UNIT OVERVIEW:
OXIDATIVE BIOLOGY OF THE ERTHROCYTE
PROTECTION AND MECHANISMS OF INJURY

Redox protection of erythrocytes (glutathione)

Energy production for redox reaction (PMPS)

Genetic disorders of erythrocyte function

Oxidative free radicals:

Fenton Reaction:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^- + HO^\bullet$$

Haber-Weiss Reaction:

$$O_2^{\bullet-} + H_2O_2 \xrightarrow{[Fe^{3+} \rightarrow Fe^{2+}]} O_2 + HO^- + HO^\bullet$$

Reaction Cycling:

$$O_2^{\bullet-} + Fe^{+3} \rightleftharpoons O_2 + Fe^{+2}$$

Macrophage / WBC's:

$$Cl^- + H_2O_2 \longrightarrow HO^- + HOCl$$

Hypochlorous acid

$$O_2^{\bullet-} + HOCl \longrightarrow O_2 + Cl^- + HO^\bullet$$

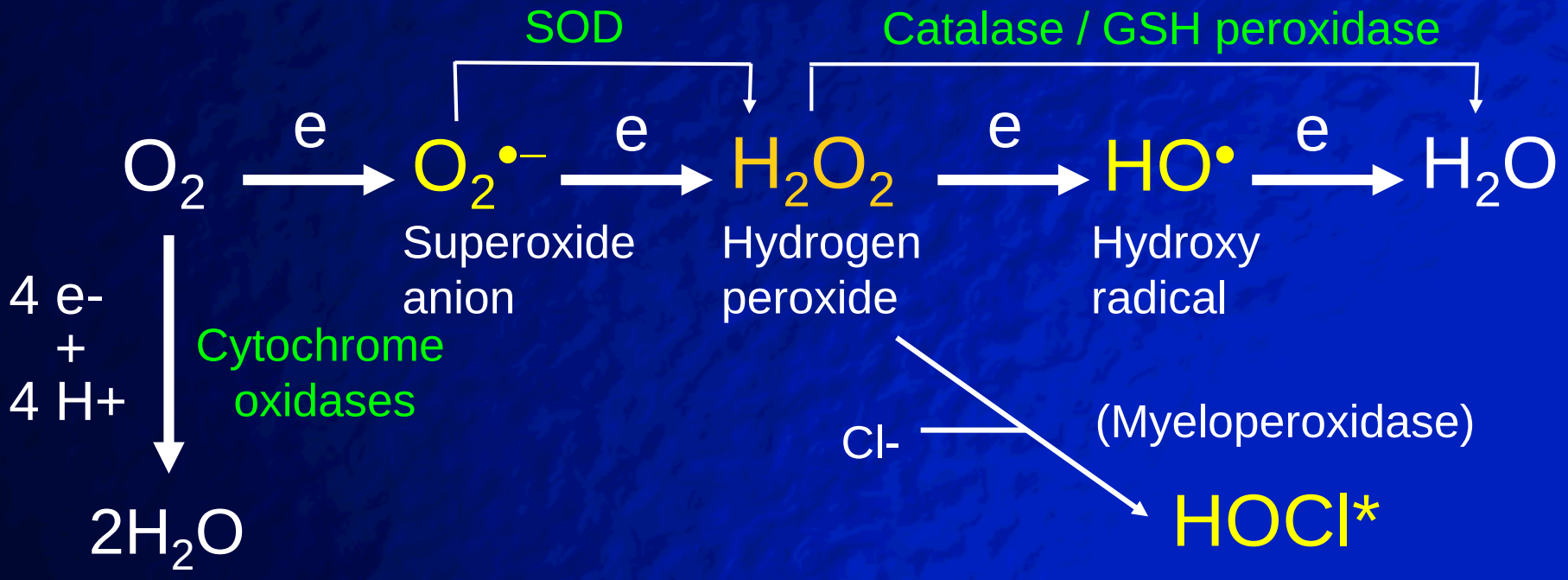
$$HOCl + Fe^{+2} \longrightarrow Fe^{+3} + Cl^- + HO^\bullet$$

$$O_2^{\bullet-} + NO\bullet \longrightarrow ONOO^-$$ (peroxynitrite - inflam./ atherosclerosis)

Nitric oxide - vasodilator, neurotransmitter

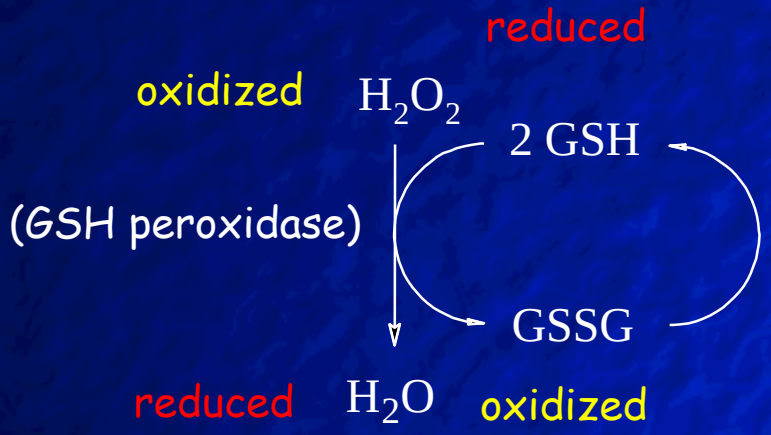
Oxidation and free radicals:

- A secondary consequence of oxidative metabolism is the potential for the production of free radicals.
- Free radical formation is exacerbated in presence of iron.
- Thus sites such as the erythrocyte must possess mech. to deal with free radical formation.



Redox protection of erythrocytes (Glutathione - GSH)

Cofactor for cellular defence against oxidative stress.



Properties of glutathione:

- Principle antioxidant of the cell.
- Tripeptide consisting of glutamate, cysteine, and glycine.
- Glutathione is particularly important for erythrocytes and liver hepatocytes. In red blood cells, GSH exists at high concentrations (5-10 mM).
- GSH is a cofactor for many cellular enzymes.
- 85-90% of GSH exists in the cytosol, while 10-15% exists in the mitochondria.
- Detoxifies reactive drug metabolites (acetaminophen).

Cellular roles of glutathione:

- Scavenging activity on free radicals.

- Maintains essential redox status of proteins by maintaining cysteine thiols in their reduced (SH) form.

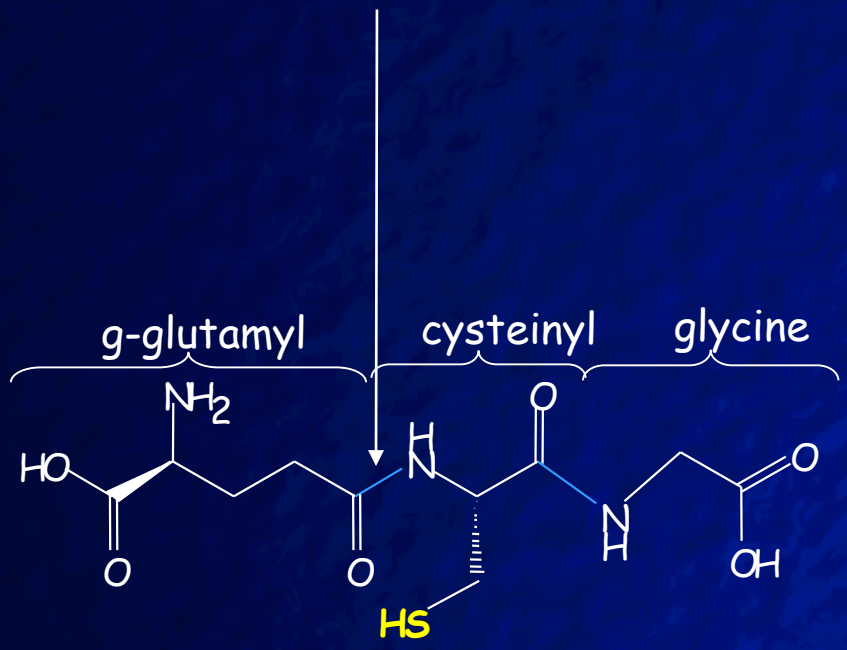
- Provides reservoir of cysteine for protein synthesis (not erythrocytes).

- Modulates processes such as DNA synthesis (not erythrocytes), immune / microtubular processes.

