Biochemistry - HLS

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Metabolism of heme

Prof. Mamoun Ahram
Hematopoietic-lymphatic system

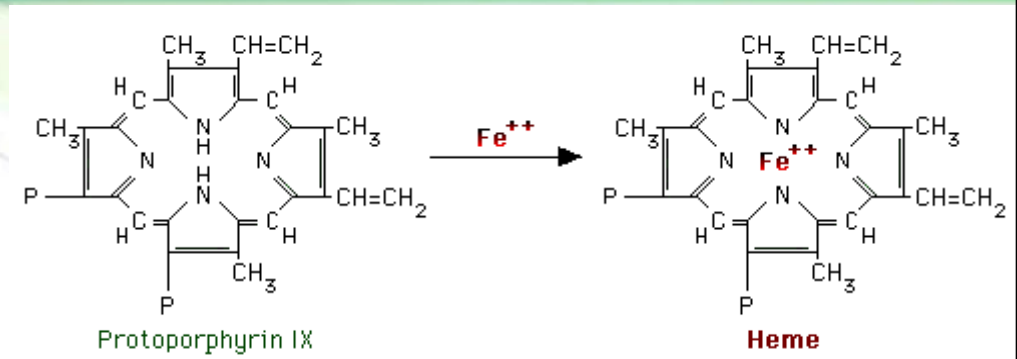
Resources

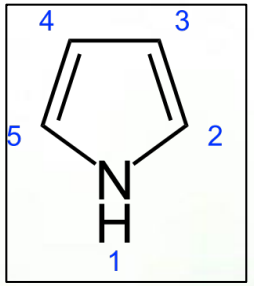
- This lecture
- Lippincott's Biochemistry, 7th edition, Ch. 21

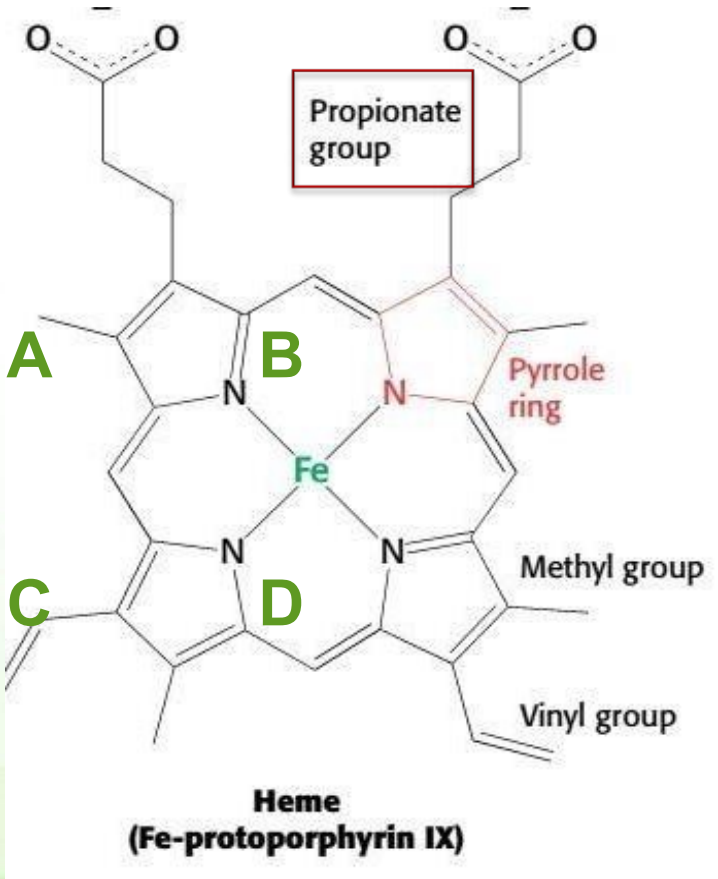
Heme structure

- It is a complex of protoporphyrin IX + Iron (Fe^{2+}) as a prosthetic group.

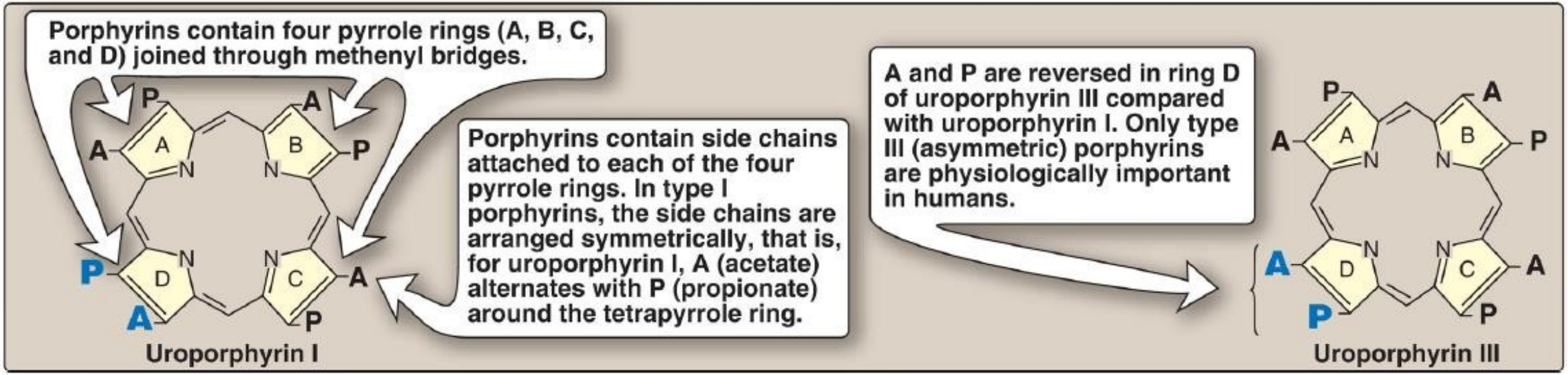
  Iron should be in the ferrous state in order the heme to be active
- The porphyrin is planar and consists of four pyrrole rings (designated A-D).
- Each pyrrole ring can bind two substituents. (Methyl and vinyl groups and they can alternate)
- Two rings have a propionate group each.
- Note: the molecule is hydrophobic.
- Fe has six coordinates of binding.





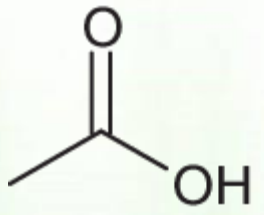


Prophyrins

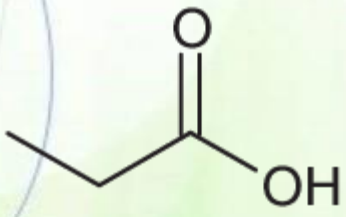


The pyrrole rings are connected to each other via methanol bridges
The substituents attached to the pyrrole rings can alternate

- Uroporphyrin I: A, P, A, P, A, P, A, P
- Uroporphyrin II: A, P, A, P, P, A, A, P



