PORPHYRIN METABOLISM

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Porphyrrins are cyclic compounds that readily bind metal ions – usually Fe\(^{2+}\) or Fe\(^{3+}\).

The most prevalent metalloporphyrin in humans is heme, which consists of one ferrous iron atom coordinated in the center of the tetrapyrrole ring of protoporphyrin IX.

Heme is the prosthetic group for hemoglobin, myoglobin, the cytochromes, catalase, and tryptophan pyrrolase.
• These heme proteins are rapidly synthesized and degraded
• For example, 6 to 7g of hemoglobin are synthesized each day to replace heme lost through the normal turnover of erythrocytes
• Coordinated with the turnover of heme proteins is the simultaneous synthesis and degradation of the associated porphyrins, and recycling of the bound iron ions
A. Structure of porphyrins

• Porphyrins are cyclic molecules formed by the linkage of four pyrrole rings through methenyl bridges

1. **Side chains:** Different porphyrins vary in the nature of the side chains that are attached to each of the four pyrrole rings

• For example, uroporphyrin contains acetate (\(-\text{CH}_2\text{-COO}^-\)) and propionate (\(-\text{CH}_2\text{-CH}_2\text{-COO}^-\)) side chains, whereas coproporphyrin is substituted with methyl (\(-\text{CH}_3\)) and propionate groups
2. Distribution of side chains: The side chains of porphyrins can be ordered around the tetrapyrrole nucleus in four different ways, designated by Roman numerals to IV.

Only type III porphyrins, which contain an asymmetric substitution on ring D are physiologically important humans.

*Note: In congenital erythropoietic porphyria, type I porphyrins, which contain a symmetric arrangement of substituents, are synthesized in appreciable quantities.
Structures of uroporphyrin I and uroporphyrin III

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

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

Acetate (A) and propionate (P) are reversed in ring D of uroporphyrin III compared with uroporphyrin I. Only type III porphyrins are physiologically important in humans.
3. **Porphyrinogens**: Porphyrin precursors exist in the chemically reduced form called porphyrinogens.

- In contrast to the porphyrins, which are colored, the porphyrinogens, such as uroporphyrinogen, are colorless.
- Porphyrinogens serve as intermediates between porphobilinogen and protoporphyrin in the biosynthesis of heme.
B. Biosynthesis of heme

• The major sites of heme biosynthesis are the liver, which synthesizes a number of heme proteins (particularly, cytochrome P450), and the erythrocyte-producing cells of the bone marrow, which are active in hemoglobin synthesis

• In the liver, the rate of heme synthesis is highly variable, responding to alterations in the cellular heme pool caused by fluctuating demands for heme proteins
• In contrast, heme synthesis in erythroid cells is relatively constant, and is matched to the rate of globin synthesis
• The initial reaction and the last three steps in the formation of porphyrins occur in mitochondria, whereas the intermediate steps of the biosynthetic pathway occur in the cytosol
• *Note: Mature red blood cells lack mitochondria and are unable to synthesize heme
• 1. Formation of δ-aminolevulinic acid (ALA): the carbon and nitrogen atoms of the porphyrin molecule are provided by two simple building blocks: glycine (a nonessential amino acid) and succinyl CoA (an intermediate in the citric acid cycle)

• Glycine and succiny CoA condense to form ALA in a reaction catalyzed by ALA synthase
• This reaction requires pyridoxal phosphate as a coenzyme, and is the rate-controlling step in hepatic porphyrin biosynthesis

a. **End product inhibition by hemin**: When porphyrin production exceeds the availability of globin (or other apoproteins), heme accumulates and is converted to hemin by the oxidation of Fe\(^{2+}\) to Fe\(^{3+}\)

• Hemin decreases the activity of hepatic ALA synthase by causing decreased synthesis of the enzyme
• *Note: In erythroid cells, heme synthesis is under the control of erythropoietin and the availability of intracellular iron

• **b. Effect of drugs on ALA synthase activity:** Administration of any of a large number of drugs, such as phenobarbital, griseofulvin or hydantoins, results in a significant increase in hepatic ALA synthase activity

• These drugs are metabolized by the microsomal cytochrome P450 monooxygenase system – a heme-protein oxidase system found in the liver
• This, in turn, causes a decrease in the concentration of heme in liver cells
• The lower intracellular heme concentration leads to an increase in the synthesis of ALA synthase (derepression), and prompts a corresponding increase in ALA synthesis
2. Formation of porphobilinogen: The dehydration of two molecules of ALA to form porphobilinogen by δ-aminolevulinic acid dehydrase is extremely sensitive to inhibition by heavy metal ions

• This inhibition is, in part, responsible for the elevation in ALA and the anemia seen in lead poisoning
Glycine + Succinyl CoA → δ-Aminolevulinate synthase → δ-Aminolevulinic acid (ALA)

