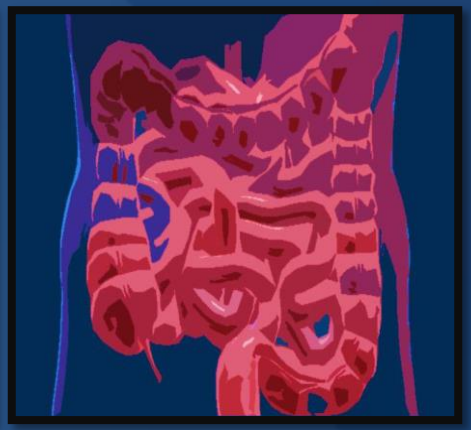
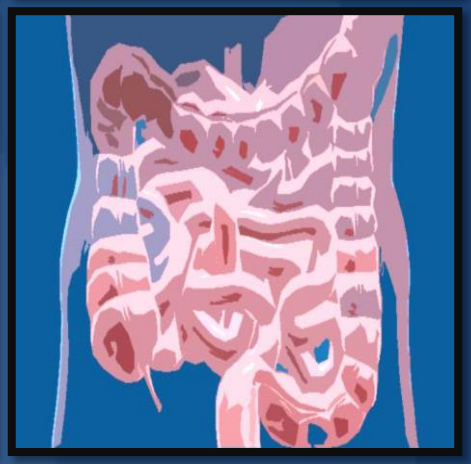
Physiology Team

GASTOINTSTINAL TRACT

LECTURE 8

BILIRUBIN METABOLISM





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OBJECTIVES

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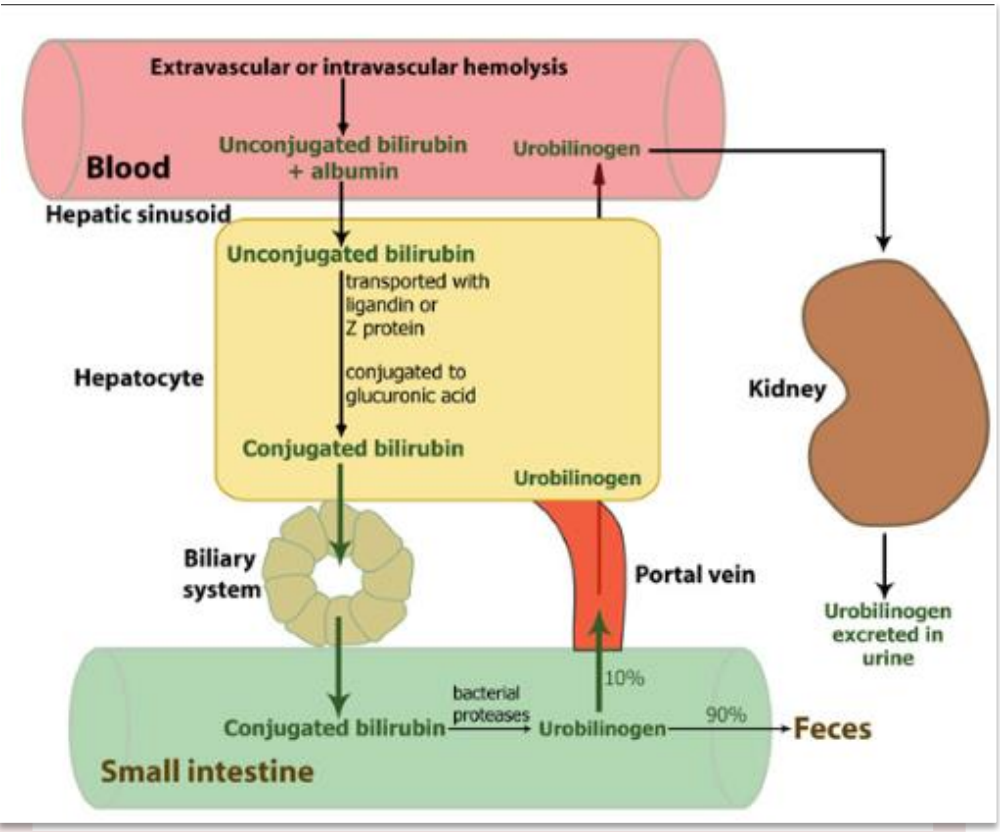
At the end of this lecture, student should be able to describe:

- 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 glucuronyl transferase.
- Other substances excreted in the bile



