BIOCHEMISTRY LECTURE

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BLOOD CHEMISTRY; BLOOD AS A TISSUE AND PORPHYRINS

BLOOD AS A TISSUE

- Blood is a liquid connective tissue
- The total blood volume makes up about 6-8 percent of the body's weight
- A 70kg person has approximately 5 to 6 litres of blood
- Normal pH of blood is 7.35(venous blood)-7.45 (arterial blood)

BLOOD COMPOSITION

- Blood consists of:

➢ Liquid plasma (volume 55-60%)

➢ Formed elements or cells (volume 40-45)

The formed elements include red blood cells, white blood cells and platelets

ERYTHROCYTES

- Red blood cells or erythrocytes are the most abundant type of blood cell
- They are involved in the transport of oxygen and carbon dioxide.
- They lack nucleus and mitochondria in order to accommodate maximum space for hemoglobin

PACKED CELL VOLUME (PCV)

- PCV also known as hematocrit is the volume (in percentage) of red blood cells in the blood

- It is normally about:

40-48% for men

And

36-42% for women

LEUKOCYTES

- Leukocytes or white blood cells have nuclei
- The major function of leukocytes is protective function, they provide immunity and thus defends the body
- Lymphocytes, neutrophils, monocytes, eosinophils and basophils are types of lymphocytes

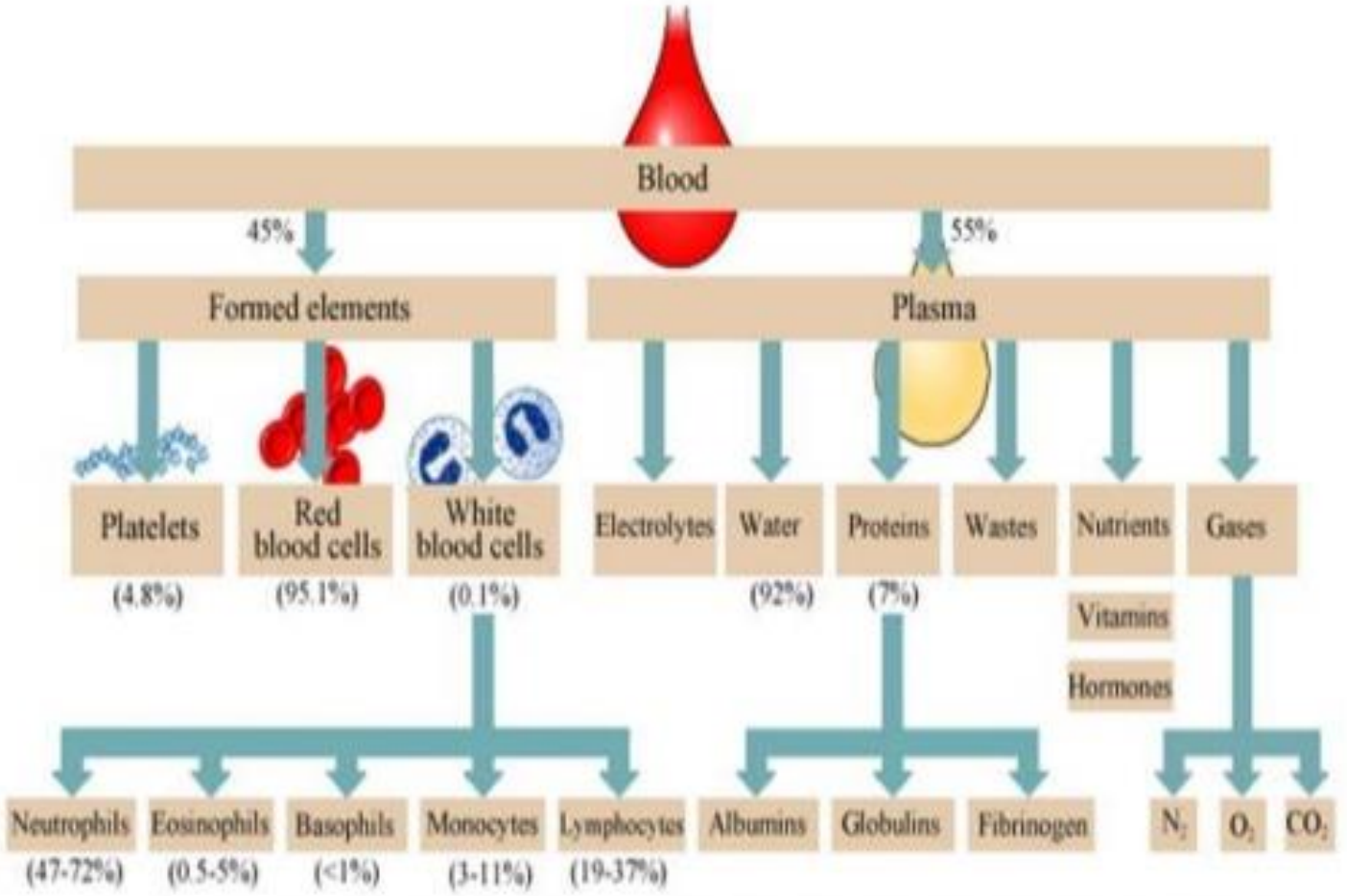
PLATELETS

- These are tiny cell fragments
- They play critical roles in blood clotting
- They are produced in the bone marrow by large cells called megakarocytes

SERUM

- When fibrinogen is removed from blood plasma as a result of coagulation, such plasma without fibrinogen is called serum. When blood is allowed to coagulate, serum is obtained, but when blood is prevented from clotting by putting in an anticoagulant such as EDTA, plasma is obtained

Blood Composition



FUNCTIONS OF BLOOD

- TRANSPORTATION of dissolved gases (Carbon dioxide and oxygen), nutrients, hormones and metabolic wastes.

