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Review

Free Radicals and Oxygen Toxicity

Douglas D. Buechter[1]

Most organisms are constantly exposed to molecular oxygen, and this has become a requirement of life for many of them. Oxygen is not totally innocuous, however, and it has long been known to be toxic to many organisms, including humans. The deleterious effects of oxygen are thought to result from its metabolic reduction to highly reactive and toxic species, including superoxide anion radical and hydroxyl radical. Peroxidation of lipids is a major consequence of exposure to these species and the cell possesses various enzymes, including superoxide dismutase and catalase, as well as cellular antioxidants which are able to scavenge oxygen free radicals and repair peroxidized lipids. These aspects of oxygen toxicity are reviewed, as well as the involvement of oxygen free radicals in the toxicity of the herbicide paraquat.

KEY WORDS: oxygen free radicals; lipid peroxidation; superoxide dismutase; catalase; paraquat.

INTRODUCTION

Living systems have evolved to survive in the presence of molecular oxygen and, for most biological systems, life depends upon its presence. Oxygen is used as the terminal electron acceptor in the oxidation of foodstuffs, being reduced to water in the process. Thus, as a result of its high oxidizing potential and because it forms nontoxic products upon final reduction, oxygen has become an indispensable component of metabolism in many organisms. Although it is critical to the maintenance of life, exposure to oxygen is not without risks. It is highly toxic at concentrations only slightly greater than that in air (1), and this toxicity may be important in several biological processes including cancer, aging, inflammation, and xenobiotic metabolism (2–11).

To explain the toxic effects of molecular oxygen, it has been proposed that partially reduced intermediates formed from the initial univalent reduction of O_2 are the reactive, toxic species. These intermediates include superoxide anion radical, hydrogen peroxide, and hydroxyl radical. The reduction may be mediated by normal metabolic pathways, exogenous compounds, or arise from exposure to electromagnetic radiation. The respiratory enzymes involved in the four electron reduction of oxygen to water and other enzymes involved in cellular redox reactions normally do not allow these intermediates to dissociate from the enzyme to become free in solution; however, it occasionally happens that "leakage" occurs and other cellular constituents are exposed to these activated species (12–14). This process may lead to extensive cellular damage, particularly loss of membrane integrity, and organisms have evolved protective systems designed to effectively convert these species to less reactive components. These systems include several enzymes, such as superoxide dismutase and glutathione peroxidase, as well as cellular anti-oxidants, such as the vitamins C and E. Thus, life in the presence of oxygen involves a delicate balance between the obligatory role of oxygen in respiration and the need to protect biological systems from the deleterious effects of the partially reduced intermediates. This review will look at some of the ways in which these intermediates may be produced, the damage they can cause in cells, the mechanisms whereby cells protect themselves against these species, and finally how the metabolism of an exogenous compound, the herbicide paraquat, can lead to the production of toxic oxygen species.

THE REDUCTION OF MOLECULAR OXYGEN

In contrast to most molecules the ground state of molecular oxygen is the triplet rather than singlet state. This state is paramagnetic and contains two unpaired electrons with parallel spins.[2] Although oxygen has great oxidizing potential, divalent reduction is relatively difficult since direct insertion of a pair of electrons would result in two electrons of the same spin occupying the same orbital. Direct divalent reduction therefore violates Pauli's exclusion principle; however, this may be circumvented in one of three ways (15):

1. The input of 23 kcal/mol will excite one of the unpaired electrons to a higher orbital and invert its spin. This leads to singlet oxygen.
2. Ligation of molecular oxygen to a metal atom con-

[1] Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143.

[2] The term "triplet" refers to the number of possible orientations of the total spin of the molecule. In the more common singlet state all electrons are paired with antiparallel spins and the molecule has zero net spin angular momentum. The three possible orientations (1, 0, −1) of triplet oxygen arise from the fact that it contains two unpaired electrons with parallel spins. Hund's Rule states that unpaired electrons of parallel spin are in a lower energy state than if their spins are opposed.