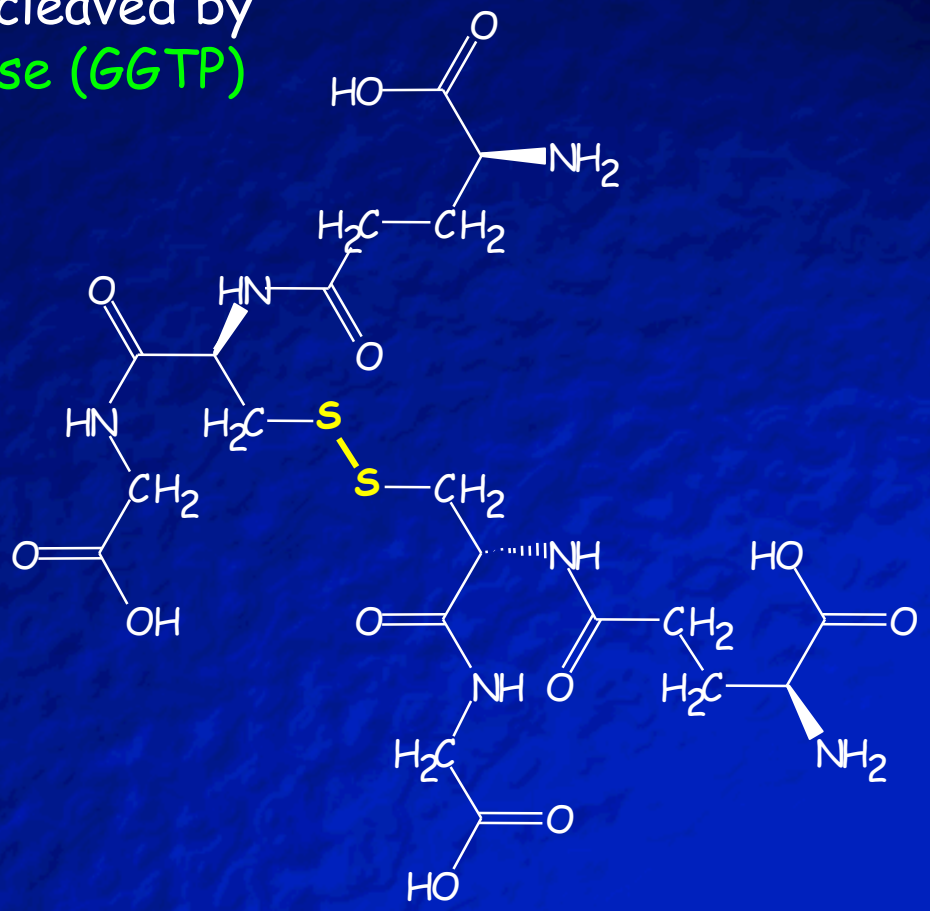
Curr. Top. In Cell. Regul., Vol 36:95-117.

GSH exits in two different forms in the cell:

This carboxyl linkage can ONLY be cleaved by the enzyme γ-glutamyl transpeptidase (GGTP)



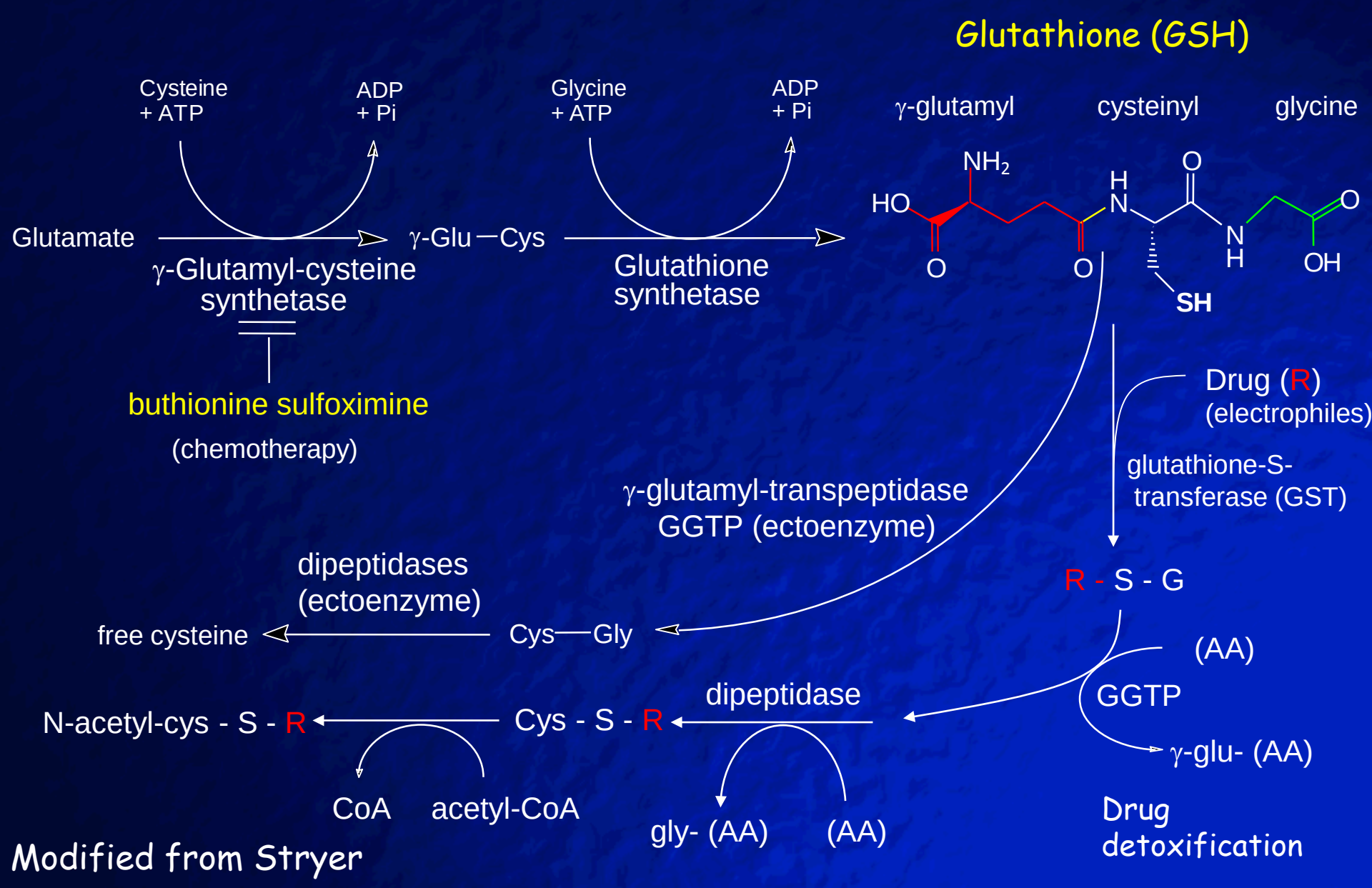
REDUCED GSH



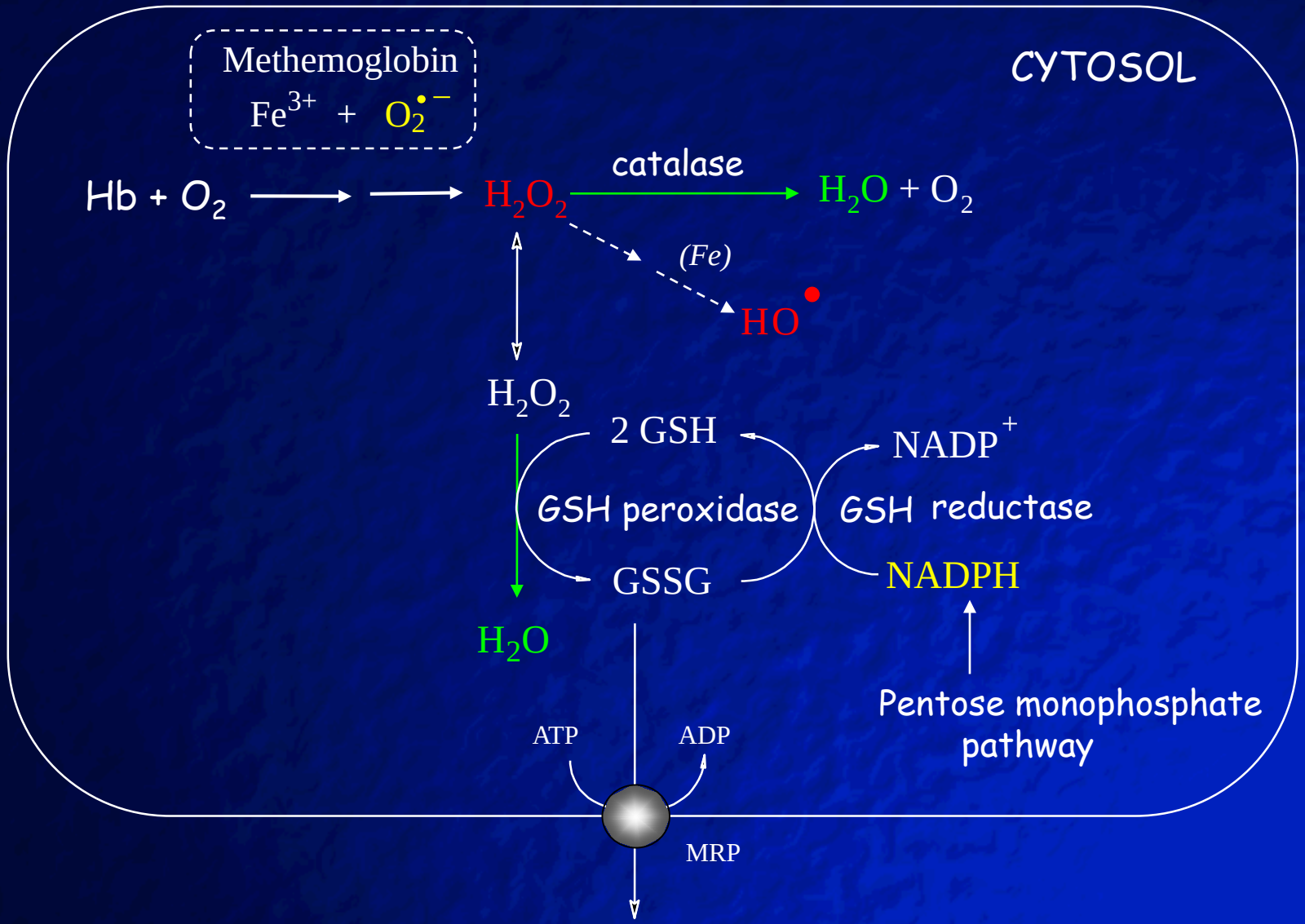
OXIDIZED GSH (GSSG)

