13: Pentose Phosphate Pathway and Nicotinamide Adenine Dinucleotide Phosphate

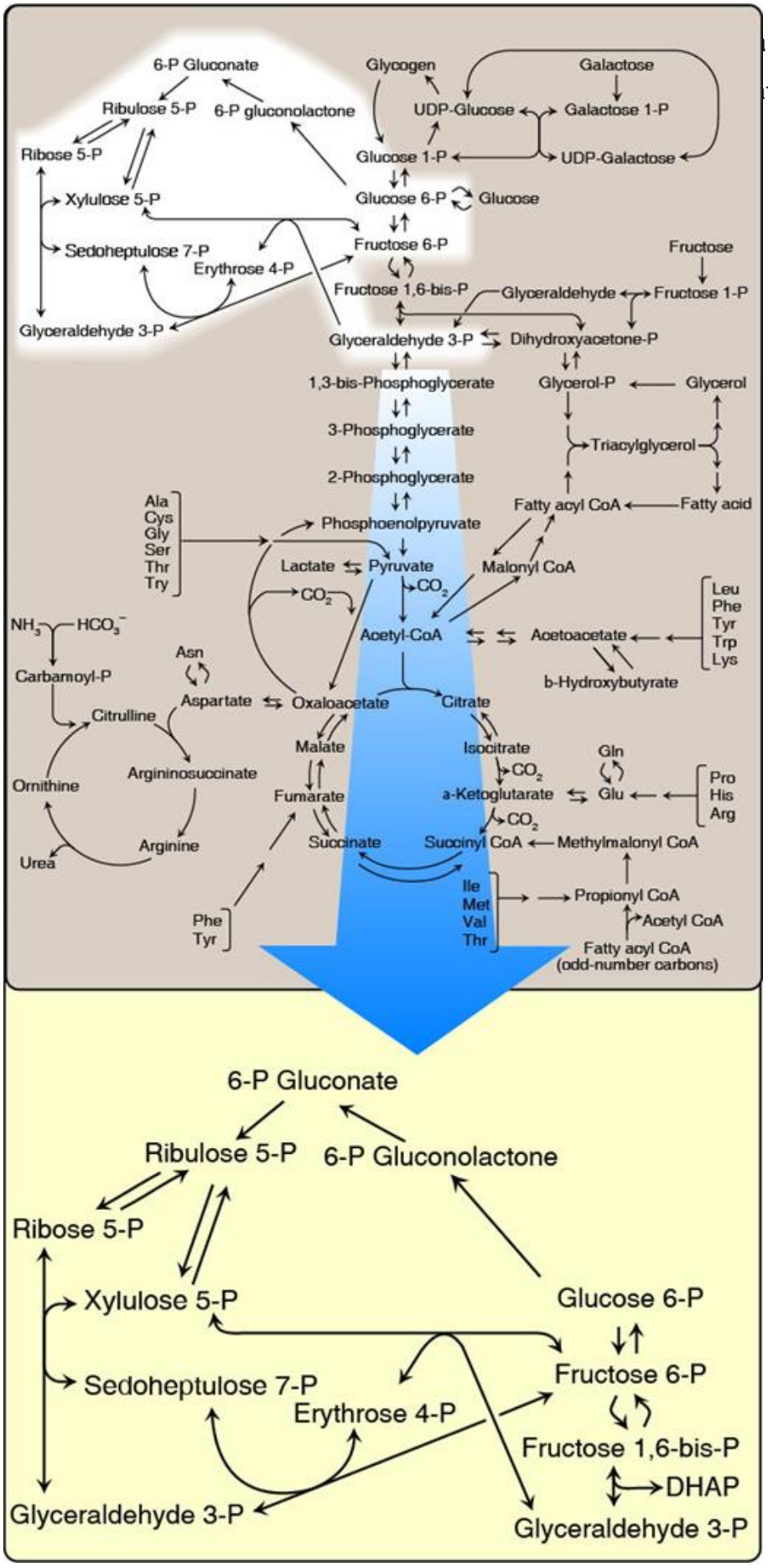
Overview

Listen

The pentose phosphate pathway, also known as the hexose monophosphate shunt, provides ribose 5-phosphate for the biosynthesis of nucleotides and is important as the body's main source of nicotinamide adenine dinucleotide phosphate (NADPH), a biochemical reductant. NADPH is the cellular source of reducing equivalents for biosynthesis of fatty acids and cholesterol and for the reduction of hydrogen peroxide (H_2O_2) formed in response to oxidative stress and as a byproduct of aerobic metabolism. Glucose-6 phosphate dehydrogenase (G6PD) catalyzes the first, rate-limiting step of the pathway; X-linked inheritance of G6PD deficiency results in insufficient NADPH, particularly in red blood cells, making them susceptible to lysis in response to oxidant stress. The pathway does not produce or consume ATP.

Reactions of this pathway occur in the cytosol and include 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 NADPHs 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 also produces ribose 5-phosphate, required for nucleotide biosynthesis (see also Chapter 22 III.), and provides a mechanism for the conversion of pentose sugars to triose and hexose intermediates of glycolysis.

FIGURE 13.1



…bolic map.

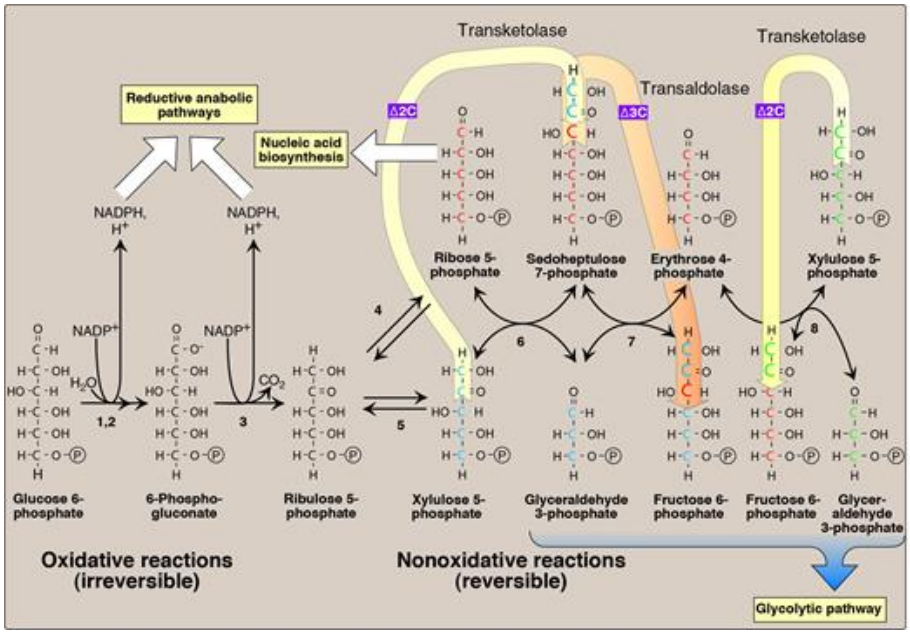
…te; DHAP = dihydroxyacetone

Irreversible Oxidative Reactions

Listen

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 also Chapter 15 III.); in the testes, ovaries, placenta, and adrenal cortex for the NADPH-dependent biosynthesis of steroid hormones (see also Chapter 18); and in red blood cells for the NADPH-dependent reduction of glutathione.

FIGURE 13.2



e and *6-phosphogluconolactone* *te isomerase*, (5) *phosphopentose* d (7) *transaldolase*. Δ2C, two carbons *se* reactions; Δ3C, three carbons are sugar + 5C sugar → 7C sugar + 3C sugar osphate; Ⓟ, phosphate; CO_2, carbon

Glucose 6-phosphate dehydrogenation

Glucose 6-phosphate dehydrogenase (G6PD) catalyzes the oxidation of glucose 6-phosphate to 6-phosphogluconolactone as the coenzyme $NADP^+$ is 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. It should be noted that insulin upregulates expression of the gene for G6PD, and flux through the pathway increases in the absorptive state (see also Chapter 24 III.).

