# Hemoglobin Metabolism

# Synopsis

- **Hemoglobin Biosynthesis**
  - ❖ **Heme Synthesis**
    - ❑ **Porphyrias (Disorders Of Heme Synthesis)**
  - ❖ **Globin Synthesis**
    - ❑ **Abnormal Hb variants/ Hemoglobinopathies (Disorders of Globin Synthesis)**
- **Hemoglobin Breakdown**
  - ❑ **Formation and Fate of Bilirubin**
  - ❑ **Hyperbilirubinemia**
- **Jaundice : Causes Types and Diagnosis**

# Hemoglobin Biosynthesis

- Hemoglobin biosynthesis includes biosynthesis of:
  - **Heme**
  - **Globin Polypeptide chains**

- Amount of Hb biosynthesized= **6.25 gm/day**

# Site For Hemoglobin Biosynthesis

## Organs Involved In Hb Biosynthesis

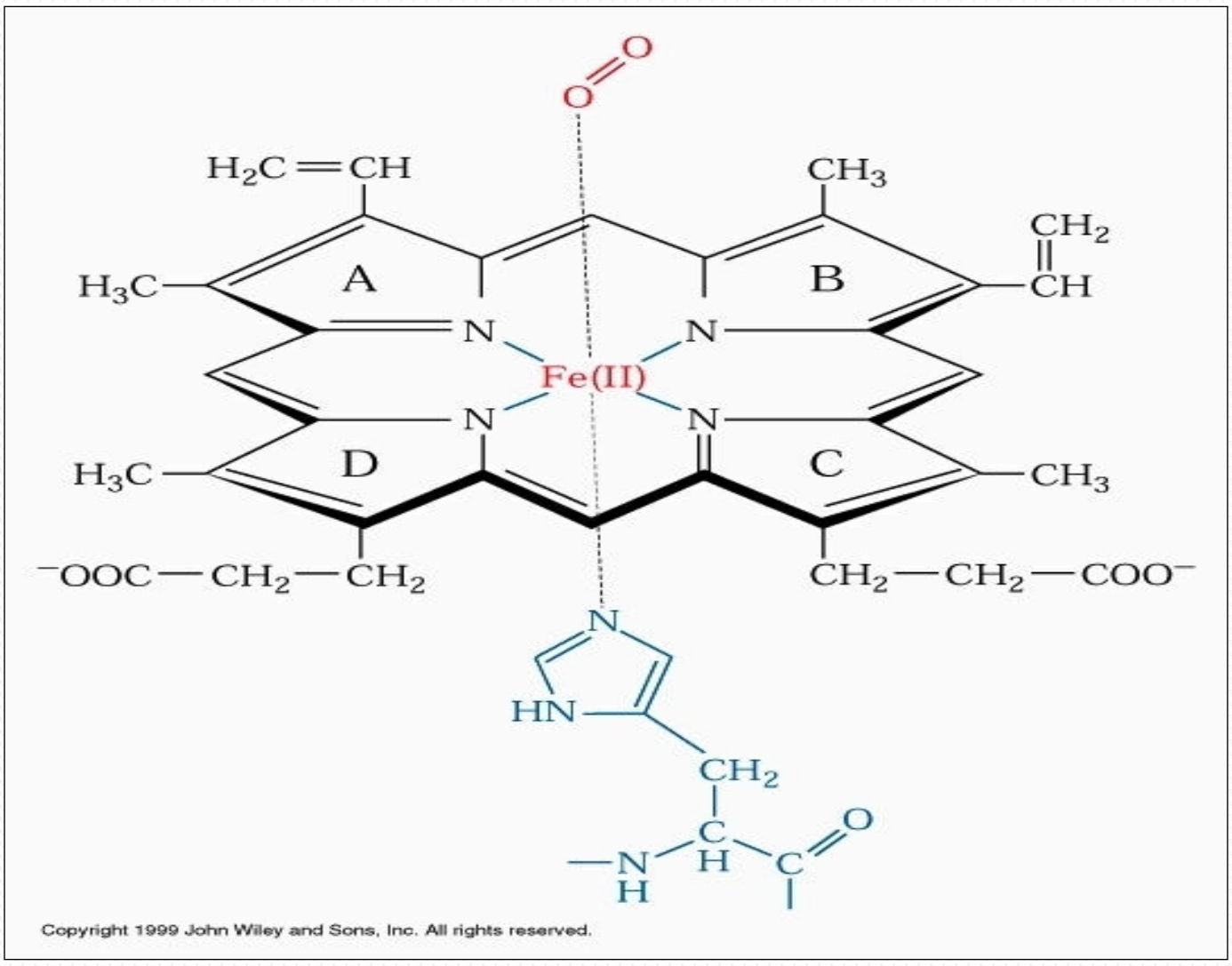
- **Bone Marrow**-
  Immature Erythrocytes – 85%
- **Liver** – 15 %

# Requirements For Hemoglobin Biosynthesis

- **Normal biosynthesis** of **Hemoglobin depends upon** an **Quality and Quantity of :**
  - **Amino acids**
  - **Minerals**
  - **Vitamins**

# Heme Biosynthesis
# OR
# Porphyrin Pathway

# Biosynthesis
# Of
# Ferroprotoporphyrin





# Site For Heme Biosynthesis

- **Organs**
- **Bone Marrow**-

  Immature Erythrocytes – 85%
- **Liver** – 15 %
  - **Cellular Site**
- **Mitochondrial Matrix**
- **Cytosol**

# Requirements For Heme Biosynthesis

- **Metabolic Precursors for Heme Biosynthesis:**
  - **Glycine** and **Succinyl-CoA**
- **Vitamins (5 Hematopoietic Vitamins):**
  - **Pantothenic acid (Vitamin B5)**
  - **Pyridoxine (Vitamin B6)**
  - **Folate (Vitamin B10)**
  - **Cyanocobalamin (Vitamin B12)**
  - **Vitamin- C (Ascorbic acid)**
- **Minerals for Heme Biosynthesis:**
  - **Iron ( $Fe^{++}$)**
  - **Copper ($Cu^{++}$)**
  - **Zinc ( $Zn^{++}$)**

# Stages and Steps Of Heme Biosynthesis

## 3 Stages Of Heme Biosynthesis

- **1. Synthesis of δ-Amino Levulinic Acid (δ ALA)**

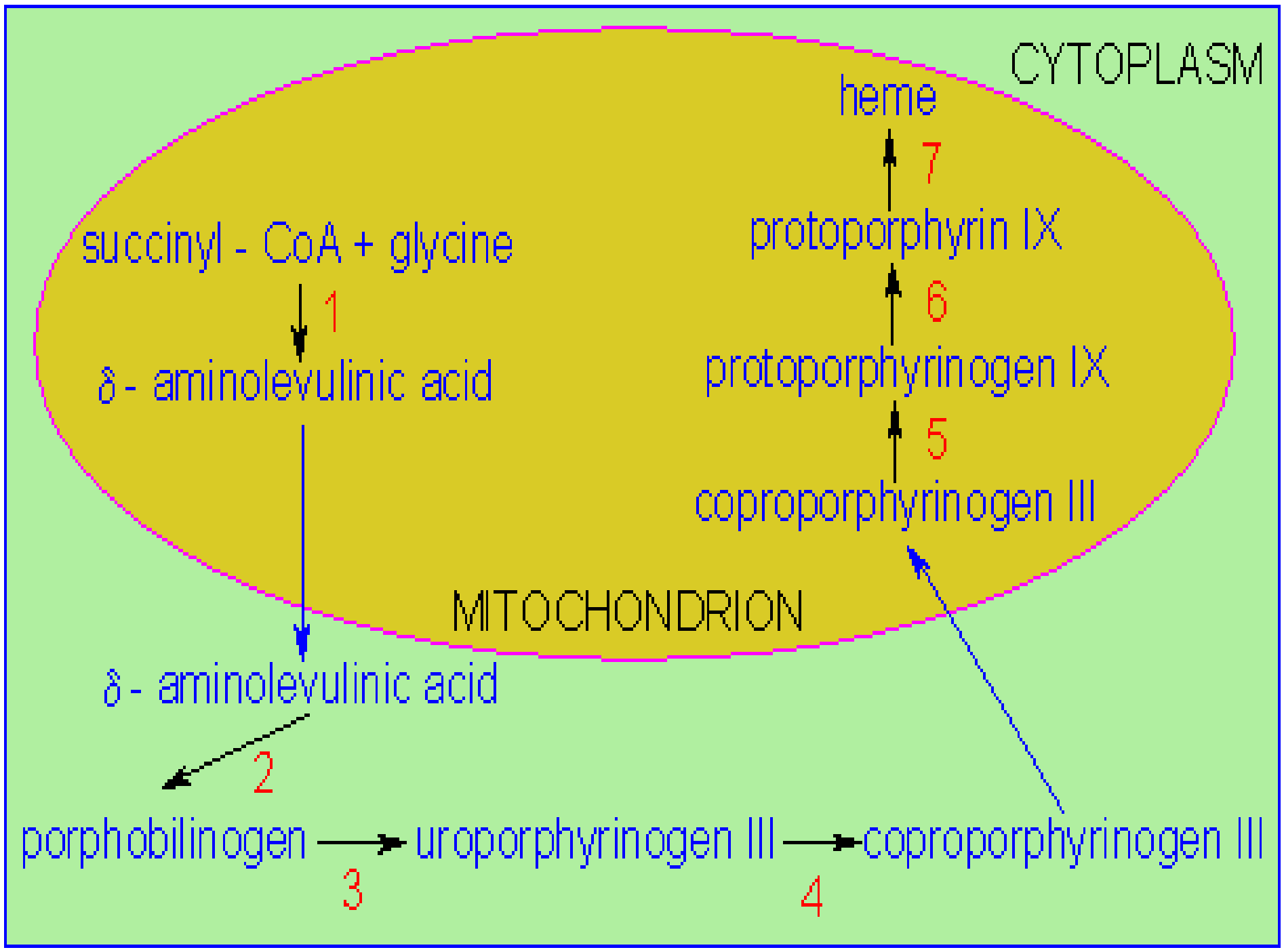
  **(In Mitochondrial Matrix)**
- **2. Synthesis of CoproPorphyrinogen–III ( CPG-III)**

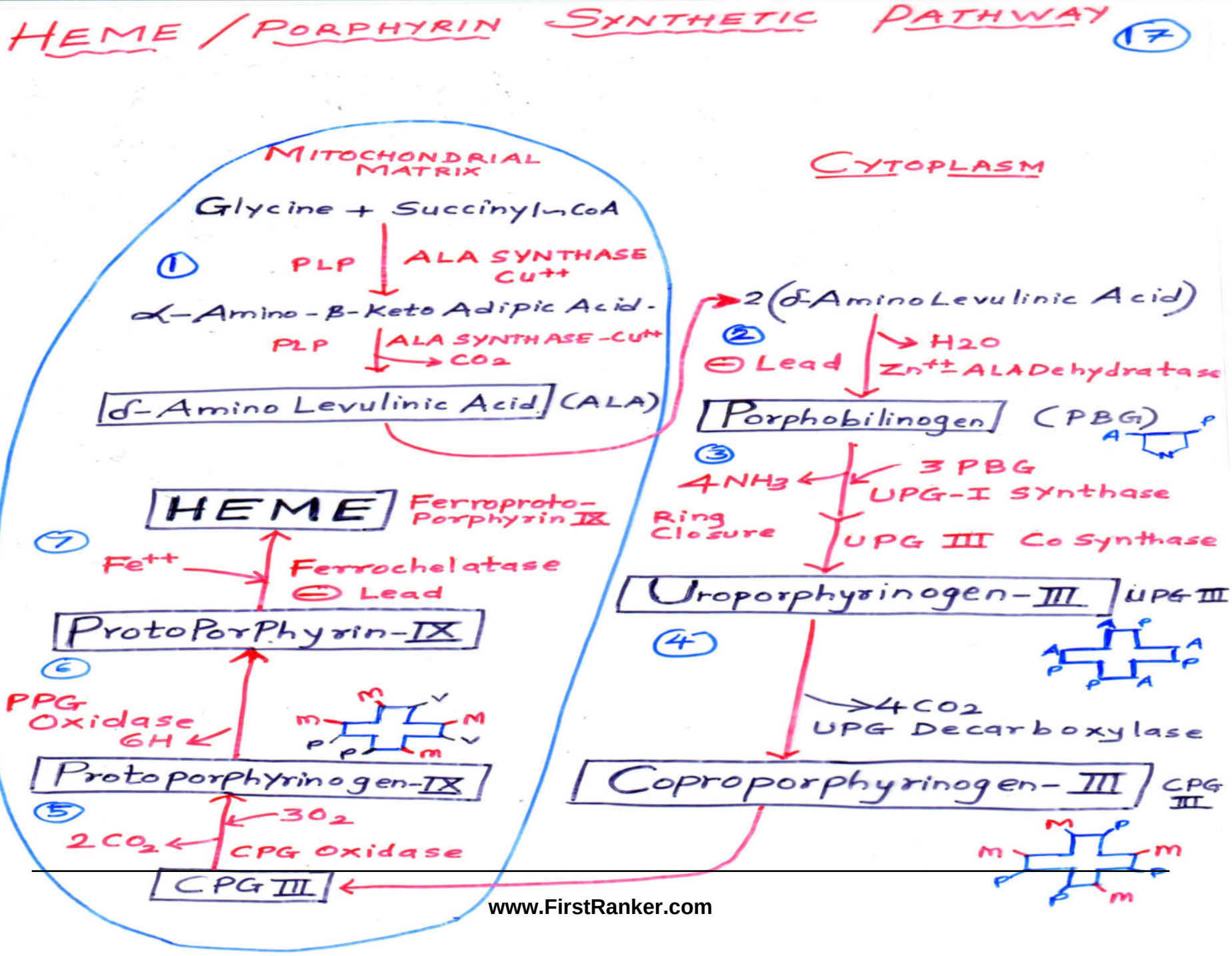
  **(In Cytoplasm)**
- **3. Synthesis of ProtoPorphyrin IX and Incorporation of $Fe^{++}$ to Form Heme**

  **(In Mitochondrial Matrix)**

- **7 steps in Heme Biosynthesis**

- Step 1 in Mitochondrial Matrix
- Steps 2,3,4 in **Cytoplasm**
- Steps 5,6 and 7 in Mitochondrial matrix

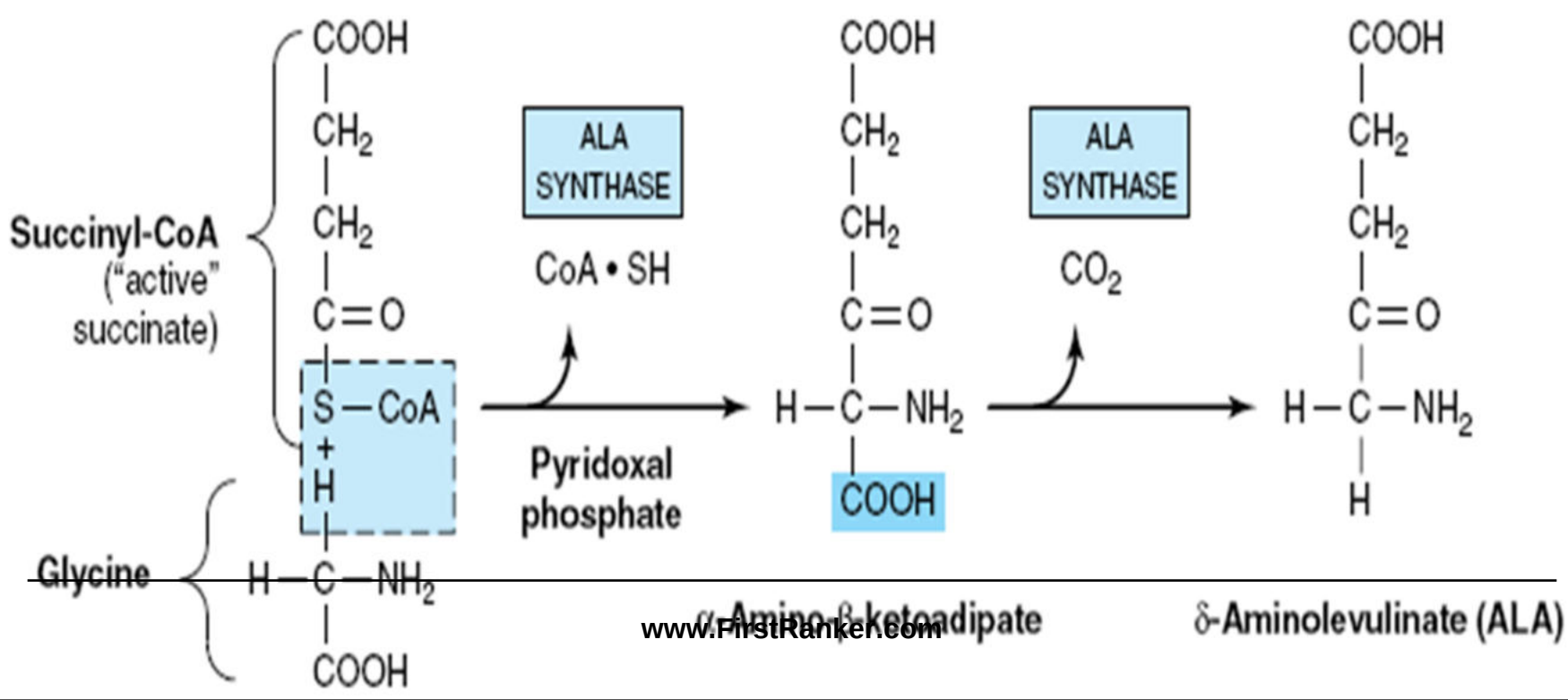


- **Important Intermediates of Heme Synthesis Pathway:**
  - **δ-Aminolevulinic acid** (ALA)
  - **Porphobilinogen** (PBG = Pyrrole derivative)
  - **Uroporphyrinogen III** ( UPG– Heme precursor)
  - **Protoporphyrin IX** (Direct Heme precursor)

# δ-Aminolevulinic Acid (ALA)

- Synthesis of Heme starts in Mitochondrial matrix
- **Succinyl-CoA and Glycine** undergo a condensation → **δ ALA**
- Reaction is catalyzed by enzyme **ALA Synthase**



# ALA Synthase requires

- **Vitamin B6 (PLP)**
- **Copper ions**

- **PLP** used in first step of Heme biosynthesis **activates Glycine.**

- Presence of **free Heme inhibits** the synthesis of an enzyme **δ- ALA Synthase**.

- This represents a **Feedback mechanism for Heme synthesis.**

- This first step is a **Rate limiting step** of Heme synthesis:

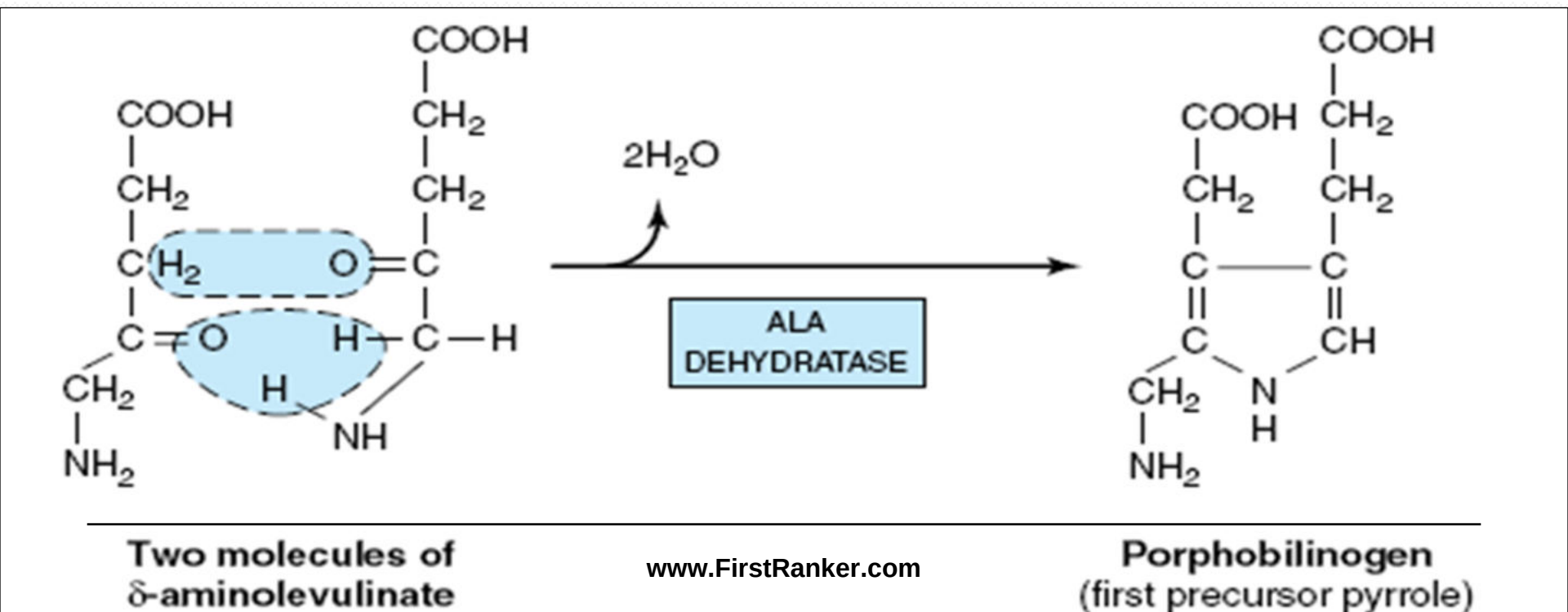
  - **Stimulated** by the presence of **Globin chains**.

  - **Inhibited by** the presence of free **Heme groups.**

- Rate of **Heme biosynthesis** has **good coordination** with **Globin synthesis.**

# Porphobilinogen (PBG)

- δALA leaves the Mitochondria → Reach **Cytoplasm**
- 2x δALA condense together to form **Porphobilinogen (PBG).**
- Reaction is catalyzed by **Porphobilinogen Synthase /(ALA dehydratase)**



- Mitochondrial **δ -Aminolevulinic acid (ALA)** is transported to the cytoplasm, where
- **ALA Dehydratase /Porphobilinogen Synthase- Zinc containing enzyme.**
- Dimerizes 2 molecules of ALA to produce
- The Pyrrole ring compound is **Porphobilinogen (PBG)**.

- PBG then biosynthesizes Porphyrin ring.
- The reactions involved for its synthesis are extremely complex but can be summarized as follows:

- The **condensation of four PBG** molecules
- Form an **asymmetric cyclic Uroporphyrinogen III**(UPG III).

- Synthesis of UPG III requires the presence of two enzymes:

  - **Uroporphyrinogen I Synthase**
  - **Uroporphyrinogen III Cosynthase**

- During UPG synthesis there involves the formation of short-lived **intermediate Hydroxy Methyl Bilane** (HMB).

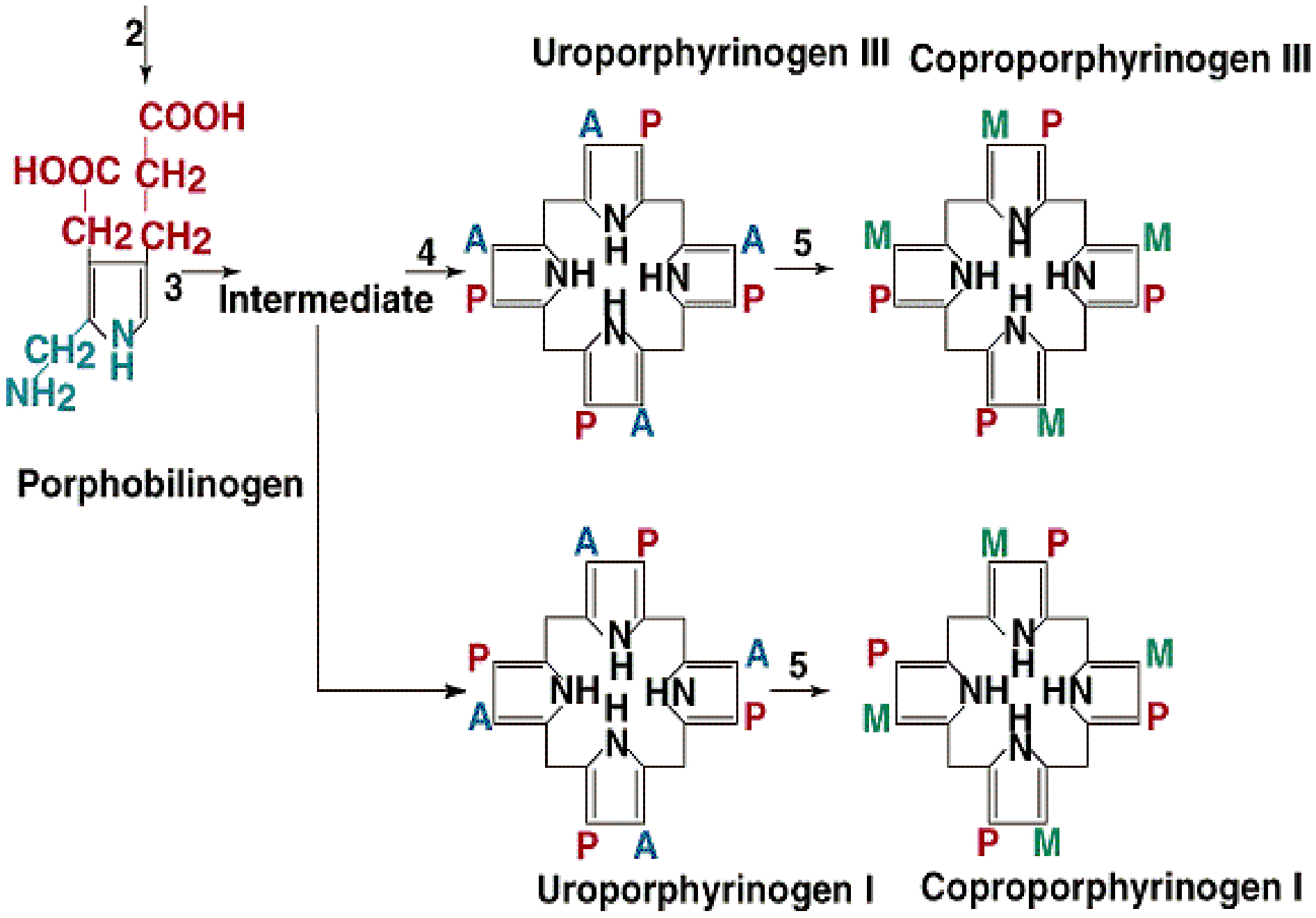
- **UPG I Synthase/PBG Deaminase /HMB Synthase**
- Transforms 4 molecules of PBG to **linear Tetrapyrrole Hydroxy Methyl Bilane (HMB)**

- **HMB** spontaneously **cyclizes to form UPG III** by **UPG III Cosynthase**

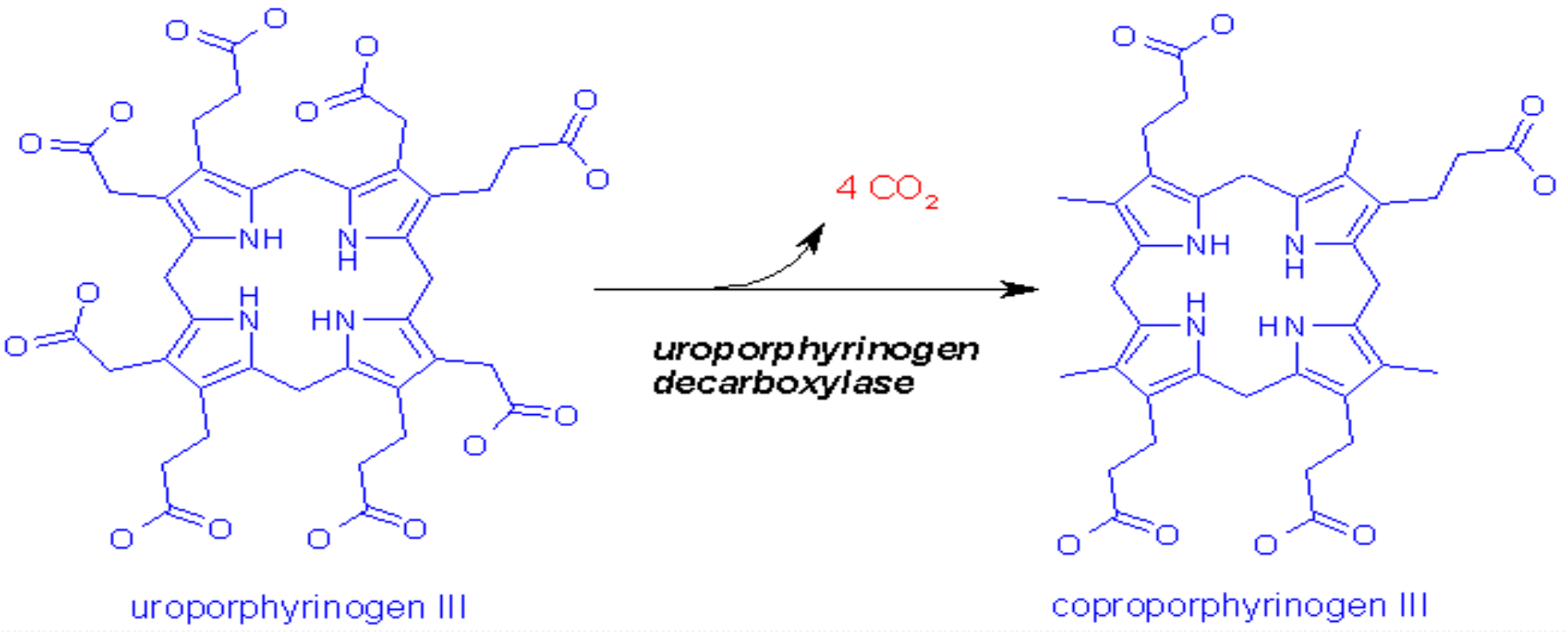
## Conversion of Uroporphyrinogen III → Coproporphyrinogen III

- 4 Acetate residues of Uroporphyrinogen III are Decarboxylated into 4 Methyl groups → Coproporphyrinogen III

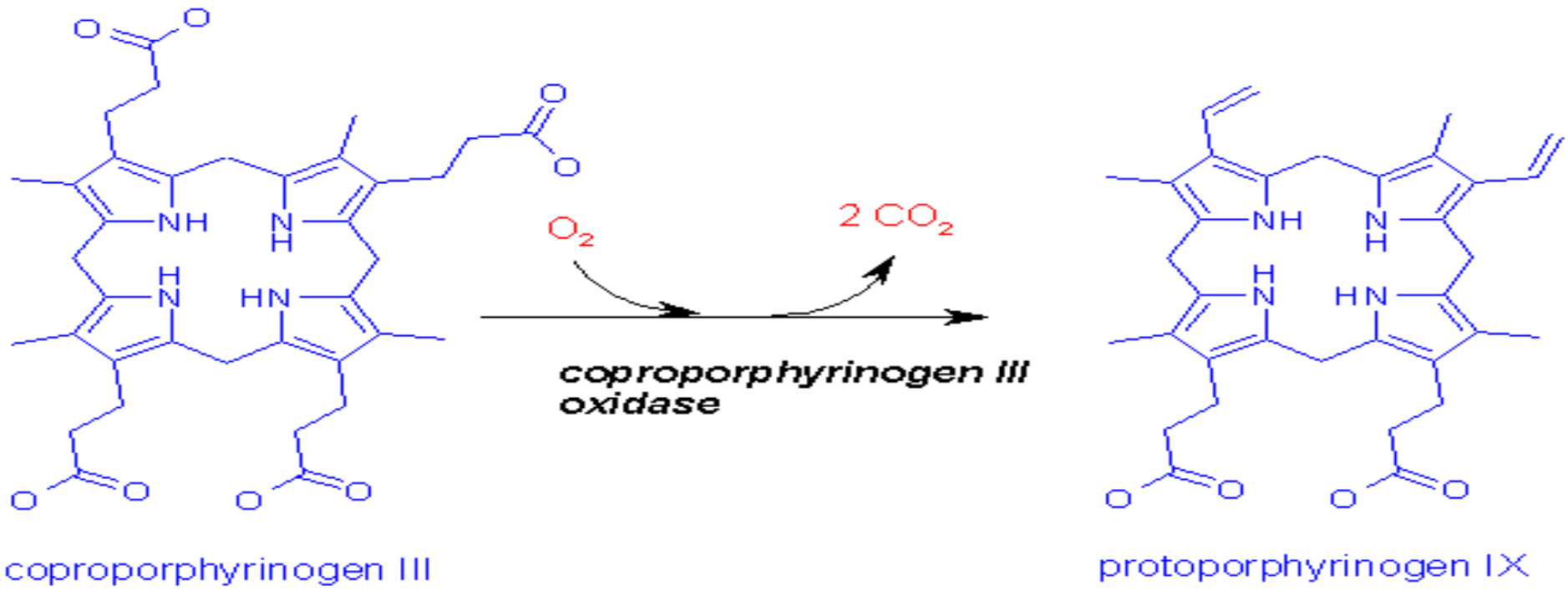
- Coproporphyrinogen III returns to the Mitochondria again.






- UPG III is converted to Coproporphyrinogen III (CPGIII) by **Decarboxylation** of the **Acetate side** chains
- To **Methyl groups** under the influence of the enzyme **Uroporphyrinogen Decarboxylase.**




- CPG III enters the Mitochondria where it is converted to Protoporphyrinogen IX (PPG IX) by an unknown mechanism.