Lead → 2 H₂O → Porphobilinogen
• 3. Formation of uroporphyrinogen: The condensation of four molecules of porphobilinogen results in the formation of uroporphyrinogen III

• The reaction requires *hydroxymethylbilane synthase* and *uroporphyrinogen synthase* (which produces the asymmetric uroporphyrinogen III)
4. Formation of heme: Uroporphyrinogen III is converted to heme by a series of decarboxylations and oxidations

• The introduction of Fe$^{2+}$ into protoporphyrin IX occurs spontaneously, but the rate is enhanced by the enzyme ferrochelatase — an enzyme that is inhibited by lead
Porphobilinogen

Hydroxymethylbilane synthase

Hydroxymethylbilane

Uroporphyrinogen III synthase

Uroporphyrinogen III
Uroporphyrinogen III

Protoporphyrin IX

\[
\begin{align*}
\text{H}_2\text{C} &= \text{CH} \\
\text{CH}_3 &\quad \text{H} \\
\text{CH}_3 &\quad \text{CH} &= \text{CH}_2 \\
\text{CH}_3 &\quad \text{CH}_3 \\
\text{CH}_3 &\quad \text{CH}_3 \\
\text{CH}_2 \cdot \text{CH}_2 \cdot \text{COO}^- \\
\end{align*}
\]

\[\text{Fe}^{2+}\]  
\[\text{Lead}\]

Ferrochelatase

\[2\text{H}^+\]

Heme (Fe\(^{2+}\) protoporphyrin IX)
Heme B biosynthesis pathway and its modulators
Pathway of Heme Biosynthesis

mitochondria

succinyl-CoA + glycine → δ-aminolevulinic acid (ALA)

HOOC–CH₂CH₂–C–CoA + H₂N–CH₂–COOH → δ-aminolevulinic acid synthase

CO₂

2x ALA → ALA dehydratase (porphobilinogen synthase)

H₉OOC–CH₂CH₂–C–CH₂–NH₂ → porphobilinogen (PBG)

PBG deaminase → hydroxymethylbilane

hydroxymethylbilane → uroporphyrinogen III synthase → uroporphyrinogen III

uroporphyrinogen III decarboxylase → coproporphyrinogen III

protoporphyrinogen oxidase → protoporphyrinogen IX

ferrochelatase → Fe²⁺, 2 H⁺ → protoporphyrin IX

2 CO₂ → coproporphyrinogen IX

zymogen oxidase
In addition to the heme $b$ found in hemoglobin, there are three different forms of heme found in cytochromes.
C. Porphyrias

• Porphyrias are caused by inherited (or occasionally acquired) defects in heme synthesis, resulting in the accumulation and increased excretion of porphyrins or porphyrin precursors.

• With the exception of congenital erythropoietic porphyria, which is a genetically recessive disease, all porphyrias are inherited as autosomal dominant disorders.
• The mutations that cause the porphyrias are heterogenous (not all are at the same DNA locus), and nearly every affected family has its own mutation
• Each porphyria results in the accumulation of a unique pattern of intermediates caused by the deficiency of an enzyme in the heme synthetic pathway
1. **Clinical manifestations:** The porphyrias are classified as **erythropoietic** or **hepatic**, depending on whether the enzyme deficiency occurs in the erythropoietic cells of the bone marrow or in the liver.

- Hepatic porphyrias can be further classified as acute or chronic.
- Individuals with an enzyme defect leading to the accumulation of tetrapyrrole intermediates show photosensitivity – that is, their skin itches and burns (**pruritis**) when exposed to visible light.
• *Note: These symptoms are thought to be a result of the porphyrin-mediated formation of superoxide radicals from oxygen
• These reactive oxygen species can oxidatively damage membranes, and cause the release of destructive enzymes from lysosomes
• Destruction of cellular components leads to the photosensitivity
• a. Chronic porphyria: Porphyria cutanea tarda, the most common porphyria, is a chronic disease of the liver and erythroid tissues

• The disease is associated with a deficiency in uroporphyrinogen carboxylase but clinical expression of the enzyme deficiency is influenced by various factors, such as hepatic iron overload, exposure to sunlight, and the presence of hepatitis B or C, or HIV infections
• Clinical onset is typically during the fourth or fifth decade of life

• Porphyrin accumulation leads to cutaneous symptoms, and urine that is red to brown in natural light, and pink to red in fluorescent light
Skin eruptions in a patient with porphyria cutanea tarda
• Urine from a patient with porphyria cutanea tarda (right) and from a patient with normal porphyrin excretion (left)
b. Acute hepatic porphyrias: Acute hepatic porphyrias (acute intermittent porphyria, hereditary coproporphyria, and varigate porphyria) are characterized by acute attacks of gastrointestinal, neurologic/psychiatric, and cardiovascular symptoms.