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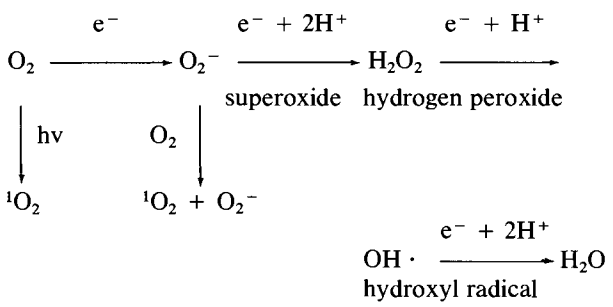
taining its own unpaired electrons (such as iron) will enable the direct divalent reduction of oxygen.

3. The spin restriction may also be bypassed by adding electrons one at a time (univalent reduction).

Each of these reactions may occur in biological systems, although enzyme-mediated divalent reduction as part of the electron transport chain is the most common. The two other processes can take place, however, and may lead to the generation of reactive oxygen species.

The reduction of molecular oxygen may be written as:



Superoxide radical (O_2^-) results from one electron reduction, while two electron reduction gives hydrogen peroxide and three electron reduction results in the hydroxyl radical. The absorption of electromagnetic radiation and the reaction of superoxide radical with molecular oxygen can both lead to the production of singlet oxygen ($^1O_2$). These intermediates (or ones generated from them) are thought to be responsible for the toxic effects of oxygen and, at least in part, other free radicals generated in the metabolism of exogenous compounds.

SUPEROXIDE AND OXYGEN TOXICITY

As mentioned above, the univalent reduction of oxygen results in the production of the superoxide anion radical (O_2^-). Superoxide production was first conclusively demonstrated in a biological system (by detection of its ESR signal) in oxidations by xanthine oxidase (16). Since that time O_2^- production has been demonstrated in several enzyme systems, including aldehyde oxidase (17), prostaglandin synthetase (13), and cytochrome P_{450} (13,18). O_2^- may also be generated by "leakage" from the electron transport chain's NADH-coenzyme Q reductase complex (17,19). The degradation of oxyhemoglobin to methhemoglobin,

$$\text{Heme-Fe}^{+2}\text{-O}_2 \rightarrow \text{O}_2^- + \text{Heme-Fe}^{+3}$$

can produce superoxide and occurs at a daily rate of 3% of the body's total hemoglobin (20). The redox cycling of exogenous compounds, such as paraquat (21), and the reduction of psoralens (22), may also lead to superoxide radical production. The production of O_2^- may also play a normal physiological role, as it has been suggested that O_2^- generation by leukocytes and macrophages during phagocytosis in the "respiratory burst" may be involved in their ability to kill bacteria (13,23). Superoxide radical also rapidly degrades hyaluronic acid and bovine synovial fluid *in vitro* (24), and this has led to the suggestion that it is involved in acute inflammation (8,13,25).

The superoxide anion radical is usually not considered to be directly responsible for the toxic effects of oxygen. But is is thought that O_2^- can lead to the production of other reactive species. In particular, the dismutation of superoxide,

$$\text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$$

followed by the metal ion catalyzed Haber–Weiss reaction (26),

$$\text{Fe}^{+3} + \text{O}_2^- \rightarrow \text{Fe}^{+2} + \text{O}_2$$
$$\text{Fe}^{+2} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{+3} + \text{OH}\cdot + \text{OH}^- \text{ (Fenton reaction)}$$

can produce the highly reactive hydroxyl radical. This species has been implicated in causing damage to DNA and proteins and in the peroxidation of lipids (19,27). The reactions which it will undergo are considered later. Reaction of O_2^- with molecular oxygen can also give rise to singlet oxygen:

$$\text{O}_2^- + {}^3\text{O}_2 \rightarrow \text{O}_2^- + {}^1\text{O}_2$$

However, this reaction is not thought to be a significant source of $^1O_2$ in biological systems, and it is possible that the reverse reaction where O_2^- scavenges $^1O_2$ may be more important (19).

