MEDICINE 438's

GIT PHYSIOLOGY

LECTURE IX: Bilirubin Metabolism

EDITING FILE

IMPORTANT | MALE SLIDES | EXTRA | FEMALE SLIDES | LECTURER'S NOTES

1 BILIRUBIN METABOLISM

Lecture Nine

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.
- Definition, classification, Causes, and pathogenesis of jaundice.
- The normal plasma concentration of total bilirubin.

Definition of Bilirubin

- Greenish yellow pigment excreted in bile, urine & feces, the major pigment present in bile is the orange compound bilirubin.
- It is water insoluble breakdown product of heme catabolism in senescent (aging) erythrocytes. Mononuclear phagocyte system (MPS)

★ 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 is highly soluble in all cell membranes (hydrophobic) and very toxic, therefore, its excretion in the bile is one of the very important functions of liver.

Serum bilirubin level is an important clinical marker of hepatobiliary excretory function.

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

Bilirubin Metabolism

1. **Formation**
2. Transport in **plasma**
3. **Hepatic** Phase
   a. Uptake
   b. Conjugation
   c. Biliary excretion
4. Excretion through **intestine**

The 4 steps are finely balanced ,Therefore:

- Reduction at any step may cause hyperbilirubinemia.
- Enhancement of the rate of production (throughput) requires induction of multiple genes, probably coordinated by nuclear receptors.

Unconjugated bilirubin

- Insoluble in water.
- Tightly complex to albumin.
- Toxic substance.
- Not filtered through renal glomeruli , not excreted in urine
- The chief form of bilirubin In the blood (represents the normal bilirubin in the blood; 0.5 mg/dl of plasma)

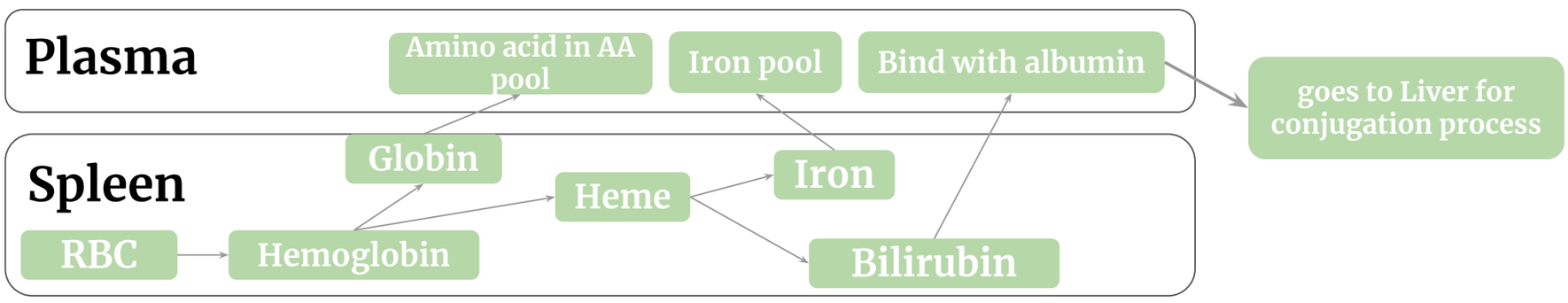
Conjugated bilirubin

- Water soluble
- Loosely bound to albumin
- Filtered through renal glomeruli and excreted in urine
- Non-toxic
- Present in low concentration in the blood

1 Formation of Bilirubin

Life span of RBCs is 60-120 days.

- Senescent RBCs are phagocytosed intravascularly or extravascularly by **reticuloendothelial system (RES)** **(Mononuclear Phagocytes System)** specially in the spleen, liver & bone marrow.
- The **hemoglobin** is first split into **globin** & **heme** In the presence of NADPH & O_2 the Heme oxygenase, with a concomitant oxidation of ferrous Fe^{2+} iron to ferric Fe^{+3}, and converts it into Biliverdin.
  - **Globin:** AA formed from breakdown of globin are stored in the body
  - **Heme:** heme ring is opened to give:
    - **Free ion** : Transported in blood by transferrin & stored in the body as a reservoir for erythropoiesis.
    - **Bile pigment (biliverdin):** reduced by **biliverdin reductase** to free bilirubin which is gradually released into the plasma.



2 Transport of Bilirubin in Plasma

★ Free bilirubin is **hydrophobic** it immediately combines with plasma proteins (mainly albumin & 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**.