= 2 reduced GSH's covalently bonded by a disulfide bridge.

Glutathione synthesis and catabolism:

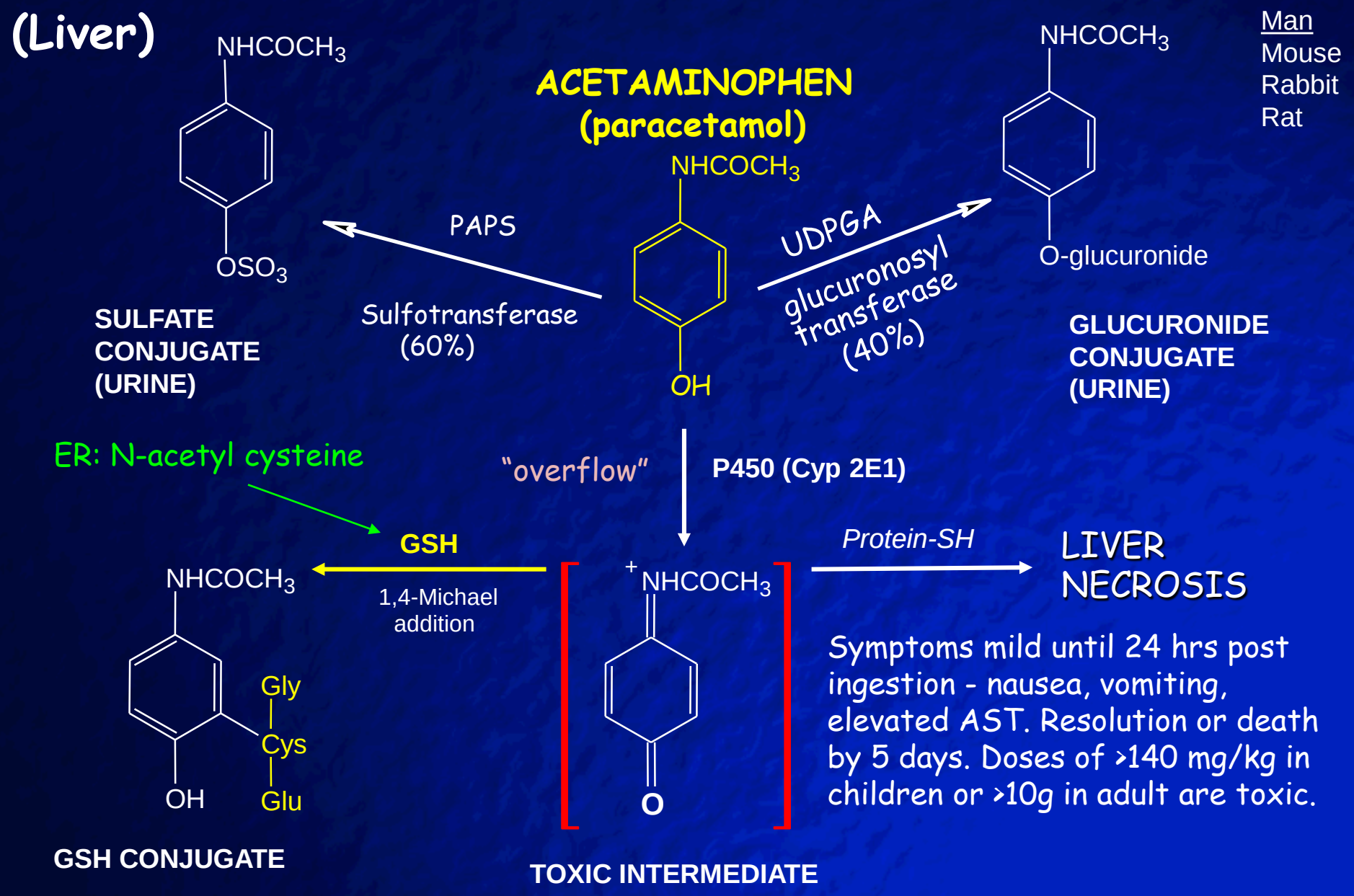


Antioxidant role of GSH in erythrocytes:



Curr. Top. Cell. Regul. 36:95-116 (2000)

Other examples of GSH-mediated protection:



Genetic disorders of erythrocyte function

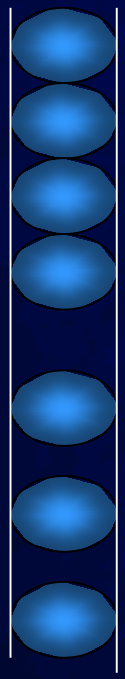
a. Genetic and environmental causes of glucose 6-phosphate dehydrogenase deficiency

b. Abnormal hemoglobins (HbS and the thalassemias)

Genetic G6PD deficiency:

- Like a number of other disorders, G6PD deficiency is X-linked
- FEMALES heterozygotes have 2 populations of red cells (wild-type and def.)
- A - 16% of Africans and Black Americans
- B - (numerous variations) Mediterranean: Greece, Turkey, Israel, Egypt, Italy.
-It is estimated that worldwide ~400 million people are deficient in G6PD!
- Type 1 <2% (Med.), Type II <10%, Type III 10-50% (type A), Type IV - normal

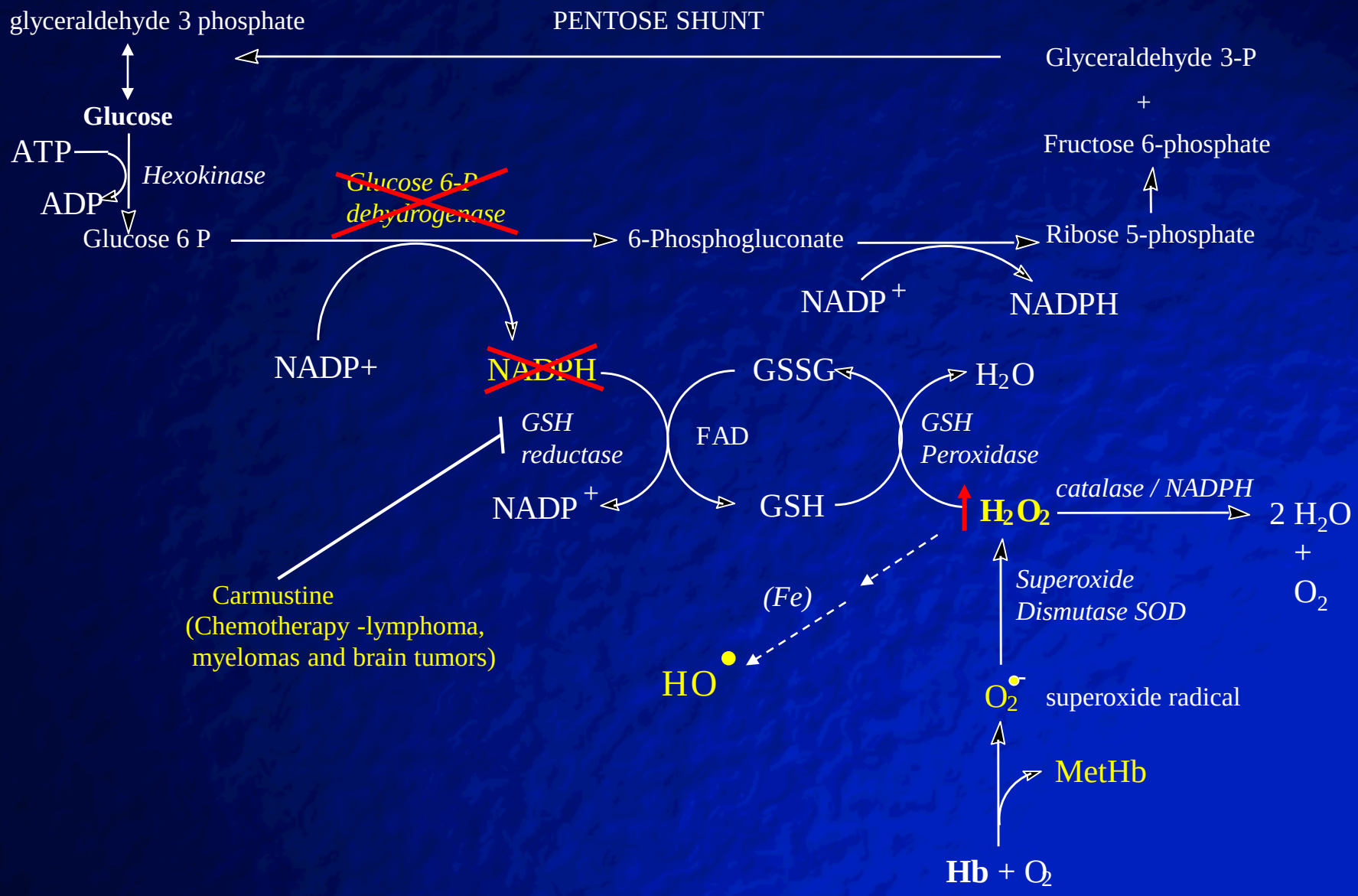
X-chromosome - long arm:



- Colour Blindness
- Glucose-6-P Dehydrogenase
- Factor VIII (Haemophilia A)
- Optic atrophy
- Xm serum groups
- Sideroblastic anemia
- Muscular dystrophy

	Percent G6PD deficient MALE population
Kurdish Jews	62%
Sardinia	30%
Saudi	13%
U.S. blacks	11%

G6PD deficiency and oxidative damage:



Structural mutations of the G6PD gene:

- More than 100 amino acid mutations identified for G6PD identified.

Two example G6PD mutations:

only 8 angst. apart in crystal structure

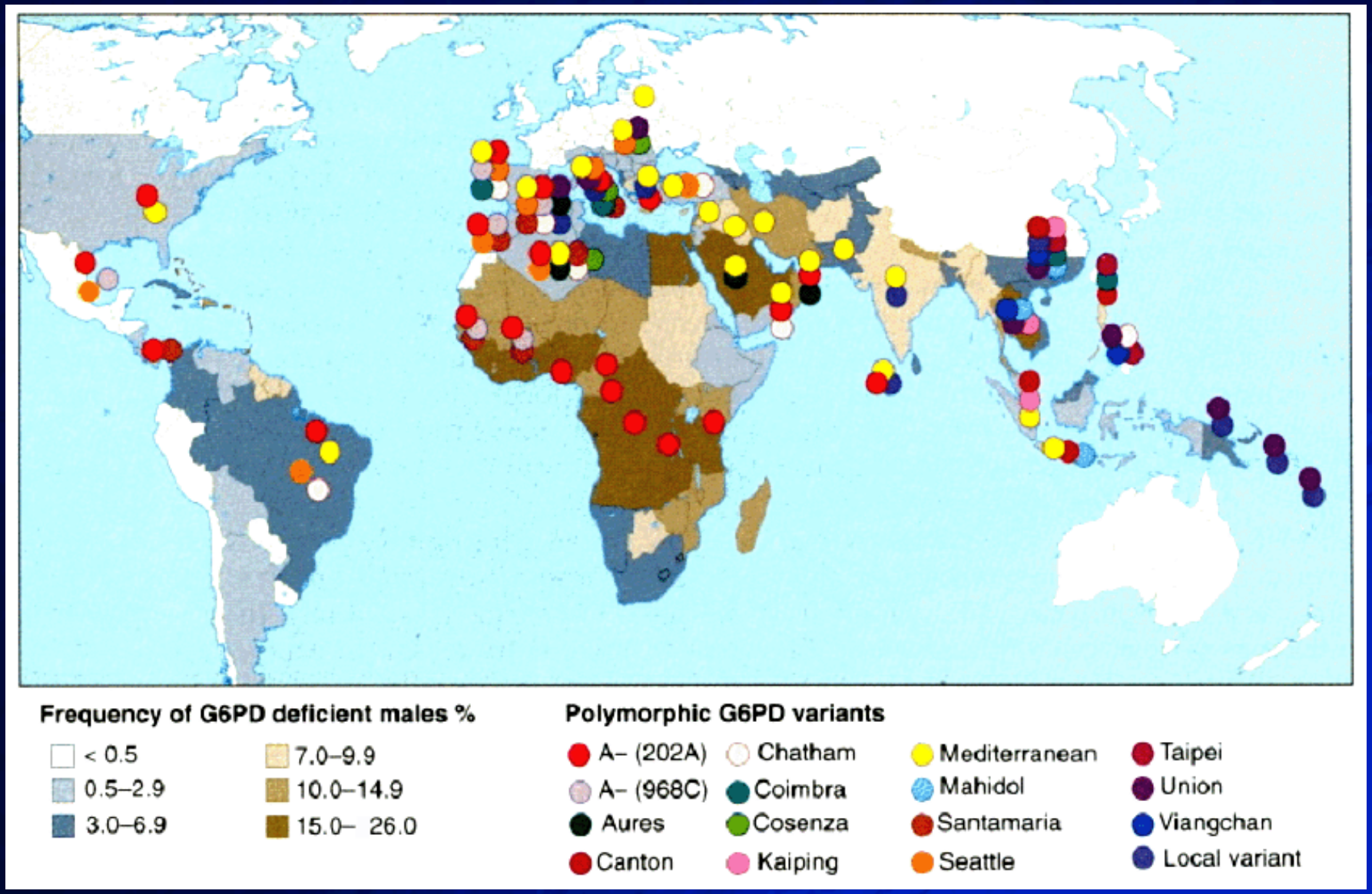
N126D mutation	V68M mutation
Asparagine 126 → Aspartate Does not affect activity G6PD A - has 85% normal activity (G6PD B = normal = 100% activity)	Valine 68 → Methionine Increases rigidity in protein affects Lysine 205 (active site that binds glucose 6 phosphate – protein folding) (G6PD A- 12% activity – both mut.)

Single letter amino acid codes:

Alanine	A	Phenylalanine	F	Proline	P	Glutamate	E
Valine	V	Tyrosine	Y	Lysine	K	Serine	S
Isoleucine	I	Tryptophan	W	Argenine	R	Threonine	T
Leucine	L	Cysteine	C	Histidine	H	Glutamine	Q
Methionine	M	Glycine	G	Aspartate	D	Asparagine	N

