Heme Metabolism

Dr Bela Goyal

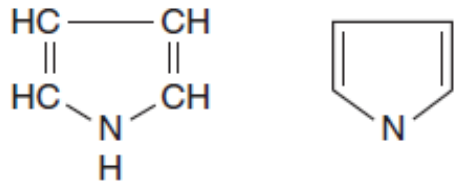
Heme Biosynthesis=porphyrins+Fe

- Disorder: Porphyria

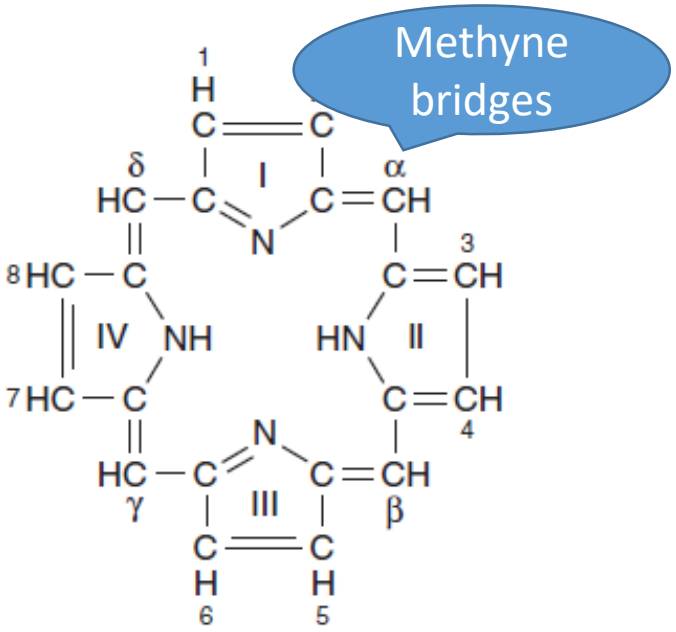
Heme Catabolism=Bile pigments+Fe

- Disorder: Hyperbilirubinemia

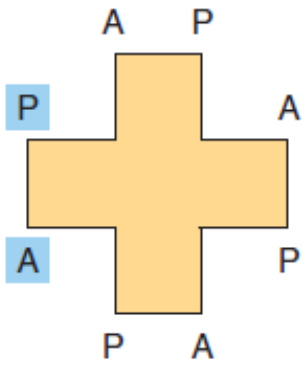
PORPHYRINS



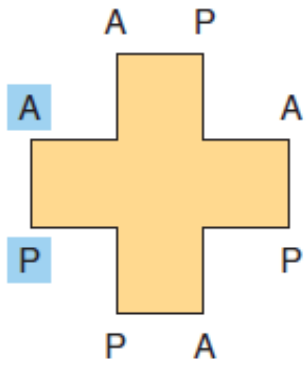
Pyrrole



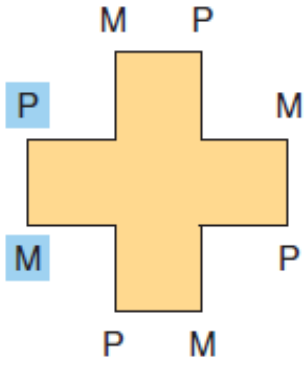
Porphyrin
($C_{20}H_{14}N_4$)



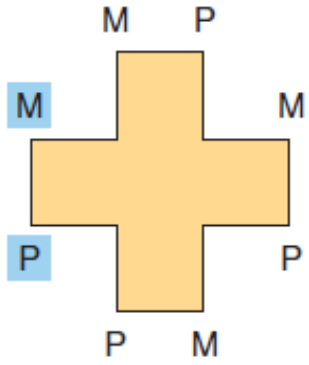
Uroporphyrin I



Uroporphyrin III

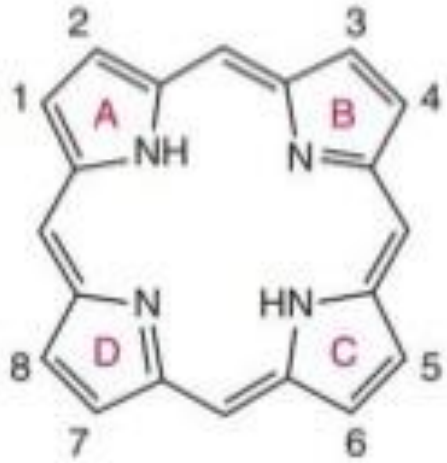


Coproporphyrin I

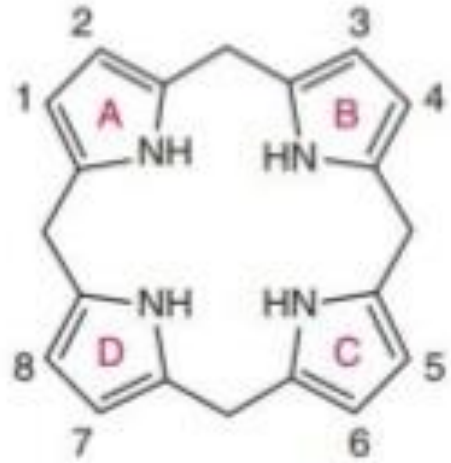


Coproporphyrin III

Porphyrinogen and Porphyrins



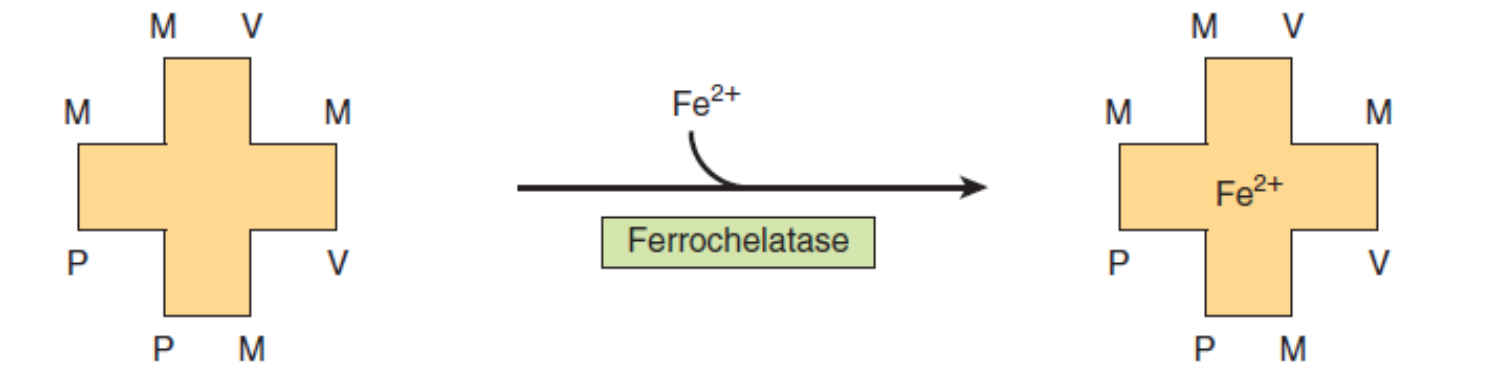
Porphyrin



Porphyrinogen

Porphyrinogens are reduced form of Porphyrins

- Porphyrinogens are reduced forms of porphyrins
- Difference- 6 hydrogens
- They are unstable in-vitro
- Spontaneously oxidised to respective porphyrins
- They can serve as intermediates of heme synthesis in situations of low oxygen tension



Protoporphyrin III
(parent porphyrin of heme)

Heme
(prosthetic group of hemoglobin)