- This reaction is catalyzed by the enzyme **Coproporphyrinogen Oxidase**.

## Coproporphyrinogen III → Protoporphyrinogen IX

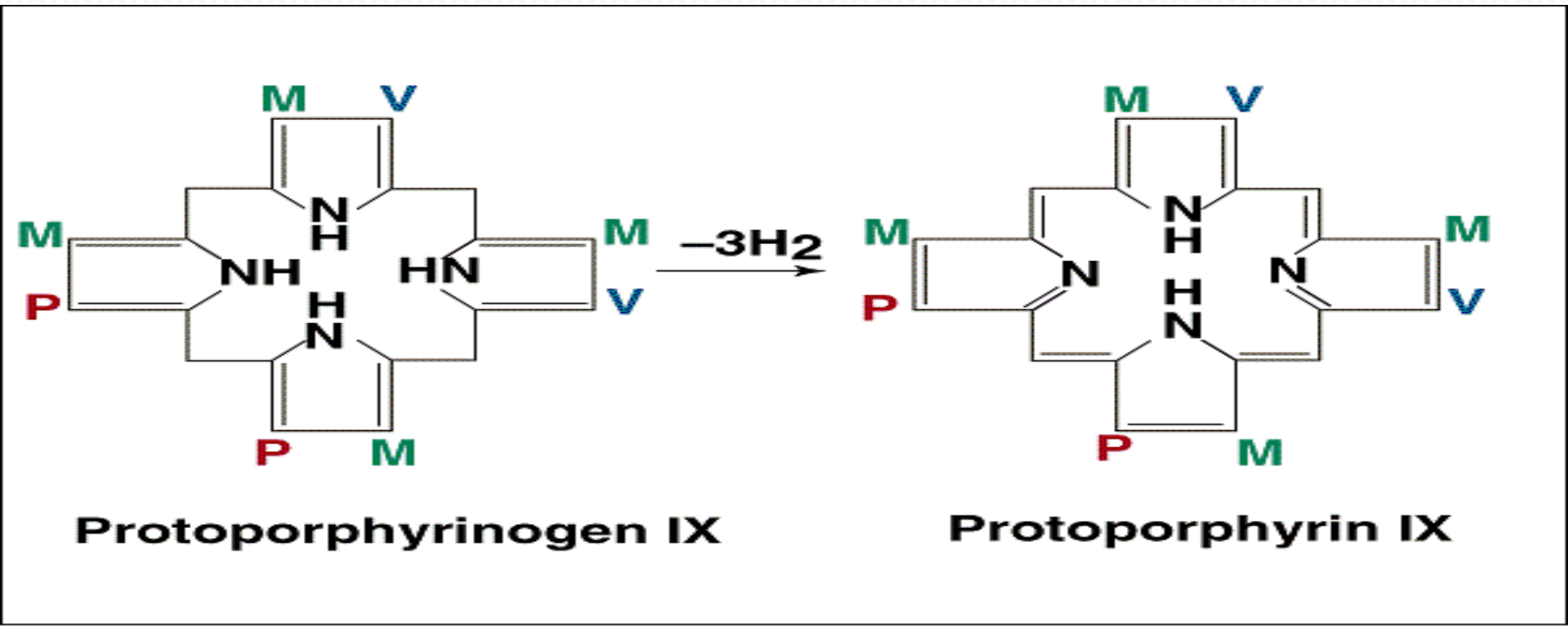
- In Mitochondria CPG III is oxidized to PPG IX.
- Two Propionyl residues transformed to Two Vinyl residues.
- Removal of **two $CO_2$ molecules**.
- Reaction catalyzed by **CPG Oxidase**.

- **Protoporphyrinogen** is a colorless compound
- It contains **Methylene bridges** in tetrapyrrole ring structure.

- **Methylene bridges** of **Protoporphyrinogen are** oxidized to **Methenyl bridges to form ProtoPorphyrin**

# Protoporphyrinogen IX → Protoporphyrin IX

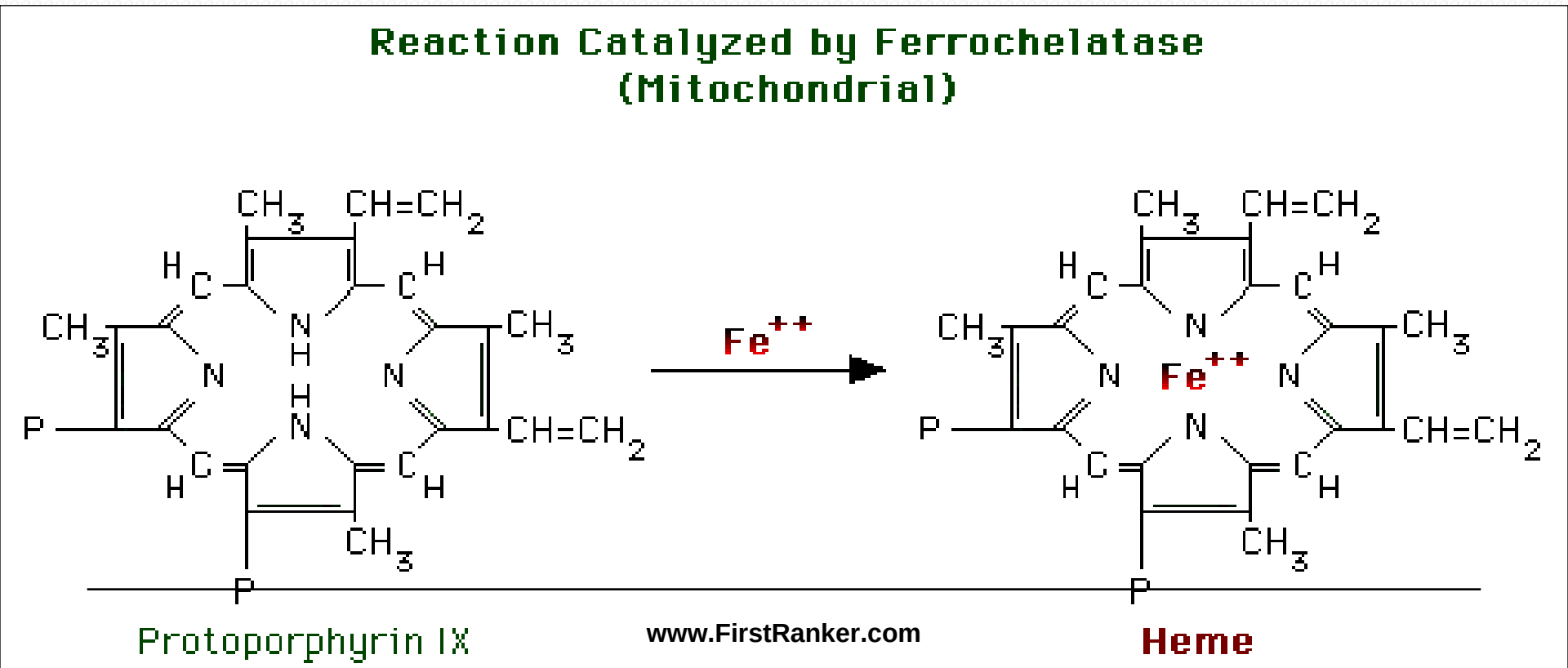
- Oxidation of protoporphyrinogen IX produces the conjugated **Methenyl bonds** of Protoporphyrin IX





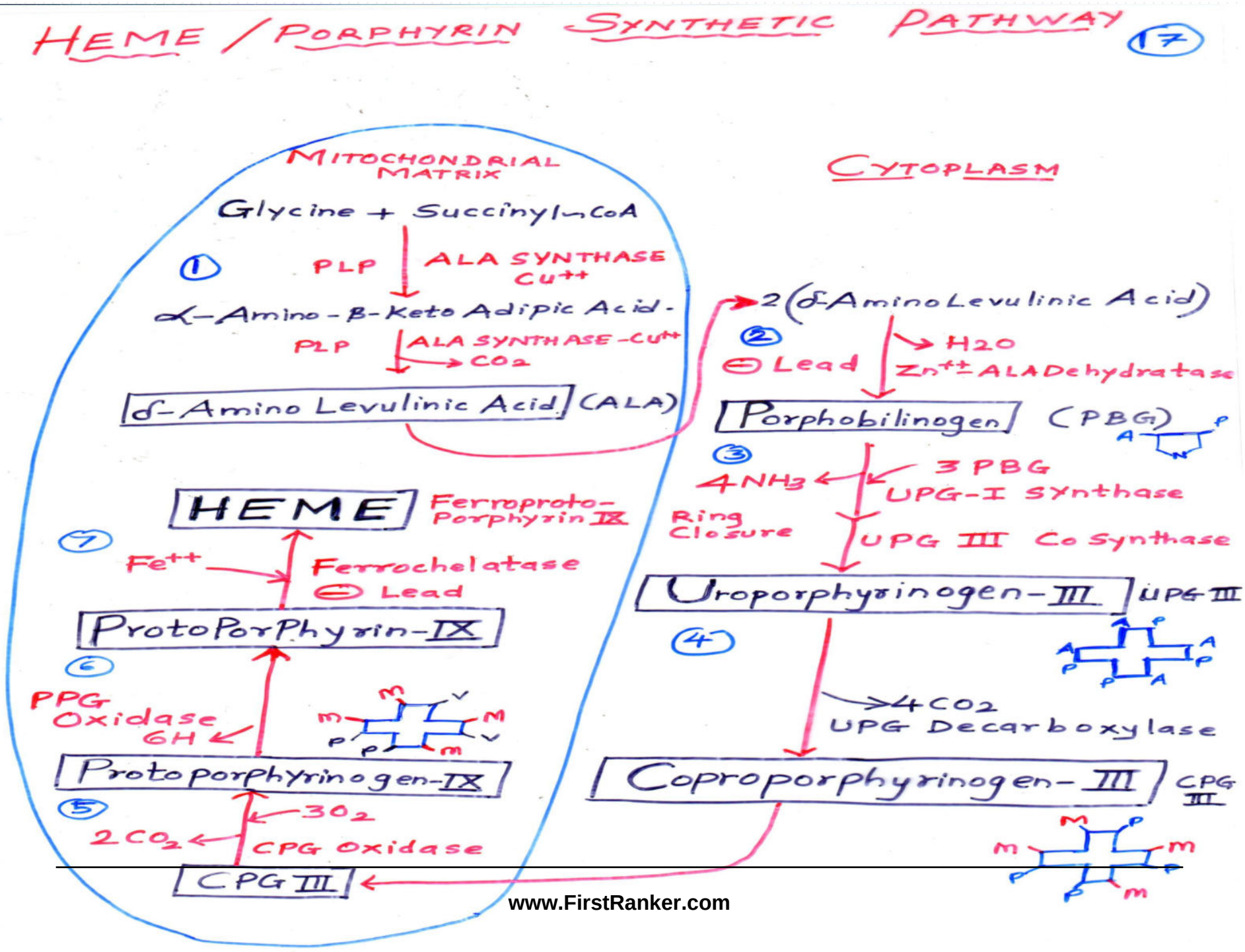

# Final Formation of Heme

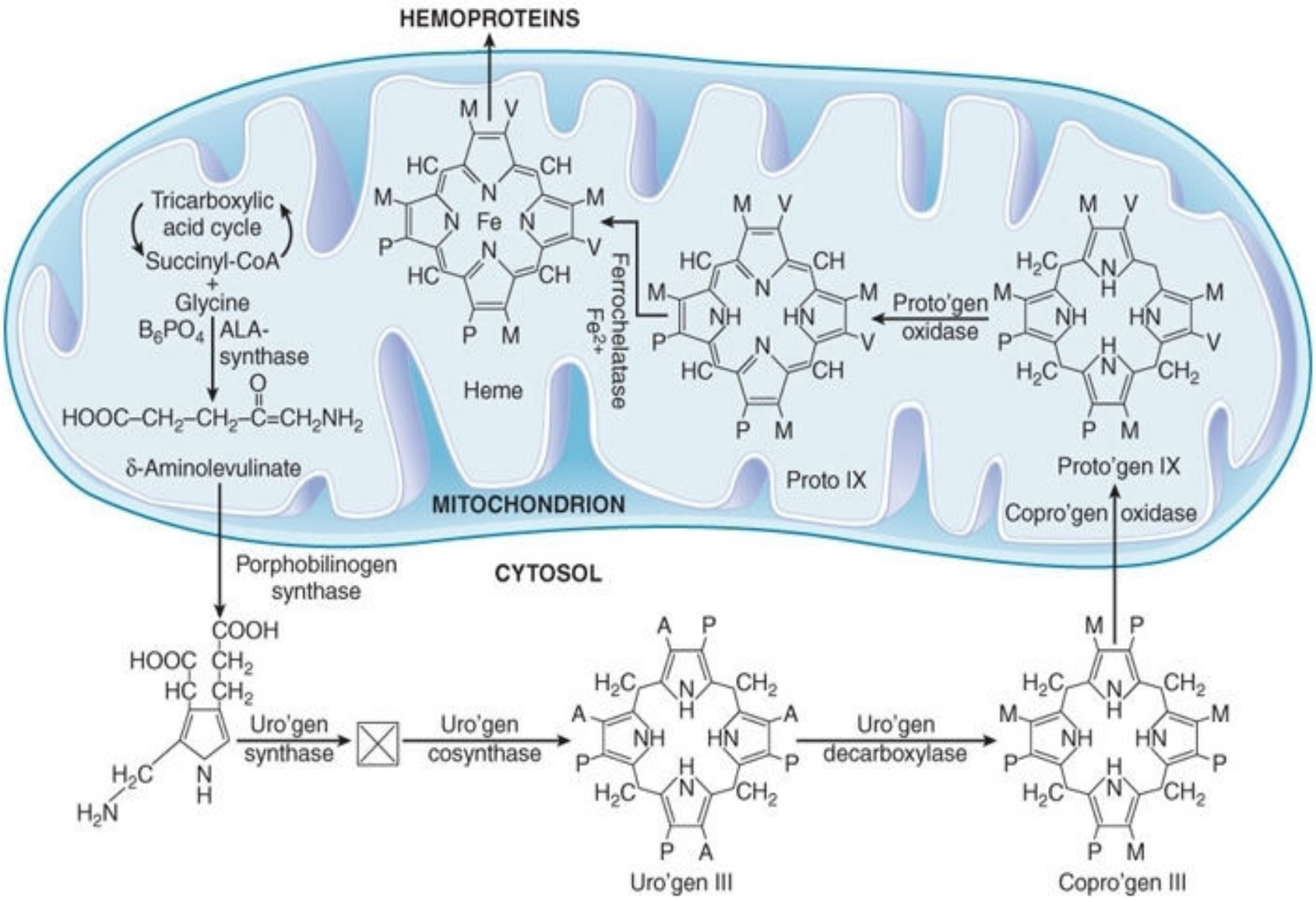
- $Fe^{2+}$ is incorporated into Protoporphyrin IX
- Reaction is catalyzed by enzyme **Ferrochelatase/Heme Synthase to Form Heme.**



- Iron is chelated within Porphyrin ring to form Heme by catalytic activity of **Ferrochelatase.**

- **Heme** is **incorporated into Proteins** to become biologically functional **Hemoproteins**.

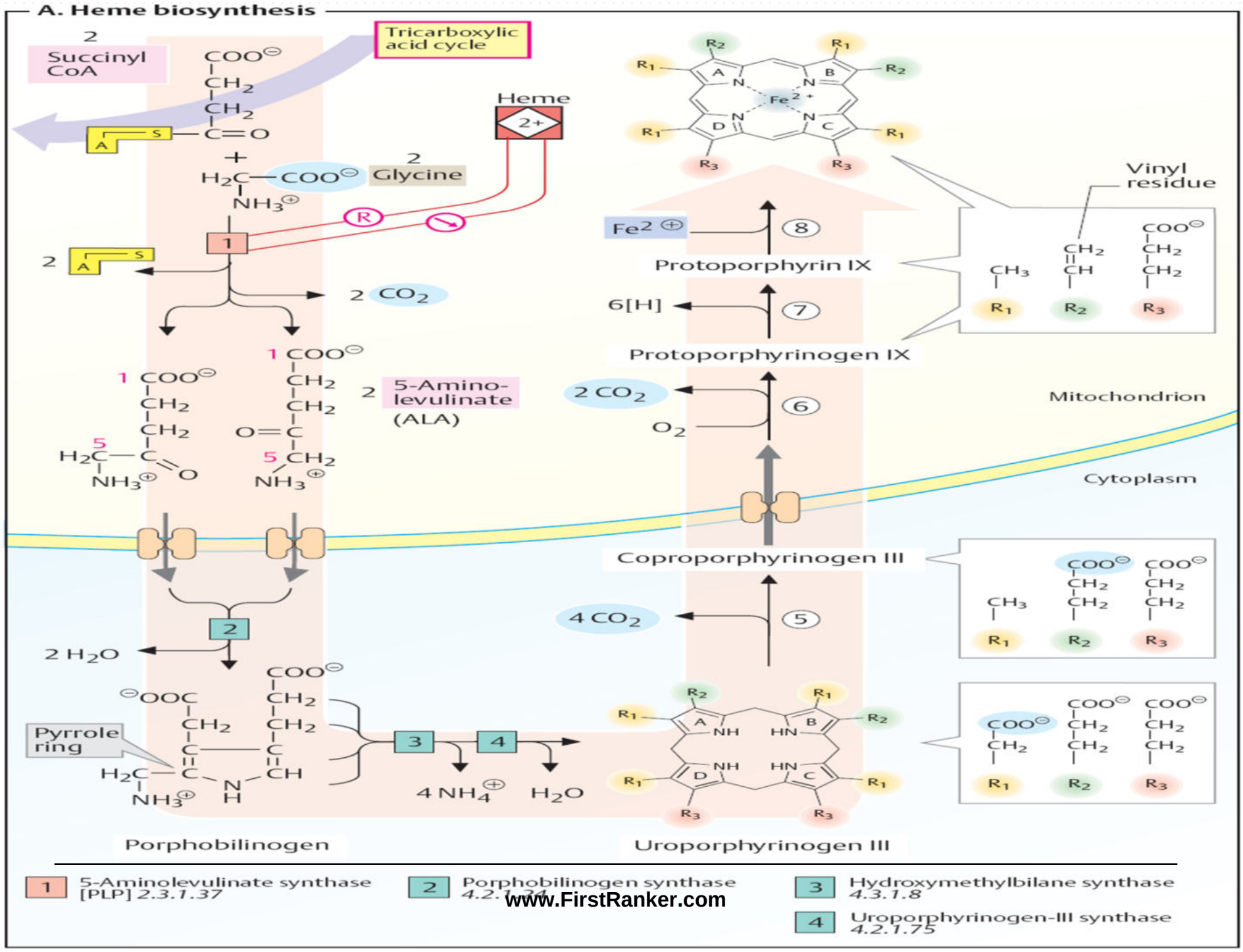





- **Heme**
- MetalloPorphyrin /Ferroprotoporphyrin
- Heme forms various: Hemoproteins
  - Hemoglobin
  - Myoglobin
  - Cytochromes
  - Catalase and Peroxidase
  - Tryptophan Pyrrolase

- **Chlorophyll** is a **Magnesium** containing **Porphyrin** present in **plants**.

- Chlorophyll is involved in **photosynthesis of plants.**



# Regulation Of Heme Biosynthesis

- **ALA Synthase** is an Allosteric **regulatory Enzyme** of Heme biosynthetic pathway.

- **ALAS 1** occurs in **Hepatocytes**
- **ALAS 2** is found in **Erythroid tissue**

- Rate of Heme biosynthesis is flexible.
- **Heme biosynthesis changes rapidly** in response to a wide variety of **external stimuli.**

# Mechanisms and Factors Regulating Heme Biosynthesis

- Feed Back Inhibition
- Repression of ALA Synthase
- Inhibition of transport of ALA Synthase from Cytosol to Mitochondrial matrix.
- Erythropoietin levels
- Iron levels

- **ALA Synthase is a key regulatory enzyme of Heme biosynthesis.**
- It is an allosteric enzyme that is inhibited by an **end product-Heme** (Feedback inhibition)
- Requires **Pyridoxal Phosphate** as a **coenzyme**

# Erythropoietin Stimulates Heme Biosynthesis

- **Erythropoietin** is a Protein produced by **Kidneys** .

- Erythropoietin **stimulates the ALA Synthase activity**.

- Erythropoietin Synthesis **increased** in **high altitude dwellers.**
- Erythropoietin **decreased** in **chronic renal failure.**

(Associated with Anemia)

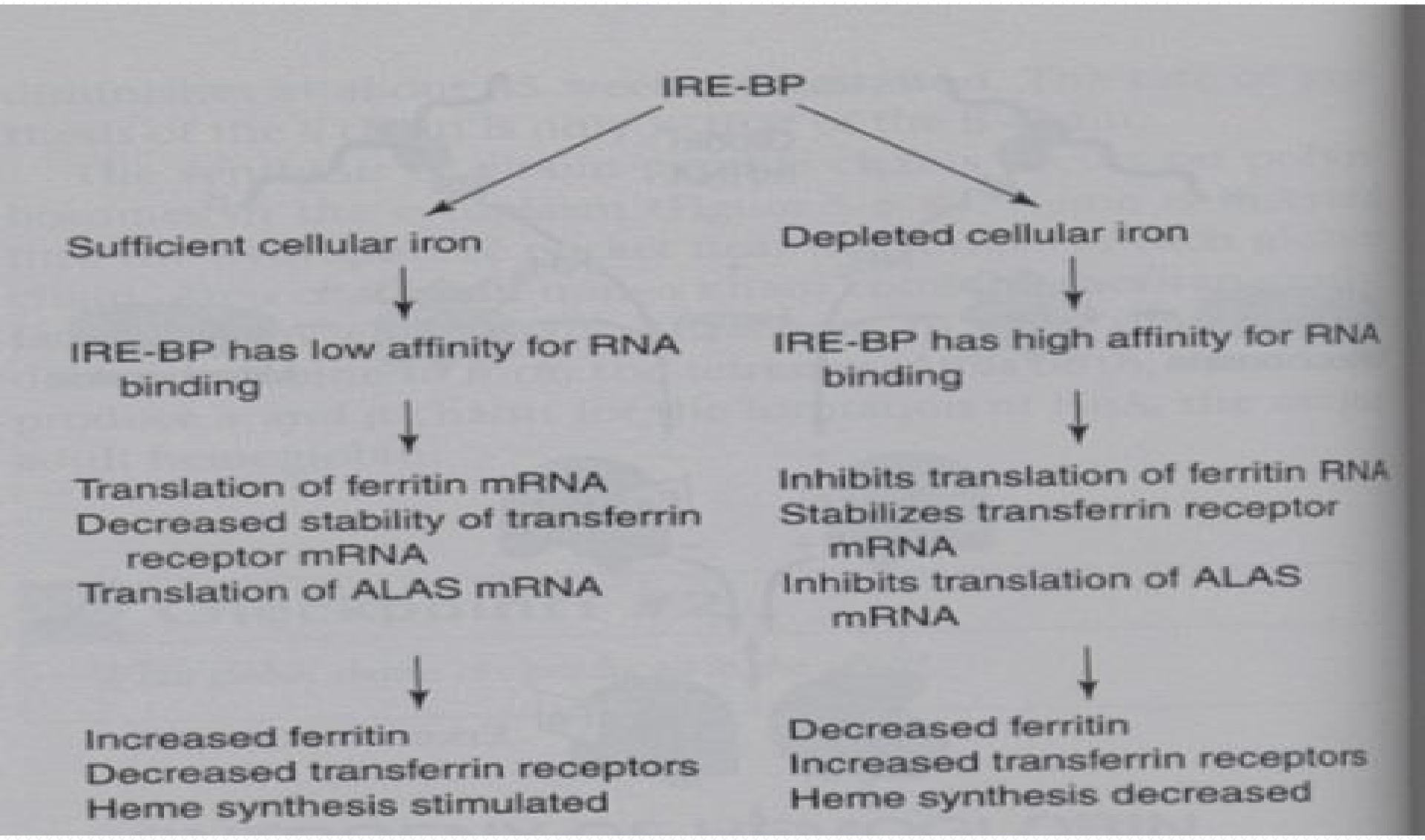
# Iron Levels Affect Heme Synthesis

- The amount of cellular Iron determines
- The affinity for Iron Responsive Element-Binding Protein(**IRE-BP**).

- When **Iron levels are low**
- There is a **high binding affinity of IRE-BP with mRNA of δ ALA.**
- Which **serves to inhibit** the translation of **δ ALA mRNA**
- Results in **decrease of Heme biosynthesis.**

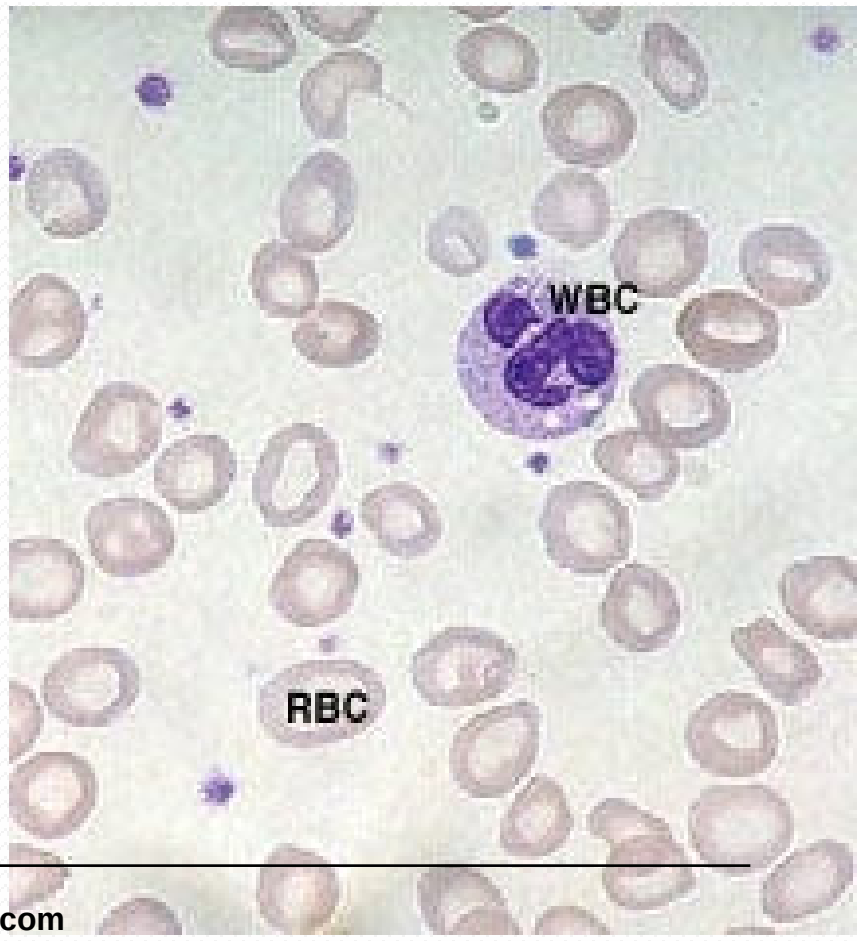
- **When Iron levels are sufficient.**
- There is a **low binding affinity** of **IRE-BP with mRNA of δ ALA.**
- Thus allowing translation of δ ALA mRNA
- **Stimulation of Heme synthesis.**

# How Iron Levels Affect Heme Synthesis




# Iron and Hemoglobin

- Iron deficient red blood cells
- **Low number** of red blood cells
- Note the **hollow and blanched** appearance of the red blood cells.



- If either **Heme or Globin synthesis is impaired**

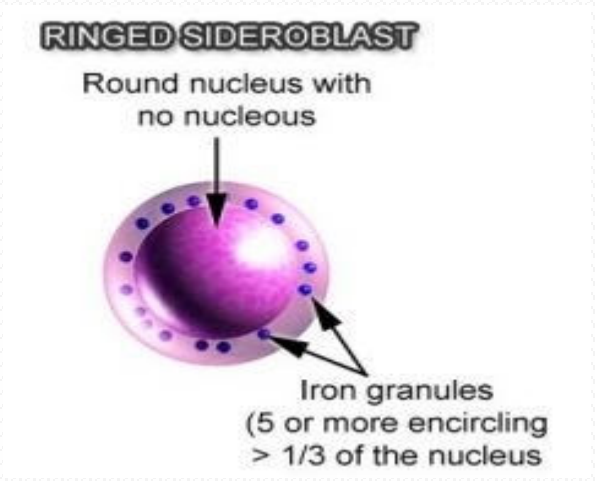
- **Iron is not utilized** and **accumulates in the RBC.**

- **When Heme biosynthesis is impaired**
- **Iron is underutilized**
- **Mitochondria or Nucleus of Erythroblasts become encrusted with Iron.**


Sideroblast

- A **Sideroblast** is a **Nucleated Erythrocyte**
- Containing **Iron granules in its cytoplasm** in the bone marrow.

# Sideroblast




- **Sideroblast** is an **Erythroblast** with **Iron granules (Pappenheimer bodies)** seen in bone marrow stained by **Prussian blue or Perl stains.**

- A **precursor Red blood cell (Immature RBC) with a ring of Iron around the nucleus** is called a **ringed Sideroblast.**

## Siderocyte

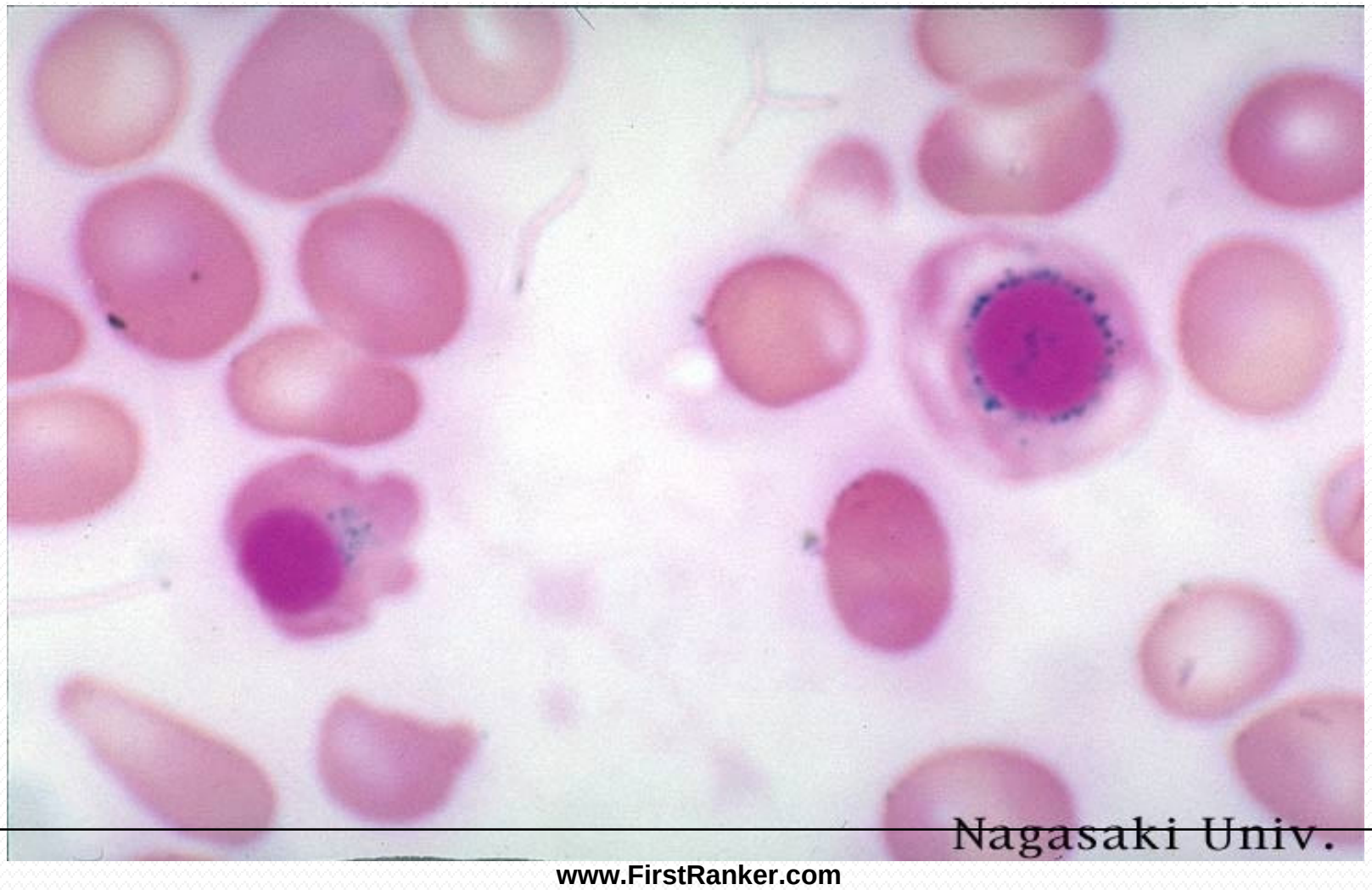
- **Siderocyte** is a **non-nucleated red cell** with Iron granules (Pappenheimer bodies)

- A **mature RBC** **with an accumulated Iron** is termed as **Siderocyte** .

