A complete blood count (CBC) is ordered when a physician suspects a problem in the cellular composition of a patient's blood. The cells within the collected blood are counted and typed using an automated analyzer, based on flow cytometry (counting cells one at a time as they flow through a detector). As each cell flows through the machine, a laser shines light at the cell, which leads to predictable light scattering and absorbance depending on the cell type. Based on the light-scattering and absorption pattern, the machine keeps track of the results of each cell that flows through the machine, leading to a very accurate count of each cell type present in the sample. The data from this analysis will include the total number of red cells per liter, the amount of hemoglobin in the red cells (in grams per liter), the hematocrit (the fraction of whole blood that consists of red blood cells), the mean corpuscular volume, the total number of white blood cells, as well as a count of the different types of white blood cells (neutrophils, lymphocytes, monocytes, eosinophils, and basophils).

II. ERYTHROCYTE METABOLISM

A. The Mature Erythrocyte

To understand how the erythrocyte can carry out its major function, a discussion of erythrocyte metabolism is required. Mature erythrocytes contain no intracellular organelles, so the metabolic enzymes of the red blood cell are limited to those found in the cytoplasm. In addition to hemoglobin, the cytosol of the red blood cell contains enzymes necessary for the prevention and repair of damage done by reactive oxygen species (see Chapter 24) and the generation of energy (Fig. 44.1). Erythrocytes can only generate adenosine triphosphate (ATP) by glycolysis (see Chapter 22). The ATP is used for ion transport across the cell membrane (primarily Na^+, K^+, and Ca^+), the phosphorylation of membrane proteins, and the priming reactions of glycolysis. Erythrocyte glycolysis also uses the Rapaport–Luebering shunt to generate 2,3-bisphosphoglycerate (2,3-BPG). Red cells contain 4 to 5 mM 2,3-BPG, compared with trace amounts in other cells. The trace amounts of 2,3-BPG found in cells other than erythrocytes is required for the phosphoglycerate mutase reaction of glycolysis, in which 3-phosphoglycerate is isomerized to 2-phosphoglycerate. As the 2,3-BPG is regenerated during each reaction cycle, it is required in only catalytic amounts. As discussed in more detail in Section IV, 2,3-BPG is a modulator of oxygen binding to hemoglobin that stabilizes the deoxy form of hemoglobin, thereby facilitating the release of oxygen to the tissues.

