



# Metabolism of heme

Prof. Mamoun Ahram  
Hematopoietic-lymphatic system

# Resources

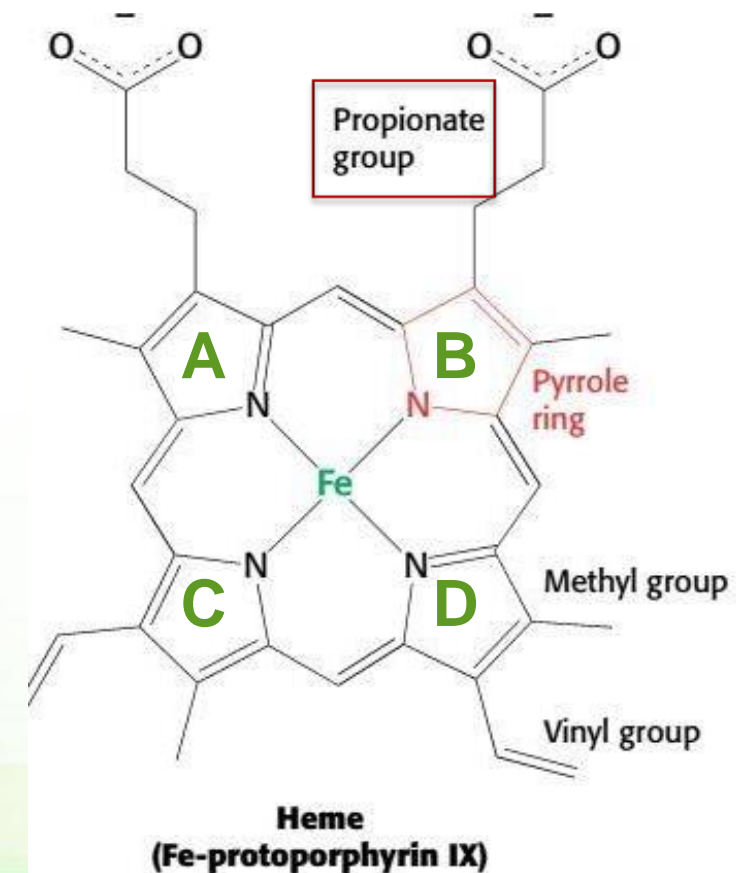
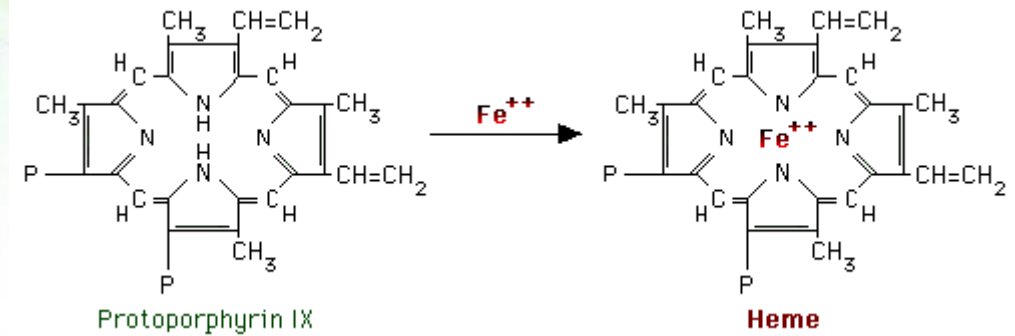
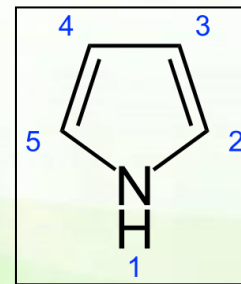


- This lecture
- Lippincott's Biochemistry, 7<sup>th</sup> edition, Ch. 21

# Heme structure



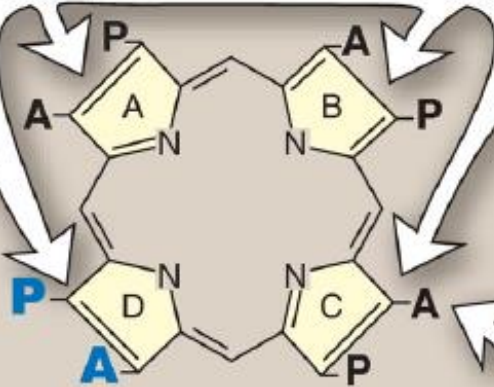
- It is a complex of protoporphyrin IX + Iron ( $\text{Fe}^{2+}$ ).
- The porphyrin is planar and consists of four pyrrole rings (designated A-D).
- Each pyrrole ring can bind two substituents.
- Two rings have a propionate group each.
- Note: the molecule is hydrophobic.
- Fe has six coordinates of binding.



# Prophyrins



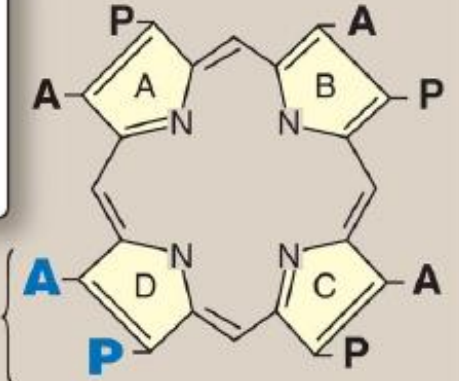
Prophyrins contain four pyrrole rings (A, B, C, and D) joined through methenyl bridges.



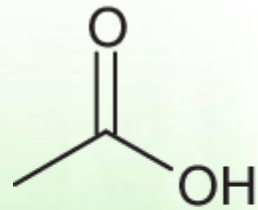
Uroporphyrin I

Prophyrins contain side chains attached to each of the four pyrrole rings. In type I porphyrins, the side chains are arranged symmetrically, that is, for uroporphyrin I, A (acetate) alternates with P (propionate) around the tetrapyrrole ring.

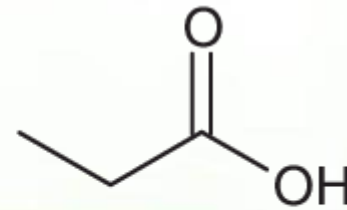
A and P are reversed in ring D of uroporphyrin III compared with uroporphyrin I. Only type III (asymmetric) porphyrins are physiologically important in humans.