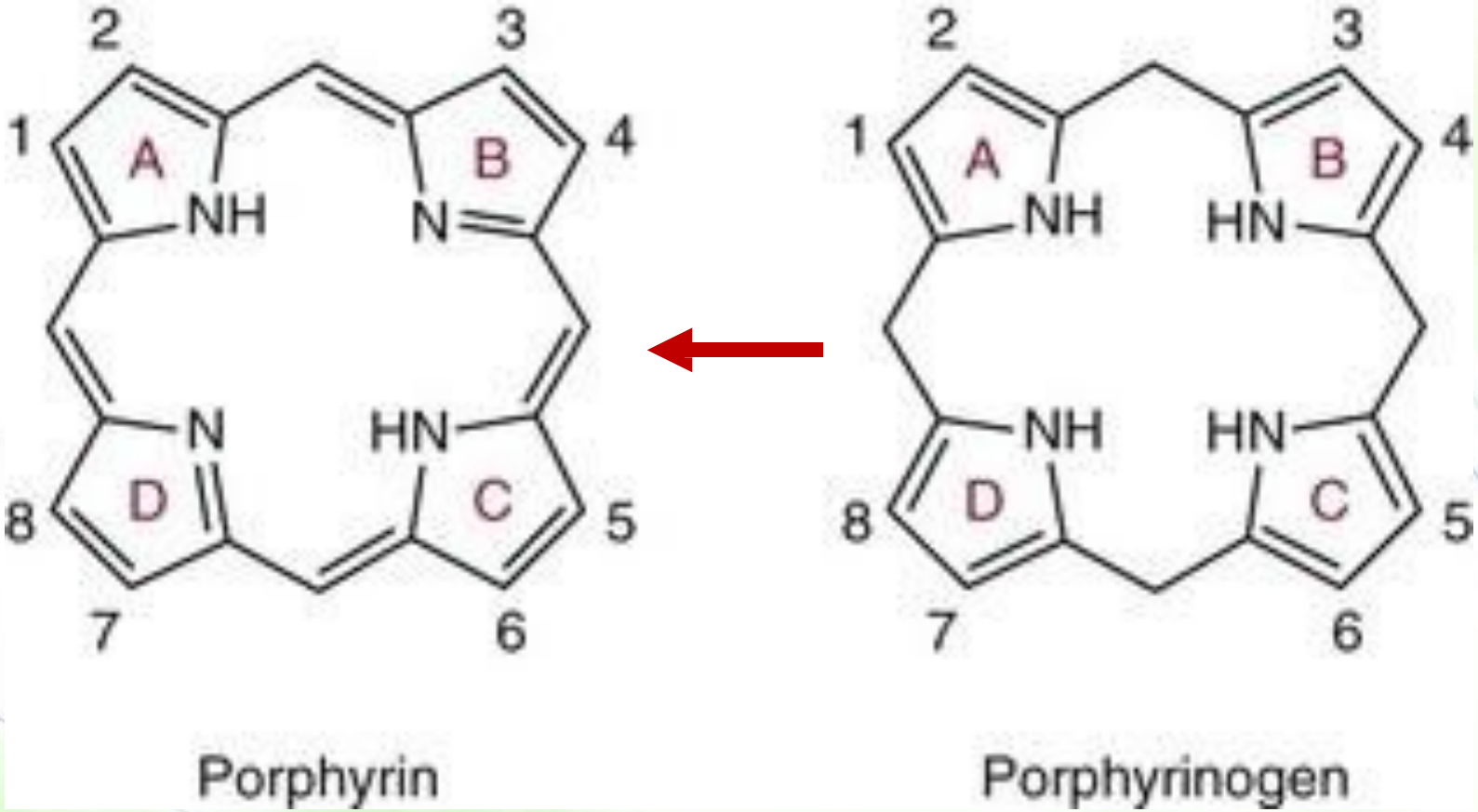
Acetate



Propionate

Porphyrinogens vs. porphyrins

Porphyrins are made of precursors and the precursors are known as porphyrinogens



Porphyrinogens are:

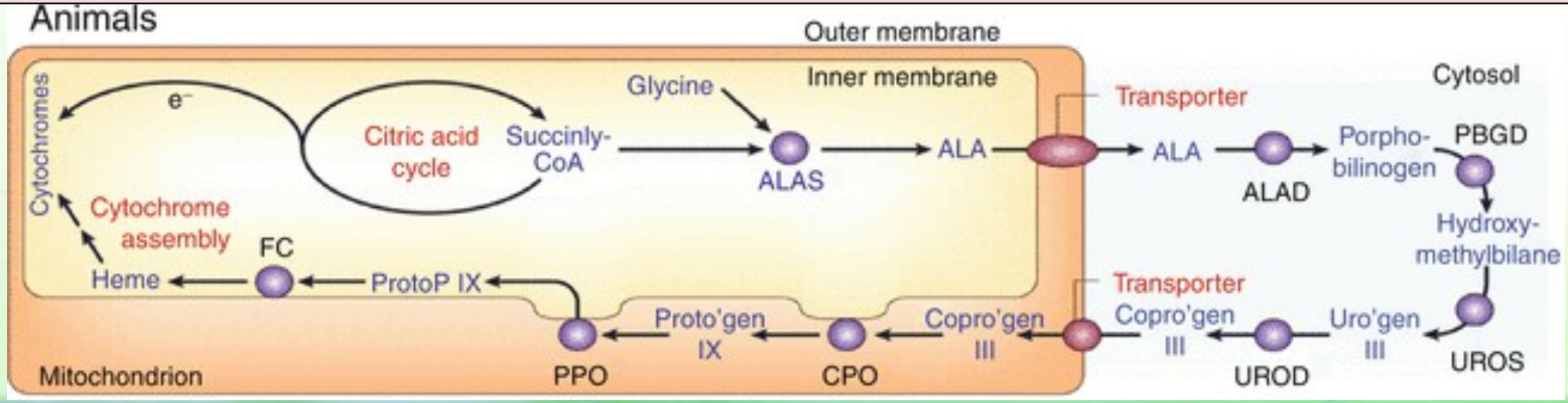
1. **Reduced (all nitrogens are reduced)**
2. **Porphyrin precursors Colorless (if it's get oxidized, it will develop a purple color)**
3. **Intermediates of heme synthesis**

Biosynthesis of heme

Sites of synthesis

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

Succinyl CoA, which is an intermediate of the citric acid cycle, conjugates with glycine into a product known as aminolevulinic acid (ALA). then ALA is transported out of the mitochondria and gets converted into several intermediates. then one of them gets back into the mitochondria and eventually it gets converted into heme



Synthesis of 5'-aminolevulinic acid (ALA)

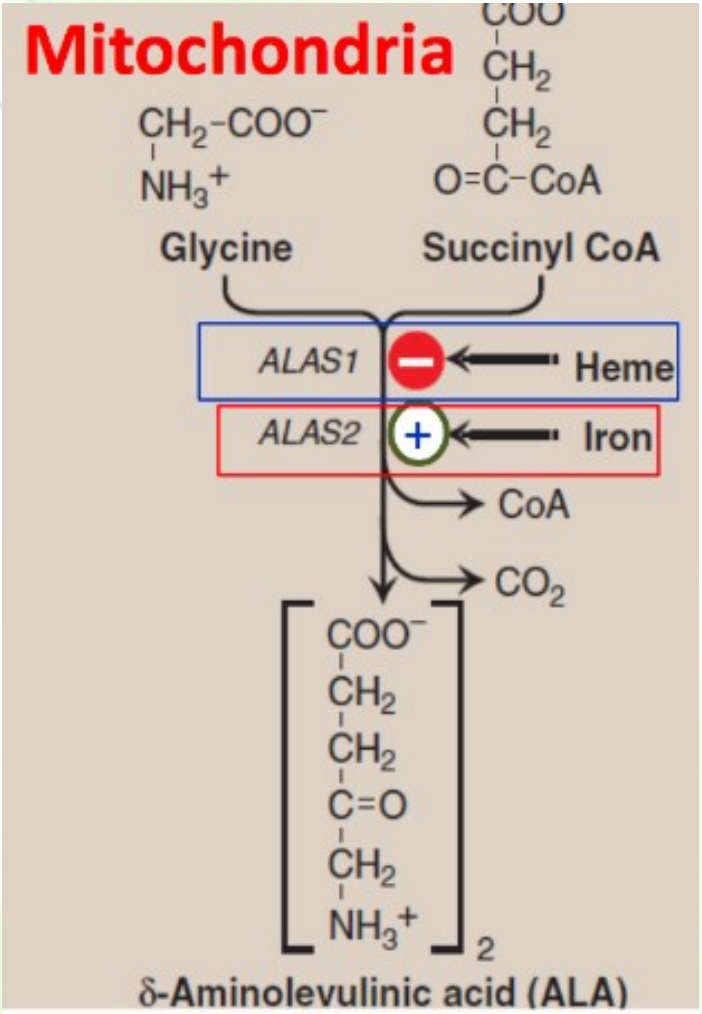
- The first reaction (probably the most important reaction) is catalyzed by 5’-aminolevulinic acid synthase, ALAS1 (expressed in all tissues inc. liver) or ALAS2 (erythroid only), which conjugates gly and succinyl CoA into ALA.
  - It is the rate limiting and committed step.
  - It requires vitamin B6 (pyridoxal phosphate).

