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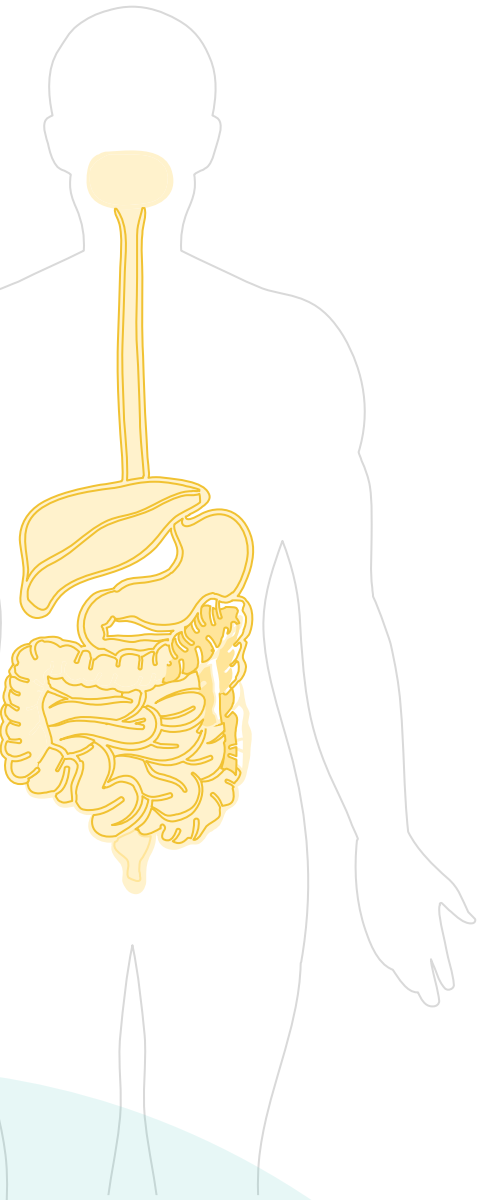

Bilirubin Metabolism

GNT Physiology

This lecture was presented by:
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Color Index:
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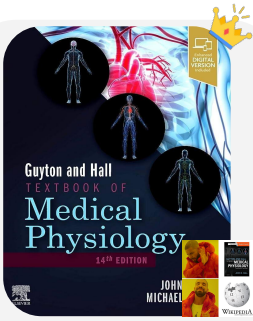
Editing file

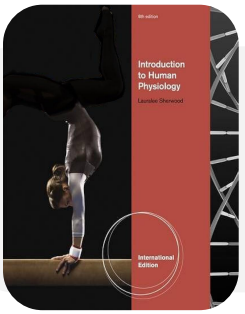
Objectives

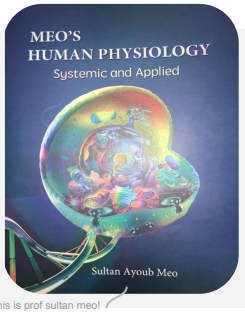
- **Definition of Bilirubin**
- **Bilirubin Metabolism: formation, Transport in Plasma, Hepatic Transport, Excretion Through Intestine**
- **Other Substances Conjugated By Glucuronyl Transferase.**
- **Differentiation Between Conjugated & Unconjugated Bilirubin**
- **Causes and Pathogenesis of Jaundice**

Resources

Only GI chapters included







This is prof sultan meo!

Click here for a helpful channel by the team!

وَلَسَوْفَ يُعْطِيكَ رَبُّكَ فَتَرْضَىٰ (5) الضحى

فلا تبتئس، فاللهُ محيطٌ بك، يعلمُ كلّ شيء، ويرى ويسمع كلَّ شيء،
هوَ الذي قدّر هذا الهمّ، وهو كفيلٌ بجبرك ورضاك

Bilirubin

Definition of Bilirubin

- **Bilirubin: is the end product of heme degradation derived from breakdown senescent (aging) erythrocytes by mononuclear phagocytes system specially in the spleen, liver and bone marrow**
- **it is water insoluble breakdown product of heme catabolism.**
- **It is the greenish yellow pigment excreted in bile, urine & feces.**
- **The major pigment present in bile is the orange compound bilirubin.**
- **Bilirubin is toxic, therefore, its excretion in the bile is one of the very important functions of the liver.**
- **Serum bilirubin level is an important clinical marker of hepatobiliary excretory function.**
- **Heme is found in hemoglobin, a principal component of RBCs**
  - **[Heme: iron + organic compound "porphyrin"].**

Heme source in body:

- 80% from hemoglobin
- 20% other hemo-protein
  - cytochrome
  - catalase
  - peroxidase
  - myoglobin

Bilirubin Metabolism

Bilirubin metabolism involves four discernible steps:

