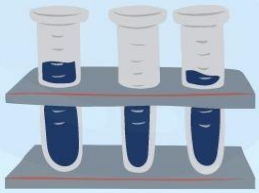
Sheet No.
6

Biochemistry



hematolymphatic system

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Let's start with a quick revision:

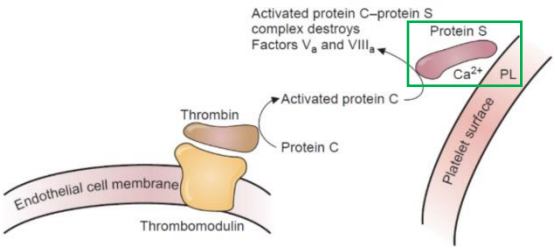
In the last lecture we talked about the cascade of reactions that ends with conversion of prothrombin to thrombin, which plays an important role in the whole cascade especially in cleaving fibrinogen to form fibrin molecules that aggregate to make the soft clot which will turn into a hard clot when the cross linking happens between the fibrin molecules (remember the role of factor XIII).

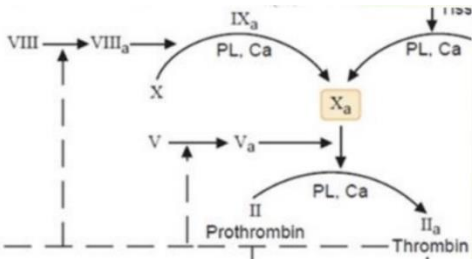
Anti-clotting factors

The anti-clotting factors are found in our body to balance the stimulation and at the same time the inhibition of the formation of blood clots.

Protein C and S

When **thrombin** is activated, it binds to a protein found on the surface of the endothelial cells called **thrombomodulin**. Once they bind, the thrombin will activate **protein C**, which forms a complex with **protein S**, this complex degrades factor V and VIII which are responsible for the stimulation of the intrinsic and extrinsic pathways.

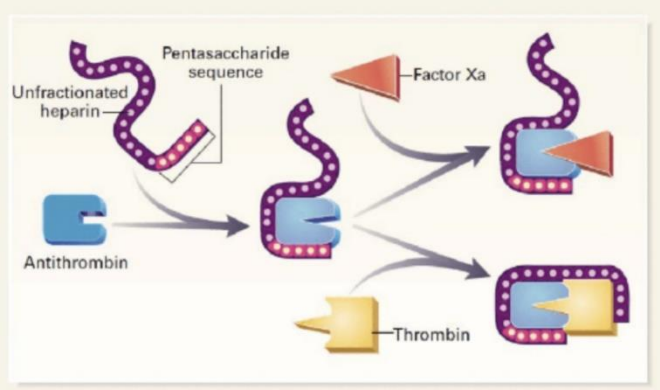


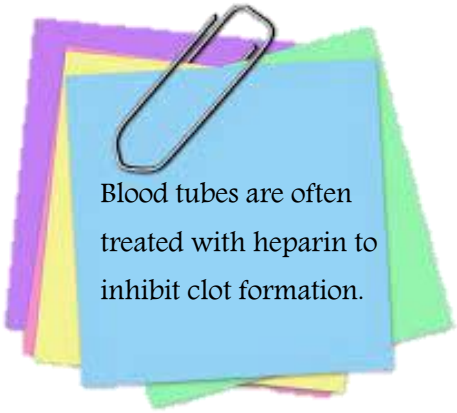


Anti-thrombin III

Also we have Anti-thrombin III which is a protease inhibitor of thrombin as well as **IXa, Xa, XIa, XIIa**, and **VIIx** when complexed with TF (tissue factor).

Heparin sulfate, is a polysaccharide synthesized by mast cells and present on the surface of endothelial cells, binds to **Anti-thrombin III**, promoting binding to its substrates, which are thrombin and the previously mentioned factors, this result in inhibiting thrombin activity and blood clot formation.





