



8 BILIRUBIN METABOLISM



GIT

Learning issues :

- Fate of red blood cells
- Metabolism of bilirubin
- Conjugation of bilirubin with glucuronic acid inside hepatocytes
- Fate of conjugated bilirubin
- Fate of urobilinogen
- Differentiation between conjugated & unconjugated bilirubin
- Other substances conjugated by glucuronyltransferase.
- Other substances excreted in the bile

The liver

Liver functions

1. Exocrine role
 - i. Synthesizes and secretes bile salt
 - ii. Secretes into the bile a bicarbonate-rich solution
 - iii. Destroy erythrocytes
2. Endocrine role
 1. Regulates circulating blood lipids.
 2. Regulates glucose level in the blood.
3. Synthesis clotting factors and plasma proteins
4. Metabolizes the organic substances & cholesterol.

Types of cell

1. **Hepatocytes:** highly specialized cell
2. **Endothelial cells:** they lack a basement membrane (to enhance the exchanging between the blood and hepatocytes)
3. **Kupffer cells:** line the hepatic sinusoids and they are part of reticuloendothelial system
4. **Stellate cells:** in inflammation(see cirrhosis lecture), they become transformed to myofibroblasts, which they secrete collagen into the space of Diss and control portal pressure by their contractions.

Portal circulation

The liver receives blood from portal vein and hepatic artery. Both, they drain into the sinusoids. And the sinusoids empty into central vein → hepatic vein → inferior vena cava.

The lymphatic system

Lymph nodes drain fluid and proteins (the protein concentration is highest in LN from the liver)

The largest space drained by lymphatic system is the perisinusoidal space.

What is bilirubin ?

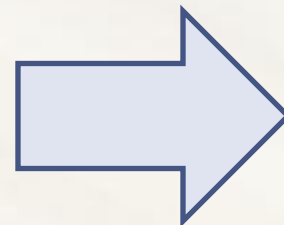
- ✓ Major end product of hemoglobin degradation .
- ✓ Highly soluble in all cell membranes.
- ✓ Very toxic therefore, its excretion in the bile is one of the very important functions of the liver.
- ✓ greenish yellow pigment excreted in bile



http://www.youtube.com/watch?v=dJ_das_mimE4

- I hope you watch it before start studying
-The duration is about 9 mins

- Life span of RBCs is 60-120 days
- Cell membrane of Senescent RBCs * rupture and they are phagocytosed and/or lysed.