Albumin + bilirubin → **bilirubin-albumin complex (Unconjugated, indirect bilirubin hemobilirubin)**

Significance of bilirubin binding to albumin:

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

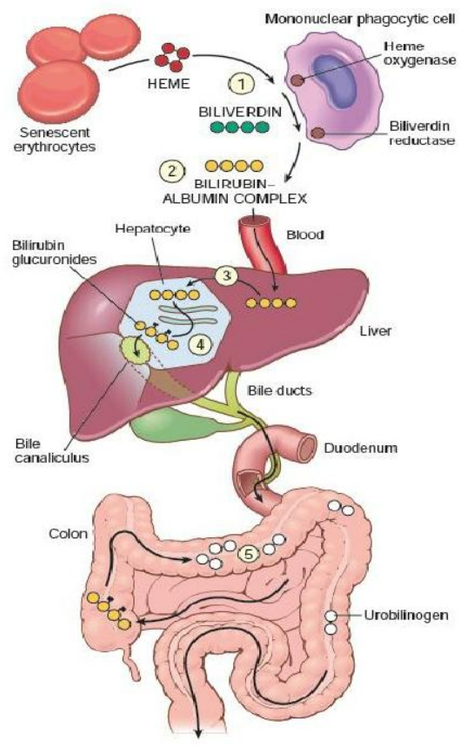


Figure 9-1

- Certain drugs (sulfonamides, salicylates) compete with bilirubin for albumin binding and displace bilirubin to enter into the brain in **neonates** 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.

3 Hepatic Phases

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 (lipid soluble) is absorbed through the hepatic cell membrane, mediated by a carrier protein (organic anion transporting protein OATP) but this process is inefficient, so there is always some unconjugated bilirubin in the veins.

Then combined with Y[1] & Z proteins that trap the bilirubin inside the cells.

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** to form bilirubin diglucuronide (**cholebilirubin**, **direct**, **conjugated bilirubin**) which is more water soluble than free bilirubin.

❖ Inherited **glucuronyl transferase deficiency** causes **jaundice.**

- **10**/**20%** conjugate with **sulphate** or **other** substances.

3. Secretion in Bile

- **Cholebilirubin** is actively secreted into the bile canaliculi through an active carrier-mediated process giving bile its color, mediated by multiple anion transporter & induced by phenobarbitone.
- its **energy-dependent**, **rate limiting step** is susceptible to impairment in liver disease.
- In normal adults this results in a daily load of **250-300 mg of bilirubin**.
- Unconjugated bilirubin is normally not excreted.

Fate of Conjugated Bilirubin

- **Majority** of conjugated bilirubin passes via the bile ducts to the intestine then colon where it is transformed by bacteria, which will deconjugate it back to bilirubin & convert it to the highly soluble colorless compound called Urobilinogen.
- **Small amount** 20% is deconjugated and converted to Urobilinogen in the small intestine & absorbed into the portal blood to the liver where it is extracted by the liver cells & conjugate again & excreted in the bile (enterohepatic circulation of bile pigments).
- **Small portion** of conjugated bilirubin returns to plasma either directly into the liver sinusoids or indirectly by absorption into the blood from the bile ducts or lymphatics. This represents 10% only this causes a small portion of the conjugated bilirubin in the ECF it bound less tightly to albumin & is excreted in the urine.

FOOTNOTES

1, Y and Z proteins are cytosolic proteins, Y protein is also called ligandin, it binds bilirubin within the hepatocytes thus preventing its regurgitation into the blood. Genetic mutation in this protein contributes to neonatal jaundice. Z protein is a fatty acid binding protein.

Fate of Urobilinogen

- **Most** of urobilinogen **(70%)** is converted into **stercobilinogen** in the large intestine, **oxidized** & 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 & re-excreted by the hepatic cells in the bile (enterohepatic circulation).
- **Small** amount 5% of urobilinogen escapes to the general circulation & excreted by the kidneys in urine where it is **oxidized** to **urobilin** when the urine is exposed to air.

Other Substances Conjugated By Glucuronyl Transferase

- **Glucuronyl transferase system** in smooth ER catalyzes the formation of 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 **e.g phenobarbitone**.

Substances That Increase Glucuronyl Transferase Activity

- Several substances as barbiturates, antihistamines & anticonvulsants can cause marked proliferation of the smooth ER in 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.