Uroporphyrin III

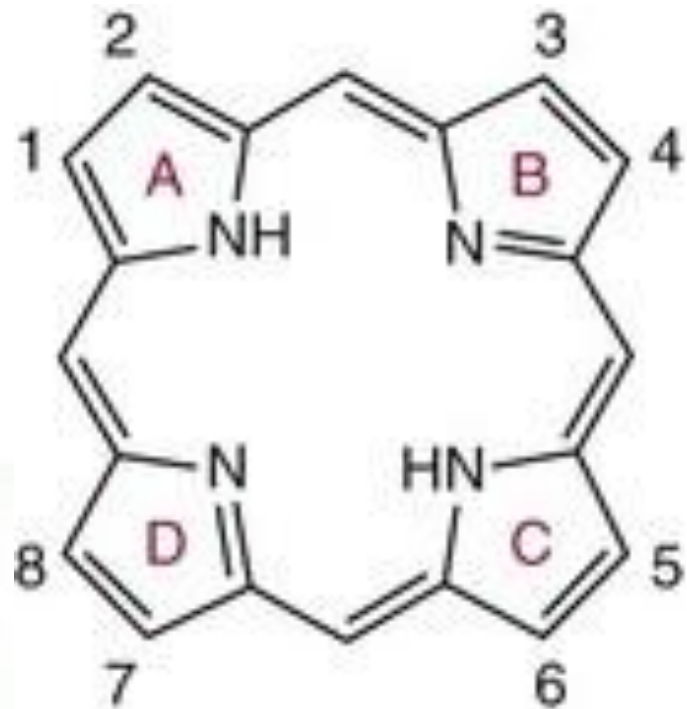


Acetate

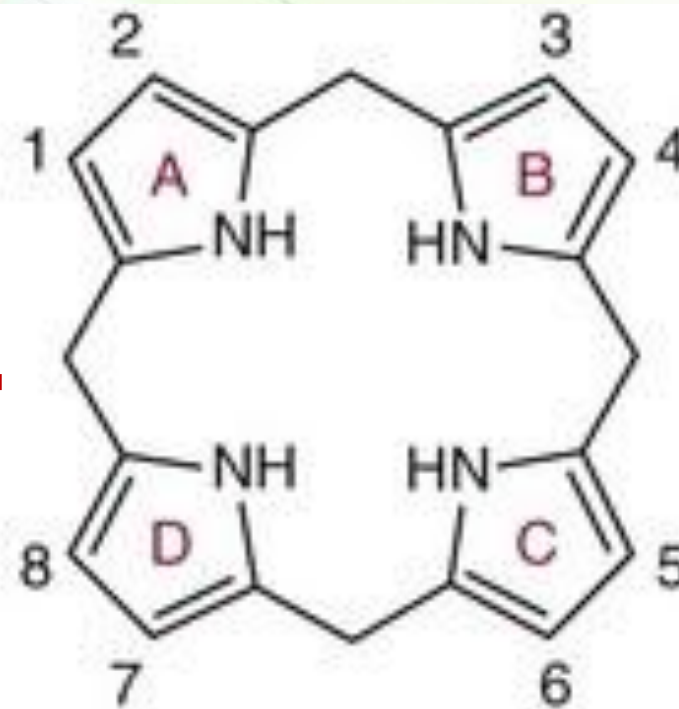


Propionate

# Porphyrinogens vs. porphyrins



Porphyrin



Porphyrinogen

**Reduced**  
**Porphyrin precursors**  
**Colorless**  
**Intermediates of heme synthesis**

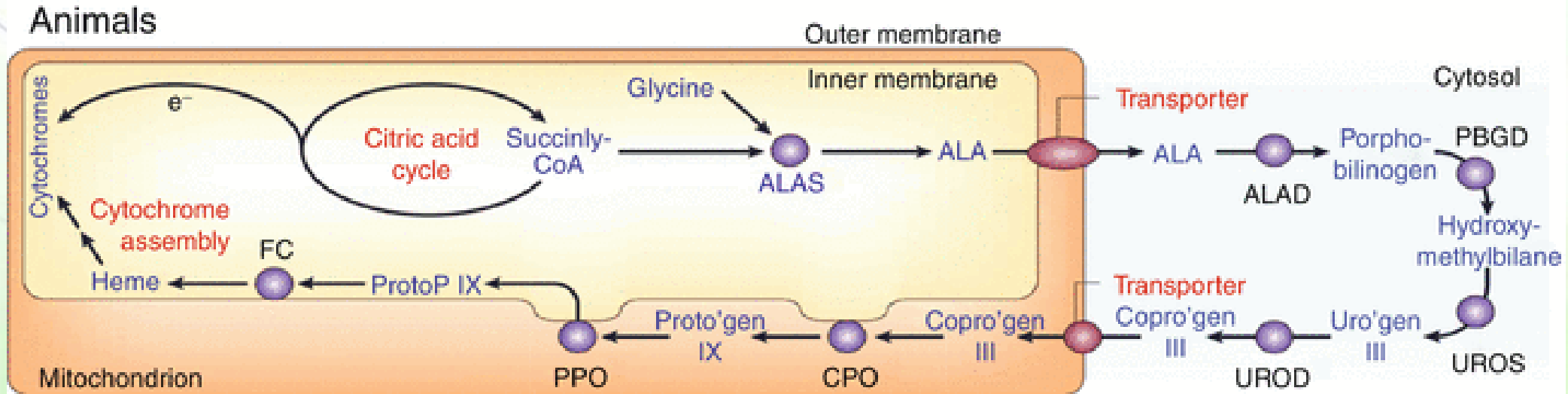


# Biosynthesis of heme

# Sites of synthesis



- The major sites of heme biosynthesis are:
  - Liver, which synthesizes a number of hemoproteins (particularly the CYP proteins)
    - The rate of heme synthesis is highly variable
  - Erythrocyte-producing cells (Hb synthesis)
    - Relatively constant production and matches the rate of globin synthesis, but synthesis is regulated at multiple points.
- Synthesis occurs in mitochondria → cytosol → mitochondria

