The human body contains about 4.5-5.0 g of iron. The share of blood hemoglobin from this amount (if you take all the iron in the body for 100%) accounts for 60-70%, myoglobin – 3-5%, ferritin – 20% (from 17 to 23%), transferrin – about 0.18%, functional iron of tissues – up to 5%. The iron content in the body is mainly regulated by the intensity of absorption in the intestines of iron coming from food. Excess of it is not absorbed. The need for iron increases sharply with anemia of various origins. Iron is absorbed in the intestine as an inorganic divalent Fe2 + ion after its release from complexes with proteins. In cells of the intestinal mucosa, iron already in the trivalent form of Fe3+combines with the protein apoferritin to form a stable ferritin complex. Further transport of iron to the sites of hematopoiesis is carried out in a complex with β1-globulins of blood serum (the complex is called transferrin) or iron is combined with apoferritin of tissues, where it is deposited as ferritin. In some diseases (for example, hemochromatosis), excess iron is deposited in the cells of the macrophage system in the form of hemosiderin – a metabolically inert compound of iron with protein.
Sources of iron for synthetic purposes are food products, as well as iron, which is released during the constant breakdown of red blood cells in the liver and spleen (about 25 mg per day). Prosthetic groups of food chromoproteins (hemoglobin, myoglobin), including chlorophyll proteins, are not used for the synthesis of ironoproteins of the body, because after digestion, the non-protein component of heme is oxidized to hematin, which, like chlorophyll, is not absorbed in the intestine. Usually, these pigments are released with the contents of the colon in unchanged form or in the form of decomposition products under the action of intestinal bacteria enzymes. Consequently, gem-containing food compounds are not used as a source of porphyrin core, and the synthesis of a complex pyrrole complex in the body proceeds from low-molecular precursors de novo.

Porphyrins are complex chemical compounds that are large heterocyclic organic ring structures. The complex ring structures of porphyrins are composed of four modified pyrrole (5-membered organic ring) subunits connected by methine (carbon with one double bond and two single bonds: =CH-) bridges. The naturally occurring porphyrins of biological significance are the hemes. Hemes in biological systems consist of ferrous iron (Fe^{2+}) complexed with four nitrogens of the specific porphyrin molecule identified as protoporphyrin IX. The major function of heme in humans is its role in the coordination of O_2 molecules in hemoglobin. There are three structurally distinct hemes in humans identified as heme *a*, heme *b*, and heme *c* (discussed below). In addition to the heme *b* of hemoglobin and its role in oxygen transport, hemes are critical for the biological functions of several enzymes such as the cytochromes of oxidative phosphorylation and the xenobiotic metabolizing enzymes of the cytochrome P450 family (CYP).
Aside from its importance as the prosthetic group of hemoglobin and the cytochromes, heme is clinically significant because a number of genetic disease states are associated with deficiencies of the enzymes used in its biosynthesis and catabolism. Some of these disorders are readily diagnosed because they cause δ-aminolevulinic acid, (ALA) and other abnormally colored heme intermediates to

appear in the circulation, the urine, and in other tissues such as teeth and bones. Some disorders of heme biosynthesis are more insidious such as the various **porphyrias**. In addition to the clinical consequences of defects in heme biosynthesis, defects in heme catabolism can lead to potentially lethal elevations in the primary catabolic by product, bilirubin (see below)

An important feature of the intermediates in heme biosynthesis, as well as in heme degradation, is their chromophoric character, some are colored while others are not. An easy way to distinguish which will have a color and which will not is to look at the suffix of the compound name. All heme intermediates and degradation products that end in **-ogen** (e.g. phorphobilinogen) will be colorless while those that end in **-in** (e.g. bilirubin) will be colored.

