Physiology Team 439

MED439
KING SAUD UNIVERSITY

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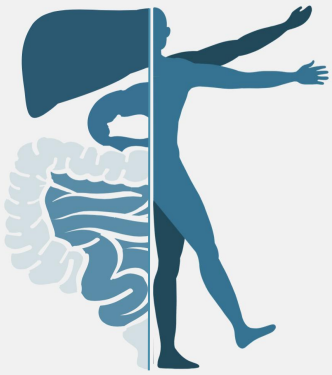


Bilirubin Metabolism

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

Color index:

- Important.
- Girls slide only.
- Boys slide only.
- Dr's note.
- Extra information.

Editing File

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.

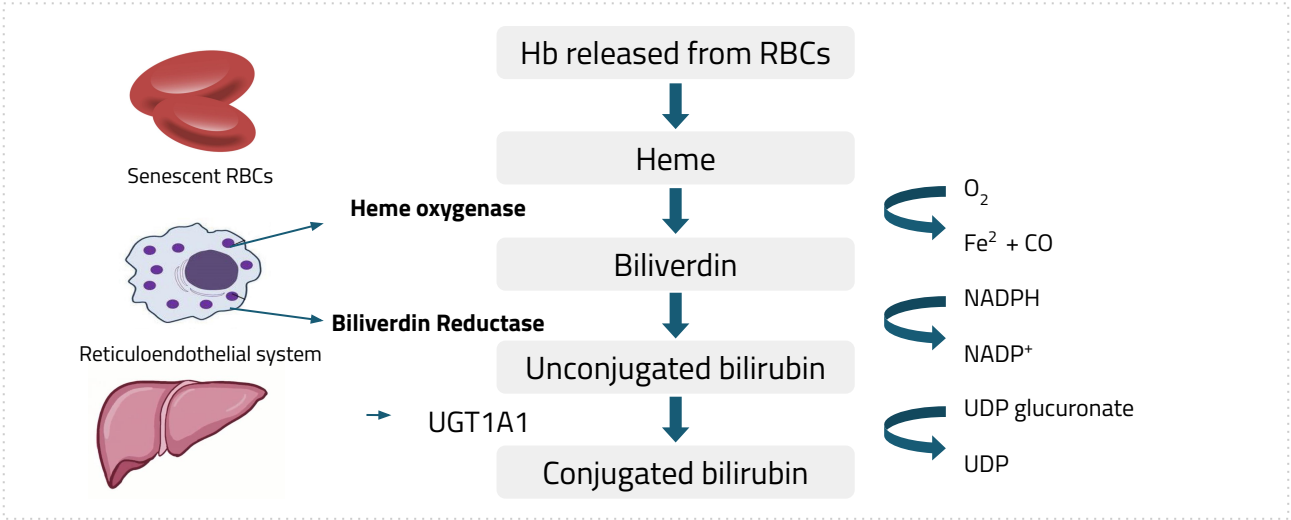
1 Bilirubin Formation

- 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 & 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.

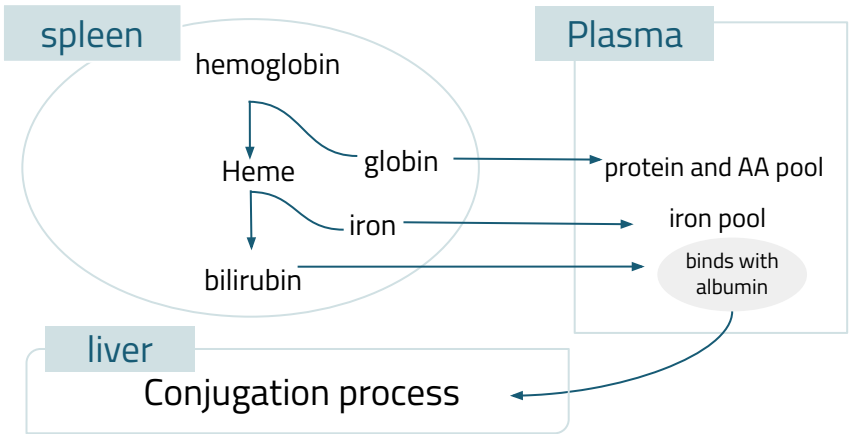
The Hemoglobin is first split into:

Heme
- Free iron — Transported in the blood by transferrin and stored in the body as a reservoir for erythropoiesis.
- Biliverdin — Bile pigments:Reduced by biliverdin reductase to free bilirubin which is gradually released into the plasma.

