Porphyrin Metabolism

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BSB 535E

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- D. Final fates of bilirubin

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- A. Bilirubin accumulation and jaundice
- B. Types of jaundice

VI. Summary

Learning objectives:

1. Describe the functional roles of heme in biological systems.
2. State the key regulatory step in porphyrin metabolism and specify how it is being regulated.
3. Explain the importance of anaplerotic reactions in porphyrin synthesis.
4. Outline the steps involved in porphyrin synthesis and specify the key enzymes involved.
5. State how lead poisoning affects porphyrin metabolism and discuss how people are usually exposed to lead.
6. State key symptoms/signs of lead poisoning.
7 Specify the molecular basis of acute intermittent porphyria (AIP) and list its key symptoms/signs.
8. Explain why some drugs worsen acute intermittent porphyria and how it should be treated.
9. Specify the molecular basis of congenital erythropoietic porphyria and porphyria cutanea tarda and describe their key symptoms/signs.
10. Explain why do individuals with photosensitive porphyrias avoid sunlight.
11. Describe how heme is broken down and how heme catabolites are transported.
12. State the difference between biliverdin and bilirubin.
13. Explain why does a bruise change color over time.
14. Explain the difference between direct and indirect bilirubin and name other synonyms of them.
15. State the final fates of heme catabolites.
16. Name 3 types of jaundice and discuss how they are characterized clinically.

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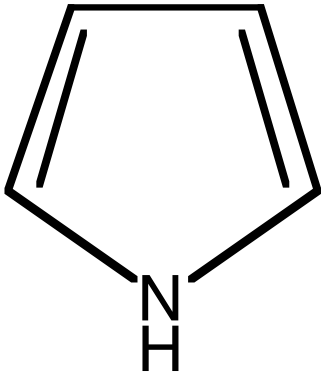
I. Overview of porphyrin metabolism

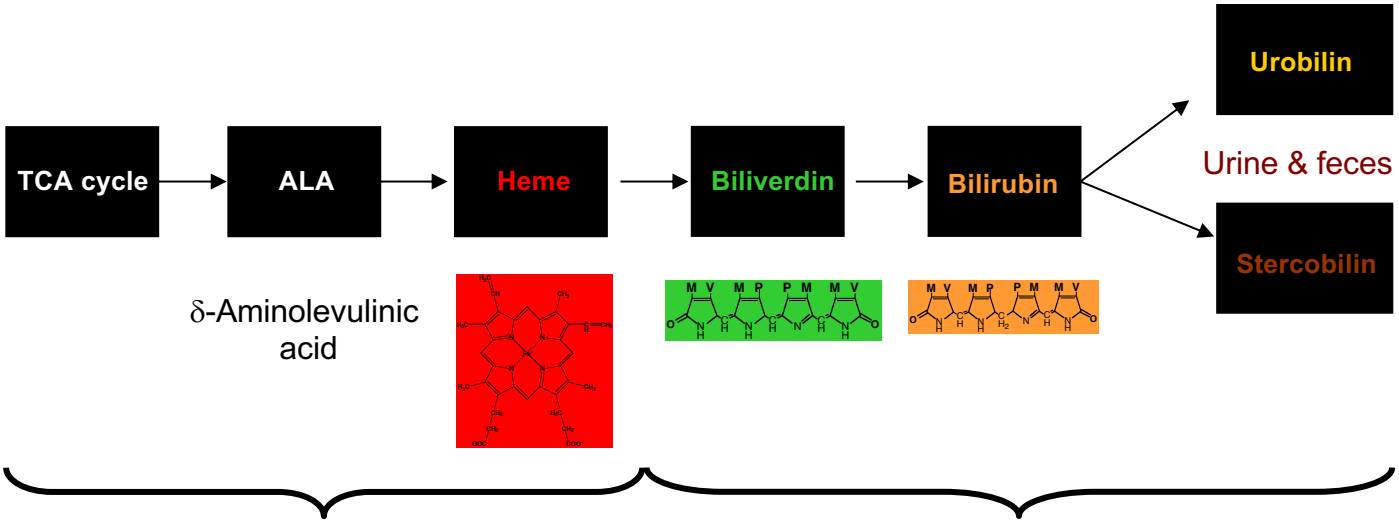
A. Pyrrole structure

Pyrrole
the basic structure in porphyrins



This is the pyrrole ring, of which there are four in each molecule of heme.

B. Porphyrin input-output



Here is an overview of both the synthesis and catabolism of heme. Initially, the TCA cycle intermediate, succinyl CoA, reacts with glycine to produce ALA (δ-aminolevulinic acid), the first intermediate in porphyrin biosynthesis. Through a series of steps, eight ALA molecules are converted to heme, a tetrapyrrole that is a red chromophore.

Heme is catabolized by cleavage of the tetrapyrrole ring to a linear tetrapyrrole, biliverdin, which is a green chromophore. Biliverdin is converted to the orange-colored bilirubin, which comes in two important forms, the water-insoluble unconjugated bilirubin and the water-soluble conjugated bilirubin. These two are measured frequently in medicine for differential diagnoses. Bilirubin ends up as urobilins in the urine and stercobilin in feces.

C. Hemoproteins

Heme
The major porphyrin in man
A prosthetic group of many proteins

- Myoglobin
- Hemoglobin
- Cytochromes of the ER (P450 & b_5)
- Cytochromes of mitochondria (P450, a, a_3, b, c_1, c)
- Catalase

Heme is made in cells that use it. It is not transported. A majority of heme, about 70%, is made in bone marrow and it goes into hemoglobin. About 15% of heme is made for liver cytochrome P450 enzymes which are present in the ER and mitochondria. That's why liver is the key site for many metabolic pathways and for drug clearance. In the ER, there is also cytochrome b_5, which is involved in the desaturation of polyunsaturated fatty acids.

As for the rest, they go into myoglobin in muscle cells, mitochondrial cytochromes of all cell types (except red blood cells which do not contain mitochondria) and catalases in many cells. Myoglobin is the major oxygen transporter in skeletal muscle and gives muscle its red color. Mitochondrial cytochromes are all components of the inner membranes where they participate in the electron transport chain. Catalase is also a heme-containing protein, and it is responsible for catalyzing the conversion of hydrogen peroxide to water and oxygen in free-radical defense. Overall, the heme components of these hemoproteins differ structurally by having different side chains.

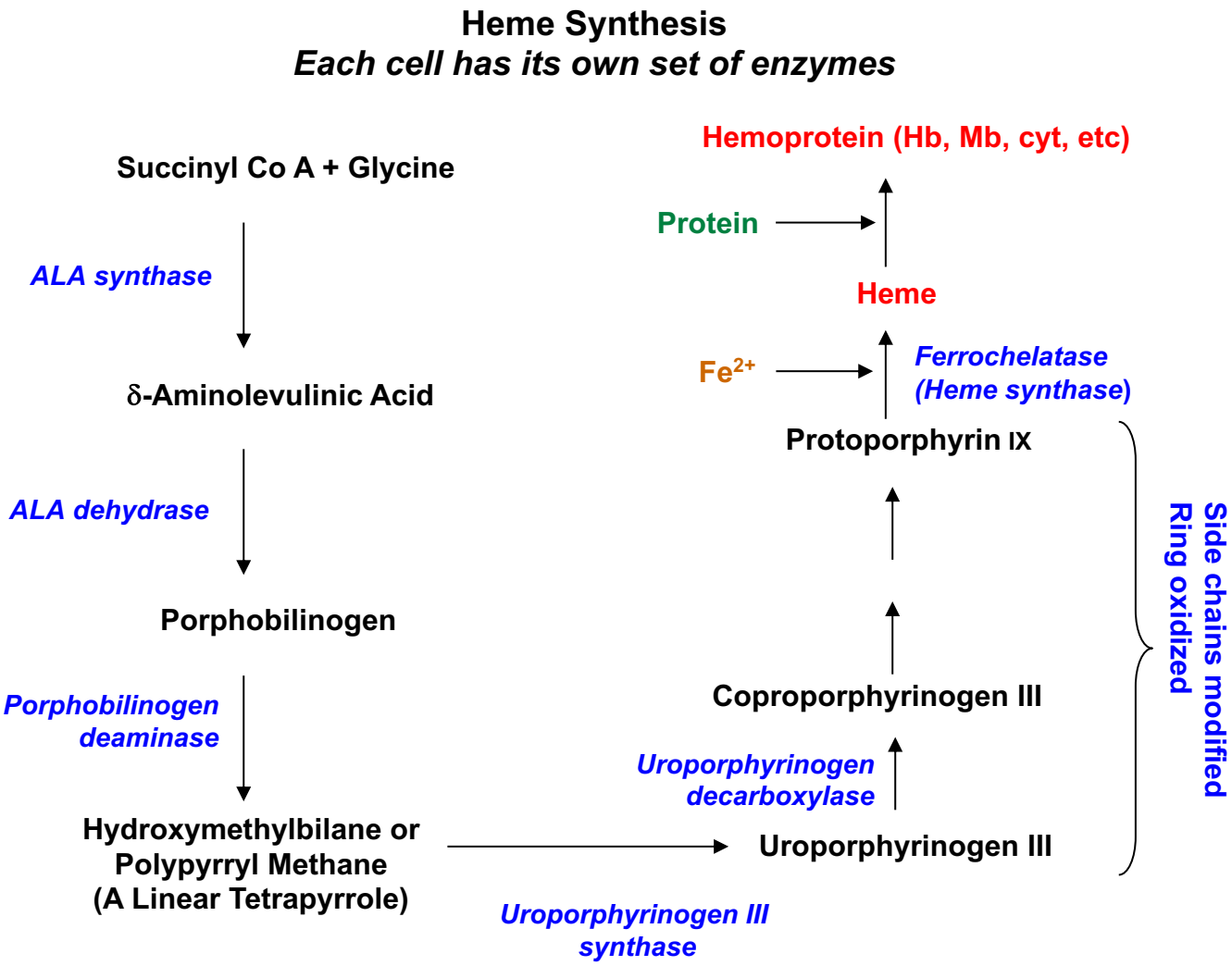
Prosthetic group

A tightly-bound, non-peptide component of proteins

Heme + Apoprotein ---> Holoprotein (aka Hemoprotein (Hb, Mb))

Porphyrins such as heme are prosthetic groups, because they are cofactors that are tightly bound to their proteins. The combination of a prosthetic group, such as heme, and an apoprotein is referred to as a holoprotein.

D. Heme synthesis overview

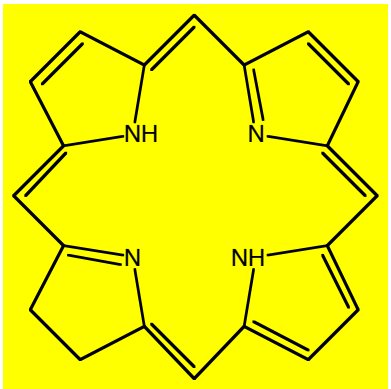


Heme synthesis begins with the condensation of succinyl CoA with glycine. This reaction is catalyzed by δ-aminolevulinic acid synthase (ALA synthase or δ-aminolevulinate synthase) and it represents the key regulatory step in heme synthesis. The first tetrapyrrole ring that is formed is uroporphyrinogen III, the side chains of which are modified to form protoporphyrin IX. Ferrous ion (Fe^{2+}) is then inserted into protoporphyrin IX by ferrochelatase to form heme.

Tetrapyrrole ring

Conjugated double bonds make it a chromophore

The tetrapyrrole ring is the basic ring structure of all mature porphyrins. Note that the ring contains a conjugated double bond system.



