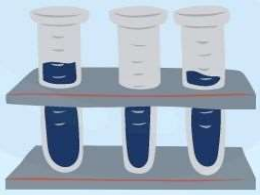
Sheet No. 7

Biochemistry



hematolymphatic system

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Corrector:

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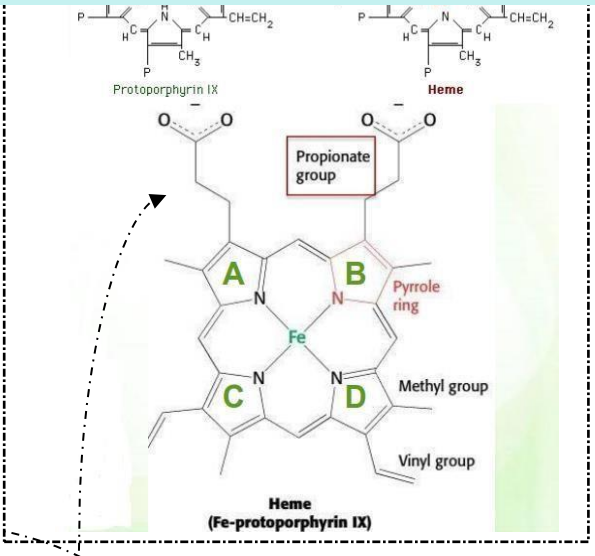
Metabolism of Heme

- ✓ Our discussion on hemoglobin is not over yet. We are aware of the fact that hemoglobin consists of both protein and non-protein components. This sheet will dive into the details of metabolism (i.e. synthesis and catabolism) of the non-protein component, a component that goes by the name "heme". (Note: Heme is also found in cytochrome P450 enzymes, as well as enzymes of the electron transport chain)

Heme Structure

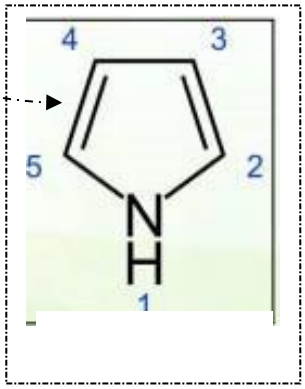
- ✓ Before delving into the structure of heme, we need to familiarize ourselves with the term **"porphyrin"**. Briefly, porphyrins are a family of planar molecules that can readily bind metal ions. (They are hydrophobic overall)
- ✓ Heme consists of:
  - A. A specific porphyrin (protoporphyrin IX)
  - B. A metal ion (Fe^{+2}) bound in the center of the porphyrin



A. Porphyrin Part

In general, porphyrins consist of 4 pyrrole rings (designated A – D) linked to each other.

- ✓ Each pyrrole ring is conjugated to two groups (substituents). The different combinations and arrangements of these conjugated groups give rise to the different types of porphyrins.
- ✓ In the case of heme, we have a specific type of porphyrin, called protoporphyrin IX. The conjugated groups in heme include: **methyl groups, vinyl groups, and two propionate groups**. (Notice how two of the rings have one propionate group each)



Pyrrole Ring

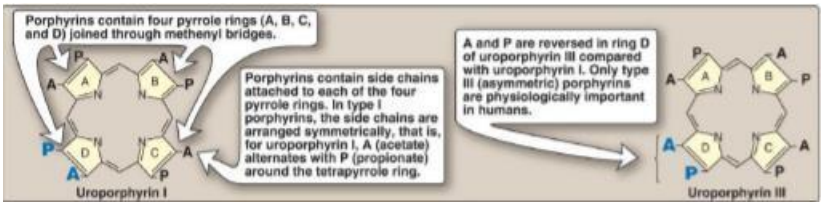
- ✓ Apart from the charged propionate groups (which are hydrophilic), heme is considered **hydrophobic** overall. Thus, heme is located in the hydrophobic pockets of hemoglobin such that its propionate groups extend outwards where they contact the hydrophilic amino acids on the surface of the protein.

B. Metal Ion Part

The protoporphyrin IX molecule in heme binds an iron atom (Fe^{+2}) in the center. Fe^{+2} has 6 coordinates of binding.

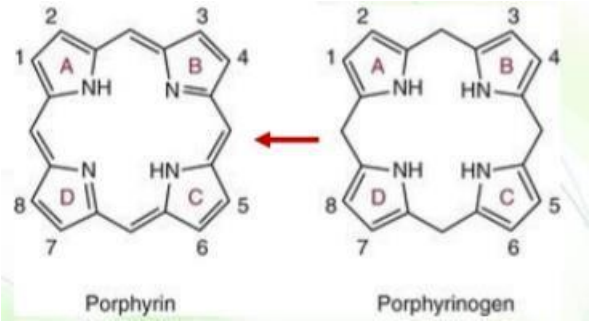
A LITTLE CHAT ABOUT PORPHYRIN

- Different porphyrin molecules are classified based on their arrangement of ACETATE(A) and PROPIONATE (P) molecules



- So if the all of the rings are in the oreder (A,P,A,P...) its called a uroporphyrin i molecule but if one of the pyrrole rings go Rogue which is ring D in our examle then (A,P,P,A..) then the entire molecule changes to uroporphyrin iii

- NOTE: porphyrins are made from porphyrinogens which differ in the fact that they are: reduced and colorless so when they are oxidized they turn to a purple color (purple=porphyrin) hence their name.



