Oxidative Chemistry and Physiology

Most cells within the body require a continual flow of oxygen to remain healthy, and many organ systems and metabolic processes within the body are at least partially focused on delivery of oxygen to cells. Oxygen transport is complicated by the fact that oxygen is poorly dissolvable in water (so much so that only about .003% of the blood plasma can be saturated with oxygen).

Oxygen transport is not only complex, but also risky. Atmospheric (or "molecular") oxygen, symbolized as O_2, is itself a free radical. (Free radicals are defined as molecules with at least one unpaired electron in their atomic structure.) To understand the risk that accompanies all oxygen metabolism, it is necessary to look further into the issue of unpaired electrons and free radicals.

Free Radicals

Within their atomic structure, all elements, including oxygen, have unique combinations of protons, neutrons, and electrons. The atomic structure of elements, and the function of their components, are reviewed in Chapter 3 (page 112, under "Elements"). Most molecules in the body have orbital regions that are occupied by a pair of electrons with opposite spin. "Spin" refers to the rotation of an electron around an axis, much like the earth rotates around an axis that passes through the north and south poles. The spin of an electron provides it with the properties of a magnet. Like magnets, electrons can "attract" each other when their spins move in opposite directions (clockwise versus counterclockwise). Oppositional spin allows electrons to share the same orbital with a moderate degree of stability by virtue of this magnetic attraction.

Free radical molecules with unpaired electrons do not enjoy this electromagnetic stability. In order to gain more stability, unpaired electrons in their outermost shells react readily to pair up with opposite-spin electrons in other molecules. The reactivity of free radicals can also lead to a series of chain reactions in which the net result is a propagation of more free radicals.

Molecular Oxygen

Molecular oxygen (*Figure 5-6*) contains two unpaired electrons and is technically classified as a free radical. However, because the two unpaired electrons in molecular oxygen have a parallel (versus opposite) spin, the molecule is not as reactive as might be expected of a free radical.

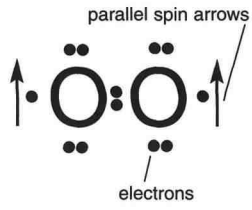

Figure 5-6
Molecular oxygen

Radical and Non-Radical Forms of Oxygen

Through a variety of enzymatic and non-enzymatic processes, molecular oxygen can be transformed into other radical and non-radical states. In most of these states, oxygen is highly reactive with other molecules, even if it lacks an unpaired electron and is classified as a non-radical. A list of radical and non-radical forms of oxygen is presented in *Table 5-2*.

Oxygen Metabolism

Within the body, the fate of molecular oxygen (O_2) is to be ultimately converted into water (H_2O). In route to that destination, oxygen can be converted into several unusually reactive forms. These forms include superoxide anion radical ($O_2^{-\bullet}$) and hydroxyl radical ($OH^{\bullet}$). Xanthine oxidase (XO), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPO) are unique enzymes in their ability to help regulate these conversion processes. Also critical in this regulation are enzymatic cofactors (like copper, zinc, and manganese for SOD and selenium for GPO), as well as intracellular concentrations of free iron that can stimulate conversion of hydrogen peroxide to hydroxyl radical through the Fenton reaction. A summary diagram of these events is presented in *Figure 5-7*, on page 200.

Direct Damage Caused By Oxidative Stress

In one sense, the complexity and risk associated with oxygen metabolism means that the body is constantly under "oxidative stress." In this context, "oxidative stress" simply means the inescapable presence of free radicals that accompanies oxygen metabolism. Without this "oxidative stress," many essential life-support functions in the body could not take place. These functions include inflammatory response to infection or trauma, elimination of fat-soluble toxins from the body, and production of energy within the mitochondria of cells.

