Pentose Phosphate Pathway and Nicotinamide Adenine Dinucleotide Phosphate 13

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I. OVERVIEW

The pentose phosphate pathway (or, hexose monophosphate shunt) occurs in the cytosol. It includes an irreversible oxidative phase, followed by a series of reversible sugar–phosphate interconversions (Fig. 13.1). In the oxidative phase, carbon 1 of a glucose 6-phosphate molecule is released as carbon dioxide (CO_2), and one pentose sugar-phosphate plus two reduced nicotinamide adenine dinucleotide phosphates (NADPH) are produced. The rate and direction of the reversible reactions are determined by the supply of and demand for intermediates of the pathway. The pentose phosphate pathway provides a major portion of the body's NADPH, which functions as a biochemical reductant. It also produces ribose 5-phosphate, required for nucleotide biosynthesis (see p. 293), and provides a mechanism for the conversion of pentose sugars to triose and hexose intermediates of glycolysis. No ATP is directly consumed or produced in the pathway.

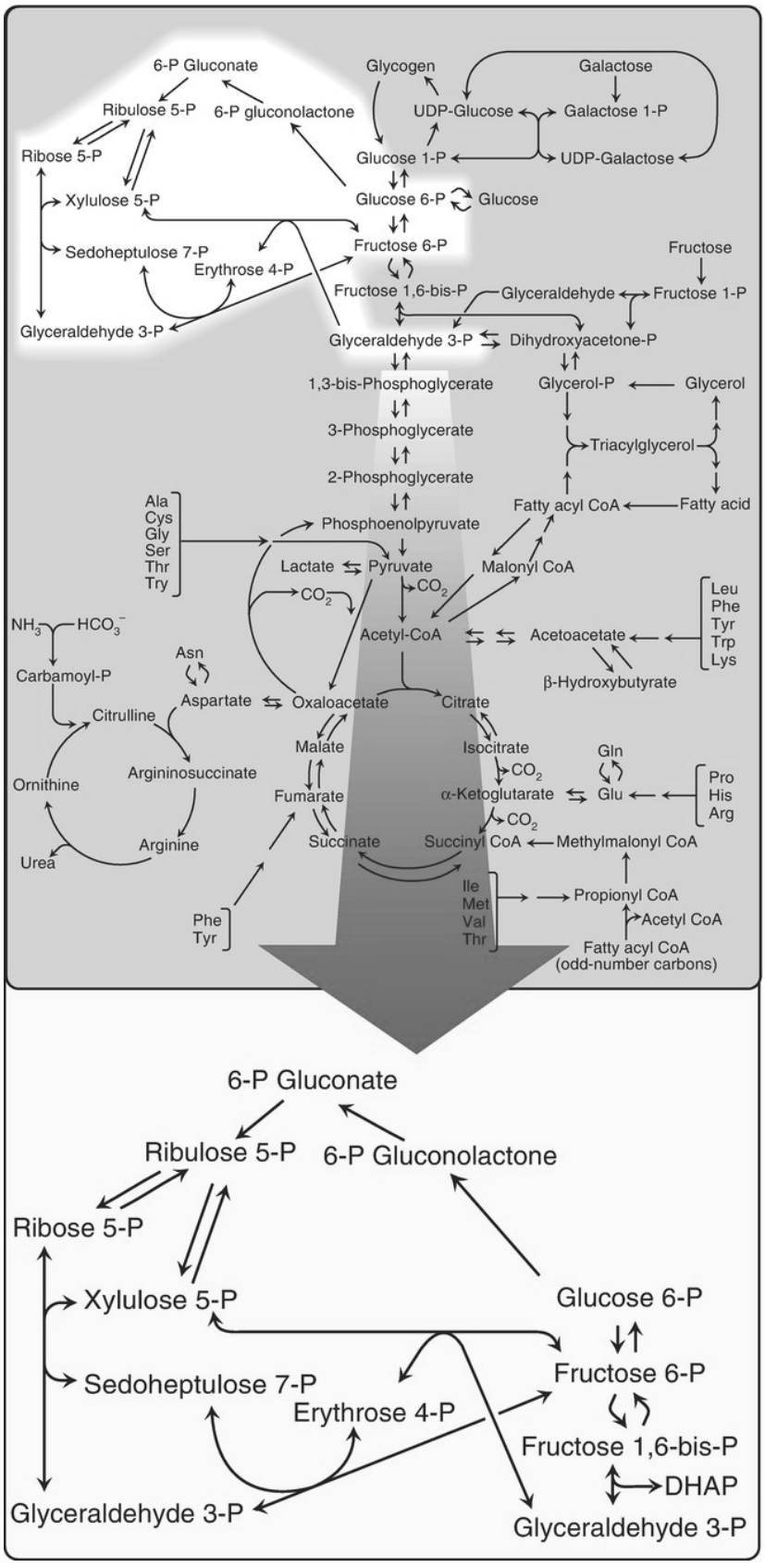
6-P Gluconate
Ribulose 5-P
6-P gluconolactone
Ribose 5-P
Xylulose 5-P
Sedoheptulose 7-P
Erythrose 4-P
Glyceraldehyde 3-P
Glycogen
Galactose
UDP-Glucose
Galactose 1-P
Glucose 1-P
UDP-Galactose
Glucose 6-P
Glucose
Fructose 6-P
Fructose
Fructose 1,6-bis-P
Glyceraldehyde
Fructose 1-P
Glyceraldehyde 3-P
Dihydroxyacetone-P
1,3-bis-Phosphoglycerate
Glycerol-P
Glycerol
3-Phosphoglycerate
Triacylglycerol
2-Phosphoglycerate
Fatty acyl CoA
Fatty acid
Ala
Cys
Gly
Ser
Thr
Try
Phosphoenolpyruvate
Lactate
Pyruvate
Malonyl CoA
CO2
NH3
HCO3−
Acetyl-CoA
Acetoacetate
Leu
Phe
Tyr
Trp
Lys
β-Hydroxybutyrate
Carbamoyl-P
Asn
Aspartate
Oxaloacetate
Citrate
Citrulline
Malate
Isocitrate
Gln
Ornithine
Argininosuccinate
Fumarate
α-Ketoglutarate
Glu
Pro
His
Arg
Arginine
Urea
Succinate
Succinyl CoA
Methylmalonyl CoA
Ile
Met
Val
Thr
Propionyl CoA
Acetyl CoA
Phe
Tyr
Fatty acyl CoA
(odd-number carbons)
6-P Gluconate
Ribulose 5-P
6-P Gluconolactone
Ribose 5-P
Xylulose 5-P
Glucose 6-P
Sedoheptulose 7-P
Fructose 6-P
Erythrose 4-P
Fructose 1,6-bis-P
DHAP
Glyceraldehyde 3-P
Glyceraldehyde 3-P

Figure 13.1 Pentose phosphate pathway shown as a component of the metabolic map. [Note: See Fig. 8.2, p. 92 for a more detailed map of metabolism.] P = phosphate; DHAP = dihydroxyacetone phosphate.

II. IRREVERSIBLE OXIDATIVE REACTIONS

The oxidative portion of the pentose phosphate pathway consists of three irreversible reactions that lead to the formation of ribulose 5-phosphate, CO_2, and two molecules of NADPH for each molecule of glucose 6-phosphate oxidized (Fig. 13.2). This portion of the pathway is particularly important in the liver, lactating mammary glands, and adipose tissue for the NADPH-dependent biosynthesis of fatty acids (see p. 186); in the testes, ovaries, placenta, and adrenal cortex for the NADPH-dependent biosynthesis of steroid hormones (see p. 237); and in red blood cells (RBC) for the NADPH-dependent reduction of glutathione (see p. 148).