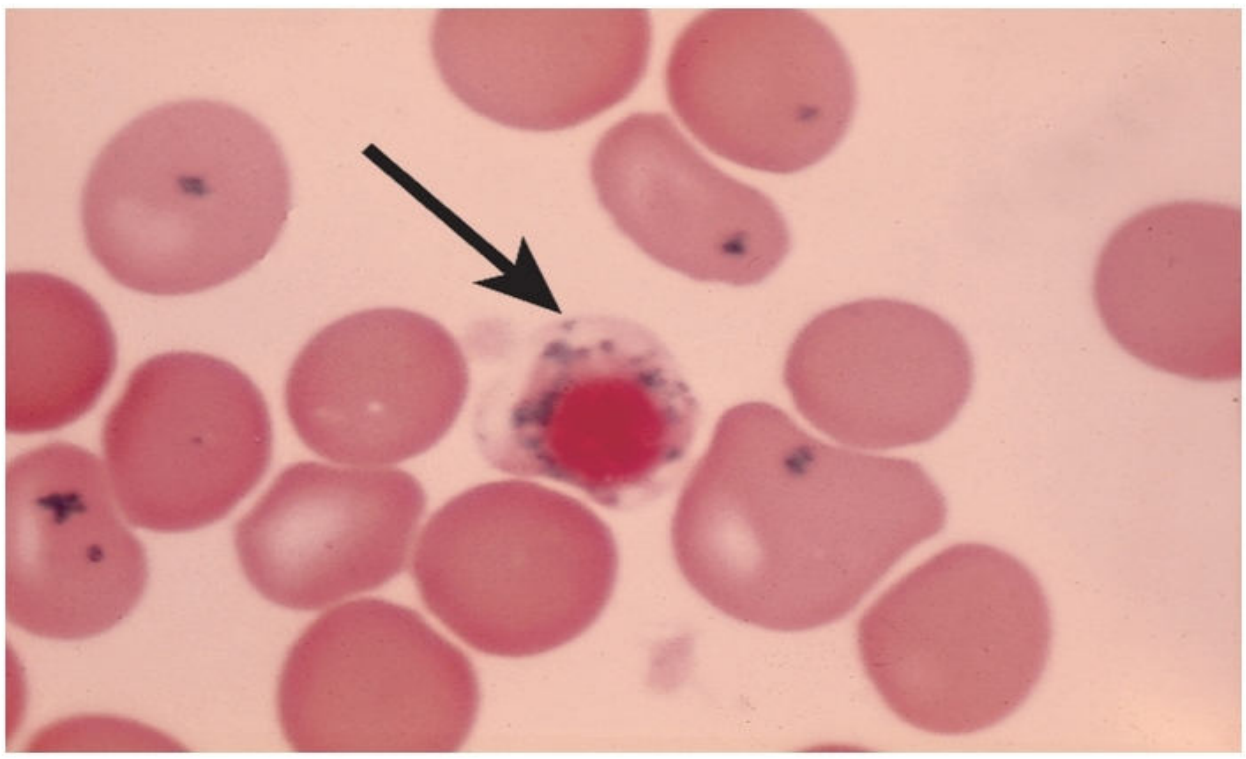
- Siderocytes are abnormally increased in :
  - **Sideroblastic Anemia**
  - **Hemosiderosis**
  - **Hemoglobinopathies**

- The Iron within **Sideroblasts and Siderocytes** can be visualized by staining with **Prussian blue stain.**

# Ringed Sideroblast



# RINGED SIDEROBLASTS AND SIDEROCYTE



# Effect Of Drugs and Other Substances On Heme Biosynthesis

- Certain Drugs and Steroid Hormones **increases Heme biosynthesis**.

- Ingestion of drugs like **Phenobarbitals ,Insecticides,** certain chemical carcinogen,
- **Markedly increases ALA Synthase (ALAS1 of Hepatocytes) activity.**
- This increases production of Heme.

- The **biosynthesized Heme in response to drug administration**
- **Is used** for production of **Cytochrome P450**
- **Cyt P450** –A **Hemoprotein** is responsible for drug detoxification.

# Lead Poisoning Affects Heme Biosynthesis and Causes Anemia

- Following **Enzymes** of Heme biosynthetic pathway are **inhibited by Lead ions ($Pb^{2+}$)** in cases of **Lead poisoning.**
  - **ALA Dehydratase / Porphobilinogen Synthase**
  - **Ferrochelatase / Heme Synthase**

- **Thus Lead poisoning**
- **Inhibit Heme biosynthesis and**
- Leads to **Anemia.**

# Porphyrins

- **Porphyrins** are chemically cyclic **tetra Pyrrole ring structures with substituted groups**.

- Porphyrins **has conjugated ring system**
- Alternate single and double bonds (**Methenyl bonds**).

- **Porphyrins** are **colored and Fluorescent compounds** with Methenyl bridges/ Methyne bonds in it.

- The **double bonds in Porphyrins absorb visible light** and **appear colored compounds.**

- The **Conjugated bonds of Porphyrins** in **UV light** shows **fluorescence intense reddish pink color.**

## Types Of Porphyrins

## Based on arrangements of Substituted groups on Tetrapyrrole Rings

# Types Of Porphyrins

- Type I Porphyrins
- Type III Porphyrins

- **Type I Porphyrins** has **symmetric arrangements** of **substituted groups** in tetra pyrrole ring structure.

- **Type III Porphyrins** have **asymmetrical distribution** of the **substituted groups** in tetra pyrrole rings.

- **Type III Porphyrins** are **most predominant** in biological system.

- **ProtoPorphyrin IX** is of **type III Porphyrins**
- Fischer placed **ProtoPorphyrin** in 9th series of 15 possible isomers.

- **In Disorders of Porphyrias**
  - **Porphyrins** are **abnormally elevated**
  - In blood and excreted in urine.

# Porphyrias

# Gk- Porphyria= Purple

# Porphyrias Disorders Due To Defective Heme Biosynthesis

## Porphyrinurias

# What Are Porphyrias?

- The **Porphyrias** are **group of disorders**
- Associated to **defective Heme biosynthesis**.

# Basic Cause Of Porphyrias

- **Metabolic block** in Heme biosynthesis leads to **Porphyrias.**

- **Defect in any one Enzyme of Heme biosynthesis**

- **Defect in Enzyme of Heme Biosynthesis may be:**
  - **Inherited**
  - **Acquired**

- **Most** of the **Porphyrias** are of **Autosomal Dominant inheritance** .

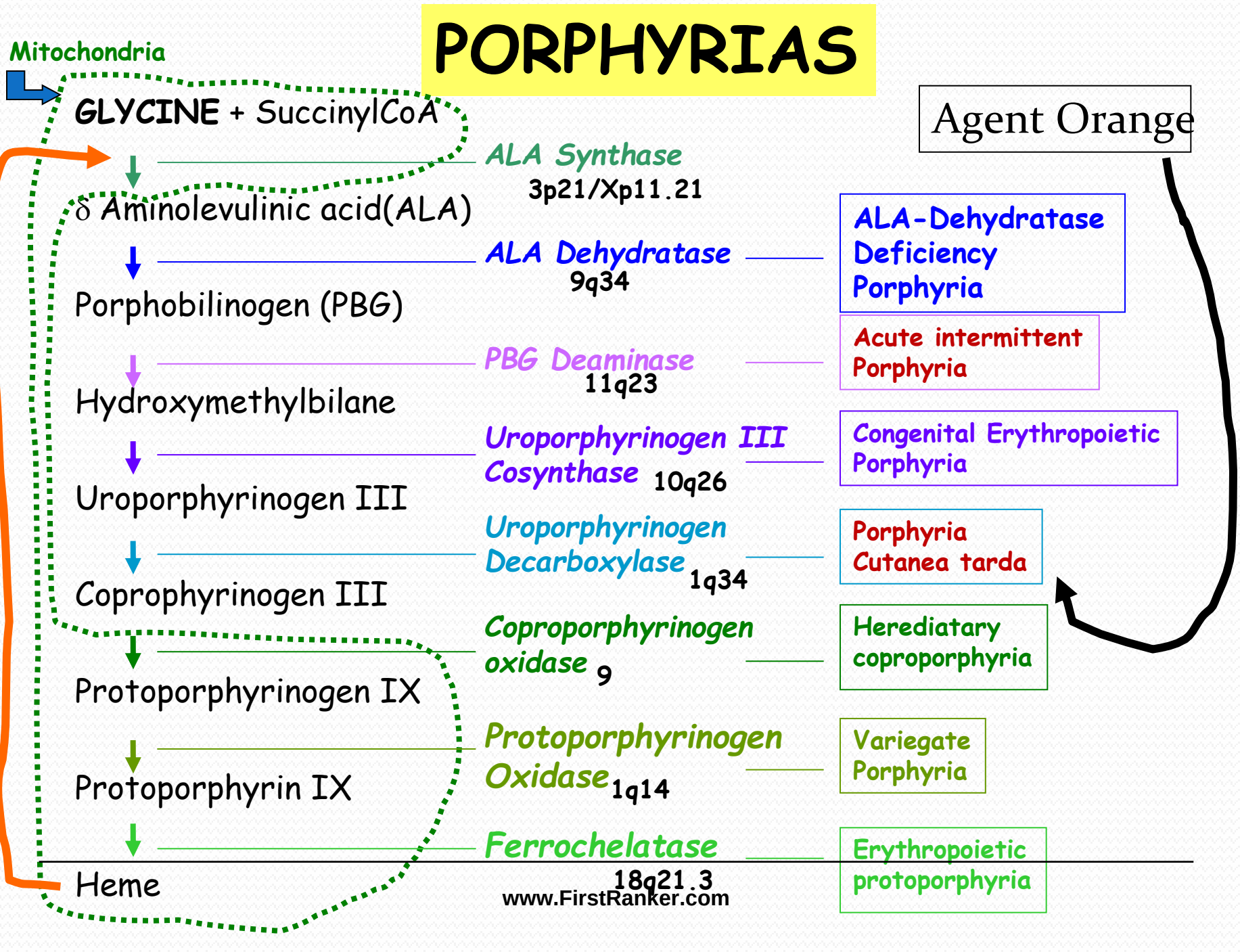
# Types and Classification Of Porphyrias

# 6 Common Types Of Porphyrias

| S.No | Type Of Porphyrias | Enzyme Defect |
|---|---|---|
| 1 | Acute Intermittent Porphyria (AIP) | UPG I Synthase/ PBG Deaminase |
| 2 | Erythropoietic Porphyria | UPG III Cosynthase |
| 3 | Cutanea Tarda | UPG Decarboxylase |
| 4 | Coproporphyria | CPG Oxidase |
| 5 | Variegate Porphyria | PPG Oxidase |
| 6 | Protoporphyria | Ferrochelatase |

# Pneumonic To Remember
# 6 Type Of Porphyrias

- **All Elephants Can Catch Ved Pathak.**



## CLASSIFICATION OF THE PORPHYRIAS

| Acute porphyria | Non-acute porphyria |
|---|---|
| ALA-D deficient porphyria | Porphyria cutanea tarda |
| Acute intermittent porphyria | Erythropoietic protoporphyria* |
| Variegate porphyria | Congenital erythropoietic porphyr |
| Hereditary coproporphyria | |

# Different Basis For Classification Of Various Types Of Porphyrias

## On Basis Of Cause

- Primary/Congenital Porphyrias
- Secondary/Acquired Porphyrias

## On Basis Of Organ

- Hepatic Porphyria
- Erythropoietic Porphyria

- **Inherited Porphyria**

- **Erythropoietic Porphyria -** results from excessive production of Porphyrins in the bone marrow.
- **Hepatic Porphyria** - results from excessive production of Porphyrins in the Liver.

- **Acquired Porphyria**

- **Lead Intoxication** - interferes with Protoporphyrin synthesis

- **Chronic Alcoholic Liver disease**

# On Basis Of Symptoms

- Neurological Porphyrias/Acute Porphyrias
  - Acute Intermittent Porphyria
  - Coproporphyria
  - Variegate Porphyria
  - Autonomic Dysfunction
  - Abdominal pain
  - Chest pain
  - Confused Thoughts
  - Depression and Psychosis

- **Photosensitive Porphyria/Chronic Porphyria**
  - **Erythropoietic Porphyria**
  - **Cutanea Tarda**
- Porphyrins below skin exposed to sunlight shows
  - **Redness**
  - **Swelling**
  - **Itching**
  - **Burning Sensation**

# Biochemical Alterations And Consequences Of Porphyrias

# Enzyme Defect Of Heme Pathway

Blocks the Reaction ↓ Of Heme Pathway

Accumulates Porphyrins Intermediates of Heme Pathways

↓

High levels of Porphyrin in blood ,Tissues and Urine (Porphyrias)

- **Porphyrinogens** are **oxidized** to **Porphyrins.**
- Porphyrins are **coloured pigments.**
- Porphyrins are **more stable products.**
- **Accumulate in blood, tissues and get excreted out through urine**

# Effects of Accumulated Porphyrins and their Precursors

- **Porphyria Sufferers Shows**

- **Severe Anemia**
- **Neurological Disturbances**
- **Extreme sensitivity to sunlight**

- **Porphyria sufferers has no normal Heme biosynthesis.**
- **No normal Hb to transport Oxygen to cells.**
- **Hence suffer from Anemia.**

- Accumulation of Porphyrinogens in Brain and Skin can lead to:

- **Neurological symptoms**

- **Photosensitivity**

- **Enzyme block early** in **Porphyrin pathway** prior to formation of Porphyrinogens.
  - **Accumulates ALA and PBG**
  - **Exhibits abdominal pain** and **neuropsychiatric symptoms.**

- **Enzyme block occur later** in the Porphyrin pathway
- **Accumulates** Porphyrinogens-**CPG/PPG** beneath skin.
- **Causes Photosensitivity** when Porphyrins exposed to light about 400 nm.

- The Porphyrins have no useful function
- Act as highly reactive oxidants, damaging to tissues.

- **Porphyrins** get **excited at 400 nm**.
- Shows sharp absorption band near 400nm (**Soret band**)

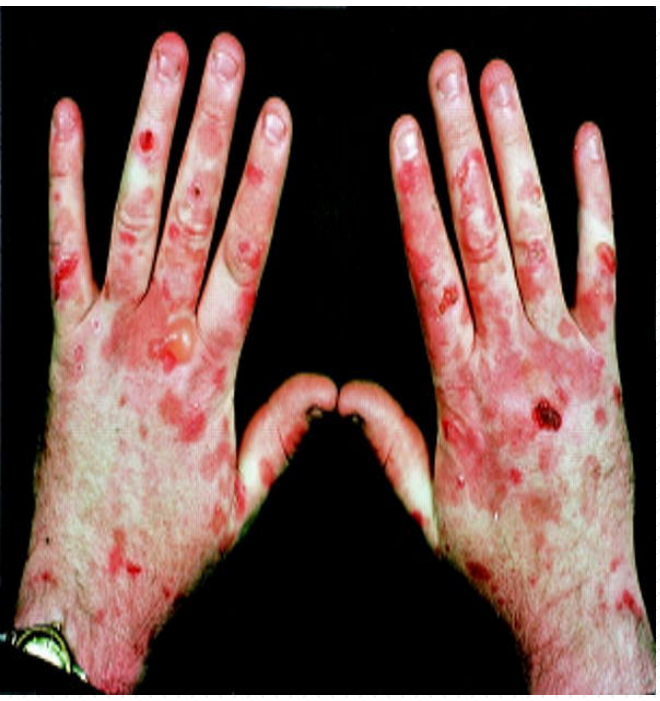
- **Porphyrins reacts with molecular Oxygen**
- To **form highly reactive oxygen free radicals**.
- **Injure Lysosomes** and other cellular organelles.

- Damaged Lysosomes **release their degradative enzymes** .
- Causing variable degrees of **skin damage including scarring**.

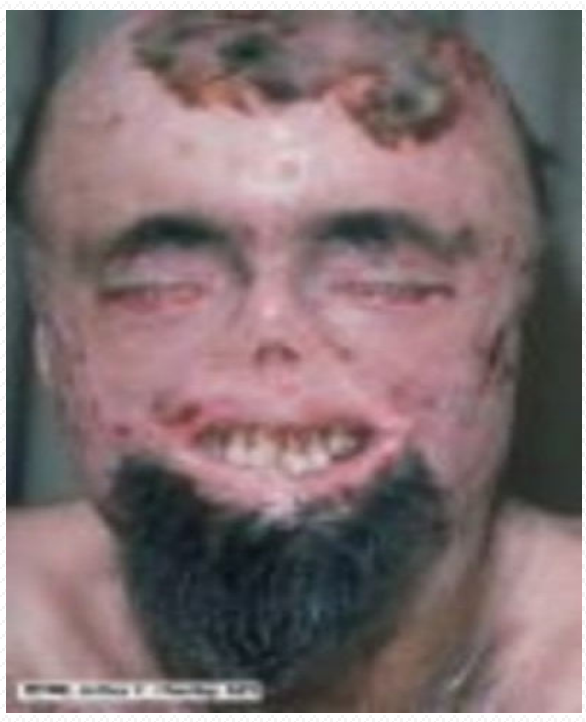
- **Porphyrias are characterized by**
  - **Extreme sensitivity to light** (exposure to sunlight causes **vesicular erythema**),
  - **Excretes reddish-brown urine**
  - **Possess reddish-brown teeth, and ulcers**

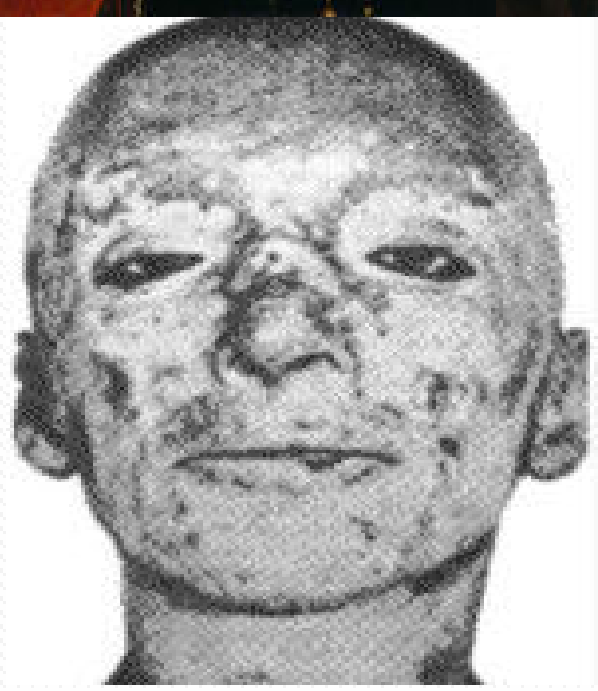
- **Destruction of cartilage and bone**
- Causing the **deformation of the nose, ears, and fingers.**
- **Mental aberrations,** such as hysteria, manic-depressive psychosis, and delirium.

- Porphyrias are cruelly referred to as a **Vampire's disease.**
- Thought to be a cause of the madness of **King George III.**
- Can be caused by lead poisoning: The **fall of the Roman Empire!**



# Porphyria





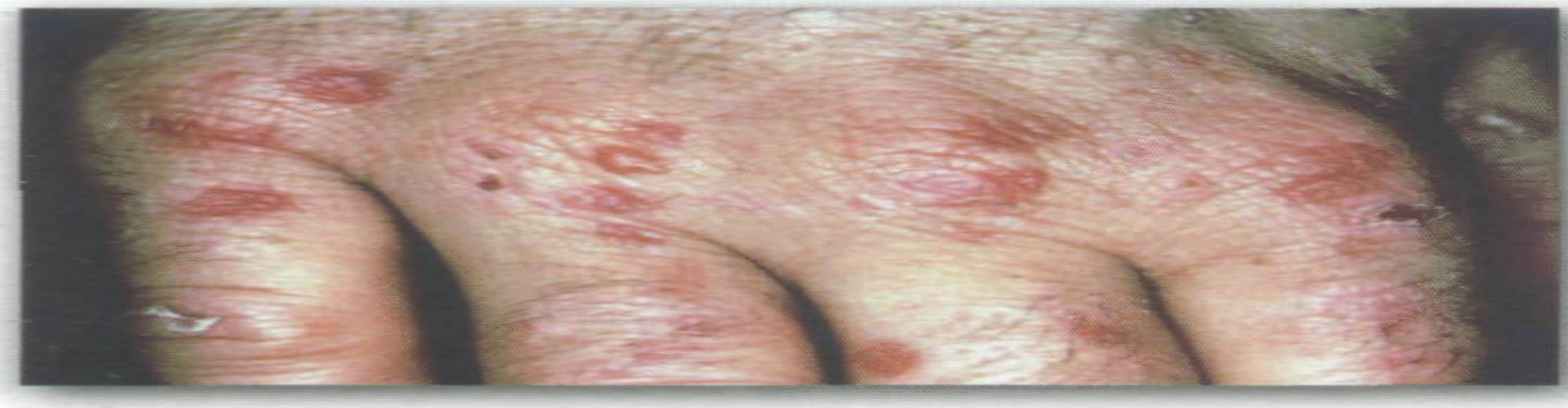


**Figure 21.5**
Skin eruptions in a patient with porphyria cutanea tarda.

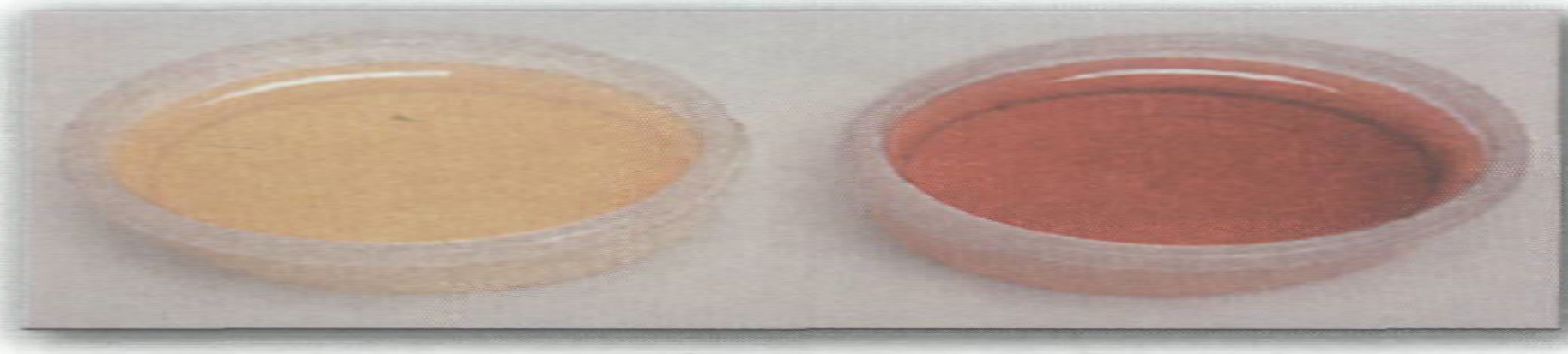


**Figure 21.6**
Urine from a patient with porphyria cutanea tarda (right) and from a patient with normal porphyrin excretion (left).

# Diagnosis Of Porphyrias

- Porphyrias are rare, but frightening conditions:
- **Hard to diagnose and there is no cure for Porphyrias.**

# Porphyrins Excreted In Urine and Feces

- **Uroporphyrin** excreted in **urine.**
- **ProtoPorphyrin** excreted in **feces.**
- **Coproporphyrin** excreted either in **urine /feces.**

- Porphyrins are Colored and Fluorescent.

- Porphyrias are diagnosed by analysis of Porphyrins in the laboratory.
  - **Spectrophotometry**
  - **Fluorometry**
- **Woods lamp-** Fluorescence in aqueous layered viewed.

- Based on quantitative **Ehrlich's reagent**
  - **Watson Schwartz**
  - **Hoesch Test**

- Defective enzymes of Porphyrias can be assayed by various methods.
- **Enzyme Assay- HPLC**.

# Acute Intermittent Porphyria (AIP)

- **Acute Intermittent Porphyria**

- The **most common type of Porphyria**.
- **Autosomal Dominant** trait.
- Symptoms **more common** in **females** than males.

- **Acute Intermittent Porphyria**

- **Type Of Porphyria-**
  - **Acute/Hepatic/ Neurological Porphyria**

- **Enzyme Defect Of AIP**

- **UPG I Synthase/PBG Deaminase**

- **Biochemical Alteration In AIP**
- No conversion of **PBG to UPG III.**
- **PBG and δ-ALA** are abnormally elevated in **blood, tissues and urine.**

# Manifestations Of AIP

- **δ ALA and PBG accumulates in CNS.**
- **This causes** excitation of visceral pain fibers
- Leads to **acute pain crises.**

- **δ ALA blocks** the **action of GABA.**
- Possess neurological symptoms.

- **Symptoms** of AIP are **Acute and Intermittent.**

- **Symptoms** does **not occur before puberty** and **shown** at **Adolescence.**

(Due to Steroidal Hormone action)

- **Person with AIP has**
- **Affected GIT, Heart and Brain.**
  - Abdominal colic pain
  - No abdominal tenderness
  - Vomiting, Constipation
  - Tachycardia, Hypertension
  - Neuro toxicity
  - Behavioral changes, seizures

- **AIP** symptoms gets **aggravated during:**
  - **Infections**
  - **Fasting**
  - **Intake of drugs**

- **Diagnostic Test For AIP**

- **Watson and Schwartz Test** using **Woods lamp** ( UV lamp)
- Detects urine Porphobilin.

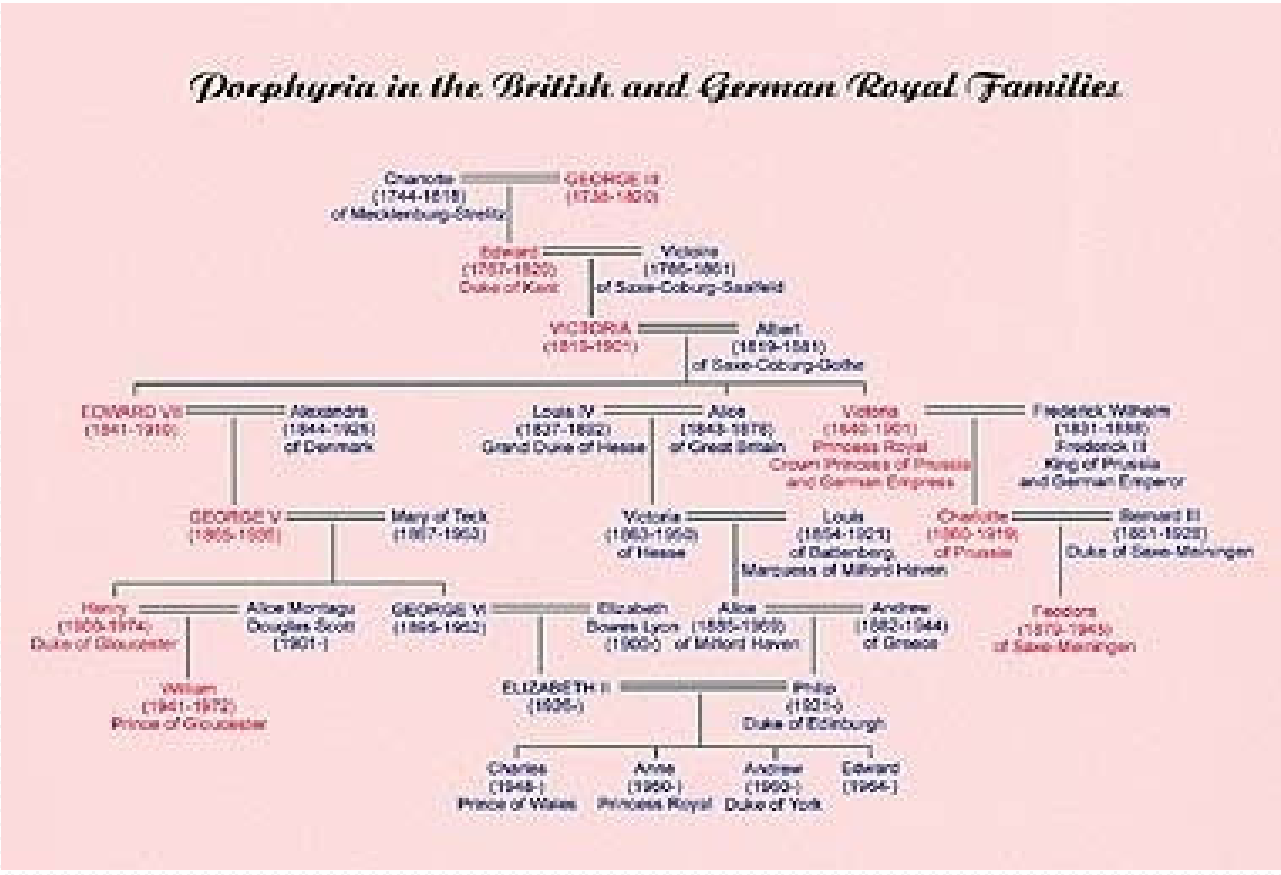
# Treatment of AIP

- Infusion of Hematin
- Represses ALA Synthase synthesis.

- **Administration Of Glucose.**
- High cellular Glucose prevents induction of ALA Synthase.

- **Use of Sunscreens** that filter out visible light,
- Can be used in **management of Photosensitive Porphyrias**

# The Madness of Inbreeding




King George III : Severe abdominal pain, mental confusion, dark urine.

