

Bilirubin Metabolism (Lecture 8)

Excretory function of bile

- Bile is a medium for excretion of many substances as bile pigments, cholesterol, many drugs, toxins and various organic substances as copper and zinc. These substances are then eliminated in the feces.
- One of these substances excreted in bile is the greenish yellow pigment bilirubin. This is a major end product of hemoglobin degradation.
- It's highly soluble in all cell membranes & is also very toxic. Therefore, its excretion in the bile is one of the very important functions of the liver.

FATE OF RED BLOOD CELLS

- Life span of RBCs in blood stream is 60-120 days.
- Senescent RBCs become too fragile to exist longer in the circulatory system, their cell membranes rupture and they are phagocytosed and/or lysed.
- Normally, lysis occurs extravascularly in the reticuloendothelial system subsequent to RBC phagocytosis.
- Lysis can also occur intravascularly (in blood stream).
- The hemoglobin is first split into globin & heme.
- The AA formed from breakdown of globin are stored in the body.

Metabolism of bilirubin

The heme ring is opened to give:

1. Free iron that is transported in the blood by transferrin and stored in the body as a reservoir for erythropoiesis.
2. Bile pigments:
 - ❖ The 1st pigment is biliverdin but it is rapidly reduced by biliverdin reductase to free bilirubin which is gradually released into the plasma.
 - ❖ The free bilirubin is hydrophobic, immediately combines with plasma proteins (mainly albumin and globulin) forming a water soluble compound called hemobilirubin (unconjugated bilirubin) which is rapidly transported to hepatocytes for further metabolism. Even when bound to albumin it's called free bilirubin.

- ❖ Bilirubin is absorbed through the hepatic cell membrane, mediated by a carrier protein (receptor) & combined with Y & Z proteins that trap the bilirubin inside the cells.
- ❖ Thereafter, in the liver cells hemobilirubin dissociates into protein and free bilirubin.
- ❖ About 80% of bilirubin conjugates with glucuronic acid catalyzed by the enzyme glucuronyl transferase. This enzyme is located primarily in the smooth endoplasmic reticulum.
- ❖ Each bilirubin molecule reacts with 2 uridine diphospho-glucuronic acid (UDPGA) molecules to form bilirubin diglucuronide (cholebilirubin, conjugated bilirubin) which is more water soluble than the free bilirubin.
- ❖ 10% conjugate with sulphate to form bilirubin sulphate,
- ❖ The final 10% conjugate with other substances.
- ❖ These forms of bilirubin are actively secreted by the liver cells by an active transport process into the bile canaliculi.
- ❖ The color of bile is due to bilirubin.
- ❖ In normal adults this results in a daily load of 250-300 mg of bilirubin.
- ❖ Normal plasma concentrations are less than 1 mg / dL.

Fate of conjugated bilirubin

- A small portion of the conjugated bilirubin returns to the plasma either directly into the liver sinusoids or indirectly by absorption into the blood from the bile ducts or lymphatics.
- This causes a small portion of the bilirubin in the extracellular fluid always to be of the conjugated type rather than of the free type.
- Some of conjugated bilirubin escapes into the blood where it is bound less tightly to albumin & is excreted in the urine.
- Small amount of bilirubin glucuronide is deconjugated and absorbed by the small intestine into the portal blood to the liver where it is extracted by the liver cells and conjugate again and excreted in the bile (enterohepatic circulation of bile pigments).
- The intestinal mucosa is relatively impermeable to conjugated bilirubin but permeable to unconjugated bilirubin.

➤ The majority of cholebilirubin passes via the bile ducts to the intestine where it is transformed through bacterial action into urobilinogen which is highly soluble.

Fate of urobilinogen

✓ Most of urobilinogen (70%) in the intestine is converted into stercobilinogen, which is oxidized and excreted in the feces in the form of stercobilin that causes dark brown color of the feces.

✓ Some of urobilinogen (20 %) of urobilinogen is reabsorbed through the intestinal mucosa into the portal vein and passes to the liver and re-excreted by the hepatic cells in the bile (enterohepatic circulation of urobilinogen).

✓ Small amount of urobilinogen escapes to the general circulation and excreted by the kidneys in the urine where it is oxidized to urobilin when the urine is exposed to air.

Differentiation between conjugated & unconjugated bilirubin

By van den Bergh reaction:

1- If the bilirubin is of the conjugated type:

An immediate reaction occurs with van den Bergh reagent (which gives a colorimetric change), and the reaction is called a direct van den Bergh reaction.

* Conjugated bilirubin + Diazo reagent \longrightarrow Purple color

2- If the bilirubin is of the unconjugated (free) type:

One must add ethanol to the plasma. This precipitates the protein and frees albumin from its protein complex so that it can combine with van den Bergh reagent. This causes the colorimetric changes to be much stronger, and the additional result is called the indirect van den Bergh reaction.

* Unconjugated bilirubin + Ethanol \longrightarrow Free bilirubin

* Free bilirubin + Diazo reagent \longrightarrow Purple color.

3- Biphasic van den Bergh reaction:

It occurs when blood contains both conjugated and unconjugated bilirubin.

In this case purple color appears without adding alcohol and is intensified after adding it.

It should be noted that:

Transport of bilirubin in plasma occurs in two forms:

1- Unconjugated bilirubin

- It is indirect reacting bilirubin-hemobilirubin.
- It is the chief form of bilirubin in the blood.
- It bound to albumin.
- It is not filtered through renal glomeruli and hence not present in urine.

2- Conjugated bilirubin

- It is direct reacting bilirubin-cholebilirubin.
- It is present in low conc. in the blood.
- It bound to glucuronic acid.
- It is filtered through renal glomeruli & excreted in urine.

Other Substances Conjugated by Glucuronyl Transferase

❖ The glucuronyl transferase system in the smooth endoplasmic reticulum catalyzes the formation of the glucuronides of a variety of substances in addition to bilirubin.

❖ The list includes steroids & various drugs. These other compounds can compete with bilirubin for the enzyme system when they are present in appreciable amounts.

❖ In addition several barbiturates, antihistamines, anticonvulsants and other compounds can cause marked proliferation of the smooth endoplasmic reticulum in the hepatic cells, with a concurrent increase in hepatic glucuronyl transferase activity.

❖ Phenobarbital has been used successfully for the treatment of a congenital disease in which there is a relative deficiency of glucuronyl transferase (type 2 UDP-glucuronyl transferase deficiency).

Other Substances Excreted in the Bile

❖ Cholesterol & alkaline phosphatase are excreted in the bile.

- In patients with jaundice due to intra or extra hepatic obstruction of the bile duct, the blood levels of these 2 substances usually rise.
- A much smaller rise is generally seen when the jaundice is due to non obstructive hepatocellular disease.

❖ Adrenocortical, other steroid hormones & a number of drugs are excreted in the bile and subsequently reabsorbed (enterohepatic circulation).