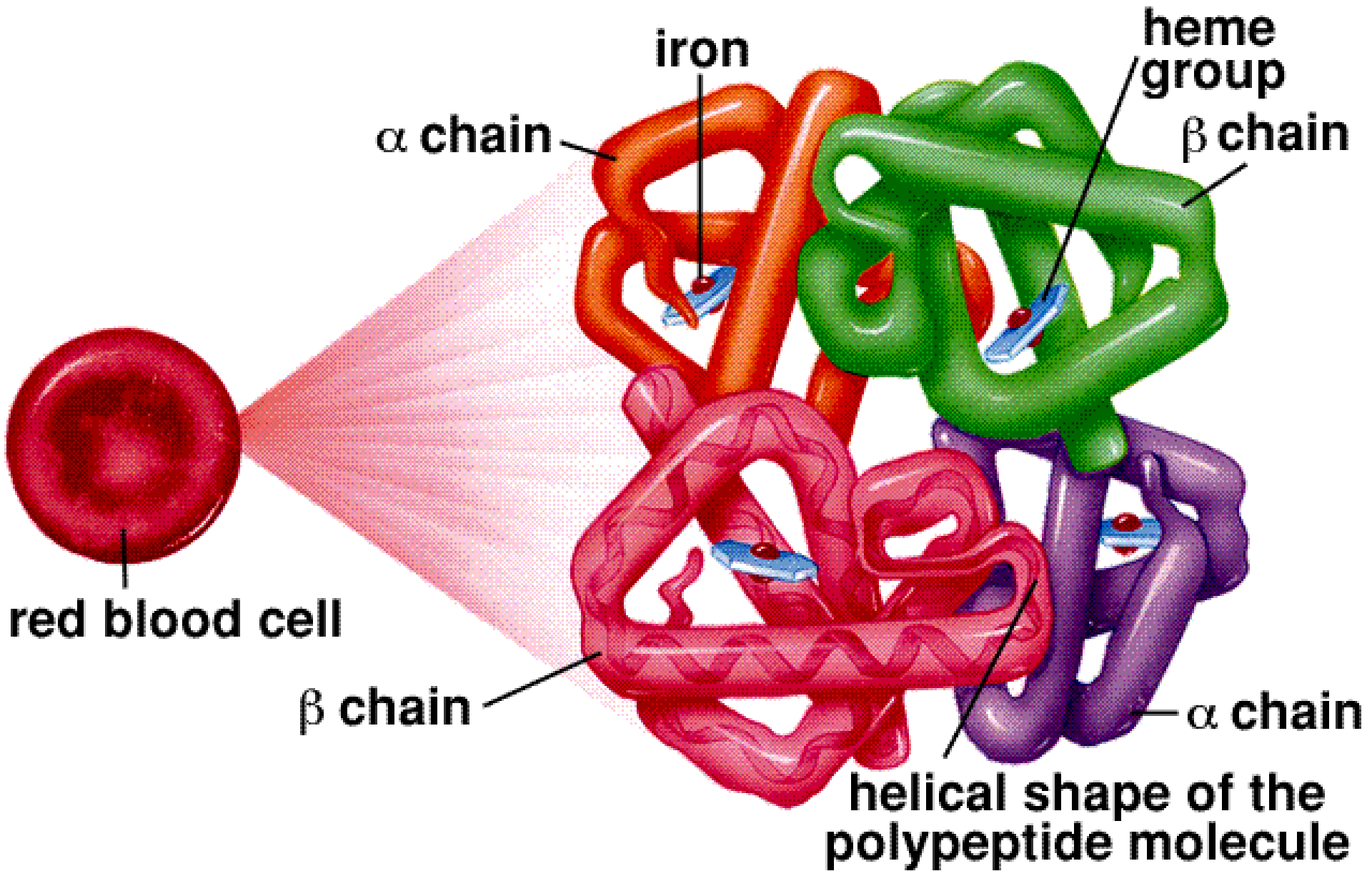
BLOOD

HEMOGLOBIN

- Introduction of hemoglobin
- Structure of hemoglobin
- Types of hemoglobin
- **DERIVATIVES OF HAEMOGLOBIN**
- ***PORPHYRINS***

INTRODUCTION OF HEMOGLOBIN

Hemoglobin Molecule



Haemoglobin

- Red coloring matter of the blood
  - Conjugated protein
  - Haem is the prosthetic group
  - Globin is the protein part
- It is composed of two identical alpha and two identical Beta chain.
- To each of these four chains a haeme molecule is attached

- Normal concentration of Hb is 14 to 16gm %
- About 750 gms of Hb in total circulation
- About 6.25Gm of Hb are produced and destroyed in the body each day.

Hb is present in R.B.C but R.B.C cannot synth

- Globin :-Because it lacks nucleus –So cannot synth proteins
- Haem:-Because it lacks mitochondria,required for synth of porphyrins.

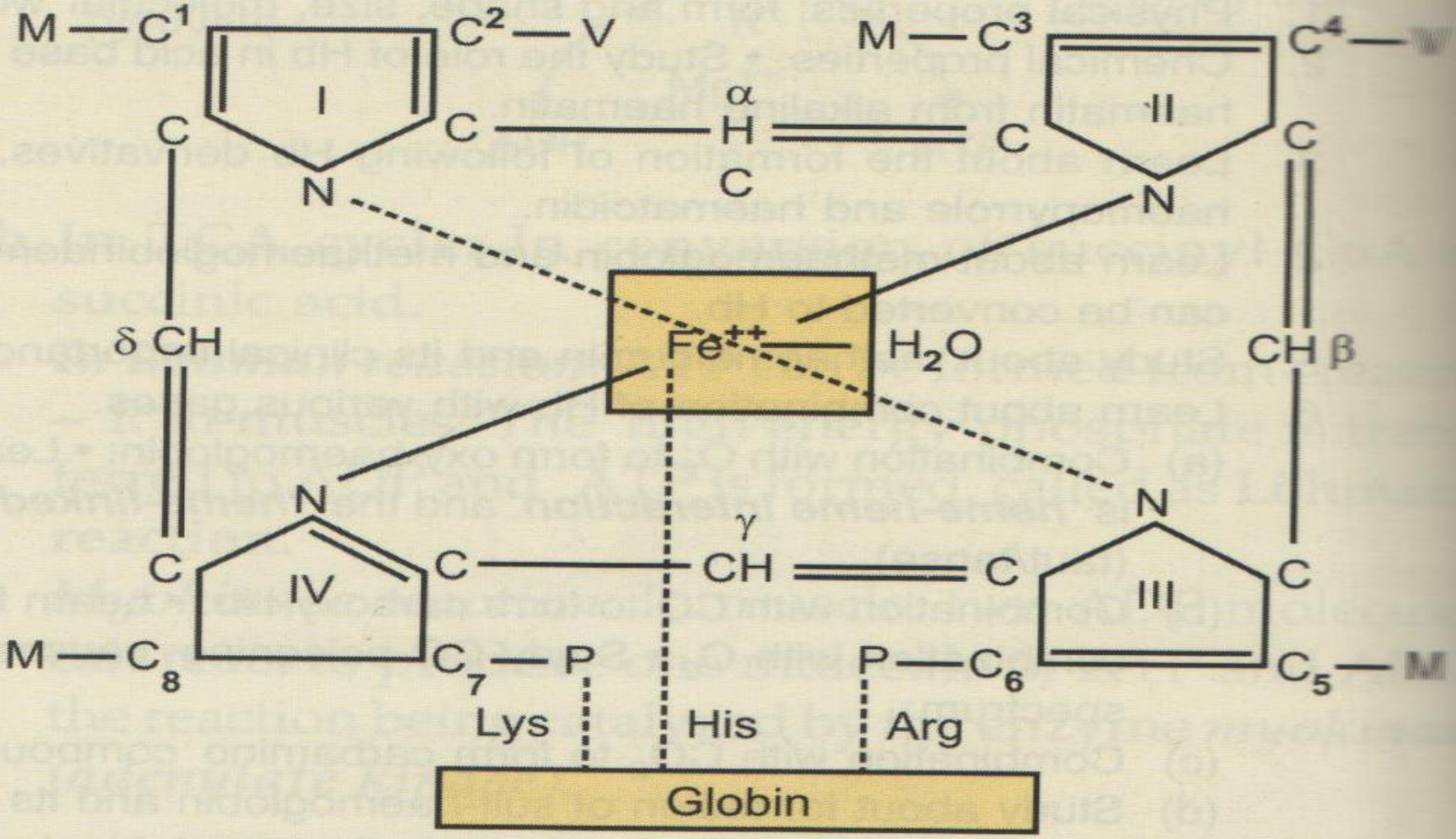
Structure of hemoglobin

- Can be studied under two headings:

A. Structure of heme

B. Structure of globin

- Structure of heme



M = Methyl — CH_3
V = Vinyl — $CH=CH_2$
P = Propionic acid — CH_2 — CH_2 — COOH

Structure of Heame

- Fe-Porphyrin compound .
- Porhyrins have tetra pyrrole structure
- Four pyrole rings are combined through –CH=bridge called Methyne or Methylidine bridges.
- The outer carbons of four pyrrole rings which are not linked with methyne bridges are number 1 to 8.
- The methyne bridges are numbered as α,β, γ,δ

- The propionic Acid group of 6 and 7 position of Haem of III and IV pyrroles are also linked to Arg and Lys of Polypeptide chain.

Fe: (Ferrous)

- Four linkages with Nitrogens of four pyrrole rings.
- Fifth attachment is with imidazole ring of histadine of polypeptide chain.
- Sixth valance is satisfied by its linkage to a molecule of water in deoxygenated hemoglobin.
- When hemoglobin is oxygenated, water is displaced by oxygen.

- The Heam pocket of α submits are of size just adeqate for entry of oxygen molecule but the entry of O_2 in haem pockets of β submit is blocked by a valine residue,in deoxygenated state.
- In oxygenated state this valine residue is remove and oxygen can enter.

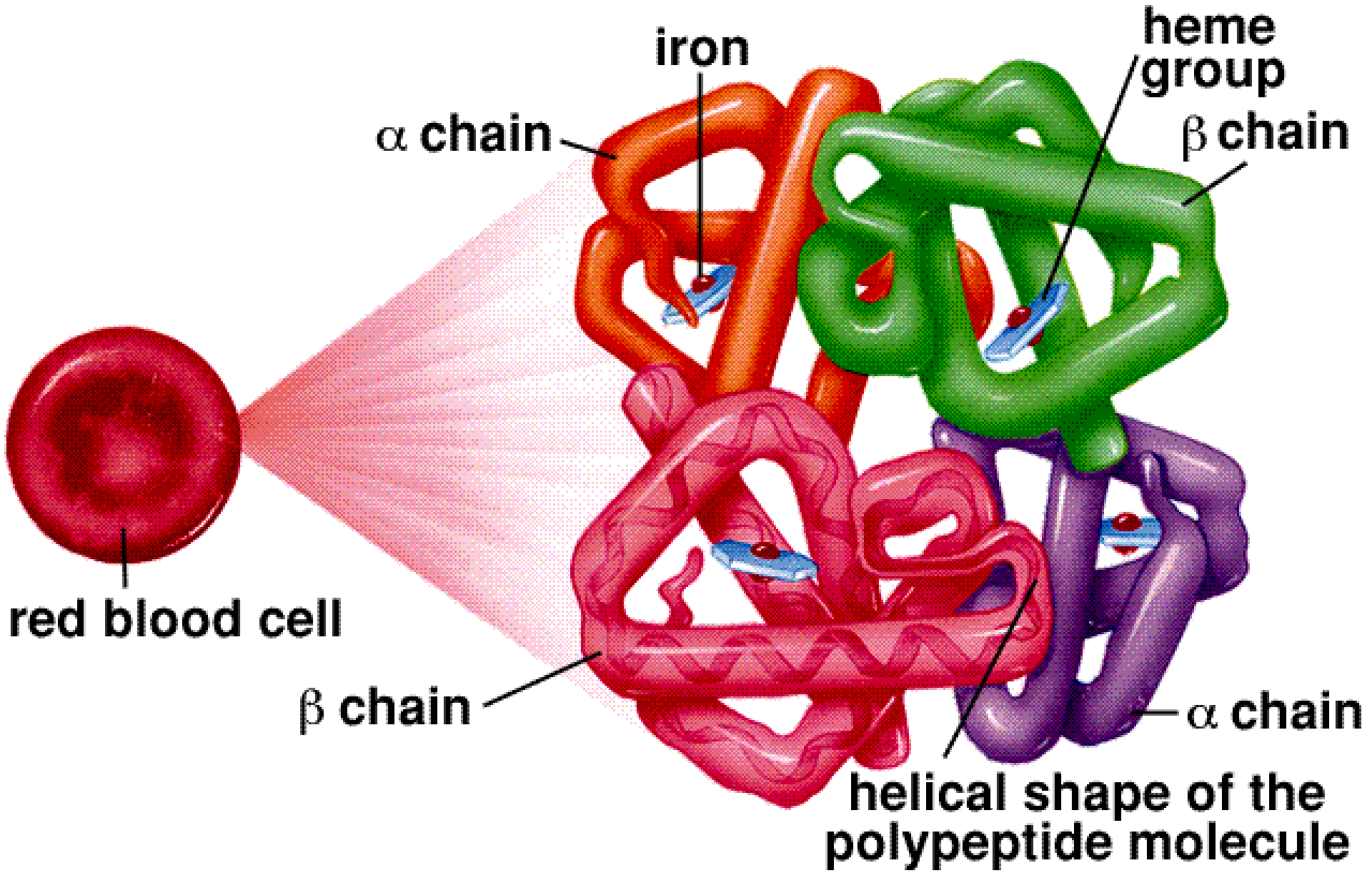
(Haem-Haem interaction)

- Haem-Pockets:

Hb contain hydrophobic a. acid internally and hydrophilic a.acid on surfaces – so waxy inside and soapy outside making it solouble in water but impermeable to water.

GLOBIN

Hemoglobin Molecule



GLOBIN :

- This contains four polypeptide chains, thus hemoglobin is a tetramer consisting of four sub-units or monomers.
- Each monomer consists of one Heme part attached to one of the four polypeptide sub-units groups and four polypeptide chains in each molecule of hemoglobin.

- Polypeptide chain of globin has relative high content of His and lysine, and a small amount of isoleucine

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TYPES OF HEMOGLOBIN:

- HEMOGLOBIN A
- HEMOGLOBIN F
- HEMOGLOBIN A2
- HEMOGLOBIN A3
- EMBRYONIC HEMOGLOBIN
- Glycosylated Hb.

Hb-A, ADULT HEMOGLOBIN:

- Hemoglobin A consists of two Alpha and two Beta chains.
- The alpha chain consists of 141 amino acids and the beta chains consists of 146 amino acids.

- The alpha and beta chains are fit together in such a way that the hemoglobin molecule is nearly spherical.
- The centre of molecule has a cavity which is filled with water
- It is about 90-97 %

$Hb-A_2$

- Minor component of normal adult Hb
- 2-3%
- It contains 2α and 2δ chains
- Electro phoretically it is slowly migrating fraction .

- HEMOGLOBIN A 3
- about 3-10% of the total
- appears to an altered form of Hb A.
- Found chiefly in matured blood cells
- Electrophoretically fast fraction .

Embryonic Hb

Occurs in first three months of Embryonic life

They include

Gower I. Two epsilon ε and two zeta chains

Gower II.Two zeta and two gama chains

FETAL HEMOGLOBIN Hb F:

- It consists of two alpha and two gamma chains.
- Less than 1% in adults.
- At the age of 4-6 months , gamma chain formation is normally replaced by beta chain formation.

- The affinity of HbF for Oxygen is more due to the absence of 2,3 BPG,due to lack of some positive charge a.a that are responsible for binding of 2,3 BPG
- Foetus can`t inspire oxygen from atmosphere, so has to preserve it.

- Affinity to oxygen is more as compared to adult Hb
- Delivery power of oxygen is less
- Persistence of Hb- F after one year is pathological

Table 11.1: Differentiation of Hb-A from Hb-F

	Hb-A	Hb-F
1.	***Polypeptide chains:*** $\alpha_2\beta_2$	$\alpha_2\gamma_2$
2.	***Behaviour with alkali:*** Denatured by alkali	Resistant to alkali denaturation
3.	***Electrophoresis:*** At pH 8.9 Hb-A moves ahead of Hb-F.	Hb-F moves behind Hb-A
4.	***BPG-content:*** BPG ↑	BPG ↓
5.	***Affinity to O_2:*** Affinity of O_2 less	Affinity to O_2 ↑
6.	***Delivery of O_2:*** Delivery power of O_2 ↑ (unloading)	Delivery power of O_2↓ decreased
7.	***Concentration:*** ***At birth*** $Hb\text{-}A_1$-85% Hb-F-15% Hb-F disappears by end of first year ***Persistence of Hb-F after one year is pathological*** ***In adult*** $Hb\text{-}A_1$-90 to 95%	Present in foetal life Disappears after one year

Glycosylated hemoglbin HbAIc:

- Hb to which glucose is bound .
- Normal –upto 6%
- Increased in poorly controled dibetes mellitus
- Level above 9% -poor control of blood sugar level above 12% - very poor control.
- The level of HbAIc reflect the average blood glucose level over the past three months.
- Glucose is attached non-enzymatically to the valine of β chain

- Hb A1c at 6% correlates to an average glucose level over 3 months of 135mg /dl
- Each 1% above 6% Hb A1c represents an increase of 35mg/dl of glucgose.
- Detecting an 8% concentration of Hb A1c would equal to glucose level of

  135 + 70 or 205 mg /dl average over past 3 months.