# Enzyme Defects Responsible for The Porphyrias

| Type | Enzyme Involved | Major Symptoms | Laboratory tests |
|---|---|---|---|
| **Acute intermittent Porphyria** | Uroporphyrinogen synthase | Abdominal pain Neuropsychiatric | **urinary Porphobilinogen ⇑** |
| **Congenital Erythropoietic Porphyria** | Uroporphyrinogen III Cosynthase | Photosensitivity | **urinary uroporphyrin ⇑** **Porphobilinogen ⇔** |
| Porphyria cutanea tarda | UPG Decarboxylase | Photosensitivity | urinary uroporphyrin ⇑ Porphobilinogen ⇔ |
| Variegate Porphyria | PPG Oxidase | Photosensitivity Abdominal pain Neuropsychiatric | urinary uroporphyrin ⇑ fecal coproporphyrin ⇑ fecal Protoporphyrin ⇑ |
| **Erythropoietic protoporphyria** | Ferrochelatase | Photosensitivity | **fecal Protoporphyrin ⇑** **red cell Protoporphyrin ⇑** |

## THE HEME BIOSYNTHETIC PATHWAY

| Product | Enzyme | Diseases resulting from deficiencies in enzyme activity |
|---|---|---|
| Glycine + succinyl CoA | | |
| | Aminolevulinic acid (ALA) synthase* | X-linked hereditary sideroblastic anemia |
| Delta ALA | | |
| | ALA dehydratase | ALA dehydratase-deficient porphyria |
| Porphobilinogen | | |
| | Porphobilinogen deaminase | Acute intermittent porphyria |
| Hydroxymethylbilane | | |
| | Uroporphyrinogen III synthase | Congenital erythropoietic porphyria |
| Uroporphyrinogen ⟹ Uroporphyrin I and III | | |
| | Uroporphyrinogen decarboxylase | Porphyria cutanea tarda |
| Coproporphyrinogen ⟹ Coproporphyrin I and III | | |
| | Coproporphyrinogen oxidase | Hereditary coproporphyria |
| Protoporphyrinogen | | |
| | Protoporphyrinogen oxidase | Variegate porphyria |
| Protoporphyrin | | |
| | Ferrochelatase + $Fe^{2+}$ | Erythropoietic protoporphyria |
| Heme + Globin + Apoprotein | | |
| Hemoglobin, Cytochromes | | |



* Erythroid and ubiquitous isoenzymes are encoded by genes on the X chromosome and chromosome 3, respectively. Mutations in the former cause sideroblastic anemia

# Globin Biosynthesis

- The biosynthesis of Hemoglobin **Globin Polypeptide chains** is under **genetic control**.
- Using Protein synthetic machinery.
- Globin chain biosynthesis **occurs in cytosol** on **Polyribosomes.**

# Number And Types Of Globin Chains Biosynthesized In Human Life

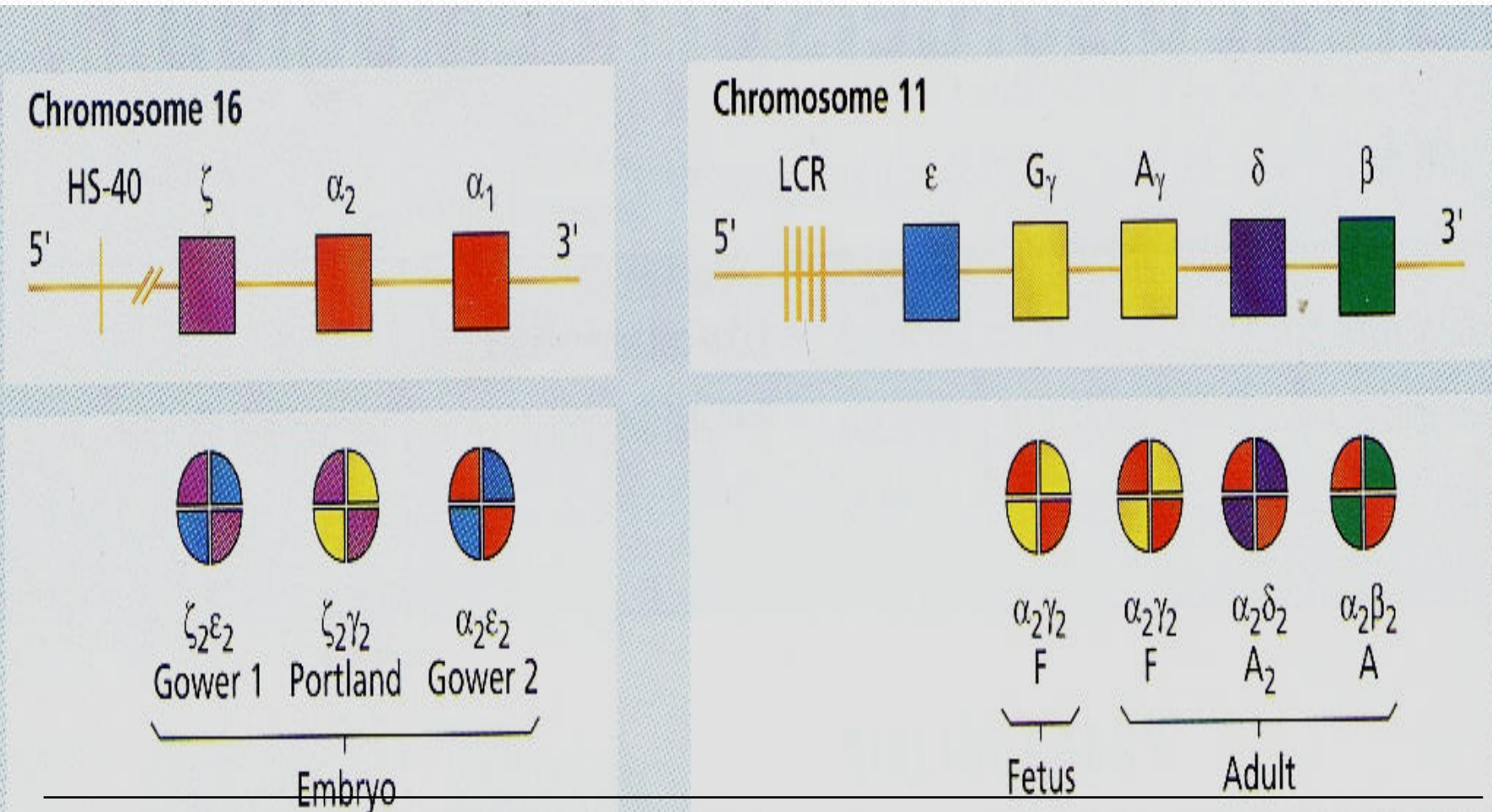
- **6 different types of Globin chains** are associated with Normal Hb variants :
  - **α Globin**
  - **β Globin**
  - **γ Globin**
  - **δ Globin**
  - **ε Globin**
  - **ζ Globin**

- To biosynthesize these **6 types of Globin chains**

- Human being normally carry **8 functional Globin genes**

- Arranged **in two duplicate gene clusters.**

# Globin Gene Clusters



- The **β-like cluster located** on the short arm of **chromosome 11**

- The **α-like cluster is located** on the short arm of **chromosome 16**

- Globin polypeptide chain biosynthesis begins in the **yolk sac**

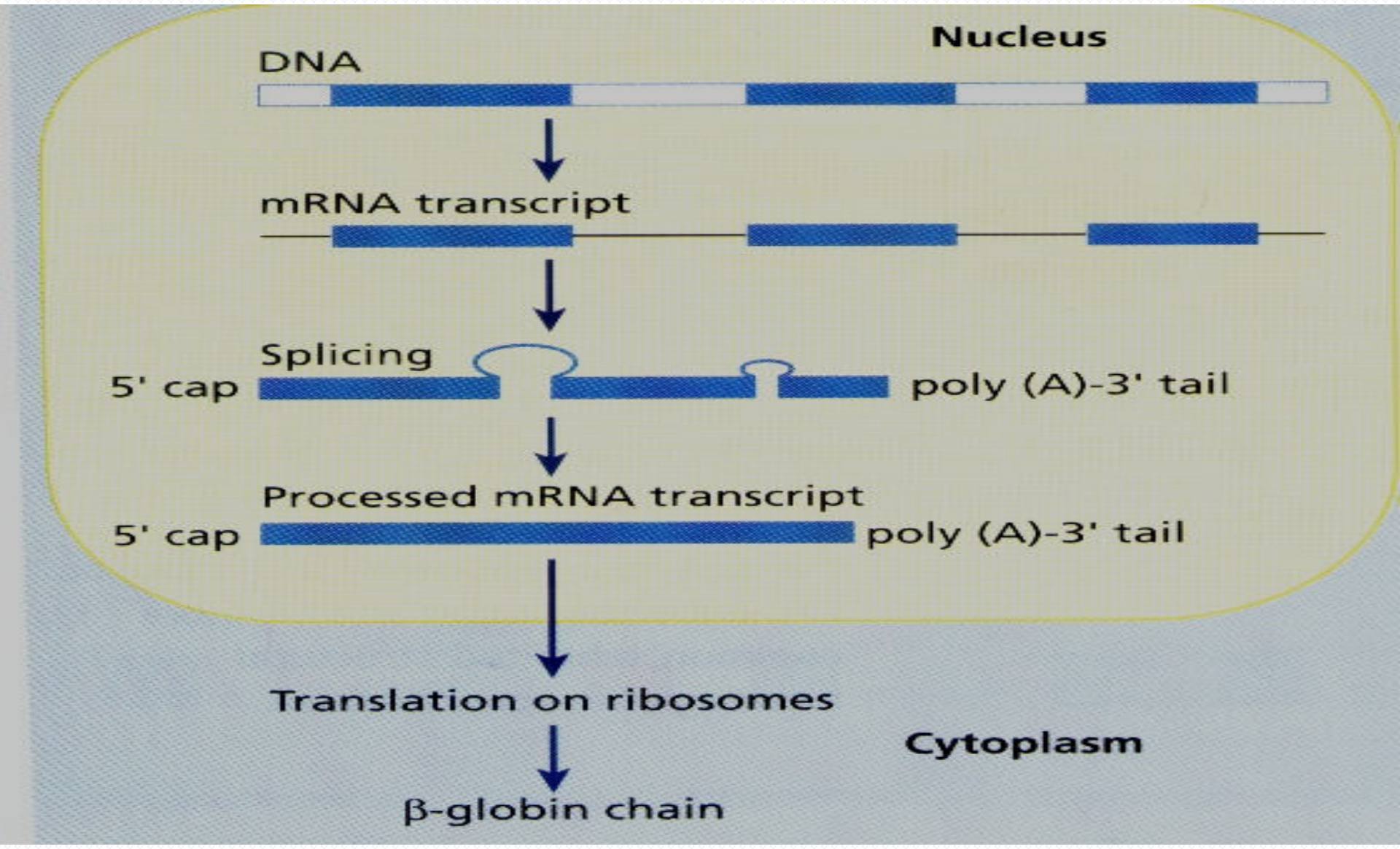
- At about **3 weeks' of gestation**.

# Ontogeny of Globin Synthesis

# Ontogeny of Globin Synthesis

| Time | Region | Type of Globin Gene | Normal Hb Variant Type of Hb |
|---|---|---|---|
| **3 weeks of Gestation** | Yolk Sac | ζ & ε | (Hb Gower I ( ζ ε)$_2$ |
| **5 weeks of Gestation** | Yolk Sac | Γ & α | Hb Portland (ζ γ)$_2$<br>Hb Gower II (αε)$_2$ |
| **6-30 weeks of Gestation** | Liver & spleen | α & γ & β | Hb F (α γ)$_2$ |
| **30 weeks of Gestation** | Liver | δ | Hb A$_2$ (α δ)$_2$ |
| **At Birth** | Bone Marrow | α & γ & β | Hb A (α β)$_2$<br>Hb F (α γ)$_2$ |

# Synthesis of Globin




# Primary Structure Of Globin

- The primary structure of globin refers to the **amino acid sequence** of the various chain types.

- **Numbering** from the **N-terminal end** identifies the position of individual amino acids.

- The **specific number** ,and **sequence** of **amino acids** in Globin chains

- Is very important for the **normal structure and function of Hemoglobin**.

## Secondary Structure of Globin

- The secondary structure of all Globin chain types comprised of:
- **9 Non-helical sections** joined by **8 Helical sections.**

- The **Helical sections of Globin Chains** are identified by the letters **A-H**

- While the non helical are identified by a pair of letters corresponding to the adjacent helices
- e.g. **NA** (N-terminal end to the start of A helix), **AB** (joins the A helix to the B helix) etc.

## Tertiary Structure of Globin

- The **secondary structure is further folded and bended** on its own to form **3 dimensional subunit**.
- To form a **Tertiary structure of Globin.**

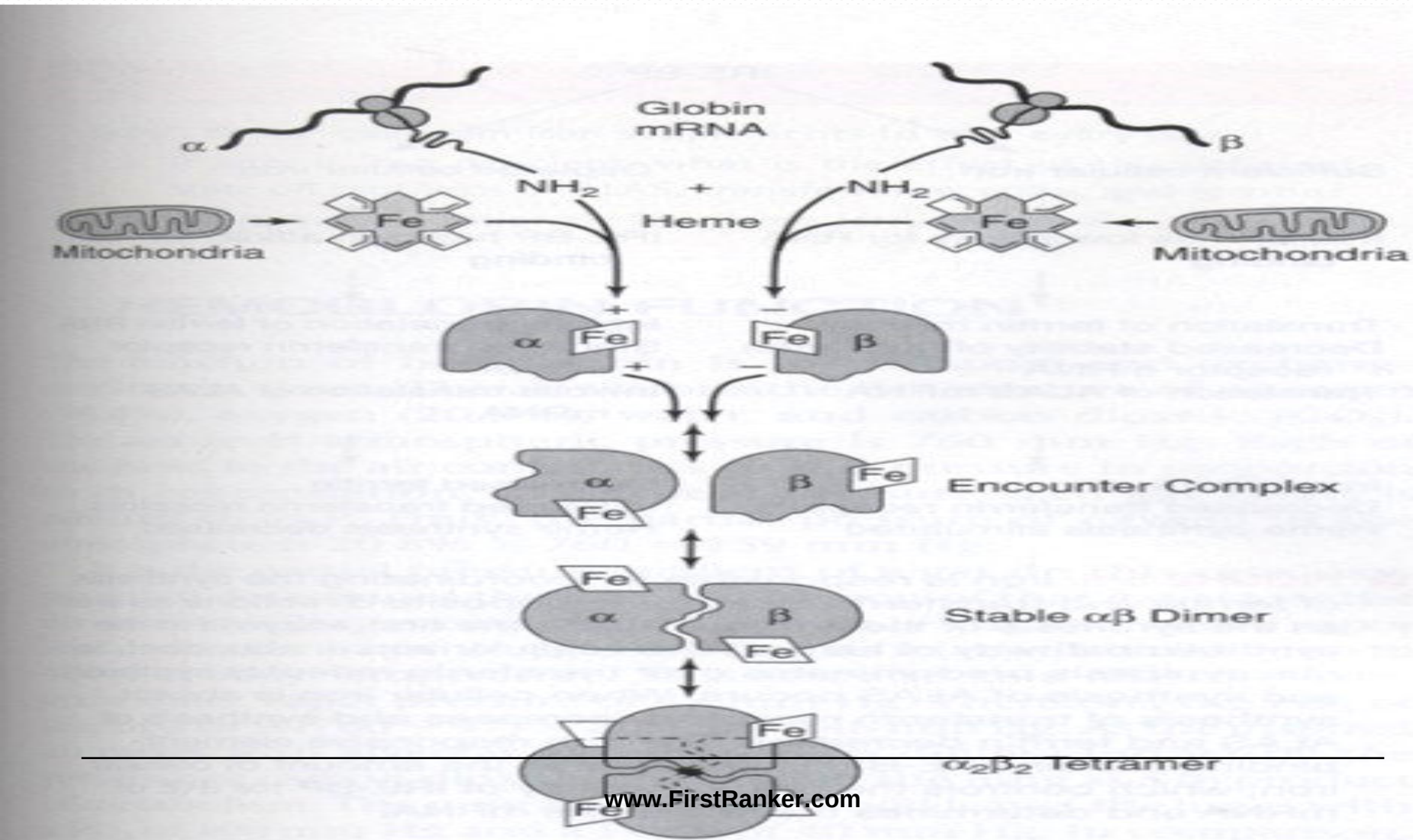
- **Heme gets incorporated** in the Heme pocket formed **inside each Globin subunit.**

## Quaternary Structure Of Hb

- **It is native conformational state of Hb/ Functional form of Hb.**

- **Four subunits of tertiary structure** ,non covalently linked
- To form **quaternary level of organization of Hb.**

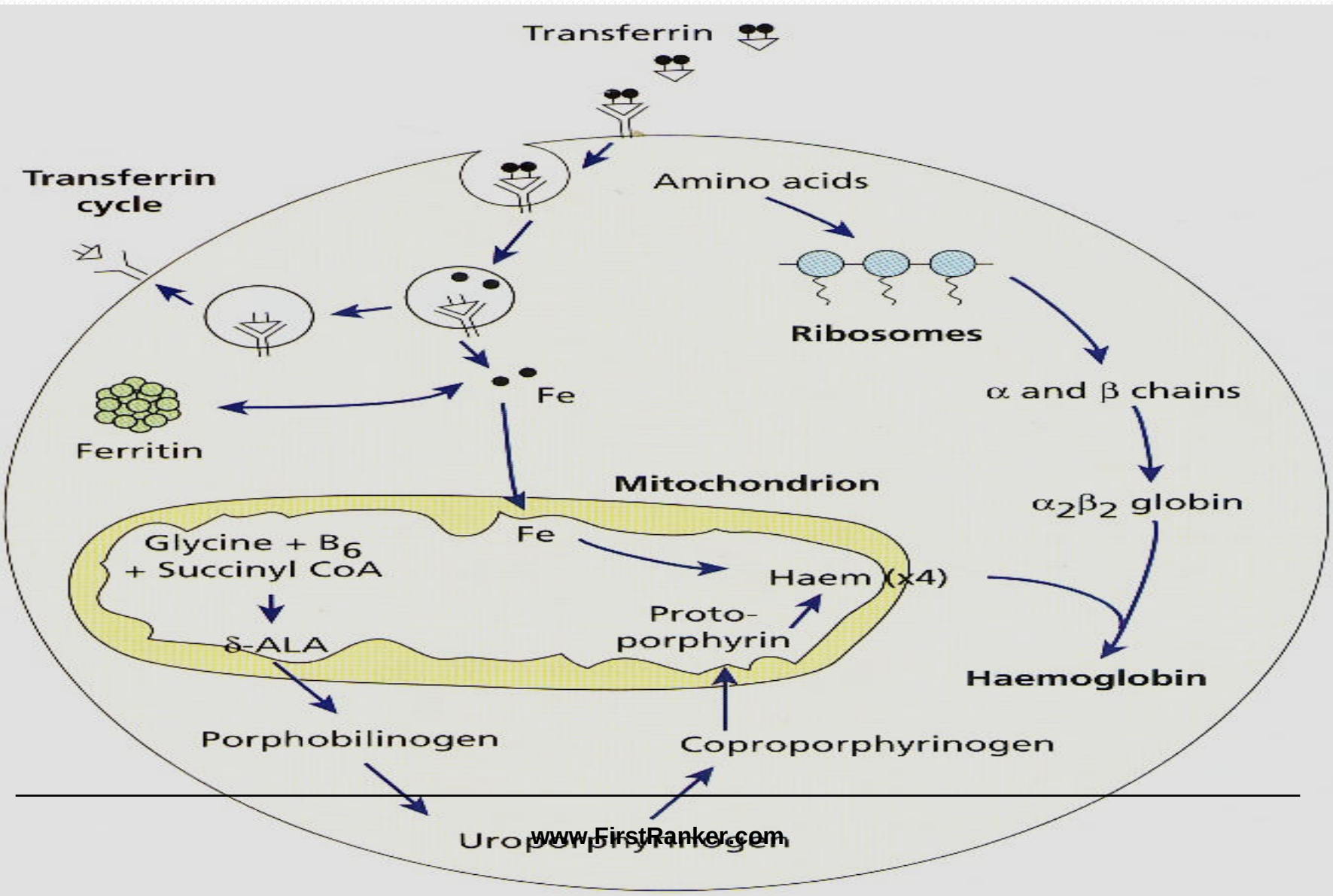
# Assembly Of Hemoglobin



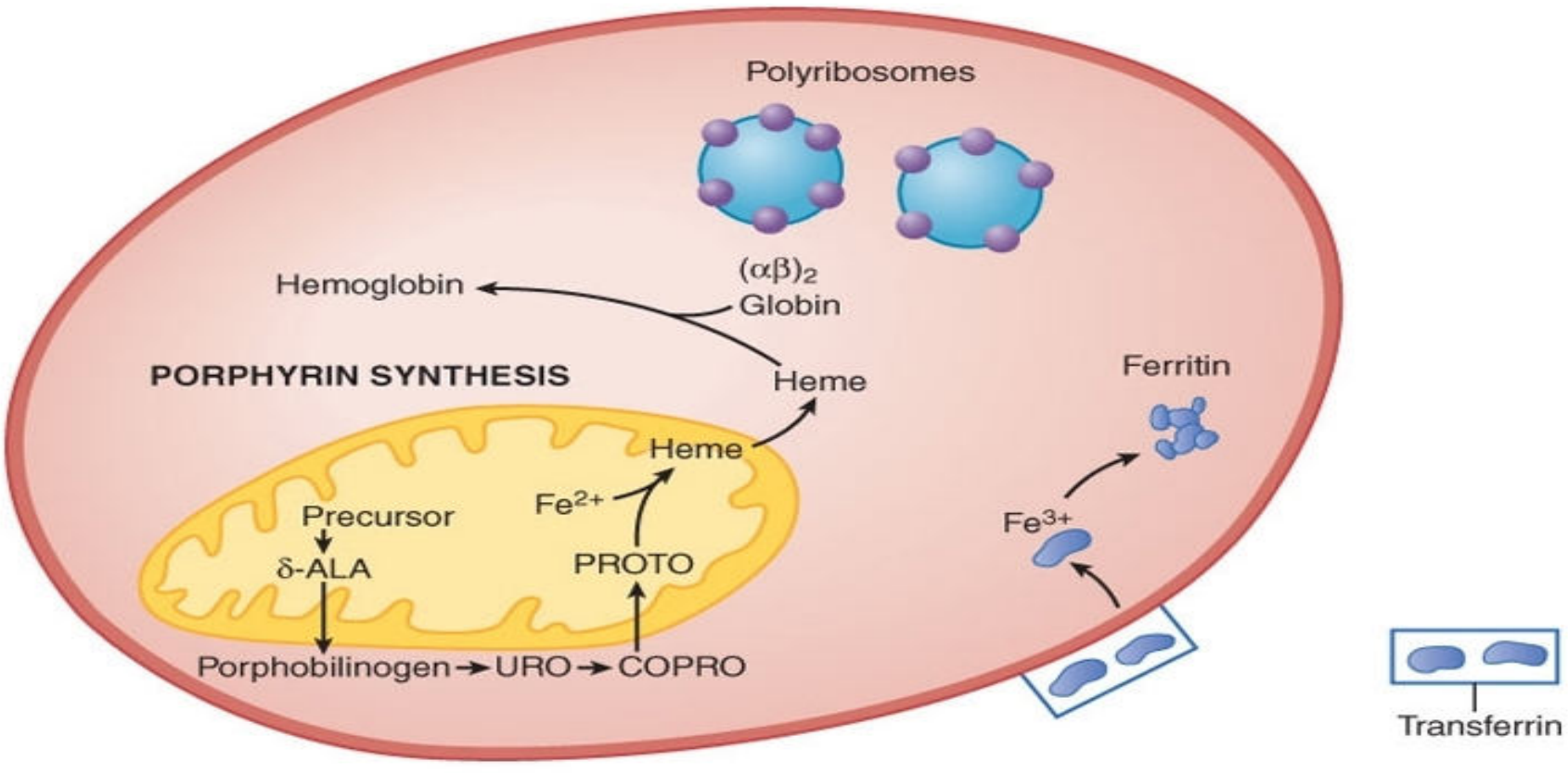
- Although **Heme and Globin synthesis occur separately** within developing red cell precursors,

- Their **rates of synthesis are carefully coordinated** to ensure **optimal efficiency of Hb assembly**.

# Synthesis of Hemoglobin



# HEMOGLOBIN SYNTHESIS




- **Normal structure of Hb** includes the **structure and proportion** of **Globin chains**

- Which are **necessary for the normal function of Hemoglobin**

- **Decreased Concentration of Hemoglobin** in the red blood cells
- Caused due to any abnormality
- Results into a **clinical situation called Anemia**

# Mechanisms Regulating Hemoglobin Synthesis

- **Formation of Hemoglobin is regulated by several mechanisms:**

- The rate of **Globin biosynthesis** is **directly related** to the **rate of Heme biosynthesis.**

- **Heme stimulates Globin biosynthesis** by

- **Inactivating an inhibitor of Globin translation**.

- **Negative feedback of Heme.**
- High concentrations of Heme
- Prevent the mitochondrial import of the first enzyme in Heme synthesis, δ ALA Synthase (δ ALAS).

- **The Concentration Of Iron**
- An **Iron Responsive Element-binding protein** (IRE-BP) binds to mRNA
- Iron Response Elements (IRE-BP) affects the translation of the mRNA for δ ALAS, Ferritin and Transferrin receptors.

- Low Iron Levels = Low Heme Synthesis

- Sufficient Iron Levels= Adequate Heme Synthesis

# Disorders Associated To Globin Chain Synthesis Of Hemoglobin

## Hemoglobinopathies Caused By Abnormal Hb Variants

# What are Abnormal Hb variants ?

- **Abnormal Hb variants** are Hemoglobin's with:
  - **Normal Heme and Altered Globin Chain**
- **Abnormal Hb variants are structurally abnormal**
- The **Abnormal Hb variants may be abnormal functionally**

- Approximately **400 abnormal Hb variants** are detected out.

- But **not all abnormal Hb variants affect** the normal **function of Hb.**

# Basic Cause For Formation Of Abnormal Hb Variants

# Mutations In Globin Genes

- **Altered /Mutated Globin Genes leads to form Abnormal Hb variants.**

# Abnormal Hb Variants

- Occurs due **to Mutation** in **Globin Genes.**
- Leads to **defective Globin chain synthesis.**

# Formation Of Abnormal Hb Variants Leads To Hemoglobinopathies

## What are Hemoglobinopathies?

- **Hemoglobinopathies** are **genetic disorders associated to**

- **Structurally and Functionally Abnormal Hemoglobin variants.**

- **Structurally and Functionally Abnormal Hb variants** in human body leads to **Hemoglobinopathies**

# Types Of Abnormal Hemoglobin Variants and Hemoglobinopathies

- **Broadly two types of Hb abnormalities**

# Qualitative Hb Abnormalities:

- **Mutations** in **Structural Globin** genes

**e.g. HbS-Sickle cell anemia.**

# Quantitative Hb Abnormalities:

- **Mutations in Regulatory Globin Genes**

**e.g. α Thalassemia**
**β Thalassemia**

- **Qualitative**

# Abnormal Hb variants:

- Caused due to **mutations in structural Globin gene**.
- Has **altered amino acid sequence in Globin** polypeptide chain.
- Has **altered Globin subunits** in Hb structure
- But Has **Normal Heme Structure.**

# Examples of Common Abnormal Hb variants And Corresponding Hemoglobinopathy Due to Structural Globin Gene Mutations OR Symptomatic Abnormal Hb Variants

| Abnormal Hb Variants | Globin Gene/Chain Altered | Amino acid Altered In Globin Chain |
|---|---|---|
| **Hb S** Sickle cell Hb | **β** | 6 GLU → VAL |
| **Hb C** Cooley's Hb | **β** | 6 GLU → LYS |
| **Hb D** Punjab Hb | **β** | 121 GLU → GLN |
| **Hb E** | **β** | 26 GLU → LYS |
| **Hb M** | **α** | 87 HIS → TYR |
| Hb has $Fe^{+3}$ | | Proximal |

- **If noted most common abnormal Hb variants has:**
- Altered **β Globin genes** and **β Globin chains.**
- **Substitution** of Polar amino acid **"GLUTAMATE"** with **another amino acid.**

# Non Symptomatic Abnormal Hb Variants

## Abnormal Hb Variants

- Hb P
- Hb Q
- Hb N
- Hb J

# Consequences Of Abnormal Hb Variants

## Presence of Symptomatic Abnormal Hb Variants In RBCs

- **Alters normal structure and function of Hb**
- **Alters morphology of RBC's**
- **Make RBC's fragile.**
- **Causes Hemolysis**, reduces Hb content and affects its function.
- **Leads to Hemolytic Anemia**

- Increases **Unconjugated serum Bilirubin**
- **Causes Hemolytic Jaundice**
- **Possess Splenomegaly** - Increased function of Spleen to remove defective Erythrocytes from the blood circulation.

# Detection Of Abnormal Hb Variants

- **CBC and Blood Film Evaluation**
- **Solubility Test**
- **Electrophoresis**

(Cellulose Acetate and Citrate Agar)

- **DNA Technology-** PCR based techniques:
  - **DNA Finger Print Technique**
  - **Hybridization Technique**

## Hemoglobinopathy-Antenatal Diagnosis

- **Check the Test partners** of heterozygous or affected individuals
- **Antenatal diagnosis from DNA** is obtained by chorionic villus sampling, or by **Amniocentesis**

# Common Abnormal Hb Variant Causing Hemoglobinopathy

## Sickle Cell Hemoglobin (HbS)

- **Hb S** is most commonly occurring abnormal Hb variant.

# Hb S leads to Hemoglobinopathy

# Sickle Cell Anemia

# Biochemical Defect TO Form HbS

- **Formation of**

**Sickle Cell Hemoglobin (HbS)**

- Is a classic example of **point mutation (Transversion)**

- **Point mutation is a substitution of Nitrogen base** in a normal **β Globin gene sequence**.

# Substitution Of Nitrogen Base Which Forms Hb S

- **Altered** Nitrogen base sequence in **Beta Globin gene.**
- There is substitution of **Nitrogen base Thymine to Adenine (T to A).**
- On transcription of mRNA it has altered codon, **GAG to GUG**
- **Altered** amino acid substitution in the **beta Globin chain.**
- **Glutamate substituted by**

- On translation at **6th position of β Globin chain** polar amino acid **GLU** is **substituted by** non polar amino acid **VAL** .

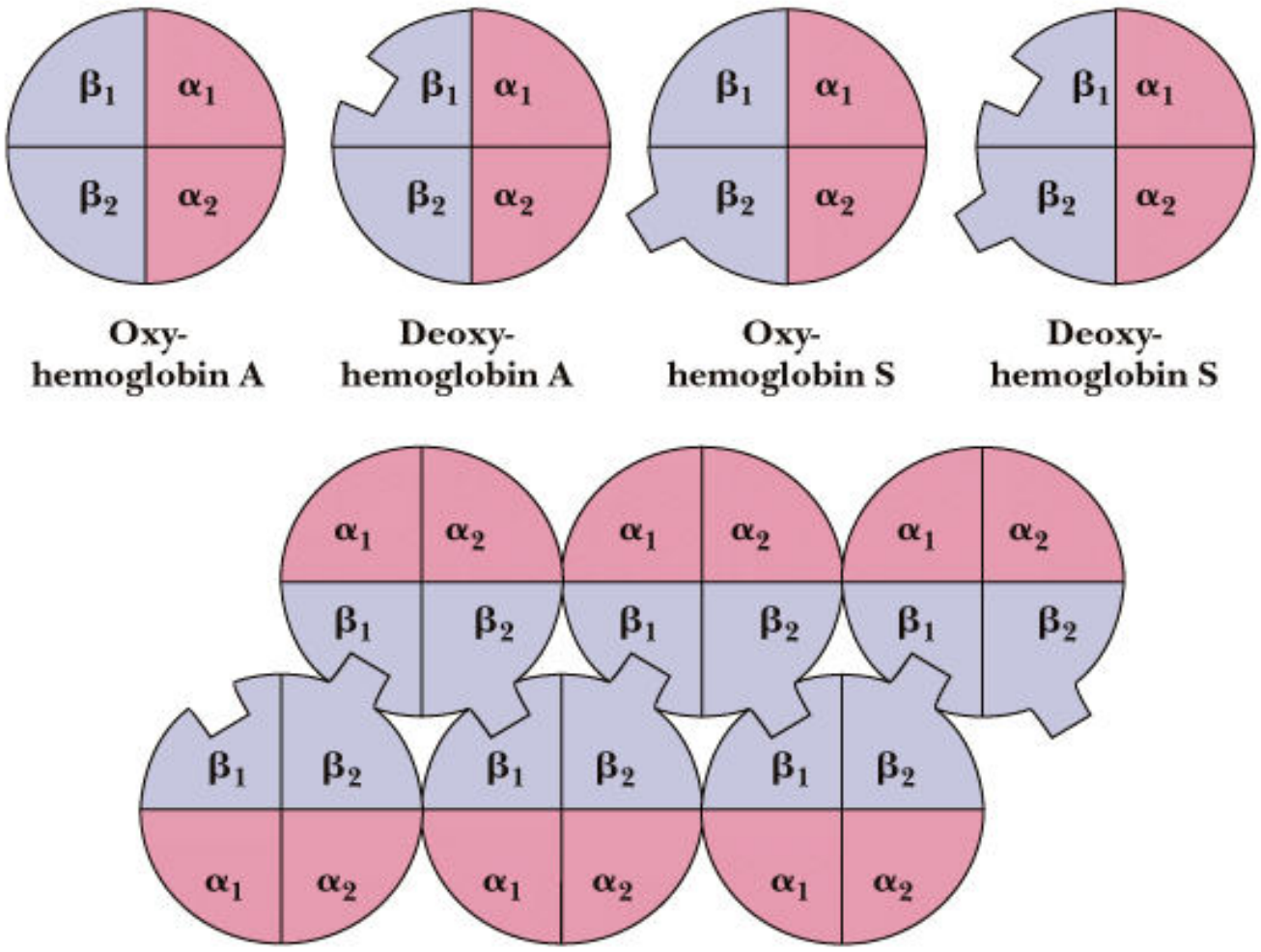
- **This transforms HbA1 to HbS.**

# Deoxystate of HbS Affects Solubility

- HbS – Is sickle cell Hb.
- Altered **HbS affects the solubility of Hb** at Deoxystate in RBCs.

- **HbS at low oxygen tension** / deoxy state **forms Deoxy HbS**.
- DeoxyHb S looses its polarity /solubility
- Deoxy HbS forms **protrusion on the β globin chain**.
- **Sticky patch** appears on HbS at deoxy state.

Garrett & Grisham: Biochemistry, 2/e
Figure 15.40




Deoxyhemoglobin S polymerizes into filaments

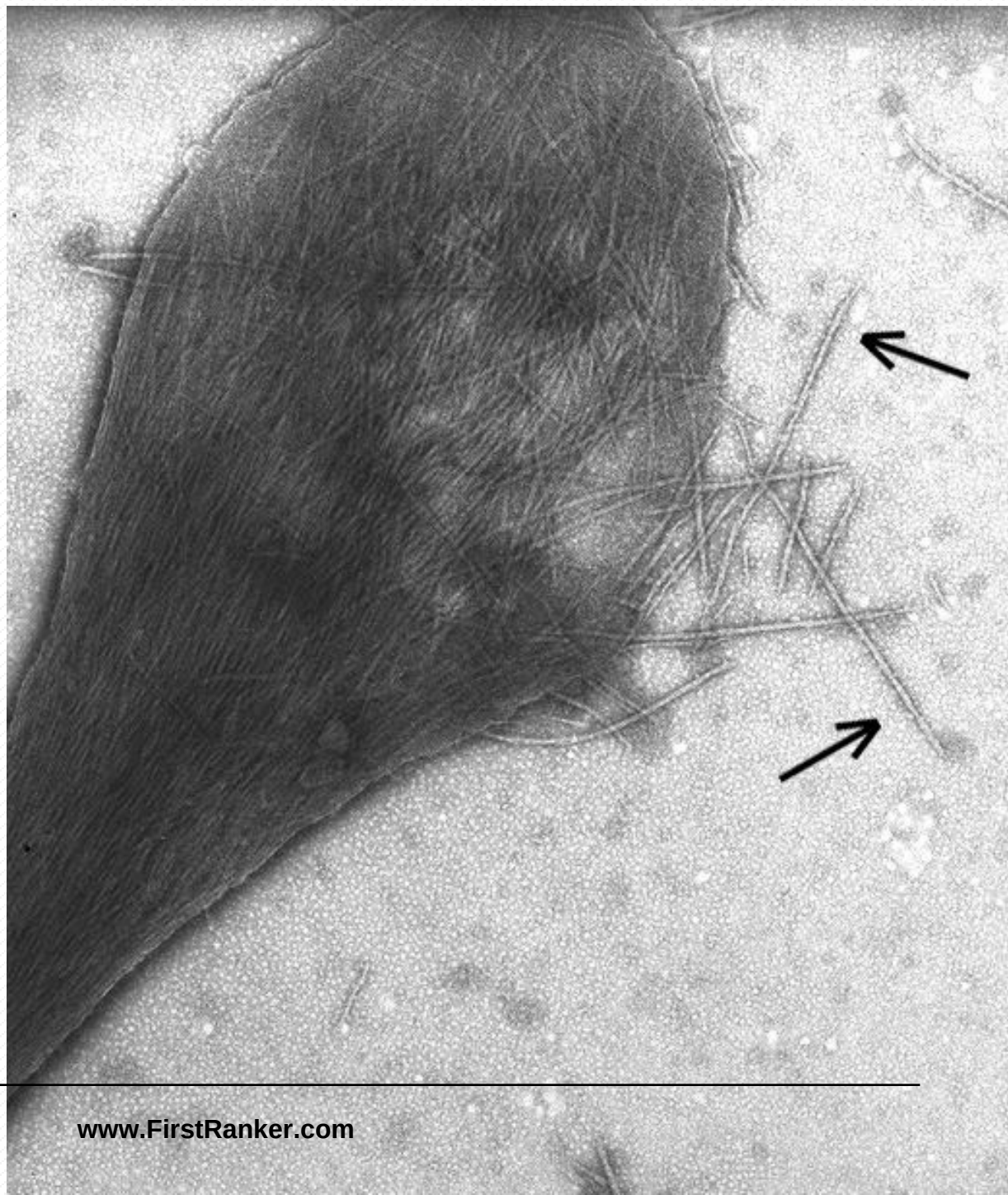
Saunders College Publishing

- Each **Hb S fits into this complementary site** of another β globin chain.
- Many **Hb S polymerizes** inside the RBC's
- Forming a **network of fibrous polymers.**

- HbS aggregates into long, rigid polymers are called **Tactoids**.

