Oxygen Is Toxic!

Irwin Fridovich

We, whose lives depend upon a continual supply of oxygen, do not easily comprehend its toxicity. Our apparent comfort at the ambient level of oxygen is due to elaborate defenses against its very considerable toxicity. These defenses are adequate to the task ordinarily faced, but can be overwhelmed when the degree of oxygen exposure is significantly increased.

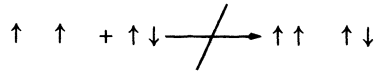
This truth is forcibly exhibited by exposure to levels of oxygen higher than the fifth of an atmosphere in which we normally function. A few hours of breathing pure oxygen at one atmosphere pressure causes tracheitis, and after a few days the lung damage is severe enough to cause death, ironically from anoxia. Rats exposed to 2.5 atmospheres of oxygen convulse and die in a few hours. The toxicity of hyperbaric oxygen is universal, having been demonstrated with bacteria, fungi, plants, and animals. Mammalian cells, in culture, are adversely affected by the oxygen in air. Thus, cultured human diploid W.I.-38 cells do much better under 1% O_2 than under 20% O_2.

There are many microorganisms which are killed by exposure to air. These sensitive obligate anaerobes lack defenses against oxygen toxicity, and they can do nicely without them so long as they restrict their lives to the islands of anaerobiosis which exist, even in our oxygenated planet—in the deep waters, reducing muds, and large intestines of higher animals. Why, then, is oxygen toxic, and what are the defenses which allow aerobes, such as we, to prosper in its presence?

THE SPIN RESTRICTION

The reactions of oxygen with organic substances are exothermic; yet, such reactions do not occur at perceptible rates at ordinary temperature. Molecular oxygen is actually much less reactive than we might anticipate, and the explanation for its sluggishness must be sought in its special electronic arrangement. Oxygen exhibits a paramagnetism which is very unusual for any gas. Given that there are 6 outer shell electrons on each oxygen atom, we have 12 electrons to arrange in O_2. An electronic orbital is filled when it contains two electrons, and their spins must be opposite or paired. Paired electrons do not interact with a magnetic field because their individual magnetic fields are oppositely oriented and mutually cancelling, but unpaired electrons do. The paramagnetism of O_2 means that it contains unpaired electrons. In fact, 10 of its electrons are disposed as pairs in five filled orbitals, whereas each of the remaining two electrons resides in a different orbital, and their spins are parallel or unpaired. Virtually all stable organic compounds contain only paired electrons.

Now, consider an organic reductant which has a pair of electrons to give up to O_2. This incoming electron pair could not be accommodated into the partially filled orbitals on O_2, because two electrons with parallel spins would end up in one of these orbitals and that is not magnetically feasible. This hypothetical situation can be illustrated as follows:



Of course, inversion of the spin or one of the electrons on oxygen during its interaction with the organic donor would allow the electron transfer to occur. But spin inversion is a slow process compared with the lifetime of collisional complexes and is, therefore, not likely to occur while the O_2 and the reductant are close enough to react.

The necessity for spin inversion presents a barrier to the reactivity of O_2. This barrier to reaction, here referred to as a spin restriction, accounts for the unreactivity of O_2. An indication of the effectiveness of this spin restriction can be gained by comparing ground state oxygen with electronically excited singlet oxygen. Singlet oxygen is not paramagnetic because excitation of ground state oxygen results in a spin inversion.

There are two readily accessible forms of singlet oxygen, designated $O_2(^1\Delta g)$ and $O_2(^1\Sigma g)$; these contain 22 and 37 kcal of excitation energy respectively. They can be made by transfer of excitation energy from a dye which, in turn, was excited by absorbing a photon of light. $O_2(^1\Sigma g)$ has a very brief lifetime in water; longer-lived $O_2(^1\Delta g)$ has been much studied (Foote 1976). Singlet oxygen reacts with histidine at a rate of 6×10^7 m^{-1} sec^{-1} at 20°C, whereas ground state oxygen does not react at all at this temperature.