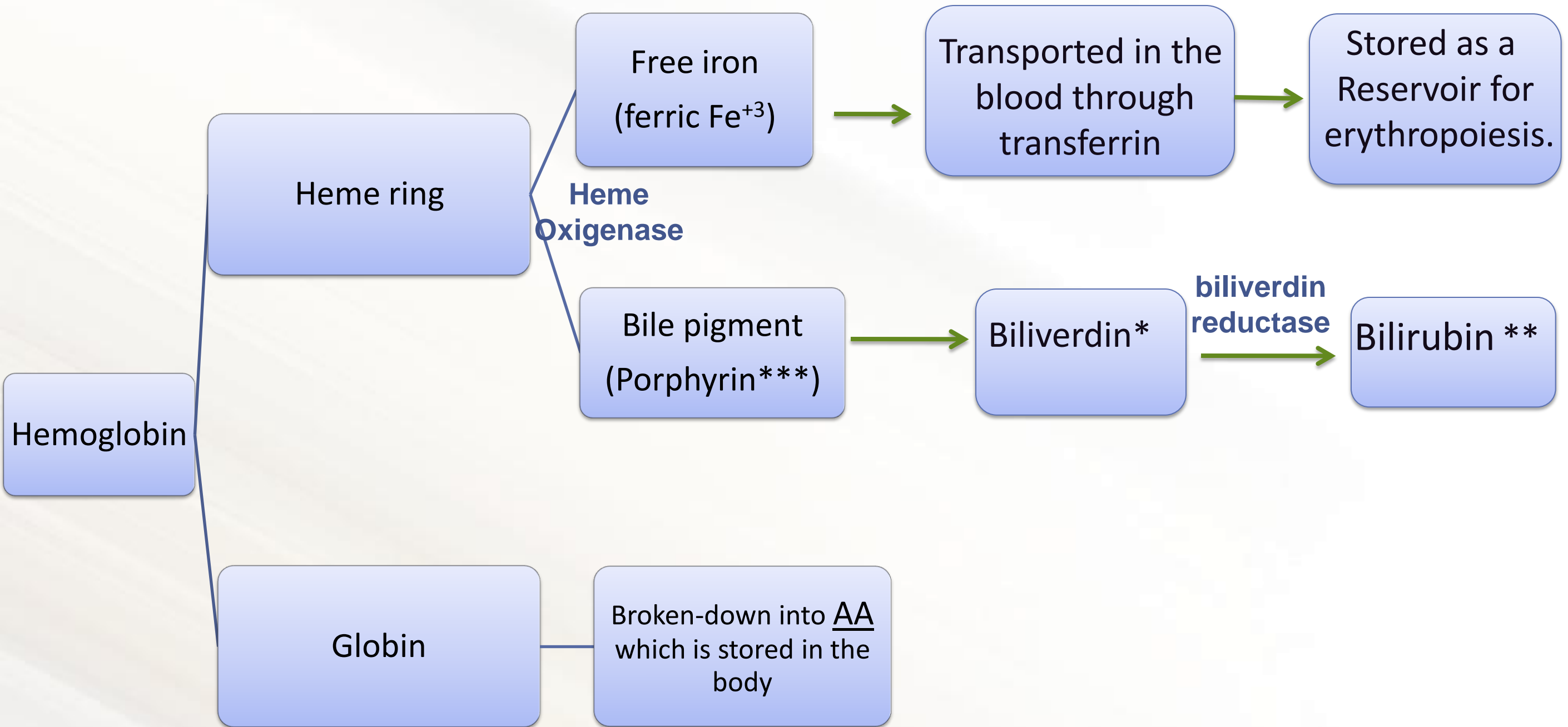


Lysis occurs

Extravascularly
(in reticulo-
endothelial system

Intravascularly
(in blood stream).

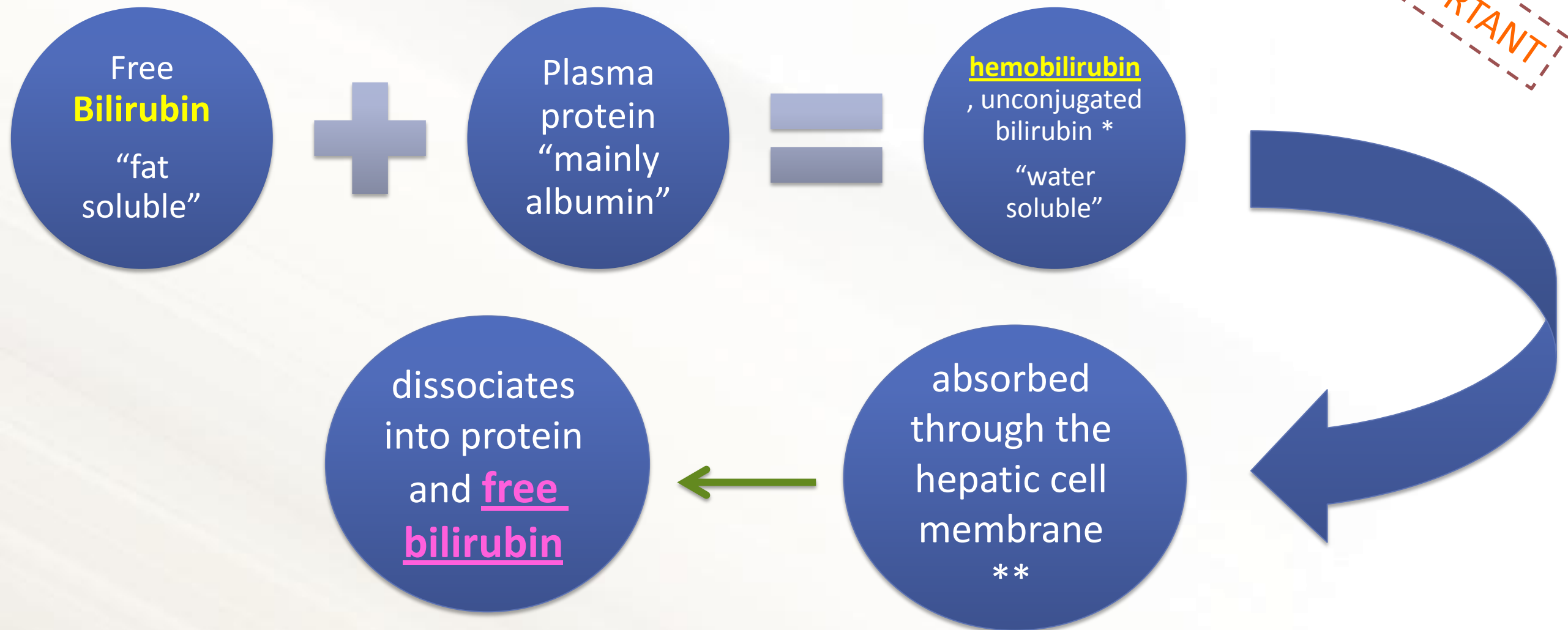
* Senescent RBCs are those who become too too fragile to exist longer in the circulatory system



