3) THE THREAT OF OXIDANT INJURY

All human things are subiect to decay.
John Dryden

The treatment of critically ill patients is dominated by the notion that promoting the supply of oxygen to the vital organs is a necessary and life-sustaining measure. Oxygen is provided in a liberal and unregulated fashion, while the tendency for oxygen to degrade and decompose organic (carbon-based) matter is either overlooked or underestimated. In contrast to the notion that oxygen protects cells from injury in the critically ill patient, the accumulated evidence overthe past 15 years suggests that oxygen is responsible for the cell injury that accompanies critical illness. Oxygen's ability to act as a lethal toxin has monumental implications for the way we treat critically ill patients.

The Oxidation Reaction

An oxidation reaction is a chemical reaction between oxygen and another chemical species. Because oxygen removes electrons from other atoms and molecules, oxidation is also described as the loss of electrons by an atom or molecule. The chemical species that removes the electrons is called an oxidizing agent or oxidant. The companion process (i.e., the gain of electrons by an atom or molecule) is called a reduction reaction, and the chemical species that donates the electrons is called a reducing agent. Because oxidation of one atom or molecule must be accompanied by reduction of another atom or molecule, the overall reaction is often called a redox reaction.
When an organic molecule (a molecule with a carbon skeleton) reacts with oxygen, electrons are removed from carbon atoms in the molecule. This disrupts one or more covalent bonds, and as each bond ruptures, energy is released in the form of heat and light (and sometimes sound). The organic molecule then breaks into smaller fragments. When oxidation is complete, the parent molecule is broken down into the smallest molecules capable of independent existence. Because organic matter is composed mainly of carbon and hydrogen, the end-products of oxidation are simple combinations of oxygen with carbon and hydrogen: carbon dioxide and water.

OXYGEN METABOLISM

Oxygen is a weak oxidizing agent, but some of its metabolites are potent oxidants capable of producing widespread and lethal cell injury (1). The mechanism whereby oxygen metabolism can produce more powerful oxidants than the parent molecule is related to the atomic structure of the oxygen molecule, which is described below.

THE OXYGEN MOLECULE

Oxygen in its natural state is a diatomic molecule, as shown by the familiar O2symbol at the top of Figure 3.1. The orbital diagram to the right of the O2symbol shows how the outer electrons of the oxygen

molecule are arranged. The circles in the diagram represent orbitals. (An orbital is an energy field that can be occupied by electrons. It is distinct from an orbit, which is a path that represents a specific point in space and time.) The arrows in the diagram represent electrons that are spinning in the same or opposite directions (indicated by the direction of the arrows). Note that one of oxygen's orbitals contains two electrons with opposing spins, and the other two orbitals each contain a single electron spinning in the same direction. The orbital with the paired electrons is obeying one of the basic rules of the quantum atom: An electron orbital can be occupied by two electrons if they have opposing spins. Thus, the two outermost orbitals that contain single electrons are only half full, and their electrons are unpaired. An atom or molecule that has one or more unpaired electrons in its outer orbitals is called a free radical (2). (The term free indicates that the atom or molecule is capable of independent existence—it is free-living.)

Free radicals tend to be highly reactive chemical species by virtue of their unpaired electrons. However, not all free radicals are highly reactive. This is the case with oxygen, which not highly reactive molecule despite having two unpaired electrons.

The reason for oxygen's sluggish reactivity is the directional spin of its two unpaired electrons. No two electrons can occupy the same orbital if they have the same directional spin. Thus, an electron pair cannot be added to oxygen because one orbital would have two electrons with the same directional spin, which is a quantum impossibility. This spin restriction limits oxygen to single electron additions, and this not only increases the number of reactions needed to reduce molecular oxygen to water, but it also produces more highly reactive intermediates.

THE METABOLIC PATHWAY

Oxygen is metabolized at the very end of the electron transport chain, where the electrons and protons that have completed the transport process are left to accumulate. The complete reduction of molecuiar oxygen to water requires the addition of four electrons and four protons, as shown in the reaction sequence in Figure 3.1. Each metabolite in this sequence is accompanied by an orbital diagram to demonstrate the changes occurring at each point in the pathway.