II. Heme synthesis

A. Steps involved in heme synthesis

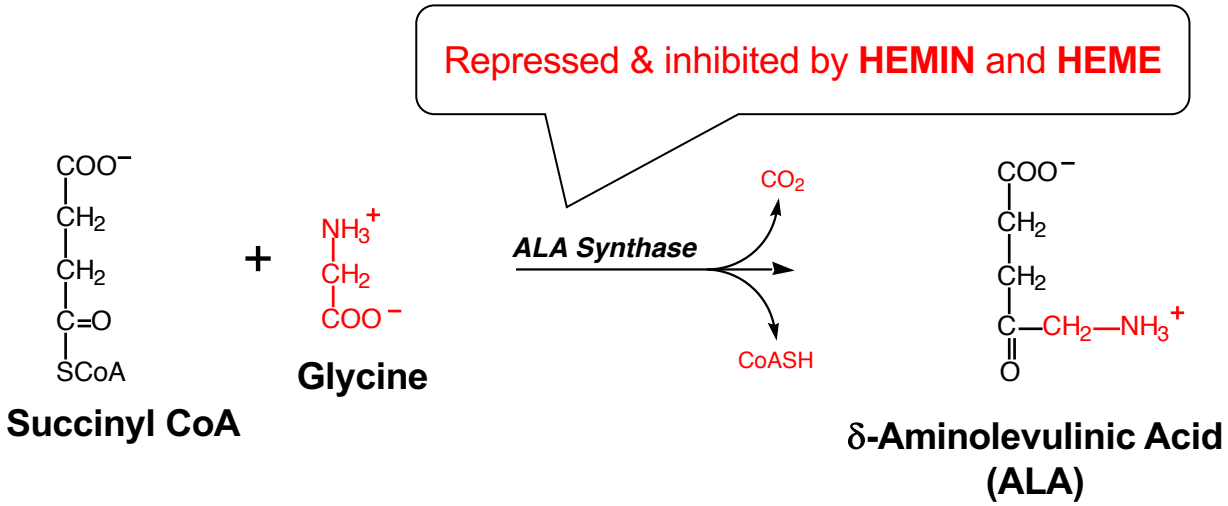
i. ALA synthesis: the rate-limiting step

The **rate-limiting step** of porphyrin biosynthesis is catalyzed by **ALA synthase**. This enzyme decarboxylates glycine and combines it with the succinate portion of succinyl CoA. Here, we see another example where a reaction is driven by a high-energy thioester of coenzyme A. Once ALA is produced, it is committed to transit the rest of the porphyrin biosynthetic pathway.

ALA synthase is allosterically regulated by hemin and heme, end products of the pathway. As shown, hemin is the oxidized form of heme in which the iron has been converted from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) state. Hemin can no longer function as an oxygen carrier, but it is an important regulator of ALA synthase. The reaction catalyzed by ALA synthase requires pyridoxal phosphate. Pyridoxal phosphate is made from vitamin B_6 (pyridoxine), and it is required by a number of enzymes, especially transaminases.

δ-Aminolevulinate (ALA) synthase

- Catalyzes the rate-limiting step in porphyrin synthesis
- Reactants are succinyl CoA (TCA) and glycine
- Requires PLP (Pyridoxal Phosphate)
- Hemin and heme repress and allosterically inhibit ALA synthase

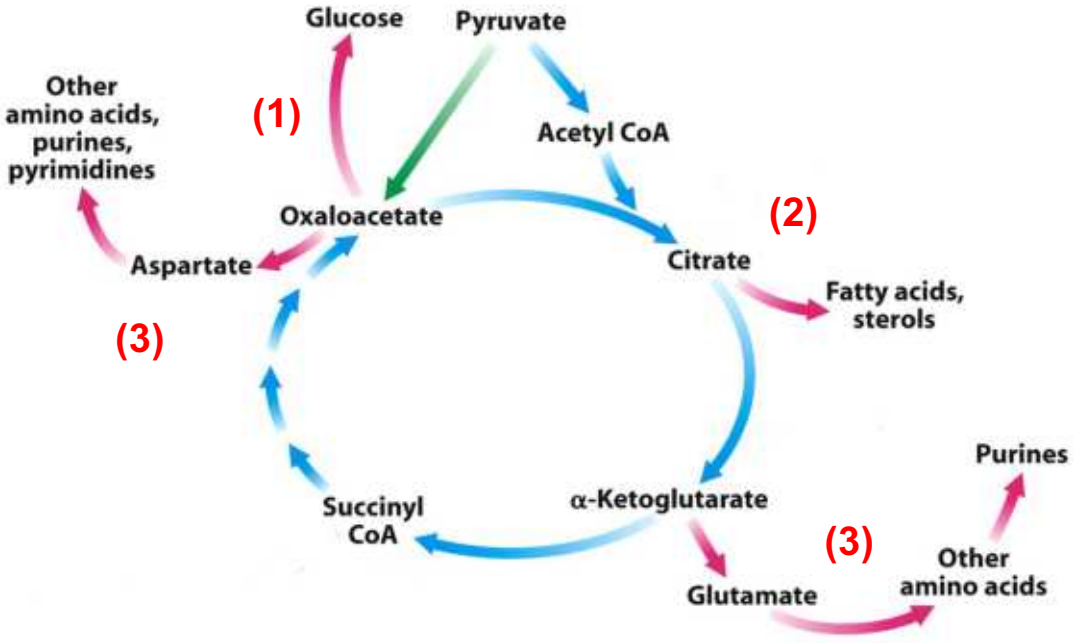


Heme (Fe^{2+}) Ferrous form → (- e^-) → Hemin (Fe^{3+}) Ferric form

- Hemin is the oxidized form of heme.
- Hemin does not carry O_2, but it can function as an important regulator of ALA synthase.

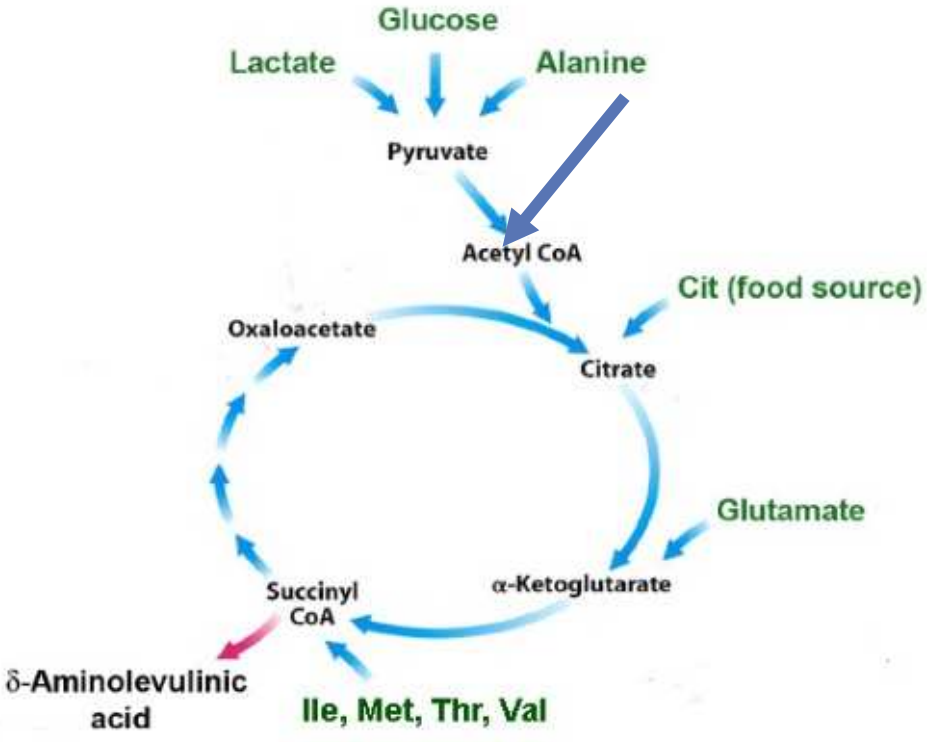
ii. Anaplerotic reactions provide precursors for heme synthesis

Anaplerotic reactions and the synthesis of glucose, fatty acids, steroids, amino acids, purines, and pyrimidines



The TCA cycle supplies intermediates that can be used for the synthesis of glucose (1), fatty acids, steroids (2), amino acids, purines, and pyrimidines (3). These TCA cycle intermediates can be replenished by anaplerotic reactions using pyruvate, oxaloacetate, and other biomolecules.

Anaplerotic reactions are required for porphyrin synthesis

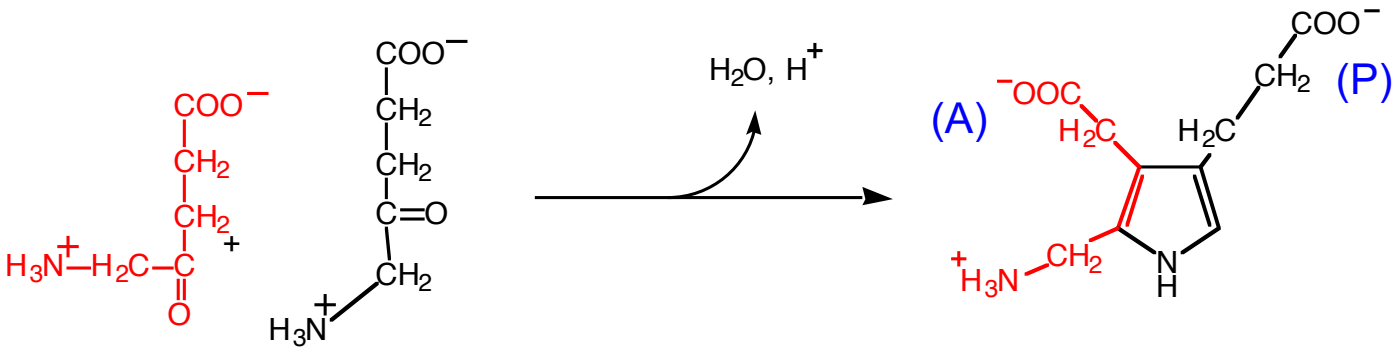


For porphyrin synthesis, succinyl CoA can be produced via anaplerotic reactions from a number of sources, including pyruvate (formed from glucose, lactate, and alanine), citrate, glutamate, and branched-chain amino acids. For each molecule of heme synthesized, 8 molecules of succinyl CoA are required.

iii. Porphobilinogen synthesis

Two ALA molecules react with one another in a reaction catalyzed by ALA dehydrase, which removes water and a proton to produce porphobilinogen, which contains a pyrrole ring. Its propionyl and acetyl side chains will later be modified. Both ALA and porphobilinogen are neurotoxins, and it is important to keep them at low levels in metabolic systems. In addition, this enzyme requires zinc, which is linked via two sulfhydryl groups. Moreover, this enzyme is sensitive to lead inhibition.

δ-Aminolevulinate dehydrase converts ALA to porphobilinogen



ALA **ALA** **Porphobilinogen**

Note acetate (A) and propionate (P) - two acidic side chains.

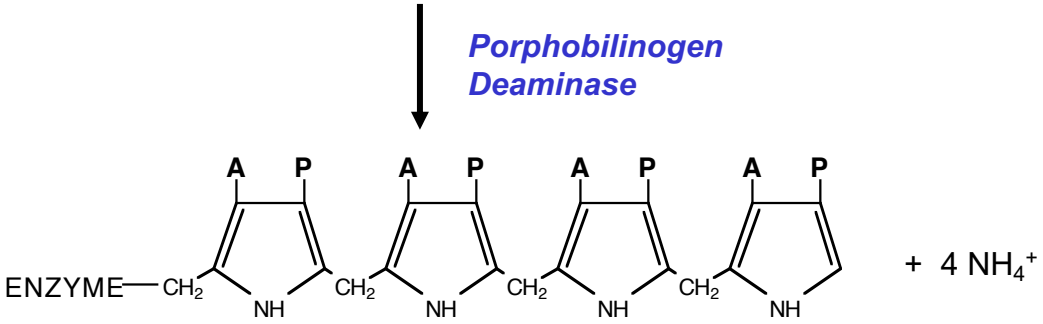
ALA dehydrase contains functionally important **sulfhydryl** groups, as well as **zinc**, which is apparently linked to them.