- This makes **HbS relatively insoluble and non functional**.

EM of Red Blood Cell showing ‘Tactoids’



- **Tactoids stiffen** and **distort** the **red blood cells.**

- RBC's **changes morphology** and appear **sickled/crescent shaped.**

- **Thus HbS Leads To**

- **Sickling** of Erythrocytes and **hemolysis**.

- **Sickled** Erythrocytes may return to their **original shape** when oxygenated.

# Effect of Sickled RBC's And its Associated Complications

- **HbS Causes**
- **Reduction of RBC life span to just 20 days.**
  - Sickling of RBC's
  - Distortion and lysis of RBC's
  - Hemolysis

- Sickling distorts and **make RBC's fragile.**
  - After **several sickling episodes of RBC's**
  - There is **irreversible damage to RBC membrane.**

- Thus Sickling Of RBC's causes **Sickle Cell Anemia/Hemolytic Anemia.**

- Sickled cells are **phagocytized** by **macrophages**.

- In the spleen, Liver or bone marrow.

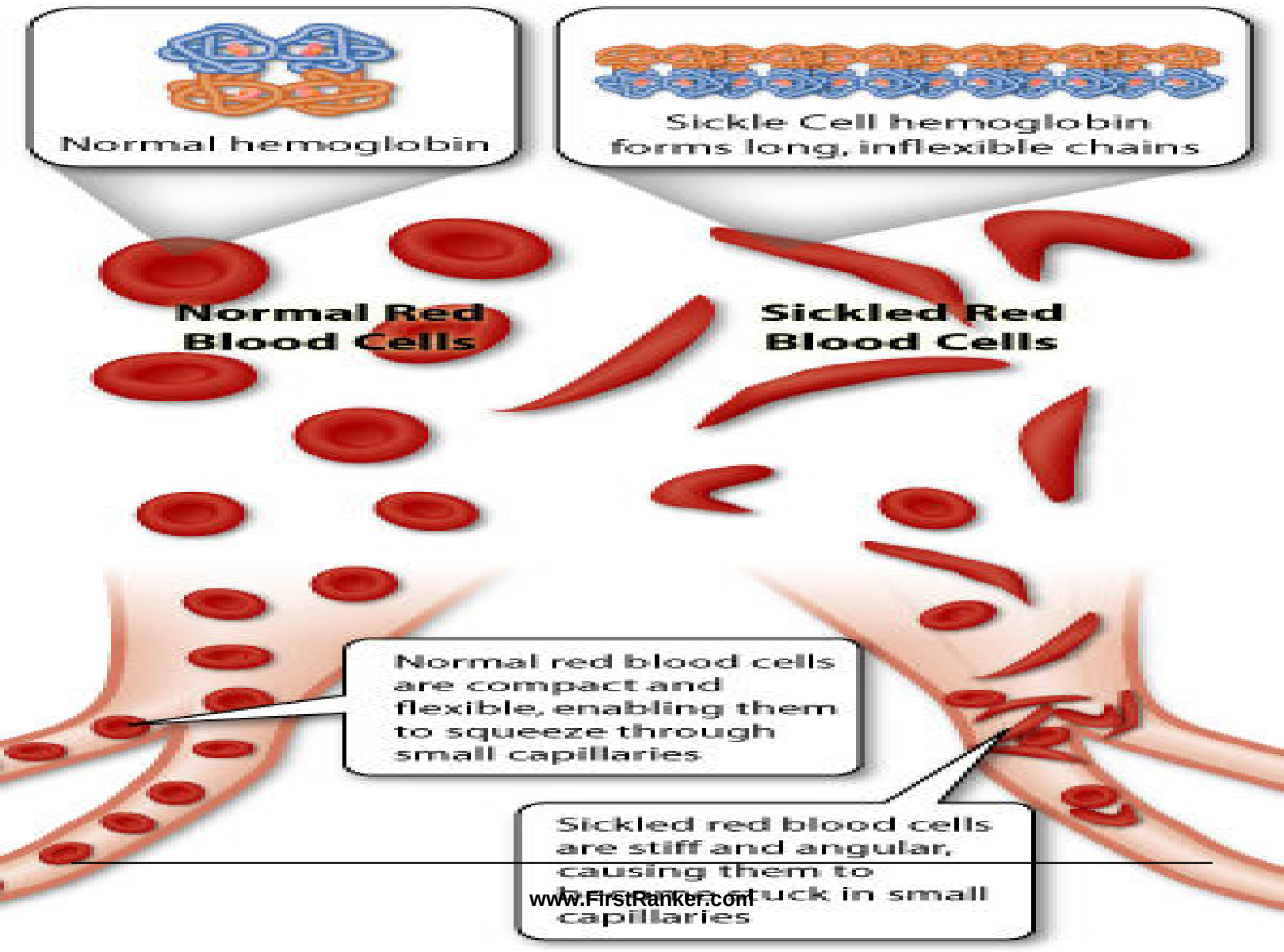
- **Sickling** of RBC's makes **blood viscous**

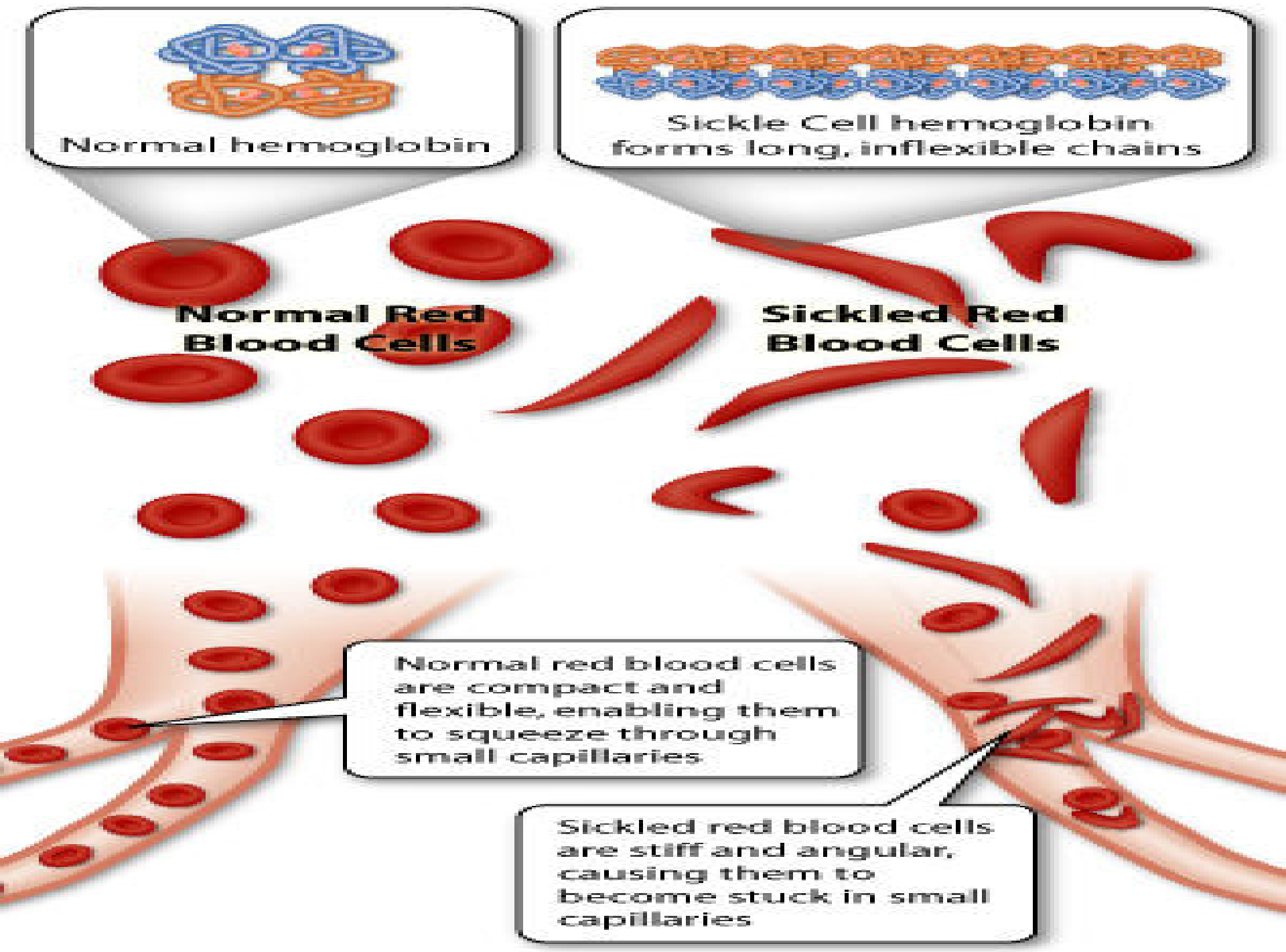
- **Lowers the rate of blood circulation.**

- Sickled cells has **increased** tendency to **adhere to blood vessels**.

- Rigid sickled cells **unable to squeeze out through small capillaries**

- Sickled cells get trapped in **small capillaries** and **block** them.






- **Sickling** produces **localized Anoxia/Tissue Hypoxia**
- **Oxygen deprivation** in the tissues.
- **Lowers ATP production in cells**.
- **Anoxia** in turn **leads to increased sickling** process.

- Sickling causes **pain and infarction** (death) of cells in tissues.

- Sickling causes **Spleen Dysfunction**

- Making the spleen non functional

- Sickling Increases susceptibility towards **tissue infection**

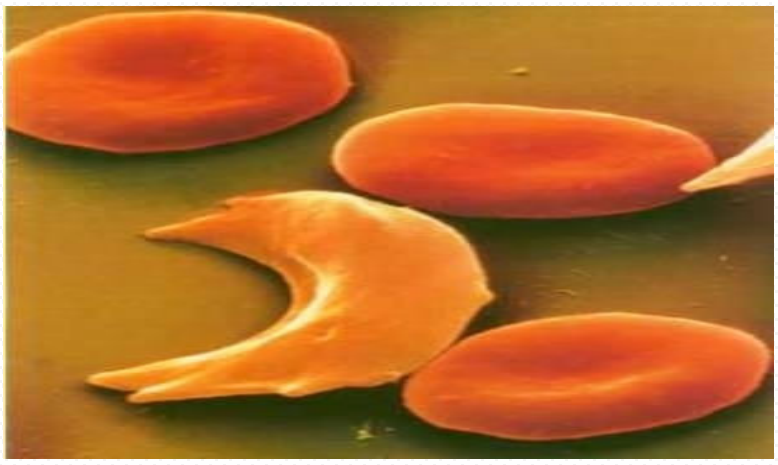
- **Premature death** of individuals **before 20 years** due to infections.

# Factors Increasing Sickling Of RBC's

- Extent of RBC's sickling is related to
  - **Amount of Hb S** present in Erythrocytes.

- **Conditions Creating Hb S in Deoxy state:**
  - **Decreased $pO_2$**
  - **Increased $pCO_2$**
  - **Decreased pH**
  - **Increased 2,3 BPG**
  - **Dehydration**

# Sickle Cell Anemia



- **Sickle Cell Anemia** is a **genetic disorder due to presence of abnormal Hb variant HbS**
- **It is a Commonest type of a Hemoglobinopathy**
- **It is a type of Hemolytic Anemia due to Sickling of Erythrocytes**

# Inheritance of Sickle Cell Anemia

- Sickle Cell Disease is an **Autosomal recessive disorder**

# Prevalence and Incidence

- **Prevalence of HbS**
- Tropical areas
- Africa
- South America
- **Incidence of HbS**
- 1:5000 births.

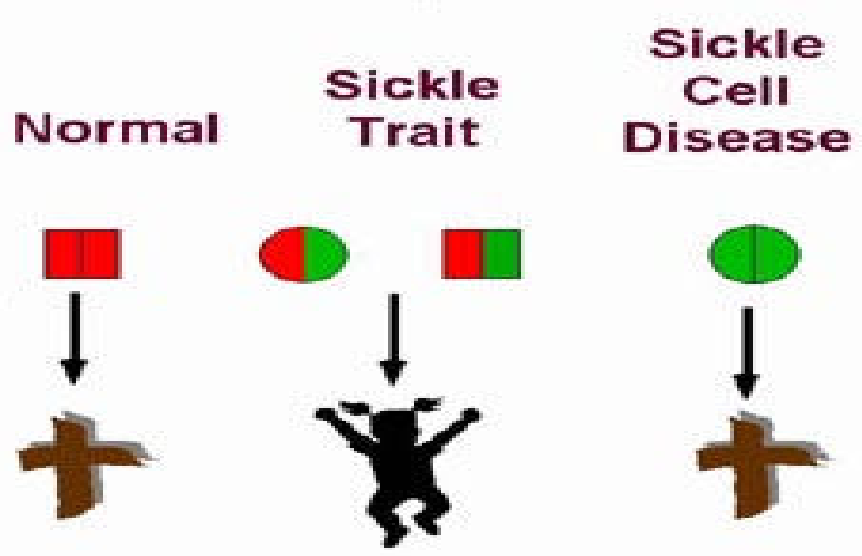
# Biochemical Defect To Cause Sickle Cell Anaemia

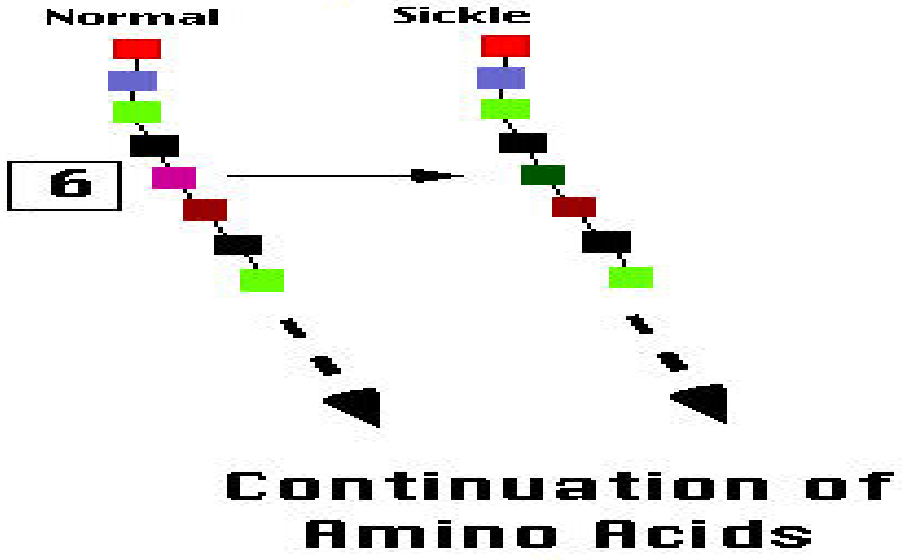
- Sickle Cell Anemia is caused by a **point mutation** in structural **beta Globin gene**

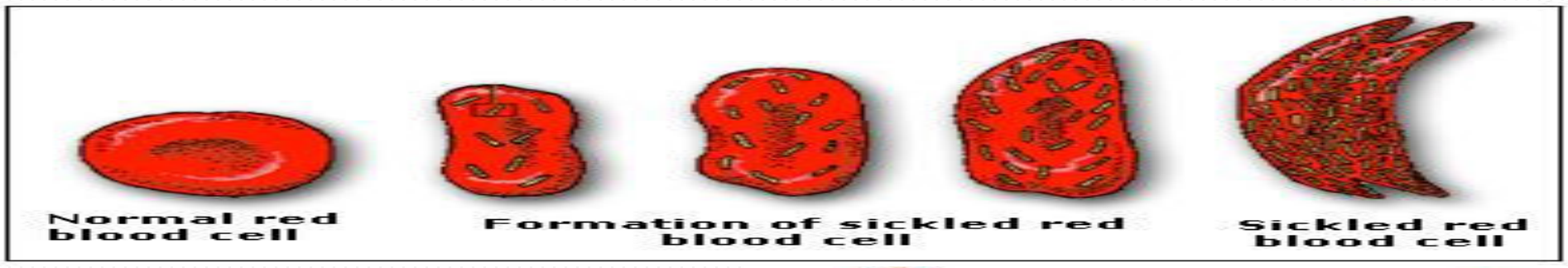
- Characterized by **the presence of abnormal HbS** in Erythrocytes.

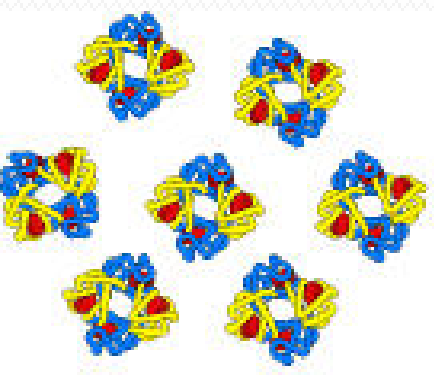
- Hb S in **Deoxy state** promotes formation of **hard, sticky, sickled-shaped red blood cells – Sickling of RBCs.**

# Types of Sickle Cell Anemia

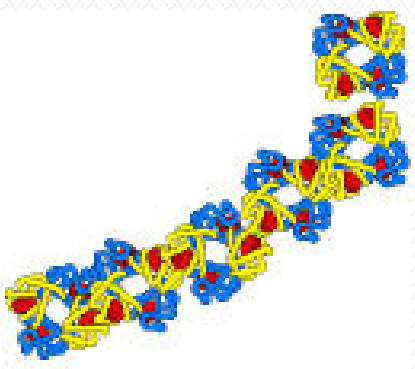


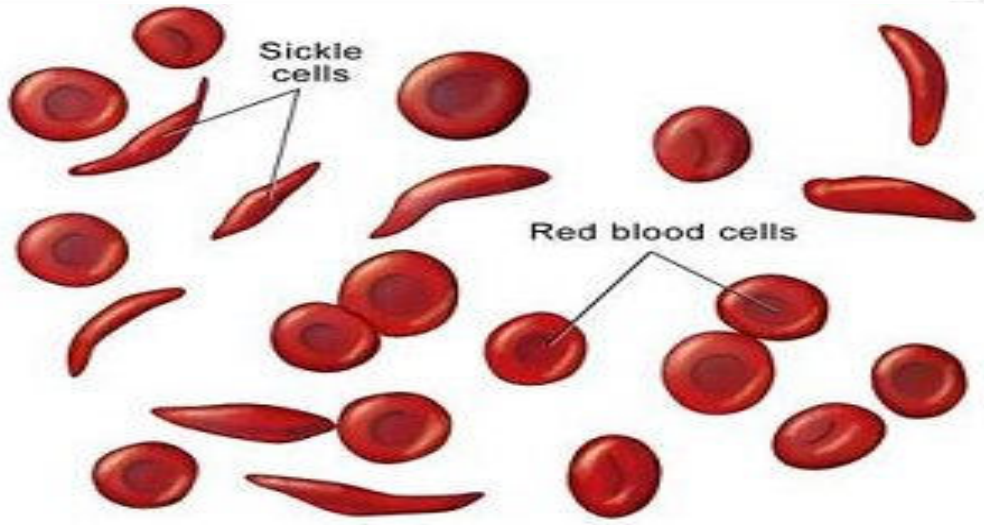



NORMAL HEMOGLOBIN



CLUMPED HEMOGLOBIN




# HbSS

- HbSS is sickle cell disease
- **Homozygous state**
- Full blown disease
- **100% HbS concentration**.

- **Both** β Globin **genes** of **2 chromosomes** are **mutated**.
- β Globin chain has alteration at **6 Glu to Val**

## HbAS

- HbAS is **sickle cell trait**
- **Heterozygous state.**
- **50% HbA1 and 50 % HbS**
- Symptoms are **mild and less severe.**
- Fatality can be delayed.

- **Sickle cell trait** offers **protection** from **Malarial parasites-** Plasmodium falciparum.

# Hb SC Disease

- Another **red cell sickling disease**
- Individual has **mutant genes** for both **Hb S** and **Hb C.**
- Has significant **clinical variability**
- **Less severe anemia**
- **Less painful crises.**

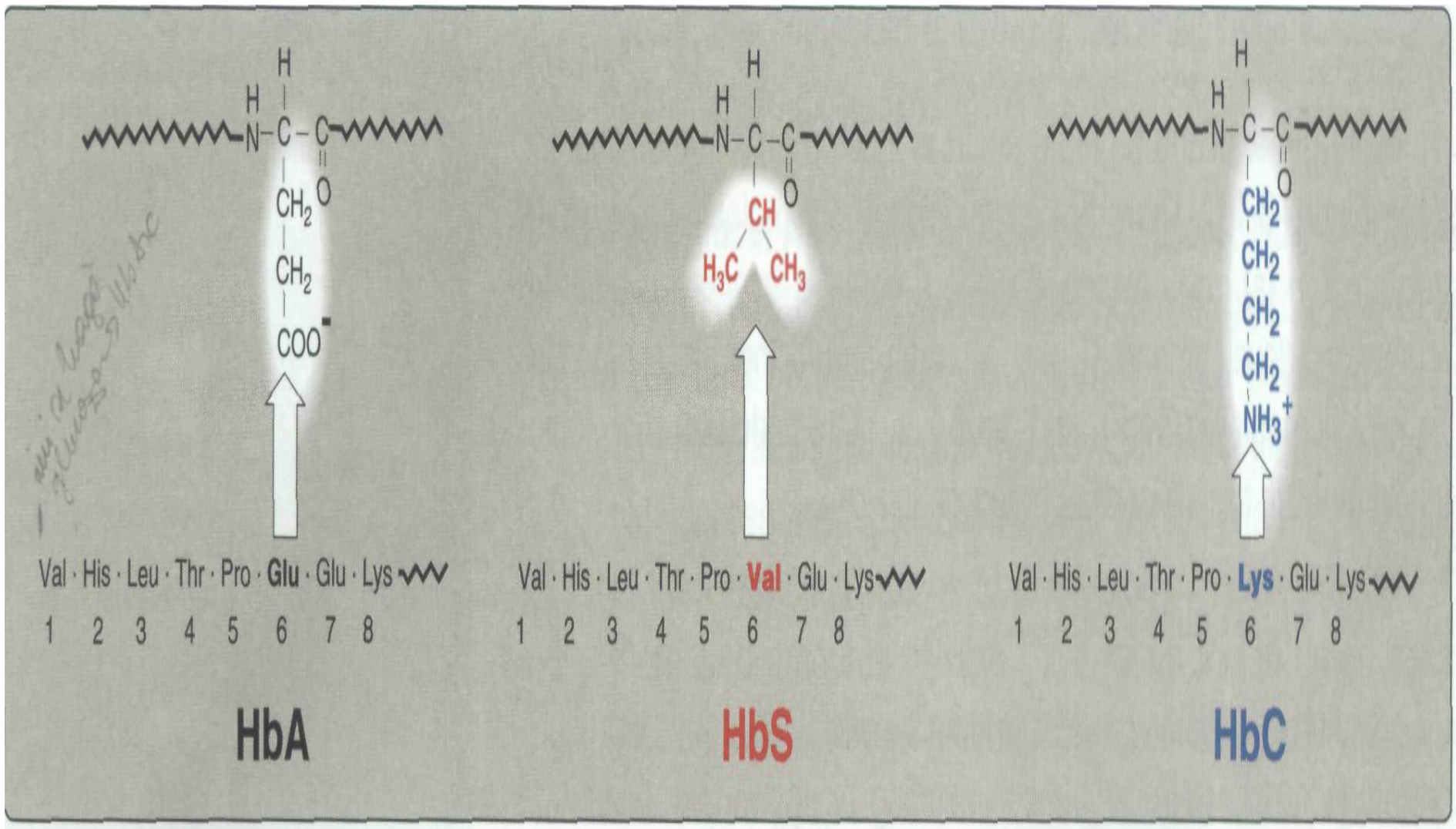



**Figure 3.19**
Amino acid substitutions in HbS and HbC.

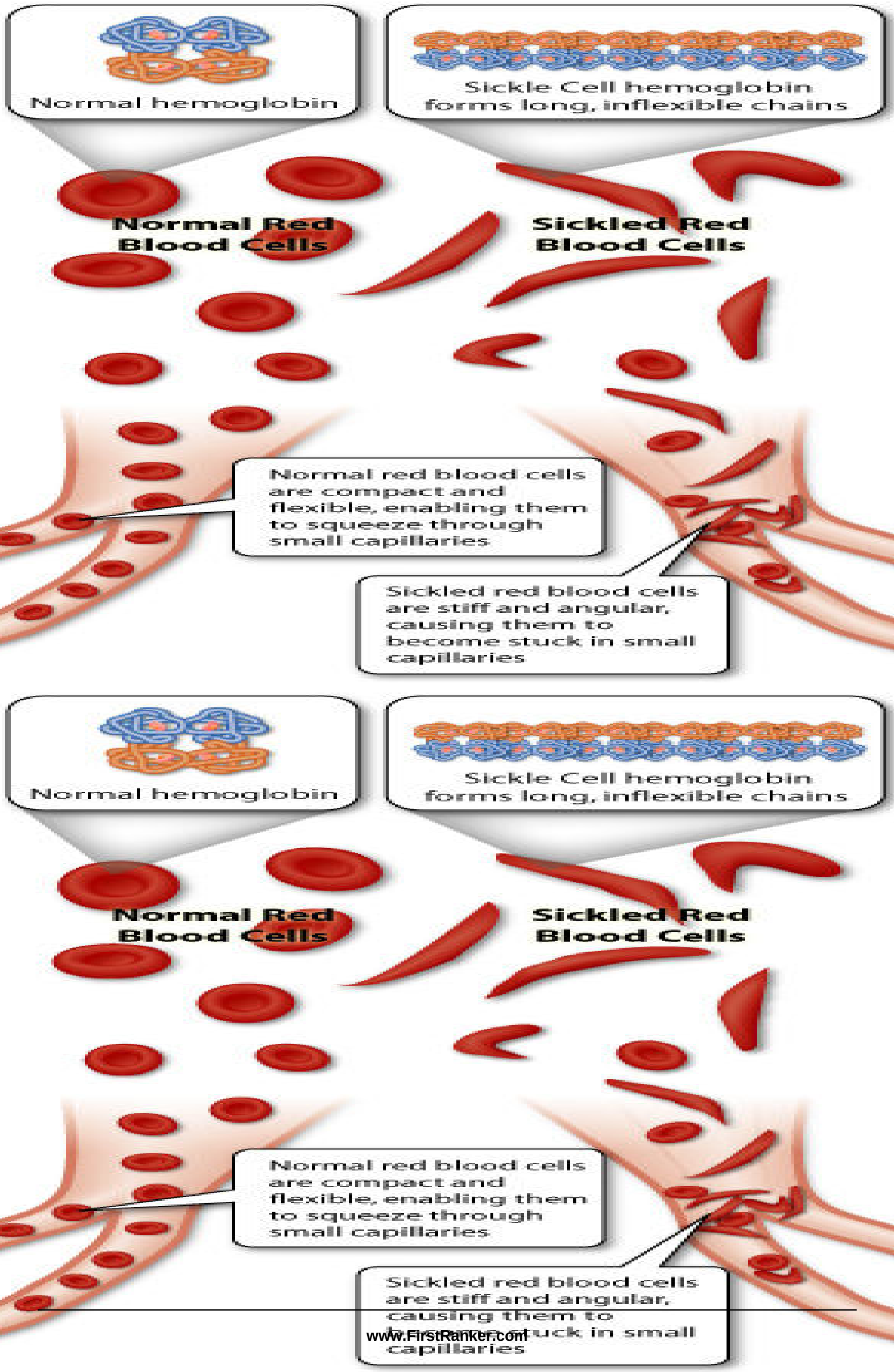
# Effects And Complications Of Sickle Cell Anemia

- **Sickle Cell Anemia Leads To**

- **Hemolytic Anemia**
- **Hemolytic Jaundice**

## Sickle Cell Anemia
## Main Clinical Features

- Hemolysis
- Occlusion of blood vessels by sickled red cells




Normal hemoglobin
Sickle Cell hemoglobin forms long, inflexible chains
Normal Red Blood Cells
Sickled Red Blood Cells
Normal red blood cells are compact and flexible, enabling them to squeeze through small capillaries
Sickled red blood cells are stiff and angular, causing them to become stuck in small capillaries

- Hemolysis /Lysis of Sickled RBC
- Low Oxygen transport to tissues (Hemolytic Anemia)
- Hemolytic Jaundice
- Tissue Hypoxia
- Tissue Infarction
- Tissue Infection
- Painful Crisis
- Fatality in severe cases

| Site of Sickling | Clinical Features | Management |
|---|---|---|
| **Bone** | Painful crises | Pain relief and hydration Hydroxyurea |
| **Lung** | Acute chest syndrome | Transfusion regimen, pain relief and hydration |
| **Brain** | Stroke | Transfusion regimen. |
| **Heart** | Myocardial infarction | Transfusion regimen, pain relief and hydration |
| **Spleen** | Acute splenic sequestration | Transfusion, pain relief and hydration |
| **Spleen** | Hyposplenism | Pneumovax |
| **Retina** | Proliferative retinopathy | Retinal surveillance. Laser |

- **'Tactoids' form at low oxygen tension**
- **Stiff Sickled red cells occlude small blood vessel**
- Tissue Hypoxia and Infarction
- Tissue Infections
- Symptoms are more severe
- Fatality confirmed

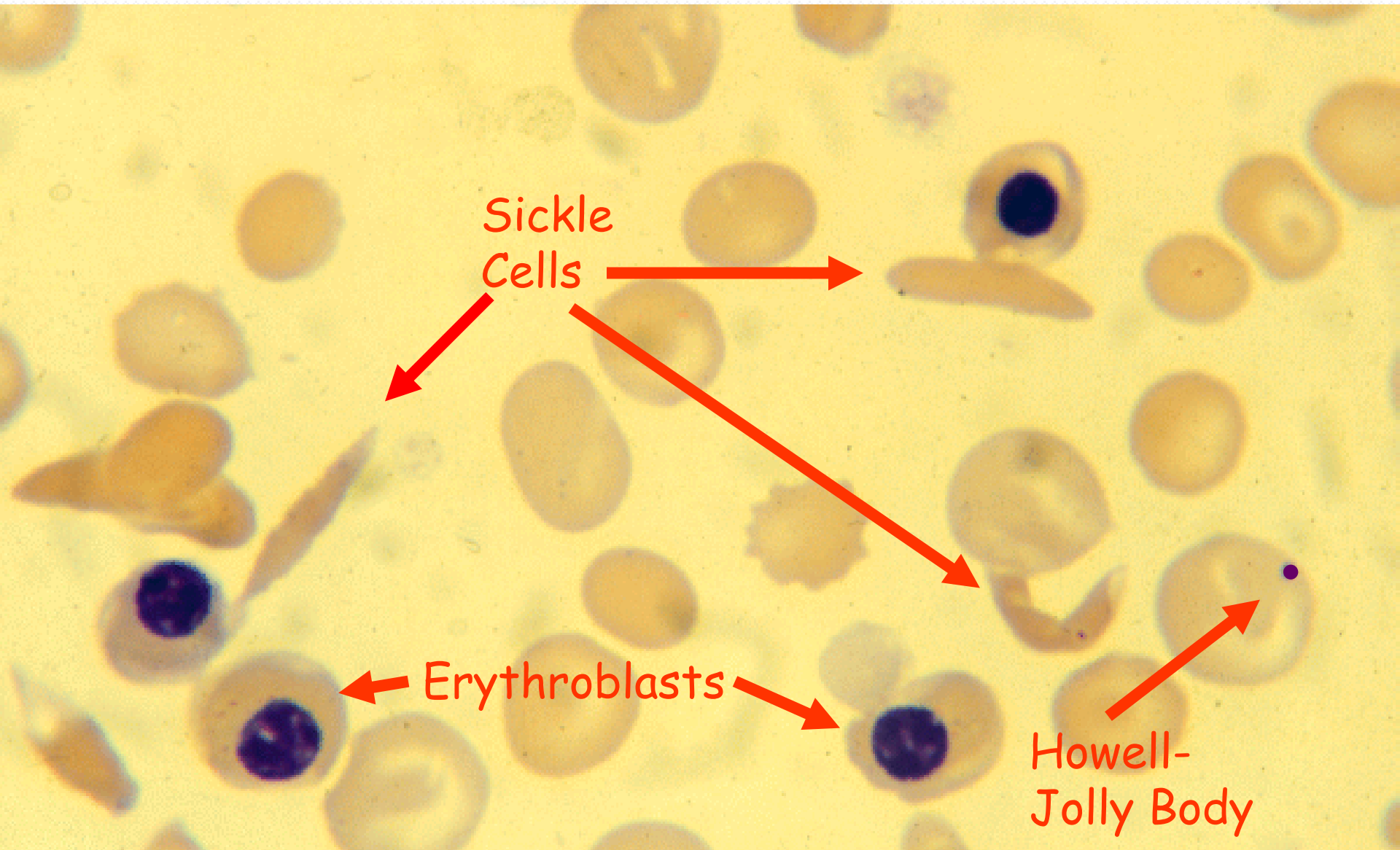
- Sudden death during intensive training
- **Hematuria, Isosthenuria** (Renal Papillary Necrosis)

- Infected RBC has **incomplete life cycle of parasites.**
- **Sickled** erythrocytes efficiently **phagocytized** and **destroyed.**
- **Low $K^+$** ion concentration in Hb S is **unfavorable** for **malarial parasites to develop.**

## Diagnosis of Sickle Cell Anemia

- **Sickling Test-**
- Using **Sodium Dithionite reducing agent** on blood smear
- Watch microscopically for sickled RBC's.

