# BIOSYNTHESIS & REGULATION OF HEME IN HEMOGLOBIN – An Overview

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# What is Heme?

- Heme is a cyclic Tetra-pyrrole (Protoporphyrin) with Ferrous Iron in the middle of the ring,
- Heme is the Prosthetic group of:
  - Hemoglobin,
  - Myoglobin,
  - Cytochromes,
- Catalase and Peroxidase are enzymes that contain Heme,

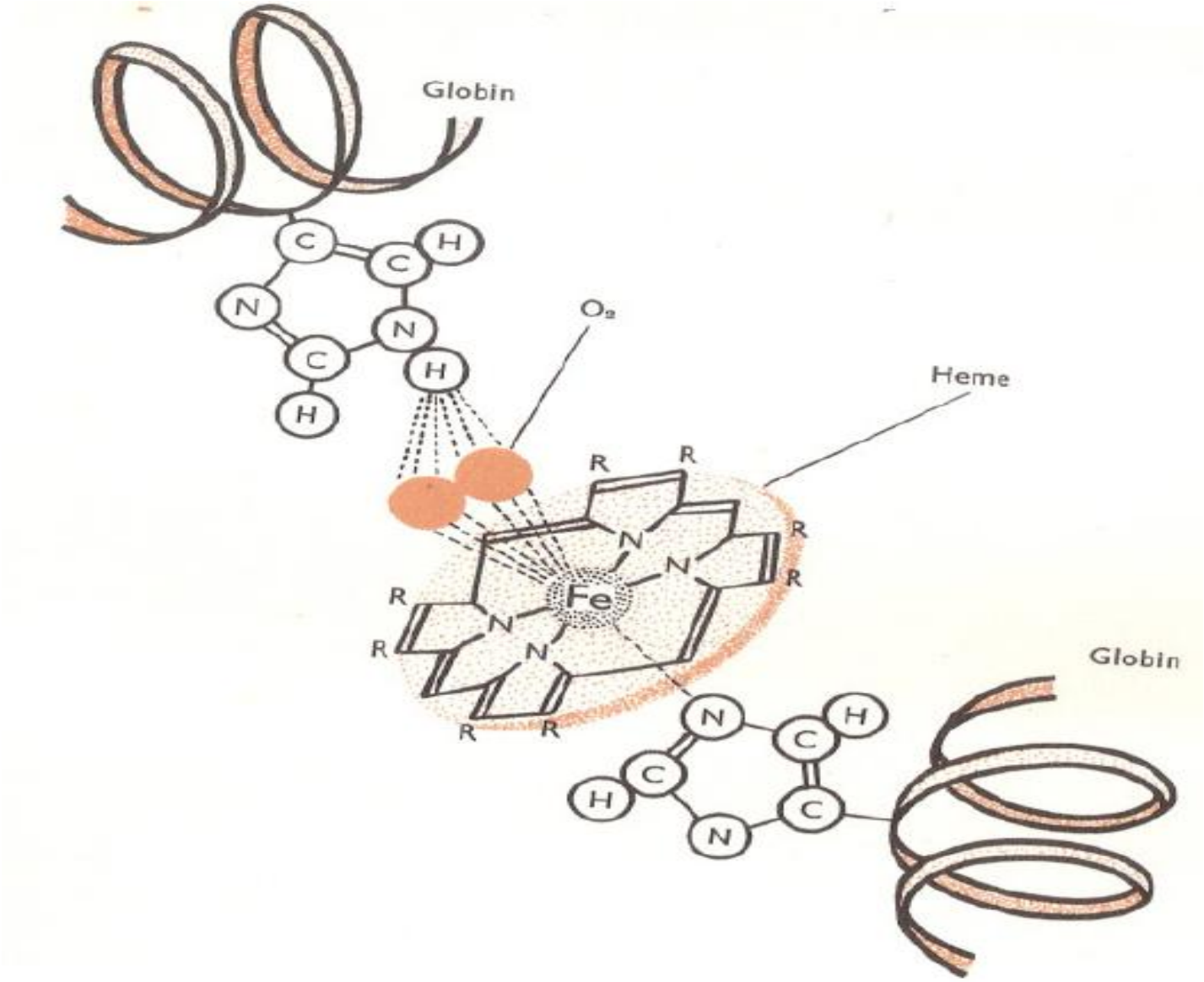
## Outline the basic structure of Adult Hb

- **Adult Hemoglobin (Hb)** consist of 4-Subunits (Tetramer) held by non-covalent interactions;
- **Hb $A_1$** ($\alpha_2$ $\beta_2$) is major (98%) form of Hb A in adults;
  - Two alpha and Two beta subunits,
- Each subunit consist of: Heme (Ferro-Protoporphyrin) and Globin protein;
- **Heme** = **Protoporphyrin IX** and **Ferrous ion** ($Fe^2$);
- Globin protein folds around Heme to form protective Hydrophobic pocket;