Superoxide radical in aqueous solution will accept a proton to form the hydroperoxyl radical ($HO_2\cdot$) with a pKa of 4.45 (28). It is also a weak oxidant, although it can act as a weak reductant (19) and will reduce heme-Fe^{+3} to heme-Fe^{+2} as well as reduce free ferric iron or iron chelated to other species such as EDTA (29,30). The reduction of Fe^{+3} to Fe^{+2} may then result in an increase in the production of hydroxyl radicals by the Fenton reaction. O_2^- will also oxidize ascorbate to give hydrogen peroxide and the ascorbate radical. Although these reactions have all been shown to occur *in vitro,* their biological significance is poorly understood.

Several laboratories in the 1940s and 50s isolated copper-containing proteins from a variety of sources including human and bovine blood and horse liver (31–33). The function of these proteins remained unknown until it was shown that human erythrocuprein (a copper-containing protein from human blood) possessed superoxide dismutation activity (31,34) and it was proposed that these proteins, the superoxide dismutases, act to protect cells from the superoxide radical by catalyzing the dismutation of superoxide (35). The dismutation of superoxide to hydrogen peroxide and oxygen occurs spontaneously in aqueous solution and this nonenzymatic reaction can produce singlet oxygen,

$$2\text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + {}^1\text{O}_2$$

which will then decompose to the ground state:

$$^1\text{O}_2 \rightarrow {}^3\text{O}_2 + \text{hv}$$

This reaction is a potential source of reactive singlet oxygen and, although its significance has been questioned (19), it has been suggested (36) that the role of superoxide dismutase (SOD) is to convert superoxide to triplet oxygen before it has a chance to go to the singlet state. The enzymatic pathway is thought to proceed by the following mechanism (33,37):

$$\text{E-(metal)}^n + \text{O}_2^- \rightarrow \text{E-(metal)}^{n-1} + \text{O}_2$$
$$\text{E-(metal)}^{n-1} + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{E-(metal)}^n + \text{H}_2\text{O}_2$$

Eukaryotes contain two types of SOD, a copper and zinc containing cytosolic enzyme and a manganese enzyme located in mitochondria (33,38). Exposure of *E. coli* to hyperbaric oxygen results in increased cellular levels of SOD, and this increase correlates with enhanced resistance to high pO_2 (39). Numerous studies of this sort have been conducted (40–43), and although SOD does not always protect against the toxic effects of oxygen (see below) it does appear that it constitutes a first line of defense against superoxide radicals.

The dismutation of superoxide produces hydrogen peroxide and hydrogen peroxide can be reduced to the highly reactive hydroxyl radical (OH·). It might be expected then that the dismutation of superoxide would actually lead to enhanced toxicity of O_2^-. However, most cells also contain a hemoprotein (or in some cases a flavoprotein) called catalase (44) which will catalyze the decomposition of hydrogen peroxide to water and oxygen:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Catalase is found in all organs (particularly erythrocytes) and is usually localized in peroxisomes (17,45–47). The human enzyme has four subunits each containing an iron protoporphyrin and is believed to work by the following mechanism (48):

$$E\text{-}Fe^{+3} + H_2O_2 \rightarrow \text{Compound I}$$
$$\text{Compound I} + H_2O_2 \rightarrow E\text{-}Fe^{+3} + 2H_2O + O_2$$

Catalase may also catalyze the oxidation of other exogenous substrates, including hydroxylamine, formic acid, methanol, and ethanol (48):

$$\text{Compound I} + AH_2 \rightarrow E\text{-}Fe^{+3} + 2H_2O + A$$

In addition, to catalase the enzyme glutathione peroxidase is also important in regulation of the cellular levels of hydrogen peroxide and other peroxides. There are two types of glutathione peroxidases, selenium dependent and nonselenium dependent. Both enzymes will catalyze the following reaction (49–51):

$$GSH + H_2O_2 \rightarrow GSSG + H_2O$$

The enzyme is specific for glutathione, but appears able to accept a wide variety of peroxides other than hydrogen peroxide (52). It is found at high levels in the cytosol and mitochondria of both liver cells and erythrocytes (17). Glutathione disulfide produced by this enzyme can then be reduced back to glutathione by glutathione reductase (53):