- **Lead** is a powerful inhibitor of this enzyme, because it replaces the zinc.
- A dietary **zinc deficiency** will impair this enzyme, leading to anemia.

iv. Hydroxymethylbilane synthesis

Porphobilinogen deaminase forms a linear tetrapyrrole

4 Porphobilinogens



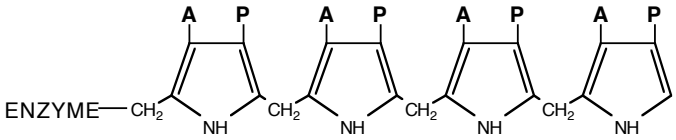
(Hydroxymethylbilane or Polypyrryl methane)
A linear tetrapyrrole

Note the symmetry of the acetate and propionate side chains

Porphobilinogen deaminase synthesizes hydroxymethylbilane or polypyrryl methane from four molecules of porphobilinogen. Each porphobilinogen is deaminated, and free ammonia is produced. Hydroxymethylbilane is a linear tetrapyrrole. The acetyl and propionyl side chains are symmetric at this point and can be read as APAPAPAP.

v. Uroporphyrinogen III synthesis

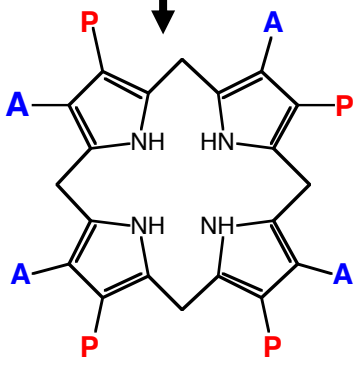
Uroporphyrinogen III synthase forms the tetrapyrrole ring



Hydroxymethylbilane

Note the asymmetry

A P A P A P P A



Uroporphyrinogen III

A = Acetate
P = Propionate

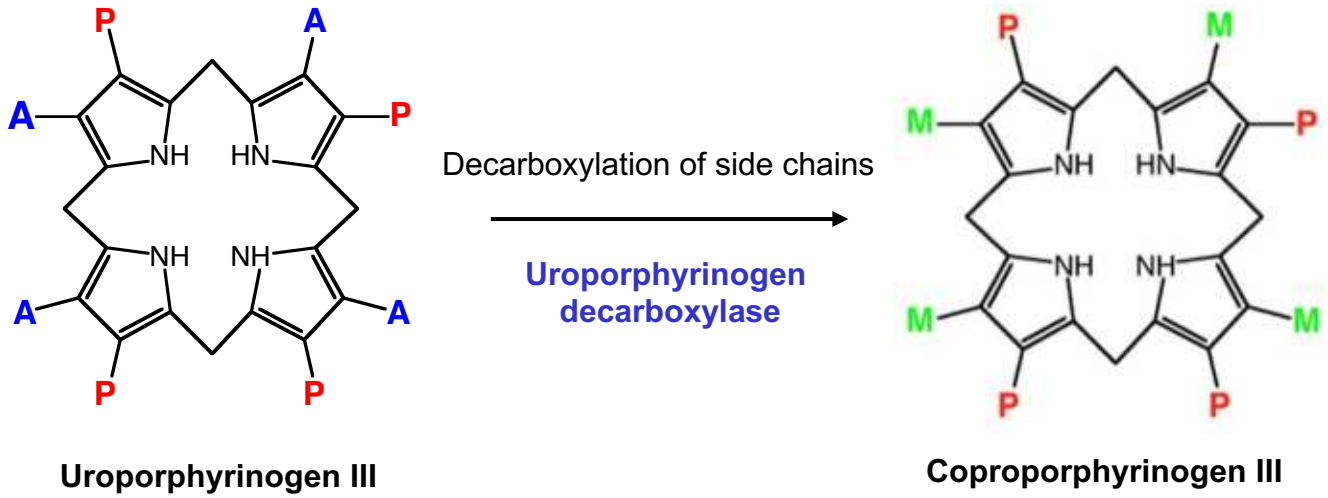
- Colorless (double bonds are not conjugated)
- Abundant in nature

Uroporphyrinogen III synthase converts hydroxymethylbilane to the colorless uroporphyrinogen III. Uroporphyrinogen III is the first ring form of tetrapyrrole. This intermediate is asymmetric, because the side chains are not in the same linear order as in hydroxymethylbilane. Reading from the upper left corner of the structure, it may be seen that the order is APAPAPPA.

vi. Coproporphyrinogen III synthesis

Uroporphyrinogen III is then acted on by uroporphyrinogen decarboxylase to form coproporphyrinogen III. In the process, the side-chain acetyl groups are converted to methyl groups.

Coproporphyrinogen III synthesis



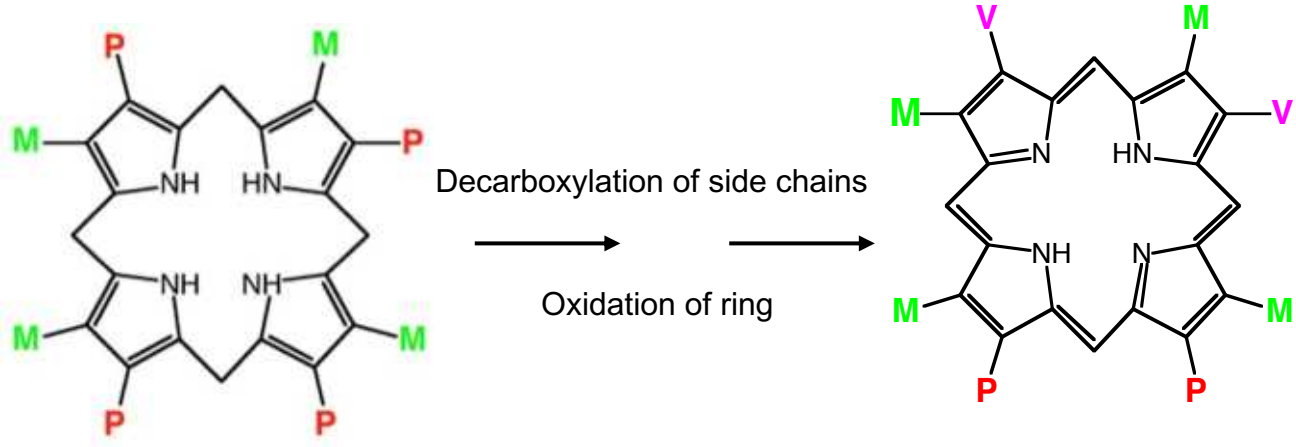
Uroporphyrinogen III **Coproporphyrinogen III**

Side-chain modifications

$-CH_2-COO^-$ (Acetyl (A)) $\xrightarrow{O_2}$ $-CH_3$ (Methyl (M)) + CO_2

vii. Protoporphyrin IX synthesis

Protoporphyrin IX synthesis



Coproporphyrinogen III

Protoporphyrin IX
(Heme without ferrous iron)

Side-chain modifications

$-CH_2-CH_2-COO^-$ $\xrightarrow{O_2}$ $-CH=CH_2$ + CO_2

Propionyl (P) **Vinyl (V)**

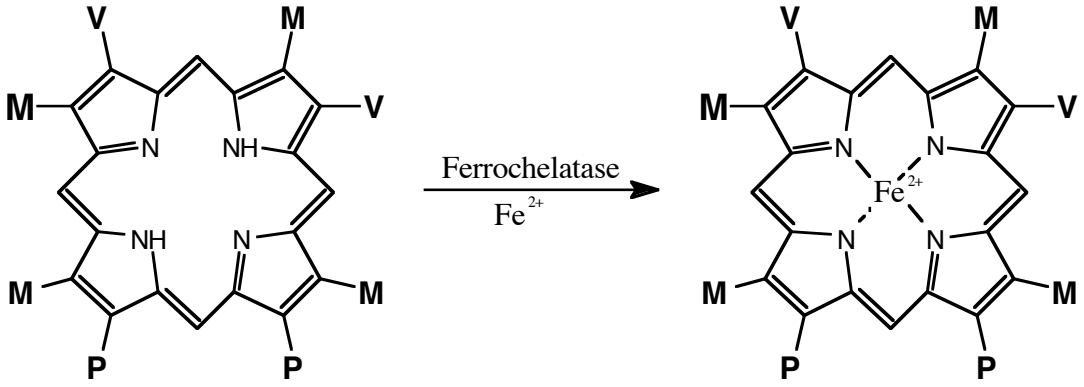
➡Notes:
- •No iron yet
- •Fewer charged groups
- •Ring is conjugated

In the next couple of reactions, coproporphyrinogen III is converted to protoporphyrin IX, which is heme without iron. Two of the acidic propionyl groups are converted to uncharged vinyl groups. In addition, oxidation reactions result in a conjugated ring system (alternating double and single bonds). Cytochrome P450 enzymes in mitochondria carry out these oxidation reactions.

viii. Formation of heme

In the final step, the enzyme **ferrochelatase** inserts a ferrous ion (Fe^{2+}) into **protoporphyrin IX** to synthesize **heme**. In addition to forming bonds with the 4 pyrrole nitrogen atoms, the Fe^{2+} ion in the middle can form two additional bonds. In hemoglobin, the fifth bond or fifth coordination site is bound to a histidine residue while the sixth bond enables the binding of an oxygen molecule.

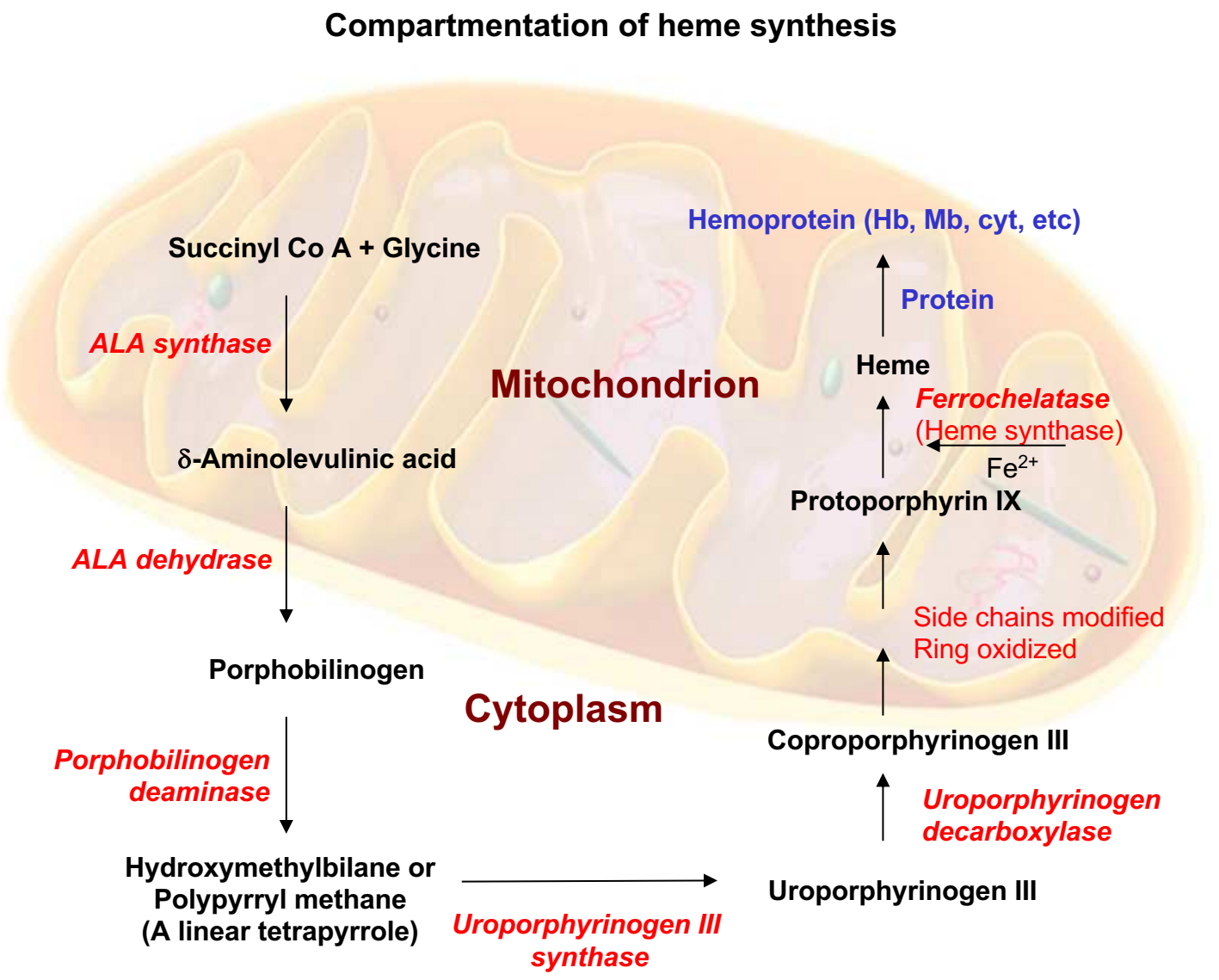
Ferrochelatase completes the heme synthesis



