TOXICITY OF OZONE, OXYGEN, AND RADIATION[1]

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Reviews have appeared on the general problem of oxygen toxicity (1) and the effects of oxygen at high pressure (2). The limited toxicology of ozone has been reviewed by Stokinger (3) and Nasr (3a), and volumes have appeared concerning radiobiology. This paper examines specifically the similarities and dissimilarities of these three agents in light of what is hoped to be a unifying hypothesis. We shall examine the evidence for the occurrence of toxicity via free radicals produced by each agent. Such a hypothesis has been proposed by Gershman (4) for oxygen and by Stokinger (3) for ozone. Consequently, this review is in part an examination of the interactions and fate of free radicals in biological systems.

In all three cases, the exposure of mammals to critical concentrations of these agents evokes a spectrum of effects. Oxygen and ozone interact at low concentrations predominately with the lung, and much of the literature consequently involves this tissue. Radiation studies of the lung are unfortunately relatively sparse, especially at the molecular level. Neuroendocrine effects are evoked by oxygen at high pressure and will be dealt with as space and relevancy permit. The diversity of response to these agents, especially to increased pressures of oxygen, provides the reviewer with difficulty in treading a clear path through the mass of literature. Consequently, we have chosen to emphasize the molecular events occurring at the cellular level, in the hope that this view will clarify the basic principles involved.

FREE RADICAL SPECIES PRODUCED BY OXYGEN, OZONE, AND RADIATION

It is important to recognize that free radicals in cells are not biological curiosities, but occur in the oxidoreductions essential for the maintenance of life and the generation of usable energy. The electronic configuration of oxygen provides both a large oxidation potential and a slow reaction rate (4,

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5). Molecular oxygen appears to react most readily with activated species which involve mostly free radical rather than ionic mechanisms. The original one electron reduction of oxygen suggested by Michaelis (6) has been borne out by electron spin resonance studies (7), and more recently by detailed studies of the reaction mechanism of oxygenases (8). While it is unlikely that oxygen free radicals occur per se, it is certain that reactions via free radical species are required. This suggests that a normal flux of free radicals exists in all living systems.

Aqueous solutions of ozone decompose into HO· and H· free radicals, with hydroxyl ions catalyzing the reaction (8a). Molecular oxygen can be reduced through H· and HO_2· to H_2O_2, which in turn is ultimately reduced to H_2O, again via H· and HO· free radicals. Irradiation of water produces solvated electrons, H·, HO·, and HO_2· free radicals, the relative proportions of which are dependent upon the trace contaminants in solution and the pH. At physiological pH ranges it appears that H·, HO·, and HO_2· are the dominant species, since the pK_a of the solvated electron is far removed. Consequently, it appears that all of the same species of free radicals can be generated by interactions with any of the above agents.

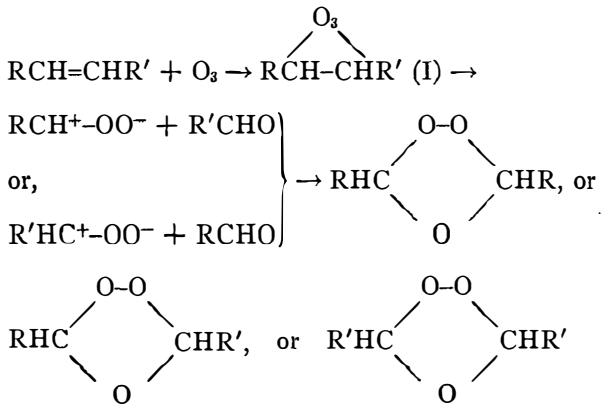
The HO· and HO_2· free radicals can abstract hydrogen readily from complex organic molecules leading to a number of more complex free radicals. Some of these species, especially sulfur-containing compounds, will be discussed separately. The occurrence of complex organic free radicals is the focus for the integration of the direct versus indirect theories of radiation damage (9, 10). Primary products of the interaction of ionizing radiation with cells can include alkyl free radicals of organic molecules. Molecular oxygen can react with this alkyl free radical to form a hydroperoxyl free radical. The latter is relatively stable and can abstract a hydrogen from water to form a semistable hydroperoxide, or propagate further reactions with other organic molecules by abstraction of hydrogen.