* Is the first pigment

** gradually released into plasma

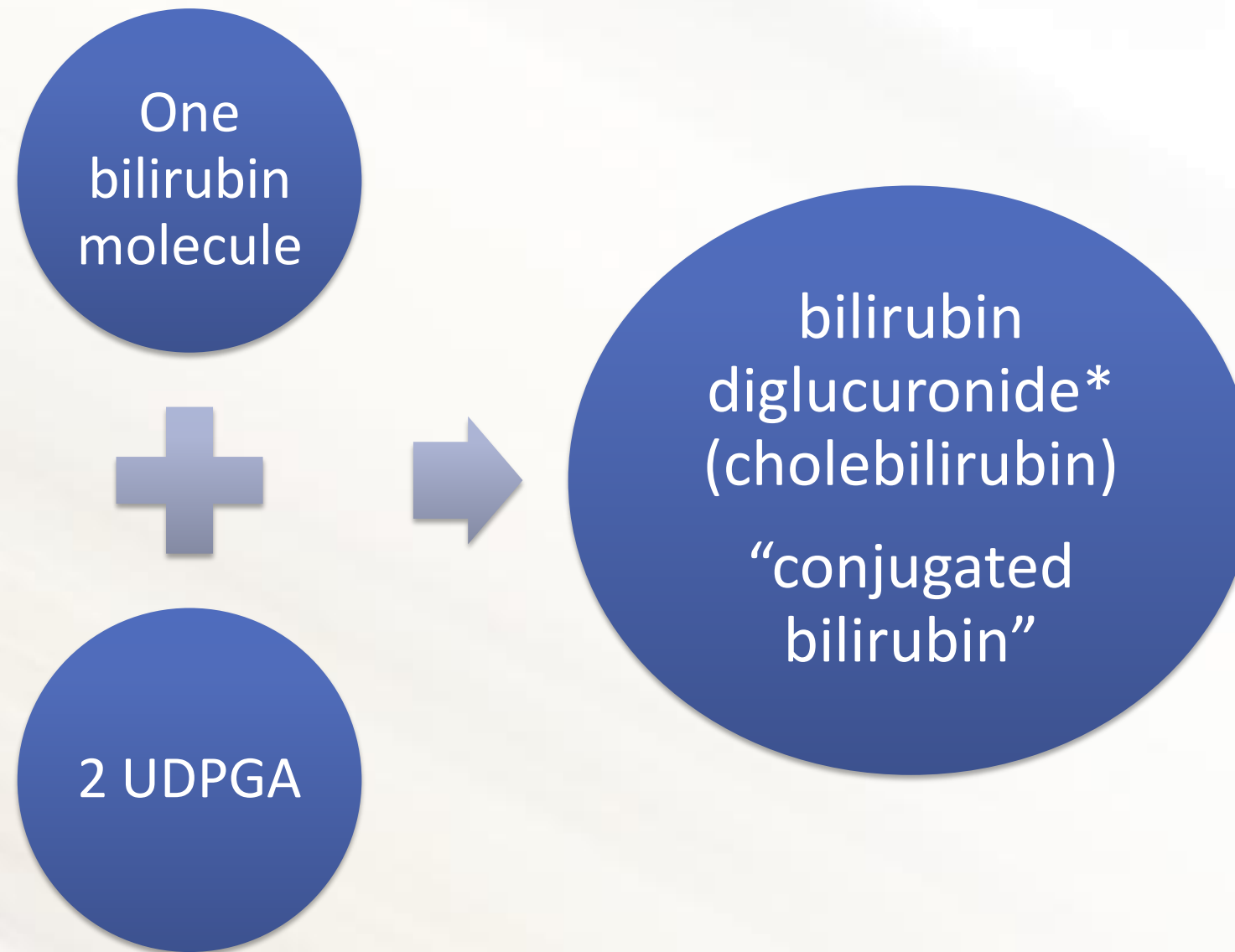
*** Is chemical compound found attached to iron in heme.