As this single example illustrates, singlet oxygen is vastly more reactive than ground state oxygen because it does not face the spin restriction. If atmospheric oxygen were as reactive as $O_2(^1\Delta g)$, life would be impossible; indeed, accumulation of any organic matter would then be unlikely. The spin restriction accounts for much of the difference in reactivity between ground state and $O_2(^1\Delta g)$.

THE UNIVALENT PATHWAY

The spin restriction applies to the divalent reduction of O_2. It can be circumvented by combining the O_2 with a transition metal which is itself paramagnetic. This is the course followed by many oxidases which contain Cu(II) or Fe(II) or other metals. Another way to avoid the spin restriction is to add electrons to O_2 one at a time. Such univalent pathways for the reduction of oxygen are actually quite common, and therein lies an explanation for the toxicity of oxygen.

Thus, the reduction of O_2 to $2H_2O$ requires four electrons, and the univalent pathway involves intermediates which are much more reactive than O_2 itself. It is these intermediates of oxygen reduction which are the cause of oxygen toxicity. The following reactions illustrate the univalent pathway of oxygen reduction:

a. $O_2 + e^- \rightarrow O_2^-$

b. $O_2^- + e^- + 2H^+ \rightarrow H_2O_2$

c. $H_2O_2 + e^- + H^+ \rightarrow H_2O + OH\cdot$

d. $OH\cdot + e^- + H^+ \rightarrow H_2O$

Reaction (a) yields the superoxide anion radical (O_2^-); reaction (b) yields hydro-

The author is with the Department of Biochemistry, Duke University Medical Center, Durham, NC 27710.

gen peroxide, and reaction (c) yields the hydroxyl radical (OH·).

Biological Production of Oxygen Radicals

H_2O_2 has long been known to be a product of the biological reduction of O_2. O_2^- and OH·, however, have usually been associated with the effects of ionizing radiation on aqueous solutions, rather than with normal aerobic metabolism. But O_2^- and OH· are really produced by living cells! Several enzymatic oxidations have been shown to generate O_2^-; the autoxidations of several common biochemicals, including epinephrine, leucoflavine, hydroquinones, reduced ferredoxins, and hemoglobin all produce O_2^-, and mitochondria, chloroplasts, and even whole cells of the phagocyte line have been shown to liberate O_2^-.

Whenever O_2^- is generated in an aqueous system, H_2O_2 is soon also produced—because O_2^- is not stable relative to O_2 plus H_2O_2, and it spontaneously dismutes to give those products. O_2^- is actually the conjugate base of the weak acid HO_2·, whose pK is 4.88. The spontaneous conversion of O_2^- to H_2O_2 plus O_2 must, then, take account of dismutation reactions involving both O_2^- and HO_2·. Thus, the reactions and their rate constants which must be considered are:

e. $HO_2\cdot + HO_2\cdot \rightarrow H_2O_2 + O_2$

$k_2 = 7.6 \times 10^5\ M^{-1}\ sec^{-1}$

f. $HO_2\cdot + O_2^- + H^+ \rightarrow H_2O_2 + O_2$

$k_2 = 8.5 \times 10^7\ M^{-1}\ sec^{-1}$

g. $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$

$k_2 = <1.0\ M^{-1}\ sec^{-1}$

Since reaction (f) is the most rapid of the dismutation reactions, the net spontaneous dismutation would be most rapid at pH 4.8, where there would be equivalent concentrations of HO_2· and O_2^-. In contrast, reaction (g) is, for all practical purposes, trivially slow. Hence, as the pH is raised above 4.8, the net dismutation becomes progressively lower. Nevertheless, over the pH range of concern to biochemists, the dismutation of superoxide radical is always quite rapid, and superoxide, therefore, always gives rise to H_2O_2. O_2^- and H_2O_2 would, then, both be present, and they can cooperate in the production of OH· and of $O_2(^1\Delta g)$. The manner in which this occurs is not quite clear, but the end results are definite.