Heme Synthesis

Site For Heme Biosynthesis

- **Organs**

- **Bone Marrow**-

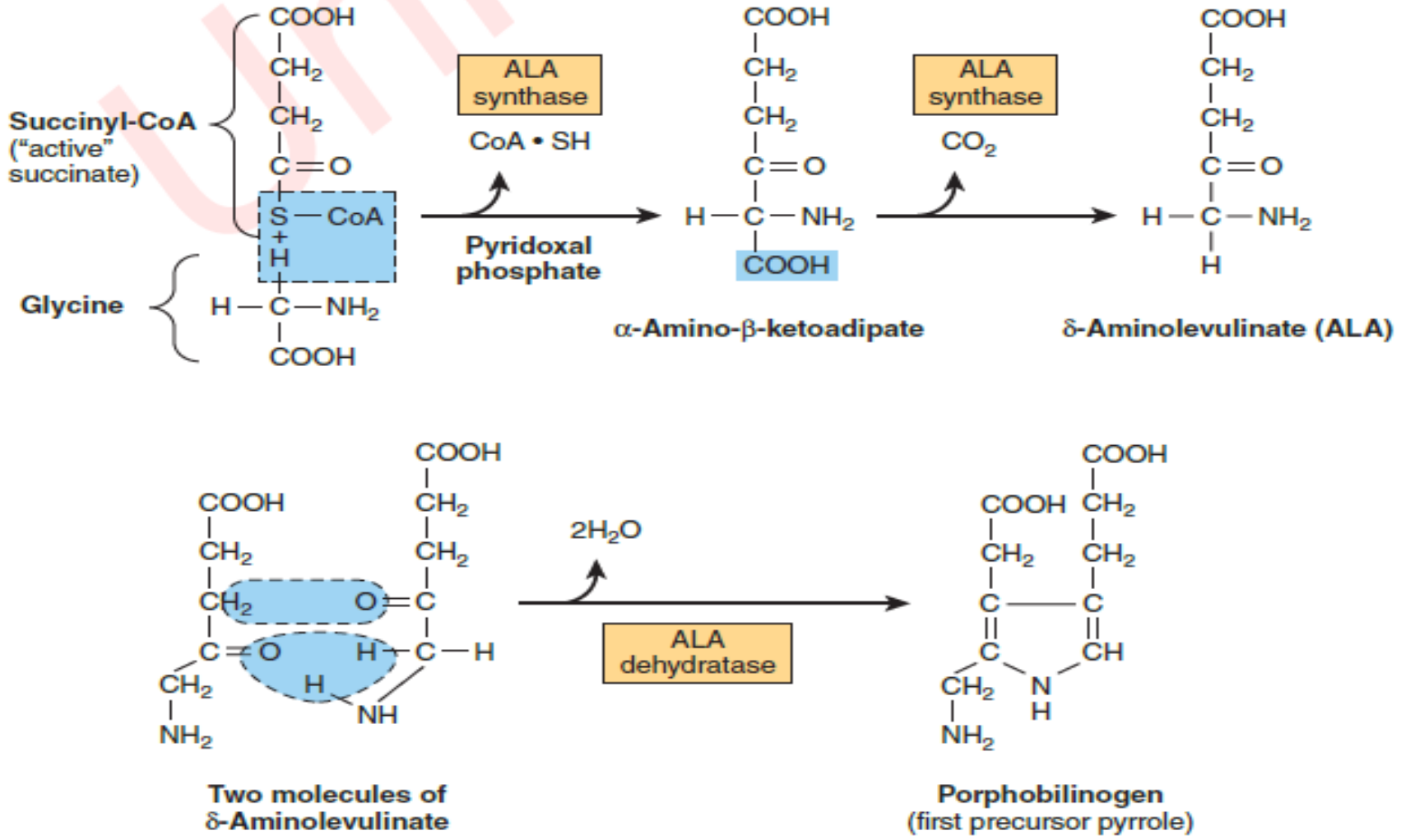
  Immature Erythrocytes – 85%

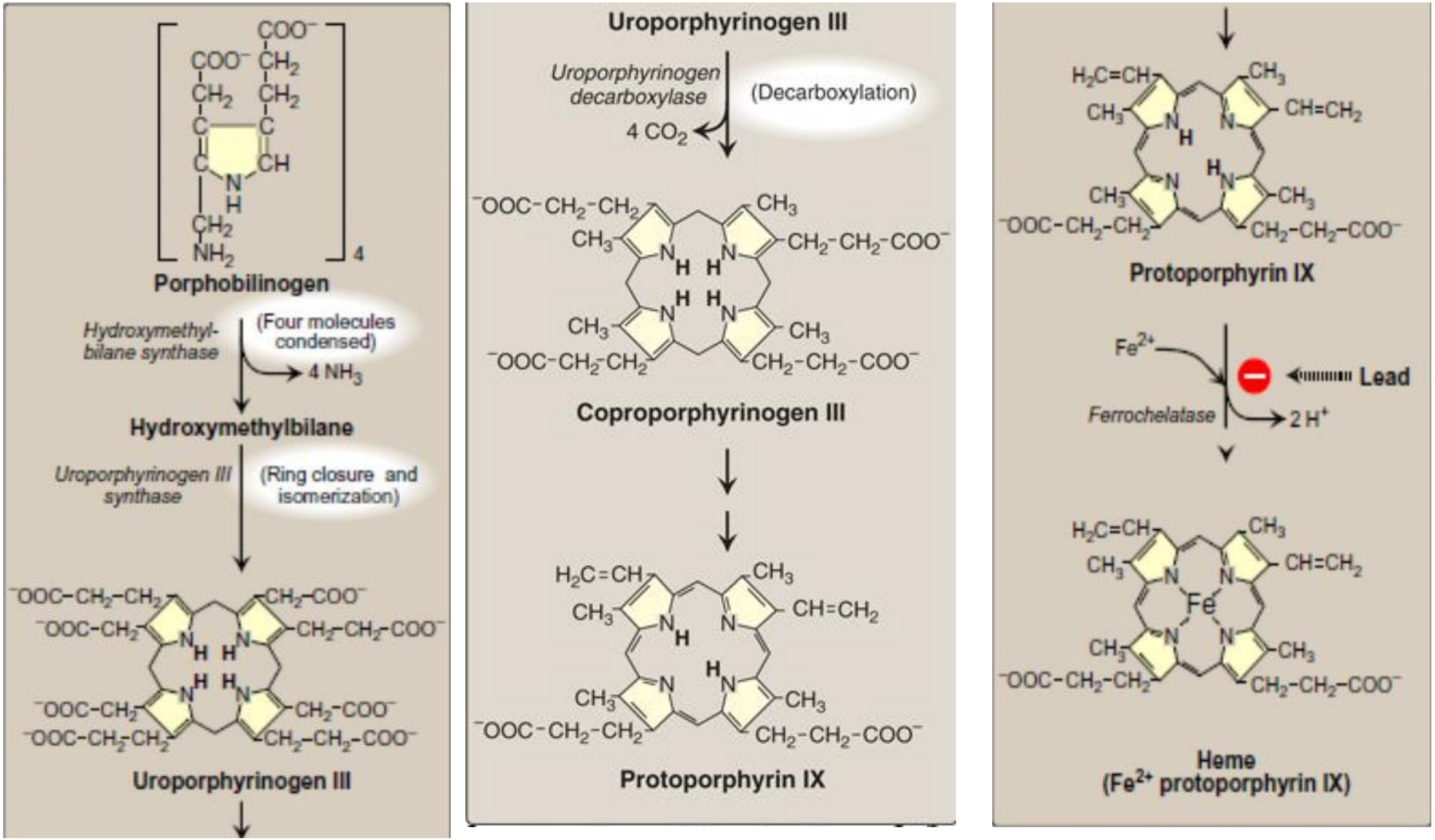
- **Liver** – 15 %

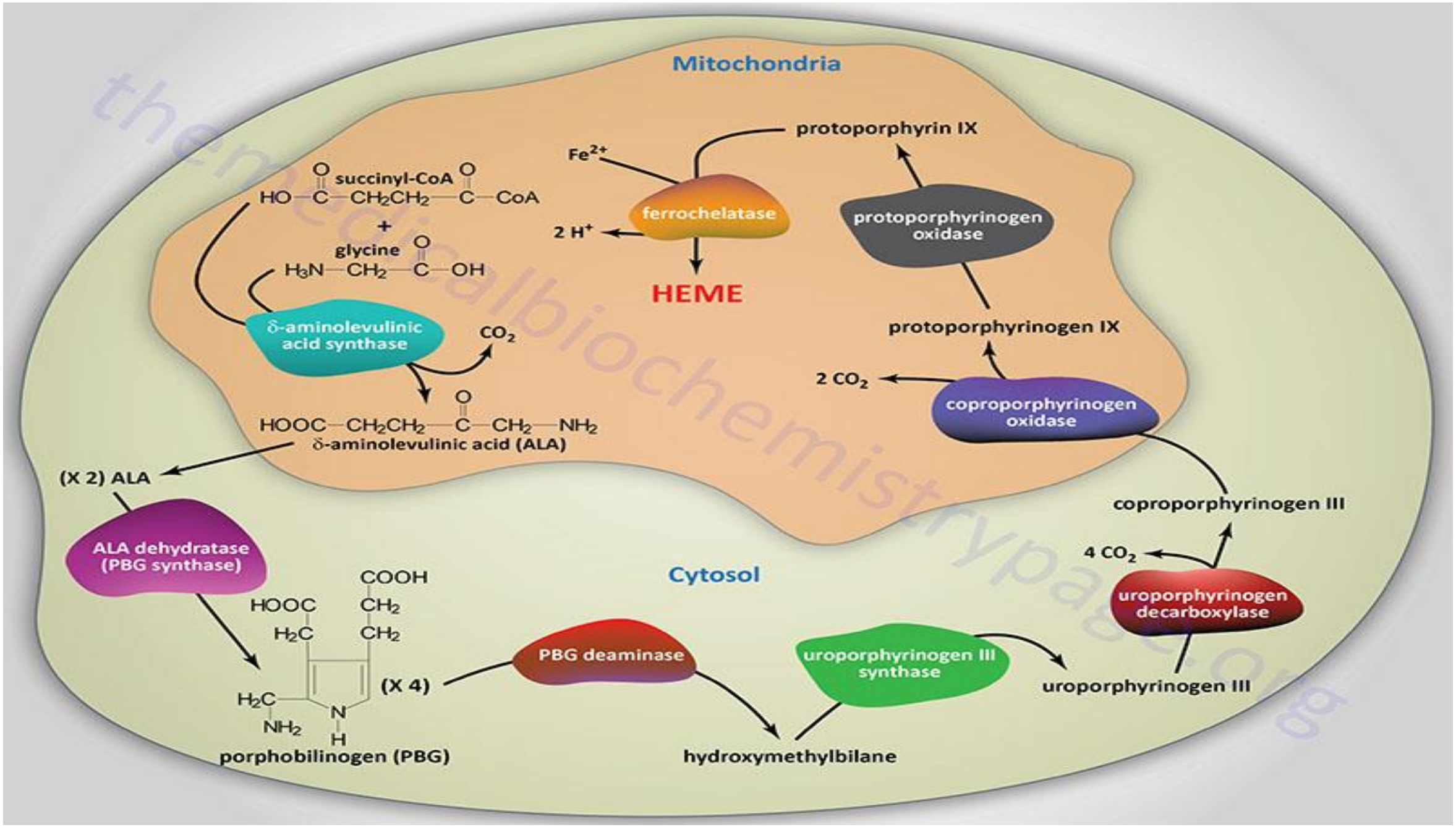
- **Cellular Site**

- **Mitochondrial Matrix**
- **Cytosol**

First and second step

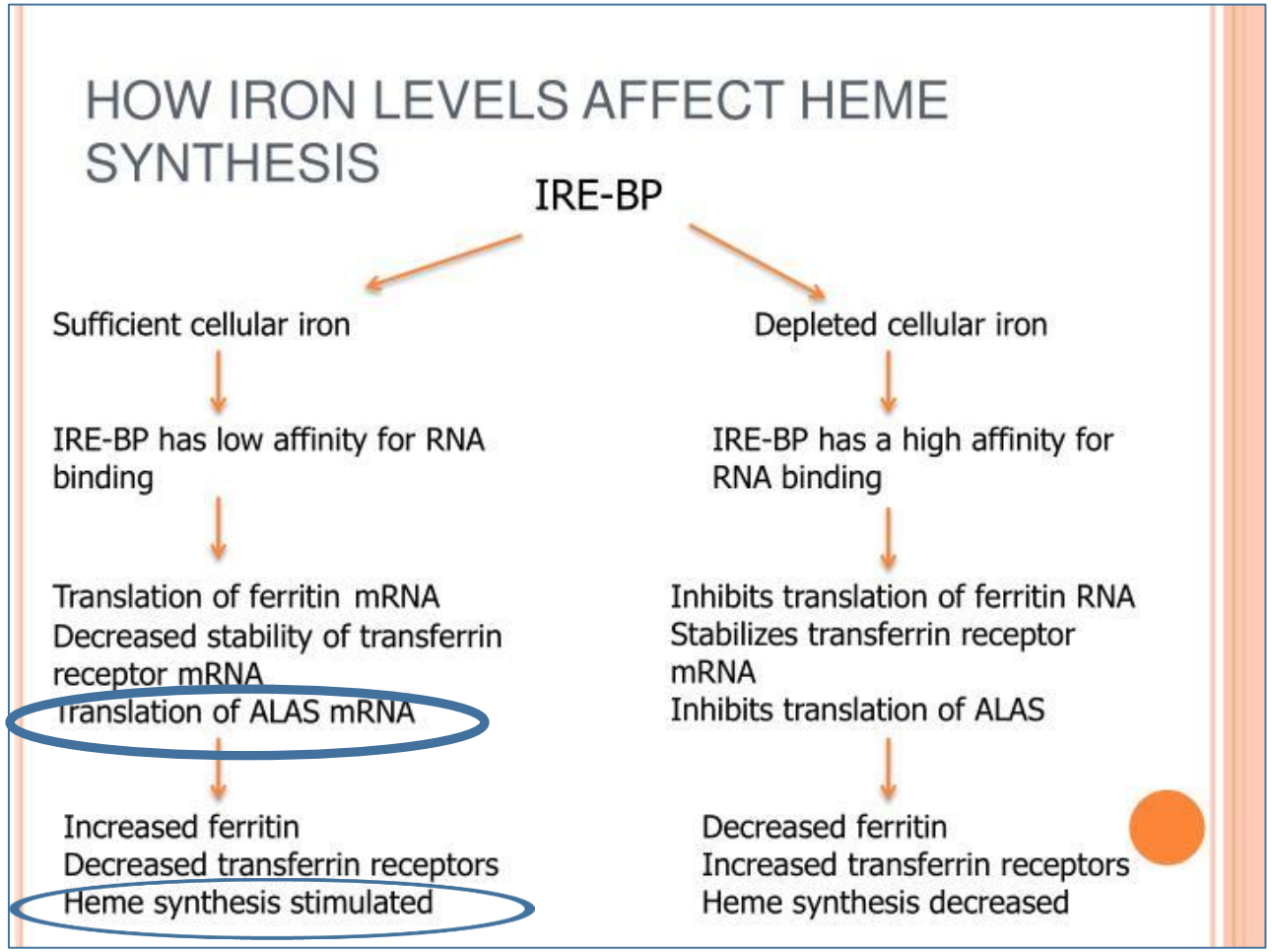



Porphobilinogen
Hydroxymethyl-bilane synthase
(Four molecules condensed)
4 NH3
Hydroxymethylbilane
Uroporphyrinogen III synthase
(Ring closure and isomerization)
Uroporphyrinogen III
Uroporphyrinogen III
Uroporphyrinogen decarboxylase
(Decarboxylation)
4 CO2
Coproporphyrinogen III
Protoporphyrin IX
Protoporphyrin IX
Fe2+
Lead
Ferrochelatase
2 H+
Heme
(Fe2+ protoporphyrin IX)


Mitochondria
succinyl-CoA
HO C CH2CH2 C CoA
+
glycine
H3N CH2 C OH
δ-aminolevulinic acid synthase
CO2
HOOC CH2CH2 C CH2 NH2
δ-aminolevulinic acid (ALA)
(X 2) ALA
ALA dehydratase (PBG synthase)
Fe2+
ferrochelatase
2 H+
HEME
protoporphyrin IX
protoporphyrinogen oxidase
protoporphyrinogen IX
2 CO2
coproporphyrinogen oxidase
coproporphyrinogen III
4 CO2
uroporphyrinogen decarboxylase
uroporphyrinogen III
Cytosol
uroporphyrinogen III synthase
hydroxymethylbilane
PBG deaminase
(X 4)
porphobilinogen (PBG)

