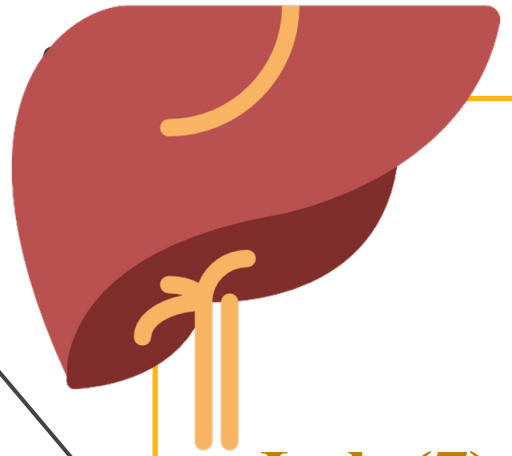
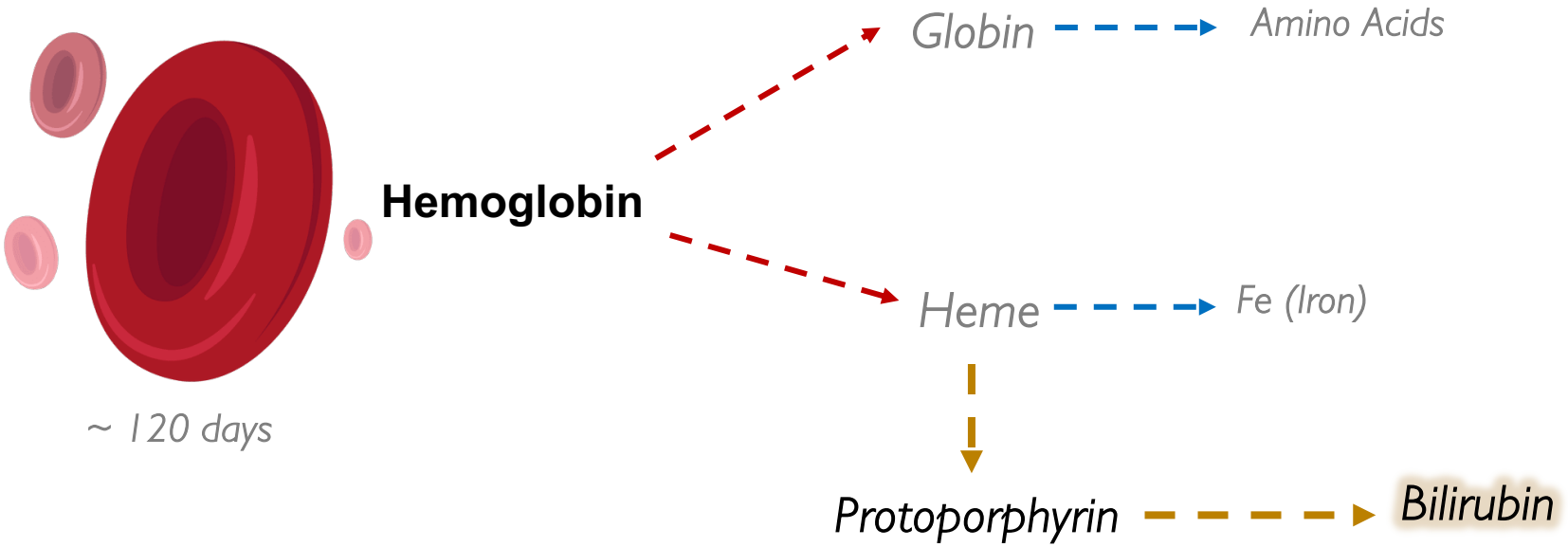


Blood Biochemistry BCH 471 [Practical]

Lab (7) Estimation of Serum Bilirubin (Total & Direct)