The so-called "oxygen effect" observed on irradiation of tissues is complex, but primarily related to the reaction of molecular oxygen with organic free radicals (9, 10). Peroxidation is possible, and the formation of peroxides of diverse nature has been studied. The drastic alteration by molecular oxygen of the electron spin resonance spectra of the paramagnetic centers generated on anaerobic irradiation of biological compounds can be explained for the most part by the reaction with molecular oxygen to form peroxides. It should be recalled that tumors are often cited as tissues having marked "oxygen effects." These tumors are generally solid and hence anoxic in their interiors (11). Saturation of the surrounding tissue results in an increase of the internal tumor oxygen pressure to nearly normal levels.

Alper & Howard-Flanders (11a) and Elkind et al. (11b) originally observed that increasing the cellular partial pressure of oxygen above that required for adequate respiration had little effect on the radio-sensitivity of normal tissue. Christensen et al. (11c) have observed about a 12 to 13 per cent increase in radiosensitivity of mice, indicating that increasing the cellu-

lar partial pressure of oxygen to hyperbaric levels does affect the primary radiation response. The increased sensitivity to radiation, though small, is in agreement with a predominantly free radical propagated mechanism of damage by oxygenated species.

Ozone reacts with olefins in nonpolar solvents to form ozonides. Two mechanisms have been proposed; Criegee (12) suggested



Storey et al., (13) suggest that ozonides are also formed by the reaction of the aldehyde with the molozonide (I), as well as by the Criegee mechanism. The ozonide can decompose via free radical species. Alkanes and alcohols are oxidized by ozone even at −78° (14). Radical or ion pairs are intermediaries. Hydroperoxides can result and the ultimate products of the reaction are peracetic acid, formaldehyde, acetone, and hydrogen peroxide. Amines are oxidized to amine oxides by electrophilic attack of ozone (15). Modification of egg albumin on ozone treatment may be related to amine oxidation, tyrosine hydroxylation, or free radical crosslinking, since an unfolding of tyrosine residues and insolubility occurred (16). Unfortunately, we are not certain that the egg albumin used in these experiments was lipid free and that the alterations were not due to lipid peroxidation. Many of the ozone intermediaries from such reactions can decompose via free radicals. Some of the products such as aldehydes from the ozonolysis of polyunsaturated fatty acids are indistinguishable from the products of the peroxidation.

Direct evidence for ozone involvement with lipid peroxidation has been found in the formation of malonaldehyde from ozone-treated human red blood cells (16a). Malonaldehyde is the principal product of the oxidation of polyunsaturated fatty acids and is commonly used as an index of lipid oxidation. Electron spin resonance spectra of ozone-linoleic acid reaction mixtures have been interpreted as evidence for free radical intermediaries (16b). Diene conjugation, another indicator of lipid oxidation of polyunsaturated fatty acids (16c), has been reported in lipid extracts of the lungs of

ozone-exposed mice (16d). It appears likely that ozone does promote lipid oxidation and probably involves free radical intermediaries. Whether the other reactions of ozone occur in cells is not known, since the reactions have been studied in organic solvents.

Before dismissing anhydrous reactions such as those described above for ozone, one should recall the experiments of Whitting and co-workers on the vitamin E deficient rat (17–20). They found that the rate of oxidation of polyunsaturated fatty acids in muscle could be adequately described from studies of the rate of oxidation of anhydrous films of the polyunsaturated fatty acids. These observations tend to imply that the reactions of oxygen with the lipid phase components of cell membranes may occur under essentially anhydrous conditions.