*Table 5-2. **Radical and non-radical forms of oxygen***

Oxygen radicals		Oxygen non-radicals	
$O_2^{-\bullet}$	Superoxide anion radical	$^1O_2$	Singlet Oxygen
$HO^{\bullet}$	Hydroxyl radical	H_2O_2	Hydrogen peroxide
$ROO^{\bullet}$	Peroxyl radical	ROOH	Organic/fatty acid hydroperoxides
$HO_2^{\bullet}$	Hydroperoxyl radical	O_3	Ozone

The term "oxidative stress" can also be defined, however, as a situation in which there is excessive risk associated with oxygen metabolism. This excessive risk occurs whenever oxygen metabolism is insufficiently supported within the body. The lack of support can involve lack of enzymatic activity, lack of enzymatic cofactors, imbalanced production of free radicals, excessive presence of free iron, lack of "antioxidant" nutrients, etc. For the remainder of this chapter, we will adopt this second definition of oxidative stress, namely, excessive risk associated with unsupported oxygen metabolism.

Air Pollution and Oxidative Stress

Air pollution has long been associated with excessive presence of oxygen free radicals. In atmospheric chemistry, processes in which free radicals are formed through interaction of molecules with light are referred to as primary photochemical reactions. In these reactions, a photon of light is absorbed by an airborne molecule, creating, photoelectrically, an unpaired electron state. Two key photochemical reactions include 1) the conversion of nitrogen dioxide into nitric oxide (a nitrogen free radical), and 2) the multi-step conversion of ozone into hydroxyl radical (an oxygen free radical) (*Figure 5-8*, on the following page.). Emission of agricultural and industrial pollutants into the atmosphere has repeatedly been show to induce primary photochemical reactions and to result in environmental problems like photochemical smog. As described above, these smogs depend heavily on excessive formation of free radicals.

Common Air Pollutants

Toxins contributing to these air pollution problems include aldehydes (e.g., formaldehyde) and ketones (e.g., acetone or methyl ethyl ketone) derived from solvents like benzene and toluene; by-products of fossil fuel combustion (e.g., sulfur and nitric oxides); and by-products of herbicide production (e.g., dioxin). All of these toxins have also been shown to exist in the U.S. food supply. The issue of photochemical smog and primary photochemical reactions has been thoroughly reviewed by Hippeli and Elstner.[74]

Oxidative Stress in the Body

Like excessive production of free radicals in the earth's atmosphere, excessive production of free radicals in the body has been shown to have a long list of unwanted consequences. Most of these consequences involve damage to tissue structure. In this section of the chapter, we review the kinds of structural damage that have been associated with overproduction of free radicals and oxidative stress.

