HEME METABOLISM

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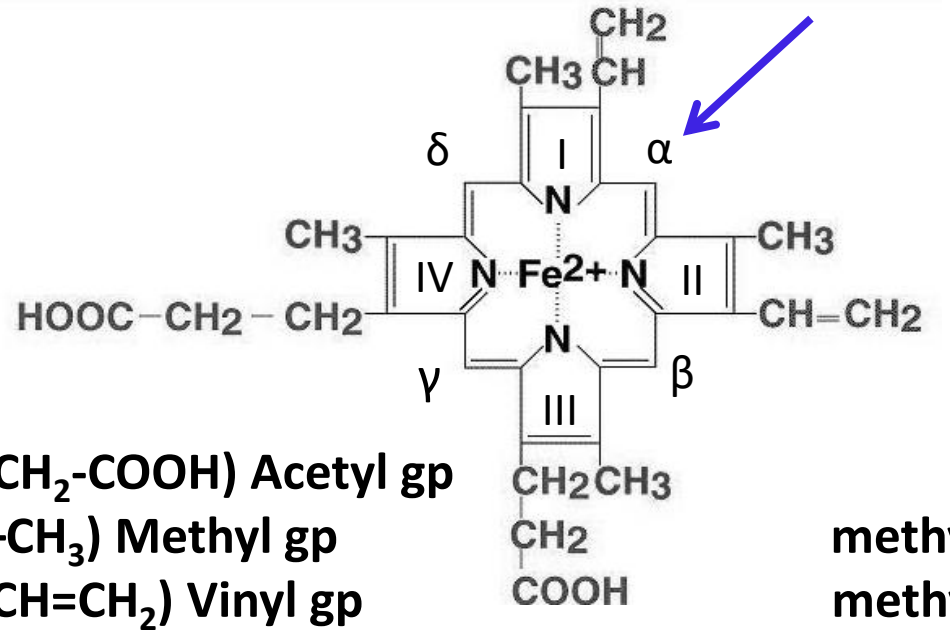
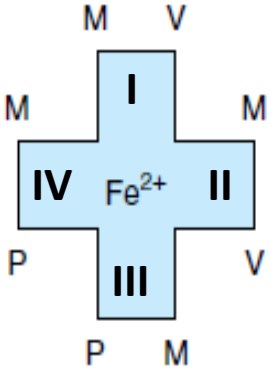
Shuzan Ali

Chemistry of heme

ferrous protoporphyrin III

[4 pyrrole units linked by methenyl bridges (-CH=)]





A: (–CH_2-COOH) Acetyl gp

M: (–CH_3) Methyl gp

V: (–CH=CH_2) Vinyl gp

P: (–CH_2-CH_2-COOH) Propionate gp

methyl gp: (-CH_3)

methylene bridge: (-CH_2-)

methenyl bridge: (-CH=)

Site of heme synthesis:

***Occurs in most mammalian cells, <u>except mature erythrocytes</u> (absent mitochondria)**

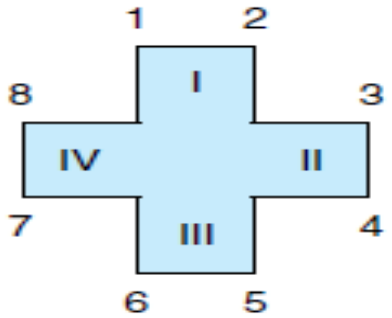
***Mainly bone marrow and liver (mitochondria & cytoplasm)**

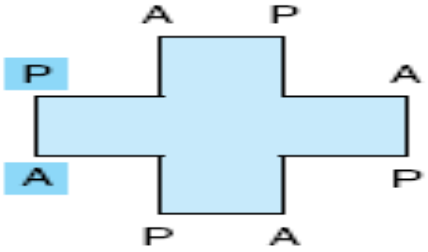
N.B. Iron porphyrin (heme), Mg porphyrin (chlorophyll)

Structure of porphyrins:

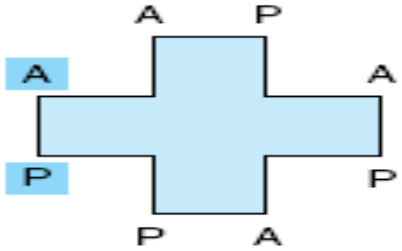
- 4 pyrrole rings connected by methenyl bridges, as follow:

1. **Side chains**: **Uroporphyrin: acetate (A) and propionate (P)**
   Coproporphyrin: methyl (M) and propionate (P)
   Protoporphyrin: methyl (M), propionate (P) & vinyl (V)
2. **Distribution of side chains**: Only type III porphyrins, contain asymmetric substitution on ring D & are important in humans.
3. **Porphyrinogens** (**colorless precursors**) & are intermediates between porphobilinogen & protoporphyrin in heme synthesis.
4. Porphyrins are colored