Superoxide Radical '

The first reaction adds one electron to oxygen, and produces the superoxide radical.

Note the superscript dot on the superoxide symbol. This signifies an unpaired electron, and is the conventional symbol for a free radical. The superoxide radical has one unpaired electron, and thus is less of a free radical than oxygen. Superoxide is neither a highly reactive radical nor a potent oxidant (3). Nevertheless, it has been implicated in conditions associated with widespread tissue damage, such as the reperfusion injury that follows a period of ischemia (2). The toxicity of superoxide may

be caused by the large daily production, which is estimated at 1 billion molecules per cell, or 1.75 kg (4 lb) for a 70-kg adult

Hydrogen Peroxide

The addition of one electron to superoxide creates hydrogen peroxide, a strong oxidizing agent (and the source of acid rain in the atmosphere).

Hydrogen peroxide is very mobile, and crosses cell membranes easily. It is a powerful cytotoxin and is well known for its ability to damage endothelial cells. It is not a free radical, but it may have to generate a free radical (a hydroxyl radical) to express its toxicity.
Hydrogen peroxide is loosely held together by a weak oxygen-oxygen bond (this bond is represented by the lower orbital in the orbital diagram for hydrogen peroxide). This bond ruptures easily, producing two hydroxyl radicals, each with one unpaired electron. An electron is donated to one of the hydroxyl radicals, creating one hydroxyl ion (OH-) and one hydroxyl radical (-OH). The electron is donated by iron in its reduced form, Fe(II), which serves as a catalyst for the reaction. Iron is involved in many free radical reactions, and is considered a powerful pro-oxidant. The role of transition metals in free radical reactions is discussed again later in the chapter. ,,

Hydroxyl Radical

The iron-catalyzed dissociation of hydrogen peroxide proceeds as follows:

$$H2O2 + Fe(II) \_ OH- + \bullet OH + Fe(III) \qquad (3.3)$$

(Note that Roman numerals are used instead of plus signs to designate the oxidation state of iron, as recommended by the International Union of Chemistry.) The hydroxyl radical is the ace of free radicals. It is one of the most reactive molecules in biochemistry and often reacts with another chemical species within five molecular diameters from its point of origin (2). This high degree of reactivity limits the mobility of the hydroxyl radical, and this may serve as a protective device to limit the toxicity of the hydroxyl radical. However, the hydroxyl radical is always dangerous because it can oxidize any molecule in the human body

Hypochlorous Acid

The metabolism of oxygen in neutrophils has an additional pathway (not shown in Figure 3.1) that uses a myeloperoxidase enzyme to chlorinate hydrogen peroxide, creating hypochlorous acid (hypochlorite).

$$H202 + 2CL- \_ 2HOCL \qquad (3.4)$$

When neutrophils are activated, the conversion of oxygen to superoxide increases twentyfold. This is called the respiratory burst, which

is an unfortunate term because the increased O2consumption has nothing to do with energy metabolism. When the increased metabolic traffic reaches hydrogen peroxide, about 40% is diverted to hypochlorite production and the remainder forms hydroxyl radicals (6). Hypochlorite is the active ingredient in household bleach. It is a powerful germicidal agent and requires only milliseconds to produce lethal damage in bacteria (7).

Water

The final reaction in oxygen metabolism adds an electron to the hydroxyl radical and produces two molecules of water.

'OH + OH- + e- + 2H+_2H20 (3-5)

Therefore, the metabolism of one molecule of oxygen requires four chemical reactions, each involving the addition o a single electron. This process, then, requires four reducing equivalents (electrons and protons).
Under normal conditions, about 98% of the oxygen metabolism is completed, and less than 2% of the metabolites escape into the cytoplasm (3). This is a tribute to cytochrome oxidase, which carries on the reactions in a deep recess that effectively blocks any radical escape. This degree of suppression is necessary because of the ability of free radicals to start chain reactions (see next section).