Globin — The AA formed from breakdown of globin are stored in the body.

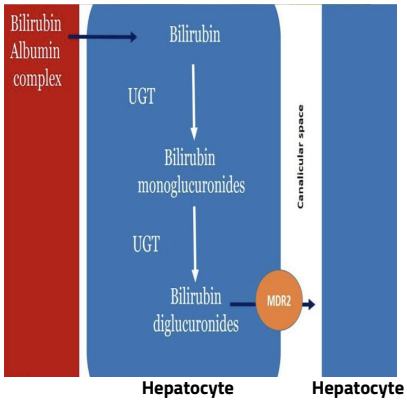


Hemoglobin Degrading and Bilirubin Formation



RBCs phagocytosis > Free hemoglobin > Hemoglobin by oxygenation (oxygenase) give rise to it iron and biliverdin > biliverdin will be converted into bilirubin by reductase.

2 Transport of bilirubin in plasma



- 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) 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

Unconjugated, indirect bilirubin, hemobilirubin

Significance of bilirubin binding to albumin:

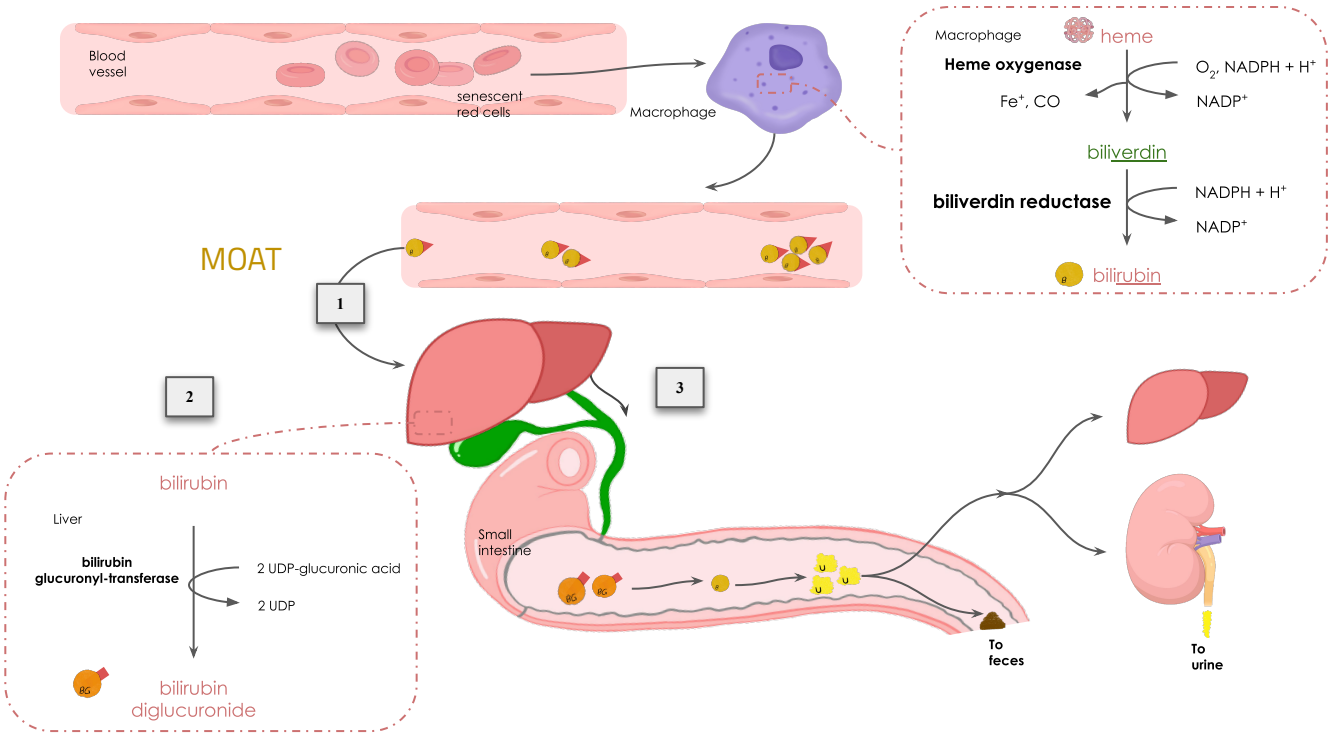
- Increase the solubility of whole molecule.
- Prevent unconjugated bilirubin freely come into other tissue, cause damage.