Porphyrias leading to accumulation of ALA and porphobilinogen, such as acute intermittent porphyria, cause abdominal pain and neuropsychiatric disturbances.
• Symptoms of the acute hepatic porphyrias are often precipitated by administration of drugs such as barbiturates and ethanol, which induce the synthesis of the heme-containing cytochrome P450 microsomal drug oxidation system.

• This further decreases the amount of available heme, which, in turn, promotes the increased synthesis of ALA synthase.
c. Erythropoietic porphyrias: The erythropoietic porphyrias (congenital erythropoietic porphyria and erythropoietic protoporphyria) are characterized by skin rashes and blisters that appear in early childhood. The diseases are complicated by cholestatic liver cirrhosis and progressive hepatic failure.
2. Increased ALA synthase activity: One common feature of the porphyrias is a decreased synthesis of heme.

- In the liver, heme normally functions as a repressor of ALA synthase
- Therefore, the absence of this end product results in an increase in the synthesis of ALA synthase (derepression)
- This causes an increased synthesis of intermediates that occur prior to the genetic block
The accumulation of these toxic intermediates is the major pathophysiology of the porphyrias

3. **Treatment**: During acute porphyria attacks, patients require medical support, particularly treatment for pain and vomiting.

- The severity of symptoms of the porphyrias can be diminished by intravenous injection of hemin which decreases the synthesis of *ALA synthase*.
- Avoidance of sunlight and ingestion of β-carotene (a free-radical scavenger) are also helpful.
Degradation of heme

• After approximately 120 days in the circulation, red blood cells are taken up and degraded by the reticuloendothelial (RE) system, particularly in the liver and spleen.

• Approximately 85 percent of heme destined for degradation comes from red blood cells, and fifteen percent is from turnover of immature red blood cells and cytochromes from extraerythroid tissues.
• **1. Formation of bilirubin:** The first step in the degradation of heme is catalyzed by the microsomal *heme oxygenase system* of the RE cells

• In the presence of NADPH and O\(_2\), the enzyme adds a hydroxyl group to the methenyl bridge between two pyrrole rings, with a concomitant oxidation of ferrous iron to Fe\(^{3+}\)

• A second oxidation by the same enzyme system results in cleavage of the porphyrin ring
• Ferric iron and carbon monoxide are released, resulting in the production of the green pigment **biliverdin**

• Biliverdin is reduced, forming the red-orange **bilirubin**

• Bilirubin and its derivatives are collectively termed **bile pigments**

• **2. Uptake of bilirubin by the liver:** Bilirubin is only slightly soluble in plasma and, therefore, is transported to the liver by binding covalently to albumin
• *Note: Certain anionic drugs, such as salicylates and sulfonamides, can displace bilirubin from albumin, permitting bilirubin to enter the central nervous system (CNS)

• This causes the potential for neural damage in infants

• Bilirubin dissociates from the carrier albumin molecule and enters a hepatocyte, where it binds to intracellular proteins, particularly the protein ligandin
3. Formation of bilirubin diglucuronide: In the hepatocyte, the solubility of bilirubin is increased by the addition of two molecules of glucuronic acid.

*Note: This process is referred to as conjugation.

The reaction is catalyzed by *bilirubin glucuronyltransferase* using UDP-glucuronic acid as the glucuronate donor.

*Note: Bilirubin conjugates also bind to albumin, but much more weakly than does unconjugated bilirubin.
Bilirubin diglucuronide
• 4. Excretion of bilirubin into bile: Bilirubin diglucuronide is actively transported against a concentration gradient into the bile canaliculi and then into the bile

• This energy-dependent, rate-limiting step is susceptible to impairment in liver disease

• Unconjugated bilirubin is normally not excreted.
5. Formation of urobilins in the intestine:
Bilirubin diglucuronide is hydrolyzed and reduced by bacteria in the gut to yield urobilinogen, a colorless compound.

- Most of the urobilinogen is oxidized by intestinal bacteria to stercobilin, which gives feces the characteristic brown color.

