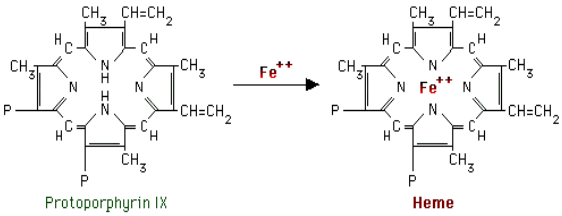
Metabolism of heme

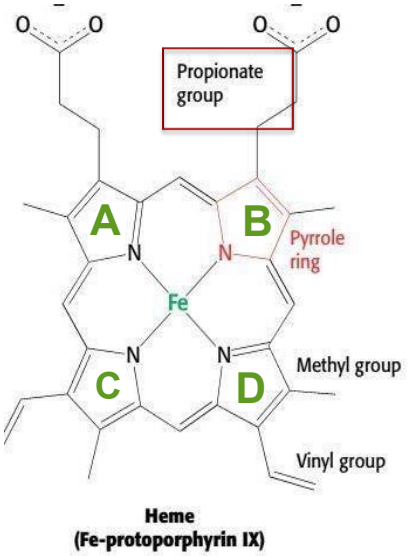
In this lecture, we're going to talk about biosynthesis and catabolism of heme.



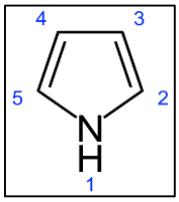
Heme structure

What was mentioned in the slide:

- It is a complex of protoporphyrin IX + Iron (Fe^{+2}).
- The porphyrin is planar and consists of four pyrrole rings (designated A-D).
- Each pyrrole ring can bind two substituents.
- Two rings have a propionate group each.
- Note: the molecule is hydrophobic. Fe has six coordinates of binding.



What was mentioned in the lecture:



heme is basically a protoporphyrin IX molecule with an iron atom bound in the center of the molecule, heme is a major part of hemoglobin, myoglobin, cytochrome p450 enzymes and enzymes of the electron transport chain.

Heme belongs to a group of molecules known as porphyrins, these porphyrin molecules (overall) are hydrophobic, planar, and they are composed of four rings joined by methylene (-CH=) bridges, these rings are known as pyrrole rings (pyrrole rings are heterocyclic unsaturated cyclic molecules containing nitrogen, as you can see in the figure), the four pyrrole rings are designated as A, B, C and D, each pyrrole ring in porphyrins can be conjugated to two groups, in case of heme, the groups are methyl ($-CH_3$), vinyl ($-CH=CH_2$) as well as two groups of propionate ($-CH_2-CH_2-COO^-$) and notice that these propionate groups are charged, we've already talked about heme being conjugated with hemoglobin saying that the planar molecule fits into the hydrophobic pocket of hemoglobin while the propionate groups extend to the outside contacting the hydrophilic amino acids on the surface of the hemoglobin.

Biosynthesis of heme

Sites of synthesis

What was mentioned in the slide:

- The major sites of heme biosynthesis are:

○ Liver, which synthesizes a number of hemoproteins (particularly the CYP proteins)

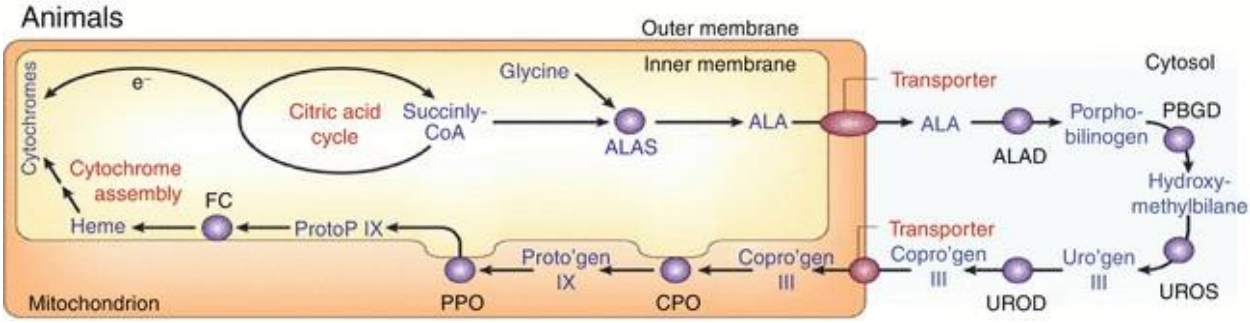
▪ The rate of heme synthesis is highly variable

○ Erythrocyte-producing cells (Hb synthesis)

▪ Relatively constant production and matches the rate of globin synthesis, but synthesis is

▪ regulated at multiple points.

• Synthesis occurs in mitochondria → cytosol → mitochondria



What was mentioned in the lecture:

so let's start talking about synthesis of heme, there are two major tissues that are responsible for the majority of heme synthesis, these are liver and erythrocyte producing cells, in the liver heme is used for production of hemoproteins such as cytochrome p450 enzymes, the rate of heme synthesis is highly variable depending on the presence of certain drugs or as needed (remember from the pharmacology course, some drugs cause inducing of the liver enzymes, this requires increased synthesis of heme) on the other hand, in erythrocyte producing cells, the production of heme is constant.

inside cells, synthesis of heme starts in the mitochondria then it continues in the cytosol and then it returns to end in the mitochondria, in the mitochondria, what you have is succinyl Coenzyme A ($^{-}OOC\text{-}CH_3\text{-}CH_3\text{-}CO\text{-}CoA$) which is an intermediate of the citric acid cycle (that, as everybody knows, occurs in the mitochondrial matrix), succinyl CoA is conjugated in the mitochondria with glycine which is an amino acid having the structure ($^{+}H_3N\text{-}CH_2\text{-}COO^{-}$) into a product known as aminolevulinic acid (ALA) this ALA then is transported out of the mitochondria to the cytosol, where it gets converted into several intermediates and then one of them gets into back into the mitochondria and

eventually it gets converted to heme.