Bilirubin Metabolism

- Fate of RBCs
- Metabolism of Bilirubin
- Conjugation of bilirubin with glucuronic acid inside hepatocytes
- Fate conjugated bilirubin
- Fate of urobilinogen
- Substances conjugated by glucuronyl transferase
- Differentiation between conjugated & unconjugated bilirubin

BILIRUBIN

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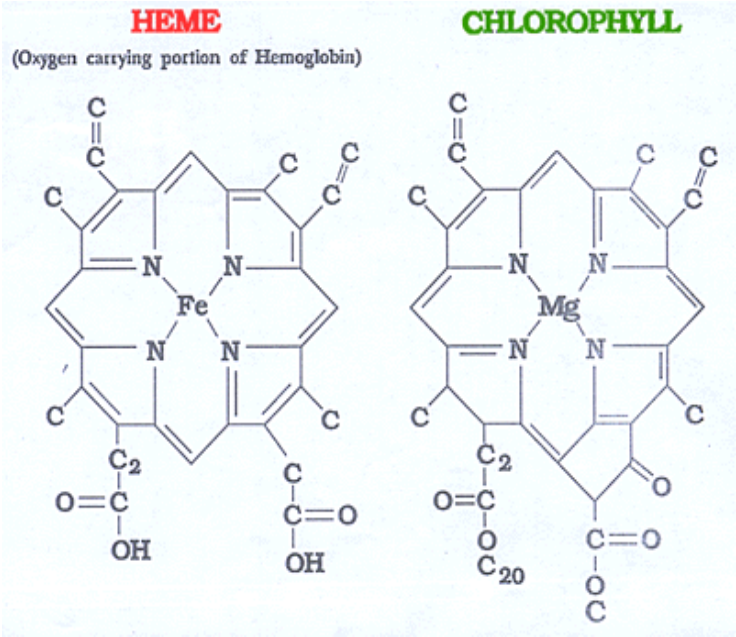
- Bilirubin is the greenish yellow pigment excreted in bile.

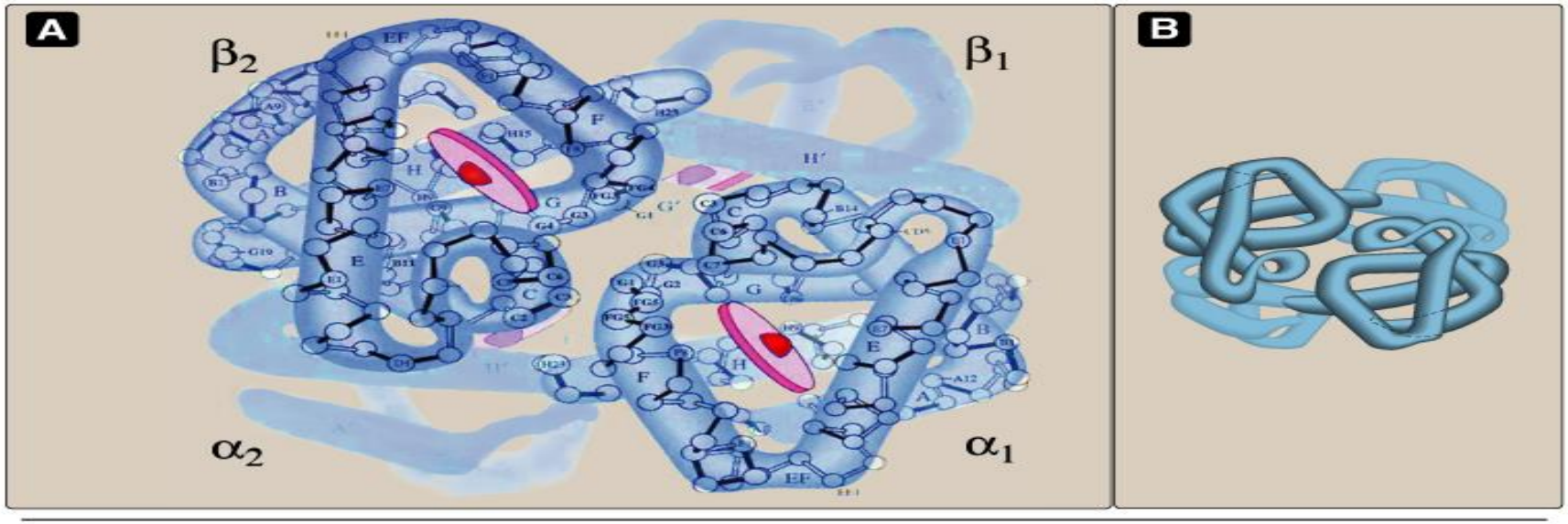
  Bile contents are: Bilirubin - Bile acids (Salts) – Cholestrole. So, bilirubin is one of the bile pigments.