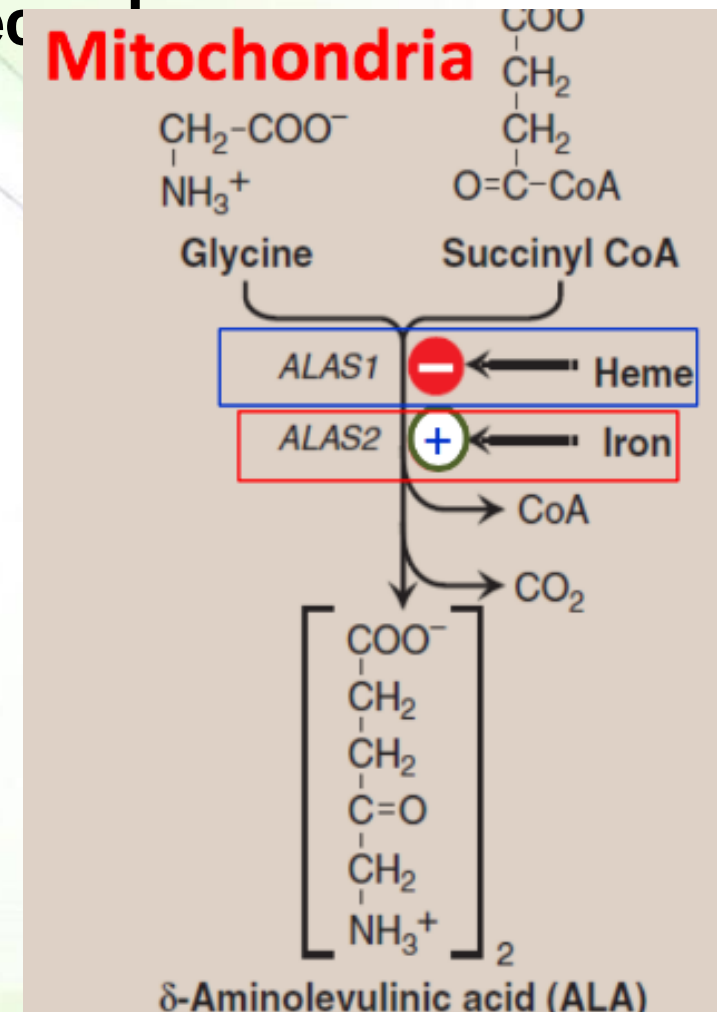


# Synthesis of 5'-aminolevulinic acid (ALA)



- The first reaction is catalyzed by 5'-aminolevulinic acid synthase, ALAS1 (all tissues inc. liver) or ALAS2 (erythroid), which conjugates gly and succinyl CoA into ALA.
  - It is the rate limiting and committed step.
  - It requires vitamin B6 (pyridoxal phosphate).
- ALA moves out of mitochondria to cytosol.

Re-  
rec

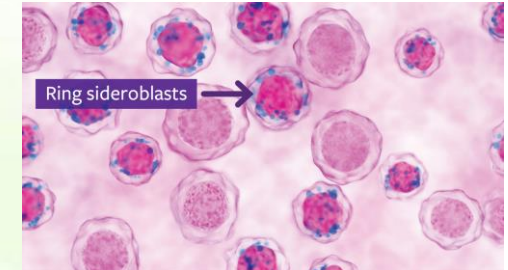
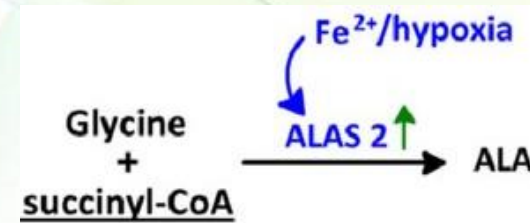




# ALA synthase isoenzymes



- ALAS2 is regulated by level of iron.
  - Loss of function mutations in result in X-linked sideroblastic anemia.
  - Iron accumulates in the erythroid marrow and deposits as mitochondrial non-ferritin iron **ring sideroblasts**.



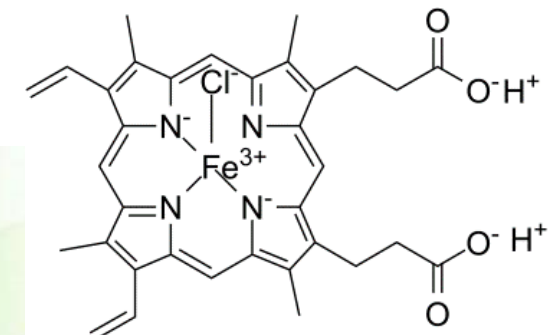
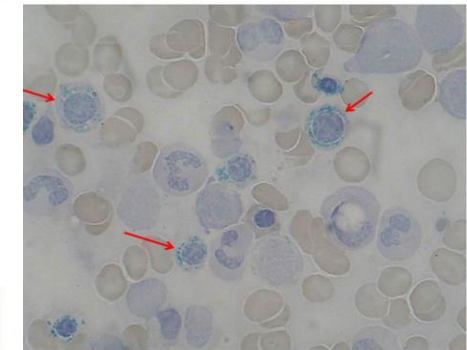
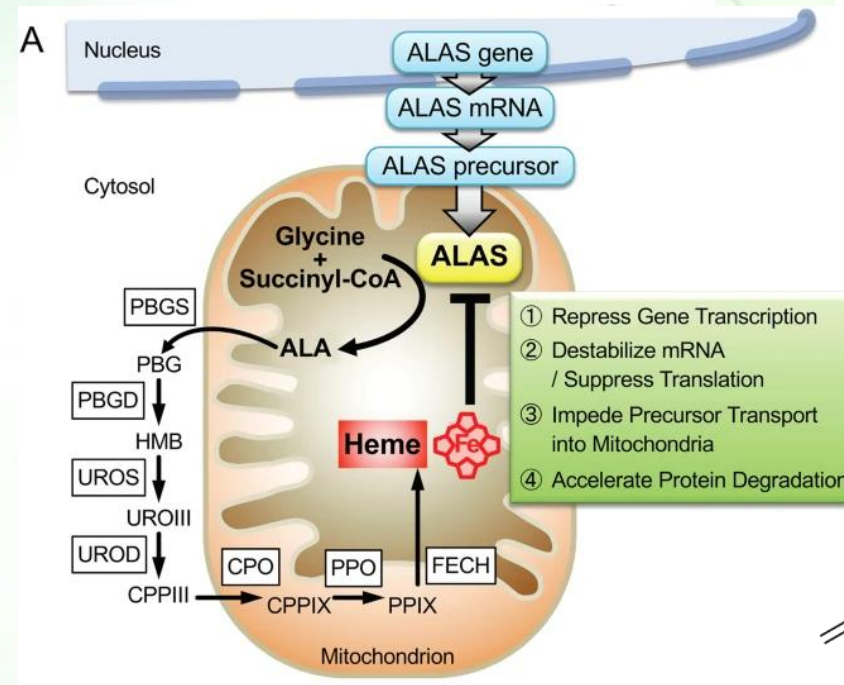
- ALAS1 is regulated by

- **hemin:**

- Reduces synthesis and stability of mRNA
    - Inhibits mitochondrial import of ALAS1
    - Induces protein degradation