Proposed Scheme

The superoxide radical is mobile but not toxic, whereas the hydroxyl radical is toxic but not mobile. Combining the advantages of each oxidant yields a scheme that has the mobile oxidant serving as a transport vehicle that can reach distant places. Once at the desired location, this metabolite could then generate hydroxyl radicals to produce local damage (3). This scheme is intuitively satisfying, regardless of its validity.

FREE RADICAL REACTIONS

The damaging effects of oxidation are largely the result of free radical reactions. This section describes the two basic types of free radical reactions: those involving free radicals and nonradicals and those involving two free radicals.

RADICAL AND NONRADICAL

When a free radical reacts with a non radical,the non radical loses an electron and is transformed into a free radical. Therefore, the union of a radical and a nonradical begets another radical (thus illustrating the survival value of the free radical). Because free radicals are often

highly reactive in nature. This type of radical-regenerating reaction tends to become repetitive, creating a series of self-sustaining reactions known collectively as a chain reaction (3). The tendency to produce chain reactions is one of the most characteristic features of free radical reactions. A fire is one example of a chain reaction involving free radicals, and fires illustrate a very important feature of chain reactions: the tendency to produce widespread damage. A chain reaction that is capable of producing widespread organ damage is described below.

Lipid Peroxidation

The rancidity that develops in decaying food is the result of oxid4ative changes in polyunsaturated fatty acids (8). This same process, called lipid peroxidation, is also responsible for the oxidative damage of membrane lipids. The lipophilic interior of cell membranes is rich in polyunsaturated fatty acids (e.g., arachidonic acid) and the characteristic low melting point of these fatty acids may be responsible for the fluidity of cell membranes. Oxidation increases the melting point of membrane fatty acids and reduces membrane fluidity. The membranes eventually lose their selective permeability and become leaky, predisposing cells to osmotic disruption (8).
The peroxidation of membrane lipids proceeds as shown in Figure 3.2. The reaction sequence is initiated by a strong oxidant such as the hydroxyl radical, which removes an entire hydrogen atom (proton and electron) from one of the carbon atoms in a polyunsaturated fatty acid. This creates a carbon-centered radical (C-), which is then transformed into an oxygen-centered peroxy radical (COO-) that can remove a hydrogen atom from an adjacent fatty acid and initiate a new series of reactions. The final propagation reaction creates a self-sustaining chain reaction that will continue until the substrate (i.e., fatty acid) is exhausted, or until something interferes with the propagation reaction. (The latter mechanism is the basis for the antioxidant action of vitamin E, which is described later.)

Implications

Free radical reactions have been implicated in the pathogenesis of more than 100 diseases (9), but it is not clear whether oxidant injury is a cause or a consequence of disease (9,10). However, a chain reaction is an independent process (i.e., independent of the initiating process), and if it causes tissue injury it becomes an independent pathologic process (a primary illness).

RADICAL AND RADICAL

Two radicals can react by sharing electrons to form a covalent bond. This eliminates the free radicals but does not necessarily eliminate the risk of toxicity. In the example below, the product of a radical-radical reaction is much more destructive than both radicals combined.

Nitric Oxide Transformation

Nitric oxide has been placed in a category of its own as a free radical because of its beneficial actions as a vasodilator, neurotransmitter, and bactericidal agent (11). The regard for nitric oxide has been so favorable that it was named "Molecule of the Year" by Science Magazine in 1992. = However, despite its favorable profile, nitric oxide can become a toxin in the presence of superoxide. The reaction of superoxide with nitric oxide generates a powerful oxidant called peroxynitrite, which is 2000 times more potent than hydrogen peroxide as an oxidizing agent (12). Peroxynitrite can either cause direct tissue damage or can decompose and produce hydroxyl radicals and nitrogen dioxide.

$$NO^- + O_2 \_ ONOOO^- \text{ (peroxynitrite)}$$

$$ONOOOH \_ -OH + NO_2$$

The transformation of nitric oxide into a source of oxidant injury demonstrates how free radicals can promote oxidant damage indirectly.