Free Radicals in Oxygen Toxicity

Gershman (4) first proposed a unifying theory of oxygen toxicity based upon the concept that the adverse effects of oxygen at pressures from 0.2 to 10 atm are primarily due to free radical induced damage. Gershman (4) proposed that the free radical induced damage resulted from chain reactions leading to destructive oxidations. The source of the initial free radicals was proposed to be from the radiolysis of water, the univalent reduction of oxygen, or free radicals arising from normal metabolism. Antioxidant mechanisms such as that of α-tocopherol curtail chain reactions of this type and protect against the uncontrolled oxidations mediated by free radicals. Gershman (4) suggests that antioxidant defenses were built by aerobic animals as protection against its normal environment at an oxygen pressure of 0.2 atm. These antioxidant mechanisms are thought to be capable of control rather than abolition of the destructive oxidations, and hence cannot prevent completely the unwanted oxidations. A small but significant rate of oxidation thus can proceed despite the presence of an adequate antioxidant defense system. This small continuous oxidation may be a significant factor in aging and exposure to increased oxygen pressures, ionizing radiation, and ozone has been likened to an accelerated aging process. The symptoms of vitamin E deficiency can be regarded as the toxicity of oxygen at 0.2 atm, caused by the decrease or loss of antioxidant defenses.

Substantial evidence has been accumulated for this theory, particularly at oxygen pressures of 1 atm or greater. Some of the protective agents against oxygen are also protective against ionizing radiation (21). Thyroidectomy and fasting provide protection by decreasing metabolism and thus the flux of free radicals that are potentially misdirected to oxidation (4, 22). The depression of appetite and growth on chronic exposure to increased oxygen pressures may be an adaptive mechanism. Force feeding of quail exposed to oxygen, however, failed to affect the survival time in oxygen (23). The principal congener of vitamin E, α-tocopherol, is thought to be the main biological lipid phase antioxidant, and exerts protection against hemolysis, convulsions, and mortality of mice at 3 atm oxygen (24–28). Tocopherol administration also protected against oxidative reactions in brain

and red blood cells during exposure to oxygen, as judged by a decrease in the malonaldehyde content of the exposed tissue (24, 28). Sulfhydryl enzymes are the most sensitive enzymes to ionizing radiation (29), inactivation by ozygen at high pressures (1), and peroxidizing lipid (30).

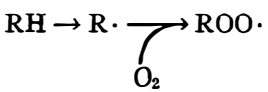
Evidence for the operation of the free radical mechanism at oxygen pressures less than 1 atm but greater than 0.2 atm is less convincing. Brooksby et al. (31) observed a decrease in the reduced glutathione content of rats, Roth (32) found equivocal hematological results, and the Gemini flights (33) indicated that the red cell mass of the pilots was reduced possibly through an inadvertent vitamin E deficiency (32). There is a sudden increase in the sensitivity of mammals to oxygen pressures between 450 and 760 torr which may reflect a steep increase in the reaction rate (21). A dependence on the pressure of oxygen is to be expected since oxygen is a reactant in the processes. The complexity of biological response to increased oxygen pressures can in part be explained by the mass action evoked by increasing the oxygen concentration. Alveolar and lung capillary walls are the most severely affected since they are exposed to the highest concentration of oxygen. Other tissues are exposed to lower concentrations, since once hemoglobin is saturated (at an alveolar partial pressure of oxygen of about 100 torr) the additional oxygen reaching the tissues will be relatively small, as it would reach the tissue only in physical solution in plasma (about 3×10^{-5} ml oxygen per ml of blood per torr increase of oxygen partial pressure) (34).

Vitamin E Deficiency, Normal Atmosphere Toxicity

Let us consider the evidence accumulated for the interaction of free radicals in antioxidant deficiency under normal atmospheres or the toxicity of oxygen at 0.2 atm.

The symptoms of antioxidant deficiency have been largely attributed to the free radical mediated peroxidation of structural lipids in cell membranes, and to the random destruction of essential proteins in free radical chain reactions. Deprivation of animals of vitamin E evokes these symptoms, and addition of certain synthetic antioxidants to the diet in appropriate amounts competely reverses the symptoms (35, 36). The sulfur amino acid and selenium content of the diet is also known to be involved in the alleviation of some, but not all, of the vitamin E deficiency symptoms (37–39). Sulfur amino acids function as potent antioxidants and potential regulators of cell metabolism. Selenium is incorporated into selenocysteine and selenomethionine, both of which are very potent aqueous phase free radical scavengers (40–42) and have recently been shown to be catalysts of the sulfhydryl exchange reaction (43).

