24 Oxygen Toxicity and Free Radical Injury

***O_2 is both essential to human life and* toxic.** *We are dependent on O_2 for oxidation reactions in the pathways of adenosine triphosphate (ATP) generation, detoxification, and biosynthesis. However, when O_2 accepts single electrons, it is transformed into highly reactive* **oxygen radicals** *that damage cellular lipids, proteins, and DNA. Damage by reactive oxygen radicals contributes to cellular death and degeneration in a wide range of diseases (Table 24.1).*

***Radicals** are compounds that contain a single electron, usually in an outside orbital. Oxygen is a **biradical**, a molecule that has two unpaired electrons in separate orbitals (Fig. 24.1). Through a number of enzymatic and nonenzymatic processes that routinely occur in cells, O_2 accepts **single electrons** to form **reactive oxygen species (ROS)**. ROS are highly reactive oxygen radicals, or compounds that are readily converted in cells to these reactive radicals. The ROS formed by reduction of O_2 are the radical **superoxide (O_2^-)**, the nonradical **hydrogen peroxide (H_2O_2)**, and the **hydroxyl radical (OH•)**.*

*ROS may be generated nonenzymatically, or enzymatically as accidental byproducts or major products of reactions. Superoxide may be generated nonenzymatically from CoQ, or from metal-containing enzymes (e.g., **cytochrome P450, xanthine oxidase,** and **NADPH oxidase**). The highly toxic hydroxyl radical is formed nonenzymatically from superoxide in the presence of **Fe^{3+}** or **Cu^+** by the **Fenton reaction,** and from hydrogen peroxide in the **Haber–Weiss reaction.***

*Oxygen radicals and their derivatives can be deadly to cells. The hydroxyl radical causes oxidative damage to proteins and DNA. It also forms **lipid peroxides** and **malondialdehyde** from membrane lipids containing **polyunsaturated fatty acids.** In some cases, free radical damage is the direct cause of a disease state (e.g., tissue damage initiated by exposure to ionizing radiation). In **neurodegenerative diseases,** such as Parkinson's disease, or in ischemia-reperfusion injury, ROS may perpetuate the cellular damage caused by another process.*

*Oxygen radicals are joined in their destructive damage by the free radical nitric oxide (NO) and the reactive oxygen species **hypochlorous acid (HOCl)**. NO*

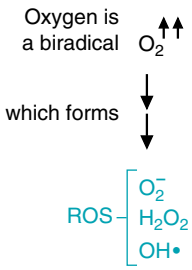


Fig 24.1. O_2 is a biradical. It has two antibonding electrons with parallel spins, denoted by the parallel arrows. It has a tendency to form toxic reactive oxygen species (ROS), such as superoxide (O_2^-), the nonradical hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH•).

Table 24.1. Some Disease States Associated with Free Radical Injury

Atherogenesis	Cerebrovascular disorders
Emphysema bronchitis	Ischemia/reperfusion injury
Duchenne-type muscular dystrophy	Neurodegenerative disorders
Pregnancy/preeclampsia	Amyotrophic lateral sclerosis (Lou Gehrig's disease)
Cervical cancer	Alzheimer's disease
Alcohol-induced liver disease	Down's syndrome
Hemodialysis	Ischemia/reperfusion injury following stroke
Diabetes	Oxphos diseases (Mitochondrial DNA disorders)
Acute renal failure	Multiple sclerosis
Aging	Parkinson's disease
Retrolental fibroplasia	

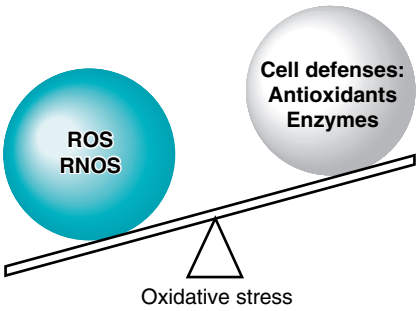


Fig 24.2. Oxidative stress. Oxidative stress occurs when the rate of ROS and RNOS production overbalances the rate of their removal by cellular defense mechanisms. These defense mechanisms include a number of enzymes and antioxidants. Antioxidants usually react nonenzymatically with ROS.

combines with O_2 or superoxide to form reactive ***nitrogen oxygen species (RNOS),*** *such as the nonradical peroxynitrite or the radical* ***nitrogen dioxide.*** *RNOS are present in the environment (e.g., cigarette smoke) and generated in cells. During phagocytosis of invading microorganisms, cells of the immune system produce O_2^-, HOCl, and NO through the actions of* ***NADPH oxidase, myeloperoxidase, and inducible nitric oxide synthase,*** *respectively. In addition to killing phagocytosed invading microorganisms, these toxic metabolites may damage surrounding tissue components.*

Cells ***protect*** *themselves against damage by ROS and other radicals through* ***repair processes, compartmentalization*** *of free radical production,* ***defense enzymes,*** *and* ***endogenous and exogenous antioxidants (free radical scavengers).*** *The defense enzyme* ***superoxide dismutase (SOD)*** *removes the superoxide free radical. Catalase and glutathione peroxidase remove hydrogen peroxide and lipid peroxides.* ***Vitamin E, vitamin C,*** *and* ***plant flavonoids*** *act as* ***antioxidants.*** ***Oxidative stress*** *occurs when the rate of ROS generation exceeds the capacity of the cell for their removal (Fig. 24.2).*

The basal ganglia are part of a neuronal feedback loop that modulates and integrates the flow of information from the cerebral cortex to the motor neurons of the spinal cord. The neostriatum is the major input structure from the cerebral cortex. The substantia nigra pars compacta consists of neurons that provide integrative input to the neostriatum through pigmented neurons that use dopamine as a neurotransmitter (the nigrastriatal pathway). Integrated information feeds back to the basal ganglia and to the cerebral cortex to control voluntary movement. In Parkinson's disease, a decrease in the amount of dopamine reaching the basal ganglia results in the movement disorder.

In ventricular fibrillation, rapid premature beats from an irritative focus in ventricular muscle occur in runs of varying duration. Persistent fibrillation compromises cardiac output, leading to death. This arrythmia can result from severe ischemia (lack of blood flow) in the ventricular muscle of the heart caused by clots forming at the site of a ruptured atherosclerotic plaque. However, **Cora Nari's** rapid beats began during the infusion of TPA as the clot was lysed. Thus, they probably resulted from reperfusing a previously ischemic area of her heart with oxygenated blood. This phenomenon is known as ischemia–reperfusion injury, and it is caused by cytotoxic ROS derived from oxygen in the blood that reperfuses previously hypoxic cells. Ischemic–reperfusion injury also may occur when tissue oxygenation is interrupted during surgery or transplantation.

THE WAITING ROOM

Two years ago, **Les Dopaman** (less dopamine), a 62-year-old man, noted an increasing tremor of his right hand when sitting quietly (resting tremor). The tremor disappeared if he actively used this hand to do purposeful movement. As this symptom progressed, he also complained of stiffness in his muscles that slowed his movements (bradykinesia). His wife noticed a change in his gait; he had begun taking short, shuffling steps and leaned forward as he walked (postural imbalance). He often appeared to be staring ahead with a rather immobile facial expression. She noted a tremor of his eyelids when he was asleep and, recently, a tremor of his legs when he was at rest. Because of these progressive symptoms and some subtle personality changes (anxiety and emotional lability), she convinced Les to see their family doctor.

The doctor suspected that her patient probably had primary or idiopathic parkinsonism (Parkinson's disease) and referred Mr. Dopaman to a neurologist. In Parkinson's disease, neurons of the substantia nigra pars compacta, containing the pigment melanin and the neurotransmitter dopamine, degenerate.

Cora Nari had done well since the successful lysis of blood clots in her coronary arteries with the use of intravenous recombinant tissue plasminogen activator (TPA)(see Chapters 19 and 21). This therapy had quickly relieved the crushing chest pain (angina) she experienced when she won the lottery. At her first office visit after discharge from the hospital, Cora's cardiologist told her she had developed multiple premature contractions of the ventricular muscle of her heart as the clots were being lysed. This process could have led to a life-threatening arrhythmia known as ventricular fibrillation. However, Cora's arrhythmia responded quickly to pharmacologic suppression and did not recur during the remainder of her hospitalization.

I. O_2 AND THE GENERATION OF ROS

The generation of reactive oxygen species from O_2 in our cells is a natural everyday occurrence. They are formed as accidental products of nonenzymatic and enzymatic

reactions. Occasionally, they are deliberately synthesized in enzyme-catalyzed reactions. Ultraviolet radiation and pollutants in the air can increase formation of toxic oxygen-containing compounds.

A. The Radical Nature of O_2

A radical, by definition, is a molecule that has a single unpaired electron in an orbital. A free radical is a radical capable of independent existence. (Radicals formed in an enzyme active site during a reaction, for example, are not considered free radicals unless they can dissociate from the protein to interact with other molecules.) Radicals are highly reactive and initiate chain reactions by extracting an electron from a neighboring molecule to complete their own orbitals. Although the transition metals (e.g., Fe, Cu, and Mo) have single electrons in orbitals, they are not usually considered free radicals because they are relatively stable, do not initiate chain reactions, and are bound to proteins in the cell.

The oxygen atom is a biradical, which means it has two single electrons in different orbitals. These electrons cannot both travel in the same orbital because they have parallel spins (spin in the same direction). Although oxygen is very reactive from a thermodynamic standpoint, its single electrons cannot react rapidly with the paired electrons found in the covalent bonds of organic molecules. As a consequence, O_2 reacts slowly through the acceptance of single electrons in reactions that require a catalyst (such as a metal-containing enzyme).

O_2 is capable of accepting a total of four electrons, which reduces it to water (Fig. 24.3). When O_2 accepts one electron, superoxide is formed. Superoxide is still a radical because it has one unpaired electron remaining. This reaction is not thermodynamically favorable and requires a moderately strong reducing agent that can donate single electrons (e.g., CoQH· in the electron transport chain). When superoxide accepts an electron, it is reduced to hydrogen peroxide, which is not a radical. The hydroxyl radical is formed in the next one-electron reduction step in the reduction sequence. Finally, acceptance of the last electron reduces the hydroxyl radical to H_2O.