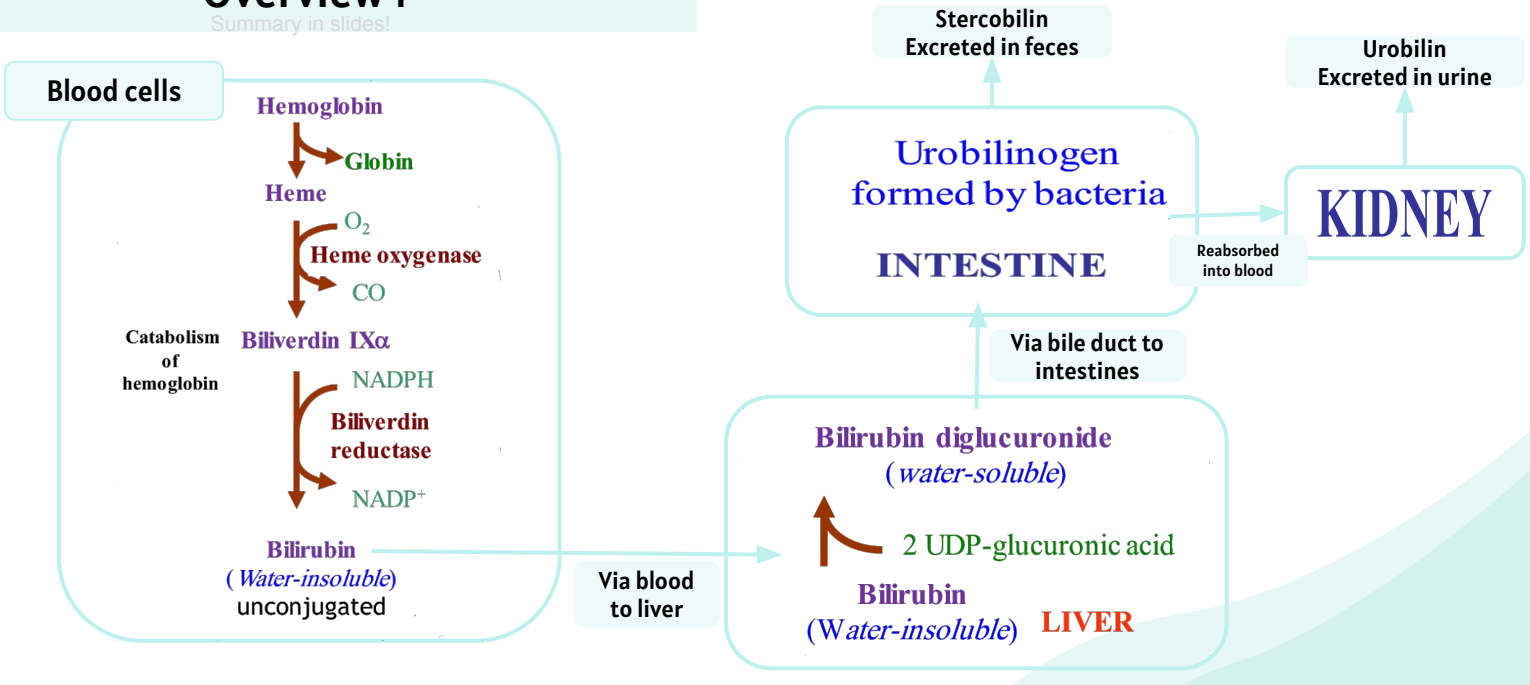
1. **Bilirubin Formation**
2. **Transport of bilirubin in plasma**
3. **Hepatic phase:**
   - Uptake
   - Conjugation
   - Biliary excretion
4. **Excretion through intestine**

The four steps are finely balanced. Therefore:

- Reduction at any step may cause hyperbilirubinemia. Impairment in any step, for example: excessive bilirubin formation.
- Enhancement of the throughput requires induction of multiple genes, coordinated by nuclear receptors.

Overview !

Summary in slides!



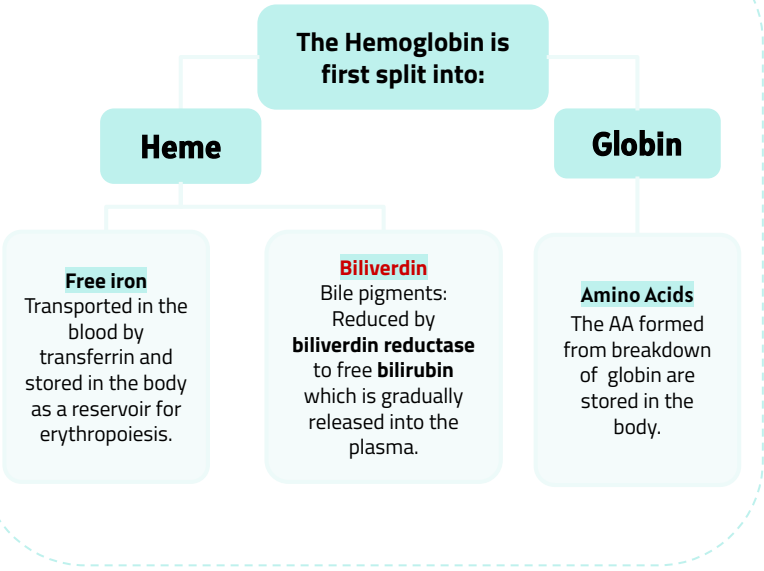
Bilirubin Metabolism cont.