Heparin is glycosaminoglycan (sugar+ protein)

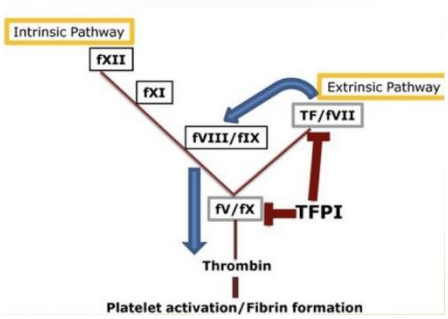
Tissue Factor pathway inhibitor

Tissue factor pathway inhibitor (TFPI) is a protein found in plasma lipoproteins (ex. Cholesterol, lipid transporter) and bound to the vascular endothelium.

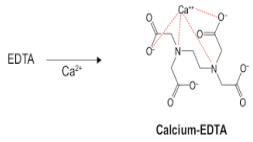
Note the role of vascular endothelial cells in inhibiting the blood clotting.

- It binds to and inhibits factor Xa.
- The Xa-TFPI complex then interacts with the TF-VIIa complex and inhibits its activation of factors X and IX.
- Protein S binds to TFPI localizing it to membrane surfaces and enhancing the inhibition of Xa. Notice the green square in page 2.

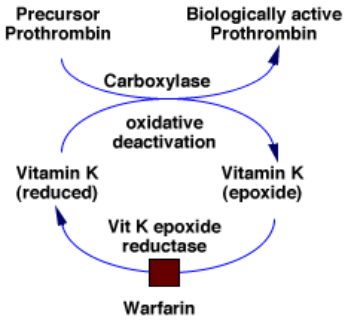
TFPI also inhibits Xa-activated Va resulting in inhibition of the pro-thrombinase complex, so we have inhibition in both: intrinsic and extrinsic pathways at the same time.



Anti-coagulants



Blood clotting can be prevented by addition of Ca^{+2} chelators* and vitamin K antagonists such as the anticoagulant drug warfarin, which inhibits reduction of vitamin K and thereby prevents the synthesis of active prothrombin and factor VII, IX, and X.



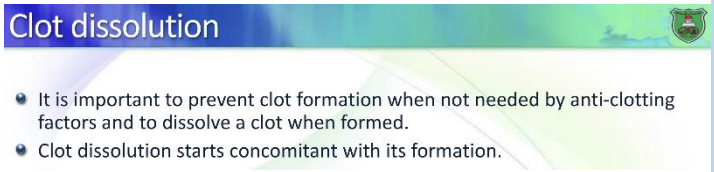
*chelators: factors that bind to metals hijacking them (like EDTA).

Remember that Ca^{+2} is important in the coagulation process.

Some blood tubes also contain EDTA to inhibit blood coagulation.

Degradation of the fibrin clot

We have different factors and proteases that play a major role in the removal of blood clots.



The fibrinolytic system

This system causes lysis of the fibrin mesh.

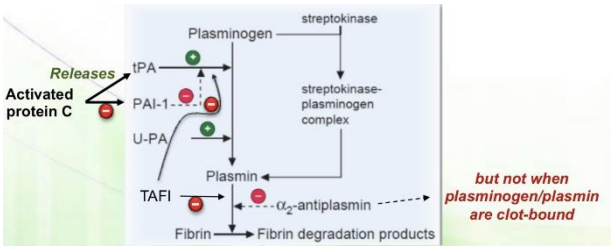
Plasmin is the major protease (serine protease), formed from plasminogen, and is responsible for fibrinolysis where it binds to the lysine residues of fibrin and catalyzes its hydrolysis.

- Plasminogen has a high affinity for fibrin clot, and the activation of plasminogen to plasmin happens more efficiently inside the clot.
- So plasminogen is found soluble in the blood, but it's not activated, because it's not bound to the clot, once its bound, activation happens causing degradation of the clot. PROTECTIVE MECHANISM

????How plasminogen activation happens????