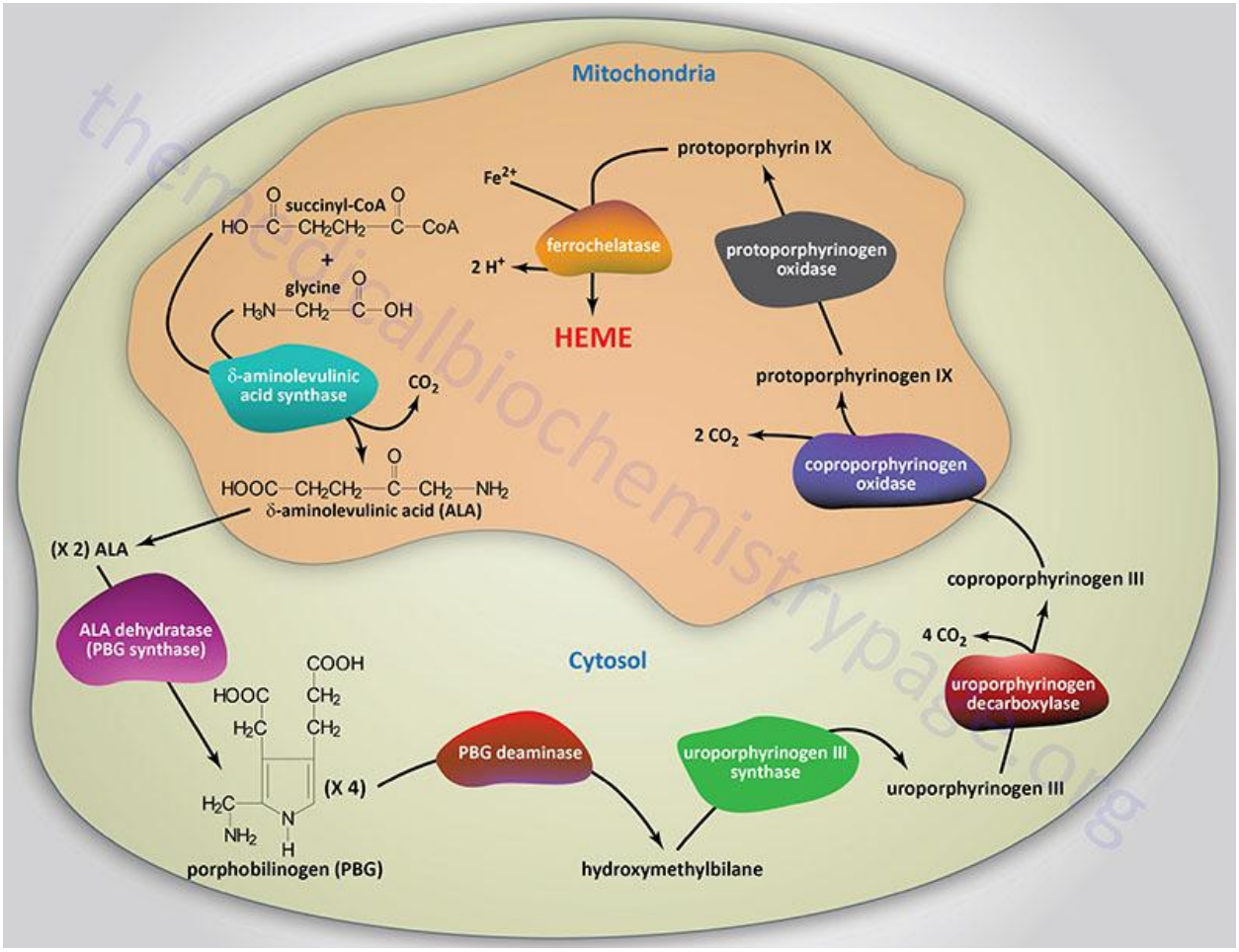
Synthesis of Porphobilinogen and Heme

The first reaction in heme biosynthesis takes place in the mitochondria and involves the condensation of one glycine and one succinyl-CoA by the pyridoxal phosphate-requiring (vitamin B6) enzyme, δ-aminolevulinic acid synthase (ALAS) forming the compound δ-aminolevulinic acid. Delta-aminolevulinic acid (ALA) is also called 5-aminolevulinic acid. This reaction is both the rate-limiting reaction of heme biosynthesis, and the most highly regulated reaction (see Regulation below). Due to the loss of ALAS activity in vitamin B_6 deficiency there is an associated microcytic hypochromic anemia where the erythroblasts are histopathologically associated with siderosomes.