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).

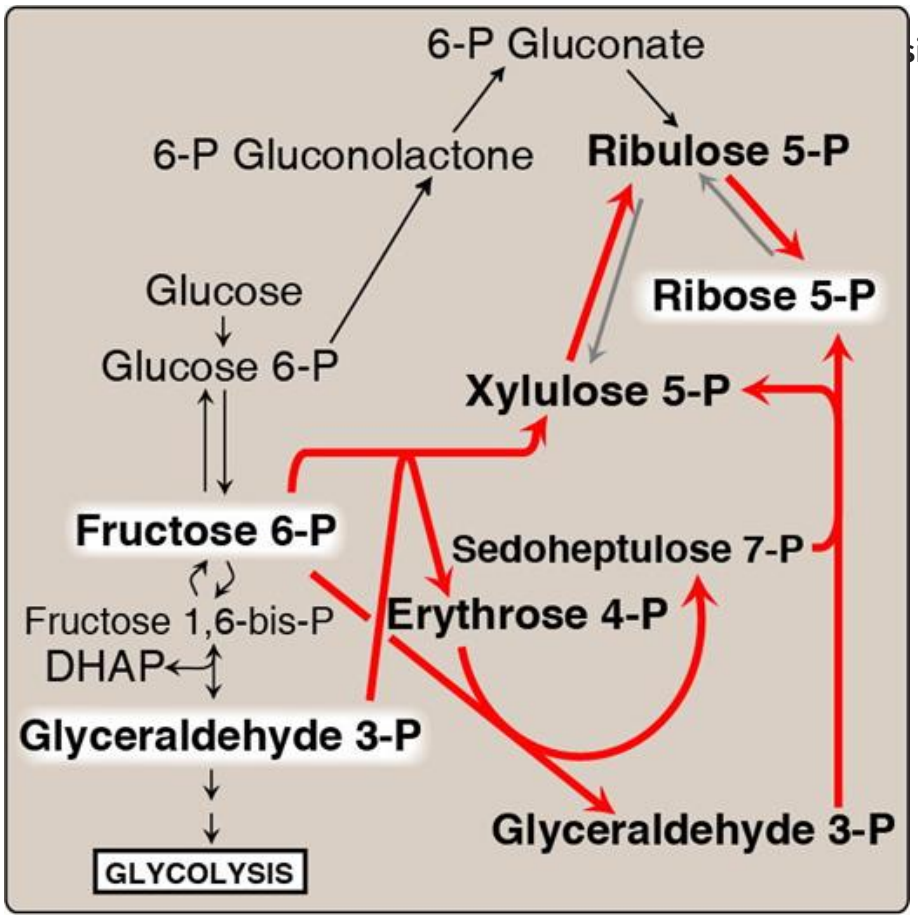
Reversible Nonoxidative Reactions

Listen

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 also Chapter 22 III.) or to intermediates of glycolysis, fructose 6-phosphate and glyceraldehyde 3-phosphate.

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. 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



is.

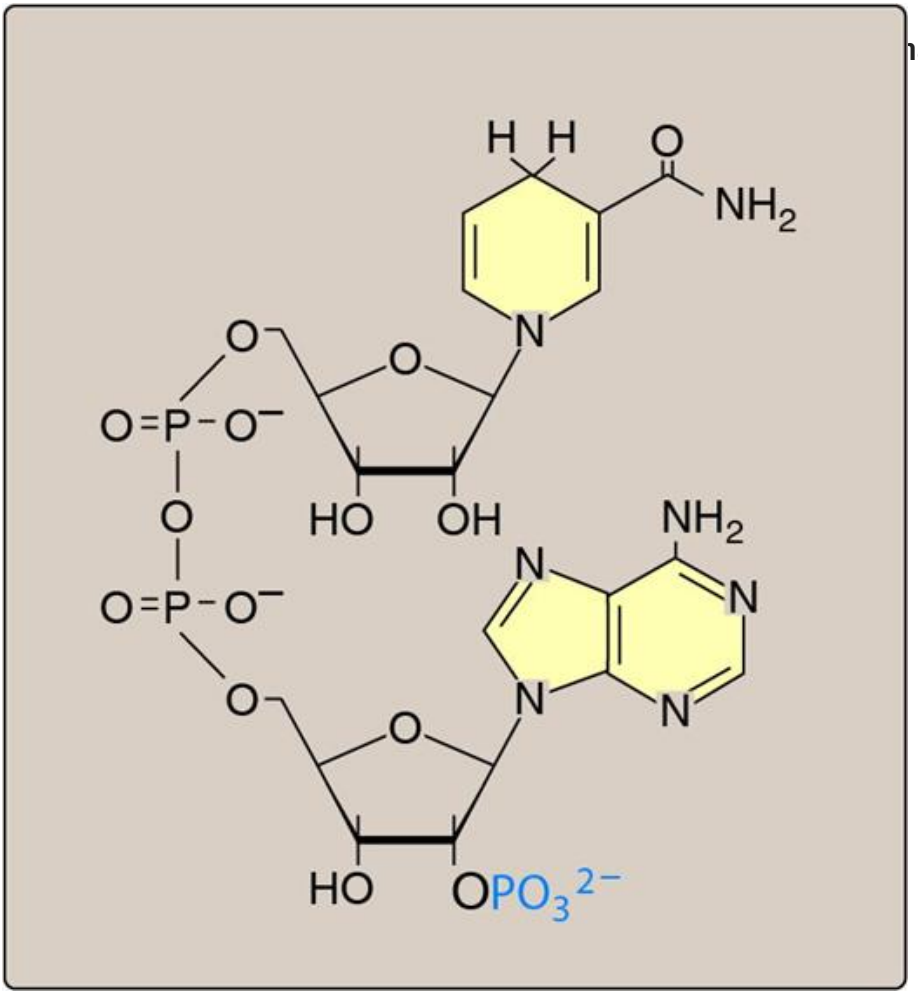
In addition to transketolase, TPP is required by the multienzyme complexes pyruvate dehydrogenase (see also Chapter 9 II.), α-ketoglutarate dehydrogenase of the tricarboxylic acid cycle (see also Chapter 9 II.), and branched-chain α-keto acid dehydrogenase of branched-chain amino acid catabolism (see also Chapter 20 III.).

Uses of NADPH

Listen

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 summarizes some important NADPH-specific functions in reductive biosynthesis and detoxification reactions.

FIGURE 13.4



nate (NADPH).

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 Chapter 6 V.). 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 Chapter 6), which, therefore, can be used in reactions requiring an electron donor, such as fatty acid (see Chapter 16 III.), cholesterol, and steroid hormone synthesis (see also Chapter 18 III. and VII.).

Reduction of H_2O_2

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 generated 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. ROS can also be generated in the killing of microbes by white blood cells (see section D., on page 165).

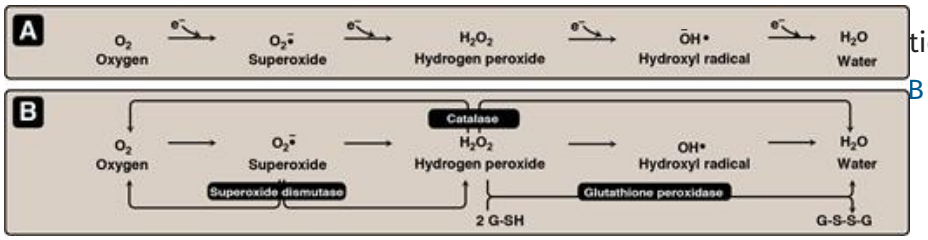
Enzymes that catalyze antioxidant reactions

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 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.