- PROTECTION

  Platelets in the blood minimize blood loss when a blood vessel is damaged

  White blood cells protect the body against infectious diseases

FUNCTIONS OF BLOOD

- REGULATION

  Blood regulates pH and electrolyte composition of the body

  blood regulates body temperature

PORPHYRINS

OUTLINE

- Biosynthesis of heme
- Regulation of heme biosynthesis
- Disorders of heme biosynthesis
- Catabolism of hemoglobin
- Fate of bilirubin
- Jaundice

PORPHYRINS

- Porphyrins are cyclic compounds formed by fusion of four pyrrole rings linked by methenyl (=CH-) bridges.

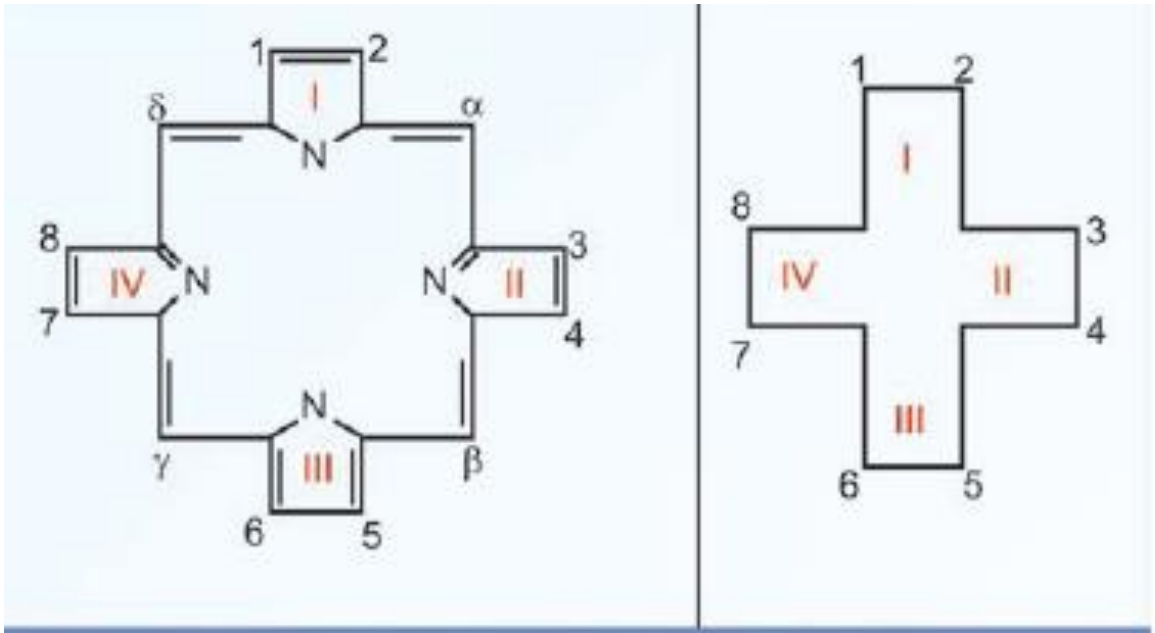
- Heme is a ferroprotoporphyrin; it consists of Fe^{2+} attached to the center of tetrapyrrole ring, called protoporphyrin IX.

- Heme is a prosthetic group for hemoglobin, myoglobin, the cytochromes, catalase.

- Porphyrin is also found in plants; chlorophyll, the photosynthetic green pigment in plants is Magnesium-porphyrin complex.

PORPHYRINS

Pyrrole ring



The pyrrole rings are numbered I to IV; the bridges named as alpha to delta and the possible sites of substitutions are denoted from 1 to 8. (For brevity, the bridges and double bonds are sometimes omitted, as shown on the right).

Porphyrin ring

BIOSYNTHESIS OF HEME

- Heme is mainly synthesized in the liver and immature red blood cells

- The pathway is partly cytosolic and partly mitochondrial. The first step and last three steps are mitochondrial, while other steps are cytosolic.

BIOSYNTHESIS OF HEME

STEP1: FORMATION OF DELTA AMINO LEVULINIC ACID (ALA)

- Glycine and succinyl CoA condense to form ALA in a reaction catalyzed by ALA synthase

- This reaction is the rate limiting step of heme biosynthesis

- ALA synthase requires pyridoxal phosphate(PLP) as coenzyme. Thus deficiency of vitamin B6 (Pyridoxine), reduces ALA and heme synthesis. This leads to decrease in hemoglobin content of blood (anaemia).