By the help of plasminogen activator enzymes:

1- Tissue plasminogen activator (TPA)
2- Urokinase plasminogen activator



These two needs activated protein C to be activated.

Thrombin activates protein C, which form a complex with protein S, causing cleavage to factors and thrombin.

So activated thrombin stimulate the activation of its inhibitors (protein C). PROTECTIVE MECHANISM

- Activated protein C causes the release of TPA and inhibits their inhibitors (PAI).
- If activated plasmin is found soluble in the blood, it interacts with Anti-plasmin. But when it is hiding inside the clot it is protected from anti-plasmin.

أول ما يطلع البلازمن من الكلوت بهجم عليه الانتي بلازمن عشان ما يتفاعل مع اشي ثاني.

Thrombin activatable fibrinolysis inhibitor (TAFI) is a carboxypeptidase that removes the N-terminal lysine residues and prevent fibrinolysis.

- Streptokinase, a regulatory protein isolated from streptococci, allows auto-activation of plasminogen in blood, resulting in degradation of fibrinogen as well as fibrin. (It is clinically injected to remove clots by activating plasminogen…)

Urokinase

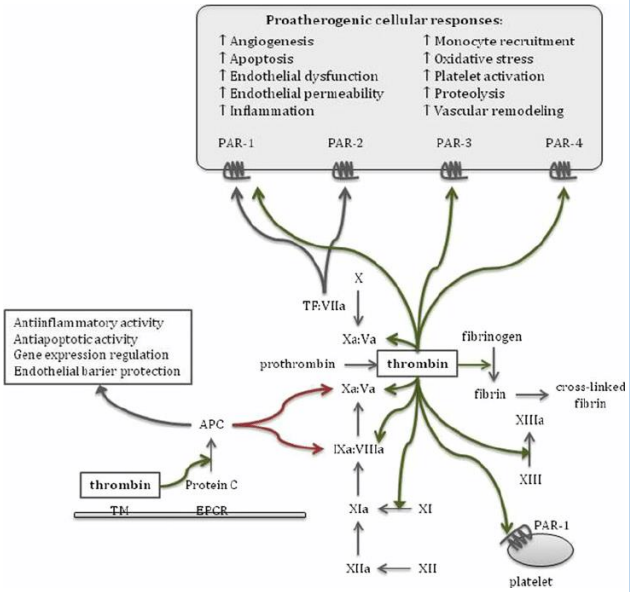
Urokinase is a protease that is formed from the zymogen pro-urokinase.

It is a potent plasminogen activator, and is used clinically.

Roles of thrombin

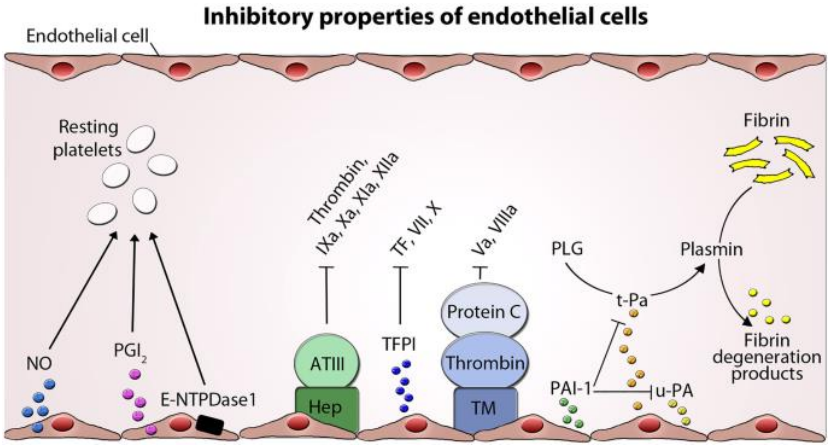
As we said, thrombin is a major player, when activated, it interacts with its receptors on the platelet surface (G-protein coupled receptors), causing activation of the signaling pathway→ platelet recruitment→ adhesion→ and aggregation. So thrombin works in:

1. **Platelet recruitment**
2. **Amplification of the coagulation complex.**
3. **Formation of soft clot.**
   - Proteolytic cleavage of fibrinogen.
4. **Formation of hard clot.**
   - Activation of factor XIII.
5. **Attenuation of its own activity.**
   - Activation of protein C.
6. **Other actions.**
   - Binding to its receptor on the surface of platelets induces vascular remodeling (angiogenesis: formation of new blood vessels) and inflammation.



Roles of endothelial cells in coagulation

These endothelial cells work as a negatively charged barrier, inhibiting the interaction of platelets with the sub-endothelial collagen and TF (tissue factor on the surface of endothelium) by repulsion force.



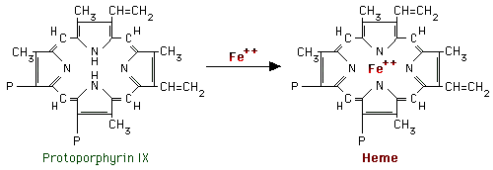
- ECs release NO, prostacyclin (PGI2), and ADPase, which inhibit platelet adhesion and aggregation.
- Membrane-bound heparin sulfate binds to antithrombin III (ATIII) inactivating several coagulation factors.
- ECs express tissue factor pathway inhibitor (TFPI), which inhibits tissue factor (TF) and, consequently, factors VII and X.
- Thrombomodulin (TM) binds thrombin activating protein C, which degrades factors Va and VIIIa.
- ECs balance fibrin accumulation and lysis by releasing plasminogen activators, t-PA and u-PA, and their inhibitor (PAI).

Now we will start with a new concept.

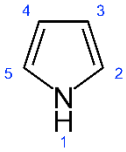
Take a break, fill your coffee and come back

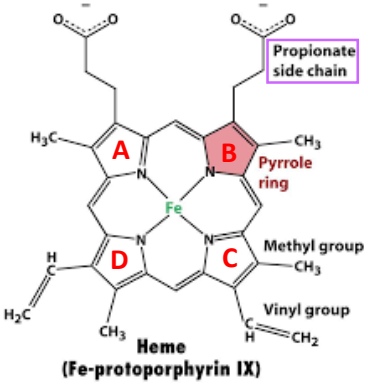
Metabolism of heme

Heme structure



- It is a complex of protoporphyrin IX + iron (Fe^{+2}).
- The porphyrin is planar and consists of four pyrrole rings, so it's a tetrapyrrole (designated A–D).
- Each pyrrole ring can bind two substituents
  2 binds methyl, vinyl
  2 binds methyl, propionate
- Its hydrophobic (forms clusters if released)
- Fe has six coordinates of binding





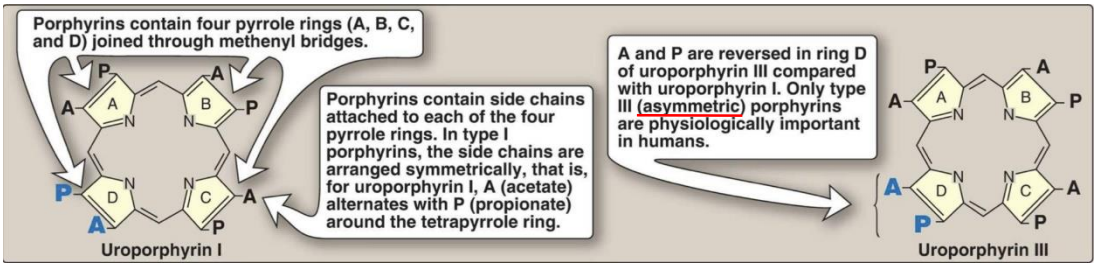
The iron is very precious, so we need to balance between heme synthesis and heme degradation. (We will discuss them in the upcoming lectures)

Porphyrins

This part is just a quick review for some definitions.