DERIVATIVES OF HAEMOGLOBIN

1. Action of Acids and Alkalies :

   Acid haematin and alkali haematin

2. Haemin

   Chemically it is Haematin hydrochloride ,prepared by boiling oxy-Hb with Na Cl and glacial a.acid

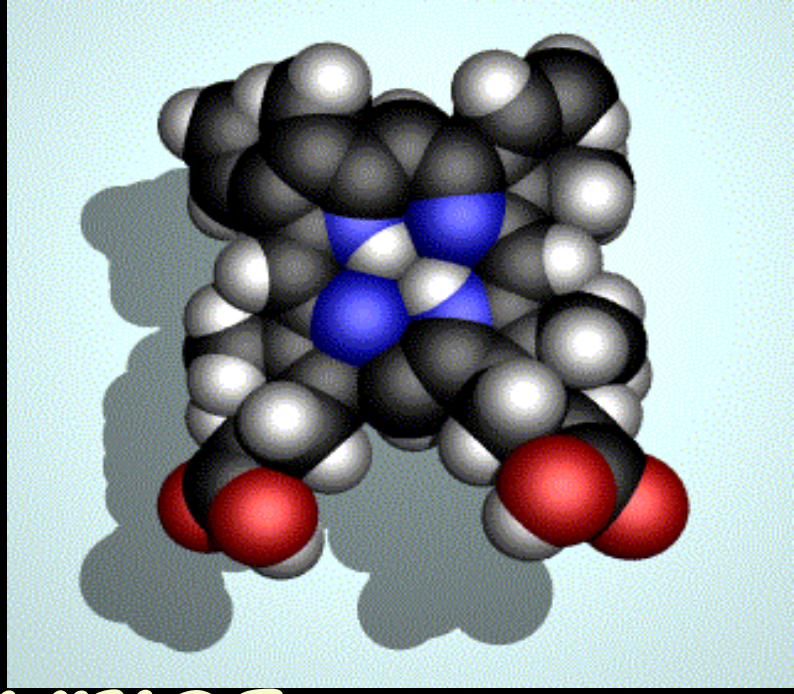
3. Haemochromogen

4. Haematoidin

5. Haematoporphyrin

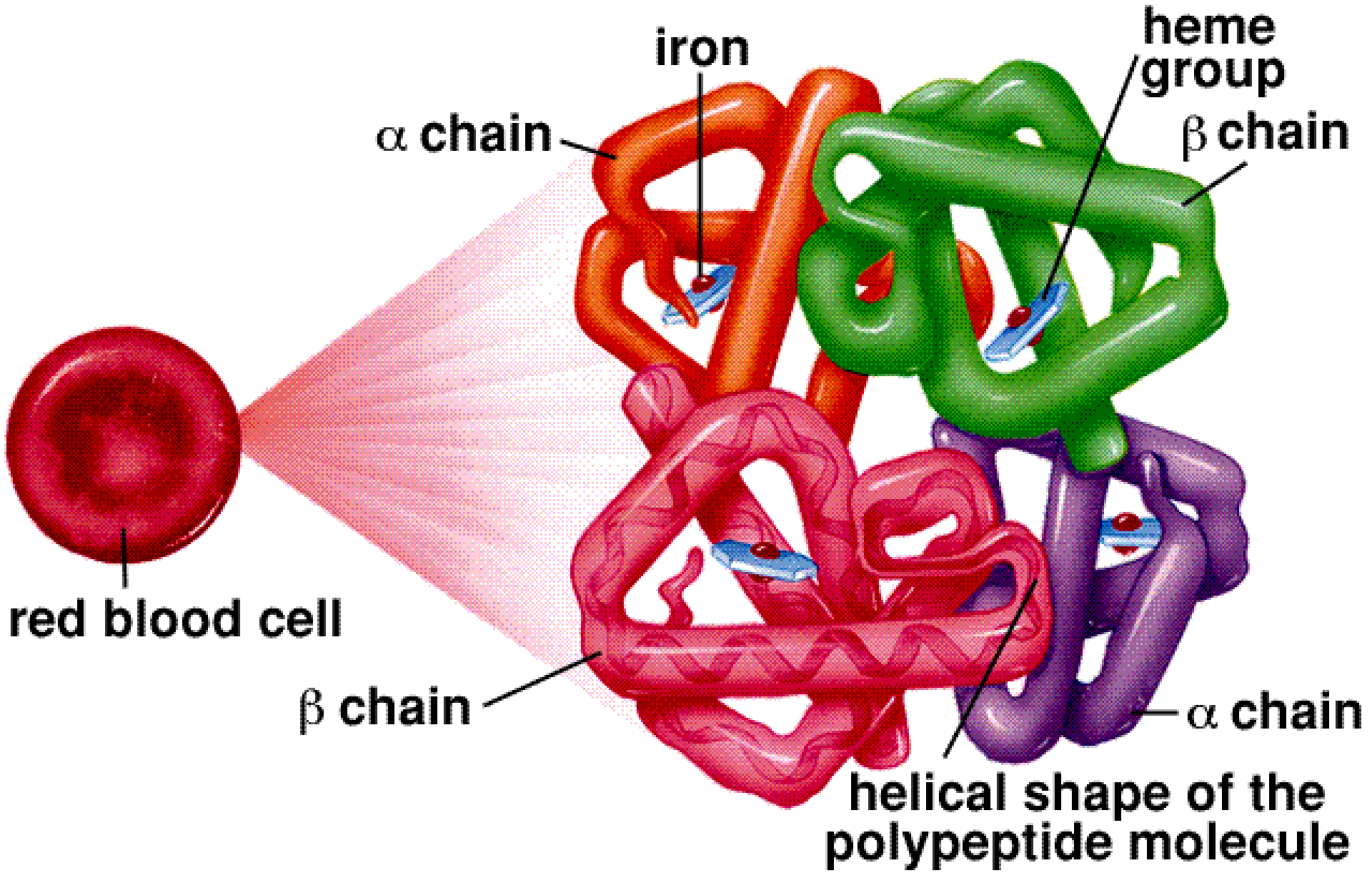
6. Methaemoglobin

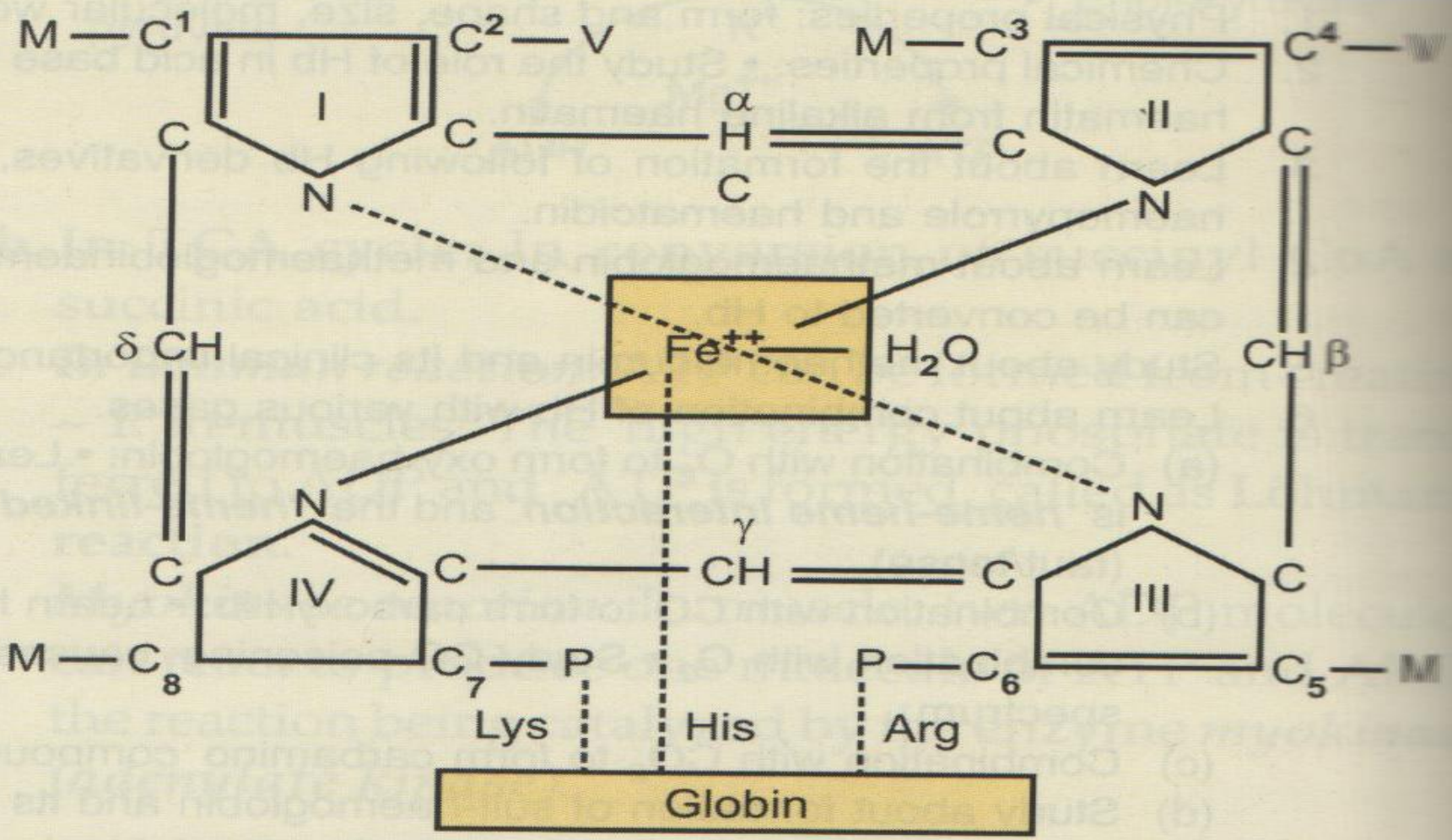
7.Methaemalbumin



Porphyrins, porphyrias & Synthesis of Heme

Hemoglobin Molecule





M = Methyl — CH_3
V = Vinyl — $CH=CH_2$
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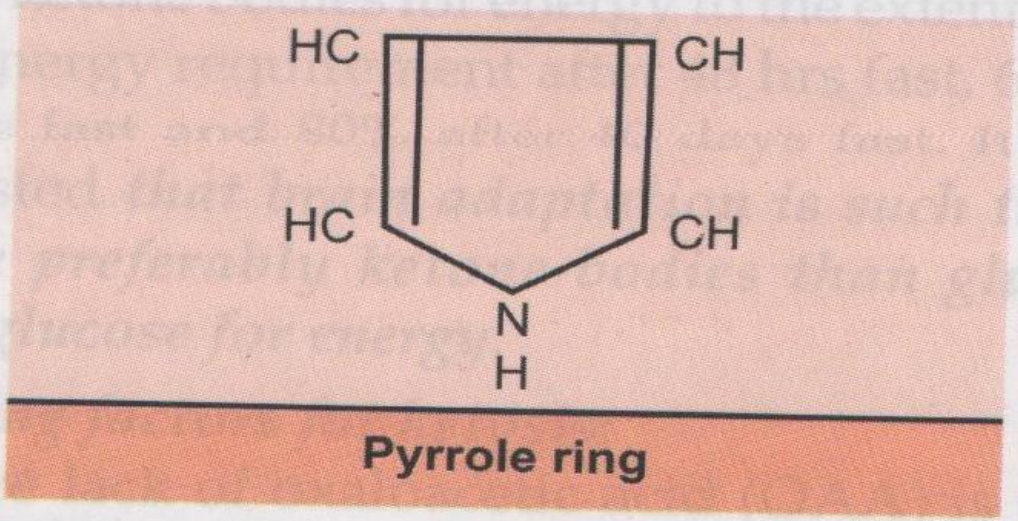
PORPHYRINS

INTRODUCTION

- The porphyrins are complex structures consisting of 4 pyrrole rings, united by ***"methyne" bridges (or methylidene bridges)***



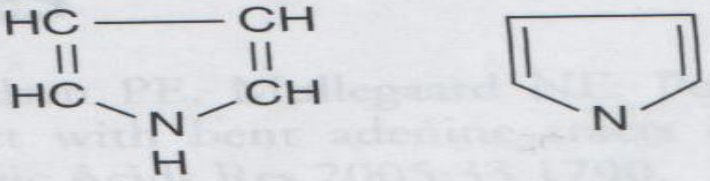
Pyrrole ring

- The nitrogen of 4 pyrrole rings can form complex with metallic ions such as Fe^{++} and Mg^{++}.
- They form the prosthetic groups of conjugated proteins, viz.

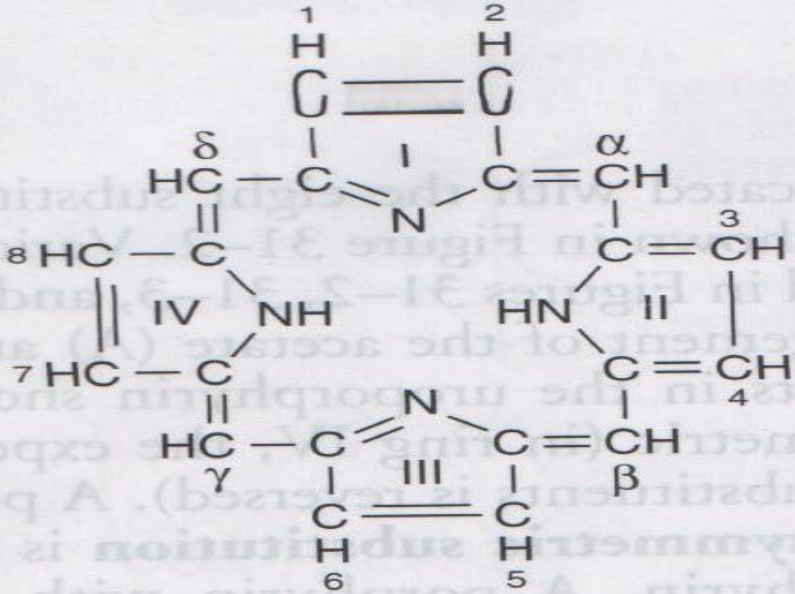
Table 31–1. Examples of some important human and animal hemoproteins.[1]

Protein	Function
Hemoglobin	Transport of oxygen in blood
Myoglobin	Storage of oxygen in muscle
Cytochrome *c*	Involvement in electron transport chain
Cytochrome P450	Hydroxylation of xenobiotics
Catalase	Degradation of hydrogen peroxide
Tryptophan pyrrolase	Oxidation of tryptophan

[1]The functions of the above proteins are described in various chapters of this text.



Pyrrole



Porphyrin
($C_{20}H_{14}N_4$)

Figure 31–1. The porphyrin molecule. Rings are labeled I, II, III, and IV. Substituent positions on the rings are labeled 1, 2, 3, 4, 5, 6, 7, and 8. The methyne bridges (—HC═) are labeled α, β, γ, and δ. The numbering system used is that of Hans Fischer.

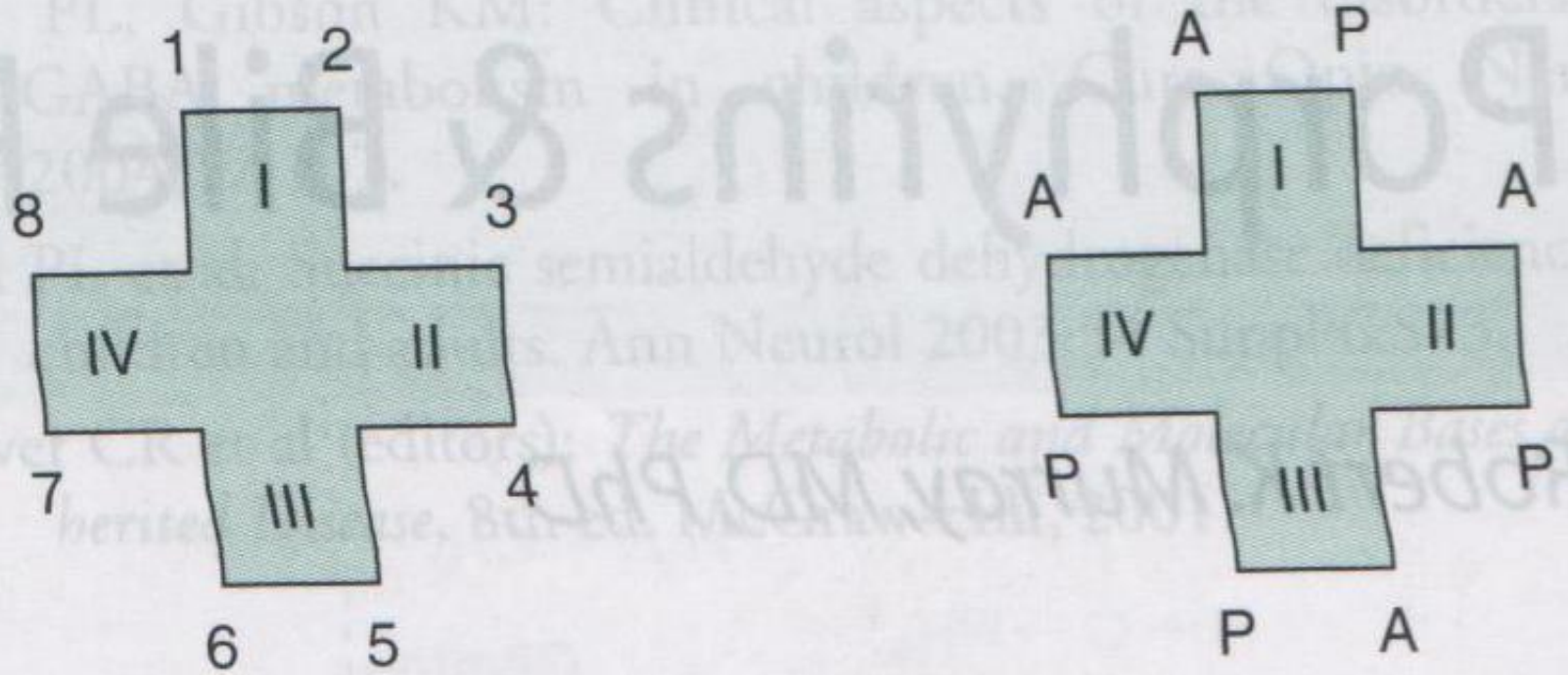
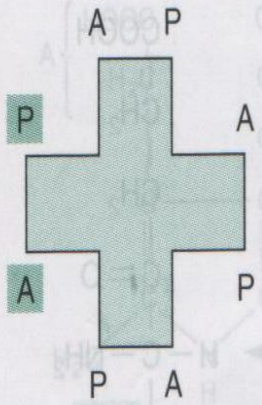
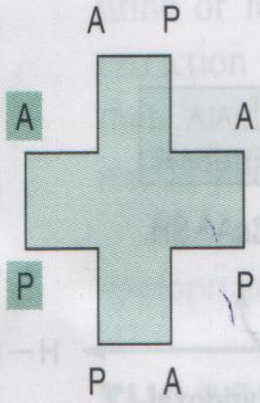


Figure 31–2. Uroporphyrin III. A (acetate) = $—CH_2COOH$; P (propionate) = $—CH_2CH_2COOH$. Note the asymmetry of substituents in ring IV (see text).

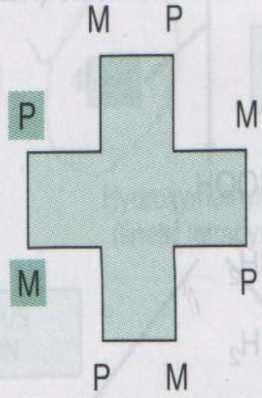


Uroporphyrin I

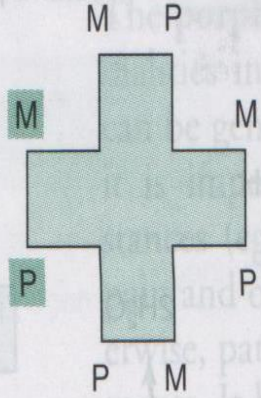


Uroporphyrin III

Uroporphyrins were first found in the urine, but they are not restricted to urine.