B. Characteristics of Reactive Oxygen Species

Reactive oxygen species (ROS) are oxygen-containing compounds that are highly reactive free radicals, or compounds readily converted to these oxygen free radicals in the cell. The major oxygen metabolites produced by one-electron reduction of oxygen (superoxide, hydrogen peroxide, and the hydroxyl radical) are classified as ROS (Table 24.2).

Reactive free radicals extract electrons (usually as hydrogen atoms) from other compounds to complete their own orbitals, thereby initiating free radical chain reactions. The hydroxyl radical is probably the most potent of the ROS. It initiates chain reactions that form lipid peroxides and organic radicals and adds directly to compounds. The superoxide anion is also highly reactive, but has limited lipid solubility and cannot diffuse far. However, it can generate the more reactive hydroxyl and hydroperoxy radicals by reacting nonenzymatically with hydrogen peroxide in the Haber–Weiss reaction (Fig 24.4).

Hydrogen peroxide, although not actually a radical, is a weak oxidizing agent that is classified as an ROS because it can generate the hydroxyl radical (OH•). Transition metals, such as Fe^{2+} or Cu^{+}, catalyze formation of the hydroxyl radical from hydrogen peroxide in the nonenzymatic Fenton reaction (see Fig. 24.4.).

The two unpaired electrons in oxygen have the same (parallel) spin and are called antibonding electrons. In contrast, carbon–carbon and carbon–hydrogen bonds each contain two electrons, which have antiparallel spins and form a thermodynamically stable pair. As a consequence, O_2 cannot readily oxidize a covalent bond because one of its electrons would have to flip its spin around to make new pairs. The difficulty in changing spins is called the **spin restriction.** Without the spin restriction, organic life forms could not have developed in the oxygen atmosphere on earth because they would be spontaneously oxidized by O_2. Instead, O_2 is confined to slower one-electron reactions catalyzed by metals (or metalloenzymes).

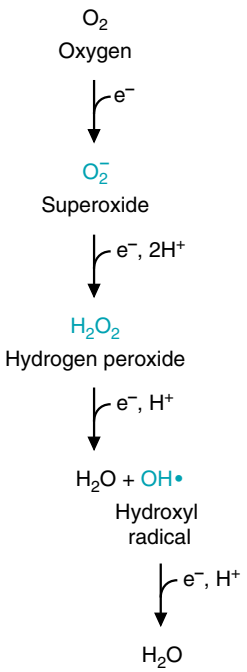


Fig 24.3. Reduction of oxygen by four one-electron steps. The four one-electron reduction steps for O_2 progressively generate superoxide, hydrogen peroxide, and the hydroxyl radical plus water. Superoxide is sometimes written $O_2^{-\cdot}$ to better illustrate its single unpaired electron. H_2O_2, the half-reduced form of O_2, has accepted two electrons and is, therefore, not an oxygen radical.

To decrease occurrence of the Fenton reaction, accessibility to transition metals, such as Fe^{2+} and Cu^{+}, are highly restricted in cells, or in the body as a whole. Events that release iron from cellular storage sites, such as a crushing injury, are associated with increased free radical injury.

Table 24.2. Reactive Oxygen Species (ROS) and Reactive Nitrogen–Oxygen Species (RNOS)

Reactive Species	Properties
O_2^- Superoxide anion	Produced by the electron transport chain and at other sites. Cannot diffuse far from the site of origin. Generates other ROS.
H_2O_2 Hydrogen peroxide	Not a free radical, but can generate free radicals by reaction with a transition metal (e.g., Fe^{2+}). Can diffuse into and through cell membranes.
OH• Hydroxyl radical	The most reactive species in attacking biologic molecules. Produced from H_2O_2 in the Fenton reaction in the presence of Fe^{2+} or Cu^+.
RO•, R•, R-S• Organic radicals	Organic free radicals (R denotes remainder of the compound.) Produced from ROH, RH (e.g., at the carbon of a double bond in a fatty acid) or RSH by OH• attack.
RCOO• Peroxyl radical	An organic peroxyl radical, such as occurs during lipid degradation (also denoted LOO•)
HOCl Hypochlorous acid	Produced in neutrophils during the respiratory burst to destroy invading organisms. Toxicity is through halogenation and oxidation reactions. Attacking species is OCl^-
$O_2^{\downarrow\uparrow}$ Singlet oxygen	Oxygen with antiparallel spins. Produced at high oxygen tensions from absorption of *uv* light. Decays so fast that it is probably not a significant in vivo source of toxicity.
NO Nitric oxide	RNOS. A free radical produced endogenously by nitric oxide synthase. Binds to metal ions. Combines with O_2 or other oxygen-containing radicals to produce additional RNOS.
$ONOO^-$ Peroxynitrite	RNOS. A strong oxidizing agent that is not a free radical. It can generate NO_2 (nitrogen dioxide), which is a radical.

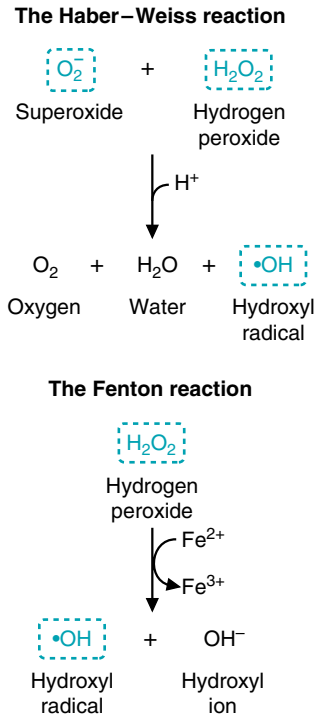


Fig 24.4. Generation of the hydroxyl radical by the nonenzymatic Haber–Weiss and Fenton reactions. In the simplified versions of these reactions shown here, the transfer of single electrons generates the hydroxyl radical. ROS are shown in blue. In addition to Fe^{2+}, Cu^+ and many other metals can also serve as single-electron donors in the Fenton reaction.

Because hydrogen peroxide is lipid soluble, it can diffuse through membranes and generate OH• at localized Fe^{2+}- or Cu^+-containing sites, such as the mitochondria. Hydrogen peroxide is also the precursor of hypochlorous acid (HOCl), a powerful oxidizing agent that is produced endogenously and enzymatically by phagocytic cells.

Organic radicals are generated when superoxide or the hydroxyl radical indiscriminately extract electrons from other molecules. Organic peroxy radicals are intermediates of chain reactions, such as lipid peroxidation. Other organic radicals, such as the ethoxy radical, are intermediates of enzymatic reactions that escape into solution (see Table 24.2).

An additional group of oxygen-containing radicals, termed RNOS, contain nitrogen as well as oxygen. These are derived principally from the free radical nitric oxide (NO), which is produced endogenously by the enzyme nitric oxide synthase. Nitric oxide combines with O_2 or superoxide to produce additional RNOS.

C. Major Sources of Primary Reactive Oxygen Species in the Cell

ROS are constantly being formed in the cell; approximately 3 to 5% of the oxygen we consume is converted to oxygen free radicals. Some are produced as accidental by-products of normal enzymatic reactions that escape from the active site of metal-containing enzymes during oxidation reactions. Others, such as hydrogen peroxide, are physiologic products of oxidases in peroxisomes. Deliberate production of toxic free radicals occurs in the inflammatory response. Drugs, natural radiation, air pollutants, and other chemicals also can increase formation of free radicals in cells.

1. CoQ GENERATES SUPEROXIDE

One of the major sites of superoxide generation is Coenzyme Q (CoQ) in the mitochondrial electron transport chain (Fig. 24.5). The one-electron reduced form of CoQ (CoQH•) is free within the membrane and can accidentally transfer an electron to dissolved O_2, thereby forming superoxide. In contrast, when O_2 binds to cytochrome oxidase and accepts electrons, none of the O_2 radical intermediates are released from the enzyme, and no ROS are generated.

With insufficient oxygen, **Cora Nari's** ischemic heart muscle mitochondria were unable to maintain cellular ATP levels, resulting in high intracellular Na^+ and Ca^{2+} levels. The reduced state of the electron carriers in the absence of oxygen, and loss of mitochondrial ion gradients or membrane integrity, leads to increased superoxide production once oxygen becomes available during reperfusion. The damage can be self-perpetuating, especially if iron bound to components of the electron transport chain becomes available for the Fenton reaction, or the mitochondrial permeability transition is activated.

2. OXIDASES, OXYGENASES, AND PEROXIDASES

Most of the oxidases, peroxidases, and oxygenases in the cell bind O_2 and transfer single electrons to it via a metal. Free radical intermediates of these reactions may be accidentally released before the reduction is complete.

Cytochrome P450 enzymes are a major source of free radicals "leaked" from reactions.

Because these enzymes catalyze reactions in which single electrons are transferred to O_2 and an organic substrate, the possibility of accidentally generating and releasing free radical intermediates is high (see Chapters 19 and 25). Induction of P450 enzymes by alcohol, drugs, or chemical toxicants leads to increased cellular injury. When substrates for cytochrome P450 enzymes are not present, its potential for destructive damage is diminished by repression of gene transcription.

Hydrogen peroxide and lipid peroxides are generated enzymatically as major reaction products by a number of oxidases present in peroxisomes, mitochondria, and the endoplasmic reticulum. For example, monoamine oxidase, which oxidatively degrades the neurotransmitter dopamine, generates H_2O_2 at the mitochondrial membrane of certain neurons. Peroxisomal fatty acid oxidase generates H_2O_2 rather than FAD(2H) during the oxidation of very-long-chain fatty acids (see Chapter 23). Xanthine oxidase, an enzyme of purine degradation that can reduce O_2 to O_2^-or H_2O_2 in the cytosol, is thought to be a major contributor to ischemia–reperfusion injury, especially in intestinal mucosal and endothelial cells. Lipid peroxides are also formed enzymatically as intermediates in the pathways for synthesis of many eicosanoids, including leukotrienes and prostaglandins.

3. IONIZING RADIATION

Cosmic rays that continuously bombard the earth, radioactive chemicals, and x-rays are forms of ionizing radiation. Ionizing radiation has a high enough energy level that it can split water into the hydroxyl and hydrogen radicals, thus leading to radiation damage to the skin, mutations, cancer, and cell death (Fig. 24.6). It also may generate organic radicals through direct collision with organic cellular components.