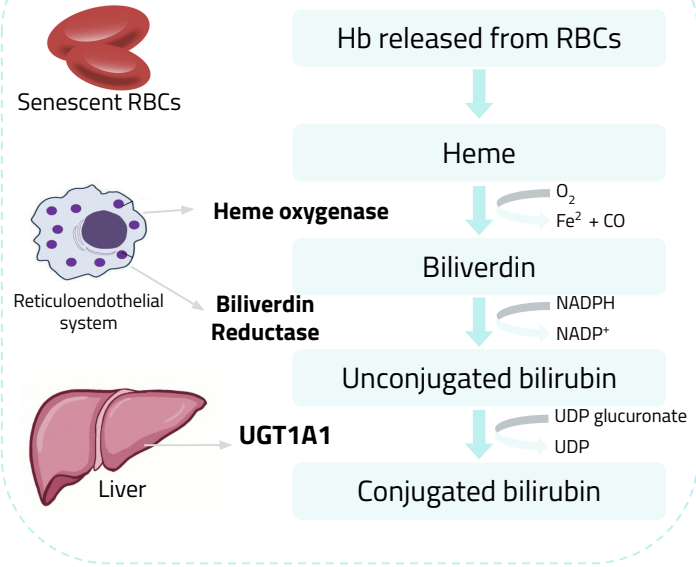
1	2	3	4
Bilirubin Formation	**Transport of bilirubin in plasma**	**Hepatic phase:** • Uptake • Conjugation • Biliary excretion	**Excretion through intestine**

Life span of RBCs is 60-120 days.

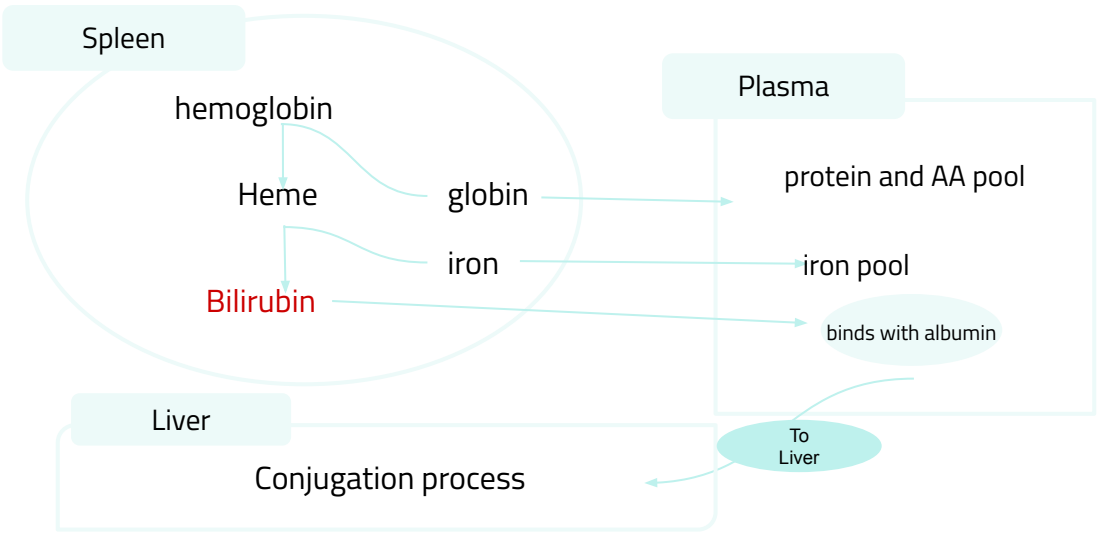
Senescent RBCs are phagocytosed intravascularly (inside blood vessel) or extravascularly (outside blood vessel) in the reticulo-endothelial system.

The hemoglobin is first split into globin(protein)& heme. In the presence of NADPH and O2 , the **Heme oxygenase** enzyme hydroxylates Heme, with a concomitant oxidation of ferrous Fe2+ iron to ferric Fe+3 , and converts it into Biliverdin.





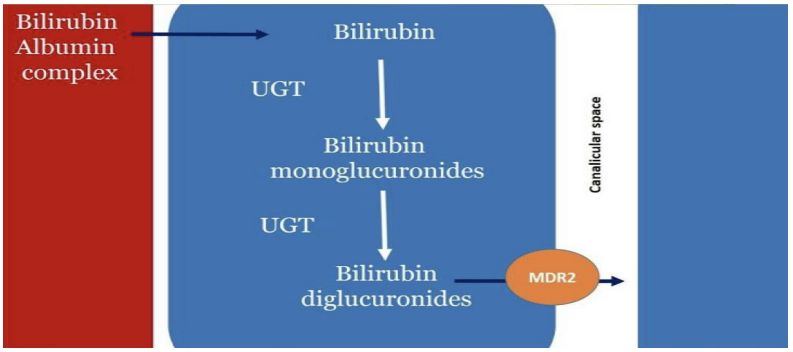
Hemoglobin Degrading and Bilirubin Formation



Bilirubin Metabolism cont.

