UNIVERSITY OF PAPUA NEW GUINEA

SCHOOL OF MEDICINE AND HEALTH SCIENCES

PBL SEMINAR

BIOSYNTHESIS AND REGULATION OF HEME IN HEMOGLOBIN – An Overview

- Heme is a cyclic tetrapyrrole with Iron (Ferrous) located in the middle of the ring
- Heme is the Prosthetic group of Hemoglobin, Myoglobin and Cytochromes.
- Enzymes, such as Catalase and Peroxidase, contain Heme.

Briefly outline the pathway for the Biosynthesis of Heme:

- Pathway for Biosynthesis of Heme can conveniently be separated into Two Stages:
  - **Stage 1:** Involves biosynthesis of Porphobilinogen (**Fig. 1**);
  - **Stage 2:** Involves conversion of Porphobilinogen to Heme (**Fig. 2**).

- **Stage 1: Formation of Porphobilinogen (Fig.1)**:
  - Starting compounds are:
    - Succinyl-CoA, derived from the Citric Acid Cycle (TCA cycle) in mitochondria,
    - Glycine, a non essential amino acid
    - Pyridoxal phosphate (B6-Phosphate) needed for activation of Glycine.

  - **First reaction occurs in the Mitochondria**:
    - **Glycine and Succinyl-CoA converted to δ-Aminolevulinic acid also called 5-Aminolevulinic-acid (ALA);**
      - **Reaction catalyzed by Aminolevulinic acid Synthase (ALA Synthase)**, with **Pyridoxal phosphate as Cofactor**
    - **It is the rate-controlling step and the most highly regulated reaction in Heme biosynthesis**;
    - ALA Synthase is regulate by amount of Heme in the cell

  - **Second reaction occurs in the Cytosol**:
    - ALA formed in the mitochondria is transported to the Cytosol,
    - **ALA Dehydratase (Porphobilinogen Synthase)** catalyses the formation of Porphobilinogen from Two molecules of ALA.

- **Stage 2: Conversion of Porphobilinogen to Heme (Fig. 2**):
  - Involves condensation of Four Molecules of Porphobilinogen to form **Uroporphyrinogen I and then Uroporphyrinogen III**;
  - Reactions are catalyzed by **Uroporphyrinogen I synthase and Uroporphyrinogen III cosynthase respectively**;
  - In cytosol Uroporphyrinogen III is **Decarboxylated to form Coproporphyrinogen III; catalyzed Uroporphyrinogen Decarboxylase;**
  - In the next step **Coproporphyrinogen III is transported into Mitochondria**, where it is **converted to Protoporphyrinogen IX by Coproporphyrinogen Oxidase,**

- **Protoporphyrinogen IX is converted to Protoporphyrin IX by Protoporphyrinogen Oxidase**;
- In mammalian liver: conversion of Coproporphyrinogen to Protoporphyrin requires molecular oxygen;
  - **Note**: Protoporphyrin IX has a completely Conjugated Ring system, which is responsible for the characteristic Red Color of Heme

- **Final reaction occurs in the Mitochondria and involves insertion of Ferrous ion (Fe^{2+}) in the middle of Protoporphyrin IX Ring to form Heme;**
- **Reaction is catalyzed by Ferrochelatase (Heme Synthase)**

Localization of Heme biosynthesis:

- 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 in cells: Mitochondria and Cytosol;
- **Figure 3** shows compartmentalization of Heme biosynthesis in cells

Regulation of Heme Biosynthesis (See Fig. 4):

- Principle sites of biosynthesis of Heme are Hepatocytes and Erythroid cells

- **In hepatocytes**:
  - Heme is required for incorporation into the Cytochromes (examples, the P_{450} class of cytochromes that are important for detoxification)
  - Numerous Cytochromes of the Oxidative-Phosphorylation pathway contain Heme
  - Rate-limiting step in Heme synthesis in Hepatocytes is the ALA Synthase reaction, which is the committed step in Heme biosynthesis;
  - Fe^{3+} oxidation product of Heme is called Hemin;
  - Hemin acts as feedback inhibitor of ALA Synthase
  - Hemin also inhibits transport of ALA Synthase from Cytosol (its' site of synthesis) into the Mitochondria (its' site of action) as well as represses synthesis of the enzyme