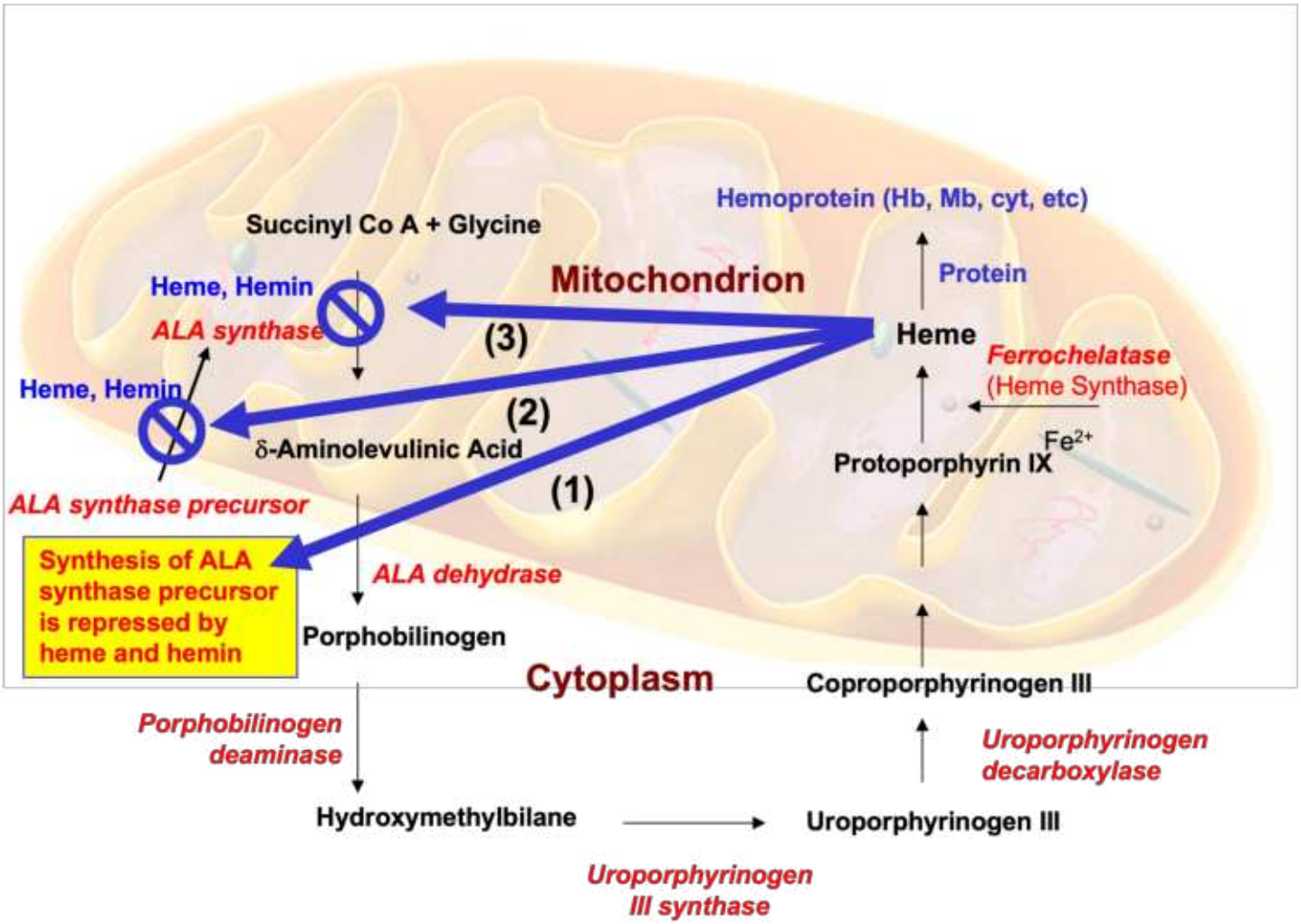
Protoporphyrin IX Heme

B. Compartmentation of heme synthesis

Porphyrinogenesis begins in the mitochondrion, continues in the cytoplasm, and ends in the mitochondrion. Once ALA is synthesized, it must be transported out of the mitochondrion into the cytoplasm where a series of reactions result in the formation of coproporphyrinogen III. This intermediate is then transported back into the mitochondrion where heme synthesis is completed. Heme is then incorporated into various apoproteins to make hemoproteins such as hemoglobin, myoglobin, and cytochromes.



C. Regulations of heme synthesis



Excess heme and hemin play multiple regulatory roles in porphyrin biosynthesis via product inhibition. Both heme and hemin repress the biosynthesis of ALA synthase by reducing the synthesis of its precursor (1). In addition, they both inhibit the transport of the ALA synthase precursor into the mitochondrion where posttranslational modification occurs (2). Lastly, they both allosterically inhibit ALA synthase (3).

III. Heme synthesis related diseases

A. Lead poisoning

Lead poisoning

A few facts:

- Because of the lack of clear symptoms, lead poisoning is difficult to recognize in its early phases
- One of the most difficult environmental health problems to control
- In children, it can cause anemia and a profound encephalopathic crisis
- In adults, it may manifest itself as fatigue, abdominal pain, and/or arthralgia
- Seriously damaging and may lead to death.
- Biochemical effects leading to anemia:
    1. Inactivates ALA dehydrase
    2. Inactivates ferrochelatase indirectly, resulting in the formation of zinc protoporphyrin

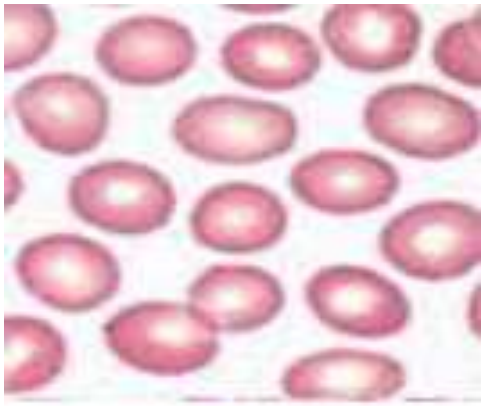
Lead poisoning is a serious public health problem. In the past, lead was used quite liberally in paints and gasoline. Children are especially susceptible because they ingest paint fragments or breathe the dust of old paint while playing on the floor or outside. The symptoms of lead poisoning are very difficult to differentiate in the early phase. The major pathogenic problem with lead poisoning involves the central nervous system and can lead to behavioral problems as well as encephalopathic crises and even death.

Regarding the porphyrinogenic pathway, lead inhibits two enzymes: ALA dehydrase and ferrochelatase. This, of course, provides a logical explanation for the anemia often seen in lead poisoning. Lead can bind covalently to sulfhydryl groups of ALA dehydrase and cause large conformational changes, leading to its irreversibly denaturation. Clinically, if one suspects lead poisoning, then blood levels of lead can be measured. The mechanism by which lead inhibits ferrochelatase is unclear. Zinc protoporphyrin can also be used in the diagnosis, because this is produced when lead inhibits ferrochelatase.

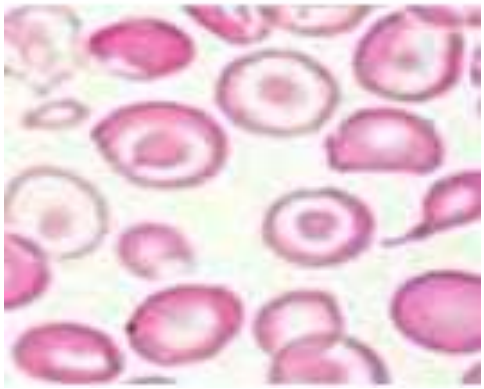
Lead poisoning gives rise to hypochromic microcytic anemia, which is characterized by red blood cells that are fewer in number, smaller in volume (MCV < 80 fL), and paler in color. Basophilic stippling of red blood cells is commonly associated with lead poisoning. It is characterized by the appearance of small dots at the periphery of erythrocytes due to aggregated ribosomes. Such stippling may also appear with severe anemia, other heavy metal poisoning, exposure to some chemicals, and septicemia.