# Sickle Cell Anemia – Blood film




- **Electrophoresis**

- Hb S is confirmed with **Cellulose Acetate Electrophoresis**

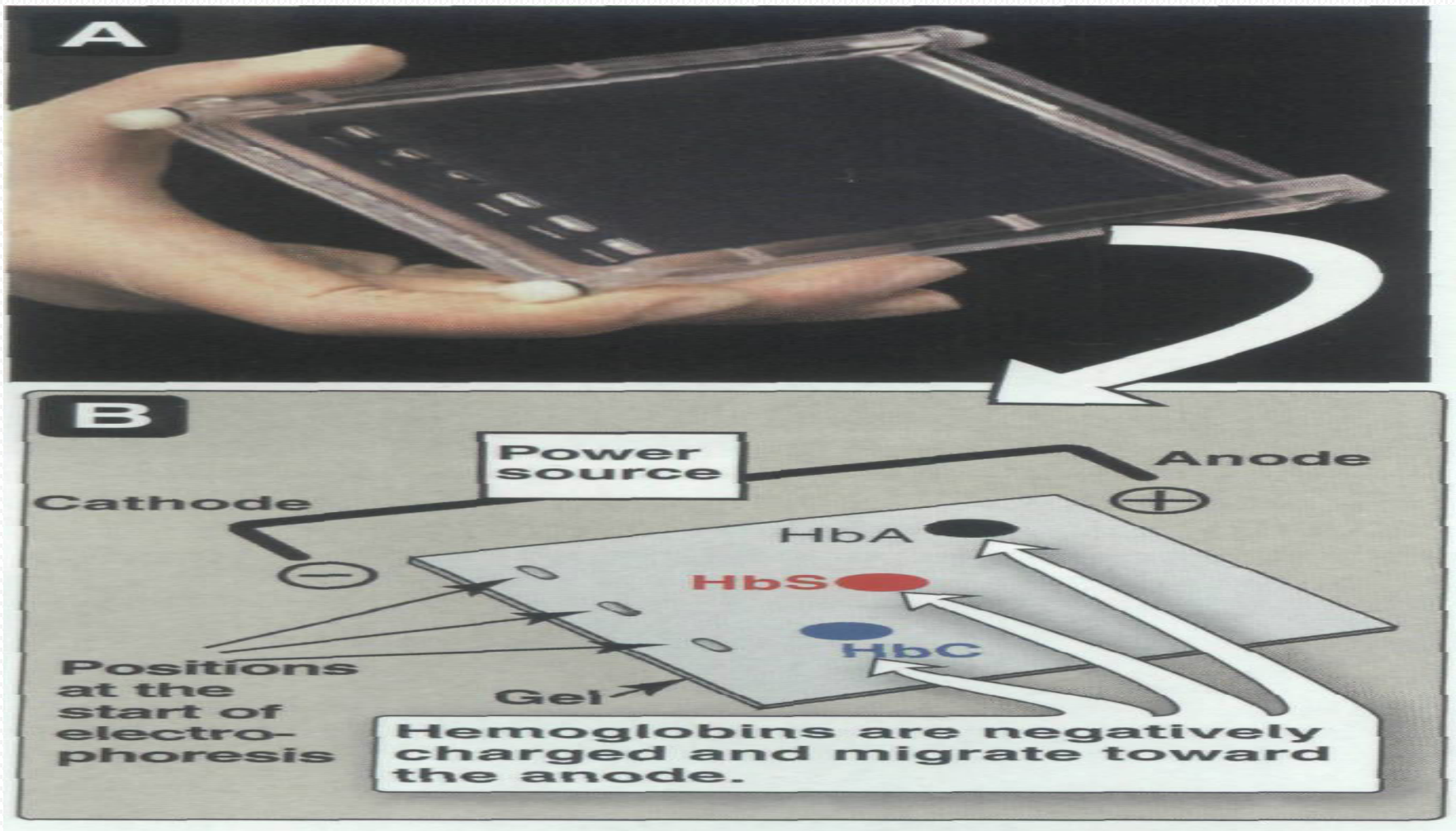
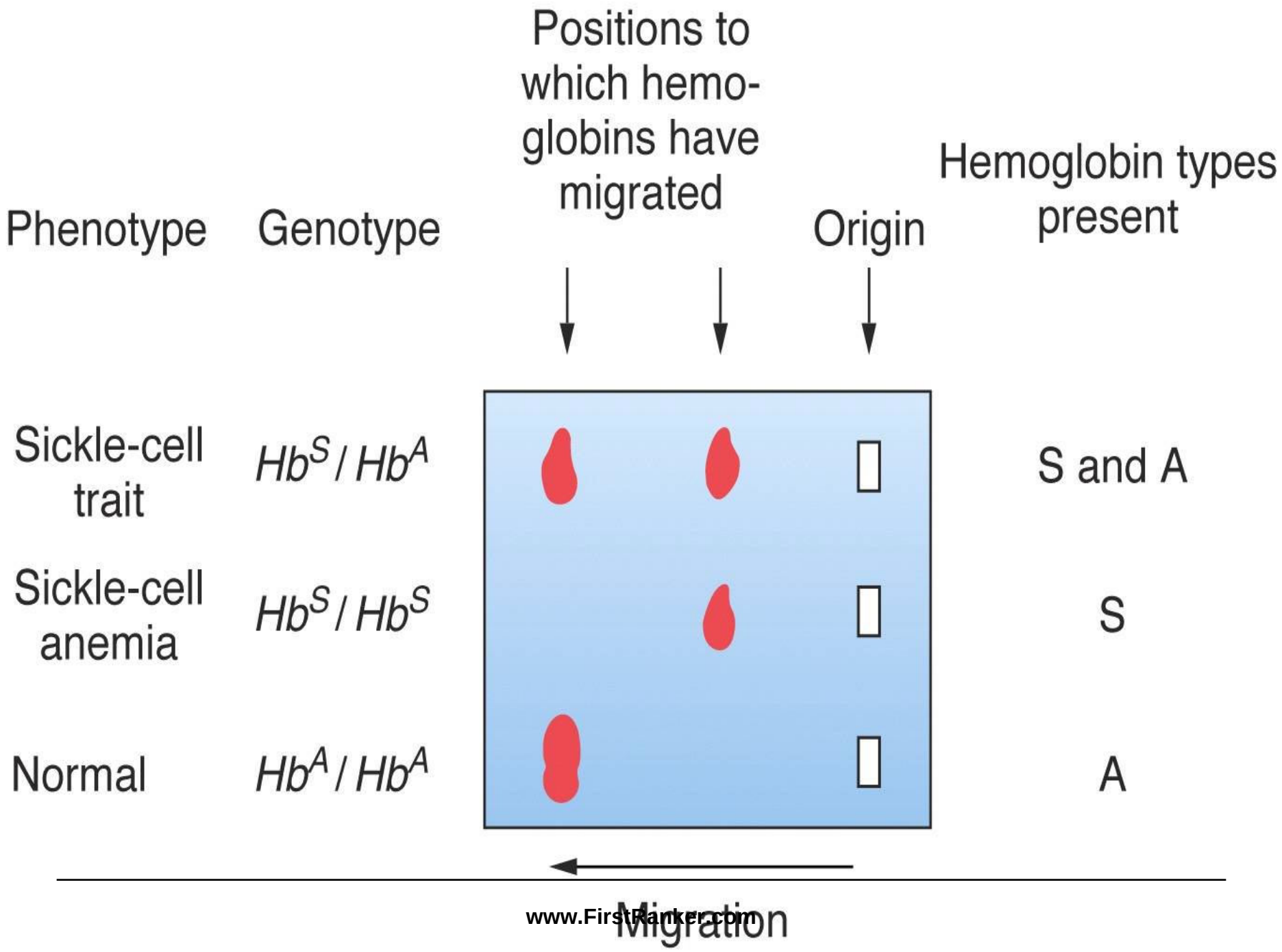



**Figure 3.20**
A. Photograph of a gel prior to electrophoresis. B. Diagram of hemoglobins A, S, and C after electrophoresis.



- At Alkaline pH during Electrophoresis.
- Hb S is **less negatively charged** than Hb A1.

- Hb S moves in a position between Hb A1 and Hb A2.

- Hb S **migrates more slowly** towards anode than Hb A1.

- Altered mobility of HbS is **due to absence of negatively charged Glutamate residue.**

## Sickle Cell Anemia - Treatment

- Adequate **hydration**
- **Analgesics** to relive pain
- Aggressive **Antibiotic therapy** to arrest the infection.

- Ingestion of **0.01 M of Potassium or Sodium Cyanate.**

- Prevents sickling of RBC's and its complications.

- Opiates and hydration for painful crises
- Pneumococcal vaccination
- Retinal surveillance

- **Hydroxyurea** an antitumor drug
- Used in therapy of Sickle cell anemia.
  - Increases circulating levels of Hb F
  - **Decreases Sickling**
  - **Decreases painful crises**
  - **Reduces mortality**

- **Blood Transfusion** for serious manifestations
- Support with **Folate, Iron chelation.**

---



- **Stem cell transplant**

# Thalassemias

- Thalassemia's are **Hemoglobinopathies**
- Caused due to **defect/mutations in Regulatory Globin genes** of Globin chain synthesis.

- Individual suffering from Thalassemia's has

- **Structurally** and **functionally** **unfavorable abnormal Hb variants**.

- Thalassemias are **Autosomal recessive** blood disorders.

- **Thalassemias are** Characterized with Anemia

- **Thalassemias** mostly occur in regions of **Mediterranean sea.**
- Also termed as **Mediterranean Anemia.**

- **Thalassemias are also prevalent**

- In Arab,Americans, and Asians
- In populations where Malaria is endemic

# Causes Of Thalassemias

- **Thalassemias due to Regulatory Gene mutations is a quantitative abnormality** of Hemoglobin.

- Mutations in **Regulatory Genes** of Globin chain synthesis.
- **Suppression of Globin chain synthesis.**

- **Reduced/Absent** of **one or more of Globin polypeptide chain synthesis** of Hb.

- Globin chains has normal amino acid sequence.

- **Alpha Thalassemia** – reduced alpha chain synthesis

- **Beta Thalassemia** – reduced beta chain synthesis

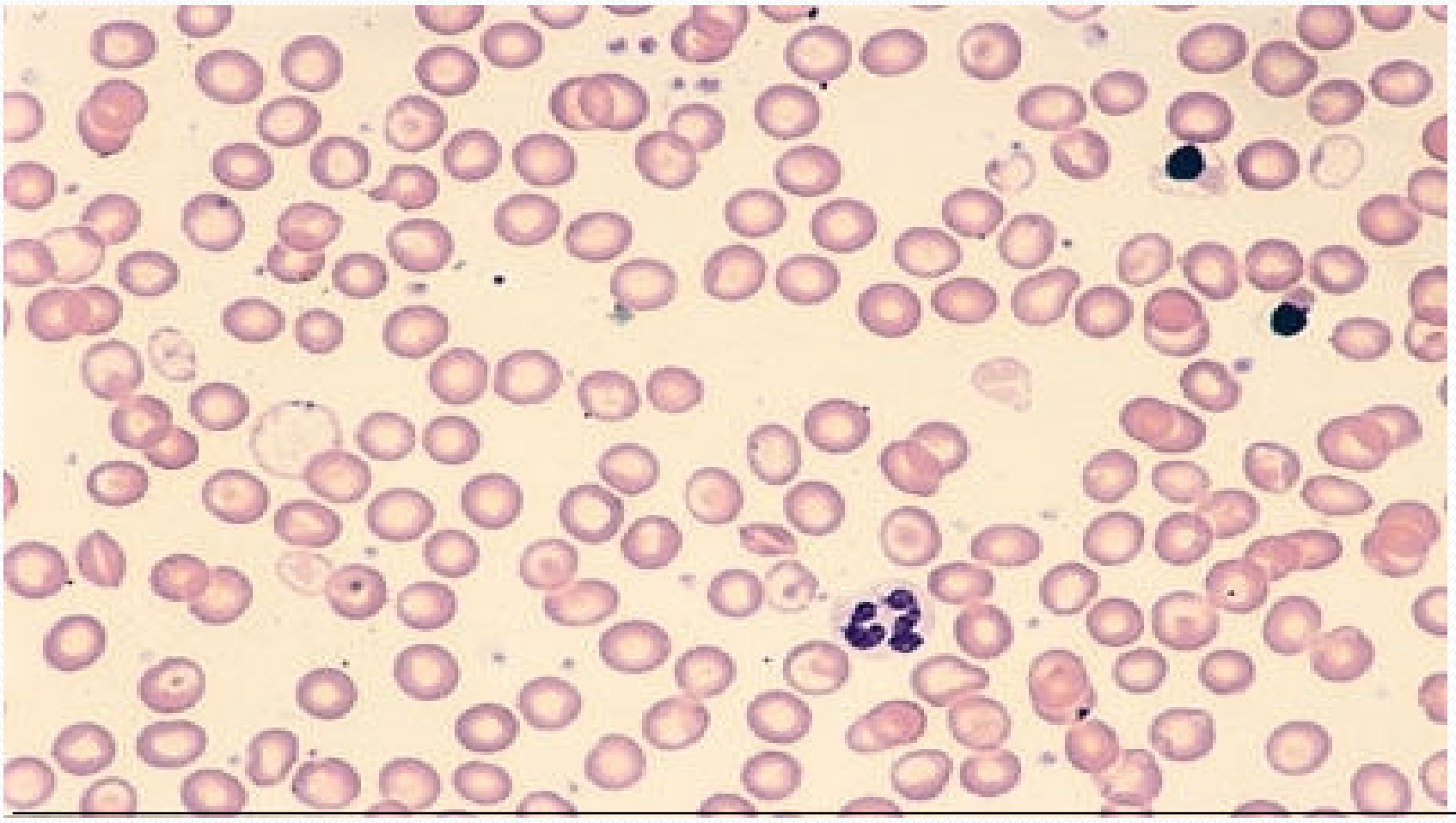
- **$\beta^+$ Reduced production of β chains**
- **$\beta^o$ complete absence of β chains**

- **Compensatory** Globin chain **synthesis** occurs in Thalassemias.

- **Unbalanced production** of **Globin chains** in Thalassemias causes

- **Erythrocytes** to be **small, hypochromic** and sometimes **deformed**.

# Blood Picture Of Thalassemia



- There occurs intracellular **accumulation of unmatched Globin chains** in the developing Erythrocytes
- **Precipitation of the Proteins**, which leads to cell destruction in the bone marrow.

- **Infective Erythropoiesis.**
- **Mature** functional **RBC's do not reach** the **peripheral blood to carry oxygen.**

# Types of Thalassemia

- **Thalassemia Minor**
- **Heterozygous State**
- Asymptomatic

- **Thalassemia Major**
- **Homozygous Type**
- **Lethal at birth** or in childhood.
- Has many **complications**
- **Early** and **Continuous treatment** of β Thalassemia **allows survival** to young adulthood .

# α-Thalassemia

- **Suppression of α Globin genes**
- No/reduced α globin chains synthesis.
- **Compensatory more β/γ globin chains synthesized.**

- **Abnormal Hb in α-Thalassemia**
  - **HbH – $\beta^4$**
  - **Hb Bart - $\gamma^4$**
- Affect normal function of Hb
- Anemia
- Fetal death

# Types Of Alpha Thalassemias

| | |
|---|---|
| αα/αα | Normal |
| αα/α- | Mild microcytosis |
| αα/- -<br>α-/α- | Mild microcytosis |
| α-/- - | Hemoglobin H disease |
| - -/- - | Hemoglobin Barts Disease – Hydrops Fetalis |

# Silent Carriers of α Thalassemia

- Out of 4 α Gene there is **missing of only 1 α Gene.**
- Remaining **3 α genes produces sufficient α chains for normal Hb production.**
- **1-2 % of Hb Bart** in cord blood.

# α Thalassemia Trait

- **2 α Genes are deleted**
- Shows **mild microcytic hypochromic anemia**
- Occasionally Hb H inclusions
- Cord blood contains **2-10% of Hb Bart.**

- **Hb H inclusions** can be seen in RBC's after **supra vital stain.**
- Cord blood contains 10-20% Hb Bart.

# Hb H Disease

- Type of α Thalassemia where **3 α genes absent**
- Hb H present-Tetramer of β chains.
- Alters shape of RBC's
- Shorten RBC life span.
- Moderate hemolytic anemia.

# Hb Bart Disease

- **Most clinically severe form α Thalassemia.**
- Where **all 4 α genes deleted**
- **Total absence of α chain biosynthesis**

- Hb Bart major Hb found- tetramer of γ chains.
- **Hydrops Fetalis**

**(Fetal Anemia causes Edema)**

- **Hb Bart has extremely high Oxygen affinity**
- Allows Oxygen transport **but no release at tissues.**
- Hypoxia
- **Still born infants/ die shortly after birth.**

# Beta Thalassemia

Beta Thalassemia

- Suppression of β Globin gene.
- **Reduced/ no** production of beta **globin chains**.
- Compensatory **γ/δ Globin chains biosynthesized.**

- Abnormal Hb in beta Thalassemia:
  - **HbF (α2γ2)**
  - **HbA2(α2δ2)**

## Types Of Beta Thalassemia

- **Beta Thalassemia minor**
  – heterozygous (or trait)
- **Beta Thalassemia major**
  - homozygous

# Beta Thalassemia Trait

- No symptoms
- Mild microcytic anemia

# Beta Thalassemia Major

- No beta chain produced (no HbA)
- **Cooley's Anemia**
- Homozygous disease

# Beta Thalassemia Major

- **Crippling disease of childhood**
- **Persistent HbF in age above 1 year**
- Reduced unloading of oxygen at tissues.
- Premature destruction of RBC's.
- Severe **hypochromic microcytic anemia** occurs gradually in the first year of life
- Bone Marrow expansion

- **Hypersplenism**
- **Hepatosplenomegaly**

- MCV low
- Severe Anemia
- Reticulocytosis
- Extreme **Poikilocytosis** (Different Shape ) and **Anisocytosis** (Different Size).

# HPFH

- **Hereditary Persistence of Fetal Hb (HPFH)**
- It is a **Genetic heterogeneous disorder**
- **Caused due to deletions of Genes in chromosome 11.**
- **Exhibits total absence of β and δ Globin chain synthesis.**
- **Hb F(α2 γ2) is the predominant** Hb present.

- **HPFH patients are asymptomatic**

- **If they are sedentary and slow workers.**

## Diagnosis Of Thalassemia's

- PCR based methods.

- Gene Mapping

- For **families** that **carry a Thalassemia trait.**

- **Genetic counseling** and **genetic testing** is **recommended**

## Treatment Of β Thalassemias

- Repeated / frequent blood transfusions.

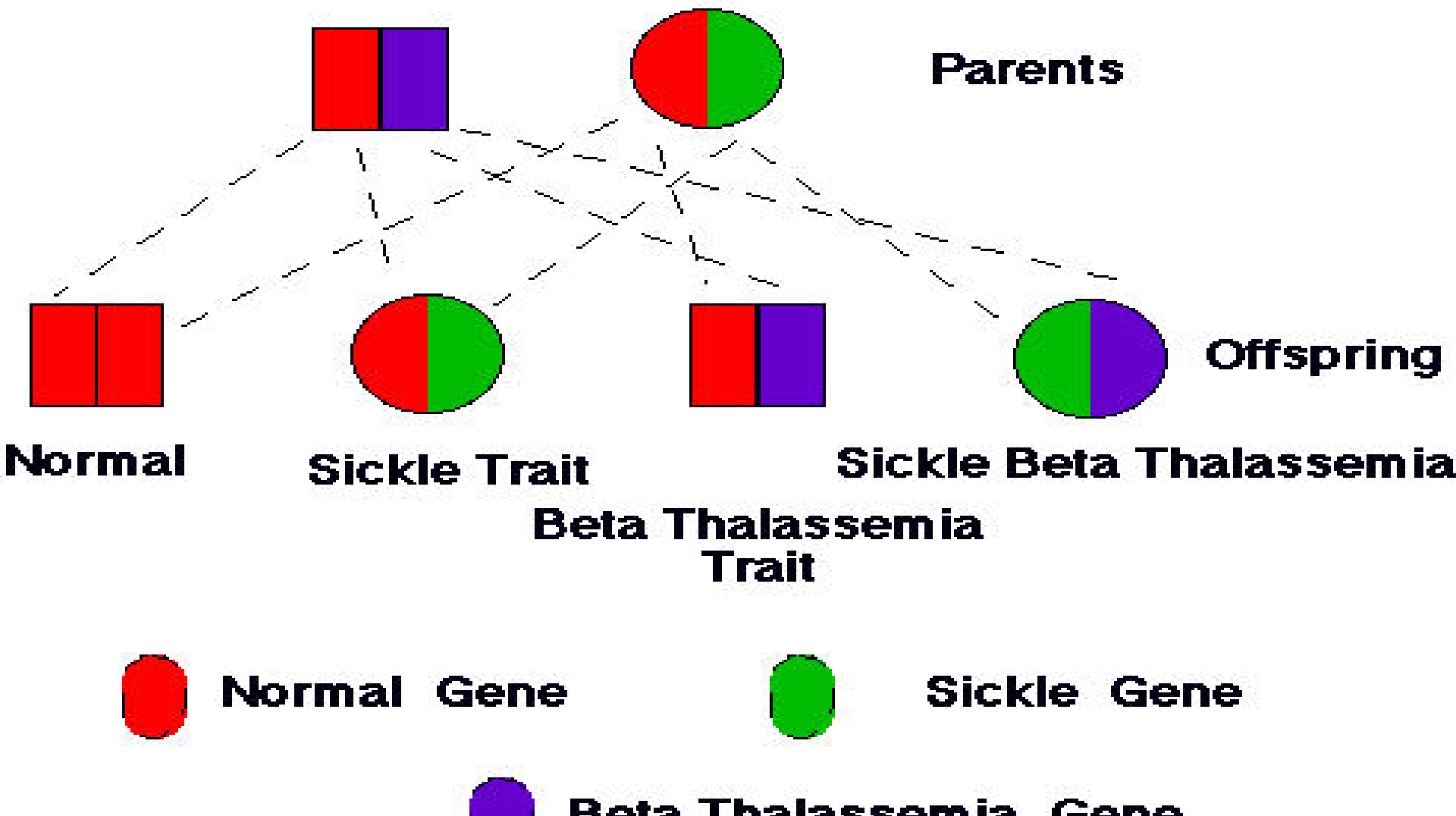
**(After every 3- 4 months)**

- Due to **repeated blood transfusions** in patients of **Beta Thalassemia.**
- **There exhibits Iron overload**
- Iron toxicity is noted since **Iron is one way element**
- **Iron** once entered in blood do not get excreted out.
- Iron gets **accumulated in functional tissues.**
- **Tissue dysfunctions, Growth failure and death** occurs before puberty due to **Iron toxicity**.
- However **Iron chelation- Reduces Iron toxicity.**

- **Folate supplementation** for promotion of Erythropoiesis
- **Azacytidine** – Drug used with limited success

- Bone marrow transplantation
- Stem cell transplant
- Gene therapy

## Inheritance of Sickle Beta Thalassemia




## Abnormal Hb Variants With Increased Oxygen Affinity

- Hb Bart
- Hb H
- Hb Chesapeake
- Hb Rainier

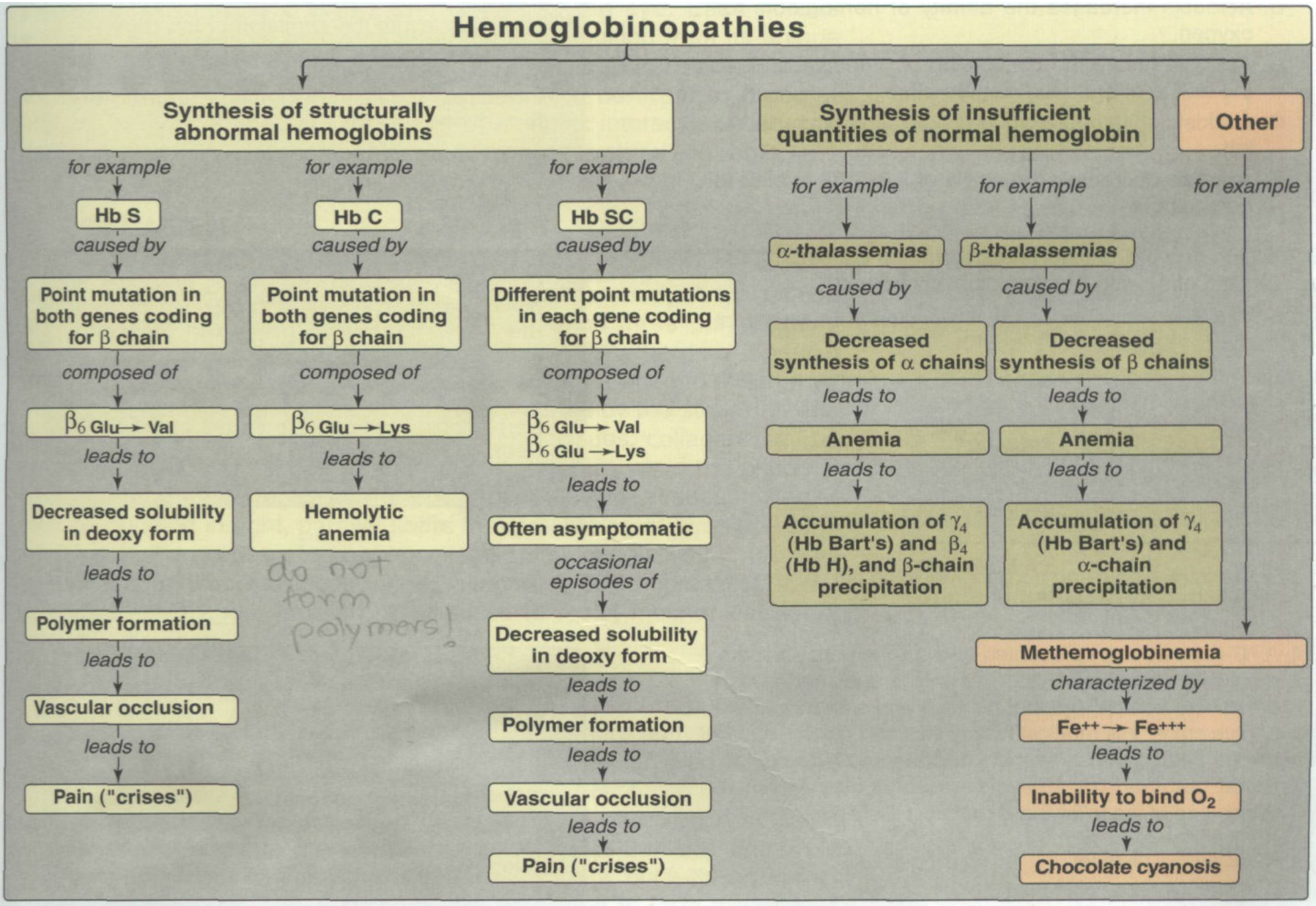



**Figure 3.26**
Key concept map for hemoglobinopathies.

# Catabolism/Breakdown Of Hb
# OR
# Formation and Fate Of Bilirubin
# OR
# How Bilirubin is Formed and Excreted ?

- Catabolism of Hemoglobin begin **after destruction of RBC's**.

- RBC destruction is normally the result of **senescence (Old/Aged)**.

- Red cell destruction usually occurs after a mean **life span of 120 days.**

- The old red blood cells are removed **Extravascularly** by **Macrophages** of R.E System.

- **Reticuloendothelial system** (RES), specially of **Spleen, Bone marrow and Liver** are involved in RBC destruction.

- **Essentials for Erythrocyte Membrane Integrity**
- Continuous supply of **Glucose** to Erythrocytes
- **Continuous and uninterrupted Glycolysis** in RBCs
- Continuous **minimal ATP** production in RBCs

- RBC aging is characterized by:
- **Decreased Glycolytic** enzyme activity
- Which leads to **decreased Glycolysis and ATP production**
- Subsequent loss of deformability and membrane integrity of RBCs.

- Each day ~ 1% of the RBCs are removed and replaced.

- Approximately **2- 3 million old RBCs removed** and **same amt of new red blood cells enter** the circulation per second.
- This maintains constant RBC count in blood.

- 80-90% of aged RBC destruction is **Extravascular**
- Occurs mainly in the Macrophages, Mononuclear phagocytic cells of Spleen

- Spleen is grave yard for RBC's

- Small amount occurring in the RE system of Liver and Bone marrow.

- 10-20 % of RBC destruction is **Intravascular,** occurring in the lumen of the blood vessels.

- Senescent /old RBC's in RES are lysed to release its contents- Hemoglobin (Hb)

# Hb is degraded to:

**Globins** → Amino Acids →Recycled → Metabolism

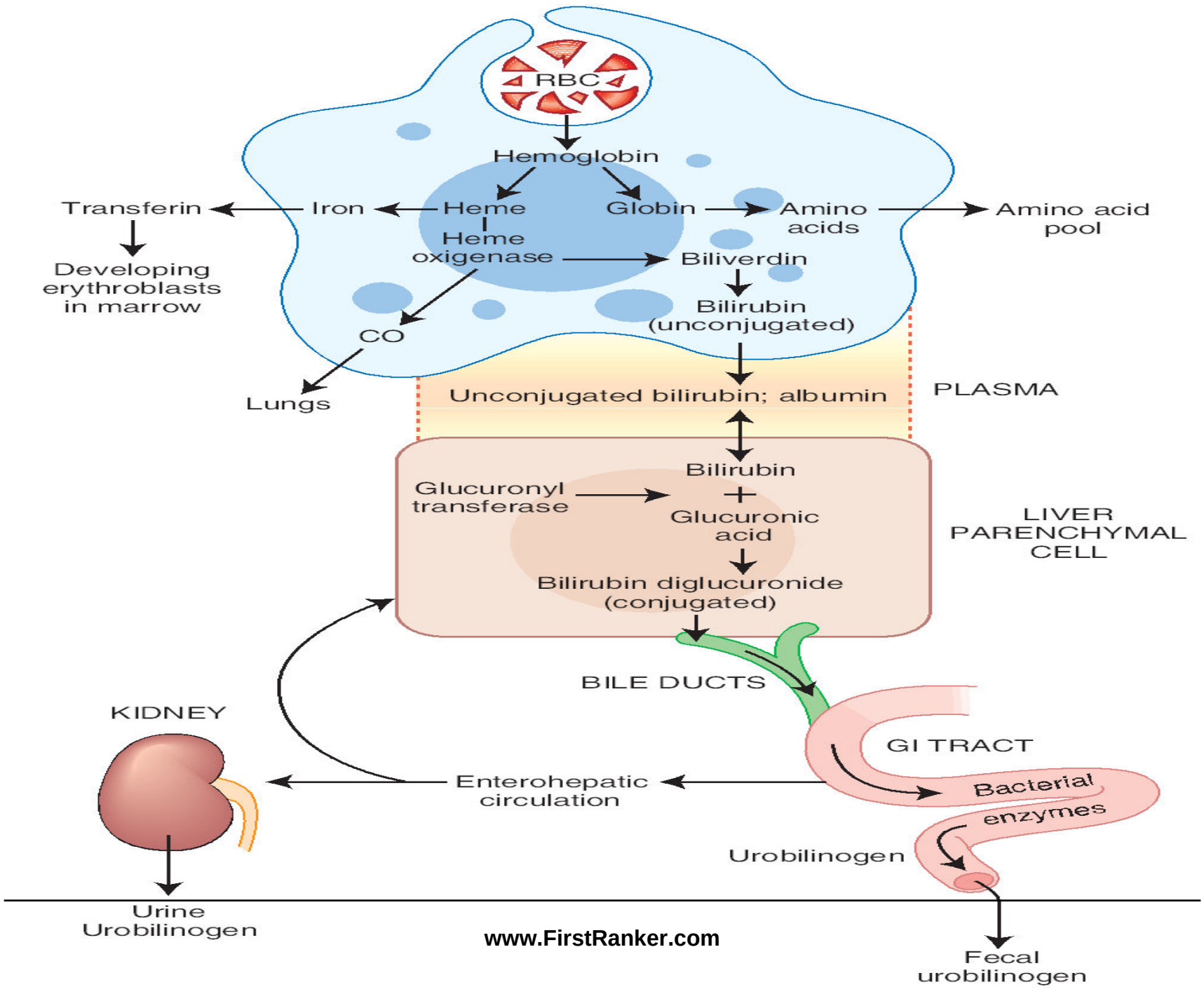
**Heme** → **Bilirubin** excreted out of the body.