There are two forms of ALAS encoded by two different genes. ALAS1 is considered a house-keeping gene and is expressed in all cells. ALAS2 is an erythroid-specific form of the enzyme and is expressed only in fetal liver and adult bone marrow. The ALAS1 gene is located on chromosome 3p21.2 and is composed of 12 exons that generate four alternatively spliced mRNAs that collectively encode two distinct precursor proteins. ALAS1 isoform 1 is composed of 640 amino acids and ALAS1 isoform 2 is composed of 657 amino acids. The ALAS2 gene is located on the X chromosome (Xp11.21) and is composed of 13 exons that generate three alternatively spliced mRNAs, each of which encode a distonct protein isoform. ALAS2 isoform a is composed of 587 amino acids, ALAS2 isoform b is composed of 550 amino acids, and ALAS2 isoform c is composed of 574 amino acids. Inherited defects in the ALAS2 gene result in the disorder called **X-linked sideroblastic anemia, XLSA**. Sideroblasts are erythroblasts with non-heme iron-containing conjugates (predominantly associated with the mitochondria), called siderosomes. XLSA has also been called congenital sideroblastic anemia, hereditary sideroblastic anemia, hereditary iron-loading anemia, X-linked hypochromic anemia, hereditary hypochromic anemia, and hereditary anemia.

Following synthesis, the mitochondrial ALA is transported to the cytosol, where ALA dehydratase (also called porphobilinogen synthase) dimerizes two molecules of ALA to produce the pyrrole ring compound porphobilinogen. Functional ALA dehydratase is a multi-subunit complex composed of eight identical subunits (homooctamer) and is also a clinically significant zinc– (Zn^{2+}) requiring enzyme. The ALA dehydratase gene (symbol: ALAD) is located on chromosome 9q32 and

is composed of 14 exons that generate three alternatively spliced mRNAs, each of which encode a distinct protein isoform. Mutations in the ALAD gene result in the autosomal recessive hepatic porphyria called ALAD deficient porphyria, ADP.
The next step in the pathway involves the head-to-tail condensation of four molecules of porphobilinogen to produce the linear tetrapyrrole intermediate, hydroxymethylbilane. The enzyme for this condensation is porphobilinogen deaminase (PBG deaminase). This enzyme is also called hydroxymethylbilane synthase or uroporphyrinogen I synthase. The PBG deaminase gene (official symbol: HMBS, for hydroxymethylbilane synthase) is located on chromosome 11q23.3 and is composed of 15 exons that generate four alternatively spliced mRNAs encoding different isoforms of the enzyme. PBG deaminase isoform 1 is composed of 361 amino acids, PBG deaminase isoform 2 is composed of 344 amino acids, PBG deaminase isoform 3 is composed of 321 amino acids, and PBG deaminase isoform 4 is composed of 304 amino acids. PBG deaminase isoform 2 is an erythroid cell-specific form of the enzyme. Once produced, hydroxymethylbilane has two main fates, one is due to enzymatic action, the other is non-enzymatic. Non-enzymatic alteration in hydroxymethylbilane is a cyclization to the compound called uroporphyrinogen I. The latter fate of hydroxymethylbilane is of significance only in patients with defects in enzymes downstream of PBG deaminase. Of significance to patients harboring a defective heme biosynthetic enzyme is the fact that defects prior to hydroxymethylbilane synthesis **ARE NOT** associated with photosensitivity, whereas, defects from this point on **ARE** associated with photosensitivity. Defects in the PBG deaminase gene result in the autosomal dominant hepatic porphyria called **acute intermittent porphyria**, AIP.
The most important fate of hydroxymethylbilane is the regulated, enzymatic conversion to uroporphyrinogen III, the next intermediate on the pathway to heme synthesis. This step is mediated by the enzyme, uroporphyrinogen-III synthase. The uroporphyrinogen-III synthase gene (symbol: UROS) is located on chromosome 10q26.2 and is composed of 18 exons that generate five alternatively spliced mRNAs, each of which encode a distinct protein isoform. Defects in the UROS gene are associated with the autosomal recessive erythroid porphyria called **congenital erythropoietic porphyria**, **CEP**