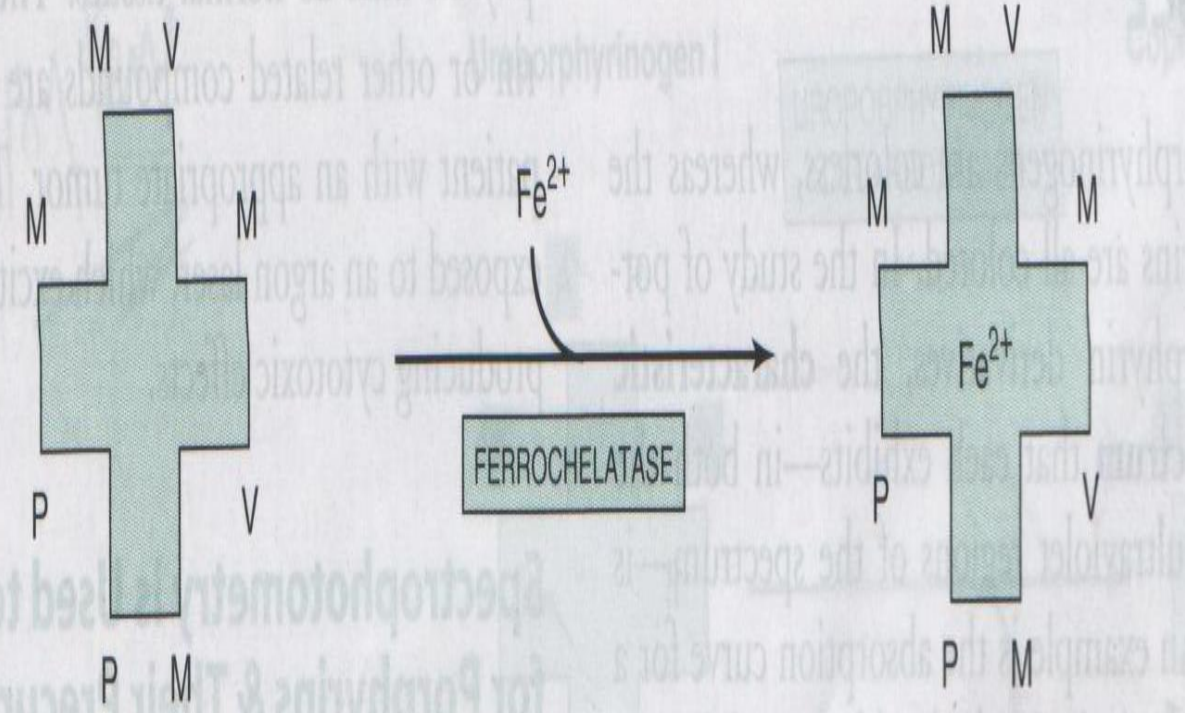
Coproporphyrin I



Coproporphyrin III

Coproporphyrins were first isolated from feces, but they are also found in urine.

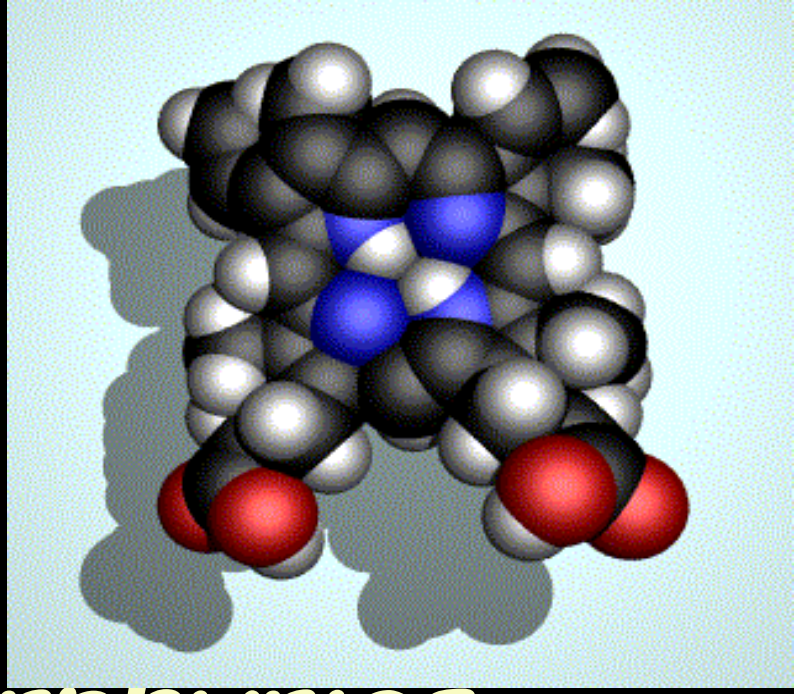
Figure 31–3. Uroporphyrins and coproporphyrins. A (acetate); P (propionate); M (methyl).



Protoporphyrin III (IX)
(parent porphyrin of heme)

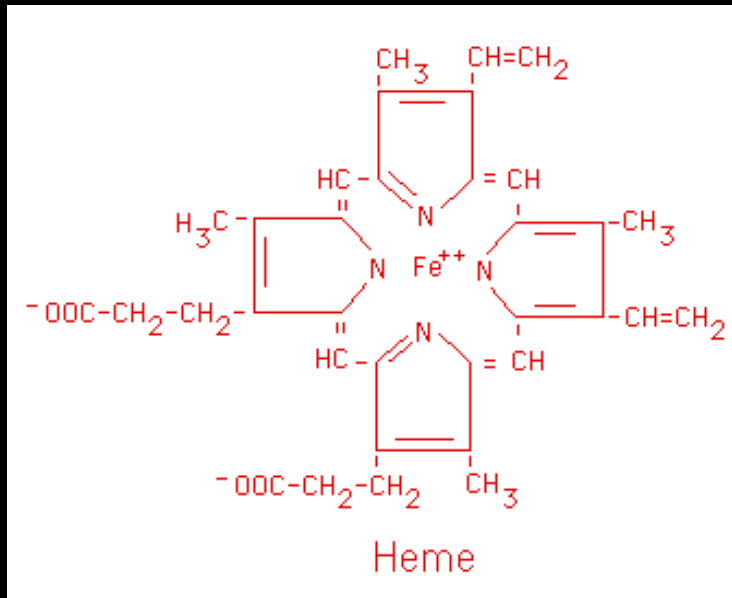
Heme
(prosthetic group of hemoglobin)

Figure 31–4. Addition of iron to protoporphyrin to form heme. V (vinyl) = $-CH=CH_2$.



Porphyrins, porphyrias & Synthesis of Heme

Porphyrins are cyclic compounds formed by joining together of four pyrrole rings through methyne bridges (–H-C=).



Heme

--CH_2—COO (Acetate)

--CH_2—CH_2—COO (Propionate)

--CH_3-- (methyl)

--CH = CH_2 (Vinyl)

--CH_2 = (Methyne Bridge)

--CH_2– (Methylene Bridge)

HEME SYNTHESIS

- The major sites of heme synthesis are
- Liver
- Erythroid tissues i.e

Erythrocyte producing cells of the bone marrow.

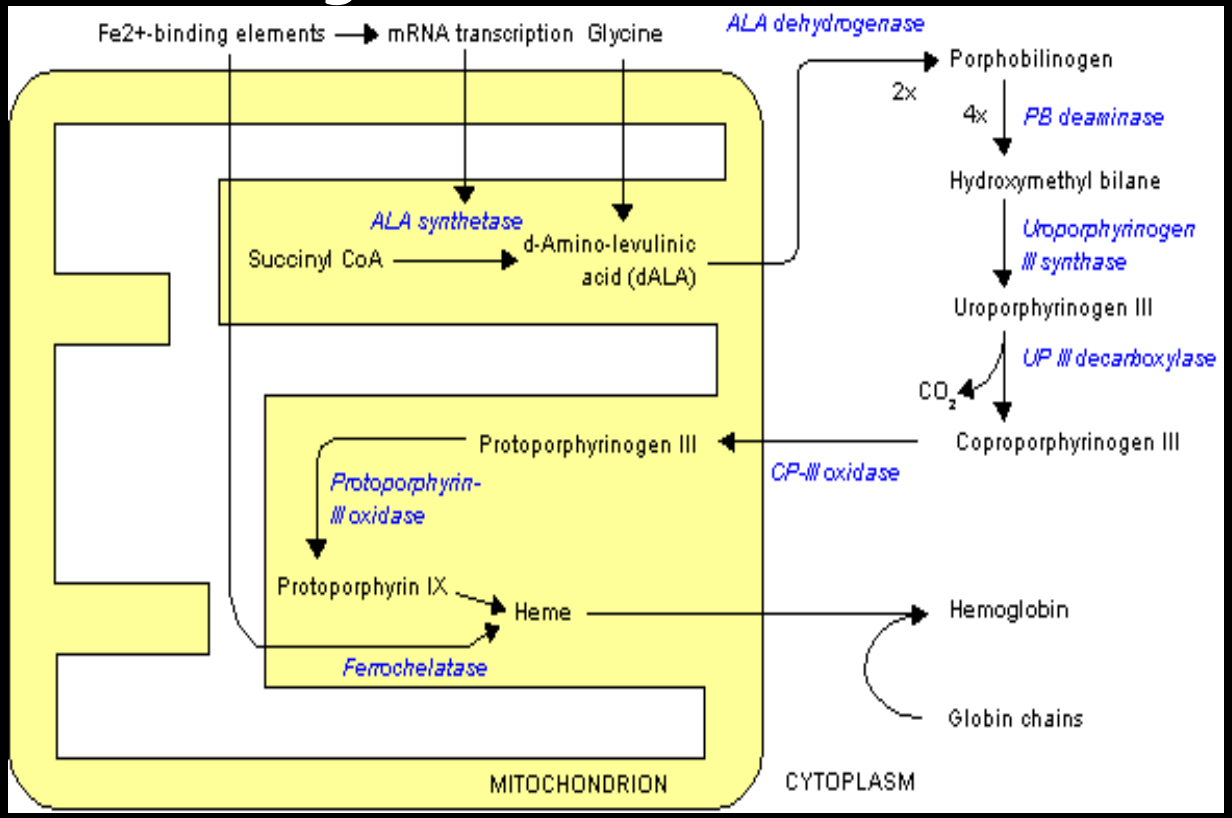
* No synthesis in matured RBC because they do not contain mitochondria

TWO COMPONENTS ARE REQUIRED FOR HEME SYNTHESIS

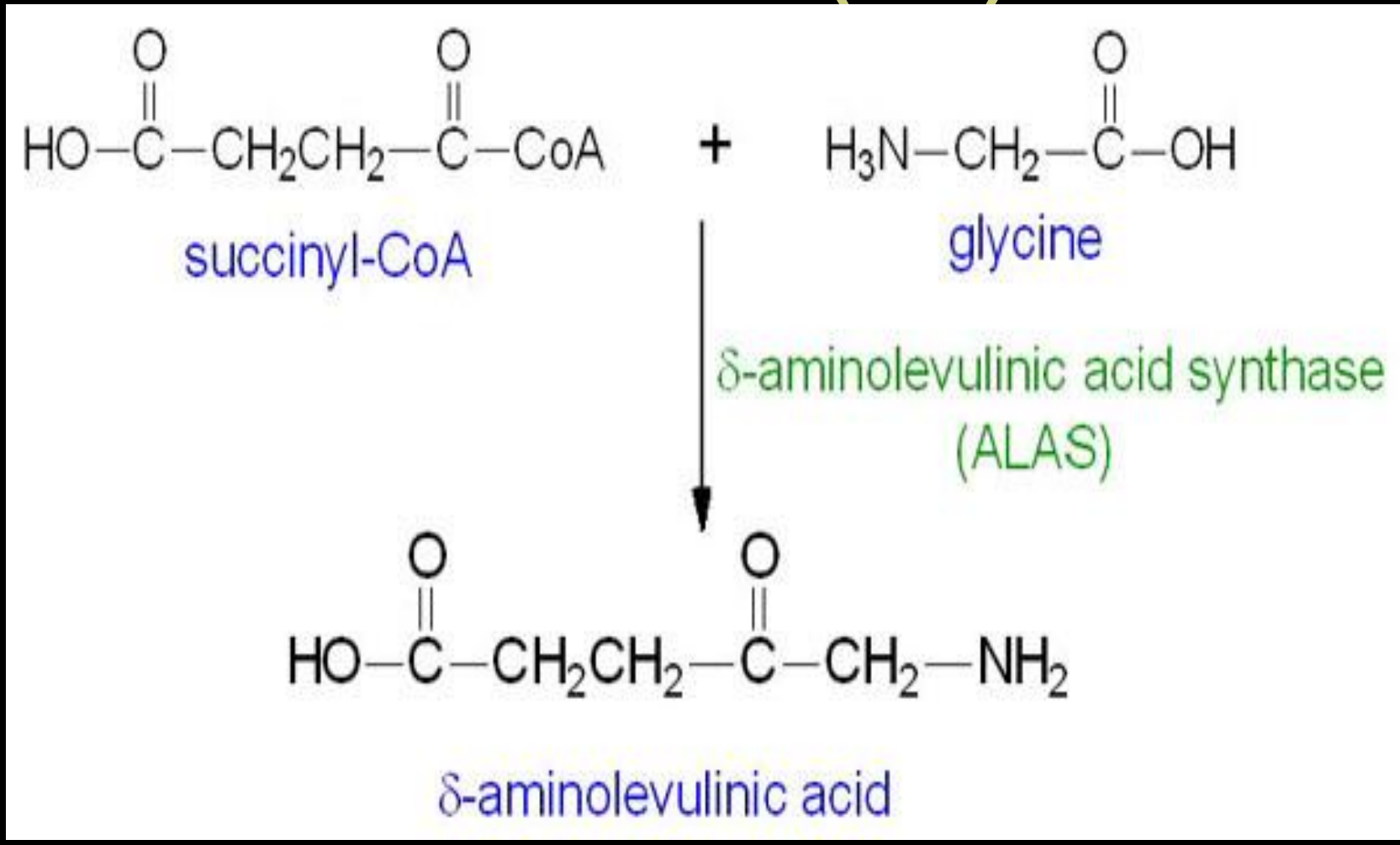
- Synthesis of porphyrins
- Iron

BIOSYNTHESIS OF PORPHYRINS AND HEME

- Porphyrin synthesis occurs in several stages which are given below: -



1. FORMATION OF DELTA-AMINOLEVULINIC ACID (ALA)

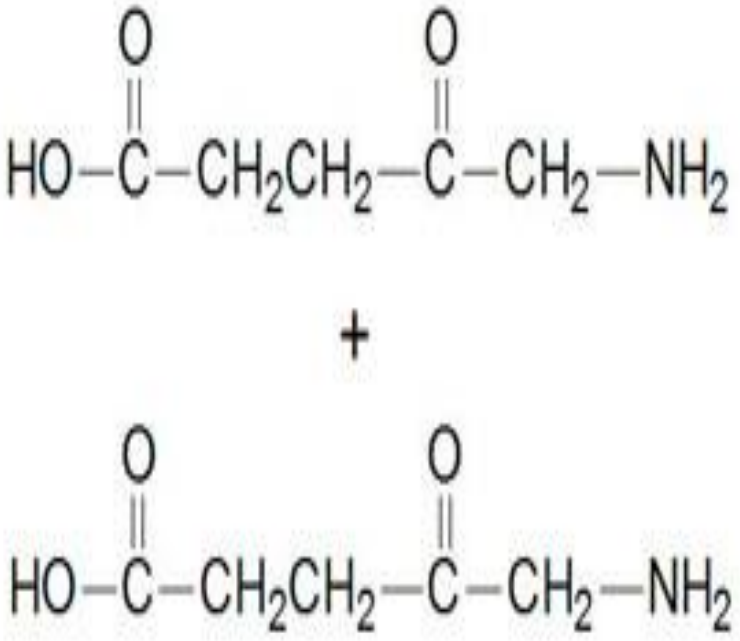


1. FORMATION OF DELTA-AMINOLEVULINIC ACID (ALA)

- Succinyl-CoA is produced either by Krebs citric acid cycle or by metabolic degradation of valine and isoleucine.
- ENZYME: ALA Synthase
- REACTION: MITOCHONDRIA
- ALA Synthase is inhibited by heme
- Main rate limiting enzyme

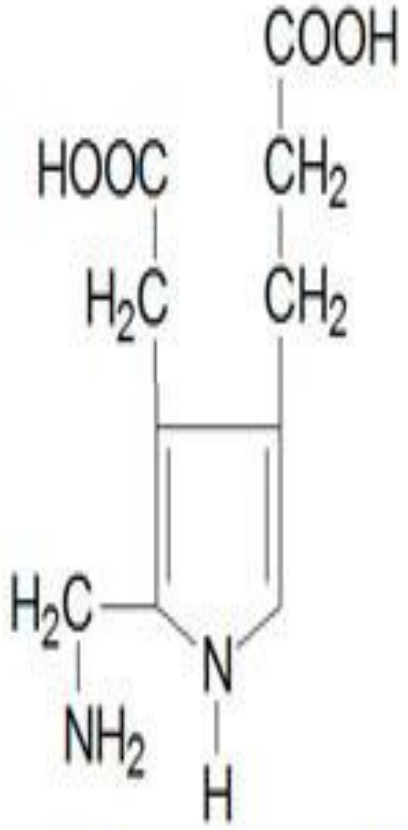
And require B6_P

2. FORMATION OF



δ-aminolevulinic acid, ALA

ALA dehydratase
(PBG synthase)

