



AL-MUSTAQBAL UNIVERSITY COLLEGE

Department of Medical laboratory Techniques Department

Clinical Biochemistry

(Estimation of Bilirubin) 4stg



Lecturer : M. Sc. Hadeel luay kareem

M.Sc. Mohammed Ali

M.Sc: Sahbaa Majeed kadhem

Liver function tests:

also known as liver chemistries, help determine the health of liver by measuring the levels of proteins, liver enzymes, and bilirubin in blood, include :

1- Tests deal with liver damage: (Alanine aminotransferase (**ALT**) or (**GPT**) ,and Aspartate aminotransferase (**AST**)or (**GOT**))

2-Test deal with cholestasis : Alkaline phosphatase (**ALP**),and g-glutamyltransferase (**GGT**)

3- Tests deal with liver damage: conjugated and un conjugated **bilirubin**.

Bilirubin:

is a waste product from the breakdown of red blood cells. It's ordinarily processed by the liver. It passes through the liver before being excreted through stool.

A damaged liver can't properly process bilirubin. This leads to an abnormally high level of bilirubin in the blood. A high result on the bilirubin test may indicate that the liver isn't functioning properly.

Bilirubin metabolism begins with breakdown of **RBC** in reticulo-endothelial system.

Hemoglobin released from **RBC** and broken down to heme and globin molecules . Heme is then catabolized to form biliverdin, which is transformed to bilirubin, this form of bilirubin is called unconjugated (indirect) bilirubin .

In the liver , indirect bilirubin (**IDB**) is conjugated with a glucuronide resulting in conjugated (direct) bilirubin (**DB**).

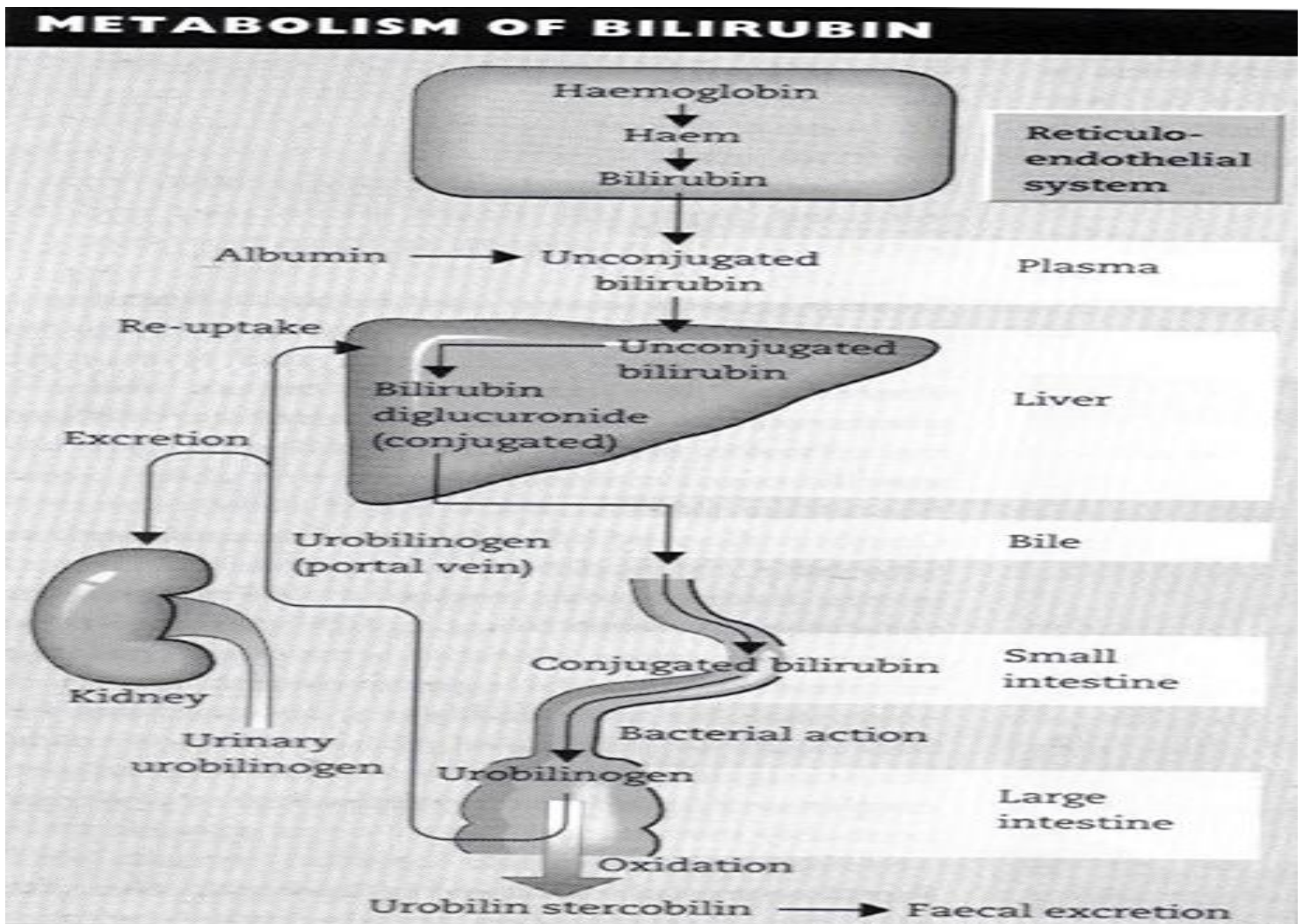
DB is then excreted from the liver cells into the intrahepatic canaliculi, which eventually lead to the hepatic ducts, the common bile duct and the bowel.