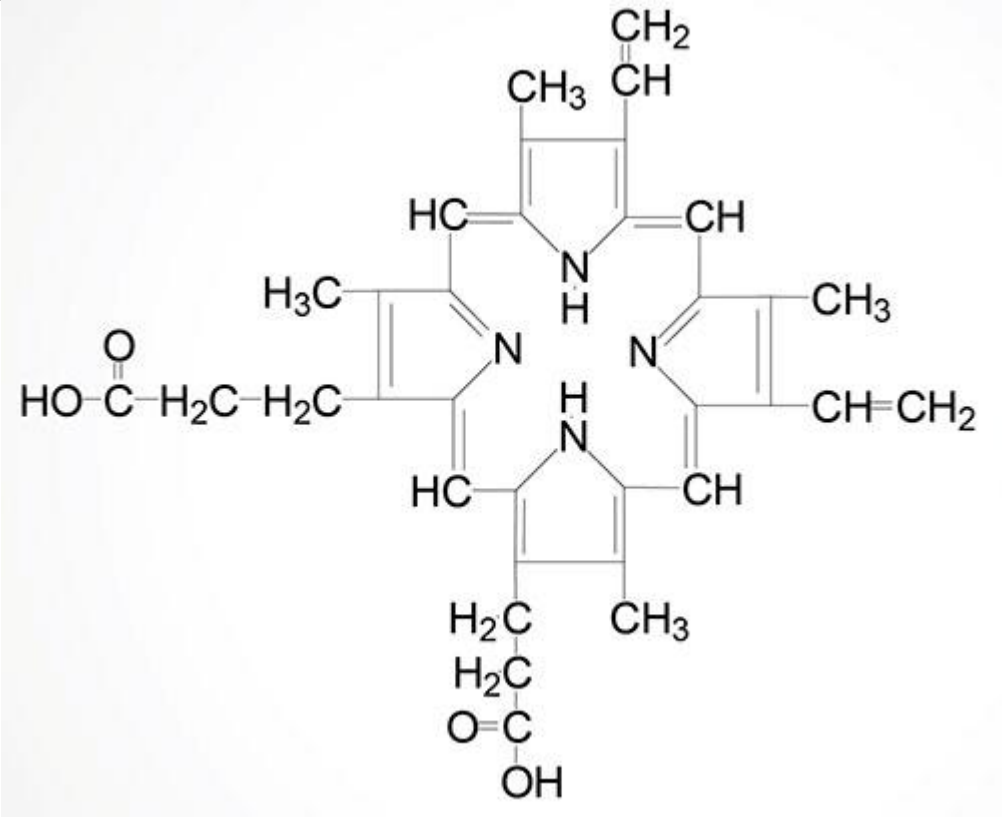


Pathway of Heme Biosynthesis. Heme biosynthesis begins in the mitochondria from glycine and succinyl-CoA, continues in the cytosol, and ultimately is completed within the mitochondria. The heme that it produced by this biosynthetic pathway is identified as heme *b*. PBG: porphobilinogen; ALA: δ-aminolevulinic acid.

In the cytosol, the acetate substituents of uroporphyrinogen (normal uroporphyrinogen III or abnormal uroporphyrinogen I) are all decarboxylated by the enzyme uroporphyrinogen decarboxylase (gene symbol: UROD). The resultant products have methyl groups in place of acetate and are known as coproporphyrinogens, with coproporphyrinogen III being the important normal intermediate in heme synthesis. The UROD gene is located on chromosome 1p34.1 and is composed of 10 exons that encode a protein is 367 amino acids. Mutations in the UROD gene are associated with various forms of the autosomal dominant porphyria called **porphyria cutanea tarda**, **PCT**. Mutations in the UROD gene that are directly associated with the pathogenesis of PCT are only associated with the type II version of the disorder. The type I and type III forms of PCT are most likely the result of multifactorial effects that secondarily negatively affect the function of the UROD enzyme.

Following its synthesis, coproporphyrinogen III is transported to the interior of the mitochondrion, where two propionate residues are decarboxylated, yielding vinyl substituents on the two pyrrole rings. The colorless product is protoporphyrinogen IX. These reactions are catalyzed by oxygen-dependent coproporphyrinogen-III oxidase (gene symbol: CPOX). The CPOX gene is located on chromosome 3q11.2 and is composed of 10 exons that encode a precursor protein of 454 amino acids.

Mutations in the CPOX gene result in the autosomal dominant acute hepatic porphyria called **hereditary coproporphyria**, **HCP**
In the mitochondrion, protoporphyrinogen IX is converted to protoporphyrin IX (structure shown below) by protoporphyrinogen IX oxidase. The protoporphyrinogen IX oxidase gene (symbol: PPOX) is located on chromosome 1q23.3 and is composed of 19 exons that generate six alternatively spliced mRNAs. These six mRNAs encode four distinct protein isoforms. The oxidase reaction requires molecular oxygen and results in the loss of six protons and six electrons, yielding a completely conjugated ring system, which is responsible for the characteristic red color of the hemes. Mutations in the PPOX gene result in the autosomal dominant acute hepatic porphyria called **variegate porphyria**, VP.
The final reaction in heme synthesis also takes place in the mitochondrion and involves the insertion of the ferrous iron (Fe^{2+}) atom into the ring system generating heme *b*. The enzyme catalyzing this reaction is known as ferrochelatase. The ferrochelatase gene (symbol: FECH) is located on chromosome 18q21.31 and is composed of 12 exons that generate two alternatively spliced mRNAs. Ferrochelatase isoform a is composed of 429 amino acids and isoform b is composed of 423 amino acids. Defects in the ferrochelatase gene are associated with the autosomal dominant erythroid porphyria called **erythropoietic protoporphyria, EPP.**