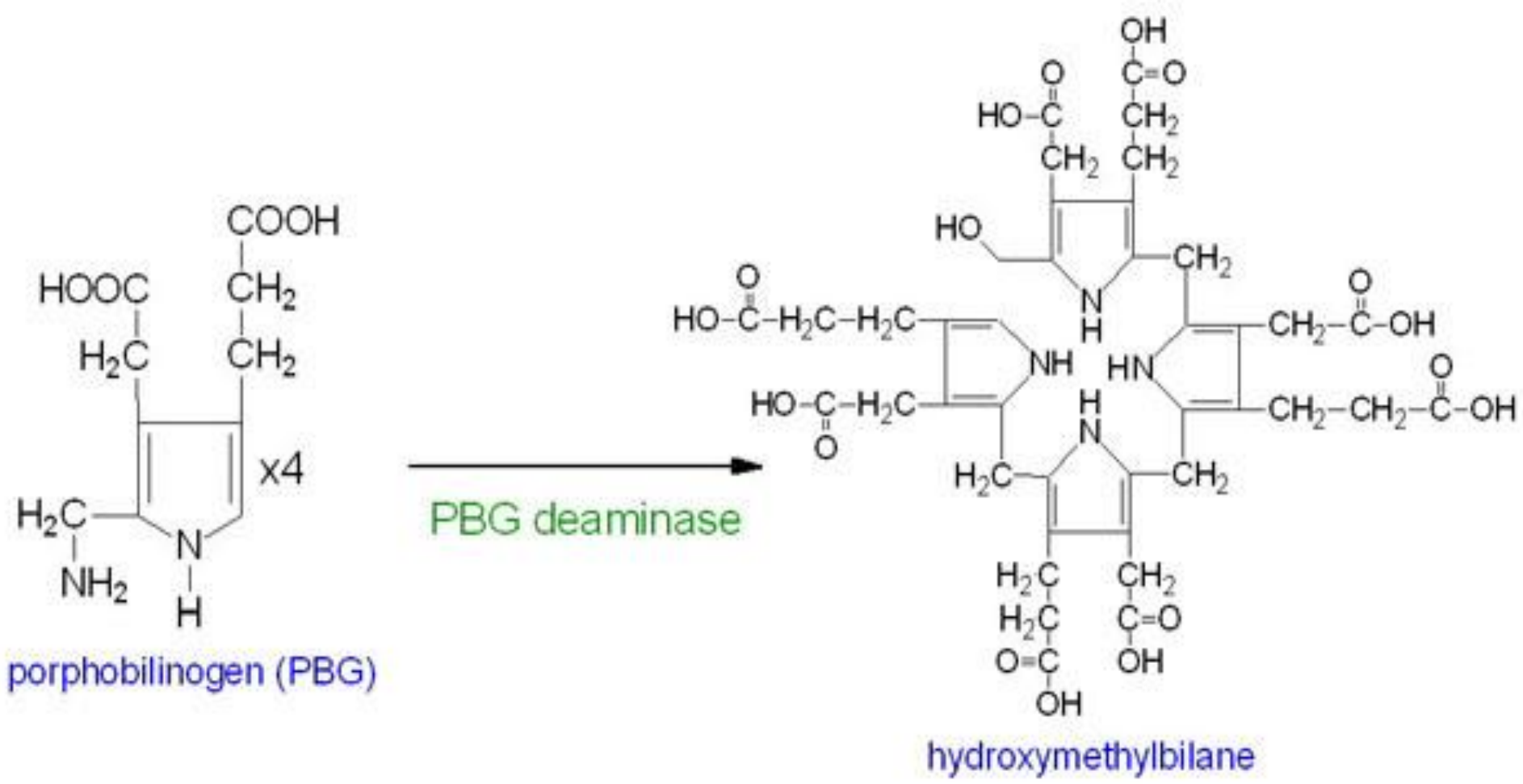


porphobilinogen, PBG

2. FORMATION OF PORPHOBILINOGEN

- This compound is a monopyrrole and its formation takes place by the condensation of two molecules of ALA.
- ENZYME: ALA DEHYDRATASE and also needs reduced glutathione.
- REACTION: CYTOSOL.
- Zn containing enzyme inhibited by lead and require cu++
- Second rate limiting enzyme inhibited by heme.

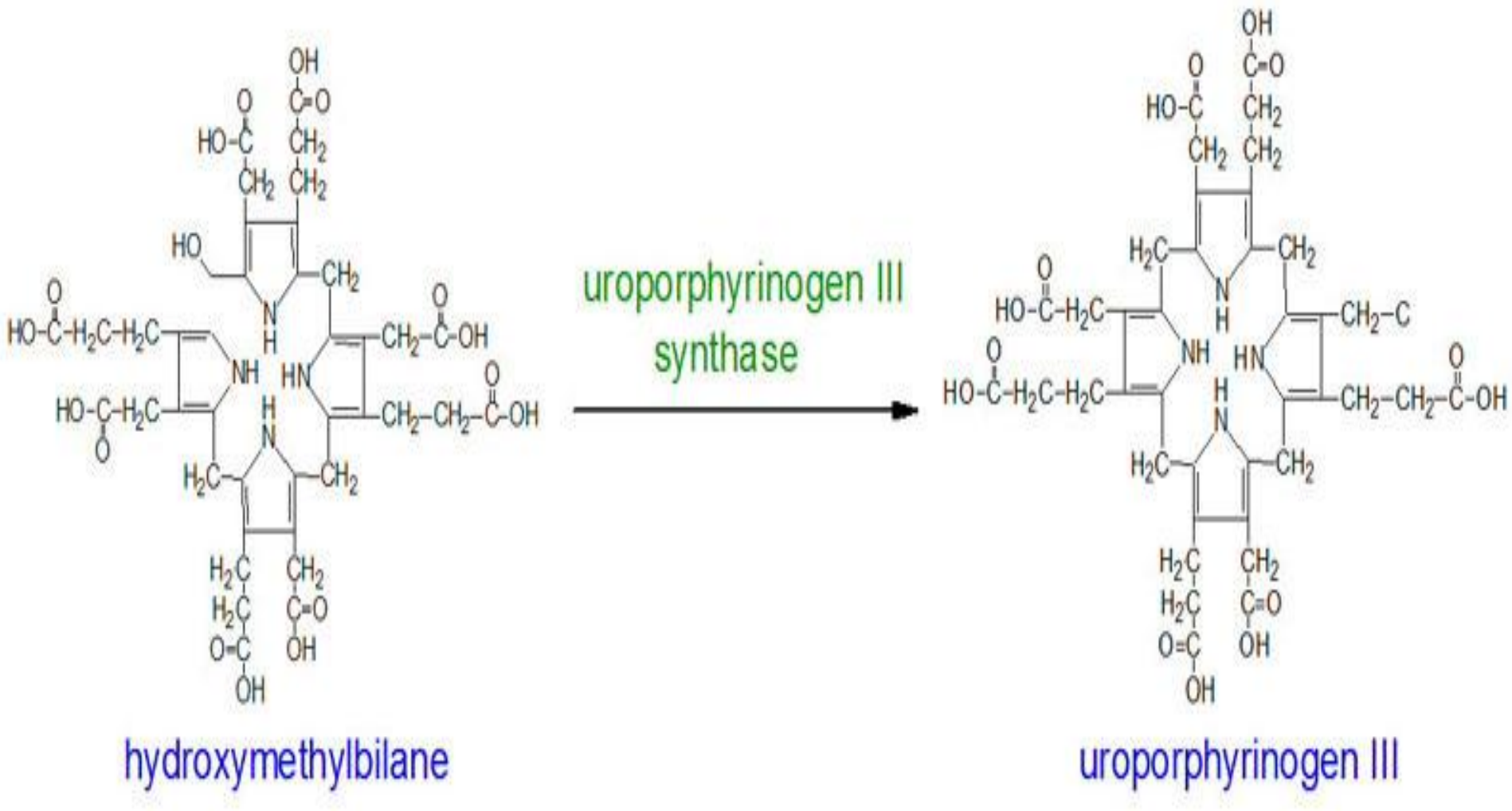
3. FORMATION OF HYDROXYMETHYLBILANE OR UROPORPHYRINOGEN-I



3. FORMATION OF HYDROXYMETHYLBILANE

- Porphobilinogen is converted to hydroxymethylbilane or uroporphyrinogen-1
- ENZYME :UROPORPHYRINOGEN I SYNTHASE or Hydroxy methyl bilane synthase or porphobilinogen Deaminase.
- REACTION: CYTOSOL
- It is a linear tetrapyrrole

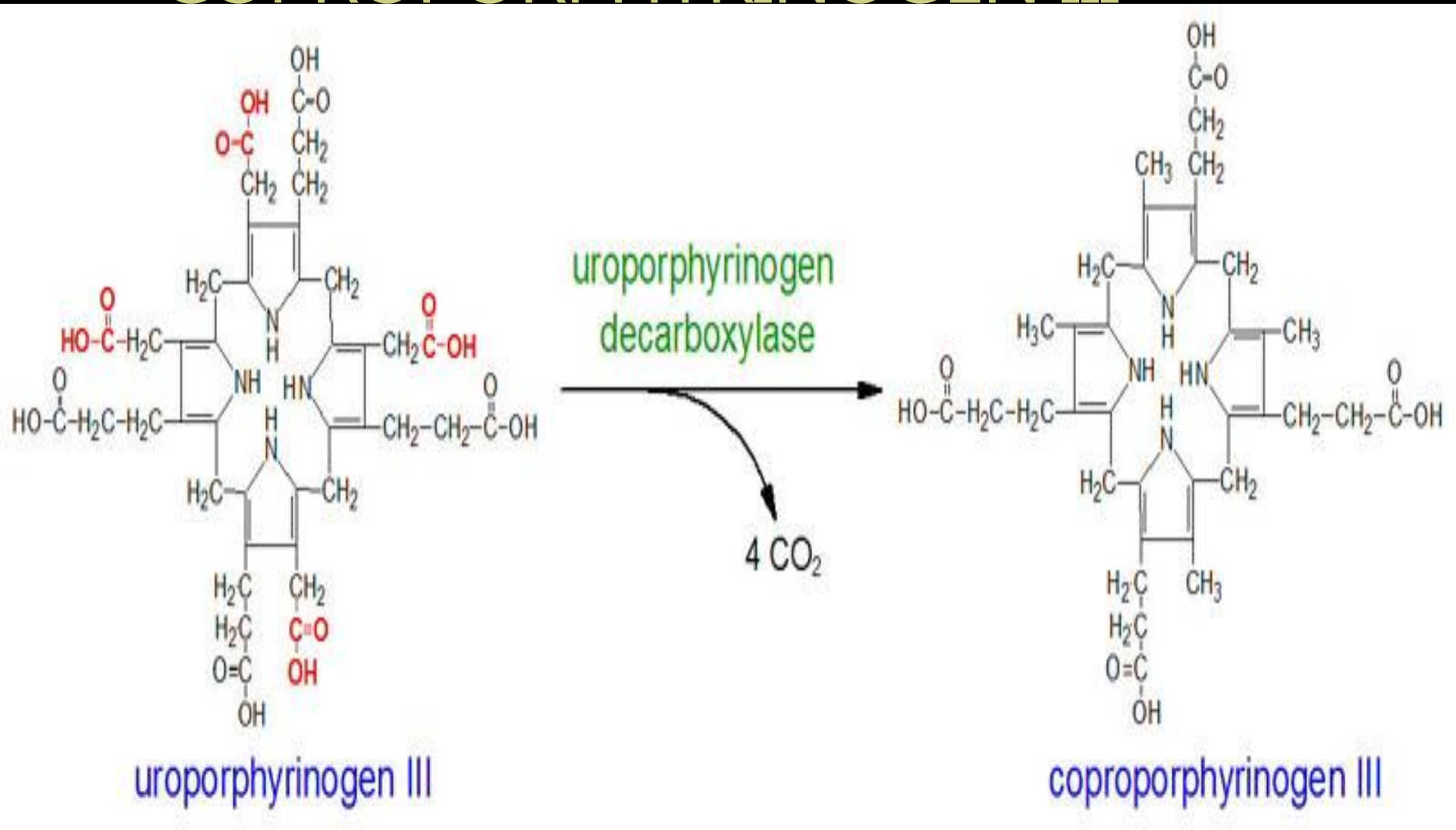
4. FORMATION OF UROPORPHYNOGEN III



4. FORMATION OF UROPORPHYNOGEN III

- Uroporphrinogen III is the **first cyclic tetrapyrrole** derivative; it is formed from hydroxymethylbilane by the concomitant action of ISOMERASE with DEAMINASE resulting in reversal of acetate and propionate in pyrrole ring IV.
- ENZYME:UROPORPHYRINOGEN III COSYNTHASE
- REACTION :CYTOSOL

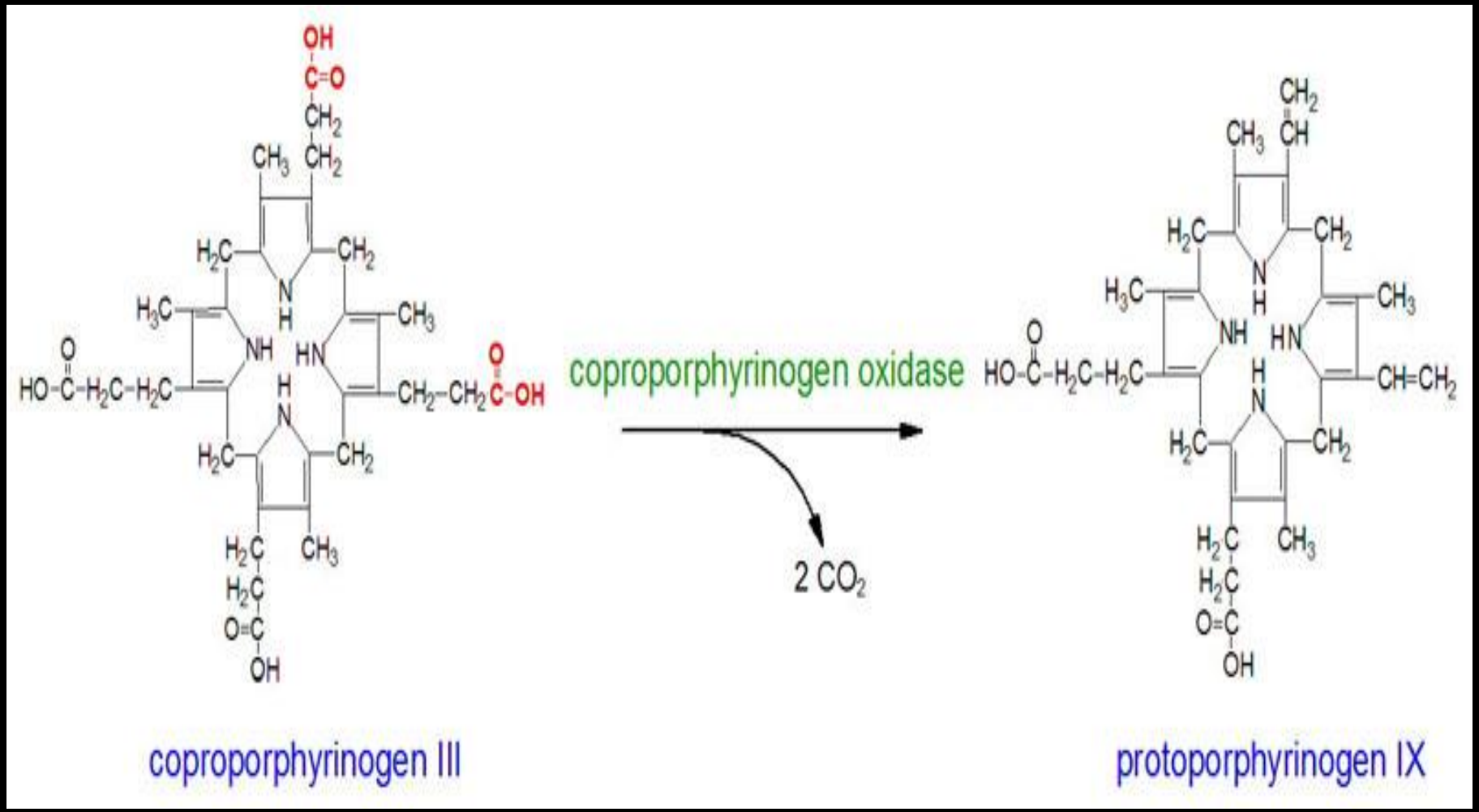
5. FORMATION OF COPROPORPHYRINOGEN III



5. FORMATION OF COPROPORPHYRINOGEN III

- REACTION:CYTOSOL
- ENZYME:UROPORPHYRINOGEN III DECARBOXYLASE
- In this reaction all the acetate groups of uroporphyrinogen III lose CO_2 and are changed to methyl substituents.

6. FORMATION OF PROTOPORPHYRINOGEN III OR IX.



6. FORMATION OF PROTOPORPHYRINOGEN III OR IX.

- The coproporphyrinogen III formed in the **CYTOSOL** enters the **MITOCHONDRIA** where it is converted to protoporphyrinogen IX.

  ENZYME:COPROPORPHYRINOGEN-OXIDASE.

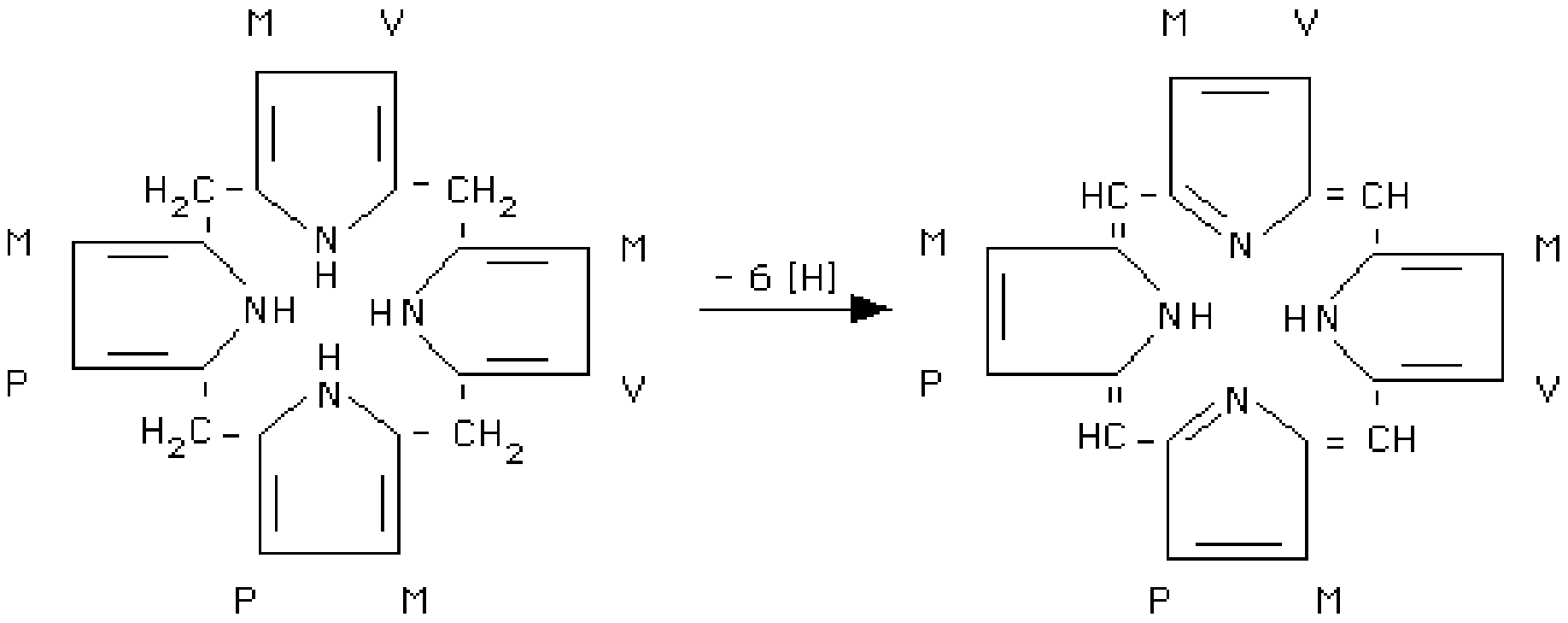
  In this reaction there is decarboxylation and oxidation of two propionic acid side chains.