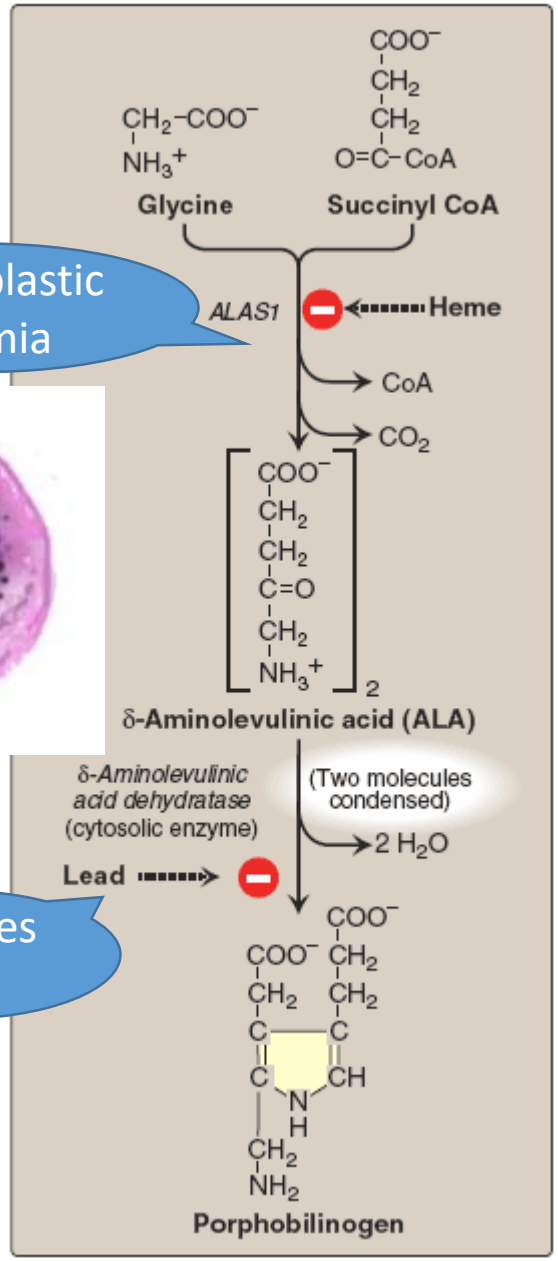
Regulation of heme synthesis

- Allosteric regulation by heme
- Erythropoetin
- Iron

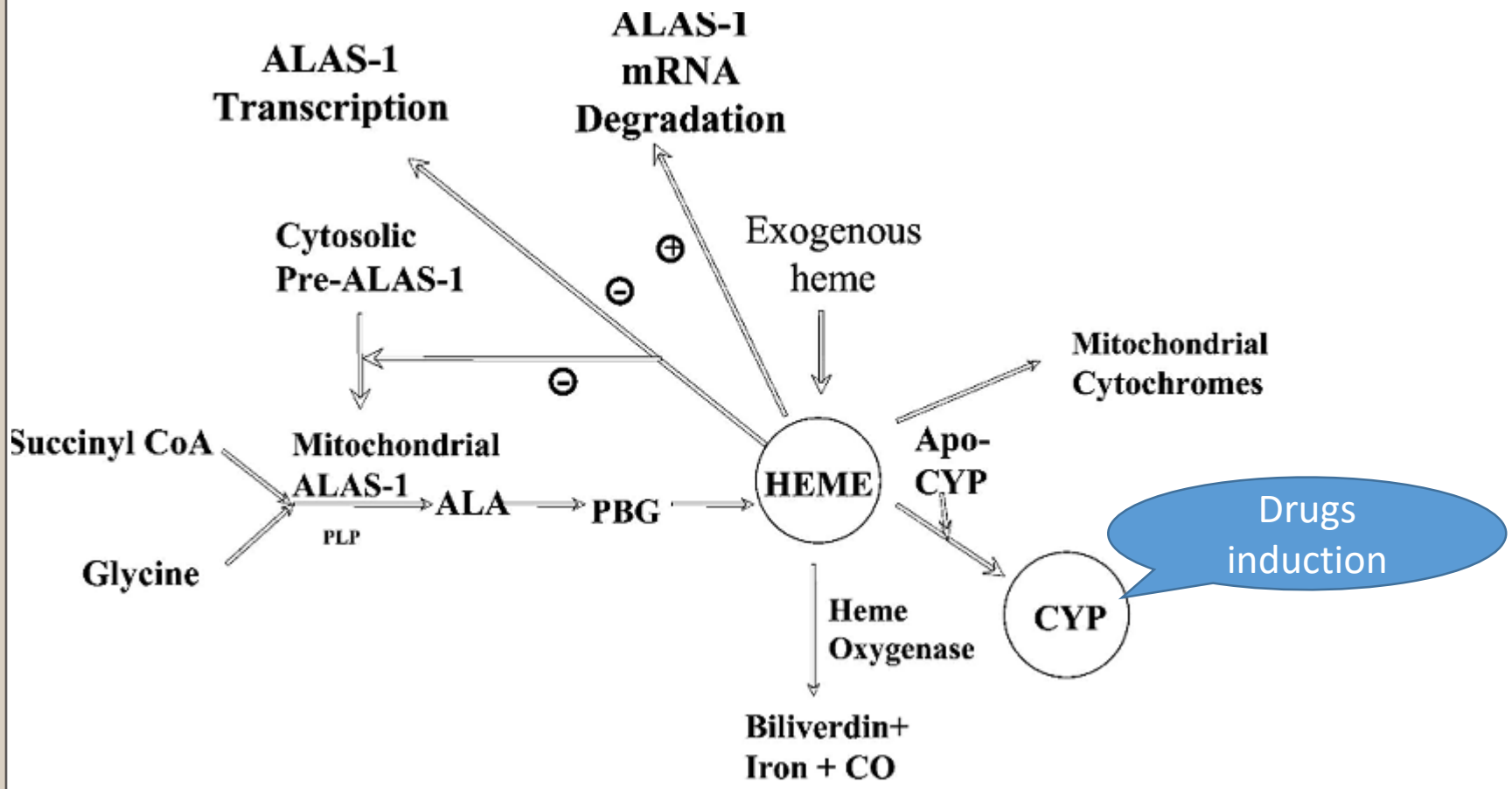


Sideroblastic anemia


Sideroblast



Replaces Zn



Drugs induction

Disorders of heme synthesis: Porphyrias

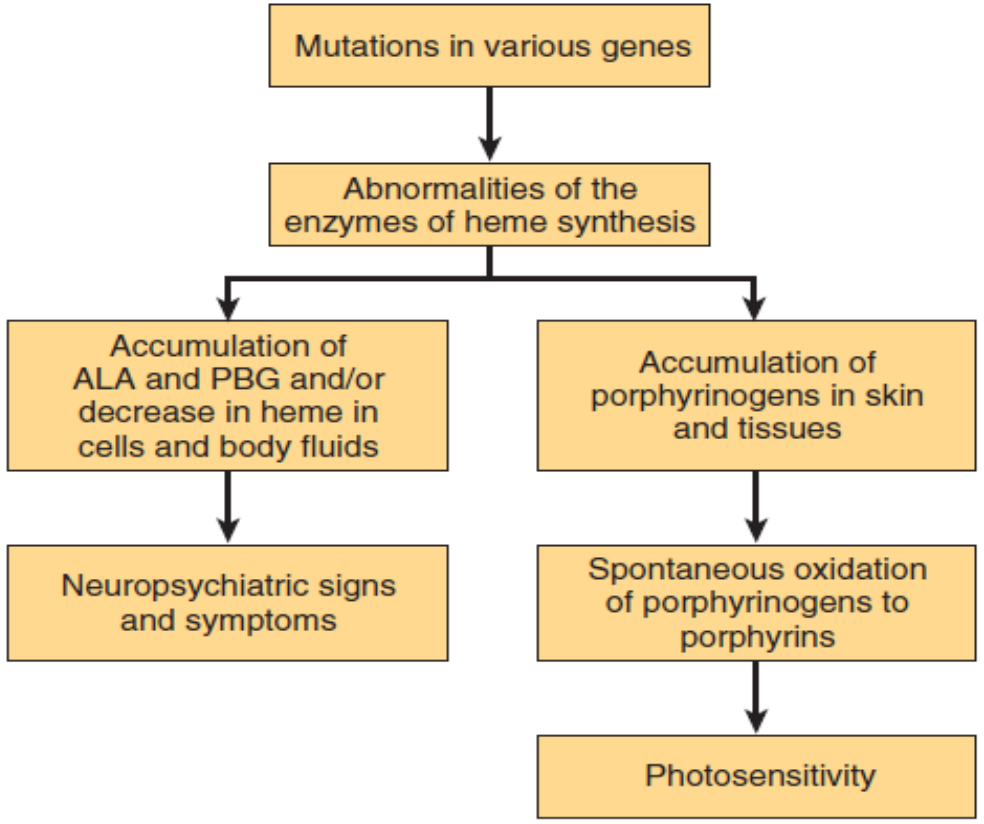
- **Acquired or Genetic**

- An example of an acquired defect is **lead poisoning**.
- Lead can inactivate ferrochelatase and ALA dehydratase by combining with essential thiol groups.
- Signs include elevated levels of protoporphyrin in erythrocytes and elevated urinary levels of ALA and coproporphyrin.

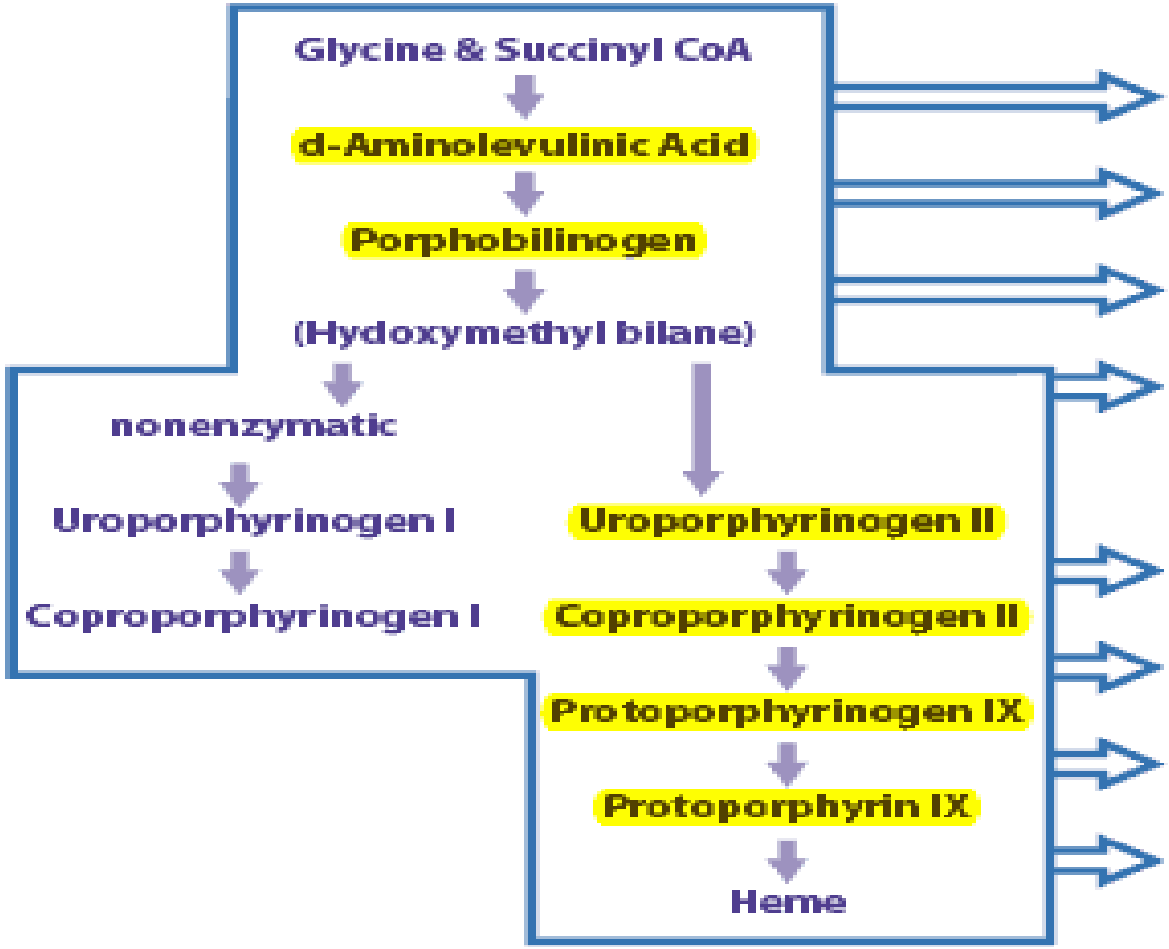
Porphyrias

- Porphyrias are classified as erythropoietic or hepatic
- Hepatic porphyrias can be further classified as chronic or acute.
- enzyme defect prior to the synthesis of the tetrapyrroles manifest abdominal and neuropsychiatric signs
- enzyme defects leading to the accumulation of tetrapyrrole intermediates show photosensitivity

Biochemical basis of Porphyrias



Porphyrias



ENZYME	DISEASE
d-Aminolevulinic Acid Synthase	X-linked sideroblastic anemia
a-Aminolevulinic Acid Dehydratase	ALAD Porphyria
Porphobilogen Deaminase	Acute Intermittent Porphyria
Uroporphyrinogen III Cosynthase	Congenital Erthropoietic Porphyria
Uroporphyrinogen Decarboxylase	Porphyria Cutanea Tarda, Hepatoerythropoietic Porphyria
Coproporphyrinogen Oxidase	Herediatary Coproporphyria
Protoporphyrinogen Oxidase	Variegate Porphyria
Ferrochelatase	Erythropoietic Protoporphyria

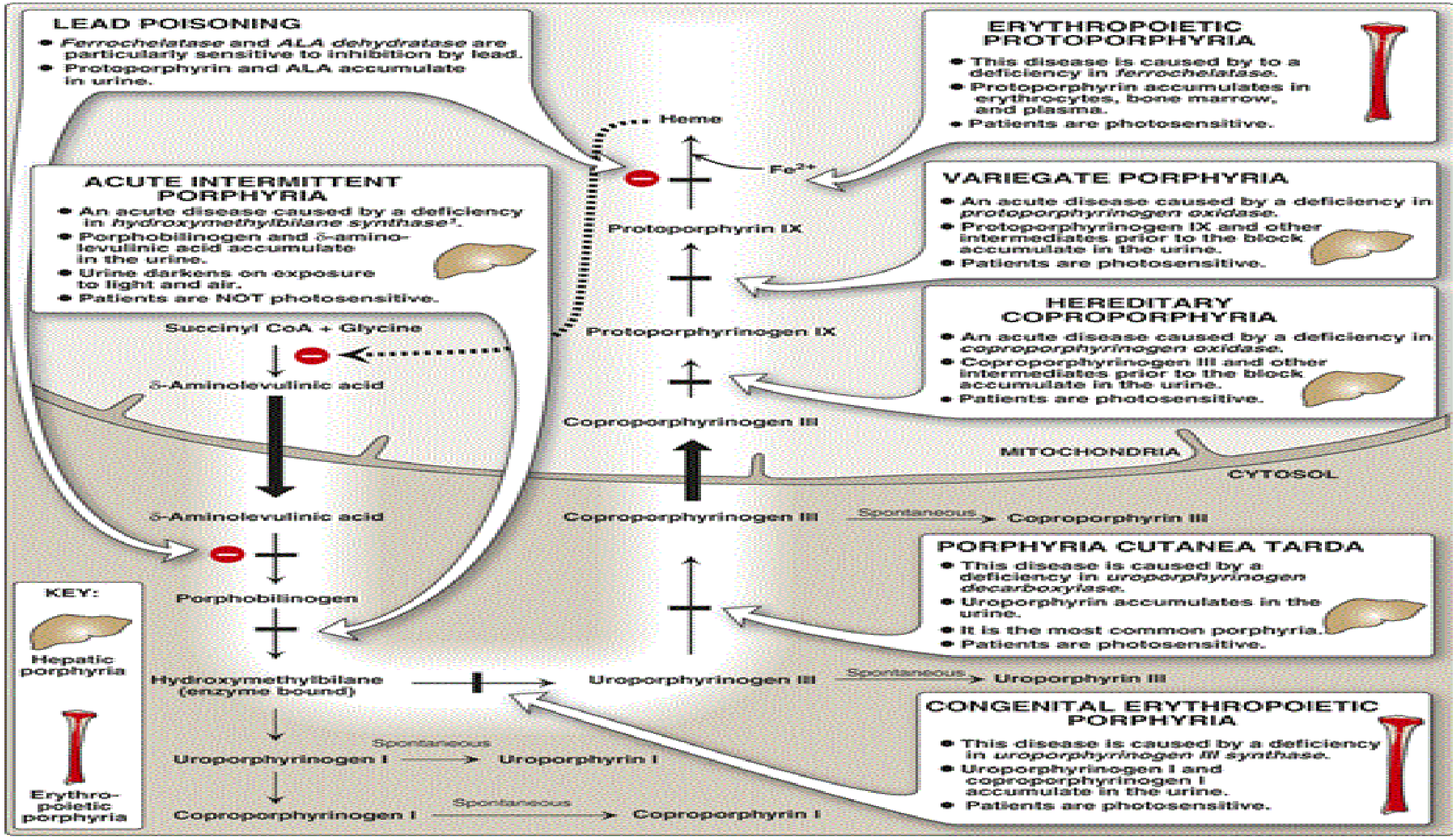
All people in pub(AP PB)

U sing Cute(US C)

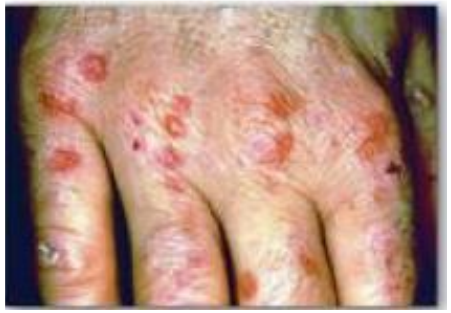
U Dance with Pace(UD P)

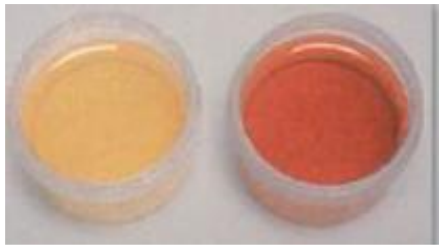
Put out variety

Feels enthusiastic


LEAD POISONING
Ferrochelatase and ALA dehydratase are particularly sensitive to inhibition by lead.
Protoporphyrin and ALA accumulate in urine.
ERYTHROPOIETIC PROTOPORPHYRIA
This disease is caused by to a deficiency in ferrochelatase.
Protoporphyrin accumulates in erythrocytes, bone marrow, and plasma.
Patients are photosensitive.
Heme
Fe2+
ACUTE INTERMITTENT PORPHYRIA
An acute disease caused by a deficiency in hydroxymethylbilane synthase1.
Porphobilinogen and δ-amino-levulinic acid accumulate in the urine.
Urine darkens on exposure to light and air.
Patients are NOT photosensitive.
Protoporphyrin IX
VARIEGATE PORPHYRIA
An acute disease caused by a deficiency in protoporphyrinogen oxidase.
Protoporphyrinogen IX and other intermediates prior to the block accumulate in the urine.
Patients are photosensitive.
Protoporphyrinogen IX
HEREDITARY COPROPORPHYRIA
An acute disease caused by a deficiency in coproporphyrinogen oxidase.
Coproporphyrinogen III and other intermediates prior to the block accumulate in the urine.
Patients are photosensitive.
Coproporphyrinogen III
Succinyl CoA + Glycine
δ-Aminolevulinic acid
MITOCHONDRIA
CYTOSOL
δ-Aminolevulinic acid
Coproporphyrinogen III
Spontaneous
Coproporphyrin III
PORPHYRIA CUTANEA TARDA
This disease is caused by a deficiency in uroporphyrinogen decarboxylase.
Uroporphyrin accumulates in the urine.
It is the most common porphyria.
Patients are photosensitive.
Porphobilinogen
KEY:
Hepatic porphyria
Hydroxymethylbilane (enzyme bound)
Uroporphyrinogen III
Spontaneous
Uroporphyrin III
CONGENITAL ERYTHROPOIETIC PORPHYRIA
This disease is caused by a deficiency in uroporphyrinogen III synthase.
Uroporphyrinogen I and coproporphyrinogen I accumulate in the urine.
Patients are photosensitive.
Spontaneous
Uroporphyrinogen I
Uroporphyrin I
Erythro-poietic porphyria
Spontaneous
Coproporphyrinogen I
Coproporphyrin I

- **Chronic hepatic porphyria:** Porphyria cutanea tarda, the most common porphyria, is a chronic disease of the liver





- **b. Acute hepatic porphyrias:** ALA dehydratase deficiency porphyria, acute intermittent porphyria, hereditary coproporphyria, and variegate porphyria

- acute attacks of gastrointestinal (GI), neuropsychiatric, and motor symptoms that may be accompanied by photosensitivity

- Symptoms of the acute hepatic porphyrias are often precipitated by use of drugs, such as barbiturates and ethanol???

