Clinical Science Review

The Expanding Role of Oxygen Free Radicals in Clinical Medicine

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In 1969 McCord and Fridovich discovered superoxide dismutase, which converts the oxygen free radical O_2^- to hydrogen peroxide H_2O_2. In the presence of excess O_2^-, H_2O_2 may then undergo further reduction to the highly toxic hydroxyl radical, $OH^\bullet$. Since the description of this enzymatic process, there has been explosive growth in related biochemical research, which has now percolated through to clinical investigation. The hypoxanthine-xanthine oxidase system originally used as a radical production model has a close counterpart in the ischemia-reperfusion phenomenon purported to cause diseases of heart, brain and gastrointestinal tract, and free radicals are now known to have a critical role in postphagocytic bacterial killing. Prototypic deficiency diseases such as chronic granulomatous disease are now recognized. Some evidence indicates that excess states such as perhaps Batten's disease also occur, and environmental influences such as selenium and vitamin E deficiency may augment free radical levels. Many disorders including microvasculopathies, noncardiogenic pulmonary edema, glomerulopathies and radiation damage may owe part of their proximate pathogenesis to free radicals. Control of tissue free radical levels is now pharmacologically feasible and perhaps justified for specific diseases.

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For a medical student of 20 years ago, it may have seemed strange to contemplate the teleology of why humans were blessed with the enzyme xanthine oxidase—a seemingly poor joke of divine engineering to convert the very soluble and harmless products of purine catabolism into the very insoluble and execrable uric acid—a source of misery to all afflicted organisms excepting rheumatologists and urologists. The more curious of these medical students may have noticed that molecular oxygen was a reactant in the conversion from hypoxanthine to xanthine, and from xanthine to uric acid, and that a single conversion did not donate the usual four electrons converting O_2 to $2H_2O$, but only a single electron that would have to make O_2^- or $HO_2^\bullet$, known as the oxygen free radical, superoxide. Biochemists had long been aware of reactions in which fewer than four electrons would be transferred: one electron gave superoxide, two gave $O_2^=$ or H_2O_2 (hydrogen peroxide) and three gave $O^=$ and O^-, or H_2O and $OH^\bullet$ (hydroxyl radical). These species, however, were thought to "go away" in a fashion that would be of no biologic significance.

This was at least the case until 1969 when a little-noticed paper by McCord and Fridovich appeared in the *Journal of Biological Chemistry* entitled "Superoxide Dismutase. An Enzymatic Function for Erythrocuprein (Hemocuprein)."[1] These authors showed that the further reduction of O_2^- to H_2O_2 was catalyzed by a copper-containing enzyme that they named superoxide dismutase. A paper from the same laboratory the following year showed that the xanthine-xanthine oxidase or hypoxanthine-xanthine oxidase system could also generate $OH^\bullet$.[2] Although there was some dispute about how this conversion occurred, it was finally established that the three electron-reduced oxygen moieties occurred by metal catalysis through the Fenton reaction as follows[3]:

$$\begin{aligned}
HX + O_2 &\xrightarrow{XO} X + O_2^- \\
2\,O_2^- + 2H^+ &\xrightarrow{SOD} O_2 + H_2O_2 \\
O_2^- + Me^{n+} &\longrightarrow O_2 + Me^{(n-1)+} \\
Me^{(n-1)+} + H_2O_2 &\longrightarrow Me^{n+} + OH^- + OH^\bullet,
\end{aligned}$$

where HX = hypoxanthine, Me = metal catalyst, SOD = superoxide dismutase, X = xanthine and XO = xanthine oxidase.