*This reaction requires molecular oxygen

7. FORMATION OF PROTOPORPHYRIN III

Oxidation of Protoporphyrinogen IX to Protoporphyrin IX

The reaction is catalyzed by protoporphyrinogen IX oxidase.



Protoporphyrinogen IX

Protoporphyrin IX

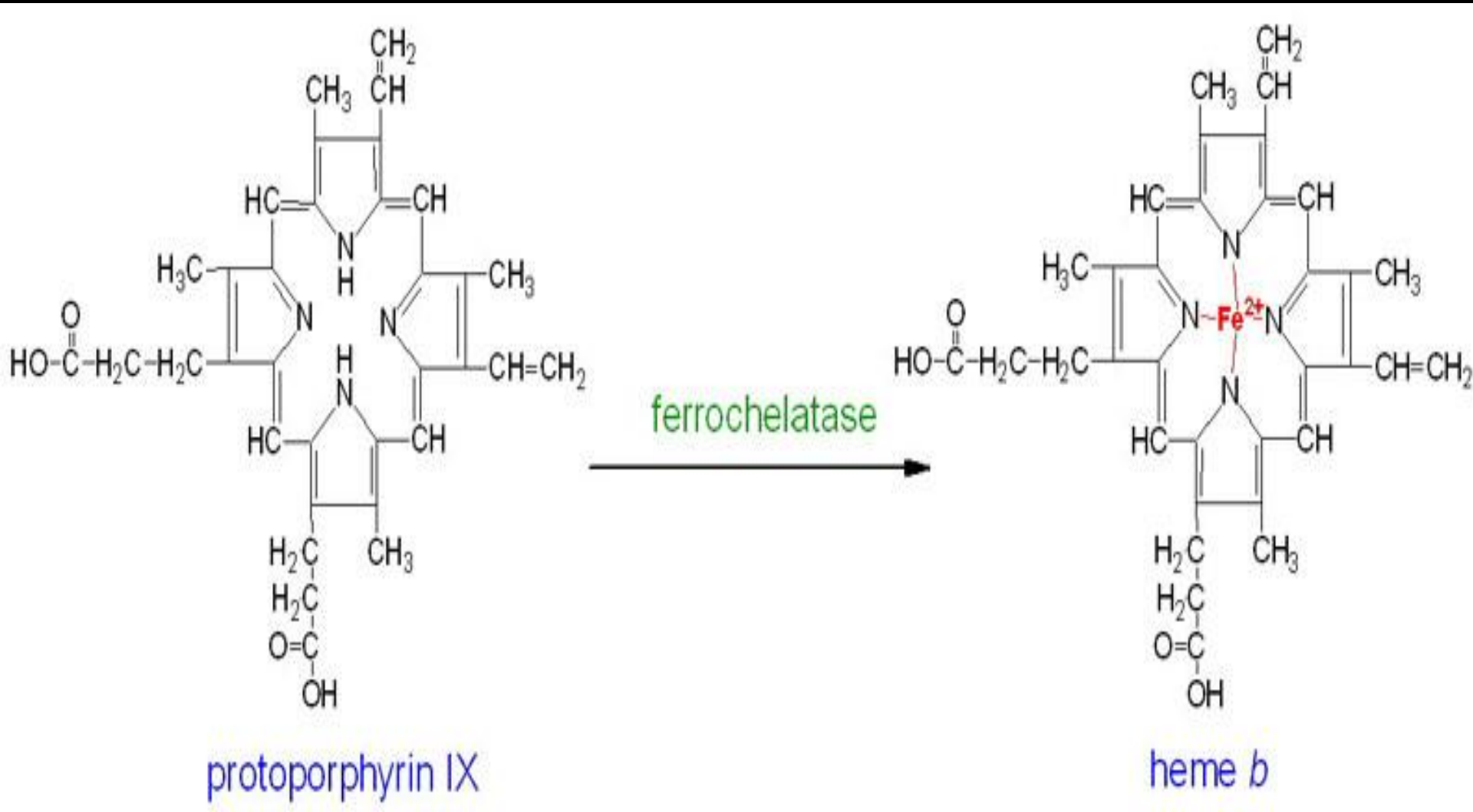
7. FORMATION OF PROTOPORPHYRIN III

- Protoporphyrinogen III gives off 6 H+ to form protoporphyrin III.
- ENZYME:PROTOPORPHYRINOGEN III OXIDASE
- REACTION: MITOCHONDRIA.

CONVERSION OF PORPHYRINOGENS TO PORPHYRINS

- Porphyrinogens are colorless compounds. In porphyrinogens the 4 pyrrole rings are attached to each other by methylene ($-CH_2-$) bridges and N of each pyrrole ring has one H attached to it. Porphyrinogens are readily converted by auto-oxidation to their respective porphyrins which are colored compounds.

8. FORMATION OF HEME

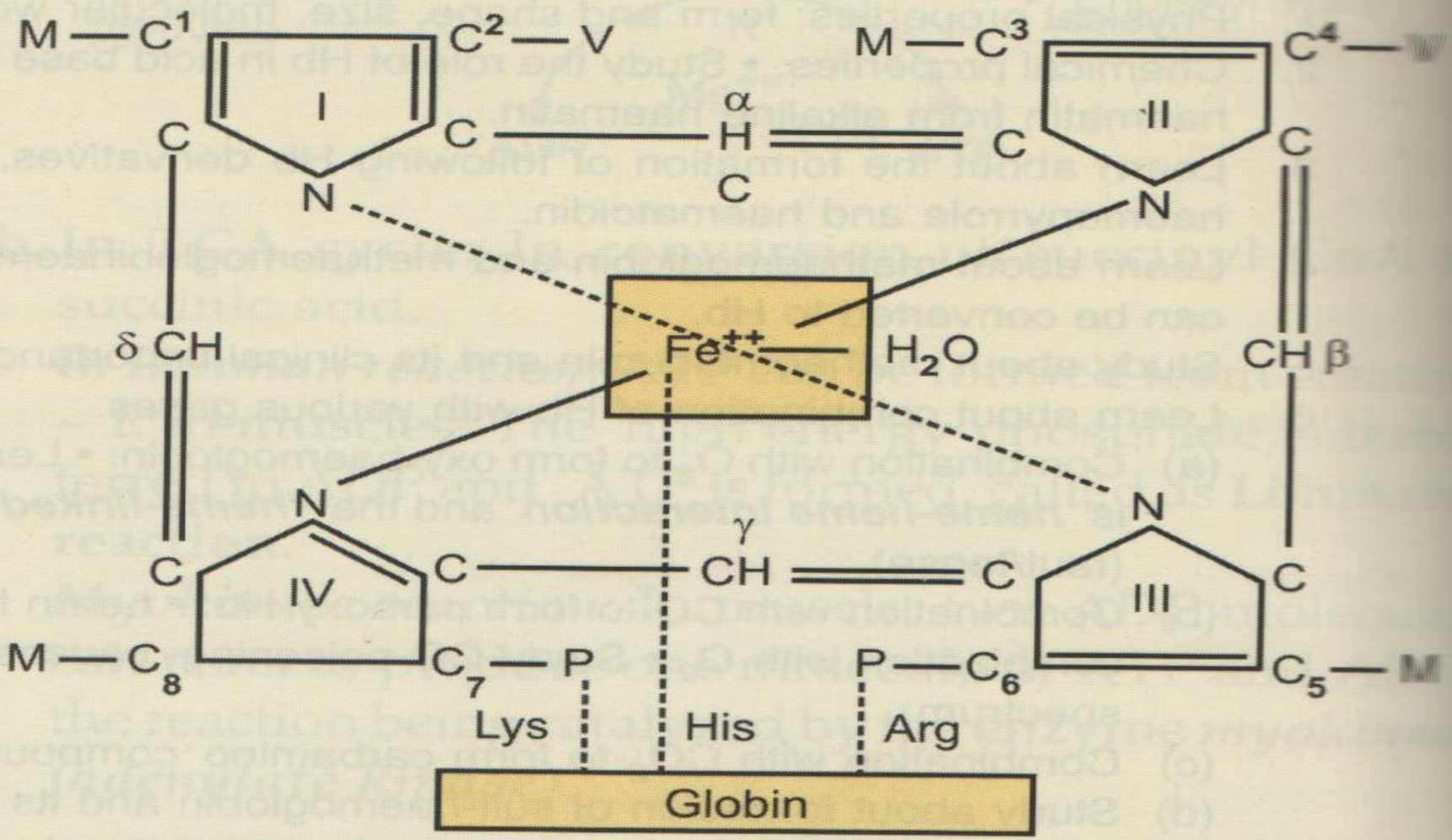


8. FORMATION OF HEME

- It takes place by the reaction,
  PROTOPORPHYRIN IX + Fe^{2+} → HEME

Fe+2 is provided by transferin

- REACTION: MITOCHONDRIA
- ENZYME:HEME SYNTHASE(ferrochelatase)
  Reduced glutathione is needed for this reaction.
- Inhibited by lead



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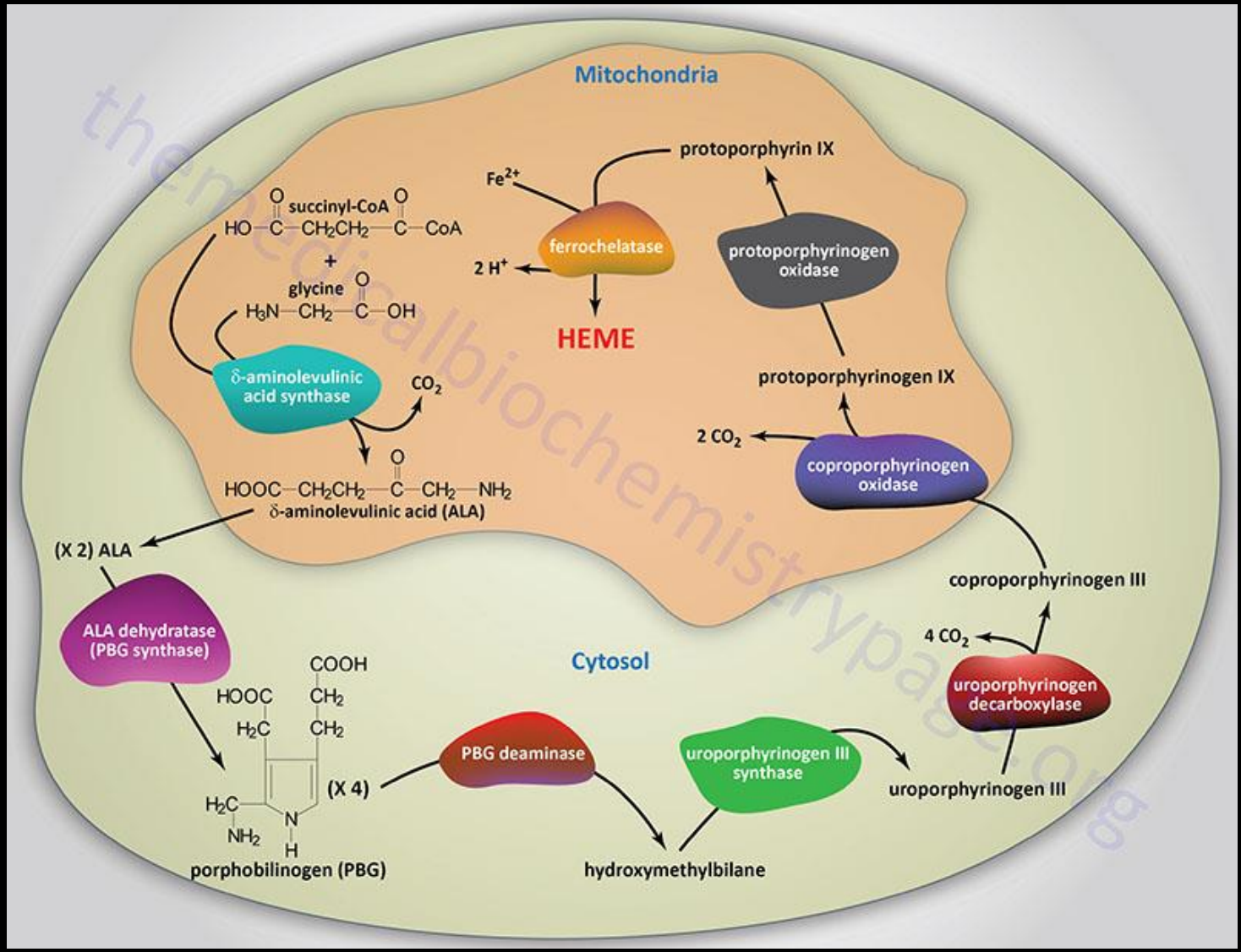

Mitochondria
protoporphyrin IX
Fe2+
ferrochelatase
2 H+
HEME
protoporphyrinogen oxidase
protoporphyrinogen IX
succinyl-CoA
glycine
δ-aminolevulinic acid synthase
CO2
δ-aminolevulinic acid (ALA)
2 CO2
coproporphyrinogen oxidase
coproporphyrinogen III
(X 2) ALA
ALA dehydratase (PBG synthase)
4 CO2
uroporphyrinogen decarboxylase
Cytosol
PBG deaminase
(X 4)
uroporphyrinogen III synthase
uroporphyrinogen III
porphobilinogen (PBG)
hydroxymethylbilane

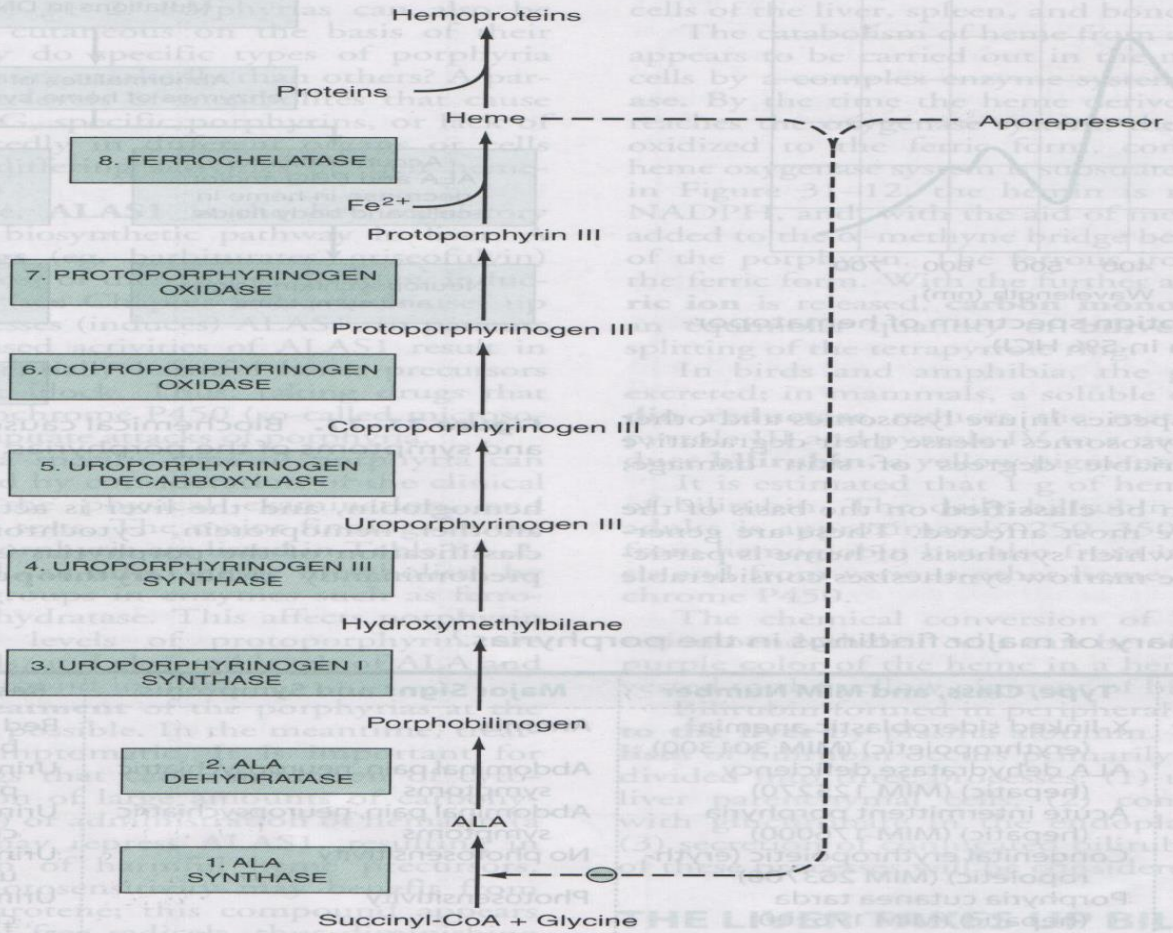


Figure 31–9. Intermediates, enzymes, and regulation of heme synthesis. The enzyme numbers are those referred to in column 1 of Table 31–2. Enzymes 1, 6, 7, and 8 are located in mitochondria, the others in the cytosol. Mutations in the gene encoding enzyme 1 causes X-linked sideroblastic anemia. Mutations in the genes encoding enzymes 2–8 cause the porphyrias, though only a few cases due to deficiency of enzyme 2 have been reported. Regulation of hepatic heme synthesis occurs at ALA synthase (ALAS1) by a repression-derepression mechanism mediated by heme and its hypothetical aporepressor. The dotted lines indicate the negative (⊖) regulation by repression. Enzyme 3 is also called porphobilinogen deaminase or hydroxymethylbilane synthase.

REGULATORY INFLUENCES AND EFFECTS OF INHIBITORS

1.Effect of oxygen

2.Enzyme inhibition

3. Drugs

Other factors:

- Lead
- Glucose
- Hypoxia
- Steroids
- Iron
- Haematin

Porphyrias

- These are rare, inherited or acquired defects in heme synthesis resulting in the accumulation and increased excretion of porphyrins or porphyrin precursors
- Each porphyria results in the accumulation of a unique pattern of intermediates caused by the deficiency of an enzyme in the hme synthetic pathway.