Antioxidant chemicals

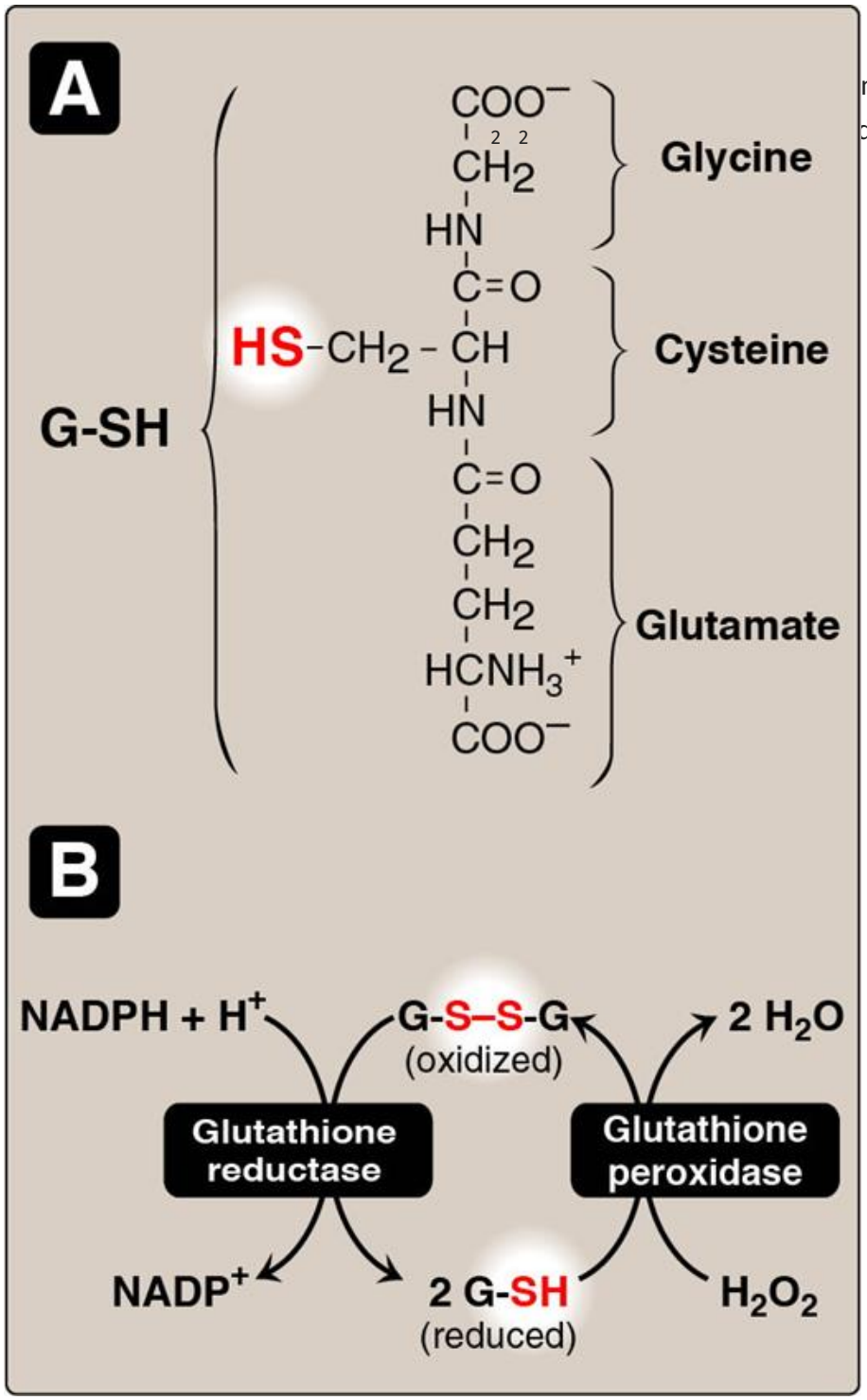
A number of intracellular reducing agents, such as ascorbate or vitamin C, vitamin E, and β-carotene, are able to reduce and, thereby, detoxify ROS in the laboratory. Consumption of foods rich in these antioxidant compounds has been correlated with a reduced risk for certain types of cancers as well 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 (see also Chapter 28).

FIGURE 13.5



tions of antioxidant enzymes. G-SH = B for the regeneration of G-SH.)

FIGURE 13.6



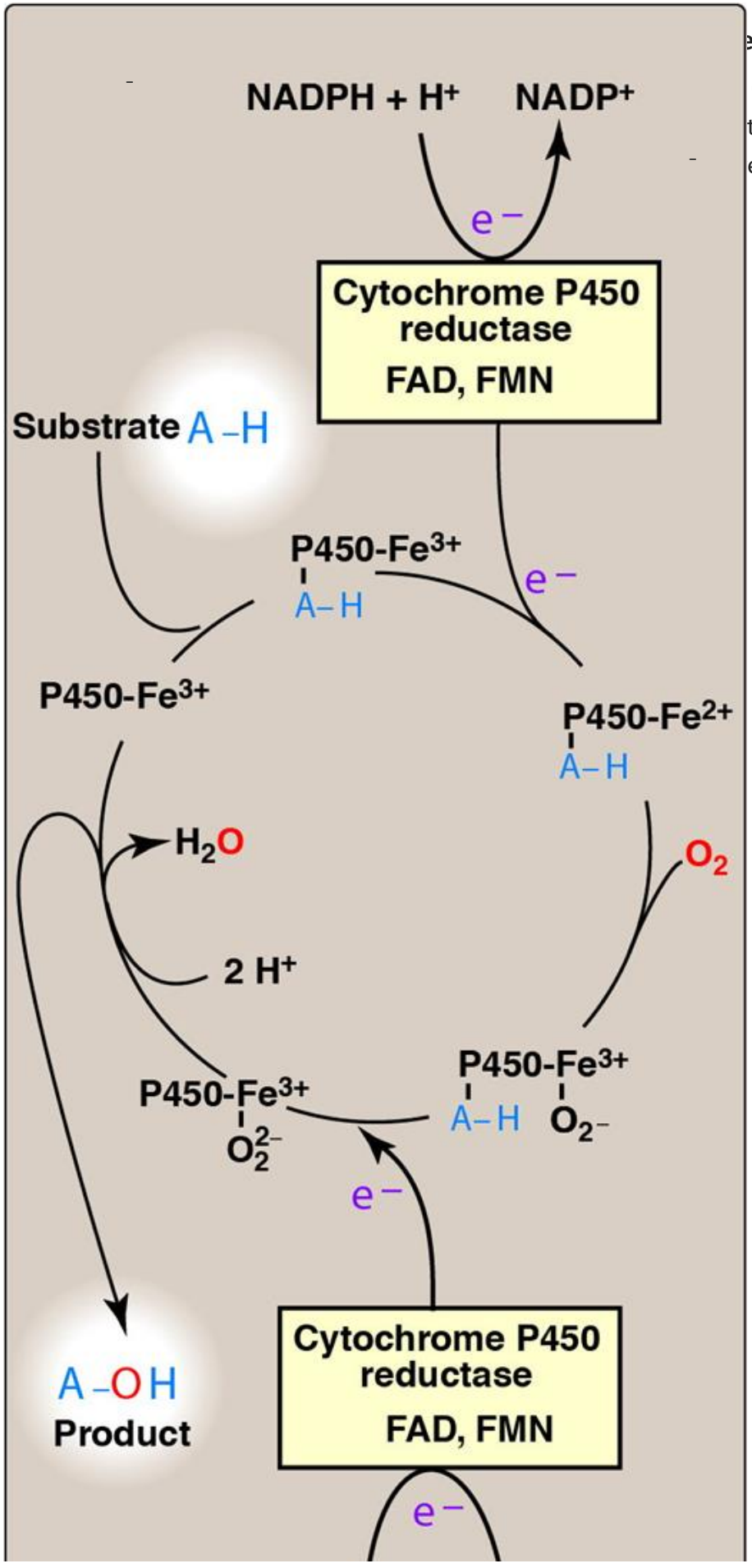
cysteine through a γ-carboxyl, rather
nine dinucleotide phosphate (NADPH)
d glutathione.