Lead poisoning leads to hypochromic microcytic anemia and basophilic stippling

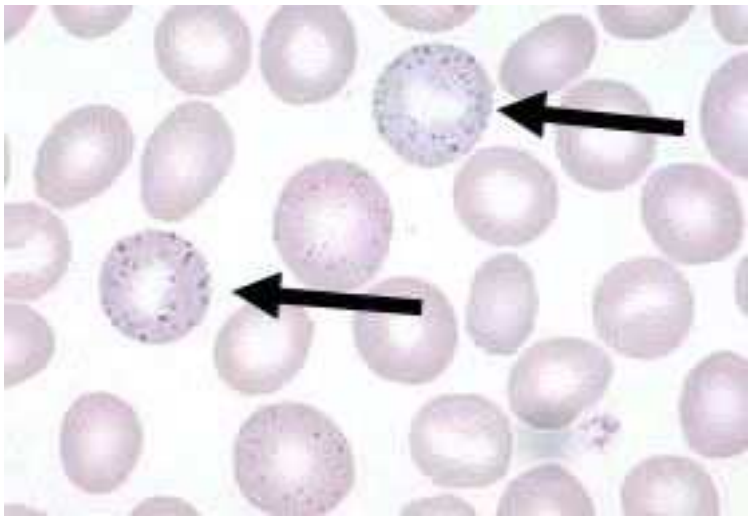
Normal



Microcytic anemia

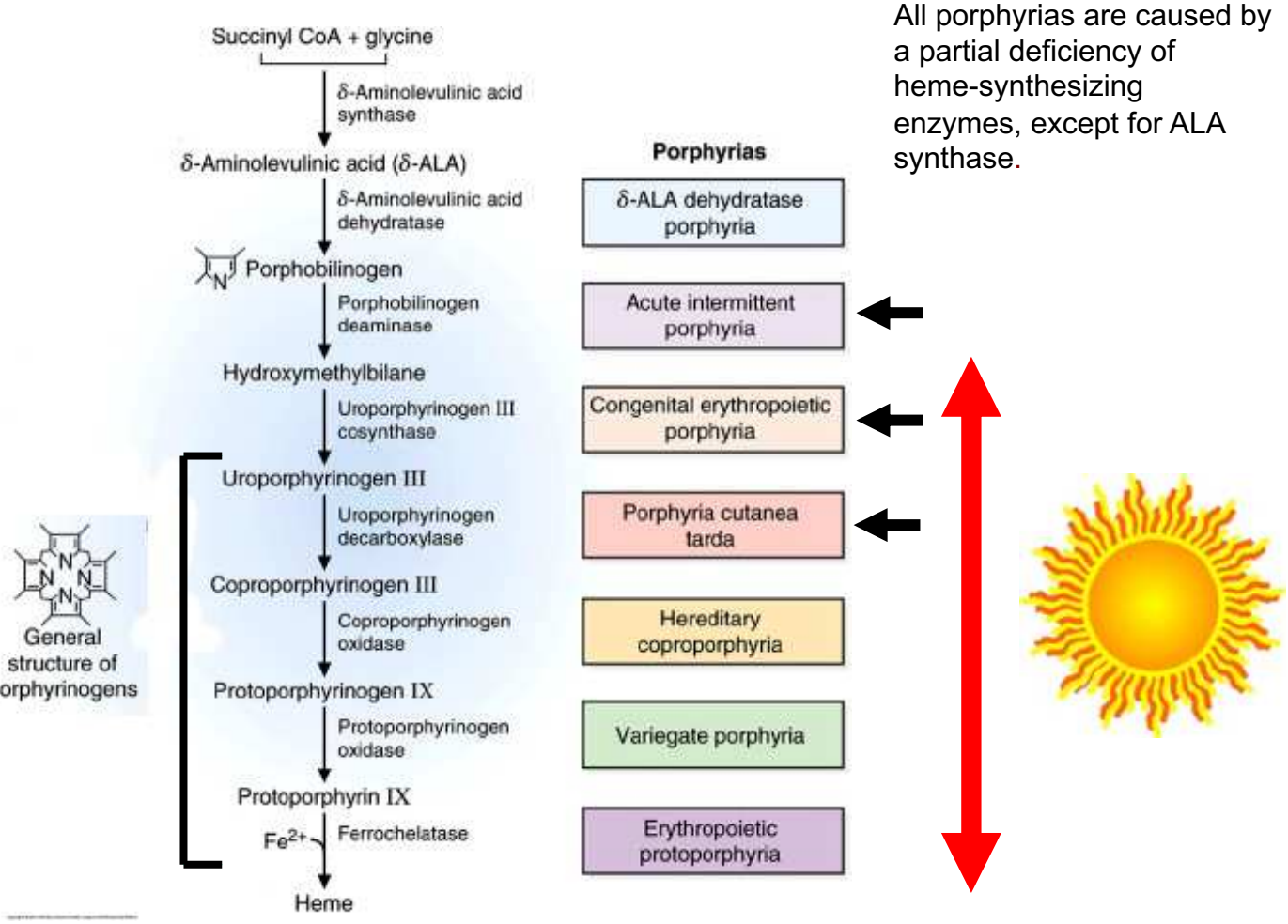


Basophilic stippling



B. Porphyrias

All porphyrias are caused by a partial deficiency of heme-synthesizing enzymes, except for ALA synthase.



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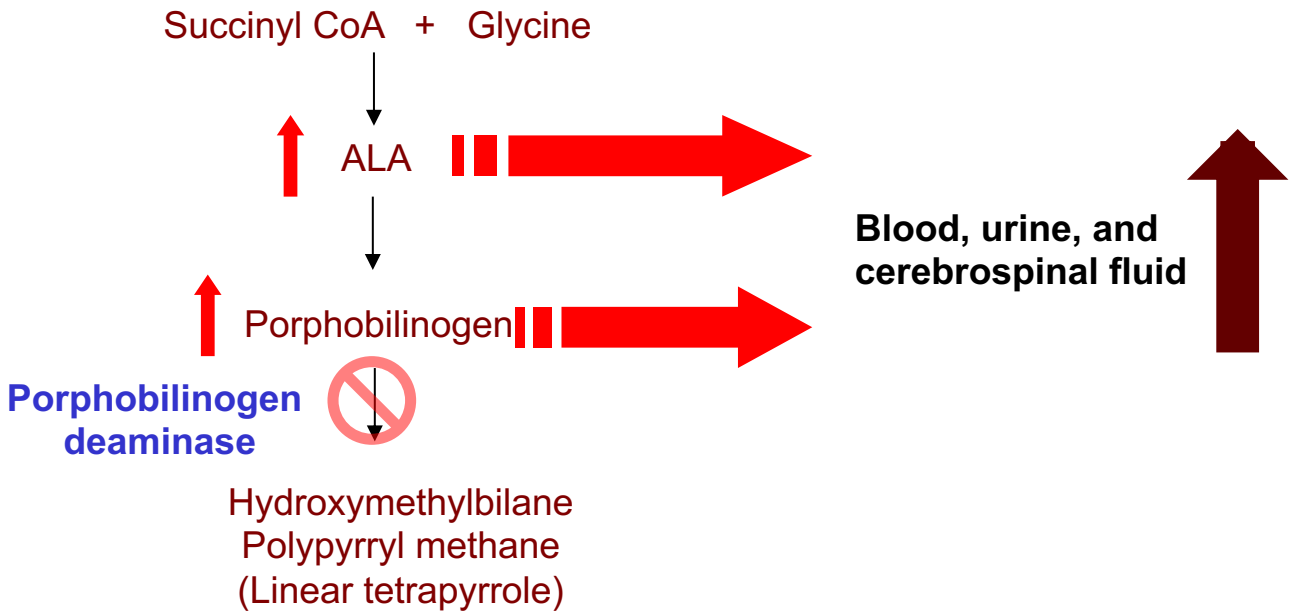
Genetic defects in enzymes involved in heme synthesis, with the exception of ALA synthase, lead to porphyria. Of clinical significance is the fact that all of the porphyrias, **except** ALA dehydrase and acute intermittent porphyrias, induce **photosensitivity**. In addition, other frequent symptoms include neuropsychiatric problems and abdominal pain. Porphyrias are relatively rare and are sometimes incorrectly diagnosed as psychiatric disorders. There are many medical horror stories about patients who have had unrecognized defects in this pathway and were passed from one physician to another without a proper diagnosis.

i. Acute intermittent porphyria

Acute intermittent porphyria mechanism

- Cause: partial deficiency (50%) of **porphobilinogen deaminase** (autosomal dominant trait)
- ALA and porphobilinogen accumulate
- Symptoms are not caused by lack of heme, but by accumulation of porphobilinogen (PBG) and ALA



The mechanism of **acute intermittent porphyria** (**AIP)** is relatively simple. It is caused by a defect in **porphobilinogen deaminase**, the enzyme that converts porphobilinogen to the linear tetrapyrrole, methylbilane. Both ALA and porphobilinogen (PBG) accumulate. At high concentrations, these compounds are **toxic** by unknown mechanisms to the nervous system. As a result, most of the symptoms and signs are related to a dysfunctional nervous system. The urine contains porphobilinogen, which can form porphyrins upon exposure to light, giving rise to port wine-colored (red-colored) urine.

Lina Rebeiz's Road to Medical School

https://www.youtube.com/watch?v=SM6BFzum8yI

Acute intermittent porphyria (AIP)

•Sufferers typically have a series of mysterious, ultimately incapacitating illnesses
•Attacks come and go without warning & may last for days or months and become chronic
•Main symptoms/signs center around the nervous system

Nervous trembling
Altered consciousness
Intractable abdominal pain
Terrible insomnia
Port-wine colored urine
No photosensitivity

•Patients are often misdiagnosed as having a psychiatric disorder
•Treatment with certain drugs are potentially fatal; for example:

barbiturates
phenytoin
tranquilizers
antipsychotics
hypnotics

The Good News:
90% with the genetic trait never have symptoms

So, how would one go about diagnosing this disorder? The abdominal pain and other symptoms/signs are commonly associated with other disorders. Elevated PBG and ALA in the urine would give a very good clue. One can also check the level of porphobilinogen deaminase in red blood cells. There would be no symptoms of photosensitivity, and none of the porphyrins would be elevated.

5 P's of AIP

•Painful abdomen
•Port wine-colored urine
•Polyneuropathy
•Psychological disturbances
•Precipitated by drugs

How some drugs worsen AIP

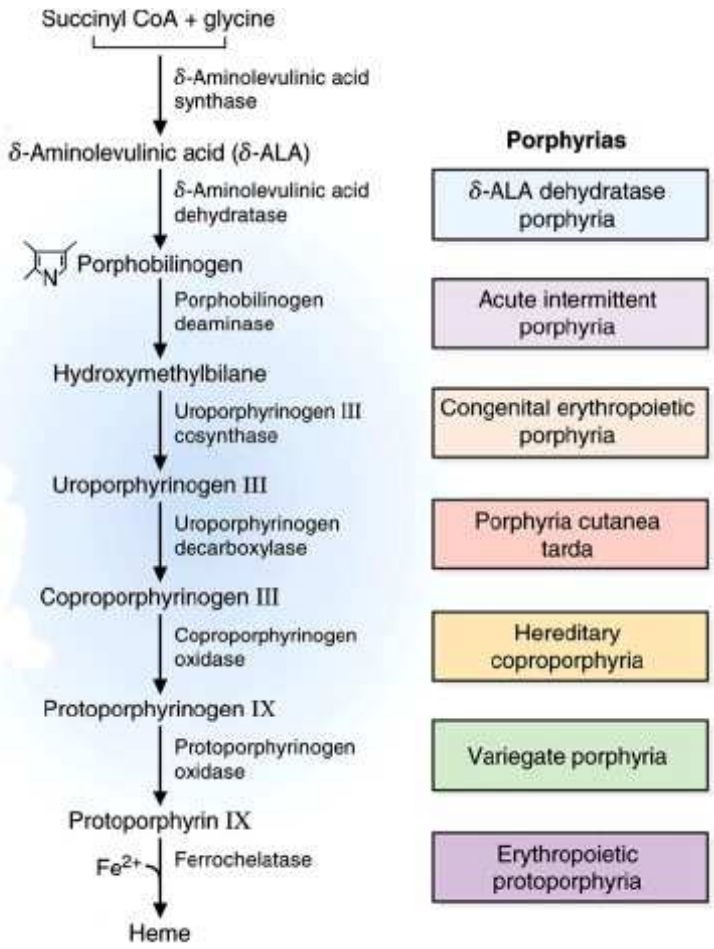
- The cytochrome P450 enzymes contain 65% of heme in the liver. They are used in drug metabolism.

- Relative to other porphyrinogenic enzymes, uroporphyrinogen III synthase is lower in the liver than other tissues.

- Drugs induce the synthesis of apoproteins of cytochrome P450 enzymes and deplete free heme pool in the liver.

- Heme depletion derepresses ALA synthase, causing accumulation of both ALA and porphobilinogen (PBG).

- This can prove fatal in some individuals.



Some drugs, such as barbiturates, tranquilizers, and antipsychotics stimulate ALA synthase, which along with the defective porphobilinogen deaminase, markedly increase the concentrations of δ-aminolevulinic acid and porphobilinogen. Elevated levels of these compounds would then further exacerbate nervous system dysfunction.

The drugs in question induce the synthesis of cytochrome P450 apoproteins. These enzymes are the major mechanism for metabolizing drugs. Since there is such an abundance of these enzymes in the liver, the increased demand for heme can deplete the heme pool. Since heme both inhibits ALA synthase allosterically and acts as a repressor of its synthesis, so the lowered amount of heme results in activation of ALA synthase. Since porphobilinogen deaminase is defective, both δ-aminolevulinic acid (ALA) and porphobilinogen accumulate to toxic levels.