STEP 2: FORMATION OF PORPHOBILINOGEN (PBG)

- ALA dehydratase , an enzyme that is inhibited by lead catalyzes the condensation of two molecules of ALA to form PBG

STEP 3: FORMATION OF UROPORPHYRINOGEN (UPG)

- PBG deaminase or Hydroxymethylbilane (HMB) synthase catalyzes the condensation of four molecules of PBG to form a linear tetrapyrrole, called HMB.
- HMB cyclises sponteneously to form Uroporphyrinogen I, which is converted to Uroporphyrinogen III by Uroporphyrinogen III cosynthase

STEP 4: SYNTHESIS OF COPROPORPHYRINOGEN (CPG)

- UPG decarboxylase catalyzes the decarboxylation of UPGIII to form CPG III
- In this reaction, the acetyl groups in UPG are decarboxylated to methyl groups

STEP 5: SYNTHESIS OF PROTOPORPHYRINOGEN (PPG)

CPG III is oxidized to PBGIII by coproporphyrinogen oxidase. In this reaction, two propionyl side chains are oxidatively decarboxylated to vinyl groups

STEP 6: GENERATION OF PROTOPORPHYRIN (PP)

- Protoporphyrinogen III is oxidized by Protoporphyrinogen III oxidase to protoporphyrin III. In this reaction, methylene bridges are oxidized to methenyl bridges with double bonds

STEP 7: GENERATION OF HEME

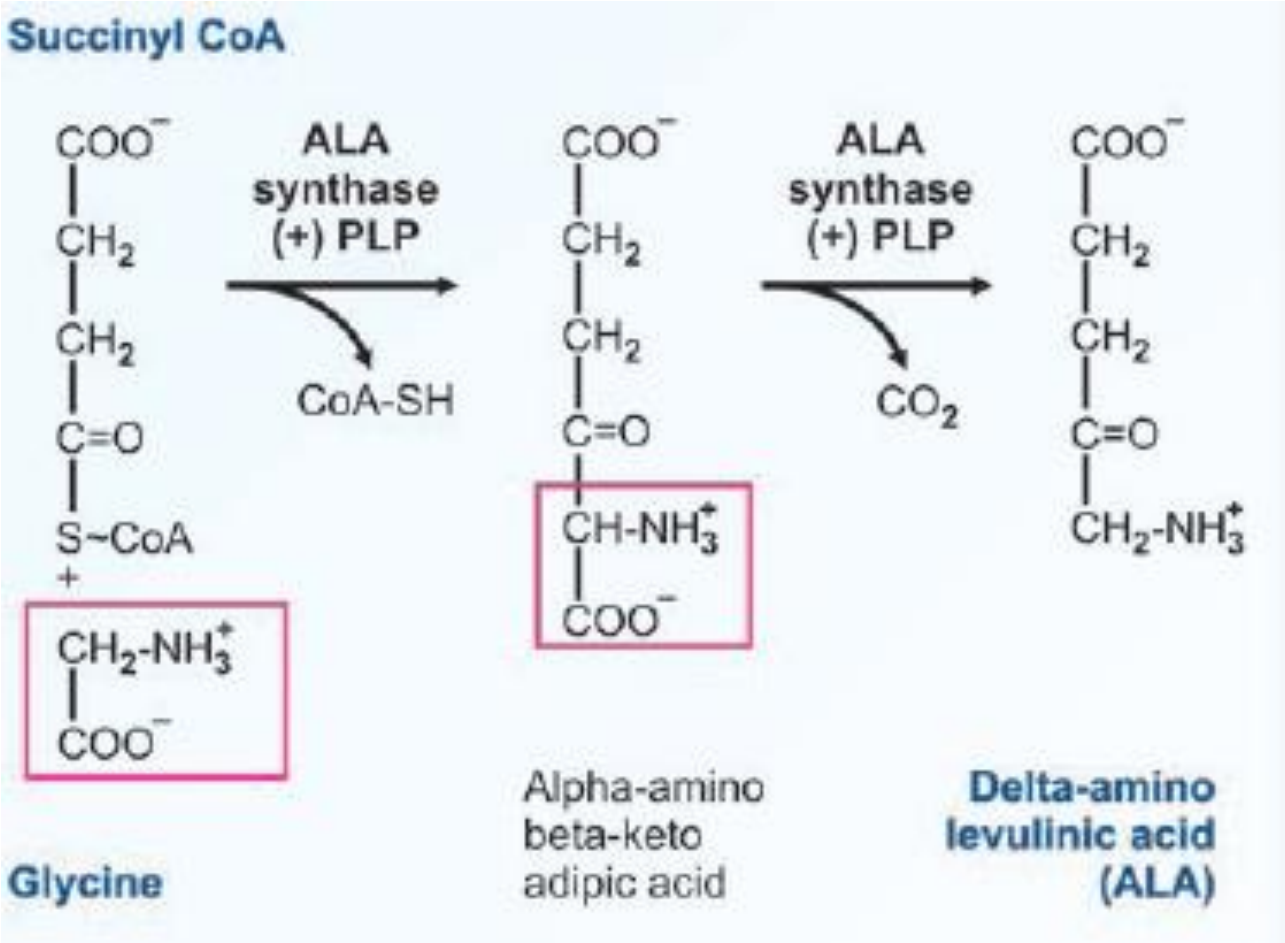
- This involves the attachment of ferrous ion Fe^{2+} to protoporphyrin III, as catalyzed by heme synthase, also known as ferrochelatase. This enzyme is subject to inhibition by lead.