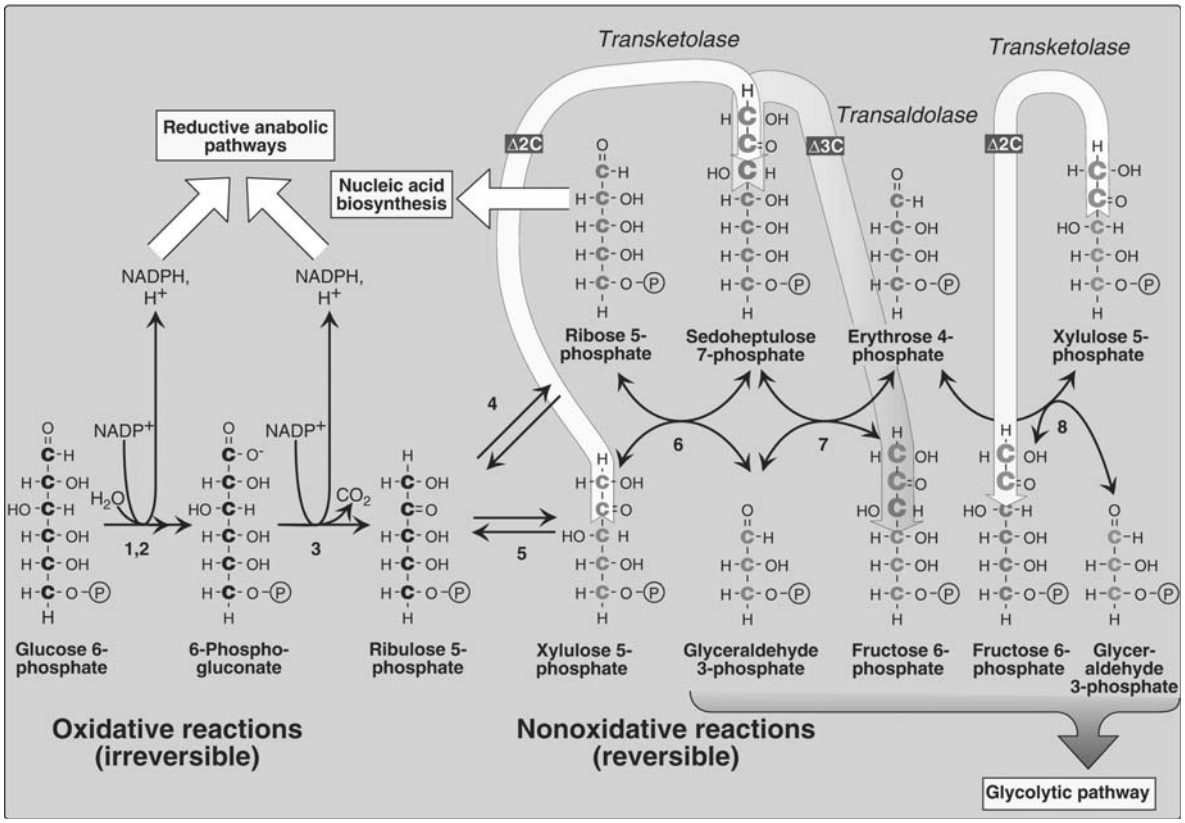

Figure 13.2 Reactions of the pentose phosphate pathway. Enzymes numbered above are: (1, 2) ***glucose 6-phosphate dehydrogenase*** and ***6-phosphogluconolactone hydrolase***, (3) ***6-phosphogluconate dehydrogenase***, (4) ***ribose 5-phosphate isomerase***, (5) ***phosphopentose epimerase***, (6, 8) ***transketolase*** (coenzyme: thiamine pyrophosphate), and (7) ***transaldolase***. Δ2C = two carbons are transferred from a ketose donor to an aldose acceptor in ***transketolase*** reactions; Δ3C = three carbons are transferred in the ***transaldolase*** reaction. This can be represented as: 5C sugar + 5C sugar 7C sugar + 3C sugar 4C sugar + 6C sugar. NADP(H) = nicotinamide adenine dinucleotide phosphate; = phosphate; CO_2 = carbon dioxide.

A. Glucose 6-phosphate dehydrogenation

Glucose 6-phosphate dehydrogenase (***G6PD***) catalyzes the oxidation of glucose 6-phosphate to 6-phosphogluconolactone as the coenzyme $NADP^+$ gets reduced to NADPH. This initial reaction is the committed, rate-limiting, and regulated step of the pathway. NADPH is a potent competitive inhibitor of ***G6PD***, and the ratio of NADPH/$NADP^+$ is sufficiently high to

substantially inhibit the enzyme under most metabolic conditions. However, with increased demand for NADPH, the ratio of NADPH/$NADP^+$ decreases, and flux through the pathway increases in response to the enhanced activity of ***G6PD***. [Note: Insulin upregulates expression of the gene for ***G6PD***, and flux through the pathway increases in the absorptive state (see p. 323).]

B. Ribulose 5-phosphate formation

6-Phosphogluconolactone is hydrolyzed by ***6-phosphogluconolactone hydrolase*** in the second step. The oxidative decarboxylation of the product, 6-phosphogluconate, is catalyzed by ***6-phosphogluconate dehydrogenase.*** This third irreversible step produces ribulose 5-phosphate (a pentose sugar–phosphate), CO_2 (from carbon 1 of glucose), and a second molecule of NADPH (see Fig. 13.2).

III. REVERSIBLE NONOXIDATIVE REACTIONS

The nonoxidative reactions of the pentose phosphate pathway occur in all cell types synthesizing nucleotides and nucleic acids. These reactions catalyze the interconversion of sugars containing three to seven carbons (see Fig. 13.2). These reversible reactions permit ribulose 5-phosphate (produced by the oxidative portion of the pathway) to be converted either to ribose 5-phosphate (needed for nucleotide synthesis; see p. 293) or to intermediates of glycolysis (that is, fructose 6-phosphate and glyceraldehyde 3-phosphate). For example, many cells that carry out reductive biosynthetic reactions have a greater need for NADPH than for ribose 5-phosphate. In this case, ***transketolase*** (which transfers two-carbon units in a thiamine pyrophosphate [TPP]-requiring reaction) and ***transaldolase*** (which transfers three-carbon units) convert the ribulose 5-phosphate produced as an end product of the oxidative phase to glyceraldehyde 3-phosphate and fructose 6-phosphate, which are glycolytic intermediates. In contrast, when the demand for ribose for nucleotides and nucleic acids is greater than the need for NADPH, the nonoxidative reactions can provide the ribose 5-phosphate from glyceraldehyde 3-phosphate and fructose 6-phosphate in the

absence of the oxidative steps (Fig. 13.3).

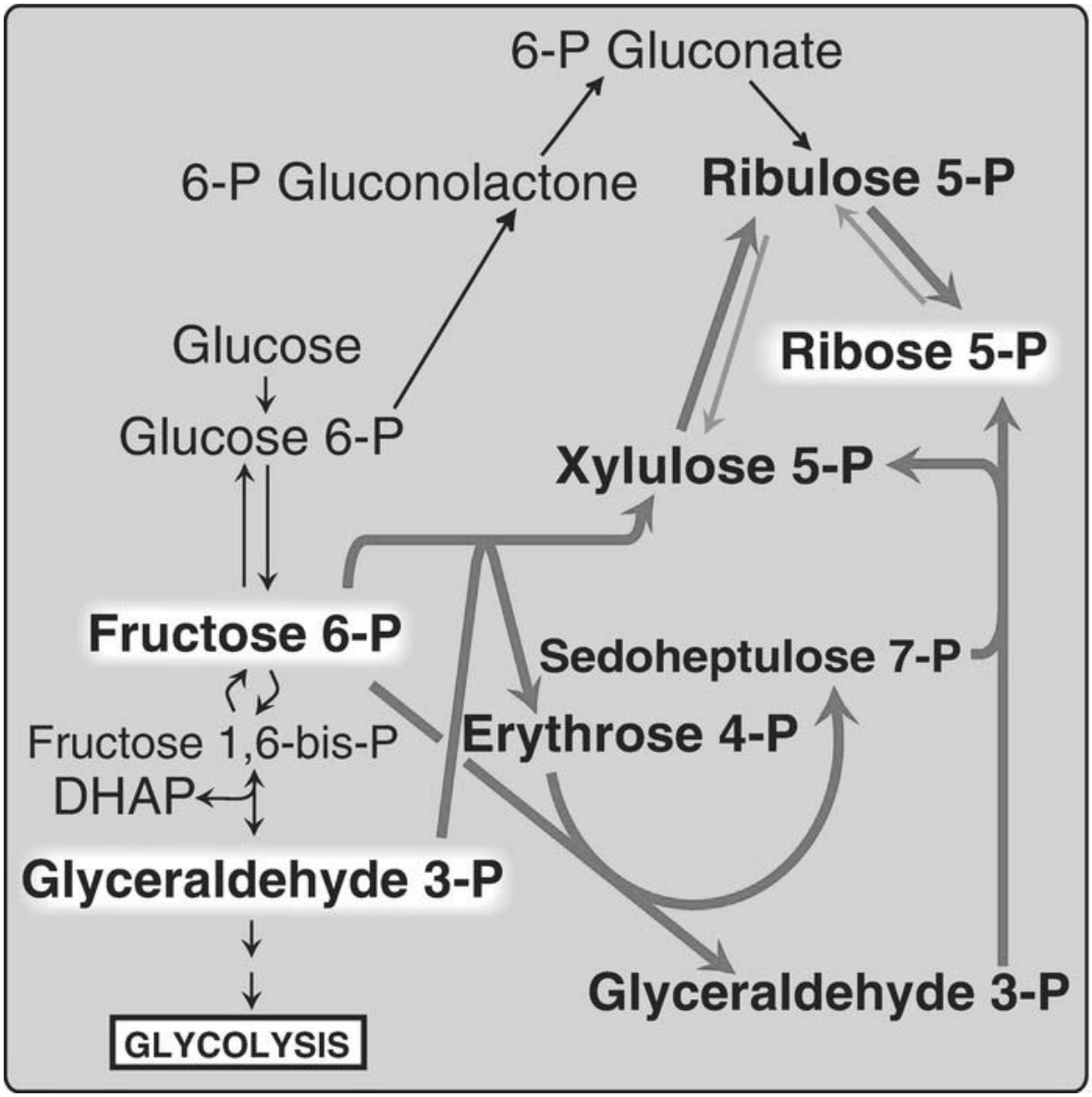

Figure 13.3 Formation of ribose 5-phosphate from intermediates of glycolysis. P = phosphate; DHAP = dihydroxyacetone phosphate.

In addition to ***transketolase***, TPP is required by the multienzyme complexes ***pyruvate dehydrogenase*** (see p. 110), ***α-ketoglutarate dehydrogenase*** of the tricarboxylic acid cycle (see p. 112), and ***branched-chain α-keto acid dehydrogenase*** of branched-chain amino acid catabolism (see p. 266).