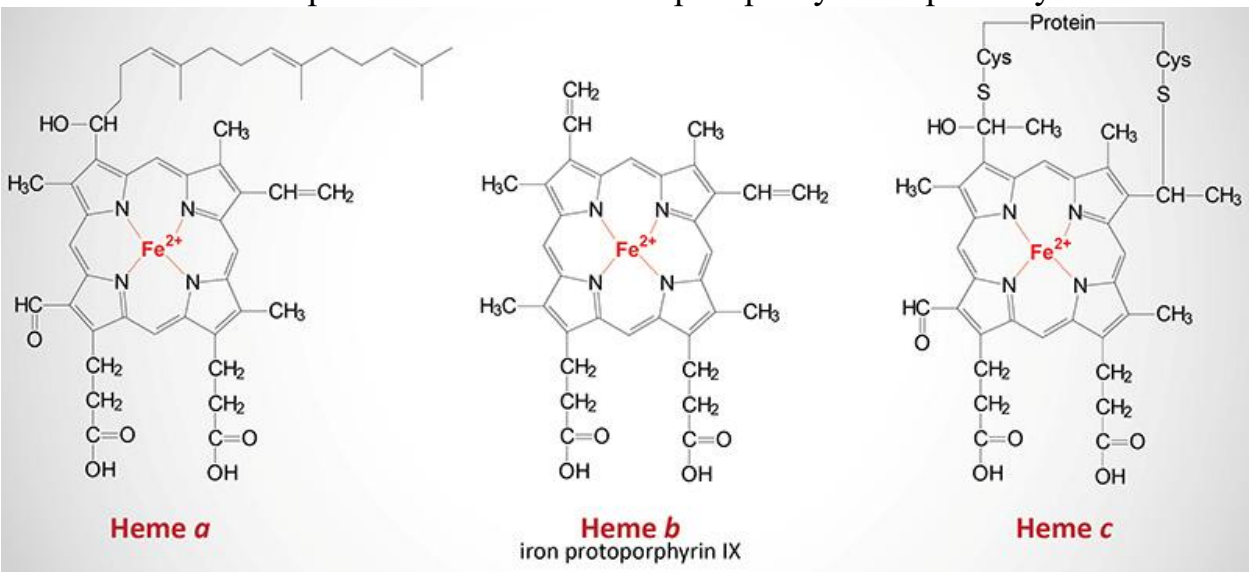


Heavy Metal Inhibition of Heme Metabolism
The enzymes ferrochelatase, ALA synthase and ALA dehydratase (a sulfhydryl containing enzyme) are sensitive to inhibition by heavy metal poisoning, with inhibition of ferrochelatase being the most sensitive and most significant to the clinical manifestations of heavy metal poisoning. A characteristic of lead poisoning is an increase in ALA in the circulation in the absence of an increase in heme.

Indeed, due to the inhibition of ferrochelatase and the associated loss of heme synthesis, the feed-back inhibition of ALA synthase is no longer active leading to increased ALA synthesis even in the presence of a heavy metal such as lead. The consequences of heavy metal inhibition of ferrochelatase are an acquired porphyria (as opposed to an inherited disease) referred to as **plumbism** (so-called because the symbol for lead is Pb). Due to the lack of enzymatic incorporation of iron into protoporphyrin IX, by heavy metal inhibited ferrochelatase, erythroblasts will acquire siderosomes. Siderosomes are histologically observable structures that result from iron deposition on mitochondria. In addition, zinc atoms will non-enzymatically incorporate into protoporphyrin IX forming Zn-protoporphyrin (ZPP). Zn-protoporphyrin imparts a fluorescence capability that can be visualized by observing blood under appropriate wavelength light which causes the erythroid cells to "glow". This same phenomenon is observable in patients with iron deficient anemia. Indeed, the measurement of ZPP is used as a screening tool for both heavy metal (lead) poisoning and iron deficiency.