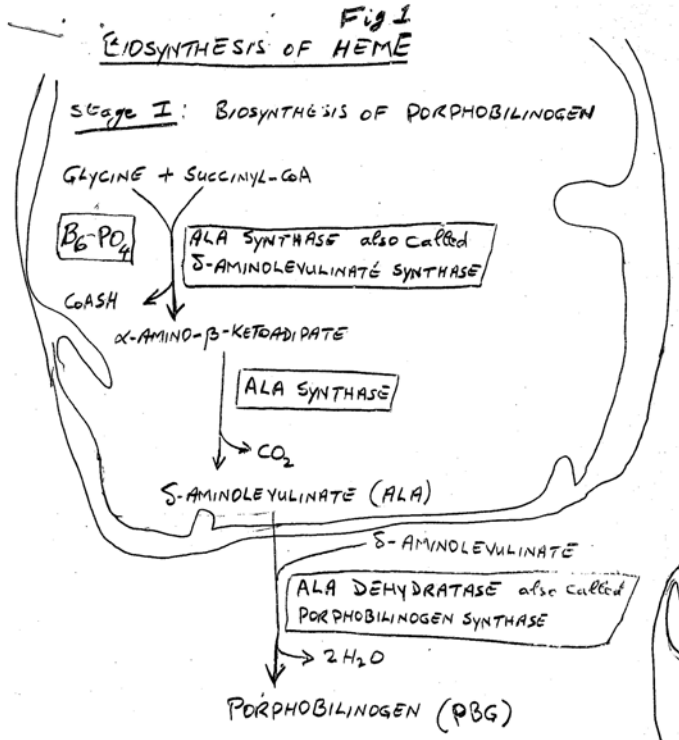
- **In Erythroid cells:**
  - Heme is synthesized for incorporation into Hb and occurs only upon differentiation when synthesis of Hb proceeds;
  - Heme and Hb syntheses do not occur in mature RBC
  - Heme (and Hb) must, therefore, survive for the life of the red cell (normally 120 days)
  - In Reticulocytes (immature red cells) Heme stimulates protein synthesis;
  - Control of Heme biosynthesis in RBC occurs at numerous sites other than at the level of ALA Synthase;
  - Control has been show to be exerted on Ferrochelatase (that catalyzes insertion Ferrous ion into Protoporphyrin IX), and on Porphobilinogen Deaminase.

Some factors that can affect the biosynthesis of Heme:

- ALA Dehydratase (Porphobilinogen Synthase) is a Zinc-containing enzyme and is sensitive to inhibition by Lead, as can occur in Lead poisoning.
- Ferrochelatase and ALA Synthase are highly sensitive to inhibition by heavy metal poisoning;

- Characteristic of Lead poisoning is an increase in ALA in the blood circulation in the absence of an increase in Porphobilinogen;

- Many drugs, when administered to humans, can result in a marked increase in Hepatic ALA Synthase;
  - Some of these 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 the intracellular Heme concentration;
  - This later event causes a depression of ALA Synthase with a corresponding increased 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 the disorders called Porphyrias;