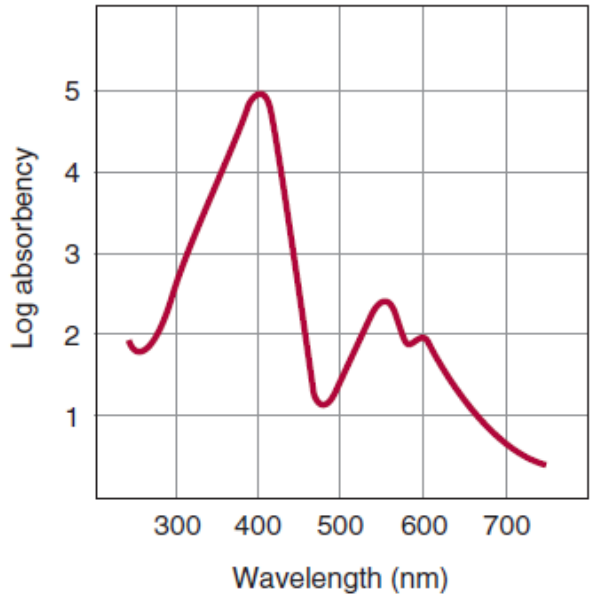
- **Erythropoietic porphyrias:** The chronic erythropoietic porphyrias (congenital erythropoietic porphyria and erythropoietic protoporphyria)

- skin rashes and blisters that appear in early childhood.

Porphyrins Excreted In Urine and Feces

- **Uroporphyrin** excreted in **urine.**
- **ProtoPorphyrin** excreted in **feces.**
- **Coproporphyrin** excreted either in **urine /feces.**

PORPHYRINS ARE COLORED & FLUORESCE



- **porphyrinogens are colorless,**
- **Porphyrins are colored.**

- The sharp absorption band **near 400 nm**, a distinguishing feature shared by all porphyrins, is termed the **Soret band**
- Porphyrins dissolved in strong mineral acids or in organic solvents and illuminated by ultraviolet light emit a strong red **fluorescence**
- **Enzyme Assay- HPLC**

- **cancer phototherapy**

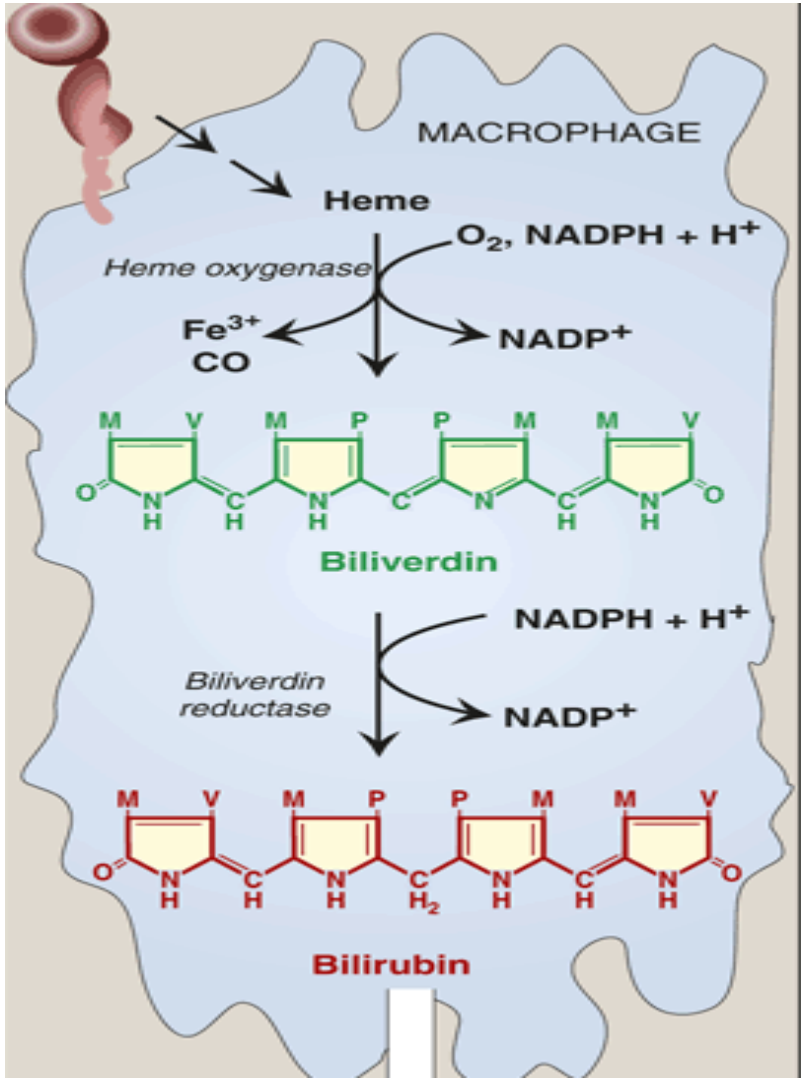
Treatment:

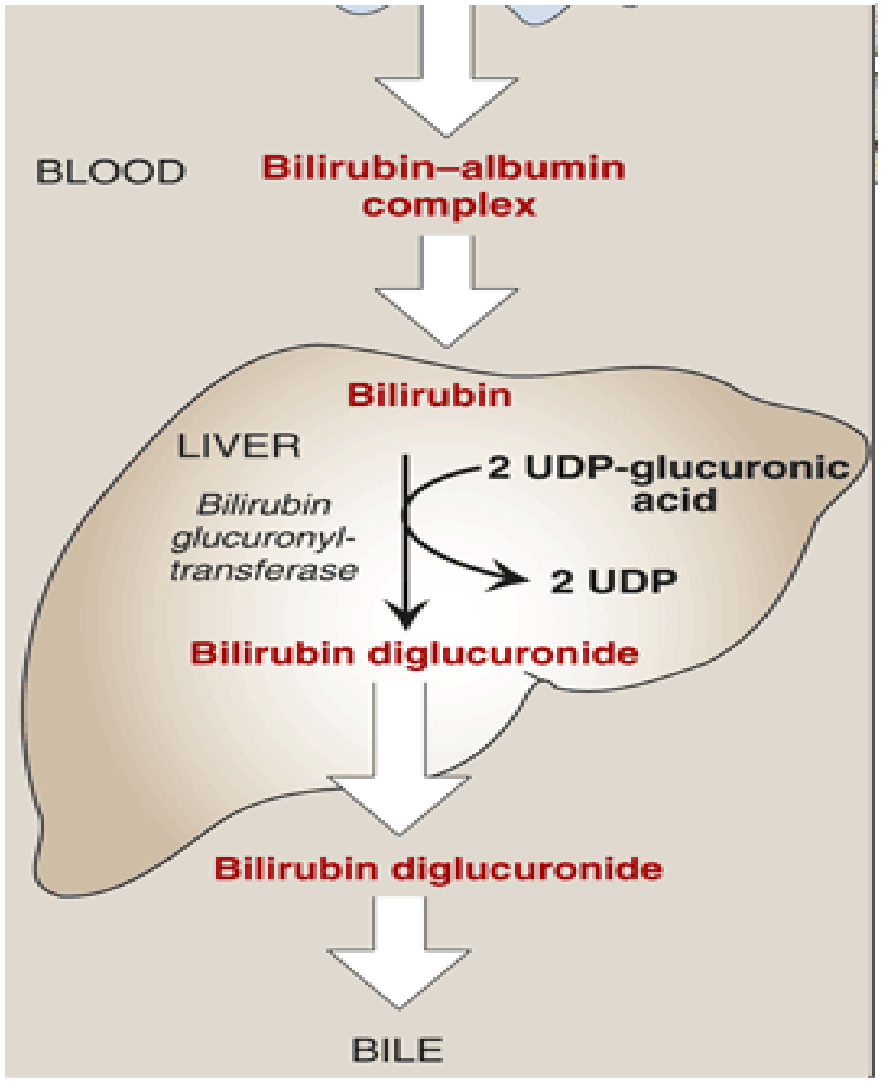
- During acute porphyria attacks, patients require medical support, particularly **treatment for pain and vomiting**.
- The severity of acute symptoms of the porphyrias can be diminished by intravenous **injection of hemin** and glucose, which decreases the synthesis of ALAS1.
- Protection from **sunlight**,
- ingestion of **β-carotene** (a free-radical scavenger),
- and **phlebotomy** are helpful in porphyrias with photosensitivity.

- Porphyrias are cruelly referred to as a **Vampire's disease.**
- Thought to be a cause of the madness of **King George III.**
- Can be caused by lead poisoning: The **fall of the Roman Empire!**