Heme Biosynthesis

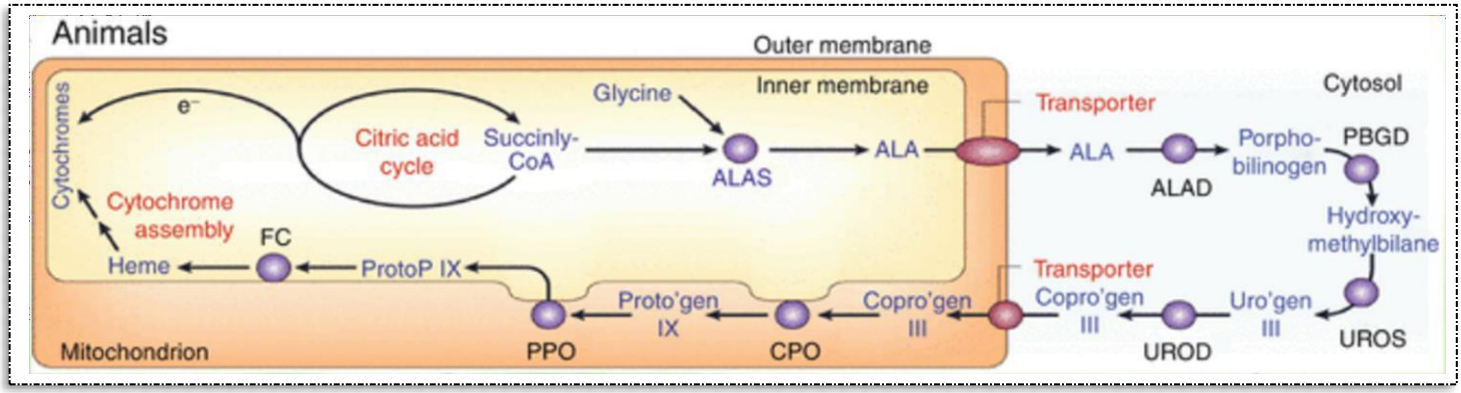
Two major sites are responsible for synthesis of the majority of heme in the body

Liver: it synthesizes a number of hemoproteins (particularly the CYP450 enzymes). The rate of heme synthesis in the liver is highly variable, and occurs in parallelism with the tissue's needs. (E.g. presence of drugs may affect this rate).

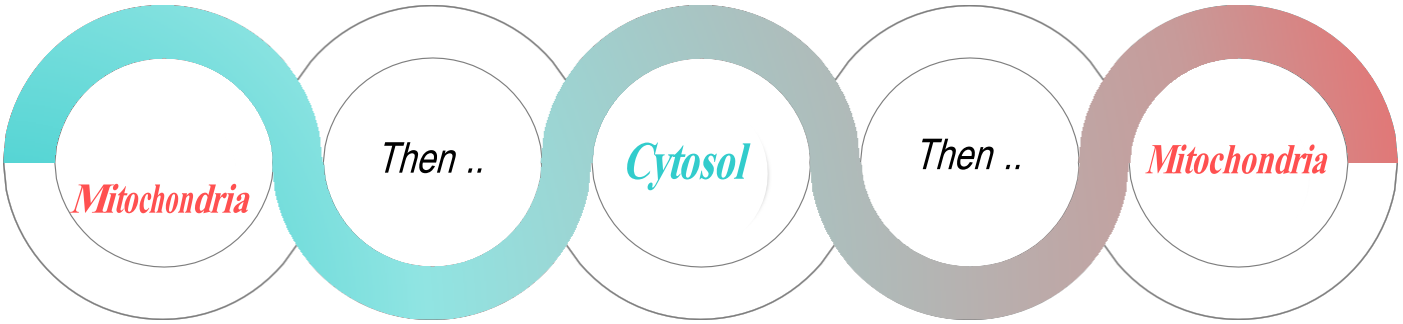
Erthyrocyte-producing Cells: they synthesize heme for hemoglobin. The rate of heme synthesis here is relatively constant and matches the rate of globin synthesis, but synthesis is regulated at multiple points.

✓ At the cellular level, synthesis of heme begins in the mitochondria, proceeds in the cytosol, and then returns to the mitochondria to ultimately produce the final product: heme.

✓ The following image illustrates the reactions occurring in heme synthesis:



✓ The following diagram provides a summary of the overall process illustrated in the previous image:



Succinyl CoA (an intermediate of the citric acid cycle) condenses with **glycine** to form **aminolevulinic acid (ALA)**. ALA is subsequently transported outside the mitochondria and into the cytosol.

ALA undergoes multiple reactions in the cytosol, being converted from one intermediate to another. The last intermediate in the cytosol is transported into the mitochondria.

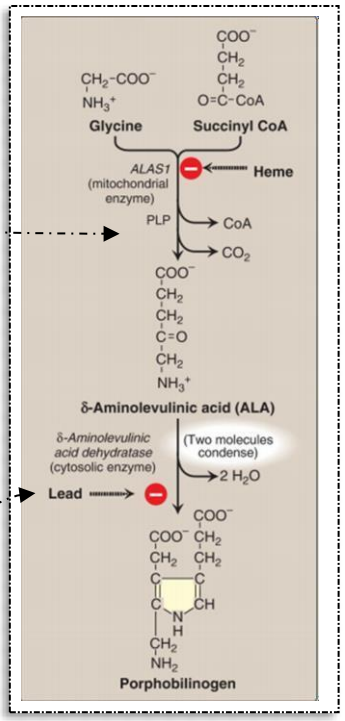
The intermediate that returns to the mitochondria is eventually converted into the ultimate product: **Heme**

✓ Let's discuss more details regarding the reactions:

First Reaction

The first reaction is catalyzed by ĵ -aminolevulinic acid synthase (ĵ-ALAS or 5'-ALAS), and occurs in the mitochondria. There are two isoforms of ALAS:

1. ALAS1: Found in all tissues including the liver
2. ALAS2: Predominantly found in erythrocyte-producing cells



✓ ALAS condenses glycine with succinyl CoA, producing ĵ-ALA.

✓ This step is both the rate-limiting and committed step of heme synthesis, and therefore, is slow and highly regulated.

✓ This reaction requires the active form of vitamin B6 (pyridoxal phosphate).

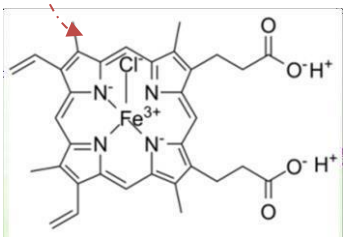
✓ Regulation of ALAS:

- ALAS2 is regulated by the presence of iron.
- ALAS1 is regulated by hemin and drugs.