1	2	3 Hepatic phase:	4
Bilirubin Formation	**Transport of bilirubin in plasma**	• Uptake • Conjugation • Biliary excretion	**Excretion through intestine**

- The free bilirubin is **hydrophobic** (water insoluble), immediately combines with plasma proteins (mainly albumin and globulin).
- forming a water soluble compound (hemobilirubin, **unconjugated**, indirect bilirubin all three names have the same meaning" be familiar with them") which is rapidly transported to hepatocytes for further metabolism.
- Even when bound to albumin it's called free bilirubin. Unconjugated bilirubin even if it is attach to protein



Albumin + **Free bilirubin** ⇌ **Bilirubin-Albumin Complex**
Unconjugated, indirect bilirubin, hemobilirubin

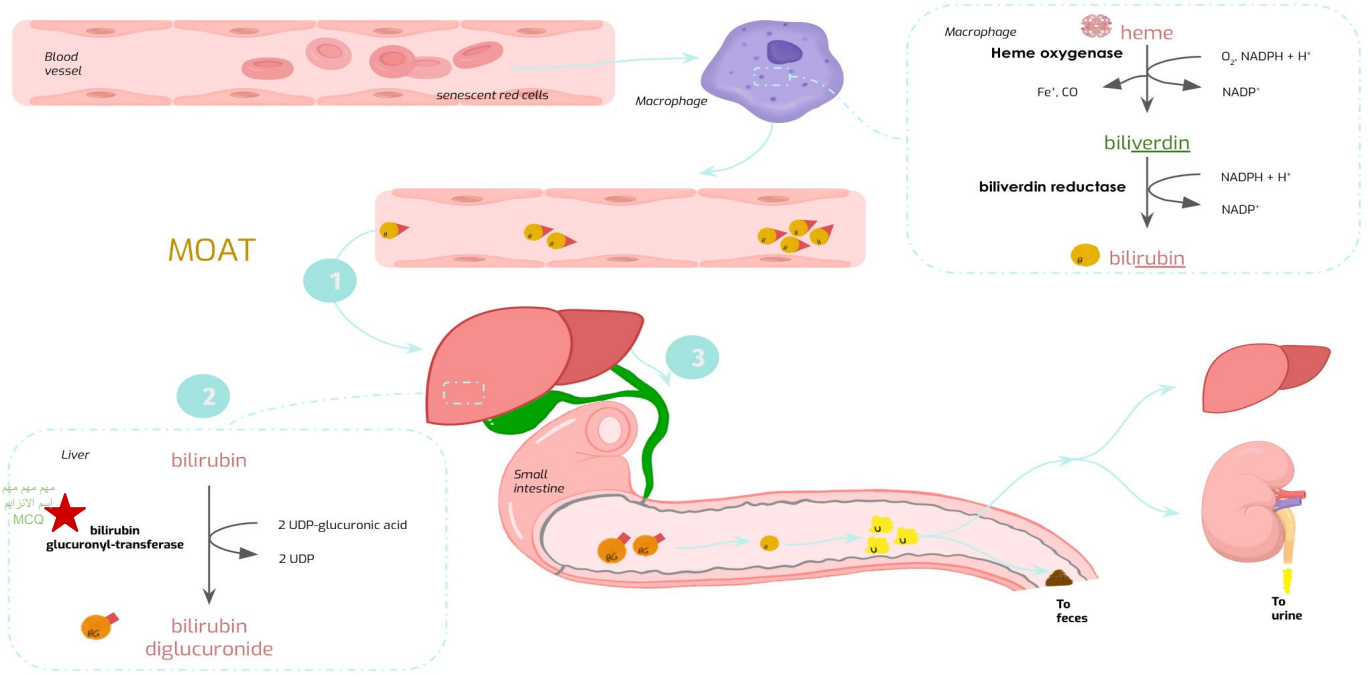
Significance of bilirubin binding to albumin:

- Prevent unconjugated bilirubin freely come into other tissue, cause damage.
- Increase the solubility of whole molecule.

- N.B: Certain drugs as sulfonamides and salicylates compete with bilirubin for albumin binding and displace bilirubin to enter into the brain ("these drugs prevent the attachment to plasma proteins so that makes bilirubin less water soluble so pass the cell membrane easily") in neonates and increase the risk of kernicterus.
- **kernicterus (a type of brain damage that can result from high levels of bilirubin in a baby's blood** for that reason when baby has jaundice we worry about developing this disorder!).It can cause **cerebral palsy** and **hearing loss**.

Bilirubin Metabolism cont.

1	2	3	4
Bilirubin Formation	**Transport of bilirubin in plasma**	**Hepatic phase:** • Uptake • Conjugation • Biliary excretion	**Excretion through intestine**



Hepatic phase:
On coming in contact with the hepatocyte surface , unconjugated bilirubin is preferentially metabolized which involved 3 steps:

1 Hepatic uptake
From the blood

Bilirubin is absorbed through the hepatic cell membrane, mediated by a **carrier protein receptor** Multiple organic anion transporting protein (MOAT) & **combined with "Y & Z" proteins that trap** تسحب the bilirubin inside the cells. but this process is inefficient, therefore, there is always some unconjugated bilirubin in the veins), and conjugates most of it with glucuronic acid and only 10% with sulfate.