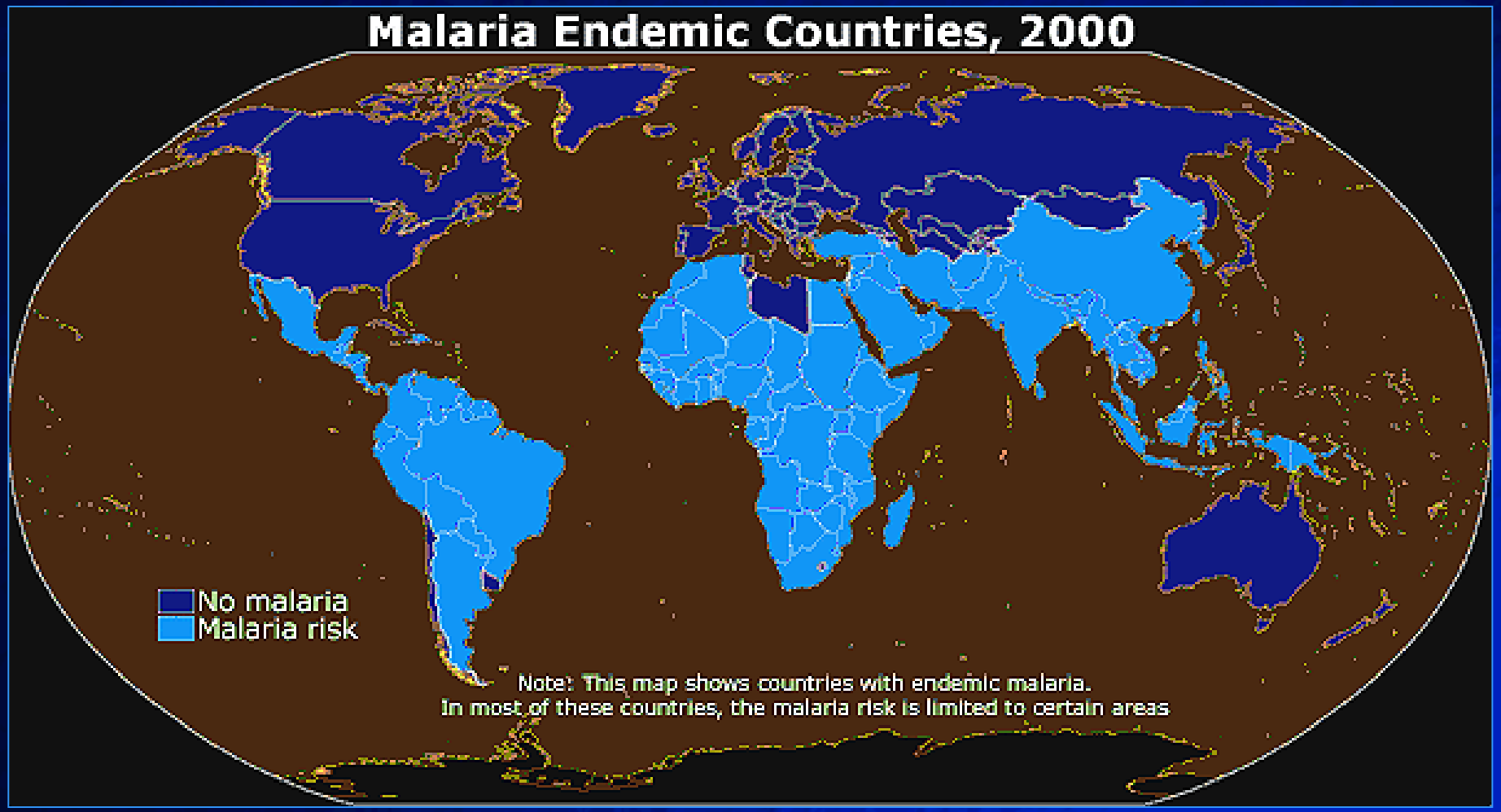
J. Biol. Chem. 275, 9256-62 (2000), Febs Lett. 366, 61-4 (1995)

Global G6PD deficiency and polymorphisms:



Luzzatto & Notaro, 2001. Science. 293:442-3.

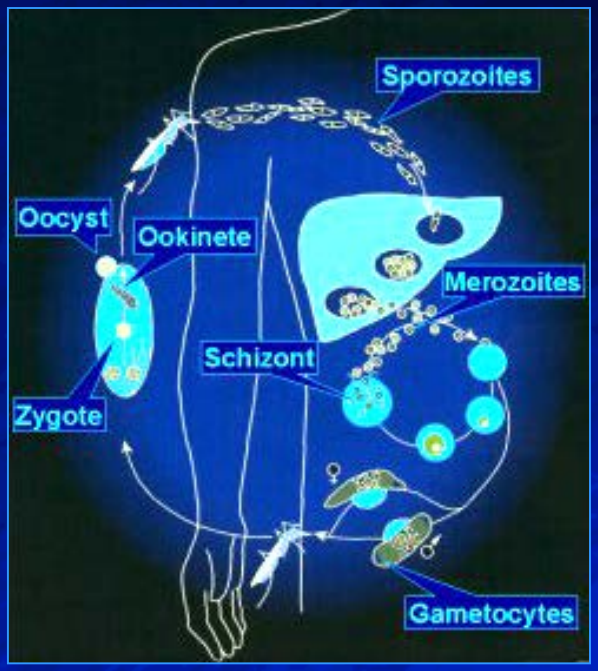
Epidemiology of Malaria:



Malaria - Mala (bad), Aria (air):

8000 BC Introduction of agriculture in Middle East & Africa, promoting conditions for spread of malaria

5700 BC Ancestral Plasmodium falciparum



1200 BC Heterozygote for G6PD exhibit malarial resistant (mis-sense mutations of small in-frame deletions)

476 AD Fall of the Western Roman Empire (malarial contribution)

Luzzatto & Notaro, 2001. Science. 293:442-3.

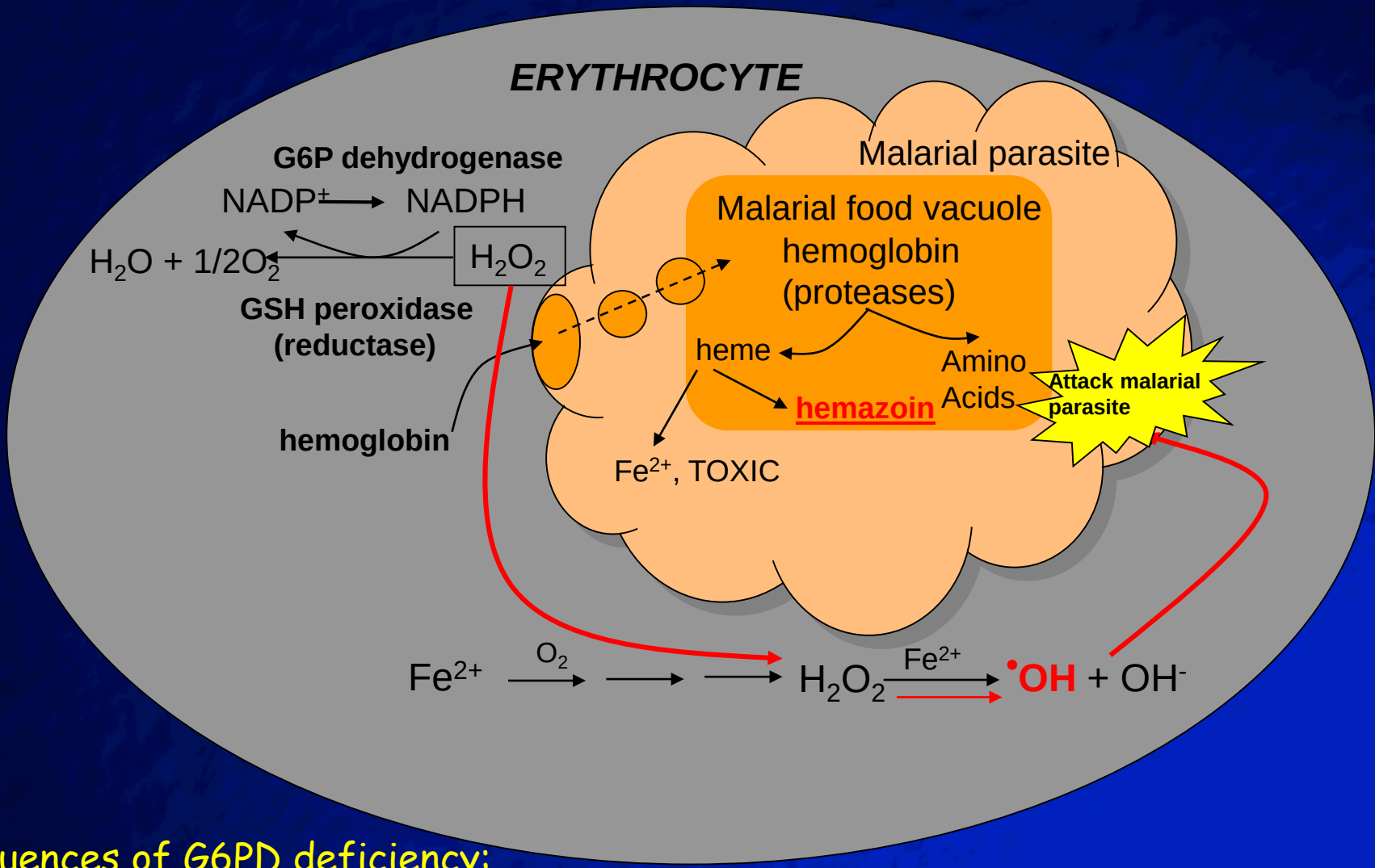
Infection of red cells by malarial parasite:

- Plasmodium Falciparum (the most serious and prevalent form of malaria) is a protozoan parasite carried by mosquitoes. The parasite attacks red blood cell hemoglobin using a specialized food vacuole. 60-80 percent of the hemoglobin content in infected cells can be consumed by the parasite.

- Normal Red Cell – Low glucose utilization
  - Low lactate formation
- Infected Red Cell – High glucose utilization

  Malarial parasite needs ATP for RNA/DNA/Protein synthesis. ATP is taken from the erythrocyte by the synthesis of parasitic hexokinase.