N.B: Certain drugs as sulfonamides and salicylates compete with bilirubin for albumin binding and displace bilirubin to enter into the brain (make bilirubin less water soluble so pass the cell membrane easily "not attach to protein") in neonates (because their BBB is still not will develop) and increase the risk of **kernicterus** (a type of brain damage that can result from high levels of bilirubin in a baby's blood). It can cause **cerebral palsy** and **hearing loss**. Depend on the area where they are precipitated in the brain.

3 Hepatic phase:

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

1. Hepatic uptake
2. Conjugation
3. Secretion in Bile



1 Hepatic Uptake: Bilirubin displace from albumin ? ما يقدر يدخل الخلية معه

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 Bilirubin Conjugation :

- 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)

- Cholebilirubin is more water soluble than free bilirubin.
- Inherited glucuronyl transferase deficiency causes jaundice. hyperbilirubinemia
- 20% conjugate with sulphate or other substances

- In the blood: bilirubin is unconjugated, hemobilirubin, indirect > attach to plasma protein.
- Inside the liver: bilirubin is conjugated, cholebilirubin, direct.
- deficiency in glucuronyl transferase cause impairment to bilirubin metabolism.

3 Bilirubin 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.

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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.

Other Substances Conjugated By Glucuronyl Transferase

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- 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. Can cause jaundice.

ليه نتكلم عن هذه المواد؟
لأنه لو هي موجودة Will compete with bilirubin for conjugation with glucuronic acid.

Substances That Increase Glucuronyl Transferase Activity

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- 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 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.

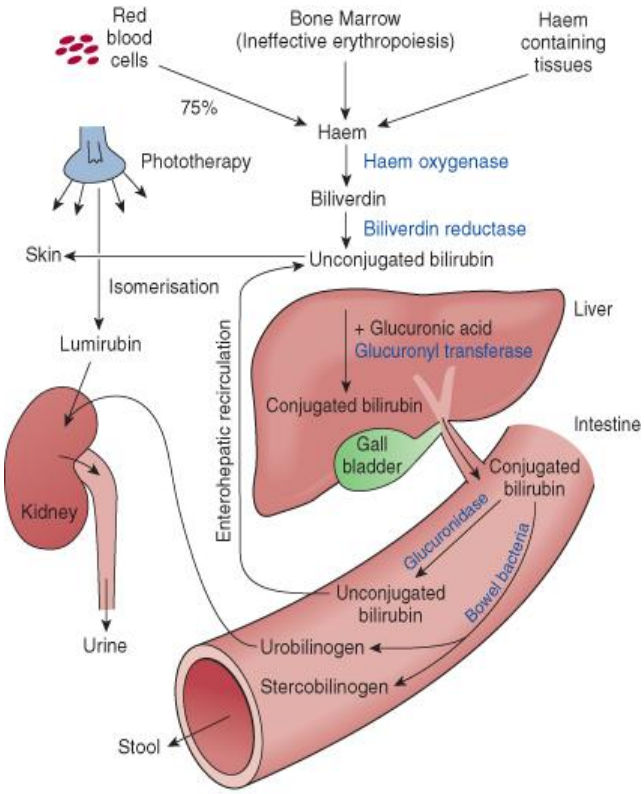
Fate of conjugated bilirubin

- **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 represents 10% only. 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 amoun**t 20% 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).

