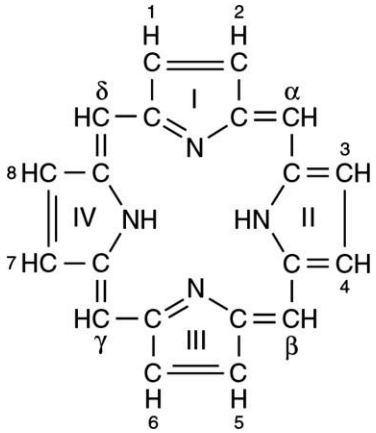
Porphyrins and Bile Pigments

The biochemistry of the porphyrins and of the bile pigments are closely related topics. Heme is synthesized from porphyrins and iron, and the products of degradation of heme are the bile pigments and iron.

Porphyrins are cyclic compounds formed by the linkage of four **pyrrole rings** through **methyne** (=HC–) bridges (also called **methenyl** bridges). In the naturally occurring porphyrins, various side chains replace the eight numbered hydrogen atoms of the pyrroles.



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

- ***Roman numerals*** *designate the pyrrole rings.*
- ***Arabic numbers*** *indicate positions at which substituents may be attached (1 through 8).*
- ***Greek letters*** *denote methyne bridges (=HC–).*

The common substituents are often abbreviated as follows:

A = acetic acid ($–CH_2–COOH$)
M = methyl ($–CH_3$)
P = propionic acid ($–CH_2–CH_2–COOH$)
V = vinyl ($–CH=CH_2$)

Names of Porphyrins:

The names of the porphyrins of interest consist of a **word** and a **number**, e.g., uroporphyrin III. The word denotes the kinds of substituents found on the ring, and the number denotes how they are arranged.

There are three important **words**:

- ✓ **Uroporphyrin** contains A and P only
- ✓ **Coproporphyrin** contains M and P only (A has been decarboxylated to M)
- ✓ **Protoporphyrin** contains M and P and V (**some** P has been decarboxylated to V)

There are two important **numbered series**, **I** and **III**.
(Series II and IV do not occur in natural systems).

- ✓ In series I the substituents repeat in a regular manner, e.g., APAPAPAP (starting with ring I).
- ✓ In series III the order of substituents in ring IV is reversed: APAPAP**PA**.

If three kinds of groups are present, as in the protoporphyrins, its immediate precursor is variously referred to as protoporphyrin III or protoporphyrin IX (being the 9^{th} isomer to be discovered).

Water Solubility of Porphyrins:
Depends on number of carboxylate groups, $-COO^-$

- ✓ Uroporphyrins, 8 carboxylates (more soluble).
- ✓ Coproporphyrins, 4 carboxylates.
- ✓ Protoporphyrins, 2 carboxylates (less soluble).

This determines routes of excretion. Water soluble compounds are excreted in urine via kidney. While, water insoluble compounds are excreted in feces via GIT.

NOTE:
Porphyrins form complexes with metal ions that bind to the nitrogen atom of each of the four pyrrole rings.
Examples:

- ✓ **Iron porphyrins** such as the **heme** of hemoglobin.
- ✓ **Magnesium porphyrin** of **chlorophyll,** the photosynthetic pigment of plants.

Functions of Heme:

Heme proteins serve diverse functions including (but not limited to):

Protein	**Protein Function**
Hemoglobin	Transport of oxygen in blood
Myoglobin	Storage of oxygen in muscle
Cytochrome *c*	Involvement in the electron transport chain
Cytochrome P_{450}	Hydroxylation of xenobiotics
Catalase	Degradation of hydrogen peroxide
Tryptophan pyrrolase	Oxidation of tryptophan

Biosynthesis of Heme:

Site of synthesis

- ✓ Heme biosynthesis occurs in **most mammalian cells** **except** mature erythrocytes, which lack mitochondria. Approximately 85% of heme synthesis occurs in **bone marrow**, and the majority of the remainder in **hepatocytes.**
- ✓ Within the cells, part of the biosynthesis reactions occurs in the **mitochondria** and part in the **cytoplasm**.

Reactions 1, 6, 7, and 8 take place in mitochondria.
Reactions 2, 3, 4, and 5 take place in cytoplasm.