$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$$

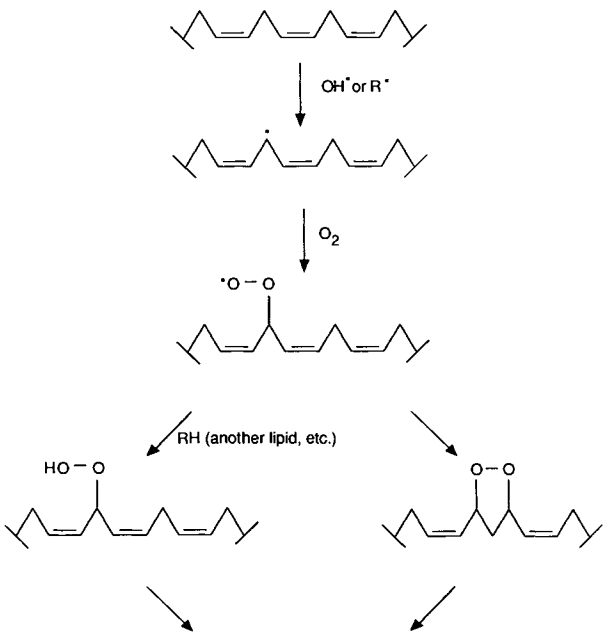
The main line of defense against oxygen toxicity appears to be the combined actions of superoxide dismutase, catalase, and glutathione peroxidase. However, neither superoxide nor hydrogen peroxide appears to be directly responsible for oxygen toxicity (17,54). As mentioned earlier the species that is assigned this role most frequently is the hydroxyl radical, produced *via* the Haber–Weiss reaction. The following section discusses this species and the damage it may cause.

THE HYDROXYL RADICAL AND LIPID PEROXIDATION

Although the combined actions of SOD and catalase are capable of reducing the superoxide radical to water, this pathway leads to the production of hydrogen peroxide which may then become free in solution. Hydrogen peroxide may be reduced by free iron (or certain types of iron chelates) to the hydroxyl radical (OH·), and this is the species which is most often cited as the initiator of lipid peroxidation.

Lipid peroxidation is thought to occur by the mechanism shown in Scheme I (55). Any radical (R·) can initiate peroxidation and it has been demonstrated that peroxidation can arise from the $\cdot CCl_3$ radical, a product of the metabolic reduction of carbon tetrachloride (56). Once a lipid radical has been formed it can react with molecular oxygen to become "fixed" or it may be repaired by reaction with a cellular thiol (usually glutathione). The reaction with oxygen eventually leads to a lipid hydroperoxide which initially was thought to be irreparable but, as discussed earlier, it appears that glutathione peroxidase can reduce a variety of peroxides, including lipid peroxides (57). The initially formed lipid peroxide radical may abstract a hydrogen atom from a second molecule of lipid which can then react with oxygen in a process that is capable of being repeated in a chain reaction. Alternatively, the peroxide radical can react intramolecularly to form an endoperoxide. Both the lipid hydroperoxide and the endoperoxide will decompose in a complex series of reactions to several possible products. The most notable of these products are various aldehydes (especially malonaldehyde) which themselves have been implicated as damaging species in lipid peroxidation (58,59). Fe^{+2}, and to a lesser extent Fe^{+3}, in free heme, hemoglobin, myoglobin, or in the cytochromes can catalyze this decomposition (60,61).