What Is the enterohepatic circulation of bile pigments ? 20% of urobilinogen

- **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

- **Most** of urobilinogen (70%) is converted into stercobilinogen in the large intestine , oxidized by bacteria and excreted in the feces as **stercobilin** that causes dark brown color of the feces.

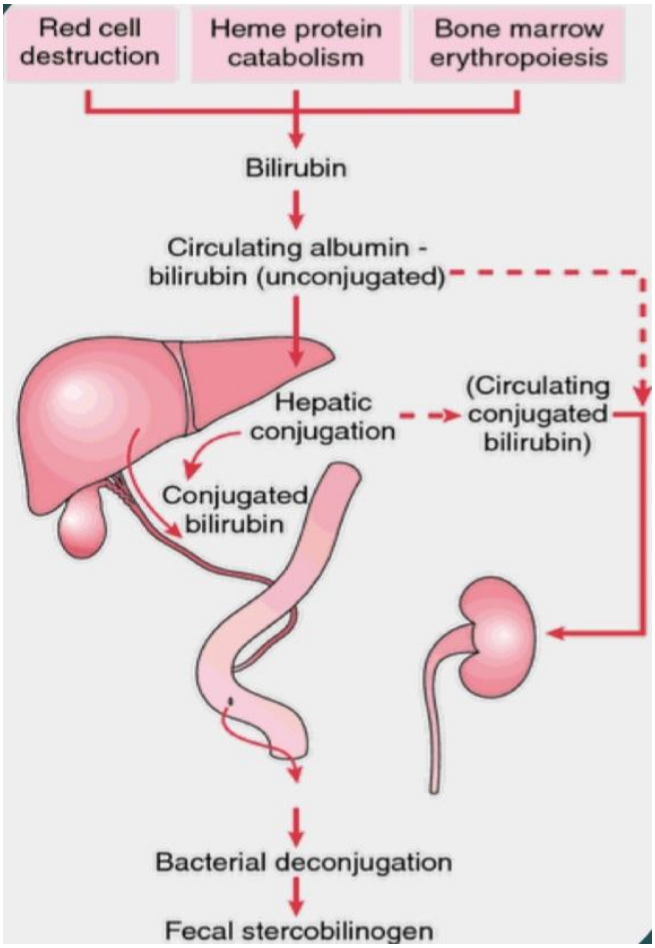
- **Some** of urobilinogen (20 %) is reabsorbed through the intestinal mucosa into the portal vein and re-excreted by the hepatic cells in the bile (enterohepatic circulation)

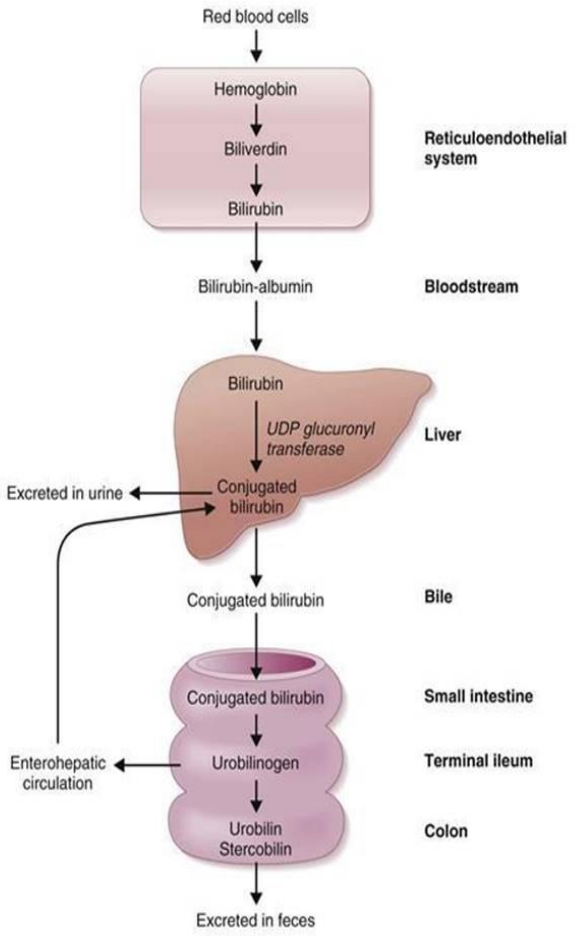
- **Small** amount 5% of urobilinogen escapes to the general circulation and excreted by the kidneys in the urine where it is oxidized to urobilin when the urine is exposed to air. Because it is water soluble and not attach to plasma protein > may be exerted in the kidneys