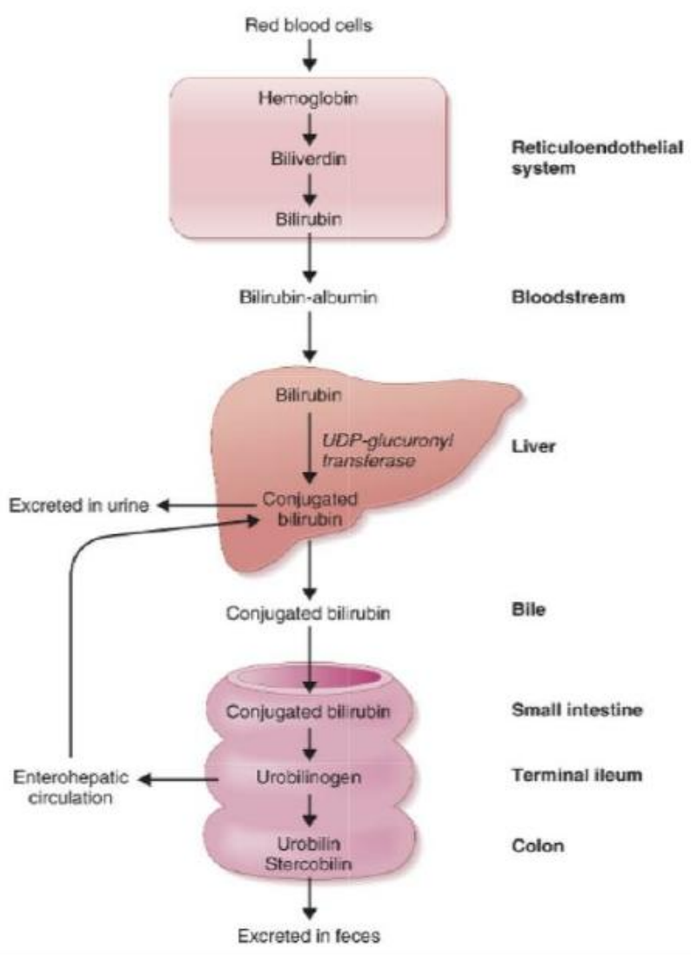


Figure 9-2

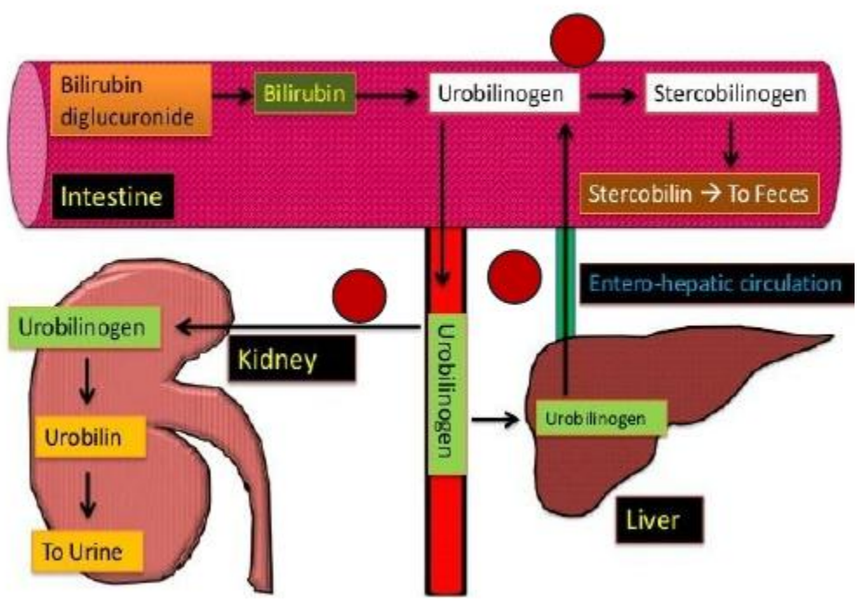


Figure 9-3 Urobilinogen fate

Types of Bilirubin in Serum

- **Direct bilirubin:** is conjugated (water soluble) bilirubin, it reacts rapidly with reagent (direct reacting).

- **Indirect bilirubin:** is unconjugated (water insoluble) bilirubin because it is less soluble, it reacts more slowly with reagent (reaction carried out in methanol).

- In this case both conjugated and unconjugated bilirubin are measured given **total bilirubin**.

Unconjugated is calculated by subtracting direct from total.

Total bilirubin = D + ID

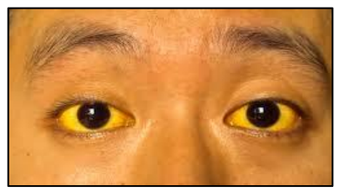
Total bilirubin (1-1.5 mg/dL) – conjugated = unconjugated.