- **Drugs:**

**Drugs → ↑CYP450 → ↓heme → ↑ALAS1 synthesis**

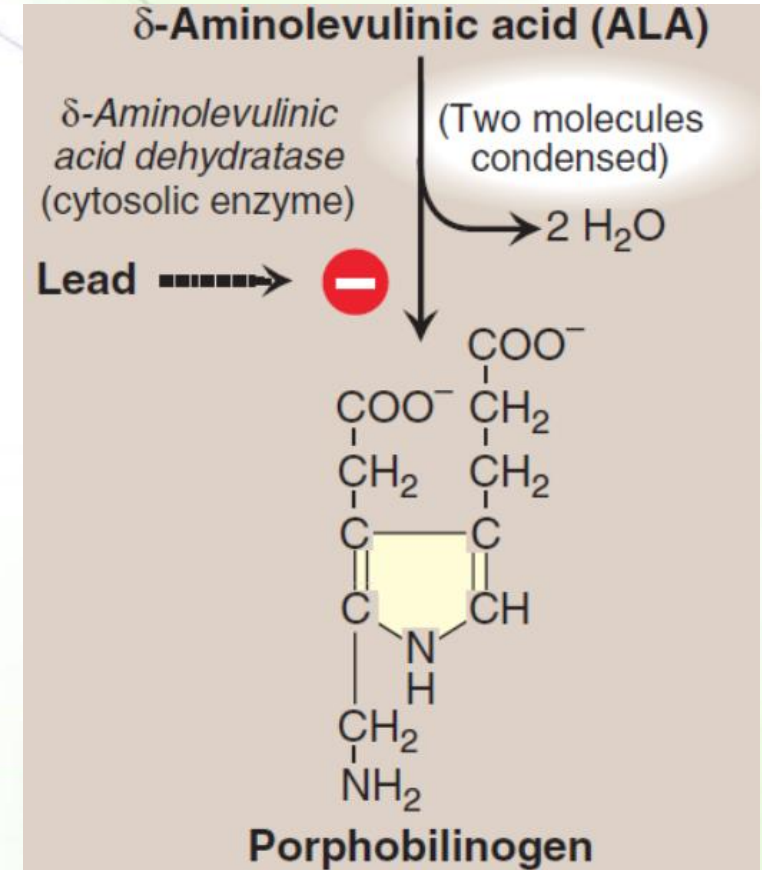


# Synthesis of porphobilinogen



- ALA moves out of mitochondria to cytosol where porphobilinogen is formed by condensing 2x ALA by zinc-containing ALA dehydratase (porphobilinogen synthase).
- The enzyme is sensitive to inhibition by heavy metal ions (for example, lead) that replace the zinc.
- This inhibition causes
  - increase in ALA
  - lead poisoning-associated anemia

## Re-record



# Subsequent reactions



## Re-record

- 4x PBG → hydroxymethylbilane → *cyclic* uroporphyrinogen III → coproporphyrinogen III → *mitochondria* → protoporphyrinogen IX → *oxidized* protoporphyrin IX → (+Fe<sup>+2</sup>) heme.
- The last reaction is spontaneous, but can be catalyzed by ferrochelatase.

### LEAD POISONING

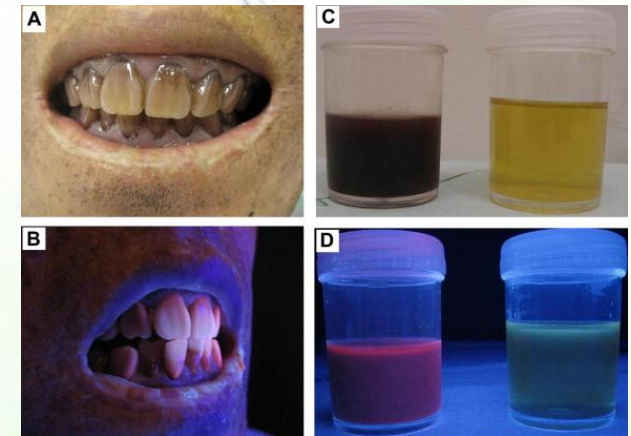
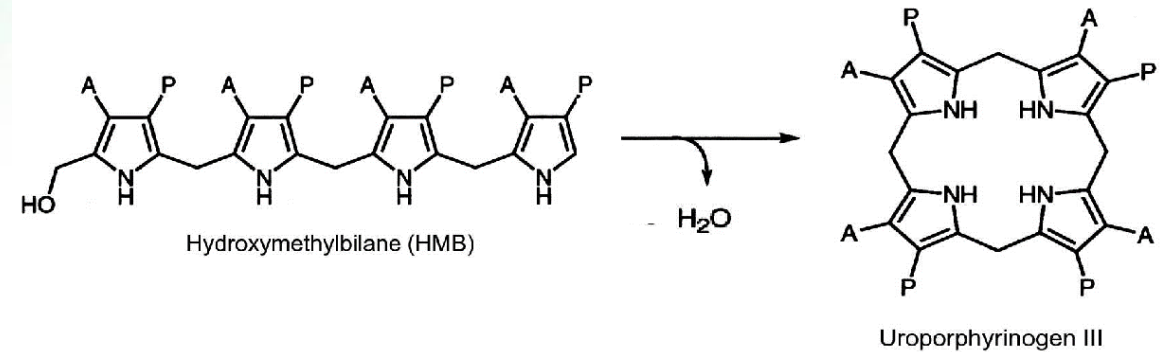
- Ferrochelatase and ALA dehydratase are inhibited by lead.
- Protoporphyrin and ALA accumulate in the urine in lead poisoning.



# Porphyrias



- Porphyrias: inherited or acquired disorders caused by a deficiency of enzymes in the heme biosynthetic pathway resulting in elevations in the serum and urine content of intermediates in heme synthesis.
- Porphyria = purple.
- These disorders are classified according to:
  - **Affected tissue (site of expression):**
    - Erythroid
    - Hepatic (acute or chronic)
  - **Manifestations**
    - **Not photosensitive**
      - Abdominal and neuropsychiatric
    - **Photosensitive**
      - Tetrapyrrole-dependent
      - Skin itching and burns
        - ↑Superoxide radicals





**LEAD POISONING**

- *Ferrochelatase* and *ALA dehydratase (ALAD)*<sup>1</sup> are particularly sensitive to inhibition by lead.
- Protoporphyrin and ALA accumulate in urine.
- *ALAD* deficiency porphyria is a very rare AR acute hepatic porphyria.

**ERYTHROPOIETIC PROTOPORPHYRIA (EPP)**

- This chronic AD and AR disease is caused by a deficiency in *ferrochelatase*.
- Protoporphyrin accumulates in erythrocytes, bone marrow, and plasma.
- Patients are photosensitive.

**ACUTE INTERMITTENT PORPHYRIA (AIP)**

- This acute AD disease is caused by a deficiency in *hydroxymethylbilane synthase*<sup>2</sup>.
- Porphobilinogen and ALA accumulate in the urine.
- Urine darkens on exposure to light and air.
- Patients are not photosensitive.

**VARIEGATE PORPHYRIA (VP)**

- This acute AD disease is caused by a deficiency in *protoporphyrinogen oxidase*.
- Protoporphyrinogen IX and other intermediates prior to the block accumulate in the urine.
- Patients are photosensitive.

**HEREDITARY COPROPORPHYRIA (HCP)**

- This acute AD disease is caused by a deficiency in *coproporphyrinogen III oxidase*.
- Coproporphyrinogen III and other intermediates prior to the block accumulate in the urine.
- Patients are photosensitive.