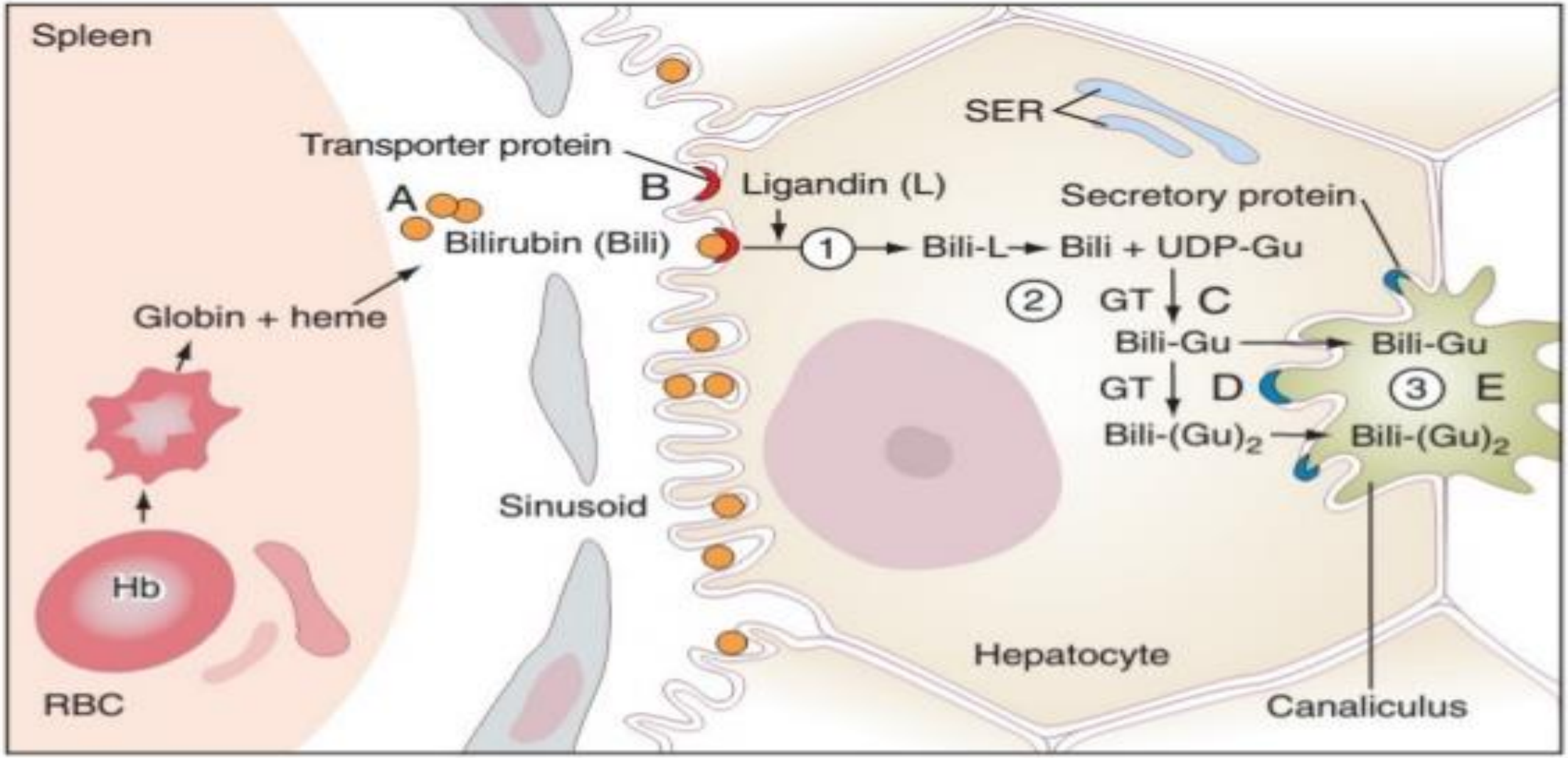
Heme catabolism

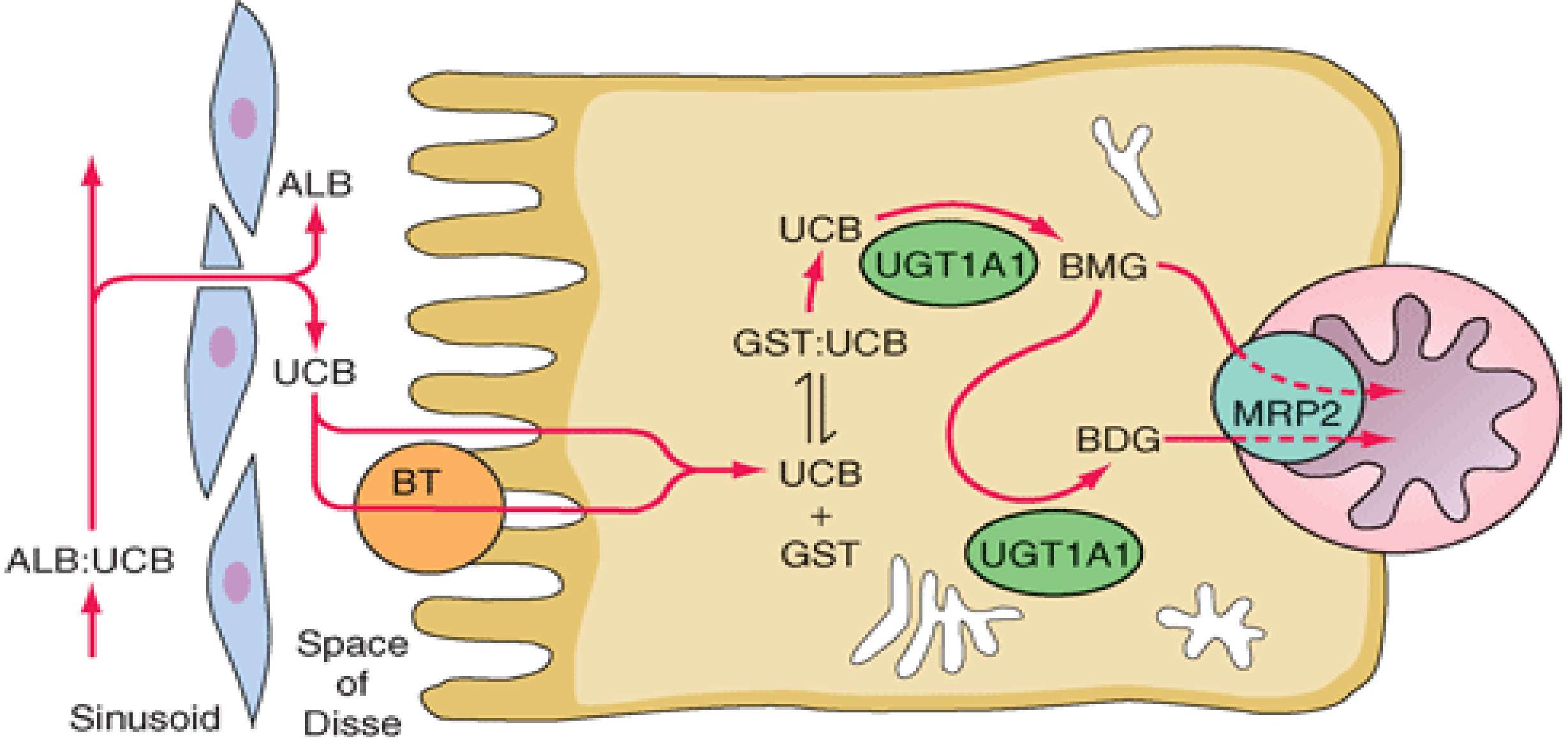
Heme Catabolism





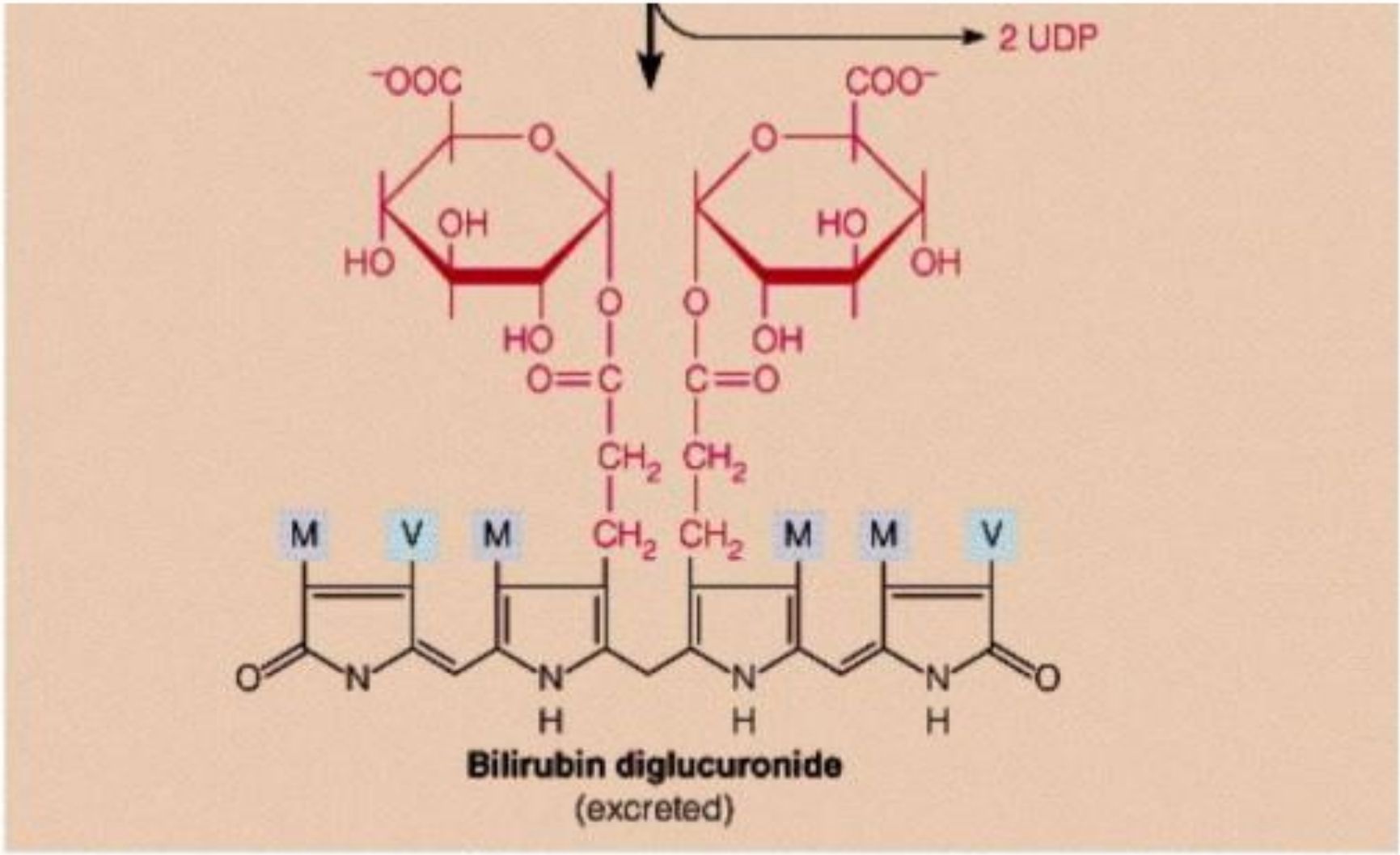
Bilirubin Metabolism

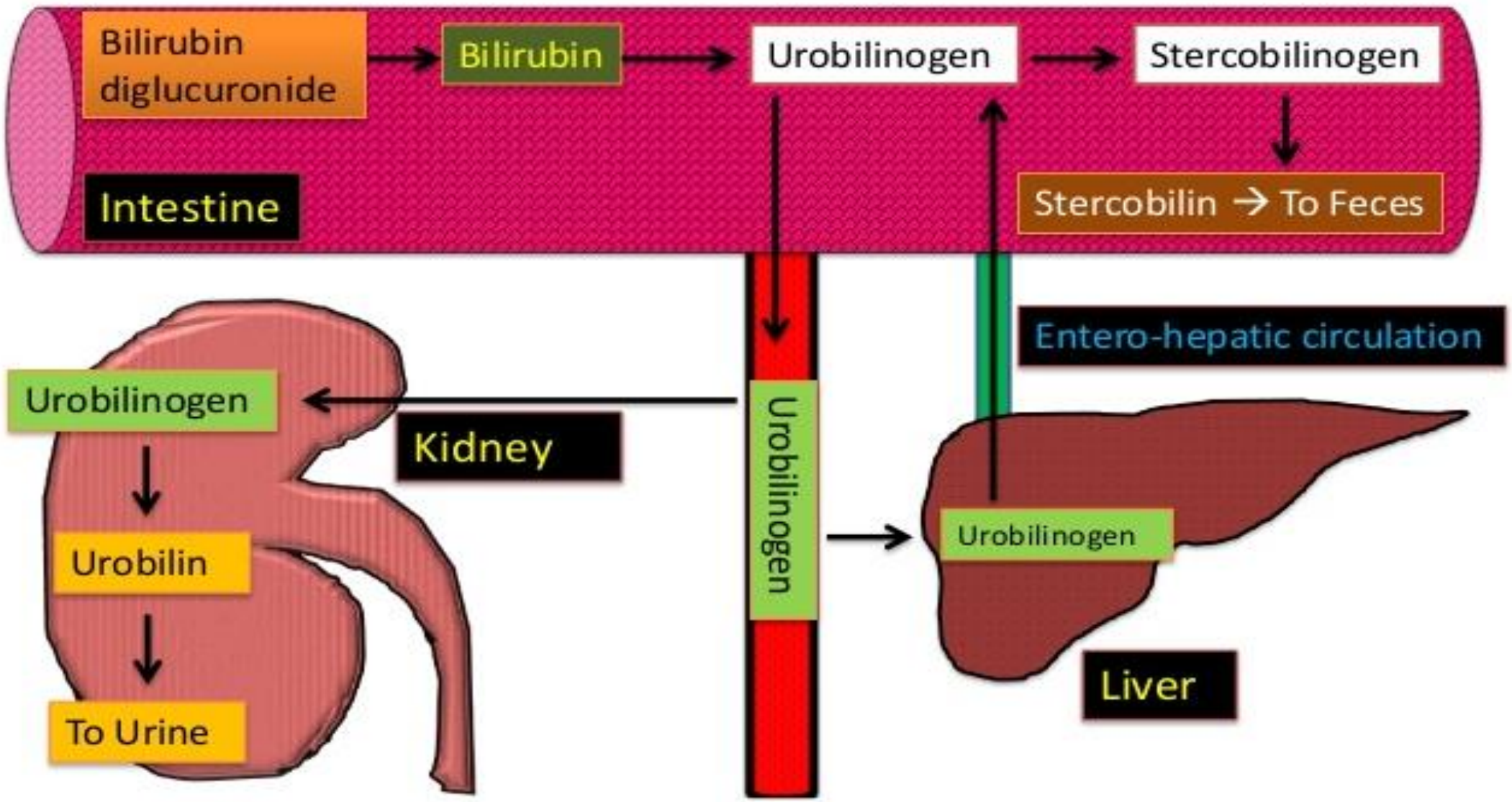


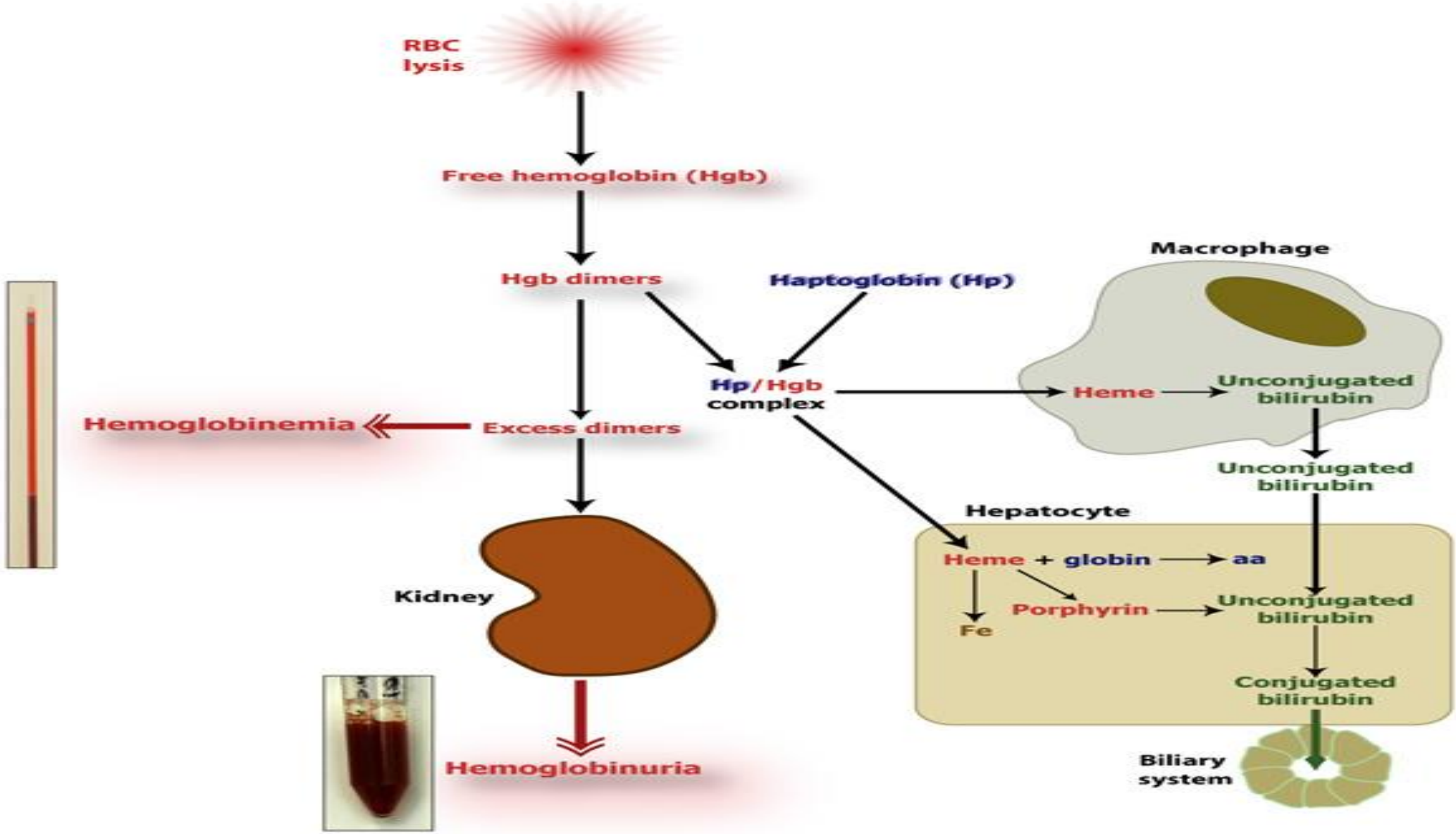


Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

Structure of Conjugated Bilirubin



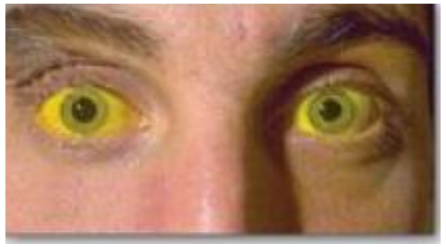

Bilirubin diglucuronide
Bilirubin
Urobilinogen
Stercobilinogen
Intestine
Stercobilin → To Feces
Entero-hepatic circulation
Urobilinogen
Kidney
Urobilinogen
Urobilin
To Urine
Urobilinogen
Liver


RBC lysis
Free hemoglobin (Hgb)
Hgb dimers
Haptoglobin (Hp)
Macrophage
Hp/Hgb complex
Heme
Unconjugated bilirubin
Hemoglobinemia
Excess dimers
Unconjugated bilirubin
Hepatocyte
Heme + globin
aa
Kidney
Porphyrin
Unconjugated bilirubin
Fe
Conjugated bilirubin
Biliary system
Hemoglobinuria

S. No	Type Of Bilirubin	Normal Ranges
1	Total Bilirubin Direct+ Indirect	0.2- 1 mg %
2	Unconjugated/Indirect Bilirubin	0.2-0.8 mg%
3	Conjugated / Direct Bilirubin	0- 0.2 mg%

Hyperbilirubinemia(>1.0 mg/dL)

Jaundice/Icterus



- **Jaundice** is a **pathological/Clinical condition/Symptom**

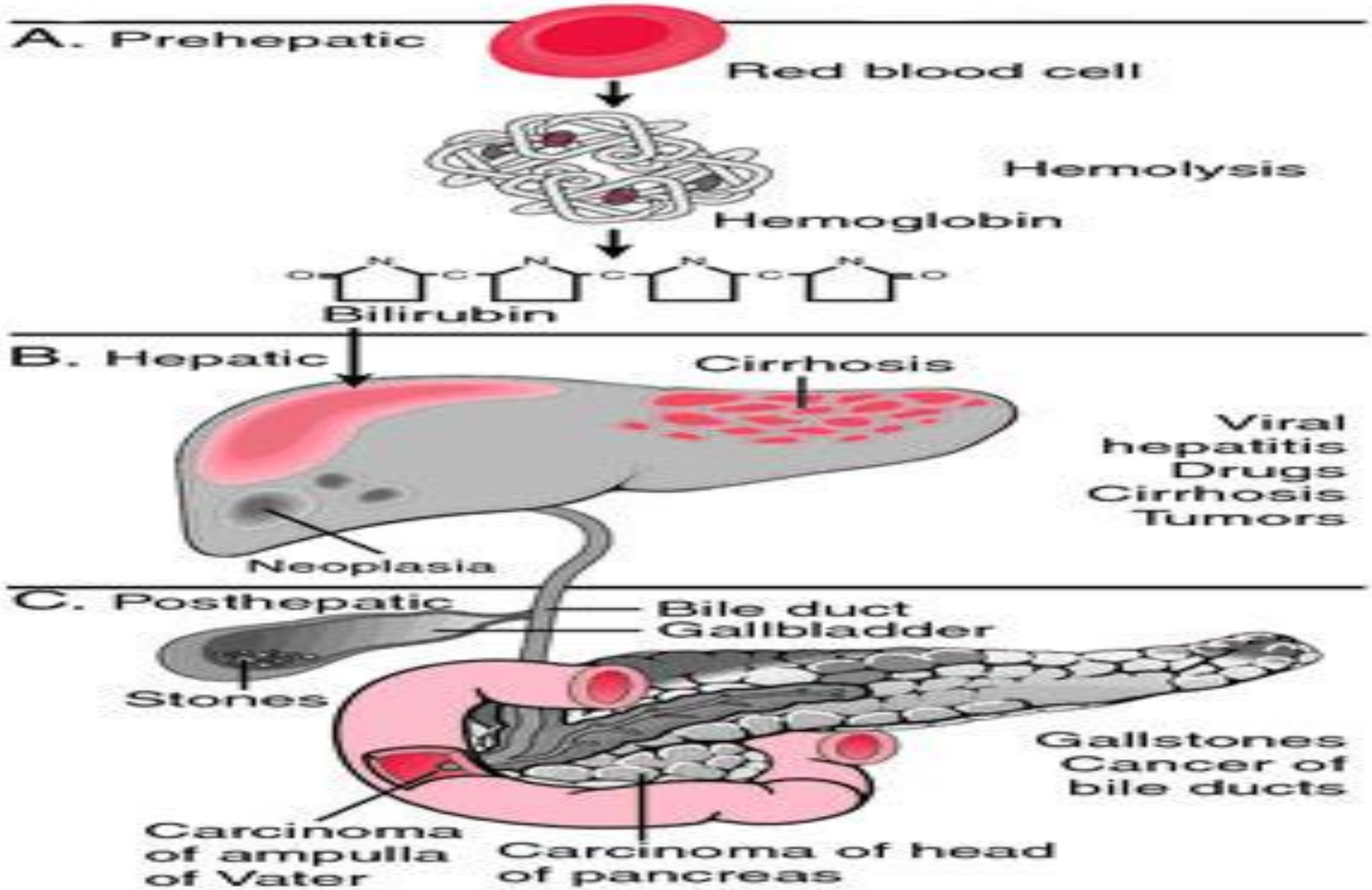
- yellowish discoloration of tissue resulting from the deposition of bilirubin