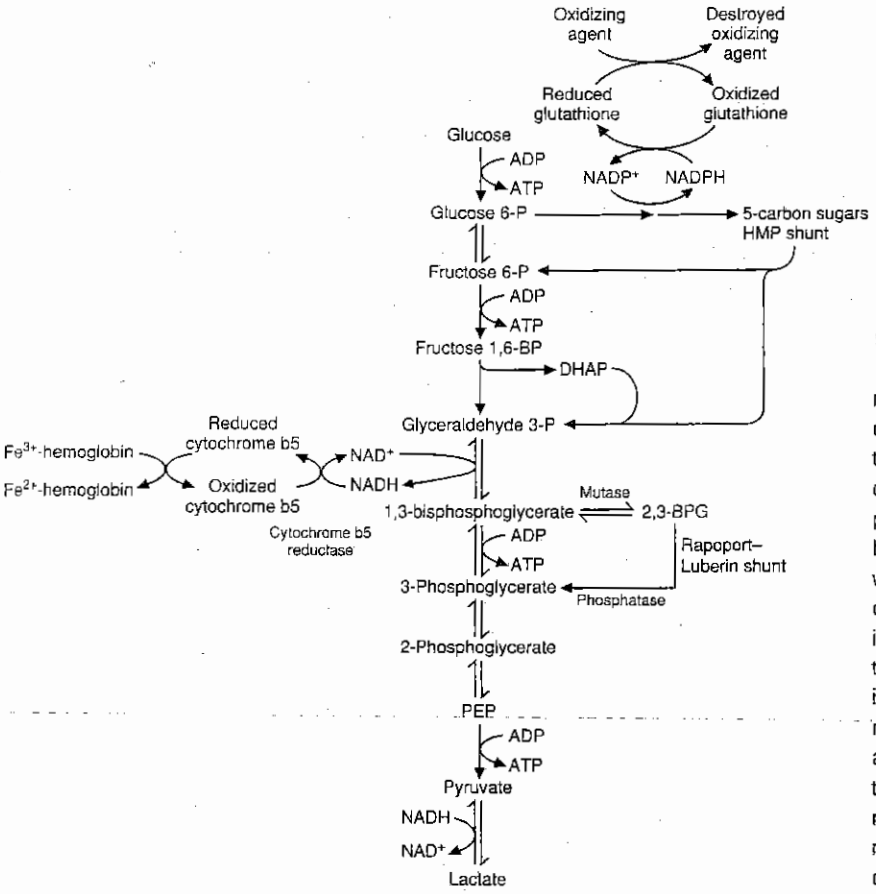


FIG. 44.1. Overview of erythrocyte metabolism. Glycolysis is the major pathway, with branches for the hexose monophosphate shunt (for protection against oxidizing agents) and the Rapoport–Luebering shunt (which generates 2,3-bisphosphoglycerate, which moderates oxygen binding to hemoglobin). The NADH generated from glycolysis can be used to reduce methemoglobin (Fe^{3+}) to normal hemoglobin (Fe^{2+}), or to convert pyruvate to lactate, so that NAD^+ can be regenerated and used for glycolysis. Pathways that are unique to the erythrocyte are indicated in red. See text for abbreviations.

To bind oxygen, the iron of hemoglobin must be in the ferrous (+2) state. Reactive oxygen species can oxidize the iron to the ferric (+3) state, producing methemoglobin. Some of the NADH produced by glycolysis is used to regenerate hemoglobin from methemoglobin by the NADH-cytochrome b_5 methemoglobin reductase system. Cytochrome b_5 reduces the Fe^{3+} of methemoglobin. The oxidized cytochrome b_5 is then reduced by a flavin-containing enzyme, cytochrome b_5 reductase (also called methemoglobin reductase), using NADH as the reducing agent.

Approximately 5% to 10% of the glucose metabolized by red blood cells is used to generate NADPH by way of the hexose monophosphate shunt. The NADPH is used to maintain glutathione in the reduced state. The glutathione cycle is the red blood cell's chief defense against damage to proteins and lipids by reactive oxygen species (see Chapter 24).

The enzyme that catalyzes the first step of the hexose monophosphate shunt is glucose 6-phosphate dehydrogenase (G6PD). The lifetime of the red blood cell correlates with G6PD activity. Lacking ribosomes, the red blood cell cannot synthesize new G6PD protein. Consequently, as the G6PD activity decreases, oxidative

An inherited deficiency in pyruvate kinase leads to hemolytic anemia (an anemia caused by the destruction of red blood cells; hemoglobin values typically drop to 4 to 10 g/dL in this condition). Because the amount of ATP formed from glycolysis is decreased by 50%, red blood cell ion transporters cannot function effectively. The red blood cells tend to gain Ca^{2+} and lose K^+ and water. The water loss increases the intracellular hemoglobin concentration. With the increase in intracellular hemoglobin concentration, the internal viscosity of the cell is increased to the point that the cell becomes rigid and, therefore, more susceptible to damage by shear forces in the circulation. Once they are damaged, the red blood cells are removed from circulation, leading to the anemia. However, the effects of the anemia are frequently moderated by the twofold to threefold elevation in 2,3-BPG concentration that results from the blockage of the conversion of phosphoenolpyruvate to pyruvate. Because 2,3-BPG binding to hemoglobin decreases the affinity of hemoglobin for oxygen, the red blood cells that remain in circulation are highly efficient in releasing their bound oxygen to the tissues.