Production of ROS by xanthine oxidase in endothelial cells may be enhanced during ischemia–reperfusion in **Cora Nari's** heart. In undamaged tissues, xanthine oxidase exists as a dehydrogenase that uses NAD^+ rather than O_2 as an electron acceptor in the pathway for degradation of purines (hypoxanthine ↔ xanthine ↔ uric acid (see Chapter 41). When O_2 levels decrease, phosphorylation of ADP to ATP decreases, and degradation of ADP and adenine through xanthine oxidase increases. In the process, xanthine dehydrogenase is converted to an oxidase. As long as O_2 levels are below the high K_m of the enzyme for O_2, little damage is done. However, during reperfusion when O_2 levels return to normal, xanthine oxidase generates H_2O_2 and O_2^- at the site of injury.

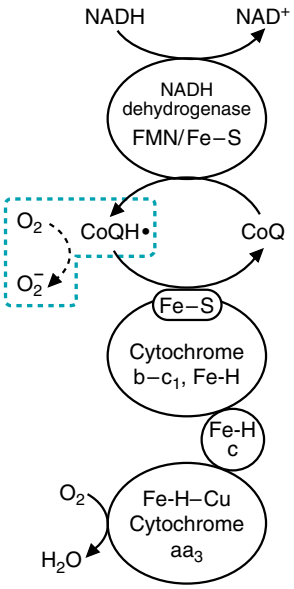


Fig 24.5. Generation of superoxide by CoQ in the electron transport chain. In the process of transporting electrons to O_2, some of the electrons escape when CoQH• accidentally interacts with O_2 to form superoxide. Fe-H represents the Fe-heme center of the cytochromes.

Carbon tetrachloride (CCl_4), which is used as a solvent in the dry-cleaning industry, is converted by cytochrome P450 to a highly reactive free radical that has caused hepatocellular necrosis in workers. When the enzyme-bound CCl_4 accepts an electron, it dissociates into CCl_3· and Cl·. The CCl_3· radical, which cannot continue through the P450 reaction sequence, "leaks" from the enzyme active site and initiates chain reactions in the surrounding polyunsaturated lipids of the endoplasmic reticulum. These reactions spread into the plasma membrane and to proteins, eventually resulting in cell swelling, accumulation of lipids, and cell death.

Les Dopaman, who is in the early stages of Parkinson's disease, is treated with a monoamine oxidase B inhibitor. Monoamine oxidase is a copper-containing enzyme that inactivates dopamine in neurons, producing H_2O_2. The drug was originally administered to inhibit dopamine degradation. However, current theory suggests that the effectiveness of the drug is also related to decrease of free radical formation within the cells of the basal ganglia. The dopaminergic neurons involved are particularly susceptible to the cytotoxic effects of ROS and RNOS that may arise from H_2O_2.

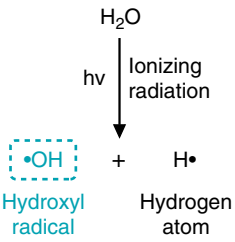


Fig 24.6. Generation of free radicals from radiation.

II. OXYGEN RADICAL REACTIONS WITH CELLULAR COMPONENTS

Oxygen radicals produce cellular dysfunction by reacting with lipids, proteins, carbohydrates, and DNA to extract electrons (summarized in Fig. 24.7). Evidence of free radical damage has been described in over 100 disease states. In some of these diseases, free radical damage is the primary cause of the disease; in others, it enhances complications of the disease.

A. Membrane Attack: Formation of Lipid and Lipid Peroxy Radicals

Chain reactions that form lipid free radicals and lipid peroxides in membranes make a major contribution to ROS-induced injury (Fig. 24.8). An initiator (such as a hydroxyl radical produced locally in the Fenton reaction) begins the chain reaction. It extracts a hydrogen atom, preferably from the double bond of a polyunsaturated fatty acid in a membrane lipid. The chain reaction is propagated when O_2 adds to form lipid peroxyl radicals and lipid peroxides. Eventually lipid degradation occurs, forming such products as malondialdehyde (from fatty acids with three or more double bonds), and ethane and pentane (from the ω-terminal carbons of 3 and 6 fatty acids, respectively). Malondialdehyde appears in the blood and urine and is used as an indicator of free radical damage.

Peroxidation of lipid molecules invariably changes or damages lipid molecular structure. In addition to the self-destructive nature of membrane lipid peroxidation, the aldehydes that are formed can cross-link proteins. When the damaged lipids are the constituents of biologic membranes, the cohesive lipid bilayer arrangement and stable structural organization is disrupted (see Fig. 24.7). Disruption of mitochondrial membrane integrity may result in further free radical production.

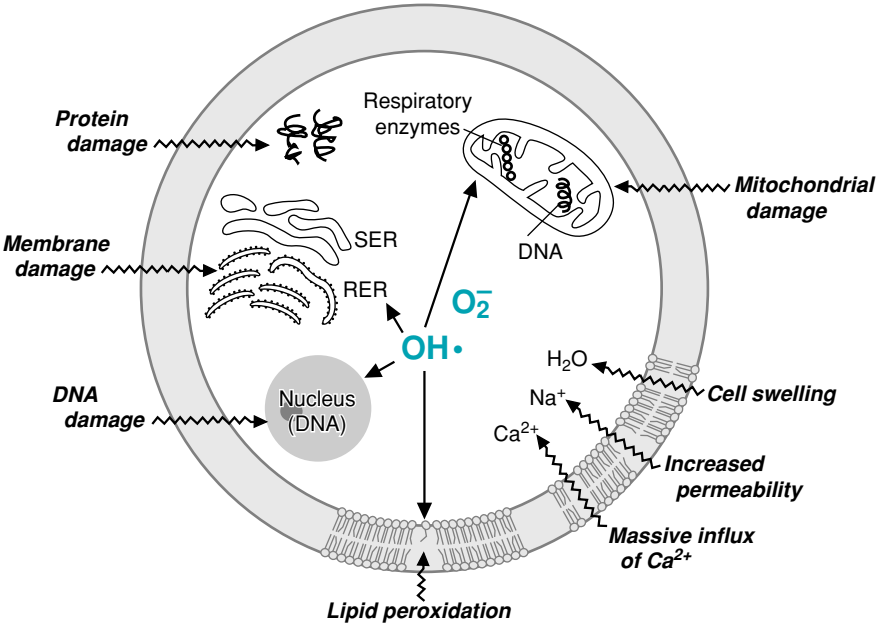


Fig 24.7. Free radical–mediated cellular injury. Superoxide and the hydroxyl radical initiate lipid peroxidation in the cellular, mitochondrial, nuclear, and endoplasmic reticulum membranes. The increase in cellular permeability results in an influx of Ca^{2+}, which causes further mitochondrial damage. The cysteine sulfhydryl groups and other amino acid residues on proteins are oxidized and degraded. Nuclear and mitochondrial DNA can be oxidized, resulting in strand breaks and other types of damage. RNOS (NO, NO_2, and peroxynitrite) have similar effects.

The appearance of lipofuscin granules in many tissues increases during aging. The pigment lipofuscin (from the Greek "lipos" for lipids and the Latin "fuscus" for dark) consists of a heterogeneous mixture of cross-linked polymerized lipids and protein formed by reactions between amino acid residues and lipid peroxidation products, such as malondialdehyde. These cross-linked products are probably derived from peroxidatively damaged cell organelles that were autophagocytized by lysosomes but could not be digested. When these dark pigments appear on the skin of the hands in aged individuals, they are referred to as "liver spots," a traditional hallmark of aging. In **Les Dopaman** and other patients with Parkinson's disease, lipofuscin appears as Lewy bodies in degenerating neurons.

Evidence of protein damage shows up in many diseases, particularly those associated with aging. In patients with cataracts, proteins in the lens of the eye exhibit free radical damage and contain methionine sulfoxide residues and tryptophan degradation products.

B. Proteins and Peptides

In proteins, the amino acids proline, histidine, arginine, cysteine, and methionine are particularity susceptible to hydroxyl radical attack and oxidative damage. As a consequence of oxidative damage, the protein may fragment or residues cross-link with other residues. Free radical attack on protein cysteine residues can result in cross-linking and formation of aggregates that prevents their degradation. However, oxidative damage increases the susceptibility of other proteins to proteolytic digestion.

Free radical attack and oxidation of the cytsteine sulfhydryl residues of the tripeptide glutathione (γ-glutamyl-cysteinyl-glycine; see section V.A.3.) increases oxidative damage throughout the cell. Glutathione is a major component of cellular defense against free radical injury, and its oxidation reduces its protective effects.

C. DNA

Oxygen-derived free radicals are also a major source of DNA damage. Approximately 20 types of oxidatively altered DNA molecules have been identified. The nonspecific binding of Fe^{2+} to DNA facilitates localized production of the hydroxyl radical, which can cause base alterations in the DNA (Fig. 24.9). It also can attack the deoxyribose backbone and cause strand breaks. This DNA damage can be repaired to some extent by the cell (see Chapter 12), or minimized by apoptosis of the cell.

III. NITRIC OXIDE AND REACTIVE NITROGEN-OXYGEN SPECIES (RNOS)

Nitric oxide (NO) is an oxygen-containing free radical which, like O_2, is both essential to life and toxic. NO has a single electron, and therefore binds to other compounds containing single electrons, such as Fe^{3+}. As a gas, it diffuses through the cytosol and lipid membranes and into cells. At low concentrations, it functions physiologically as a neurotransmitter and a hormone that causes vasodilation. However, at high concentrations, it combines with O_2 or with superoxide to form additional reactive and toxic species containing both nitrogen and oxygen (RNOS). RNOS are involved in neurodegenerative diseases, such as Parkinson's disease, and in chronic inflammatory diseases, such as rheumatoid arthritis.

A. Nitric Oxide Synthase

At low concentrations, nitric oxide serves as a neurotransmitter or a hormone. It is synthesized from arginine by nitric oxide synthases (Fig 24.10). As a gas, it is able to diffuse through water and lipid membranes, and into target cells. In the target cell, it exerts its physiologic effects by high-affinity binding to Fe-heme in the enzyme guanylyl cyclase, thereby activating a signal transduction cascade. However, NO is rapidly inactivated by nonspecific binding to many molecules, and therefore cells that produce NO need to be close to the target cells.