Knowing the level of each type of bilirubin is of diagnostic importance.

Major Differences Between Unconjugated and Conjugated Bilirubin

Feature	Unconjugated bilirubin (Hemobilirubin)	Conjugated bilirubin (Cholebilirubin)
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

Jaundice(Hyperbilirubinemia, Icterus)



- It is the **yellow** coloration of the skin, sclera, mucous membranes and deep tissues.
- **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 40mq/dL of blood.
- Subclinical jaundice (occult jaundice) occur when the Bilirubin level is from 1 to 2 mg/dl .
- The skin usually begins to appear jaundiced when the concentration of total bilirubin in the plasma is greater than 2 -2.5 mg/dl.

Classification Of Jaundice

1. Pre-hepatic (hemolytic) jaundice
2. Hepatic (hepatocellular) jaundice
3. Post-hepatic (obstructive) jaundice

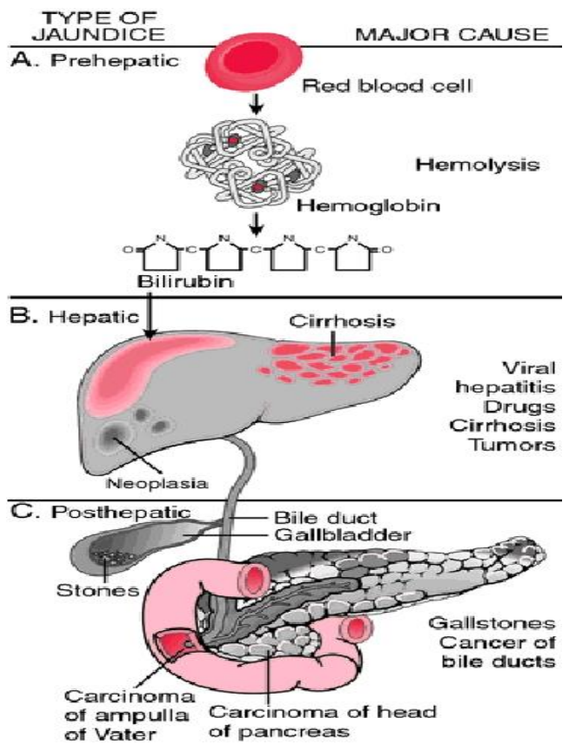


Figure 9-4

Prehepatic: Hemolytic Jaundice

- ★ The excretory function of the liver is not impaired.
- ★ It results from excess production of bilirubin (beyond the livers ability to conjugate it) following hemolysis of erythrocytes (RBC).
- ★ Excess RBC lysis is commonly the result of:
  - Autoimmune disease
  - Hemolytic disease of the newborn
  - Rh- or ABO- incompatibility
  - Structurally abnormal RBCs (Sickle cell disease)
  - Breakdown of extravasated blood

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[1].

- The urine is free from bilirubin (acholuric jaundice).
- The stools appear darker than the normal color due to excessive stercobilin formation

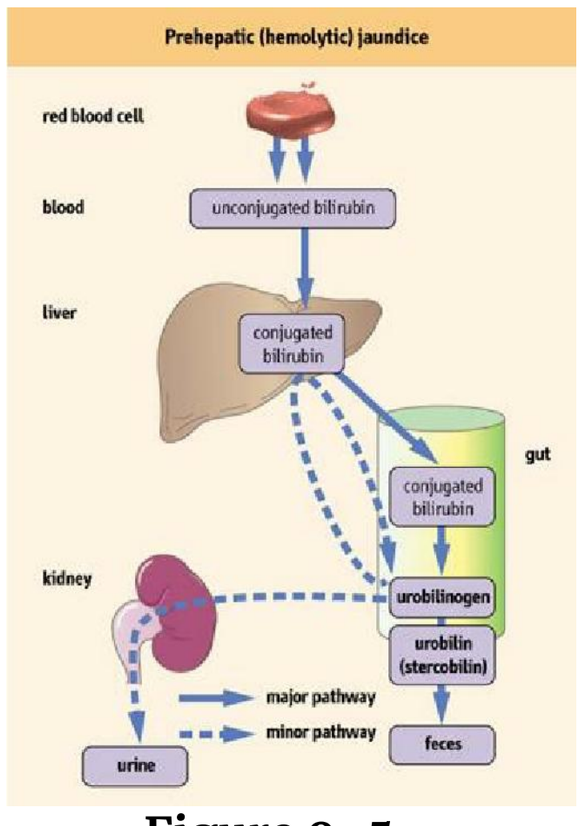


Figure 9-5

FOOTNOTES

1. Remember that unconjugated bilirubin has strong affinity to albumin and form a tight complex together. The kidney glomeruli is equipped with a basement membrane that prevents passage of albumen, hence there is no bilirubin in urine.