Congenital methemoglobinemia, the presence of excess methemoglobin, is found in people with an enzymatic deficiency in cytochrome b_5 reductase or in people who have inherited hemoglobin M. In hemoglobin M, a single amino acid substitution in the heme-binding pocket stabilizes the ferric (Fe^{3+}) oxygen. Individuals with congenital methemoglobinemia appear cyanotic but have few clinical problems. Methemoglobinemia can be acquired by ingestion of certain oxidants such as nitrites, quinones, aniline, and sulfonamides. Acquired methemoglobinemia can be treated by the administration of reducing agents, such as ascorbic acid or methylene blue.

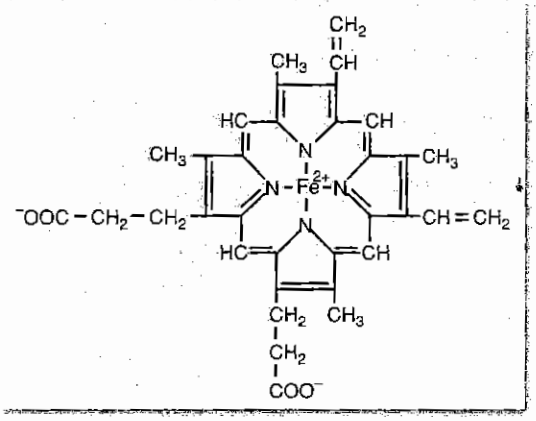


FIG. 44.2. Structure of heme. The side chains can be abbreviated as MVMVMPPM. M, methyl (—CH_3); P, propionyl (—CH_2—CH_2—COO^-); V, vinyl (—CH=CH_2).

G6PD deficiency is the most common enzyme deficiency in humans, probably, in part, because individuals with G6PD deficiency are resistant to malaria. The resistance to malaria counterbalances the deleterious effects of the deficiency. G6PD-deficient red cells have a shorter life span and are more likely to lyse under conditions of oxidative stress. When soldiers during the Korean War were given the antimalarial drug primaquine prophylactically, approximately 10% of the soldiers of African ancestry developed spontaneous anemia. Because the gene for G6PD is found on the X chromosome, these men had only one copy of a variant G6PD gene.

All known G6PD variant genes contain small in-frame deletions or missense mutations. The corresponding proteins, therefore, have decreased stability or lowered activity, leading to a reduced half-life or life span for the red cell. No mutations have been found that result in complete absence of G6PD. Based on studies with knockout mice, those mutations would be expected to result in embryonic lethality.

Q: Pyridoxine (vitamin B_6) deficiencies are often associated with a microcytic, hypochromic anemia. Why would a B_6 deficiency result in small (microcytic), pale (hypochromic) red blood cells?

damage accumulates, leading to lysis of the erythrocyte. When red blood cell lysis (hemolysis) substantially exceeds the normal rate of red blood cell production, the number of erythrocytes in the blood drops below normal values, leading to hemolytic anemia.

B. The Erythrocyte Precursor Cells and Heme Synthesis

1. HEME STRUCTURE

Heme consists of a porphyrin ring coordinated with an atom of iron (Fig. 44.2). Four pyrrole rings are joined by methenyl bridges (–CH–) to form the porphyrin ring (see Fig. 7.12). Eight side chains serve as substituents on the porphyrin ring, two on each pyrrole. These side chains may be acetyl (A), propionyl (P), methyl (M), or vinyl (V) groups. In heme, the order of these groups is M V M V M P P M. This order, in which the position of the methyl group is reversed on the fourth ring, is characteristic of the porphyrins of the type III series, the most abundant in nature.

Heme is the most common porphyrin found in the body. It is complexed with proteins to form hemoglobin, myoglobin, and the cytochromes (see Chapters 7 and 21), including cytochrome P450 (see Chapter 24).

