP Glucuronosyl Transferase

Types of bilirubin

- Direct bilirubin: Conjugated with glucoronic acid
- Indirect bilirubin: unconjugated, insoluble in water
- Total bilirubin sum of the direct and indirect of bilirubin.

Types of Jaundice

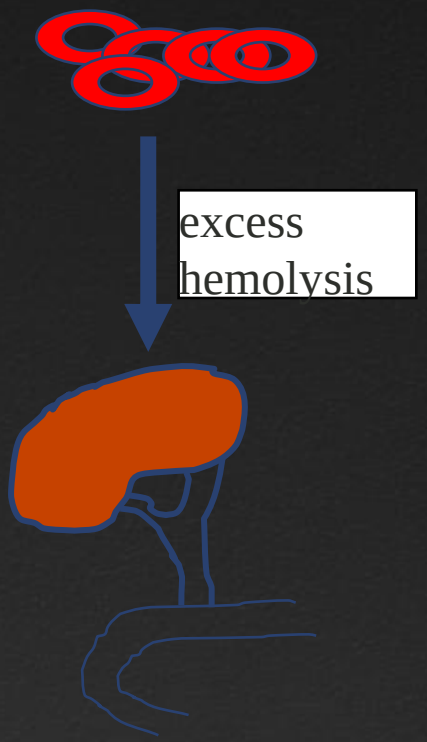
Jaundice is the discoloration of skin and *sclera* of the eye, which occurs when bilirubin accumulates in the blood at a level greater than approximately 2.5 mg/dl. Jaundice occurs because red blood cells are being broken down too fast for the liver to process, because of disease in the liver, or because of bile duct blockage.

The causes of jaundice may be classified as:

- Pre-Hepatidc Jaundice
  - ✓ ***haemolytic disease***
- Hepatic Jaundice
  - ✓ ***Cirrhosis of the liver***
  - ✓ ***Infective Hepatitis***
  - ✓ ***Neonatal Jaundice)***
- Post-Hepatic Jaundice
  - ✓ ***Cholecystitis.***

Pre-Hepatic Jaundice

A. Hemolytic anemia



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

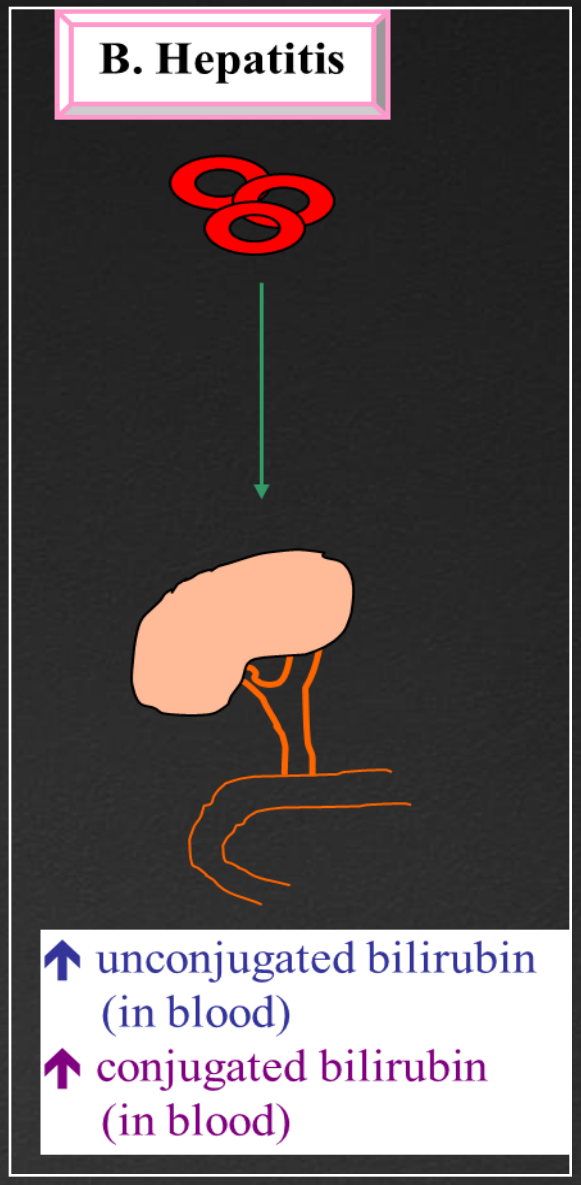
↑ unconjugated bilirubin (in blood)

upper normal range conjugated bilirubin (released to bile duct)

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 caniculi of 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 which are made in the liver and of cholesterol, but the raising of antibodies to infection usually leads to raised total proteins level.



Cirrhosis (in the absence of infection)

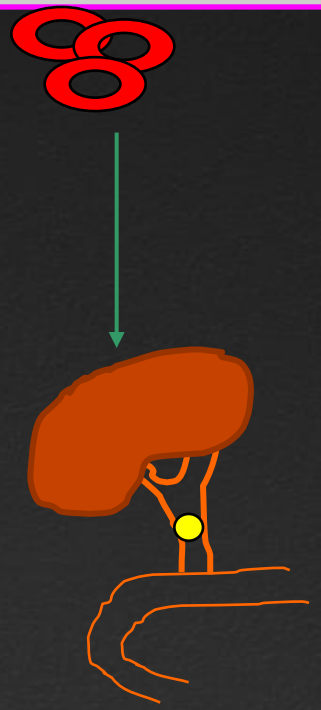
Destruction of liver cells will lead to a reduced conjugating capacity with a raised serum level of indirect (and of total) bilirubin but with a low level of direct bilirubin and an abnormally high release, into the blood, of the enzymes: AST, ALT and ALP. Synthesizing power will be diminished and hence low levels of total protein, albumin and cholesterol. The insoluble unconjugated bilirubin will not be excreted in the urine, and bilirubin will be absent in severe cases.

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, with a 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 to the condition known as **Kernicterus**, and permanent **Brain Damage**.

Post-Hepatic Jaundice

C. Biliary duct stone



Cholecystitis

Here, 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 azre normal. If the bile ducts are obstructed, direct bilirubin will build up, escape from the liver, and end up in the blood. If the levels are high enough, some of it will appear in the urine. Only direct bilirubin appears in the urine. Increased direct bilirubin usually means that the biliary (liver secretion) ducts are obstructed. This test is useful in determining if a patient has liver disease or a blocked bile duct.

Normal unconjugated bilirubin (in blood)

↑ conjugated bilirubin (in blood)

Principle

- Bilirubin in serum is coupled with diazotized Sulfanilic acid to form azobilirubin .
- The water soluble conjugated bilirubin (*direct bilirubin*)reacts easily with reagents such as diazotized sulphanilic acid .
- while the water insoluble unconjugated bilirubin(*indirect bilirubin)* requires a solubilising reagent, such as ***Caffeine***, in order to react with the diazotized sulphanilic acid.
- In this experiment, the direct bilirubin is estimated in the absence of the solubilising agent and then further bilirubin estimatin in the presence of the solubilising 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

Thank you