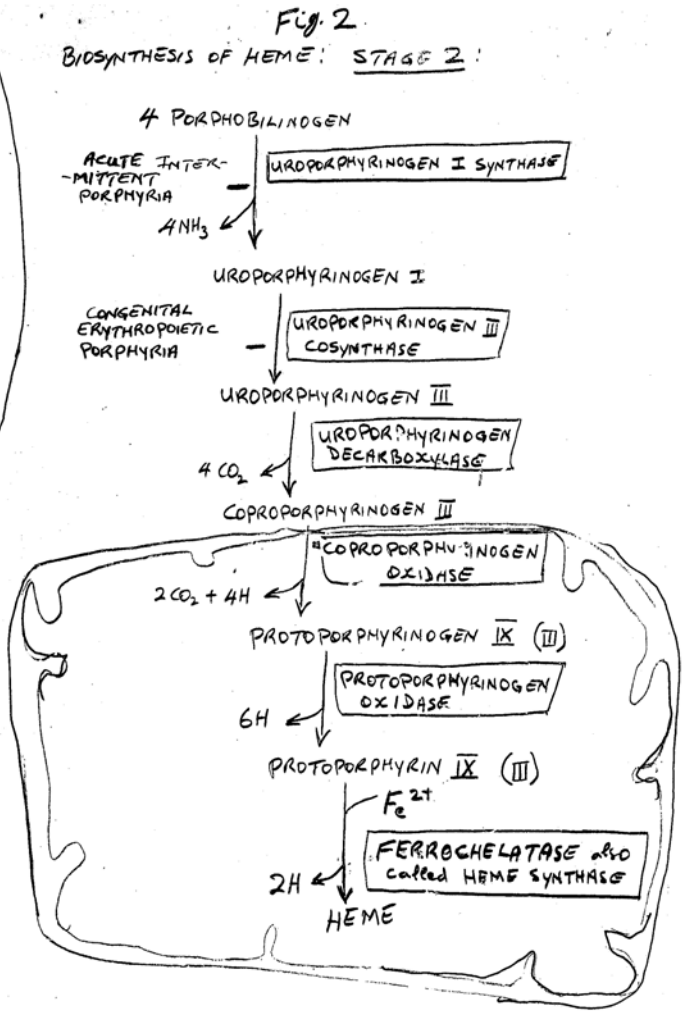


Fig. 3

LOCALIZATION OF HEME SYNTHESIS

Enzymes involved in the biosynthesis of heme were isolated from the liver, bone marrow, intestinal mucosa, nucleated erythrocytes and kidney. The formation of porphobilinogen from 5-aminolevulinic acid and further reactions up to coproporphyrinogen proceed in the cytoplasm. Synthesis of 5-aminolevulinic acid, as well as oxidation and decarboxylation of coproporphyrinogen and incorporation of iron into the molecule, take place in mitochondria.

Enzymes required: E_1 – pyridoxal phosphate, E_2 – δ-aminolevulinate synthase, E_3 – porphobilinogen synthase, E_4 – uroporphyrinogen synthase, E_5 – uroporphyrinogen cosynthase, E_6 – uroporphyrinogen decarboxylase, E_7 – coproporphyrinogen oxidase, E_8 – ferrochelatase.

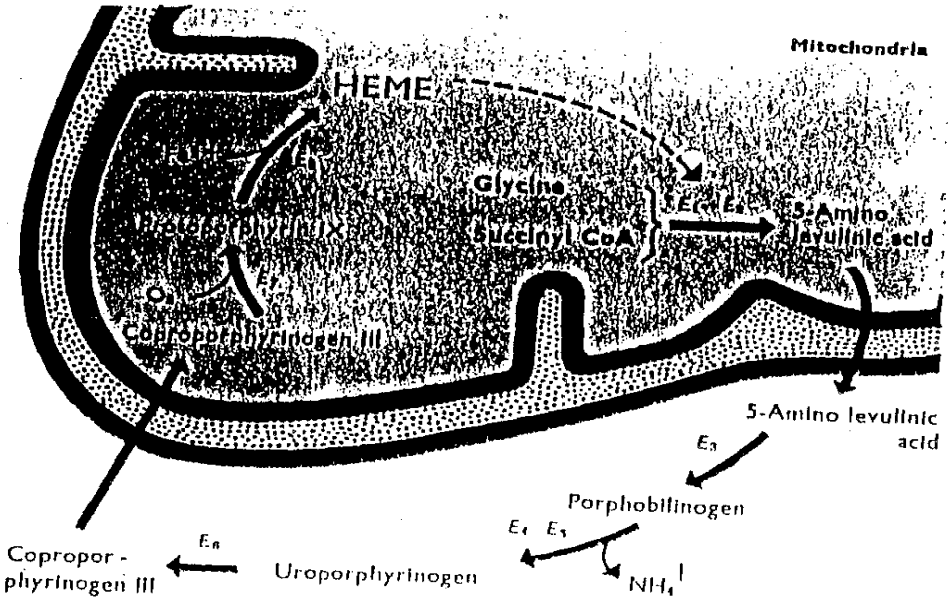


Fig 4

REGULATION OF HEMOGLOBIN BIOSYNTHESIS

The inhibition of 5-aminolevulinate synthase by protohemin IX is most important in the regulation of porphyrin biosynthesis. When a sufficient amount of globin that could be bound to protoheme IX (and give rise to hemoglobin) is not present, a spontaneous oxidation to protohemin IX inhibiting further prophyrin synthesis occurs. The Fe^{3+} ions are thought to support the formation of the globin. Free Fe^{3+} ions are present if their quantity exceeds that required for binding to protoheme IX. In this case the formation of globin is stimulated. This regulation probably maintains an equilibrium between the individual precursors of hemoglobin but does not affect the amount of circulating hemoglobin.