2. SYNTHESIS OF HEME

Heme is synthesized from glycine and succinyl CoA (Fig. 44.3), which condense in the initial reaction to form δ-aminolevulinic acid (δ-ALA) (Fig. 44.4). The enzyme that catalyzes this reaction, δ-ALA synthase, requires the participation of pyridoxal phosphate, as the reaction is an amino acid decarboxylation reaction (glycine is decarboxylated; see Chapter 39).

The next reaction of heme synthesis is catalyzed by δ-ALA dehydratase, in which two molecules of δ-ALA condense to form the pyrrole, porphobilinogen (Fig. 44.5). Four of these pyrrole rings condense to form a linear chain and then a series of porphyrinogens. The side chains of these porphyrinogens initially contain acetyl (A) and propionyl (P) groups. The acetyl groups are decarboxylated to form methyl groups. Then the first two propionyl side chains are decarboxylated and oxidized to vinyl groups, forming a protoporphyrinogen. The methylene bridges are subsequently oxidized to form protoporphyrin IX (see Fig. 44.3). Heme is red, and is responsible for the color of red blood cells and of muscles that contain a large number of mitochondria.

بعض المراجع والسورسات

Sources of Lecture 7

(Metabolism in mature erythrocyte & Genetic Deficiences)

1- Supplement from Marks on Erythrocyte metabolism (2 pages) will be supplied

2- slides :- will be supplied

3- Lippincot [chapter, Pentose phosphate Pathway (chap. 13)]

V- Glucose-6-P Dehydrogenase deficiency

Pages 152 - 154

4. Pyruvate kinase Deficiency

chapter 8 (Glycolysis)