Thus, an enzymatic oxidation, known to be producing both O_2^- and H_2O_2, was shown to generate a very reactive radical which could generate ethylene from methional. This reaction radical could be scavenged by alcohols (Beauchamp and Fridovich 1970). O_2^- and H_2O_2 do not individually react with methional or with alcohols but OH· does. This reactive radical, presumably OH·, was made only in the combined presence of O_2^- and H_2O_2. A reaction, proposed by Haber and Weiss to help account for the decomposition of H_2O_2 by iron salts, was advanced in explanation of these observations. It is now frequently called the Haber-Weiss reaction:

h. $O_2^- + H_2O_2 \rightarrow OH^- + OH\cdot + O_2$

There have been numerous other cases in which a very reactive radical, with the properties of OH·, was generated from O_2^- and H_2O_2. In all of these cases, the Haber-Weiss reaction has been invoked. Yet, it is likely that reaction (h), as written, has no reality, but that its overall occurrence depends upon efficient catalysis by complexes of iron or other transition metals. One could envision catalysis of the Haber-Weiss reaction as follows:

i. $Fe(III) + O_2^- \rightarrow Fe(II) + O_2$

j. $Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + OH\cdot$

O_2^- is known to reduce ferric complexes, and reaction (j) is the well-known Fenton reaction. Certainly iron complexes are plentiful in all cells, and reactions (i) and (j) could account for the Haber-Weiss reaction.

Singlet Oxygen

OH· reacts rapidly with virtually all organic compounds, and its production within living things, as a consequence of the interactions of O_2^- and H_2O_2, would be reason enough for oxygen toxicity. But there is yet another danger. An enzymatic oxidation, known to be producing both O_2^- and H_2O_2, was found to cause the peroxidation of polyunsaturated fatty acids. This peroxidation was dependent upon both O_2^- and H_2O_2, and it was prevented by scavengers of $O_2(^1\Delta g)$, not by scavengers of OH. Furthermore, this enzymatic oxidation reaction converted dimethylfuran to the same product as was obtained by exposing this $O_2(^1\Delta g)$-scavenger to photochemical source of $O_2(^1\Delta g)$, and this conversion was dependent upon both O_2^- and H_2O_2 (Kellogg and Fridovich 1975).

It, thus, appears that $O_2(^1\Delta g)$, as well as OH·, can be produced by the interaction of O_2^- and H_2O_2. $O_2(^1\Delta g)$ is a powerful oxidant, but more discriminating and therefore potentially more dangerous than OH. Thus, $O_2(^1\Delta g)$ reacts rapidly at carbon-carbon double bonds and attacks polyunsaturated fatty acids with relish. Unsaturated fatty acids are particularly plentiful in cell and organelle membranes, and lipid peroxidation readily proceeds by a free radical chain reaction, wherein one initiating event can lead to the oxidation of many molecules of the fatty acid (Barber and Bernheim 1967). $O_2(^1\Delta g)$ could, thus, cause a disproportionately great damage to cell membranes. It is obvious that the production of OH· and of $O_2(^1\Delta g)$, within living things, must be avoided, and to the extent that their production is unavoidable, the consequences of their reactions must be minimized.

PASSIVE DEFENSE: MULTIVALENT OXYGEN REDUCTION

The spin restriction favors the univalent pathway of oxygen reduction, but the intermediates of this pathway are dangerously reactive. The spin restriction can be circumvented by a reaction pathway in which O_2 is combined with a paramagnetic transition metal such as Cu(II) or Fe(II). There are enzymes which use metal cations or special organic substances, such as flavins, at their active sites to accomplish the multivalent reduction of O_2 without the release of intermediates.