Synthesis of heme

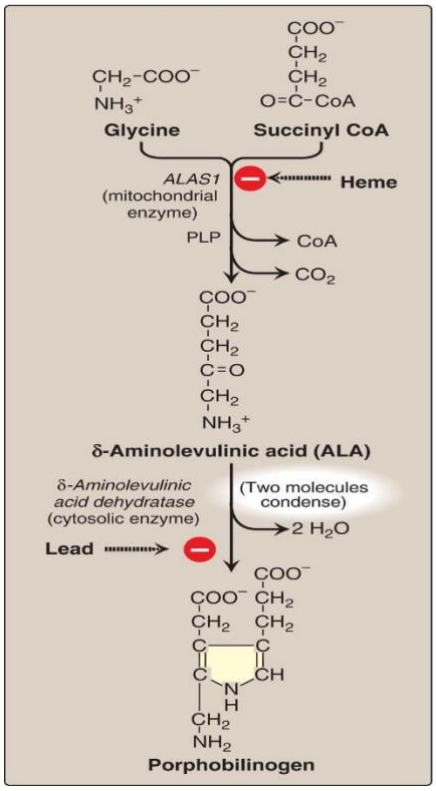
What was mentioned in the slide:

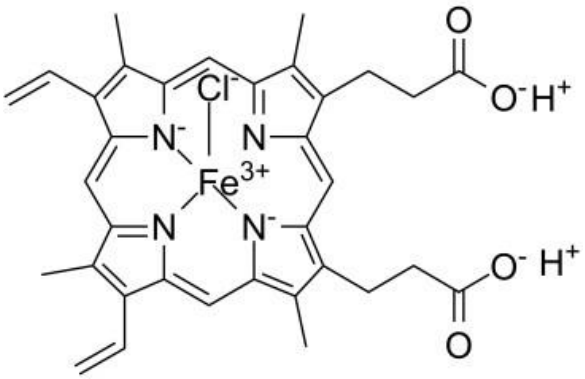
• The first reaction is catalyzed by 5'-aminolevulinic acid synthase, ALAS1 (all tissues inc. liver) or ALAS2 (erythrocytes), which conjugates gly and succinyl CoA into ALA.

• It is the rate limiting and committed step.

• It requires vitamin B6 (pyridoxal phosphate).

- ALA moves out of mitochondria to cytosol where porphobilinogen is formed by 2X ALA.
- ALAS2 is regulated by level of iron. ALAS1 is regulated by he min and drugs.
- Reduced synthesis and stability of mRNA
- Inhibition of mitochondrial transport





What was mentioned in the lecture:

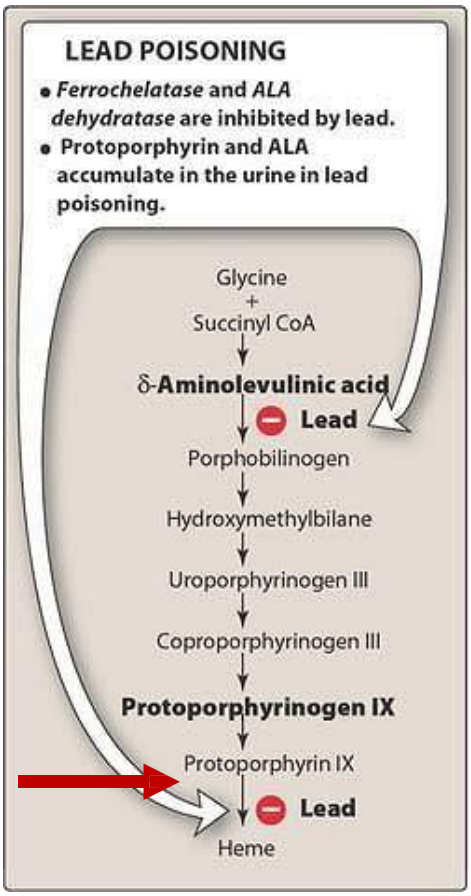
the very first reaction in heme synthesis is catalyzed by an enzyme known as 5'-aminolevulinic acid synthase (ALAS), there are two isoforms of this synthase: ALAS1 which is present in all tissues including the liver and ALAS2 which is predominantly found in erythrocyte precursors, this ALA synthase conjugates, as we said, both glycine ($^{+}H_3N\text{-}CH_2\text{-}COO^{-}$) and succinyl CoA ($^{-}OOC\text{-}CH_2\text{-}CH_2\text{-}CO\text{-}CoA$) into aminolevulinic acid ($^{+}H_3N\text{-}CH_2\text{-}CO\text{-}CH_2\text{-}CH_2\text{-}COO^{-}$) releasing CO_2, this reaction is the rate limiting (the rate of this reaction determines the rate of heme synthesis) and committed step (aminolevulinic acid cannot procced to any metabolic pathway except heme synthesis), so it is highly regulated and slow as a result of that, it requires vitamin B_6 (pyridoxine) as a cofactor, as we said, after formation of ALA, it moves out of the mitochondria into the cytosol through a certain transporter, where it can be converted into porphobilinogen so what you will have is that two molecules of ALA would conjugate to each other forming porphobilinogen and porphobilinogen continues with the other steps that we'll talk about in the next slides.
what's important about the enzymes ALA synthase 1 and ALA synthase 2 is that ALA synthesis 2 is regulated by iron, so iron dictates the activity of the enzyme in erythrocyte precursors, however, ALAS1 is regulated by hemin and drugs, ALAS1 is the liver specific enzyme, so what hemin and drugs do is that they they control not only the rate of transcription of gene that codes for ALA1, but also the alter the stability of the mRNA that codes for this enzyme, they also inhibit mitochondrial transport of ALA.
the structure of hemin is illustrated above in the figure, it differs from the structure of heme in having iron in the ferric (Fe^{+3}) state rather than the ferrous (Fe^{+2}) state, and it's also bound with a chloride ion.