✓ Drugs and hemin regulate ALAS1 activity through different mechanisms:

- They affect the rate of transcription (synthesis) and the stability of its mRNA (degradation)
- They affect mitochondrial transport.

Hemin differs from heme in that it contains a ferric (Fe^{+3}) rather than a ferrous (Fe^{+2}) iron in the center, and this ferric iron is bound to a chloride ion. (**Extra:** Where does hemin come from? When there is excess heme in the cell, it starts to accumulate instead of being incorporated into proteins. This excess "free" heme becomes oxidized forming hemin. The presence of hemin indicates that heme synthesis is outpacing the synthesis of proteins into which heme is incorporated. Thus, hemin inhibits ALAS1 activity to slow down heme synthesis).



✓ ALA moves out of the mitochondria to the cytosol where porphobilinogen (PBG) is formed by the condensation of 2 ALA molecules **(Second reaction in heme synthesis)**. It is catalyzed by ALA dehydratase.(contains zinc (so lead inhibits it))

Other Reactions

✓ Four PBG molecules condense (in the cytosol) and eventually form uroporphyrinogen III. (Extra: PBG is first converted into hydroxymethylbilane by hydroxymethylbilane synthase -also known as PBG deaminase- and then into uroporphyrinogen III)

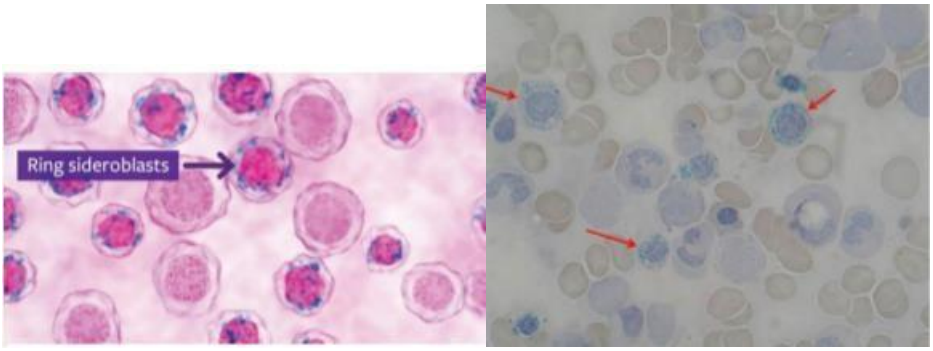
✓ Uroporphyrinogen III is then converted into coproporphyrinogen III which is then transported back into the mitochondria, where it is converted into protoporphyrinogen IX, and then into protoporphyrin IX.

✓ **The last reaction:** Iron (Fe^{+2}) binds to protoporphyrin IX, forming heme. This reaction is

spontaneous, but can be catalyzed by ferrochelatase. (Image of reactions is in the next page)

SOME NOTES ABOUT THE PREVIOUS PAGE

ALAS2 is regulated by level of iron. Loss of function due to mutations in 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 which does so in several ways:

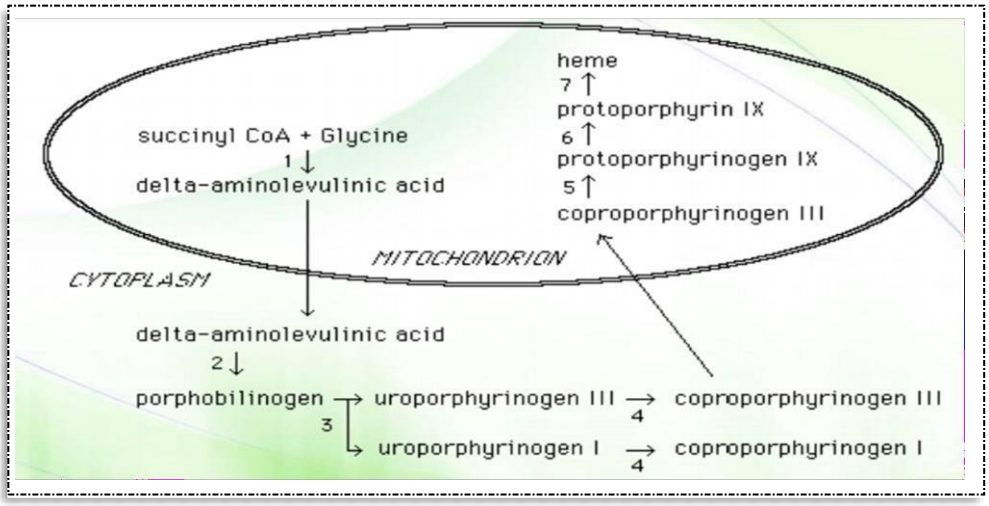
-Reduces synthesis and stability of mRNA

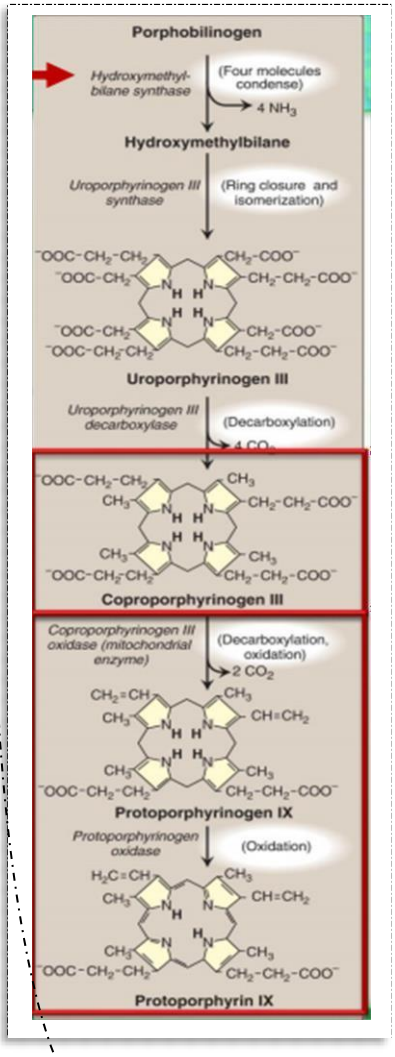
-Inhibits mitochondrial import of ALAS1

-Induces protein degradation

Note: increased levels of drugs in the body leaves the cyp450 enzyme in the liver more active and reducing heme level in hepatocytes which in turn dives the transcription of more ALAS1

