HEMATOLOGY
&LYMPH SYSTEM

Biochemistry sheet

Number	6
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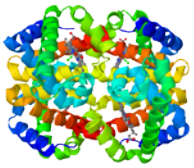


Heme Synthesis

In the previous lecture we talked about HEME SYNTHESIS and we said that the rate limiting step is the condensation of Glycine + Succinyl CoA to produce **ALA** by **ALAS** [in the **mitochondria**] .Also, we said that ALAS is encoded by two different genes (there are two isoform), each one's regulation is different from the other; they are controlled by different mechanisms:

1) ALAS1: is present in the liver and inhibited by hemin; feedback inhibition (when it access porphyrin, heme will be converted to hemin). This enzyme inducible by drugs which require monoxygenation, these drugs enhance consumption of heme; decrease concentration on heme which leads to increase synthesis of ALAS1.
2) ALAS2: is present in erythroid cells and regulated by ***erythropoietin*** and ***iron*** (which is an inhibitor).

The second enzyme in this reaction is **ALA dehydratase** (porphobilinogen synthase) which present [in the **cytosol**], (this enzyme is sensitive to Pb, so in lead "heavy metal" poisoning; it will be inhibited and ALA will accumulate and this is the cause of anemia in lead poisoning), it condensates 2 ALA to give a Pyrrole (porphobilinogen) ring. **So we need 8 gly AND 8 succinyl CoA to make one porphyrin or Hb**.

After that, 4 porphobilinogens (PBG) condense by **hydroxymethylbilane synthase**; it will bind them together in linear fashion. Then another enzyme **uroporphyrinogen III synthase** forms the closed Tetrapyrrole (porphyrin) ring.

From the past papers

Note the arrangement of the substituents in uroporphyrinogen III: acetyl, propionate, acetyl, propionate, acetyl, propionate, **propionate**, acetyl (**asymmetric**). Uroporphyrinogen I is symmetrical, it is the same as III but with the reverse location of the last two. Uroporphyrinogen is not colored because it is reduced, if it exposed to light it will be oxidized and become uroporphyrin which is colored (reddish).

Which of the following is true about uroporphyrinogen: (a) can be synthesized directly from PBG by cosynthase (b) result from direct conversion of coproporphrinogen

Uroporphyrinogen lll undergoes decarboxylation which means that all acetate groups will be converted to methyl and become Coproporphyrinogen. Also, some uroporphyrinogen l and coproporphyrinogen l are formed but they are excreted; they are produced in excess in erythropoietic porphyria and excreted.

IMPORTANT

Coproporphyrinogen lll then enters [the **mitochondria** (**remember** that the first reaction was in mitochondria; Heme synthesis don't take place in mature RBCs because it need mitochondria)] and by the microsomal enzyme **oxidase**, two propionates are decarboxylated to vinyl and it becomes Protoporphyrinogen (reduced+ colorless) lX which will be oxidized in the mitochondria to give the colored Protoporphyrin (oxidized +colored). **And this oxidation is the only oxidase that occurs here.**