Lipid peroxidation appears to be a common biological occurrence (19). It can cause a loss of membrane function, an impairment of membrane enzymes, and eventually cell death (62). The free radicals or reactive aldehydes generated



Scheme I

in this process may also react with other sensitive cellular components such as nucleic acids and proteins (63). This process appears to be a major reason for the toxicity of molecular oxygen and, although there is a clear correlation between oxygen toxicity and lipid peroxidation, it has been difficult to conclusively identify the species that is directly responsible for this toxicity. Free iron and an iron-EDTA complex can reduce superoxide to the hydroxyl radical (26) and OH· can also be produced upon the absorption of radiation by water (16,64). The iron chelating agent, desferrioxamine, will block iron-EDTA initiation of lipid peroxidation in liposomes (65). Urate will also protect against lipid peroxidation *in vitro,* presumably by scavenging either OH· or singlet oxygen (66), although such studies must be interpreted with caution as free radical trapping agents will often react with a variety of free radicals. Cellular levels of glutathione also correlate with the degree of lipid peroxidation (51), although again it is not clear whether glutathione is reacting with OH· or is acting to "repair" lipid peroxides.

The effect of superoxide dismutase on lipid peroxidation is often confusing. In some preparations SOD enhances membrane damage while in others it decreases the damage (67). If sufficient iron is present then the enhanced production of H_2O_2 by SOD may increase the level of lipid peroxidation; however, since O_2^- can reduce Fe^{+3} to Fe^{+2}, decreasing the level of O_2^- will decrease reduction of Fe^{+3} and the production of OH· (*via* the Haber–Weiss reaction) will decline. The amount of catalase present in the preparation (catalase activity is often difficult to completely remove) is also probably an important factor.

The role of OH· as the initiating species in lipid peroxidation has recently been questioned (29,30,68). If the Haber–Weiss reaction is responsible for the production of OH· (which then initiates lipid peroxidation), decreasing the amount of H_2O_2 should decrease the production of OH· and decrease lipid peroxidation. It has been found, however, that adding catalase or inhibiting endogenous catalase with azide has no effect on the rate of lipid peroxidation in a microsomal preparation (29). It was also found that addition of exogenous H_2O_2 also had no effect, but that the addition of H_2O_2 and azide actually decreased the amount of lipid peroxidation. Other studies have found that exogenous Fe^{+3}-EDTA or the radical scavenger dimethyl sulfoxide also did not effect lipid peroxidation (30,68). These studies have led to the suggestion that initiation of peroxidation involves an oxo-iron chelate rather than the free hydroxyl radical (30). Some chelates, such as ferric-ADP, would be effective in initiating peroxidation while others would not, even though all may lead to an increase in the production of OH·

It should be noted that because the hydroxyl radical is such a reactive species, and would be expected to have an extremely short life in aqueous solution, proving its involvement in lipid peroxidation is very difficult. Indeed, it is possible that hydroxyl radicals are so reactive that they cannot be responsible for lipid peroxidation, since they would not have time to diffuse to a membrane before reacting. In this regard it is feasible that superoxide (or H_2O_2), which is less reactive, may be able to diffuse throughout the cell and result in the "transport" of OH· to various areas of the cell. The observation that radical scavengers or the addition of compounds which should increase the production of OH· (such as iron chelates) may not effect the extent of lipid peroxidation is consistent with OH· not being the initiating species; however, it also may be that the local generation of OH· very close to a lipid may not be affected in these cases. Generation of OH· in the bulk of the cytosol may not always lead to damage as these hydroxyl radicals may be rapidly quenched. However, if hydroxyl radicals are produced close to a lipid they may rapidly react with the lipid before a free radical scavenger; therefore, the scavenger may not greatly effect the extent of peroxidation (69,70).

In summary, while these studies do question the role of hydroxyl radicals in lipid peroxidation they are not conclusive enough to completely discount its proposed role in initiation. Clearly, a highly reactive species resulting from the univalent reduction of molecular oxygen is responsible for oxygen toxicity. The exact nature of this species is not known, but its central role in lipid peroxidation and oxygen toxicity is evident.