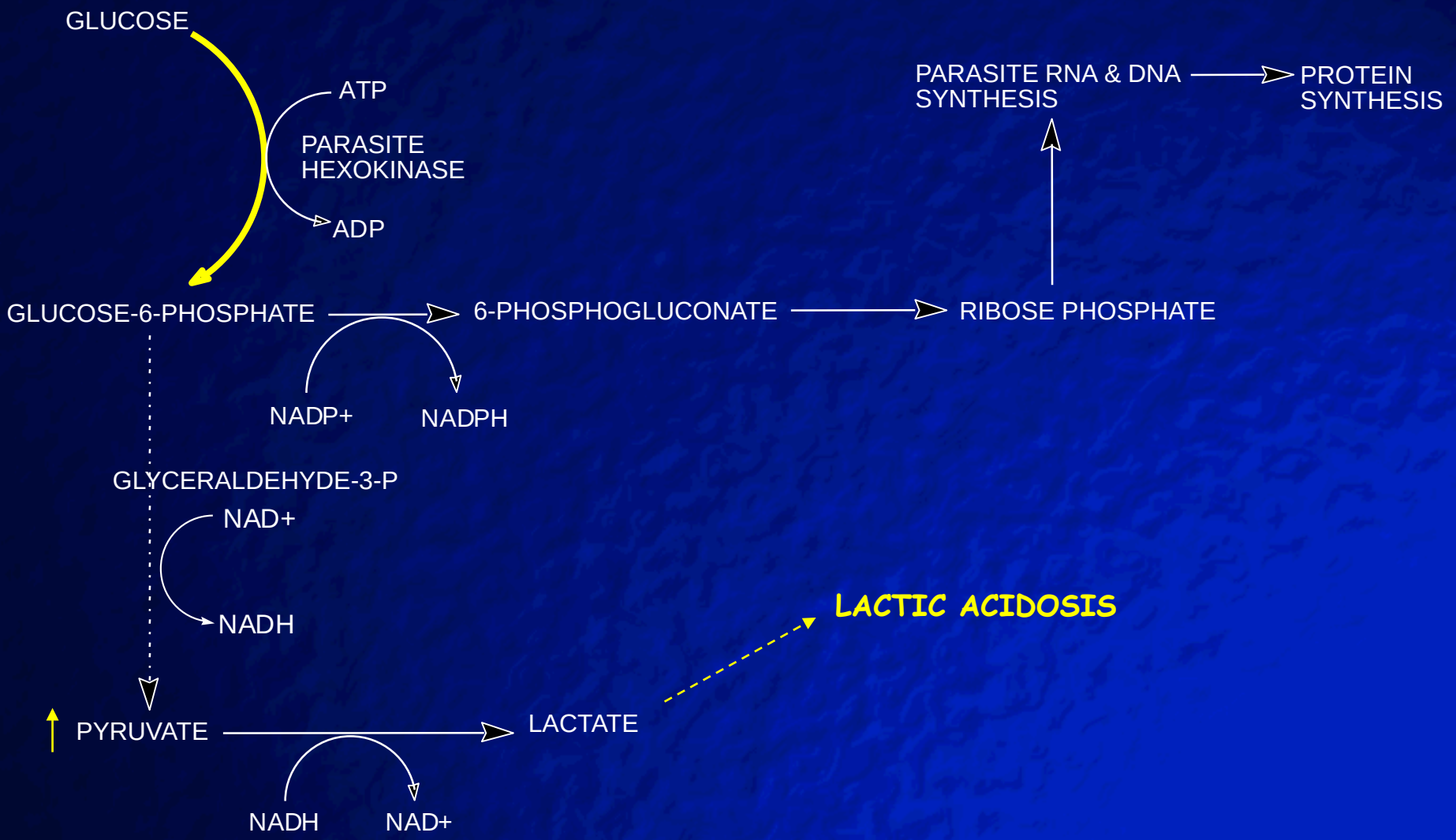
G6PD deficiency combats malaria:



Consequences of G6PD deficiency:

- Lower GSH:GSSG ratio in the cell, leading to higher levels of reactive oxygen species (ROS) in the cell.
- Result: Less than ideal environment for survival of malarial parasite.

Malarial parasite biochemistry in red cells:



Parasite biochemistry - cellular targets:

1. Infected red cells show increased glycolysis (30x).
   This can result in lactic acidosis due to pyruvate buildup (coma).

2. Red cell ATP → hypoxanthine → parasite purines → RNA/DNA

3. Makes ATP by glycolysis (not citric acid cycle). Mitochondrial electron transport chain is for pyrimidine synthesis but acidosis inhibits erythrocyte glycolysis.

4. Degrades hemoglobin to release amino acids → parasite protein synthesis (hemozoin)

5. Also makes NADPH through its own G6PDH (also glutamate dehydrogenase)

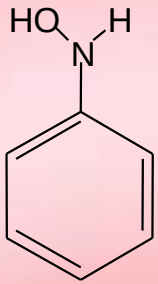
Voet, Biochemistry; Pharmacol. Ther. 81, 91-110 (1999).

DRUG INDUCED HEMOLYTIC ANEMIA and G6PD:

Aniline

* Individuals with G6PD deficiency particularly susceptible to this mechanism of toxicity

Liver P450



MODEL DRUG METABOLITE (Phenylhydroxyamine)

ERYTHROCYTE

Oxyhemoglobin

Fe^{2+}

NADP+

NADPH REDUCTASE

NADPH

(O_2)

REDOX CYCLE

(H_2O)

NAD+

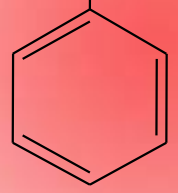
Methemoglobin Reductase

NADH

Fe^{3+}

Methemoglobin

+



(Nitrosobenzene)

$2H^+$ $O_2^{\bullet -}$ SUPEROXIDE ANION

Disproport. (via SOD)

H_2O_2 HYDROGEN PEROXIDE

Fe II/Cu I

$^{\bullet}OH$ HYDROXY RADICAL

Membrane Damage
Eventual Hemolysis

catalase

$H_2O + 1/2O_2$

2 GSH

GSH peroxidase

GSSG

Scavenged by :
Vitamins C/E, GSH, etc.

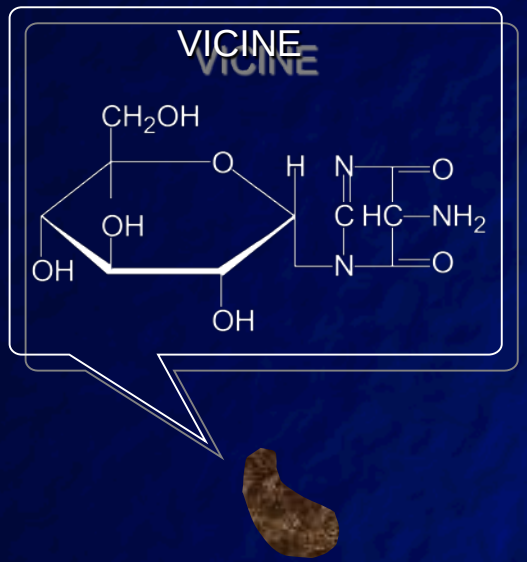
Drugs which induce hemolytic anemia in G6PD deficient individuals

In the modern world, individuals with G6PD deficiency typically exhibit few ill effects, until:

Acetanilide
Aminopyrine
Aspirin
Chloroquine
Dapsone
Dimercaprol
Furazolidine
Mepacrine
Methylene Blue
Naphthalene
Nitrofurantoin
Pamaquin, Pantaquin
Phenacetin
Phenylhydrazine
Primaquine *
Probenecid
Salicylates
Sulfa drugs
Toluidine blue

Reference: New Engl J Med. 324, 169-74 (1990).

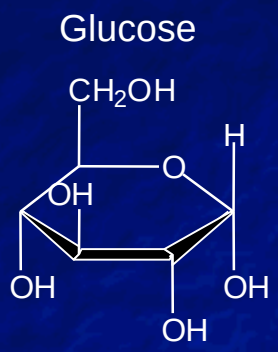
Dietary G6PD deficiency (Favism):



FAVA BEAN

Vicine is found in uncooked fava beans. This toxic glycoside increases the body's need for NADPH and strains GSH stores.