Thus, most of the oxygen consumed by aerobes is actually reduced by cytochrome *c* oxidase; this enzyme contains heme and copper at its active site and reduces O_2 to water. There are several blue-colored copper-containing oxidases that also accomplish the tetravalent reduction of O_2 to water. And there are many flavin-containing oxidases, which carry out the divalent reduction of O_2 to H_2O_2. These enzymes, which appear to be specifically designed to circumvent the spin restriction, consume O_2 without imposing a load of oxygen radicals on the cell, and they must be reckoned as part of the cell's defense against oxygen toxicity. Indeed, were all oxygen reduction accomplished by enzymes, such as cytochrome *c* oxidase

which can carry out its tetravalent reduction, there would be no problem of oxygen toxicity. However, some production of O_2^-, by the univalent reduction of O_2, appears to be unavoidable in respiring cells. Since this is the case, active defenses are indispensable.

ACTIVE DEFENSE: BY ENZYMATIC SCAVENGING OF H_2O_2

H_2O_2, whether generated directly by divalent reduction of oxygen or indirectly by the dismutation of superoxide radicals, must not be allowed to accumulate. This is prevented by two classes of related enzymes, the catalases and the peroxidases, which catalyze the divalent reduction of H_2O_2 to $2H_2O$. Catalases can use H_2O_2 itself as the source of electrons for that reduction, whereas peroxidases use a variety of other reductants.

Hematin is the prosthetic group for most catalases and peroxidases, and their actions appear to be closely related. Indeed, catalase can act as a peroxidase when the concentration of H_2O_2 is low and electron donors such as formate or ethanol are present. The catalatic and peroxidatic reactions respectively are:

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

l. $H_2R + H_2O_2 \rightarrow R + 2H_2O$

(The enzymatic properties of catalases and peroxidases have been reviewed by Saunders et al. 1964, Deisseroth and Dounce 1970, Aebi and Suter 1972, and Sies 1974.)

The relative stability of H_2O_2 and the existence of redundant defenses against it tend to mask the importance of any one of the reactions that scavenge H_2O_2. Thus, there are respiring microorganisms which lack both catalase and peroxidase. Under ordinary conditions, they live in mixed cultures with cells which do contain these enzymes or in soils which contain inorganic catalysts for the decomposition of H_2O_2. Such microorganisms excrete H_2O_2, much as all respiring cells excrete CO_2. There are acatalasic mutants in humans and in mice (Aebi and Suter 1972).

This does not imply that catalase is not important. Indeed, acatalasics do display their defect when exposed to exogenous H_2O_2, such as that produced around the gum line by oral microflora, and cells from acatalasics, in culture, are killed by dilute H_2O_2, which does not harm normal cells.

Furthermore, the lack of catalase can be compensated for by an increase in peroxidase. There is a peroxidase which is abundant in mammalian tissues and which uses glutathione as the reductant for H_2O_2. This is a fascinating enzyme in that it contains selenium, and it has been shown to be important in scavenging H_2O_2 in erythrocytes (Cohen and Hochstein 1963). Duck erythrocytes normally contain less than 1% as much catalase as human erythrocytes, but this potential defect is compensated by elevated levels of glutathione and of glutathione peroxidase (Aebi and Suter 1972). In mammals which contain high levels of catalase in their red blood cells, specific tissues can do with very little catalase because the blood circulating through such tissues can serve to remove and decompose the H_2O_2 excreted by that tissue. Thus, humans contain high levels of catalase in liver, kidney, and blood but very little in brain, thyroid, testis, and other tissues. Removal of H_2O_2 from certain tissues is, thus, as surely a function of the circulating blood as is the removal of CO_2.

Catalase is well suited to the scavenging of H_2O_2 over a wide range of concentrations. Thus, when the level of H_2O_2 is very low, the enzyme functions as a peroxidase, and when it is high, as a catalase. Of course, the peroxidatic function requires an electron donor. Methanol, ethanol, nitrite, or formate serve this purpose in vitro, but the compound used in vivo is not known. Indeed, the localization of liver catalases in peroxisomes, along with the peroxide-producing enzymes, and in mitochondria, where most of the oxygen reduction occurs, seems to assure that catalase will be exposed to high local concentrations of H_2O_2 so that it can act in the catalatic mode.