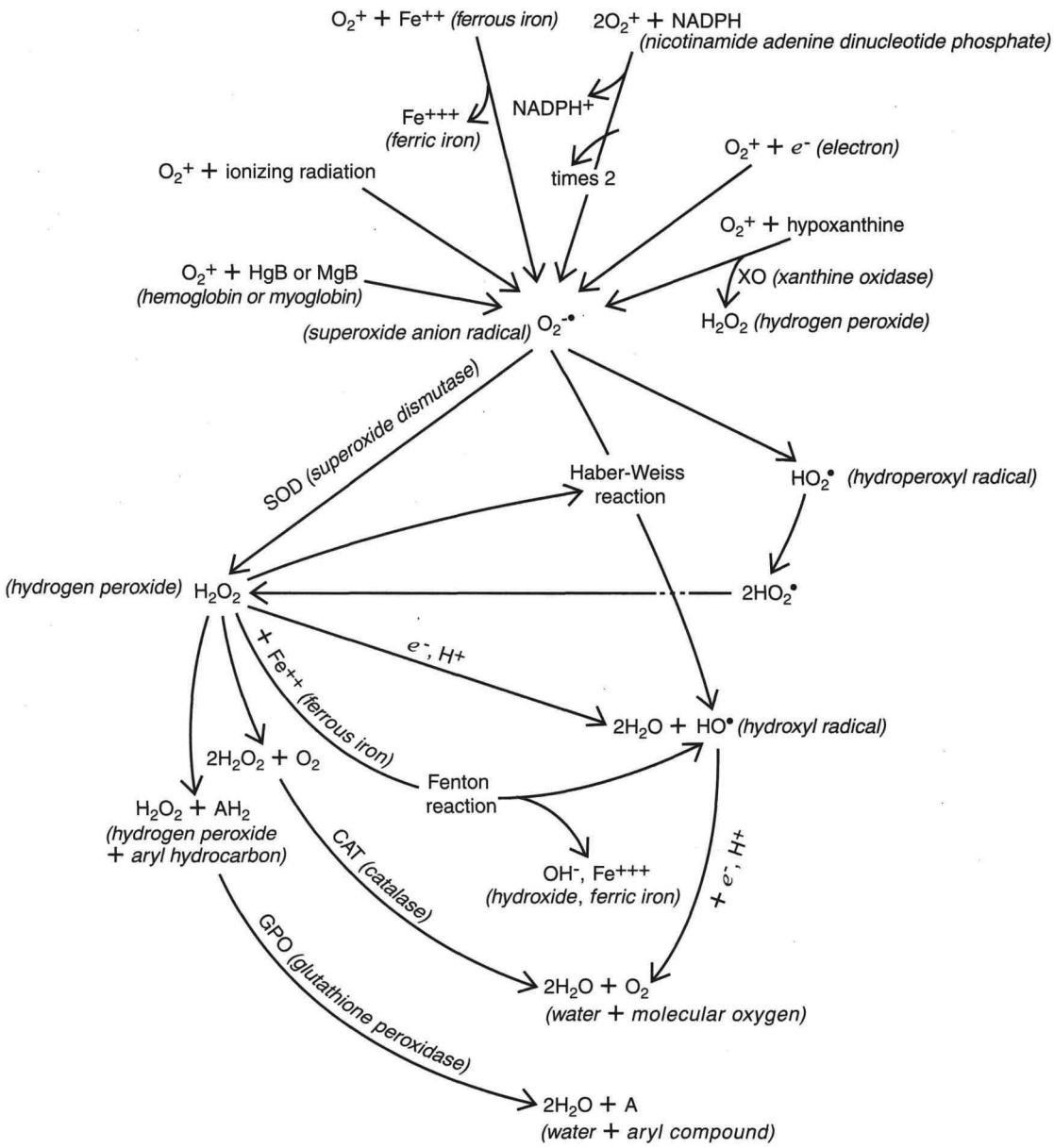

Figure 5-7
Oxygen metabolism

Damage to Plasma Membranes

Because of their high unsaturated fatty acid content, plasma membranes (the outermost membranes of cells that enclose all of their fluids and contents) are unusually susceptible to oxidative stress. Many researchers view oxidative damage to plasma membrane fats as a gateway event in the ability of oxygen to further disrupt cellular function.[75] Oxidative damage to lipids in the plasma membrane is often initiated by a hydroxyl free radical and its ability to convert a plasma lipid into a lipid carbon-centered radical. (Molecular oxygen itself can react with membrane lipids to form lipid carbon-centered radicals, as well as perhydroxy radicals.) Hydroxyl radicals are able to accomplish this feat by removing a hydrogen atom from a methylene group in the lipid's hydrocarbon chain (*Figure 5-9*, on the following page). The presence of double bonds in unsaturated fatty acids