BIOSYNTHESIS OF HEME

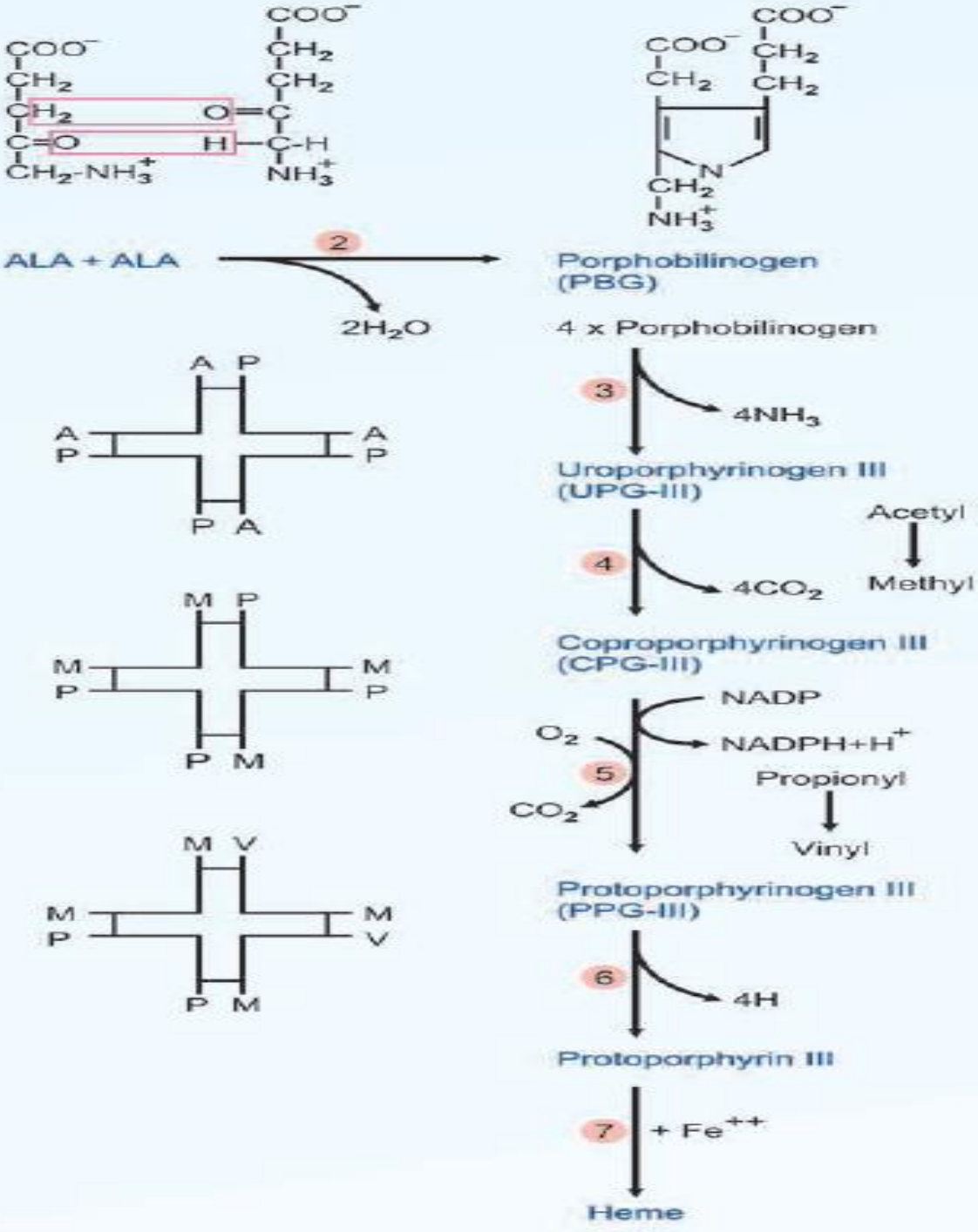
- Note that porphyrins are in two main forms; I and III isomers. The III isomers are also called IX isomers. They are the most predominant forms in biological systems.

- The I isomers are symmetrical while the III isomers are asymmetrical with respect to ring IV