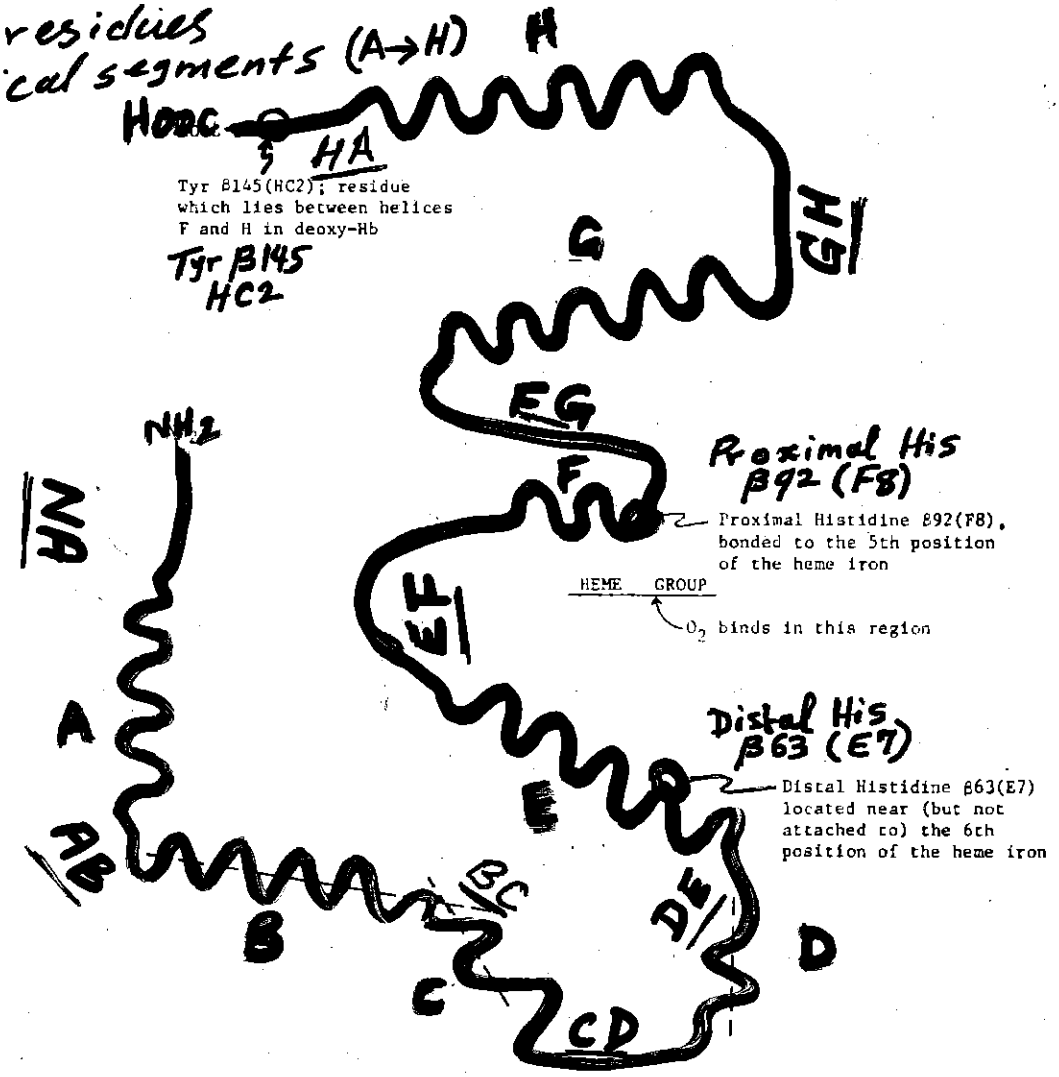
Pages 102 - 103

Please note, the slides that appear v. dark after photocopy are all from your book Lippincot. You can see them clearly in the book.

SEC. STRUCTURE OF β-chain of Hb

Figure 81. Secondary Structure of the β-Chain of Human Hemoglobin

146 residues
8 helical segments (A→H)



The helical regions (labeled A-H, after Kendrew), N- and C-termini, and the histidines located near the heme group are indicated. The axes of the B, C, and D helices are indicated by dashed lines.

The α-helical regions are terminated by
1- Presence of Proline
or 2- β-bends and loops stabilized by H-bonds and ionic bonds
Electrostatic Interactions or salt bridges

b.

c.

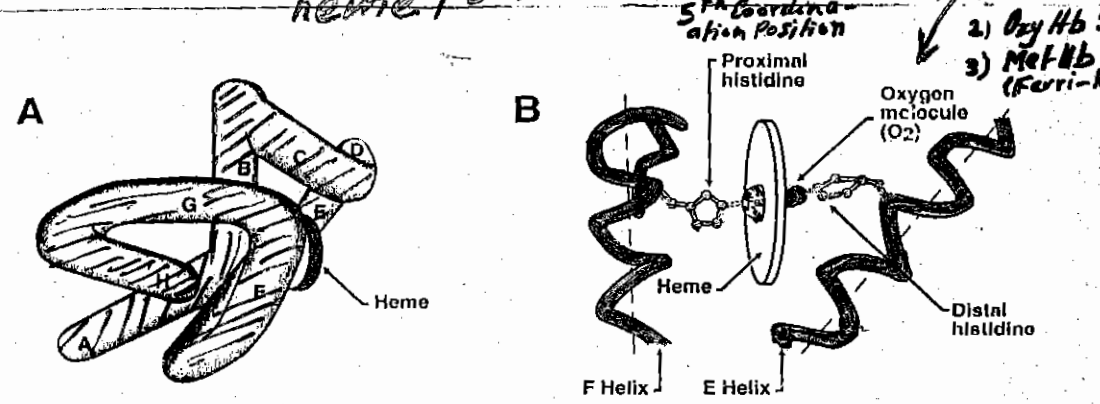
6. a.