IDB is insoluble in water but soluble in chloroforms while **DB** is soluble in water

DB reacts in aqueous solution (direct reaction), whereas **IDB** requires an solubilizer ,such as alcohol and has therefore termed indirect reaction.

Both give purple azo-bilirubin with diazotized sulphanilic acid.

Total serum bilirubin (**TB**) level is the sum of the **DB** and **IDB** .



Causes of elevated conjugated bilirubin:

- 1- Sickle cell anemia
- 2-pregnancy
- 3-Cancer
- 4-cirrhosis of the liver
- 5-blood poisoning,drugs
- 6-Scarring of the bile duct
- 7-Alcoholic liver disease

What are the causes of low bilirubin?

There are no health conditions that cause low levels of bilirubin. But taking certain substances can reduce it temporarily.

- 1-Caffeine
- 2- Non-steroidal anti-inflammatory drugs (NSAIDs) called salicylates, such as aspirin
barbiturates

Interfering factor:

- 1-Blood hemolysis and lipidmia can produce false results.
- 2- Certain food (ex.carrots) may increase the yellow hue in serum thus causing falsely increased bilirubin levels by spectrophotometric methods.
- 3- Prolonged fasting raises bilirubin level as does anorexia.
- 4- Drugs that may increase levels of **TB** include:-
Anabolic steroids , antibiotics , nicotic acid and ascorbic acid .
- 6-Drugs that may decrease levels of **TB** include:-
Caffeine , Penciline and high doses of salicylates.

Normal values:

TB : 0.2 – 1.0 mg / dl , DB : 0.0 -0.2 mg/dl

Procedure:

1-Total bilirubin :

Working solution : Mix **20 R1**Vol with **1 R3** Vol

	Blank	sample
sample	50 µl	50 µl
R1	1 ml	
Working solution		1 ml

Mix and incubate 5 min and read A sample at 555nm.

2- direct bilirubin :

Working solution : Mix **20 R2**Vol with **1 R3** Vol

	Blank	sample
sample	50 µl	50 µl
R2	1 ml	
Working solution		1 ml

Mix and incubate 5 min and read A sample at 555nm.

Calculation:

$$C_{\text{(total or direct bilirubin)}} = \frac{A(\text{sample})}{A(\text{standard})} * n$$

*A standard (total) = 0.09 & n= 76

*A standard (direct) = 0.048 & n= 26