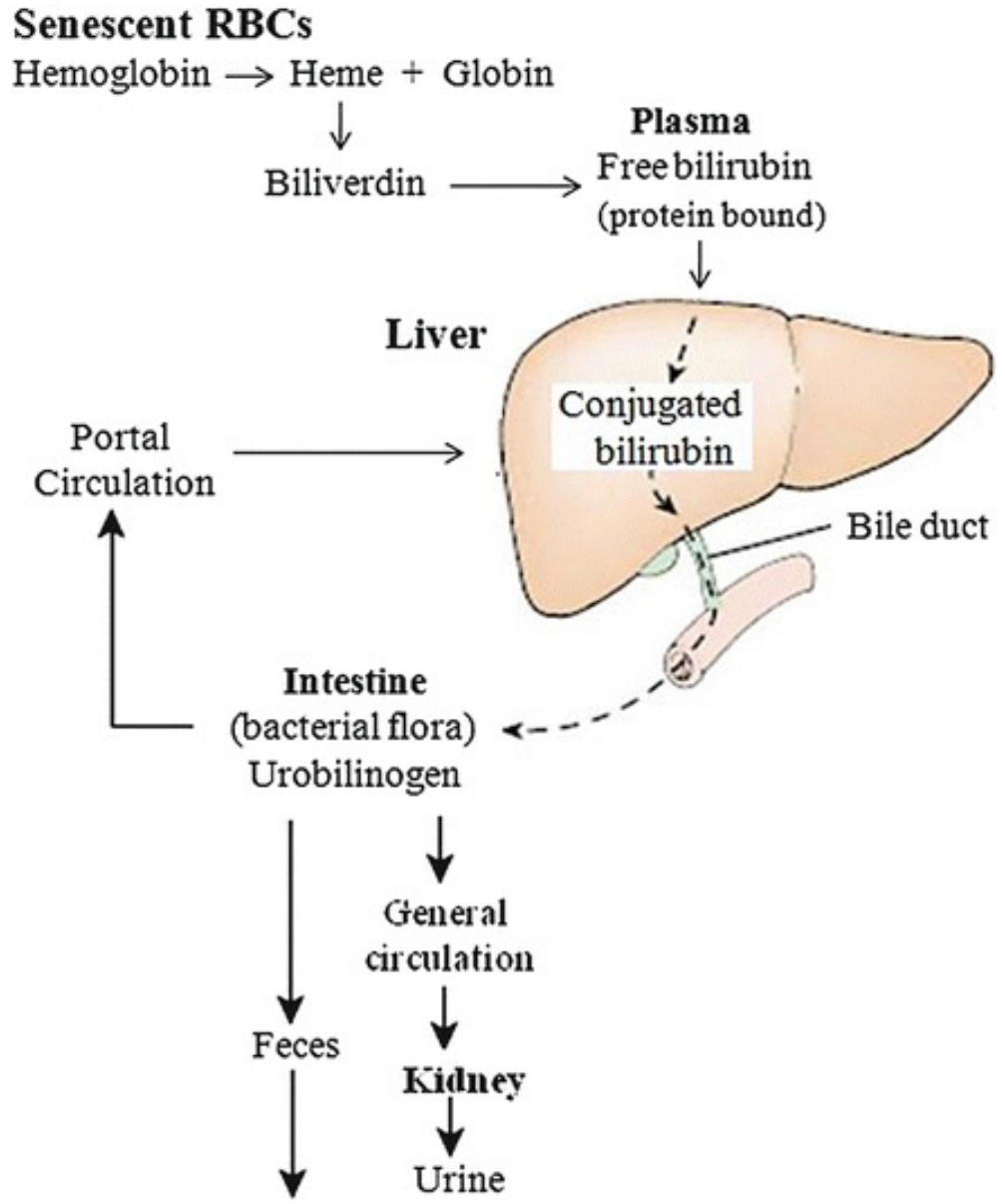
Bilirubin

- **Heme** is formed from **hemoglobin**, a principal component of red blood cells.
- **Bilirubin** is the **yellow** breakdown product of normal heme catabolism.
- **Bilirubin** is excreted in bile, and its levels are elevated in certain diseases.
- It is responsible for the yellow color of bruises and the yellow discoloration in **jaundice.**



