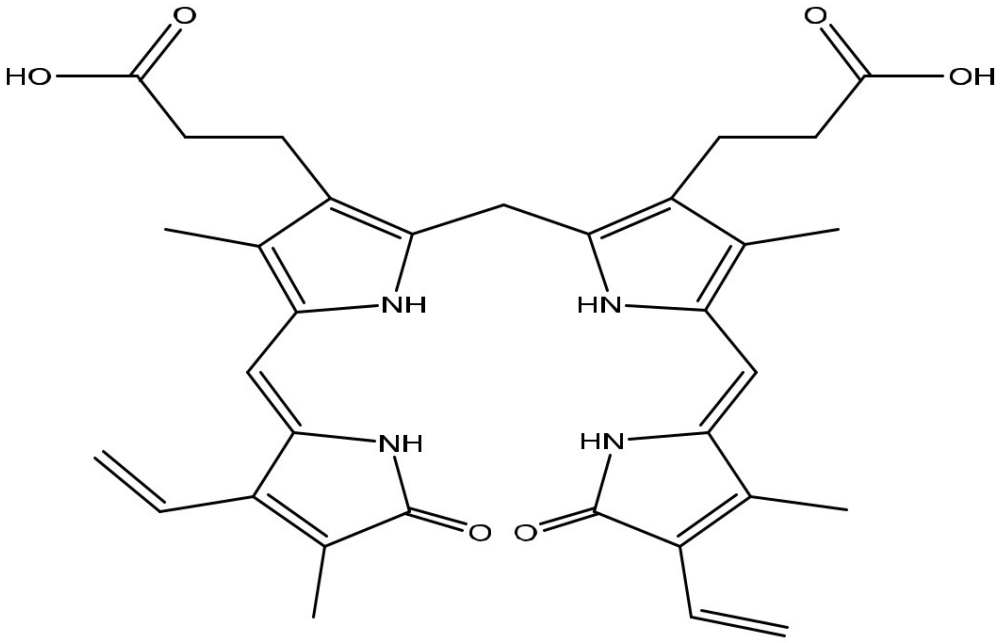
)The eleventh Experiment:

SERUM BILIRUBIN

Bilirubin results from the breakdown of hemoglobin in the RBCs and is a byproduct of hemolysis (ie, RBC destruction). It is produced by the reticuloendothelial system. Removed from the body by the liver, which excretes it into the bile, bilirubin gives the bile its major pigmentation. Usually, a small amount of bilirubin is found in the serum. A rise in serum bilirubin levels occurs when there is excessive destruction of RBCs or when the liver is unable to excrete the normal amounts of bilirubin produced.



Bilirubin

There are two forms of bilirubin in the body: indirect or unconjugated bilirubin (less soluble in water), which is protein bound; and direct or conjugated bilirubin (soluble in water), which circulates freely in the blood until it reaches the liver, where it is conjugated with glucuronide transferase and then excreted into the bile. An Increase in protein-bound bilirubin (unconjugated bilirubin) is more frequently associated with increased destruction of RBCs (hemolysis); an increase in free-flowing bilirubin is more likely seen in dysfunction or blockage of the liver. A normal level of total bilirubin rules out any significant impairment of the excretory function of the liver or excessive hemolysis of red cells.

Only when total bilirubin levels are elevated will there be a call for differentiation of the bilirubin levels by conjugated and unconjugated types.

Medical Importance

1. Bilirubin is a powerful antioxidant like ascorbic acid. It is effective against soluble peroxides.
2. However free bilirubin has high affinity for membrane lipids which can interfere with function of nervous system.
3. Heme oxygenase exist in two isoforms. Heme oxygenase-I and heme oxygenase-II, In diabetes heme oxygenase activity is modified.

Jaundice (Icterus) :

It is most common known disease of bilirubin metabolism in which skin and sclera of eye acquires yellow color due to excessive bilirubin in blood. Normal blood plasma bilirubin level is 1 mg/dl.

In jaundice the plasma bilirubin level is high. Hence excess bilirubin diffuses into tissues and turns them yellow. Further, excess bilirubin leads to a condition known as hyperbilirubinemia. Thus the characteristic signs of jaundice are hyperbilirubinemia and yellow colored skin and sclera.

Based on clinical causes jaundice is classified into pre hepatic jaundice, hepatic jaundice and post hepatic jaundice.

Pre hepatic or hemolytic jaundice

It is due to excessive breakdown of erythrocytes. This leads to increased production of bilirubin. But liver cells are unable to conjugate all bilirubin formed. Hence unconjugated bilirubin level of plasma is elevated. Excessive breakdown of RBC occurs in:

1) hemglobinopathies.
2) hereditary spherocytosis.
3) incompatible blood transfusion .
4) malaria.

Hepatic or hepatocellular jaundice

It is due to damaged hepatocytes. Poisons like chloroform, carbon tetrachloride, phosphorus, antibiotics, amanita mushroom poison and hepatitis virus can damage parenchymal cells of liver.

In cirrhosis also liver cell damage occurs. Damaged hepatocytes are unable to perform functions. So in hepatic jaundice liver cells are unable to conjugate or secrete bilirubin though the production of bilirubin is as usual. If conjugation of bilirubin is impaired unconjugated bilirubin in plasma is elevated .

Post hepatic or obstructive jaundice

It is due to obstruction of bile duct. Gall stones and cancer of head of pancreas can cause obstruction of bile duct. Due to blockage of bile duct conjugated bilirubin secreted by liver returns to blood. Hence in obstructive jaundice conjugated bilirubin in plasma is elevated.

- Cholestatic jaundice is term used to indicate all forms of extra hepatic or post hepatic obstructive jaundice.
- ✓ A routine examination measures only the total bilirubin.

Procedure :

Pipette into well identified test tubes:	Total bilirubin		Direct bilirubin	
	Blank	Test	Blank	Test
Reagent R1			1.5	1.5
Reagent R2	1.5	1.5		
D.W		--		
Reagent R3 nitrate		50µL		50µL
Mix				
specimen	100µL	100µL	100µL	100µL

Mix well and start a timer when adding specimen .

Read absorbance at 555 nm(530-580) against blanks.

T.B reading after ≥ 3 min at 37 C° or ≥ 5 min at R.T .

D.B reading at **exactly** 3 min at 37 C° or 5 min R.T.

Mg/dl x 17.1 = µmol/L

Calculation :

Procedure n :

$$\frac{\textbf{A}\ \text{sample} - \textbf{A}\ \text{sample Blank}}{\textbf{A}\ \text{standard} - \text{A standard Blank}} \times \textbf{C}\ \text{standard} = \mu\text{g/dl bilirubin}$$

Normal value :

Adult and child > 5 days	**T.B** (mg/dL)	**D.B** (mg/dL)
> 5 days-60 years	**0.3 - 1.2**	**< 0.2**
60-90 years	**0.2 - 1.1**	**< 0.2**
0 -1 day	**< 8.0**	**---**
1 -2 day	**< 12.0**	**---**
3 – 5 day	**< 16.0**	**---**