Rate limiting step is slow because it’s highly regulated

It’s the committed step because once you have the formation of ALA, it has to go forward till the end and the formation of heme

This step occurs in the mitochondria

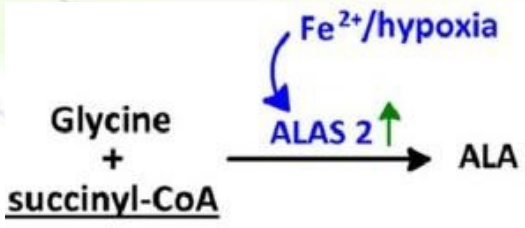
- ALA moves out of mitochondria to cytosol.

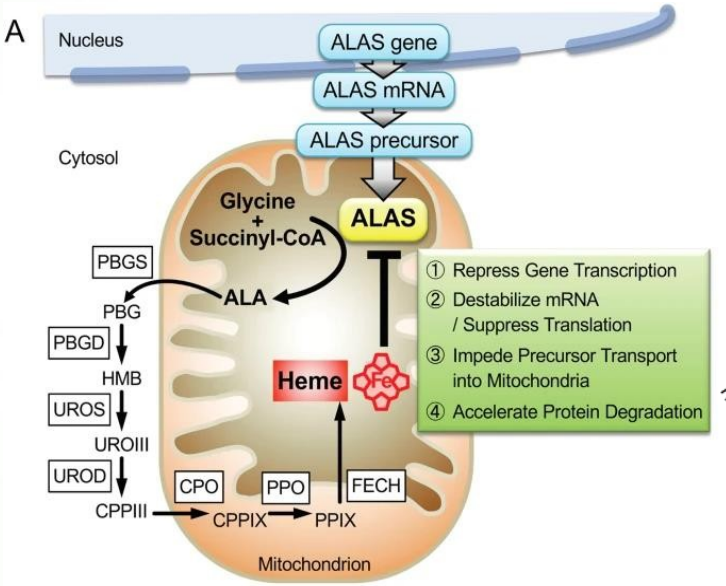


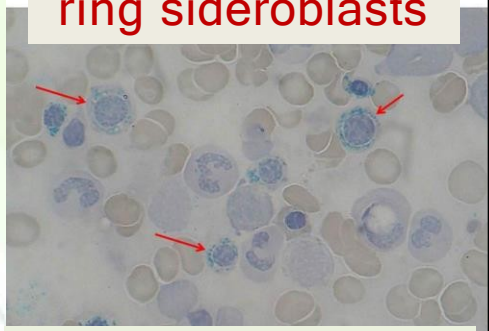
ALA synthase isoenzymes

- ALAS2 is regulated by level of iron and by hypoxia
  - Loss of function mutations result in X-linked sideroblastic anemia.
    - Iron accumulates in the erythroid marrow and deposits as mitochondrial non-ferritin iron **ring sideroblasts**.
- ALAS1 is regulated by
  - Hemin: Has iron in the ferric stated and linked to chloride (it regulates ALAS1 by 4 mechanisms)
    - It reduces the transcription of ALAS1
    - Reduces synthesis and stability of mRNA
    - Inhibits mitochondrial import of ALAS1
    - `Induces protein degradation
  - Drugs:

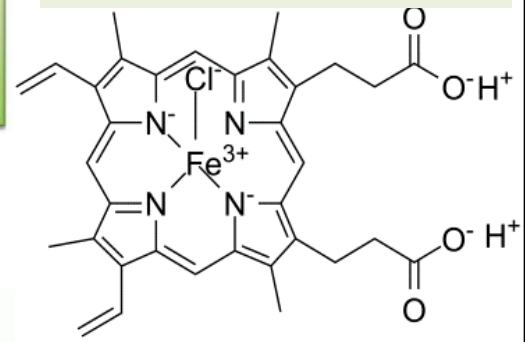
Drugs → ↑CYP450 → ↓heme → ↑ALAS1 synthesis







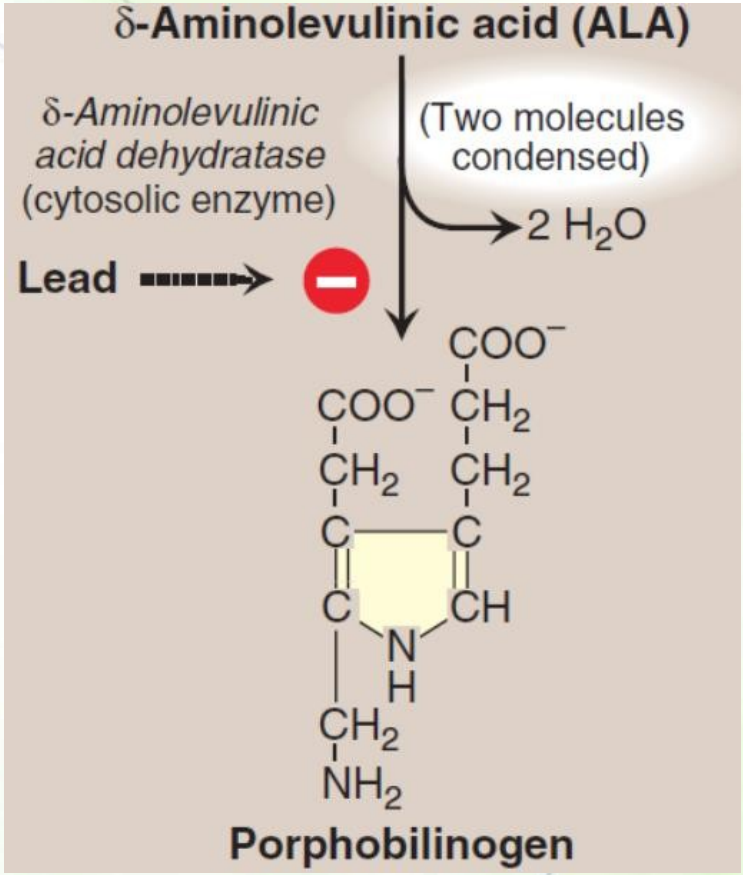
Hemin



Drugs require CYP450 for detoxification or modification which results in increasing the synthesis of CYP540, now since there is high demand for heme, this would reduce the level of heme in hepatocytes which induces ALAS1 synthesis at the transcriptional level

Synthesis of porphobilinogen

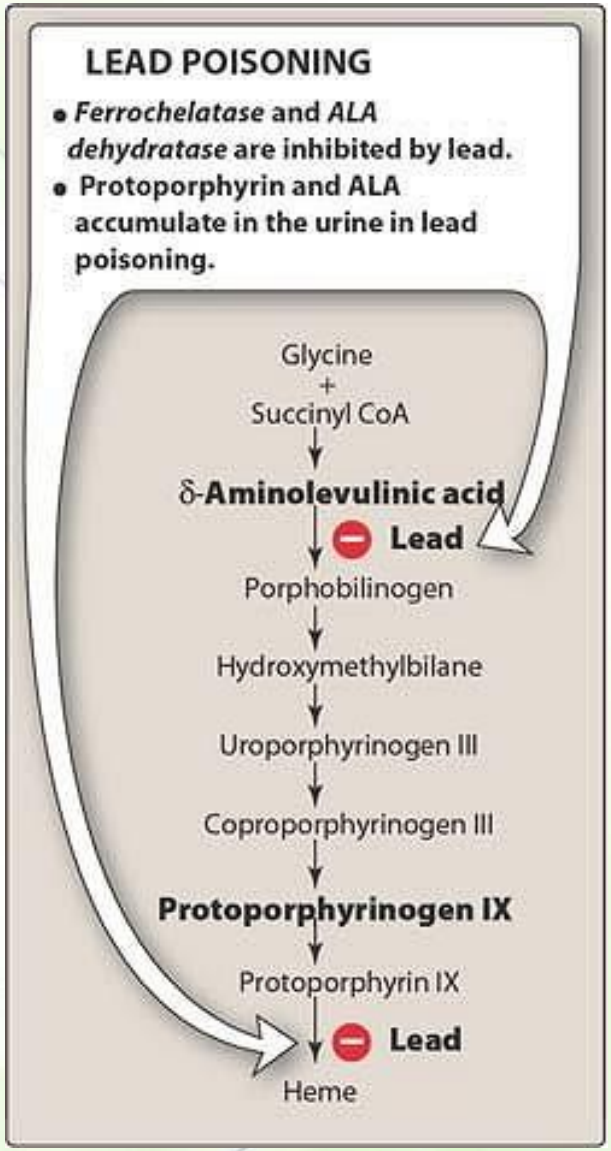
- ALA moves out of mitochondria to cytosol where porphobilinogen is formed by condensing 2x ALA by zinc-containing ALA dehydratase (porphobilinogen synthase).
- The enzyme is sensitive to inhibition by heavy metal ions (for example, lead) that replace the zinc.
- This inhibition causes
  - increase in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