SINGLET OXYGEN

As discussed earlier, singlet oxygen can be produced by the absorption of energy (usually electromagnetic) by ground-state molecular oxygen. Singlet oxygen has a half-life of 2 μsec in aqueous solution (71) and may be detected either photometrically (72–74) or by trapping with singlet oxygen scavengers (75–77). The role of $^1O_2$ in the biologically relevant reactions of activated oxygen species is not well understood. $^1O_2$ is rapidly quenched by β-carotene, and it has been suggested that this is an important biological function of β-carotene (78). $^1O_2$ has been claimed to be an initiator of lipid peroxidation (75,79), although this has been difficult to conclusively demonstrate. Most of the methods to detect singlet oxygen in biological systems rely on quenching with compounds such as azide or on the enhancement of the measured effect in D_2O [the half-life of $^1O_2$ is increased to 20 μsec in D_2O (80,81)]; however, it is not clear that azide is specific for $^1O_2$ (as mentioned earlier it will inhibit catalase) and D_2O will have an effect on the rate of any reaction in which an exchangeable proton is being removed in the slow step. The role of singlet oxygen in oxygen toxicity, in radiation damage, and in the toxicity (or activity) of radiosensitizers (such as the psoralens) is difficult to prove; however, it is likely that it plays an important role in many instances.

PROTECTIVE MECHANISMS

Superoxide dismutase, catalase, and the combined actions of glutathione peroxidase and glutathione reductase constitute the major mechanism that the cell utilizes to protect itself against oxygen free radical toxicity. There are, however, several other mechanisms that may play important roles in protection. These include (82):

1. Maintenance of low O_2 tension.
2. Compartmentalization.
3. Maintenance of structural integrity.
4. Miscellaneous peroxidases.
5. Cellular anti-oxidants.

Biological systems are able to regulate cellular pO_2 by a variety of mechanisms involving the respiratory, circulatory, and blood systems. These mechanisms will not be discussed

here (for a review see ref. 83); however, it should be stressed that these systems are probably the primary method of controlling cellular exposure to oxygen. In a related fashion, cells are also capable of limiting exposure of sensitive structures to oxygen by compartmentalization. The most obvious example of this is the ability to "hold" oxygen within the electron transport chain until it is reduced completely to water; however, compartmentalization is also apparent in the observation that erythrocytes, which often generate large amounts of superoxide, also contain high concentrations of SOD and glutathione peroxidase (17).

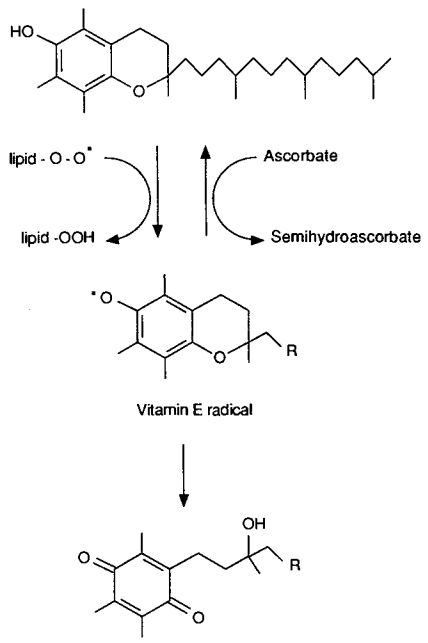
The maintenance of structural integrity, particularly of membranes, is also important in protecting against lipid peroxidation. Tissue samples show a relatively slow rate of lipid peroxidation if they are not damaged; however, upon homogenation the rate of peroxidation increases dramatically. In this regard the role of cholesterol as a membrane stabilizing agent may be important as well as the role of vitamin E which, when incorporated into liposomes, also appears to have a stabilizing function (84,85).