- Protoporphyrin-IX contains 4 Pyrrole rings,
- Ferrous ion ($Fe^{2+}$ ) can form **Six** bonds,
- **$Fe^{2+}$** forms 4-coordinate bonds with Protoporphyrin,
- **Fifth**-coordinate bond of **$Fe^{2+}$** is with Imidazole group of Histidine (Proximal Histidine) on Globin,
- The Sixth position on **$Fe^{2+}$** comes very close to but does not bond with Distal Histidine on Globin,
  - Position may be occupied by Oxygen in Oxy-Hb,
  - It may also be occupied by Carbon Monoxide or Hydrogen Cyanide (HCN), when Hb is poisoned;
- **Fig. 1: Schematic representation of a Subunit**

# Fig. 1: Schematic diagram of Hemoglobin Structure

(Musil, Novakova, Kunz, 1977)



## Outline the pathway for Biosynthesis of Heme

- Heme biosynthesis can be separated into Two Stages:
- Stage 1: Biosynthesis of Porphobilinogen (Fig. 1);
- Stage 2: Conversion of Porphobilinogen to Heme (Fig. 2);

- **Stage 1:** Compounds required are:
  - **Succinyl-CoA**, from TCA cycle in mitochondria,
  - **Glycine**, a non essential amino acid,
  - **Pyridoxal Phosphate** (biologically active form of Vitamin B6) needed for activation of Glycine;

- First reaction occurs in Mitochondria: (**Fig. 1**)
  - **Delta-Aminolevulinic Acid** (ALA) is formed when Glycine interacts with Succinyl-CoA ;
    - Reaction catalyzed by **Aminolevulinic Acid Synthase** (**ALA Synthase**), with Pyridoxal phosphate (B6-phosphate) as Cofactor
  - This is the rate-controlling step and most highly regulated reaction in Heme biosynthesis;
  - ALA Synthase is regulate by amount of Heme in cell

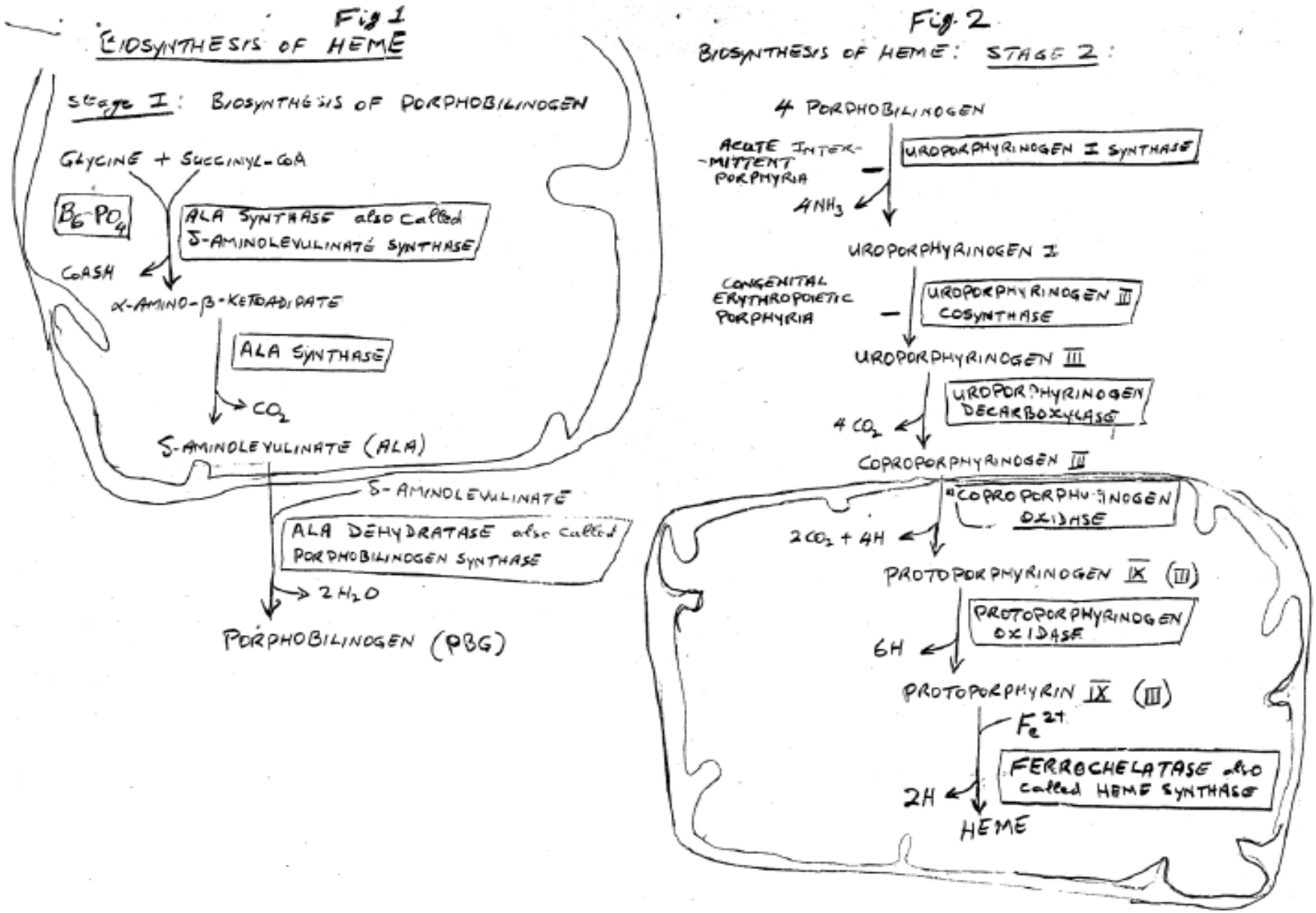
- Second reaction occurs in the Cytosol: (**Fig. 1**)
  - **ALA** in mitochondria is transported to Cytosol,
  - **Two ALA** are converted to **Porphobilinogen**;
    - Reaction catalyzed by **ALA Dehydratase** (Porphobilinogen Synthase);