Fatty acids having a methylene-interrupted polyunsaturation are prone to hydrogen abstraction from the methylene group followed by reaction with molecular oxygen and the formation of a hydroperoxide via the chain reaction (44).



$$RH + ROO\cdot \rightarrow ROOH + R\cdot$$

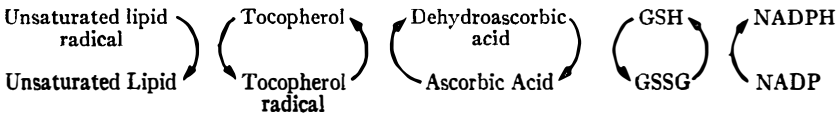
Hydroperoxides contribute to the chain propagation by homolytic decomposition and carbon-carbon scission. Heavy metals catalyze lipid peroxidation by initiation of chain reactions and decomposition of hydroperoxides (45). Hematin is abundant in biological systems, and functions as an efficient catalyst in the decomposition of hydroperoxides (46).

The consequences of lipid oxidation *in vitro* are disastrous, and suggestive of many of the changes observed in oxygen high pressure, ozone and radiation exposure, as well as vitamin E deficiency. Swelling and lysis of red blood cells, mitochondria, microsomes, and lysosomes can be initiated by lipid oxidation. Red blood cells of antioxidant-deficient animals are susceptible to hemolysis by dialuric acid or H_2O_2 (47). Lipid peroxidation of red blood cells precedes hemolysis, and involves primarily the phosphatidyl ethanolamine fraction of the membrane which contains the majority of the unsaturated fatty acids of the membrane. Peroxidized rat liver mitochondria lose hematin compounds, and succinoxidase and NADPH-cytochrome c reductase are inactivated (48). Oxidative phosphorylation of vitamin E deficient rabbit liver mitochondria is also depressed (49). Cytochrome components of isolated electron transport particles show increased random destruction accompanying lipid peroxidation (50). The swelling and lysis of mitochondria by a variety of free radical initiators, F^{+2} ion, ascorbate, and oxidized and reduced glutathione have been investigated, and peroxidation has been shown to precede lysis in all cases (51). Lysosomes peroxidize by either ionic free radical initiators or by irradiation (52, 53). The pathology of nutritional muscular dystrophy in tocopherol-deficient rabbits implicates peroxidation-caused release of the degradative enzymes from lysosomes (54).

Peroxides and free radicals, as well as irradiation, are powerful inhibitors of sulfhydryl enzymes (30). Proteins are crosslinked and insolubilized by reaction with oxidizing lipid mixtures, and the destruction of amino acids is of the same order as that caused by ionizing radiation (55–57). The main product of polyunsaturated lipid oxidation, malonaldehyde, is also inhibitory, reacting with ribonuclease (58a) and deoxyribonucleic acids to form crosslinked polymers (58). Malonaldehyde-reacted deoxyribonucleic acid is less functional in transcription than the native form (59).

Complex interactions which result in synergism can occur between antioxidants. Synergists may suppress the tendency of antioxidants to decompose peroxides and may also chelate heavy metals. In many cases they may merely regenerate the original antitoxidant. Ascorbate, citrate, and gluta-

thione may be an important series of *in vivo* synergists. Tappel et al. (50) found marked synergism in the inhibition of hematin-catalyzed oxidation of linoleic acid by this mixture. Such synergism may exist by *in vivo* by the formation of redox couples (50) such as



The terminal oxidation of the sequence is suggested to function through glutathione reductase. Glutathione reductase and glutathione are widely distributed in tissues and thus could function as a means of transporting unwanted electrons from tocopherol to the pyridine nucleotide pool. A similar mechanism has been proposed for the protection of the red blood cell against oxidation (60), and a number of unusual diseases have been identified which are associated with one or more defects in the maintenance of an adequate, reduced glutathione pool (61).