The generation of free radicals is complemented by natural and xenobiotic scavengers of free radicals that by their own

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ABBREVIATIONS USED IN TEXT

DMSO = dimethyl sulfoxide
MLP = maximum life-span potential
NADPH = the reduced form of nicotinamide-adenine dinucleotide phosphate
SMR = specific metabolic rate

radical conversion serve to neutralize $OH^{\bullet}$. Such substances include glucose, dimethyl sulfoxide (DMSO), mannitol, phenobarbital, ascorbic acid, vitamin E and many other substances, perhaps including uric acid, the end product of the xanthine-xanthine oxidase system.[4]

The establishment of the enzymatic machinery for generating and disposing of free radicals combined with their highly reactive and perhaps toxic properties to organic molecules rapidly led to an explosion of interest in them from the perspectives of measurement techniques,[5-7] biochemistry[8-12] and clinical significance. In this review I shall consider only the last of these with regard to the salutary and toxic effects of these fascinating reactants. Although many of the references noted are from conference literature and require confirmation, the breadth of work in this new area demonstrates common threads of activity of free radicals in many organ systems that, in the aggregate, suggest future directions of clinical research in the field.

With the recognition that free radicals produce a variety of potentially untoward effects in the test tube, such as ester cleavage, DNA and RNA depolymerization, lipid peroxidation, nucleophilic attack of saturated compounds, hyaluronic acid depolymerization and enhancement of arachidonate cascades through prostaglandins, prostacyclins, thromboxanes, chemotactic factors and leukotrienes, basic information began to percolate slowly into the clinical arena in the early to mid-1970s. In 1976, *Index Medicus* introduced a subject heading of "Free Radicals," which contained five references. This grew to a peak of 32 to 36 entries from 1981 through 1985, and the abstracts of major clinical research organizations have had large recent increases in the number of presentations relating to free radicals.

Prototypic Free Radical Deficiency Disease

Chronic Granulomatous Disease

In 1967 Holmes, Quie, Windhorst and Good first reported cases of chronic granulomatous disease, which they characterized as an "inborn abnormality of phagocytic function" leading to normal phagocytosis of bacteria but without bacterial killing.[13] The following year, these investigators reported that granulocytes of chronic granulomatous disease undergoing phagocytosis had reduced O_2 consumption.[14] The significance of this finding had to await the classic discovery by Babior, Kipnes and Curnutte in 1973 that O_2 consumption by leukocytes undergoing phagocytosis results in the production of large amounts of O_2^-,[15] and that free radicals have a direct role in bacterial killing. Gabig, Kipnes and Babior later found that this O_2^- production from the "oxidative burst" of phagocytosis was mediated by a plasmalemmal enzyme that was termed NADPH (the reduced form of nicotinamide-adenine dinucleotide phosphate) oxidase.[16] Thus, the defect of patients with chronic granulomatous disease that leads to frequent serious infection is directly attributable to a deficiency of O_2^- production.

Lest one gain the false impression that impaired bactericidal action of phagocytes is simply attributable to defective free radical production, it should be pointed out that this is not the case for the Chédiak-Higashi syndrome. In this syndrome, as in chronic granulomatous disease, patients suffer from repeated and severe infections. Phagocytosis is normal in both, yet in the Chédiak-Higashi syndrome the free radical production is increased,[17,18] unlike the situation in chronic granulomatous disease where it is decreased. In the Chédiak-Higashi syndrome, the defect apparently has to do with the failure of phagocytes to deliver peroxidase to the phagosomes where local levels of free radicals bring about bactericidal activity.[17]

Possible Prototypic Free Radical Excess Disease

Batten's Disease—Ceroid Lipofuscinosis

In 1903 F. E. Batten described the familial cerebral degeneration syndrome[19] that was later characterized by neuronal deposition of lipofuscin[20] in specific patterns in the perikaryon. A clue to its complex etiology might have been seen in the fact that although its worldwide incidence is 1 per 100,000 live births, its incidence in Finland is 1 per 13,000,[21] a point to which I will return.