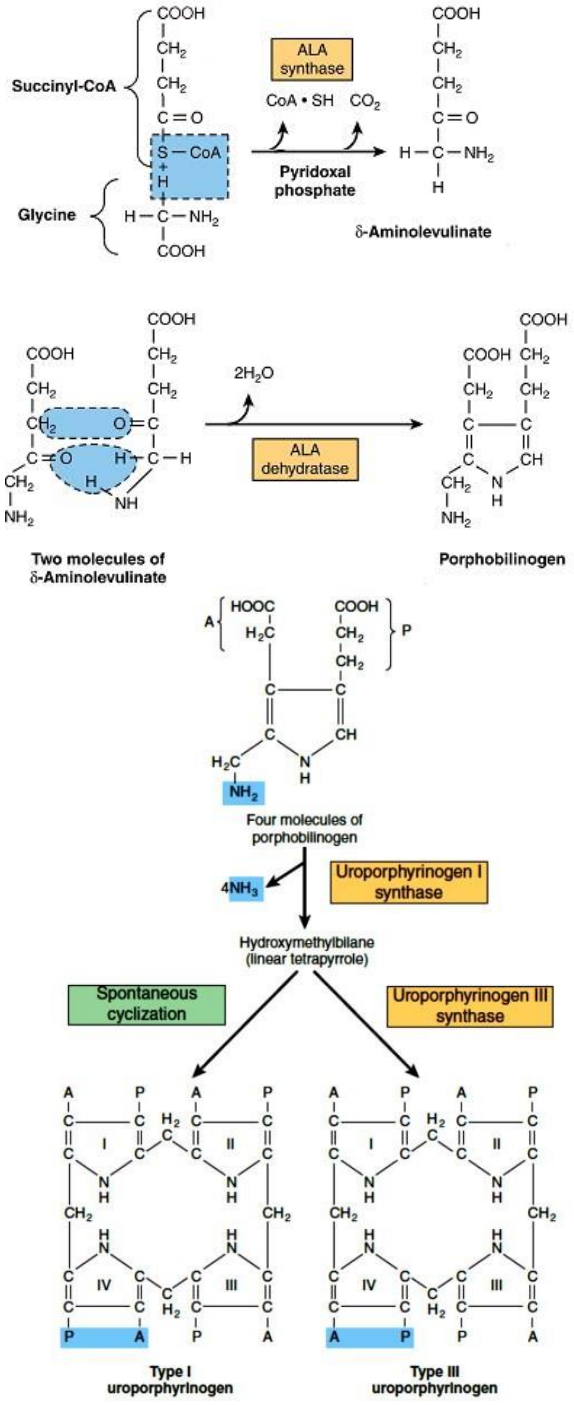
Reaction of Heme Biosynthesis

1. Heme biosynthesis is initiated by the formation of **δ- aminolevulinic acid (ALA)** from glycine and succinyl-CoA, catalyzed by the *mitochondrial* **ALA synthase**. This reaction is the **rate-limiting reaction** of heme synthesis in all tissues, and it is therefore tightly regulated. The reaction is pyridoxal phosphate-dependent.

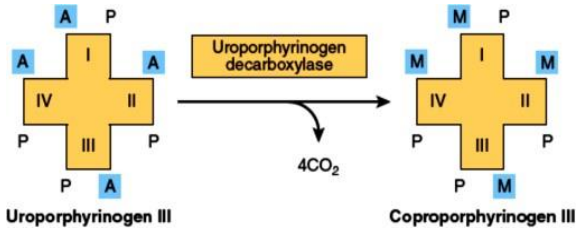
2. **Two ALA molecules** are joined together to form porphobilinogen (the first pyrrole) by the *cytoplasmic* enzyme, **ALA dehydratase**. ALA dehydratase is sensitive to inhibition by **lead,** as can occur in lead poisoning.

3. Condensation of four molecules of porphobilinogen catalyzed by the *cytoplasmic* **hydroxymethylbilane synthase** (uroporphyrinogen I synthase) forms **hydroxymethylbilane.** The four pyrrole rings in porphyrin are interconnected by methylene (-CH_2-) bridges derived from α-carbon of glycine.

4. **Hydroxymethylbilane** undergo cyclization reaction which is catalyzed by **uroporphyrinogen III synthase** to form **uroporphyrinogen III**. Hydroxymethylbilane can also cyclize spontaneously to form **uroporphyrinogen I**, but under normal conditions the uroporphyrinogen formed is almost exclusively the type III isomer. The type-I isomers of porphyrinogens are, however, formed in excess in certain porphyrias.