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

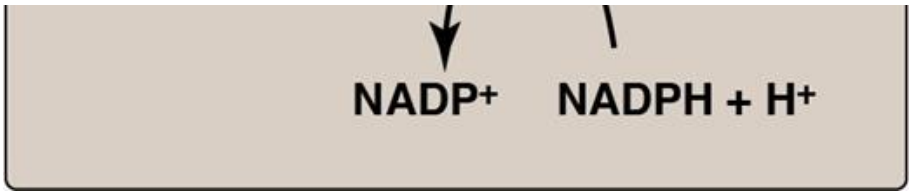
$$R\text{–}H + O_2 + NADPH + H^+ \rightarrow R\text{–}OH + H_2O + NADP^+$$

FIGURE 13.7



ed).

(NADPH) to flavin adenine
tase and then to the heme iron (Fe) of
e from FAD to an iron–sulfur protein



where R may be a steroid, drug, or other chemical. 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.

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 Chapter 18 VII.). The liver uses this same system in bile acid synthesis (see Chapter 18 V.) and the hydroxylation of cholecalciferol to 25-hydroxycholecalciferol (vitamin D_3; see Chapter 28 XII.), and the kidney uses it to hydroxylate vitamin D_3 to its biologically active 1,25-dihydroxylated form.

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 or 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 twofold. 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 Chapter 14 III.), which will significantly increase the compound's solubility (phase II). It should be noted that polymorphisms (see Chapter 34) in the genes for CYP enzymes can lead to differences in drug metabolism.

White blood cell phagocytosis and microbe killing

Phagocytosis is the ingestion by receptor-mediated endocytosis of microorganisms, foreign particles, and cellular debris by 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.

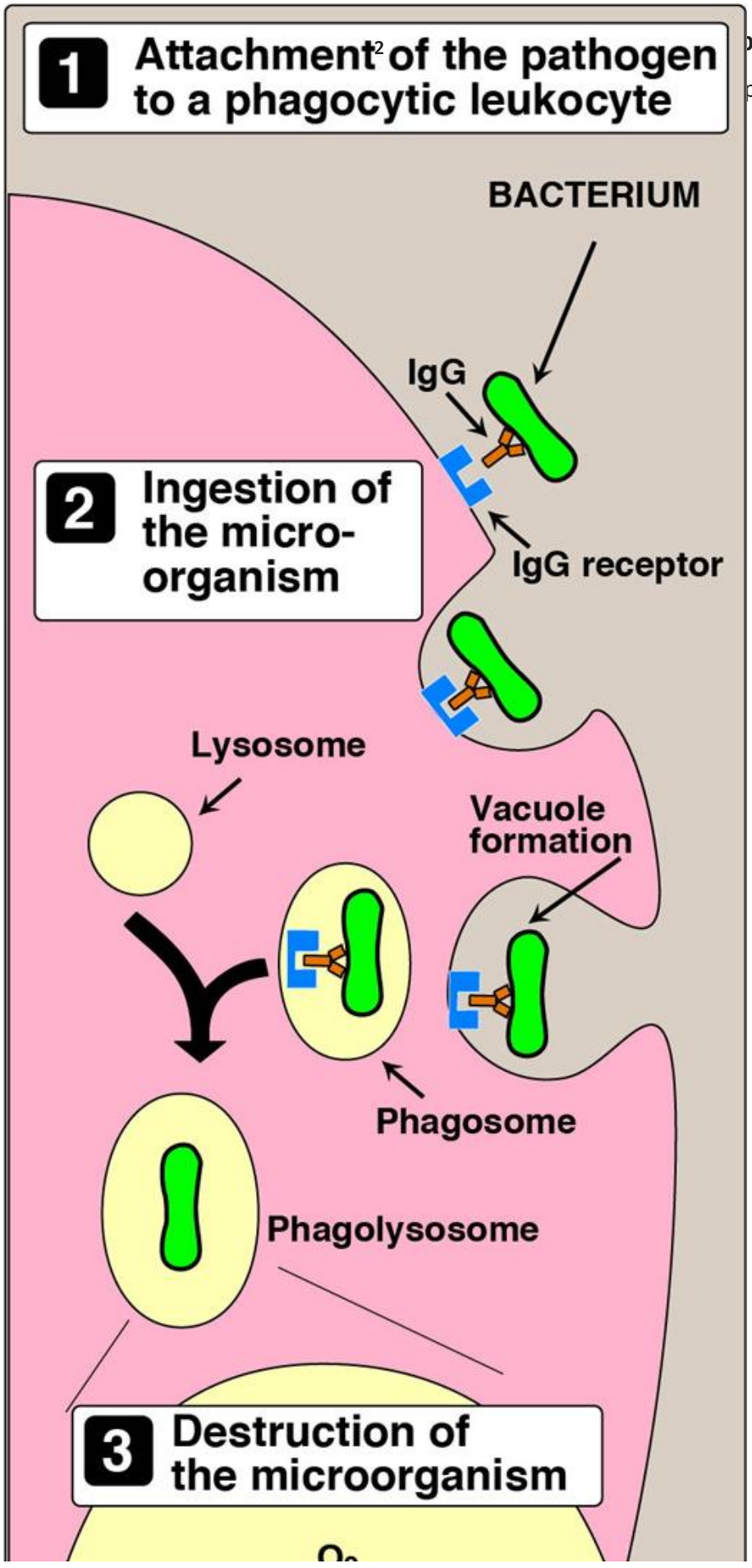
Oxygen independent

Oxygen-independent mechanisms use pH changes in phagolysosomes and lysosomal enzymes to destroy pathogens.