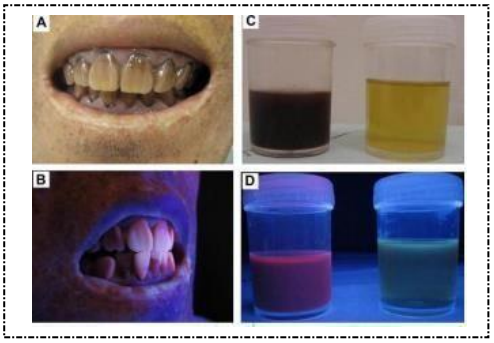
- ✓ Note: ALA dehydratase and ferrochelatase are inhibited by lead. This is among the reasons why lead is toxic to the body.
- ✓ In erythrocytes, synthesis is also regulated at ferrochelatase and porphobilinogen deaminase (PBG Deaminase = Hydroxymethylbilane synthase). (This note is present in the slides but was not mentioned by the professor).
- ✓ Here is an additional image from the slides that summarizes the heme synthesis pathway:



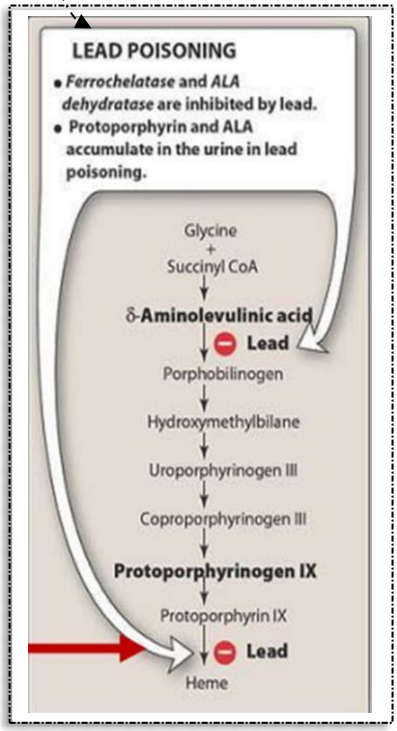


Porphyrias

- ✓ Porphyrias are 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 means "purple", and porphyrias are characterized by a purple discoloration of the lips, teeth, gums, skin and urine.

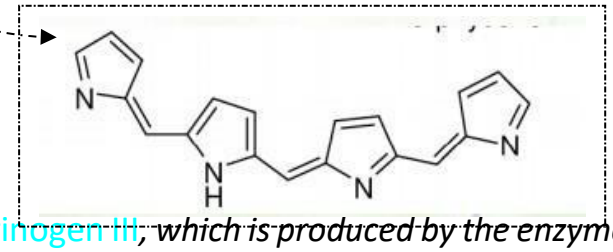


- ✓ These diseases are classified according to the affected tissue into:
  1. Erythroid
  2. Hepatic (acute or chronic)
- ✓ Porphyrias differ in their manifestations:
  - Photosensitive or not photosensitive (is the patient photosensitive or not?) (Tetrapyrrole-dependent)
  - The presence of abdominal or neuropsychiatric signs