BIOSYNTHESIS OF HEME



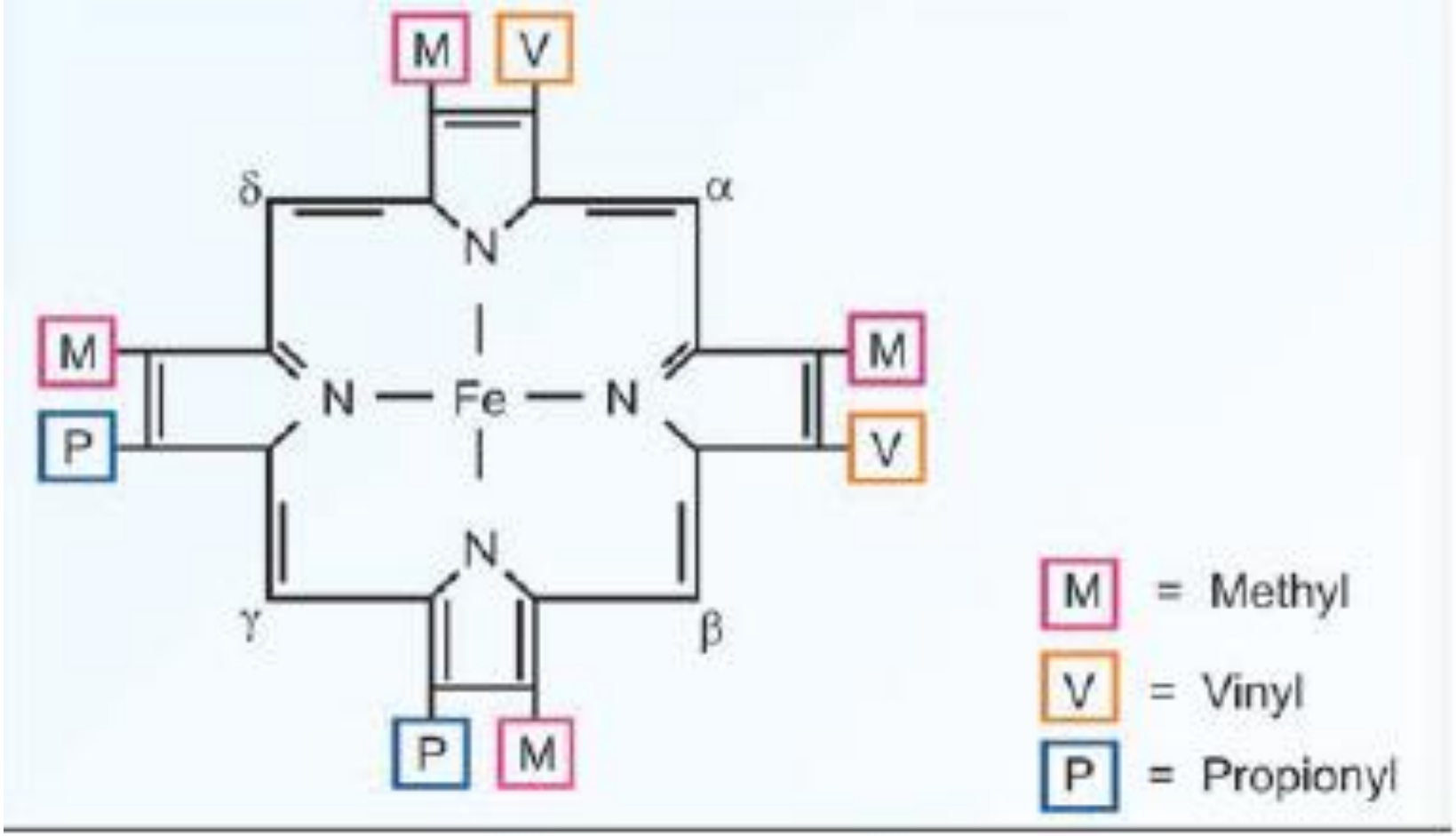
STEP 1


ALA + ALA
2
$2H_2O$
Porphobilinogen (PBG)
4 x Porphobilinogen
3
$4NH_3$
Uroporphyrinogen III (UPG-III)
Acetyl
Methyl
4
$4CO_2$
Coproporphyrinogen III (CPG-III)
NADP
O_2
$NADPH+H^+$
5
Propionyl
CO_2
Vinyl
Protoporphyrinogen III (PPG-III)
6
4H
Protoporphyrin III
7
$+ Fe^{++}$
Heme

KEY

2 = ALA dehydratase
3 = PBG deaminase and UPG-III co-synthase
4 = uroporphyrinogen decarboxylase
5 = Copro porphyrinogen oxidase
6 = Protoporphyrinogen oxidase
7 = Heme synthase or Ferrochelatase
A = acetyl; P = propionyl; M = methyl; V = vinyl.

STRUCTURE OF HEME



REGULATION OF HEME SYNTHESIS

- The rate limiting enzyme of heme synthesis, ALA Synthase is regulated as follows:

a. Heme inhibits synthesis of ALA synthase

b. Hematin is formed from heme, when there is excess of free heme, Fe^{2+} in heme is oxidized to Fe^{3+}, thus forming hematin. ALA synthase is allosterically inhibited by hematin.

C. Effect of drugs on ALA synthase activity: drugs like barbiturates, griseofulvin, hydantoins are metabolised by cytochrome P450 Monooxygenase system (which contains heme) of the liver. In response to these drugs, the synthesis of ALA synthase increases to provide heme for incorporation into the cytochrome P450 that is rapidly used up.

REGULATION OF HEME SYNTHESIS

- Ferrochelatase and ALA dehydratase are inhibited by lead. This explains the mechanism by which lead poisoning causes anaemia. The inhibition of these enzymes by lead reduces the body's ability to make heme and hemoglobin, thus reducing hemoglobin concentration of the blood.

DISORDERS OF HEME BIOSYNTHESIS (PORPHYRIAS)

- Porphyrias are groups of inborn errors of metabolism associated with biosynthesis of heme.

- Porphyrias are classified as erythropoietic or hepatic, depending on whether the enzyme deficiency occurs in the erythropoietic cells or in the liver.

HEPATIC PORPHYRIAS

- **Porphyria cutanea tarda:**
- A chronic disease caused by a deficiency of uroporphyrinogen decarboxylase.
- Uroporphyrin accumulates in the urine
- It is the most common Porphyria
- Patients are photosensitive, their skin itches and burn (pruritis) when exposed to visible light.

Porphyria cutanea tarda:

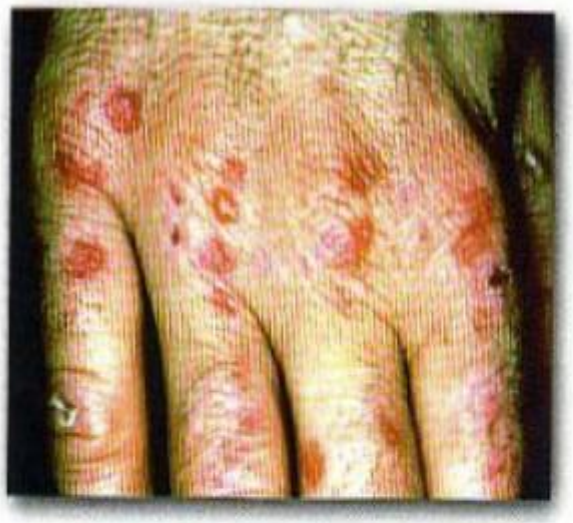


Figure 21.5
Skin eruptions in a patient with porphyria cutanea tarda.

Figure 21.6
Urine from a patient with porphyria cutanea tarda (right) and from a patient with normal porphyrin excretion (left).