- It is a major end product of hemoglobin degradation.
- It is highly soluble in all cell membranes (Hydrophobic) & is also very toxic. Therefore, its excretion in the bile is one of the very important functions of the liver.
- Essential function of the liver → get rid of bilirubin

- Porphyrins are cyclic compounds that readily bind metal ions- usually **Fe^{2+}** or **Fe^{+3}** which can carry **O_2**.
- Porphyrins are heterocyclic macrocycles composed of **four modified pyrrole subunits** interconnected at their **α carbon** atoms via methine bridges (=CH-).
- The most prevalent porphyrin in the human is **heme**, which consists of one ferrous (Fe^{2+}) iron ion coordinated in the center of tetrapyrrole ring of protoporphyrin IX.



- Structure of Hemoglobin showing the polypeptides backbone that are composed of **four** subunits: **2 α and 2 β subunits**. Every subunit is consisted of **one ferrous** (Fe^{2+}) iron ion coordinated in the center porphyrin compound.



Fate of Red Blood Cells

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- The Origin of bilirubin is from RBCs.
- 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 reticulo-endothelial system subsequent to RBC phagocytosis.
- Lysis can also occur intravascularly (in blood stream).
- The hemoglobin is first split into **globin** & **heme**.
- The AA (amino acids) formed from breakdown of globin are stored in the body.
- Globin is Amino Acid utilized by the body for further protein synthesis (Stored in the body.)
- Heme ring → Iron & Pigment

Metabolism of bilirubin

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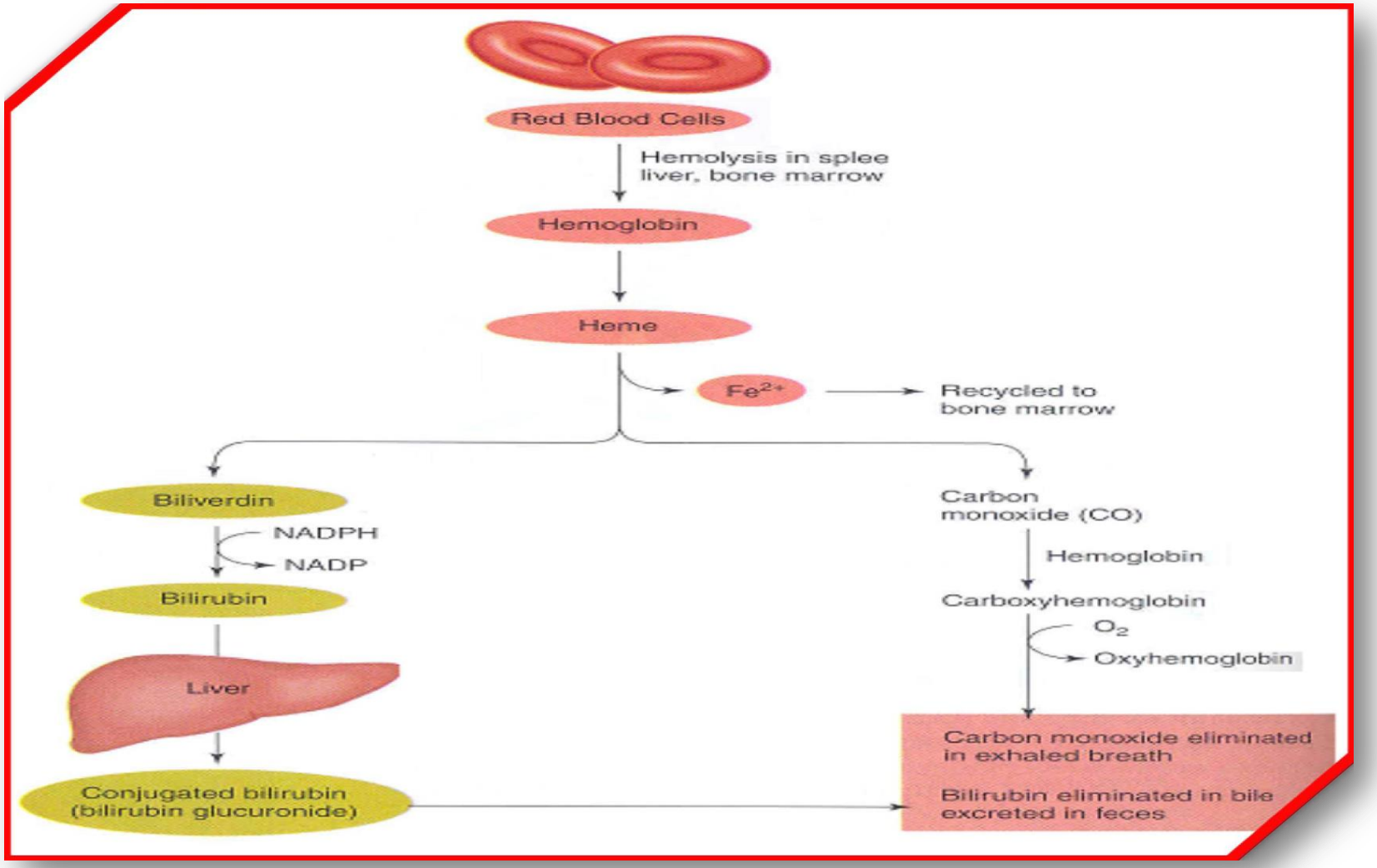
The heme ring is opened to give:

Free Iron	Bile Pigments
that is transported in the blood by transferrin and stored in the body as a reservoir for erythropoiesis. (Used for further hemoglobin synthesis)	The 1st pigment is biliverdin but it is rapidly reduced by biliverdin reductase to free bilirubin which is gradually released into the plasma.

In the presence of NADPH and O_2, the **Heme oxygenase** enzyme hydroxylates Heme, with a concomitant oxidation of ferrus Fe^{2+} iron to ferric Fe^{+3} , and converts it into Biliverdin.

- The free bilirubin is hydrophobic, immediately combines with plasma proteins (mainly albumin and globulin) forming a water soluble compound called hemobilirubin (unconjugated bilirubin) because not conjugated with any substances. 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.


Red Blood Cells
Hemolysis in splee
liver, bone marrow
Hemoglobin
Heme
Fe2+
Recycled to
bone marrow
Biliverdin
NADPH
NADP
Bilirubin
Liver
Conjugated bilirubin
(bilirubin glucuronide)
Carbon
monoxide (CO)
Hemoglobin
Carboxyhemoglobin
O2
Oxyhemoglobin
Carbon monoxide eliminated
in exhaled breath
Bilirubin eliminated in bile
excreted in feces

80% of Bilirubin	• conjugates with glucuronic acid catalyzed by the enzyme glucuronyl transferase in the smooth endoplasmic reticulum. **HOW?** 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% of Bilirubin	conjugate with sulphate to form bilirubin sulphate.
10% of Bilirubin	conjugate with other substances.

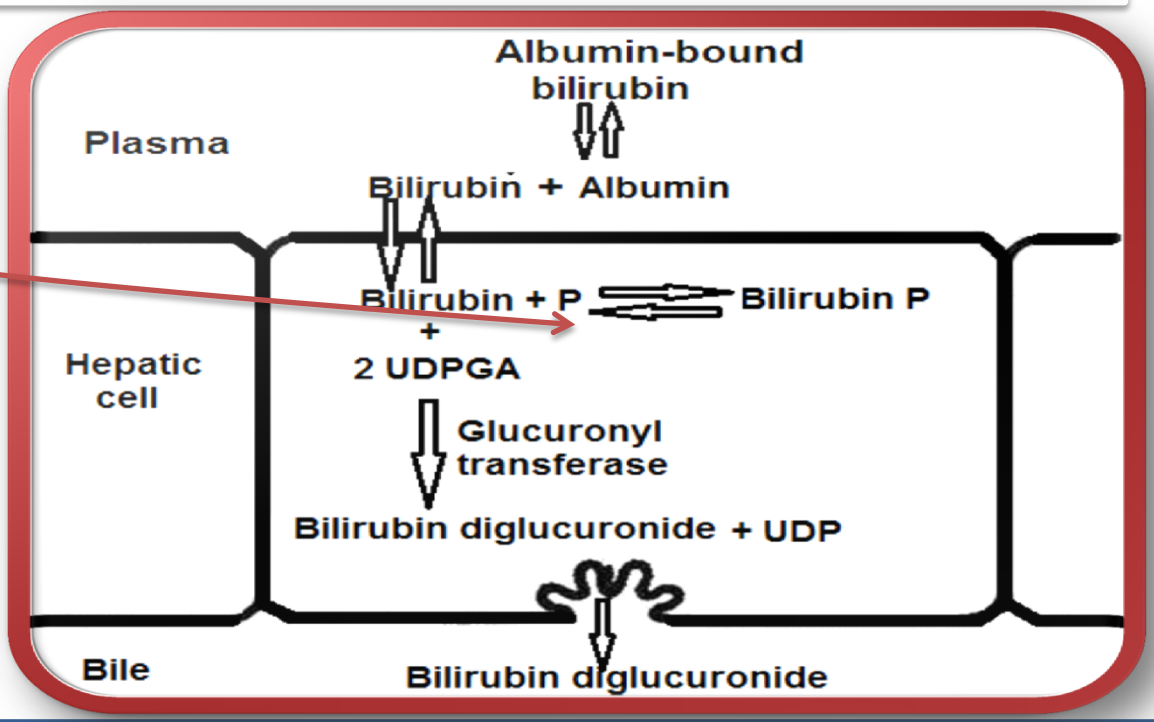
- These forms of bilirubin are actively secreted by the liver cells by an active transport process into the bile canaliculi. (**Note:** : the unconjugated bilirubin is normally not secreted.)
- The color of bile is due to bilirubin.
- In normal adults this results in a daily load of 250-300 mg of bilirubin.
- Normal plasma concentrations are less then 1 mg/dL.