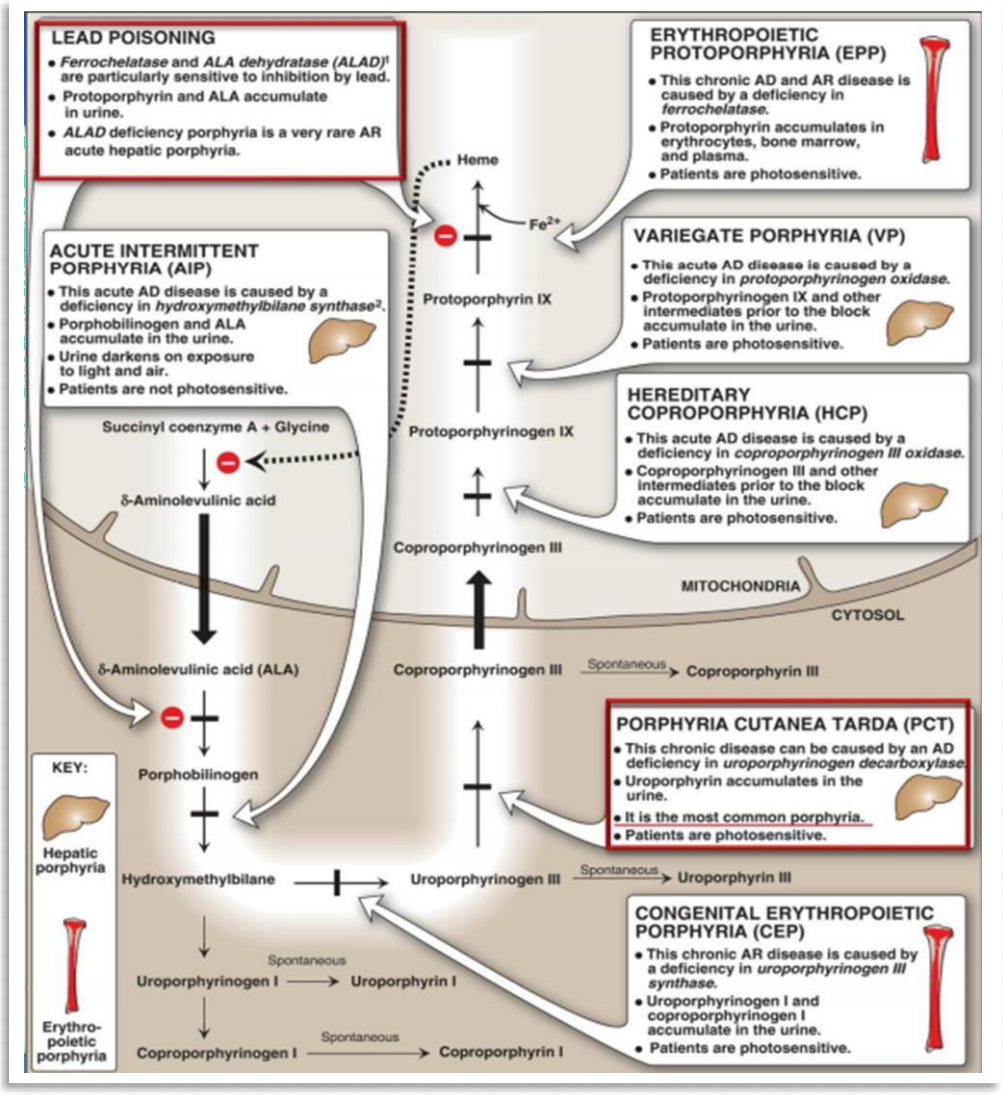


Porphyrias and Photosensitivity

Porphyrias are classified as photosensitive or photo-insensitive depending on whether there is accumulation of photoactive intermediates or not, and this in turn depends on the step that is defective in heme synthesis. The accumulated compounds responsible for photosensitivity are those containing the tetrapyrrole structure (4 linked pyrrole rings). This structure is photoactive as it interacts with/absorbs sunlight, and causes photosensitivity.



- ✓ ***Part of the lecture video is missing, and the following italicized paragraphs are based on Doctor017 sheets, with rephrasing and other additions:***
- ✓ *The first intermediate to exhibit a tetrapyrrole structure in the heme synthesis pathway is:* uroporphyrinogen III*, which is produced by the enzyme* uroporphyrinogen III synthase*, and is therefore photoactive. All of the intermediates afterwards also possess the tetrapyrrole structure, and are thus photoactive as well).*
- ✓ *If the defect in the pathway occurs before* uroporphyrinogen III *production (that is, if the defect is in* uroporphyrinogen decarboxylase *or any of the preceding enzymes), the resulting condition will not cause photosensitivity, but instead will present with abdominal and neuropsychiatric symptoms.*
- ✓ *If the defect occurs after* uroporphyrinogen III *production, the patient will be photosensitive due to accumulation of tetrapyrrole-containing intermediates.*
- ✓ *From the adjacent image: "The doctor said to focus on the following things:*

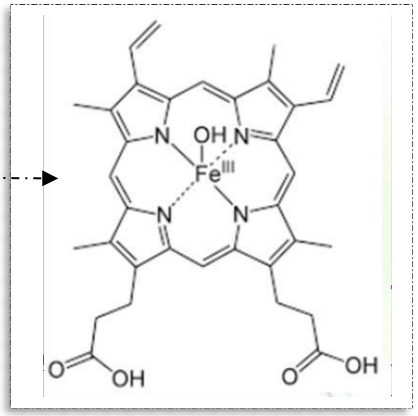


1. *Porphyria Cutanea Tarda/ 2. Acute intermittent porphyria/ 3.Whether the disorder causes photosensitivity or not/ 4. Deaminase, ferrochelatase and ALA synthase enzymes from previous pages./also on EPP on the top right (said can be dominant or recessive) (PCT is dominant)*

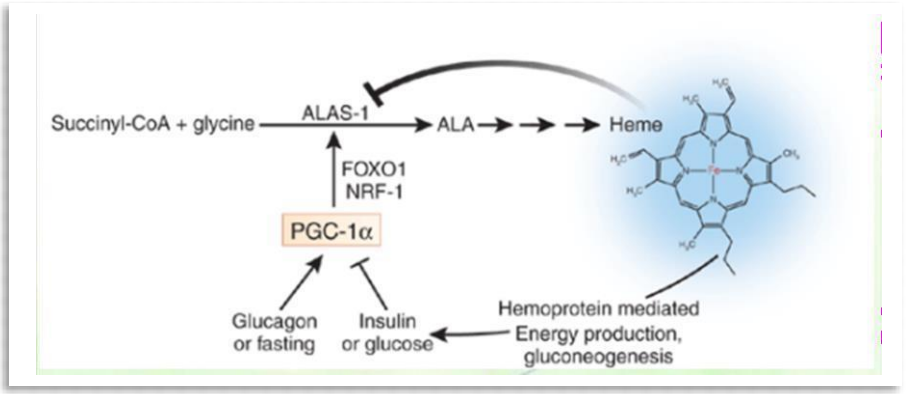
Hepatic porphyria heme ↓ ↑ALAS1 synthesis ↑intermediate ↓ ↑toxicity Drugs??
(doc said this mutation leads to more intermediates by negative feedback because heme will be deficient)(this is made worse by drugs in the liver ofc)

Treatment of Porphyrias

1. Porphyrias can be treated by giving patients hemin (or hematin), which strongly inhibit the activity of ALAS. Hematin resembles hemin in that it contains ferric (Fe^{+3}) iron, but differs in that this ferric iron is bound to a hydroxyl group (-OH) rather than chloride. (Recall: heme has ferrous (Fe^{+2}) iron)



2. Porphyrias can also be treated by giving patients glucose, or by preventing them from fasting. How?



- ✓ When fasting, glucose levels fall, and the hormone glucagon is secreted. Glucagon induces the production of /activates a transcription factor known as PGC-1a, which induces gluconeogenic genes for the synthesis of gluconeogenic enzymes in order to produce glucose. PGC-1a also happens to induce the synthesis of ALAS1 (think of this as some sort of by-product or side-result). The increase in ALAS1 will increase heme synthesis. Now, in a patient with porphyria, fasting will exacerbate acute porphyria attacks because the increase in ALAS1 levels (due to glucagon and PGC-1a) will stimulate the heme synthesis pathway, which is defective in this patient, causing more accumulation of intermediates.
- ✓ On the other hand, when we give patients glucose, insulin is secreted, and it antagonizes the effect of glucagon, reducing the synthesis of/ inhibiting PGC-1a, and thus, decreasing synthesis of ALAS1. The end result is less accumulation of intermediates of heme synthesis.