- Urobilinogen is excreted in either urine (where it is converted to yellow Urobilin in the urine, after exposure to air in the urine) or fesses (after it is converted to Stercobilin which is responsible for the brown color of fesses).

Urobilinogen Normally in urine, but not responsible of urine color. Urobilin or urochrome هي المسؤولة عنه

Summary of Bilirubin Formation and Excretion





Review: Normally, If the liver conjugated all of the bilirubin.

I. 10% will return back to blood (which is why we see some conjugated bilirubin in serum normally)

II. 90% will go on to Small intestine, of which:
   A. 70% become stercobilin
   B. 20% become urobilinogen, of which:
      1. 15% reconjugated and secreted in next cycle.
      2. 5% leak to kidneys

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
- in this case both conjugated and unconjugated bilirubin are measured given total bilirubin. Unconjugated will calculated by subtracting direct from total and so called indirect

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.
Usually, 90% of total bilirubin is unconjugated and 10% conjugated.

Major Differences Between Unconjugated and Conjugated Bilirubin *

Feature	Unconjugated bilirubin (Hemobilirubin) Indirect or Bilirubin Glucuronide	Conjugated bilirubin (Cholebilirubin) Direct or Free 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 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 *

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.

Hyperbilirubinemia (Jaundice, Icterus)

It is the yellow coloration of the skin, sclera, mucous membranes and deep tissues
this tissue have high affinity for bilirubin. So bilirubin precipitate in this tissue, this tissue appear jaundice

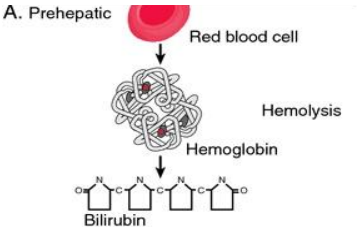
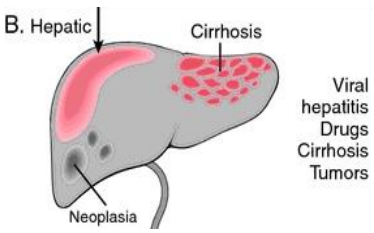
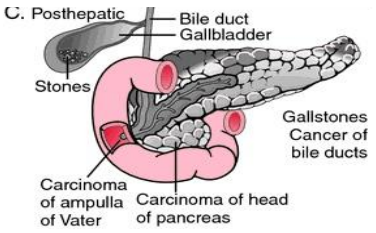
The usual cause is large quantities of bilirubin in the ECF, either free or conjugated bilirubin

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.

The skin usually begins to appear jaundiced when the concentration of total bilirubin in the plasma is > than 2 -2.5 (3) mg/dl

Bilirubin level from 1 to 2 mg/dl is called subclinical (occult مخفية jaundice)

Classification of Jaundice

Prehepatic (hemolytic) jaundice	Hepatic (hepatocellular) jaundice	Posthepatic (obstructive) jaundice
		
Excessive hemolysis of RBCs	Defect in bilirubin uptake or excretion by the liver	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)

Prehepatic (Hemolysis) Jaundice

Bilirubin: Free - High **Conjugated - Normal**

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).

Excess RBC lysis is commonly the result of:

1) Autoimmune disease.
2) Hemolytic disease of the newborn. (thalassemia,Spherocytosis and anemia)
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)

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.