(More than 1 mg/dl >>>> cause Jaundice)

① The major source of bilirubin is breaking down of hemoglobin.
② RBCs producing by bone marrow then go to the circulation.
③ When RBCs stack over liver, spleen or bone marrow…etc.
④ It will activate macrophages then the macrophages will breakdown the RBCs.
⑤ Normal RBCs goes for extravascular hemolysis not intravascular hemolysis.

P= Phosphate

Conjugation of bilirubin with glucuronic acid inside hepatocytes



Fate of conjugated bilirubin

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- 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. The two forms (Conjugated and Unconjugated)found in the circulation.

- 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. Because the conjugated form **hydrophilic** while unconjugated form **hydrophobic** .**(Small amount)**

- Some (Small amount) of conjugated bilirubin escapes into the blood where it is bound less tightly to albumin & is excreted in the urine.

The majority (Not small amount) of conjugated bilirubin passes via the bile ducts to the intestine where it is transformed through bacterial action into urobilinogen which is highly soluble.
*the majority pass to the intestine.
*Excreted in the urine because of it's highly soluble but not responsible for the color of the urine.

Fate of urobilinogen

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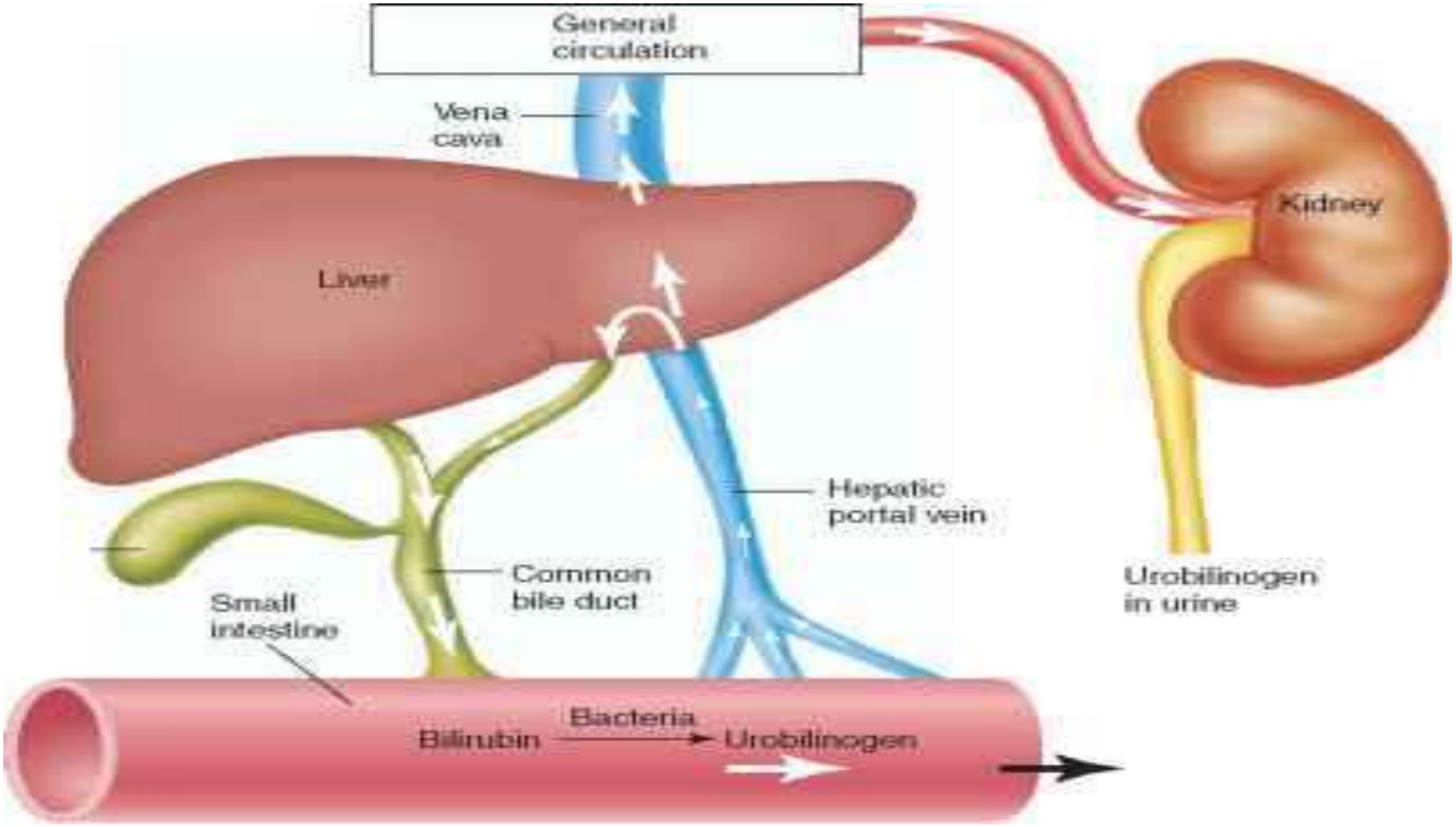
70% of urobilinogen (Most of urobilinogen)	in the intestine is converted (by bacteria) into stercobilinogen, oxidized and excreted in the feces as stercobilin that causes dark brown color of the feces.
20% of urobilinogen	urobilinogen is reabsorbed through the intestinal mucosa into the portal vein and passes to the liver and reexcreted by the hepatic cells in the bile (enterohepatic circulation of urobilinogen).
10% 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.