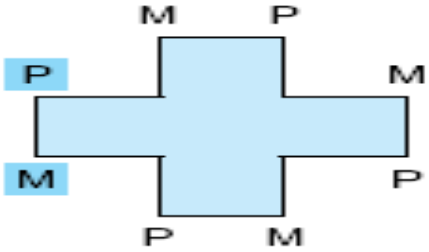




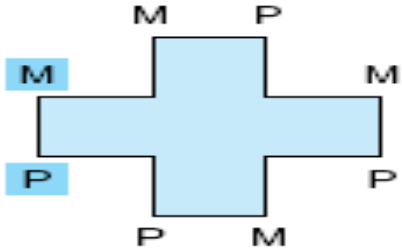
Uroporphyrin I



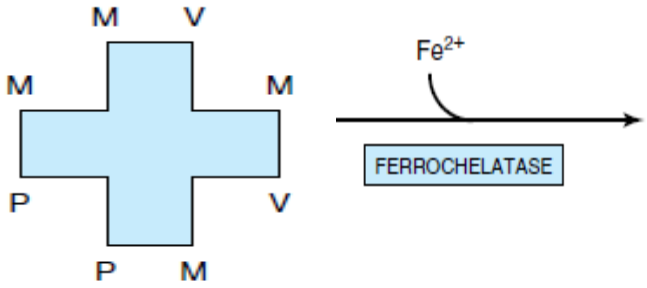
Uroporphyrin III



Coproporphyrin I



Coproporphyrin III

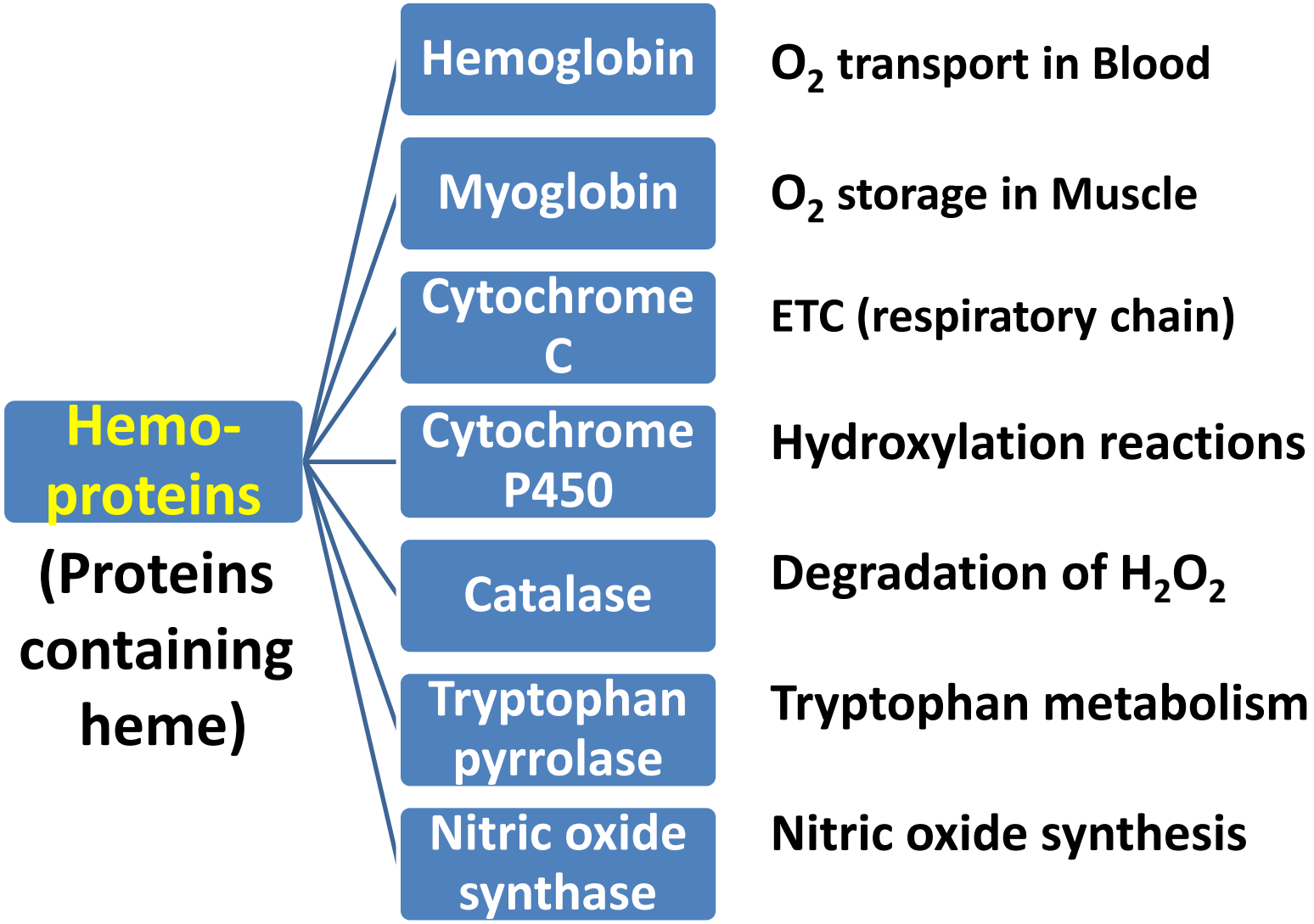


Protoporphyrin III (IX) Heme

A: ($-CH_2$-COOH) Acetyl gp **M: ($-CH_3$) Methyl gp**

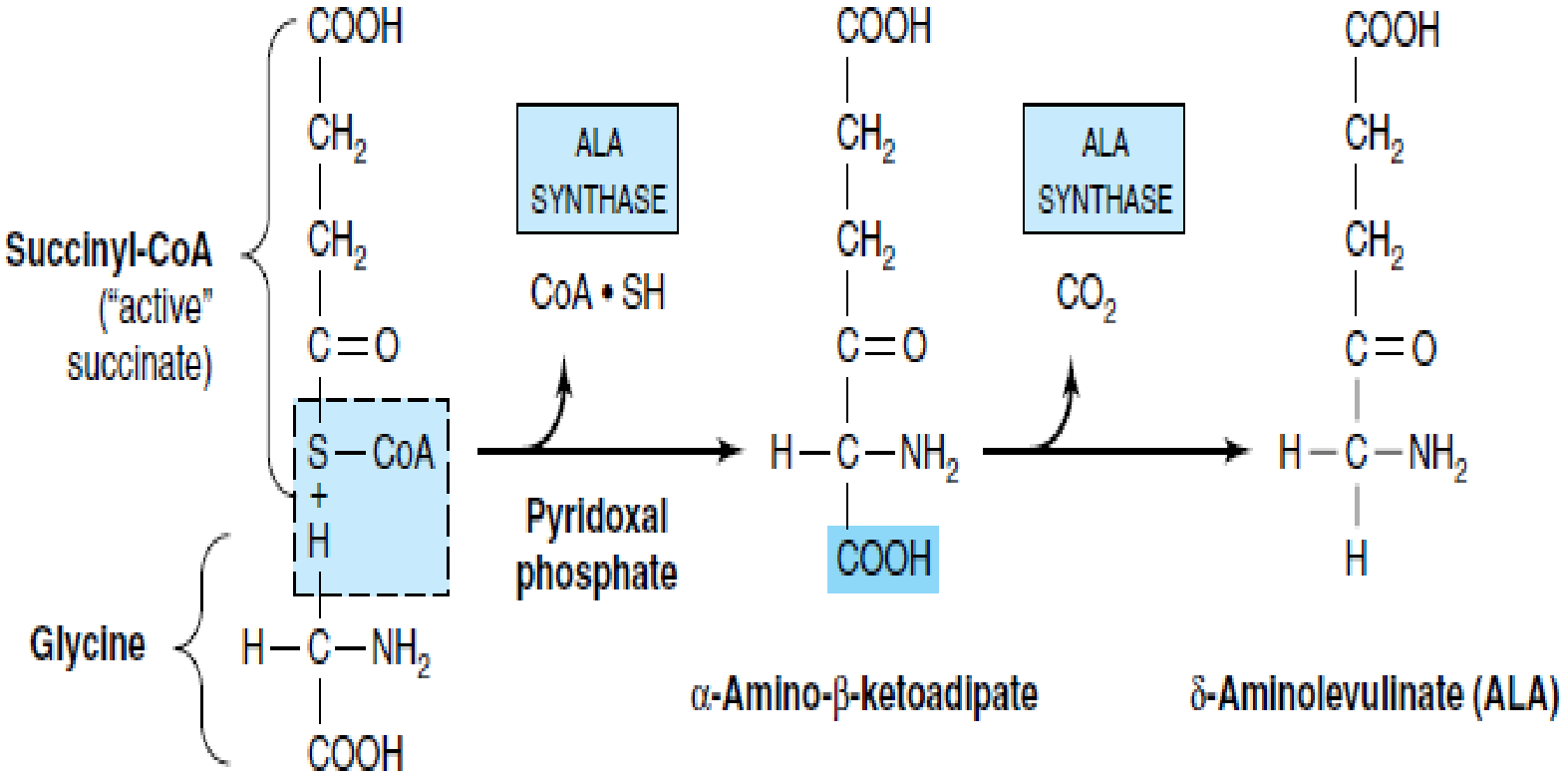
P: ($-CH_2$-CH_2-COOH) Propionate gp **V: ($-CH=CH_2$) Vinyl gp**

Heme is prosthetic gp of Hb, myoglobin & cytochromes



Biosynthesis of heme

1-Format. of δ-aminolevulinic acid (ALA) in mitochondria

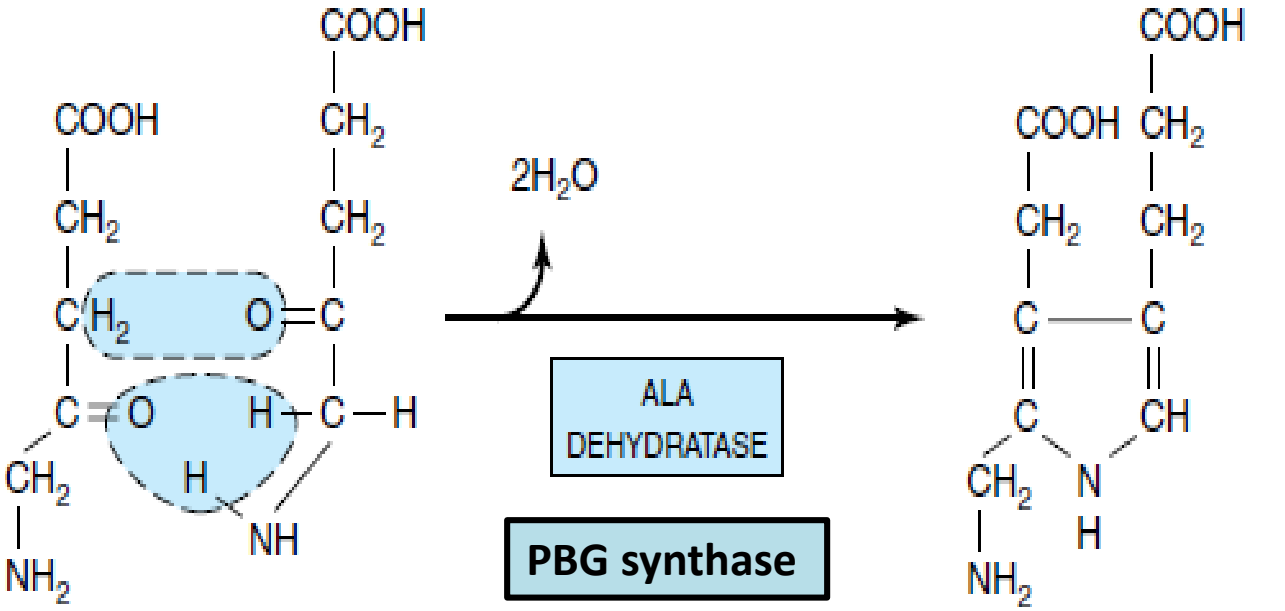


Take care: ALA **synthase** not ALA **synthetase**

ALA synthase I (hepatic) and II (erythroid; in red cell precursors).

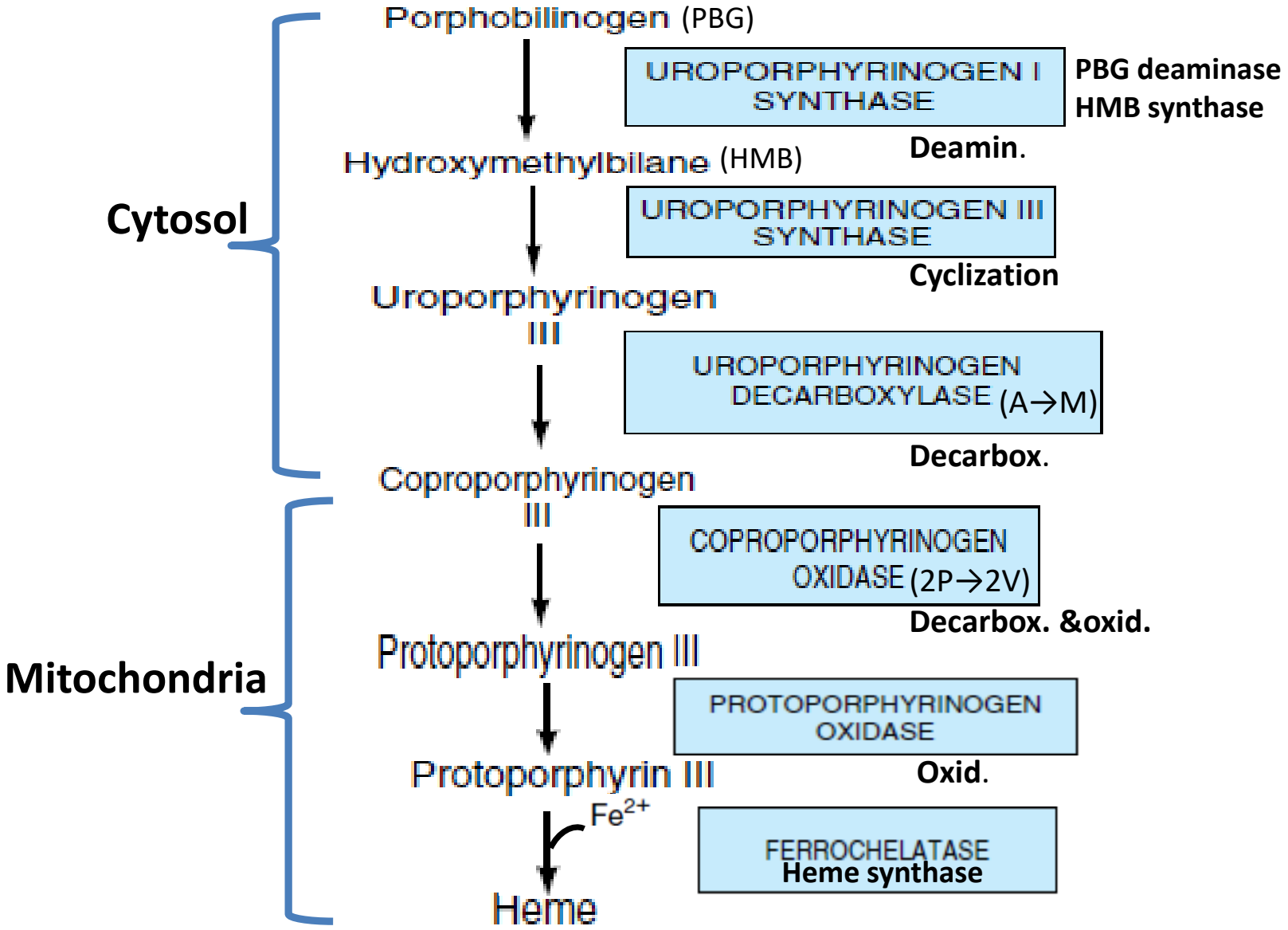
Only type I is regulated by heme.