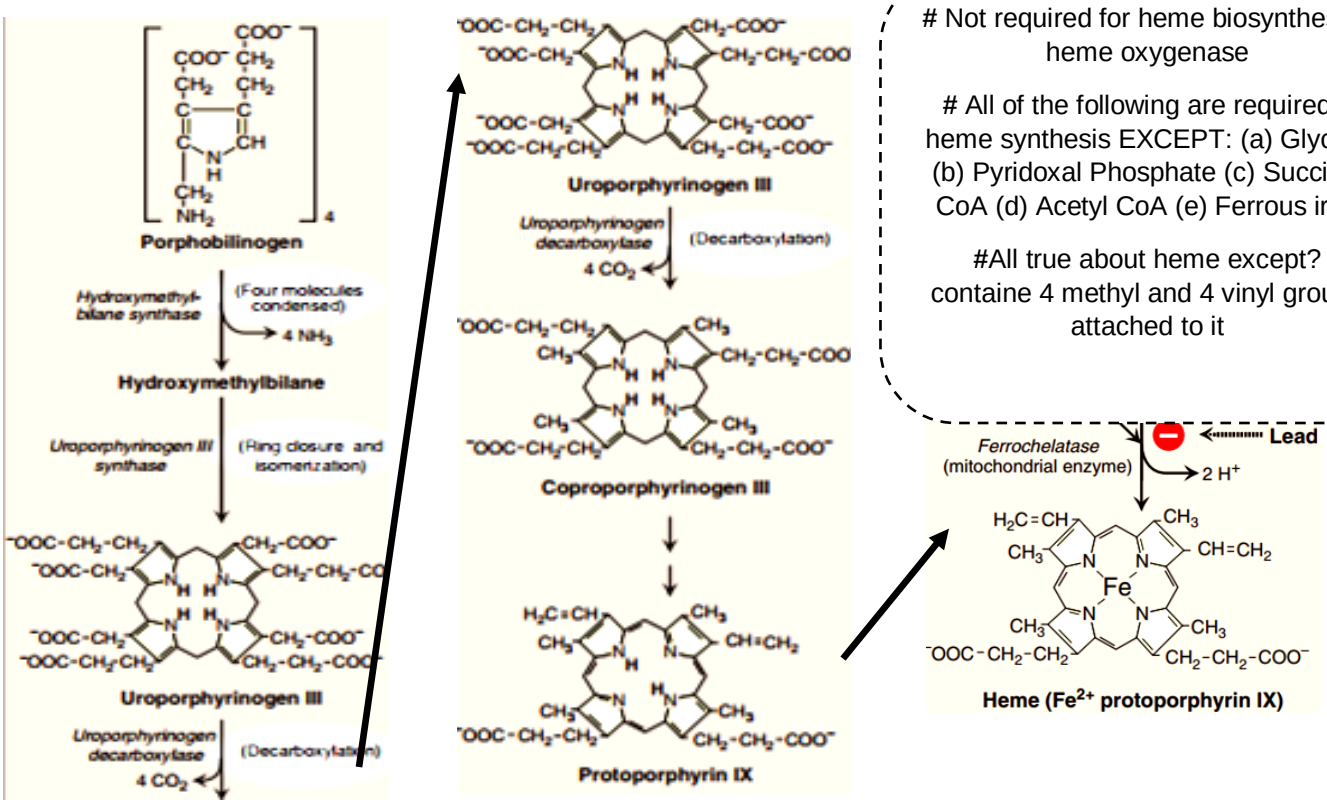
Protoporphyrin can bind non-enzymatically with iron but faster by **ferrochelatase** (sensitive to lead, so in Pb poisoning, it will be inhibited and Protoporphyrin will accumulate) to give Ferroprotoporphyrin (**Heme**). If there is an increase in synthesis and it reaches a higher level than globin this compound will accumulate and the ferrous become ferric, Ferrous in aqueous solution fastly become ferric and we call it Hemin.

From the past papers

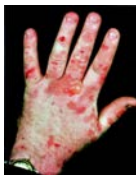
Not required for heme biosynthesis? heme oxygenase

All of the following are required in heme synthesis EXCEPT: (a) Glycine (b) Pyridoxal Phosphate (c) Succinyl CoA (d) Acetyl CoA (e) Ferrous iron

#All true about heme except? containe 4 methyl and 4 vinyl groups attached to it



Disorders



Porphyria:-

Deficiency of one of the enzymes in heme synthesis pathway (inherited or acquired), it is not common, lead to accumulation and increased excretion of porphyrin or precursors (ALA or PBG). All inherited as autosomal dominant except erythropoietic porphyria as AR. Mutations are very heterogeneous..

Porphyria is classified as a) erythropoietic b) hepatic (acute or chronic)

If there was accumulation of ALA or PBG, the patient will not suffer from **photosensitivity** but once tetrapyrrole was formed; if accumulate in a later stages (the enzyme defects leading to accumulating of tetrapyrrole intermediates) the patient will suffer from it because colored porphyrin makes ROS which damages lysosomes and releases its enzymes, as a result the patient will suffer from itching and erythema.

The most common acquired form of porphyria is **lead** poisoning (which works in two places) which affects two enzymes we already talked about, ALA dehydratase & ptotoporphyrin ferrochelatase. It leads to accumulation of both; ALA & protoporphyrin. It causes anemia due to Hb loss and energy production will be lower due to cytochrome decrease.

From the past papers

Lead poisoning affects an enzyme of heme synthesis that might be producing? porphobilinogen

Lead poisoning will result in accumulation of: (a) Porphobilinogen (b) δ-aminolevulinic acid and protoporphyrin IX (c) Uroporphyrinogen (d) Hydroxymethylbilane (e) δ-aminolevulinic acid and coproporphyrinogen

If the defect was **before** tetrapyrrole synthesis, the patient will complain of abdominal pain and neuropsychiatric signs (Photosensitivity and hepatic damage in some).