**PORPHYRIA CUTANEA TARDA (PCT)**

- This chronic disease can be caused by an AD deficiency in *uroporphyrinogen decarboxylase*.
- Uroporphyrin accumulates in the urine.
- It is the most common porphyria.
- Patients are photosensitive.

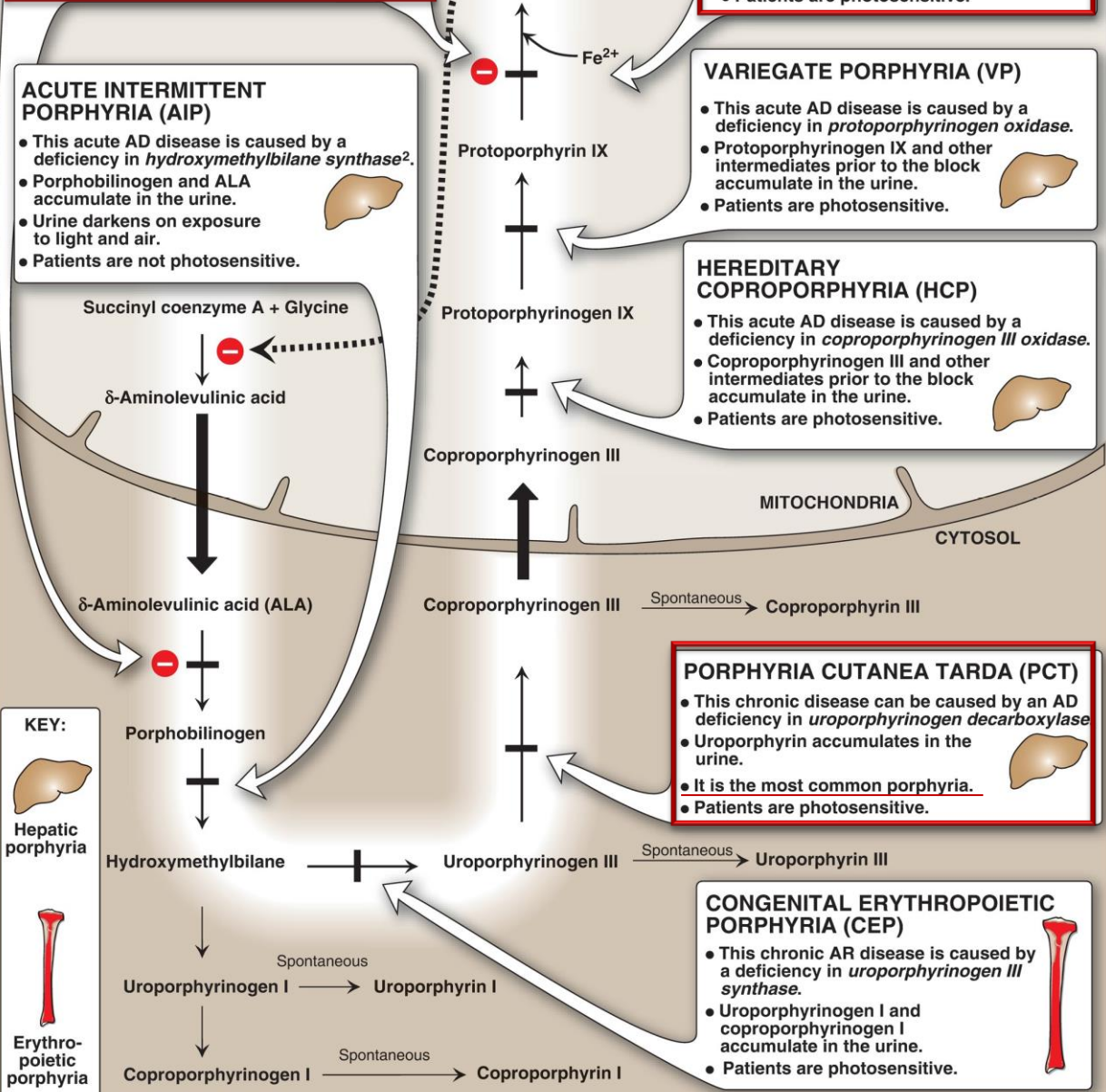
**CONGENITAL ERYTHROPOIETIC PORPHYRIA (CEP)**

- This chronic AR disease is caused by a deficiency in *uroporphyrinogen III synthase*.
- Uroporphyrinogen I and coproporphyrinogen I accumulate in the urine.
- Patients are photosensitive.

**KEY:**

Hepatic porphyria

Erythropoietic porphyria



**Hepatic porphyria**

↓

↓ heme

↓

↑ALAS1 synthesis

↓

↑intermediate

↓

↑toxicity

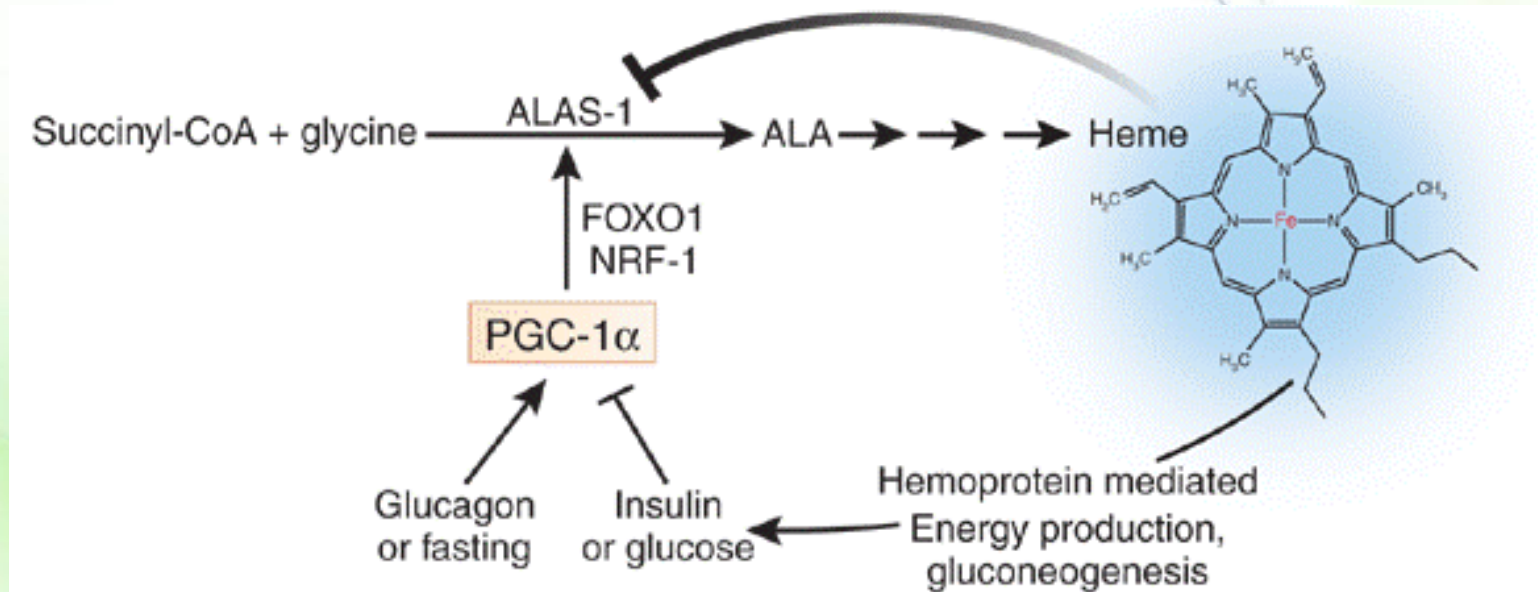
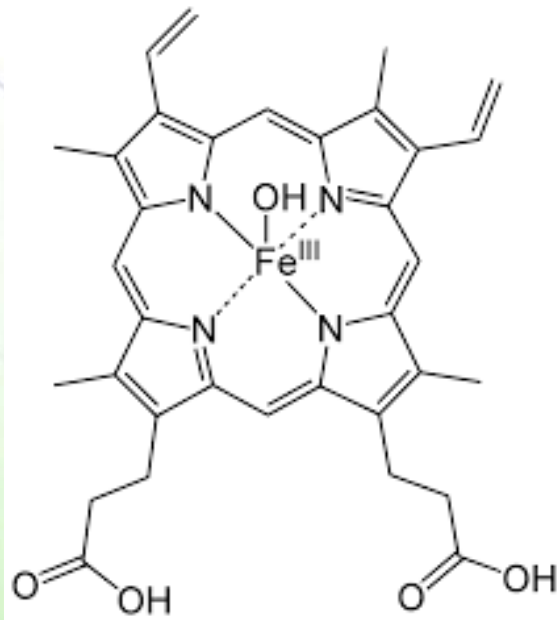
↓