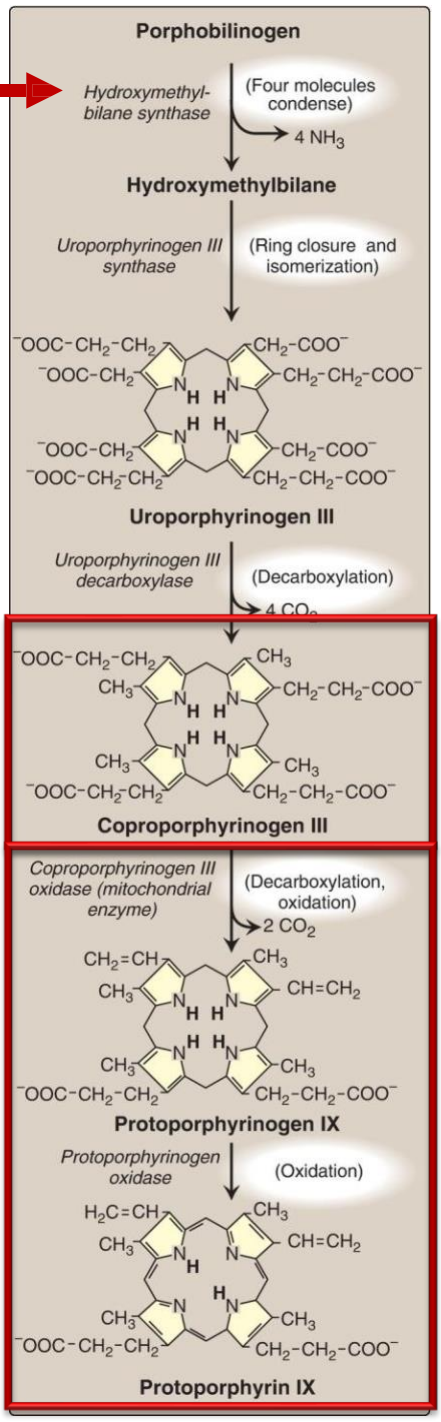
More reactions

What was mentioned in the slide:

- 4X PBG form uroporphobilinogen III, then coproporphyrnogen III .

- Coproporphyrnogen III moves back into mitochondria.
- The last reaction is spontaneous, but can be catalyzed by ferrochelatase.
- In erythrocytes, synthesis is regulated at ferrochelatase and porphobilinogen deaminase **(→).**

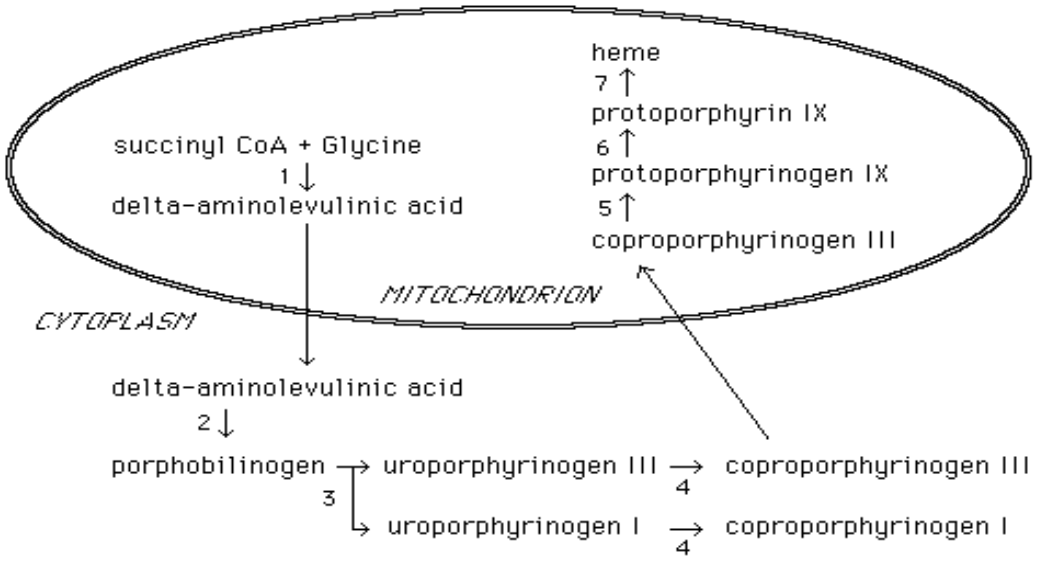




What was mentioned in the lecture:

what you have next is that four molecules of porphobilinogen molecules conjugate with each other forming a molecule known as uroporphyrinogen III, this uroporphyrinogen III is then converted (through decarboxylation (removal of CO_2) and oxidation) to a molecule known as coproporphyrinogen III which goes back into the mitochondria, in which it gets converted into protoporphyrinogen IX, then protoporphyrinogen IX is attached to an iron ion forming heme, this attachment of iron is mediated by an enzyme known as ferrochelatase.

there are two reactions that are important: the first one that is catalyzed by ALA Synthase, and the other one is the last step that is catalyzed by ferrochelatase, both of these reactions are inhibited by lead (Pb), that's one reason why lead is toxic to humans.