The body has three different tissue-specific isoforms of NO synthase, each encoded by a different gene: neuronal nitric oxide synthase (nNOS, isoform I), inducible nitric oxide synthase (iNOS, isoform II), and endothelial nitric oxide synthase (eNOS, isoform III). nNOS and eNOS are tightly regulated by Ca^{2+} concentration to produce the small amounts of NO required for its role as a neurotransmitter and hormone. In contrast, iNOS is present in many cells of the immune system and cell types with a similar lineage, such as macrophages and

Nitroglycerin, in tablet form, is often given to patients with coronary artery disease who experience ischemia-induced chest pain (angina). The nitroglycerin decomposes in the blood, forming NO, a potent vasodilator, which increases blood flow to the heart and relieves the angina.

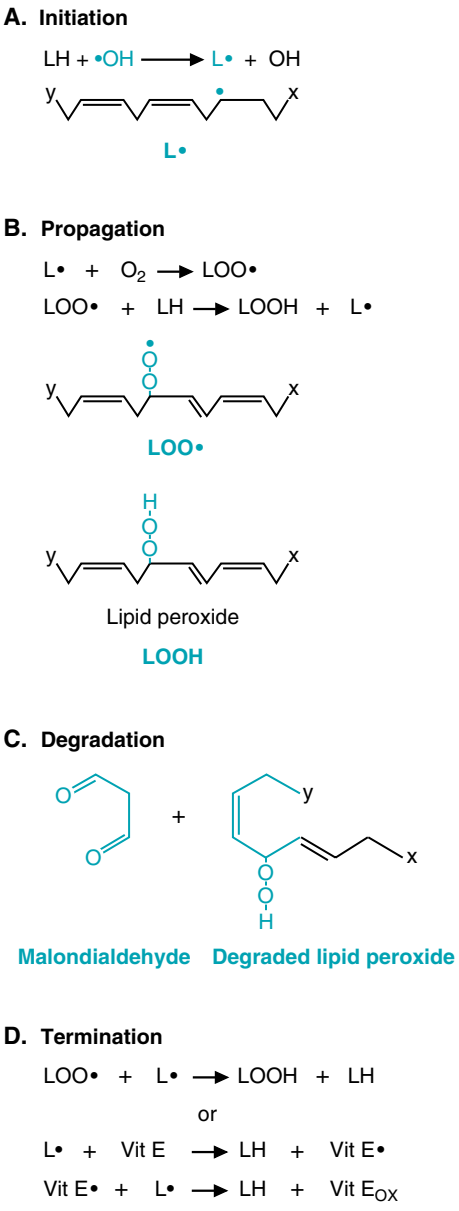


Fig 24.8. Lipid peroxidation: a free radical chain reaction. **A.** Lipid peroxidation is initiated by a hydroxyl or other radical that extracts a hydrogen atom from a polyunsaturated lipid (LH), thereby forming a lipid radical (L•). **B.** The free radical chain reaction is propagated by reaction with O_2, forming the lipid peroxy radical (LOO•) and lipid peroxide (LOOH). **C.** Rearrangements of the single electron result in degradation of the lipid. Malondialdehyde, one of the compounds formed, is soluble and appears in blood. **D.** The chain reaction can be terminated by vitamin E and other lipid-soluble antioxidants that donate single electrons. Two subsequent reduction steps form a stable, oxidized antioxidant.

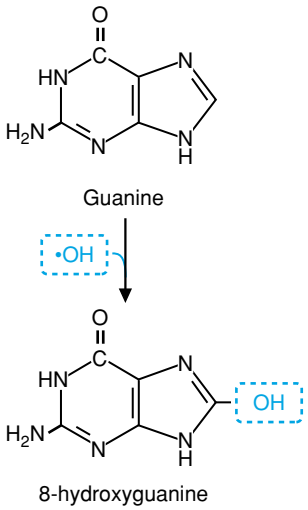


Fig 24.9. Conversion of guanine to 8-hydroxyguanine by the hydroxy radical. The amount of 8-hydroxyguanosine present in cells can be used to estimate the amount of oxidative damage they have sustained. The addition of the hydroxyl group to guanine allows it to mispair with T residues, leading to the creation of a daughter molecule with an A-T base pair in this position.

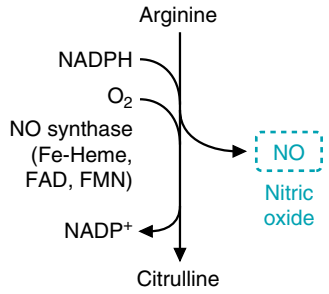


Fig 24.10. Nitric oxide synthase synthesizes the free radical NO. Like cytochrome P450 enzymes, NO synthase uses Fe-heme, FAD, and FMN to transfer single electrons from NADPH to O_2.

brain astroglia. This isoenzyme of nitric oxide synthase is regulated principally by induction of gene transcription, and not by changes in Ca^{2+} concentration. It produces high and toxic levels of NO to assist in killing invading microorganisms. It is these very high levels of NO that are associated with generation of RNOS and NO toxicity.

B. NO Toxicity

The toxic actions of NO can be divided into two categories: direct toxic effects resulting from binding to Fe-containing proteins, and indirect effects mediated by compounds formed when NO combines with O_2 or with superoxide to form RNOS.

1. DIRECT TOXIC EFFECTS OF NO

NO, as a radical, exerts direct toxic effects by combining with Fe-containing compounds that also have single electrons. Major destructive sites of attack include Fe-S centers (e.g., electron transport chain complexes I-III, aconitase) and Fe-heme proteins (e.g., hemoglobin and electron transport chain cytochromes). However, there is usually little damage because NO is present in low concentrations and Fe-heme compounds are present in excess capacity. NO can cause serious damage, however, through direct inhibition of respiration in cells that are already compromised through oxidative phosphorylation diseases or ischemia.

2. RNOS TOXICITY

When present in very high concentrations (e.g., during inflammation), NO combines nonenzymatically with superoxide to form peroxynitrite ($ONOO^-$), or with O_2 to form N_2O_3 (Fig. 24.11). Peroxynitrite, although not a free radical, is a strong

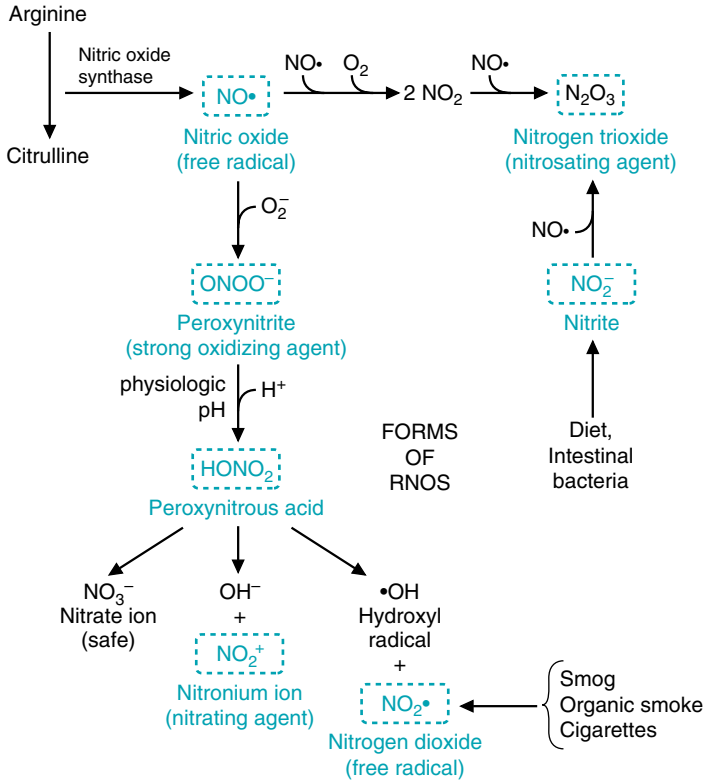


Fig 24.11. Formation of RNOS from nitric oxide. RNOS are shown in blue. The type of damage caused by each RNOS is shown in parentheses. Of all the nitrogen–oxygen-containing compounds shown, only nitrate is relatively nontoxic.

oxidizing agent that is stable and directly toxic. It can diffuse through the cell and lipid membranes to interact with a wide range of targets, including protein methionine and -SH groups (e.g., Fe-S centers in the electron transport chain). It also breaks down to form additional RNOS, including the free radical nitrogen dioxide (NO_2), an effective initiator of lipid peroxidation. Peroxynitrite products also nitrate aromatic rings, forming compounds such as nitrotyrosine or nitroguanosine. N_2O_3, which can be derived either from NO_2 or nitrite, is the agent of nitrosative stress, and nitrosylates sulfhydryl and similarly reactive groups in the cell. Nitrosylation will usually interefere with the proper functioning of the protein or lipid that has been modified. Thus, RNOS can do as much oxidative and free radical damage as non–nitrogen-containing ROS, as well as nitrating and nitrosylating compounds. The result is widespread and includes inhibition of a large number of enzymes; mitochondrial lipid peroxidation; inhibition of the electron transport chain and energy depletion; single-stranded or double-stranded breaks in DNA; and modification of bases in DNA.

NO_2 is one of the toxic agents present in smog, automobile exhaust, gas ranges, pilot lights, cigarette smoke, and smoke from forest fires or burning buildings.

IV. FORMATION OF FREE RADICALS DURING PHAGOCYTOSIS AND INFLAMMATION

In response to infectious agents and other stimuli, phagocytic cells of the immune system (neutrophils, eosinophils, and monocytes/macrophages) exhibit a rapid consumption of O_2 called the respiratory burst. The respiratory burst is a major source of superoxide, hydrogen peroxide, the hydroxyl radical, hypochlorous acid (HOCl), and RNOS. The generation of free radicals is part of the human antimicrobial defense system and is intended to destroy invading microorganisms, tumor cells, and other cells targeted for removal.

A. NADPH Oxidase

The respiratory burst results from the activity of NADPH oxidase, which catalyzes the transfer of an electron from NADPH to O_2 to form superoxide (Fig. 24.12). NADPH oxidase is assembled from cytosol and membranous proteins recruited into the phagolysosome membrane as it surrounds an invading microorganism.