**Drugs??**

# Treatment



- Hemin (or hematin) strongly inhibits the activity of ALAS.
- Glucose: by decreasing synthesis of ALAS1 by inhibiting the transcription factor, PGC-1 $\alpha$ , in the liver, which reduces the synthesis of gluconeogenic genes and the ALAS1 gene resulting in accumulation of heme intermediates.
- Fasting (hypoglycemia) exacerbates acute porphyria attack.





# Catabolism of heme

# Challenges



- RBCs are the largest storage place of heme.
- Erythrocytes are mainly destroyed by macrophages in the spleen and bone marrow, releasing hemoglobin, which is degraded to heme and globin.
- The protein is metabolized into amino acids.
- 6 g/day of hemoglobin are turned over, but
  - First, the porphyrin ring is hydrophobic.
  - Second, iron must be conserved.



# Heme degradation

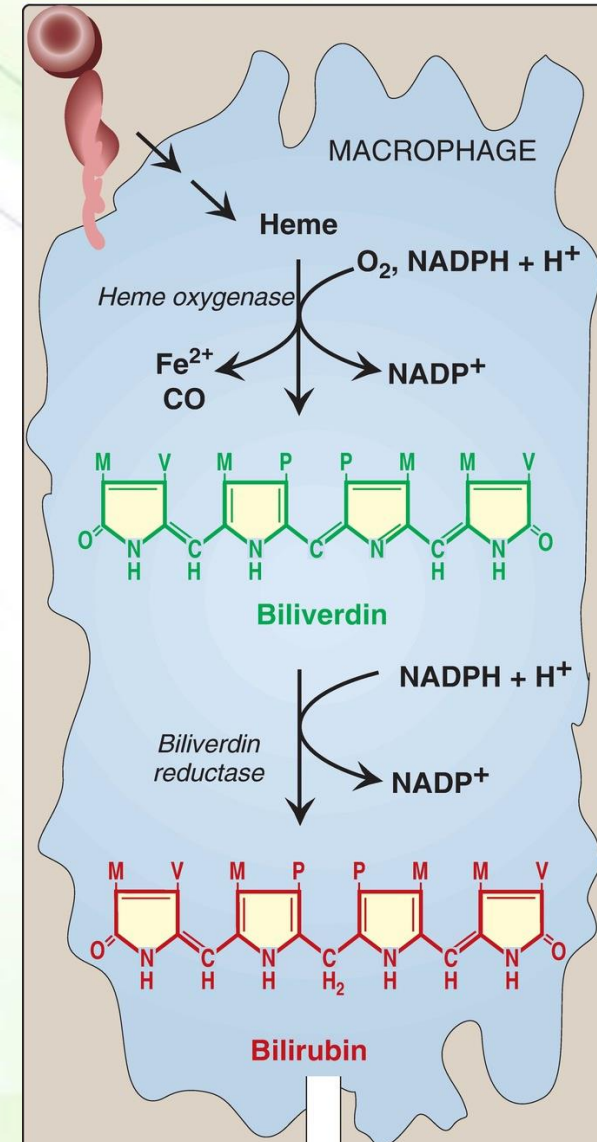


- The roles of heme oxygenase and NADPH
- The production of CO
- The world of colors