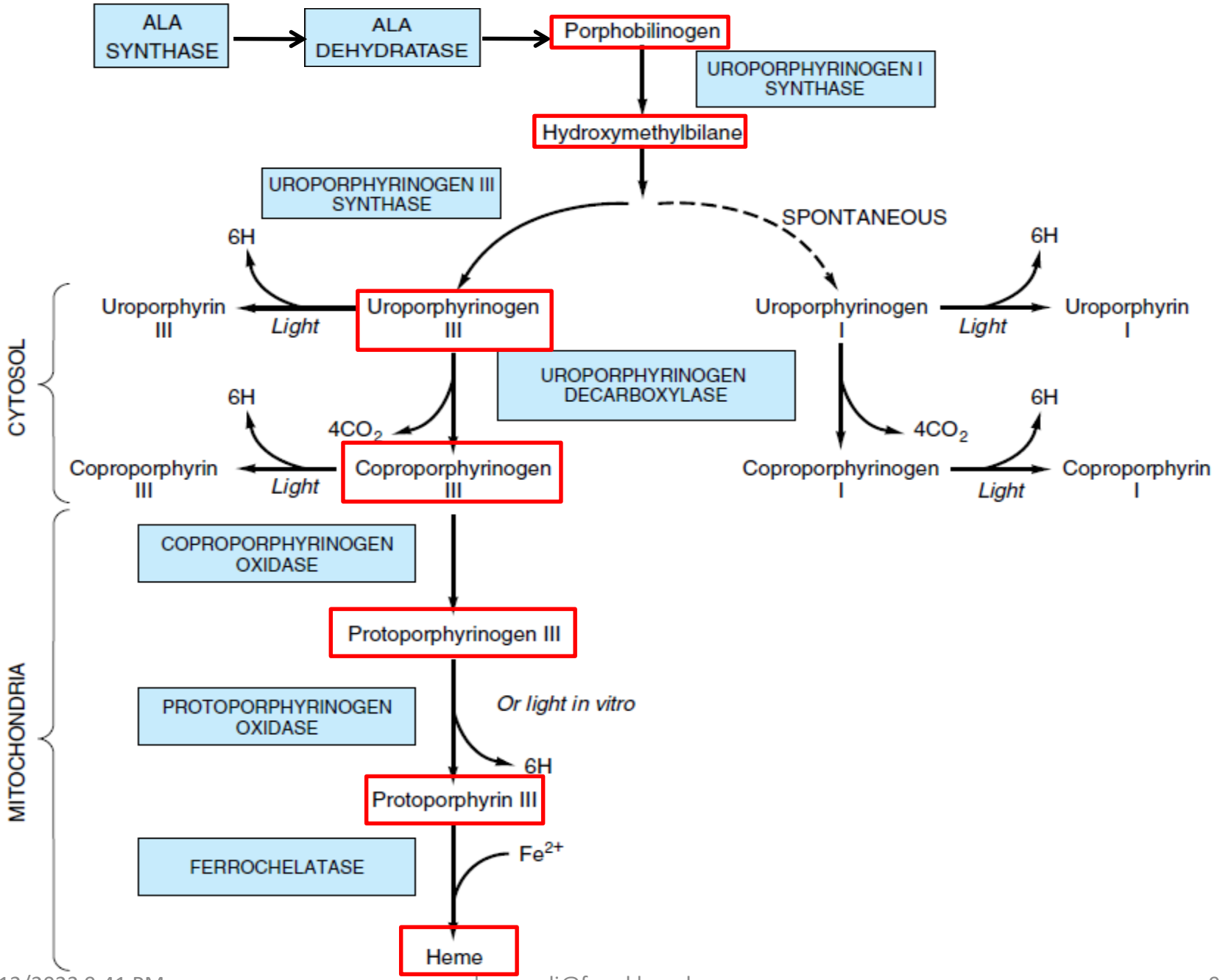
2-Format. of porphobilinogen (PBG): in cytosol



Two molecules of δ-aminolevulinate

Porphobilinogen
(first precursor pyrrole)


Cytosol
Porphobilinogen (PBG)
UROPORPHYRINOGEN I SYNTHASE
PBG deaminase
HMB synthase
Deamin.
Hydroxymethylbilane (HMB)
UROPORPHYRINOGEN III SYNTHASE
Cyclization
Uroporphyrinogen III
UROPORPHYRINOGEN DECARBOXYLASE (A→M)
Decarbox.
Coproporphyrinogen III
Mitochondria
COPROPORPHYRINOGEN OXIDASE (2P→2V)
Decarbox. &oxid.
Protoporphyrinogen III
PROTOPORPHYRINOGEN OXIDASE
Oxid.
Protoporphyrin III
Fe2+
FERROCHELATASE
Heme synthase
Heme


ALA SYNTHASE
ALA DEHYDRATASE
Porphobilinogen
UROPORPHYRINOGEN I SYNTHASE
Hydroxymethylbilane
UROPORPHYRINOGEN III SYNTHASE
SPONTANEOUS
6H
6H
Uroporphyrin III
Light
Uroporphyrinogen III
Uroporphyrinogen I
Light
Uroporphyrin I
CYTOSOL
UROPORPHYRINOGEN DECARBOXYLASE
6H
6H
4CO2
4CO2
Coproporphyrin III
Light
Coproporphyrinogen III
Coproporphyrinogen I
Light
Coproporphyrin I
COPROPORPHYRINOGEN OXIDASE
Protoporphyrinogen III
MITOCHONDRIA
PROTOPORPHYRINOGEN OXIDASE
Or light in vitro
6H
Protoporphyrin III
FERROCHELATASE
Fe2+
Heme

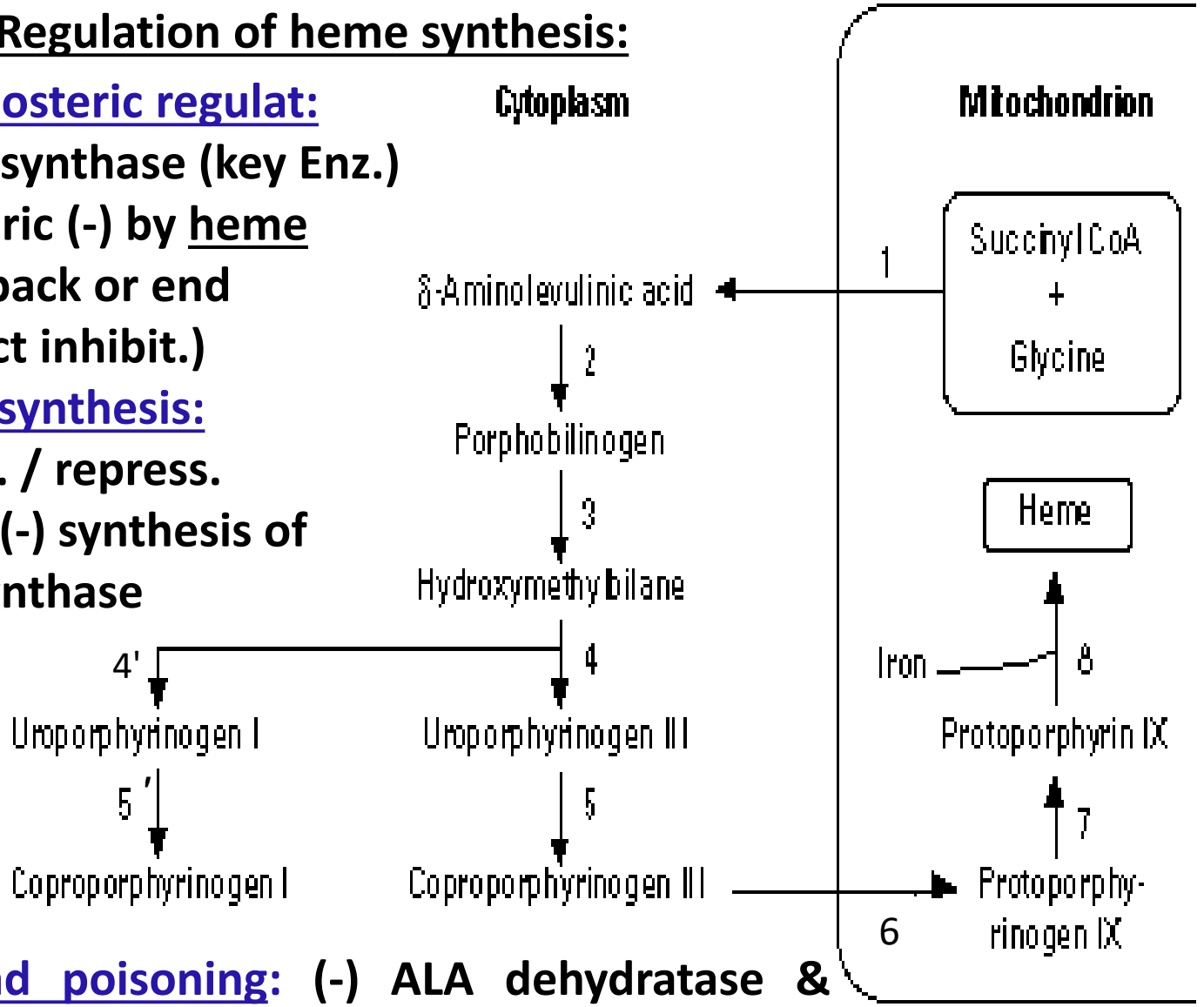
Regulation of heme synthesis:

1. **Allosteric regulat:**

δ-ALA synthase (key Enz.) allosteric (-) by heme (feed back or end product inhibit.)