Superoxide is released into the intramembranous space of the phagolysosome, where it is generally converted to hydrogen peroxide and other ROS that are effective against bacteria and fungal pathogens. Hydrogen peroxide is formed by superoxide dismutase, which may come from the phagocytic cell or the invading microorganism.

In patients with chronic granulomatous disease, phagocytes have genetic defects in NADPH oxidase. NADPH oxidase has four different subunits (two in the cell membrane and two recruited from the cytosol), and the genetic defect may be in any of the genes that encode these subunits. The membrane catalytic subunit β of NADPH oxidase is a 91-kDa flavocytochrome glycoprotein. It transfers electrons from bound NADPH to FAD, which transfers them to the Fe–heme components. The membranous α-subunit (p22) is required for stabilization. Two additional cytosolic proteins (p47phox and p67phox) are also required for assembly of the complex. Rac, a monomeric GTPase in the Ras subfamily of the Rho superfamily (see Chapter 9), is also required for assembly. The 91-kDa subunit is affected most often in X-linked chronic granulatomous disease, whereas the α-subunit is affected in a rare autosomal recessive form. The cytosolic subunits are affected most often in patients with the autosomal recessive form of granulomatous disease. In addition to their enhanced susceptibility to bacterial and fungal infections, these patients suffer from an apparent dysregulation of normal inflammatory responses.

B. Myeloperoxidase and HOCl

The formation of hypochlorous acid from H_2O_2 is catalyzed by myeloperoxidase, a heme-containing enzyme that is present only in phagocytic cells of the immune system (predominantly neutrophils).

Myeloperoxidase **Dissociation**

$$H_2O_2 + Cl^- + H^+ \rightarrow HOCl + H_2O \rightarrow {}^-OCl + H^+ + H_2O$$

Myeloperoxidase contains two Fe heme-like centers, which give it the green color seen in pus. Hypochlorous acid is a powerful toxin that destroys bacteria within seconds through halogenation and oxidation reactions. It oxidizes many Fe and S-containing groups (e.g., sulfhydryl groups, iron-sulfur centers, ferredoxin, heme-proteins, methionine), oxidatively decarboxylates and deaminates proteins, and cleaves peptide bonds. Aerobic bacteria under attack rapidly lose membrane

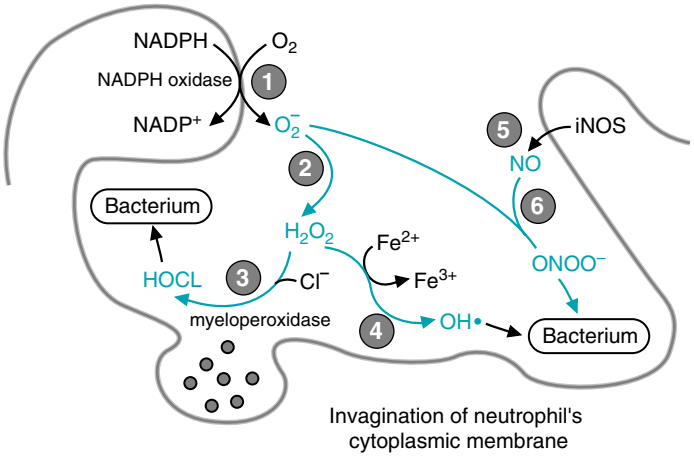


Fig 24.12. Production of reactive oxygen species during the phagocytic respiratory burst by activated neutrophils. (1) Activation of NADPH oxidase on the outer side of the plasma membrane initiates the respiratory burst with the generation of superoxide. During phagocytosis, the plasma membrane invaginates, so superoxide is released into the vacuole space. (2) Superoxide (either spontaneously or enzymatically via superoxide dismutase [SOD]) generates H_2O_2. (3) Granules containing myeloperoxidase are secreted into the phagosome, where myeloperoxidase generates HOCl and other halides. (4) H_2O_2 can also generate the hydroxyl radical from the Fenton reaction. (5) Inducible nitric oxide synthase may be activated and generate NO. (6) Nitric oxide combines with superoxide to form peroxynitrite, which may generate additional RNOS. The result is an attack on the membranes and other components of phagocytosed cells, and eventual lysis. The whole process is referred to as the respiratory burst because it lasts only 30 to 60 minutes and consumes O_2.

transport, possibly because of damage to ATP synthase or electron transport chain components (which reside in the plasma membrane of bacteria).

C. RNOS and Inflammation

When human neutrophils of the immune system are activated to produce NO, NADPH oxidase is also activated. NO reacts rapidly with superoxide to generate peroxynitrite, which forms additional RNOS. NO also may be released into the surrounding medium, to combine with superoxide in target cells.

In a number of disease states, free radical release by neutrophils or macrophages during an inflammation contributes to injury in the surrounding tissues. During stroke or myocardial infarction, phagocytic cells that move into the ischemic area to remove dead cells may increase the area and extent of damage. The self-perpetuating mechanism of radical release by neutrophils during inflammation and immune complex formation may explain some of the features of chronic inflammation in patients with rheumatoid arthritis. As a result of free radical release, the immunoglobulin G (IgG) proteins present in the synovial fluid are partially oxidized, which improves their binding with the rheumatoid factor antibody. This binding, in turn, stimulates the neutrophils to release more free radicals.

During **Cora Nari's** ischemia (decreased blood flow), the ability of her heart to generate ATP from oxidative phosphorylation was compromised. The damage appeared to accelerate when oxygen was first reintroduced (reperfused) into the tissue. During ischemia, CoQ and the other single-electron components of the electron transport chain become saturated with electrons. When oxygen is reintroduced (reperfusion), electron donation to O_2 to form superoxide is increased. The increase of superoxide results in enhanced formation of hydrogen peroxide and the hydroxyl radical. Macrophages in the area to clean up cell debris from ischemic injury produce nitric oxide, which may further damage mitochondria by generating RNOS that attack Fe-S centers and cytochromes in the electron transport chain membrane lipids. Thus, the RNOS may increase the infarct size.

V. CELLULAR DEFENSES AGAINST OXYGEN TOXICITY

Our defenses against oxygen toxicity fall into the categories of antioxidant defense enzymes, dietary and endogenous antioxidants (free radical scavengers), cellular compartmentation, metal sequestration, and repair of damaged cellular components. The antioxidant defense enzymes react with ROS and cellular products of free radical chain reactions to convert them to nontoxic products. Dietary antioxidants, such as vitamin E and flavonoids, and endogenous antioxidants, such as urate, can

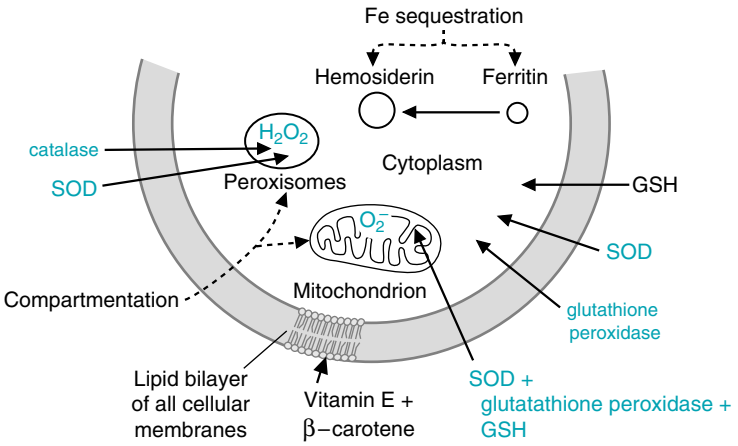


Fig 24.13 Compartmentation of free radical defenses. Various defenses against ROS are found in the different subcellular compartments of the cell. The location of free radical defense enzymes (shown in blue) matches the type and amount of ROS generated in each subcellular compartment. The highest activities of these enzymes are found in the liver, adrenal gland, and kidney, where mitochondrial and peroxisomal contents are high, and cytochrome P450 enzymes are found in abundance in the smooth ER. The enzymes superoxide dismutase (SOD) and glutathione peroxidase are present as isozymes in the different compartments. Another form of compartmentation involves the sequestration of Fe, which is stored as mobilizable Fe in ferritin. Excess Fe is stored in nonmobilizable hemosiderin deposits. Glutathione (GSH) is a nonenzymatic antioxidant.

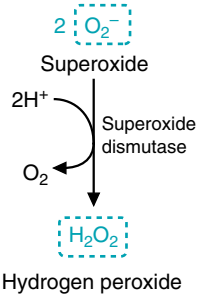


Fig 24.14. Superoxide dismutase converts superoxide to hydrogen peroxide, which is nontoxic unless converted to other ROS.

terminate free radical chain reactions. Defense through compartmentation refers to separation of species and sites involved in ROS generation from the rest of the cell (Fig. 24.13). For example, many of the enzymes that produce hydrogen peroxide are sequestered in peroxisomes with a high content of antioxidant enzymes. Metals are bound to a wide range of proteins within the blood and in cells, preventing their participation in the Fenton reaction. Iron, for example, is tightly bound to its storage protein, ferritin and cannot react with hydrogen peroxide. Repair mechanisms for DNA, and for removal of oxidized fatty acids from membrane lipids, are available to the cell. Oxidized amino acids on proteins are continuously repaired through protein degradation and resynthesis of new proteins.

A. Antioxidant Scavenging Enzymes

The enzymatic defense against ROS includes superoxide dismutase, catalase, and glutathione peroxidase.

1. SUPEROXIDE DISMUTASE (SOD)

Conversion of superoxide anion to hydrogen peroxide and O_2 (dismutation) by superoxide dismutase (SOD) is often called the primary defense against oxidative stress because superoxide is such a strong initiator of chain reactions (Fig 24.14). SOD exists as three isoenzyme forms, a Cu^+-Zn^{2+} form present in the cytosol, a Mn^{2+} form present in mitochondria, and a Cu^+-Zn^{2+} form found extracellularly. The activity of Cu^+-Zn^{2+} SOD is increased by chemicals or conditions (such as hyperbaric oxygen) that increase the production of superoxide.

In the body, iron and other metals are sequestered from interaction with ROS or O_2 by their binding to transport proteins (haptoglobin, hemoglobin, transferrin, ceruloplasmin, and metallothionein) in the blood, and to intracellular storage proteins (ferritin, hemosiderin). Metals also are found bound to many enzymes, particularly those that react with O_2. Usually, these enzymes have reaction mechanisms that minimize nonspecific single-electron transfer from the metal to other compounds.