Subsequent reactions

- 4x PBG (porphobilinogen) → hydroxymethylbilane (linear molecule and gets cyclized) → *cyclic* uroporphyrinogen III → coproporphyrinogen III → *mitochondria* → protoporphyrinogen IX (precursor) → *oxidized* protoporphyrin IX (gets attached to iron) → (+ Fe^{+2}) heme.
  - The last reaction is spontaneous, but can be catalyzed by ferrochelatase. (This enzyme is sensitive to lead and can be inhibited by lead)

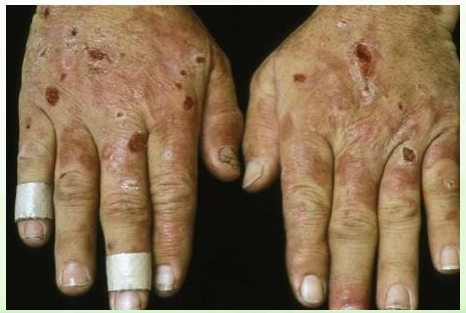
Lead poisoning leads to inhibition of formation of porphobilinogen and accumulation of ALA (so no intermediates formation, but it is not the case because we still have low levels of intermediates and some enzymatic activity) but lead also results in inhibition of the very last reaction (so will terminate the synthesis of heme)

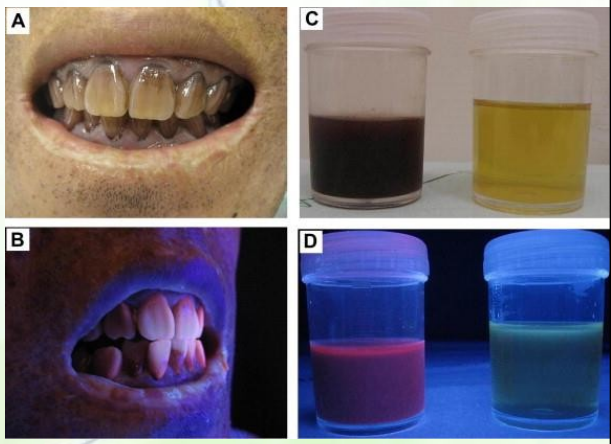


Porphyrias

Mutations of the genes of the enzymes that catalyze any of the reactions would lead to accumulation of intermediates and finally inhibition of the synthesis of the heme

- Porphyrias: inherited or acquired disorders caused by a deficiency of enzymes in the heme biosynthetic pathway resulting in elevations in the serum and urine content of intermediates in heme synthesis.
- Porphyria = purple. (Some of the intermediates can turn into purple color)
- These disorders are classified according to:
  - Affected tissue (*site of expression*): There are variations of how these enzymes are regulated, expressed and needed in different tissues and the conditions (drugs …)
    - Erythroid
    - Hepatic (acute or chronic) Acute or chronic depending on the circumstances of the patient (ingestion of certain types of food, consumption of alcohol, taking drugs ,,etc.)
  - Manifestations Depending on which enzyme is inhibited
    - Not photosensitive Check next slide
      - Abdominal and neuropsychiatric
    - Photosensitive
      - Tetrapyrrole-dependent Skin
      - itching and burns
        - ↑ Superoxide radicals





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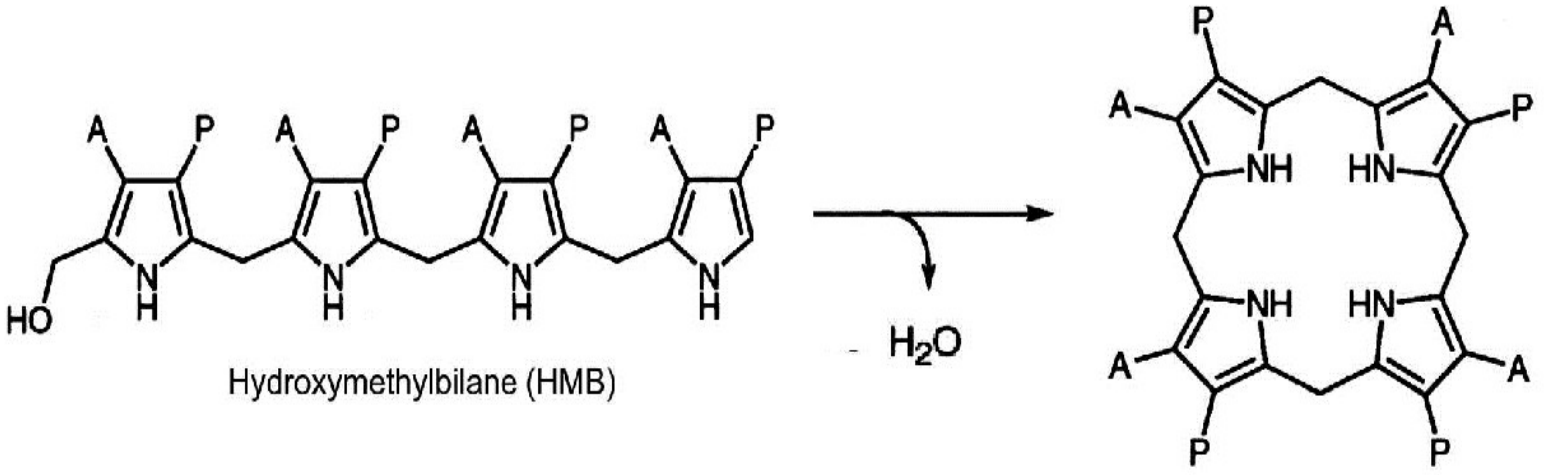