Oxygen dependent

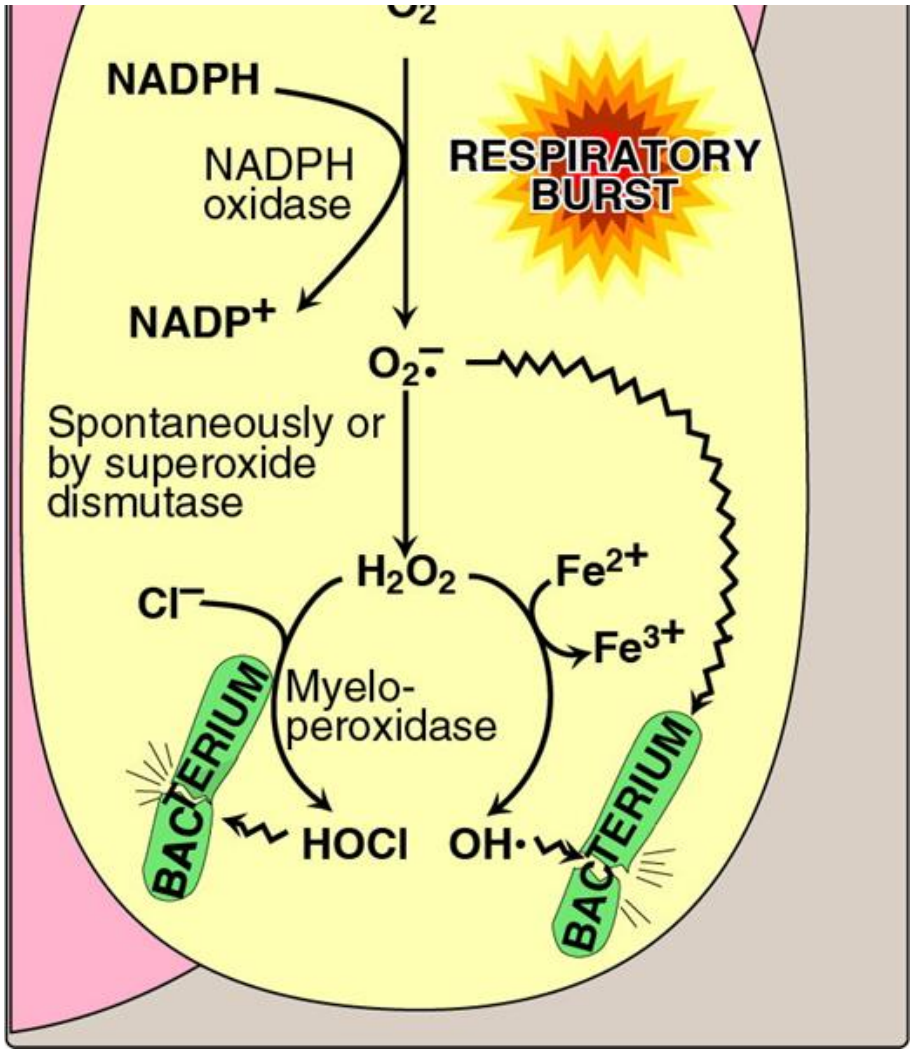
Oxygen-dependent mechanisms include the enzymes NADPH oxidase and myeloperoxidase (MPO) that work together in killing bacteria (Fig. 13.8). Overall, the MPO system is the most potent of the bactericidal mechanisms. An invading bacterium is recognized by the immune system and attacked by antibodies that 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^-), a free radical ROS, as NADPH is oxidized. The rapid consumption of O_2 that accompanies its formation 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^-.])

FIGURE 13.8



ıial killing.

phosphate; O_2^- = superoxide; H_2O_2 =



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^- 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•), an ROS, or be fully reduced to H_2O by catalase or glutathione peroxidase. Deficiencies in MPO do not confer increased susceptibility to infection because peroxide from NADPH oxidase is bactericidal.

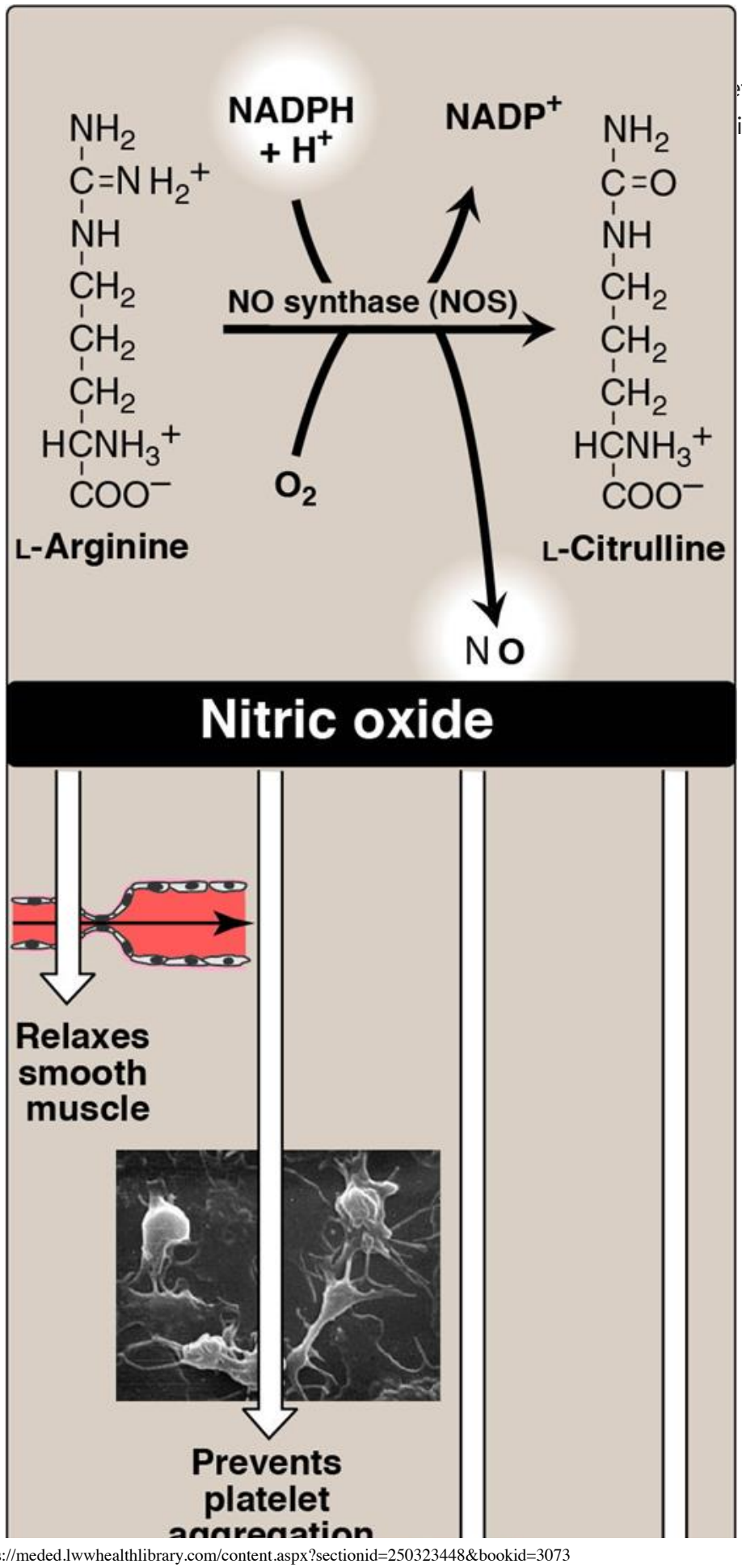
Nitric oxide synthesis

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 to 10 seconds) because it reacts with O_2 and is converted into nitrates and nitrites including peroxynitrite (O = NOO^-), a reactive nitrogen species (RNS). Note that 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.