Binding of O_2 Is Reversible

$O_2 + Fe^{2+} \longleftrightarrow Fe-O_2 \xrightarrow{X} Fe^{3+} + O_2^-$

Hydrophobic heme pocket

6th position
1) Deoxy Hb: Empty
2) Oxy Hb: O_2
3) Met Hb (Ferri-Hb) H_2O



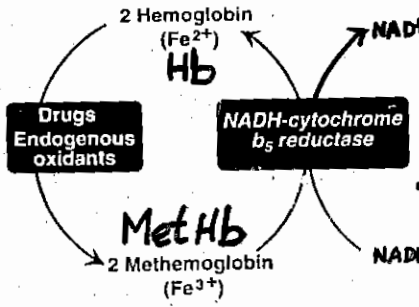
⇒ Formation of Methemoglobin:-

$Fe^{2+} \longrightarrow Fe^{3+}$ in Hb or Mb

Causes

1- Drugs + chemicals
2- Endogenous production of H_2O_2 + free radicals
3- Inherited defect in α- or β-chain → HbM

→ Reduction of Methemoglobin:-



NADH-Cyt b5 reductase
Activity is half of adults in newborns. More susceptible to HbM producing drugs

→ Role of Methemoglobin in Cyanide Poisning:-

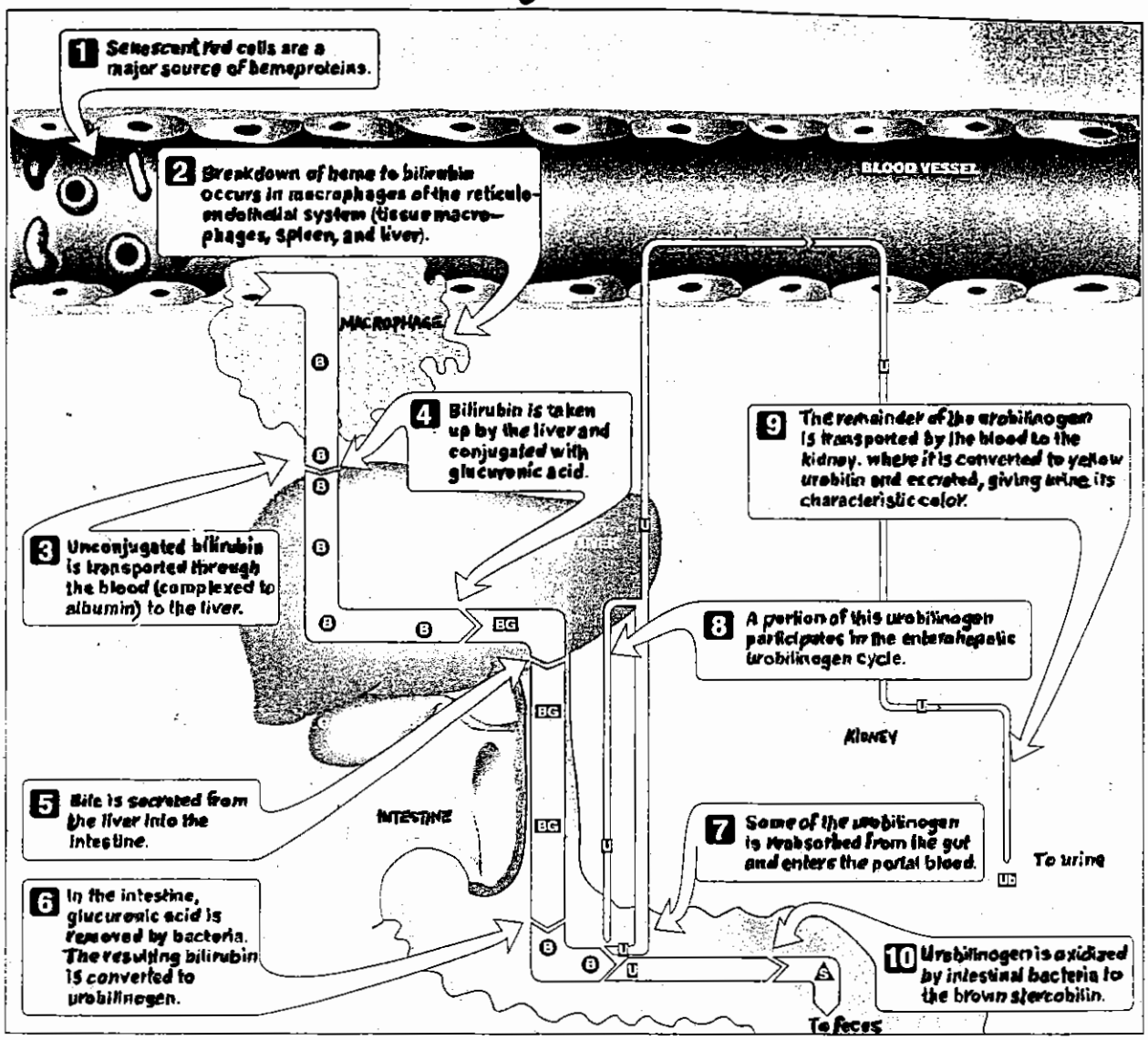
Treatment with Hb-M producing drugs to form some HbM to bind CN^- and protects complex IV of respiratory chains from poisning.