Types of Bilirubin

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



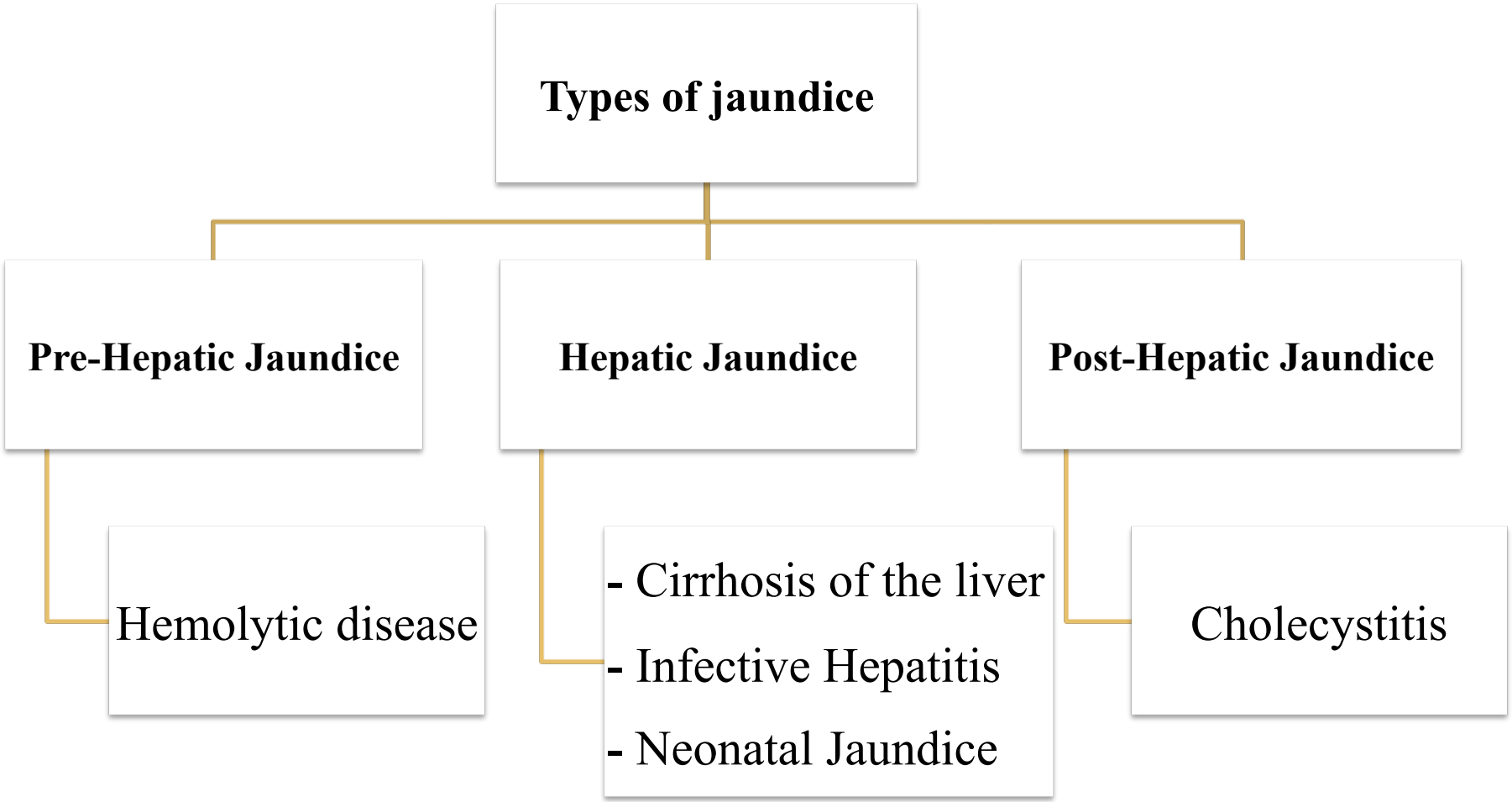
Notes:

1. **About 200 mg** per day of unconjugated bilirubin are transported to the liver.
2. Disturbances in the powers of conjugated and/or excretion of the liver of this yellow compound will lead to raised levels in serum.