Stomach Acid (low pH)



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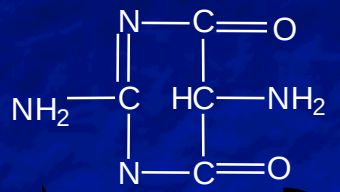
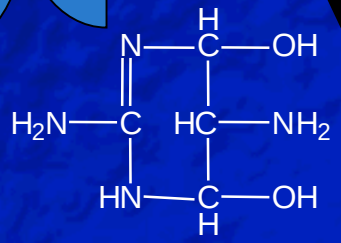


$O_2^{\bullet -}$

O_2

H_2O_2

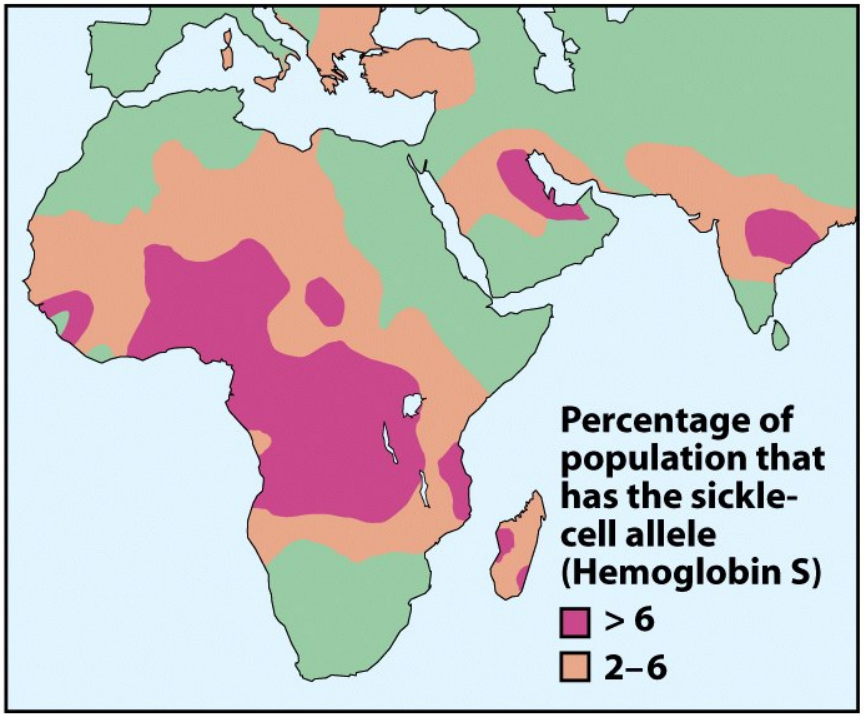
Reductase



Divicine
(reduced)

Toxicity may result in hemolytic anemia

Sickle cell disease and malaria demographics:



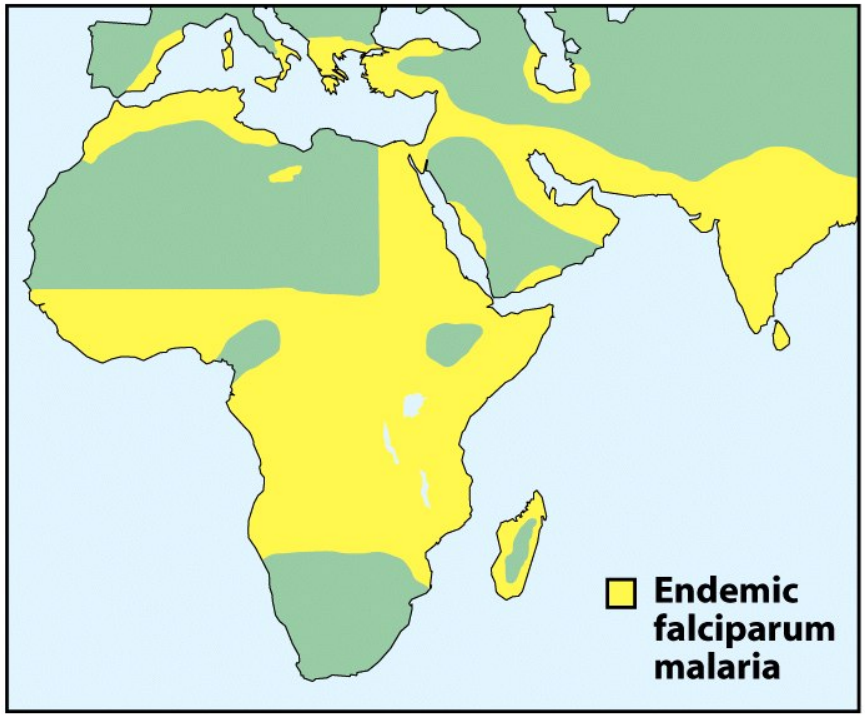
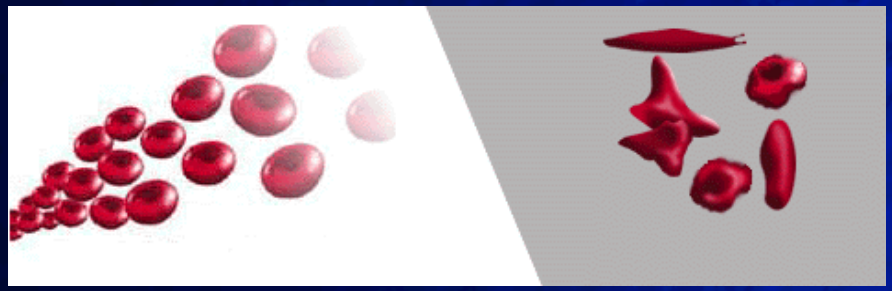


Figure 7-26
Biochemistry, Sixth Edition



http://www.kie.berkeley.edu/ned/data/E01-980311-003/E01-980311-003.html

The geographical prevalence of the sickle cell allele strongly correlates to that of malaria. This suggests that an evolutionary relationship exists between the sickle cell allele and malaria. A similar correlation has been shown between malaria and G6PD deficiency.

Sickle Cell Disease: Epidemiology

A inherited human disease independently identified in African and Hispanic populations.

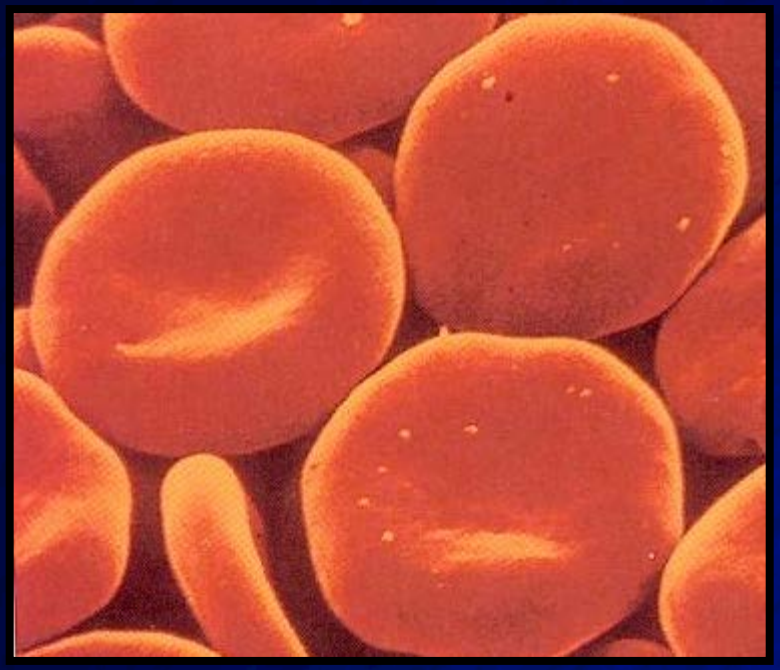
Sickle cell carrier (heterozygote) distributions:

As high as 25% in some West Africans populations,

10% in Afro-Caribbean populations,

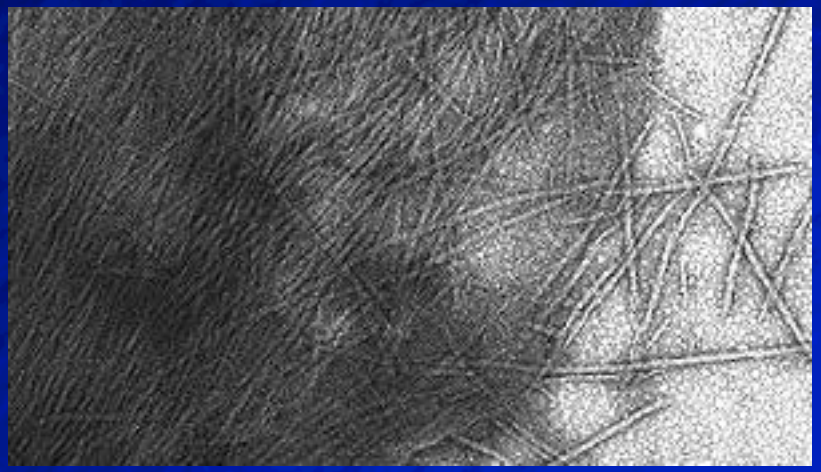
8% in African Americans,

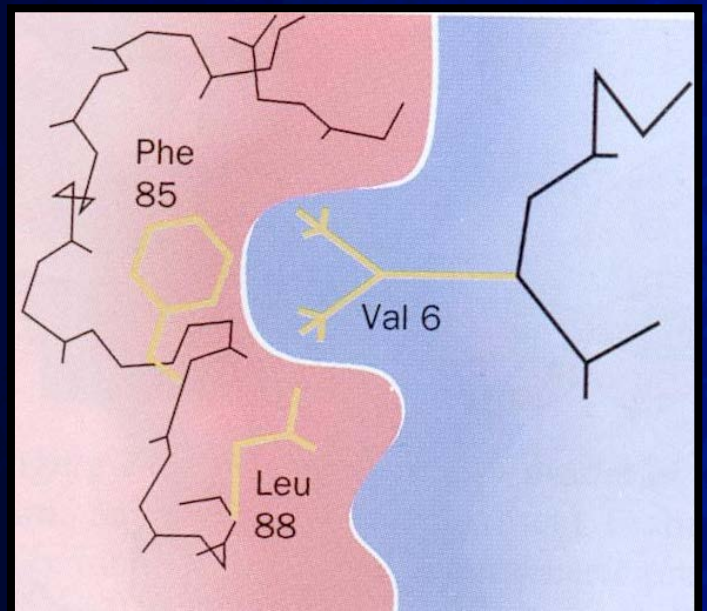
Pakistan, Indian and Cypriot populations report carrier frequencies of approximately 1%