It can be

- 1.acquired
- 2. Genetic

Main Symptoms are

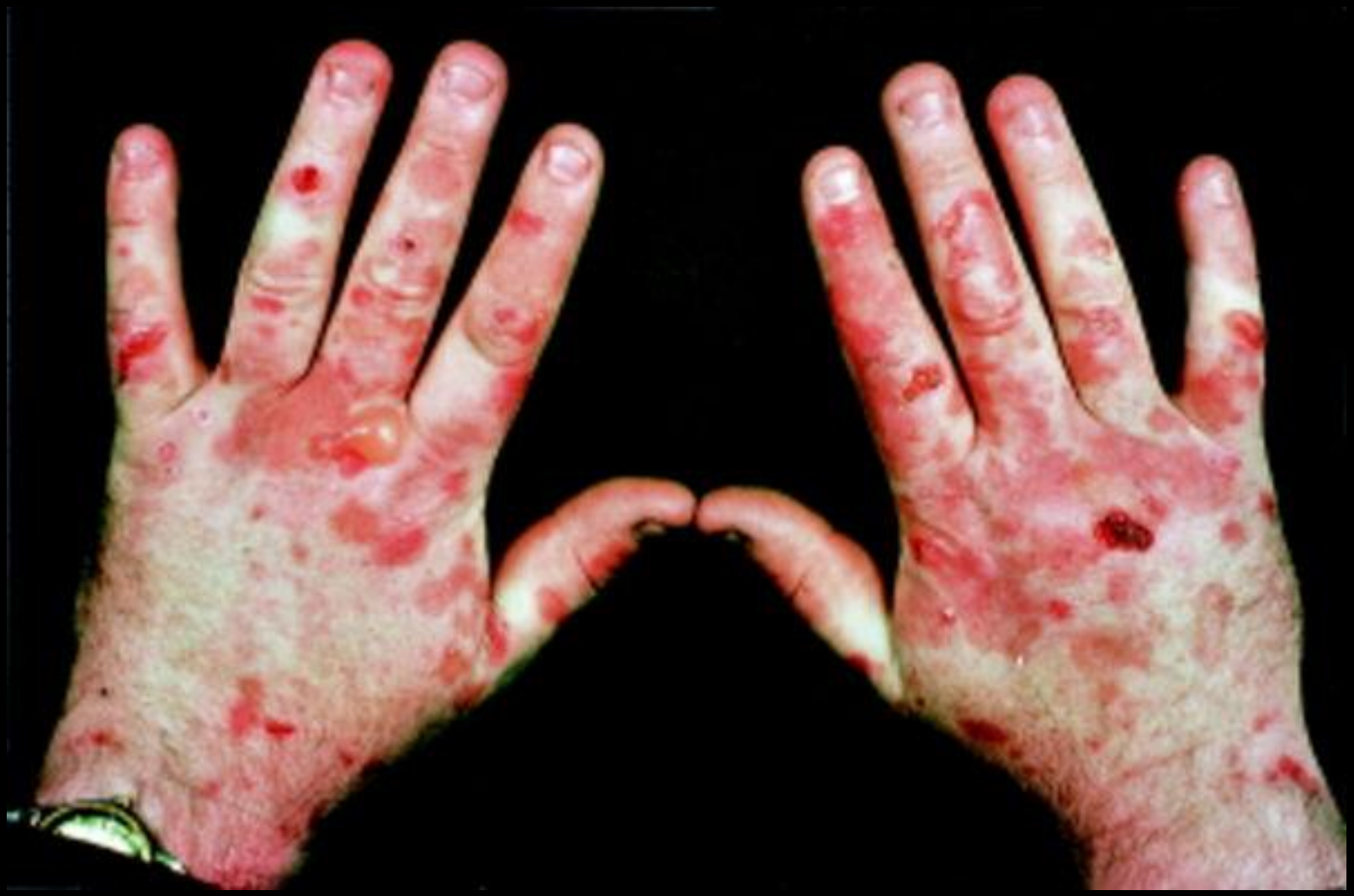
- 1. Abdominal pain
- 2. Neuro psychiatric symptoms and
- 3. . **Photosensitity**

If the enzyme lesion occurs early in the pathway, prior to the formation of porphyrinogens (Enz-3), only ALA and PBG will accumulate
There will be only abdominal pain and Neuropsychiatric symptoms
No photosensitivity
(Acute intermittent porphyia)

- If enzyme block occurs later in the pathway, resuls in accumulation of porphyrinogens.
- Their oxidation product, porphyrin, wil cause phtosensitivity also

Cause of Photosensitivity

- Porphyrins are " Excited" when exposed to light
- Excited porphyrins react with mol oxygen to from oxygen radicals
- Ox- Radicals injures lysosomes and other organelles, which release their degrative enzymes, causing skin damage and scarring




Porphyria
www.medindia.net

CAUSE OF NEUROPSYCHIATIRIC SYMPTOMS

- Due to depleted heme levels, there is reduced activity of tryptophan pyrolase resulting in acculmulation of tryptophan and 5-hydroxy tryptamin, resulting in increased synthesis of serotonin

X

Tryptophan

Tryptophan Pyrrolase Niacin+Acetyl-CoA

Hydrolase

Serotonin

- Neuropsychiatric symptoms include

Anxiety, depression ,hellucintions paralysis of limbs (even qudriplegia)

G.I Symptoms include

Anoroxia, Vomiting ,Diarrhoea,Constipation and Abdominal pain.

These symptoms are due to accumulation of various intermediates of heme synthesis.

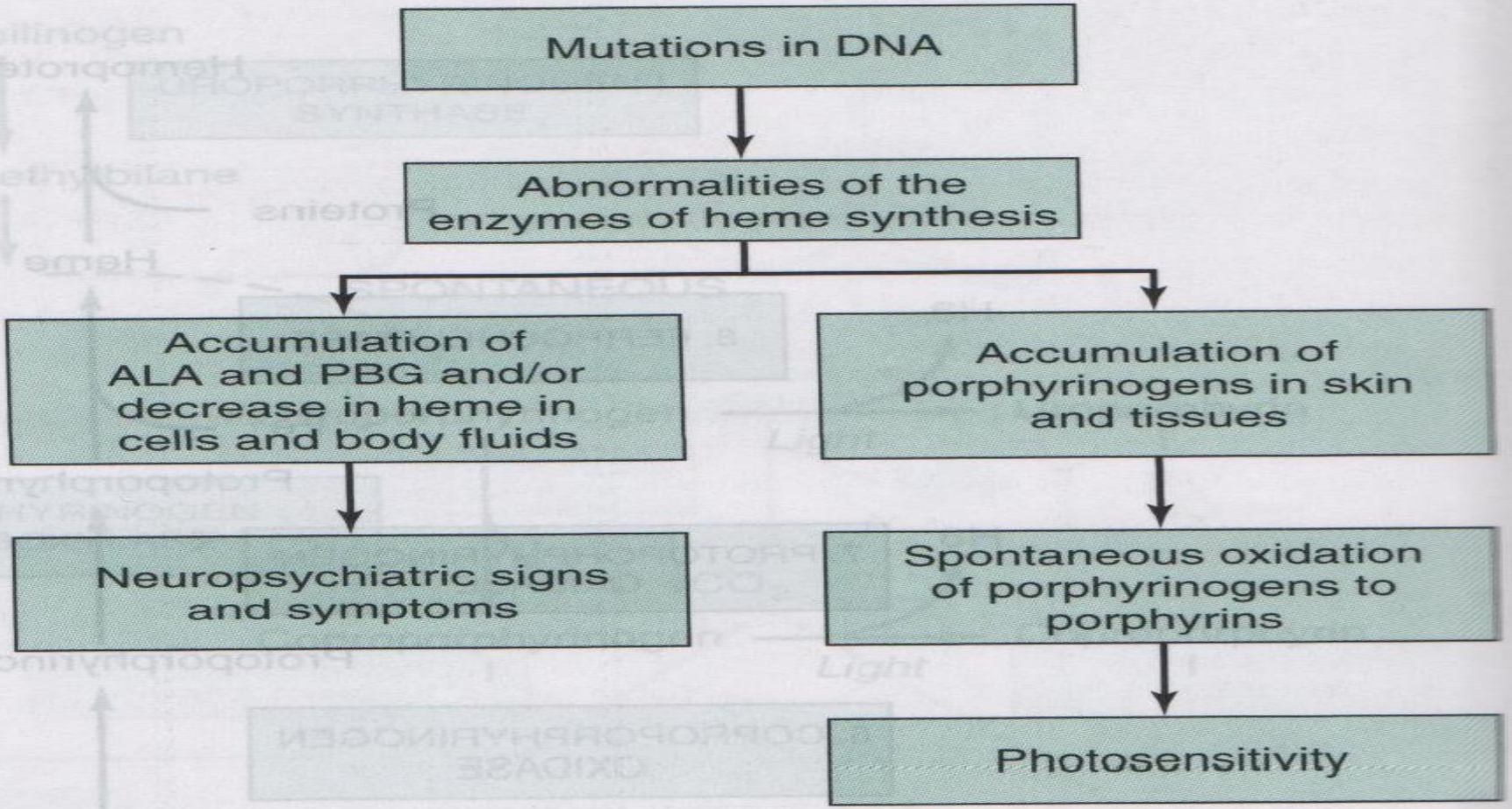


Figure 31–11. Biochemical causes of the major signs and symptoms of the porphyrias.

hemoglobin, and the liver is active in the synthesis of another hemoprotein, cytochrome P450. Thus, one classification of the porphyrias is to designate them as predominantly either **erythropoietic** or **hepatic;** the

- Classification of Porphyrias

1. A: Genetic

   From deficeincy of enzymes (3 to 8)

   B: Acquired

   Drugs e.g Barbiturales, grisofulvin,

   Lead etc.

   By these drugs there is utilization of heme by induction of Cyt –P 450. The diminish heme level activates ALA S , causing increased heme synth , resulting in increased intermediates of heme synthesis.

2. On the basis of organs or cells that are most affected

A: Erythropoietic

When the enzyme deficincy occurs in the erothropoeitic cells of the bone marrow e.g

I) Congenital erothropoeitic porphyria (Chronic)
II) Erothropoeitic proto porphyia (Chronic)

B: Hepatic

- : When the enzyme deficiency occurs in the liver.

Can be further classified as

1. Chronic : porphyria cutanea tarda

II. Acute:

- ALA dehydratase deficincy
- Acute intermitted porphyrai
- Heriditary coproporphyria
- Variegata porphyiai

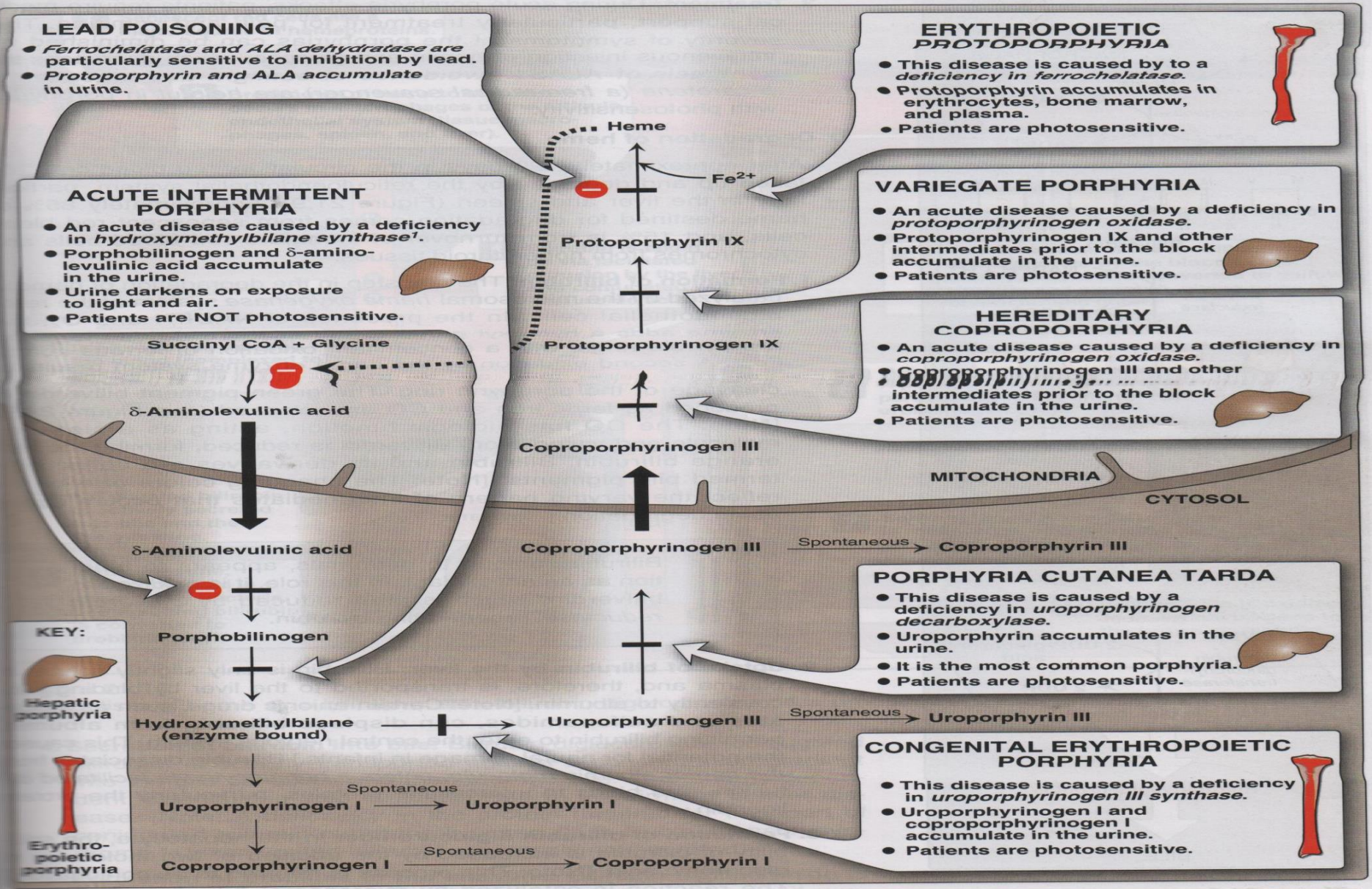


Figure 21.8
Summary of heme synthesis. [1]Also referred to as *porphobilinogen deaminase*.

DIAGNOSIS

WHEN PORHYRINS, DISSOLVED IN STRONG MINERAL ACIDS OR IN ORGANIC SOLVENTS, ARE ALLUMINATED BY U.V.LIGHT, THEY EMIT A STRONG RED FLOURESCENCE, DUE TO THE PRESENCE OF DUOUBLE BONDS.THESE DOUBLE BONDS ARE ABSENT IN PORPHYRINOGENS

5

- This fluorescence is so characteristic that it is often used to detect small amounts of free phorphyrins .

Treatment

- Treatment at Gene level may be come possible
- Mainly symptomatic
- Avoid drugs which cause induction of cytochrome 450
- Ingestion of large amount of carbohydrates
- Administration of β-carotene to lessen the free Radicals and sunscreens for photosensitivity.

Clinical Application

- Cancer phototherapy

- Tumor cells take more porphyrins than do the normal cells .
  - Hemeto phorphyrins and other related compounds are administered
- The tumors are then exposed to argon lasser , which excite the porphyrins, producing cyto toxic effects .

Heme Degradation
AND
Bile Pigments Metabolism

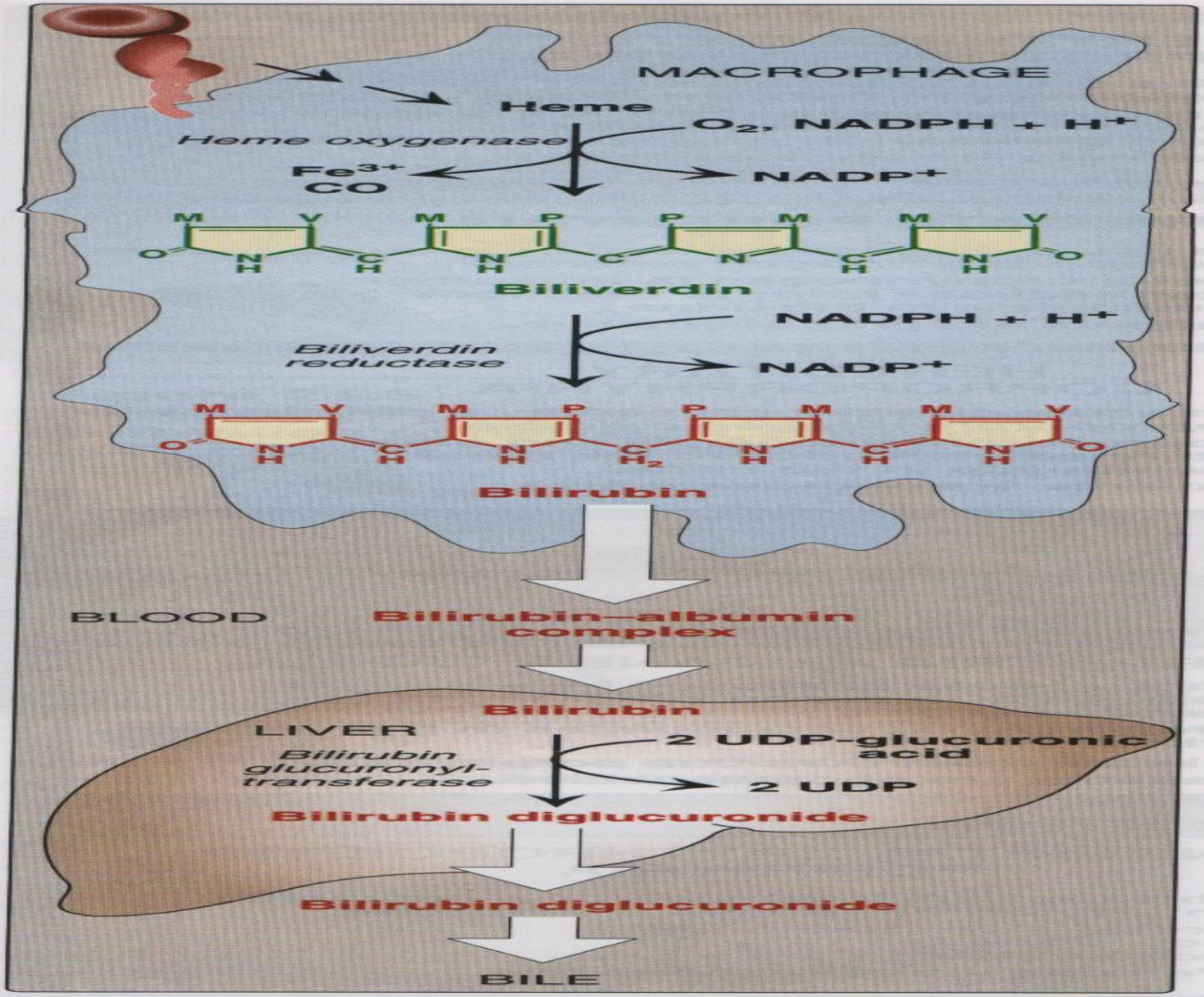


Figure 21.9
Formation of bilirubin from heme.
UDP = uridine diphosphate.