2. Enz synthesis:

induct. / repress. Heme (-) synthesis of ALA synthase

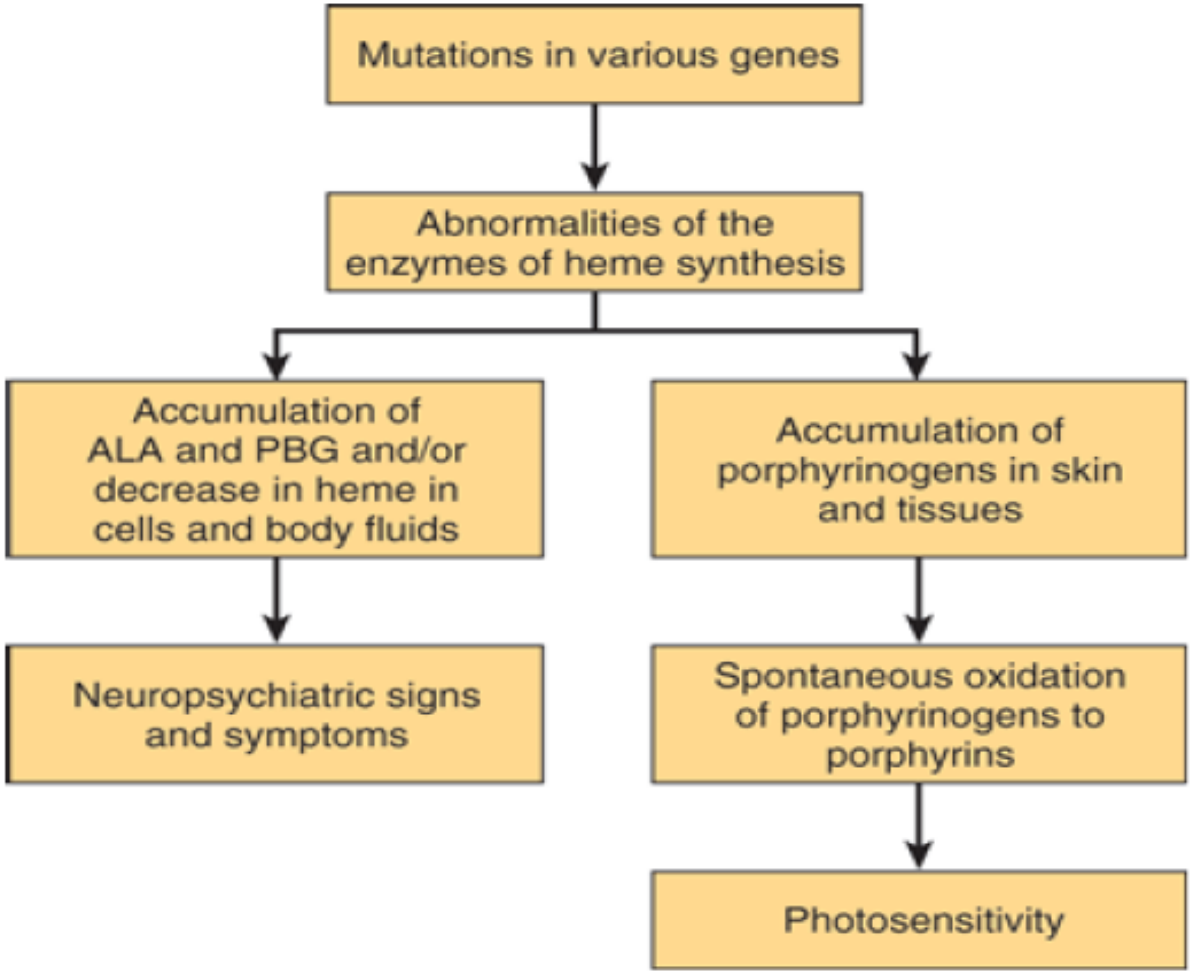


3. Lead poisoning: (-) ALA dehydratase & Ferrochelatase (heme synthase).

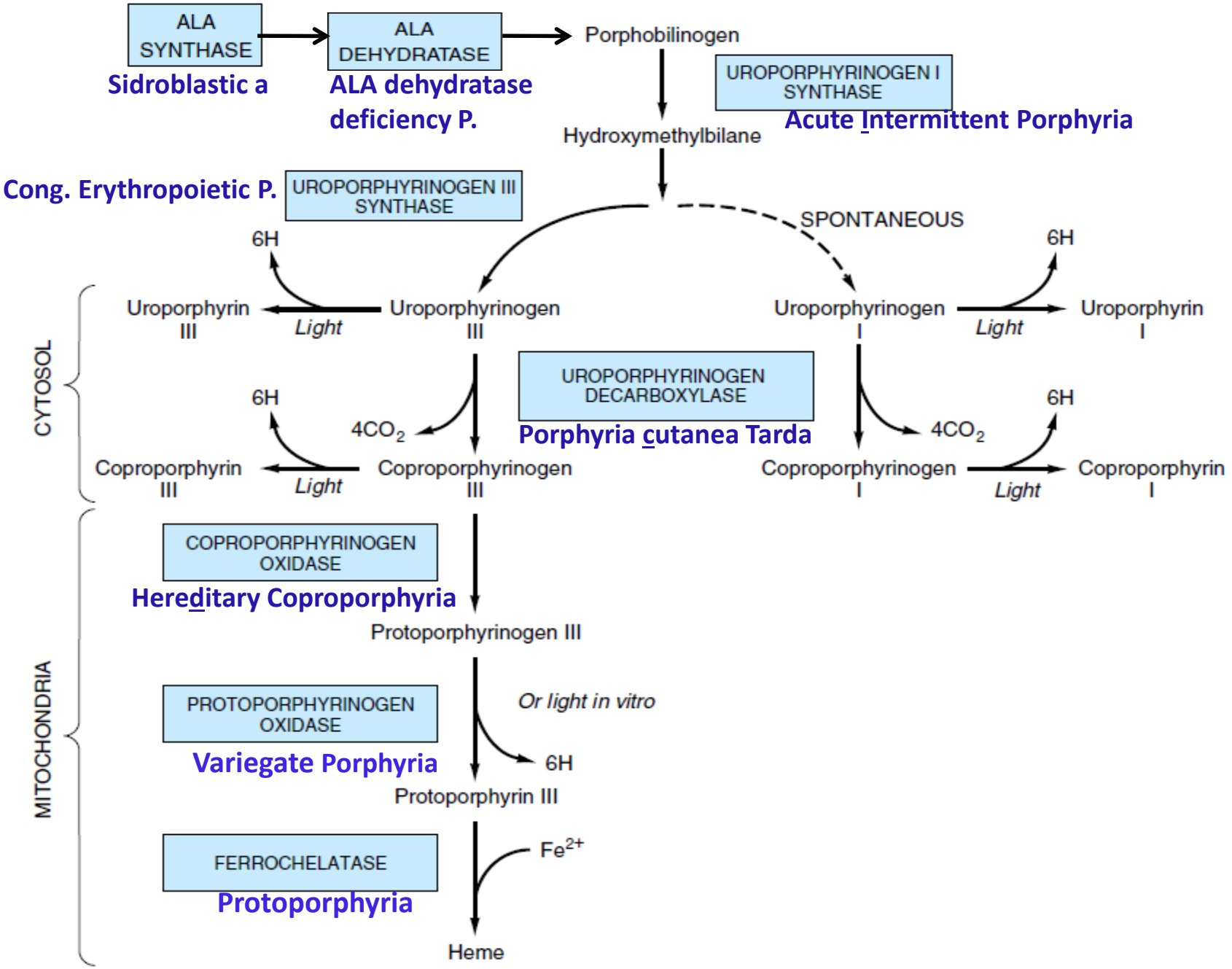
Porphyria

Definition

Porphyria is A metabolic disease due to congenital deficiency of one of the enzymes of **heme synthesis**. This leads to accumulation of the metabolic products before the site of the deficient enzyme. The symptoms depend on the site of the defect.