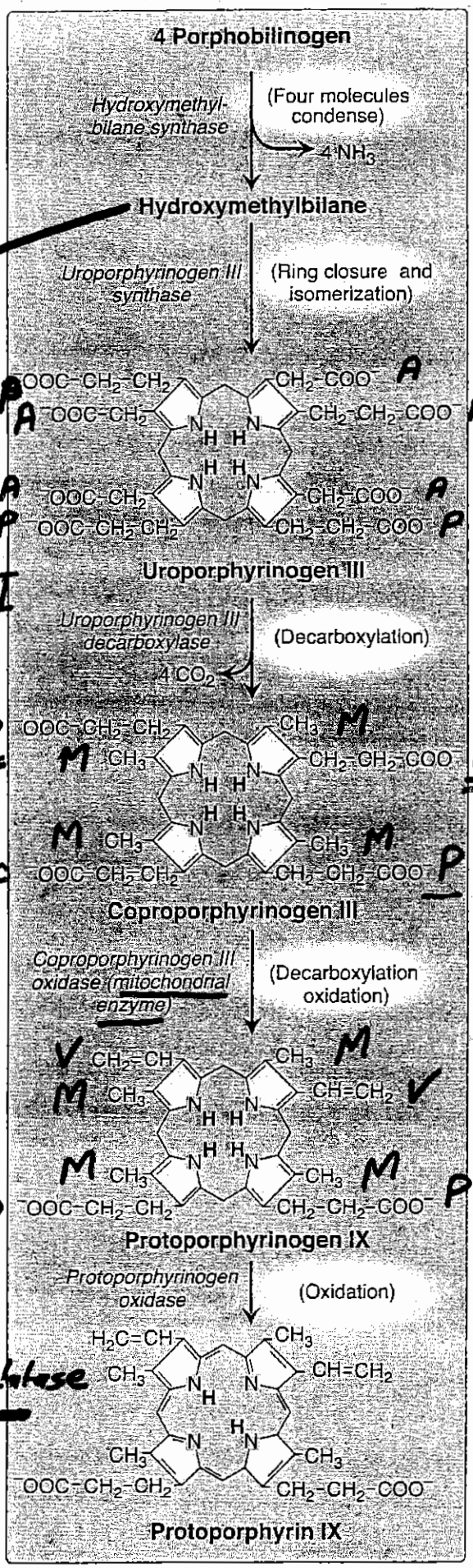
• Treatment of Methemoglobinemia:-

• With methylene blue or Ascorbate (less effective)
• Not effective in G6PD-deficiency

(because Methyle blue requires NADPH from G6PD)

CATABOLISM of HEME





Uroporphyrinogen I

Coproporphyrinogen I (excreted)

Uro I and Copro I are produced in excess in Erythropoietic Porphyria and excreted in urine

Mitochondria

Ferrochelatase

HEME

Fe^{2+}