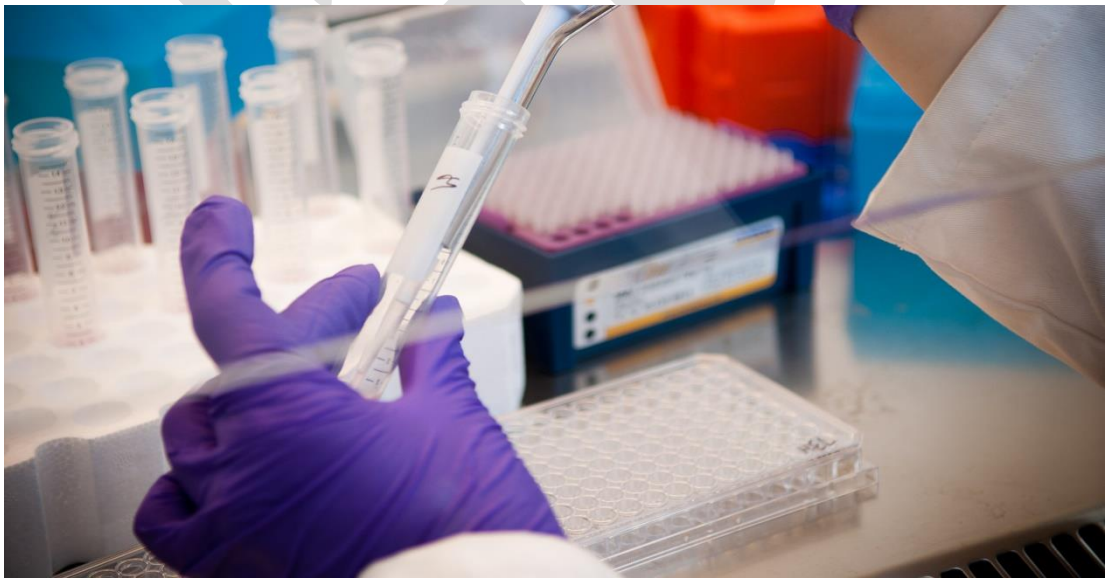
Al-Rasheed University College
Department of Dentistry

practical Biochemistry

For the second class

lab 6

Serum Bilirubin



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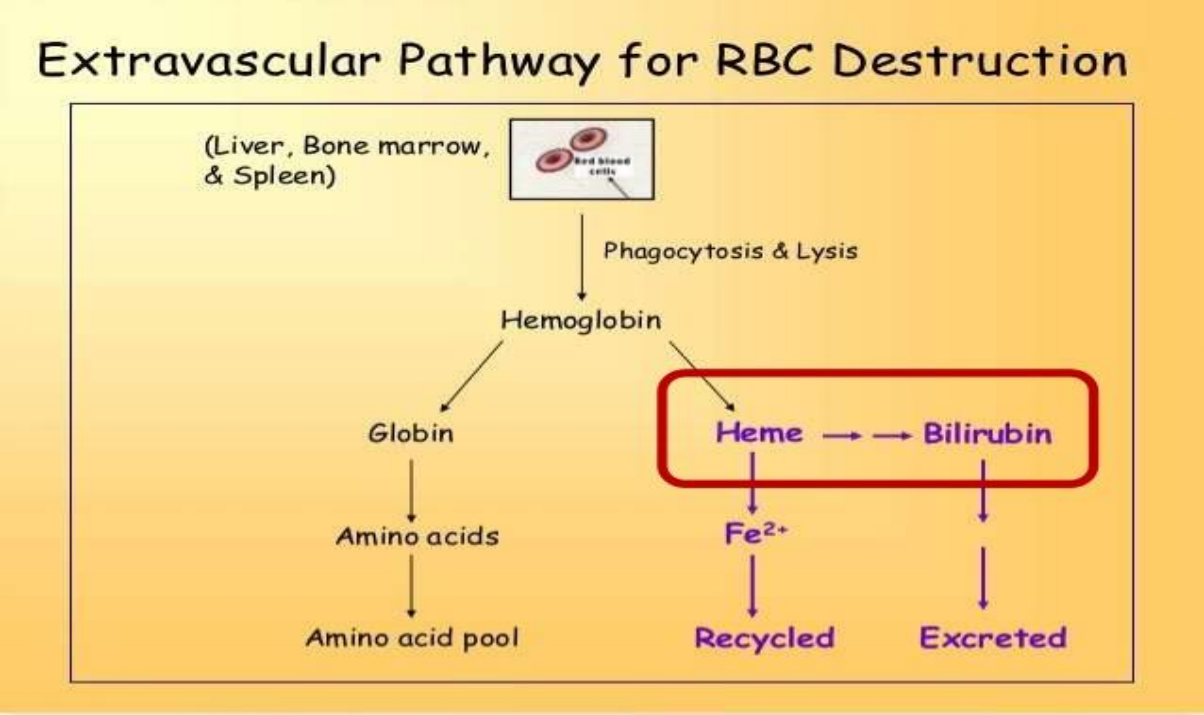
lab 7