Porphyrias are autosomal dominent except congenital erythropoietic P. is autosomal recessive


ALA SYNTHASE
ALA DEHYDRATASE
Porphobilinogen
Sidroblastic a
ALA dehydratase deficiency P.
UROPORPHYRINOGEN I SYNTHASE
Acute Intermittent Porphyria
Hydroxymethylbilane
Cong. Erythropoietic P.
UROPORPHYRINOGEN III SYNTHASE
SPONTANEOUS
6H
6H
Uroporphyrin III
Light
Uroporphyrinogen III
Uroporphyrinogen I
Light
Uroporphyrin I
CYTOSOL
UROPORPHYRINOGEN DECARBOXYLASE
Porphyria cutanea Tarda
6H
6H
4CO2
4CO2
Coproporphyrin III
Light
Coproporphyrinogen III
Coproporphyrinogen I
Light
Coproporphyrin I
COPROPORPHYRINOGEN OXIDASE
Hereditary Coproporphyria
Protoporphyrinogen III
MITOCHONDRIA
PROTOPORPHYRINOGEN OXIDASE
Variegate Porphyria
Or light in vitro
6H
Protoporphyrin III
FERROCHELATASE
Protoporphyria
Fe2+
Heme

Deficient Enzyme	Diseases	Clinically	Lab
1. ALA synthase	X-linked sidroblastic anemia (not P.)	anemia	↓ red cell count & Hb
2. ALA dehydratase	ALA dehydratase deficiency P. (rare)	Abd. Pain, neuropsych.	Urine ALA +ve
3. Uroporphyrinogen I synthase	Acute Intermittent P.		Urine PBG and uroporphyrin +ve
4. Uroporphyrinogen III synthase	Congenital Erythropoietic P.	PhotoS.	Uroporphyrin +ve, PBG -ve
5. Uroporphyrinogen decarboxylase	P. Cutanea Tarda		
6. Coproporphyrinogen oxidase	Hereditary Coproporphyria	PhotoS., Abd. pain & neuropsych.	Urine PBG & uroporphyrin & fecal protoporphyrin +ve
7. Protoporphyrinogen oxidase	Variegate porphyria		Urine PBG & fecal protoporphyrin +ve
8. Ferrochelatase 3, 5 & 8 most common	Protoporphyria	PhotoS.	fecal and Red cell protoporphyrin +ve

Treatment of Porphyrias

1. Avoid drugs that induce cytochrome P 450
2. Ingestion of large amount of CHO (glucose) or hematin (oxidized heme) to repress ALA S1
3. Administration of β-carotenes (antioxidant).
4. Sunscreen that filters visible light.

NB. Porphyrias are not common & the treatment is only symptomatic hoping for future treatment at the gene level.

NB. Lead poisoning; ↑ protoporphyrins in red cells, ↑ urinary ALA & coproporphyrins

Catabolism of heme:

***bile pigments (bilirubin & biliverdin)** are the end products of heme catabolism

***occurs in macrophages of RES system (liver, spleen & bone marrow).**

Rate of catabolism: 6 g Hb /per day, form 250 mg bilirubin

The formation of bile pigments; 3 stages:

1-In reticulo-endothelial system:

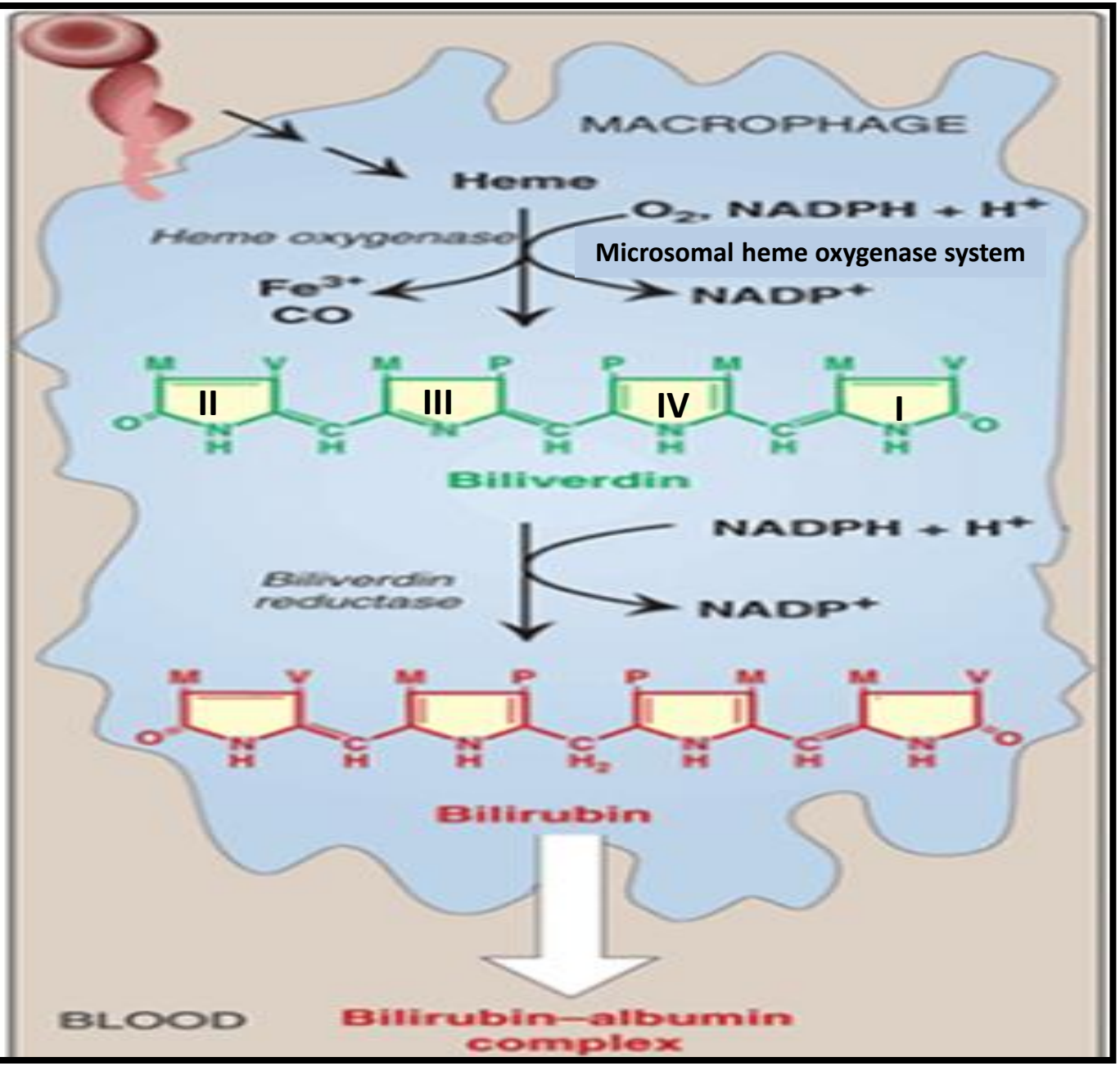
2-in the liver:

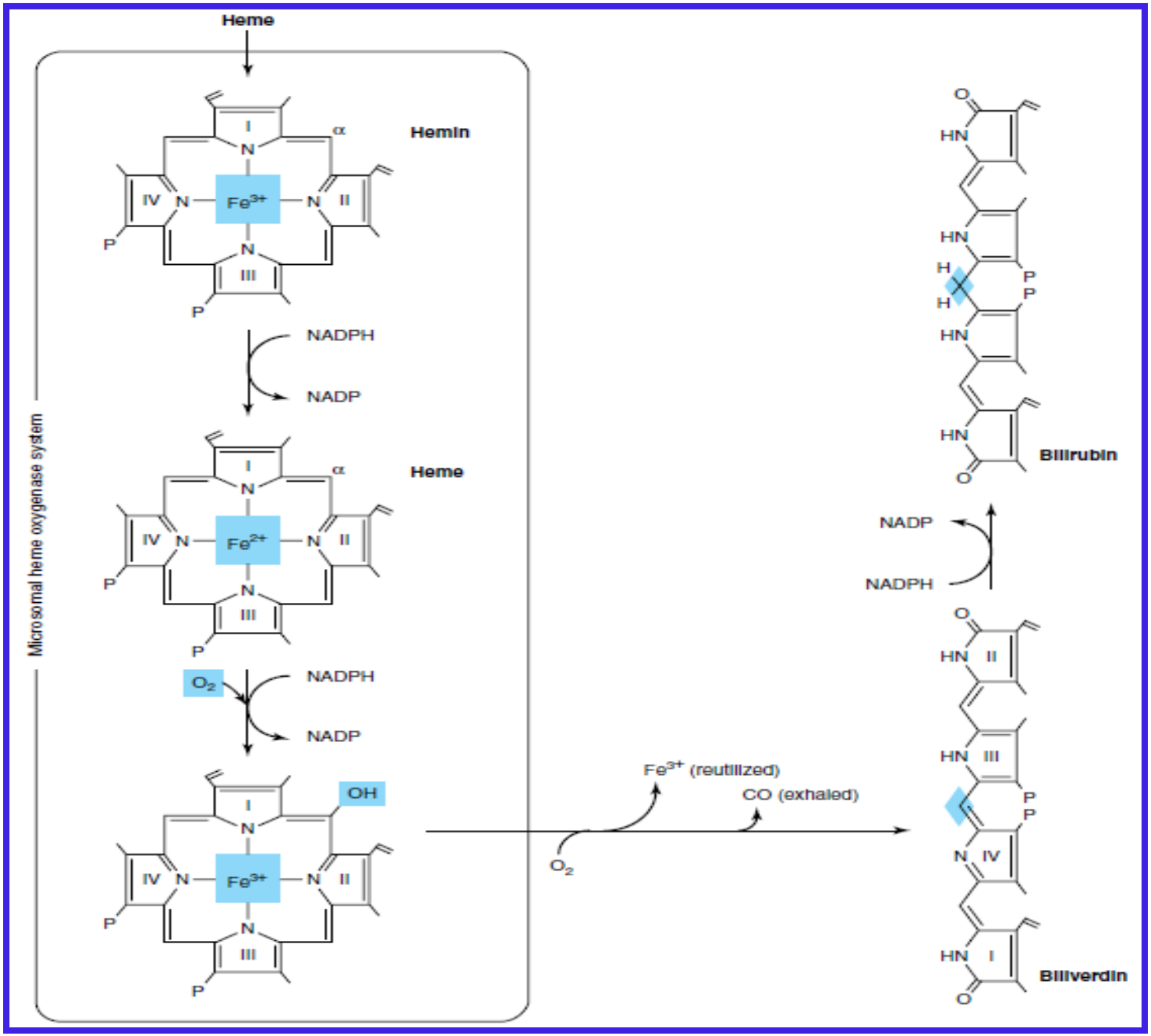
3-In the intestine:

Step 1
RES

a. Oxidat. Of methenyl C bet pyrrole 1 & 2 (α) → release of Fe_3^+ & CO with format. of biliverdin (green)

b. Reduct. of Methenyl C bet pyrrole 3 & 4 (ϒ) → methylene C → bilirubin (yellow)



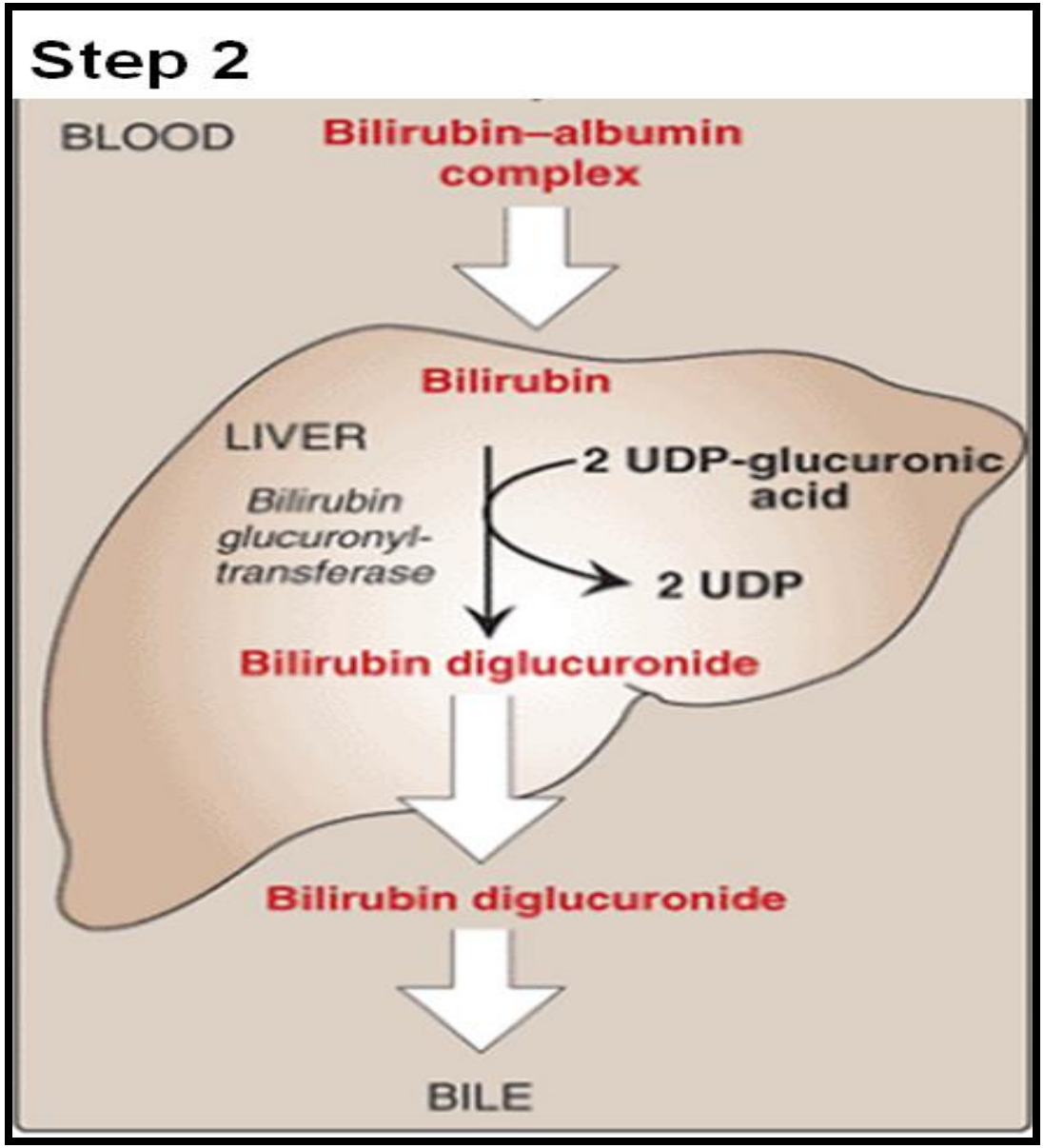

Heme
Hemin
NADPH
NADP
Heme
O_2
NADPH
NADP
OH
Microsomal heme oxygenase system
Fe^{3+} (reutilized)
CO (exhaled)
O_2
Bilirubin
NADP
NADPH
Biliverdin