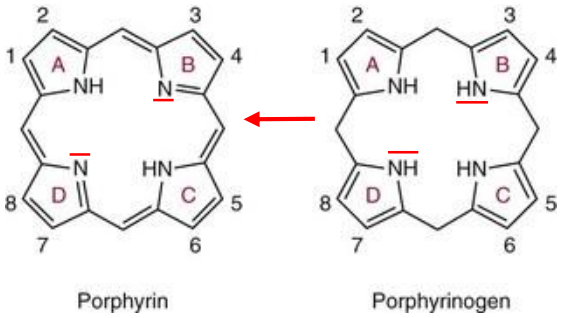
Remember:

Porphyrin I has alternated branches on the rings (AP AP AP AP), but porphyrin III has switched branches on ring D (AP AP PA AP). Look at the picture below (counting B→C→D→A)



Porphyrinogen vs. porphyrins

porphyrinogen	porphyrin
Porphyrin precursors	The active form
Reduced (lines in red)	oxidized
colorless	Produce color
Intermediates of heme synthesis	



Biosynthesis of heme

Sites of synthesis

Almost all tissue can produce heme (e.g. muscles, stomach cells, neurons…), but the main sites for heme biosynthesis are:

- **Liver**, which synthesizes several hemoproteins (particularly the CYP proteins, needed for detoxification).

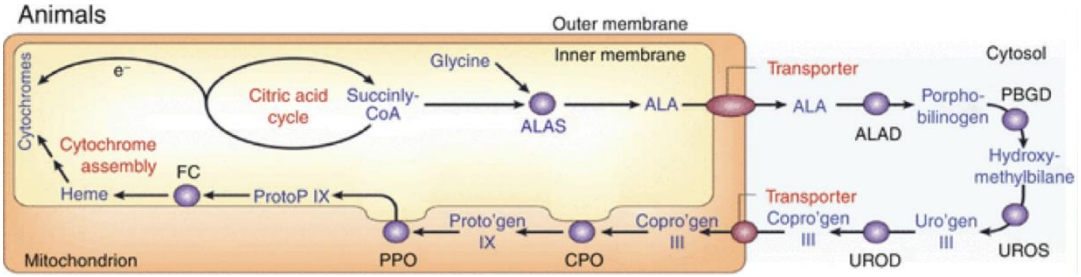
  The rate of heme synthesis is highly variable.

  (Heme synthesis is highly controlled and regulated, depending on food, drugs that are taken).

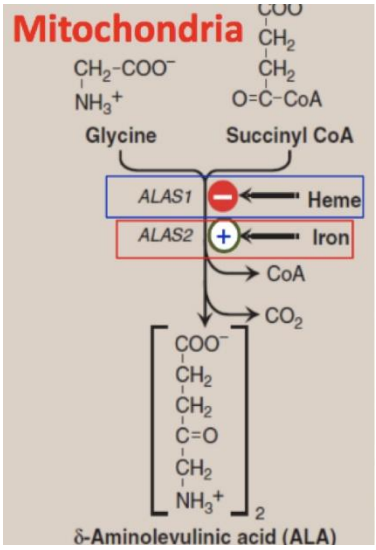
- **Erythrocyte-producing cells** (Hb synthesis, for the formation of RBCs)

  Relatively constant production and matches the rate of globin synthesis, yet synthesis is regulated at multiple points. (Relatively constant regulation)

Synthesis occurs in mitochondria→ cytosol→ mitochondria.



Synthesis of 5′-aminolevulinic acid (ALA)



The first reaction is catalyzed by 5′-aminolevulinic acid synthase (ALAS), which conjugates glycine and succinyl CoA into ALA –this occurs in the mitochondria-.

