SCHOOL OF MEDICINE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
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CLINICAL BIOCHEMISTRY FOR BMLT 3 & BDS 4

PORPHYRINS AND PORPHYRIN DISORDERS

- Heme is a component (Prosthetic group) of several important proteins (Haemoglobin, Myoglobin), and enzymes (Catalase, Cytochromes)
- Heme is made up of Protoporphyrin and Iron (Fe)
- Some genetic disease states are associated with deficiencies of enzymes in Heme biosynthesis
- Some of the disease conditions are readily diagnosed because they cause the compound Delta-Amino-Levulinic Acid (ALA) and abnormally coloured Heme intermediates to appear in blood circulation, urine and in other tissues such as teeth and bones.
- Some disorders of Heme biosynthesis are more Insidious such as the various Porphyrias.

How is Haem synthesized?

- Biosynthesis of Heme occurs mainly in the bone and liver.
- Enzymes (about 8) involved in biosynthesis of Heme are located in the Mitochondria and Cytoplasm of cells.
- The pathway includes at lease 8 different enzymes located in the mitochondria and cytoplasm of the cell.
- Schematic diagram of the pathway for heme biosynthesis and enzymes involved are presented in **Fig. 1 & Table 1**

Fig. 1:
Schematic diagram showing summary of pathway for biosynthesis of Heme

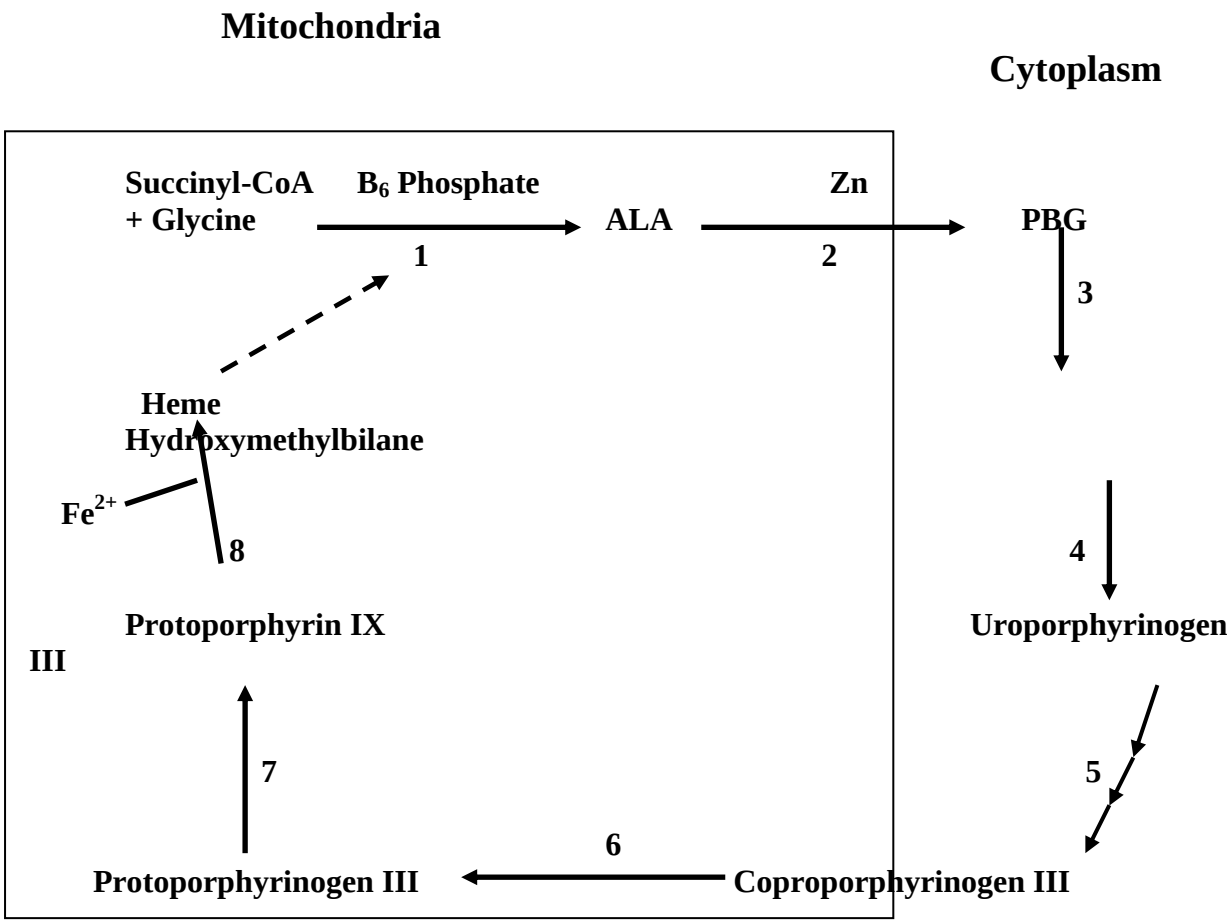

TABLE 1: ENZYMES INVOLVED IN THE BIOSYNTHESIS OF HEME AND THE CORRESPONDING DISEASE CONDITIONS ASSOCIATED WITH DEFICIENCY OF THESE ENZYMES:

	ENZYME	**Disorder due to enzyme defect**	**Primary Symptom**
1.	ALA Synthase		
2.	PBG Synthase (ALA Dehydratase)	PBG synthase deficiency Porphyria (ADP)	Neurovisceral
3.	PBG Deaminase (Uroporphyrinogen 1 synthase)	Acute Intermittent Porphyria (AIP). Results in excessive accumulation of ALA & PBG	Abdominal pain and neurological disorder
4.	Uroporphyrinogen III synthase	Congenital Erythropoietic Porphyria (CEP). Results in excessive accumulation of an excretion of uroporphyrinogen I.	Photosensitivity, severe skin lesions and haemolytic anaemia
5.	Uroporphyrinogen decarboxylase	Porphyria Cutanea Tarda (PCT).	Photosensitivity, and dermatologic problems
6.	Coproporphyrinogen Oxidase	Hereditary Coproporphyria (HCP)	Neurovisceral, Photosensitivity
7.	Protoporphyrinogen Oxidase	Variegate Porphyria (VP)	Neurovisceral, Photosensitivity
8.	Ferrochelatase	Erythropoietic Protoporphyria (EPP)	Photosensitivity

Reaction sequence can be represented as follows:

- First reaction in Heme biosynthesis occurs in mitochondria
    - Involves condensation of Succinyl-CoA and Glycine to form Delta-Amino-Levulinic Acid (ALA)
    - Enzyme is called: Delta Amino-Levulinic Acid Synthase **(ALA synthase)**
    - Vitamin B_6 (B_6-Phosphate, Pyridoxal phosphate) is the co-enzyme
    - This is the Rate-limiting reaction of Heme biosynthesis, and the most highly regulated reaction.

- Second reaction occurs in the Cytosol:
    - ALA is transported to Cytosol,
    - ALA Dehydratase (also called **PBG synthase**) catalyses the conversion of 2 ALA to form Porphobilinogen (PBG),
    - **ALA Dehydratase (PBG synthase) is a metallo-enzyme that requires Zinc as an activator**

- Third reactions occurs in Cytosol:
    - Involves conversion of Porphobilinogen (PBG) to Hydroxymethylbilane
    - Enzyme is **PBG Deaminase** (also called **Uroporphyrinogen I synthase**)

- Next reaction occurs in Cytosol:
    - Hydroxymethylbilane is converted to **Uroporphyrinogen III**
    - Enzyme is **Uroporphyrinogen III synthase**, which is a Holoenzyme that is made up of Uroporphyrinogen synthase plus a protein known as Uroporphyrinogen III cosynthase)

- Next reaction occurs in Cytosol:
    - Uroporphyrinogen III is Decarboxylated to form mainly the compound known as Coproporphyrinogen III
    - Enzyme is Uroporphyrinogen Decarboxylase

- Next reaction occurs in Mitochondria:
- Coproporphyrinogen III is transported into the mitochondria, where it is converted to the colourless compound called **Protoporphyrinogen IX, in a reaction catalysed by Coproporphyrinogen Oxidase (CO)**.
- In the mitochondria, **Protoporphyrinogen IX** is converted to **Protoporphyrin IX** by **Protoporphyrinogen IX oxidase that utilizes molecular oxygen**. This reaction yields a completely conjugated ring system, which is responsible for the characteristic red colour to heme.
- The final reaction in heme synthesis also takes place in the mitochondria and involves the insertion of ferrous iron atom into the ring system. The enzyme called **Ferrochelatase** catalyses this reaction.

- **Take Note:**
- **Ferrochelatase, ALA synthase and ALA Dehydratase are particularly sensitive to lead poisoning, which is often brought about by direct ingestion of lead (eg, by eating leaded paints).**
- **A characteristic of lead poisoning is an increase in ALA in the circulation without any increase in porphobilinogen (PBG).**
- **Affected individuals become severely anaemic and excrete large quantities of Coproporphyrinogen and ALA in the urine.**

What is Porphyria?

- Porphyria is a group of metabolic disorders characterised by excessive secretions of Porphyrins in blood-forming tissue or the liver.
- There are two main types of porphyria:
    - Porphyria that occurs in the red blood cells formed in bone marrow (Erythropoietic type)
    - Porphyria that occurs in the liver (Hepatic type).

What are some of the symptoms of Porphyria?

- The symptoms occur most commonly in children and may be very severe.
- They include: blisters, red teeth, and purple or pink urine.
- If the skin is exposed to the sun, the blister formation may progress to a stage of scarring.
- The condition may be fatal, but in its mildest form the patient needs only to avoid sunlight.