Bilirubin and Jaundice

- A normal level is total bilirubin: **0.1** to **1.2** mg/dL
- Above about **2 mg/dL** in the blood, leads to disease called **Jaundice.**
- **Jaundice** is caused by a **build-up of bilirubin (yellow color)** in the blood and tissues of the body.
- **Jaundice** is the **discoloration** of skin and sclera of the eye caused by high concentration of bilirubin.

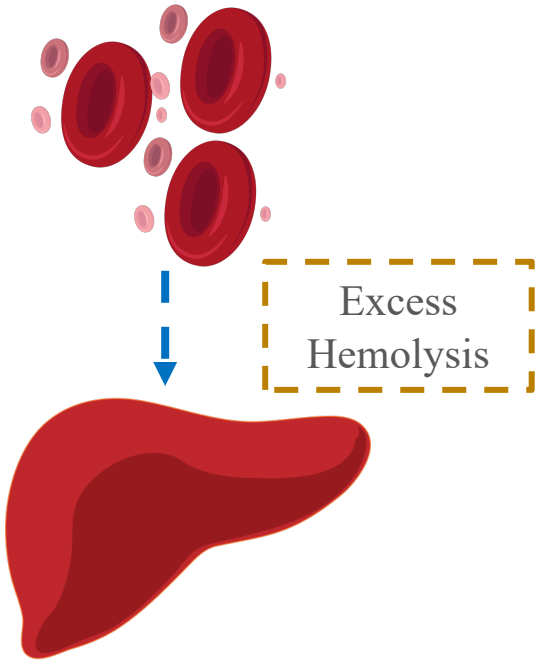
Causes of Jaundice



Pre-Hepatic Jaundice

Hemolytic disease (excess hemolysis)

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



Indirect bilirubin ► increased

Direct bilirubin ► Slightly increased

Total bilirubin ► increased

↑↑↑ UCB + ↑CB = ↑↑↑ TB

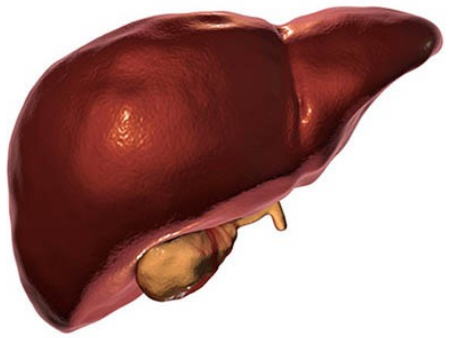
- ↑ Unconjugated bilirubin (in blood)
- Upper normal range conjugated bilirubin (released to bile duct)

Hepatic Jaundice

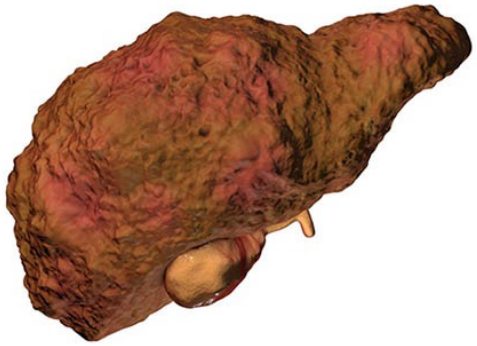
1- Cirrhosis (in the absence of infection)

- Destruction of liver cells will lead to a reduced conjugating capacity.
- **Raised** serum level of indirect (and of total) bilirubin.
- **Low** level of direct bilirubin.
- 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.