Treatment of AIP

•Withdraw offending drugs

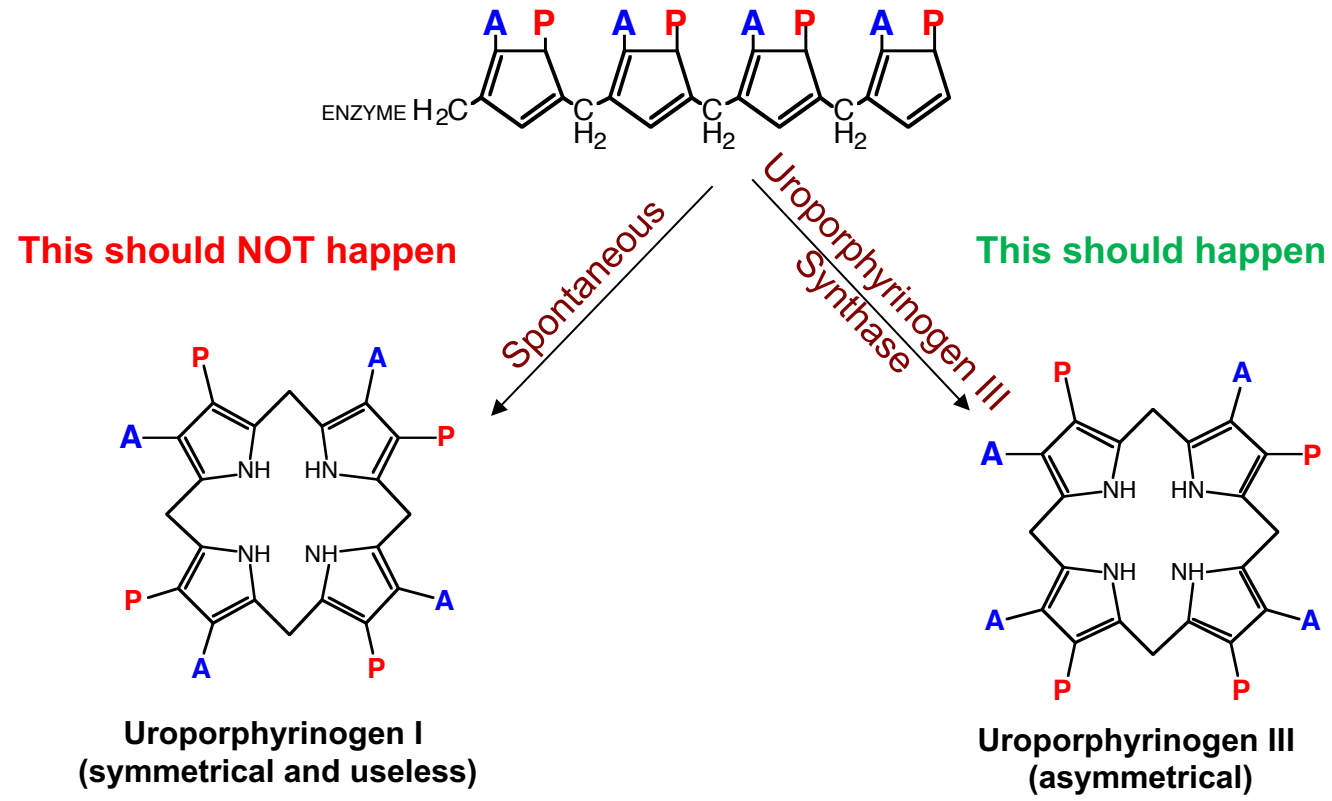
•Administer hematin (hemin). This represses and allosterically inhibits ALA synthase

•Carbohydrate-rich diet--this represses the same enzyme

ii. Congenital erythropoietic porphyria

When there is a genetic defect in uroporphyrinogen III synthase, uroporphyrinogen I is formed spontaneously. Reading from the upper left corner of uroporphyrinogen I, it may be seen that the order of the side chains is APAPAPAP. This compound will form some, but not all of the analogous intermediates as uroporphyrinogen III. However, they have no function.

Congenital erythropoietic porphyria mechanism



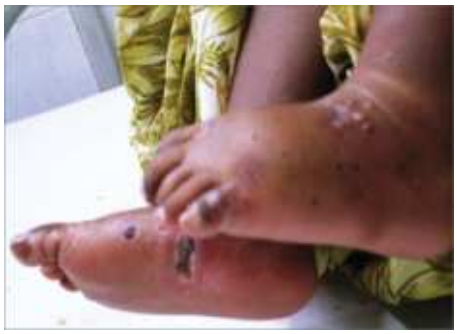
Uroporphyrinogen I (symmetrical and useless)

Uroporphyrinogen III (asymmetrical)

Defects in **uroporphyrinogen III synthase** cause **congenital erythropoietic porphyria**. There are some 36 known defects in this enzyme, each one due to the substitution of a different amino acid in the enzyme. In any case, when this enzyme is defective, methylbilane accumulates and is non-enzymatically converted to uroporphyrinogen I. Upon exposure to sunlight, uroporphyrinogen I may form free radicals which attack lysosomes, leading to the release of many hydrolytic enzymes. Once released, these enzymes destroy the cell by digesting virtually everything in the cell.

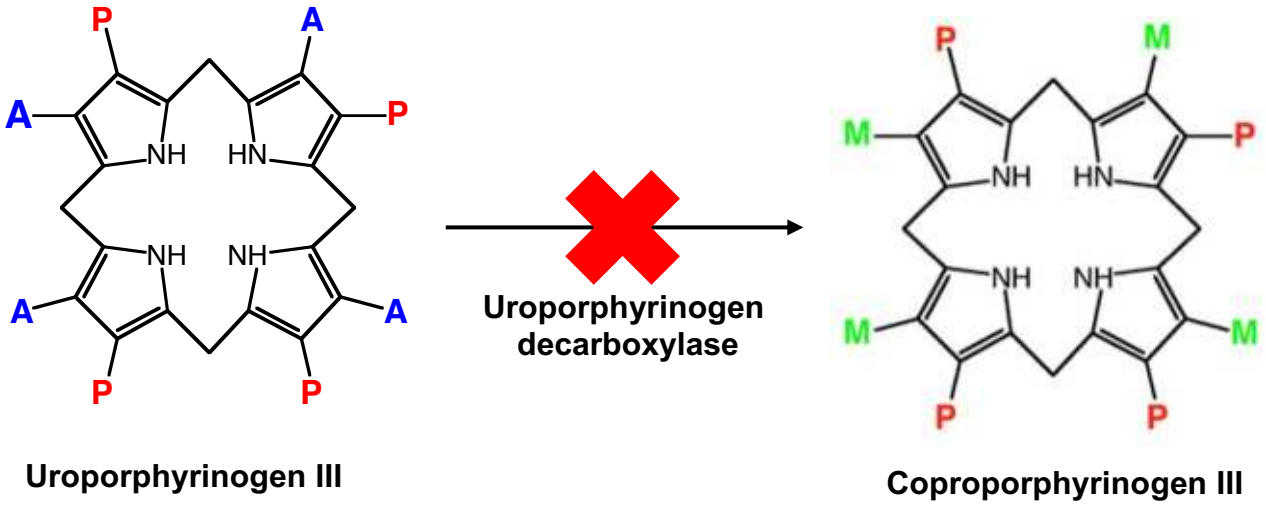
Congenital erythropoietic porphyria

- Genetically defective uroporphyrinogen III synthase
  - -36 variants known
- Uroporphyrinogen I and its metabolites accumulate
- Hypersensitivity to sunlight
  - -skin blisters
  - -ulcerating vesicles form
  - -these can become infected
  - -scarring and disfiguring is severe especially on hands and face
- Hemolytic anemia and enlargement of spleen
- Red wine-colored urine



iii. Porphyria cutanea tarda

Porphyria cutanea tarda mechanism



Uroporphyrinogen III **Coproporphyrinogen III**

Side-chain modifications

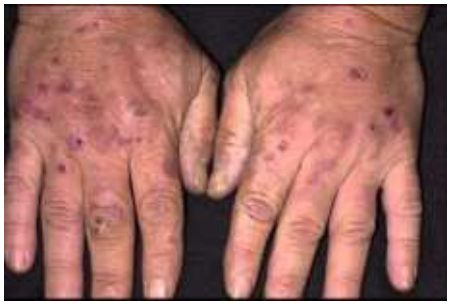
$-CH_2-COO^-$ —O_2→ $-CH_3 + CO_2$

Acetyl (A) Methyl (M)

Porphyria cutanea tarda is caused by a deficiency in **uroporphyrinogen decarboxylase**, the enzyme that decarboxylates uroporphyrinogen III to form coproporphyrinogen III. This leads to the accumulation of uropophyrinogen III and its byproducts.

Porphyria cutanea tarda

- The most common subtype of porphyrias
- Two major forms: familial (20%) and sporadic (80%)
- Uroporphyrinogen III and its metabolites accumulate
- Hypersensitivity to sunlight
- Alcohol abuse can aggravate the condition
- Often associated with hepatitis C infection
- Many individuals with this condition do not experience symptoms

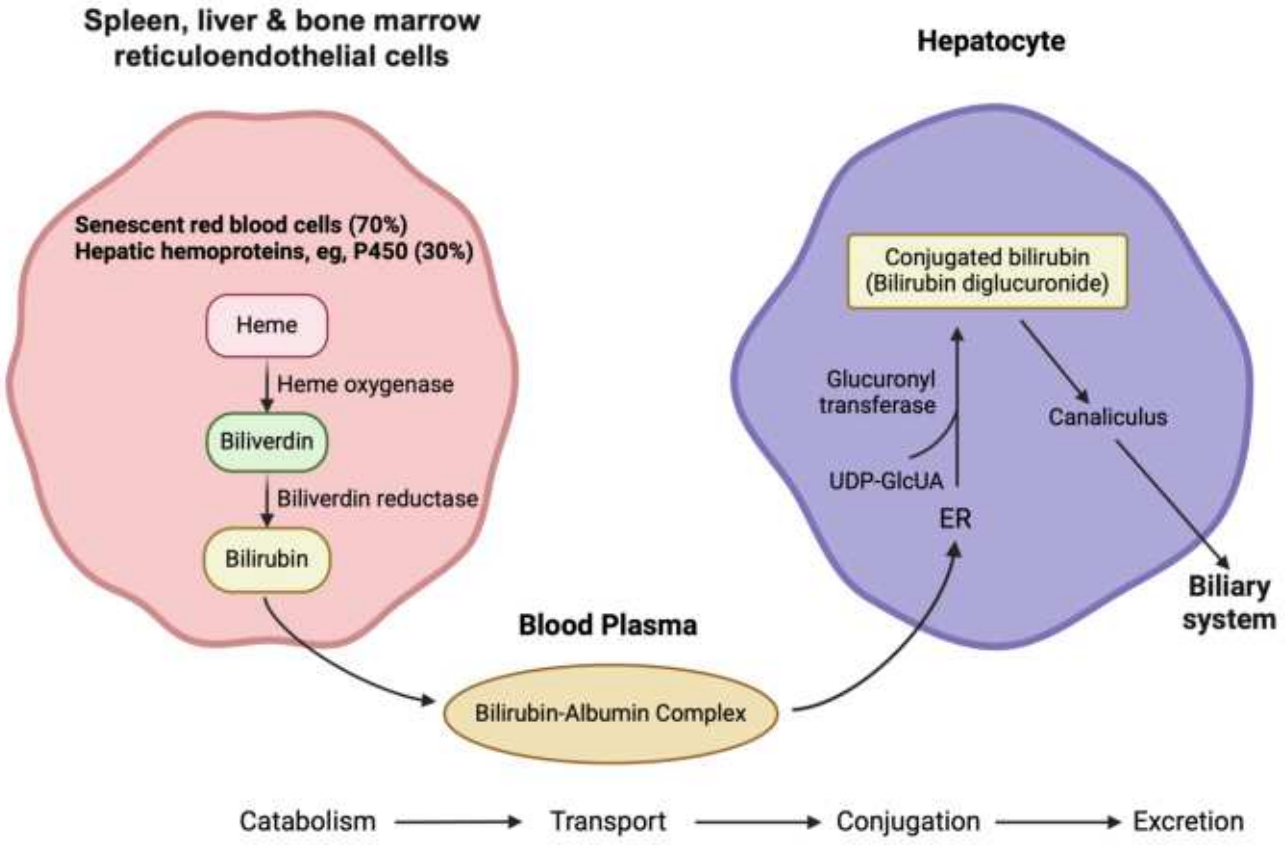


Porphyria cutanea tarda is the most common subtype of porphyrias. This disease is primarily due a deficiency in the activity of uroporphyrinogen decarboxylase. There are two forms of porphyria cutanea tarda: familial and sporadic. The familial and sporadic forms account for 20% and 80% of all cases, respectively. The familial form is due to mutations in the gene encoding uroporphyrinogen decarboxylase. The sporadic form, on the other hand, has normal uroporphyrinogen decarboxylase DNA sequence. However, exposure to external stimuli such as alcohol or hepatitis infection results in the loss of enzyme activity. The accumulation of uroporphyrinogen III and its intermediaries then causes the formation of photoactive porphyrins. This could result in skin blisters and lesions upon exposure to sunlight. Many individuals with this condition, however, never exhibit any symptoms.