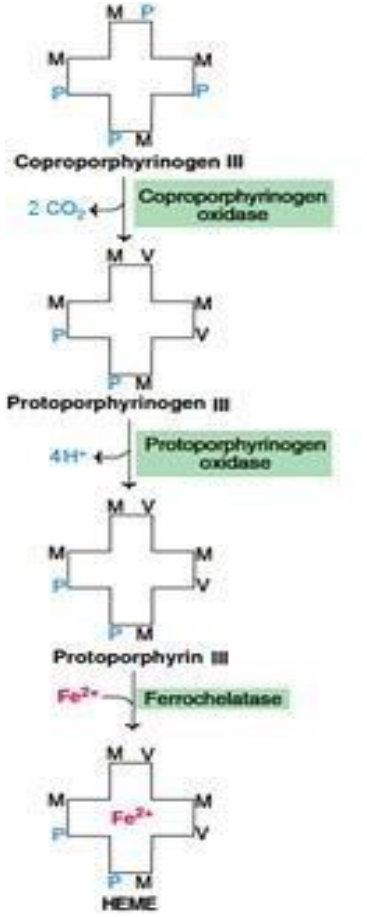
5. The *cytoplasmic* enzyme, **uroporphyrinogen decarboxylase** decarboxylates all the four acetate (A) side chains to form methyl groups (M), to form **coproporphyrinogen III**.
Uroporphyrinogen decarboxylase can also convert **uroporphyrinogen I**, if present, to **coproporphyrinogen I**.



6. The **coproporphyrinogen III** formed is acted on by the enzyme, **coproporphyrinogen oxidase**, which by oxidative decarboxylation converts two of the propionate side chains (P) to vinyl groups (V) and results in the formation of **protoporphyrinogen III**.
Coproporphyrinogen oxidase *is specific for type III coproporphyrinogen, so type I protoporphyrins generally do not occur in humans.*

7. **Protoporphyrinogen III** is next oxidized to **protoporphyrin III (also called IX)** in a reaction catalyzed by **protoporphyrinogen oxidase**.
Protoporphyrinogen oxidase oxidizes methylene groups (-CH_2-) interconnecting pyrrole rings to methenyl groups (-CH=).

8. The final step of heme biosynthesis, catalyzed by the enzyme **ferrochelatase (heme synthase)**, is the insertion of ferrous iron into **protoporphyrin III** to produce heme. Ferrochelatase is inhibited by **lead**.



Regulation of heme synthesis:

- ✓ **Feedback regulation**: heme is a feedback inhibitor of ALA synthase. It represses the synthesis and diminishes the transport of ALA synthase from cytoplasm to mitochondria.

Effects of drugs: Many drugs whose metabolism requires the hemoprotein cytochrome P_{450} increase cytochrome P_{450} biosynthesis. The resulting depletion of the intracellular heme pool induces expression of ALA synthase

- ✓ **Substrate availability**: Fe^{2+} must be available for ferrochelatase.

Porphyrinogens differ from porphyrins:

- ✓ Porphyrinogens have more hydrogen atoms and less double bonds than porphyrins.
- ✓ Porphyrinogens are colorless, However, they are easily auto-oxidized to the colored porphyrins.
- ✓ Porphyrinogens are not fluorescent while porphyrins are illuminated by ultraviolet light and emit a strong red **fluorescence.**

Disorders of heme synthesis (porphyrias)

Porphyria can be classified depending on the etiology into:
1- Genetic (defective enzyme).
2-Acquired (e.g., lead poisoning).

Porphyrias may be divided into **two** major types based on the organs most affected:

- ✓ **Erythropoietic porphyria** is a defect of porphyrin synthesis of bone marrow.
- ✓ **Hepatic porphyria** is a defect in porphyrin synthesis of the liver.

Example of acquired porphyrias

***Lead toxicity*:**

- ✓ Cause: inhibition of **ALA dehydratase** and **Ferrochelatase** by lead.
- ✓ Results: elevated levels of protoporphyrin in erythrocytes and elevated urinary levels of ALA and coproporphyrin.

Examples of hereditary porphyria

***Congenital erythropoietic porphyria*:**

- ✓ Cause: Deficiency of uroporphyrinogen III synthase.
- ✓ Results: increase in type I porphyrin.

***Acute intermittent porphyria*:**

- ✓ Cause: Deficiency of hepatic uroporphyrinogen I synthase.
- ✓ Result: increase in ALA and porphobilinogen.

NOTE:

Certain drugs (enzyme inducers e.g, barbiturates, griseofulvin) induce the production of cytochrome P_{450}. In patients with porphyria, this can precipitate an attack of porphyria (***drug-induced porphyria***) by depleting heme (the negative regulator of ALA synthase) levels. Thus, the rate of synthesis of ALA synthase is increased with the subsequent increase in levels of potentially harmful heme precursors.

Catabolism of Heme

Most of the heme which is degraded comes from **hemoglobin** in red blood cells, which have a life span of about 120 days. Human adults normally destroy about 200 billion erythrocytes per day. Thus, a 70-kg human turns over approximately **6 g of hemoglobin** daily. Since 1 g of hemoglobin yields about 35 mg of bilirubin, human adults form **250 to 350 mg of bilirubin per day**. The **globin** is degraded to its constituent amino acids, the released **iron** enters the iron pool, and all products are reused. The iron-free **porphyrin** portion of heme is also degraded.