The Various Heme Molecules

In addition to the heme *b* found in hemoglobin, there are two additional forms of heme found in cytochromes such as those involved in the process of oxidative phosphorylation. Cytochromes of the *c* type contain a modified iron protoporphyrin IX known as heme *c*. In heme *c* the 2 vinyl (C=C) side chains are covalently bonded to cysteine sulfhydryl residues of the apoprotein. Only cytochromes of the *c* type contain covalently bound heme. Heme *a* is also a modified iron protoporphyrin IX. **Heme a** is found in cytochromes of the *a* type such as those of complex IV of the oxidative phosphorylation pathway.



Regulation of Heme Biosynthesis

Although heme is synthesized in virtually all tissues, the principal sites of synthesis are erythroid cells (≈85%) and hepatocytes (accounting for nearly all the rest of heme synthesis). The differences in these two tissues and their needs for heme result in quite different mechanisms for regulation of heme biosynthesis.

In hepatocytes, heme is required for incorporation into the cytochromes, in particular, the P450 class of cytochromes (CYP) that are important for xenobiotic detoxification. In addition, numerous cytochromes of the oxidative-

phosphorylation pathway contain heme. The rate-limiting step in hepatic heme biosynthesis occurs at the ALA synthase catalyzed step, which is the committed step in heme synthesis. Heme itself functions as a co-repressor in the inhibition of ALA synthase gene expression. The Fe^{3+} oxidation product of heme is termed **hemin**. Heme itself, and hemin acts as a feed-back inhibitors on ALA synthase. Hemin also inhibits transport of ALA synthase from the cytosol (its site of synthesis) into the mitochondria (its site of action). Because certain pharmaceutical drugs are metabolized by the hepatic CYP system, which requires heme, increased utilization of heme occurs upon administration of these drugs. Of particular significance are the barbiturates. Use of barbiturates should **NEVER** be prescribed for the pain associated with certain types of porphyrias. This is because the administration of barbiturates leads to their degradation by CYP enzymes in the liver, resulting in a reduction in overall heme levels as the heme needs to be incorporated into the CYP for their function. This results in de-repression of ALA synthase with the result being an exacerbation of the symptoms of the porphyria due to increased ALA synthesis and subsequent heme biosynthesis products upstream of the defective enzyme.

In erythroid cells all of the heme is synthesized for incorporation into hemoglobin and occurs only upon differentiation when synthesis of hemoglobin proceeds. When red cells mature both heme and hemoglobin synthesis ceases. The heme (and hemoglobin) must, therefore, survive for the life of the erythrocyte (normally this is 120 days). In reticulocytes (immature erythrocytes) heme stimulates protein synthesis. Additionally, control of heme biosynthesis in erythrocytes occurs at numerous sites other than at the level of ALA synthase. Control has been shown to be exerted on ferrochelatase, the enzyme responsible for iron insertion into protoporphyrin IX, and on porphobilinogen deaminase.