IV. Heme catabolism

A. Heme catabolism overview

Heme catabolism & transport



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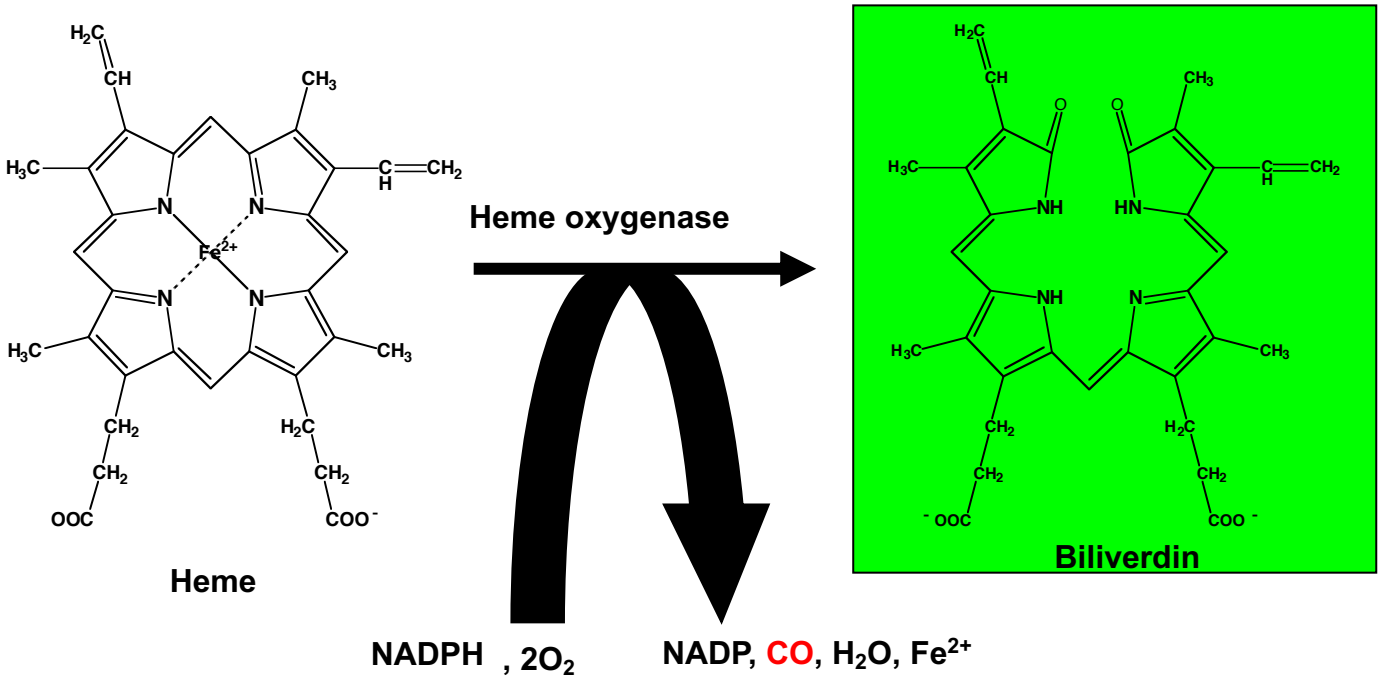
Heme is catabolized primarily in reticuloendothelial cells of the spleen, liver, and bone marrow. Old (senescent) red blood cells account for 70% of heme catabolized, with other hemoproteins accounting for the other 30%. Heme is first converted to biliverdin. Biliverdin reductase then reduces the green biliverdin to orange bilirubin. In extrahepatic tissues, bilirubin is released and is transported by albumin to hepatocytes. In the ER of hepatocytes, glucuronyl transferase then conjugates bilirubin with 2 glucuronic acid molecules from UDP-glucuronic acid (UDP-GlcUA). In contrast to bilirubin, conjugated bilirubin (bilirubin diglucuronide) is water-soluble. It is exported from hepatocytes to liver canaliculus via the MRP2 transporter and then to the biliary system, which directs it into the intestine for excretion.

B. Formation of biliverdin and bilirubin

Heme catabolic reactions

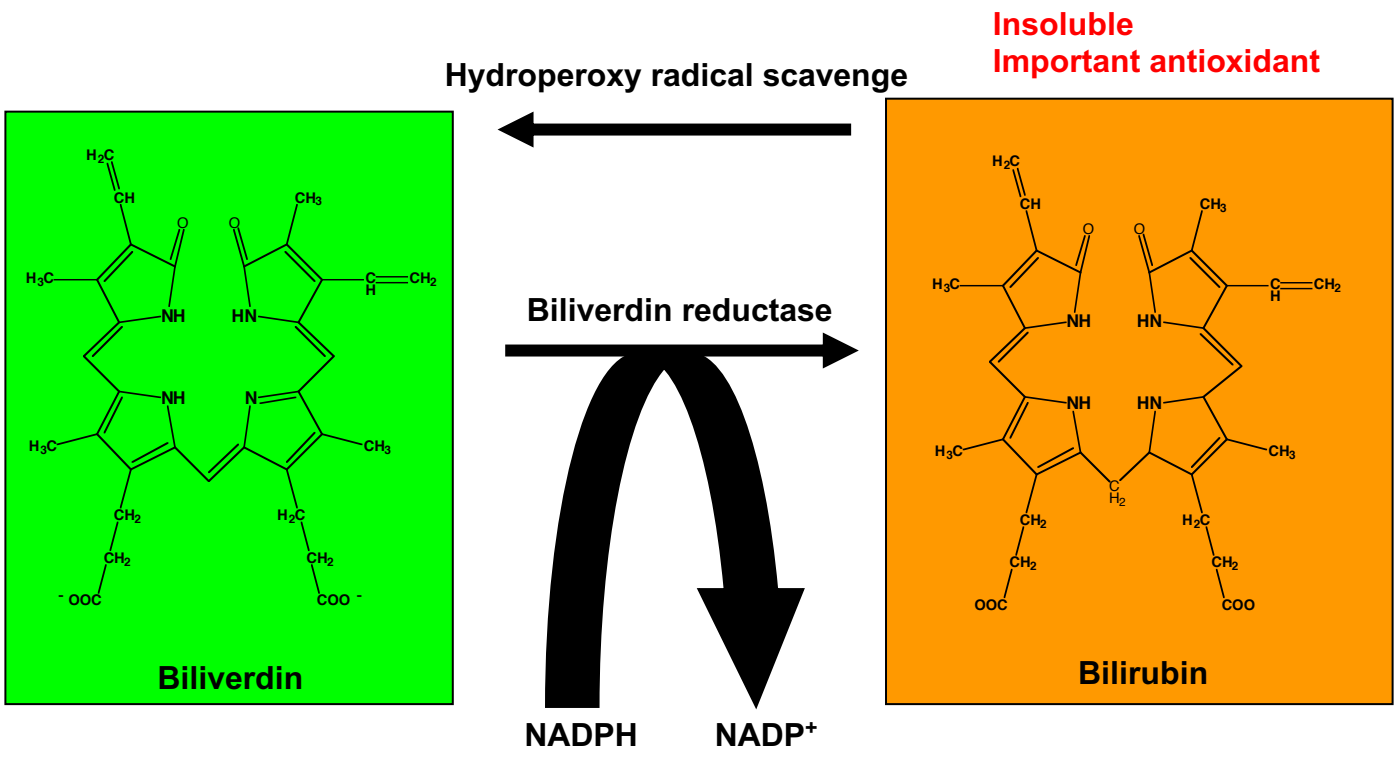


- Heme oxygenase is one of the cytochrome P450 enzymes.
- It liberates iron and carbon monoxide from heme.
- The result is the green-colored bile pigment biliverdin.

The first reaction in heme catabolism is catalyzed by heme oxygenase, which utilizes molecular oxygen to crack the ring and produce biliverdin, iron, and carbon monoxide. Heme oxygenase not only contains heme, but it also degrades it. It is one of the cytochrome P450 enzymes, which all utilize molecular oxygen as one of their substrates. Note that carbon monoxide is one of the products of the reaction. CO is very toxic, because it binds tightly to hemoglobin, preventing the transport of oxygen. It can also bind to Complex IV of the ETC, thereby preventing ATP synthesis. However, the metabolic system is designed such that normally-produced CO does not interfere with oxygen transport or ATP synthesis. The iron can be recycled, but biliverdin is destined for clearance.

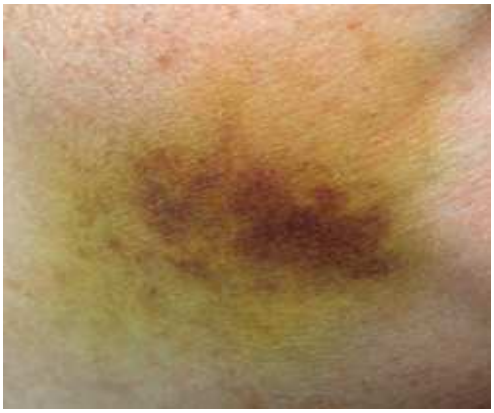
Mammals synthesize bilirubin from biliverdin via the action of biliverdin reductase. Bilirubin is then released and binds to serum albumin. Bilirubin actually comprises part of the free-radical defense system in the body. While bound to albumin in blood, each molecule can destroy two hydroperoxy radicals by donating two electrons and is oxidized back to biliverdin (see next page). Note that the last two reactions (heme oxygenase and biliverdin reductase) require NADPH. Recall that glutathione is also a part of the free radical defense mechanism, and that it requires NADPH. This means that all of these operations are dependent on the pentose phosphate pathway to produce NADPH.

Synthesis of bilirubin



- Biliverdin is the end-product of heme metabolism in birds and reptiles.
- Mammals make bilirubin, because it is an important antioxidant!
- While albumin-bound, it can scavenge two hydroperoxy radicals, forming biliverdin.

Different colors of bruises



Red/purple-Heme

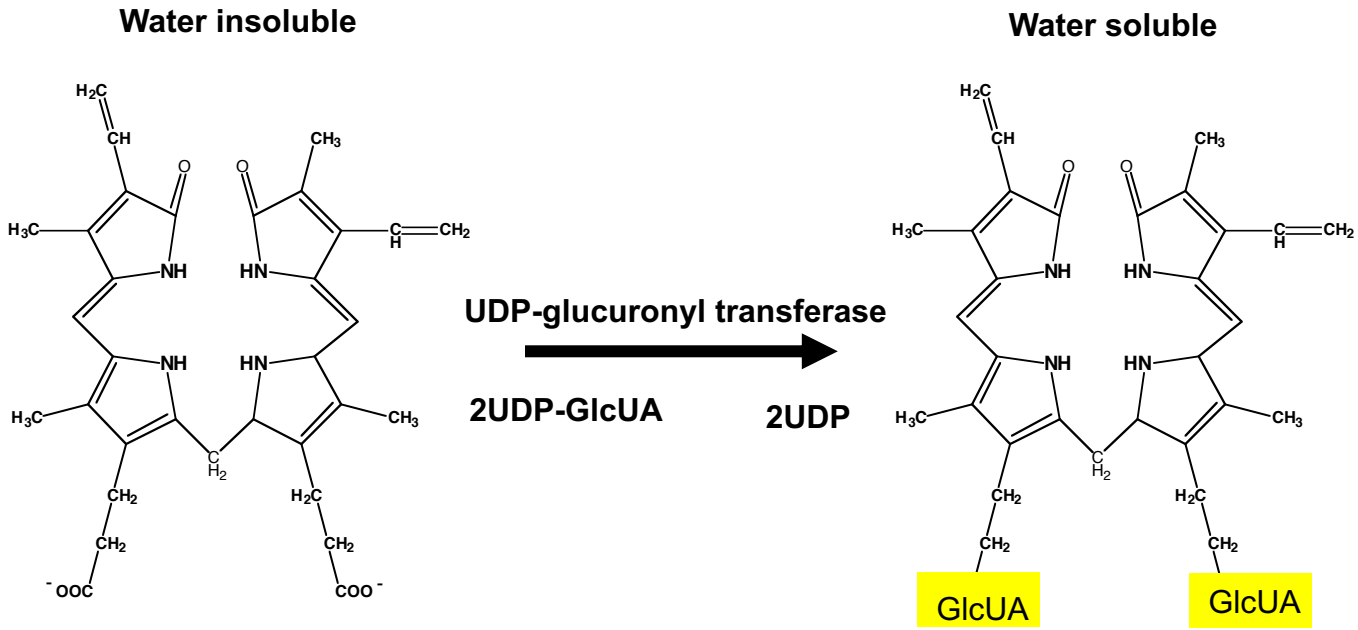
Green-Biliverdin

Orange-Bilirubin

When one's skin gets bruised, very often the color of the bruise will change from red or purple to green and then to orange or yellow. The red or purple is due to the color of heme from hemoglobin of red blood cells that leaked out from injured blood vessels. The green color is due to formation of biliverdin. Finally, the orange or yellow color is due to the formation of bilirubin. Gradually, the bruised area of the skin will return to its normal color as bilirubin is removed.