hemoglobin → biliverdin → bilirubin

bruise → healing

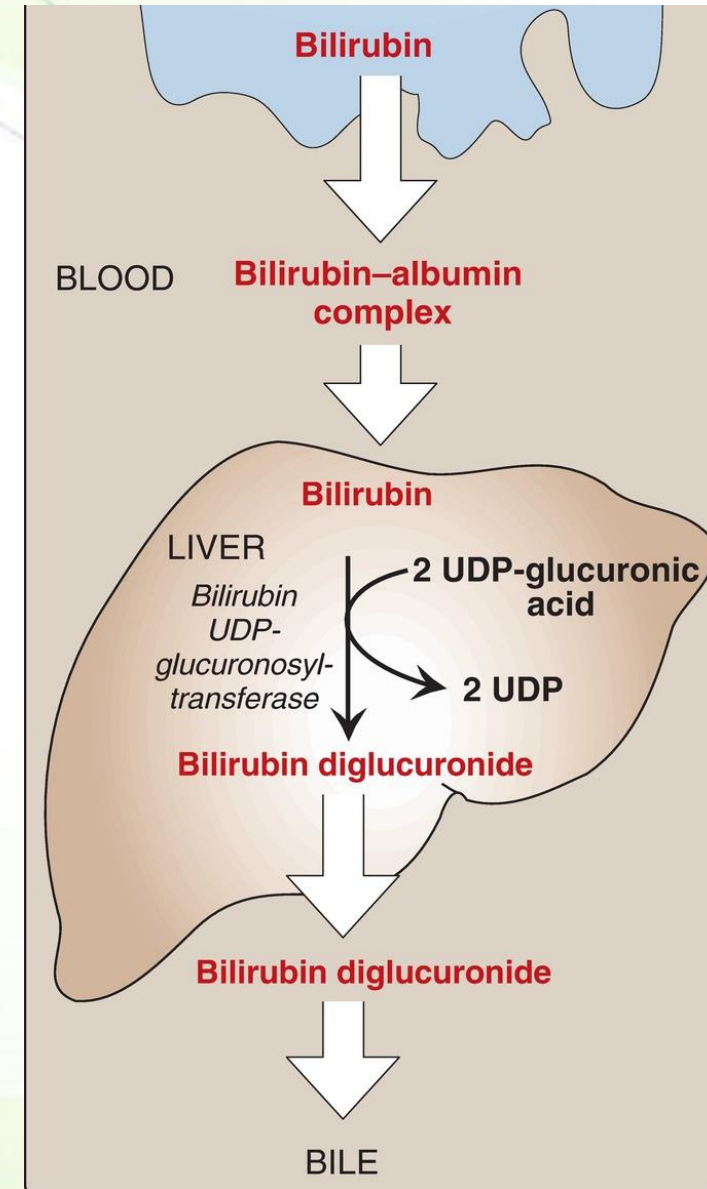
Bruise Age By Color	
Bruise Color	Bruise Age
Red (Swollen, Tender)	0 to 2 Days
Blue, Purple	2 to 5 Days
Green	5 to 7 Days
Yellow	7 to 10 Days
Brown	10 to 14 Days
No further evidence of Bruising	2 to 4 Weeks

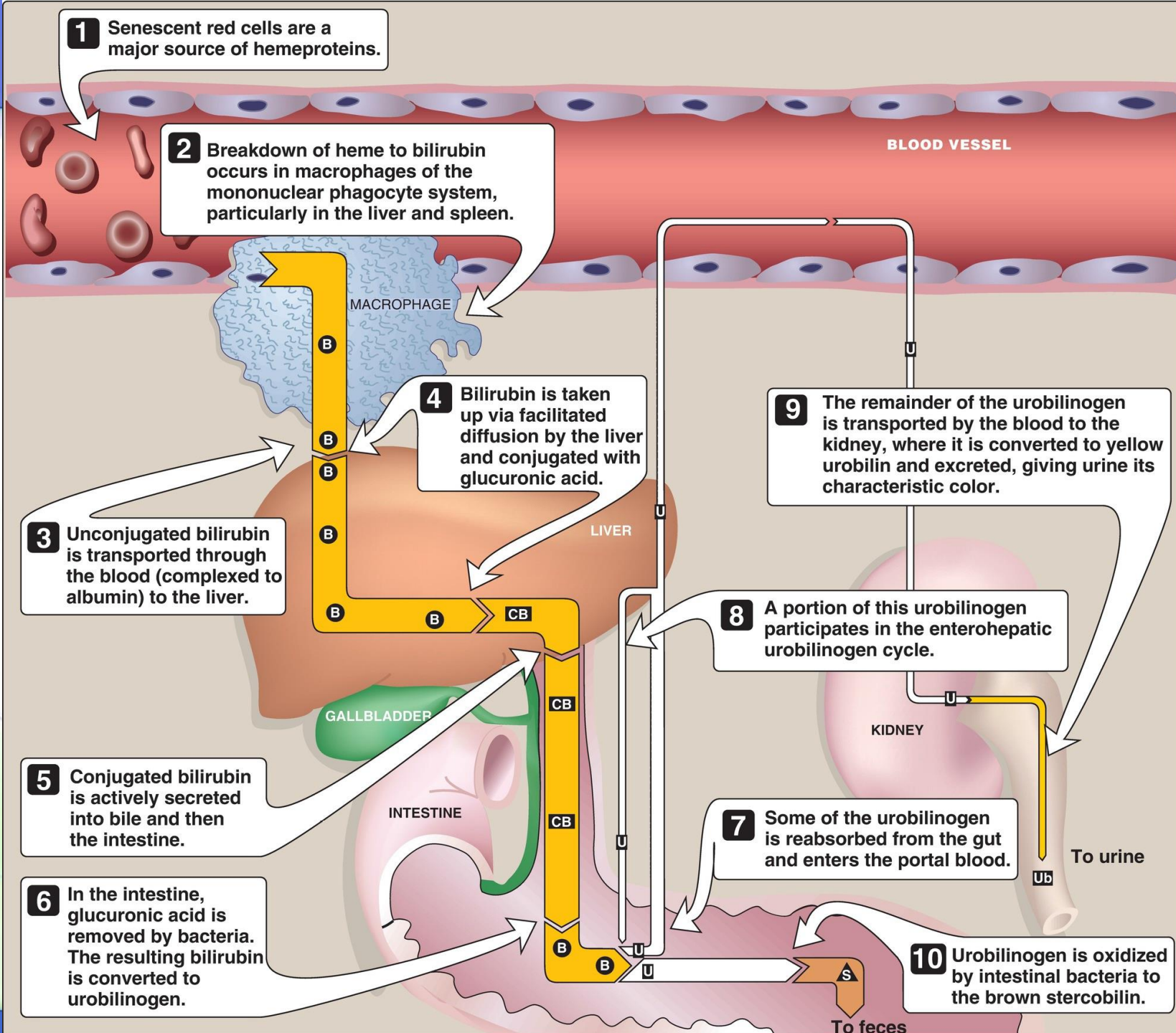


# Transport of bilirubin



- The role of albumin
  - Salicylates and sulfonamides can displace bilirubin from albumin permitting bilirubin to enter the central nervous system (CNS).
    - This may cause neural damage in infants.
- Formation of bilirubin diglucuronide.
  - Crigler-Najjar I and II and Gilbert syndrome
- Transport into bile
  - Dubin-Johnson syndrome





**1** Senescent red cells are a major source of heme proteins.

**2** Breakdown of heme to bilirubin occurs in macrophages of the mononuclear phagocyte system, particularly in the liver and spleen.

**3** Unconjugated bilirubin is transported through the blood (complexed to albumin) to the liver.

**4** Bilirubin is taken up via facilitated diffusion by the liver and conjugated with glucuronic acid.

**5** Conjugated bilirubin is actively secreted into bile and then the intestine.

**6** In the intestine, glucuronic acid is removed by bacteria. The resulting bilirubin is converted to urobilinogen.

**8** A portion of this urobilinogen participates in the enterohepatic urobilinogen cycle.

**7** Some of the urobilinogen is reabsorbed from the gut and enters the portal blood.

**9** The remainder of the urobilinogen is transported by the blood to the kidney, where it is converted to yellow urobilin and excreted, giving urine its characteristic color.