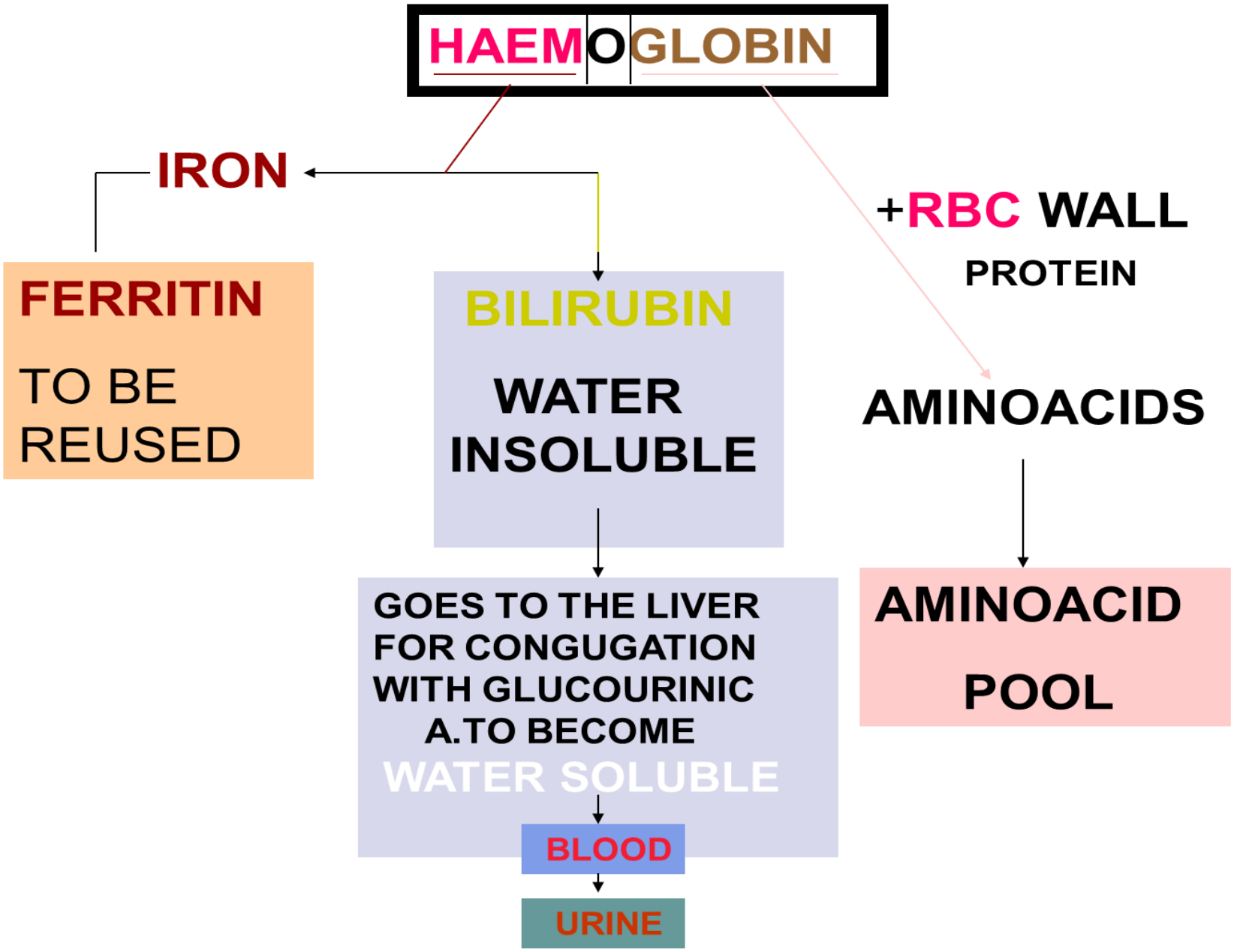
a. Uptake by facilitated transport

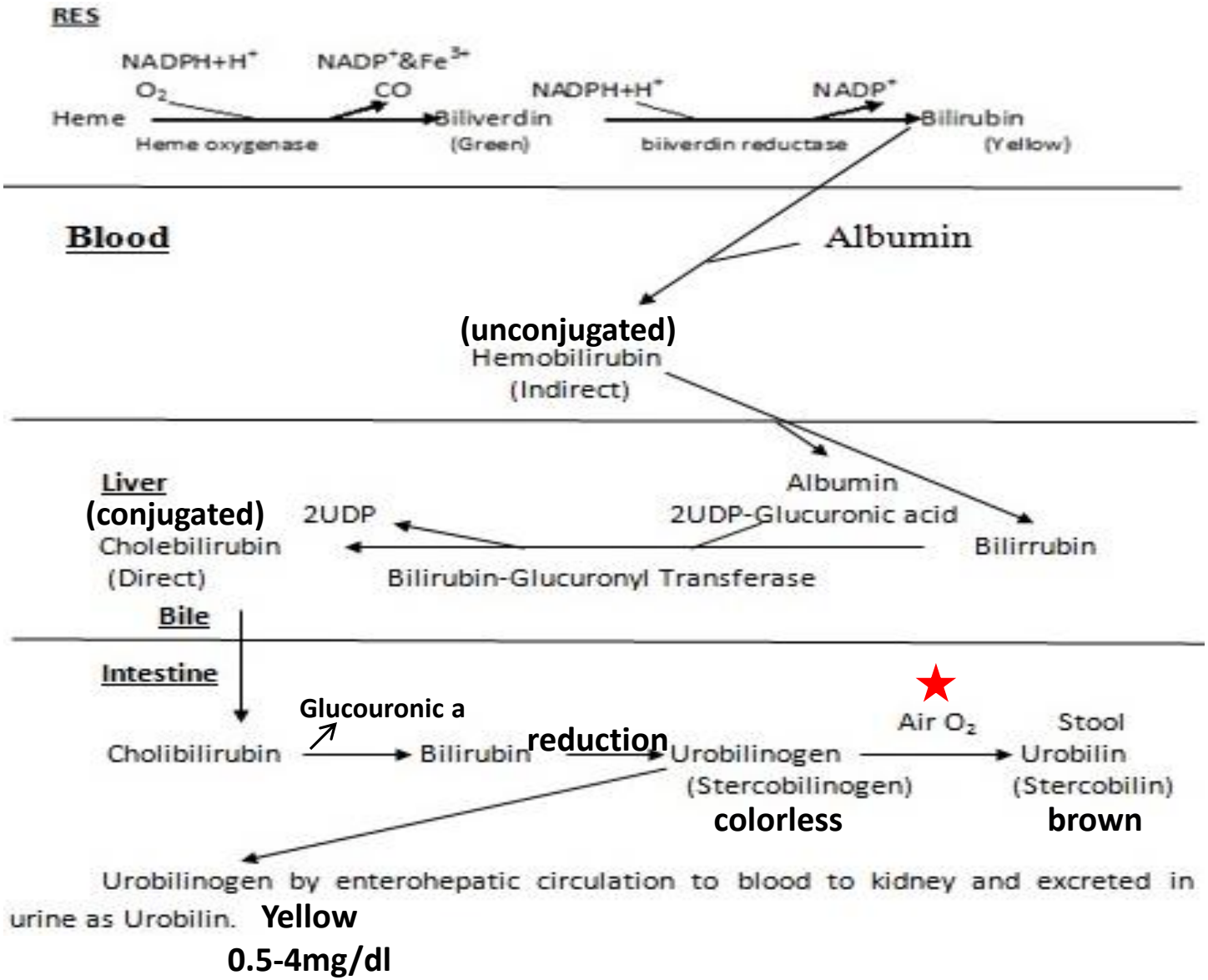
b. In the liver bilirubin binds ligandin & protein Y before conjug.

c. Conjugat. with UDP-glucouronic a by bilirubin glucuronyl transferase enz)

d. Secretion of bilirubin diglucuronide by active process




HAEMOGLOBIN
IRON
FERRITIN
TO BE REUSED
BILIRUBIN
WATER INSOLUBLE
GOES TO THE LIVER FOR CONGUGATION WITH GLUCOURINIC A.TO BECOME WATER SOLUBLE
BLOOD
URINE
+RBC WALL PROTEIN
AMINOACIDS
AMINOACID POOL


RES
NADPH+H+
O2
Heme
Heme oxygenase
NADP+&Fe3+
CO
Biliverdin
(Green)
NADPH+H+
biliverdin reductase
NADP+
Bilirubin
(Yellow)
Blood
Albumin
(unconjugated)
Hemobilirubin
(Indirect)
Liver
(conjugated)
2UDP
Albumin
2UDP-Glucuronic acid
Cholebilirubin
(Direct)
Bilirrubin
Bilirubin-Glucuronyl Transferase
Bile
Intestine
Glucouronic a
Air O2
Stool
Cholibilirubin
Bilirubin
reduction
Urobilinogen
(Stercobilinogen)
Urobilin
(Stercobilin)
colorless
brown
Urobilinogen by enterohepatic circulation to blood to kidney and excreted in urine as Urobilin.
Yellow
0.5-4mg/dl

Unconjugated and conjugated bilirubin

Points	**Unconjugated bilirubin**	**Conjugated bilirubin**
Other names	Hemobilirubin (chief in blood) Indirect bilirubin	**Chol**ebilirubin Direct bilirubin
Normal plasma level	0.2-0.6 mg/dl	0.0-0.2 mg/dl
Solubility	Water –insoluble	Water –soluble
Carriage on albumin	Carried	Not carried
Blood brain barrier	**can** pass	**cannot** pass
Excretion in urine	Not excreted	Excreted
van den Bergh React.	Indirect*	Direct*

N.B. Normal serum total bilirubin level is 0.2-0.8 mg/dl

Van den Bergh Test for Bilirubin:

Bilirubin reacts with *diazo reagent* (diazotised sulphanilic acid) to produce coloured azo pigment. At pH 5, the pigment is purple in colour. Conjugated bilirubin, being water soluble gives the colour immediately (**direct reaction).** Free bilirubin is water insoluble. It has to be extracted first with alcohol, when the reaction becomes positive (**indirect reaction).**

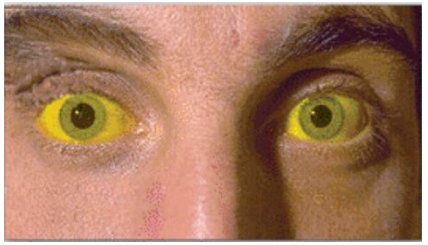
Ehrlich

Hyperbilirubinemia:

Definition

Bilirubin level in blood exceeds 1 mg/dl.

Jaundice (icterus):



Definition

Yellow color of skin, nail beds, and sclera. It is caused by deposition of bilirubin, due its increased levels in blood (hyperbilirubinemia) above 2mg/dl. Values between 1.2- 2 mg/dl are considered **Latent jaundice**.

- **Normal bilirubin production is only 250-300 mg/day.**
- **The liver has the capacity to conjugate & excrete > 3,000 mg/day**

Types of Jaundice

A-Unconj. Hyperbilirubinemia (↑ indirect):

1. **Hemolytic jaundice (acholuric jaundice;** no bilirubin in urine**)**
2. **Jaundice of newborns (Physiological jaundice)**
3. **Crigler-Najjar syndrome (conjugation)**
4. **Gilbert syndrome (uptake)**

B-Conj. hyperbilirubinemia (↑ direct): Obstructive jaundice

1. **Dubin-Johnson syndrome**
2. **Rotor syndrome**
3. **Stone or tumor obstruction**

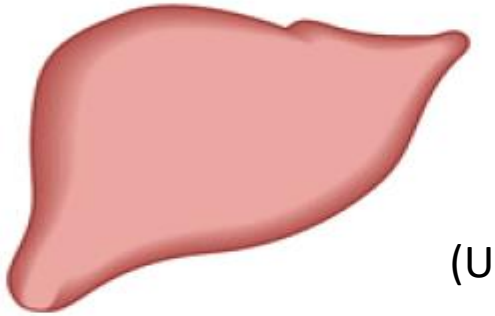
C-Both conj. and unconj. hyperbilirubinemia (↑ direct & ↑ indirect): hepatic diseases

Clinically

PRE-HEPATIC
(Vascular)

Hemolytic anemias

HEPATIC
(Liver)