ANTIOXIDANT PROTECTION

Evidence for endogenous antioxidant protection is provided by the simple observation that accelerated decay begins at the moment of death. This section presents the substances that are believed to play a major role in this protection.
An antioxidant is defined as any chemical species that can reduce or delay the oxidation of an oxidizable substrate (2). The nonenzyme antioxidants are included in Table 3.1.

ENZYME ANTIOXIDANTS

There are three enzymes that function as antioxidants, shown in Figure 3.3. Note that the reaction sequence in this figure is the same as in Figure 3.1.

Superoxide Dismutase

The discovery of superoxide dismutase (SOD) enzyme in 1969 was the first indication of free radical activity in humans, and this began the frenzy of interest in free radicals. The role of SOD as an antioxidant is not clear. In fact, SOD promotes the formation of hydrogen peroxide, which is an oxidant. How can an enzyme that promotes the formation of an oxidant be defined as an antioxidant? In fact, if SOD increases the metabolic traffic flowing through hydrogen peroxide, and the catalase and peroxidase reactions are unable to increase their activity proportionally, the hydrogen peroxide levels may rise, and in this situation SOD functions as a pro-oxidant (13). Thus, SOD is not an

antioxidant at least some of the time.

Catalase

Catalase is an iron-containing heme protein that reduces hydrogen peroxide to water. It is present in most cells, but is lowest in cardiac cells and neurones. Inhibition of the catalase enzyme does not enhance the toxicity of hydrogen peroxide for endothelial cells (14), so the role of this enzyme as an antioxidant is unclear.

Glutathione Peroxidase

The peroxidase enzyme reduces hydrogen peroxide to water by removing electrons from glutathione in its reduced form and then donating the electrons to hydrogen peroxide. Glutathione is returned to its reduced state by a reductase enzyme that transfers the reducing equivalents from NADPH. The total reaction can be written as follows:
Peroxidase reaction: H2O2 + 2GSH_ 2H20 + GSSG (3.8)
Reductase reaction: NADPH + H + GSSG_2GSH + NADP (3.9)

where GSSG and GSH are oxidized and reduced glutathione, respectively.

Selenium

The activity of the glutathione peroxidase enzyme in humans depends on the trace element selenium. Selenium is an essential nutrient with a recommended dietary allowance of 70 mcg daily for men and 55 mcg daily for women (15). Despite this recommendation, selenium is not included in most total parenteral nutrition regimens. Because the absence of dietary selenium produces measurabTe differences in glutathione peroxidase activity af^ter just 1 week (16), the routine administration of selenium seems justified. However, selenium, has no clear-cut deficiency syndrome in humans, so there is little impetus to provide selenium on a routine basis.
Selenium status can be monitored with whole blood selenium levels. The normal range is 0.5 to 2.5 mg/L. Selenium can be provided intravenously as sodium selenite (17). The highest daily dose that is considered safe is 200 mcg, given in divided doses (50 mcg intravenously every 6 hours).

NONENZYME ANTIOXIDANTS

Glutathione

One of the major antioxidants in the human body is a sulfur-con aining tripeptide glutathione (glycine-cysteine-glutamine), which is present in molar concentrations (0.5 to 10 mM/L) in most cells (18,19). Glutathione is a reducing agent by virtue of a sulfhydryl group in the cysteine residue of the molecule. It is normally in the reduced state (GSH), and the ratio of reduced to oxidized forms is 10 :1. The major

antioxidant action of glutathione is to reduce hydrogen peroxide directly to water, which diverts hydrogen peroxide from producing hydroxyl radicals. Glutathione is found in all organs, but is particularly prevalent in the lung, liver, endothelium, and intestinal mucosa. It is primarily an intracellular antioxidant, and plasma levels of glutathione are three orders of magnitude lower than intracellular levels. Glutathione does not cross cell membranes directly, but is broken down first into its constituent amino acids and then reconstituted on the other side of the membrane. It is synthesized in every cell of the body, and largely remains sequestered within cells. Exogenous glutathione has little effect on intracellular levels (20), which limits the therapeutic value of this agent.