- It is rate limiting and committed step.
- It requires vitamin B6 (pyridoxal phosphate).

ALA moves out of the mitochondria to the cytosol.

ALAS have two isoenzymes:

- ALAS1 (all tissues inc. liver)
- ALAS2 (erythroid)

Rate limiting: highly regulated.

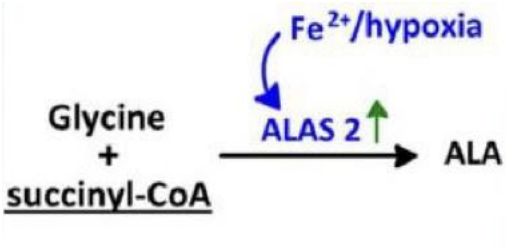
Committed: if this step happens, the rest of the reaction must be completed.

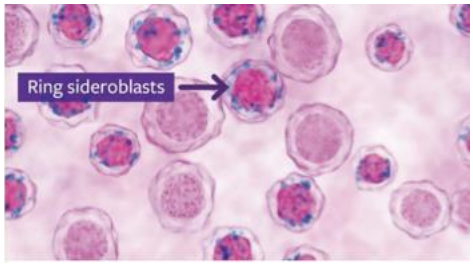
Izoenzymes: differ in their gene, tissue distribution, catalytic activity, and regulation, but they catalyze the same reaction.

ALA synthase isoenzymes

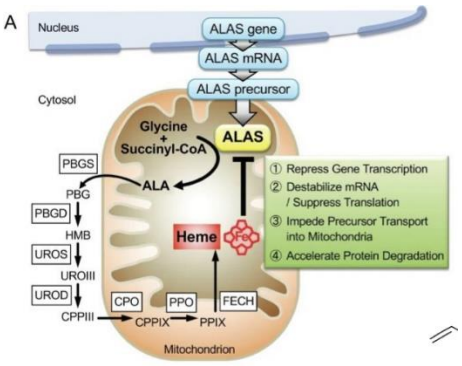
ALAS2 is regulated by the level of iron and hypoxia.

- High iron level stimulates the production of more heme to make Hg.
- Hypoxia shows low oxygen level in tissues, so we need more hg to bind oxygen.
- Loss of function mutations results in X-linked sideroblastic anemia, in which iron accumulates in erythroid marrow and deposits as mitochondrial non-ferritin iron ring sideroblasts.





ALAS1 is regulated by:



- Heme
- Hemin/ Hematin: (heme but with oxidized iron Fe^{+3})
  - Reduces gene transcription (transcriptional)
  - Reduces mRNA stability (post-transcriptional)
  - Inhibits mitochondrial import of ALAS1 (transporting)
  - Induces protein degradation (post-translational)

  Notice the levels of regulation in red.

  When heme level increases the hemin & hematin also increases.

- Drugs:

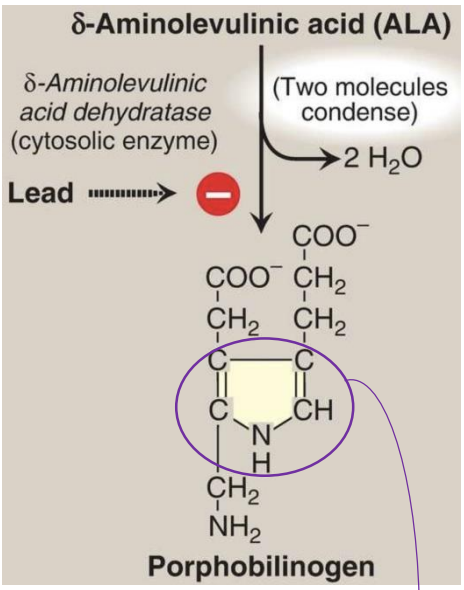
  They affect ALAS1 indirectly

  Barbiturates (which is an anti-anxiety drug), increases the liver cells need for CYP450 production, which results in consuming heme, and so activating ALAS1 synthesis to compensate.