The intracellular form of the Cu^+–Zn^{2+} superoxide dismutase is encoded by the SOD1 gene. To date, 58 mutations in this gene have been discovered in individuals affected by familial amyotrophic lateral sclerosis (Lou Gehrig's disease). How a mutation in this gene leads to the symptoms of this disease has yet to be understood. It is important to note that only 5 to 10% of the total cases of diagnosed amyotrophic lateral sclerosis are caused by the familial form.

Why does the cell need such a high content of SOD in mitochondria?

Premature infants with low levels of lung surfactant (see Chapter 33) require oxygen therapy. The level of oxygen must be closely monitored to prevent retinopathy and subsequent blindness (the retinopathy of prematurity) and to prevent bronchial pulmonary dysplasia. The tendency for these complications to develop is enhanced by the possibility of low levels of SOD and vitamin E in the premature infant.

A: Mitochondria are major sites for generation of superoxide from the interaction of CoQ and O_2. The Mn^{2+} superoxide dismutase present in mitochondria is not regulated through induction/repression of gene transcription, presumably because the rate of superoxide generation is always high. Mitochondria also have a high content of glutathione and glutathione peroxidase, and can thus convert H_2O_2 to H_2O and prevent lipid peroxidation.

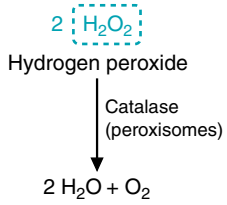


Fig 24.15. Catalase reduces hydrogen peroxide. (ROS is shown in a blue box).

Selenium (Se) is present in human proteins principally as selenocysteine (cysteine with the sulfur group replaced by Se, abbreviated sec). This amino acid functions in catalysis, and has been found in 11 or more human enzymes, including the four enzymes of the glutathione peroxidase family. Selenium is supplied in the diet as selenomethionine from plants (methionine with the Se replacing the sulfur), selenocysteine from animal foods, and inorganic selenium. Se from all of these sources can be converted to selenophosphate. Selenophosphate reacts with a unique tRNA containing bound serine to form a selenocysteine-tRNA, which incorporates selenocystiene into the appropriate protein as it is being synthesized. Se homeostasis in the body is controlled principally through regulation of its secretion as methylated Se. The current dietary requirement is approximately 70 μg/day for adult males and 55 μg for females. Deficiency symptoms reflect diminished antioxidant defenses and include symptoms of vitamin E deficiency.

2. CATALASE

Hydrogen peroxide, once formed, must be reduced to water to prevent it from forming the hydroxyl radical in the Fenton reaction or Haber–Weiss reactions (see Fig. 24.4) One of the enzymes capable of reducing hydrogen peroxide is catalase (Fig.24.15). Catalase is found principally in peroxisomes, and to a lesser extent in the cytosol and microsomal fraction of the cell. The highest activities are found in tissues with a high peroxisomal content (kidney and liver). In cells of the immune system, catalase serves to protect the cell against its own respiratory burst.

3. GLUTATHIONE PEROXIDASE AND GLUTATHIONE REDUCTASE

Glutathione (γ-glutamylcysteinylglycine) is one of the body's principal means of protecting against oxidative damage (see also Chapter 29). Glutathione is a tripeptide composed of glutamate, cysteine, and glycine, with the amino group of cysteine joined in peptide linkage to the γ-carboxyl group of glutamate (Fig. 24.16). In reactions catalyzed by glutathione peroxidases, the reactive sulfhydryl groups reduce hydrogen peroxide to water and lipid peroxides to nontoxic alcohols. In these reactions, two glutathione molecules are oxidized to form a single molecule, glutathione disulfide. The sulfhydryl groups are also oxidized in nonenzymatic chain terminating reactions with organic radicals.

Glutathione peroxidases exist as a family of selenium enzymes with somewhat different properties and tissue locations. Within cells, they are found principally in the cytosol and mitochondria, and are the major means for removing H_2O_2 produced outside of peroxisomes. They contribute to our dietary requirement for selenium and account for the protective effect of selenium in the prevention of free radical injury.

Once oxidized glutathione (GSSG) is formed, it must be reduced back to the sulfhydryl form by glutathione reductase in a redox cycle (Fig. 24.17). Glutathione reductase contains an FAD, and catalyzes transfer of electrons from NADPH to the disulfide bond of GSSG. NADPH is, thus, essential for protection against free radical injury. The major source of NADPH for this reaction is the pentose phosphate pathway (see Chapter 29).

B. Nonenzymatic Antioxidants (Free Radical Scavengers)

Free radical scavengers convert free radicals to a nonradical nontoxic form in nonenzymatic reactions. Most free radical scavengers are antioxidants, compounds

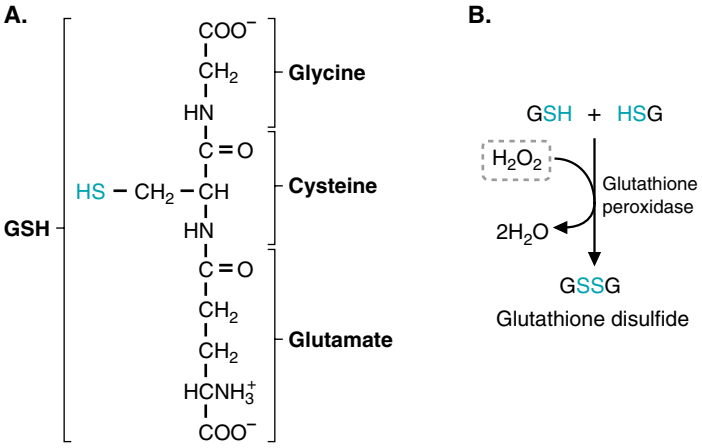


Fig 24.16. Glutathione peroxidase reduces hydrogen peroxide to water. A. The structure of glutathione. The sulfhydryl group of glutathione, which is oxidized to a disulfide, is shown in blue. B. Glutathione peroxidase transfer electrons from glutathione (GSH) to hydrogen peroxide.

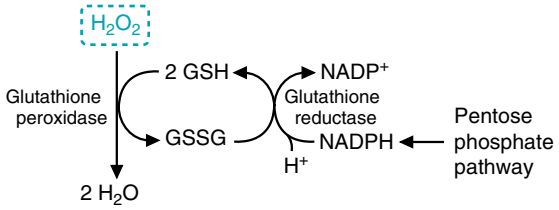


Fig 24.17. Glutathione redox cycle. Glutathione reductase regenerates reduced glutathione. (ROS is shown in the blue box).

that neutralize free radicals by donating a hydrogen atom (with its one electron) to the radical. Antioxidants, therefore, reduce free radicals and are themselves oxidized in the reaction. Dietary free radical scavengers (e.g., vitamin E, ascorbic acid, carotenoids, and flavonoids) as well as endogenously produced free radical scavengers (e.g., urate and melatonin) have a common structural feature, a conjugated double bond system that may be an aromatic ring.

1. VITAMIN E

Vitamin E (α-tocopherol), the most widely distributed antioxidant in nature, is a lipid-soluble antioxidant vitamin that functions principally to protect against lipid peroxidation in membranes (see Fig. 24.13). Vitamin E comprises a number of tocopherols that differ in their methylation pattern. Among these, α-tocopherol is the most potent antioxidant and present in the highest amount in our diet (Fig. 24.18).

Vitamin E is an efficient antioxidant and nonenzymatic terminator of free radical chain reactions, and has little pro-oxidant activity. When Vitamin E donates an electron to a lipid peroxy radical, it is converted to a free radical form that is stabilized by resonance. If this free radical form were to act as a pro-oxidant and abstract an electron from a polyunsaturated lipid, it would be oxidizing that lipid and actually propagate the free radical chain reaction. The chemistry of vitamin E is such that it has a much greater tendency to donate a second electron and go to the fully oxidized form.

2. ASCORBIC ACID

Although ascorbate (vitamin C) is an oxidation-reduction coenzyme that functions in collagen synthesis and other reactions, it also plays a role in free radical defense. Reduced ascorbate can regenerate the reduced form of vitamin E through donating electrons in a redox cycle (Fig. 24.19). It is water-soluble and circulates unbound in blood and extracellular fluid, where it has access to the lipid-soluble vitamin E present in membranes and lipoprotein particles.

3. CAROTENOIDS

Carotenoids is a term applied to β-carotene (the precursor of vitamin A) and similar compounds with functional oxygen-containing substituents on the rings, such as zeaxanthin and lutein (Fig. 24.20). These compounds can exert antioxidant effects, as well as quench singlet O_2 (singlet oxygen is a highly reactive oxygen species in which there are no unpaired electrons in the outer orbitals, but there is one orbital that is completely empty). Epidemiologic studies have shown a correlation between diets high in fruits and vegetables and health benefits, leading to the hypothesis that carotenoids might slow the progression of cancer, atherosclerosis, and other degenerative diseases by acting as chain-breaking antioxidants. However, in clinical

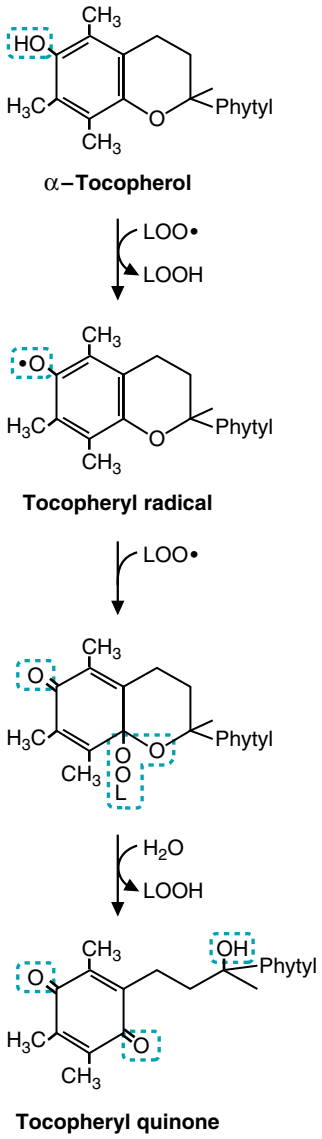


Fig 24.18. Vitamin E (α-tocopherol) terminates free radical lipid peroxidation by donating single electrons to lipid peroxyl radicals (LOO•) to form the more stable lipid peroxide, LOOH. In so doing, the α-tocopherol is converted to the fully oxidized tocopheryl quinone.