Classification of Jaundice

Hepatic: Hepatocellular Jaundice

Hyperbilirubinemia may be due to:

★ Impaired uptake of bilirubin into hepatic cells.
★ Disturbed intracellular protein binding or conjugation.[1]
★ Disturbed active secretion of bilirubin into bile canaliculi. If there is a defect in the active transporter to the bile canaliculi, then the conjugated B. will leak outside (and here it will look like Obstuctive jaundice)

Causes

- Damage of liver cells e.g., viral hepatitis, drugs, chemical, alcohol, or toxins.
- Autoimmune hepatitis. Hepatocytes will not be able to take up free bilirubin
- Genetic errors in bilirubin metabolism. (eg, enzyme)
- Genetic errors in specific proteins.

- The diseased liver cells are unable to take all the unconjugated hemobilirubin, increasing its concentration in the blood.
- There is intrahepatic biliary duct obstruction that leads to regurgitation of conjugated bilirubin to blood.
- Both types of bilirubin (conjugated & unconjugated) are present in blood in high concentration.

Clinical features

- Stools appear pale grayish in color due to deficiency of stercobilin.
- Urine appears dark brown due to filtration of excess conjugated bilirubin through the kidney.
- In this case, hyperbilirubinemia is usually accompanied by other abnormalities in biochemical markers of liver function {Alanine amine transferase (ALT/ SGPT) & Aspartate amine transferase (AST/SGOT)
- By looking at the ratio between different liver enzymes we can distinguish the cause whether it is from biliary (cholestatic) or liver (hepatic).

The main diagnostic tip is:

★ **Biliary obstruction :** the ALT goes up and down (pulsatile increase) and Bilirubin in the blood is high.
★ **Hepatic jaundice :** ALT shows persistent increase for along period of time (months).