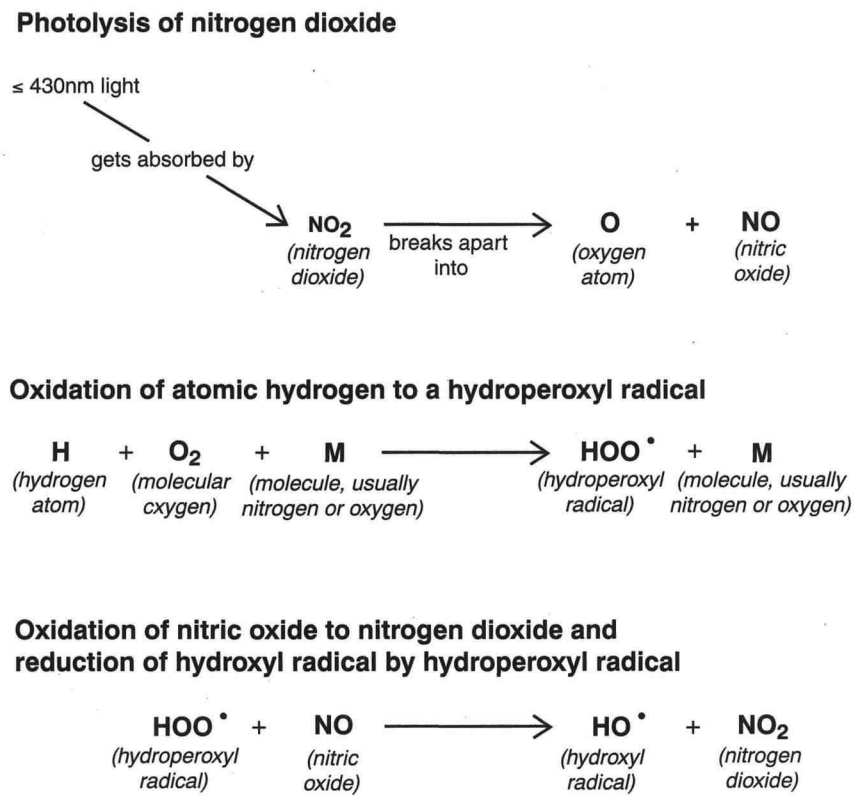

Figure 5-8
Photochemical production of free radicals

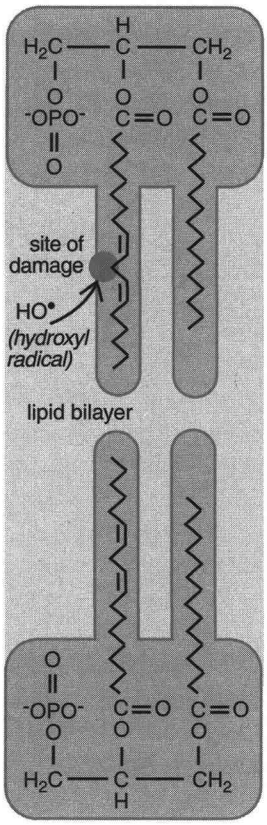

Figure 5-9
Lipid membrane damage by hydroxyl radical

makes this process easier, since double bonds weaken carbon-hydrogen bonds in carbons that lie next to the site of double-bonding. Hydroxyl radicals are the most active of all oxygen radicals. The rates at which hydroxyl radicals can react with surrounding structures approaches the speed of diffusion, although they must be in extremely close proximity (within a few angstroms) of those structures.[76]

Peroxidation of Membrane Lipids. The interaction of hydroxyl radicals (as well as other free radicals, including peroxyl radicals and alkoxyl radicals) with membrane lipids can also trigger an ongoing series of free radical reactions. For example, interaction of lipid carbon-centered radicals with molecular oxygen can generate lipid peroxyl radicals. These lipid peroxyl radicals can go on to interact with other lipid and fat components in the membrane. This chain-like series of reactions eventually ends with the formation of hydroperoxides and cyclic peroxides. Collectively, these molecules are referred to as *lipid peroxides*, and their process of formation as *lipid peroxidation*. Numerous laboratory tests have been developed to measure the presence of lipid peroxides. The chemistry of lipid peroxidation and the lab tests available for its detection have been comprehensively reviewed by Gutteridge,[77] Esterbauer[78] and Benzie.[79]

Lipid peroxidation has been shown to be a common mode of action for numerous food-related toxins. These toxins include organochlorine insecticides like endosulfan,[80] herbicides like paraquat[81] and herbicide production by-products like TCDD,[82] as well as solvents like trichloroethylene,[83] metals like aluminum,[84] heavy metals like cadmium[85] or lead,[86] and particulates like asbestos.[87] Most of the above-cited research studies on lipid peroxidation has utilized the TBARS (thiobarbituric acid reactive substances) test to measure free radical damage to membranes. TBARS results have typically been reported in nanamoles of thiobarbituric acid reactive substances per gram of protein. Increases of 25-30% in TBARS levels have been common following toxic exposure.