Serum Bilirubin

Bilirubin (BR)is a yellow-orange pigment which gives the serum its characteristics yellow color, Bilirubin is a product of heme catabolism. It's the breakdown product of normal heme catabolism, caused by the body's clearance of aged red blood cells which contain hemoglobin. At the end of their life span red blood cells are broken down by the reticuloendothelial system, mainly in the spleen. The released hemoglobin is split into globin, which enters the general protein pool and haem, which is converted to bilirubin after removal of iron, the iron is reutilized.

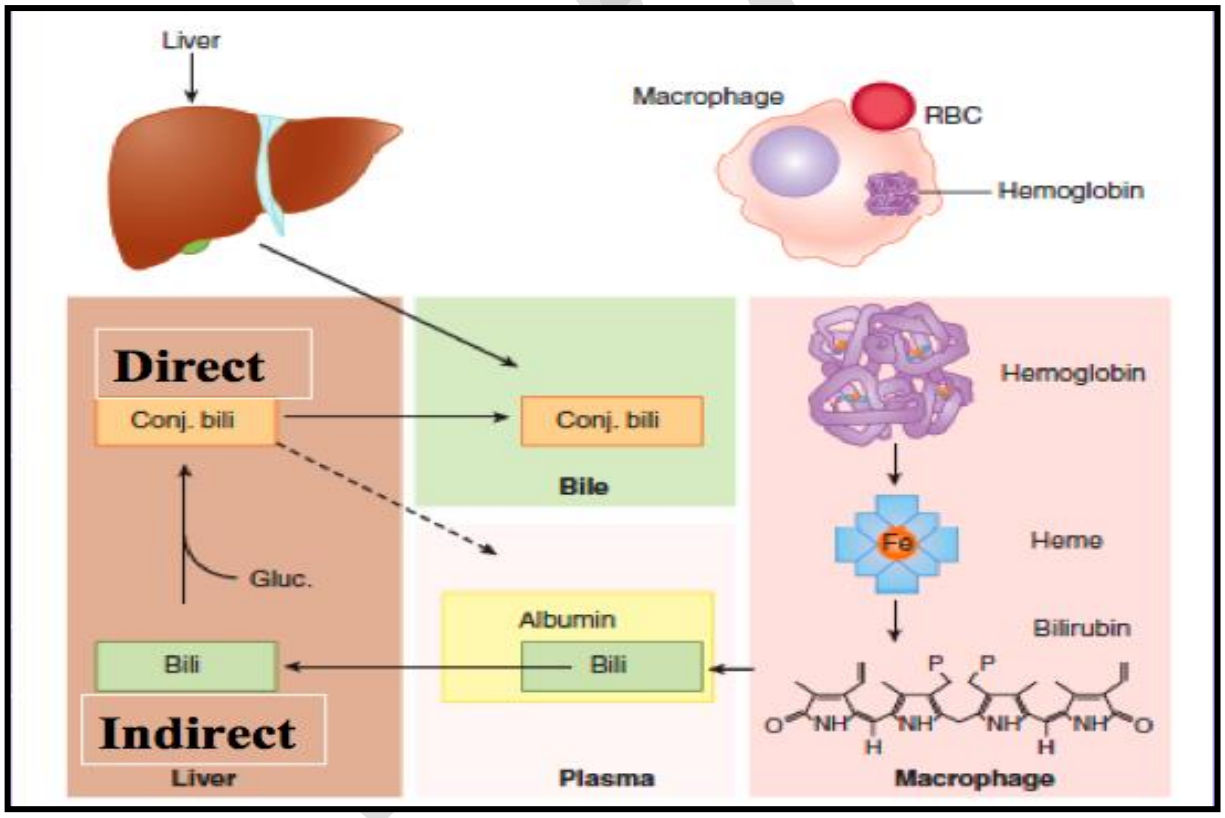
Heme is **catabolized to unconjugated bilirubin** in the **reticuloendothelial system**. Unconjugated bilirubin **is bound to albumin in the plasma** and transported bound to albumin to the **liver** and is conjugated with **glucuronic acid** in the hepatocytes; the conjugation is catalyzed by **glucuronyl transferase**.