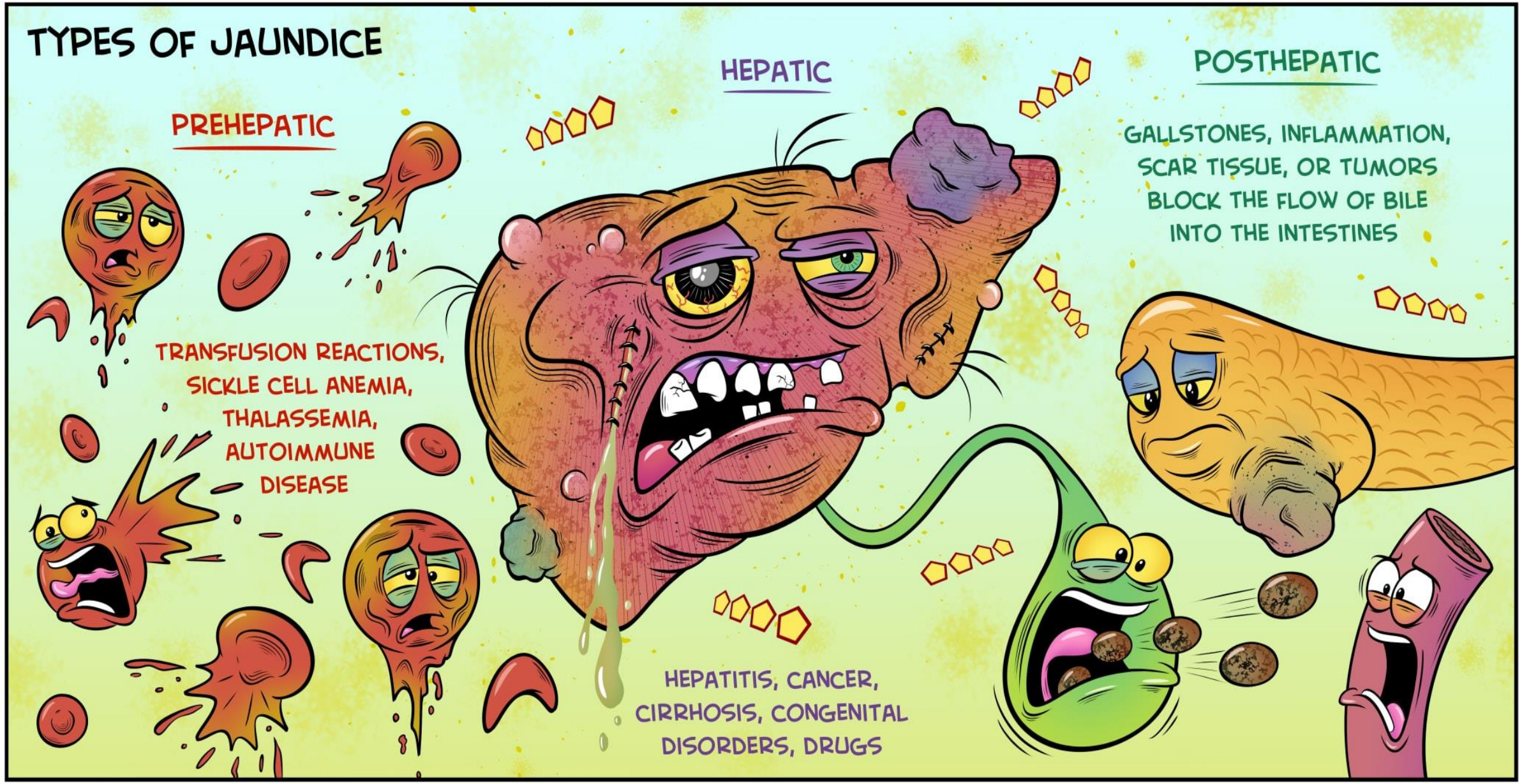
  - **Sclera of eyes (>3mg/dL)**
  - **Skin**
  - **Nails**
  - **Mucous membrane**

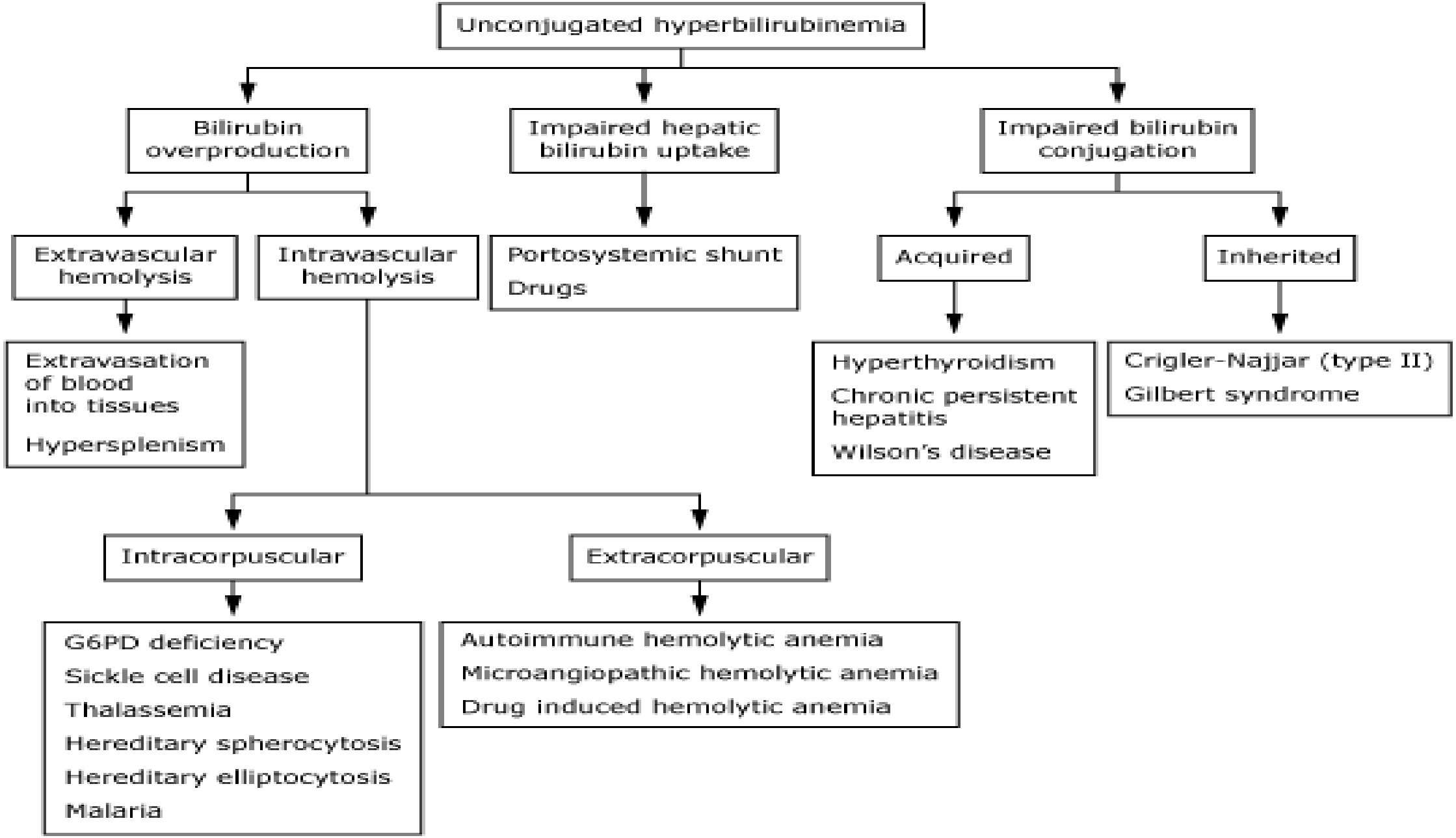
Classification

- **Anatomical**
  - Prehepatic
  - Hepatic
  - Post-hepatic(obstructive)
- **Based on type of hyperbilirubinemia**
  - Unconjugated
  - Conjugated
  - Mixed

Types of Jaundice






Unconjugated hyperbilirubinemia
Bilirubin overproduction
Impaired hepatic bilirubin uptake
Impaired bilirubin conjugation
Extravascular hemolysis
Intravascular hemolysis
Portosystemic shunt
Drugs
Acquired
Inherited
Extravasation of blood into tissues
Hypersplenism
Hyperthyroidism
Chronic persistent hepatitis
Wilson's disease
Crigler-Najjar (type II)
Gilbert syndrome
Intracorpuscular
Extracorpuscular
G6PD deficiency
Sickle cell disease
Thalassemia
Hereditary spherocytosis
Hereditary elliptocytosis
Malaria
Autoimmune hemolytic anemia
Microangiopathic hemolytic anemia
Drug induced hemolytic anemia

Alteration in metabolism of heme(Unconjugated hyperbilirubinemia)

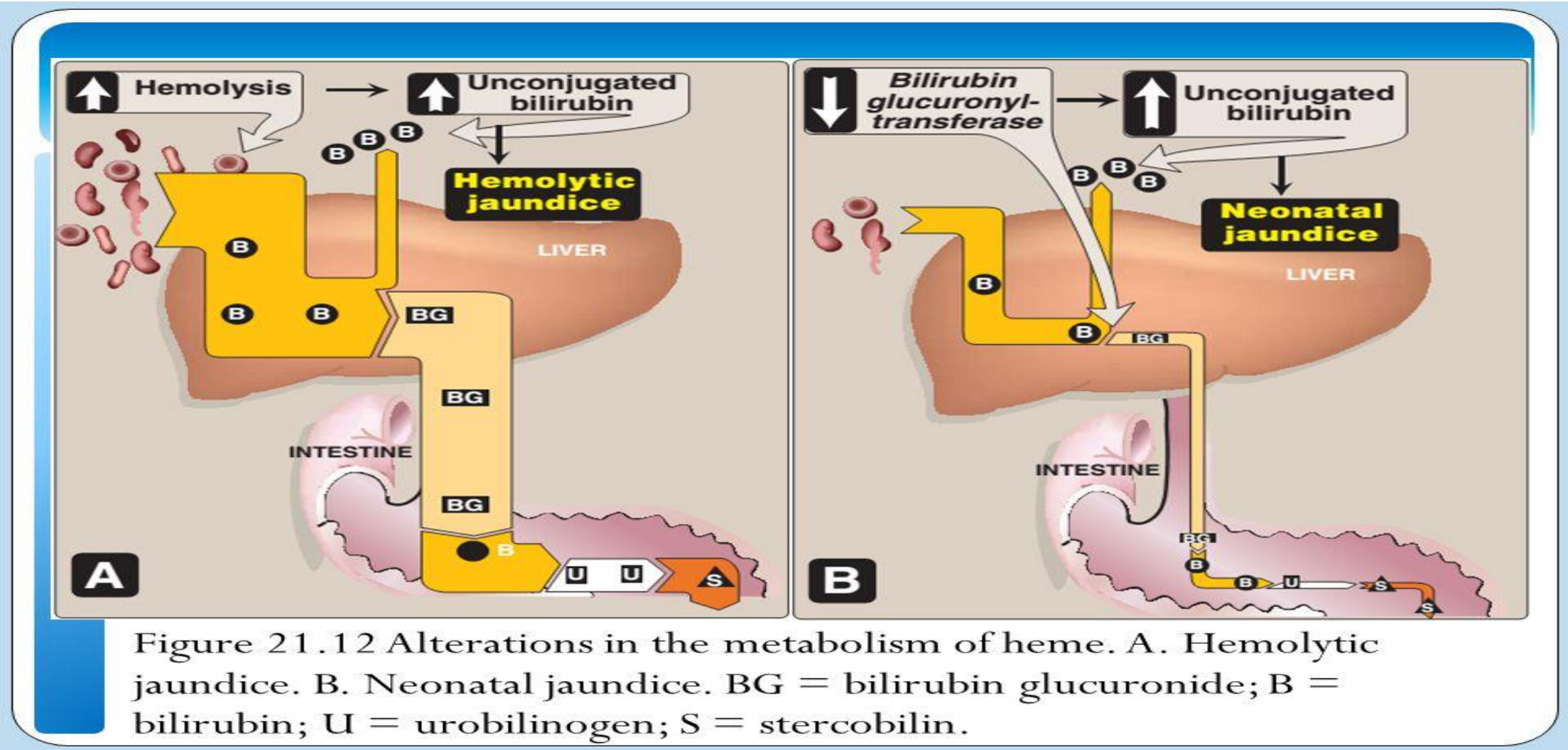


Figure 21.12 Alterations in the metabolism of heme. A. Hemolytic jaundice. B. Neonatal jaundice. BG = bilirubin glucuronide; B = bilirubin; U = urobilinogen; S = stercobilin.