Nitric oxide synthase

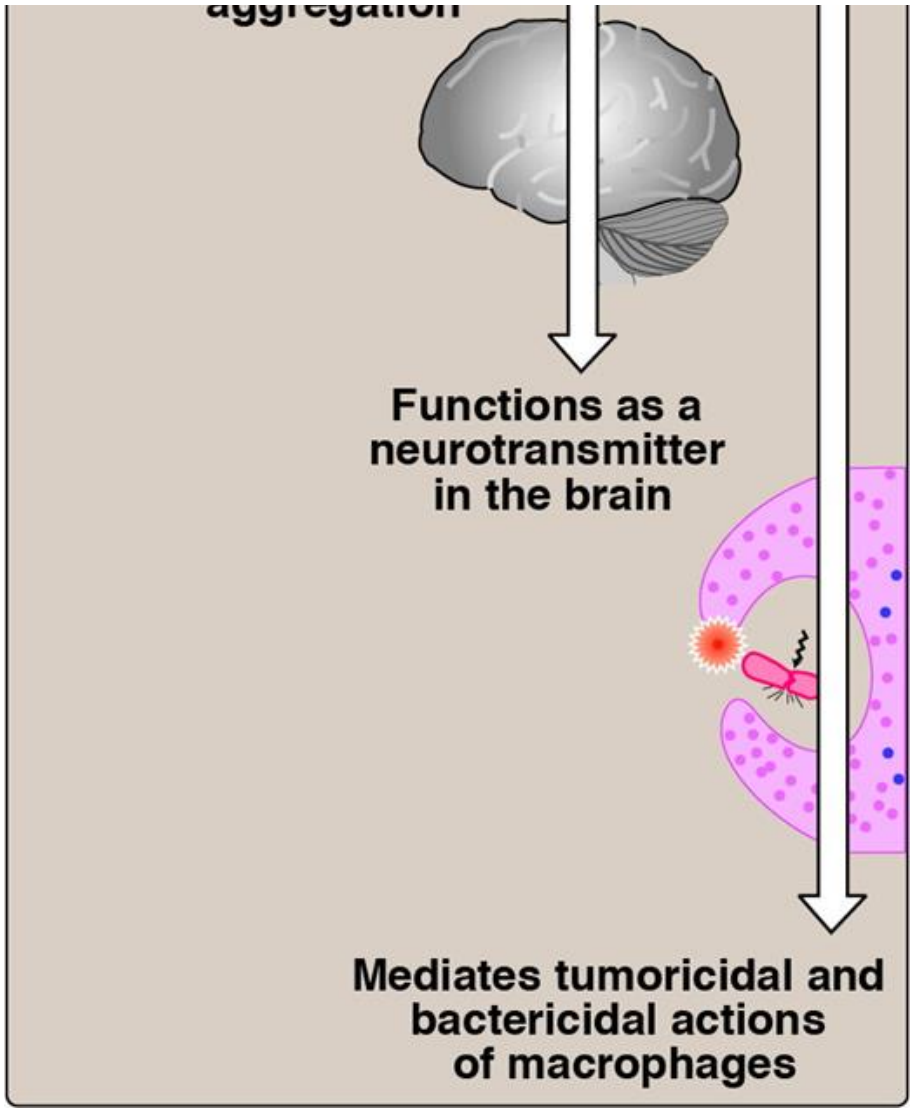
Arginine, O_2, and NADPH are substrates for cytosolic NO synthase ([NOS], Fig. 13.9). Flavin mononucleotide (FMN), FAD, heme, and tetrahydrobiopterin (see Chapter 20 V.) are coenzymes, and NO and citrulline are products of the reaction. Three NOS isozymes, each the product of a different gene, have been identified. Two are constitutive (synthesized at a constant rate), calcium (Ca^{2+})–calmodulin (CaM)-dependent enzymes (see Chapter 11 V.). 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.

FIGURE 13.9



etrahydrobiopterin are additional

ide phosphate.



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). This reaction is analogous to the formation of cyclic adenosine monophosphate (cAMP) by adenylyl cyclase (see Chapter 8 II. D.). 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 that 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.

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 radicals that combine with NO to form intermediates that decompose, producing the highly bactericidal OH• radical.

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.

G6PD Deficiency

Listen

Deficiency of G6PD, a hereditary condition that affects mostly males, is characterized by hemolytic anemia when the affected individual is exposed to an oxidant stress. The anemia is caused by the inability of red blood cells (erythrocytes) to detoxify oxidizing agents. With G6PD deficiency, less NADPH is available to maintain a pool of reduced glutathione to detoxify H_2O_2 generated in response to oxidant stress.

CLINICAL APPLICATION 13.1

Characteristics of G6PD Deficiency

Inherited as an X-linked trait, G6PD deficiency affects mostly males and is the most common disease-producing enzyme abnormality in humans. More than 400 million individuals are affected worldwide. This enzyme deficiency has the highest prevalence in persons whose ancestries come from the Middle East, tropical Africa and Asia, and parts of the Mediterranean. G6PD deficiency is actually a family of deficiencies caused by a number of different mutations in the G6PD gene. Only some of the resulting protein variants cause clinical symptoms.

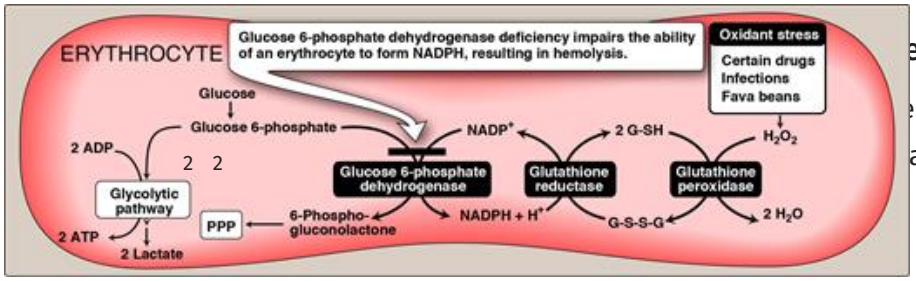
In addition to periodic bouts of hemolytic anemia in response to oxidant stress, a common clinical manifestation of G6PD deficiency is neonatal jaundice appearing 1 to 4 days after birth. The jaundice, which may be severe, typically results from increased production of unconjugated bilirubin (see Chapter 21 II.). The lifespan 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 increased resistance to malaria caused by *Plasmodium falciparum*. Infection of red blood cells by the parasite induces oxidant stress, resulting of lysis of the red blood cells, and protecting the host from developing malaria.

G6PD role in erythrocytes

Adequate G6PD activity is required for cells to form NADPH essential for the maintenance of the G-SH pool. Although G6PD deficiency occurs in all cells of the affected individual, it is most severe in erythrocytes, where the pentose phosphate pathway provides the only means of generating NADPH. Additionally, since red blood cells have no nucleus or ribosomes they cannot renew their supply of the enzyme, leaving erythrocytes particularly vulnerable to enzyme variants with diminished stability. Other tissues have an alternative pathway to produce NADPH (via $NADP^+$-dependent malate dehydrogenase [malic enzyme]; see Chapter 16 III.).

Deficiency of G6PD impairs the process of 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 red cell membranes (Fig. 13.11). Additional oxidation of membrane proteins causes erythrocyte membranes to be rigid (less deformable), and they are removed from the circulation by macrophages in the spleen and liver.

FIGURE 13.10

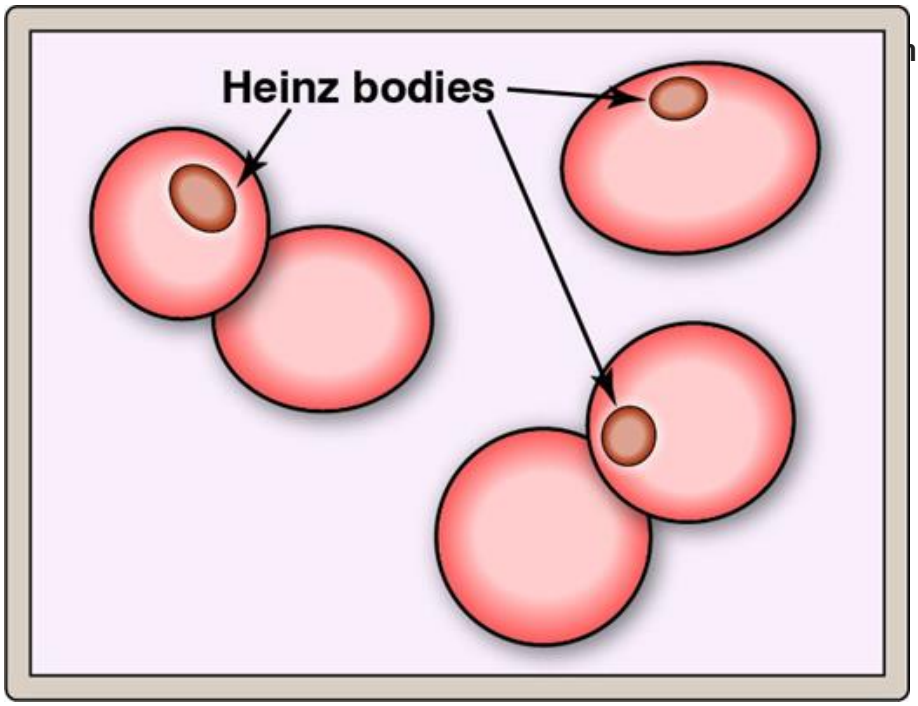


e.

ed glutathione; G-S-S-G = oxidized

ay.