Vitamin E is found in the diet in the lipid fractions of some vegetable oils and in liver, egg yolks, and cereals. It is absorbed together with lipids, and fat malabsorption results in symptomatic deficiencies. Vitamin E circulates in the blood in lipoprotein particles. Its deficiency causes neurologic symptoms, probably because the polyunsaturated lipids in myelin and other membranes of the nervous system are particularly sensitive to free radical injury.

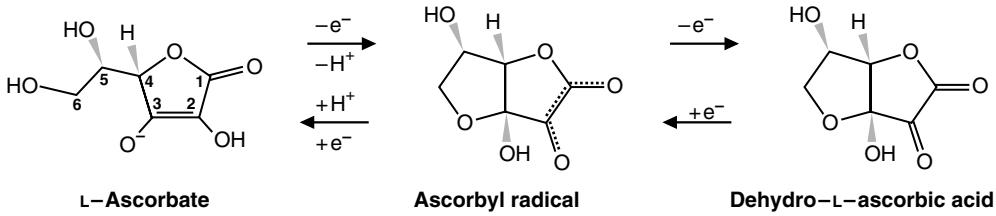


Fig 24.19. L-Ascorbate (the reduced form) donates single electrons to free radicals or disulfides in two steps as it is oxidized to dehydro-L-ascorbic acid. Its principle role in free radical defense is probably regeneration of vitamin E. However, it also may react with superoxide, hydrogen peroxide, hypochlorite, the hydroxyl and peroxyl radicals, and NO_2.

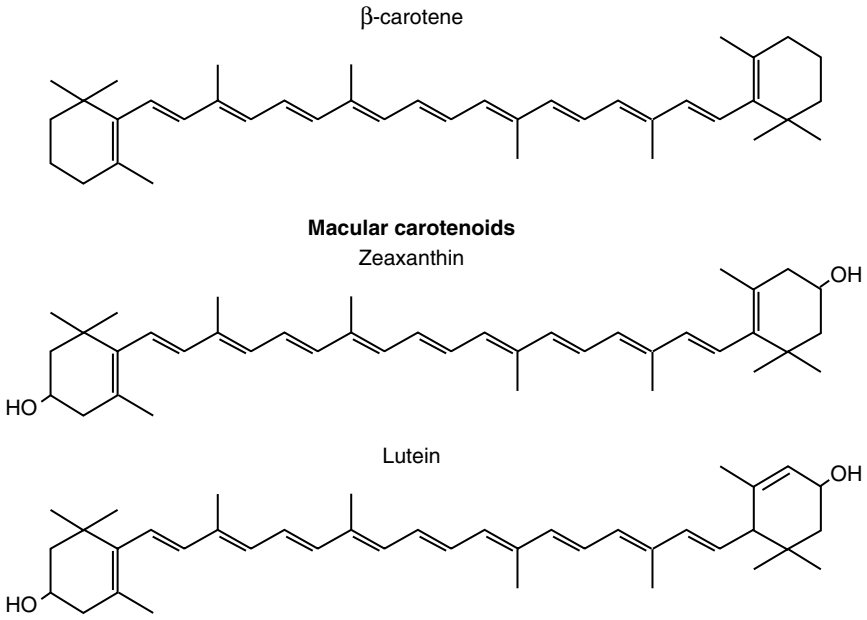


Fig 24.20. Carotenoids are compounds related in structure to β-carotene. Lutein and zeathanthin (the macular carotenoids) are analogs containing hydroxyl groups.

Epidemiologic evidence suggests that individuals with a higher intake of foods containing vitamin E, β-carotene, and vitamin C have a somewhat lower risk of cancer and certain other ROS-related diseases than do individuals on diets deficient in these vitamins. However, studies in which well-nourished populations were given supplements of these antioxidant vitamins found either no effects or harmful effects compared with the beneficial effects from eating foods containing a wide variety of antioxidant compounds. Of the pure chemical supplements tested, there is evidence only for the efficacy of vitamin E. In two clinical trials, β-carotene (or β-carotene + vitamin A) was associated with a higher incidence of lung cancer among smokers and higher mortality rates. In one study, vitamin E intake was associated with a higher incidence of hemorrhagic stroke (possibly because of vitamin K mimicry).

trials, β-carotene supplements had either no effect or an undesirable effect. Its ineffectiveness may be due to the pro-oxidant activity of the free radical form.

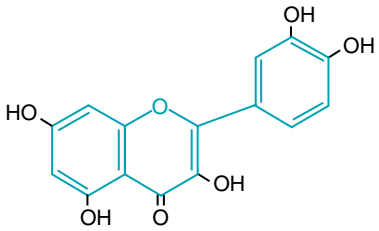
In contrast, epidemiologic studies relating the intake of lutein and zeoxanthin with decreased incidence of age-related macular degeneration have received progressive support. These two carotenoids are concentrated in the macula (the central portion of the retina) and are called the macular carotenoids.

Age-related macular degeneration (AMD) is the leading cause of blindness in the United States among persons older than 50 years of age, and it affects 1.7 million people worldwide. In AMD, visual loss is related to oxidative damage to the retinal pigment epithelium (RPE) and the choriocapillaris epithelium. The photoreceptor/retinal pigment complex is exposed to sunlight, it is bathed in near arterial levels of oxygen, and the membranes contain high concentrations of polyunsaturated fatty acids, all of which are conducive to oxidative damage. Lipofuscin granules, which accumulate in the RPE throughout life, may serve as photosensitizers, initiating damage by absorbing blue light and generating singlet oxygen that forms other radicals. Dark sunglasses are protective. Epidemiologic studies showed that the intake of lutein and zeanthin in dark green leafy vegetables (e.g., spinach and collard greens) also may be protective. Lutein and zeaxanthein accumulate in the macula and protect against free radical damage by absorbing blue light and quenching singlet oxygen.

4. OTHER DIETARY ANTIOXIDANTS

Flavonoids are a group of structurally similar compounds containing two spatially separate aromatic rings that are found in red wine, green tea, chocolate, and other plant-derived foods (Fig. 24.21). Flavonoids have been hypothesized to contribute to our free radical defenses in a number of ways. Some flavonoids inhibit enzymes responsible for superoxide anion production, such as xanthine oxidase. Others efficiently chelate Fe and Cu, making it impossible for these metals to participate in the Fenton reaction. They also may act as free radical scavengers by donating electrons to superoxide or lipid peroxy radicals, or stabilize free radicals by complexing with them.

It is difficult to tell how much dietary flavonoids contribute to our free radical defense system; they have a high pro-oxidant activity and are poorly absorbed. Nonetheless, we generally consume large amounts of flavonoids (approximately 800 mg/day), and there is evidence that they can contribute to the maintenance of vitamin E as an antioxidant.



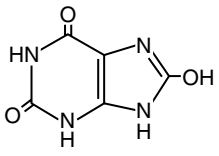
A flavonoid

Fig 24.21. The flavonoid quercetin. All flavonoids have the same ring structure, shown in blue. They differ in ring substituents (=O, -OH, and OCH_3). Quercetin is effective in Fe chelation and antioxidant activity. It is widely distributed in fruits (principally in the skins) and in vegetables (e.g., onions).

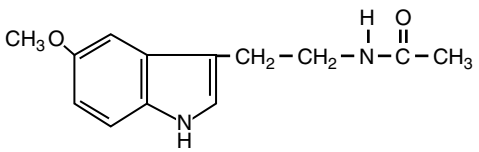
5. ENDOGENOUS ANTIOXIDANTS

A number of compounds synthesized endogenously for other functions, or as urinary excretion products, also function nonenzymatically as free radical antioxidants. Uric acid is formed from the degradation of purines and is released into extracellular fluids, including blood, saliva, and lung lining fluid (Fig. 24.22). Together with protein thiols, it accounts for the major free radical trapping capacity of plasma. It is particularly important in the upper airways, where there are few other antioxidants. It can directly scavenge hydroxyl radicals, oxyheme oxidants formed between the reaction of hemoglobin and peroxy radicals, and peroxyl radicals themselves. Having acted as a scavenger, uric acid produces a range of oxidation products that are subsequently excreted.

Melatonin, which is a secretory product of the pineal gland, is a neurohormone that functions in regulation of our circadian rhythm, light–dark signal transduction, and sleep induction. In addition to these receptor-mediated functions, it functions as a nonenzymatic free radical scavenger that donates an electron (as hydrogen) to "neutralize" free radicals. It also can react with ROS and RNOS to form addition products, thereby undergoing suicidal transformations. Its effectiveness is related to both its lack of pro-oxidant activity and its joint hydrophilic/hydrophobic nature that allows it to pass through membranes and the blood-brain barrier.



Uric acid



Melatonin

Fig 24.22. Endogenous antioxidants. Uric acid and melatonin both act to successively neutralize several molecules of ROS.

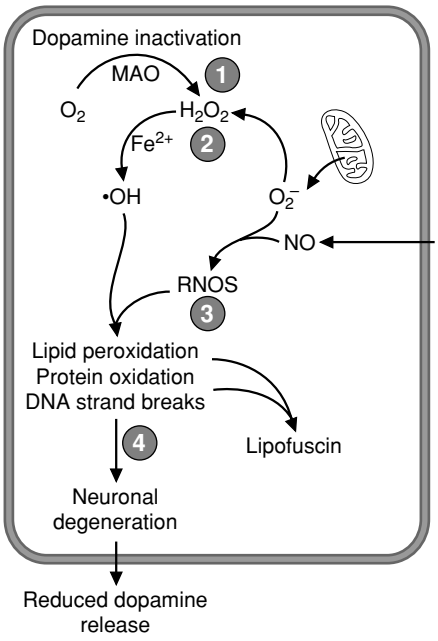


Fig 24.23. A model for the role of ROS and RNOS in neuronal degradation in Parkinson's disease. 1. Dopamine levels are reduced by monoamine oxidase, which generates H_2O_2. 2. Superoxide also can be produced by mitochondria, which SOD will convert to H_2O_2. Iron levels increase, which allows the Fenton reaction to proceed, generating hydroxyl radicals. 3. NO, produced by inducible nitric oxide synthase, reacts with superoxide to form RNOS. 4. The RNOS and hydroxyl radical lead to radical chain reactions that result in lipid peroxidation, protein oxidation, the formation of lipofuscin, and neuronal degeneration. The end result is a reduced production and release of dopamine, which leads to the clinical symptoms observed.