Chemical antioxidants function at an optimum concentration, since at high concentrations antitoxidants may become pro-oxidants by decomposing peroxides to form free radicals. Glutathione, normally considered an antioxidant, can initiate free radical formation by

$$GSH + O_2 \rightarrow HO_2\cdot + GS\cdot$$

Gershman et al. (21) found that various sulfhydryl compounds, chelating agents, trihydroxylphenone antioxidants, and cobalt salts provide protection at some pressures of oxygen but are detrimental at others. These observations may be a reflection of a requirement for an optimal concentration of antitoxidant. Adaptive changes on the part of the animal may cause the "protector" to become an "antagonist."

The susceptibility of a given tissue to oxidation will be a function of the balance of pro-oxidants and antioxidants. The relative proportions of polyunsaturated fatty acids and metal catalysis (Cu^{+2} ion and hematin) will be a function of the species, tissue lipoprotein specificity, and diet (65). Symptoms of tocopherol deficiency vary accordingly with the diet and species: muscular dystrophy in the rabbit, encephalomalacia in the chick, liver necrosis and fetal resorption in the rat, anemia in the monkey. Tissue differences may also be expected in oxygen toxicity. Diet alters cell membranes and the susceptibility to deficiency symptoms.

Selenium compounds are effective in preventing the majority of tocopherol deficiency symptoms, especially on diets low in biologically available selenium (37–39). Dietary selenium increases tocopherol transport and storage, and selenium-rich proteins may be associated with tocopherol utilization (40). Organic selenium compounds accelerate the antioxidant properties of sulfhydryl compounds (41) and decompose peroxides (42, 53). Re-

cent work indicates that their biological function may be in the catalysis of sulfhydryl exchange reactions regulating the function of enzymes which either contain sulfhydryl groups in their active center or are dependent upon disulfide linkages for conformational retention (43).

There is thus a considerable literature in support of free radical mediated reactions in antioxidant deficiency at normal oxygen pressures (0.2 atm).

Thiol Oxidation and Reduction in Toxicity

Of the many compounds potentially influenced by oxygen, ozone, and radiation, thiols have received the most attention. Thiols may, in fact, play the key role in the molecular events leading to the disruptions observed as inferred from vitamin E deficiency.

Although sulfur amino acids and sulfhydryl compounds are less effective than seleno compounds, the presence of much higher concentrations of sulfur compounds may make them quantitatively more important as antioxidants. Sulfur compounds act synergistically with tocopherol, breaking free radical chains, decomposing lipid peroxides, and repairing damage to sulfhydryl groups of proteins. The antioxidant reactions of sulfur amino acids (66) are:

Reaction with free radical intermediaries:

$$\begin{aligned} ROO\cdot + R'SH &\rightarrow R'S\cdot + ROOH \\ 2R'S\cdot &\rightarrow R'SSR' \end{aligned}$$

Peroxide decomposition:

$$\begin{aligned} R'SCH_3 + ROOH &\rightarrow R'SOCH_3 + ROH \\ R'SSR' + ROOH &\rightarrow R'SSOR' + ROH \end{aligned}$$

Molecular repair of important sulfhydryl groups:

$$\begin{aligned} 2\ R'SH + ROOH &\rightarrow R'SSR' + ROH + H_2O \\ P\text{–}S\cdot + R'SH &\rightarrow P\text{–}SH + R'S\cdot \end{aligned}$$

The inhibition of enzymes and the respiration of tissue homogenates and slices appears to be particularly involved in the oxidation of tissue sulfhydryl groups. Haugaard (1) has reviewed the role of both nonprotein and protein sulfhydryl groups in oxygen toxicity. He points out that much of the mediation of oxygen toxicity by metabolic intermediary compounds (succinate, γ-amino butyric acid, and γ-hydroxyaminobutyric acid) may be related to the provision of critical amounts of substrate necessary for the generation of reduced pyridine nucleotides and hence sulfhydryl groups.