FIGURE 13.11



hrocytes of a patient with glucose

Precipitating factors in G6PD deficiency

Male individuals who inherit a *G6PD* mutation on their lone X chromosome are considered to be hemizygous for the G6PD deficiency trait since they have only one X chromosome. Affected individuals will normally remain asymptomatic unless or until they experience a strong oxidant stress, which may be from treatment with an oxidant drug, ingestion of fava beans, or a severe infection. Lysis of red blood cells and hemolytic anemia results in G6PD-deficient individuals in response to oxidant stress-inducing agents.

Oxidant drugs

Drugs that can cause oxidant stress and produce hemolytic anemia in patients with G6PD deficiency are often in categories that begin with the letter A: some *antibiotics* (particularly sulfa drugs), some *antimalarials*, some *analgesics*, and some *antipyretics*. Only certain drugs in each category are implicated. Drug lists are available for prescribers that include usually safe agents and those best avoided by G6PD-deficient individuals.

Favism

Persons with some forms of G6PD deficiency, especially the Mediterranean variant, are particularly susceptible to the hemolytic effect of the fava or 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 do have G6PD deficiency.

Infection

Infection is a common precipitating factor of hemolysis in persons with G6PD deficiency. The inflammatory response to infection results in the generation of free radicals in macrophages. The radicals can diffuse into red blood cells and cause oxidative damage.

G6PD gene variants

The cloning and sequencing of the *G6PD* gene (see Chapter 34) have led to identification of more than 400 *G6PD* variants that result in G6PD enzyme deficiency. Some mutations do not affect enzymatic activity. Most mutations that do result in low G6PD enzyme function are missense point mutations (see Chapter 32 II.); some cause decreased catalytic activity, others decreased stability while other *G6PD* mutations alter the binding affinity for $NADP^+$ or glucose 6-phosphate. Active G6PD enzyme exists as a homodimer or tetramer. Mutations at the interface between subunits can affect enzyme stability.

The severity of hemolytic anemia in those with G6PD deficiency usually correlates with the amount of residual enzyme activity in the patient's red blood cells. G6PD variants can be classified as shown in Figure 13.12. G6PD A^- is the prototype of the moderate (class III) form of the disease. Red blood cells contain an unstable but kinetically normal G6PD, with most of the enzyme activity present in the reticulocytes and younger red cells (Fig. 13.13). The oldest red blood cells have the lowest level of G6PD 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, even in the absence of oxidative stress.

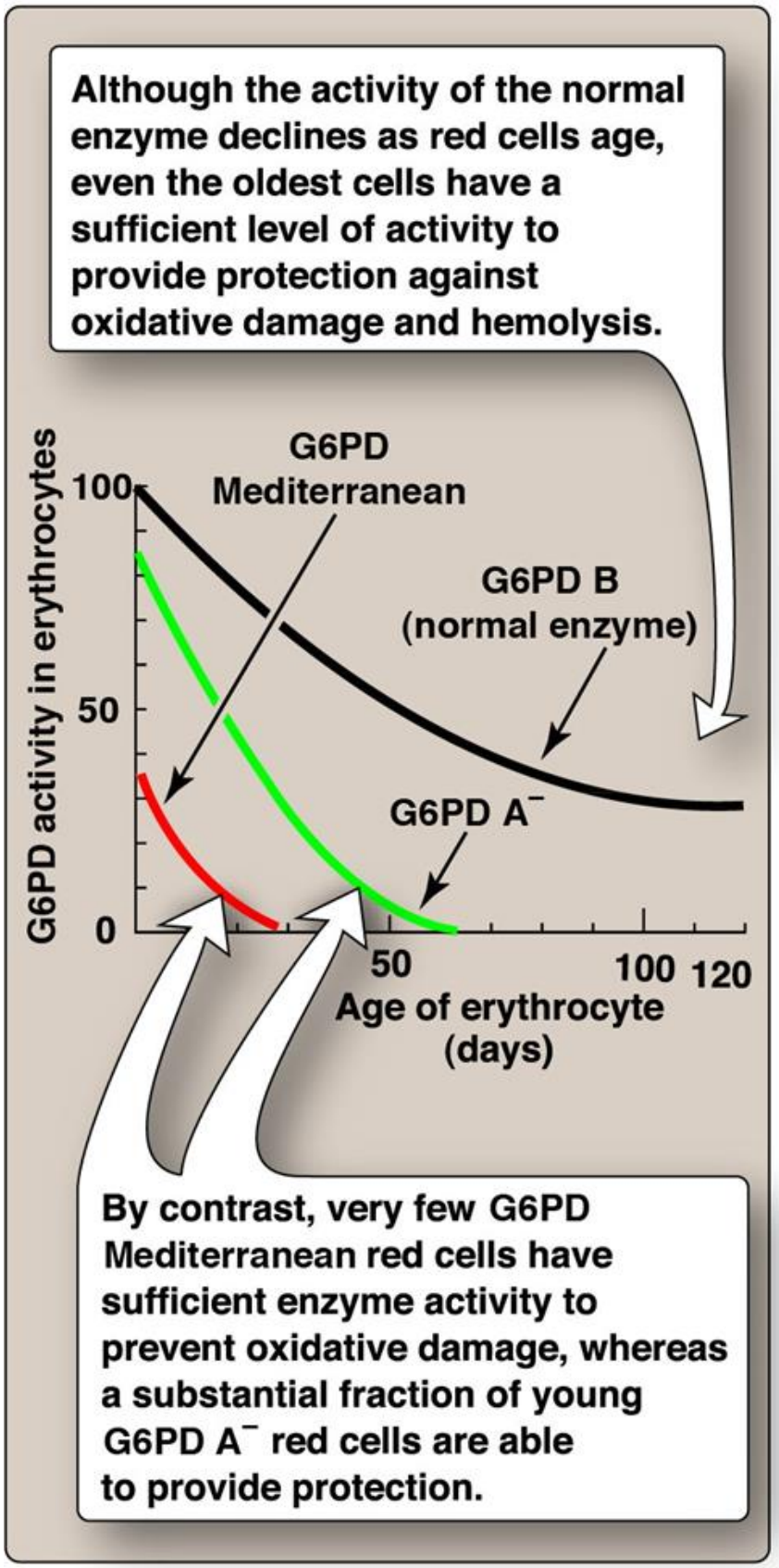
FIGURE 13.12

ficiency variants.

= most common.