* Even it's bound to albumin it is called free bilirubin.

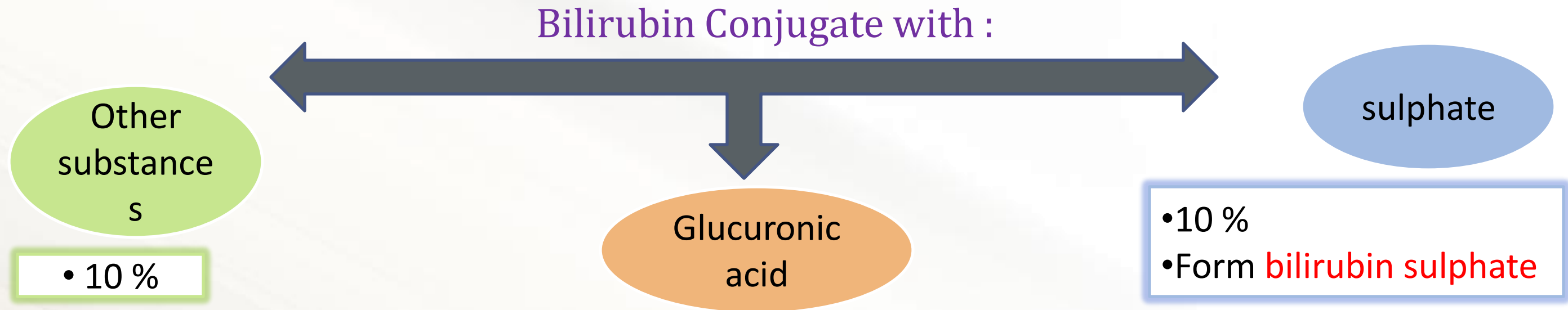
The absorption mechanism is mediated by a carrier protein (active transport**) & combined with Y & Z proteins that trap the bilirubin inside the cells.

In the smooth endoplasmic reticulum → 80% of **the free bilirubin** conjugates with glucuronic acid catalyzed by the enzyme glucuronyl transferase.



* The **conjugated bilirubin** is more **water soluble** than the free bilirubin.

Conjugated bilirubin “direct”:



• 10 %

• 80 %

• **HOW ?** One bilirubin molecule reacts with **2 uridine diphospho-glucuronic acid (UDPGA)** molecules to form bilirubin diglucuronide (**cholebilirubin**)

• more **water soluble** than the free bilirubin.

• catalyzed by the enzyme **glucuronyl transferase** in the smooth endoplasmic reticulum.

• 10 %

• Form **bilirubin sulphate**

• All these forms of bilirubin are **actively secreted** by the liver cells by an active transport process into the bile canaliculi (**Note: : the unconjugated bilirubin is normally not secreted**)

• The color of bile is yellow-green due to bilirubin

• Normal plasma concentrations are less than 1 mg/dL (**the increase cause Jaundice**)

• In normal adults this results in a daily load of 250-300 mg of bilirubin.

Fate of conjugated Bilirubin :

1/ **small portion of the conjugated bilirubin** returns to the plasma either :

Directly
into the liver sinusoids (then re-excreted in bile again)

Indirectly

- absorption into the blood from the bile ducts or lymphatics
- bound less tightly to albumin & is excreted in the urine (not responsible for the normal color of the urine, but the increase of its amount causes the change in the urine color)

❖ 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 .

2/ **Small amount** of bilirubin glucuronideis (conjugated form) → **de-conjugated*** → 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 (**entero-hepatic-circulation of bile pigments**).