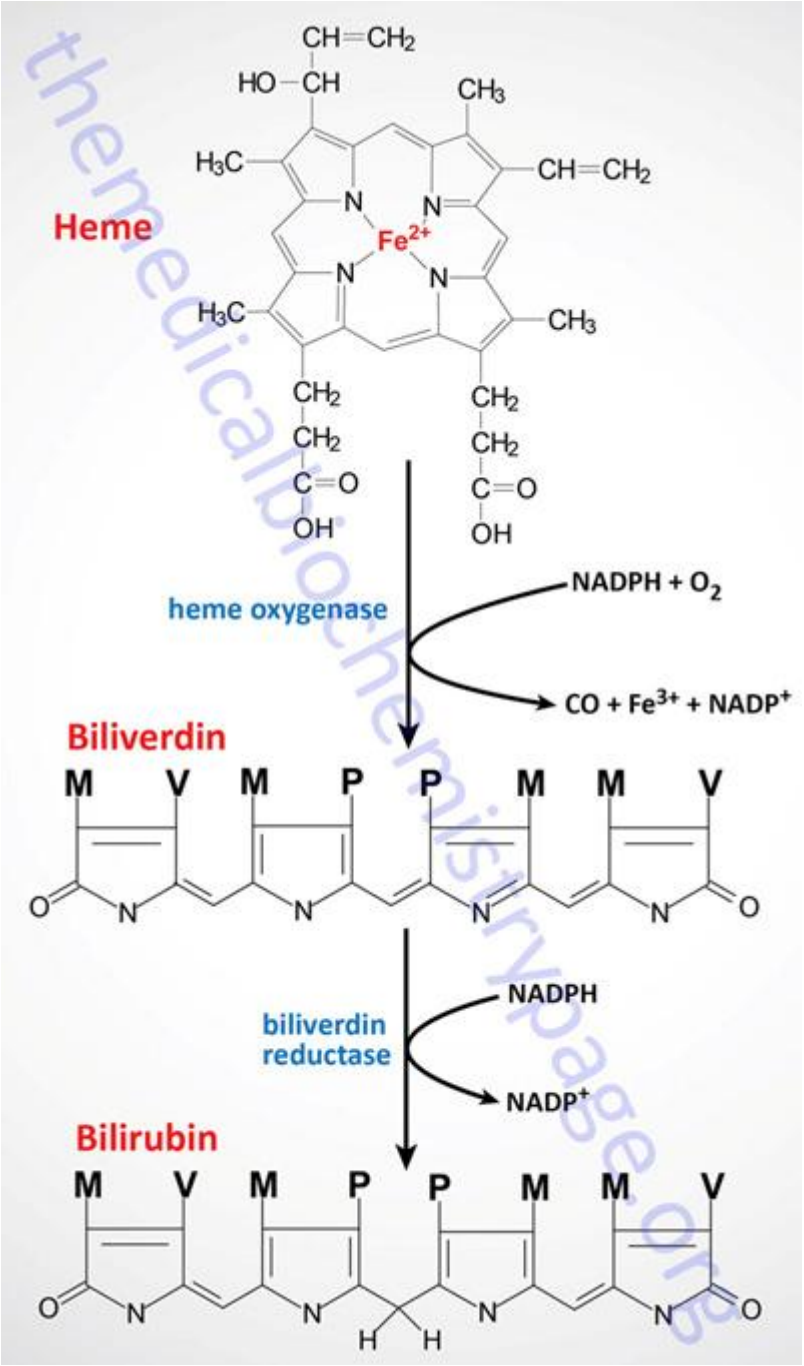
Heme Metabolism and Disposition of Bilirubin

The largest repository of heme in the human body is in red blood cells, which have a life span of about 120 days. There is thus a turnover of about 6 g/day of hemoglobin, which presents 2 problems. First, the porphyrin ring is hydrophobic and must be solubilized to be excreted. Second, iron must be conserved for new heme synthesis.

Normally, senescent red blood cells and heme from other sources are engulfed by cells of the reticuloendothelial system (phagocytic macrophages primarily of the spleen but also of the liver, lymph, and bone marrow). The globin is recycled or converted into amino acids, which in turn are recycled or catabolized as required. Heme is oxidized, with the heme ring being opened by the endoplasmic reticulum enzyme, heme oxygenase. The oxidation step requires heme as a substrate, and any hemin (Fe^{3+}) is reduced to heme (Fe^{2+}) prior to oxidation by heme oxygenase. The heme oxygenase-mediated oxidation occurs on a specific carbon producing the linear tetrapyrrole **biliverdin**, ferric iron (Fe^{3+}), and carbon monoxide (CO). This is the only reaction in the body that is known to produce CO. Most of the CO is excreted through the lungs, with the result that the CO content of expired air is a direct measure of the activity of heme oxygenase in an individual. In the next reaction a second bridging methylene (between rings III and IV) is reduced by