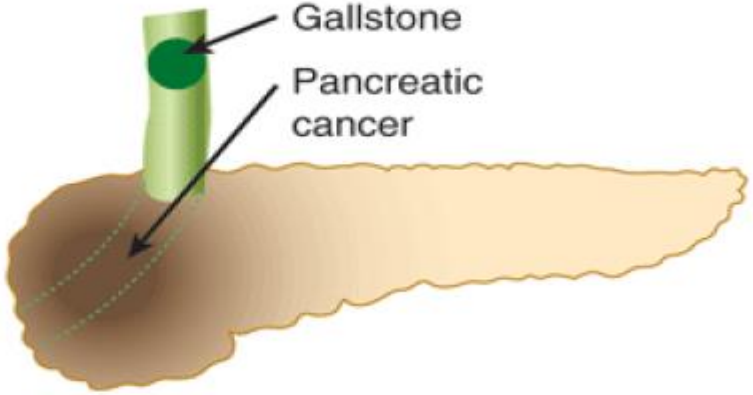


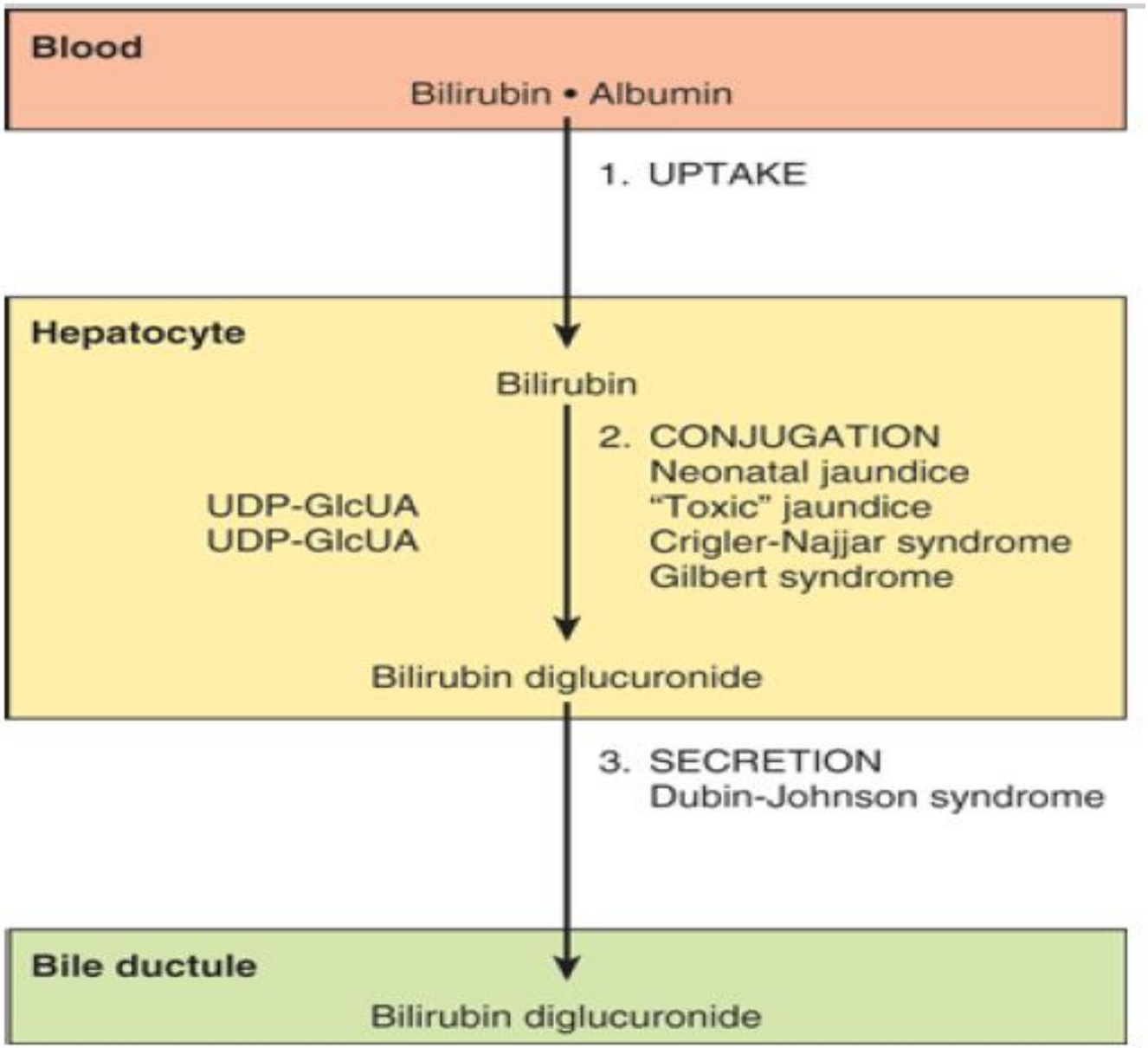
Liver diseases
(eg hepatitis, cancer)

(Uptake, Conjugat. & Secret.)

POST-HEPATIC
(Biliary system & pancreas)

(Obstructive)




Blood
Bilirubin • Albumin
1. UPTAKE
Hepatocyte
Bilirubin
2. CONJUGATION
Neonatal jaundice
"Toxic" jaundice
Crigler-Najjar syndrome
Gilbert syndrome
UDP-GlcUA
UDP-GlcUA
Bilirubin diglucuronide
3. SECRETION
Dubin-Johnson syndrome
Bile ductule
Bilirubin diglucuronide

A-Unconj. Hyperbilirubinemia

1. Hemolytic jaundice:

In massive lysis of RBCs in:

i. **Sickle cell anemia**

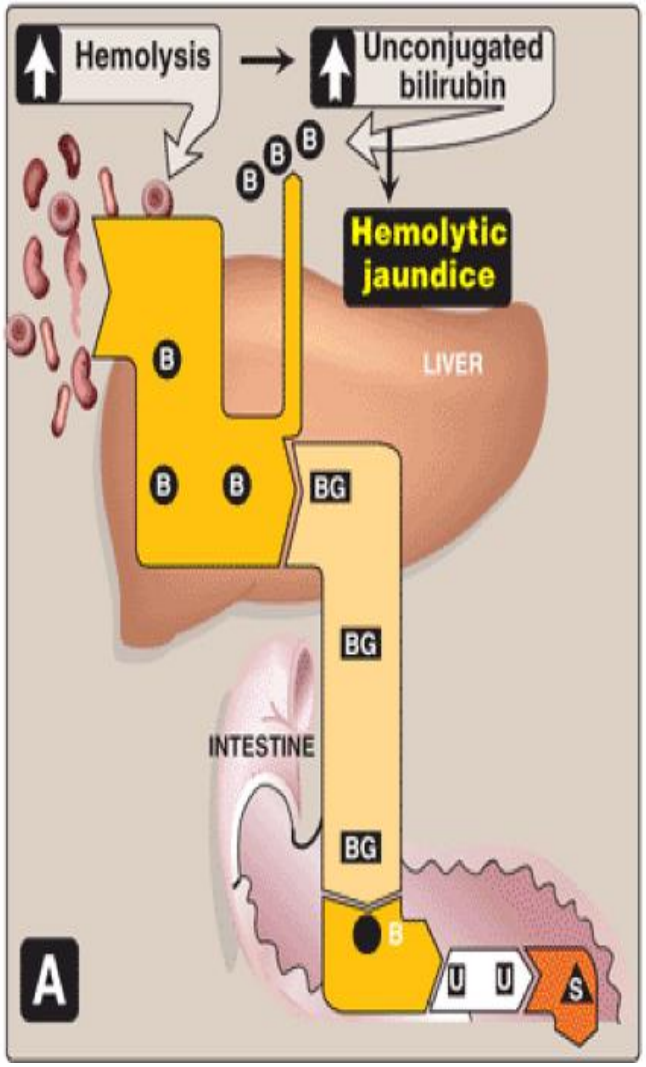
ii. **Pyruvate kinase**

iii. **G-6-PD deficiency**

***Unconjug. bilirubin is ↑ in blood**

***Urobilinogen ↑ (dark yellow urine) &**

***Sterecobilinogen ↑ (dark brown stool)**



2- Jaundice of newborns (Physiological jaundice)

*** This is transient jaundice In all newborn, after 2nd day of life.**

It is the most common cause of unconj. Jaundice.

It is due to:

a. An accelerated rate of destruction of RBCs at birth time.

b. Immature hepatic conjugation system (esp. premature)

The activity of hepatic bilirubin glucuronyltransferase is low at birth—it reaches adult levels in about 4 weeks.

*** Bilirubin does not increase above 5 mg/dl and It disappears by the 2nd week of life.**

• Elevated bilirubin, in excess of albumin binding capacity **(20 mg/dl)**, can diffuse into basal ganglia ⟶ toxic encephalopathy **(kernicterus).**

***Newborns with significantly high bilirubin are treated with:**

a. **Phototherapy (blue fluorescent light):**
bilirubin ⟶ more polar, water-soluble photoisomers (excreted into bile without conjugation).
b. Induction of glucuronyl transferase activity.
c. Frequent and effective feedings

3-Crigler-Najjar syndrome: (impaired CONJUGATION)

It is a rare autosomal recessive disease leads to **decreased activity** of UDP- glucuronyltransferase.

4-Gilbert syndrome:

It is due to **defect in hepatic uptake** of bilirubin

B. Obstructive jaundice:

*** Intrahepatic cholestasis**

1. Dubin Johnson syndrome: autosomal recessive defect in active bilirubin **secretion** into bile in neonates
2. Rotor syndrome: rare benign (no identified pathology yet)

*** Extrahepatic cholestasis:**

Obstruction of the bile duct by a **tumor or bile stones**

1. **Gastrointestinal pain and nausea,**
2. **Itching** due to bile salt retention.
3. ***Stools: pale, clay color (no Stercobilin)***
4. **Urine dark brown (direct bilirubin and bile salts→itching)**
5. ***Urinary urobilinogen is absent.***

[Prolonged obstruction of bile duct can lead to liver damage & subsequent rise in unconjugated bilirubin.]

5. ↑serum alkaline phosphatase (biliary obstruct.)

Clay colored (لون الطحينة)

C-Both conj. & unconj. hyperbilirubinemia:

a. **Damage to liver cells** (cirrhosis, hepatitis or toxins e.g. CCl4) ↑**unconjug. bilirubin** in blood (↓ conjugation)

b. **Then hepatocytes swell** blocking biliary canaliculi, ↑ **conjugated** bilirubin in blood.

- **Stercobilinogen ↓ in feces (faint stool).**
- **Dark brown Urine (Urobilinogen and bilirubin appear in urine).**
- **Both ALT & AST are elevated due to liver cell damage.**

Points		Hemolytic (Pre-hepatic)	Hepatocellular (Hepatic)	Obstructive (Post-hepatic)
Cause		RBCs Hemolysis	Liver Diseases	Biliary Obstruction
Serum	Bilirubin	↑ Hemobilirubin	↑ Hemo. & Chole.	↑ Cholebilirubin
	Hepatic Enz.	Normal Levels	↑ AST & ALT	↑ Alk. Phosphatase
Stool	Color	Dark	Faint	Clay
	Stercobilinogen	↑	↓	Trace to absent
Urine	Color	deep yellow	dark brown	dark brown
	Urobilinogen	↑	↓ if microbstruct.	absent
	Bilirubin	Absent	Present if microbstruct.	Present
Test		Indirect Bilirubin	Direct & Indirect	Direct bilirubin

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