ALA & PBG accumulation leads to:

1) Impaired function of abdominal nerve and CNS.
2) ATPase activity decreases.
3) Conduction paralysis.

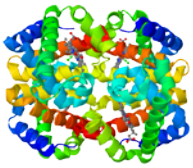
You should know that the most common genetic porphyria is **porphyria cutanea tarda** which is chronic hepatic porphyria.

The only autosomal recessive genetic porphyria is **congenital erythropoietic porphyria.** In which Insufficient uroporphyrinogen lll cosynthase leads to decrease uroporphyrinogen lll and increase in uroporphyrinogen l & consequently corporporphyrin l.

Signs and symptoms:

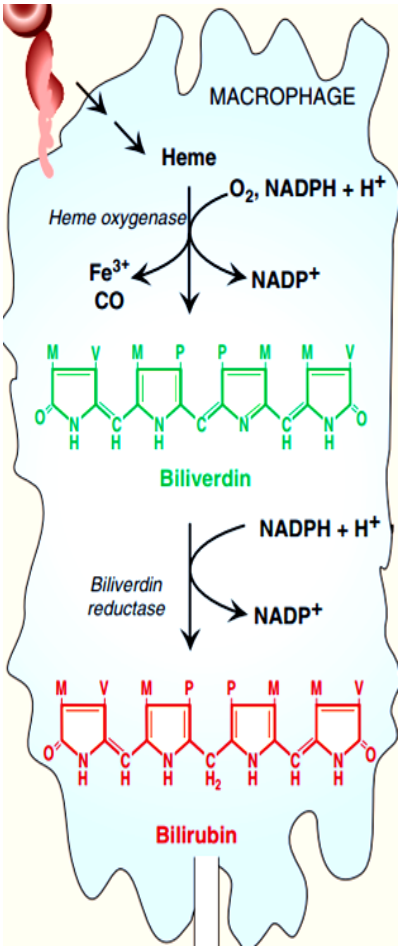
1) Premature destruction of RBCs.
2) Red urine (large amount of porphyrin l).
3) Teeth exhibit strong red fluorescence under UV light.
4) Skin is sensitive to light.

Another disorder: If there was increase in **ALA synthase** due to decrease in Hb concentration, the treatment will be administration of hemin, avoidance of sunlight and free radicals scavenger e.g. beta-carotene, vitamin C & E.



Heme Degradation

RBCs when they become old or if they were defective (have some abnormalities), they undergo certain changes in them and the membrane will not look normal, then the undergo phagocytosis by macrophages in all tissues, particularly in spleen and liver. When hemoglobin undergoes degradation it gives globin which will be converted to amino acids that reutilized, iron will be released and utilize, heme will undergo degradation.



The first enzyme to act on heme is **heme oxygenase** on the macrophage itself which breaks the closed ring by the need of NADPH and Oxygen molecule to give Biliverdin Xl, Ferrous, CO(When the bond broken alpha carbon will become CO, very little amount but it has a function; anti-inflammatory and it acts as a signaling molecule) and NADP+.

Biliverdin Xl (a potent antioxidant) a result of cleavage of the bond **(green pigment)**, it then undergoes reduction by **biliverdin Xl reductase** (also needs NADPH) to give **Bilirubin (red-orange)** [In the **macrophage**]. We see the changes in blood color when someone has a wound.

We call bilirubin and its derivatives: **bile pigments.**
Biliverdin is more soluble than bilirubin.
Bilirubin has some antioxidant activity.

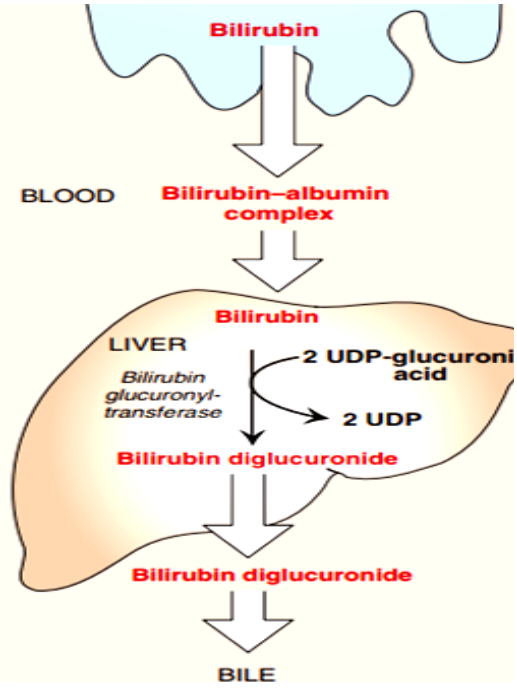
From the past papers

heme oxygenase:? require molecular oxygen

Heme oxygenase? a) Produces carbon dioxide
b) Can oxidize the membrane bridge between two pyrole rings of heme
c) Requires molecular oxygen d) Produces bilirubin
e) two of the above are correct