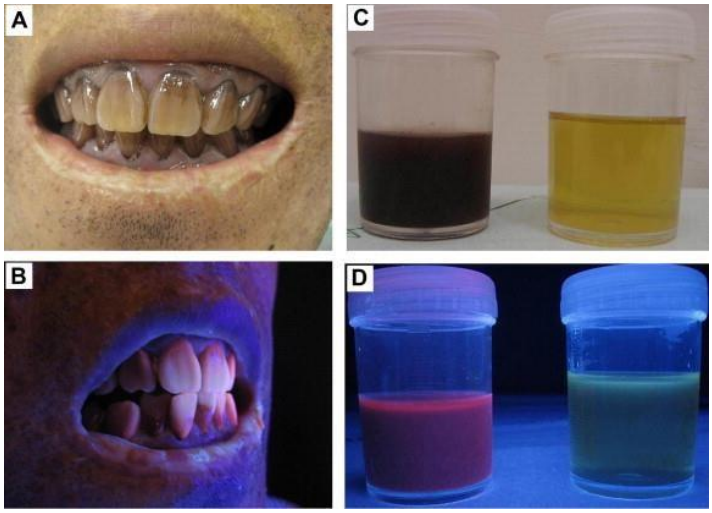
What was mentioned in the lecture:

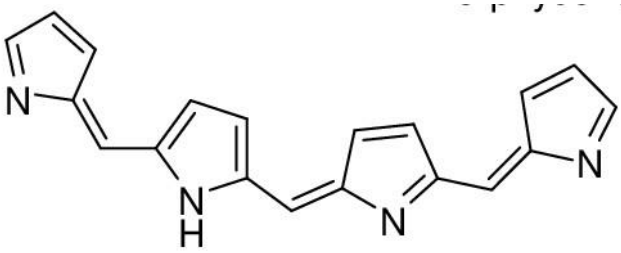
this is another look for the synthesis of heme, so the first reaction takes place in the mitochondria, then ALA is transported out to the cytosol and it gets converted into all of these intermediates, and then coproporphyrinogen goes back into the mitochondria and it gets converted to heme.

Porphyrias

What was mentioned in the slide:

- 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 = purple.
- These disorders are classified as
  - Erythroid
  - Hepatic (acute or chronic)
- They differ in manifestations
  - Photosensitive or not photosensitive
    - Tetrapyrrole-dependent
- Abdominal and neuropsychiatric signs





What was mentioned in the lecture:

Defects can occur in any one of these steps in the biosynthesis of heme, that results in the production of a group of disorders known as porphyrias, porphyrias are basically inherited or acquired disorders caused by deficiency of enzymes responsible for heme biosynthesis, that results in an increase in the level of any one of these intermediates in blood (if an enzyme is deficient, its substrate (the intermediate before it) will accumulate).
Porphyria means “purple” in Greek, so what happens with porphyrias is that patients will have their lips, teeth, gum, skin and urine being purple.
pophoryias are classified according to the affected tissue, so they can be erythroid specific or hepatic specific, hepatic specific disorders can also be acute or chronic.
Porphoryias also differ in two important manifestations: one of them is that is photosensitivity, that is , is the patient photosensitive or not, and that depends on the formation of the tetrapyrrole molecule or intermediate, so if you have the formation of a tetrapyrrole (so the deficient enzymes is involved in the pathway after formation of the tetrapyrrole intermediate, remember that a tetrapyrrole is a molecule having all four pyrrole rings attached to each other, if that occurs, then the patient would be photosensitive (sensitive to light), since these ring structures would be able to absorb light, otherwise, the patient would be photo insensitive, the other thing is the presence of abdominal or neuropsychiatric signs.

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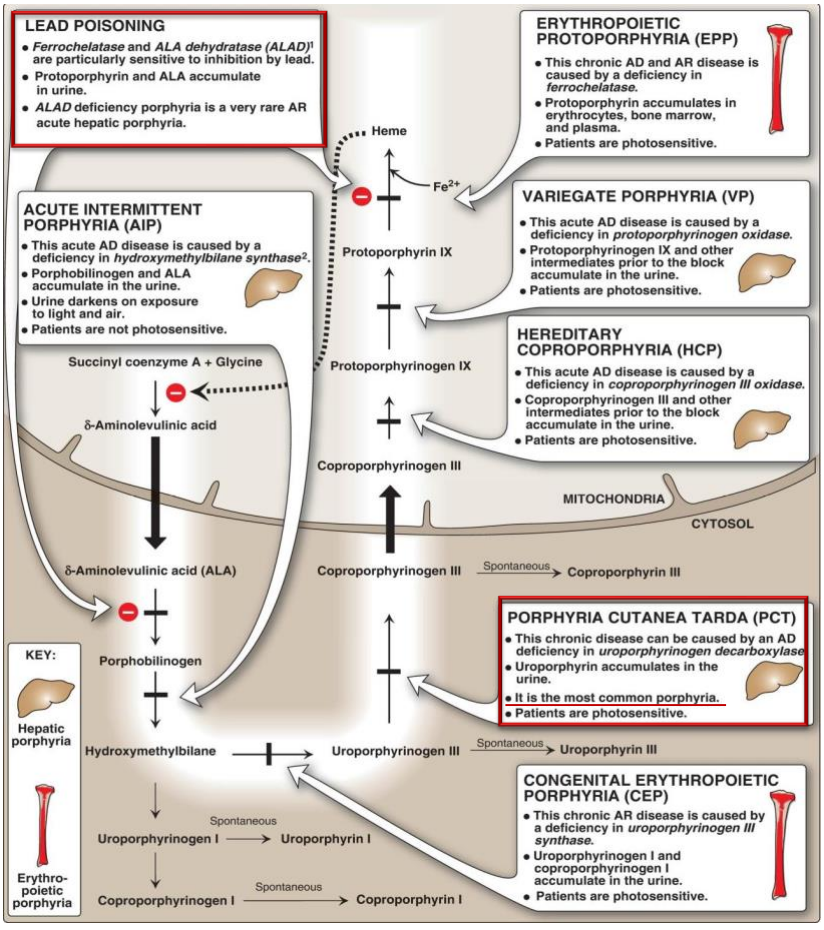
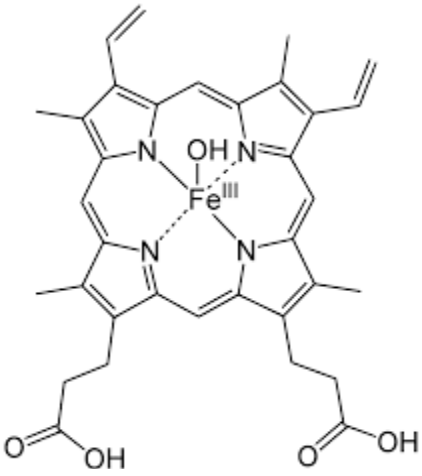