Fate of bilirubin:

① go to the circulation through liver sinusoids.
② enterohepatic circulation.
③ secreted in the urine.

Bile considered as Secretory and Excretory:

① Secretory: Contain bile salts which has many physiological functions in the body.
② Excretory: through the blood get rid of many unneeded substances.


General circulation
Vena cava
Liver
Kidney
Hepatic portal vein
Common bile duct
Small intestine
Urobilinogen in urine
Bacteria
Bilirubin
Urobilinogen

Summary of Bilirubin Formation and Excretion

Males' Slide

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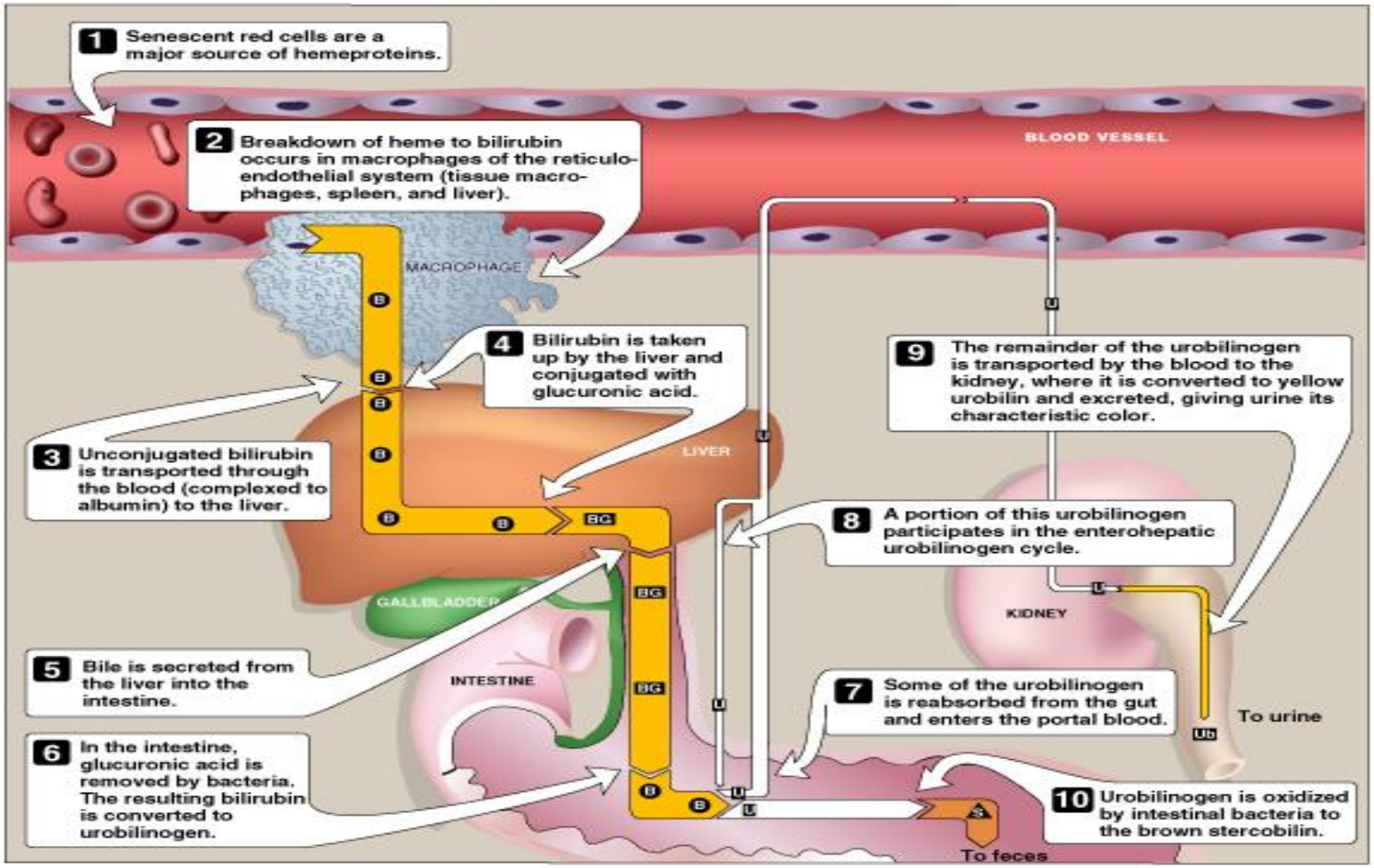