When bilirubin exits the macrophage to the blood, it forms a complex with **albumin** in blood. This binding is affected by some drugs e.g.salicylate, sulphonamides which displace bilirubin in infants (have high bilirubin) and causes neural damage. Then Bilirubin will enter the liver via FACILITATED DIFFUSION, inside the liver will be bound to another protein and there it will be solubilized by joining with glucuronic acid from UDP-glucuronic acid (doner, we need 2) to become **Bilirubin diglucuronide** (conjugated bilirubin) then it will exit the liver against a concentration gradient by ACTIVE TRANSPORT and go to the bile duct then it will be released to the intestine and there the bacteria shed out the glucuronic acid and bilirubin is converted to urobilinogen → stercobilinogen →stercobilin (gives the color of feces and if we expose this feces to the sunlight stercobilinogen will undergo oxidation and the color will be darker). Some urobilinogen goes to kidney and converted to urobilin (gives the yellowish color of urine).

SO, urobilinogen has different choices, it either goes back into the **liver** or into the **kidney** or it ends up in the **feces**.



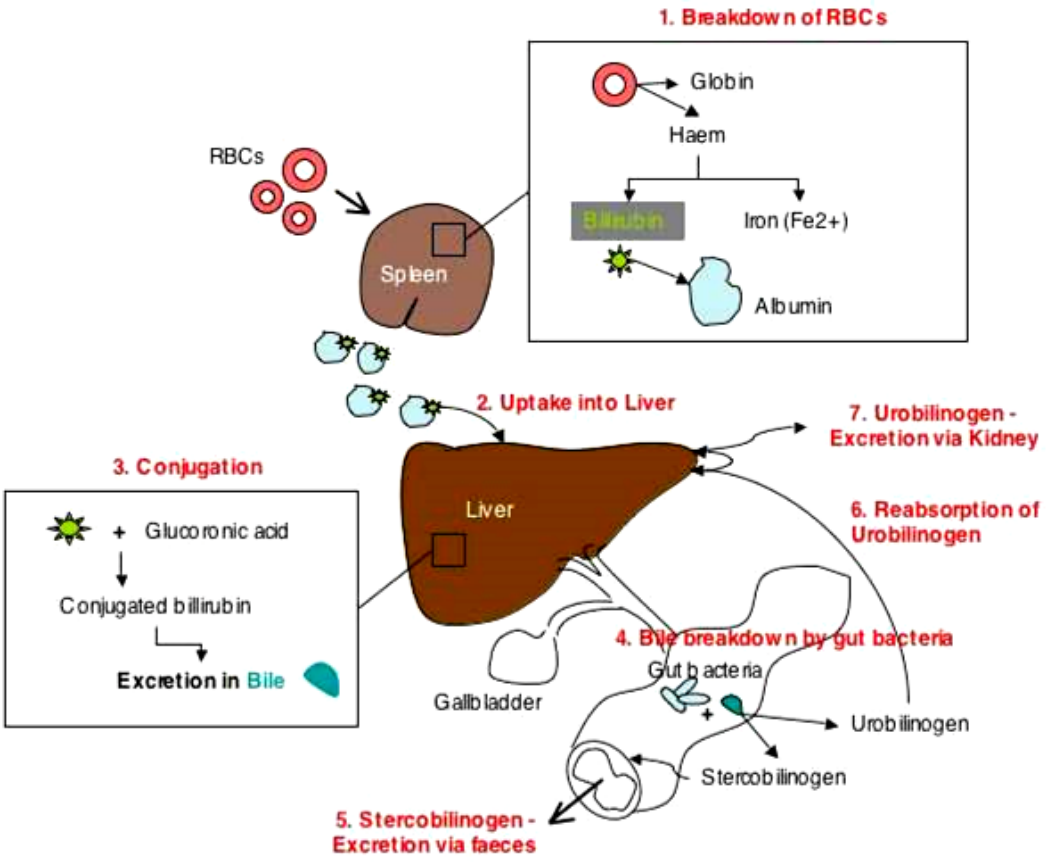
From the past papers

Wrong about bilirubin ? produced for heme by heme oxygenase system

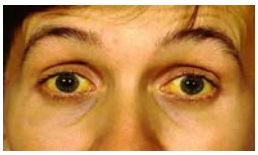
High levels of conjugated bilirubin, near-normal levels of unconjugated bilirubin and low fecal stercobilinogen is best characterized by: (a) Hepatitis (b) Hemolytic disease (c) Obstruction of the bile duct (d) Low levels of UDP- glucuronic acid (e) Any of the above