What was mentioned in the slide:

there are certain conditions that are related to heme biosynthesis, it's nice if you know

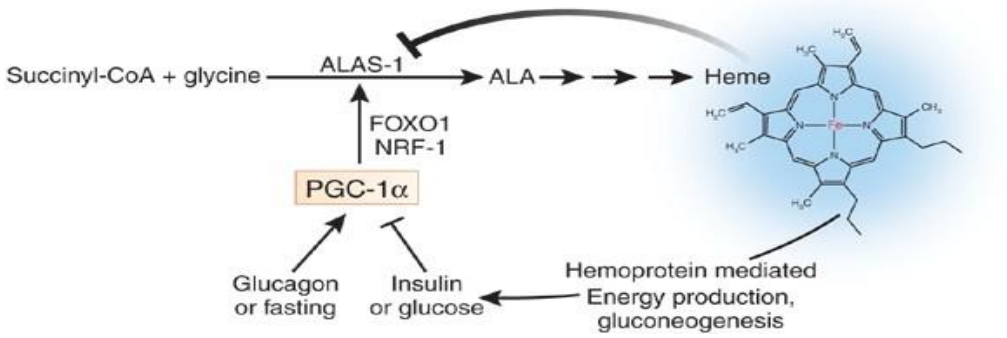
these different disorders, but we'll try to focus on two of them: the first one is related to lead poisoning (this slide is missing, I'll edit the handout when the doctor publishes the new lectures).

Treatment



What was mentioned in the slide:

- Hemin (or hematin) strongly inhibits the activity of ALAS.
- Glucose: fasting (hypoglycemia) exacerbates acute porphyria attack due to activation of the transcription factor, PGC-1α, in the liver, which induces synthesis of gluconeogenic genes and the ALAS1 gene resulting in accumulation of heme intermediates.



What was mentioned in the lecture:

how are porphyrias treated? they are treated by giving patients hemin or hematin, hemin, as we've said, is a protoporphyrin IX molecule that has an iron ion attached to it, but this iron is in the ferric state rather than the ferric state like in heme, it also has a chloride ion
hematin is identical to hemin, expect that instead of chloride ion, it has a hydroxide group.
Both hemin and hematin inhibit the ALA synthase enzyme which catalyzed the first and the most important step in the synthesis of heme.
another way of treating porphyrias is by giving patients glucose or preventing them from fasting, the principle is that what happens is that under conditions of fasting, glucagon would be released and it induces the production of a transcription factor known as PCG-1α now this transcription factor is important inducing the synthesis of glucogenic enzyme and ALA synthase as well, so by a result of preventing fasting or by giving patients glucose, you would have the production of insulin, and insulin would reduce the production of PCG-1α, resulting in reduction in the synthesis of ALA synthase and subsequently, production of heme.

Catabolism of heme

Challenges

What was mentioned in the slide:

- RBCs are the largest storage place of heme.
- Erythrocytes are mainly destroyed by macrophages in the spleen and bone marrow, releasing hemoglobin, which is degraded to heme.
- The protein is metabolized into amino acids. 6 g/day of hemoglobin are turned over, but
- First, the porphyrin ring is hydrophobic.
- Second, iron must be conserved.

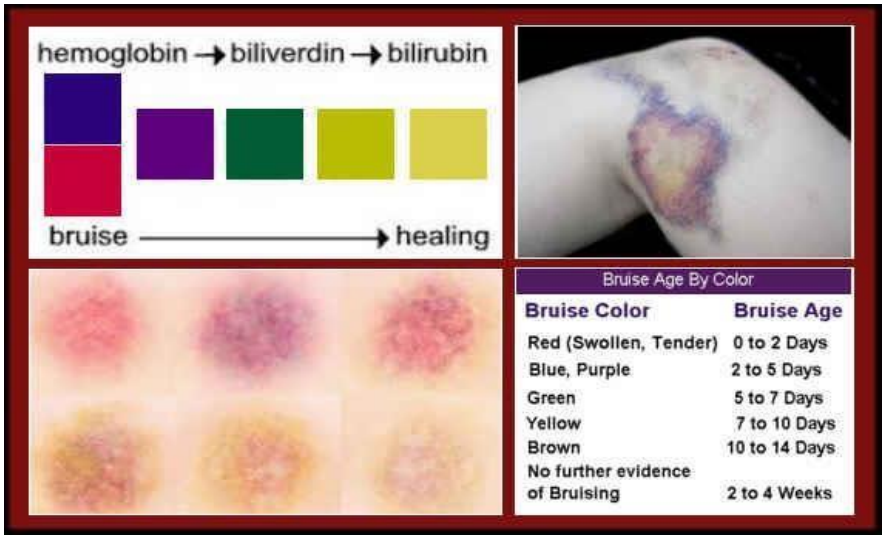
What was mentioned in the lecture:

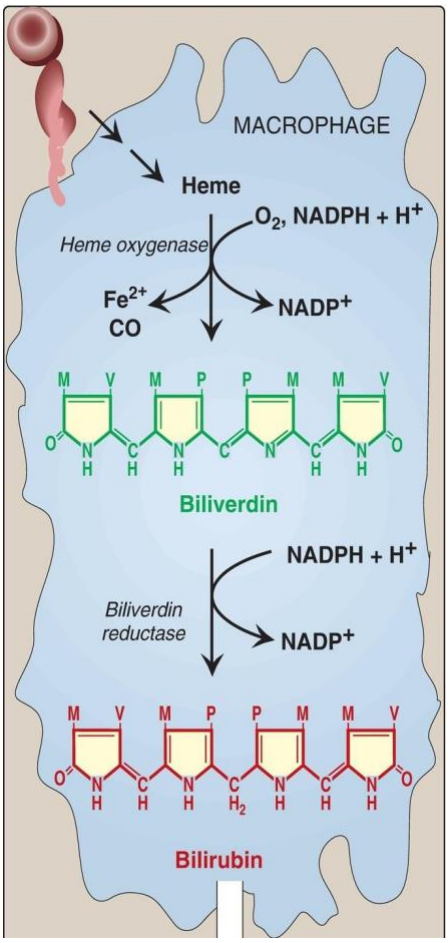
so let's go into the second half of this lecture and talk about catabolism of heme, there are a number of challenges when it comes to catabolism of heme, the first thing is that RBCs are the largest storage place of heme, and they are continuously destroyed by macrophages, releasing a lot of hemoglobin, the thing is that the globin protein is easily catabolized into amino acids and these can be used for synthesizing other proteins, but releasing heme is risky because 1-the porphyrin ring is hydrophobic, so heme must be modified chemically in order to make it more hydrophilic so that it can be excreted or at least it can be handled by the body, the second challenge is that iron must be preserved, it should not be lost as we said in the previous lecture, and this is done through a number of reactions.

Heme degradation

What was mentioned in the slide:

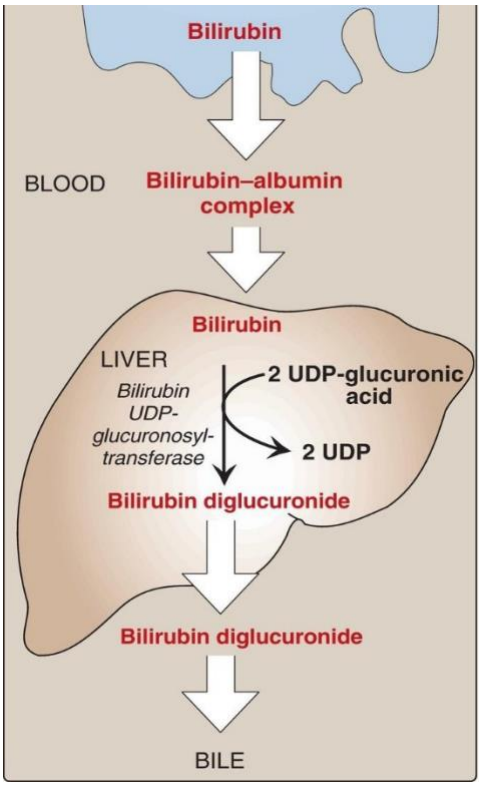
- The roles of heme oxygense and NADPH
- The production of CO
- The world of colors





What was mentioned in the lecture:

Heme degradation is done through a number of reactions, the first reaction is very important, it's catalyzed by the enzyme heme oxygenase, heme oxygenase metabolizes heme into a product known as biliverdin, this reaction requires NADPH, and in this reaction, iron is released from the heme molecule, and you have the production of carbon monoxide, actually, this is the only reaction in the body where you have production of carbon monoxide.
after biliverdin is formed, it goes through different reactions until a product known as bilirubin is formed, and what's important is that the intermediates between biliverdin and bilirubin have different colors, that's why whenever there is a bruise (a subcutaneous bleeding) in the skin, you can see that it goes through different colors, first you have a release of blood, hemolysis and then the red colored- heme is metabolized to different intermediates, each one of them has a certain color and that's why the bruise changes colors from blue to purple, green and finally yellow, all of these colors are a result of the different intermediates of heme catabolism.
It is important to know that bilirubin is yellow in color, while biliverdin is green.



Transport of bilirubin

What was mentioned in the slide:

- The role of albumin
- Salicylates 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.
- Crigler-Najjar I and II and Gilbert syndrome
- Transport into bile
- Dubin-Johnson syndrome

What was mentioned in the lecture:

As we've been saying, biliverdin is converted to bilirubin and bilirubin is then released into the blood, because bilirubin is quite hydrophobic, it cannot be transported in the watery environment of plasma, so it is carried by albumin, it binds to albumin and albumin takes it all the way to the liver.
An important thing that you need to pay attention is that some drugs, like salicylates (including aspirin) as well as sulfonamides can displace bilirubin from albumin, so that bilirubin becomes free of albumin, this allow bilirubin to readily cross the blood brain barrier and it can enter the central nervous system, in adults, that may not matter much, but in infants it can cause neural damage due to their lower content of plasma proteins, causing a condition known as kernicterus, hence these drugs are contraindicated for infants.
in the liver, what happens is that bilirubin is conjugated to glucuronic acid, so you have the formation of bilirubin diglucuronide (also referred to as conjugated bilirubin), conjugated bilirubin molecule is quite hydrophilic, it's transported into a bile and this how it is excreted.
deficiencies in either the enzymes that conjugates bilirubin with glucuronide or the transporters that transports the bilirubin diglucuronide to bile can cause certain conditions,
specifically, deficiency in the enzyme bilirubin UDP-glucuronosyl transferase, may result in diseases known as Crigel-Najjar syndrome I and II, as well as Gilbert syndrome, in these two diseases, there are accumulation of unconjugated bilirubin, while deficiency of the transporter that excretes conjugated bilirubin into bile is known as Dubin-Johnson syndrome.