IV. NADPH USES

The coenzyme NADPH differs from nicotinamide adenine dinucleotide (NADH) only by the presence of a phosphate group on one of the ribose units (Fig. 13.4). This seemingly small change in structure allows NADPH to interact with NADPH-specific enzymes that have unique roles in the cell. For example, in the cytosol of hepatocytes, the steady-state $NADP^+$/NADPH ratio is ~0.1, which favors the use of NADPH in reductive biosynthetic reactions. This contrasts with the high NAD^+/NADH ratio (~1,000), which favors an oxidative role for NAD^+. This section summarizes some important NADPH-specific functions in reductive biosynthesis and detoxification reactions.

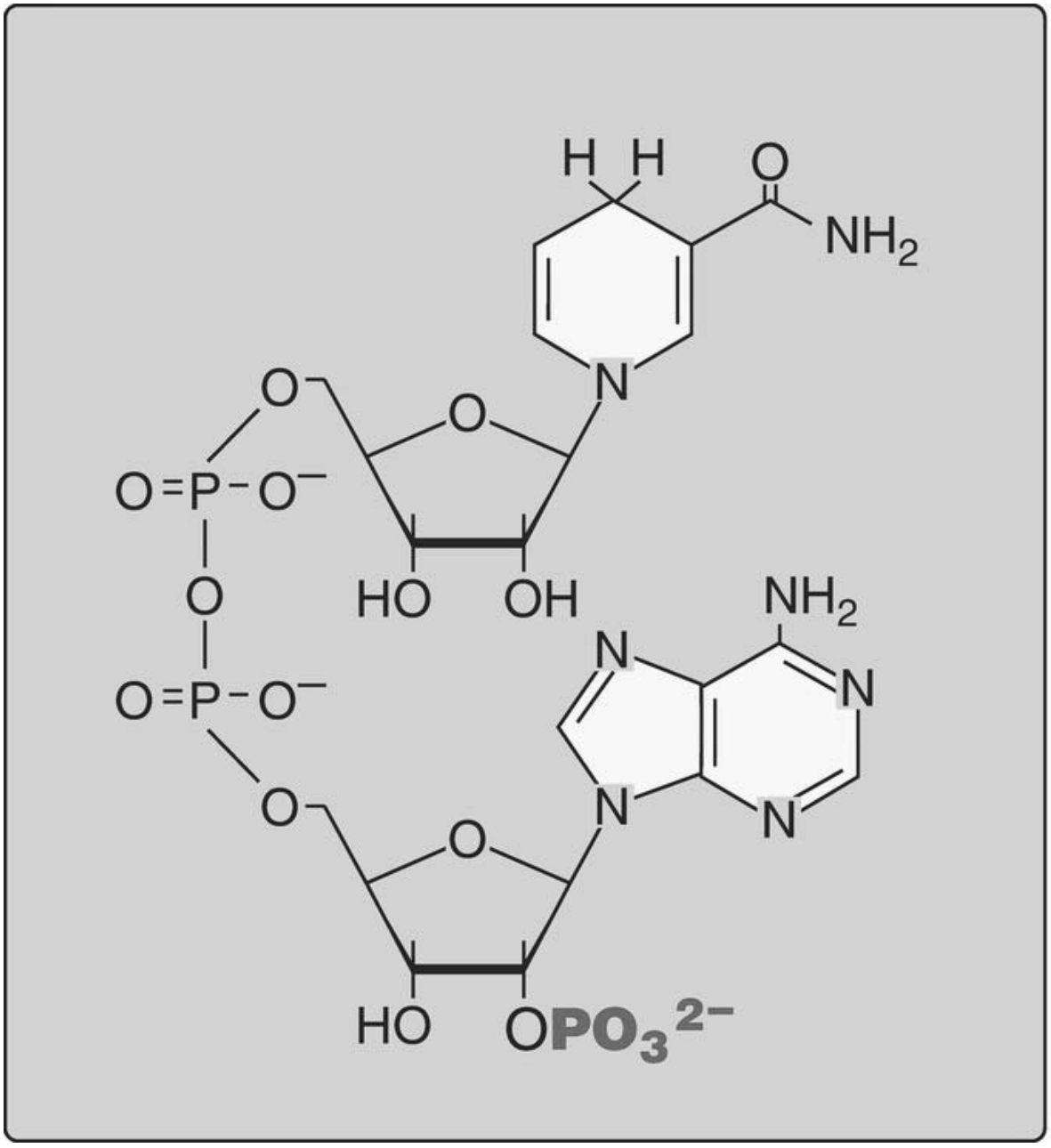

Figure 13.4 Structure of reduced nicotinamide adenine dinucleotide phosphate (NADPH).

A. Reductive biosynthesis

Like NADH, NADPH can be thought of as a high-energy molecule. However, the electrons of NADPH are used for reductive biosynthesis,

rather than for transfer to the electron transport chain as is seen with NADH (see p. 74). Thus, in the metabolic transformations of the pentose phosphate pathway, part of the energy of glucose 6-phosphate is conserved in NADPH, a molecule with a negative reduction potential (see p. 76), that, therefore, can be used in reactions requiring an electron donor, such as fatty acid (see p. 186), cholesterol (see p. 221), and steroid hormone (see p. 237) synthesis.

B. Hydrogen peroxide reduction

Hydrogen peroxide (H_2O_2) is one of a family of reactive oxygen species (ROS) that are formed from the partial reduction of molecular oxygen ([O_2], Fig. 13.5A). These compounds are formed continuously as byproducts of aerobic metabolism, through reactions with drugs and environmental toxins, or when the level of antioxidants is diminished, all creating the condition of oxidative stress. These highly reactive oxygen intermediates can cause serious chemical damage to DNA, proteins, and unsaturated lipids and can lead to cell death. ROS have been implicated in a number of pathologic processes, including reperfusion injury, cancer, inflammatory disease, and aging. The cell has several protective mechanisms that minimize the toxic potential of these compounds. [Note: ROS can also be generated in the killing of microbes by white blood cells (WBC; see D. below).]

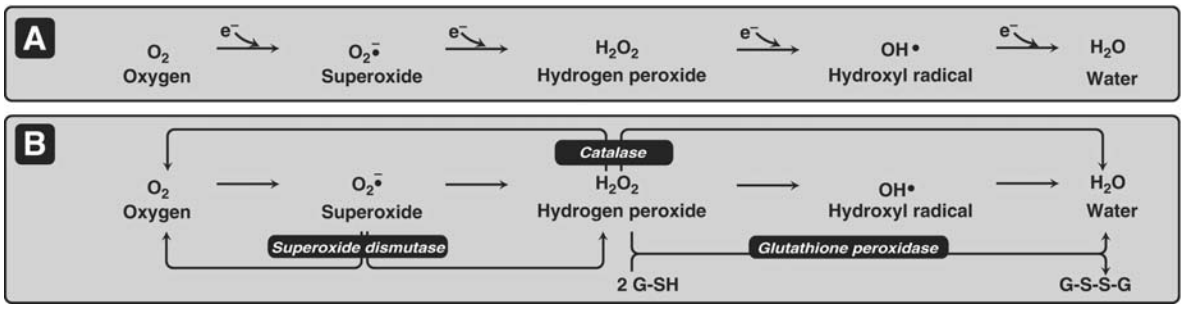

Figure 13.5 A. Formation of reactive intermediates from oxygen. e^- = electrons. B. Actions of antioxidant enzymes. G-SH = reduced glutathione; G-S-S-G = oxidized glutathione. [Note: See Fig. 13.6B for the regeneration of G-SH.]

1. Enzymes that catalyze antioxidant reactions Reduced glutathione (G-

SH), a tripeptide-thiol (γ-glutamylcysteinylglycine) present in most cells, can chemically detoxify H_2O_2 (Fig. 13.5B). This reaction, catalyzed by the selenoprotein (see p. 407) ***glutathione peroxidase***, forms oxidized glutathione (G-S-S-G), which no longer has protective properties. The cell regenerates G-SH in a reaction catalyzed by ***glutathione reductase,*** using NADPH as a source of reducing equivalents. Thus, NADPH indirectly provides electrons for the reduction of H_2O_2 (Fig. 13.6). Additional enzymes, such as ***superoxide dismutase*** and ***catalase***, catalyze the conversion of other ROS to harmless products (see Fig. 13.5B). As a group, these enzymes serve as a defense system to guard against the toxic effects of ROS.