- **Stage 2**: Conversion of Porphobilinogen to Heme (**Fig. 2**):
  - Condensation of 4 Porphobilinogen molecules to form Uroporphyrinogen-I;
  - Then Uroporphyrinogen-1 to Uroporphyrinogen-III;
    - Reactions are catalyzed by Uroporphyrinogen-I synthase and Uroporphyrinogen-III cosynthase respectively;
  - Uroporphyrinogen-III is Decarboxylated to form Coproporphyrinogen-III;
    - Catalyzed by Uroporphyrinogen Decarboxylase;
- These reactions occur in the Cytosol;

- Coproporphyrinogen-III is transported into Mitochondria, and converted to Protoporphyrinogen-IX,
  - Reaction catalyzed by Coproporphyrinogen Oxidase,
- Protoporphyrinogen-IX forms Protoporphyrin-IX,
  - Reaction catalyzed by Protoporphyrinogen Oxidase;

- **Final reaction** occurs in Mitochondria:
- Involves insertion of Ferrous ion ($Fe^{2+}$) in the middle of Protoporphyrin-IX to form Heme;
  - **Catalyzed by Ferrochelatase (Heme Synthase)**

# Fig. 2: Pathway for biosynthesis of Heme



## What cellular compartments are involved in biosynthesis of Heme?

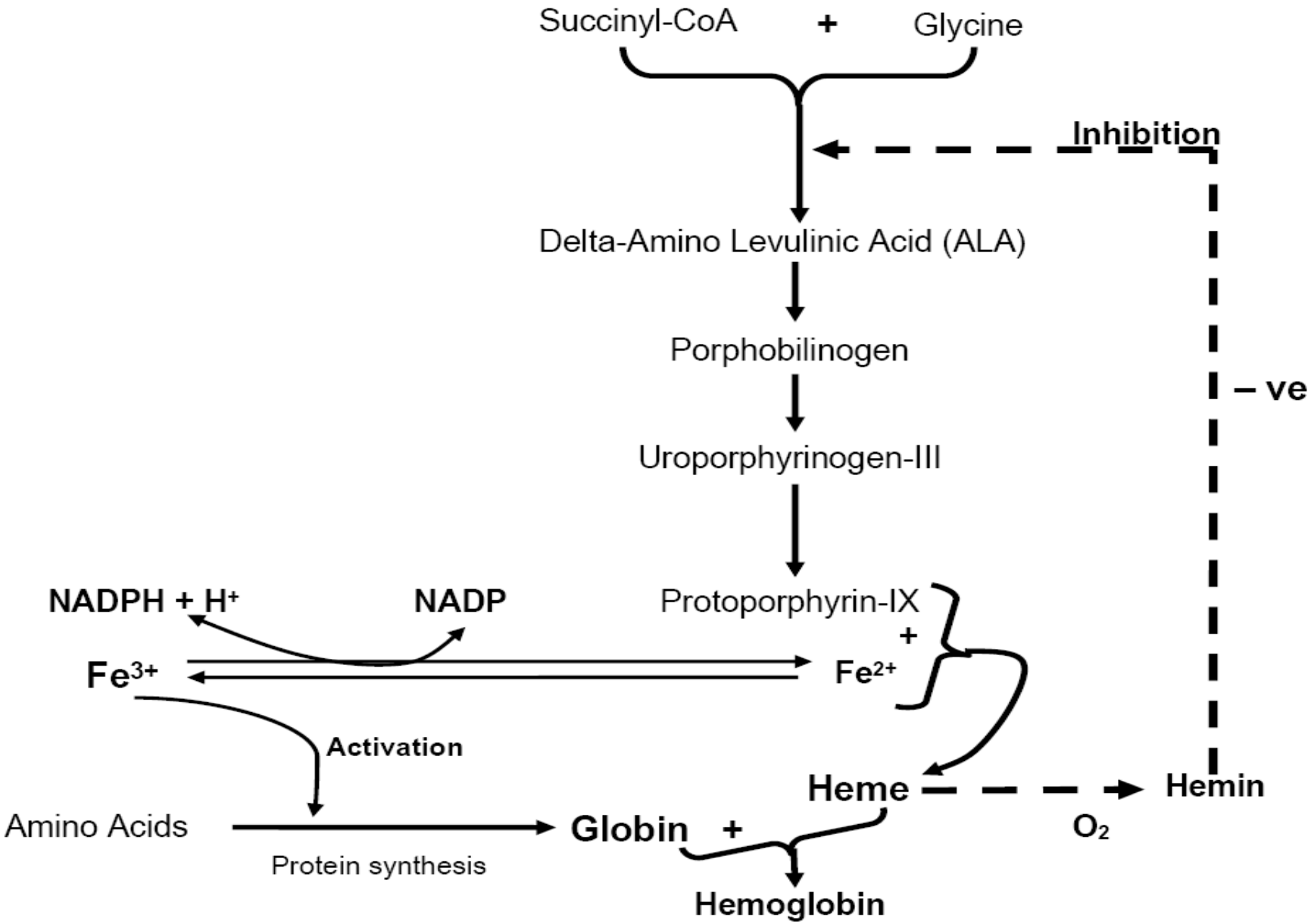
- Biosynthesis of Heme occurs in most mammalian cells that contain mitochondria,
- Mature RBC does not contain mitochondria,
- Heme biosynthesis does not occur in mature RBC,
- Heme biosynthesis occurs in two compartments
  - Mitochondria,
  - Cytosol;

## How is the biosynthesis of Heme regulated?

- Heme biosynthesis occurs in Erythroid cells and Hepatocytes,
- In Reticulocytes Heme stimulates protein synthesis;
- Control of Heme synthesis in RBC occurs at:
  - ALA Synthase;
  - Porphobilinogen Deaminase,
  - Ferrochelatase (that catalyzes insertion Ferrous ion into Protoporphyrin IX),