Acute intermittent Porphyria

- An acute disease caused by a deficiency of hydroxymethylbilane synthase.
- Porphobilinogen and δ-aminolevulinic acid accumulate in the urine.
- Urine darkens on exposure to light and air.
- Patients are not photosensitive
- Patients experience acute abdominal pain, agitation and confusion

ERYTHROPOIETIC PORPHYRIAS

- Erythropoietic Porphyrias are characterized by skin rashes and blisters that appear in the early childhood

CONGENITAL EYTHROPOIETIC PORPHYRIA

- The disease caused by a deficiency of uroporphyrinogen lll synthase.
- Uroporphyrinogen I & coproporphyrinogen I accumulate in urine
- Patients are photosensitive.

MANAGEMENT OF PORPHYRIA

- I.V injection by hematin, which decreases the synthesis of ALA synthase

- Avoidance of sunlight and ingestion of β-carotene, (free-radical scavenger) are also helpful

DIAGNOSIS OF PORPHYRIAS

- To demonstrate porphyrins, UV fluorescence is the best technique.

- When urine is observed under ultraviolet light; porphyrins if present, will emit strong red fluorescence.

CATABOLISM OF HEMOGLOBIN

- The globin chains of hemoglobin are separated, they are hydrolyzed and the amino acids are channelled into the body amino acid pool. The iron liberated from heme is reutilized.

- The porphyrin ring is broken down in reticuloendothelial cells of liver, spleen and bone marrow to bile pigments, mainly bilirubin.

CATABOLISM OF HEME

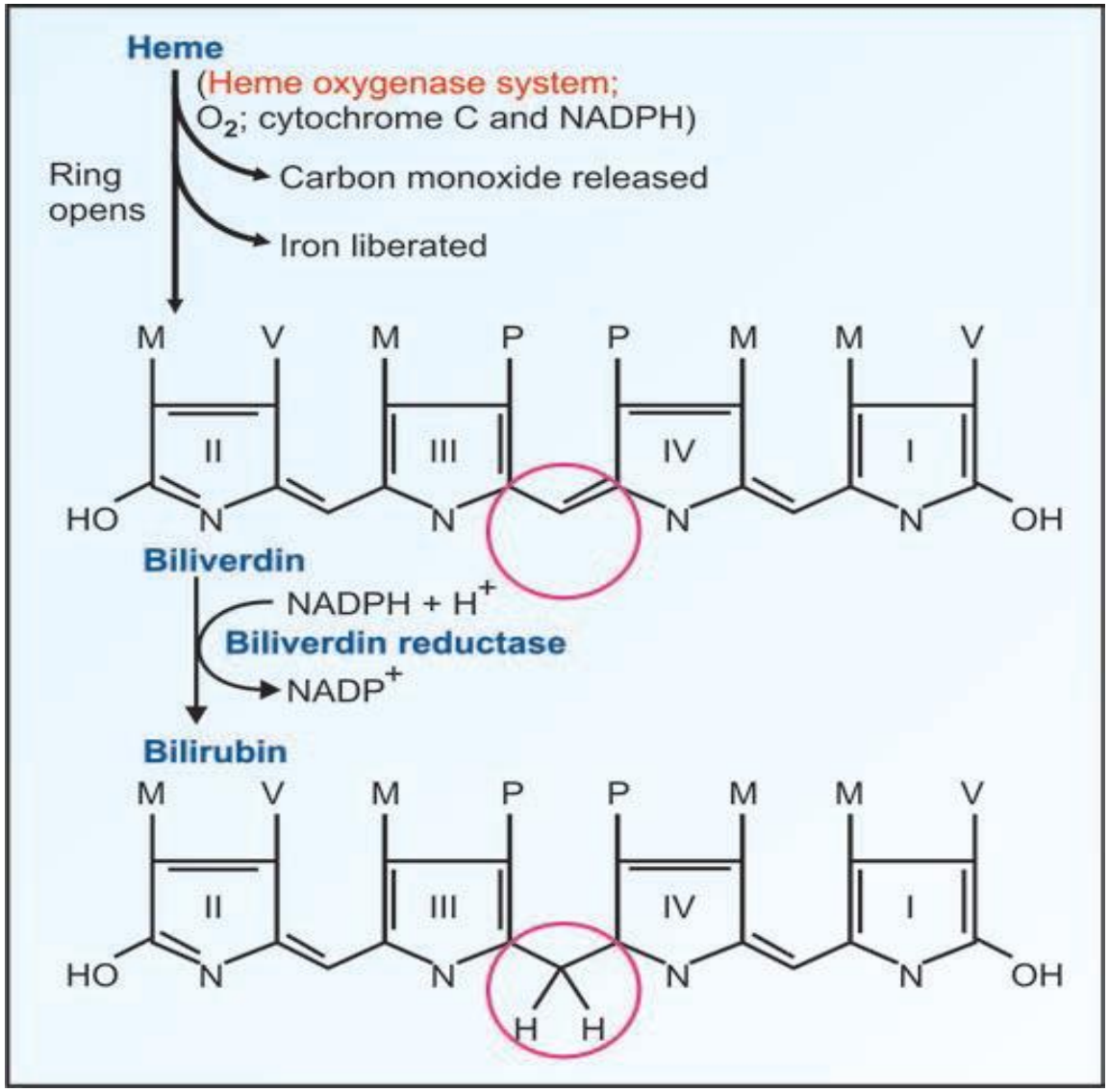
- Heme is degraded primarily by a microsomal

enzyme system; heme oxygenase. It requires

molecular oxygen and NADPH and cytochrome c. The enzyme is induced by heme.