Cholestatic Conditions that May Produce Jaundice

I. Intrahepatic

- ***A.*** Viral hepatitis
- ***B.*** Alcoholic hepatitis
- *C.* Drug toxicity
- ***D.*** Primary biliary cirrhosis
- ***E.*** Primary sclerosing cholangitis
- ***F.*** Vanishing bile duct syndrome
- ***G***.Inherited

Extra hepatic

A. Malignant

1. Cholangiocarcinoma
2. Pancreatic cancer
3. Gallbladder cancer

B. Benign

1. Choledocholithiasis
2. Postoperative biliary structures
3. Primary sclerosing cholangitis
4. Chronic pancreatitis

Causes of Isolated Hyperbilirubinemia

Indirect hyperbilirubinemia

A. Hemolytic disorders
B. Ineffective erythropoiesis
C. Drugs
D. Inherited conditions

Direct hyperbilirubinemia

A. Inherited conditions
- 1.Dubin-Johnson syndrome
- 2. Rotor's syndrome

Other causes

- Neonatal
- Breast feeding Jaundice
- Congenital

  Unconjugated

  Conjugated

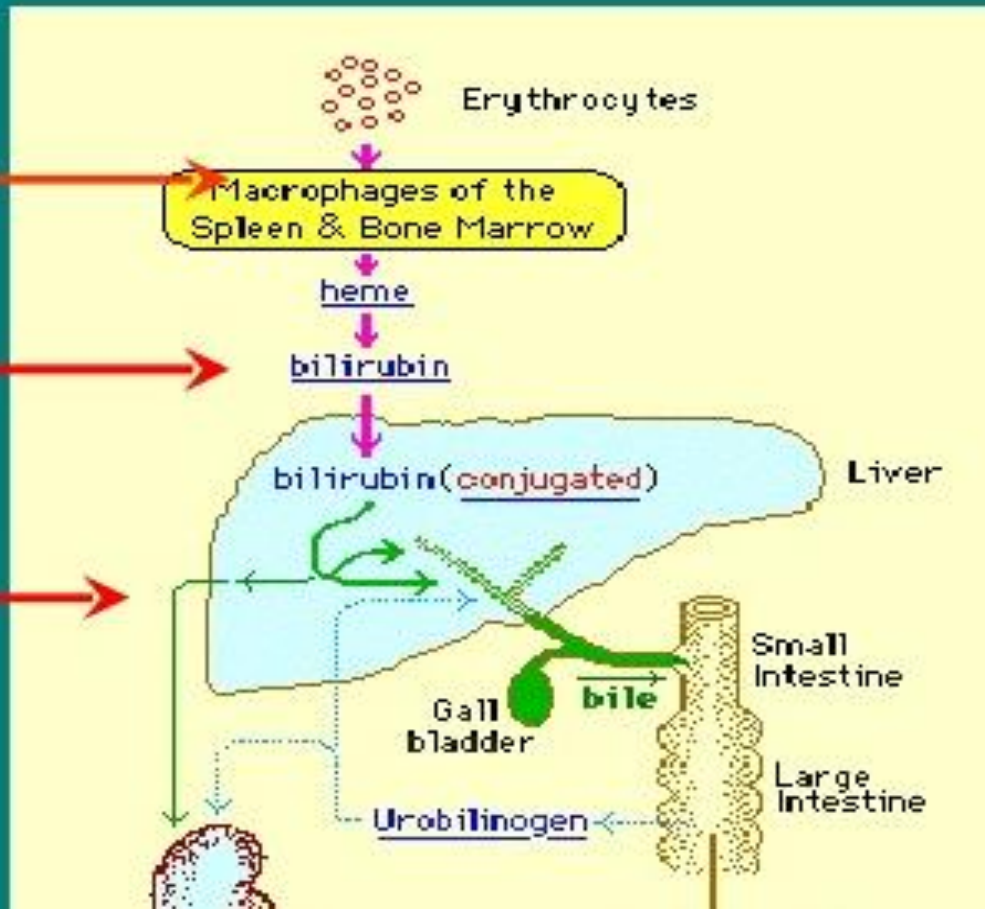
- Neonatal jaundice
  - Unconjugated bilirubin
    - Pathologic
      - Hemolytic
        - Intrinsic causes
        - Extrinsic causes
      - Non-hemolytic
    - Physiological jaundice of Neonates
  - Conjugated bilirubin
    - Hepatic
    - Post-hepatic

Mechanism of Physiologic Jaundice

Increased rbc's

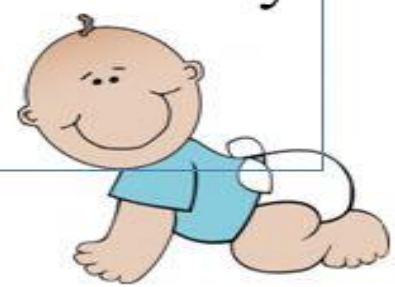
Shortened rbc lifespan

Immature hepatic uptake & conjugation



Characteristics of physiological jaundice

1. First appears between 24-72 hours of age
2. Maximum intensity seen on 4-5th day in term and 7th day in preterm neonates
3. Does not exceed 15 mg/ dl
4. Clinically undetectable after 14 days.
5. No treatment is required but baby should be observed closely for signs of worsening jaundice.



Causes of pathological Jaundice

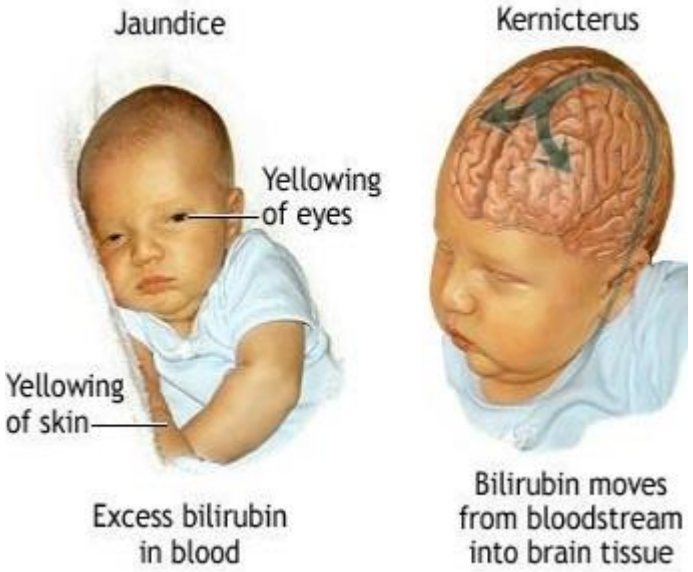
Appearing within 24 hours of age

- Hemolytic disease of NB : Rh, ABO
- Infections: TORCH, malaria, bacterial
- G6PD deficiency (**glucose-6-phosphate dehydrogenase**)

Appearing between 24-72 hours of life

- Physiological
- Sepsis
- Polycythemia
- Concealed hemorrhage
- Intraventricular hemorrhage
- Increased entero-hepatic circulation

- Clinical jaundice appears in 24 hours of age.
- Total bilirubin rises by higher than 5 mg/dl (86 umol/L) per day.
- Peak concentration of total bilirubin is more than 12 mg/dL in the term infant and 15 mg/dL in the preterm infant.



Kernicterus(chronic bilirubin encephalopathy)

The concern: Kernicterus

- Bilirubin exceeds albumin-binding capacity, crosses BBB, and deposits on basal ganglia and brainstem nuclei
- Risks increase with levels >20 mg/dl
  - Or lower levels in setting of sepsis, meningitis, hemolysis, hypothermia, hypoglycemia, or prematurity

Signs of kernicterus

- Acute sequelae:
  - Poor suck, lethargy, hypotonia, seizure
  - Then hypertonia (opisthotonus, retrocollis), fever, high-pitched cry
- Chronic sequelae:
  - Choreoathetoid CP, gaze paresis, sensorineural hearing loss, mental retardation

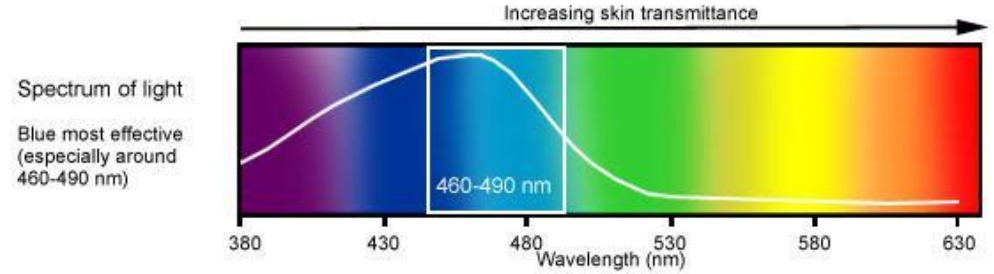
Treatment

Phototherapy

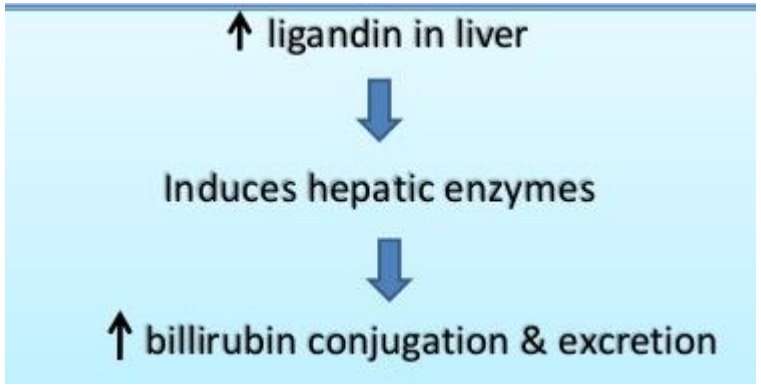
Phenobarbital

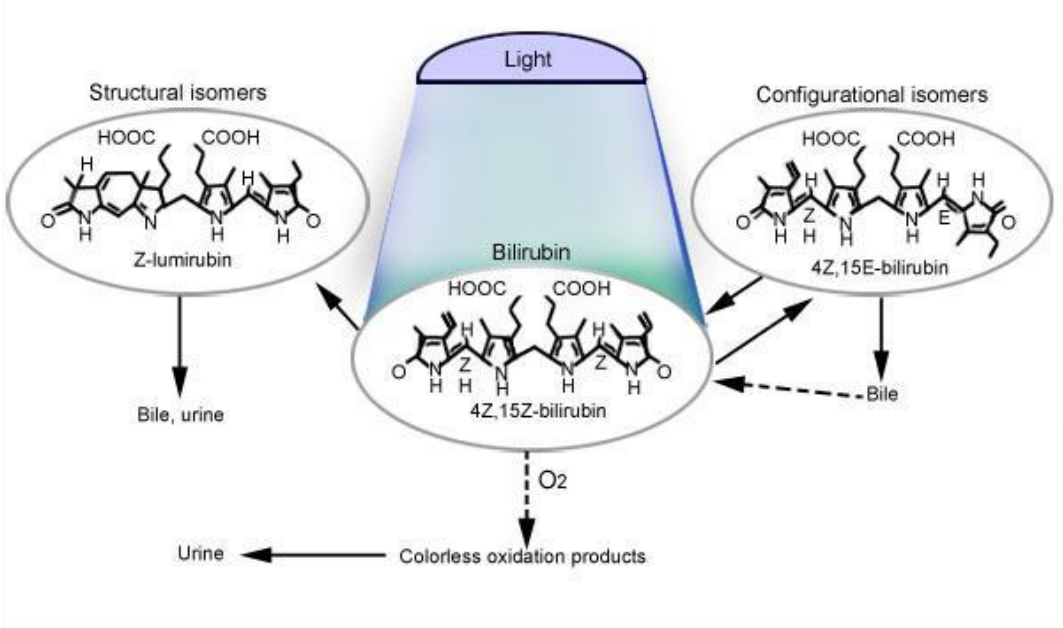
Treatment

- Phototherapy



- Phenobarbital





- Exchange Transfusion

Breastfeeding failure jaundice vs breast milk jaundice			
Diagnosis	**Timing**	**Pathophysiology**	**Clinical features**
Breastfeeding failure jaundice	First week of life	Lactation failure resulting in: • Decreased bilirubin elimination • Increased enterohepatic circulation	• Suboptimal breastfeeding • Signs of dehydration
Breast milk jaundice	Starts at age 3-5 days; peaks at 2 weeks	High levels of β-glucuronidase in breast milk deconjugate intestinal bilirubin & increase enterohepatic circulation	• Adequate breastfeeding • Normal examination

Congenital hyperbilirubinemias:

Due to abnormal uptake/ abnormal conjugation /abnormal excretion of bilirubin.

Crigler-Najjar syndrome

- AR
- Type I- total absence of UDP glucuronyl transf.
- Type II- partial def. of UDP GT.
- Unconj. Bilirubin >20mg/dl
- Kernicterus
- Death - 1yr of life

Gilbert's syndrome

- AD inheritance
- Males
- defective uptake of bilirubin by the liver
- Unconj. Bilirubin - 3 mg/dl
- harmless , no Rx

Dubin -Johnson syndrome

- AR
- Defective excretion of conj. Bilirubin into BILE
- Mutation in gene encoding MOAT protein
- Black liver jaundice

Rotor syndrome

- AR
- Exact cause??
- Abnormal excretion
- Harmless, No Rx

Diagnosis

First step first

(1) whether the hyperbilirubinemia is predominantly conjugated or unconjugated in nature

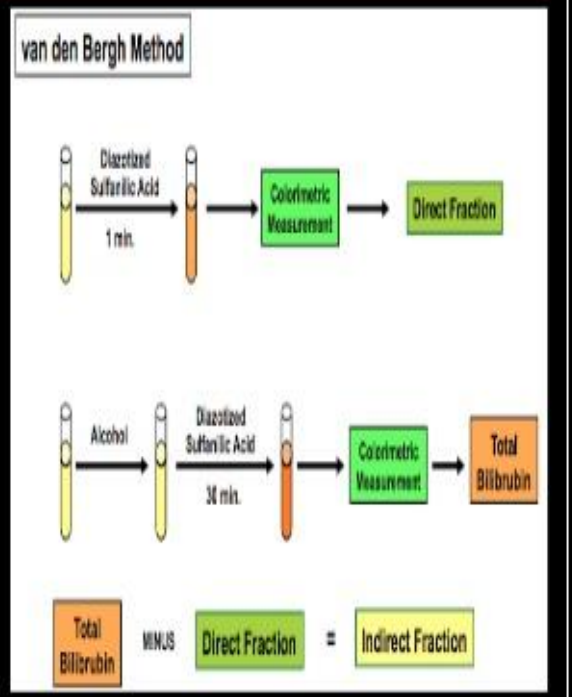
?conjugated/unconjugated hyperbilirubinemia

(2) whether other biochemical liver tests are abnormal.