Conjugated bilirubin is secreted into the **bile** and enters the duodenum. In the small bowel, some of the bilirubin is **hydrolyzed to yield unconjugated bilirubin and glucuronic acid.** Most unconjugated bilirubin is **excreted in the stool**, but some is reabsorbed and returned to the liver for re-conjugation (enterohepatic circulation).



About 80 percent of BR is derived from the **breakdown of heme** within the reticuloendothelial system. Other sources include **breakdown of immature red cells** in the bone marrow and of compounds chemically related to hemoglobin, such as myoglobin and the cytochromes.

The resulting BR is insoluble in water but soluble in lipid and in the organic solvents, it is carried by albumin and circulates in blood to reach the liver where conjugated with glucuronic acid. The conjugated BR is soluble in water and is excreted in the bile but some of it regurgitates into circulation and if exceeds 0.4 mg/100ml then appears in urine.



<u>In the liver</u>, bilirubin is **conjugated with glucuronic acid** by the enzyme **glucuronyltransferase**, making it soluble in water: the conjugated version is also often called "direct" bilirubin. **Much of it** goes into the **bile** and thus out into the **small intestine**. Though most bile acid is resorbed in the terminal ileum to participate in enterohepatic circulation, **conjugated bilirubin is not absorbed and instead passes into the colon.**

There, **colonic bacteria deconjugate and metabolize** the bilirubin into **colorless urobilinogen**, which can be **oxidized** to form **urobilin and stercobilin**: these give stool its characteristic brown color. A trace (~1%) of the urobilinogen is reabsorbed into the enterohepatic circulation to be re-excreted in the bile: some of this is instead processed by the kidneys, coloring the urine yellow.

Conjugated BR ("direct")

Reacts with **diazo reagent** (Diazotized sulphanlic acid) to give a purple color and said to have **direct Van Den Berg reaction.**

Unconjugated BR, ("indirect")

As it is **insoluble in water**, does **not react with Daizo** reagent but if methanol is added then it gives the reaction so is said to have **indirect Van Den Berg reaction.**

Bilirubin inUrine

Under **normal** circumstances, **a tiny amount of urobilinogen**, but if the **liver's function is impaired** or when **biliary drainage is blocked**, some of the **conjugated bilirubin leaks out of the hepatocytes and appears in the urine,** turning it **dark amber.**