2 Conjugation
In liver

- In the smooth ER of hepatocytes, about 80% of bilirubin conjugates with uridine diphospho-glucuronic acid (**UDPGA**).
- Each bilirubin molecule reacts with 2 UDPGA molecules **catalyzed** by the enzyme **glucuronyl transferase (UGT)**** to form bilirubin diglucuronide (cholebilirubin, direct, conjugated bilirubin all three names have the same meaning" be familiar with them")
- Cholebilirubin is more water soluble than free bilirubin.
- Inherited glucuronyl transferase deficiency causes jaundice. hyperbilirubinemia
- 20% conjugate with sulphate or other substances

3 Secretion in Bile

- Cholebilirubin (conjugated bilirubin, direct bilirubin) is actively secreted into the bile canaliculi through an active carrier mediated process via (Multidrug resistance-associated protein 2, MRP-2), giving bile its color.
- This energy-dependent, **rate – limiting step for bilirubin metabolism by hepatocytes).** (rate of secretion تحدد) is susceptible to impairment in liver disease. (bilirubin secretion in the bile)
- In normal adults this results in a daily load of 250-300 mg of bilirubin.
- Unconjugated bilirubin is normally not excreted.

Bilirubin Metabolism cont.

Female slides

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 compounds can compete with bilirubin for the enzyme system when they are present in appreciable amounts. Prevent له يصير ان conjugation, so that leads to return in circulation and appear as jaundice

Female slides

Substances That Increase Glucuronyl Transferase Activity

Several substances as barbiturates, antihistamines and anticonvulsants can cause marked proliferation of the smooth endoplasmic reticulum in the hepatic cells, with a concurrent increase in hepatic glucuronyl transferase activity.

- **Phenobarbital** عكس steroids has been used successfully for the treatment of a congenital disease in which there is a relative deficiency of 2 UDP-glucuronyl transferase. As in case of neonatal jaundice.

Male slides

1. A defect in MRP-2 causes Dubin Johnson Syndrome, that the conjugated bilirubin concentration increases.
2. A deficiency or absence of UDP-Glucuronyl transferase causes Criggler Najjar Syndrome.
3. The unconjugated bilirubin is normally not secreted

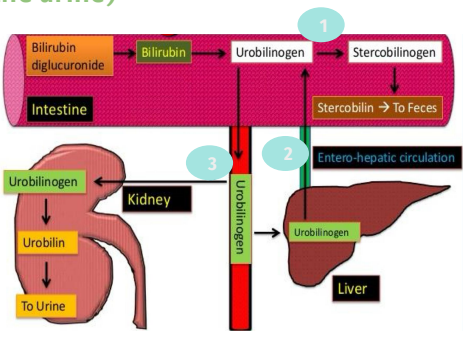
Fate of UCB & Urobilinogen

Fate of conjugated bilirubin

- **A small portion** of the(**conjugated** bilirubin it appears normally in plasma in small amounts)returns to the plasma and bound less tightly to albumin & is excreted in the urine. this causes a small portion of the bilirubin in the ECF to be of the conjugated type.
- **Small amount** is **deconjugated** in the small intestine and absorbed 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 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.

Fate of Urobilinogen

- **In the intestine, Most** of urobilinogen (70%) is converted into stercobilinogen, oxidized and excreted in the feces as **stercobilin** that causes dark brown color of the feces normally.
- **Some** of urobilinogen (20 %) is reabsorbed through the intestinal mucosa into the portal vein and re-excreted by the hepatic cells in the bile (enterohepatic circulation)
- **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. (It is normal to find urobilinogen in small quantities in the urine, but it is NOT responsible for the yellowish color of the urine)



Types of Bilirubin in the Serum

Direct bilirubin

- is conjugated (water soluble) bilirubin, it reacts rapidly with reagent (direct reacting) (direct measurement)
- Loosely bound to albumin, Non-toxic, Present in low concentration in the blood
- **Filtered** through renal glomeruli and excreted in urine

Indirect bilirubin

- is unconjugated (water insoluble) bilirubin because it is less soluble, it reacts more slowly with reagent (because it attach to plasma protein) (reaction carried out in methanol). Tightly complex to albumin, Toxic substance, (represents the normal bilirubin in the blood; 0.5 mg/dl of plasma) , **Not filtered** through renal glomeruli, is not excreted in urine
- Unconjugated will calculated by subtracting direct from total and so called indirect

Total bilirubin

- in this case both conjugated and unconjugated bilirubin are measured given total bilirubin.
- = D+ ID Knowing the level of each type of bilirubin has diagnostic important. To know the type of the jaundice
- Total bilirubin (1-1.5 mg/dL) – conjugated = unconjugated.

Major Differences Between Unconjugated and Conjugated Bilirubin

Important
Possible SAQ

Feature	Unconjugated bilirubin (Hemobilirubin) Indirect or Free Bilirubin	Conjugated bilirubin (Cholebilirubin) Direct or Bilirubin Glucuronide
Normal serum level	The chief form of bilirubin in the blood	Present in low conc. in the blood
Water solubility	Absent	Present
Affinity to lipids	Present Accumulates in organs	Absent
Binding	Bind to albumin	Bind to glucuronic acid
Reaction to reagents	Indirect (Total minus direct)	Direct
Renal excretion	Absent Because it attaches to plasma protein, which normally can't pass the bowman's capsule	Present
Affinity to brain tissue	Present (kernicterus), toxic Because it's less water soluble	Absent, less toxic Can't pass the BBB

Other Substances Excreted in the Bile

1. Cholesterol & alkaline phosphatase are excreted in the bile.

- 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.