Slides | Important | Females' Notes | Explanation | Males' Notes

Differentiation between conjugated and unconjugated bilirubin

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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. (the purple color directly appeared without adding ethanol)

Conjugated bilirubin + Diazo reagent → Purple color

2) If the bilirubin is of the unconjugated (Free) type:

Adding ethanol to the plasma precipitates the protein and frees bilirubin 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. (Indirect because the purple color appeared after adding ethanol)

- **Unconjugated bilirubin + Ethanol → Free bilirubin**
- **Free bilirubin + Diazo reagent → Purple color.**

3) Biphasic van den Bergh reaction: (Both direct and indirect)

- 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.

NOTE: conjugated and unconjugated are classified according to glucuronyl acid not according to plasma protein.

Transport of bilirubin in plasma occurs in two forms:

Important

Unconjugated 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.
- includes steroids & various drugs. These other compounds can compete with bilirubin for the enzyme system when they are present in appreciable amounts. (these drugs lead to bilirubin toxicity because of increase biirubin level in the circulation)
- 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 (used in case of Jaundice) 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**. (Increase cholesterol and alkaline phosphate level)
- 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)

- Normal serum bilirubin is 0.3-1.2 mg/dl
- 2.0-2.5 mg/dl causes Jaundice.

Main causes of Jaundice:

1. Excessive production of bilirubin
2. ↓ hepatocyte uptake
3. Impaired conjugation
4. ↓ hepatocyte excretion of bilirubin glucuronides
5. Impaired bile flow (obstruction of bile duct)

SUMMARY

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- **Bilirubin**, a yellow-colored byproduct of hemoglobin metabolism, is the major bile pigment.
- The cells **of reticuloendothelial system** degrade Hb, yielding bilirubin, which is carried in blood bound to albumin.
- The liver extracts bilirubin from blood and conjugated it with glucuronic acid to form **bilirubin glucuronide**, which is secreted into bile and accounts for bile's yellow color.
- Bilirubin glucuronide **(conjugated bilirubin**) is secreted into the intestine as a component of bile.
- In the intestinal lumen, bilirubin glucuronide is **converted back** to bilirubin, which is then converted to urobilinogen by the action of intestinal bacteria.
- A portion of the urobilinogen is recirculated to the liver, a portion is excreted in the urine, and a portion is oxidaized to urobilin and stercobilin, the compounds that give stool its dark color.