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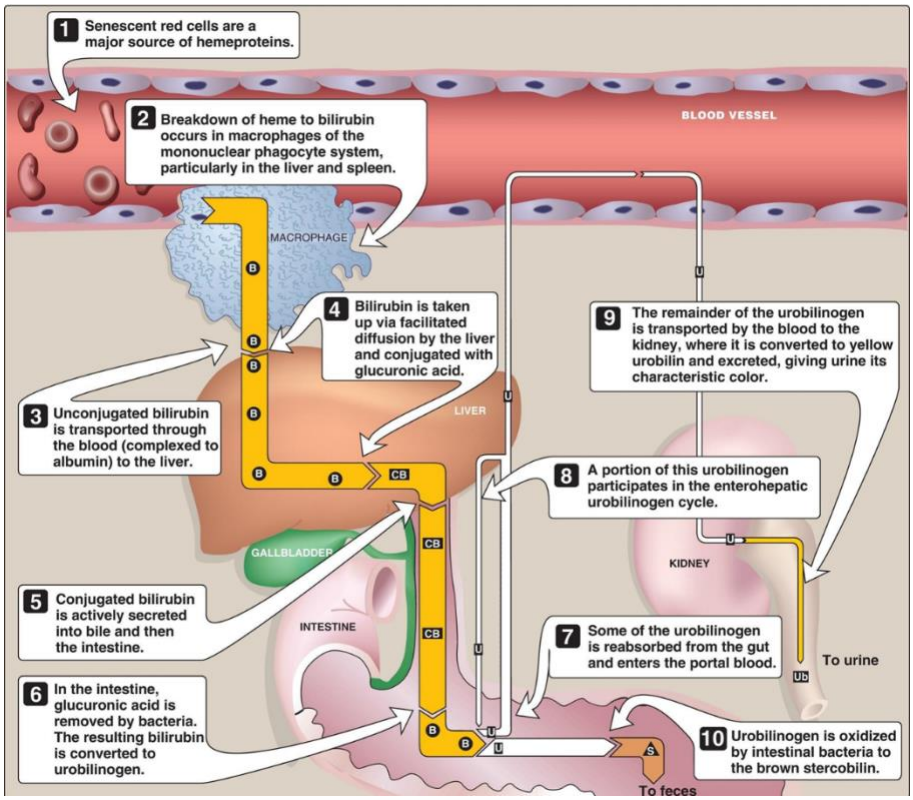


What was mentioned in the lecture:

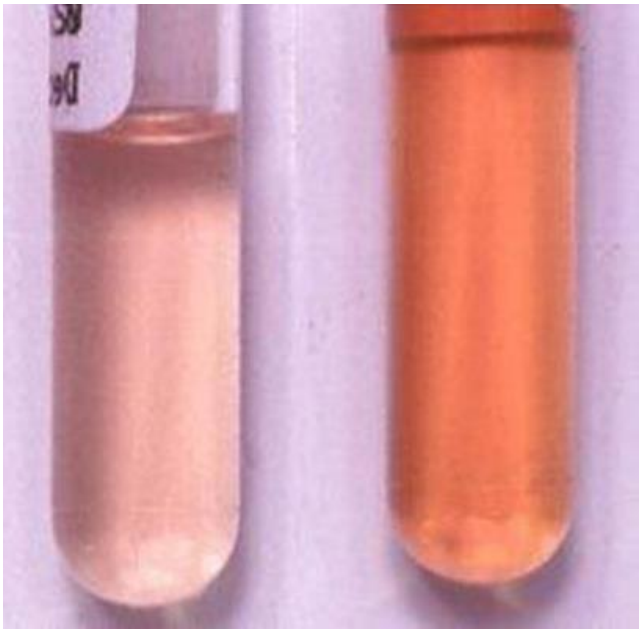
as we said, after RBCs are destroyed in the spleen, heme is released, and it is metabolized through several steps into bilirubin, bilirubin is then carried by albumin to the liver, in the liver it gets conjugated to glucuronic acid, so you have conjugated bilirubin, which is then transported into the bile where it can then be dumped into 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 can be reabsorped into the blood stream, and some remains in the small intestine, what remains from urobilinogen in the large intestine is converted to stercobilin now by gut bacteria, this stercobilin is the is a brown molecule that gives feces its color.
What leaks from urobilinogen into the blood is taken up by kidneys, and in kidneys it is converted to a final product known as urobilin, urobilin gives urine its yellowish color.

Measurement of bilirubin

What was mentioned in the slide:

- It is done via a reaction known as Van den Bergh reaction. Direct measurement of conjugated bilirubin (in water)
- Normally 4% of total bilirubin

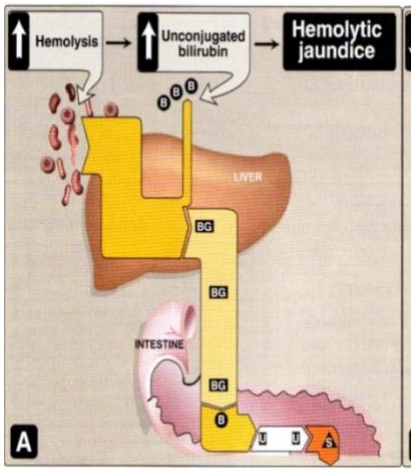
- Total measurement of bilirubin (in ethanol or methanol)
- Indirect unconjugated bilirubin = total bilirubin – direct bilirubin



What was mentioned in the lecture:

knowing the level of bilirubin in the blood is very important because it tells us a lot of information about the health of individual, specifically, it tells us much about the health of the blood, the liver and the biliary system.
Measuring levels of bilirubin can be done by a specific reaction known as Van Den Berg reaction, what's important about this reaction is that it is done in both water and ethanol or methanol, if it is done in water then it allows for the measurement of conjugated bilirubin which is the hydrophilic bilirubin, measurement of conjugated bilirubin is known as the direct bilirubin, note that conjugated bilirubin constitutes only 4% of total bilirubin.
if it is done in ethanol or methanol, this these two solvents would allow for bilirubin to be soluble, so using these solvents would allow us to measure all bilirubin molecules in a sample, including both conjugated and unconjugated.
in order to know the concentration of unconjugated bilirubin, which is known as indirect bilirubin, you just measure conjugated bilirubin using water, and total bilirubin using ethanol or methanol, and then we just subtract that from that.