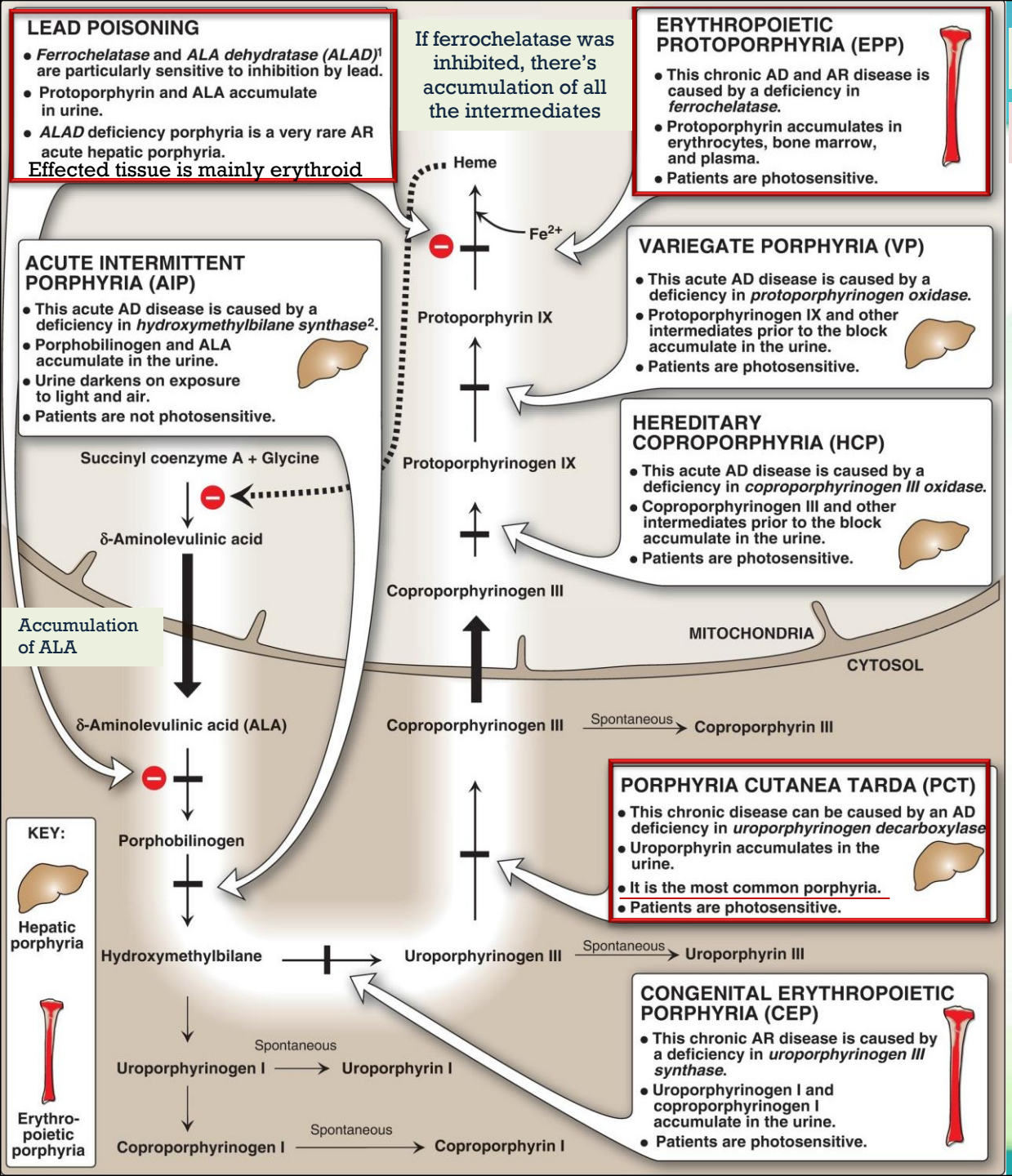
Hydroxymethylbilane (HMB)

Uroporphyrinogen III

In this reaction, the linear molecule hydroxymethylbilane is converted into the cyclic molecule uroporphinogen III (This reaction is important).

If a mutation takes place in the enzyme catalyzing this reaction or the previous reactions, this will result in conditions that are not photosensitive

Uroporphrinogen and the intermediates that are formed after can absorb light and can turn into purple, so the patient would be more photosensitive (because there's accumulation of these porphyrins in the skin and upon exposure to sunlight, there's formation of Superoxide radical that cause skin damage, cell death , skin itching, burns and blisters)



Study the 3 red boxes

These conditions can be erythroid specific or hepatic

AD = autosomal dominant
AR = autosomal recessive

Hepatic porphyria
↓
↓heme
↓
↑ALAS1 synthesis
↓
↑intermediates
↓
↑toxicity

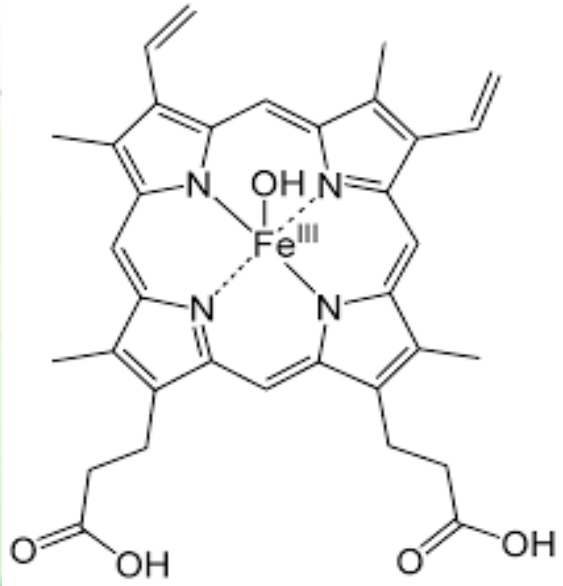
Drugs??

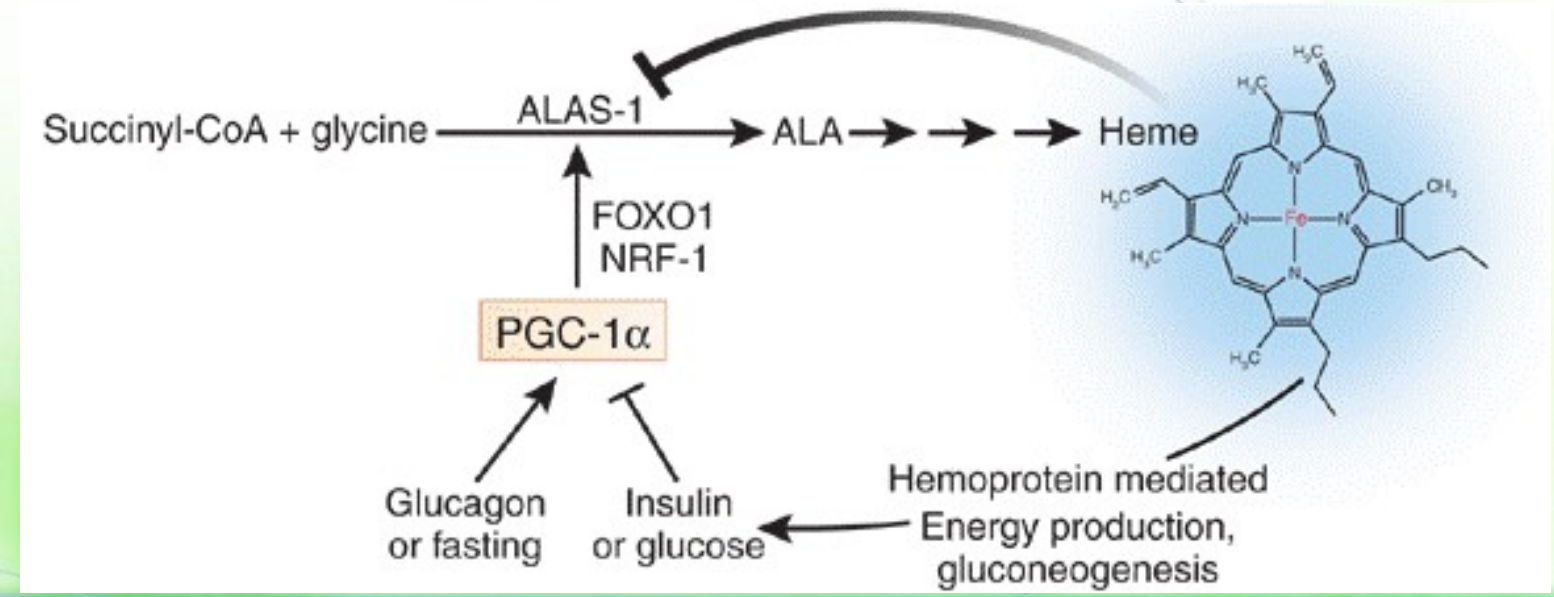
Remember: heme is a regulator of ALAS1 Enzyme

You can imagine what would happen if certain drugs were ingested by these patients

Treatment

- Hemin (or hematin) strongly inhibits the activity of ALAS. This is the best treatment
- Glucose: by decreasing synthesis of ALAS1 by inhibiting the transcription factor, PGC-1α, in the liver, which reduces the synthesis of gluconeogenic genes and the ALAS1 gene resulting in accumulation of heme intermediates. Note that glucose increases the amount of insulin and insulin inhibits the production of the transcription factor, PGC-1α
  - Fasting (hypoglycemia) exacerbates acute porphyria attack. Fasting increases the amount of glucagon which will induce the expression of PGC-1α inducing the expression of ALAS-1 resulting in an increased level of all of the intermediates





Catabolism of heme

Challenges

- RBCs are the largest storage place of heme. 2500 grams of iron in RBCs
- Erythrocytes are mainly destroyed by macrophages in the spleen and bone marrow, releasing hemoglobin, which is degraded to heme and globin.
- The (globin) protein is metabolized into amino acids which can be recycled
- 6 g/day of hemoglobin are turned over (this is a huge amount) , but

Two challenges

  - First, the porphyrin ring is hydrophobic, it's not soluble in blood so it must be dealt with delicately in order to be excreted or metabolized.
  - Second, iron must be conserved.