SUMMARY

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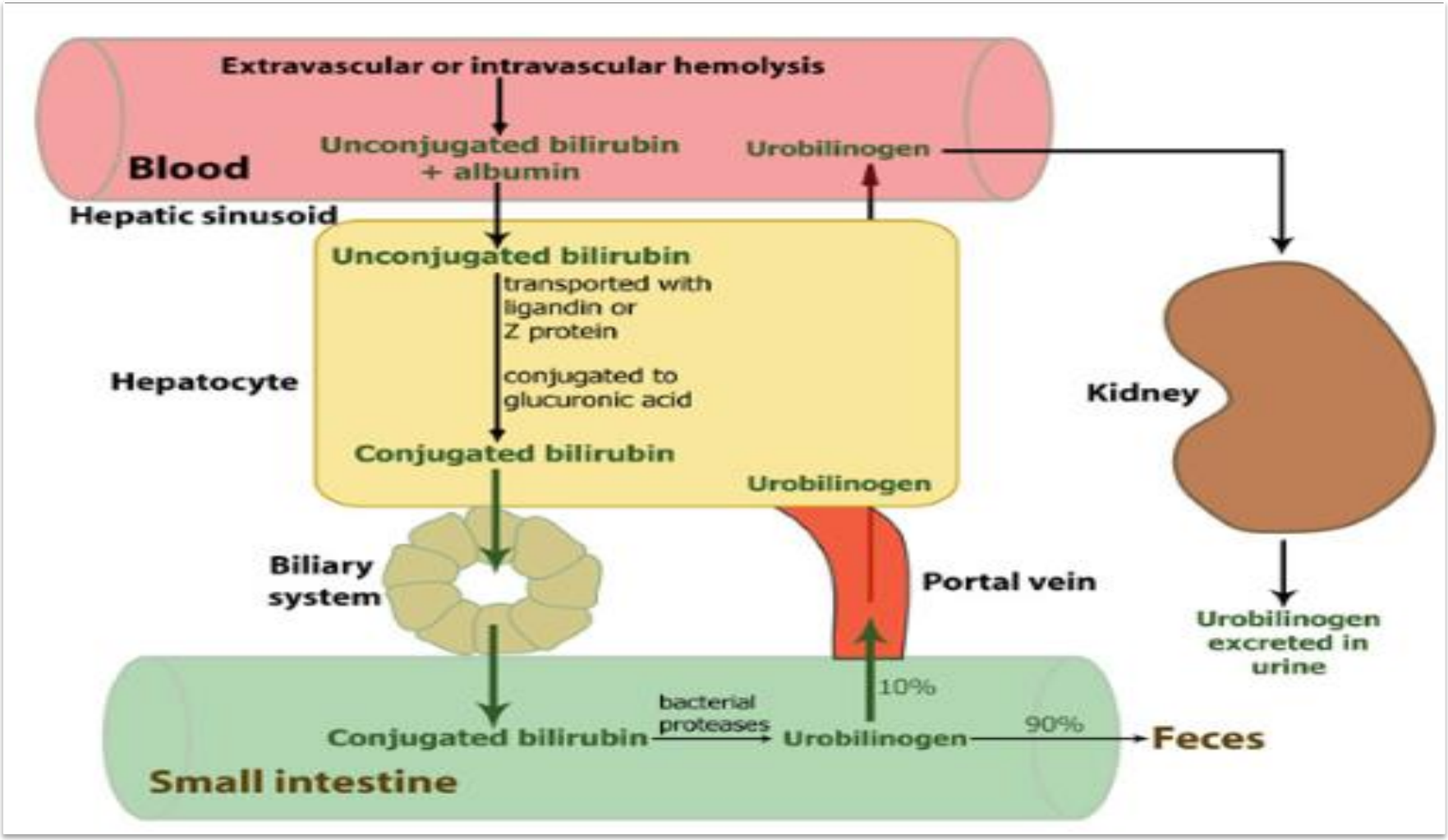

■ Slides ■ Important ■ Females' Notes ■ Explanation ■ Males' Notes

QUESTIONS

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Let's play the hidden answers Game☺

*Answers are somewhere in this slide try to answer the questions then look for the answers!

1- Bilirubin is made water soluble by:

A-Conversion into urobilinogen.

B-Oxidation to mesobiliverdin.

C-Conversion into stercobilinogen.

D-Conjugation with glucuronic.

2-In liver, bilirubin is conjugated mainly with:

A-Glycine and taurine.

B-Cholesterol.

C-Fatty acids.

D-Glucuronic acid.

3-Which of the following is not correct regarding the conjugated bilirubin:

A-Present in low conc. in the blood.

B-Toxic substance

C-Excreted in urine

D- Direct reacting bilirubin-cholebilirubin

THE END

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If there are any Problems or Suggestions,
Feel free to contact us:

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THANK YOU

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