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

Hepatic (Hepatocellular) Jaundice

Normal amount but the liver can't deal with this amount **Bilirubin: Mixed (Both can increase)**

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

- Impaired uptake of bilirubin into hepatic cells. (MOAT not working)
- Disturbed intra cellular protein binding or conjugation.(Glucuronyl Transferase not working)
- Disturbed active secretion of bilirubin into bile canaliculi.(MRP-2 not working)

The main causes of Hepatic jaundice are:

- Damage of liver cells: e.g., viral hepatitis, drugs,chemical, alcohol, or toxins.
- Ethanol induced liver injury.*
- Genetic errors in bilirubin metabolism.
- Genetic errors in specific proteins.
- Autoimmune hepatitis
- 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 UDPGTferase in liver.**

Hepatic (Hepatocellular) Jaundice Cont.

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).

increase blood concentration of both conjugated & unconjugated bilirubin.

Clinical Features:

- 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.
  Increase in liver enzymes is seen in hepatic jaundice unlike pre hepatic jaundice

Posthepatic (Obstructive) Jaundice

1- Intrahepatic bile duct obstruction

due to swelling, fibrosis, or obstruction of liver canaliculi. **The main causes are:**

1. Drugs
2. Primary biliary cirrhosis
3. Cholangitis inflammation of biliary
4. Hepatitis

2- Extrahepatic bile duct obstruction

obstruction of common bile duct which leads to compression. **The main causes are:**

1. Gall stones. (Calculus cholecystitis, Cholelithiasis).
2. Carcinoma of head of pancreas, (which is usually accompanied by high levels of serum alkaline phosphatase enzyme).Compress common bile duct decrease bile secretions and this will cause steatorrhea
3. Cholangiocarcinoma.**
4. Edema of pancreatitis. **
5. Sclerosing cholangitis **

- 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.
- Urine is free from urobilinogen. because conjugated bilirubin wasn't released to the small intestine to be converted to urobilinogen (due to obstruction)
- Stools are clay color due to absence of stercobilin.

Only in girls' slides	Prehepatic (Hemolytic)	Hepatic (Hepatocellular)	Posthepatic (Obstructive)
Unconjugated	+	+	Normal
Conjugated	Normal	+	+
Bilirubin	Indirect	Both (biphasic)	direct
AST & ALT	Normal	+	Normal
ALP & (γ 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

Liver Secretion of Cholesterol and Gallstone Formation (Cholelithiasis) Only in boys' 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.

MCQ & SAQ:

Q1:What is the form of bilirubin found normally in the stool?

A. Urobilinogen
B. Stercobilin
C. Urobilin

Q2: Gallstones will lead to which type of jaundice?

A.Prehepatic jaundice due to extrahepatic bile duct obstruction
B. Posthepatic jaundice due to extrahepatic bile duct obstruction
C. Hepatic jaundice
D. hemolytic jaundice

Q3: Which type of Bilirubin is elevated in hepatic jaundice?

A.free bilirubin
B. Conjugated bilirubin
C. Unconjugated bilirubin
D. Conjugated & unconjugated bilirubin

Q4: Which Type Of Bilirubin is water insoluble?

A.Unconjugated bilirubin
B.Conjugated bilirubin
C.Cholebilirubin
D.Cholesterol

Q5: One of the following is responsible of trapping bilirubin inside the liver

A. y and z proteins
B. glucuronyl transferase
C. albumin

Q6: Which Type Of Jaundice Have a Clay Color of the stool?

A. Prehepatic jaundice
B. Obstructive jaundice
C. Hepatic jaundice
D. hemolytic jaundice

answer key:
1: B
2: B
3: D
4: A
5: A
6: B

1- Mention 3 differences between conjugated & unconjugated bilirubin?

2- What is the fate of conjugated bilirubin?

3- Mention 3 clinical features of Hepatic jaundice?

4- Mention 3 causes of Intrahepatic bile duct obstruction?

A1: slide 10

A2: slide 8

A3: slide 13

A4: Drugs, Primary biliary cirrhosis, Cholangitis, Hepatitis.

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