C. Conjugation of bilirubin

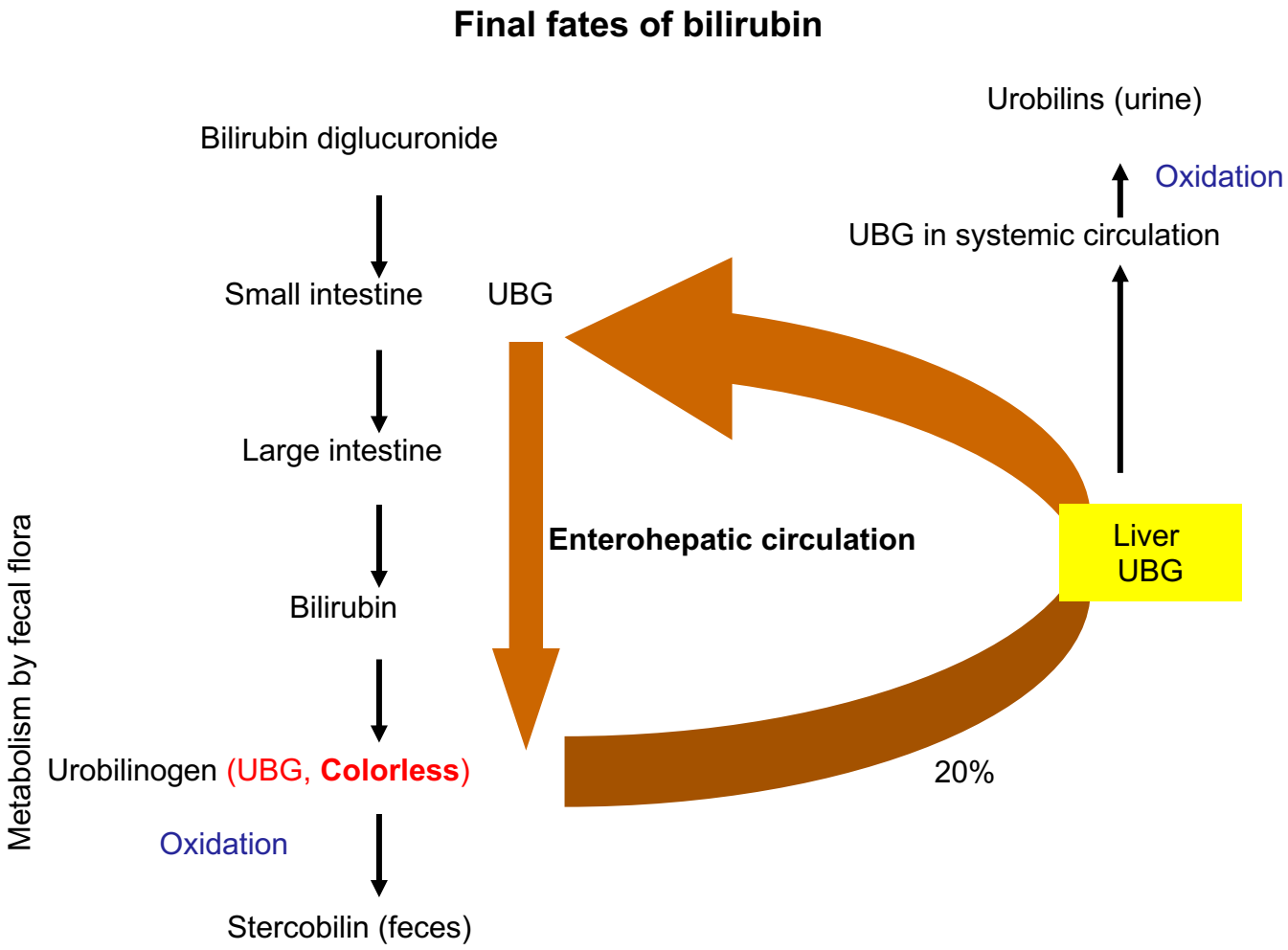
Conjugation of bilirubin



Once taken up by the liver, the water-insoluble bilirubin is conjugated with two units of glucuronic acid via its two propionyl side chains to form bilirubin diglucuronide. This reaction is catalyzed by UDP-glucuronyl transferase. The conjugation of bilirubin is an important reaction in the liver. In cases of liver damage, the capacity to carry out conjugation can be impaired, and the water-insoluble bilirubin accumulates, because it cannot be excreted at sufficient rates. It collects in blood and fatty tissues, such as subcutaneous fat, to such an extent that jaundice results.

Bilirubin, unconjugated bilirubin, and indirect bilirubin are all synonyms of the water-insoluble form. Bilirubin diglucuronide, conjugated bilirubin, and direct bilirubin are synonyms of the water-soluble form.

D. Final fates of bilirubin



Once bilirubin is conjugated (bilirubin diglucuronide), it becomes more soluble and is released into bile canaliculi via multidrug resistance-associated protein 2 (MRP2) as part of bile. Bilirubin then goes from the biliary duct into the small intestine and then into the large intestine where intestinal flora converts it back to bilirubin. It is further metabolized to the colorless urobilinogen (UBG), some of which (20%) is shuttled back to the liver via the enterohepatic circulation and is then resecreted as bile. In enterohepatic circulation, compounds are recycled between the intestine and the liver. Bile acids are also recycled in this fashion.

Urobilinogen that is not removed from the portal circulation by the liver then enters the systemic circulation where it gets oxidized. Its oxidized forms are filtered out by the kidneys, and they appear in the urine as urobilins. Several urobilins are excreted in the urine, including d-urobilin and i-urobilin. Urobilins give urine the yellowish color. In the large intestine, UBG not recycled by the enterohepatic circulation is further oxidized to form stercobilin, which then ends up in feces. Stercobilin accounts for the brownish color of human stool. It is important to pay attention to stool color. If the stool color is pale or white, it could mean biliary duct obstruction due to gallstones, hepatitis, or cancer.

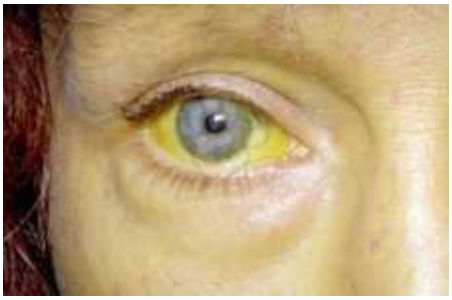
V. Heme catabolism related disease

A. Bilirubin accumulation and jaundice

Anytime the skin has a yellow color, the term jaundice applies. In jaundice, the pigment is usually unconjugated bilirubin, but sometimes it can be a carotenoid pigment if a person consumes foods containing high amounts of carotenoids. An example is β-carotene, which gives the orange color to carrots. There are hundreds of different carotenoid pigments; they are all fat soluble, and they can all accumulate and pigment the skin. Carotenoids, however, do not color the whites of the eyes, so this is a way of distinguishing between an accumulation of carotenoid pigments and bilirubin.

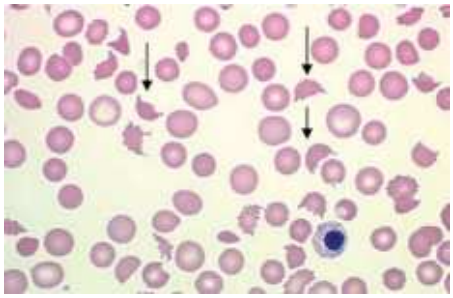
Accumulation of bilirubin and jaundice

- Jaundice means yellow skin.
- Skin may be colored yellow because of carotenoid pigments, such as β-carotene and lycopene, as well as bilirubin (hyperbilirubinemia).
- One important difference is that carotenoid pigments do not usually color the sclera of the eye.



B. Types of Jaundice

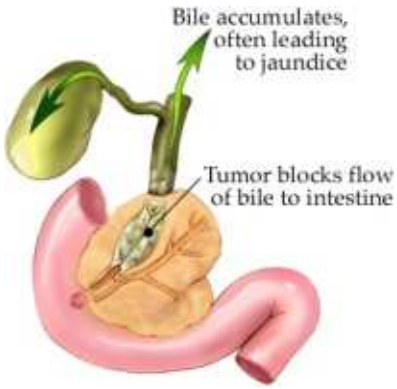
Prehepatic



• Usually due to excessive hemolysis

Hepatic

• Defective transport
• Lack of UDP-glucuronyl transferase
• Liver dysfunction



Posthepatic

• Biliary obstruction

There are three basic types of jaundice: **prehepatic**, **hepatic**, and **posthepatic**. Prehepatic jaundice is usually caused by excess hemolysis of red blood cells. Examples include sickle cell anemia and drug-induced hemolytic anemia. Here, there is so much bilirubin such that UDP-glucuronyl transferase cannot conjugate it fast enough to keep the levels low. Unconjugated bilirubin will therefore accumulate. Hepatic jaundice has a number of causes, and three of the major ones include defective transport of bilirubin, lack of UDP-glucuronyl transferase (see below), and liver dysfunction. Posthepatic jaundice is usually caused by biliary obstruction. This is sometimes caused by a tumor. For example, pancreatic cancer can cause biliary obstruction simply because the tumor can press against the duct to shut off biliary flow.

Jaundice is usually associated with liver pathology, such as hepatitis, cirrhosis, and fatty liver. In neonates, hyperbilirubinemia quite commonly shows up 1-5 days postpartum (60%), and it is much more common in premature babies (80%). Hyperbilirubinemia in the neonate can have a number of causes, some of which are pathological and can cause brain damage (kernicterus). Usually, however, it is caused by an immature liver that is not yet producing all of its enzymes in sufficient quantities. In this case, it is usually UDP-glucuronyl transferase, which after a few days or weeks, is produced in sufficient amounts, and the jaundice disappears. One of the treatments for neonatal jaundice is phototherapy. It is known that light between the wavelengths of 425-450 nm will convert bilirubin to harmless water-soluble isomers that can be excreted. Special phototherapy blankets have been designed for this treatment in premature babies.

VI. Summary

- Heme serves as a prosthetic group in hemoproteins.
- Heme synthesis occurs in all cells, using succinyl CoA and glycine.
- ALA synthase catalyzes the rate-limiting step in heme synthesis.
- ALA synthase is repressed and allosterically inhibited by heme and hemin.
- Lead blocks heme synthesis by inhibiting ALA dehydrase and ferrochelatase.
- Defects in heme synthesis lead to porphyrias.
- Heme is broken down in reticuloendothelial cells into biliverdin and then to bilirubin.
- Bilirubin is then released, bound to albumin, and transported to the liver.
- Bilirubin is conjugated with glucuronic acid and is eventually excreted as urobilins in urine and stercobilin in feces.
- Failure to clear bilirubin leads to jaundice.