Synthesis of porphobilinogen

ALA moves out of the mitochondria to the cytosol where porphobilinogen is formed by condensing 2 ALAs by zinc-containing ALA dehydratase (porphobilinogen synthase).

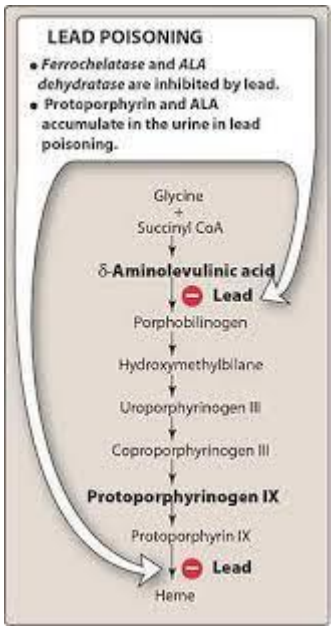
This enzyme is inhibited by lead that replace the zinc, so the enzyme becomes non-functional resulting in lead poisioning-associated anemia, and increase in ALA.



we aren't required to know the name of enzymes & reactions except the mentioned below.

Subsequent reactions

4 porphobilinogen (PBG, each of them contain one pyrrole ring) molecules form 1 hydroxymethylbilane (contains four pyrrole rings) which will be converted from the linear structure to form cyclic uroporphyrinogen III→ corpoporphyrinogen III enters the mitochondria to form protoporphyrinogen IX→ oxidized protoporphyrin IX→ with Fe^{+2} forming Heme.

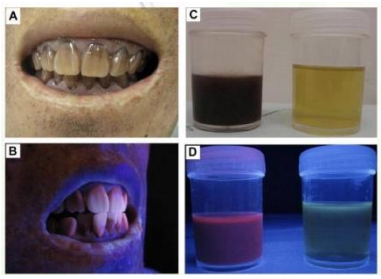


- The last reaction is spontaneous but can be catalyzed by ferrochelatase.
  This enzyme is inhibited by lead.
  SO, lead causes inhibition of the 2nd and the last reaction.

Porphyrias

The defect of any enzyme causes accumulation of all the intermediates above, so we can define porphyrias as: inherited (mainly autosomal dominant) 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 (Greek word) means purple
  The skin, teeth, gingiva will become purple, and the urine becomes dark.
  This happens especially if the patient is exposed to light.

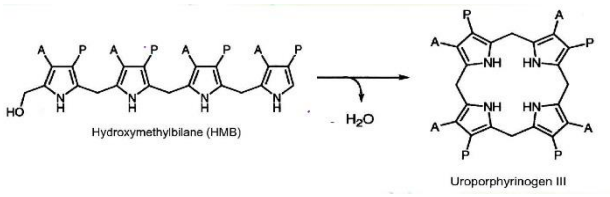


- These disorders are classified according to:
  - Affected tissue
    - Erythroid
    - Hepatic (acute or chronic)
    - Both

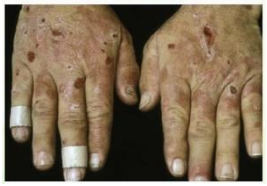
- Manifestations:
  - Not photosensitive:

    Results from accumulation of ALA, or porphobilinogen. (Don't contain tetrapyrrole rings)

    Complains from neurological disorders and severe abdominal pain.
  - Photosensitive:

    This happens because of accumulation of tetrapyrrole rings, meaning that it appears in all problems that result in accumulation of **hydroymethylbilane** or the intermediates below.



These pyrrole rings have the ability to absorb light, which result in formation of **free radicals** causing damage to cells, tissues, and also release of lysosomal enzymes (proteases) causing degradation of tissues, burn sensation, formation of skin lesions especially if the patient is exposed to light.

Don't stop until you're proud