CLINICAL COMMENTS

Les Dopaman has "primary" parkinsonism. The pathogenesis of this disease is not well established and may be multifactorial (Fig. 24.23). The major clinical disturbances in Parkinson's disease are a result of dopamine depletion in the neostriatum, resulting from degeneration of dopaminergic neurons whose cell bodies reside in the substantia nigra pars compacta. The decrease in dopamine production is the result of severe degeneration of these nigrostriatal neurons. Although the agent that initiates the disease is unknown, a variety of studies support a role for free radicals in Parkinson's disease. Within these neurons, dopamine turnover is increased, dopamine levels are lower, glutathione is decreased, and lipofuscin (Lewy bodies) is increased. Iron levels are higher, and ferritin, the storage form of iron, is lower. Furthermore, the disease is mimicked by the compound 1-methyl-4-phenylpyridinium (MPP^+), an inhibitor of NADH dehydrogenase that increases superoxide production in these neurons. Even so, it is not known whether oxidative stress makes a primary or secondary contribution to the disease process.

Drug therapy is based on the severity of the disease. In the early phases of the disease, a monoamine oxidase B-inhibitor is used that inhibits dopamine degradation and decreases hydrogen peroxide formation. In later stages of the disease, patients are treated with levodopa (L-dopa), a precursor of dopamine.

Cora Nari experienced angina caused by severe ischemia in the ventricular muscle of her heart. The ischemia was caused by clots that formed at the site of atherosclerotic plaques within the lumen of the coronary arteries. When TPA was infused to dissolve the clots, the ischemic area of her heart was reperfused with oxygenated blood, resulting in ischemic–reperfusion injury. In her case, the reperfusion injury resulted in ventricular fibrillation.

During ischemia, several events occur simultaneously in cardiomyocytes. A decreased O_2 supply results in decreased ATP generation from mitochondrial oxidative phosphorylation and inhibition of cardiac muscle contraction. As a consequence, cytosolic AMP concentration increases, activating anaerobic glycolysis and lactic acid production. If ATP levels are inadequate to maintain Na^+, K^+ -ATPase activity, intracellular Na^+ increases, resulting in cellular swelling, a further increase in H^+ concentration, and increases of cytosolic and subsequently mitochondrial Ca^{2+} levels. The decrease in ATP and increase in Ca^{2+} may open the mitochondrial permeability transition pore, resulting in permanent inhibition of oxidative phosphorylation. Damage to lipid membranes is further enhanced by Ca^{2+} activation of phospholipases.

Reperfusion with O_2 allows recovery of oxidative phosphorylation, provided that the mitochondrial membrane has maintained some integrity and the mitochondrial transition pore can close. However, it also increases generation of free radicals. The transfer of electrons from CoQ• to O_2 to generate superoxide is increased. Endothelial production of superoxide by xanthine oxidase also may increase. These radicals may go on to form the hydroxyl radical, which can enhance the damage to components of the electron transport chain and mitochondrial lipids, as well as activate the

Currently, an intense study of ischemic insults to a variety of animal organs is underway, in an effort to discover ways of preventing reperfusion injury. These include methods designed to increase endogenous antioxidant activity, to reduce the generation of free radicals, and, finally, to develop exogenous antioxidants that, when administered before reperfusion, would prevent its injurious effects. Each of these approaches has met with some success, but their clinical application awaits further refinement. With the growing number of invasive procedures aimed at restoring arterial blood flow through partially obstructed coronary vessels, such as clot lysis, balloon or laser angioplasty, and coronary artery bypass grafting, development of methods to prevent ischemia–reperfusion injury will become increasingly urgent.

mitochondrial permeability transition. As macrophages move into the area to clean up cellular debris, they may generate NO and superoxide, thus introducing peroxynitrite and other free radicals into the area. Depending on the route and timing involved, the acute results may be cell death through necrosis, with slower cell death through apoptosis in the surrounding tissue.

In **Cora Nari's** case, oxygen was restored before permanent impairment of oxidative phosphorylation had occurred and the stage of irreversible injury was reached. However, reintroduction of oxygen induced ventricular fibrillation, from which she recovered.

BIOCHEMICAL COMMENTS

Protection Against Ozone in Lung Lining Fluid The lung lining fluid, a thin fluid layer extending from the nasal cavity to the most distal lung alveoli, protects the epithelial cells lining our airways from ozone and other pollutants. Although ozone is not a radical species, many of its toxic effects are mediated through generation of the classical ROS, as well as generation of aldehydes and ozonides. Polyunsaturated fatty acids represent the primary target for ozone, and peroxidation of membrane lipids is the most important mechanism of ozone-induced injury. However, ozone also oxidizes proteins.

Although most individuals are able to protect against small amounts of ozone in the atmosphere, even slightly elevated ozone concentrations produce respiratory symptoms in 10 to 20% of the healthy population.

The lung lining fluid has two phases; a gel-phase that traps microorganisms and large particles, and a sol (soluble) phase containing a variety of ROS defense mechanisms that prevent pollutants from reaching the underlying lung epithelial cells (Fig. 24.24). When the ozone level of inspired air is low, ozone is neutralized principally by uric acid (UA) present in the fluid lining the nasal cavity. In the proximal and distal regions of the respiratory tract, glutathione (GSH) and ascorbic acid (AA), in addition to UA, react directly with ozone. Ozone that escapes this antioxidant screen may react directly with proteins, lipids, and carbohydrates (CHO) to generate secondary oxidants, such as lipid peroxides, that can initiate chain reactions. A second layer of defense protects against these oxidation and peroxidation products: β-tocopherol (vitamin E) and glutathione react directly with lipid radicals; glutathione peroxidase reacts with hydrogen peroxide and lipid peroxides, and

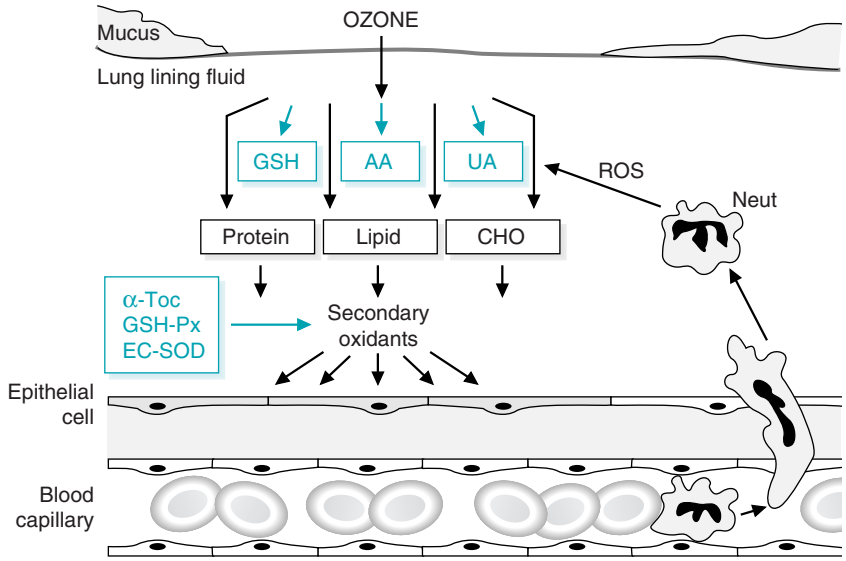


Fig 24.24. Protection against ozone in the lung lining fluid. GSH, glutathione; AA, ascorbic acid (vitamin C); UA, uric acid; CHO, carbohydrate; α-TOC, vitamin E; GSH-Px, glutathione peroxidase; ED-SOD, extracellular superoxide dismutase; *Neut*, neutrophil.

extracellular superoxide dismutase (EC-SOD) converts superoxide to hydrogen peroxide. However, oxidative stress may still overwhelm even this extensive defense network because ozone also promotes neutrophil migration into the lung lining fluid. Once activated, the neutrophils (Neut) produce a second wave of ROS (superoxide, HOCl, and NO).

Suggested References

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REVIEW QUESTIONS—CHAPTER 24

1. Which of the following vitamins or enzymes is unable to protect against free radical damage?

   (A) β-Carotene
   (B) Glutathione peroxidase
   (C) Superoxide dismutase
   (D) Vitamin B6
   (E) Vitamin C
   (F) Vitamin E

2. Superoxide dismutase catalyzes which of the following reactions?

   (A) $O_2^- + e^- + 2H^+$ yields H_2O_2
   (B) $2\ O_2^- + 2H^+$ yields $H_2O_2 + O_2$
   (C) $O_2^- + HO\bullet + H+$ yields $CO_2 + H_2O$
   (D) $H_2O_2 + O_2$ yields $4\ H_2O$
   (E) $O_2^- + H_2O_2 + H^+$ yields $2\ H_2O + O_2$

3. The mechanism of vitamin E as an antioxidant is best described by which of the following?

   (A) Vitamin E binds to free radicals and sequesters them from the contents of the cell.
   (B) Vitamin E participates in the oxidation of the radicals.
   (C) Vitamin E participates in the reduction of the radicals.
   (D) Vitamin E forms a covalent bond with the radicals, thereby stabilizing the radical state.
   (E) Vitamin E inhibits enzymes that produce free radicals.

4. An accumulation of hydrogen peroxide in a cellular compartment can be converted to dangerous radical forms in the presence of which metal?

   (A) Se
   (B) Fe
   (C) Mn
   (D) Mg
   (E) Mb

5. The level of oxidative damage to mitochondrial DNA is 10 times greater than that to nuclear DNA. This could be due, in part, to which of the following?

   (A) Superoxide dismutase is present in the mitochondria.
   (B) The nucleus lacks glutathione.
   (C) The nuclear membrane presents a barrier to reactive oxygen species.
   (D) The mitochondrial membrane is permeable to reactive oxygen species.
   (E) Mitochondrial DNA lacks histones.