In addition to glutathione peroxidase, there are other cellular peroxidases and reductases which may help protect against the harmful effects of oxygen free radicals. These include cytochrome *c* peroxidase and NADH peroxidase (19). Methionine residues in proteins can be oxidized to their sulfoxides by H_2O_2 (or by OCl^- formed in the myeloperoxide catalyzed oxidation of Cl^- by H_2O_2), and this oxidation is associated with the loss of their biological function (86). The enzyme Met(O) peptide reductase is capable of reducing methionine sulfoxide back to methionine (86). The contribution of these processes to toxicity and protection is unknown.

Several cellular anti-oxidants have been postulated to be involved in the scavenging of oxygen or organic radicals. In addition to the structural role for vitamin E, it also seems to be able to directly scavenge free radicals and help prevent lipid peroxidation. Vitamin E deficiency in chickens is associated with several disorders, including encephalomalacia and muscular dystrophy, and the administration of anti-oxidants chemically unrelated to vitamin E is capable of reversing some of these symptoms (82). Tissue homogenates from vitamin E deficient animals show an increase in the extent of lipid peroxidation, and vitamin E can protect against the toxic effects of carbon tetrachloride (82). The addition of vitamin E to liposomal preparations protects against lipid peroxidation, and it appears that it is able to act as a chain-breaking anti-oxidant, reacting with lipid peroxy radicals to give the relatively unreactive vitamin E radical as shown in Scheme II (87).

Vitamin C (ascorbic acid) may also have a protective role (88). It has been shown to react with free radicals (including the vitamin E radical, Scheme II) to give semihydroascorbate, which then may go on to oxalic acid and L-threonic acid or be reduced back to ascorbate (Scheme III) (89). There is also evidence to suggest that vitamins C and E are able to act in a synergistic fashion as anti-oxidants (90).

In addition to vitamin E and ascorbate, free glutathione and uric acid may be important in scavenging free radicals. The addition of glutathione to tissues can inhibit lipid peroxidation (51), presumably by reacting to give the glutathione

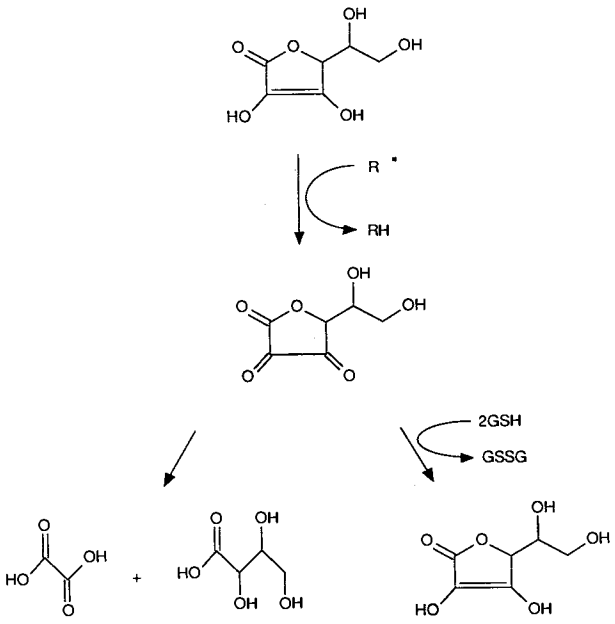


Scheme II

radical which may then react with a second glutathione radical to give glutathione disulfide. Glutathione reductase is then able to reduce this back to glutathione. As mentioned before, uric acid can also act as a radical scavenger (66), although the biological significance of this activity is not clear.

THE TOXICITY OF PARAQUAT

The generation of reactive oxygen species need not occur solely through the univalent reduction of molecular oxygen by cellular enzymes. It is also possible that exoge-



Scheme III

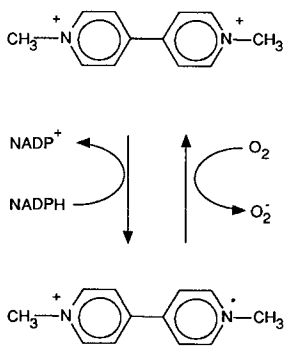
nous compounds can be metabolized to reactive species which can directly initiate lipid peroxidation or, by reaction with oxygen, lead to the generation of reactive oxygen species. One of the compounds that has been shown to exert its activity by this mechanism is the herbicide, paraquat.