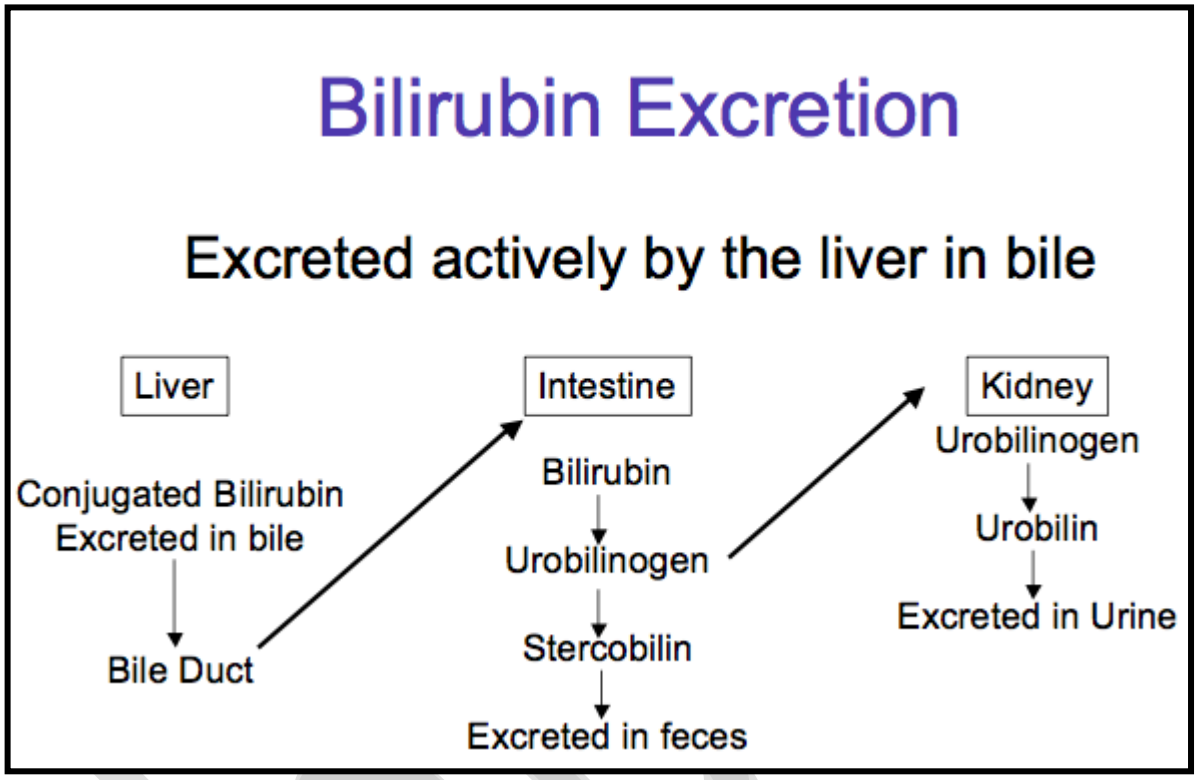
However, in disorders involving **hemolytic anemia, an increased number of red blood cells are broken down,** causing an **increase in the amount of unconjugated** bilirubin in the blood.

Because the **unconjugated** bilirubin is **not water-soluble**, one will **not see an increase in bilirubin in the urine.** Because there is no problem with the liver or bile systems, this excess unconjugated bilirubin will go through all of the normal processing mechanisms that occur (e.g., conjugation, excretion in bile, metabolism to urobilinogen, reabsorption) and will show up as an increase in urine urobilinogen. This difference between increased urine bilirubin and increased urine urobilinogen helps to distinguish between various disorders in those systems.

In adults and older children, bilirubin is measured to **diagnose and/or monitor liver diseases**, such as cirrhosis, hepatitis, or gallstones. It is also used **to evaluate people with sickle cell disease** or other **causes of hemolytic anemia** who may have episodes when excessive red blood cell destruction takes place, increasing bilirubin levels.

Bilirubin can be measured as a **total level and/or as conjugated and unconjugated levels** for these purposes. More commonly, the laboratory uses a chemical test to detect water-soluble forms of bilirubin, termed direct bilirubin, which is an estimate of the amount of conjugated

bilirubin. By subtracting this from the total bilirubin, an indirect estimate (indirect bilirubin) of unconjugated bilirubin is obtained.



Normal Range:

Total serum bilirubin = 0.3-1.1 mg/dl

Direct bilirubin = 0.1-0.4 mg/dl

Indirect bilirubin = 0.2-0.7 mg/dl

Newborn Jaundice occurs because the baby's body has more bilirubin than it can get rid of. Bilirubin leaves the body through urine and stool. In pregnant woman, the body removes bilirubin from the baby through the placenta. After birth, the baby's body must get rid of the bilirubin on its own.