Oxygen generally depresses carbohydrate metabolism, possibly as Haugaard suggests, through the loss of lipoic acid and coenzyme A to their disulfide or higher oxidation states. *Achromobacter* grew in high pressure oxygen when provided with succinate, fumarate, malate, or glutamate, but not

when 1-amino-2-propanol, acetate, lactate, citrate, or glucose served as the carbon source (67). Oxygen high pressure inhibited the sulfhydryl-containing tricarboxylic acid cycle, but not the dicarboxylic pathway supporting the importance of sulfhydryl function in the regulation of metabolism under oxygen high pressure. The radiation sensitivity of a number of species of bacteria is related to their *p*-hydroxymercuribenzoic acid binding capacity, which, in turn, is interpreted as a function of their sulfhydryl content (67a). The sulfhydryl binding sites are suggested to function in radiation repair rather than directly in radiation resistance.

Allosteric alterations through the oxidation of sulfhydryl groups is also possible, in that the oxygen binding capacity of hemoglobin is altered by loss of sulfhydryl groups, even though they are not involved directly in the oxygen binding of the molecule. A clear case for such allosteric alterations of enzymes, as well as that for reactive sites containing sulfhydryl groups, is presented in the work of Dickson & Tappel (43) on the catalytic effect of selenocystine and selenomethionine on the inactivation of ribonuclease. Ribonuclease contains a disulfide essential for native conformation, and exchange to a mixed disulfide alters the conformation and inhibits the enzyme. Glyceraldehyde-3-phosphate dehydrogenase and papain, containing sulfhydryl groups in their active sites, are activated and protected from oxygen inactivation by the presence of trace amounts of the selenocystine. A substrate displacing complex is also hypothesized with papain and selenocystine. In these experiments the concentrations of seleno compounds approached that calculated for the trace selenium content of normal cells.

Two views exist as to the fate of sulfhydryl and disulfide groups in cells exposed to radiation. Saife (69) and Harris (70) found no alteration of nonprotein sulfhydryl content on x-irradiation of Ehrlich ascites tumor cells, but Harris found a transient depression of nonprotein sulfhydryl content on exposure to high pressure oxygen. Révész & Modig (71), on the other hand, found a decrease in both nonprotein and protein sulfhydryl content and a concomitant increase in the disulfide content. On irradiation in argon there was reasonable stochiometry between the loss of sulfhydryl and the increase in disulfide content. In oxygen they failed to observe such a stochiometric relationship, but found the alterations in the redox state of sulfhydryl groups to be roughly proportional to the radiation dose. Harris & van den Brenk (72) examined the lung and liver nonprotein sulfhydryl content following exposure to oxygen high pressures or pentylenetetrazol, a drug producing convulsions and lung edema much like oxygen high pressures. Rats convulsed by either oxygen high pressures or pentylenetetrazol had similar decreases in lung nonprotein sulfhydryl content and increases in lung weight (a measure of edema). Inhibition of both convulsions and lung edema occurred on the prior administration of 5-hydroxytryptamine. Lung nonprotein sulfhydryl content did not decline when convulsions were prevented. From this work, Harris & van den Brenk postulated that direct oxidative attack on the lung was unlikely. These observations are contrasted to

the previous report of Jameison et al. (72a) from the same laboratory where high pressure oxygen oxidized the lung sulfhydryl groups to disulfide groups. The sensitivity of the oxidation state of thiols to a multitude of factors cannot be overemphasized. Furthermore, the intracellular sulfhydryl content of ozone-treated human red blood cells did not oxidize while the membrane-bound sulfhydryl enzyme acetylcholinesterase was inhibited (75a). Acetylcholinesterase is, moreover, inhibited by peroxides (75b). Ozone-treated red blood cells in which lipid peroxidation has occurred are also more susceptible to osmotic shock. The fact that ozone, H_2O_2, or oxygen high pressure fails to oxidize tissue sulfhydryl groups under conditions where membrane oxidation predominates, is not sufficient justification to dismiss sulfhydryl groups as functional antioxidants. One should recall that nonprotein sulfhydryl groups are relatively closely coupled to the redox state of cell pyridine nucleotides, and they, in turn, to the oxygen tension. The antioxidant electron transport scheme involving glutathione as an electron acceptor may be very poorly coupled, since electrons must be carried from the membrane-bound tocopherol to the aqueous phase. Furthermore, since tocopherol is incorporated into the membrane, and since unsaturated fatty acid oxidation must be contained in the lipid phase to ensure chain propagation, coupling between the relatively stable tocopherol free radical and the remainder of the chain might be extremely poor. The total flux of electrons arising from lipid oxidation might then be insignificant compared to other electron flows affecting the redox state of tissue sulfhydryl groups.