Peroxidases appear to be very important in scavenging H_2O_2. Glutathione peroxidase is widely distributed in mammalian cells and is effective at low concentrations of H_2O_2, since its Km (Michaelis constant) for H_2O_2 is 1×10^{-6}M when the level of glutathione is 1×10^{-4}M. Furthermore, it can act upon a wide range of hydroperoxides as well as upon H_2O_2 and could, thus, ameliorate the toxicity of lipid peroxides. Humans, with a genetic defect in erythrocyte glutathione peroxidase, are prone to hemolytic anemia, whereas those with a similar defect in platelets exhibit Glanzmann's thrombocytopenia.

There are many peroxidases in the biological world. They differ with respect to substrate specificity, but presumably all function to scavenge H_2O_2. Yeast mitochondria contain a cytochrome *c* peroxidase in the intermembrane space, and yeast mitochondria do liberate less H_2O_2 than do mammalian mitochondria, possibly because of the action of this enzyme. Plants contain peroxidases which act upon a wide range of compounds including phenols and arylamines (Deisseroth and Dounce 1970). A great deal is known about the structure and mechanism of these enzymes, but very little about their metabolic role.

ACTIVE DEFENSE: BY ENZYMATIC SCAVENGING OF O_2^-

There is a family of enzymes, ubiquitous among respiring cells, which have the unique property of acting upon an unstable free radical. They catalyze an oxidation-reduction in which O_2^- is both the electron donor and the electron acceptor. In this respect, the action of these enzymes upon O_2^- is completely analogous to the action of catalase upon H_2O_2. The reaction catalyzed by these enzymes is:

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Since this is a dismutation reaction, these enzymes are called superoxide dismutases. There are three distinct classes of superoxide dismutases, which are found in different places and which have different prosthetic groups, yet they all catalyze the dismutation of O_2^- with an efficiency limited only by the rate of diffusion. These enzymes appear to be essential protections against the toxicity of oxygen, and their discovery, less than a decade ago, has led to a burgeoning literature, which has already been reviewed several times (Bors et al. 1974, Fridovich 1972, 1974, 1975, Halliwell 1974). I will not attempt to review all of this information once again but rather will outline the most important aspects of this field.

A Variety of Superoxide Dismutases

The oxygen, now so abundant in our atmosphere, is a product of photosynthesis. Possibly the first organisms capable of true photosynthesis were blue-green algae. Prior to their appearance, the atmosphere of Earth must have been essentially anoxic, and the many organisms which preceded the cyanophyta must have been anaerobes. The gradual

accumulation of photosynthetic oxygen would, then, have placed all of these primitive anaerobes under a stringent evolutionary pressure. Doubtless many species, which failed to adapt, became extinct. The others developed defenses against oxygen toxicity while they evolved chemical mechanisms for exploiting the reactivity of this gas to meet their needs for energy and for biosynthetic products. Given this view of a common selection pressure, applied simultaneously to a varied biota, it is not surprising that several distinct superoxide dismutases evolved.

Thus, there are superoxide dismutases containing copper and zinc, others containing manganese, and still others containing iron. The cuprozinc superoxide dismutases are characteristic of the cytoplasms of eukaryotic cells and have been isolated from such diverse sources as yeast, bread mold, spinach, and bovine blood. The manganese and iron superoxide dismutases have been found in bacteria, blue-green algae, and mitochondria. Partial amino acid sequences have been determined for several of these enzymes, with the fascinating result that the bacterial enzymes were seen to be closely related to the mitochondrial enzymes (Bridgen et al. 1975, Steinman and Hill 1973). It has been proposed that mitochondria may have evolved as a result of an endocellular symbiosis between a prokaryote and a protoeukaryote, and vigorous arguments have been advanced both favoring and opposing this hypothesis. The striking homologies between bacterial and mitochondrial superoxide dismutases, coupled with the great differences between the mitochondrial and the cytosolic superoxide dismutases from the same species, certainly support the symbiotic origin of mitochondria.