Damage to Mitochondrial Membranes

Approximately 90% of all oxygen taken into the body ultimately finds its way into mitochondria.[88] Mitochondria are highly specialized, energy-rejuvenating components of cells. Embryologically, in their initial stages of development, all cells in the body contain mitochondria. An adult body, with approximately 30 trillion cells, contains approximately 100 quadrillion mitochondria. Mitochondria are uniquely structured to provide for oxidation (breakdown) of fats, and for recycling of the body's most critical energy storage molecule, ATP (adenosine triphosphate).

Because 90% of the body's oxygen is delivered to the mitochondria, these organelles continually face a heightened risk of oxidative stress. Approximately 4% of all oxygen in mitochondria has been estimated to undergo incomplete reduction and to routinely form oxygen radicals including superoxide radical ($O2^{-\bullet}$) and hydroxyl radical ($OH^{\bullet}$).[89] The constant formation of these oxygen radicals in close proximity to the unsaturated lipid-containing membranes of the mitochondria makes them uniquely susceptible to oxidative damage.

Because oxidative damage to the mitochondrial membranes disrupts energy-driven processes throughout the body, structural damage to mitochondrial membranes can also be regarded as disruption of the body's energetic processes. In keeping with this second way of classifying oxidative damage to mitochondrial membranes, we will review the consequences of these events beginning on page 230, under "Toxic Disruption of Mitochondrial Function"

Other Types of Direct Damage Caused by Oxidative Stress

Induction of Apoptosis. Although induction of apoptosis is reviewed earlier in this chapter, it is worth repeating the research conclusion that oxidative stress is a major mechanism for inducing apoptosis. The close connection between unsupported oxygen metabolism and increased programming of cell death makes intuitive sense. Whole-body health would clearly be benefited by sensitivity to immanent danger on the part of every cell, and by cell responses that eliminated that danger, even if these responses brought cell death. The connection between oxidative stress and apoptosis also makes sense within the context of research. Previously-cited studies with chronic fatigue syndrome (CFS) patients, for example, have moved equally in two directions. In one direction has been the confirmation of apoptosis as a relevant consideration in understanding CFS. Compared to the number of cells undergoing mitosis or simply resting, cells undergoing apoptosis are unexpectedly common in CFS. In another direction, and equally compelling, has been confirmation of mitochondrial disruption as a key component of CFS. A similar research overlap has occurred in the study of Parkinson's disease, where increased mitochondrial defects, particularly in Complex I of electron transport chain processing, have been observed alongside of observations involving increased apoptosis among nerve cells.

Adrenochrome Production. A final type of oxidative damage worthy of mention within this section is unwanted oxidation of catecholamine neurotransmitters in the nervous system. Neurotransmitters (NTs) are molecules used to

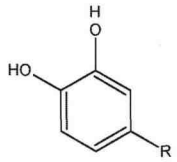

Figure 5-10
Catechol

carry messages from one nerve cell to the next. The majority of neurotransmitters are protein-related and are sometimes as simple as a single amino acid. The catecholamine neurotransmitters are a special category of NTs and include epinephrine (adrenalin), norepinephrine (noradrenalin), dopa, and dopamine. Catecholamines are named for their two common chemical characteristics: a catechol nucleus, consisting of a twice-hydroxylated benzene ring (*Figure 5-10*) and a single amine group (NH_2). This second chemical feature qualifies catecholamines as monoamines. Nerve cells and adrenal cells are the only cell types that produce catecholamines.

For the past forty years, pioneers in orthomolecular medicine, including Abram Hoffer, M.D., Ph.D., have used high-dose vitamin and mineral support of catecholamine metabolism in keeping with a metabolic theory of schizophrenia that included excessive oxidation of catecholamine neurotransmitters. Adrenolutin, for example, one catecholamine oxidation product, has been proposed as an appropriate plasma marker for overall catecholamine oxidation.[90] Adrenochrome, another oxidized form of adrenalin (epinephrine), appears to be produced in the presence of reactive oxygen molecules and may itself function as a radical.[91]

(Chapter Five continues forward at this point.)