Heme Catabolism

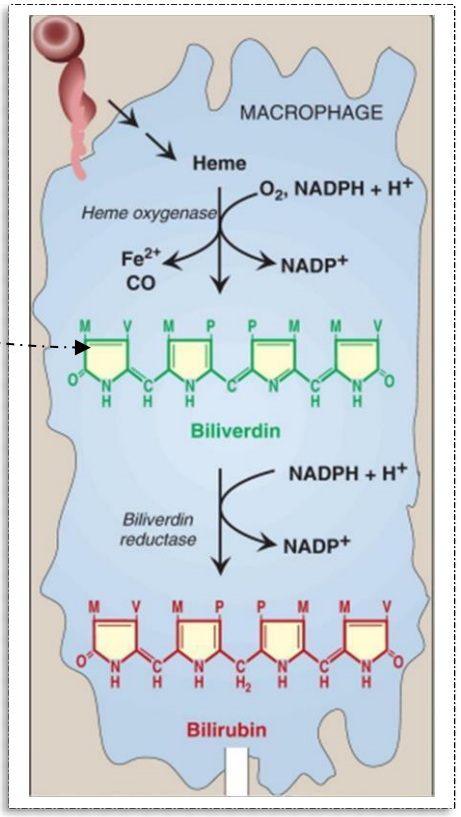
- ✓ Note: RBCs are the largest storage place of heme. They are continuously being destroyed, releasing a lot of heme (within hemoglobin).
- ✓ Erythrocytes are mainly destroyed by the macrophages in the spleen and bone marrow, releasing hemoglobin, which is degraded to heme, in addition to amino acids resulting from metabolism of the protein portion.

✓ 6 g/day of hemoglobin are turned over. The heme that results from hemoglobin metabolism cannot be released immediately, it's "risky". So, we have two challenges to face here:

1. The porphyrin ring is hydrophobic and must therefore be modified such that it becomes hydrophilic enough for the body to handle.
2. Iron must be conserved

Heme Degradation

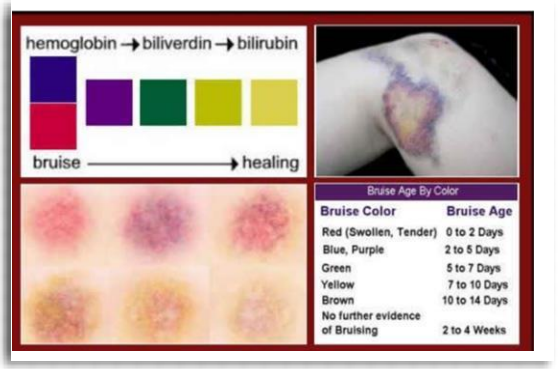
The first step in heme degradation is catalyzed by the enzyme: Heme Oxygenase (we came across this enzyme in the previous lecture).



✓ **Heme oxygenase** converts heme into **biliverdin**. During the process, it releases iron (Fe^{+2}) and carbon monoxide (CO). This is the only reaction in the body that releases CO. Note: This reaction requires NADPH.

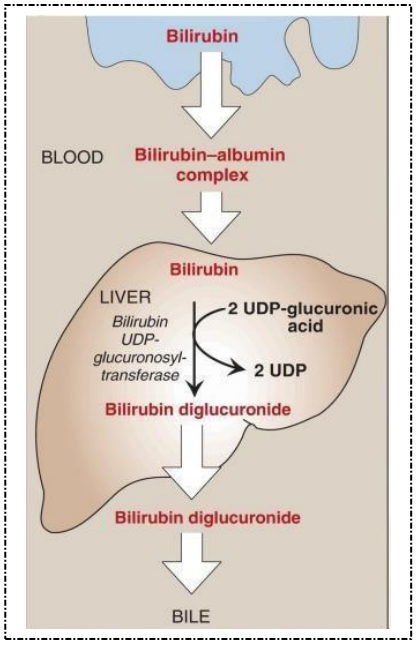
✓ **Biliverdin** then proceeds in the pathway ultimately yielding **bilirubin**.

✓ The different intermediates that result during heme degradation have different colors. This observation is well manifested in the color changes we see in **bruises**. First, blood is released, followed by hemolysis, and then the released heme is metabolized. The intermediates of heme metabolism produce different colors (From blue to purple … and finally yellow).



Transport of Bilirubin

Bilirubin is released into the blood where it binds to albumin. Albumin transports bilirubin to the liver.