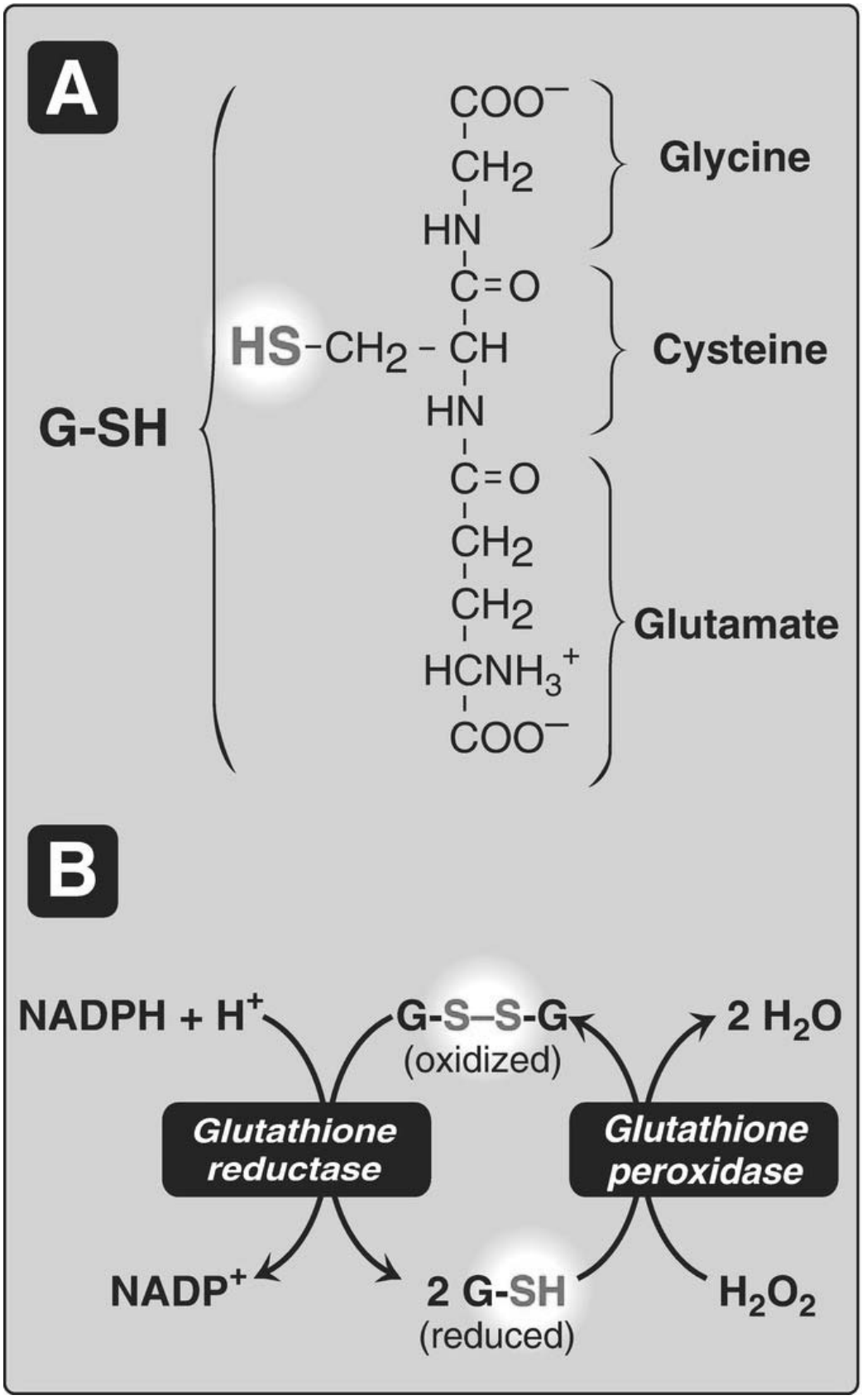
A
G-SH
COO⁻
CH2
HN
Glycine
C=O
HS-CH2-CH
HN
Cysteine
C=O
CH2
CH2
HCNH3+
COO⁻
Glutamate
B
NADPH + H+
G-S–S-G
(oxidized)
2 H2O
Glutathione reductase
Glutathione peroxidase
NADP+
2 G-SH
(reduced)
H2O2

Figure 13.6 A. Structure of reduced glutathione (G-SH). [Note: Glutamate is linked to cysteine through a γ-carboxyl, rather than an α-carboxyl.] B.The roles of G-SH and reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the reduction of hydrogen peroxide (H_2O_2) to water. G-S-S-G = oxidized glutathione.

2. Antioxidant chemicals A number of intracellular reducing agents, such as ascorbate (see p. 381), vitamin E (see p. 395), and β-carotene (see p. 386), are able to reduce and, thereby, detoxify ROS in the laboratory. Consumption of foods rich in these antioxidant compounds has been correlated with a reduced risk for certain types of cancers as well as decreased frequency of certain other chronic health problems. Therefore, it is tempting to speculate that the effects of these compounds are, in part, an expression of their ability to quench the toxic effect of ROS. However, clinical trials with antioxidants as dietary supplements have failed to show clear beneficial effects. In the case of dietary supplementation with β-carotene, the rate of lung cancer in smokers increased rather than decreased. Thus, the health-promoting effects of dietary fruits and vegetables likely reflect a complex interaction among many naturally occurring compounds, which has not been duplicated by consumption of isolated antioxidant compounds.

C. Cytochrome P450 monooxygenase system

Monooxygenases (***mixed-function oxidases***) incorporate one atom from O_2 into a substrate (creating a hydroxyl group), with the other atom being reduced to water (H_2O). In the ***cytochrome P450*** (***CYP***) ***monooxygenase*** system, NADPH provides the reducing equivalents required by this series of reactions (Fig. 13.7). This system performs different functions in two separate locations in cells. The overall reaction catalyzed by a ***CYP*** enzyme is

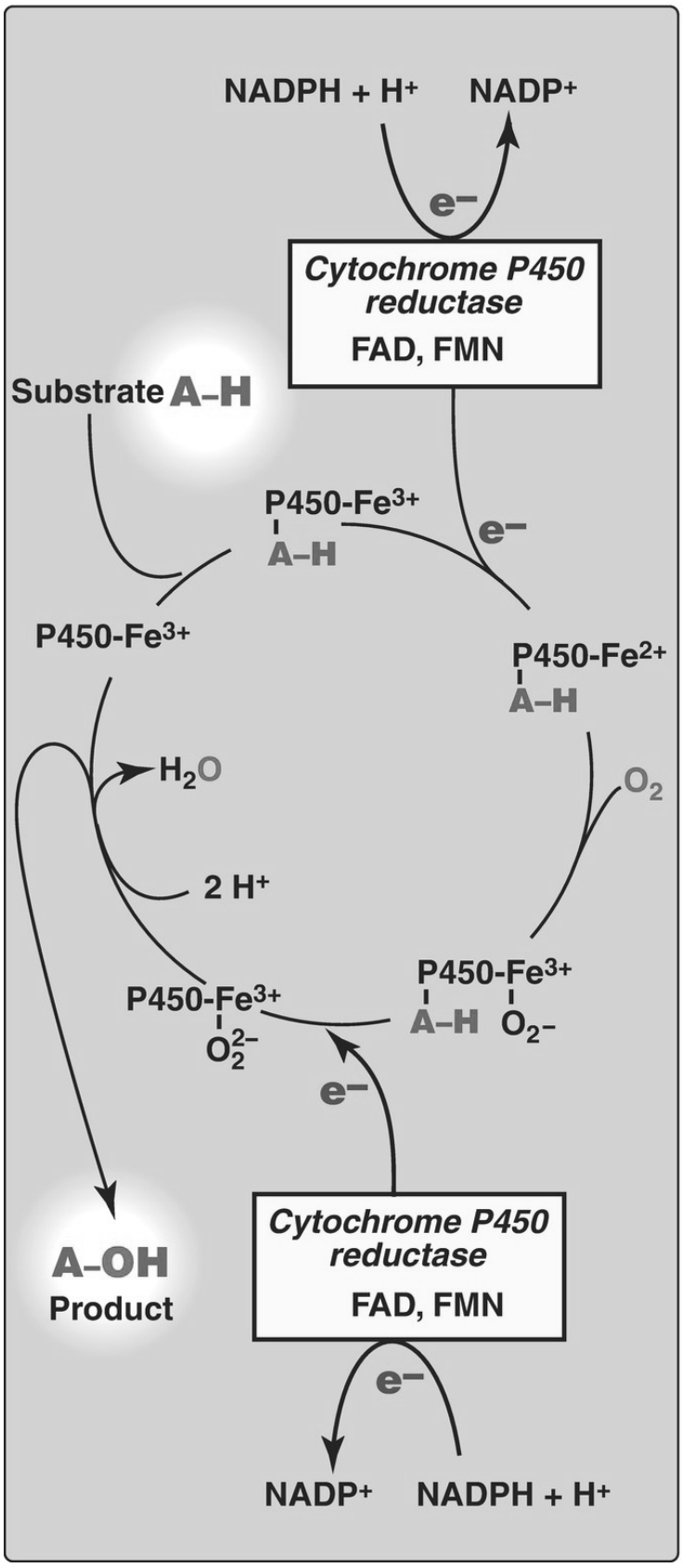
NADPH + H+
NADP+
e−
Cytochrome P450 reductase
FAD, FMN
Substrate A–H
P450-Fe3+
A–H
e−
P450-Fe3+
P450-Fe2+
A–H
H2O
O2
2 H+
P450-Fe3+
O2 2−
P450-Fe3+
A–H O2−
e−
A–OH
Product
Cytochrome P450 reductase
FAD, FMN
e−
NADP+
NADPH + H+

Figure 13.7 ***Cytochrome P450*** (***CYP***) ***monooxygenase*** catalytic cycle (simplified). Electrons (e^-) move from nicotinamide adenine dinucleotide phosphate (NADPH) to flavin adenine dinucleotide (FAD) to flavin adenine mononucleotide (FMN) of the ***reductase*** and then to the heme iron (Fe) of the microsomal ***CYP*** enzyme. [Note: In the mitochondrial system, e^- move from FAD to an iron-sulfur protein and then to the ***CYP*** enzyme.]

$$R{-}H + O_2 + NADPH + H^+ \rightarrow R{-}OH + H_2O + NADP^+$$

where R may be a steroid, drug, or other chemical. [Note: ***CYP*** enzymes are actually a superfamily of related, heme-containing ***monooxygenases*** that participate in a broad variety of reactions. The P450 in the name reflects the absorbance at 450 nm by the protein.]