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 13.13



PD) activity with cell age for the

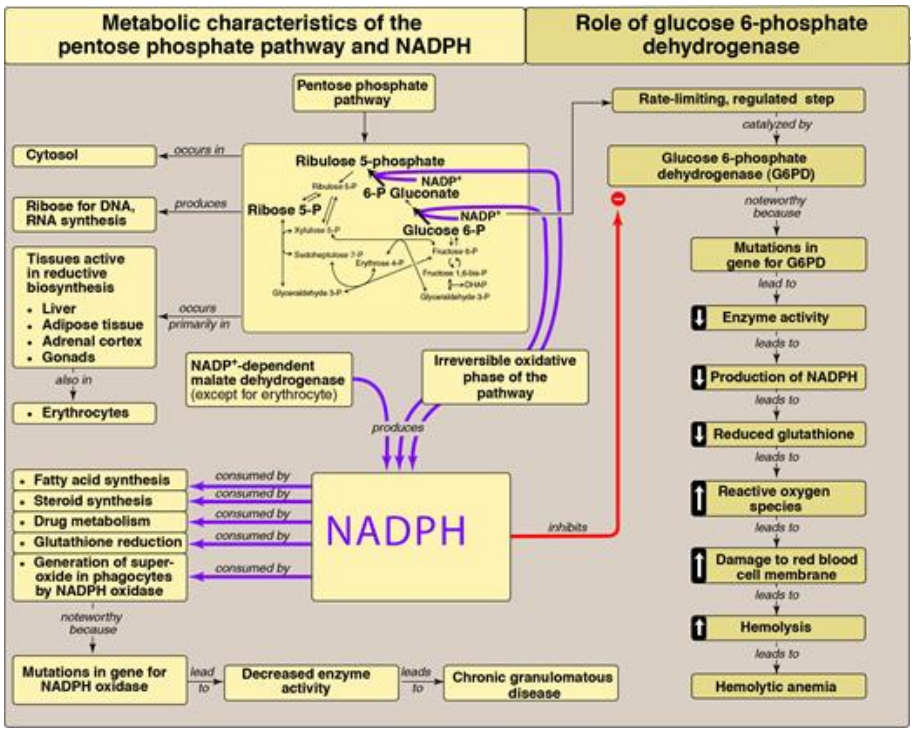
Both G6PD A^- and G6PD Mediterranean proteins represent mutant enzymes that differ from the respective normal variants by a single amino acid. Large deletions or frameshift mutations have not been identified, suggesting that complete absence of G6PD enzyme activity is most likely lethal.

Chapter Summary

Listen

- The **pentose phosphate pathway** is the main producer of **NADPH** in the body (Fig. 13.14).

FIGURE 13.14



- **No ATP** is used or consumed in the pathway.
- The pathway includes an irreversible oxidative phase followed by a series of reversible sugar–phosphate interconversions.
- **Reversible nonoxidative reactions** interconvert sugars. Ribulose 5-phosphate is converted to **ribose 5-phosphate**, required for nucleotide and nucleic acid synthesis, or to **fructose 6-phosphate** and **glyceraldehyde 3-phosphate** (glycolytic intermediates).
- The NADPH-producing **oxidative portion** of the pathway provides 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 NADPHs** are produced. The regulated step is catalyzed by **G6PD**, which is strongly inhibited by a rise in the **NADPH/NADP$^+$ ratio**.
- 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.
- NADPH is also required by erythrocytes for reduction of H_2O_2 produced as a consequence of aerobic metabolism.
- **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** xenobiotics, such as drugs and a variety of pollutants. NADPH provides the reducing equivalents for phagocytes involved in 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**.
- **G6PD deficiency, an X-linked disease that affects mostly males,** impairs **erythrocyte** ability to form NADPH essential for maintaining the G-SH pool. Erythrocytes are most affected because they do not have additional sources of NADPH. It is characterized by **hemolytic anemia** caused by the production of free radicals and peroxides after exposure to oxidant stress, including severe infection, **oxidant drugs, or fava beans**. The extent of the anemia depends on the amount of residual enzyme. Neonates with G6PD deficiency may experience prolonged **neonatal jaundice**.

Study Questions

Listen

Choose the ONE best answer.

13.1. In preparation for a trip to an area of India, a young male is given an antimalarial drug prophylactically. Several days after initiation of this therapy he develops jaundice and is diagnosed with anemia. A low level of which of the following is a consequence of the most likely enzyme deficiency and the underlying cause of the patient's presentation?

A. Glucose 6-phosphate

B. Oxidized form of nicotinamide adenine dinucleotide

C. Reduced form of glutathione

D. Ribose 5-phosphate

Correct answer = C. Glutathione (G-SH) is essential for red cell integrity and is maintained in this reduced (functional) form by nicotinamide adenine dinucleotide phosphate (NADPH)-dependent glutathione reductase. The NADPH is from the oxidative portion of the pentose phosphate pathway. Individuals with a deficiency of the regulated enzyme of this pathway, glucose 6-phosphate dehydrogenase (G6PD), have a decreased ability to generate NADPH and, therefore, a decreased ability to keep G-SH reduced. When treated with some antimalarials that induce an oxidant stress, some patients with G6PD deficiency develop a hemolytic anemia. Levels of glucose 6-phosphate are not altered. Nicotinamide adenine dinucleotide (NAD[H]) is neither produced by the pathway nor used as a coenzyme by G-SH reductase. A decrease in ribose 5-phosphate does not cause hemolysis.

13.2. Low blood pressure (hypotension), is a sign of septic shock, resulting from a severe inflammatory response to a bacterial infection. Based on this information, a likely cause of this hypotension is:

A. Activation of endothelial nitric oxide synthase causing a decrease in nitric oxide.

B. The long half-life of nitric oxide promotes long-term, excess vasoconstriction.

C. Lysine, the nitrogen source for nitric oxide synthesis, is deaminated by bacteria.

D. Bacterial endotoxin promoting iNOS synthesis causing increased NO production.

Correct answer = D. Overproduction of short-lived nitric oxide (NO) by calcium-independent, inducible nitric oxide synthase (iNOS) results in excessive vasodilation, leading to hypotension. The endothelial enzyme (eNOS) is constitutive and produces low levels of NO at a consistent rate. NOS uses arginine, not lysine, as the source of the nitrogen.

13.3. An individual who has recently been prescribed a drug (atorvastatin) to lower cholesterol levels is advised to limit consumption of grapefruit juice, because high intake of the juice reportedly results in an increased level of the drug in the blood, increasing the risk of side effects. Atorvastatin is a substrate for the cytochrome P450 enzyme CYP3A4, and grapefruit juice inhibits the enzyme. Based on this information, CYP enzymes most likely:

A. Accept electrons from reduced nicotinamide adenine dinucleotide.

B. Catalyze the hydroxylation of hydrophobic molecules.

C. Differ from nitric oxide synthase in that they contain heme.

D. Function in association with an oxidase.

Correct answer = B. The CYP enzymes hydroxylate hydrophobic compounds, making them more water soluble. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) from the pentose phosphate pathway is the electron donor. Both the CYP enzymes and the nitric oxide synthase isozymes contain heme.

13.4. In males who are hemizygous for glucose 6-phosphate dehydrogenase deficiency, pathophysiologic consequences are more apparent in red blood cells than in other cells such as in the liver. The best explanation for these findings is that:

A. Excess glucose 6-phosphate in the liver, but not in red blood cells, can be channeled to glycogen, thereby averting cellular damage.

B. Liver cells, in contrast to red blood cells, have alternative mechanisms for supplying the reduced nicotinamide adenine dinucleotide phosphate required for maintaining cell integrity.

C. Red blood cell production of ATP required to maintain cell integrity depends exclusively on the shunting of glucose 6-phosphate to the pentose phosphate pathway.

D. In contrast to liver cells, red cell glucose 6-phosphatase activity decreases the level of glucose 6-phosphate, resulting in cell damage.

Correct answer = B. Cellular damage is directly related to decreased ability of the cell to regenerate reduced glutathione, for which large amounts of reduced nicotinamide adenine dinucleotide phosphate (NADPH) are needed, and red blood cells have no means other than the pentose phosphate pathway of generating NADPH. It is decreased product (NADPH), not increased substrate (glucose 6-phosphate), that is the problem. Red blood cells do not have glucose 6-phosphatase. The pentose phosphate pathway does not generate ATP.

13.5. An essential coenzyme for several enzymes of metabolism is derived from the vitamin thiamine. The thiamine status in the body can be determined using a measurement of the activity of which enzyme?

A. Transketolase

B. Glucose-6-phosphate dehydrogenase

C. Pyruvate dehydrogenase

D. Glutathione peroxidase

Correct answer = B. Red blood cells do not have mitochondria and, so, do not contain mitochondrial enzymes such as pyruvate dehydrogenase that require the thiamine-derived coenzyme thiamine pyrophosphate (TPP). However, they do contain the cytosolic TPP-requiring transketolase, whose activity is used clinically to assess thiamine status.