- In the absent of Globin, Heme is spontaneously oxidized by Oxygen to Hemin,

- Hemin contains Ferric ion ($Fe^{3+}$ ),
  - **Hemin is the oxidation product of Heme**
- Hemin acts as feedback inhibitor of ALA Synthase,
- Hemin also inhibits transport of ALA Synthase from cytosol (site of synthesis) into mitochondria (site of action) as well as represses synthesis of the enzyme,
- Ferric ions can activate biosynthesis of Globin,
- **Fig. 2**: **Diagram on regulation of Heme biosynthesis**

# Fig. 2: Regulation of Heme biosynthesis



## State some factors that can affect biosynthesis of Heme

- **ALA Dehydratase** (Porphobilinogen Synthase) is a Zinc-containing enzyme, it is sensitive to inhibition by Lead, thus it is affected in Lead poisoning,
    - Lead poison can cause anaemia
- **Ferrochelatase** and **ALA Synthase** are highly sensitive to inhibition by heavy metal poisoning;
    - Characteristic of Lead poisoning is increase in ALA level in blood plasma in the absence of an increase in Porphobilinogen;

- Many drugs, when administered to humans, can cause increase in Hepatic ALA Synthase;
- Some of the drugs are metabolized by Cytochromes $P_{450}$ (Xenobiotic system);
- During their metabolism, the utilization of Heme by Cytochromes $P_{450}$ is greatly increased, which in turn diminishes intracellular Heme concentration;

- This later event can cause depression of ALA Synthase with corresponding decreased rate of Heme synthesis to meet the needs of the cells;
- Synthesis of ALA Synthase is feedback-inhibited by Heme;
- Several genetic defects in Heme biosynthesis have been identified that give rise to disorders called Porphyria;

# References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