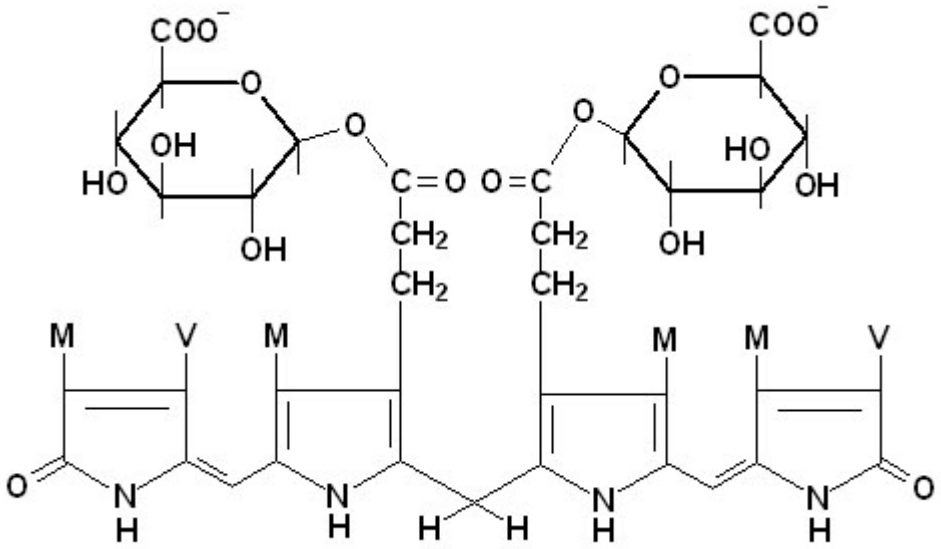
biliverdin reductase, producing **bilirubin**. There are two biliverdin reductase genes in humans identified as BLVRA and BLVRB. The enzyme encoded by the BLVRA gene is principally responsible for the catabolism of biliverdin. The enzyme encoded by the BLVRB gene catalyzes the reduction of not only biliverdin but also a variety of flavins, such as riboflavin, FAD or FMN, and methemoglobin. The enzyme encoded by the BLVRB gene is also called NADPH-dependent flavin reductase. The BLVRA gene is located on chromosome 7p13 and is composed of 11 exons generate two alternatively spliced mRNAs, both of which encode the same 296 amino acid precursor protein. The BLVRB gene is located on chromosome 19q13.2 and is composed of 5 exons that encode a protein of 206 amino acids.

Bilirubin is significantly less extensively conjugated than biliverdin causing a change in the color of the molecule from blue-green (biliverdin) to yellow-red (bilirubin). The latter catabolic changes in the structure of tetrapyrroles are responsible for the progressive changes in color of a hematoma, or bruise, in which the damaged tissue changes its color from an initial dark blue to a red-yellow and finally to a yellow color before all the pigment is transported out of the affected tissue. Peripherally arising bilirubin is transported to the liver in association with albumin, where the remaining catabolic reactions take place.



Pathway for the degradation of heme to bilirubin. The ring of heme is opened through the action of heme oxygenase which also results in the relase of the iron as the ferric form (Fe^{3+}) and also releases carbon monoxide, CO. The product of the heme oxygenase reaction is biluverdin. Biliverdin is converted to bilirubin via the action of biliverdin reductase. The various substituents on the pentameric rings of biliverdin and bilirubins are M: methyl, P: propyl, V: vinyl.

In hepatocytes, bilirubin-UDP-glucuronosyltransferase (bilirubin-UGT: a member of the large UDP glucuronosyltransferase family of enzymes) adds two equivalents of glucuronic acid to bilirubin to produce the more water soluble, bilirubin diglucuronide derivative. The increased water solubility of the tetrapyrrole facilitates its excretion with the remainder of the bile as the bile pigments.



In newborn infants, or in individuals with abnormally high red cell lysis, or liver damage with obstruction of the bile duct, the bilirubin and its precursors accumulate in the circulation; the result is **hyperbilirubinemia**, the cause of the abnormal yellowish pigmentation of the eyes and tissues known as **jaundice**. All newborn infants undergo turnover of the red blood cells that contain fetal hemoglobin (HbF) so that new red blood cells containing adult hemoglobin (HbA) can be produced. In some cases the activation of the UGT1A gene at birth is insufficient to handle all the red cell turnover resulting in neonatal jaundice, apparent around day 2 or 3. If the blood levels of bilirubin do not decline in a short period of time these infants will need to be treated by phototherapy. The blue-green wavelength light (460-490 nm) used in biliblankets is sufficient to induce breakdown of bilirubin in the skin so that it can be cleared from the blood. In rare cases the phototherapy does not work fast enough and in these cases it is appropriate to treat the infants with phenobarbital which enhances the induction of the UGT1A gene.

In normal individuals, intestinal bilirubin is acted on by bacteria to produce the final porphyrin products, urobilinogens and stercobilins, that are found in the feces. The stercobilins oxidize to brownish pigments which impart the brown to brown-black color to normal feces. Indeed, the color of the stool can be quite diagnostic since chalky clay colored feces are indicative of a defect in the hepato-biliary circulation, such as in bile obstruction. Some of the urobilinogen produced by intestinal bacteria is reabsorbed from the gut and enter the circulation. These urobilinogens are converted to the urobilins which are then excreted in the urine. Oxidation of the urobilins imparts the yellowish coloration to urine.