- The linear tetrapyrrole formed is **biliverdin** which is green in color. In mammals it is further reduced to **bilirubin, a red-yellow pigment,** by an NADPH dependent biliverdin reductase. But birds, amphibians and rabbits excrete the green biliverdin itself.

CATABOLISM OF HEME



FATE OF BILIRURIN

1. TRANSPORT TO THE LIVER

- The bilirubin formed in the reticuloendothelial cells is insoluble in water. The lipophilic bilirubin is therefore transported in plasma bound to **albumin.**

- When the albumin–bilirubin complex reaches the sinusoidal surface of the liver, the bilirubin is taken up. The uptake is a carrier mediated active process

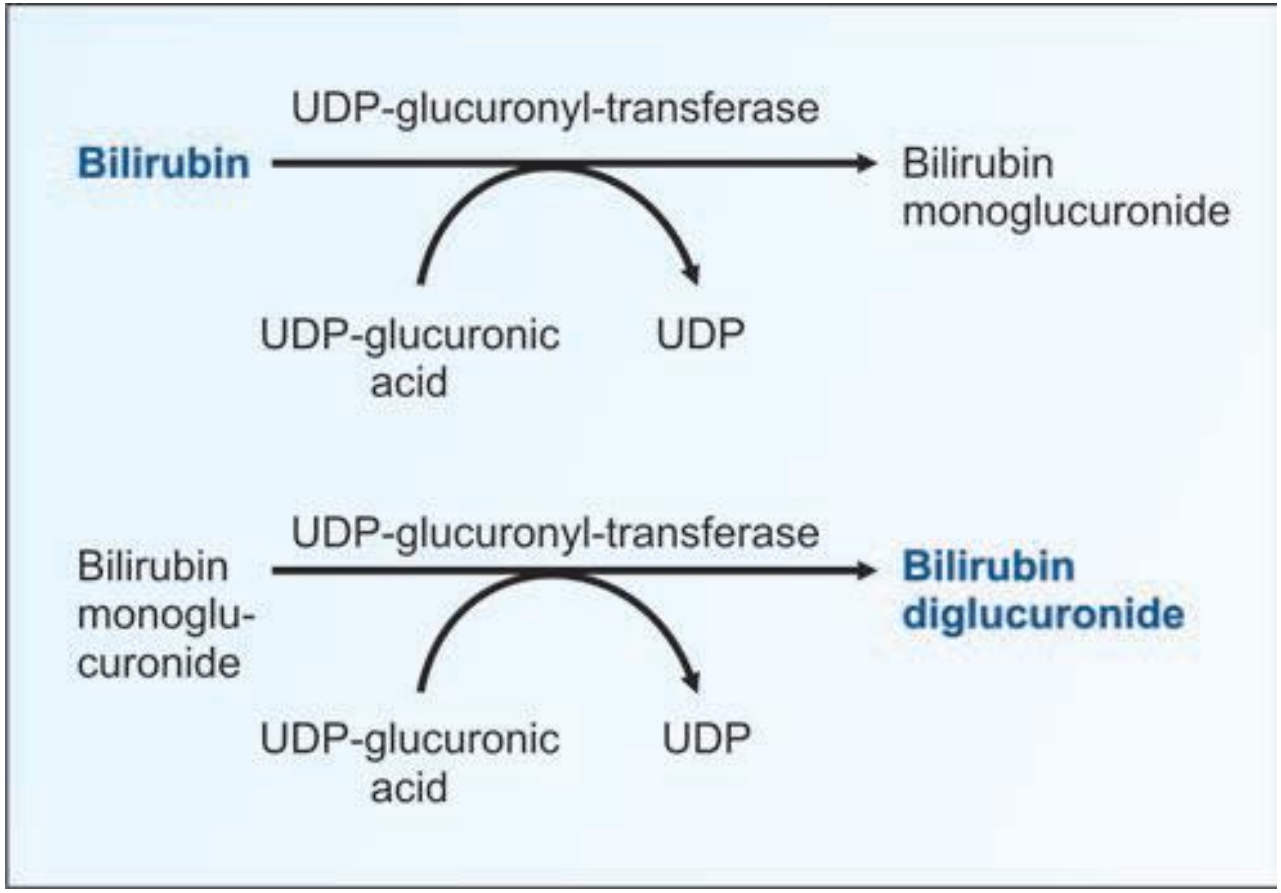
2. **CONJUGATION IN LIVER**

- Inside the liver cell, the bilirubin is conjugated

with glucuronic acid, to make it **water soluble**

- Drugs like primaquine, novobiocin, chloramphenicol, androgens and pregnanediol may interfere in this conjugation process and may cause jaundice.

CONJUGATION OF BILIRUBIN



3. **EXCRETION OF BILIRUBIN TO BILE**

- The water soluble conjugated bilirubin is excreted into the bile and this is the rate limiting step in the catabolism of heme.

- 4. **FATE OF CONJUGATED BILIRUBIN IN INTESTINE**

  The conjugated bilirubin reaches the intestine through the bile. Intestinal bacteria deconjugate the conjugated bilirubin.

  This free bilirubin is further reduced to a colourless tetrapyrrole urobilinogen (UBG) Further reduction of UBG leads to formation of stercobilinogen (SBG) .