- However, some of the urobilinogen is reabsorbed from the gut and enters the portal blood.
• A portion of this urobilinogen participates in the **enterohepatic urobilinogen cycle** in which it is taken up by the liver, and then re-excreted into the bile

• 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
JAUNDICE

• Jaundice (also called icterus) refers to the yellow color of skin, nail beds, and sclerae (whites of the eyes) caused by deposition of bilirubin, secondary to increased bilirubin levels in the blood (hyperbilirubinemia)

• Although not a disease, jaundice is usually a symptom of an underlying disorder.
Jaundiced patient, with the sclerae of his eyes appearing yellow
Jaundiced eye
• **1. Types of jaundice:** Jaundice can be classified into three major forms described below

• However, in clinical practice, jaundice is often more complex than indicated in this simple classification

• For example, the accumulation of bilirubin may be a result of defects at more than one step in its metabolism.
• **a. Hemolytic jaundice:** The liver has the capacity to conjugate and excrete over 3000 mg of bilirubin per day, whereas the normal production of bilirubin is only 300 mg/day

• This excess capacity allows the liver to respond to increased heme degradation with a corresponding increase in conjugation and secretion of bilirubin diglucuronide
• However, massive lysis of red blood cells (for example, in patients with sickle cell anemia, pyruvate kinase or glucose 6-phosphate dehydrogenase deficiency and malaria) may produce bilirubin faster than it can be conjugated.

• More bilirubin is excreted into the bile, the amount of urobilinogen entering the enterohepatic circulation is increased, and urinary urobilinogen is increased.

• Unconjugated bilirubin levels become elevated in the blood, causing jaundice.
• **b. Obstructive jaundice:** In this instance, jaundice is not caused by overproduction of bilirubin, but instead results from obstruction of the bile duct.

• For example, the presence of a hepatic tumor or bile stones may block the bile ducts, preventing passage of bilirubin into the intestine.

• Patients with obstructive jaundice experience gastrointestinal pain and nausea, and produce stools that are a pale, clay color.

• The liver "regurgitates" conjugated bilirubin into the blood (hyperbilirubinemia).
• The compound is eventually excreted in the urine
• *Note: Prolonged obstruction of the bile duct can lead to liver damage and a subsequent rise in unconjugated bilirubin
• **c. Hepatocellular jaundice:** Damage to liver cells (for example, in patients with cirrhosis or hepatitis) can cause unconjugated bilirubin levels to increase in the blood as a result of decreased conjugation
• The bilirubin that is conjugated is not efficiently secreted into the bile, but instead diffuses ("leaks") into the blood

• Urobilinogen is increased in the urine because hepatic damage decreases the enterohepatic circulation of this compound, allowing more to enter the blood, from which it is filtered into the urine
• The urine thus becomes dark in color, whereas stools are a pale, clay color
• Plasma levels of AST (SGOT) and ALT (SGPT) are elevated, and the patient experiences nausea and anorexia

• **2. Jaundice in newborns:** Newborn infants, particularly premature babies, often accumulate bilirubin, because the activity of hepatic *bilirubin glucuronyl transferase* is low at birth – it reaches adult levels in about four weeks
Activity of the enzyme that conjugates bilirubin with glucuronic acid, **UDP-glucuronyl transferase (UDPGT)**, is low in newborns and especially low in premature babies.
Neonatal jaundice – 8 weeks after birth

Answer: Hemolytic disease of the newborn

Figure – This infant presented with jaundice 8 weeks after birth. The cause was hemolytic disease of the newborn due to Rh incompatibility. The mother’s fingers are shown for contrast.
• Elevated bilirubin, in excess of the binding capacity of albumin, can diffuse into the basal ganglia and cause toxic encephalopathy (kernicterus)

• Thus, newborns with significantly elevated bilirubin levels are treated with blue fluorescent light, which converts bilirubin to more polar and, hence, water-soluble isomers
Jaundice

- Yellowning of skin
- Excess bilirubin in blood

Kernicterus

- Yellowning of eyes
- Bilirubin moves from bloodstream into brain tissue
Bilirubin staining of the thalamus and basal ganglia
Serum levels of bilirubin rise after birth in full-term infants, although usually not to dangerous concentrations.

Serum levels of bilirubin in premature infants may rise to toxic levels.
• These photoisomers can be excreted into the bile without conjugation to glucuronic acid

• *Note: Crigler-Najjar syndrome is caused by a genetic deficiency of hepatic bilirubin glucuronyl transferase
3. Determination of bilirubin concentration: Bilirubin is most commonly determined by the Van den Bergh reaction, in which diazotized sulfanilic acid reacts with bilirubin to form red azodipyrrroles that are measured colorimetrically.

In aqueous solution, the water-soluble, conjugated bilirubin reacts rapidly with the reagent (within one minute), and is said to be “direct-reading.” The unconjugated bilirubin, which is much less soluble in aqueous solution, reacts more slowly.
However, when the reaction is carried out in methanol, both conjugated and unconjugated bilirubin are soluble and react with the reagent, providing the total bilirubin value.

The "indirect-reacting" bilirubin, which corresponds to the unconjugated bilirubin, is obtained by subtracting the direct-reacting bilirubin from the total bilirubin.

*Note: In normal plasma, only about four percent of the total bilirubin is conjugated.