- There may be severe abdominal pain, vomiting, and abdominal swelling, which may be accompanied by any form of neurological disorder, such as epileptic seizures.
- In some adults heavy alcohol consumption is also sometimes associated with porphyria.

What are some of the causes of Porphyria?

- **Genetic defects that cause increased ALA synthase activity or decreased PBG Deaminase** (Uroporphyrinogen I synthase) **activity** can lead to the disease condition known as **Acute Intermittent Porphyria (AIP),**
- AIP is diagnosed by the excretion of excess porphobilinogen (a condition that is not obvious from the colour of the urine) {**See Fig. 1, enzymes 1 & 3**}

- In **Erythropoietic Protoporphyria**, bone marrow Uroporphyrinogen III Cosynthase is only present at about 30% of the normal level; the result is that massive amounts of Type I Uroporphyrinogen and its highly coloured oxidation products are found in the urine and deposited in a variety of tissues, including teeth and bones.

What are some of the Porphyrin Disorders (Porphyrias)?

- Several genetic defects in heme biosynthesis have been identified that give rise to the disorders called Porphyrias.
- There are at least eight different types of Porphyrias.
    - However let us consider the ones that are most likely to be diagnostic.

These include the following:

- **Acute intermitted porphyria (AIP) is manifested by neurovisceral symptoms.**
- **Hereditary Coproporphyria (HC) is manifested by both neurovisceral and cutaneous symptoms**
- **Variegate porphyria (VP) is also manifested by both neurovisceral and cutaneous symptoms.**
- **Porphyria cutanea tarda (PCT) is manifested by cutaneous symptoms.**

- Neurovisceral symptoms consist of autonomic neuropathies (constipation, colicky abdomen pain, vomiting, hypertension), peripheral neuropathy, seizures, delirium, coma and depression.
- Some patients are free of symptoms between attacks.

- The cutaneous manifestations are characterized by bullae formation in sun exposed areas, fragile skin, and hypertrichosis.

- Acute photosensitivity consisting of erythema and oedema is seen only with protoporphyria and not with PCT, VP, and HC.

How can Porphyrias be diagnosis?

- The key to diagnosis is suspicion of the disease, but this must be tempered by the fact that these are rare diseases.
- The first line of action is to screen the urine for the presence of porphyrin precursors, delta-amino-levulinic acid (ALA) and porphobilinogen (PBG).
- These substances are always present if the patient is having an acute neurovisceral attack.

- Most patients with latent AIP and some with latent HC and VP will have elevated levels.
- If the urine screen is negative, then one can safely eliminate porphyria as a cause of acute neurovisceral symptoms.
- Abnormal urine screens should be verified by quantitative measures of urine ALA, PBG and porphyrins.
- Most elevated urine porphyrins on screening tests are false positives, mainly due to rises in coproporphyrin excretion seen with alcohol users, liver disease, acute illness, etc, and not porphyria.
- Increased levels of porphyrins in the urine and stood can be used to demonstrate the diagnosis of HC or VP.
- Vast majority of patients with HC have extreme elevation in the levels of coproporphyrin in their stool (about 5 to 100 times normal).
- Patients with VP have elevated stool coproporphyrin and protoporphyrin.
- Patients with PCT have elevated stool iso-coproporphyrin and protoporphyrin and increased urine coproporphyrin and uroporphyrin.
- Minor elevations of urine and stool porphyrins (especially coproporphyrins) are common and non-specific.

- It is important to note the measuring the levels of enzyme activity to diagnose symptomatic porphyria is inappropriate.

- Over 90% of patients with decreased PBG-deaminase (the defect in AIP) may not excrete porphyrins and never have symptoms of porphyria.

- Also, some patients with AIP may have normal activity of RBC PBG-deaminase.

- It may be useful to measure PBG-deaminase in patients with biochemically-proven AIP to help in screening family members, but most patients with deficiencies may never have AIP.

- There is a commercial assay for the genetic defect in HC (Coproporphyrinogen Oxidase [CO]).

- However, the validity of this assay is questionable, given that it measures RBC CO activity but CO is located in the mitochondria and obviously mature red blood cells do not have mitochondria.

- This may explain why most samples sent to the laboratory for Coproporphyrinogen Oxidase estimation are reported as abnormal.

SUMMARY

- To rule out porphyria as a cause of acute neurovisceral symptoms: urine porphyrin and PBG screen can be used
- To follow-up an abnormal screen: quantitative urine for PBG, ALA and total porphyrins plus stool total porphyrins is required.
- To diagnose PCT one must perform total porphyrins on urine and stool.
- Patients with history of "porphyria": obtain records. In most patients with the diagnosis of porphyria, review of the record reveals no diagnostic abnormality. In unclear cases, repeat urine and stool studies.

Study Questions:

1. What enzyme deficiency causes acute intermittent Porphyria?
2. State the reaction catalyse by the enzyme Ferrochelatase?
3. Briefly explain the effect of lead poisoning on porphyrin biosynthesis.
4. What is the screening procedure for a suspected case of porphyria?