Heme degradation

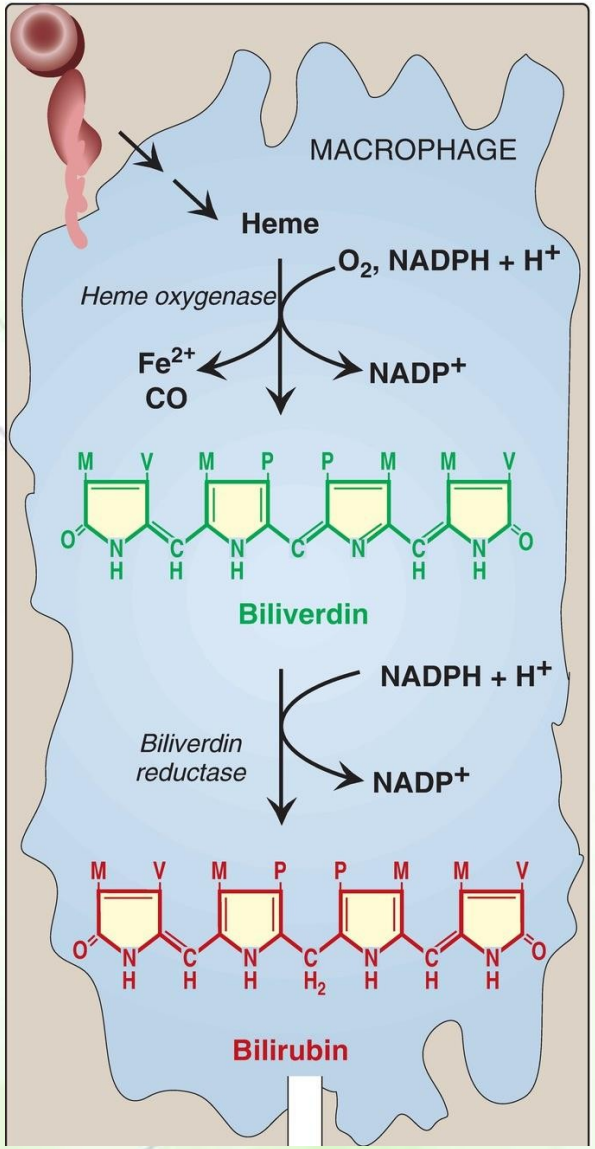
It's done through a number of reactions

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

The 1st reaction is very important, it's catalyzed by heme oxygenase which metabolizes heme into biliverdin.

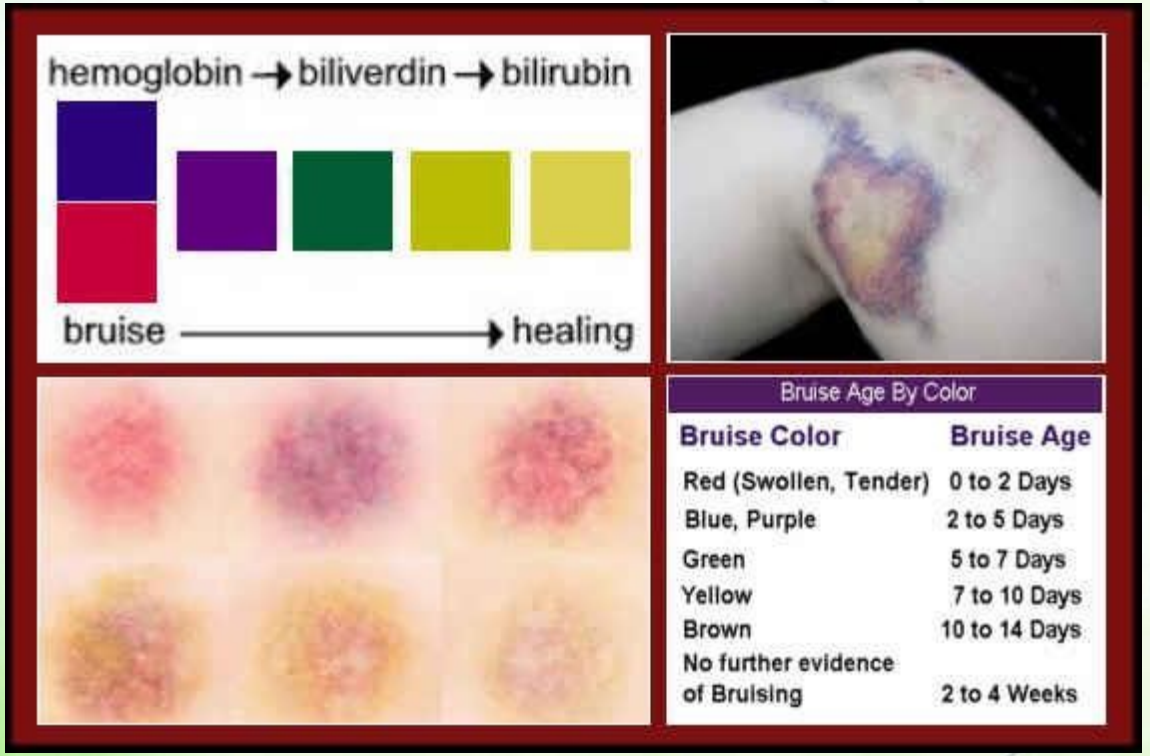
- This reaction requires NADPH
- The result is the release of iron and the production of CO (this the only reaction in the body where you have production of CO).

Then biliverdin goes through different reactions and the intermediates of these reactions have different colors, and biliverdin is converted to bilirubin which is released into the blood carried by albumin.



Heme degradation

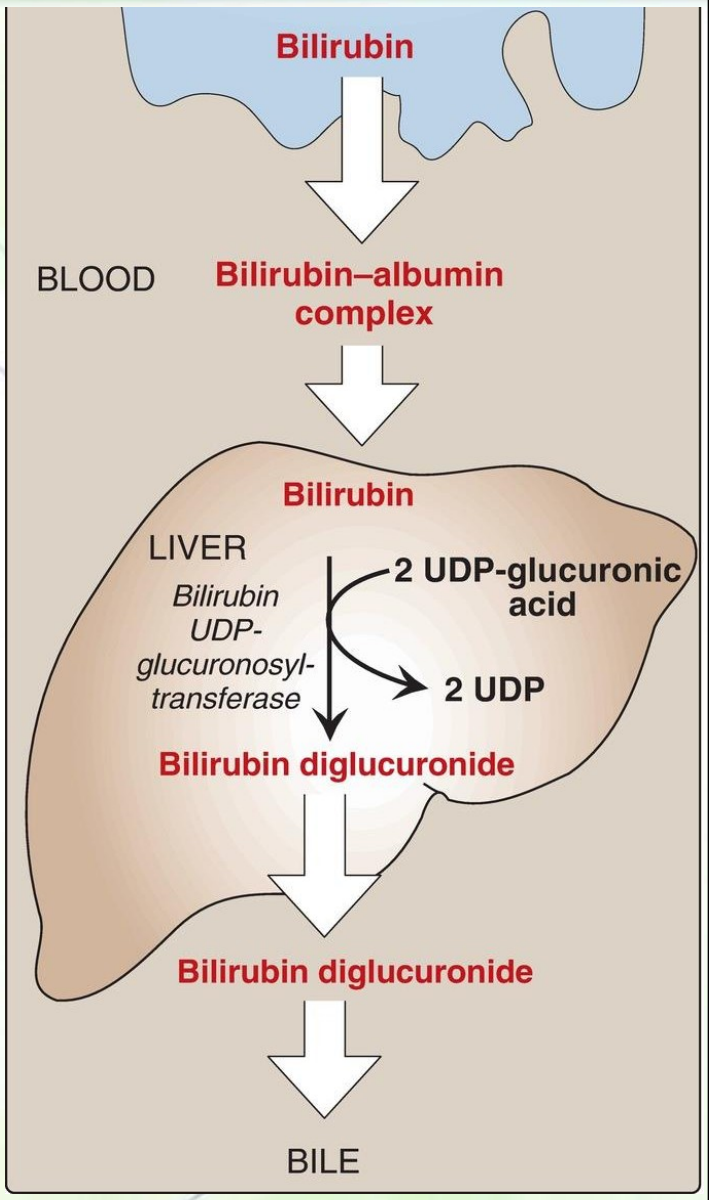
Whenever there's a bruise in the skin, it goes through different colors; first, the release of blood (hemolysis) then heme is metabolized to different intermediates with different colors (that's why bruises show different colors) blue → purple → green → yellow