1. Mitochondrial system An important function of the ***CYP monooxygenase*** system found associated with the inner mitochondrial membrane is the biosynthesis of steroid hormones. In steroidogenic tissues, such as the placenta, ovaries, testes, and adrenal cortex, it is used to hydroxylate intermediates in the conversion of cholesterol to steroid hormones, a process that makes these hydrophobic compounds more water soluble (see p. 237). The liver uses this same system in bile acid synthesis (see p. 224) and the hydroxylation of cholecalciferol to 25-hydroxycholecalciferol ([vitamin D_3] see p. 390), and the kidney uses it to hydroxylate vitamin D_3 to its biologically active 1,25-dihydroxylated form.
2. Microsomal system The microsomal ***CYP monooxygenase*** system found associated with the membrane of the smooth endoplasmic reticulum (particularly in the liver) functions primarily in the detoxification of foreign compounds (xenobiotics). These include numerous drugs and such varied pollutants as petroleum products and pesticides. ***CYP*** enzymes of the microsomal system (for example, ***CYP3A4***) can be used to hydroxylate these toxins (phase I). The purpose of these modifications is two-fold. First, it may itself activate or inactivate a drug and second, make a toxic compound more soluble, thereby facilitating its excretion in the urine or feces. Frequently, however, the new hydroxyl group will serve as a site for conjugation with a polar molecule, such as glucuronic acid (see p. 161), which will significantly increase the compound's solubility (phase II). [Note: Polymorphisms (see p. 491) in the genes for

CYP enzymes can lead to differences in drug metabolism.]

D. White blood cell phagocytosis and microbe killing

Phagocytosis is the ingestion by receptor-mediated endocytosis of microorganisms, foreign particles, and cellular debris by WBC (leukocytes) such as neutrophils and macrophages (monocytes). It is an important defense mechanism, particularly in bacterial infections. Neutrophils and monocytes are armed with both oxygen-independent and oxygen-dependent mechanisms for killing bacteria.

1. Oxygen-independent Oxygen-independent mechanisms use pH changes in phagolysosomes and lysosomal enzymes to destroy pathogens.
2. Oxygen-dependent Oxygen-dependent mechanisms include the enzymes ***NADPH oxidase*** and ***myeloperoxidase*** (***MPO***) that work together in killing bacteria (Fig. 13.8). Overall, the ***MPO*** system is the most potent of the bactericidal mechanisms. An invading bacterium is recognized by the immune system and attacked by antibodies that bind it to a receptor on a phagocytic cell. After internalization of the microorganism has occurred, ***NADPH oxidase***, located in the leukocyte cell membrane, is activated and reduces O_2 from the surrounding tissue to superoxide ($O_2^{-\bullet}$), a free radical ROS, as NADPH is oxidized. The rapid consumption of O_2 that accompanies formation of $O_2^{-\bullet}$ is referred to as the respiratory burst. [Note: Active ***NADPH oxidase*** is a membrane-associated complex containing a flavocytochrome plus additional peptides that translocate from the cytoplasm upon activation of the leukocyte. Electrons move from NADPH to O_2 via flavin adenine nucleotide (FAD) and heme, generating $O_2^{-\bullet}$. Rare genetic deficiencies in ***NADPH oxidase*** cause chronic granulomatous disease (CGD) characterized by severe, persistent infections and the formation of granulomas (nodular areas of inflammation) that sequester the bacteria that were not destroyed.] Next, $O_2^{-\bullet}$ is converted to H_2O_2 (also a ROS), either spontaneously or catalyzed by ***superoxide dismutase***. In the presence of ***MPO***, a heme-containing lysosomal enzyme present within the phagolysosome, peroxide plus chloride ions are converted to hypochlorous acid ([HOCl] the major component of household bleach), which kills the bacteria. The peroxide can also be partially reduced to the hydroxyl radical (OH•), a

ROS, or be fully reduced to H_2O by ***catalase*** or ***glutathione peroxidase***. [Note: Deficiencies in ***MPO*** do not confer increased susceptibility to infection because peroxide from ***NADPH oxidase*** is bactericidal.]

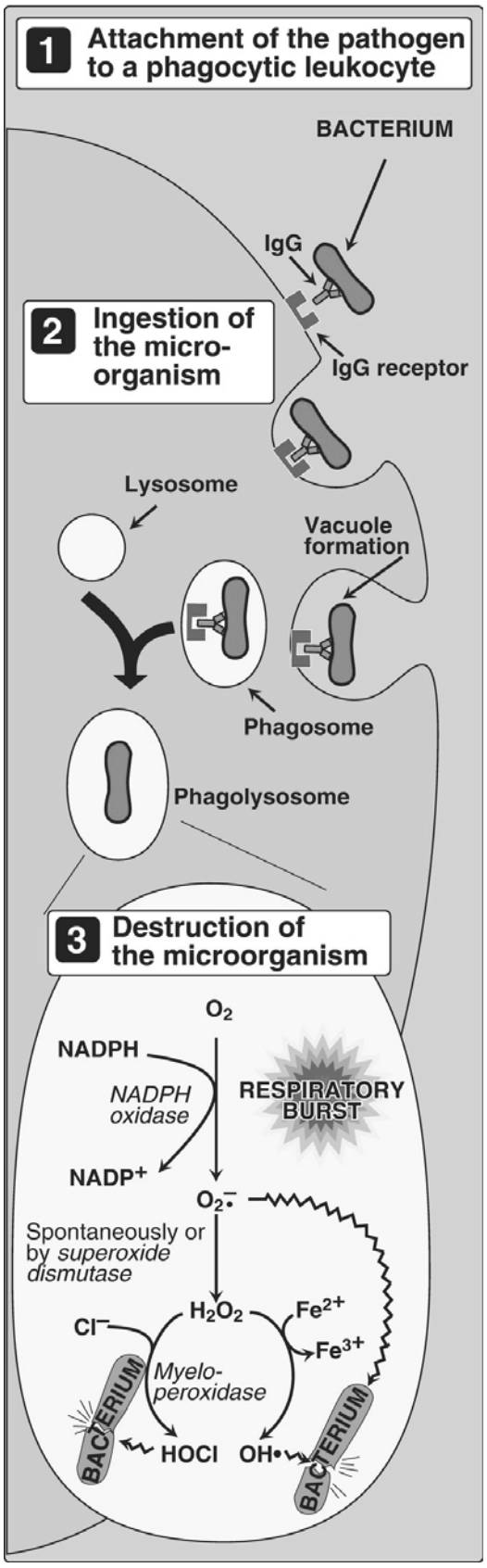
1 Attachment of the pathogen to a phagocytic leukocyte
BACTERIUM
IgG
IgG receptor
2 Ingestion of the micro-organism
Lysosome
Vacuole formation
Phagosome
Phagolysosome
3 Destruction of the microorganism
O2
NADPH
NADPH oxidase
RESPIRATORY BURST
NADP+
O2•−
Spontaneously or by superoxide dismutase
H2O2
Fe2+
Fe3+
Cl−
Myelo-peroxidase
BACTERIUM
HOCl
OH•
BACTERIUM

Figure 13.8 Phagocytosis and the oxygen (O_2)-dependent pathway of microbial killing. IgG = immunoglobulin G; NADP(H) = nicotinamide adenine dinucleotide phosphate; $O_2^{-\bullet}$ = superoxide; H_2O_2 = hydrogen peroxide; HOCl = hypochlorous acid; OH• = hydroxyl radical.

E. Nitric oxide synthesis

Nitric oxide (NO) is recognized as a mediator in a broad array of biologic systems. NO is the endothelium-derived relaxing factor that causes vasodilation by relaxing vascular smooth muscle. It also acts as a neurotransmitter, prevents platelet aggregation, and plays an essential role in macrophage function. It has a very short half-life in tissues (3–10 seconds) because it reacts with O_2 and $O_2^{-\bullet}$ and is converted into nitrates and nitrites including peroxynitrite ($O{=}NOO^-$), a reactive nitrogen species (RNS). [Note: NO is a free radical gas that is often confused with nitrous oxide (N_2O), the "laughing gas" that is used as an anesthetic and is chemically stable.]

1. Nitric oxide synthase Arginine, O_2, and NADPH are substrates for cytosolic ***NO synthase*** ([***NOS***], Fig. 13.9). Flavin mononucleotide (FMN), FAD, heme, and tetrahydrobiopterin (see p. 268) are coenzymes, and NO and citrulline are products of the reaction. Three ***NOS*** isozymes, each the product of a different gene, have been identified. Two are constitutive (synthesized at a constant rate), calcium (Ca^{2+})–calmodulin (CaM)-dependent enzymes (see p. 133). They are found primarily in endothelium (***eNOS***) and neural tissue (***nNOS***) and constantly produce very low levels of NO for vasodilation and neurotransmission. An inducible, Ca^{2+}-independent enzyme (***iNOS***) can be expressed in many cells, including macrophages and neutrophils, as an early defense against pathogens. The specific inducers for ***iNOS*** vary with cell type and include proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), and bacterial endotoxins such as lipopolysaccharide (LPS). These compounds promote synthesis of ***iNOS***, which can result in large amounts of NO being produced over hours or even days.