N-Acetylcysteine

N-Acetylcysteine, a popular mucolytic agent (Mucomyst), is a sulfhydryl-containing glutathione analog capable of passing readily across cell membranes. N-Acetylcysteine is effective as a glutathione analog in acetaminophen toxicity, which is the result of an overwhelmed glutathione detoxification pathway (see Chapter 53). Therefore, N-acetylcysteine has a proven track record as an exogenous glutathione analog. N-Acetylcysteine may prove to be a valuable antioxidant for therapeutic use. It protects the myocardium from ischemic injury, and has been successful in reducing the incidence of reperfusion injury during cardiac catheterization (21). It has also been used with some success in treating critically ill patients with acute respiratory distress syndrome and inflammatory shock syndromes (22,23).

Vitamin E

Vitamin E (alpha-tocopherol) is a lipid-soluble vitamin that functlons primarily as an antioxidant that antagonizes the peroxidative injury of membrane lipids. It is the only antioxidant capable of halting the propagation of lipid peroxidation. The mechanism for this action is shown in Figure 3.4. Vitamin E inhabits the lipophilic interior of cell membranes, where the polyunsaturated fatty acids are also located. When a propagating wave of lipid peroxidation reaches vitamin E, it is oxidized to a free radical, thereby sparing any adjacent polyunsaturated fatty acids from oxidation. The vitamin E radical is poorly reactive, and this halts the propagation of the peroxidation reactions. This action has earned vitamin E the title of a chain-breaking anti.oxidant.

The vitamin E radical is transformed back to vitamin E, and vitamin C can act as the electron donor in this reaction
Vitamin E deficiency may be common in critically ill patients (24) The normal vitamin E level in plasma is 1 mg/dL, and a level below 0.5 mg/dL is evidence of deficiency (25). Considering the important role of vitamin E as an antioxidant, it seems wise to check the vitamin E status in patients who are at risk for oxidant injury (see Table 3.2).

Vitamin C

Vitamin C (ascorbic acidj is a reducing agent that can donate electrons to free radicals and fill their electron orbitals. It is a water-soluble antioxidant, and operates primarily in the extracellular space. Vitamin C is found in abundance in the lung, where it may play a protective role in inactivating pollutants that enter the airways. The problem with vitamin C is its tendency to promote (rather than retard) the formation of oxidants in the presence of iron and copper (26-28). Vitamin C reduces iron to the Fe(II) state, and this normally aids in the absorption of iron from the intestinal tract. However, Fe(II) can promote the production of hydroxyl radicals, as described earlier Thus, vitamin C can function as a pro-oxidant by maintaining iron in its reduced or Fe(II) state. The reactions involved are as follows:

Ascorbate + Fe(III) _ Fe(II) + Dehydroascorbate (3.10)

Fe(II) + H2O2 _ -OH + OH- + Fe(III) (3.11)

Several conditions that are common in ICU patients can promote an increase in free iron. Among these are inflammation, blood transfusions, and reductions in binding proteins. The prevalence of these conditions raises serious concerns about the use of vitamin C as an exogenous antioxidant in the ICU patient population.

Plasma Antioxidants

The plasma components with antioxidant activity are listed at the very bottom of Table 3.2. Most of the antioxidant activity in plasma can be traced to two proteins that make up only 4% of total plasma protein pool (27): ceruloplasmin (the copper transport or storage protein) and transferrin. Transferrin binds iron in the Fe(III) state, and ceruloplasmin oxidizes iron from the Fe(II) to Fe(III) state. Therefore, ceruloplasmin helps transferrin to bind iron, and both proteins then act to limit free iron in the plasma. For this reason, iron sequestration has been proposed as the major antioxidant activity in plasma (24). This is consistent with the actions of Fe(II) to promote free radical production, as shown in Figure 3.1.