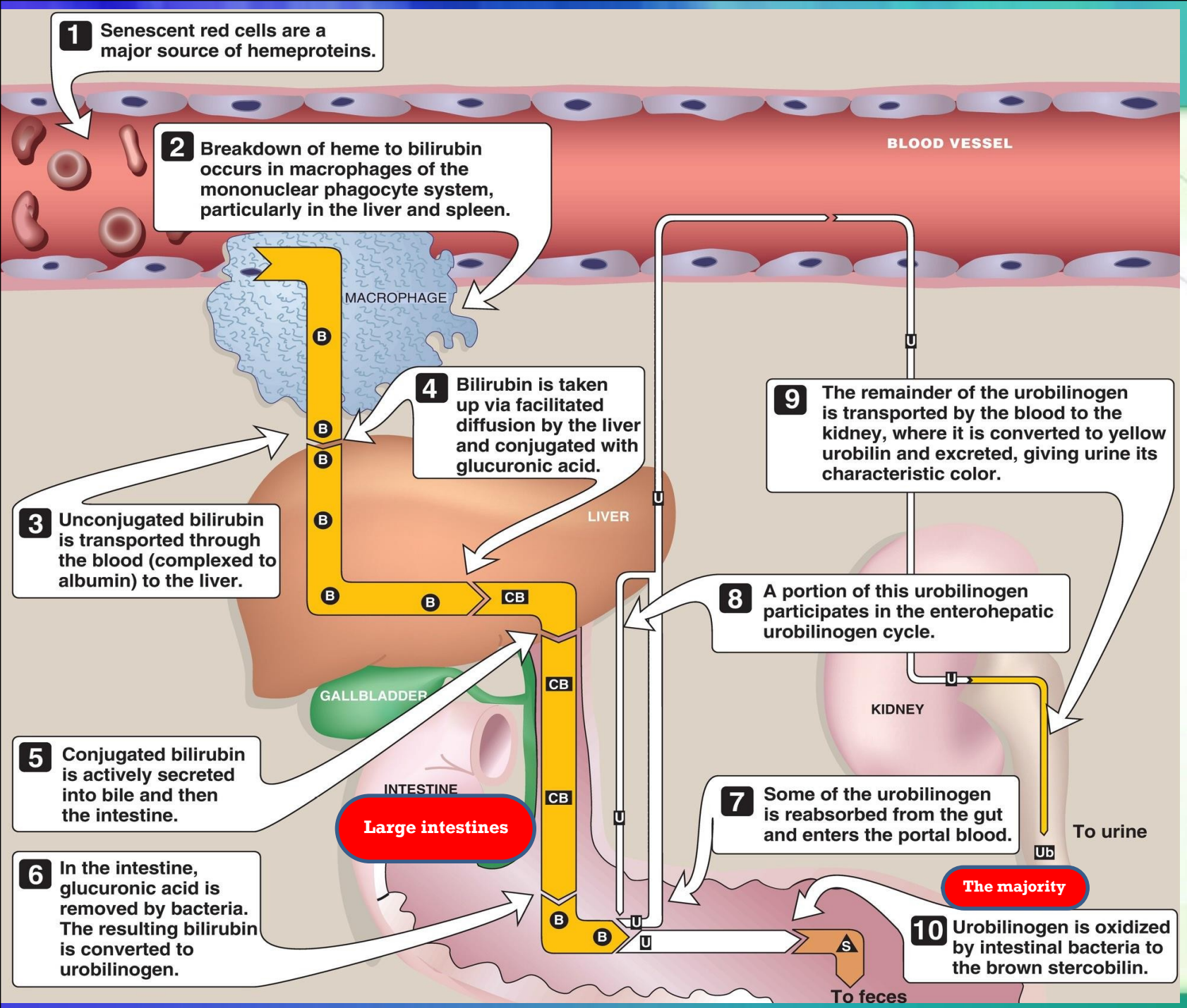


Transport of bilirubin

Biliverdin is converted to bilirubin which is released into the blood and carried by **albumin**.

- The role of albumin
  - Salicylates **(aspirin)** and sulfonamides can displace bilirubin from albumin permitting bilirubin to enter the central nervous system (CNS).
    - This may cause neural damage in infants.
- Formation of bilirubin diglucuronide. (in liver, bilirubin is conjugated into diglucuronide producing bilirubin diglucuronide which is highly hydrophilic).
  - Crigler-Najjar I and II and Gilbert syndrome (deficiency in conjugating bilirubin).
- Transport into bile
  - Dubin-Johnson syndrome (deficiency in transporting bilirubin diglucuronide into bile)





The produced bilirubin is carried by albumin to the liver.
In the liver, it gets conjugated to glucuronic acid (conjugated bilirubin), which is then transported into the bile where it can go to the large intestines.
In the large intestines, the glucuronide moieties are removed from the conjugated bilirubin producing unconjugated bilirubin again, then this bilirubin is converted in the large intestine into urobilinogen, some of this urobilinogen leak out into the bloodstream, some of it goes back to the large intestine, and some goes into the blood.
The majority of the urobilinogen is converted to stercobilin by gut bacteria, this stercobilin is brown that gives feces its color.
The urobilinogen that goes into the blood is taken up by kidneys, and in kidneys, it is converted to urobilin, urobilin gives urine its yellowish color.

Stercobilin is brown which characterizes feces

Measurement of bilirubin

Knowing the level of bilirubin is very important because it tells us a lot of information about the health of individuals, specifically the liver

- It is done via a reaction known as Van den Bergh reaction.

  Can be done in water and in (ethanol or methanol)

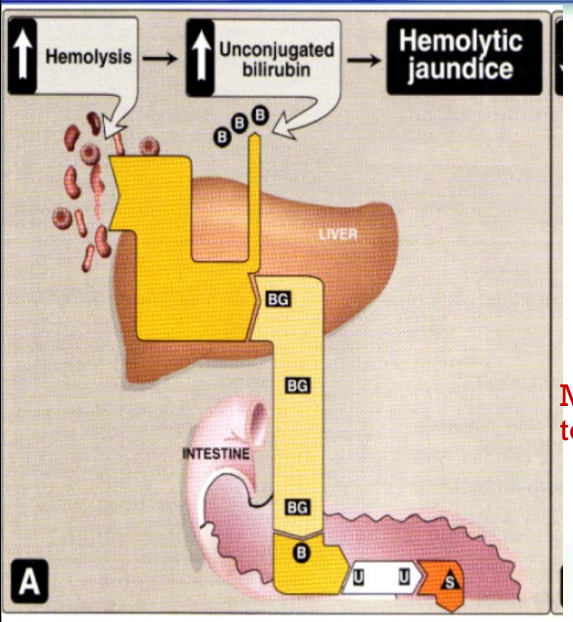
- Direct measurement of conjugated bilirubin (hydrophilic bilirubin) (in water)
  - Normally 4% of total bilirubin
- Total measurement of bilirubin (in ethanol or methanol) Indirect