- Adrenocortical, other steroid hormones & a number of drugs are excreted in the bile and subsequently reabsorbed (enterohepatic circulation) Bile is considered as secretion and excretion route for many metabolites of drugs and toxic substance.

2. Liver Secretion of Cholesterol and Gallstone Formation (Cholelithiasis)

Male slides

- Under abnormal conditions, the cholesterol may precipitate in the gallbladder, resulting in the formation of cholesterol gallstones. The amount of cholesterol in the bile is determined partly by the quantity of fat that the person eats, because liver cells synthesize cholesterol as one of the products of fat metabolism in the body. For this reason, people on a high-fat diet over a period of years are prone to the development of gallstones.

- Inflammation of the gallbladder epithelium, often resulting from low-grade chronic infection, may also change the absorptive characteristics of the gallbladder mucosa, sometimes allowing excessive absorption of water and bile salts but leaving behind the cholesterol in the bladder, and then progressing to large gallstones.

Hyperbilirubinemia (Jaundice, Icterus)

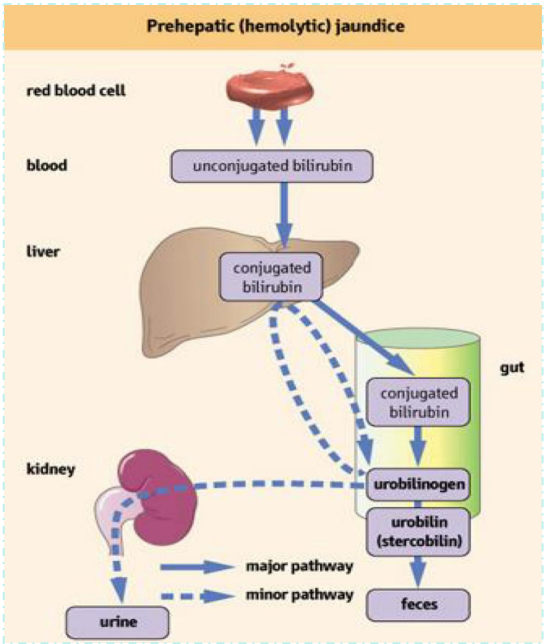
1	**It is the yellow coloration of the skin, sclera, mucous membranes and deep tissues** They have high affinity to bilirubin
2	**The usual cause is large quantities of bilirubin in the ECF, either free = (conjugated) or conjugated bilirubin.**
3	**The normal plasma concentration of total bilirubin is 0.3-1.2 mg/dl of blood.** However, in certain abnormal conditions this can rise up to 40 mg/dL of blood.
4	**The skin usually begins to appear jaundiced when the concentration of total bilirubin in the plasma is > than 2 -2.5 (3) mg/dl.** dl=100ml So that means for each 100ml it increases 1mg and this amount considered toxic
5	**Bilirubin level from 1 to 2 mg/dl is called subclinical (occult مخفية jaundice).**

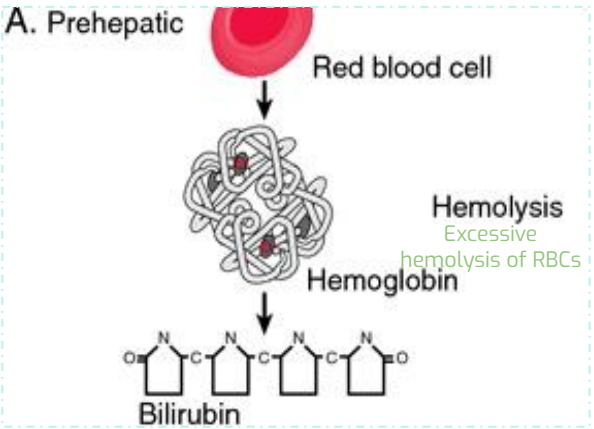
Jaundice

Classification of Jaundice

1	2	3
Prehepatic (hemolytic) jaundice	Hepatic (hepatocellular) jaundice	Posthepatic (obstructive) jaundice

Information	- In hemolytic jaundice, the excretory function of the liver is not impaired - It results from excess production of bilirubin (beyond the liver's ability to conjugate it) following hemolysis of erythrocytes (RBCs). - Therefore the plasma concentrations of free bilirubin (hemobilirubin) rises to levels much above normal but it is not filtered through the kidney, because they are unconjugated bilirubin.(not soluble) - The urine is free from bilirubin (acholuric jaundice) not change in the color but high levels of urobilinogen - The stools appear darker than the normal color due to excessive stercobilin formation
Causes	Excess RBC lysis is commonly the result of: 1) Autoimmune disease. 2) Hemolytic disease of the newborn. 3) Hemolytic anemias 4) Rh- or ABO- incompatibility 5) Structurally abnormal RBCs (Sickle cell disease). 6) Breakdown of extravasated blood. 7) Toxic substances in the circulation (e.g., snake venom)