Paraquat is an extensively used herbicide which is toxic to a wide range of plants. One of its major advantages is that it will bind very tightly to soil and be inactivated by microorganisms (91). This allows a field to be sprayed and then replanted shortly after harvest. In addition to its toxicity to plants, paraquat is also extremely toxic to man and other animals and has been responsible for several hundred deaths (92,93). Administration of paraquat leads to diffuse lung injury and the compound appears to be selectively retained by the lung (94–96).

Paraquat is readily reduced *in vivo* to give the paraquat mono-cation radical (Scheme IV) (97–99). This reduction is thought to be mediated by a soluble NADPH-reductase (97), although it can also result from the disruption of the electron transport chain, or photosystem I in plants, or via NADPH cytochrome P_{450} reductase (19,92).

One-electron-reduced paraquat will rapidly react with molecular oxygen, giving back the di-cation and also yielding superoxide radical (92,98). The di-cation may be reduced again and in this manner paraquat can serve as a continuous source of O_2^-. This redox cycling has been seen in illuminated chloroplasts (100,101) and in lung microsomes (102).

Paraquat has been shown to cause lipid peroxidation in bacteria as well as human systems (103–107). Paraquat-induced peroxidation can be prevented or decreased by vitamin E (108,109), vitamin C, glutathione, and catalase (106). In addition, administration of superoxide dismutase *in vitro* and *in vivo* will decrease the extent of lipid peroxidation and the toxicity of paraquat (106,108,110,111). Exposure of *E. coli* to paraquat will cause an increase in superoxide dismutase activity (112,113), and plants resistant to its effects contain elevated levels of SOD (19). Treatment of rats with a bacterial endotoxin known to elevate SOD has been shown to protect against paraquat-induced lung injury and these rats have a threefold longer survival time when administered a lethal dose of paraquat (114). The toxicity of paraquat is also increased by increased pO_2, and selenium-deficient rats show increased sensitivity to paraquat (109,111,115). There is also evidence that paraquat binds to DNA; it has been suggested that this is responsible for its weak mutagenicity (116).



Scheme IV

These findings suggest that paraquat toxicity is associated with the production of superoxide radicals. It was therefore proposed that paraquat toxicity was due to superoxide-stimulated lipid peroxidation (104); however, this effect has been difficult to reproduce (19) and it does not appear that all of paraquat's toxicity can be accounted for by lipid peroxidation (117). The mono-cation radical may react with other cellular components, including nucleic acids and proteins. Such reactions have not been demonstrated with paraquat; however, it is known that the reaction of free radicals with DNA can lead to strand scission (118), and that the formation of protein-centered radicals may lead to protein–protein or lipid–protein crosslinks (119–121).

CONCLUSION

Oxygen toxicity in biological systems is the result of the univalent reduction of molecular oxygen to reaction species. The exact nature of these species is not known with certainty; however, the highly reactive hydroxyl radical appears to be the most likely candidate. Most biological systems that are exposed to an oxygen atmosphere have developed protective systems to enable them to survive in the presence of these toxic species, and the observation that many obligate anaerobes lack these systems is taken as evidence for their protective role. As oxygen radicals have been implicated in cancer as well as aging, a clear understanding of their chemistry and pathology is very important in understanding, and possibly intervening in, these fundamental physiological processes.

Free radical toxicity can also result from the administration of exogenous compounds. In addition to paraquat, this mechanism has been suggested for several other compounds such as alloxan and streptozotocin (7). A clearer understanding of free radical toxicity may help in elucidating the mechanism of action of these and related compounds. This may help in the design of safer agents or of drugs that are actually administered to potentiate the toxicity of free radicals, such as the use of photosensitizers in the treatment of psoriasis and cancer.

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