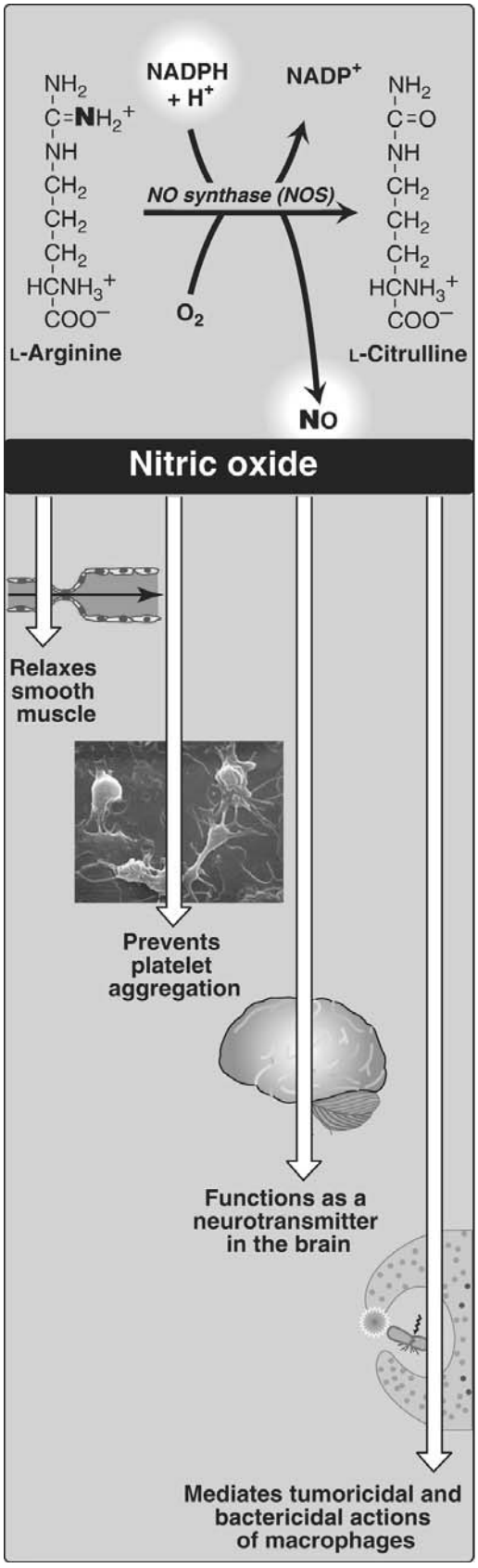
NADPH
+ H+
NADP+
NO synthase (NOS)
O2
L-Arginine
L-Citrulline
NO
Nitric oxide
Relaxes
smooth
muscle
Prevents
platelet
aggregation
Functions as a
neurotransmitter
in the brain
Mediates tumoricidal and
bactericidal actions
of macrophages

Figure 13.9 Synthesis and some actions of nitric oxide (NO). [Note: Flavin mononucleotide, flavin adenine dinucleotide, heme, and tetrahydrobiopterin are additional coenzymes required by ***NOS***.] NADP(H) = nicotinamideadenine dinucleotide phosphate.

2. Nitric oxide and vascular endothelium NO is an important mediator in the control of vascular smooth muscle tone. NO is synthesized by ***eNOS*** in endothelial cells and diffuses to vascular smooth muscle, where it activates the cytosolic form of ***guanylyl cyclase*** (or, ***guanylate cyclase***) to form cyclic guanosine monophosphate (cGMP). [Note: This reaction is analogous to the formation of cyclic adenosine monophosphate (cAMP) by ***adenylyl cyclase*** (see p. 95).] The resultant rise in cGMP causes activation of ***protein kinase G***, which phosphorylates Ca^{2+} channels, causing decreased entry of Ca^{2+} into smooth muscle cells. This decreases the Ca^{2+}–CaM activation of ***myosin light-chain kinase***, thereby decreasing smooth muscle contraction and favoring relaxation. Vasodilator nitrates, such as nitroglycerin, are metabolized to NO, which causes relaxation of vascular smooth muscle and, therefore, lowers blood pressure. Thus, NO can be envisioned as an endogenous nitrovasodilator. [Note: Under hypoxic conditions, nitrite (NO_2^-) can be reduced to NO, which binds to deoxyhemoglobin. The NO is released into the blood, causing vasodilation and increasing blood flow.]
3. Nitric oxide and macrophage bactericidal activity In macrophages, ***iNOS*** activity is normally low, but synthesis of the enzyme is significantly stimulated by bacterial LPS and by release of IFN-γ and TNF-α in response to infection. Activated macrophages form $O_2^{\bullet-}$ radicals that combine with NO to form intermediates that decompose, producing the highly bactericidal OH• radical.
4. Additional functions NO is a potent inhibitor of platelet adhesion and aggregation (by activating the cGMP pathway). It is also characterized as a neurotransmitter in the central and peripheral nervous systems.

V. G6PD DEFICIENCY

G6PD deficiency is a hereditary condition characterized by hemolytic anemia

caused by the inability to detoxify oxidizing agents. ***G6PD*** deficiency is the most common disease-producing enzyme abnormality in humans, affecting >400 million individuals worldwide. This deficiency has the highest prevalence in the Middle East, tropical Africa and Asia, and parts of the Mediterranean. ***G6PD*** deficiency is X linked and is, in fact, a family of deficiencies caused by a number of different mutations in the gene encoding ***G6PD***. Only some of the resulting protein variants cause clinical symptoms. [Note: In addition to hemolytic anemia, a clinical manifestation of ***G6PD*** deficiency is neonatal jaundice appearing 1–4 days after birth. The jaundice, which may be severe, typically results from increased production of unconjugated bilirubin (see p. 285).] The life span of individuals with a severe form of ***G6PD*** deficiency may be somewhat shortened as a result of complications arising from chronic hemolysis. This negative effect of ***G6PD*** deficiency has been balanced in evolution by an advantage in survival, an increased resistance to Plasmodium falciparum malaria. [Note: Sickle cell trait and the thalassemias also confer resistance to malaria.]

A. G6PD role in red blood cells

Diminished ***G6PD*** activity impairs the ability of the cell to form the NADPH that is essential for the maintenance of the G-SH pool. This results in a decrease in the detoxification of free radicals and peroxides formed within the cell (Fig. 13.10). G-SH also helps maintain the reduced states of sulfhydryl groups in proteins, including hemoglobin. Oxidation of those sulfhydryl groups leads to the formation of denatured proteins that form insoluble masses (called Heinz bodies) that attach to RBC membranes (Fig. 13.11). Additional oxidation of membrane proteins causes RBC to be rigid (less deformable), and they are removed from the circulation by macrophages in the spleen and liver. Although ***G6PD*** deficiency occurs in all cells of the affected individual, it is most severe in RBC, where the pentose phosphate pathway provides the only means of generating NADPH. Additionally, the RBC has no nucleus or ribosomes and cannot renew its supply of the enzyme. Thus, RBC are particularly vulnerable to enzyme variants with diminished stability. [Note: Other tissues have an alternative source of NADPH (***$NADP^+$-dependent malate dehydrogenase*** [***malic enzyme***]; see p. 186) that can keep G-SH reduced.]

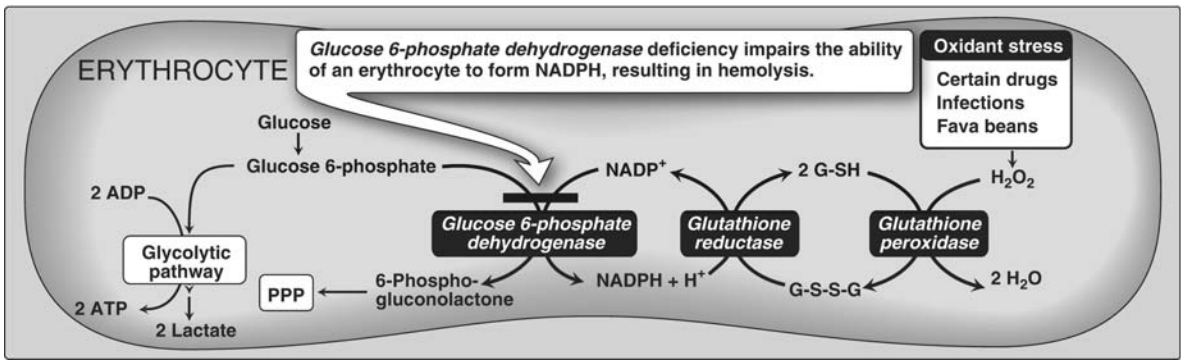

Figure 13.10 Pathways of glucose 6-phosphate metabolism in the erythrocyte. NADP(H) = nicotinamide adenine dinucleotide phosphate; G-SH = reduced glutathione; G-S-S-G = oxidized glutathione; H_2O_2 = hydrogen peroxide; PPP = pentose phosphate pathway.