Jaundice

Classification of Jaundice

1	2	3
Prehepatic (hemolytic) jaundice	Hepatic (hepatocellular) jaundice	Posthepatic (obstructive) jaundice

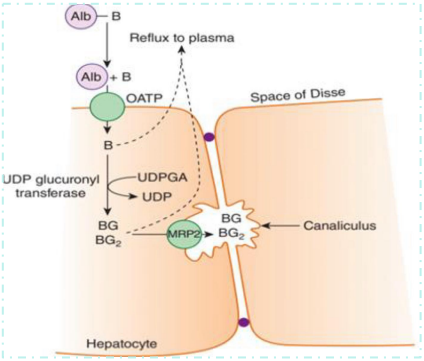
Information

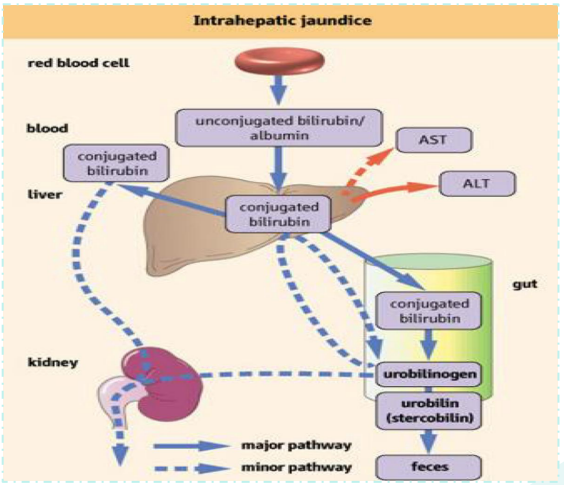
- The diseased liver cells are unable to take all the unconjugated hemobilirubin, increasing its blood concentration.
- Also, there is intrahepatic biliary duct obstruction that leads to regurgitation of conjugated bilirubin to blood. (swelling of cells and edema due to inflammation cause mechanical obstruction of intrahepatic biliary tree).
- Both types of bilirubin (conjugated & unconjugated) are present in blood in high concentration.
- Stools appear pale grayish in color due to decrease stercobilin.
- Urine appears dark brown due to filtration of excess conjugated bilirubin through the kidney. (probably by rupture of the congested bile canaliculi and direct emptying of the bile into the lymph leaving the liver).
- Hyperbilirubinemia is usually accompanied by other abnormalities in biochemical markers of liver function:
  - Alanine amine transferase (ALT, SGPT) which specific for liver function.
  - Aspartate amine transferase (AST, SGOT),
  - Alkaline phosphatase (ALP) and Gamma-glutamyltransferase (GGT) which are cellular membrane enzymes in the cholangiocytes.
  - All of these enzyme become elevated in chronic condition.

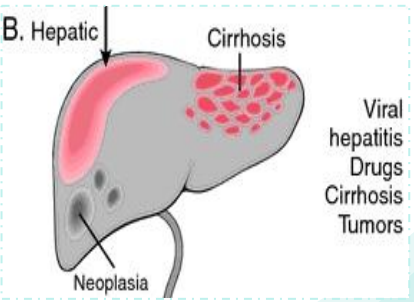
Causes

Hyperbilirubinemia (increased levels of bilirubin in the blood) may be due to:

- Damage of liver cells: e.g., viral hepatitis, drugs,chemical, alcohol, or toxins, Autoimmune hepatitis.
- Impaired uptake of bilirubin into hepatic cells.
- Disturbed intra cellular protein binding or conjugation.
- Ethanol induced liver injury.
- Genetic errors in bilirubin metabolism.
- Disturbed active secretion of bilirubin into bile canaliculi.
- Genetic errors in specific proteins.
- Gilbert syndrome: 7% of population, autosomal dominant, decreased activity of hepatic uridine diphosphate glucuronyl transferase (UDPGA) activity.
- Crigler-Najjar syndrome: Inherited disorder of bilirubin conjugation due to absence of UGT in liver.







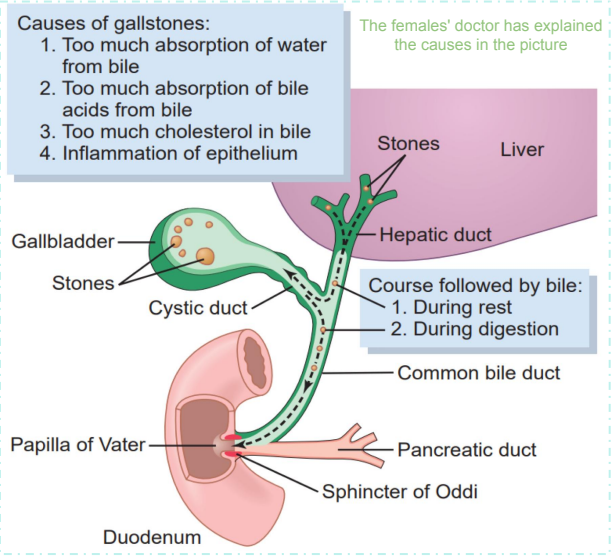
Defect in bilirubin uptake or excretion by the liver