Types and lab results of jaundice

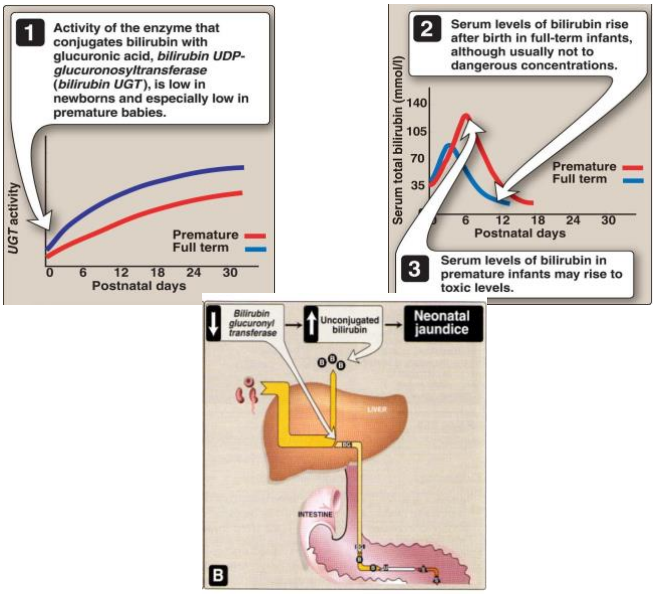


Sample	Indices		Unconjugated hyperbilirubinemia		Conjugated hyperbilirubinemia
		Normal	Hemolytic jaundice	Hepatic jaundice	Obstructive jaundice
Serum	Total Bil.	0.2-1.0 mg/dl	↑	↑	↑
	Direct (conj. Bil.)	0-0.2 mg/dl	↔	↑	↑↑
	Indirect (unconj. Bil.)	0.2-1.0 mg/dl	↑↑	↑	
	ALT/AST	Normal	Normal	↑	Normal
Urine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

What was mentioned in the lecture:

since we're talking about catabolism of heme, it's important to discuss jaundice, jaundice is basically a symptom that occurs due to a deficiency in the catabolism or transport metabolism of bilirubin, which result in yellowing of the skin and sclera.
jaundice can be due to two causes: unconjugated hyperbilirubinemia where there's increase in the amount of unconjugated bilirubin, and conjugated hyperbilirubinemia where you have high level of the conjugated bilirubin, let's look at the different markers related to jaundice in both of these types of hyperbilirubinemia.
unconjugated hyperbilirubinemia can be caused as a result of excessive hemolysis, in which you have lysing of a lot of red blood cells releasing a lot of heme, that results in a lot of heme being converted to bilirubin, so you have a lot of conjugation of bilirubin, so you have a lot of conjugated bilirubin going from liver to the intestines and you have conversion of bilirubin to urobilinogen and stercobilin, what characterizes people with hemolytic jaundice is that their stool would be very dark as a result of overproduction of stercobilin, but since the hepatic enzyme bilirubin UDP-glucuronosyl transferase enzyme is overwhelmed with bilirubin, so that some bilirubin would leak out of the liver into the blood, as a result what you will have here with hemolytic jaundice is that there is increased bilirubin in serum, mainly increased unconjugated bilirubin, but the amount of conjugated bilirubin would stay normal because little conjugated bilirubin would leak out as most of it would go into the intestines, in hemolytic jaundice urine would look darker, because there is a lor of heme, so the amount of urobilinogen and urobilin would be high in urine, also bilirubin itself would be carried to the kidneys.
you can also have what is known as hepatic jaundice, in which there is a problem in the liver itself, so what happens in hepatic jaundice is that you would have increased concentration of bilirubin in blood because there is leakage, specifically, there would be higher levels of both conjugated and unconjugated bilirubin because you have cell damage and release of both of these forms of bilirubin into the blood, also, since we have a problem in the liver, there would be increased liver enzymes (ALT: alanine transaminase, AST aspartate transaminase) in the blood, also urine would look darker because of the release of all of these forms of bilirubin into the blood which are taken to the kidneys because they cannot be excreted through the liver, stool would look either normal or lighter in color since there would be reduced bilirubin and its conjugates reaching the intestines.
another form of hyperbilirubinemia is known as conjugated hyperbilirubinemia, what happens is conjugated hyperbilirubinemia is that there is production of conjugated bilirubin, but it does not get into bile or the intestines, so this conjugated bilirubin would leak out of the liver and there would be increased level of total bilirubin in serum, specifically, there would be higher concentration, in obstructive jaundice, liver enzymes are normal and urine would look darker, because there would be a lot of bilirubin taken to the kidneys and not able to reach feces, the kidneys cannot convert bilirubin to urobilinogen and urobilin and that results in reduced amount of these two molecules in the urine, the stool would look clayish as a result of inability of the liver to transport conjugated bilirubin into the intestine, so there would be a low concentration of stercobillin in the intestines in feces.

Jaundice in newborns



What was mentioned in the lecture:

jaundice usually affects infants, this is because the enzyme bilirubin UDP-glucuronosyl transferase that conjugates bilirubin with glucuronides is not active at birth, its activity increases with time, so within like two weeks, the enzyme would be able to efficiently conjugate bilirubin with glucuronic acid.

The problem is with premature babies, in which the activity of the enzyme really really low and improves too slowly, and that results in over production of bilirubin that cannot be conjugated, this bilirubin would leak out into the central nervous system and it can damage the brain, so usually in normal infants, there would have high concentration of bilirubin but these high levels are not toxic, but in premature babies, the levels will be very high they can be very toxic, a solution is to expose infants to blue light, what blue light does is that it breaks up bilirubin into a more hydrophilic molecule that can be eliminated easily.