Indications of Indispensability

Oxygen is an environmental variable for facultative organisms; sometimes they live in its presence and sometimes in its absence. It is sensible that the defenses are called forth by the threat, which has been shown to be the case in *Escherichia coli, Streptococcus faecalis, Saccharomyces cerevisiae,* and rat lung. In these cases, and doubtless in others, increased exposure to oxygen results in increased synthesis and accumulation of superoxide dismutases. If superoxide dismutase is an indispensable defense against oxygen toxicity, then cells which have the higher levels of this enzyme, by virtue of growth in oxygen, should be more resistant to the toxicity of excess oxygen.

This is the case. Of course, the simplest approach to investigating the essentiality of superoxide dismutases would be to survey organisms and compare those which respire with those which do not for their content of this activity. This has been done to a limited extent, and the generality that respiring cells have the activity, whereas nonrespiring sensitive obligate anaerobes do not, holds true.

There are antibiotics which are more lethal in the presence of oxygen than in its absence. Streptonigrin, mitomycin-C, and porfiromycin are in this category. In the case of streptonigrin, a cycle of reduction and reoxidation, in the presence of oxygen, makes O_2^- which, in vitro, could lead to convalent breaks in DNA. If this is the mechanism of the oxygen-enhancement of the toxicity of streptonigrin, then cells which contain a high level of superoxide dismutase should be more resistant than those containing a lower level. This has been demonstrated in *E. coli* B and in *E. coli* K12.

OXYGEN-INTOLERANT MUTANTS

The thesis developed thus far leads directly to the expectation that genetic defects in superoxide dismutase, catalase, and peroxidase should result in sensitivity towards oxygen. Mutants of *E. coli* have been described (McCord et al. 1973) which contained a temperature-sensitive defect in superoxide dismutase. In these mutants, the intracellular concentration of superoxide dismutase was progressively lowered by raising the temperature of growth in the range 30° to 43°C. These mutants exhibited an inability to grow under air, which paralleled the decline in superoxide dismutase. Thus they could grow aerobically or anaerobically at 30° but could only grow anaerobically at 43°.

More recently, mutants of *E. coli* K12 were selected on the basis of intolerance for oxygen after exposure to a mutagen. These obligately anaerobic mutants fell into two categories: Either they were lacking in superoxide dismutase, catalase, and peroxidase, or they were lacking in catalase and peroxidase. These mutants were killed by brief exposure to air, but approximately one cell out of 10^4 was found able to grow in air. These revertants were selected, and they were found to fall into two categories: Either they had regained the missing enzymatic activities, or they had lost the capacity for respiration. The parallel losses of catalase, peroxidase, and the mangani-superoxide dismutase and their parallel regain by some of the spontaneous revertants certainly suggest a genetic linkage between these enzymes. They behaved as though they were under the control of the same operator gene. This makes sense because all three of these enzymes serve to protect against oxygen toxicity. The revertants which had lost the ability to respire exhibited another solution to the problem of oxygen toxicity. Thus, if the cell possessed no significant mechanisms for the reduction of oxygen, then there was no possibility of producing O_2^- and peroxide, and then the enzymatic defenses against these reactive species would be superfluous. These nonrespiring revertants exhibit a behavior which was earlier seen with a strain of *Lactobacillus plantarum,* which, although lacking the enzymatic defenses against oxygen toxicity, was nevertheless capable of growth in air, because it did not respire measurably.

ANTIOXIDANTS

The oxidation of polyunsaturated lipids is a free radical chain reaction (Barber and Bernheim 1967) which leads to the production of conjugated diene hydroperoxides and aldehydic fragments and other products as well. It is the nature of such chain reactions that a single initiating event leads to the oxidation of many molecules. It is also characteristic that the reaction chains can be broken and the oxidation process profoundly inhibited by compounds which react with the chain-propagating radicals, yielding products which are incapable of transmitting the reaction chain. Such chain-breakers are called antioxidants. Many of our processed foods contain butylated hydroxytoluene and butylated hydroxyanisole precisely to serve as antioxidants and, thus, to retard rancidification.