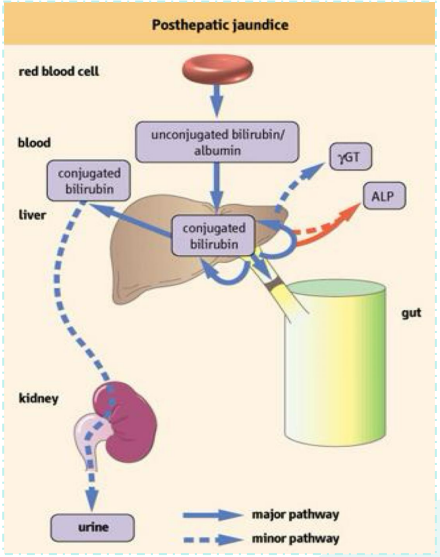
Jaundice

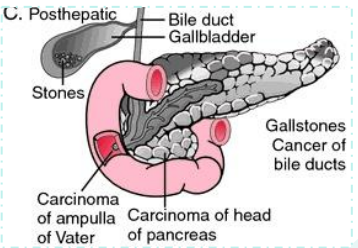
Classification of Jaundice

1	2	3
Prehepatic (hemolytic) jaundice	Hepatic (hepatocellular) jaundice	Posthepatic (obstructive) jaundice

Information	- The rate of bilirubin formation is normal. -Bilirubin enters the liver cells and become conjugated in the usual way. - The conjugated bilirubin formed simply cannot pass into small intestine and it returns back into blood, probably by rupture of the congested bile canaliculi and direct emptying of the bile into the lymph leaving the liver. - Most of the bilirubin in the plasma becomes the conjugated type rather than the unconjugated type. - In this type of jaundice, conjugated bilirubin is filtered through the kidney and appears in urine giving it dark brown color. Caused by conjugated bilirubin - Urine is free from urobilinogen. - Stools are clay color due to absence of stercobilin. - Hyperbilirubinemia is usually accompanied by other abnormalities in biochemical markers (alkaline phosphatase, ALP & y glutamyl transpeptidase, GT)
Causes	**Jaundice due to obstruction of the biliary tree:** **A- Intrahepatic bile duct obstruction e.g** 1. Drugs 2. Primary biliary cirrhosis 3. Cholangitis. 4. Hepatitis **B- Extrahepatic bile duct obstruction e.g** 1. Cholangiocarcinoma. 2. Cancer head pancreas. 3. Gallstones. 4. Edema of pancreatitis. 5. Sclerosing cholangitis







As in obstruction of biliary acid. Stone in gallbladder and obstructing bile duct or obstruction from outside like cancer head of pancreas (or any tumor)

Jaundice summary

Important
Possible SAQ

	Prehepatic (Hemolytic)	Hepatic (Hepatocellular)	Posthepatic (Obstructive)
Unconjugated	+	+	Normal
Conjugated	Normal	+	+
Bilirubin	Indirect	Both (biphasic)	direct
AST & ALT	Normal	+	Normal
ALP & yGT (y glutamyl transpeptidase)	Normal	Normal	+
Urine bilirubin (Urine color)	Absent	Present (dark brown)	Present (dark brown)
Urine urobilinogen	Present	Present	Absent Due to obstruction
Stercobilin (Stool color)	+ Darker Excessive formation of conjugated	↓ Pale grayish	Absent (Clay فاتح Color) In complete obstruction

TEST YOURSELF !

MCQ:

Q1) Which Type Of Bilirubin is water insoluble?

A) Unconjugated | B) conjugated | C)Cholebilirubin | D) Cholesterol

Q2) Which one of the following enzymes catalyzes the conjugation of bilirubin with glucuronic acid?

A) Heme oxygenase | B) Bilirubin transamylase | C) Glucuronyl hydrogenase | D) Glucuronyl transferase

Q3) What is the form of bilirubin found normally in the stool?

A) Urobilinogen | B) Stercobilin | C) Urobilin | D) Non

Q4) Primary biliary cirrhosis will lead to which type of jaundice?

A) Pre-hepatic jaundice | B) Post-hepatic jaundice | C) Hemolytic Jaundice | D) Hepatic Jaundice

Answers: Q1:A | Q2:D | Q3:B| Q4:B

SAQ:

Q1) compare Between Unconjugated and Conjugated Bilirubin

Feature	Unconjugated bilirubin	Conjugated bilirubin
Normal serum level	The chief form of bilirubin in the blood	Present in low conc. in the blood
Water solubility	Absent	Present
Affinity to lipids	Present	Absent
Binding	Bind to albumin	Bind to glucuronic acid
Reaction to reagents	Indirect (Total minus direct)	Direct
Renal excretion	Absent	Present
Affinity to brain tissue	Present (kernicterus), toxic	Absent, less toxic

Q2) compare between the types of Jaundice

	Prehepatic (Hemolytic)	Hepatic (Hepatocellular)	Posthepatic (Obstructive)
AST & ALT	Normal	+	Normal
ALP & yGT	Normal	Normal	+
Urine bilirubin (Urine color)	Absent	Present (dark brown)	Present (dark brown)
Stercobilin (Stool color)	+ Darker	↓ Pale grayish	Absent (Clay Color)

The BEST
Team Leaders
EVER..

Rafan Alhazzani

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