1. Conversion of heme to bilirubin:

- ✓ **Site:**
  - Mainly in the reticuloendothelial cells of the **liver**, **spleen**, and **bone marrow**.
- ✓ **Enzymes:**
  - ***Heme oxygenase*** cleaves heme ring between the I and II pyrrole rings producing biliverdin and carbon is released as carbon monoxide (CO).
  - ***Biliverdin reductase*** reduces the central (-CH=) bridge of biliverdin to ($–CH_2–$), producing bilirubin.

2. Transport of bilirubin in blood to the liver:

Bilirubin is only sparingly soluble in water. It must be transported in the blood by a **carrier**. The physiological carrier is serum **albumin**.

3. Uptake of bilirubin by hepatocytes:

- ✓ Hepatocytes take up bilirubin from albumin by a **large capacity**, saturable facilitated transport system. Thus, transport does not appear to be rate-limiting for the metabolism of bilirubin.
- ✓ The net uptake of bilirubin depends upon its removal by subsequent metabolism.
- ✓ Once internalized, bilirubin binds to cytosolic proteins such as glutathione *S*-transferase, to prevent bilirubin from reentering the blood stream.

4. Conversion of bilirubin to bilirubin diglucuronide (conjugation):

- ✓ Bilirubin is **nonpolar,** and would persist in cells (e.g., bound to lipids) if not converted to a more water-soluble form.
- ✓ Bilirubin is converted to a more **polar** molecule by conjugation with glucuronic acid.
- ✓ A bilirubin-specific **UDP-glucuronyl transferase** catalyzes transfer of **two** glucuronyl moieties from UDP-glucuronate to bilirubin to form **bilirubin diglucuronide** in **two steps**.

5. Secretion of bilirubin diglucuronide into bile:

- ✓ It is an **active transport** mechanism.
- ✓ Probably is rate-limiting for the entire process of **hepatic bilirubin metabolism**.
- ✓ The protein involved is a **multispecific organic anion transporter** (**MOAT)** located in the plasma membrane of the bile canaliculi.

6. Processing of bilirubin diglucuronide by intestinal bacteria:

- ✓ In the intestine, the glucuronyl moieties of the conjugated bilirubin are removed by specific bacterial β-**glucuronidases** to reform bilirubin.
- ✓ Bilirubin in the intestine is converted to urobilinogens compounds most of them are excreted into the stool as **stercobilinogen** (cause of brown color of stools) after oxidation.
- ✓ A lesser amount (of urobilinogens) is recycled to the liver and either returned to bile or excreted in urine as **urobilin** (cause of yellow color of urine) after oxidation.
- ✓ Bilirubin and its catabolic products are collectively known as the **bile pigments**, which provide the yellow tinge in normal serum, the yellow-green hue in bile, the brown in stools, and the yellow in urine.

Hyperbilirubinemia causes Jaundice

- **Hyperbilirubinemia**, a blood level that exceeds 1 mg of bilirubin per dL (17 μmol/L), may result from:
- ✓ **Production** of more bilirubin than the normal liver can conjugate, or
- ✓ **Failure** of a damaged liver **to conjugate** normal amounts of bilirubin, or
- ✓ **Obstruction** of the excretory ducts of the liver prevents the excretion of bilirubin.

- When the blood concentration reaches 2 to 2.5 mg of bilirubin per dL, it diffuses into the tissues, which turn yellow, a condition termed **jaundice (**bilirubin deposits in the skin, mucous membranes, and eyes), or **icterus** (bilirubin deposits in the blood).

NOTE:

- Hyperbilirubinemia may be classified depending on the type of bilirubin present in plasma, as:
- ✓ **Retention hyperbilirubinemia** due to overproduction of bilirubin (unconjugated).
- ✓ **Regurgitation hyperbilirubinemia,** due to reflux into the bloodstream because of biliary obstruction (conjugated).
- Only ***unconjugated*** bilirubin (due to its **hydrophobicity**), can cross the blood-brain barrier into the central nervous system causing **kernicterus** (an encephalopathy due to hyperbilirubinemia), only in retention hyperbilirubinemia.
- Only ***conjugated*** bilirubin (due to its **hydrophilicity)**, can appear in urine. Accordingly:
- ✓ **Choluric jaundice** (choluria is the presence of bile pigments in the urine) occurs only in regurgitation hyperbilirubinemia.
- ✓ **Acholuric jaundice** occurs only in the presence of an excess of unconjugated bilirubin.