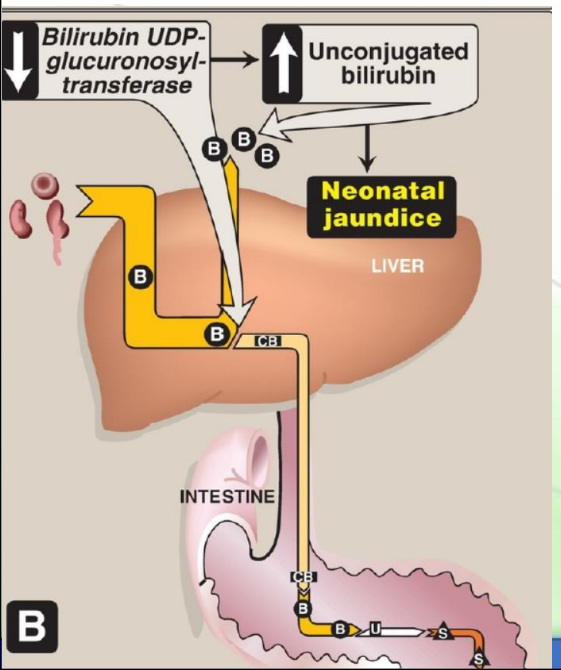
  ethanol & methanol make bilirubin soluble / It measures **all** bilirubin molecules in the sample
- unconjugated bilirubin = total bilirubin – direct bilirubin

Measured from the organic solvents (ethanol or methanol)

Measured from the water-based reaction

Types and lab results of jaundice





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

Markers related to jaundice

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

Explanation of the previous table

Jaundice is a group of disorders that are related to a deficiency in the catabolism, transport & metabolism of bilirubin and they result in yellowing of the skin in individuals.

Jaundice can be divided into two classes:

1. unconjugated hyperbilirubinemia (increase in the amount of unconjugated bilirubin)
2. conjugated hyperbilirubinemia (high level of conjugated bilirubin)

Continuation

Unconjugated hyperbilirubinemia

- In hemolytic jaundice, hemolysis is increased → releasing a lot of heme → results in a lot of heme being converted to bilirubin → so a lot of conjugation of bilirubin and this conjugated bilirubin goes from the liver to the intestines → then conversion of bilirubin to urobilinogen and sterocobilin.
- Stool is very dark as a result of overproduction of sterocobilin but since the glucoronosyltransferase enzyme is overwhelmed with bilirubin, some bilirubin will leak out of the liver into the blood.
- So, there is increased bilirubin in serum and increased unconjugated bilirubin as well, but the amount of conjugated bilirubin would stay normal because only little amount of the conjugated bilirubin will leak out, and the rest would go into the intestines.
- Urine is darker and the amount of urobilinogen and urobilin would be high in urine since a lot of urobilinogen and bilirubin will be taken into the kidneys.

- In hepatic jaundice, there is a problem in the liver → increased amount of bilirubin in the blood because there is leakage and higher levels of both unconjugated and conjugated bilirubin because there's cell damage and release of both forms of bilirubin into the blood.
- Since there's damage in the liver, liver enzymes in the blood (ALT/AST) increase
- Urine is darker because of the release of all of these forms of bilirubin into the blood which are taken into the kidneys
- Stool is either normal or lighter in color since there is reduced bilirubin and its forms reaching the intestines.

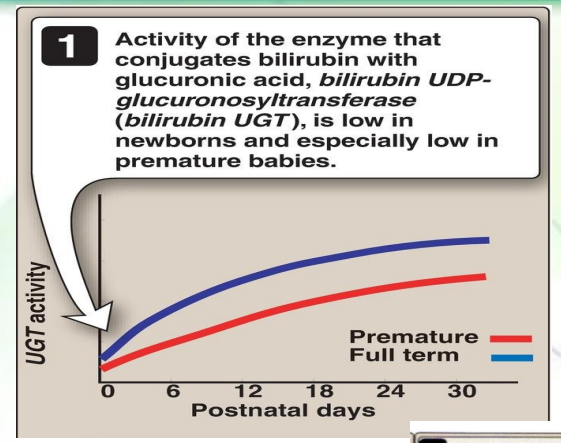
And another

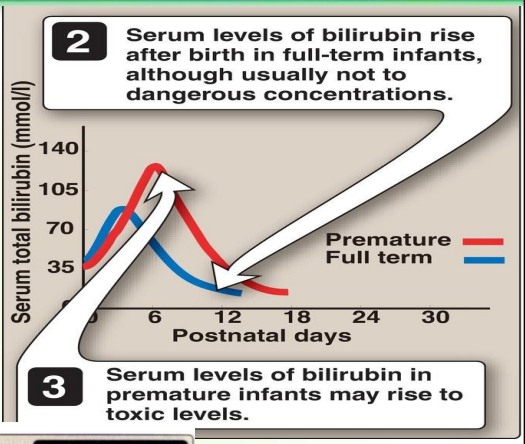
Conjugated hyperbilirubinemia

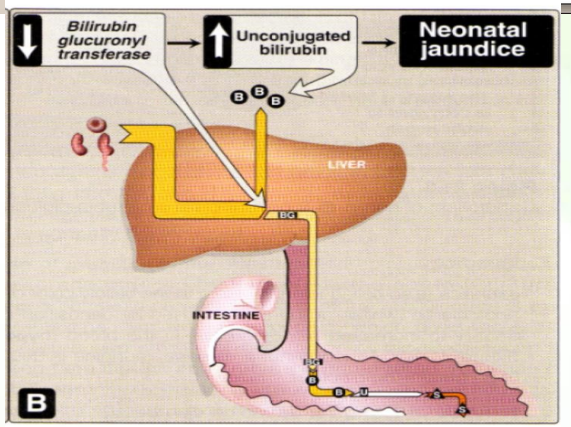
- There is a production of conjugated bilirubin, but it doesn't get into the bile or the intestines, this conjugated bilirubin would leak out of the liver and there would be an increased level of total bilirubin in the serum and higher concentration of conjugated bilirubin since it is leaking from the liver
- Liver enzymes are normal.
- Urine is darker because there's a lot of bilirubin and conjugated bilirubin taken to the kidneys which cannot convert them to urobilinogen and urobilin and that results in reduced amounts of these two molecules in the urine
- Stool is clayish as a result of the inability of the liver to transport conjugated bilirubin into the intestines so there would be low concentration of sterocobilin in the intestine and feces.

Jaundice in newborns

- Jaundice can affect infants
- The enzyme UDP-glucuronosyltransferase that conjugates bilirubin to glucoronic acid is not very active at birth and its activity increases with time (by two weeks, the liver would be able to efficiently conjugate bilirubin)
- The problem with premature babies, the activity of the enzyme is not very high and that results in overproduction of bilirubin that cannot be conjugated and this bilirubin leaks out, reaches the CNS and damage the brain.







- Usually, in normal infants, they have high concentrations of bilirubin, but these high levels are not toxic, but in premature babies, bilirubin reaches very high levels and becomes toxic.
- The solution: is to expose babies to blue light.
  - blue light breaks up bilirubin into a more hydrophilic molecule that can be eliminated easily

Genetic disorders

- Gilbert syndrome: mild, asymptomatic jaundice
- Crigler-Najjar syndrome: severe
  - Defective glucuronosyltransferase 1A1

These two conditions are caused by a defective glucoronosyltransferase 1A1.
If this enzyme is somewhat defective, it would cause Gilbert syndrome with mild symptomatic jaundice, however if the enzyme is not functioning at all, it causes a more severe syndrome known as Crigler-Najjar syndrome.

- Treatment:
  - Phototherapy (young age)

    Phototherapy works in young age, but it doesn't work after because the body gets bigger, and light would not penetrate the skin.
  - Liver transplant

    In older patients, the best treatment is liver transplant