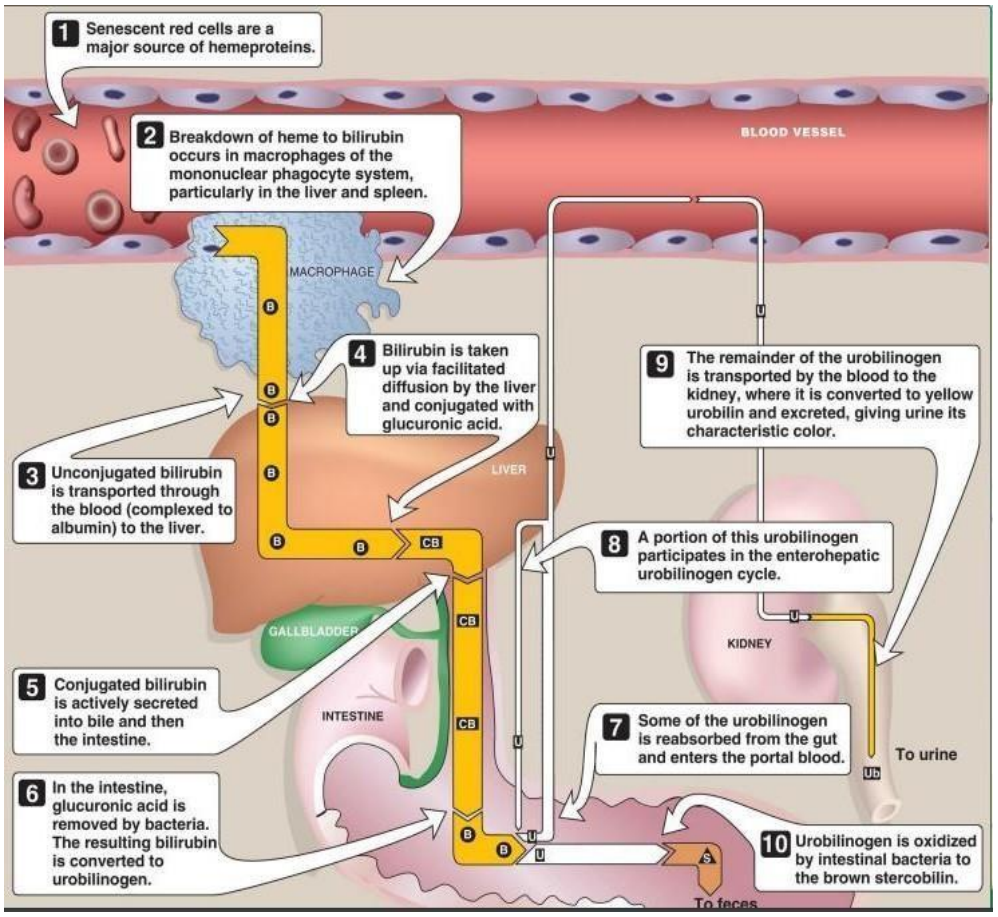


Note: Salicylates (e.g. aspirin) and sulfonamides can also bind to albumin, and can therefore displace bilirubin from albumin permitting bilirubin to enter the central nervous system. (This is especially dangerous in infants, causing neural damage. It is less dangerous in adults).

In the liver, bilirubin is conjugated to glucuronic acid, producing bilirubin diglucuronide (conjugated bilirubin). This is catalyzed by bilirubin UDP-glucuronosyl transferase. Bilirubin diglucuronide is hydrophilic, and is transported into bile.

- ✓ A defect in bilirubin conjugation causes Crigler-Najjar I and II, and Gilbert syndrome.
- ✓ A defect in bilirubin diglucuronide transport into bile causes Dubin-Johnson syndrome

- After transporting bilirubin diglucuronide into the bile, bile is dumped into the intestines.
- In the intestines, the glucuronide moieties are removed by bacteria, producing bilirubin. Bilirubin is then converted into urobilinogen.
- Urobilinogen has two fates from here:



A. The majority of urobilinogen is converted into stercobilin by bacteria, which gives feces its characteristic brown color.

B. Some of it is reabsorbed and reaches the liver. From here, it is either secreted again with bile into the intestine, or it enters the blood and is subsequently transported to the kidneys, where it is converted into urobilin, which gives urine its yellow color.

Measurement of bilirubin

- ✓ It is done via a reaction known as Van den Bergh reaction.
- ✓ This reaction can take place in an aqueous solution (water-based), or in ethanol or methanol.

1. In water: Since only conjugated bilirubin is soluble in water, it will participate in the reaction, while unconjugated bilirubin will not. This allows for direct measurement of conjugated bilirubin only. Normally, conjugated bilirubin constitutes 4% of total bilirubin. (Conjuagted bilirubin is called direct bilirubin)
2. In ethanol or methanol: Both conjugated and unconjugated bilirubin are soluble in ethanol and methanol, and thus both will participate in the reaction. This allows for measurement of total bilirubin.
   (Note: Unconjugated bilirubin is referred to as indirect bilirubin)

From the two previous tests, we can measure unconjugated bilirubin INDIRECTLY using the formula:

Indirect (unconjugated) bilirubin = Total bilirubin - Direct (Conjugated) bilirubin

Types and Lab Results of Jaundice

- ✓ Jaundice is a condition characterized by yellow discoloration of the skin due to increased bilirubin levels. It has diverse causes, all of which involve defects in bilirubin metabolism or transport.
- ✓ The table below shows various lab results obtained in different types of jaundice. Notes regarding the table will follow:

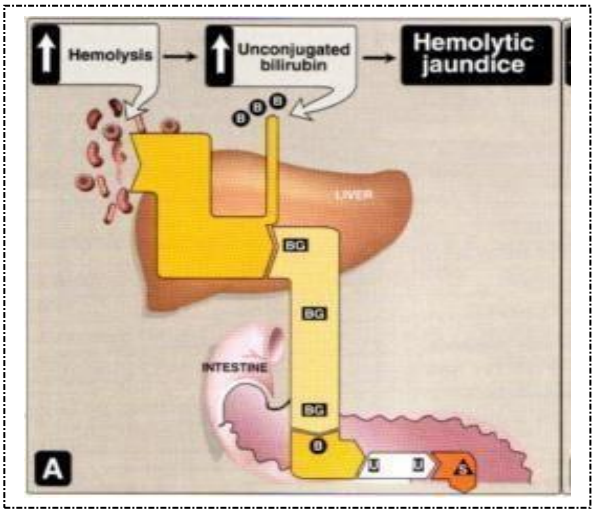
Sample	Indices		Unconjugated hyperbilirubinemia		Conjugated hyperbilirubinemia
		Normal	Hemolytic jaundice	Hepatic jaundice	Obstructive jaundice
Serum	Total Bil.	0.2-1.0 mg/dl	↑	↑	↑
	Direct (conj. Bil.)	0-0.2 mg/dl	↔	↑	↑↑
	Indirect (unconj. Bil.)	0.2-1.0 mg/dl	↑↑	↑	
	ALT/AST	Normal	Normal	↑	Normal
Urine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

- ✓ Jaundice can be classified according to the type of bilirubinemia into:
  - A. Unconjugated hyperbilirubinemia (increase in unconjugated bilirubin)
  - B. Conjugated hyperbilirubinemia (increase in conjugated bilirubin)

Unconjugated hyperbilirubinemia

It can be seen in:

1. Hemolytic Jaundice: In this case, there is excessive hemolysis of RBCs, and a large amount of bilirubin is produced. Consequently, a lot of bilirubin conjugation takes place in the liver, and so a large amount of conjugated bilirubin is released with bile, which causes increased urobilinogen and subsequent increases in both stercobilin (darker stool) and urobilin (darker urine). This large amount of bilirubin also overwhelms the capacity of liver enzymes to conjugate bilirubin. The excess unconjugated bilirubin leaks out of the liver and into the blood causing an unconjugated hyperbilirubinemia.