While sulfhydryl content of cells plays an important part in cellular regulation under oxidant stress, it is also subject to a number of other factors, such as the release of neurohormones or starvation. Because of the link between nonprotein sulfhydryl compounds (predominantly glutathione) and reduced pyridine nucleotides, variations in the oxidation-reduction state of the pyridine nucleotides will affect the sulfhydryl content. Chance and coworkers (75) have shown that a shift of pyridine nucleotides from reduced to oxidized states occurs on oxygen high pressure exposure. The exact role of glutathione in cell regulation is still unknown and much must be learned before one can explain fully all of the observations. It is interesting to note that red blood cells from vitamin E deficient rats were hemolyzed by concentration of H_2O_2 which failed to affect red cells from vitamin E supplemented animals. No marked oxidation of reduced glutathione occurred during H_2O_2 treatment, despite evidence for lipid peroxidation preceding the lysis (47).

Hess & Menzel (76) found that vitamin E deficient rats exposed to 600 torr oxygen had unusually high numbers of centrioles in their kidney proximal convoluted tubule cells. Deficient animals in air also exhibited these alterations, but not vitamin E supplemented animals. Centrioles contain large concentrations of sulfhydryl groups whose reduced state appears essential for centriole function (77).

Chromosomal breaks from ozone exposure have been found in *Vicia faba*

(76a) and human tissue cultures (76b). Pace et al. (76c) and Sachsenmaier et al. (76d) also found that ozone inhibits mitotic activity of cell cultures. Pace et al. found blebs and other surface lesions suggestive of lysosomal or cell membrane destruction.

Lastly, ozone damage to the respiration of isolated mitochondria could be reversed by ascorbate or glutathione (80).

Lipid Oxidation in Toxicity

Treatment with antiozonides, manganous 1,2-napthoquinone-2-oxime, and cobaltous 8-quinolate prevented ozone damage to tomato and tobacco plants (79). As mentioned above, Gershman also found these ions effective as oxygen protectors. Cobaltous and manganous ions are good free radical scavengers and may function in this manner.

Direct evidence for lipid oxidation in ozone atmospheres has been cited above (16a-d). Aldehydes are formed on lipid oxidation, and their appearance in ozone-exposed lungs may arise via this mechanism (76e). Alterations in cell membranes may also be related to lipid peroxidation. Brinkman et al. (76f) found that ozone breathing increased radiation-induced spherocytosis. This is most likely a cumulative effect of ozone damage and radiation-induced free radicals on the red blood cell membrane.

Indirect evidence for membrane oxidation at less than 1 atm oxygen is found in the latent damage to liver and lung lysosomes of rats exposed to 600 torr oxygen (74). Harris (73), however, found no release of leukocyte lysosomes at oxygen high pressures. The presence of latent damage on chronic oxygen exposure is akin to vitamin E deficiency, since a specific level of oxidation is required in air before the first symptom of deficiency, creatinuria, is observed (20).

The evidence for lipid oxidation in radiation and oxygen toxicity presented above indicates that many of the common effects may be related through their action on the unsaturated lipids of cell membranes. Where dissimilarities exist, they are probably associated with the differences in the efficiency of these agents at specific sites. Compare for instance the "radiation equivalents" of chromosomal breakage by ozone and x-irradiation (76a, 76b); ozone is less efficient since it does not penetrate the cell interior as well as radiation (76c). Where sufficient details are known and the balance of lipid versus aqueous phase-generated free radicals is the same, there are marked similarities between all three agents.

Convulsions under oxygen high pressures are associated with the production of lung edema. Since lung edema does not appear in animals exposed to these pressures but prevented from convulsions by 5-hydroxytryaptamine, lung edema at these pressures might not be associated with direct action of oxygen on the lung, as already noted from Harris & van den Brenk. Pulmonary edema is often noted following nonthoracic wounds in man, and the mechanisms of lung edema from this impairment have been studied. A concise review of pulmonary edema arising from nonthoracic wounds has been

provided by Simeone (81). Briefly, a center appears to exist in the preoptic portion of the hypothalamus which induces pulmonary edema by the release of some agent. A second center exists dorsal to the optic chiasma in the hypothalamus which suppresses the first center. Pulmonary edema can also be caused by the release of small amounts of lipid. Oleic acid is particularly effective (82).