❖ **NT** : the intestinal mucosa is relatively impermeable to conjugated bilirubin (**hydrophilic**) but permeable to un-conjugated bilirubin (**hydrophobic**)

CONT...

Fate of conjugated Bilirubin :

3/ **The majority of conjugated bilirubin** passes via the bile ducts to the intestine where it is transformed through **bacterial action** into **urobilinogen** which is highly soluble :

70% of urobilinogen :

- converted into **stercobilinogen**
- oxidized to **stercobilin**
- excreted in the feces (gives the dark color of stool)

20% of urobilinogen :

- reabsorbed through the intestinal mucosa into the portal vein
- passes to the liver and re-excreted by the hepatic cells in the bile (**entero-hepatic-circulation of urobilinogen**)

10% of urobilinogen :

- excreted in urine
- oxidized to **urobilin** when the urine is exposed to air.

Differentiation between conjugated and unconjugated bilirubin :

By van den Bergh reaction :

If the bilirubin is of the conjugated type :

- An immediate reaction occurs with Van den Bergh reagent (**Diazo reagent**) (which gives a colorimetric change).
- the reaction is called a **direct Van den Bergh reaction** .

Conjugated bilirubin + Diazo reagent → Purple color

If the bilirubin is of the un-conjugated type :

- Adding **ethanol** to the plasma precipitates the protein and frees bilirubin from its protein complex, then combine it with Van den Bergh reagent(**Diazo reagent**)
- (the colorimetric changes to be much stronger), the additional result is called **an indirect Van den Bergh reaction** .

Un-conjugated bilirubin + Ethanol → Free bilirubin

Free bilirubin + Diazo reagent → Purple color

The doctor said : we have to do the direct reaction first then the indirect

NOTE : conjugated and un-conjugated are classified according to **glucuronyl acid** not according to plasma protein.

CONT...

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.

يعني في حالة الدم يحتوي النوعين يظهر باللون البنفسجي علطول , و بمجرد اضافة الكحول تزيد كثافة اللون .

Transport of bilirubin in plasma occurs in two forms :

UN-CONJUGATED BILIRUBIN	CONJUGATED BILIRUBIN
Indirect reacting bilirubin-hemobilirubin	Direct reacting bilirubin-cholebilirubin
The chief form of bilirubin in the blood	Present in low conc. in the blood
Bound to albumin	Bound to glucuronic acid
Not filtered through renal glomeruli	Filtered through renal glomeruli
Not present in urine	Excreted in urine
Water insoluble	Water soluble
Toxic substance	Non-toxic substance

Other substances conjugated by glucuronyl transferse:

- ❖ 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** (ex. **Estrogen secreted in the bile**) & 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).
 - ❖ Adrenocortical, other steroid hormones & a number of drugs are excreted in the bile and subsequently reabsorbed (enterohepatic circulation).
 - ❖ **Cholesterol & alkaline phosphatase** are excreted in the bile.
- 1/ In patients with jaundice due to intra or extra hepatic obstruction of the bile duct, the blood levels of these 2 substances usually rise.
 - 2/ A much smaller rise is generally seen when the jaundice is due to non obstructive hepatocellular disease.

What is the free type of bilirubin ?

It is the un-conjugated bilirubin, that was decided based on the attachment of the bilirubin.

If with plasma protein in circulation → Free type “un-conjugated or indirect”.

If with Glucuronic acid or phosphate → “conjugated or direct”

Which will cause the urine color to change ?

↑ in conjugated bilirubin amount in the circulation which will be filtrated in kidneys.

Entro-hepatic-circulation ?

1/ small portion of the conjugated bilirubin returns **directly** to the liver sinusoids then re-excreted in bile again

2/ small portion of conjugated form → de-conjugated → absorbed by the small intestine to → the liver where it conjugate again and excreted in the bile again

3/ large portion of conjugated bilirubin transformed into **urobilinogen** → **20% of urobilinogen** reabsorbed to the liver and re-excreted by the hepatic cells in the bile

Bilirubin in urine ?

1/ small portion of the conjugated bilirubin returns **indirectly** to the circulation , then excreted in urine .

2/ large portion of conjugated bilirubin transformed into **urobilinogen** → **10% of urobilinogen** excreted in urine oxidized to **urobilin** when the urine is exposed to air.



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