✓ So, in hemolytic jaundice, we notice the following:

- Blood levels of unconjugated bilirubin increase (because the liver's capacity to conjugate is overwhelmed)
- Blood levels of conjugated bilirubin do not change (because all of the conjugated bilirubin goes to the intestine)
- Total bilirubin in blood increases (because unconjugated bilirubin levels increase)
- Urine levels of Urobilinogen and urobilin increase (explained in the previous page)

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2. Hepatic Jaundice: In this case, the problem is within the liver itself (in contrast to hemolytic jaundice, where the liver is normal, and the problem is attributed to increased RBC lysis).

✓ The liver cells are damaged, and they release their contents (which include both conjugated and unconjugated bilirubin, so both of them rise in blood), and consequently, total bilirubin rises.

✓ Also, due to damage and loss of liver cells, the liver's capacity to conjugate bilirubin also decreases, this causes an even further increase in unconjugated bilirubin levels in blood (this may be why it is classified as an "unconjugated hyperbilirubinemia". Although both conjugated and unconjugated bilirubin levels rise, the increase in unconjugated bilirubin is more profound).

✓ Liver enzymes (AST/ALT) rise in hepatic jaundice, because as mentioned, the damaged liver cells release their contents into the blood

✓ Urine is darker in this case, because both forms of bilirubin increase and will be excreted eventually in urine, imparting a darker color.

✓ However, stool may appear normal or even lighter in color (due to decreased conjugation in the liver, and therefore less amounts reach the intestines and stool).

Conjugated Hyperbilirubinemia It is seen in obstructive jaundice.

- Obstructive Jaundice: In this condition, there is normal production of conjugated bilirubin, however, its transport into bile or into the intestines is impaired.

✓ Conjugated bilirubin accumulates in the liver cells, and then leaks into blood (it has to go somewhere). Therefore, we have increased levels of conjugated bilirubin in the blood, and consequently, increased total bilirubin.

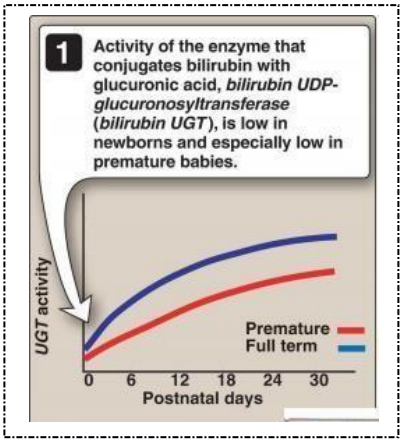
✓ Liver enzymes are normal

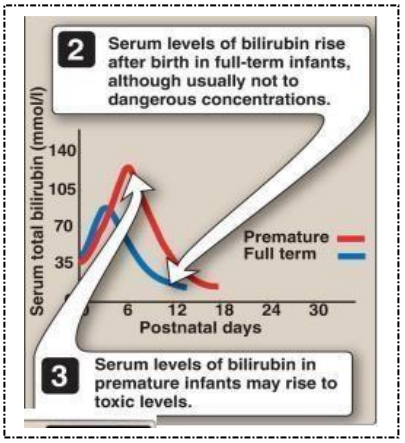
✓ Urine is darker due to the increased levels of conjugated bilirubin excreted in urine. However, urobilinogen and urobilin levels in urine are decreased. Recall that in obstructive jaundice, conjugated bilirubin does not reach the intestines (the site of urobilinogen production), and thus, less urobilinogen is formed. Since urobilin is derived from urobilinogen, its levels also decrease in urine.

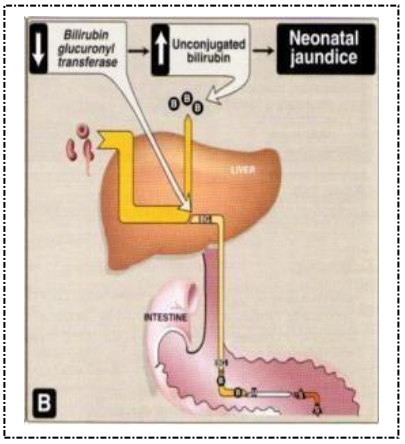
✓ Stool is clayish in color, due to decreased production of stercobilin.

Neonatal Jaundice:

- ✓ Jaundice can affect infants as well.
- ✓ At birth, the activity of the liver enzyme bilirubin UDP-glucuronosyl transferase is low. This causes increased levels of unconjugated bilirubin in infants.
- ✓ Generally, this increase does not reach toxic levels in full-term infants.
- ✓ The activity of the enzyme increases with time. Within two weeks, the enzyme is able to efficiently conjugate glucuronic acid to bilirubin.
- ✓ On the other hand, in preterm babies, bilirubin may reach toxic levels (the activity of the enzyme at birth is even less in preterm infants. Notice the adjacent graph). Consequently, unconjugated bilirubin levels rise and leak into blood, and may damage the CNS (brain).
- ✓ Treatment: We expose the baby to blue light, which converts unconjugated bilirubin into hydrophilic molecules that can be eliminated easily.







Good Luck

SOME MORE GENETIC DISORDERS (FUN)

-Gilbert syndrome: mild, asymptomatic jaundice

-Crigler-Najjar syndrome: severe

Both are caused by the same defect in glucuronosyltransferase 1A1 but in different degrees

If its slightly defective it causes gilbert syndrome but if its absent

Then causes Crigler-Najjar syndrome: severe

TREATMENT

-phototherapy in young age (doesn't work on bigger bodies cuz it doesn't penetrate the skin)

-in older age the best treatment is liver transplant

ALLAH MA3AKOM