There is a natural antioxidant. It is α tocopherol, also called vitamin E. It is a powerful inhibitor of the autoxidation of polyunsaturated fatty acids (Tappel 1962), and increased dietary intake of these fatty acids increases the need for tocopherol. A deficiency of this vitamin makes membranes more prone to oxidative attack, an effect seen most readily with erythrocytes from the deficient animal. There are serious consequences

to vitamin E deficiency, including muscular dystrophy and reproductive failure.

If O_2^- and H_2O_2 are the primary agents of oxygen toxicity and if they can secondarily generate other very reactive substances such as OH· and O_2 ('Δg), then how do we view the role of antioxidants, such as vitamin E? The defensive role of superoxide dismutases, catalases, and peroxidases may be considered the front line defenses. But no defense can be perfect. Thus even in the presence of these enzymes, there will be a low level of oxidation attack on cell components. Antioxidants would minimize the damage caused by this oxidative attack.

OXYGEN AND IONIZING RADIATION

Oxygen is known to enhance the lethality of ionizing radiation. The passage of ionizing radiation through water generates hydrated electrons, hydrogen atoms, and hydroxyl radicals. In the presence of oxygen, the hydrated electron and the hydrogen atom rapidly give rise to O_2^- while the hydroxyl radical can dimerize to H_2O_2. Along the path of an ionizing radiation in oxygenated water, we can thus expect the production of O_2^- and H_2O_2. If that ionization path is some distance from a living cell, the primary products of ionization (i.e., H·, $e^-_{aq.}$ and OH·) will be too short-lived to diffuse to that cell. O_2^- and H_2O_2, in contrast, are stable enough to diffuse to the cell and there to give rise to OH· and O_2('Δg). In this view, superoxide dismutases or catalase, added to the suspending medium, should diminish the oxygen enhancement of radiation lethality. This has been observed (Misra and Fridovich 1976).

OXYGEN AND DEFENSE AGAINST INFECTION

It would be surprising if, somewhere in the living world, there were not situations in which the propensity of oxygen for univalent pathways of reduction had not been turned to good advantage. One such situation, which has come to light, is the oxygen-dependent killing of ingested bacteria by phagocytes. Normal human phagocytes show a dramatic increase in oxygen consumption during phagocytosis. Phagocytes from persons with chronic granulomatous disease do not show this burst of oxygen consumption, and they cannot kill the ingested bacteria. Obviously, this increased oxygen consumption is important for generating lethal agents directed against the engulfed bacteria. The enhanced respiration shown by activated phagocytes has long been known to be unusual in that it is not inhibited by cyanide; whereas normal respiration is cyanide-sensitive. It is now known that the increased respiration, which accompanies phagocytosis, is due to the action of an oxidase which is localized in the cell membrane and which generates O_2^-. This O_2^- then gives rise to H_2O_2, and both the O_2^- and the H_2O_2 are, then, active in killing the engulfed bacteria. Of course the phagocytes which generate this witch's brew, to the detriment of the bacterial enemy, are also at risk, but phagocytes are expendable, like the bee which stings in defense of the hive and in so doing kills itself.

OXYGEN AND SENESCENCE

All respiring organisms are continually under threat of attack by the oxygen radicals produced by their own respiration. It is true that only a fraction of the oxygen reduction in any cell, excepting activated phagocytes, results in the production of O_2^-. It is also true that there are active enzymatic defenses against O_2^- and against H_2O_2 and passive second-line defenses in the form of antioxidants. There are certainly repair processes which can undo some of the damage done by the oxygen radicals and their reactive progeny. Yet, we may suppose that some damage is done, at a constant rate, which is not repairable.

This damage would be viewed as a chronic, low-level, cumulative oxygen toxicity, and it could be the root cause of aging. The replacement of worn individuals, senescence, and death are essential for continued life and for evolution. Maximum lifespan is variable from species to species and has certainly been determined by evolutionary selection. Nevertheless, we need some chemical explanation for the gradual wearing down, whose cumulative rate could be varied by different levels of defense and repair. Perhaps oxygen toxicity, due to the intermediates of oxygen reduction, is the root cause of that wearing down we call senescence.

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