**10** Urobilinogen is oxidized by intestinal bacteria to the brown stercobilin.

# Measurement of bilirubin



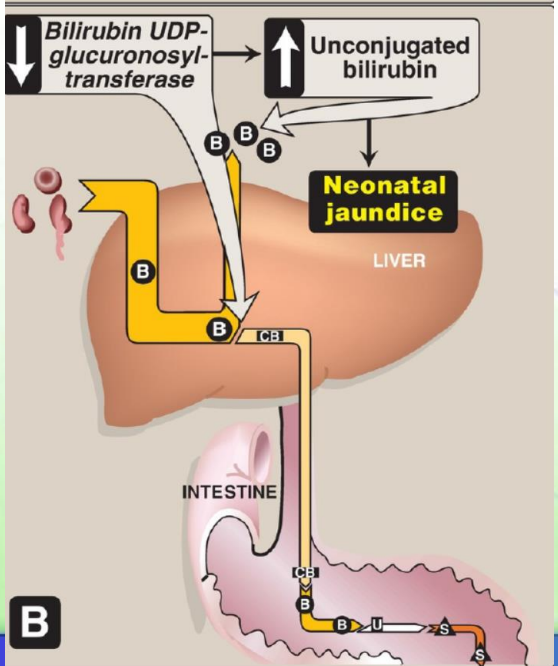
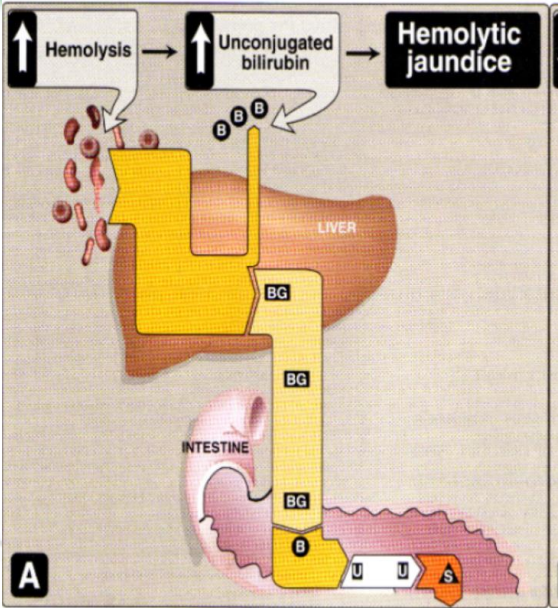
- It is done via a reaction known as Van den Bergh reaction.
- Direct measurement of conjugated bilirubin (in water)
  - Normally 4% of total bilirubin
- Total measurement of bilirubin (in ethanol or methanol)
- Indirect unconjugated bilirubin = total bilirubin – direct bilirubin



# Types and lab results of jaundice



*Jaundice: yellowing of skin, nail beds, and sclerae due to hyperbilirubinemia)*

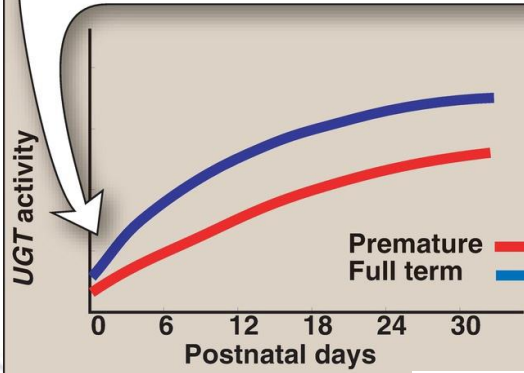


Sample	Indices	Normal	Unconjugated hyperbilirubinemia		Conjugated hyperbilirubinemia
			Hemolytic jaundice	Hepatic jaundice	Obstructive jaundice
Serum	Total Bil.	0.2-1.0 mg/dl	↑	↑	↑
	Direct (conj. Bil.)	0-0.2 mg/dl	↔	↑	↑↑
	Indirect (unconj. Bil.)	0.2-1.0 mg/dl	↑↑	↑	↔
	ALT/AST	Normal	Normal	↑	Normal
Urine	Color	Normal	Very dark	Dark	Dark
	Bilirubin	-	-	↑	↑
	Urobilinogen	Trace	↑	↑	↓ or -
	urobilin	Trace	↑		↓
Stool	Color	Normal	Dark	Lighter/normal	Clayish

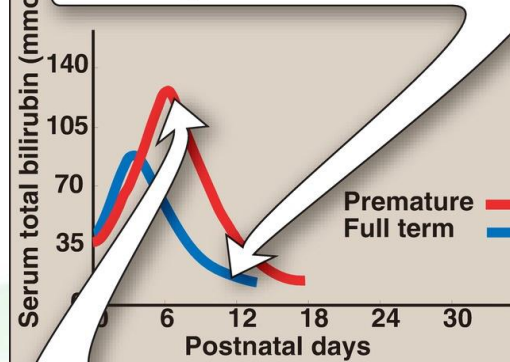
# Jaundice in newborns



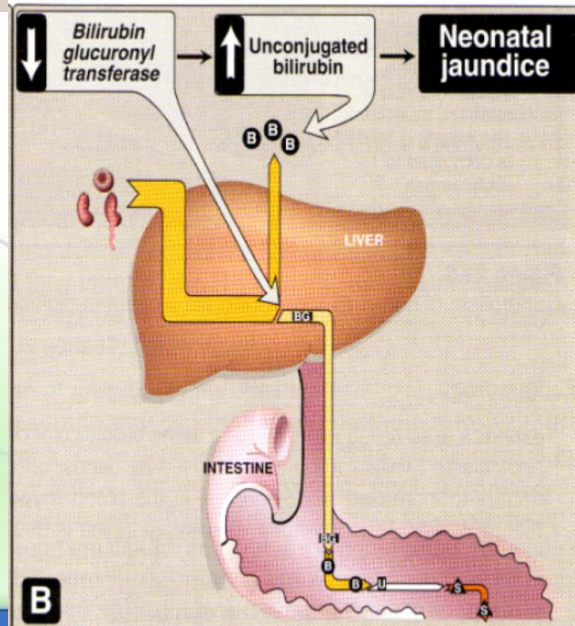
**1** Activity of the enzyme that conjugates bilirubin with glucuronic acid, *bilirubin UDP-glucuronosyltransferase (bilirubin UGT)*, is low in newborns and especially low in premature babies.



**2** Serum levels of bilirubin rise after birth in full-term infants, although usually not to dangerous concentrations.



**3** Serum levels of bilirubin in premature infants may rise to toxic levels.



# Genetic disorders



- Gilbert syndrome: mild, asymptomatic jaundice
- Crigler-Najjar syndrome: severe
- Defective glucuronosyltransferase 1A1
- Treatment:
  - Phototherapy (young age)
  - Liver transplant