- **$Fe^{2+}$ → Transported as Transferrin**
- Iron stored as **Ferritin** and reused in the next Heme biosynthesis

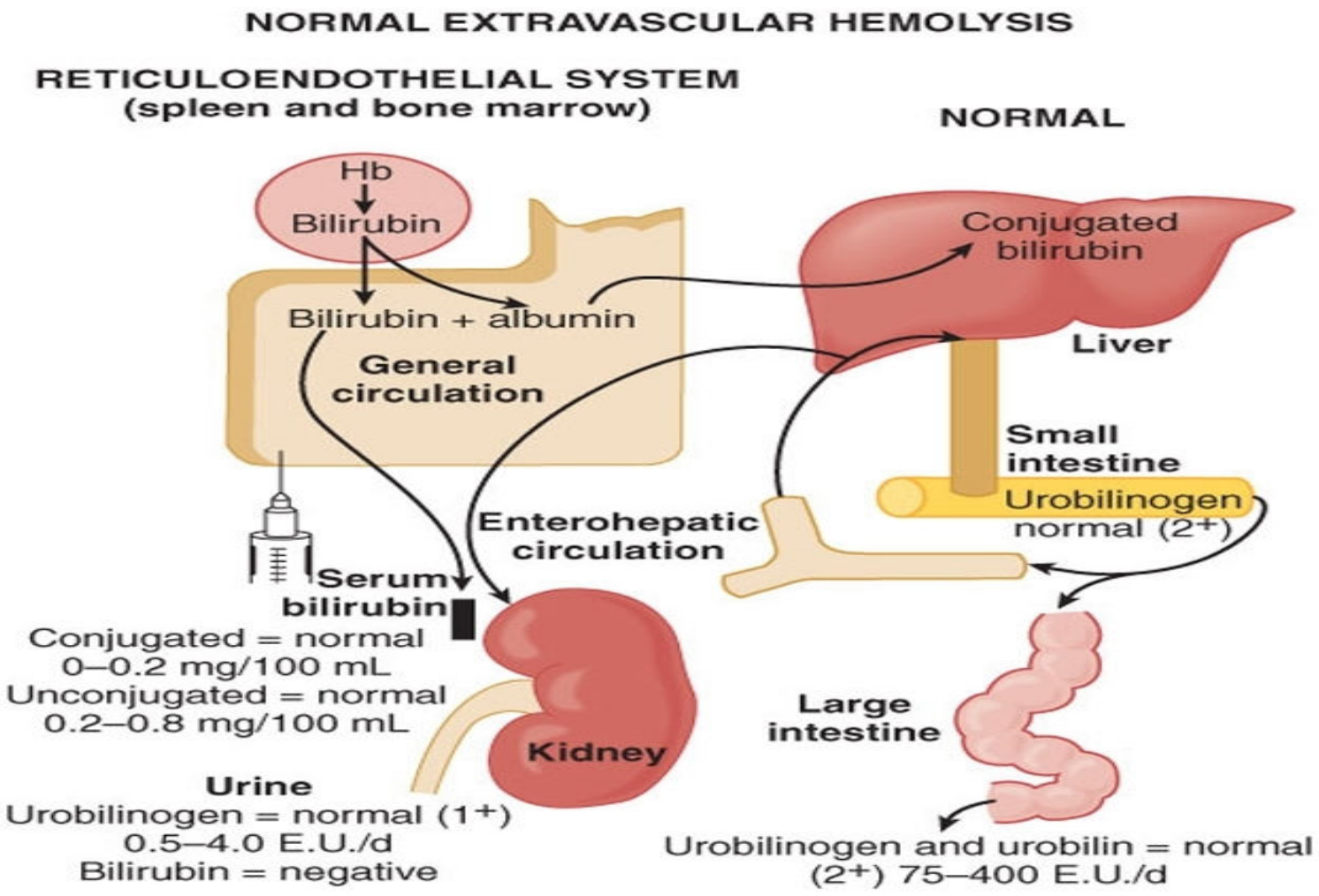
- Not only Hb but other Hemoproteins containing Heme groups are degraded by the same pathway.

# Extravascular Erythrocyte Destruction

- Extravascular Erythrocyte Destruction is a **normal pathway**.
- **80-90% Erythrocytes destructed in this manner.**
- Outside the circulatory system.
- Inside the phagocytic cells of **Spleen, Liver and Bone marrow.**



# Extravascular destruction of RBCs






Hemoglobin → heme + globin → plasma protein and amino acid pool

Histiocyte

Biliverdin + CO + Fe (lungs) → transferrin + Fe → Bone marrow

Bilirubin

plasma albumin

Bilirubin-Albumin (unconjugated)

liver

Bilirubin diglucuronide (conjugated)

bile duct to duodenum

Urobilinogen → Blood → kidney → Urobilinogen (urine)

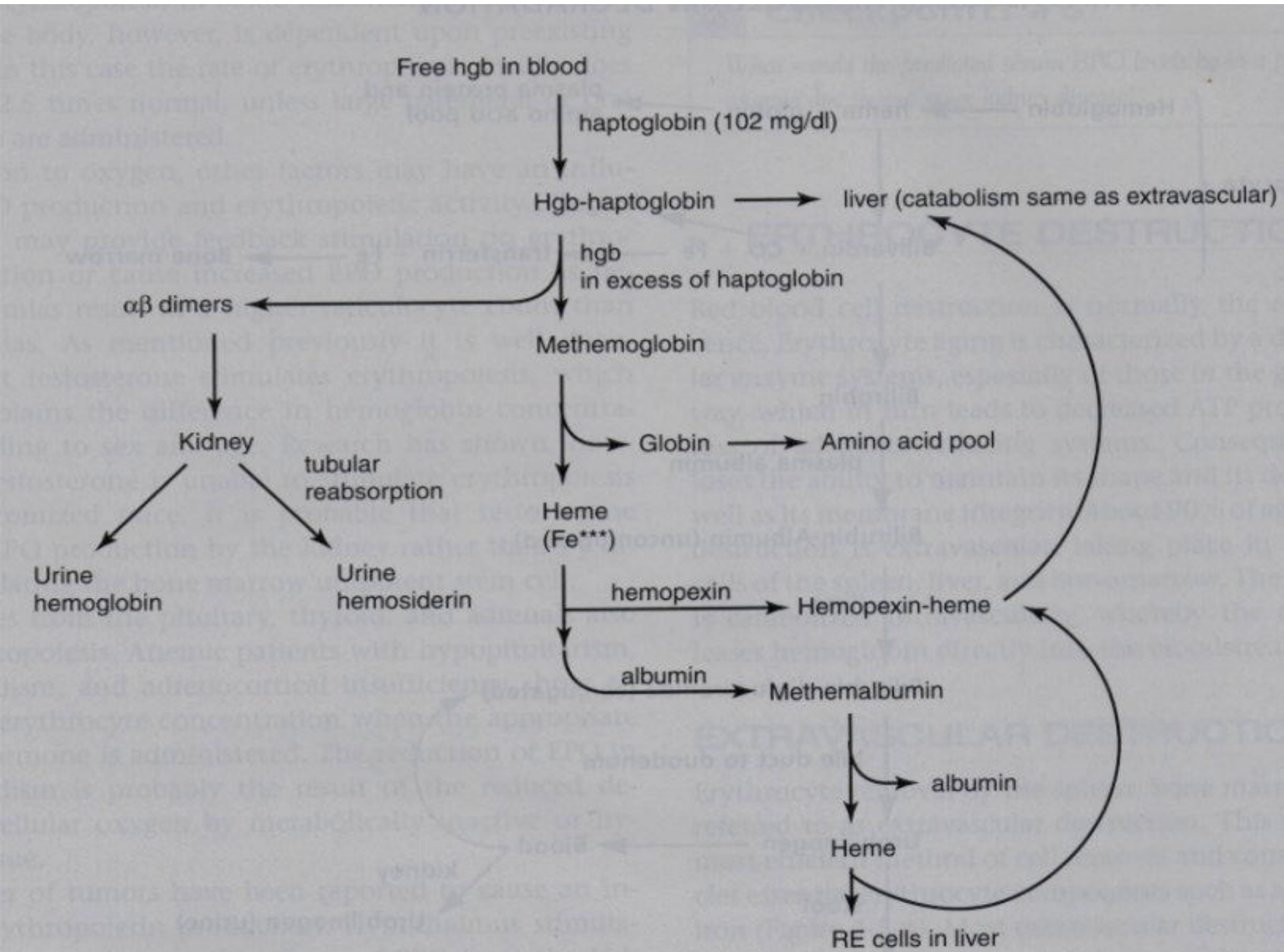
stool

Urobilinogen + stercobilinogen

# Intravascular Erythrocyte Destruction

- Erythrocytes destructed **in circulatory system.**
- **Normally 10 -20%** erythrocytes destructed in this manner.
- Hb is directly released into blood stream.

- **Hb in blood is bound to Haptoglobin.**
- Prevents renal excretion of plasma Hb
- **Circulating Hb is removed by Liver.**



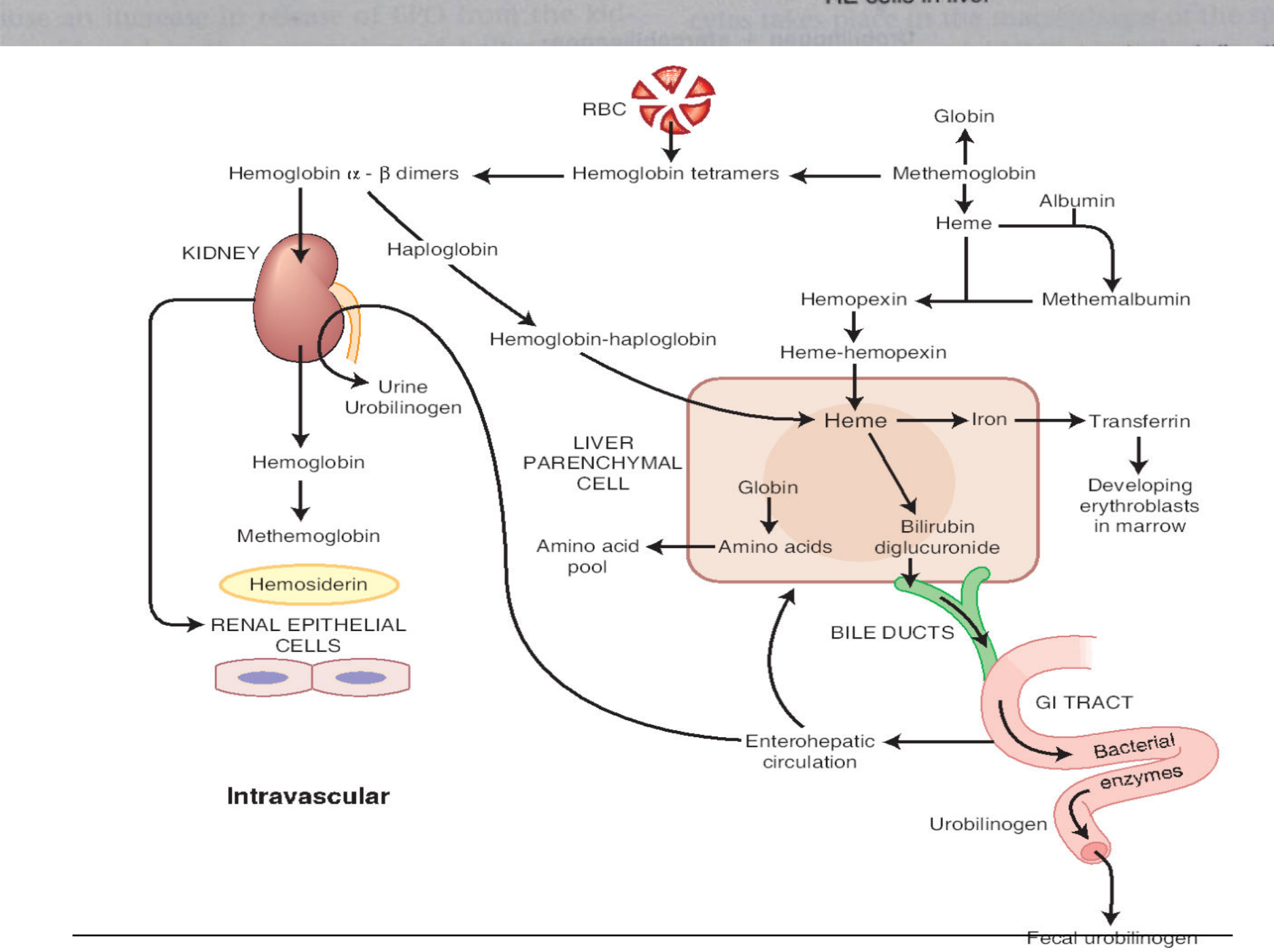






Figure 4.4 Intravascular hemolysis: increased bilirubin, decreased haptoglobin, but free hemoglobin present.


- Globin chains are broken down to amino acids

- Which are reutilized for general protein synthesis in the body.

- **Heme** part is **catabolized** to **Bilirubin** and **excreted out** of the body.

- Microsomal enzyme **Heme Oxygenase of RE cells** acts on Heme
- Requires NADPH+$H^+$ as a coenzyme
- **Cleaves α Methenyl bridge** of cyclic tetrapyrrole ring of Porphyrin .
- **Forms Biliverdin** – A linear tetra Pyrrole ring structure.

- **Iron** is released in Ferrous is oxidized to **Ferric** and transported by Transferrin.

- **CO** released is expired out.

- Enzyme **Biliverdin Reductase**
- Reduces **Methenyl bridges** of Biliverdin **to Methylene bridges.**

- Reduces Biliverdin (Green bile pigment ) to Bilirubin ( Yellow bile pigment).

- **NADPH+H$^+$** is used as reducing equivalent for this reduction reaction by **Biliverdin Reductase.**

# Albumin Transports Unconjugated Bilirubin Through Blood.

- **1 gram of Hb** yields **35 mg of Bilirubin.**
- Daily **250-300 mg** of Bilirubin is produced by an adult.

- Bilirubin formed in RE cells of a Spleen after Heme catabolism and released in the blood circulation is:
  - **Non polar**
  - **Insoluble**
  - **Free or Unconjugated Bilirubin**

- **Albumin** a polar moiety helps in **transporting this non polar Bilirubin**

- Through aqueous phase of blood circulation **up to Liver.**

- Albumin has two binding sites for Bilirubin.
  - **High affinity binding site**
  - **Low affinity binding site**

- Bilirubin **first tightly binds to high affinity binding site of Albumin.**

- **25 mg of Bilirubin** tightly binds with Albumin **in 100 ml blood.**

- **Bilirubin bound with Albumin**
- **Prevents urinary excretion of Bilirubin in urine.**

- **Drugs** like Sulfonamides, Penicillin, Salicylates
- **Compete with Bilirubin for its binding to Albumin**.

- **Hypoalbuminemia affects transport and excretion of Bilirubin.**

- **Hypoalbuminemia** may **lead to retention of Unconjugated Bilirubin** in blood circulation.

- May cause **Bilirubin Encephalopathy**.

- **Facilitated transport system** helps in **uptake of Bilirubin in sinusoidal surface of Hepatocytes**.

- **Ligandin and Protein Y** of Hepatocytes

- Prevent efflux of Bilirubin back into blood stream.

# Conjugation Of Bilirubin In Liver

- **Non polar Bilirubin entered in Liver**
- Undergoes conjugation reaction
- **Conjugating agent** is two molecules of **UDP-Glucuronic acid**

- In presence of **conjugating enzyme** **UDP Glucuronyl Transferase**

- Forms **Conjugated Bilirubin-Bilirubin Diglucuronide.**

- **Conjugated Bilirubin** is:
  - Polar
  - Soluble form
  - Readily excretable form.

- Conjugated Bilirubin is carried through Bile via common bile duct and excreted in the intestine

- **Secretion of Bilirubin into the bile** occurs by an **active transport mechanism.**

- Conjugated Bilirubin reaches terminal ileum and large intestine.

- Glucuronides are removed by specific bacterial enzymes- **β Glucuronidase**.

- Bilirubin is reduced to colorless compound **Stercobilinogen** in intestine.

- Small amount of Stercobilinogen is reabsorbed and re excreted through the Liver – **Enterohepatic circulation**.

- Stercobilinogen is partly reabsorbed enters in blood circulation is excreted in urine as Urobilinogen and Urobili

- Most of the **Stercobilinogen** of intestine is **oxidized** to

- **Stercobilin a orange yellow colored compound.**

- Stercobilin is a major excretable form of Bilirubin in feces.

# Futher Fate of Bilirubin

- Bilirubin (Bil) is released from RES into the blood.
- BUT! **Bil is only poorly soluble in plasma**, and therefore during transport it is bound to **albumin (Unconjugated Bilirubin").**

↓

**LIVER**

- In the hepatocytes, **Bil is conjugated** by 2 molecules of **glucuronic acid → bilirubin diglucuronide (soluble in water, „conjugated Bil")**

↓

**BILE**

↓

**INTESTINE**

- ~~Bilirubin is reduced to **urobilinogen** and **stercobilinogen**~~

BLOOD

Bilirubin-albumin complex

LIVER

2 UDP-glucuronic acid

*Bilirubin glucuronyl-transferase*

2 UDP

Bilirubin diglucuronide

Bilirubin diglucuronide

BILE

BLOOD CELLS

Hemoglobin

Globin

Heme

$O_2$

**Heme oxygenase**

$CO$

Biliverdin IXα

NADPH

**Biliverdin reductase**

$NADP^+$

Bilirubin (*water-insoluble*)

***via blood to the liver***

**Stercobilin excreted in feces**

**Urobilin excreted in urine**

Urobilinogen formed by bacteria

**INTESTINE**

**KIDNEY**

***reabsorbed into blood***

***via bile duct to intestines***

Bilirubin diglucuronide (*water-soluble*)

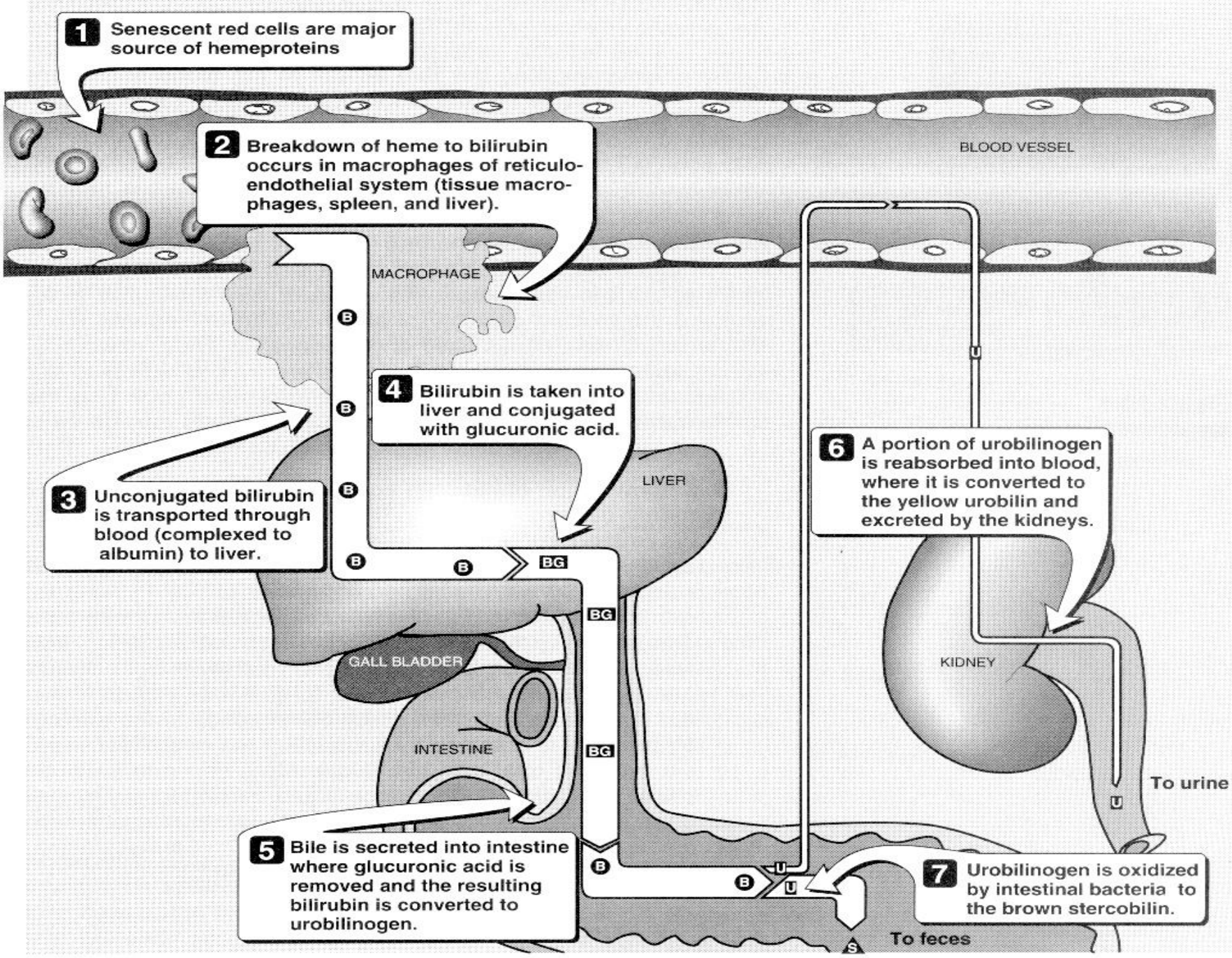
2 UDP-glucuronic acid

Bilirubin (*water-insoluble*)

**LIVER**



# Catabolism of Hemoglobin

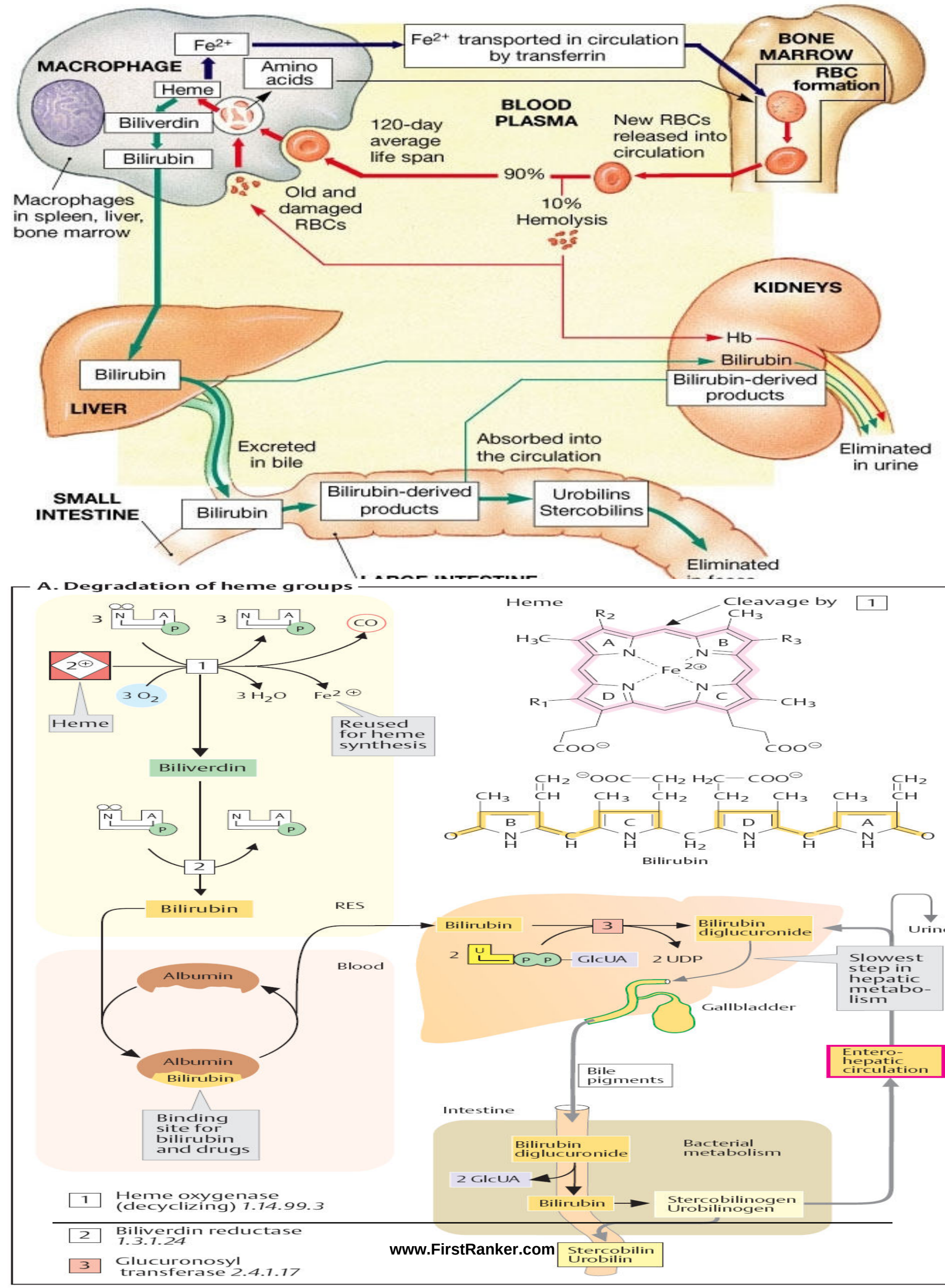



# Normal Red Cell Breakdown

**Hemoglobin**

- **Heme**
  - **Iron**
    - **Transferrin**
      - Erythroblast
  - **Protoporphyrin**
    - **CO**
      - **Expired air**
    - **Bilirubin**
      - (free)
      - Liver conjugation
      - **Bilirubin Glucuronides**
        - **Stercobilin(ogen)**
          - **Feces**
        - **Urobilin(ogen)**
          - **Urine**
- **Globin**
  - **Amino acids**

# Red Blood Cell Turnover



# What Is Bilirubin ?

- **Bilirubin is**

- **Metabolic waste, an end product of Heme catabolism**
- Formed in cells of **RE system mainly in Spleen**
- Richly **present in Bile**
- **Yellow** colored **Bile pigment**
- Carried through Bile **for its excretion through feces.**

- Recently a research study has depicted

- **Bilirubin has an Antioxidant capacity.**

- **Bilirubin** is **majorly excreted out** through feces in the form of **Stercobilin.**

(Yellow Orange pigment)

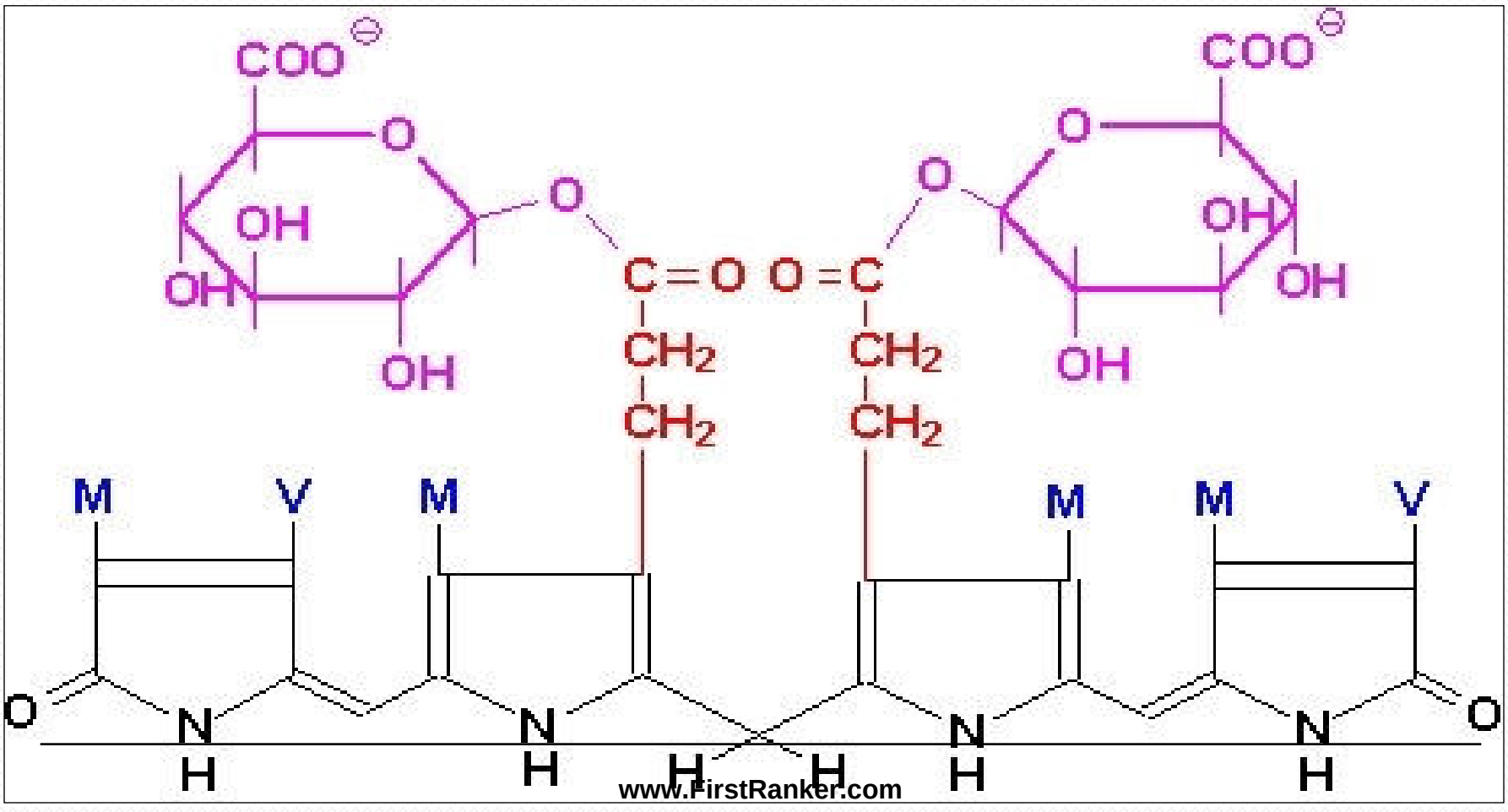
# Types Of Bilirubin

- **Free/ Unconjugated /Indirect Bilirubin**
  - **Non polar** / insoluble form
  - **Formed in RES**- Spleen , Liver
  - Present in blood circulation **before entry into Liver cells.**

- **Bilirubin Diglucuronide /Conjugated/ Direct Bilirubin**

  - **Polar/ Soluble form** of Bilirubin
  - Unconjugated Bilirubin is transformed to **conjugated Bilirubin in Liver.**

**Bilirubin-diglucuronide = Conjugated Bilirubin is soluble in water → "Direct Bilirubin"**



- Conjugated Bilirubin is formed after **conjugation reaction**,
- Conjugated with **Glucuronate**
- **Conjugated Bilirubin is readily mixed with bile and excreted out through feces.**

# Normal Levels
# Serum Bilirubin

| S. No | Type Of Bilirubin | Normal Ranges |
|---|---|---|
| 1 | Total Bilirubin Direct+ Indirect | 0.2- 1 mg % |
| 2 | Unconjugated/Indirect Bilirubin | 0.2-0.8 mg% |
| 3 | Conjugated / Direct Bilirubin | 0- 0.2 mg% |

- In **normal healthy** conditions there is
- **No conjugated Bilirubin in circulating blood.**

- **When Is Conjugated Bilirubin is present in blood?**
- During obstruction in common bile duct (CBD)

**(Obstructive Jaundice)**

# Forms of Bilirubin

- **Bilirubin - In Bile**
- **Urobilin – In Urine**
- **Stercobilin - In Stool**

# Hyperbilirubinemia

# Hyperbilirubinemia

- Increased levels of serum **Total Bilirubin**
- **Above 1 mg% is termed as Hyperbilirubinemia.**

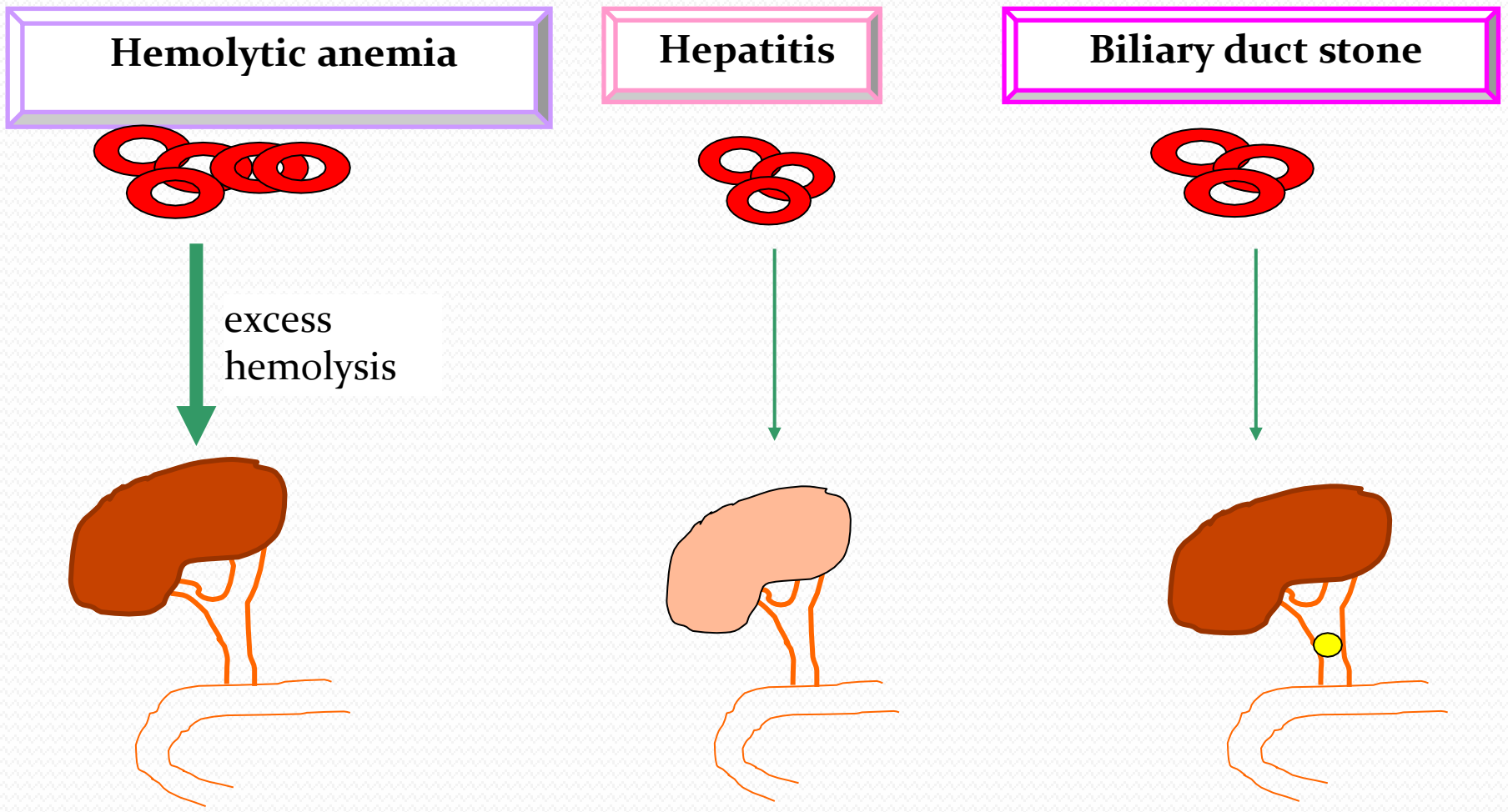
# Types Of Hyperbilirubinemia

- Unconjugated Hyperbilirubinemia
- Conjugated Hyperbilirubinemia
- Biphasic Hyperbilirubinemia.