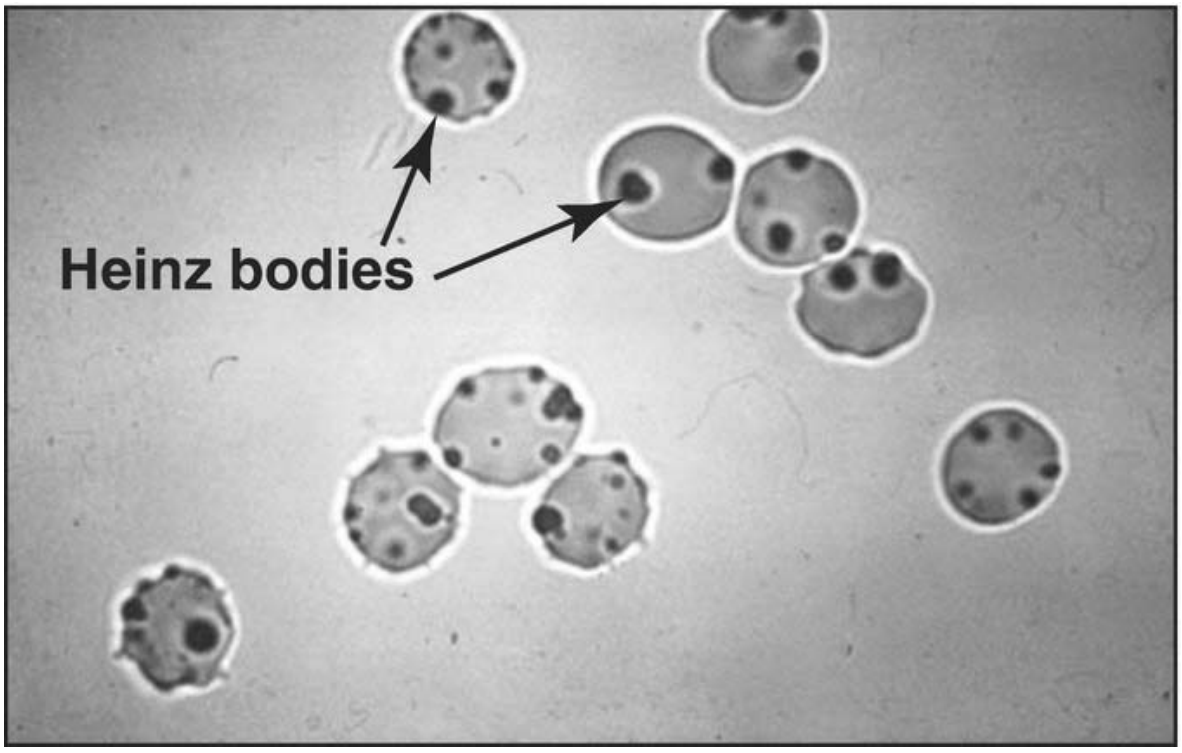

Figure 13.11 Heinz bodies in the erythrocytes of a patient with ***glucose 6-phosphate dehydrogenase*** deficiency.

B. Precipitating factors in G6PD deficiency

Most individuals who have inherited one of the ***G6PD*** mutations do not show clinical manifestations (that is, they are asymptomatic). However, some patients with ***G6PD*** deficiency develop hemolytic anemia if they are treated with an oxidant drug, ingest fava beans, or contract a severe infection.

1. Oxidant drugs Commonly used drugs that produce hemolytic anemia in patients with ***G6PD*** deficiency are best remembered from the mnemonic AAA: antibiotics (for example, sulfamethoxazole and chloramphenicol), antimalarials (for example, primaquine but not chloroquine or quinine), and antipyretics (for example, acetanilide but not acetaminophen).
2. Favism Some forms of ***G6PD*** deficiency, for example, the Mediterranean variant, are particularly susceptible to the hemolytic effect of the fava (broad) bean, a dietary staple in the Mediterranean region. Favism, the hemolytic effect of ingesting fava beans, is not observed in all individuals with ***G6PD*** deficiency, but all patients with favism have ***G6PD*** deficiency.
3. Infection Infection is the most common precipitating factor of hemolysis in ***G6PD*** deficiency. The inflammatory response to infection results in the generation of free radicals in macrophages. The radicals can diffuse into the RBC and cause oxidative damage.

C. Variant G6PD properties

Almost all ***G6PD*** variants are caused by point mutations (see p. 449) in the gene for ***G6PD***. Some mutations do not affect enzymic activity. However, other mutations result in decreased catalytic activity, decreased stability, or an alteration of binding affinity for $NADP^+$ or glucose 6-phosphate. [Note: Active ***G6PD*** exists as a homodimer or tetramer. Mutations at the interface between subunits can affect stability.] The severity of the disease usually correlates with the amount of residual enzyme activity in the patient's RBC. For example, variants can be classified as shown in Figure 13.12. ***G6PD A***$^-$ is the prototype of the moderate (class III) form of the disease. The RBC contain an unstable but kinetically normal ***G6PD***, with most of the enzyme activity present in the reticulocytes and younger RBC (Fig. 13.13). Therefore, the oldest RBC have the lowest level of enzyme activity and are preferentially removed in a hemolytic episode. Because hemolysis does not affect younger cells, the episodes are self-limiting. ***G6PD Mediterranean*** is

the prototype of a more severe (class II) deficiency. Class I mutations (rare) are the most severe and are associated with chronic nonspherocytic hemolytic anemia, which occurs even in the absence of oxidative stress.

Class	Clinical symptoms	Residual enzyme activity
I	Very severe (chronic, nonspher-ocytic hemolytic anemia)	<10%
*II	Severe (acute hemolytic anemia)	<10%
*III	Moderate	10%–60%
IV	None	>60%

Figure 13.12 Classification of ***glucose 6-phosphate dehydrogenase*** (***G6PD***) deficiency variants. [Note: Class V variants (not shown) result in overproduction of ***G6PD***.] * = most common.

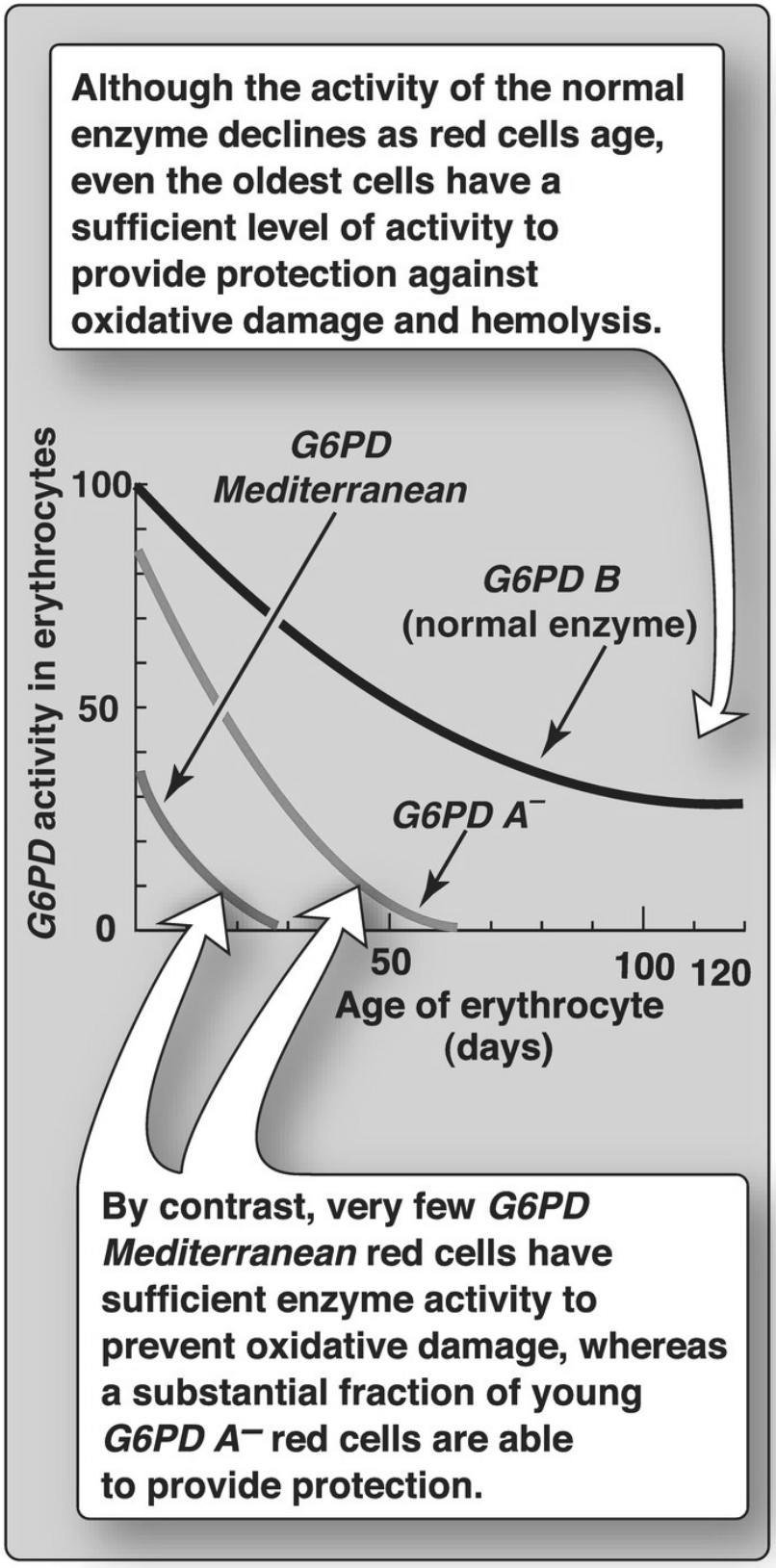
Although the activity of the normal enzyme declines as red cells age, even the oldest cells have a sufficient level of activity to provide protection against oxidative damage and hemolysis.
G6PD activity in erythrocytes
100
50
0
G6PD Mediterranean
G6PD B (normal enzyme)
G6PD A⁻
50
100
120
Age of erythrocyte (days)
By contrast, very few G6PD Mediterranean red cells have sufficient enzyme activity to prevent oxidative damage, whereas a substantial fraction of young G6PD A⁻ red cells are able to provide protection.