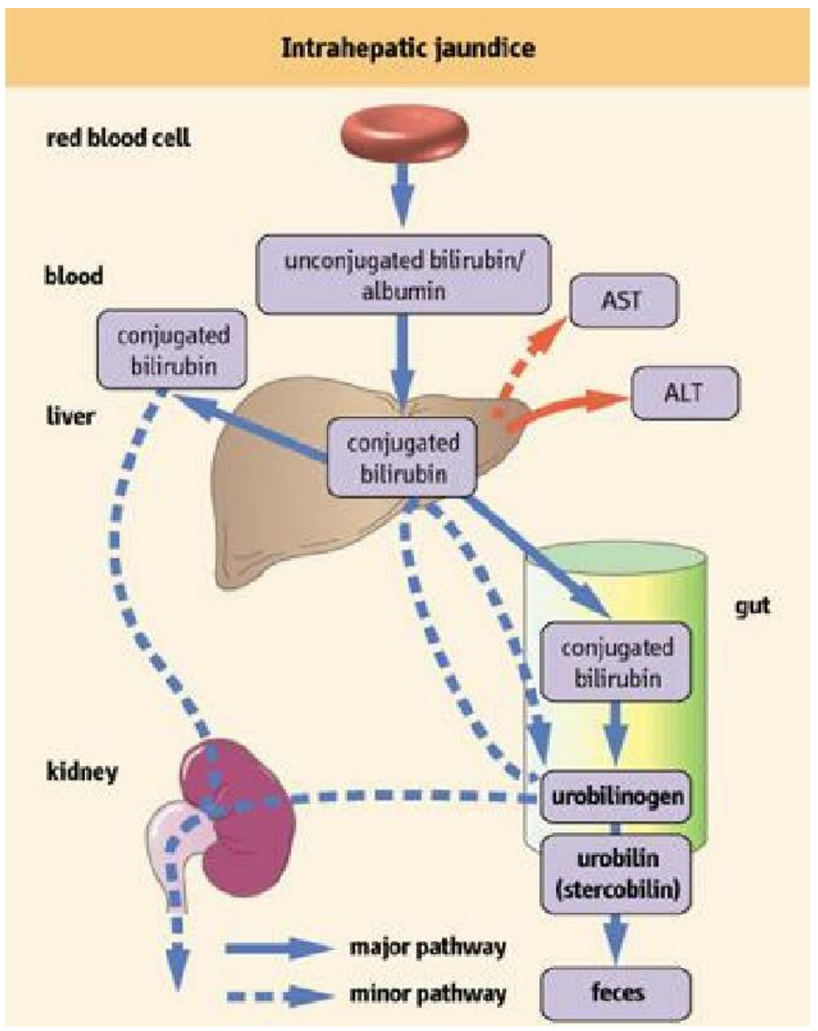


Figure 9-6

FOOTNOTES

1. e.g. if there is a defect in Glucuronyl transferase enzyme .

Classification of Jaundice (continued)

Posthepatic: Obstructive Jaundice

Causes

★ Intrahepatic bile duct obstruction such as in the liver canaliculi e.g
- Drugs
- Primary biliary cirrhosis
- Cholangitis.
- Hepatitis

★ Extrahepatic bile duct obstruction e.g
- Gallstones.
- Carcinoma of the head of the pancreas (usually accompanied by high ALP levels)
- Cholangiocarcinoma
- Edema of pancreatitis
- 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 cannot pass into small intestine and it returns back into blood.
- 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.
- Stools are clay color due to absence of stercobilin.

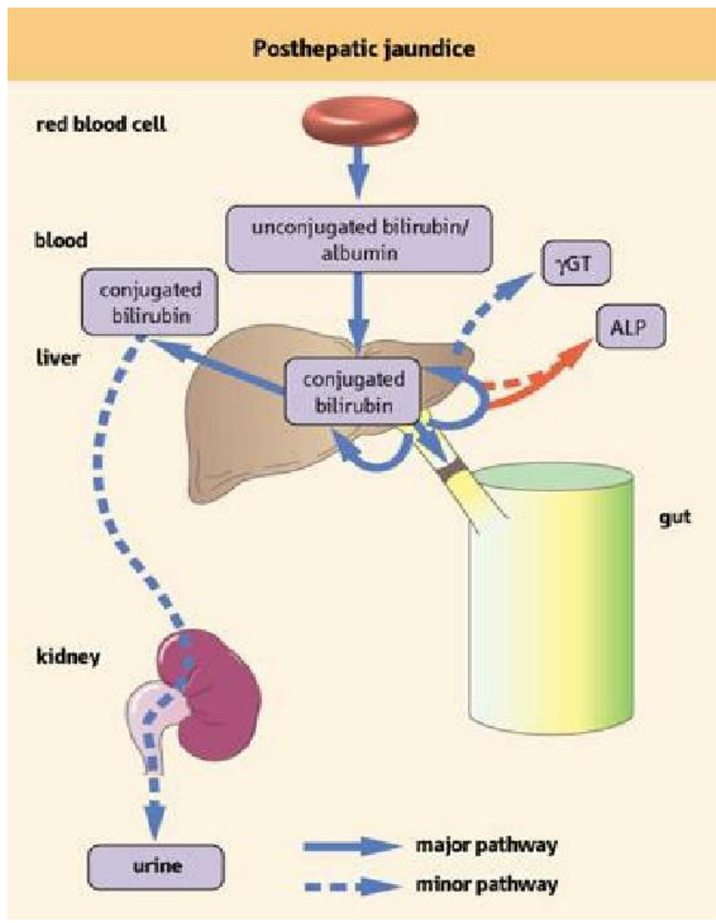


Figure 9-7

There is a special endocrine tumor called VIPoma or Verner-Morrison syndrome, this tumor result in excessive secretion of VIP which acts on colonic epithelial cells to increase active secretion of potassium resulting in a secretory diarrhea.

	Prehepatic hemolytic	Hepatic Hepatocellular	Posthepatic Obstructive
Unconjugated	Increased	Increased	Normal
Conjugated	Normal	Increased	Increased
Bilirubin	Indirect	Both	Direct
AST & ALT	Normal	Increased	Normal
ALP & yGT (y glutamyl transpeptidase)	Normal	Normal	Increased
Urine bilirubin	Absent	Present (dark brown)	Present (dark brown)
Urine urobilinogen	Present	Present	Absent
Stercobilin	Darker	Pale grayish	Absent (Clay colored)

Liver Secretion Of Cholesterol And Gallstone Formation (Cholethiasis)

- 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.