Conjugation of bilirubin:

First of all, UDP-glucuronate formed from UDP-glucuse by a dehydrogenase, addition of glucurnic acid done one by one (monoglucuronide… diglucuronide) is it in solubilize form? We know that all soluble compounds are toxic so they undergo conjugation in the liver to be able to be solubilize and then excreted. Then 2 UDP-glucuronate reacts with bilirubin to give bilirubin-diglucuronate



Jaundice



The yellow color of skin, nail beds and sclera (white of the eyes) caused by deposition of bilirubin, secondary to increased bilirubin levels in the blood. It's a symptom of underlying diseases in which bilirubin is high.

Bilirubin test is one of the routinely measurement for tissues and the normal level is 1 mg, when it becomes 2-3mg deposition will occur in the white of the eye, skin, nail buds… etc.

Most of the 1 mg is unconjugated bilirubin and a small portion is conjugated.

Types of jaundice: Jaundice can be classified into three forms:

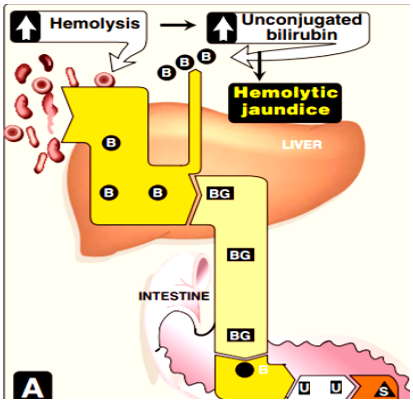
1) **Hemolytic jaundice**

The liver can conjugate up to 3000mg/day bilirubin & the daily production of bilirubin is 300mg/day (10 times more)

In massive hemolysis as in HBS, PK deficiency and G6PD deficiency, the **unconjugated bilirubin will increase** and exceeds the capacity so the

1) Excretion in bile
2) Urobilinogen in urine
3) Blood unconjugated bilirubin

All will increase everywhere; urine, blood….



Hepatocellular (hepatic) jaundice

Other types of jaundice were **excretion of bilirubin decreases** due to liver damage, results in:

1) Capacity of conjugation will be affected so the unconjugated bilirubin increase
2) Increased urobilinogen in urine because the liver uptake will decrease
3) Decreased secretion conjugated bilirubin

2) Obstructive (post hepatic) jaundice

Due to bile duct obstruction (stone,tumer…), so bilirubin undergo conjugation but it cannot pass normally in its way. So the conjugated bilirubin will increase.

Neonatal jaundice

The conjugation capacity is low because the activity of the enzyme that conjugates bilirubin with glucuronic acid (UDPGT) is low in newborn especially in a premature baby, the enzyme undergoes maturation after 4 weeks. So serum levels of bilirubin rise after birth in full-term babies, usually not too dangerous concentration, but it may rise to a toxic levels in premature infants.

The enzyme may be deficient and we have varies form of this deficiency, there are varying degrees of **transferase** deficiency (the enzyme which does the conjugation), **Cliglar najjar I & II** are more severe than **Gilbert** which is mild. In Dubin johnnson & Rotor syndromes there is a defect in excretion of conjugated bilirubin.

Laboratory test for bilirubin level → **Van der Bergh rxn**, it determines the amount of conjugated bilirubin by using diazotized sulfanilic acid which reacts and gives red azodipyrrole (direct-reacting bilirubin→ conjugated), when we add methanol we measure the total bilirubin (direct + indirect). To measure the unconjugated, we subtract Direct from total.

Phototherapy means exposing bilirubin to blue fluorecent light to becomes polar and water soluble.

وَأَنْ لَيْسَ لِلإِنسَانِ
إِلاَّ مَا سَعَى

"اللَّهُمَّ انْفَعْنَا بِمَا عَلَّمْتَنَا، وَعَلِّمْنَا مَا يَنْفَعُنَا، وَزِدْنَا عِلْمًا إِلَى عِلْمِنَا"