Normal RBCs

Sickled RBCs

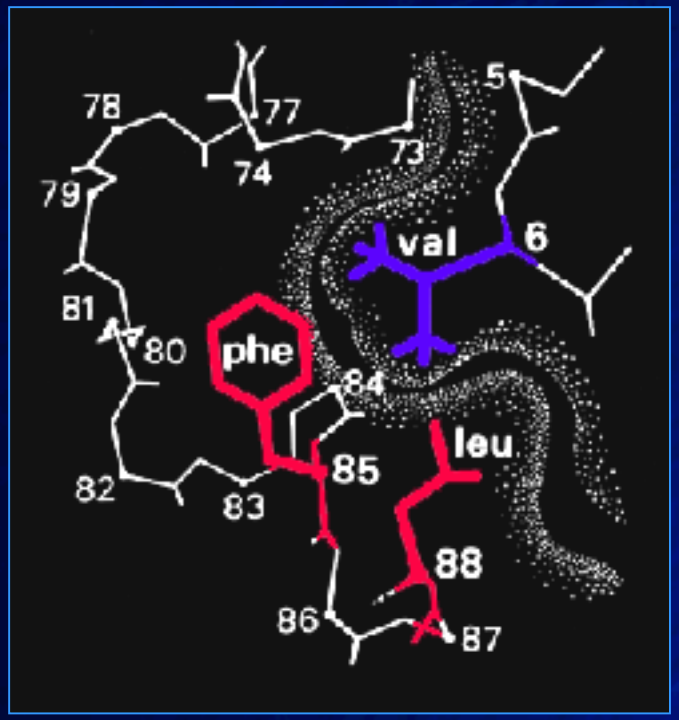


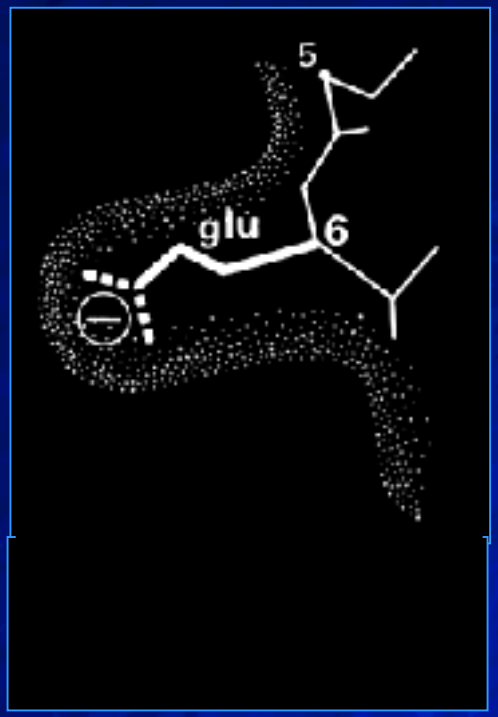


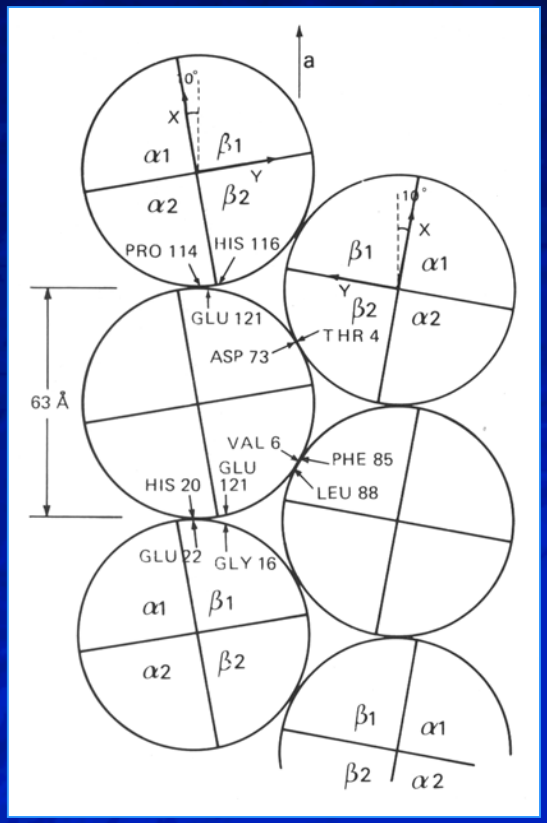
Amino acid mutation (Glu→Val)
At position 6 of the beta chain

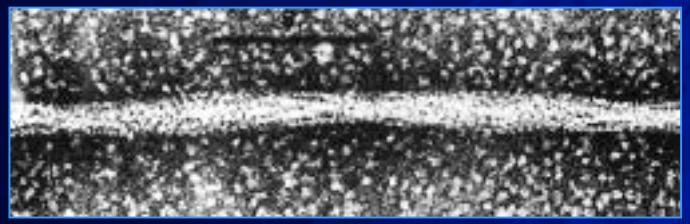
(Voet, 'Biochemistry')

Hemoglobin S: Mechanisms









14-stranded polymer
(electron micrograph)

Sickle cell hemoglobin is more readily oxidized and sickle cells generate greater quantities of superoxide radicals:

- Individuals homozygous for HbS exhibit a full blown sickling phenotype. Heterozygous individuals show little phentoypic effect to under normal circumstances.

- The defect originates in a mutation leading to replacement of Glu by Val at position 6 in the hemoglobin beta chain. Sickling can also result from beta chain Glu121Lys mutation, a site that lies close to position 6.

- In its deoxygenated state, HbS hemoglobin can aggregate to form long rod-like helical fibers, which ultimately cause deformation of the red cells (sickling). This happens because Val can pack into a pocket between helices E and F of separate beta chain.

Sickle cell erythrocytes age more rapidly:

- The abnormal sickled red blood cells are removed from the circulation at sites such as the spleen; resulting in anemia.

- There is no satisfactory treatment (hydroxyurea) and early death often results. Less serious consequences occur in heterozygotes, i.e., individuals with sickle-cell trait: these survive longer. The sickle Hb allele persisted due to survival advantage (greater resistance) of heterozygotes to malarial parasite. This is due to the ability of HbS to slow the growth of the malarial parasite during the red-cell phase of its life cycle. This results from the fact that when the HbS-containing RBCs pass through the capillaries they sickle, causing them to lose K^+ and killing the malaria parasite which grows better in high levels of K^+.

Sickle cell erythrocytes age more rapidly:

In vivo, sickling is triggered by:

Conditions that prolong capillary transit (i.e. abnormal adherence to the endothelium).

Implication: RBCs supersaturated with deoxyHb S will not sickle if the lag time for fiber formation is longer than the transit time from the peripheral capillaries to the lung alveoli.

Treatment :

Management of vaso-occlusive crisis (stroke)
Management - chronic pain syndromes
Management of chronic anemia
Prevention and treatment of primary infections

Genetic diseases II: Abnormal hemoglobins - release $O_2^{\cdot-}$ more readily and erythrocytes age more rapidly (ANEMIA)

Thalassemia:

- Group of diseases resulting from inherited defects in the rate of synthesis of one of the types of polypeptide of hemoglobin
- Such defects lead in turn to ineffective erythropoiesis, hemolysis and a variable degree of anemia
- In alpha thalassemia the defect is in the production of alpha chains and there is concomitant excessive beta- and gamma-chain production leading to the formation of various abnormal hemoglobins
- In beta thalassemia the formation of beta chains is defective with an excessive synthesis of alpha chains and the continued production of fetal hemoglobin

In these patients, transfusion therapy can cause iron overload as blood transfusions are a part of clinical management (monitor and iron chelation therapy). Other measures such as splenectomy and allogeneic hematopoietic stem cell transplantation may be employed.