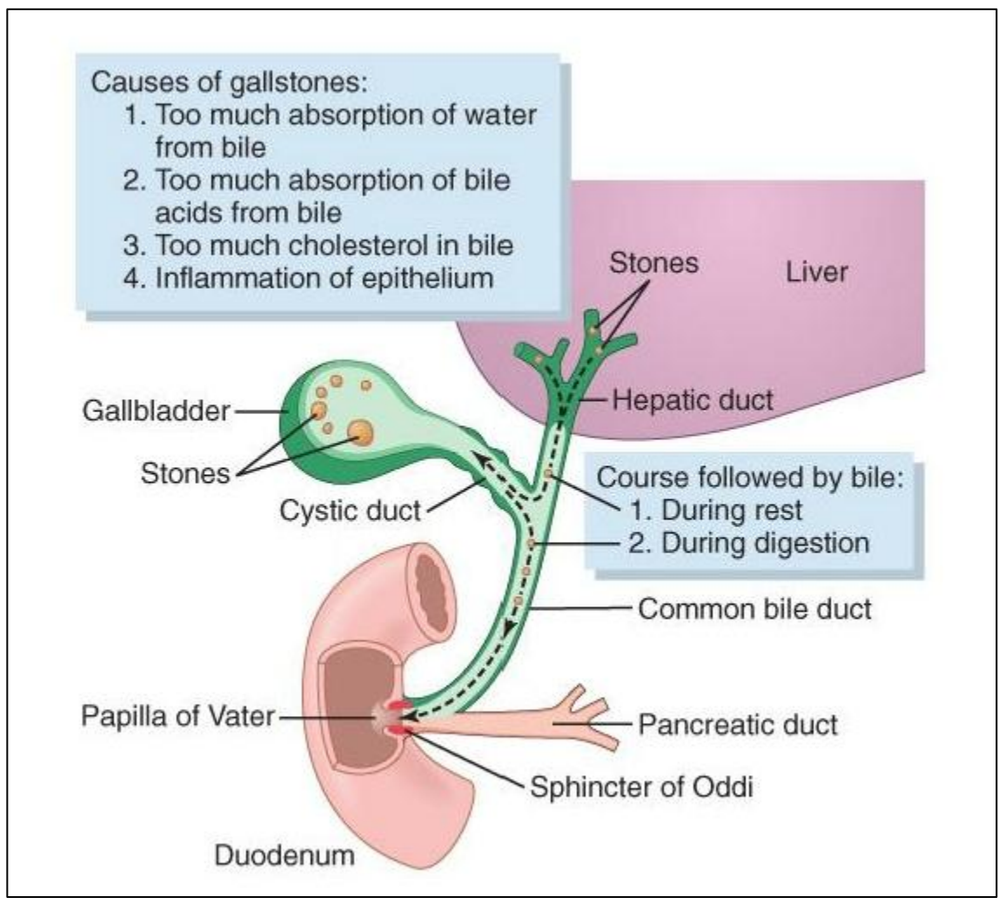


Figure 9-8

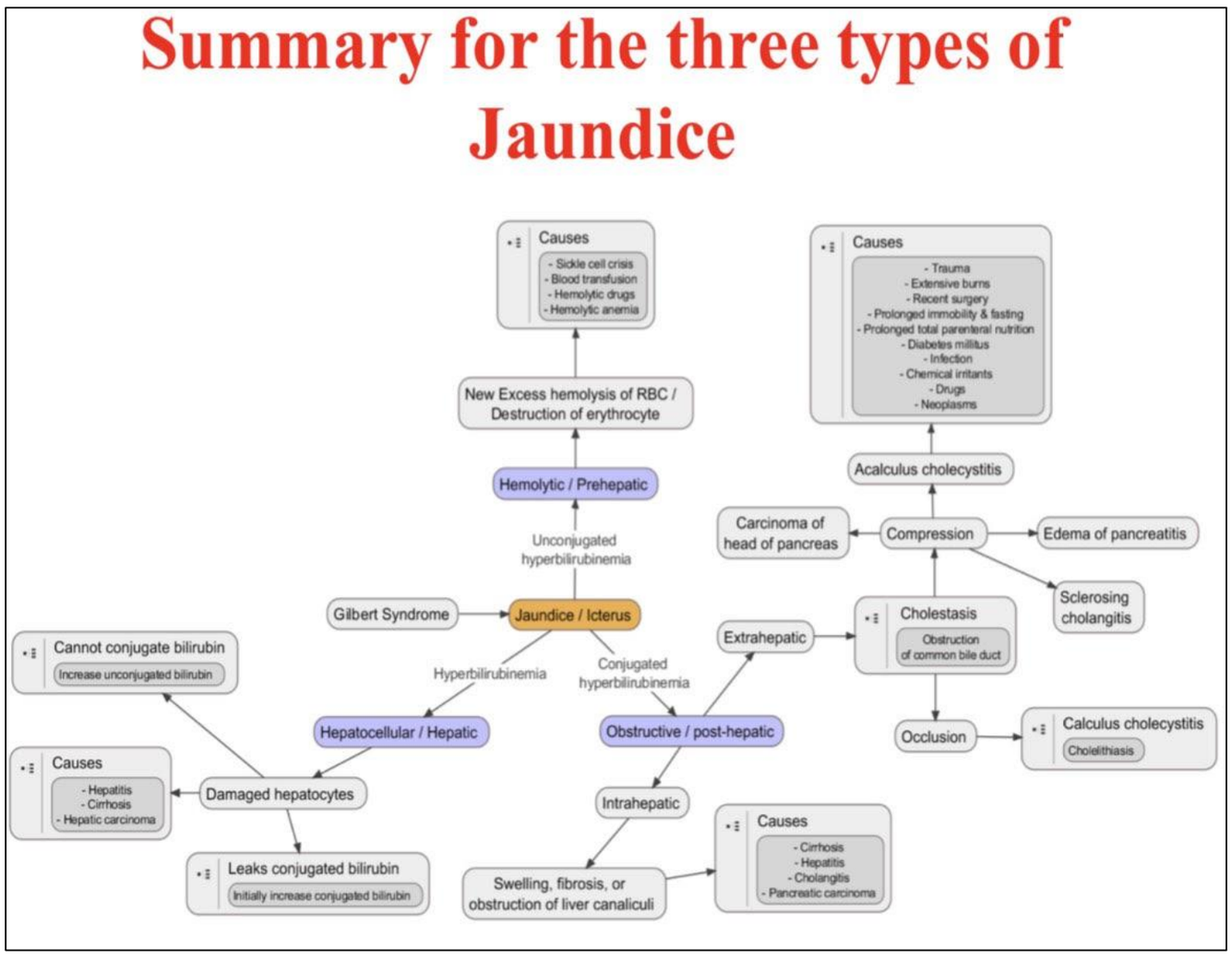


Figure 9-9

QUIZ

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1. Khalid, a 24 year-old engineer, developed yellowish skin and sclera after using a beta-lactam for a respiratory tract infection, in laboratory analysis the the total bilirubin level was 10 mg/dl, and he has been diagnosed with sickle cell disease, what is the type of jaundice does this patient have?

A) Pre-hepatic
B) Hepatic
C) Obstructive
D) Post-hepatic

2. What is the most abundant type of bilirubin will be found in serum analysis of Khalid?

A) Conjugated
B) Direct
C) Cholebilirubin
D) Free bilirubin

3. Primary biliary cirrhosis will lead to which type of jaundice?

A) Post-hepatic jaundice due to intrahepatic bile duct obstruction
B) Hemolytic jaundice
C) Post-hepatic due jaundice to Extrahepatic bile duct obstruction
D) Hepatic jaundice

4. What is the form of bilirubin found normally in the stool?

A) Urobilinogen
B) Stercobilin
C) Urobilin

SHORT ANSWER QUESTIONS

1. How is the bilirubin formed?
2. Define jaundice.
3. Mention 3 differences between conjugated & unconjugated bilirubin?
4. What is the fate of bilirubin?

ANSWERS

1. Senescent RBCs are phagocytosed intravascularly or extravascularly by RES, hemoglobin split into globin & heme, then heme gives free iron & biliverdin, which is reduced by biliverdin reductase to bilirubin.
2. Yellow coloration of the skin, sclera, mucous membranes & deep tissues when the bilirubin serum level exceed the normal range 0.2-1.3 mg/dl.
3. Table in page 6
4.
   a. Majority of conjugated will be deconjugated & to bilirubin & convert it to highly soluble compound called urobilinogen through bacterial action.
   b. Small portion of conjugated will return to plasma and is bound less tightly to albumin & is excreted through in urine.
   c. Small portion of deconjugated & converted to urobilinogen will enter the enterohepatic circulation & will be conjugated & excreted again it the bile.

ANSWER KEY: A, D, A, B

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REFERENCES

- Guyton and Hall Textbook of Medical Physiology
- Ganong's Review of Medical Physiology