Measurement of bilirubin

VAN DEN BERGH TEST

- **Reagent** used – **Diazo reagent (Mixture of Sulphanilic acid, Hydrochloric acid & sodium Nitrite)**
- Test – 2 types
  - **Direct**
  - **Indirect.**



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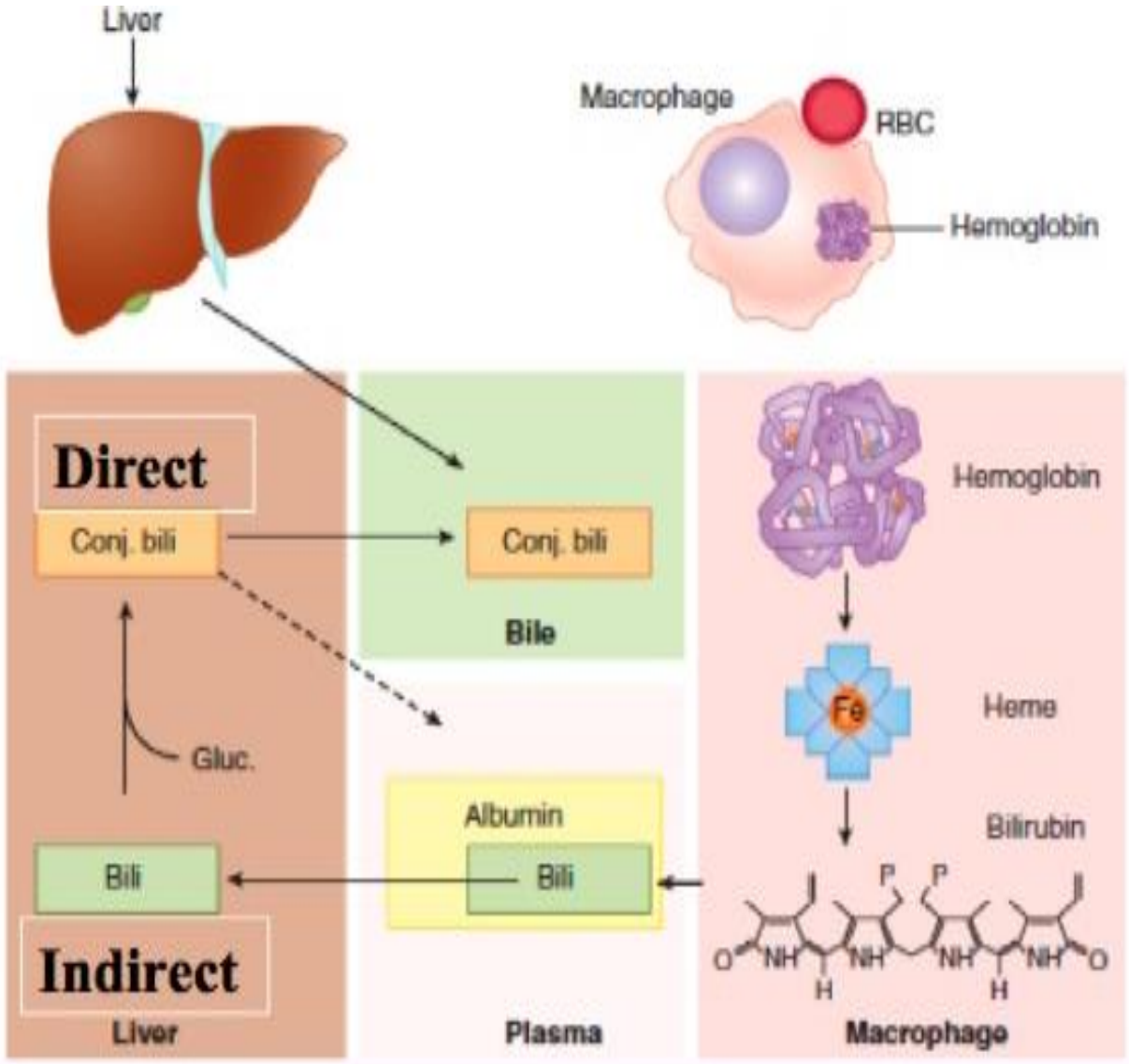
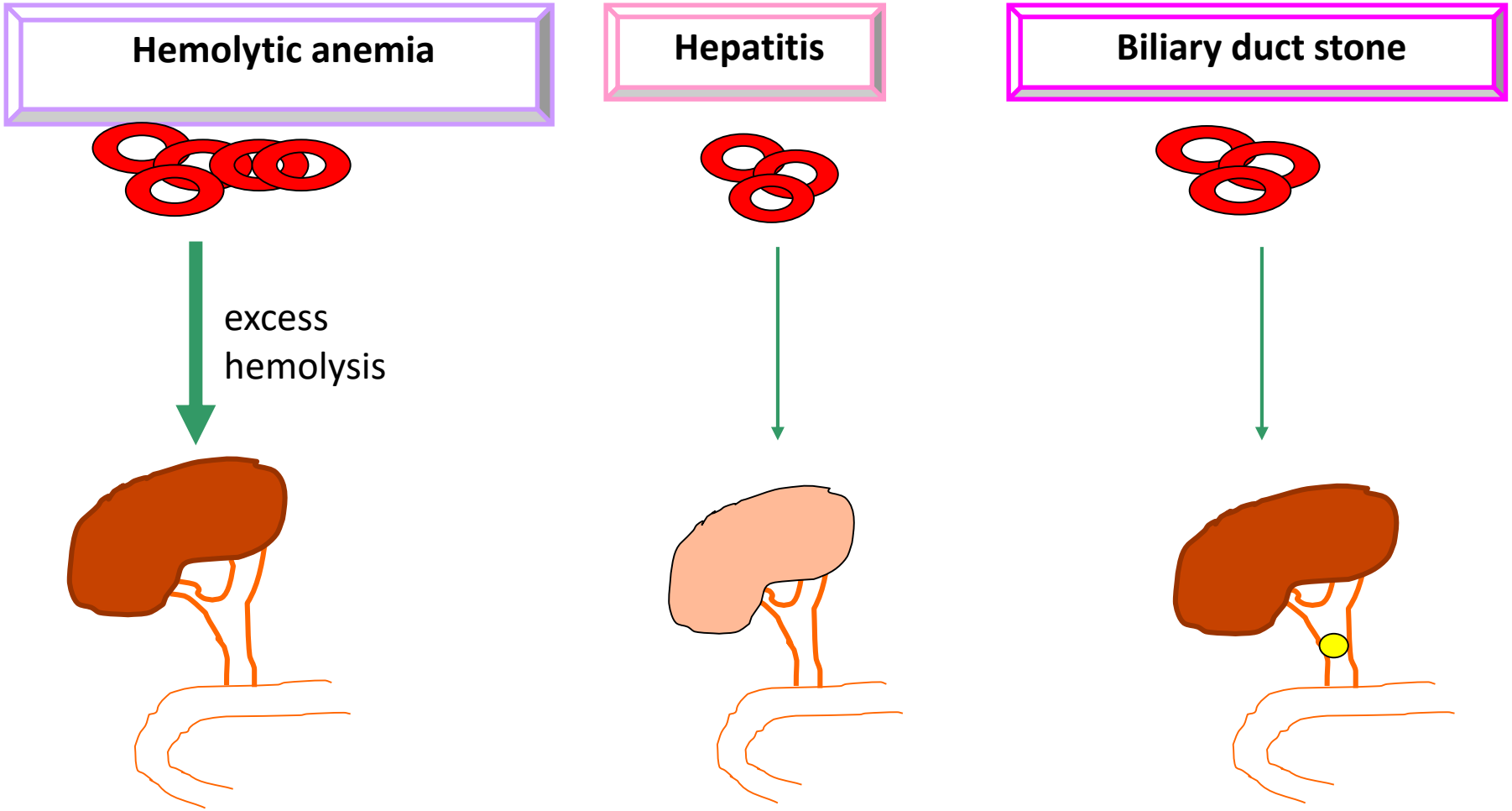


S.No	Results of Vanden Bergh	Type of Hyperbilirubinemia/ Jaundice
1	**Direct Vanden** Bergh's Reaction **Positive**	Conjugated Hyperbilirubinemia **Obstructive Jaundice**
2	**Indirect Vanden** Bergh's Reaction **Positive**	Unconjugated Hyperbilirubinemia. **Hemolytic Jaundice**
3	**Both Direct and Indirect Vanden** Bergh's Reaction **positive**	Biphasic Hyperbilirubinemia means Both conjugated and Unconjugated Bilirubin increased. **Hepatic Jaundice.**

Causes of Hyperbilirubinemia



- ↑ Unconjugated Bilirubin (in blood)
- ↑ conjugated Bilirubin (released to bile duct)

- ↑ Unconjugated Bilirubin (in blood)
- ↑ conjugated Bilirubin (in blood)

- ↑ conjugated Bilirubin (in blood)

δ bilirubin: *delta fraction* or *biliprotein*

- **conjugated bilirubin that is covalently linked to albumin**
- **an important fraction in cholestasis and hepatobiliary disorders**
- **formed in serum when hepatic excretion of bilirubin glucuronides is impaired**
- **the clearance rate approximates the half-life of albumin, 12–14 days, rather than the short half-life of bilirubin, about 4 hours.**

- **that some patients with conjugated hyperbilirubinemia do not exhibit bilirubinuria during the recovery phase**

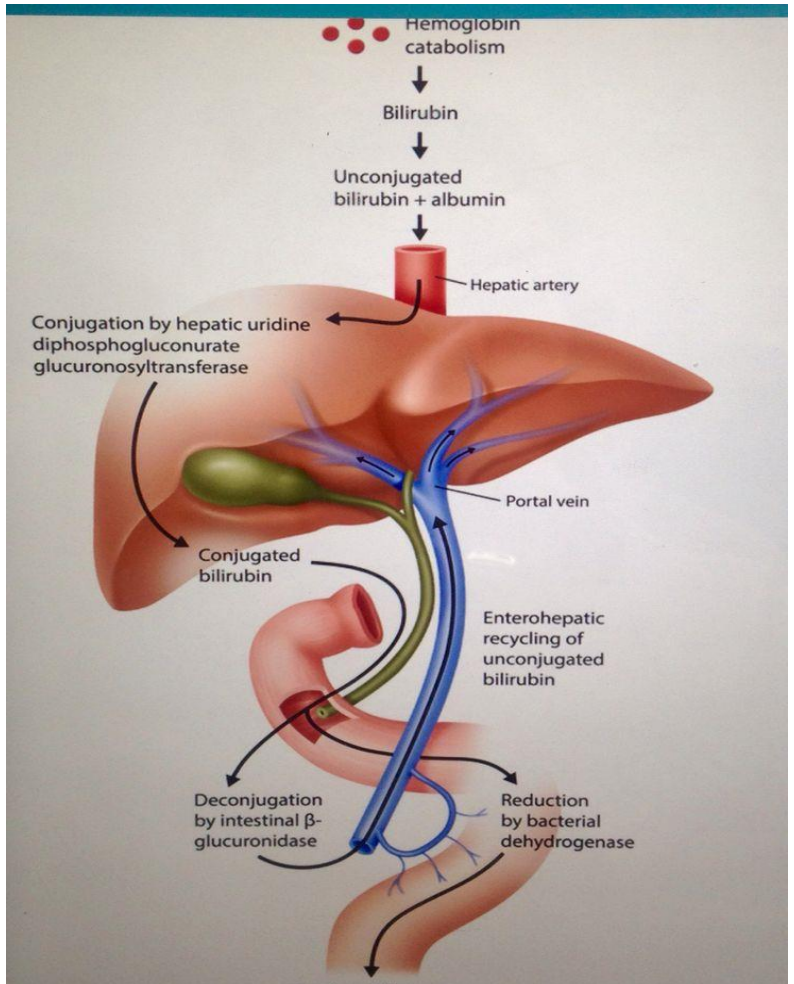
- **that the elevated serum bilirubin level declines more slowly than expected in some patients who otherwise appear to be recovering satisfactorily.**

Approach to the Patient: Hyperbilirubinemia

(1) overproduction of bilirubin

(2) impaired uptake, conjugation, or excretion of bilirubin;

(3) regurgitation of unconjugated or conjugated bilirubin from damaged hepatocytes or bile ducts.



Causes of Isolated Hyperbilirubinemia

Indirect hyperbilirubinemia

A. Hemolytic disorders

B. Ineffective erythropoiesis

C. Drugs

D. Inherited conditions

Direct hyperbilirubinemia

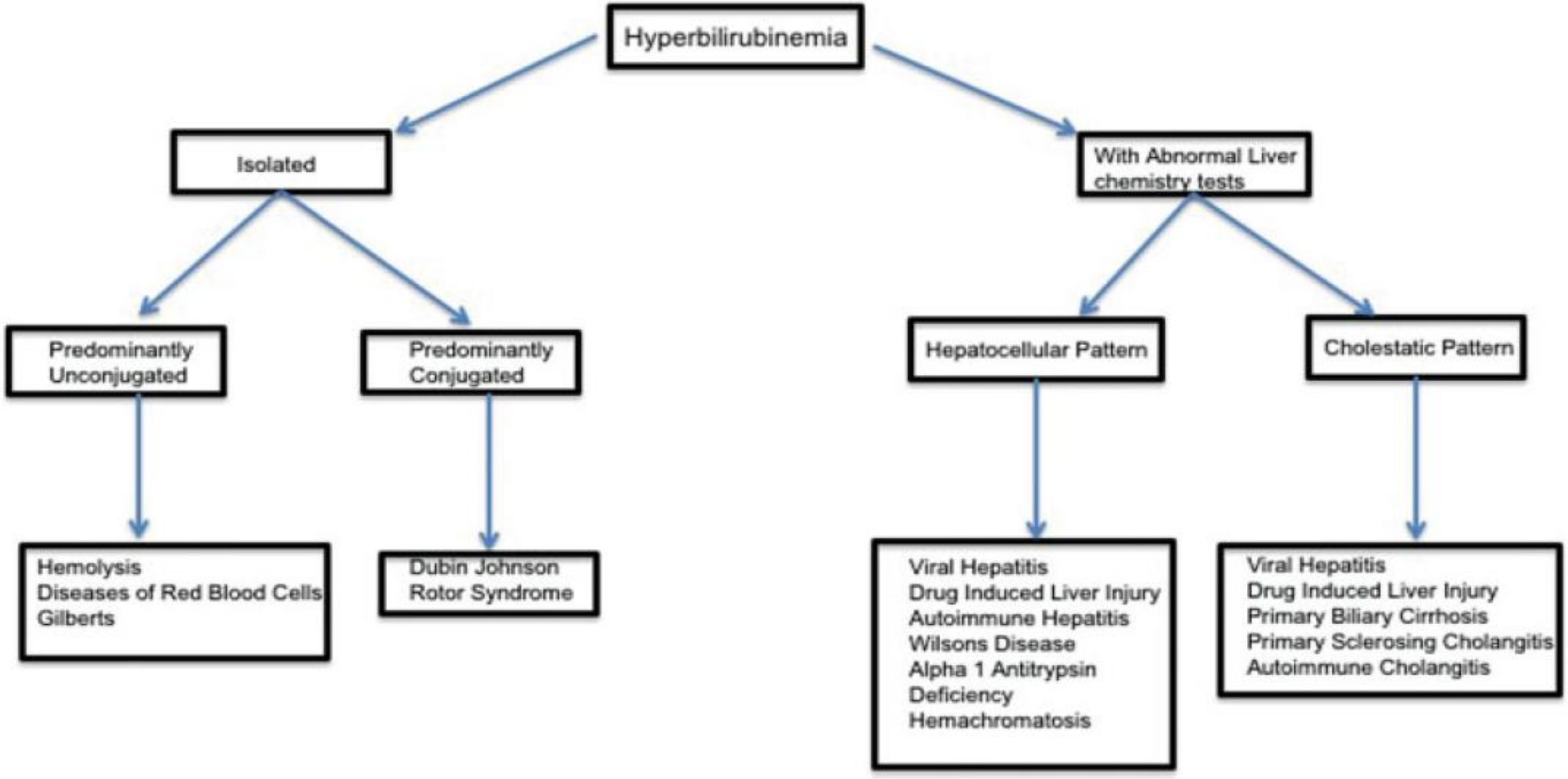
A. Inherited conditions

- 1.Dubin-Johnson syndrome
- 2. Rotor's syndrome

Comparison between 3 types of jaundice

	Hemolytic J aundice	Obstructive J aundice	Hepatic J aundice
Bilirubin	Unconjugated ↑	Conjugated ↑	Both ↑
VonDenBerg	Indirect +	Direct +	Biphasic
Serum enzymes	ALT,AST,ALP normal	ALP ↑ ↑ ALT,AST ↑	ALT,AST ↑ ↑ ALP ↑
Bilirubin In urine	Not excreted	excreted	excreted
urobilinogen	Excreted ↑	Normal or ↓	Normal or ↓

Overview of approach to hyperbilirubinemia



Integrated

- Medicine-Adult Jaundice

- Pediatrics-neonatal, congenital,breast feeding jaundice