OXIDANT STRESS

The risk and severity of oxidation-induced tissue injury are determined by the balance between oxidant and antioxidant activities. When oxidant activity exceeds the neutralizing capacity of the antioxidants, the excess or unopposed oxidant activity can promote tissue injury. This condition of unopposed biological oxidation is known as oxidant stress (29).

PREDISPOSING CONDITIONS

Any imbalance in the activities of oxidants and antioxidants can result in unopposed oxidation. The box plots in Figure 3.5 show the effects of two conditions that promote oxidant-antioxidant imbalance on the level of oxidant stress in humans. The index of oxidant stress in this study is the activity of lipid hydroperoxides in urine, measured as spontaneous chemiluminescence and reported in counts per minute (CPM). Healthy, nonsmoking adults (the control group) show the lowest level of oxidant activity. The effects of an increase in oxidant burden iS shown in a group of heavy smokers (one puff of a cigarette contains roughly one billion free radicals). The effects of a deficiency in antioxidant protection are shown for a group of patients with human immunodeticiency virus (HIV) infection (glutathione deficiency is common in HIV infections) (30). Each of the predisposing conditions is accompanied by a significant increase in oxidant activity in comparison to the activity in the healthy, control subjects. Note also that the HIV patients have ongoing oxidant stress when they are symptom-free.

This supports the notion that oxidant stress is a cause and not a consequence of pathologic organ injury.

CLINICAL DISEASE

As mentioned earlier, oxidants have been implicated in the pathogenesis of more than 100 clinical diseases (9); the ones most likely to be encountered in the ICU are listed in Table 3.2. Unopposed biological oxidation has been documented in each of these clinical conditions (9,10,30-35). This does not establish a causal role for oxidation (this will require evidence that antioxidant therapy can improve outcome), but the tendency for oxidation to cause independent and progressive tissue damage (e.g., in chain reactions) is reason enough to suspect that oxidant-induced injury plays a role in these illnesses.

Inflammation

Most of the clinical conditions in Table 3.2 are accompanied by inflammation, and the conditions with multiorgan involvement are often associated with a progressive, systemic inflammatory response. As a result, inflammation has been proposed as a principal offender in pathologic forms of oxidant injury. The release of free radicals from activated neutrophils and macrophages creates an oxidant-intense environment, and the ability of host cells to withstand this oxidative assault may be the important factor in determining the clinical course of inflammatory conditions. In the desirable world, leukocyte-derived oxidants would annihilate all invading microbes, but would not affect the host cells. In the undesirable world, the inflammatory oxidants would destroy both the invader and the host. This proposed scheme is intuitively appealing, and emphasizes the value of providing antioxidants routinely in inflammatory illnesses.

Antioxidant Therapy

The value of maintaining antioxidant protection is not a debatable issue because loss of antioxidant defenses is a known cause of tissue destruction; the best example of this is the accelerated decay that occurs after death. Therefore, antioxidant supplements should be considered as a routine measure in patients who spend more than a few days in the ICU. In patients with any of the clinical conditions in Table 3.2, it would be wise to monitor some of the endogenous antioxidants (vitamin E, vitamin C, and selenium). A reduced antioxidant level in blood (or any body fluid) may not indicate a deficiency state (it may indicate that the antioxidant is being used), but would certainly be an indication to provide supplements. The real value of antioxidant therapy will be determined by clinical studies, some of which are currently in progress.

METABOLIC SUPPORT

The tendency for aerobic metabolism to generate toxins has significant implications for the approach to metabolic support in critically ill patients. When metabolically-generated oxidants overwhelm the boay's antioxidant defenses, the common practice of supporting metabolism by promoting the availability of oxygen and nutrients serves only to generate more toxic metabolites. The proper maneuver here is to support the antioxidant defenses. This approach adds another dimension to the concept of metabolic support by considering the output side of metabolism. Remember that metabolism is an engine (i.e., an energy converter) and like all engines, it has an exhaust that contains noxious byproducts of combustion. The exhaust from an automobile engine adds pollutants to the atmosphere; likewise, the exhaust from a metabolic engine adds pollutants to the "biosphere."