↑↑↑ UCB + ↓CB = ↑↑↑ TB



Healthy liver

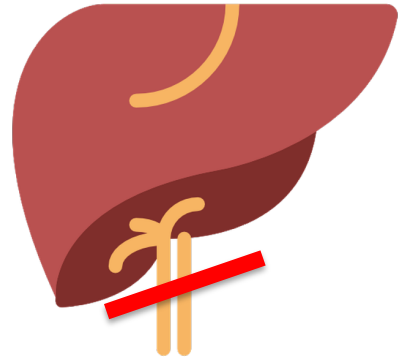


Cirrhotic liver

Hepatic Jaundice

2- Hepatitis (in the presence of infection)

- 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.
- The serum level of **unconjugated** bilirubin will be **normal**.
- **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.



- Normal unconjugated bilirubin (in blood)
- ↑ conjugated bilirubin (in blood)

Hepatic Jaundice

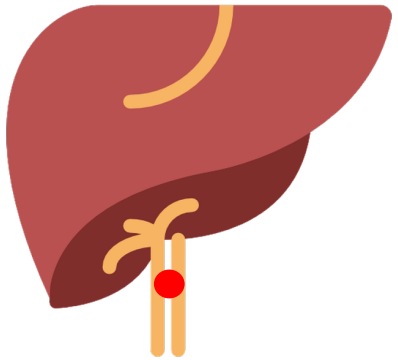
3- Neonatal Jaundice

- Conjugating enzymes in the liver are often **absent at birth.**
- **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.
- The conjugation process is delayed and the serum level of indirect bilirubin rises towards **20 mg/dl**
- Can be treated by **phototherapy** or an exchange **blood transfusion.**
- Deposition of the insoluble unconjugated bilirubin into basal ganglia of the brain leads to **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**.



- Normal unconjugated bilirubin (in blood)
- ↑ conjugated bilirubin (in blood)

Practical Part 💉

Objective

- To estimate the amount of bilirubin in serum.

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 sulfanilic acid.**
- while the water insoluble unconjugated bilirubin (indirect bilirubin) requires a solubilizing reagent, such as **methanol**, in order to react with the **diazotized sulphanilic 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.

Conjugated bilirubin (direct bilirubin) + diazotized sulfanilic acid → azobilirubin

Unconjugated bilirubin (indirect bilirubin) + diazotized sulfanilic acid $\xrightarrow{\text{Methanol}}$ **azobilirubin**

Method

- Label 4 tubes (or cuvette) and pipette the following:

Solutions	Total Bilirubin		Direct Bilirubin	
	TB	TT	DB	DT
Sulfanilic acid reagent	0.5 ml	0.5 ml	1 ml	1 ml
Sodium nitrate reagent	--	0.02 ml	--	0.02 ml
Mix and let stand at least 1 min but no longer than 3 min., then add:				
Sample	0.05 ml	0.05 ml	0.05 ml	0.05 ml
After exactly 1 min. read the absorbance of Test and Test Blank (of Direct bilirubin only) at 546 nm against distilled water. For Total bilirubin add:				
Methanol	0.5 ml	0.5 ml		
Mix and let stand for 5 min and read absorbance of Test and Test Blank (of Total bilirubin) at 546 nm against distilled water				

* **TB** (total Blank), **TT** (total test), **DB** (direct Blank), **DT** (direct test).

When handling sulfanilic acid reagent, wear protective gloves/protective clothing/eye protection/face protection.

Calculations

- **Concentration of direct bilirubin** = (abs. DT- abs. DB) x 25 = ……mg /dl

Normal range: Up to 0.5 mg/dl

- **Concentration of total bilirubin** = (abs. TT- abs. TB) x 25 = ……mg /dl

Normal range: Up to 1 mg/dl

- **Concentration of indirect bilirubin** = Conc. of total bilirubin – Conc. of direct bilirubin= ……mg /dl

Normal range: 0.1-0.4 mg/dl