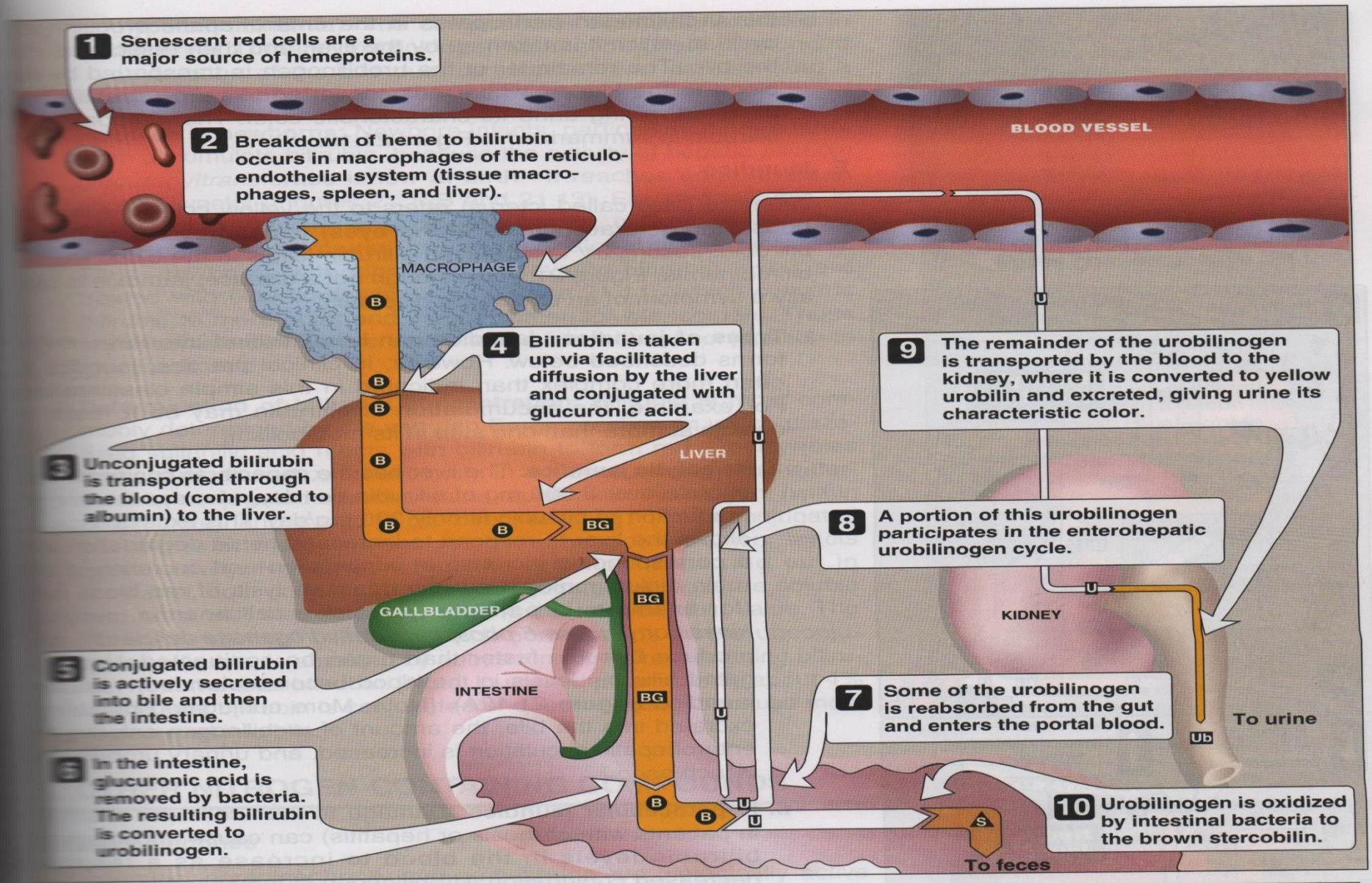


Figure 21.10
Catabolism of heme B = bilirubin; BG = bilirubin diglucuronide; U = urobilinogen; Ub = urobilin; S = stercobilin.

- Bilirubin formed from Hb, and not passed through the liver cell is called

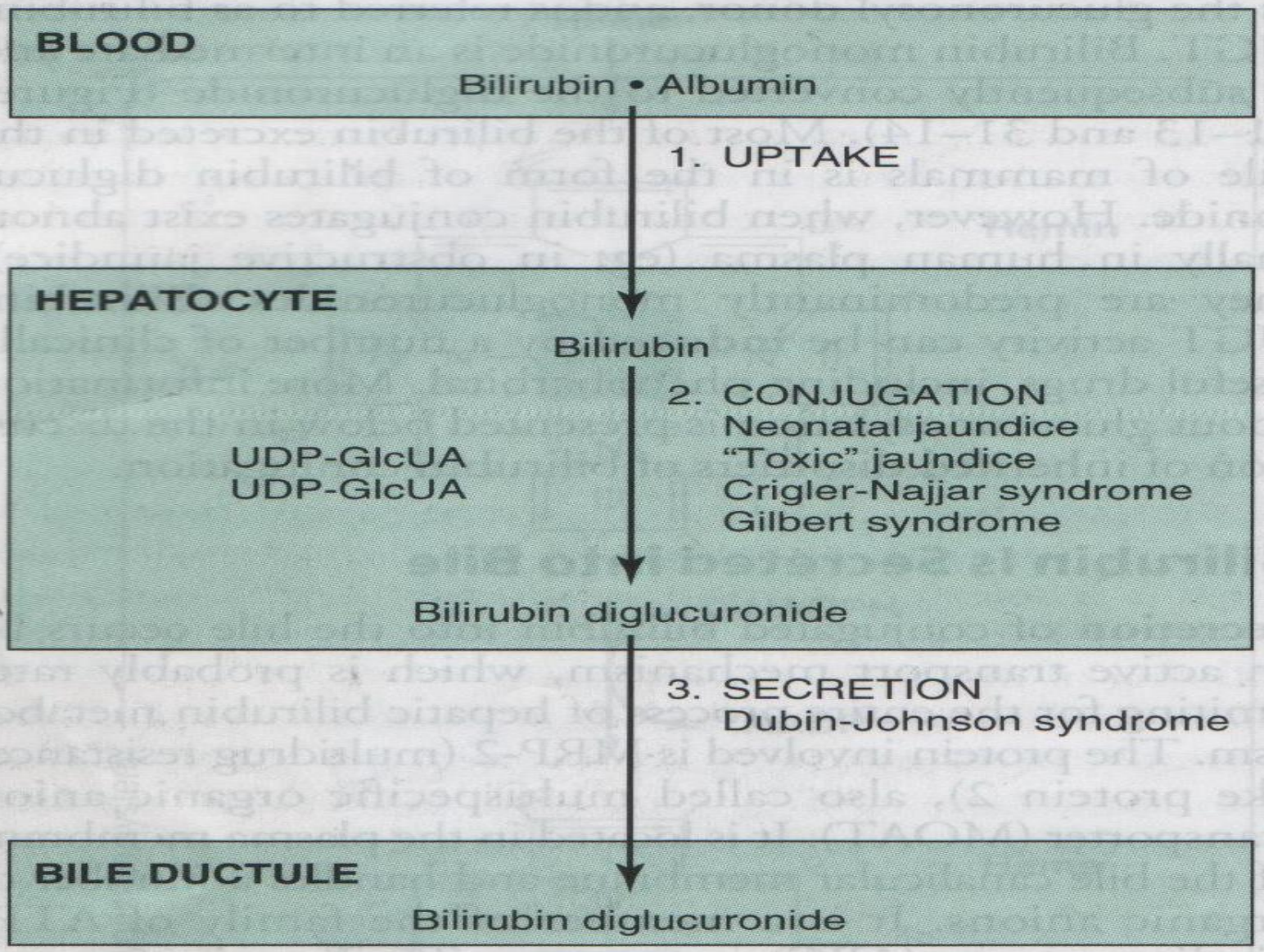
  Unconjugated or indirect Bilirubin

  Because Alcohol is required to be added to give color with Diazo reagent in vanden Bergh reaction.

Bilirubin which has passed through liver cells and undergose conjugation is calledConjugated or Direct Bilirubin ,Because it gives color directly, with out adding Alcohol.

- Bilirubin consists of an open chain of Four Pyrrole rings. In Heme these four rings are connected in a larger ring.
- Bilirubin function as antioxidant so level of Bilirubin is invesally related to heart attack.
- From 6gm of Hb breakdown per day about 250mg of Bilirubin is formed from Myoglobin & other heme containing proteins, another 50mg of Bilirubin is fromed.
- So total formation of Bilirubin is 300mg /day.

- Unconjugated bilirubin enters the neurons of basalganglia, cerebellum and medulla causing necrosis of nerve cells probably by interfering with cellular respiration
- Uncojugated bilirubin cannot pass through G.filtrate and does not appear in the urine.


BLOOD
Bilirubin • Albumin
1. UPTAKE
HEPATOCYTE
Bilirubin
2. CONJUGATION
Neonatal jaundice
"Toxic" jaundice
Crigler-Najjar syndrome
Gilbert syndrome
UDP-GlcUA
UDP-GlcUA
Bilirubin diglucuronide
3. SECRETION
Dubin-Johnson syndrome
BILE DUCTULE
Bilirubin diglucuronide

Hemolytic disease of new born (erythroblastasis fetalus)

- -Incompatibility b/w maternal and fetal blood group.
- -Rh +ve foetus may produce antibodies in Rh –ve mother.
- -The first child often escape
- -In second pregnancy, the Rh antibodies(IgG) will pass from mother to foetus and will destroy foetal RBC.
- -Child will born with sever anaemia.
- -When blood bilirubin level >20mg/dl, it is no more bound to albumin.
- -Increased free bilirubin is deposited in the brain, basal ganglia(kernicterus)

- Causing mental retardation, fits, toxic encephalitis and spasticity.
- Toxic effects of bilirubin may be due to inhibition of vital enzymes, including ATP ase in the brain mitochondria.
- There may be uncoupling of oxidative phosphorylation.

- Treatment

1. Exchange transfusion.
2. Barbiturates--→(phenobarbitone)
3. Phototherapy.

Insoluble (Z Z) form of bilirubin is converted to more soluble (Z E and E E) form, which can be excreted in the urine, with out conjugation

- Prevention
- By immuno globulines

- Neotal Jaundice

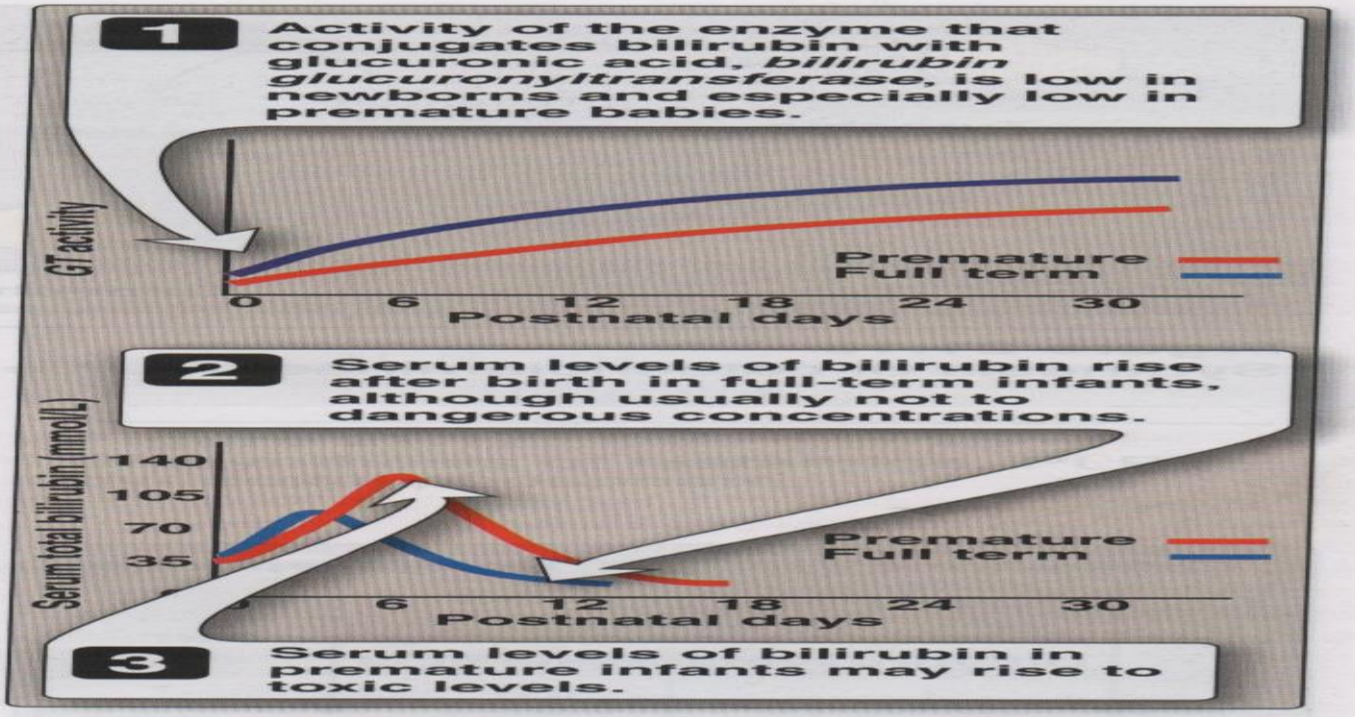
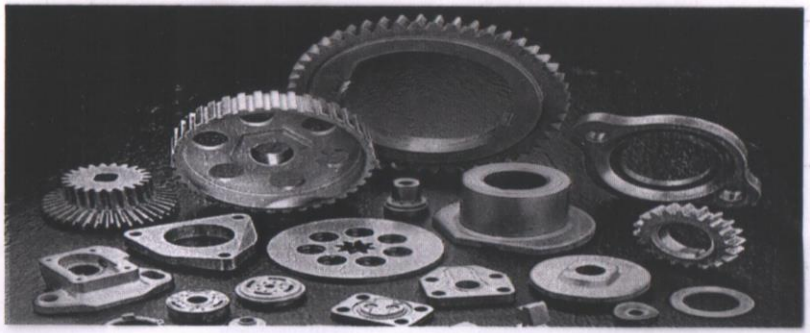


Figure 21.13
Neonatal jaundice. *GT = glucuronyl-transferase.*

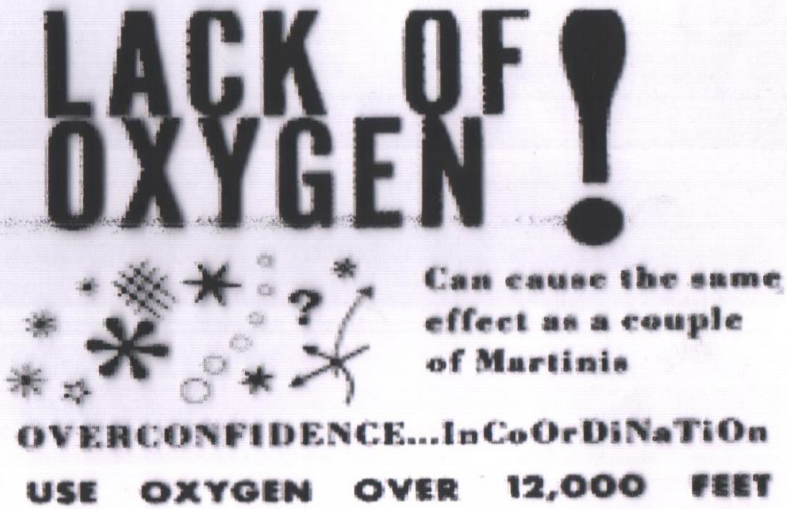
Figure 21.14
Phototherapy in neonatal jaudice

Factors affecting the synthesis of Hemoglobin :



- 1-Metals
- 2-Vitamins
- 3-Protein Diet

- 4- Hormones
- 5- Hypoxia and Erythropoeitin
- 6- Cyclic AMP



HEMOGLOBINOPATHIES

- Hemoglobinopathies are a family of genetic disorders caused by a production of a structurally abnormal hemoglobin molecule, synthesis of insufficient quantities of normal hemoglobin or rarely both.

Some of the hemoglobinopathies are:

- Hemoglobin S disease – Sickle cell anaemia
- Hemoglobin C
- Hemoglobin E
- Hemoglobin M
- Thalessemia

Hemoglobin S Disease (Qualitative) (sickle cell anemia)

- The alpha chains are normal and the beta chains are abnormal.
- In Hb-S the glutamic acid at position-6 in the beta chain is replaced by valine.
- Sickle cell trait (hetrozygous) 60% Hb-A and 40% Hb-S.
- Sickle cell disease(homozygous)100% Hb-S.
- Sickleing of RBC can block blood capillaries as sickle cells become rigid and stick at a branching or narrow part of a vessel producing infarcts.

Sickling and tissue anoxia

- The replacement of the charged glutamate with the nonpolar valine forms a protrusion on the β chain (Sticky patch) that fits in to complementory site (cleft) on the β chain of another hemoglobin molecule in the cell.
- The cleft develops in deoxygenated state (even in normal Hb).