Objections to the free radical or enzyme inhibition hypotheses of oxygen toxicity were made because the rapidity of onset of convulsions at high pressure seemed to be more rapid than could be accounted for on the basis of enzyme inhibition. Injection of a γ-aminobutyric and γ-aminohydroxybutyric acid prevented both convulsions and edema. Chance et al. (75) and Harris (70) have found a rapid shift of pyridine nucleotides toward the oxidized forms on oxygen high pressure exposure as mentioned above. The oxidation state of glutathione appears to be closely linked to that of the pyridine nucleotides. Gamma-aminobutyric acid, and its precursor γ-hydroxybutyric acid, may prevent convulsion by the provision of reducing equivalents via the γ-aminobutyric shunt. Short term reactions to oxygen high pressure may be due to substrate deficiencies versus chance free radical effects.

Veninga has presented a provocative short review comparing ozone and ionizing radiation much as we have here. He points out a number of events associated with low levels of ozone, such as increases in 5-hydroxytryptamine in the urine of ozone-exposed frogs and the release of 5-hydroxytryptamine from the platelets of rabbits exposed to ozone at levels below apparent lung edema. Similar changes in 5-hydroxytryptamine have been found on x-irradiation (84), hypoxia (85), and ultraviolet irradiation (86). The lungs also contained significantly higher amounts of 5-hydroxytryptamine after ozone exposure. The neurological effects reviewed by Stockinger (3) following ozone inhalation in man may be more involved in the release of 5-hydroxytryptamine than in transport of ozone or complexes of ozone per se. Transport appears especially unlikely in view of the negative experiments with transfused animals (86a). Certainly this area of ozone toxicology deserves more attention, considering the potential effects upon human ecology as well as pathology.

It is difficult to explain the adaptation of laboratory animals to toxic levels of ozone and oxygen following exposure to sublethal concentrations of these agents (3). One finds it unlikely that a small amount of free radicals could be a protective agent. Kydd (86b) has recently suggested a physiological explanation that prior exposure to edematous agents causes an adaptive change in the lymphatic drainage of the lung, thereby increasing the osmotic pressure at which edema occurs. Adaptation thus need not require the invocation of any special "inducible" antioxidant system.

Veninga (83) has also provoked some additional thoughts by reporting that a special strain of black mice, C_{57}, had reduced litters, increased blepharoptosis, and unlimited growth of incisors following exposure of 0.2 ppm of ozone during fetal development. Nine per cent of the impregnated

females lost their litters in the control population compared to 34 per cent in the ozone-treated groups, mainly by fetal resorption. Fetal resorption in rats is a typical sign of vitamin E deficiency. Could this observation be related to a marginal vitamin E status of the dam during pregnancy? Certainly the sparsity of long term experiments on ozone indicates the need for a better understanding of the molecular events involved in ozone toxicity.

Mengel (87) has pointed out that peroxidation of the red cell has wider implications than just the mechanism of oxygen toxicity, being perhaps applicable to paroxysmal nocturnal hemoglobinuria, acanthocytosis (a-beta-lipoproteinemia), hemolytic anemia of the premature, peroxidative damage from "oxidant drugs," erythropoietic protoporphyria, and congenital non-spherocytic hemolytic anemia. Other dysfunctions in the mechanism required for maintenance of the reduced glutathione content of the red cell or other membrane structures are likely to be discovered and explained by the application of this hypothesis. The increasing concentration of ozone and other oxidants such as nitrogen dioxide and peroxyacetyl nitrate in the atmosphere from pollution may demonstrate new symptoms of the overall phenomenon of antitoxidant deficiency. Air pollution induced emphysema and other obstructive respiratory diseases may occur in greater prevalency because of the peroxidation of lung lipids by ozone and other air pollutants.

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