Figure 13.13 Decline of erythrocyte ***glucose 6-phosphate dehydrogenase*** (***G6PD***) activity with cell age for the three most commonly encountered forms of the enzyme.

D. G6PD molecular biology

The cloning of the gene for ***G6PD*** and the sequencing of its DNA (see Chapter 34) have permitted the identification of mutations that cause ***G6PD*** deficiency. More than 400 ***G6PD*** variants have been identified, a finding that explains the numerous biochemical and clinical phenotypes that have been described. Most mutations that result in enzymic deficiency are missense mutations (see p. 449) in the coding region. Both ***G6PD A***$^{-}$ and ***G6PD Mediterranean*** represent mutant enzymes that differ from the respective normal variants by a single amino acid. Large deletions or frameshift mutations have not been identified, suggesting that complete absence of ***G6PD*** activity is likely lethal.

VI. CHAPTER SUMMARY

The pentose phosphate pathway includes an irreversible oxidative phase followed by a series of reversible sugar–phosphate interconversions (Fig. 13.14). No ATP is directly consumed or produced in the pathway. The reduced nicotinamide adenine dinucleotide phosphate (NADPH)-producing oxidative portion of the pathway is important in providing reducing equivalents for reductive biosynthesis and detoxification reactions. In this part of the pathway, glucose 6-phosphate is irreversibly converted to ribulose 5-phosphate, and two NADPH are produced. The regulated step is catalyzed by ***glucose 6-phosphate dehydrogenase*** (***G6PD***), which is strongly inhibited by a rise in the NADPH/$NADP^+$ ratio. Reversible nonoxidative reactions interconvert sugars. This part of the pathway converts ribulose 5-phosphate to ribose 5-phosphate, required for nucleotide and nucleic acid synthesis, or to fructose 6-phosphate and glyceraldehyde 3-phosphate (glycolytic intermediates). NADPH is a source of reducing equivalents in reductive biosynthesis, such as the production of fatty acids in liver, adipose tissue, and the mammary gland; cholesterol in the liver; and steroid hormones in the placenta, ovaries, testes, and adrenal cortex. It is also required by red blood cells (RBC) for hydrogen peroxide reduction. Reduced glutathione (G-SH) is used by ***glutathione peroxidase*** to reduce the peroxide to water. The oxidized glutathione (G-S-S-G) produced is reduced by ***glutathione reductase***, using NADPH as the source of electrons. NADPH provides reducing equivalents for the mitochondrial ***cytochrome P450 monooxygenase*** system, which is used in steroid hormone synthesis in steroidogenic tissue, bile acid synthesis in the liver, and vitamin D activation in the liver and kidneys. The microsomal system uses NADPH to detoxify foreign compounds (xenobiotics), such as drugs and a variety of pollutants. NADPH provides the reducing equivalents for phagocytes in the process of eliminating invading microorganisms. ***NADPH oxidase*** uses molecular oxygen (O_2) and electrons from NADPH to produce superoxide radicals, which, in turn, can be converted to peroxide by ***superoxide dismutase***. ***Myeloperoxidase*** catalyzes the formation of bactericidal hypochlorous acid from peroxide and chloride ions. Rare genetic defects in ***NADPH oxidase*** cause chronic granulomatous disease

characterized by severe, persistent, infections and granuloma formation. NADPH is required for the synthesis of nitric oxide (NO), an important free radical gas that causes vasodilation by relaxing vascular smooth muscle, acts as a neurotransmitter, prevents platelet aggregation, and helps mediate macrophage bactericidal activity. NO is made from arginine and O_2 by three different NADPH-dependent ***NO synthases*** (***NOS***). The endothelial (***eNOS***) and neuronal (***nNOS***) isozymes constantly produce very low levels of NO for vasodilation and neurotransmission, respectively. The inducible isozyme (***iNOS***) produces large amounts of NO for defense against pathogens. ***G6PD*** deficiency impairs the ability of the cell to form the NADPH that is essential for the maintenance of the G-SH pool. The cells most affected are RBC because they do not have additional sources of NADPH. ***G6PD*** deficiency is an X-linked disease characterized by hemolytic anemia caused by the production of free radicals and peroxides following administration of oxidant drugs, ingestion of fava beans, or severe infections. The extent of the anemia depends on the amount of residual enzyme. Class I variants, the most severe (and least common), are associated with chronic nonspherocytic hemolytic anemia. Babies with ***G6PD*** deficiency may experience neonatal jaundice.

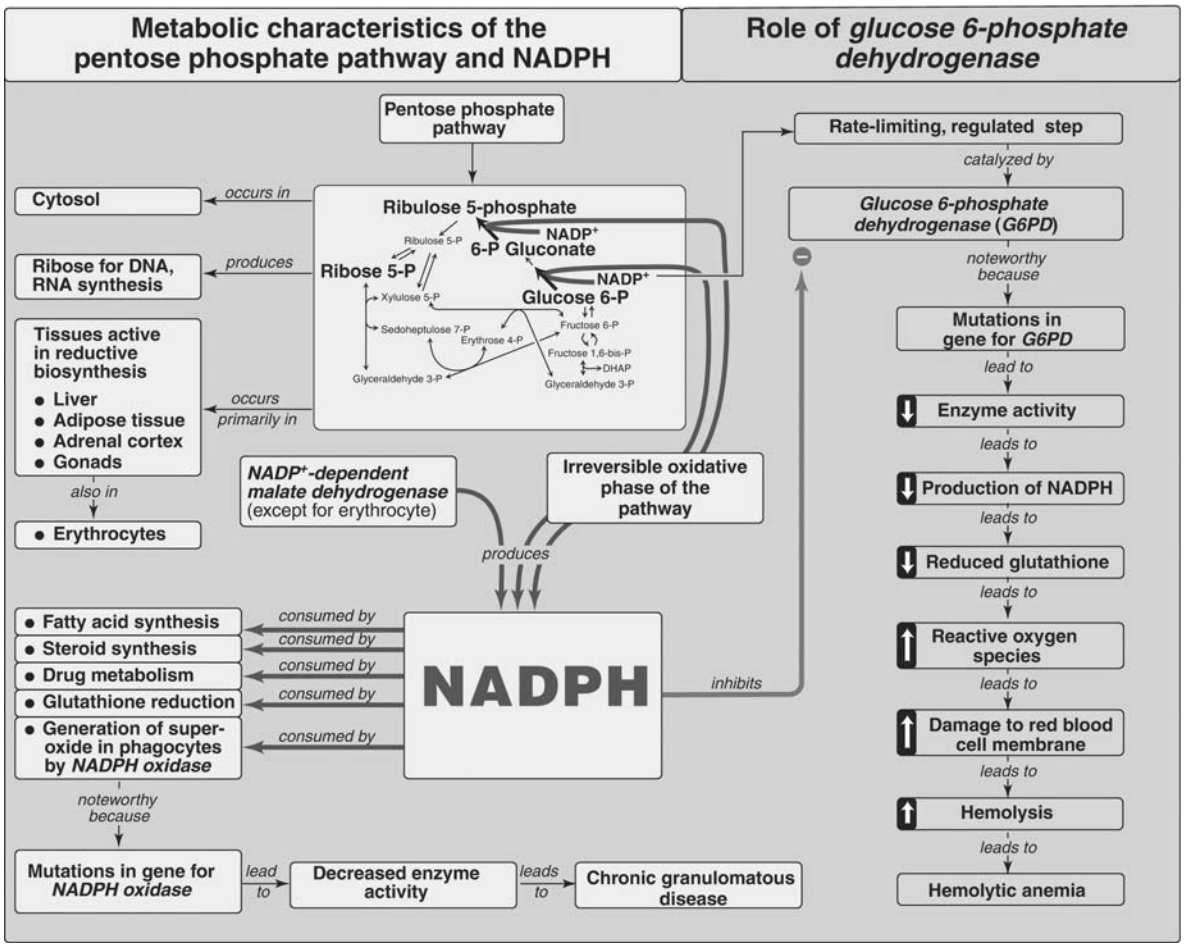

Figure 13.14 Key concept map for the pentose phosphate pathway and nicotinamide adenine dinucleotide phosphate (NADPH).

Study Questions

Choose the ONE best answer.

3.1. In preparation for a trip to an area of India where chloroquine-resistant malaria is endemic, a young man is given primaquine prophylactically. Soon thereafter, he develops a hemolytic condition due to a deficiency in glucose 6-phosphate dehydrogenase. A less-than-normal level of which of the following is a consequence of the enzyme deficiency and the underlying cause of the hemolysis?

A. Glucose 6-phosphate