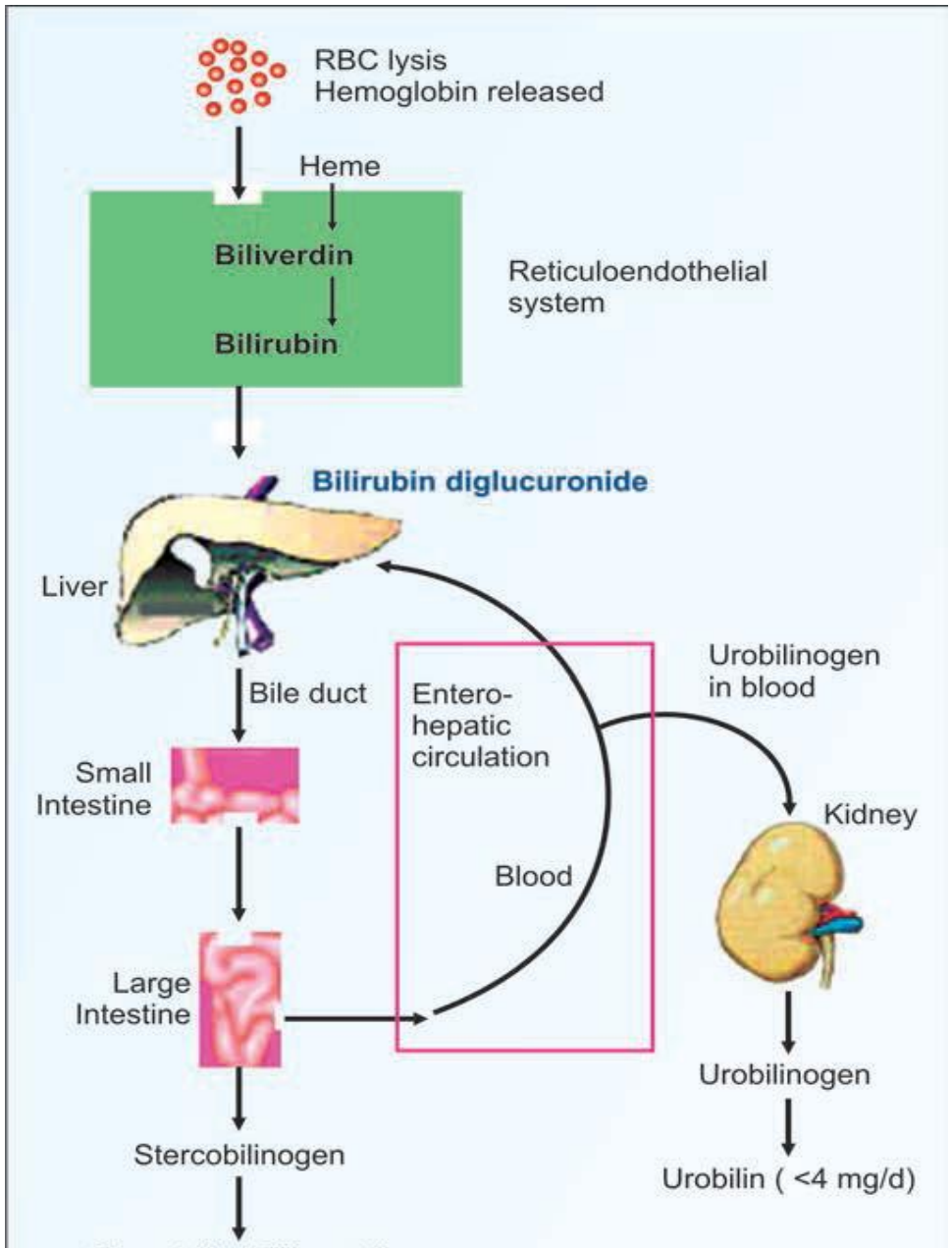
  The SBG is mostly excreted through feces.20% of the UBG is reabsorbed from the intestine and returned to the liver by portal blood. The UBG is again re-excreted (enterohepatic circulation).

5. FINAL EXCRETION

- UBG and SBG are both colorless compounds but are oxidized to coloured products, urobilin or stercobilin respectively by atmospheric oxidation.

- Both urobilin and stercobilin are present in urine as well as in feces. Urobilin is mostly excreted in urine, while stercobilin is is mostly excreted in feces. The normal colour of feces is due to stercobilin.

FATE OF BILIRUBIN



JAUNDICE

- If the plasma bilirubin level exceeds 1 mg/dl, the condition is called **hyperbilirubinemia.** Levels between 1 and 2 mg/dl are indicative of **latent jaundice.**

- **When the bilirubin level exceeds 2 mg/dl, it** diffuses into tissues producing yellowish discoloration of sclera, conjunctiva, skin and mucous membrane resulting in **jaundice.**

JAUNDICE

- **In Hemolytic Jaundice** results from massive lysis of RBCs (as seen in patients with sickle cell anemia, or malaria). Bilirubin produced faster than it can be conjugated.

- So more bilirubin is excreted in the bile, **Uncojugated** bilirubin level in blood is increased

OBSTRUCTIVE JAUNDICE

- This results from obstruction of the bile duct (due to hepatic tumor or bile stone that may block the bile duct), preventing passage of bilirubin in the intestine.

- Patients with obstructive jaundice suffer from gastrointestinal pain and nausea and produce stools that are a pale ,clay color

- The liver regurgitates bilirubin into the blood. The compound is excreted in the blood.

HEPATOCELLULAR JAUNDICE

- Damage of the liver cells (as seen in Liver cirrhosis or hepatitis) can cause increased in the unconjugated bilirubin level in the blood, this is called hepatocellular jaundice.

JAUNDICE

Patient with jaundice; sclera of the eye appears yellow