# Causes of Hyperbilirubinemia

- **Over production Of Bilirubin**
- **Less excretion of Bilirubin**
  - Leads to retention of Bilirubin in blood causing **Hyperbilirubinemia.**

# Causes of Hyperbilirubinemia




↑ Unconjugated Bilirubin (in blood)
↑ conjugated Bilirubin (released to bile duct)

↑ Unconjugated Bilirubin (in blood)
↑ conjugated Bilirubin (in blood)

↑ conjugated Bilirubin (in blood)

## Causes of Hyperbilirubinemia

1. Conditions which **form excess Bilirubin than the excreting capacity of body**.

- **Abnormal excessive intravascular hemolysis**
- Overproduction of Bilirubin from Heme catabolism.
- More than the capacity of Liver to conjugate and excrete
- **Leads to Unconjugated Hyperbilirubinemia.**

2.Conditions which **block excretion of Bilirubin out of the body.**

- **Failure** of **diseased Liver** to **conjugate and excrete Bilirubin** through bile.
- In viral hepatitis
- **Leads to biphasic Hyperbilirubinemia.**

- **Obstruction to the flow of bile**
- Due to obstruction in bile duct.
- Regurgitation of bile in systemic blood circulation.
- **Leads to conjugated Hyperbilirubinemia.**

## 3.Congenital / Inherited defects in uptake and excretion of Bilirubin by Liver

- Leads to **congenital Hyperbilirubinemia.**

## 4.Hypoalbuminemia / high drug concentration in blood

- Affects Bilirubin transportation by Albumin
- Retains Bilirubin in blood
- **Leads to Unconjugated Hyperbilirubinemia.**

# Diagnosis of Hyperbilirubinemia

Diagnosis of Hyperbilirubinemia

- **Vanden Bergh's Reaction**
- **Quantitative estimation** of serum:
  - **Total Bilirubin**
  - **Unconjugated Bilirubin**
  - **Conjugated Bilirubin**

# Types of Vanden Bergh's Reaction

- **Direct Vanden Bergh's Reaction**

- Estimates serum **Conjugated Bilirubin** (soluble form)

- **Serum Conjugated Bilirubin** + **Diazo Reagent** = **Pink Azobilirubin**

- In the Direct Vanden Bergh Reaction soluble form of a Bilirubin is **directly and immediately reacted with the Diazo Reagent**.
- Conjugated Bilirubin **requires no solubilizing agent.**
- Hence **Conjugated Bilirubin** is also termed as **Direct Bilirubin**

- **Indirect Vanden Bergh's Reaction**
- Estimates Serum **Unconjugated Bilirubin** (Insoluble form)
- **Serum Unconjugated Bilirubin** + **Methanol**/Surfactant (solubilizing agent)+ **Diazo Reagent** = Pink Azobilirubin

- In an **Indirect Vanden Bergh Reaction**
- Insoluble form of Unconjugated Bilirubin is first solubilized with a **solubilizing agent (Methanol)**
- Then the solubilized form reacts with Diazo reagent to form a pink Azobilirubin complex
- Unconjugated Bilirubin **indirectly reacts with the Diazo Reagent**.

- Since Unconjugated Bilirubin **requires solubilizing agent for reaction with Diazo reagent.**

- Hence Unconjugated Bilirubin is also termed as **Indirect Bilirubin**

# Results and Significance Of Vanden Bergh's Reaction

| S.No | Results of Vanden Bergh | Type of Hyperbilirubinemia/ Jaundice |
|---|---|---|
| 1 | **Direct Vanden** Bergh's Reaction **Positive** | Conjugated Hyperbilirubinemia **Obstructive Jaundice** |
| 2 | **Indirect Vanden** Bergh's Reaction **Positive** | Unconjugated Hyperbilirubinemia. **Hemolytic Jaundice** |
| 3 | **Both Direct and Indirect Vanden** Bergh's Reaction **positive** | Biphasic Hyperbilirubinemia means Both conjugated and Unconjugated Bilirubin increased. **Hepatic Jaundice.** |

# Significance Of Vanden Bergh's Reaction

I. **Quantitatively Estimates serum** Total, conjugated and Unconjugated **Bilirubin**.

II. **From the levels of serum total Bilirubin- Diagnoses Jaundice**

III. From the serum levels of Direct and Indirect Bilirubin levels- **Differentiate- Type of Jaundice**

IV. From the values of serum Bilirubin- **Indicate-Severity of Jaundice**

# Jaundice/Icterus Condition

- **Jaundice** is a **pathological/Clinical condition**
- **Characterized by Hyperbilirubinemia**

- In **Jaundice Total Serum Bilirubin** levels are **more than 2.5 mg %.**

- High circulating Bilirubin more than 2.5 mg% in blood and tissues causes jaundice.

- **Jaundice is a yellow discoloration to:**
  - **Skin**
  - **Sclera of eyes**
  - **Nails**
  - **Mucous membrane**

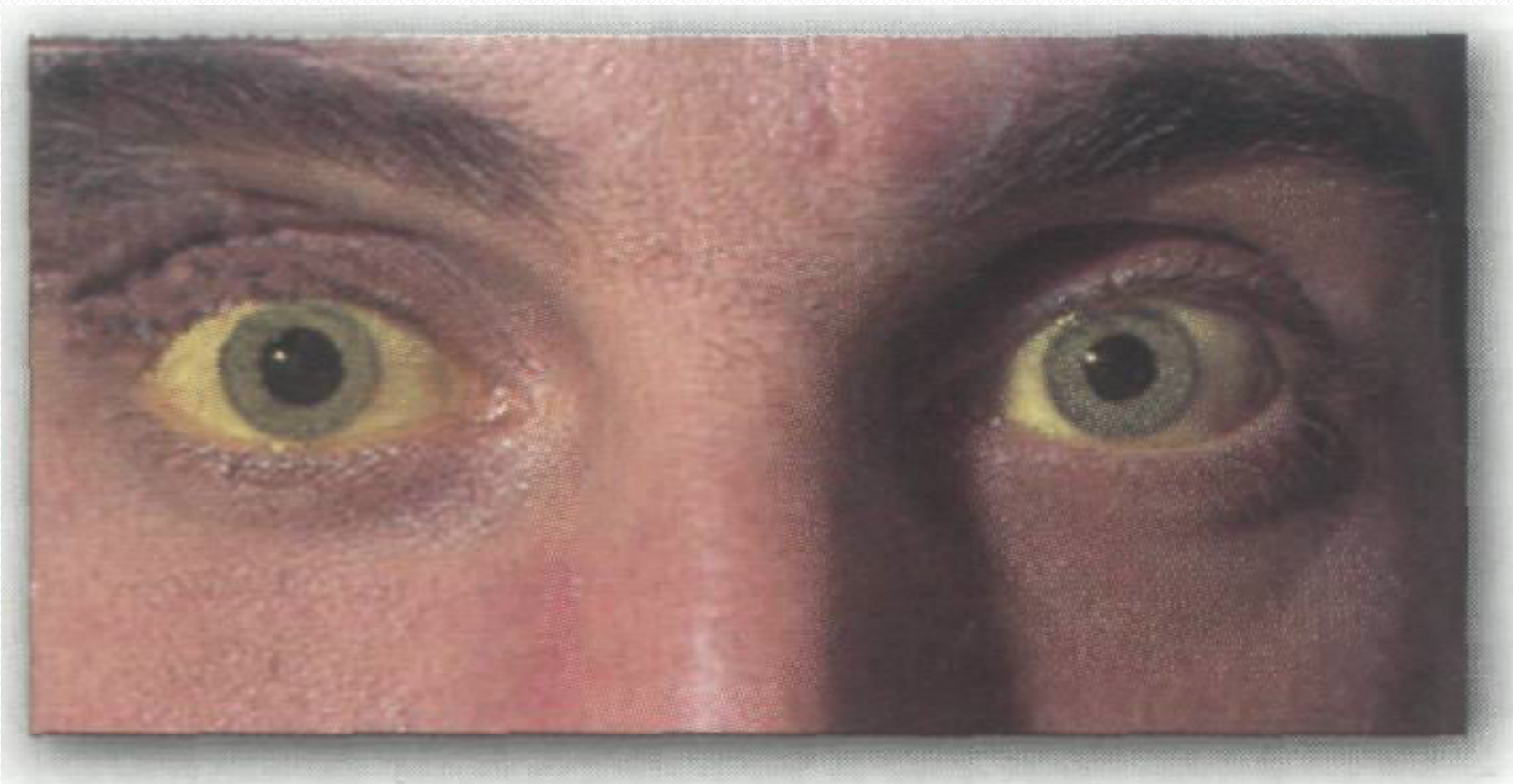


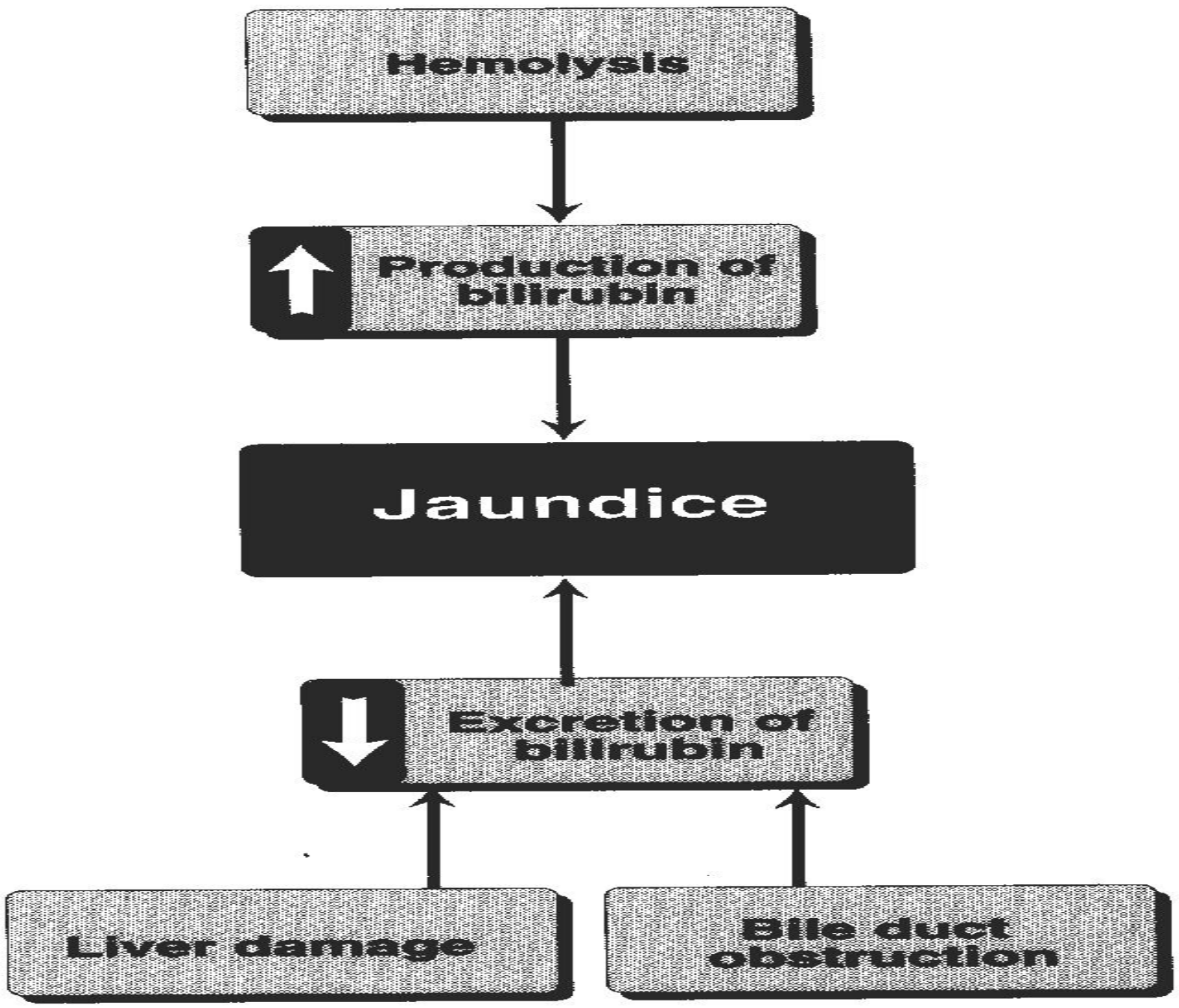
**Figure 21.10**
Jaundiced patient, with the sclerae of his eyes appearing yellow.

# Causes and Types Of Jaundice



- **Basic Cause and Consequence Of Jaundice**

- **Defect in Heme catabolism**
  - **Overproduction of Bilirubin**
- **Defect in Bile excretion**
  - **Less excretion of Bilirubin**
- **Retains Bilirubin in blood and body**




# Types Of Jaundice

- **Hemolytic/Pre Hepatic /Retention/ Acholuric Jaundice**
- **Hepatic/Infectitious Jaundice**
- **Obstructive/Post Hepatic/ Regurgitation /Choluric/Cholestatic Jaundice.**

- **Neonatal Physiological Jaundice**
- **Neonatal Pathological Jaundice**
  - **Erythroblastosis Foetalis**
  - **Breast Feeding Jaundice**
  - **Breast Milk Jaundice**

- **Sub Clinical Jaundice/ Latent Jaundice-**
  - Serum Bilirubin levels between **1-3 mg%**

- **Clinical Jaundice-**
  - Serum Bilirubin levels more than **3 mg%.**

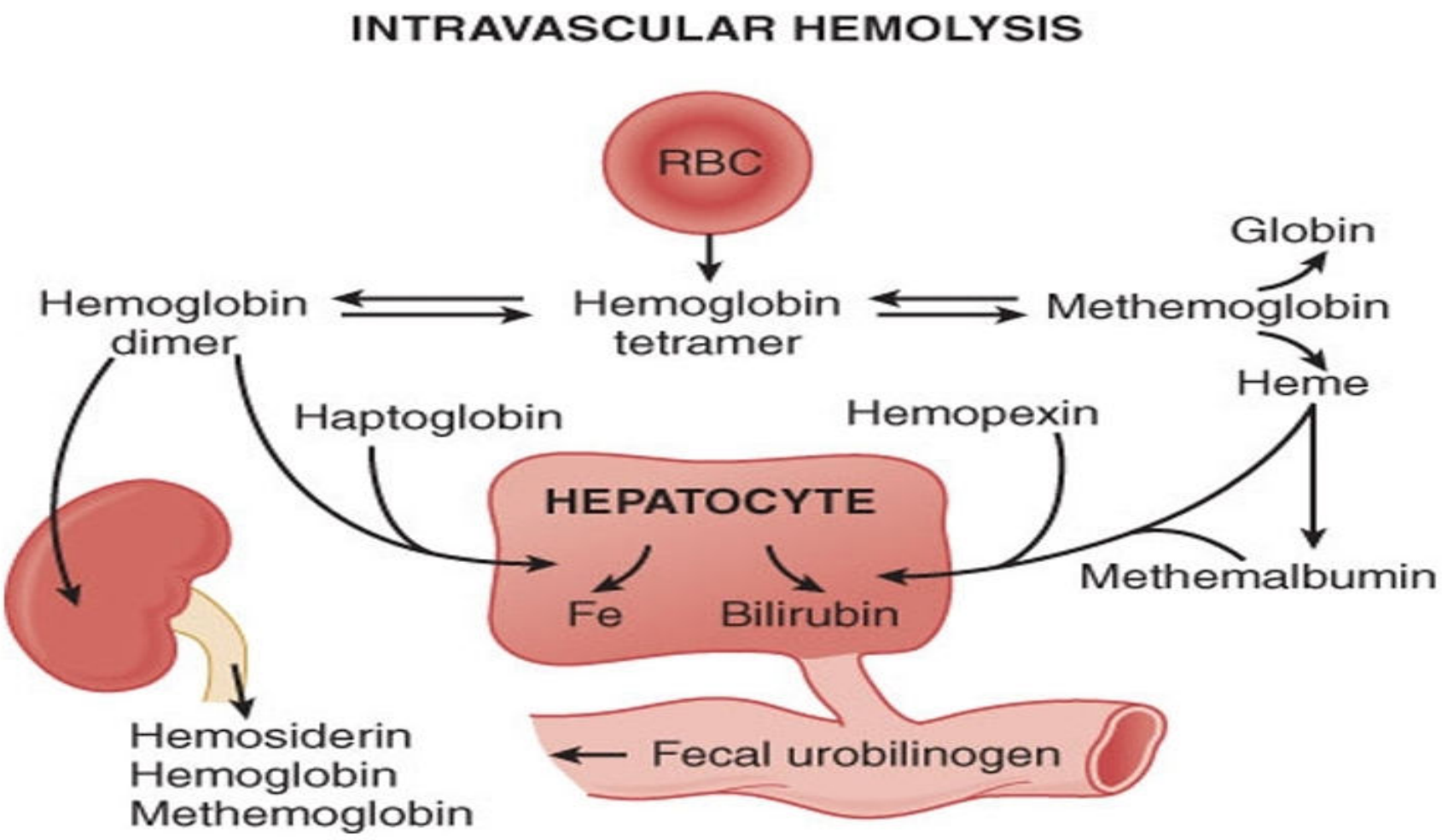
## Hemolytic/ Prehepatic /Retention Jaundice

- Due to **abnormal excessive intravascular hemolysis(premature) .**
- Characterized by **Unconjugated Hyperbilirubinemia.**

# Healthy Body RBC Destruction

- Normal human body brings approx. 200 billion RBC lysis per day
  - 160 billion is Extravascular Lysis
  - 40 billion is Intravascular lysis

# Intravascular destruction of RBCs



# Conditions Causing Abnormal Excessive Intravascular Hemolysis

- Sickle Cell Anemia
- Thalassemia's
- Glucose-6-Phosphate Dehydrogenase deficiency
- Mismatched blood transfusion
- Malaria
- Burns
- Spherocytosis
- Drug interactions

- **Excessive abnormal intravascular hemolysis**
- **Increased Heme catabolism**
- **Increased Unconjugated Bilirubin in blood**
- Levels of the Unconjugated Bilirubin **more than normal capacity of Liver to conjugate and excrete (3 gm/day)**.

- Unconjugated Bilirubin **is in queue to enter Liver** for conjugation and excretion.

- Thus there occurs retention of **Unconjugated Bilirubin in blood.**

# Laboratory Findings In Hemolytic Jaundice.

## Blood Investigations

- **Results of Vanden Bergh's Reaction**
- Total Bilirubin Increased
- Indirect Bilirubin Increased
- Direct Bilirubin Normal

# Urine Investigations

- **Ehrlich's Test for urine Urobilinogen** – Positive
- Urine Urobilin increased.
- **Hays sulfur test for Bile salts-** Negative
- **Fouchet's Test for Bilirubin-** Negative

# Stool Appearance

- **Dark brown color feces** in Hemolytic Jaundice
- Due to **more Stercobilin excreted out in feces.**

# Bone Marrow Examination

- **Hyperplasia of Bone marrow**
- **Reticulocytosis in Peripheral blood smear**
- Immature form of RBC's increased in blood circulation.

# Hepatic/ Infectitious Jaundice

- Liver Parenchymal damage
- Due to Viral Hepatitis
- **Biphasic Hyperbilirubinemia**.

# Conditions Affecting Liver Parenchymal Damage

- **Viral infection of Liver**
  ( Viral Hepatitis)
- **Liver Cirrhosis- Alcoholism**
- **Drug Effects:**
  - **Rifampicin** -affect cellular **uptake of Bilirubin** by Liver cells
  - **Novobiocin** -affect **conjugation of Bilirubin** in Liver.

# Causes Of Biphasic Hyperbilirubinemia in Hepatic Jaundice

- In Hepatitis **damage** and **inflammation** of Liver **parenchymal cells.**
- This **impairs** and **delays** the **conjugation** and **excretion** of Bilirubin by Liver.
- This retains **Unconjugated Bilirubin in blood.**

- **Inflammation of Hepatocytes** and **intra hepatic obstruction** in hepatitis
- **Leaks out conjugated Bilirubin** in blood.
- Thus biphasic Hyperbilirubinemia is noted.

- In **Hepatic Jaundice** **there is a Marginal increase**
- In **both** serum **Unconjugated** and **Conjugated** Bilirubin

# Laboratory Findings In Hepatic Jaundice

## Blood Investigations

- **Results of Vanden Bergh's Reaction**
  - Total Bilirubin increased
  - Indirect Bilirubin increased (Marginal)
  - Direct Bilirubin increased (Marginal)
- Serum **SGPT/ALT and SGOT/AST activity increased.**

# Urine Investigations

- Ehrlich's Test for urine Urobilinogen – Normal
- Urine Urobilin normal.
- **Hays sulfur test for Bile salts-** may be positive in severe cases.
- **Fouchet's Test for Bilirubin**-may be positive in severe cases.

# Stool Appearance

- Normal/ slightly pale colored feces in **Hepatic Jaundice**.

# Obstructive/ Post hepatic / Regurgitative Jaundice

- Due to **obstruction in bile flow to reach small intestine**.
- Characterized with **Conjugated Hyperbilirubinemia.**

# Conditions Causing Obstructive Jaundice

- **Obstruction** of **Bile duct** due to **Gall stones in Common Bile Duct(CBD).**
- **Narrowing of bile duct** due to surgery.
- **Tumor of head of Pancreas.**
- **Enlargement** of **lymph glands near Gall bladder /bile duct.**

# Causes Of Conjugated Hyperbilirubinemia In Obstructive Jaundice.

- **Partial/ Complete Obstruction of bile duct**
- **Regurgitation of bile** into systemic circulation
- Bile contains bile salts and bile pigment- Bilirubin .
- **Hydrophilic Conjugated Bilirubin is now in blood circulation.**

- **In Obstructive Jaundice**
- **Bile get excreted out through urine.**
- **Bile salts and conjugated Bilirubin present in urine.**
- **Dark yellow colored urine noted** due to presence of Bilirubin occurs **in Obstructive Jaundice patients.**

# Laboratory Findings In Obstructive Jaundice.

## Blood Investigations

- **Results of Vanden Bergh's Reaction**
- Total Bilirubin increased
- Indirect Bilirubin normal
- **Direct Bilirubin increased**
- **Serum ALP activity increased.**

# Urine Investigations

- **Ehrlich's Test for urine Urobilinogen** – Negative
- Urine Urobilin decreased.
- **Hays sulfur test for Bile salts- Significantly Positive**
- **Fouchet's Test for Bilirubin- Significantly Positive**

# Stool Appearance

- **Clay colored stools due to absence of Stercobilin** in feces of **Obstructive Jaundice**.
- **Fatty stools** due to excretion of Lipids in feces

(Absence of Bile salts in intestine).

# Neonatal Physiological Jaundice

## Neonatal - Physiological Jaundice

- Noted in **premature, low birth weight infants**.
- After 1- 7 days of birth.

# Causes

- **Immature hepatic system** in premature born infants
- **Poor uptake and conjugation of Unconjugated Bilirubin** from blood by **Liver**
- **Low levels of Conjugating Enzyme UDP- Glucuronyl Transferase**
- **Delays the conjugation** and **excretion of Bilirubin**

# Consequences

- Physiological Jaundice exhibits **Unconjugated Hyperbilirubinemia**
- Serum Bilirubin may raise up to **20 mg % or more**

- Unconjugated Bilirubin is **hydrophobic**
- It can easily **cross blood brain barrier to** enter central nervous system.
- **Leading to Kernicterus (Bilirubin Encephalopathy)**

- **Bilirubin accumulates** in **Neurons of Basal Ganglia,**
- **Hippocampus Cerebellum**
- **Medulla of Brain**.
- This causes **necrosis of nerve cells and brain damage.**

## Symptoms

- Fits/ Convulsions
- Mental Retardation
- Encephalitis
- Spasticity
  **(Skeletal muscle tightness and stiffness)**

# Treatment

- Phototherapy at 450 nm.

- Exchange transfusions of blood

(When Serum Bilirubin > 20 mg%)

- During **Phototherapy** baby is **exposed to uv light**

- The **Bilirubin is transformed to solubilized form of Bilirubin**

- Which is readily excretable out through urine.

- Blue / white UV light induces **isomerization**
- Of non polar , insoluble form of Unconjugated **Bilirubin –Z isomer**
- **To water soluble, polar form of Bilirubin- E isomer.**

- The **phototherapy** should be **exposed to child's skin**
- **Breast feed** the child **every 2 to 3 hours (10 to 12 times a day).**

- **Feeding prevents dehydration** and helps Bilirubin to excrete out the body.
- Phototherapy will continue until **the baby's serum Bilirubin levels are low enough to be safe**.

# Neonatal-Pathological Jaundice

# Erythroblastosis Foetalis

- **Hemolytic condition in neonates**
- **Extrinsic Cause of Hemolysis**
- Exhibits **Unconjugated Hyper Bilirubinemia**

## Cause

- **Caused due to Rh incompatibility**
  - **When Rh – ve mother conceives Rh + ve baby.**
  - **This causes excessive hemolysis of Erythrocytes at the time of birth.**

# Neonatal Pathological Jaundice

# Non-Hemolytic Cause

## Breast Feeding Failure Jaundice

- Jaundice caused in new born infants.
- **Due to insufficient/lack in breast feeding of milk**

- **Infants born by cesarean section** are at **higher risk** for this condition.

- Due to **no normal Lactation phase**.

- **Inadequate quantities of milk reached to infants body.**
- **Decreases body fluids ,lowers bowel movements ,impair to remove Bilirubin** from an infants body.

- **Proper feeding prevents dehydration**
- **Helps Bilirubin to excrete out** of the body **without its retention.**

- Condition of breast feeding Jaundice **can be ameliorated** by
- **Frequent breast feeding sessions of sufficient duration(10-12 /day)**
- This **stimulate adequate milk production by mothers breast**.

- Extra fluids are helpful for babies who have not been getting enough breast milk.
- Nursing more often (up to 12 times a day)
- Will increase the baby's fluid levels

- Can cause the Bilirubin level to drop.

# Breast Milk Jaundice

# Breast Milk Jaundice

## Non Organic Cause

- Breast milk Jaundice is **more of a biochemical problem**
- **Probably caused by factors/chemicals present in the Breast milk.**
- These may **block certain Proteins/ Enzymes in the infant Liver that metabolize Bilirubin**.

- Breast Milk Jaundice tends to run in families
- It occurs equally often in Males and Females
- **Affects 0.5 % to 2.4% of all newborns.**

# Hypothesized Mechanisms

- Increased levels of **Epidermal Growth Factor (EGF)** in **Breast milk.**
- **Increased Bilirubin uptake from the gut (enterohepatic circulation)** in breast fed babies.
- In a new born Liver, activity of Glucuronyl Transferase is only at **0.1-1% of adult levels**
- Conjugation of Bilirubin in infants is reduced in comparison to adults.
- Further inhibition of Bilirubin conjugation by **other agents leads** to increased levels of Bilirubin in the blood.

- Breast-milk of some women contains a **metabolite of Progesterone** called **3-Alpha-20-beta Pregnanediol.**

- **This metabolite inhibits** the action of the **conjugating enzyme** **Uridine Di Phospho (UDPGA) Glucuronyl Transferase.**
- **This brings poor conjugation and subsequent excretion of Bilirubin.**

- An enzyme in breast milk called **Lipoprotein Lipase** produces increased concentration of **non esterified free fatty acids**
- That **inhibit Hepatic UDP Glucuronyl Transferase**
- Which again leads to decreased conjugation and subsequent excretion of Bilirubin.

- **Mothers taking drugs** like **Novobiocin,** Steroidal derivatives **or Rifampicin**
- **Drugs secreted through breast milk**
- Infant fed by this milk has **drug inhibitory effect on Bilirubin metabolism.**
- **Delay in Bilirubin uptake and conjugation in infants Liver.**
- Leads to Unconjugated Hyperbilirubinemia.

- **Management Of Breast Milk Jaundice**
- Temporary stoppage of breast milk feeding
- Till the drug is cleared away from the breast fed milk.

# Congenital Hyperbilirubinemia

## Congenital Hyperbilirubinemia

- **Genetic defects** in Bilirubin **uptake, conjugation and excretion of Bilirubin**
- Leads to elevated levels of Bilirubin in infants blood and body tissues.

| Congenital Disorders | Defect | Type Of Hyperbilirubinemia |
|---|---|---|
| Gilbert's Syndrome | Defect in uptake of Bilirubin by Liver cells | Unconjugated Hyperbilirubinemia |

| Congenital Disorders | Defect | Type Of Hyperbilirubinemia |
|---|---|---|
| Crigler Najjar Syndrome-I | **Complete absence of** enzyme UDP-Glucuronyl Transferase | Unconjugated Hyperbilirubinemia |

| Congenital Disorders | Defect | Type Of Hyperbilirubinemia |
|---|---|---|
| Crigler Najjar Syndrome-II | **Partial absence of** enzyme UDP-Glucuronyl Transferase | Unconjugated Hyperbilirubinemia |

| Congenital Disorders | Defect | Type Of Hyperbilirubinemia |
|---|---|---|
| **Dubin Johnson's Syndrome/**<br><br>**Black Liver Jaundice** | **Defect in Hepatic excretion** of conjugated Bilirubin | Conjugated Hyperbilirubinemia<br><br>Deposition of Bilirubin in Liver |

| Congenital Disorders | Defect | Type Of Hyperbilirubinemia |
|---|---|---|
| **Rotors Syndrome** | Unknown Autosomal Recessive Inheritance | Conjugated Hyperbilirubinemia<br><br>Deposition of Bilirubin in Liver |

# Conditions Causing Unconjugated Hyperbilirubinemia

# Conditions Causing Unconjugated Hyperbilirubinemia

- Hemolytic Jaundice
- Hepatic Jaundice
- Neonatal/ Physiological Jaundice
- Breast Milk Jaundice
- Gilbert's Syndrome
- Crigler Najjar Syndrome
- Hypoalbuminemia
- High Drug Concentration lowering Albumin activity.

# Conditions Causing Conjugated Hyperbilirubinemia

- Obstructive Jaundice
- Hepatic Jaundice
- Dubin Johnson's Syndrome
- Rotors Syndrome

# Differential Diagnosis Of Jaundice

| Parameters | Hemolytic Jaundice | Hepatic Jaundice | Obstructive Jaundice |
|---|---|---|---|
| Serum Bilirubin | Indirect Bilirubin/ Unconjugated Bilirubin increased | Biphasic Both Direct and Indirect Bilirubin Increased | Direct Bilirubin/ Conjugated Bilirubin Increased |
| Urine Urobilinogen | Increased | Normal or Decreased | Absent or Decreased |
| Urine Bilirubin and Bile Salts | Absent | May present in small amounts | Present in high amounts |
| Fecal Stercobilin Color Of Feces | Increased Dark color feces | Normal or Decreased | In traces or absent Clay colored feces |
| Serum ALT activity | Normal | Significantly Increased | May be slightly increased |
| Serum ALP | Normal | May slightly | Significantly |

# Questions

- **SHORT NOTES**
- Outline of Heme Biosynthesis
- Porphyrias: Types , Causes and Consequences
- Acute Intermittent Porphyria (AIP).

- Catabolism of Hb /Fate & Formation of Bilirubin.
- Jaundice & its types.
- Vanden-Berg's Reaction and its significance.

- Name the enzymes of Heme Biosynthesis inhibited during lead poisoning. What is its consequence?
- Comparison between abnormal Hb derivatives & abnormal Hb variants.

- Abnormal Hb variants
- Hemoglobinopathies
- Sickle Cell Anemia
- Thalassemias
- α Thalassemia
- β Thalassemia

- Name the Bile pigments & give the significance of their presence in blood & urine.
- Laboratory reports of Obstructive Jaundice.
- When and Why Bilirubin comes in Urine Explain?

- Neonatal Jaundice
- Breast Milk and Breast Feeding Jaundice
- Congenital Hyperbilirubinemias

- Write the defect & type of Hyperbilirubinemia in
  - Crigler's Najjar Syndrome-I
  - Crigler's Najjar Syndrome-II
  - Gilbert's Disease.
  - Dubin Johnson Syndrome.

# THANK YOU