- Micro infarcts causing
- Auto spleenectomy (Pneumocci and influenza)
- Pains in limbs after exercise
- Micro infarcts in kidneys and brain

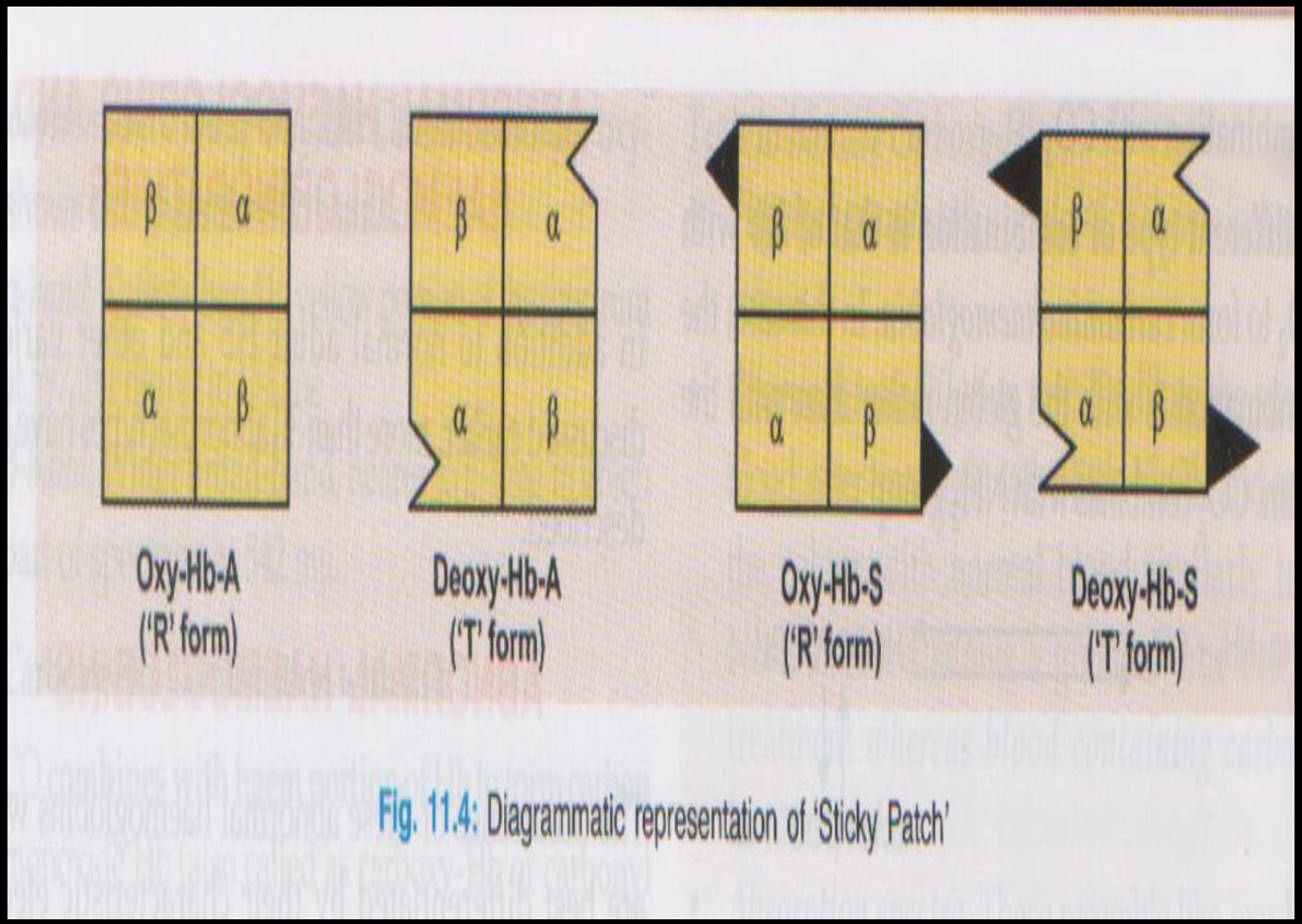


Fig. 11.4: Diagrammatic representation of 'Sticky Patch'

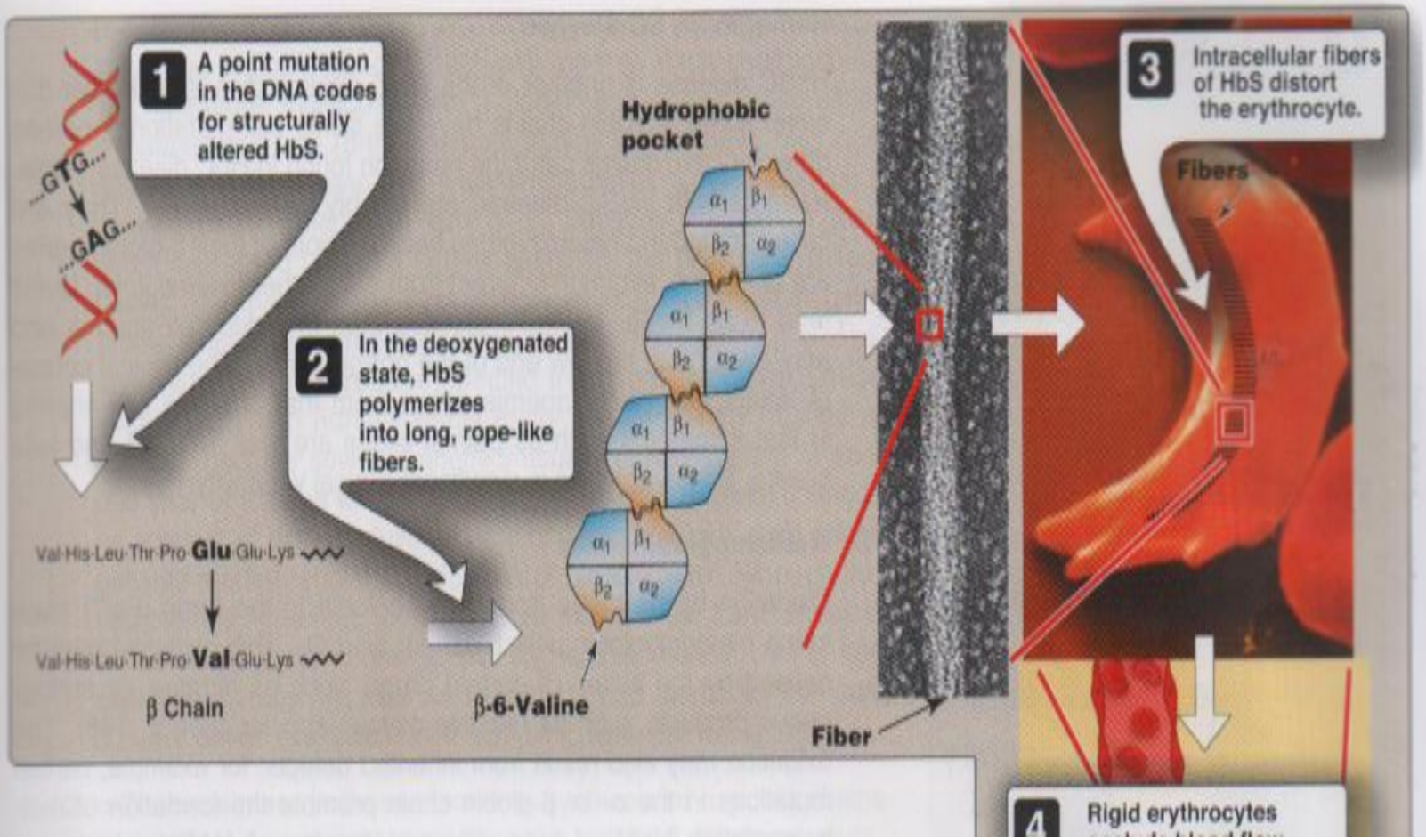

1 A point mutation in the DNA codes for structurally altered HbS.
...GTG...
...GAG...
2 In the deoxygenated state, HbS polymerizes into long, rope-like fibers.
Hydrophobic pocket
Val·His·Leu·Thr·Pro·Glu·Glu·Lys
Val·His·Leu·Thr·Pro·Val·Glu·Lys
β Chain
β-6-Valine
Fiber
3 Intracellular fibers of HbS distort the erythrocyte.
Fibers
4 Rigid erythrocytes

Figure 3.20
Molecular and cellular events leading to sickle cell crisis.
HbS = hemoglobin S.

Sickle Cell Disease:

Val – His – Thr – Leu – Pro – Glu – Glu – Lys – >>>>> α

Val – His – Leu – Thr – Pro – Val – Glu – Lys – >>>>> β

In DeOxygenated state HbS forms long, rope like fibers

Elongated Erythrocytes occlude Blood flow in the Capillaries

Micro infarction produce tissue Anoxia resulting in Tissue damage & Pain

Clinical Aspects

- Protection from Malaria
- Increased incidence of Salmonella infection.

COMPLICATIONS

- Anaemia
- Increase risk of severe bacterial infection due to loss of functioning spleen
- Stroke due to narrowing of blood vessels of the brain.
- Cholilithiasis and cholecustitis due to excess bilirubin production of and prolong Hemolysis
- Chronic kidney failure due to sickle cell Nephropathy.

PROGNOSIS

- About 90 % of people survive to age of 20 and 50% survive beyond the fifth decade.

Prenatal Diagnosis

Can be done using

- first fetal blood sampleing
- later, chorion villus biopsy and direct analysis of globin genes.

The error rate in experience centre is now well under 1%.

DIAGNOSIS

- Complete blood count with Hb-6-8 g/dl with high reticulocytes count .
- Haemoglobin –Electrophoresis –Abnormal Hb form can be detected.

TREATMENT

- Folic Acid and Penicilin daily from birth to five years of age due to immature immune system
- Frequent blood transfusion
- Hydroxyurea ⟶ First approved drug, which reactivate fetal hemoglobin production in place of Hemoglobin-S
- Bone marrow transplant

- The continued blood transfusion will eventually lead to iron overload , which must be treated with chelation therapy to avoid organ failure.
- New treatment include, the use of Desferal, to replace the chelation treatment, using desferal delivered by infusion under the skin through a battery-operated pump.
- Bone Marrow Transplant
- Gene Therapy

Prevention Efforts

- Pre marital screening to make sure that the couple are not both carrier
- Provision of prenatal testing
- Reduction of marriages between relatives

THALESSEMIA: (Quantitative)

- In thalessemia there is defect in the synthesis of one or more of the sub-units of Hb.
- The R.B.Cs are hypochromic and microcytic.
- According to the decreased synthesis of alpha and beta chains of hemoglobin,the disease might be alpha and beta thalessemia respectively.

Alpha Thalessemia

Two alpha genes are inherited from each parent.

-Synthesis of α chains are repressed ,so there is compensatory increased in the synthesis of other chains of which the cell is capable, either β or γ chain.

- One gene deletion…no hemolytic disorder (silent carrier).
- Two genes deletion… little clinical effects (alpha thalessemia trait).
- Three genes deletion…hemolytic anemia (hypocromic microcytic) RBC shows ppt.(Heinz bodies).
- Four genes deletion…Incompetible with life and the feotus die in uterus. (Hemoglobin Bart or hydorps fetalis)

Beta thalessemia

One beta gene inherited from each parent.

Rarely deletion of these genes result in hereditary persistence of Hb-F.

Types of Beta Thalessemia

- **Beta Thalessemia minor or trait:**

One of gene is abnormal.(fewer or no symptoms).

- **Beta Thalessima intermedia:**

mild mutation of both of beta genes.(mild-sever anemia, dose not require frequent transfusions.

- **Beta Thalessemia major:**

  Both genes are defective (Major or cooley anemia)

  Sever anemia appears at the age of 4-6 months, b/c gamma chain formation is not replaced by beta chain formation.

  Hb-A, Hb-A2 and Hb-F are 0, 4-10% and 90-96%.

. Sign of mutation of both genes. Sever form. Require frequent transfusion for survival.

Lab Diagnosis

- 1. Blood smear shows microcytic, Hypochromic Anemia . Hb level may be very low
- 2. Electrophoresis

Prenatal Diagnosis

- Can be done using first fetal blood sampleing and later, chorion villus biopsy and direct analysis of globin genes. The error rate in experience centre is now well under 1%.

Management and Treatment.

- Thalassemia minor:- No need for any Treatment , Since carriers are usually symptomless. Thallasemia Major :- Severe life thretening anemia, requires life long blood transfussions.

- The continued blood transfusion will eventually lead to iron overload , which must be treated with chelation therapy to avoid organ failure.
- New treatment include, the use of Desferal, to replace the chelation treatment, using desferal delivered by infusion under the skin through a battery-operated pump.
- Bone Marrow Transplant
- Gene Therapy

Prevention Efforts

- Pre marital screening to make sure that the couple are not both carrier
- Provision of prenatal testing
- Reduction of marriages between relatives

Methemoglobinemias

- Disorder characterized by the presence of higher than normal level of Methemoglobin.
- (meth Hb i.e, Ferric $^{3+}$ rather than Ferrorus $^{2+}$)
- It has a decreased ability to bind oxygen.
- Binding of oxygen to methemoglobin results in an increased affinity of oxygen to the three other heme sites.
- This leads to an overall reduced ability of R.B.C to release oxygen to the tissues causing hypoxia.

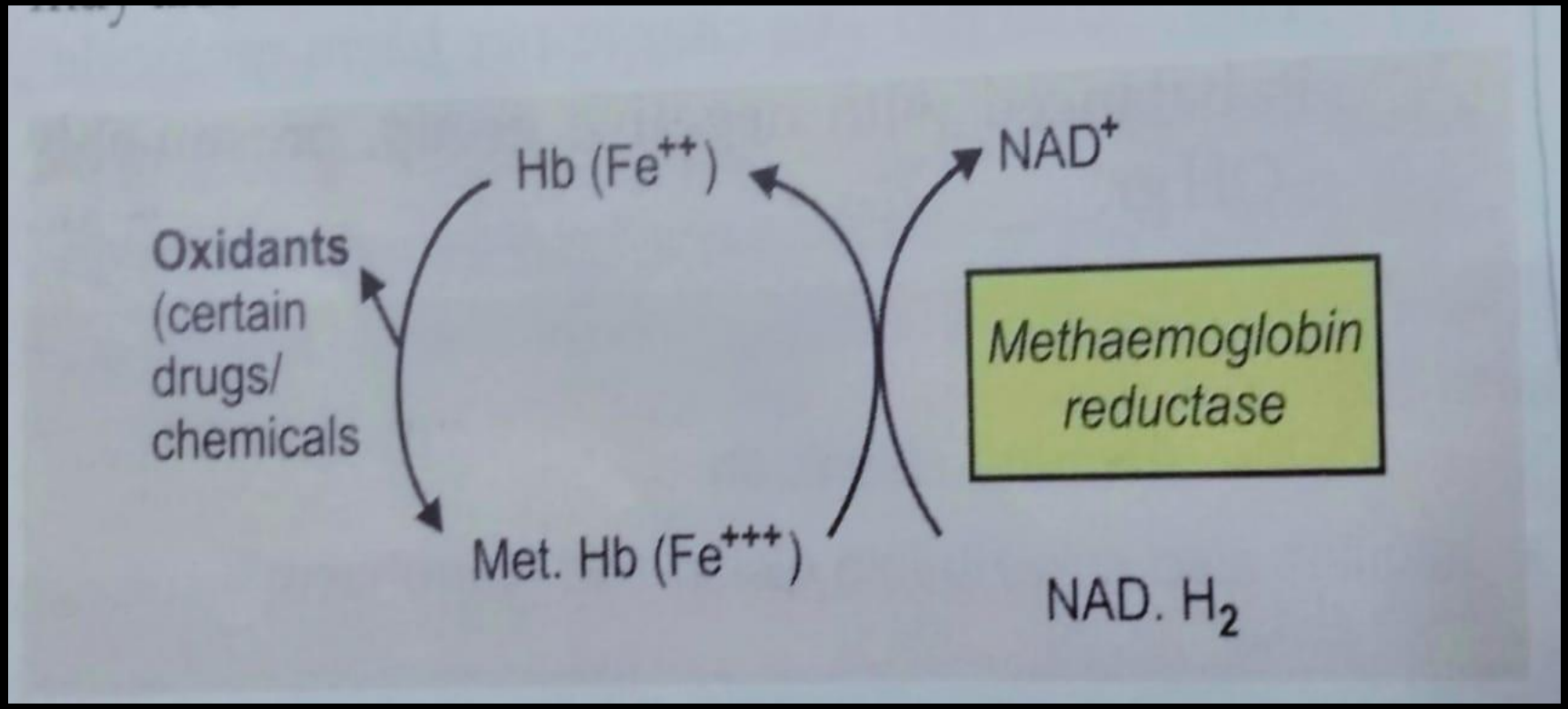
Methemoglobinemias

- May be caused by
- Certain drugs e.g Nitrates
- Reactive oxygen species
- Certain Mutations in α or β globin chain
- NADH-Methemoglobin Reductase deficiency

Effects:

- Chocolate cynosis
- Tissue hypoxia causing anxiety, headache and Dyspnoea.
- Coma and Death may occur

MECHANISM OF RECONVERSION OF METHAEMOGLOBIN TO HEMOGLOBIN



- Diphorase I, which is NADPH-Dependent.
- IV injection of glucose or methylene.
- Ascorbic Acid

JAUNDICE

- Yellow discoloration of scleral,conjunctivae, mucous membrane and skin due to Increased Bilirubin level
- Jaundice is visible, when serum Bilirubin exceeds 2.4 mg/dl.
- Although not a disease itself, jaundice is usually a symptom of underlying disorder.

Normal Value

Total Bilirubin = 0.1---1.9mg /dl

Direct Bilirubin =0---0.4mg/dl

(1mg /dl =17.1 µ mole /L)

Classification of Jaundice (Rolleston and McNee)

A. Haemolytic or pre-Hepatic Jaundice.

B. Hepatocellular or hepatic jaundice.

C. Post hepatic or Obstructive jaundice.

A. Haemolytic or pre-Hepatic Jaundice.

- Increased breakdown of Hb
- Rate of bilirubin formation is faster than the liver can conjugate it .
- Normal production is 300 mg /day and liver can conjugate up to 3000 mg/day.
- Lipid soluble so increased chances of deposition in brain .
- No bilirubin in urine.

CAUSES

Principally there are two categories

Intrinsic :

-Abnormalities within the R.B.C by

-Haemoglobino pathies

-Hereditary spherocytosis

-G-6- PD deficiency

Extrinsic :

Factors external to R.B.C

- Incompatible blood transfusion
- H.D.N
-Auto-Immune diseases
-Malaria

B. **Hepatocellular or hepatic jaundice:**

Disease of the parenchymal cells of liver. This may be divided into 3 groups

1.Conditions in which there is defective conjugation:

a. Reduction in the number of functioning liver cells, e.g, in Chronic hepatitis . In this all liver functions are impaired . OR

b. There may be a specific defect in the conjugation process

-Gilbert`s disease,

-Crigler-Najjar syndrome etc.

Other liver functions are normal.

II.-Conditions such as viral hepatitis and toxic jaundice:

There is extensive damage to liver cells, associated with considerable degree of intrahepatic obstruction resulting in appreciable absorption of conjugated bilirubin.

- **III.Cholestatic Jaundice:**
- This occurs due to drugs, (drug-induced) such as chlorpromazine and some steriods in which there is mainly intrahepatic obstruction
- Liver functions being essentially normal.

C. **Post hepatic or obstructive jaundice:**

Obstructive to the flow of bile in extrahepatic ducts e.g.

-Gall stones

-Ca head of Pancrease

There is GI Pain, Nausea, pale , Clay color stool.

Liver "Regurgitate Conjugated bilirubin in the blood , which is excreted in the urine.

- **II. Rich`s classification of jaundice :**
- According to this classification jaundice is divided into mainly two groups

A. Retention jaundice :

In which there is impaired removal of bilirubin from the blood or excessive amount of Bilirubin is produced and not cleared fully by liver cells.

This group includes haemolytic jaundice and those conditions characterized by impaired conjugation of bilirubin.

B. Regurgitation jaundice:

- In which there is excess of conjugated bilirubin and it includes obstructive jaundice and those liver conditions in which there is considrable degree of intrahepatic obstruction (cholestasis)