Physiologic jaundice, occurs because their organs aren't yet able to get rid of excess bilirubin very well. This type of jaundice usually appears about 24 hours after birth. It gets worse until the third or fourth day, and then it goes away in about a week.

In rare cases, jaundice may be caused by other things, such as an infection, a problem with the baby's digestive system, or a problem with the mom's and baby's blood types (Rh incompatibility). Your baby may have one of these problems if jaundice appears less than a day after birth.

In newborns with jaundice, bilirubin is measured to investigate the cause. Excessive unconjugated bilirubin damages developing brain cells in infants and may cause mental retardation, learning and developmental disabilities, hearing loss, or eye movement problems. It is important that an elevated level of bilirubin in a newborn be identified and quickly treated. In both physiologic jaundice of the newborn and hemolytic disease of the newborn, only unconjugated (indirect) bilirubin is increased. In the much less common cases of damage to the liver (neonatal hepatitis and biliary atresia), conjugated (direct) bilirubin elevations are present as well, often providing the first evidence that one of these less common conditions is present.

The baby will need treatment if the bilirubin level is above the normal range for newborns. He or she will be put under a type of fluorescent light to treat the jaundice. This is called **phototherapy** . The skin absorbs the light, which changes the bilirubin so that the body can more easily get rid of it. The treatment is usually done in a hospital. But babies sometimes are treated at home.

Clinical Significance

Increased serum bilirubin may result from:

1) Increased destruction of hemoglobin (hemolysis).
2) Decreased excretion (or retention) due to either hepatocellular or excretory duct disease of the liver.

Jaundice, is a yellowish pigmentation of the skin, the conjunctival membranes over the sclerae (whites of the eyes), and other mucous membranes caused by high blood bilirubin levels.

Jaundice is manifested clinically when **total serum bilirubin exceeds 2 mg/dl**.This hyperbilirubinemia subsequently causes increased levels of bilirubin in the extracellular fluid. Concentration of bilirubin in blood plasma is normally below 1.2 mg/dL(<25µmol/L).

Jaundice is often seen in liver disease such as **hepatitis or liver cancer**. It may also indicate leptospirosis or obstruction of the biliary tract, for example by gallstones or pancreatic cancer, or less commonly be congenital in origin (e.g., biliary atresia).

Jaundice is classified into:

1. **Pre-hepatic jaundice** , is due to:
   - **A.** Overproduction of bilirubin,as in:

1- Hemolytic anemia. 2- Incompatible blood transfusion.

B. Hemolytic disease of the newborn due to Rh-incompatibility where total serum bilirubin may exceed 20mg/dl and may cross blood brain barrier causing **kernicterus**.

C. Transport Defects

Either at the level of carrying capacity of the blood as in the case of neonates exposed to sulphonamides or the defect is at the level of uptake by the liver (Gilberts Disease)or at the level of conjuction (Griggler-Najar's syndrome).

2. Hepato-cellular jaundice

Disease of the parenchymal cells of the liver may impair conjugation and excertion of bilirubin to various degrees:

A. Congenital: Criggler-Najar's syndrome (Impaired conjugation)
Dubin-Johnsons syndrome (Impaired excretion).

B. Premature enzymatic system as in physiological jaundice of the newborn.

C. Hepatitis:

1. Due to toxic agents: paracetamol, alcohol.

2. Due to infective agents: viral hepatitis, Septicemia.

3. Post-hepatic (obstructive, cholestatic):

May be medical **(Intra-hepatic)** or surgical **(extra-hepatic).**

Intrahepatic cholestasis occurs in infectious hepatitis, cirrhosis, intrahepatic carcinoma and on intake of drugs such as phenothiazines and methyl testosterone. While extra-hepatic cholestasis is caused by mechanical obstruction of the biliary tree mostly due to gallstones or carcinoma of the head of pancreas.