Armed with the knowledge that the Fenton reaction for $OH^{\bullet}$ production requires metal ions and previous data perhaps incriminating decreased elimination of free radicals in Batten's disease, Gutteridge and co-workers studied free metals and antifree radical activity in the cerebrospinal fluid of these patients.[21] Nonprotein bound iron levels in the cerebrospinal fluid of patients with Batten's disease was 74% higher than in controls, and their total antioxidant or scavenging activity was only 38% of normal. Although the picture is not complete, one might hypothesize that with increased free radical production from the Fenton reaction and decreased natural scavenger activity, patients with Batten's disease may have extensive brain destruction from lipid peroxidation. This deficiency of antioxidant activity associated with a disease that is prevalent in Finland is consistent with the contention that environmental influences may play a role in free radical formation or decreased clearance.

Role of Selenium in Protection of Excess Free Radicals

Veterinarians have long been aware of a variety of animal diseases that they have attributed to selenium-deficient diets including liver necrosis in rats, white muscle disease in sheep, hepatosis dietetica and mulberry heart disease in pigs, gizzard myopathy in turkeys and pancreatic atrophy in chicks.[22,23]

It is now apparent that the selenium-containing enzyme, glutathione peroxidase, which harmlessly produces a radical of reduced glutathione by destroying H_2O_2, is deficient in animals and humans ingesting selenium-poor diets.[24,25] With the loss of glutathione peroxidase, the antioxidant protective mechanisms are depleted, opening the way for $OH^{\bullet}$ damage in a variety of tissues. In mulberry heart disease, capillary thrombi may be one of the earliest changes noted.[23] This dietary importance of selenium may in part explain the 7.7-fold increased incidence of Batten's disease in Finland, an area of selenium-poor soils. One must still be cautious regarding this possible link, however, because the effect of

dietary selenium repletion is unknown except in specific animal diseases.[23]

Capillaropathies and Venulopathies

Arfors and colleagues, and now many other groups, have shown that the microcirculation in a variety of tissues is a target for free radical damage induced by systems that generate hypoxanthine-xanthine oxidase.[26,27] Using the hamster cheek pouch preparation with intravital microscopy, these workers found that local superfusion with hypoxanthine (0.96 mmol per liter) and xanthine oxidase (0.05 units per ml) results within five minutes in massive postcapillary venular extravasation of fluorescein isothiocyanate-dextran 150 through discrete sites. These "leaky sites" are presumed to be located at areas where endothelial cell barriers are disrupted, and they are known to occur near zones where granulocytes adhere. Moreover, the hyaluronic acid gel phase of the interstitium that retards macromolecular movement has been shown in in vitro studies to become more fluid, thereby augmenting macromolecular flux into the interstitium and secondarily allowing for increased edema formation by augmenting interstitial oncotic pressure.[28] These edemogenic changes in vascular permeability were greatly reduced by the prior addition of 50 μg per ml of superoxide dismutase, 50 μg per ml of catalase, 10 mmol per liter of DMSO and 10 mmol per liter of L-methionine to the superfusate. Significant protection against increased hyaluronic acid fluidity was afforded by the prior addition of 5 to 10 mmol per liter of mannitol, ethanol or DMSO.

Ischemic Cardiomyopathy

The hypoxanthine-xanthine-xanthine oxidase system appears to have a direct role in the generation of tissue injury brought about by ischemia, and two tissues where this is most apparent are the heart and the gut. The xanthine-xanthine oxidase system when used to treat sarcoplasmic vesicles induces damage in the form of increased Ca^{++} permeability and depressed Ca^{++} uptake at low pH.[29] In addition, rats subjected to coronary ligation are afforded significant sparing of myocardium when treated with the various scavengers or antioxidants such as α-tocopherol, diphenyl-*p*-phenylene-diamine, selenium or the cyclooxygenase inhibitor, diclofenac sodium.[30] Most impressive are the recent data from Downey and colleagues that pretreatment of dogs with 400 mg of allopurinol, a xanthine oxidase inhibitor, reduces by more than 50% the size of the no-flow zones in canine myocardium subjected to coronary embolization.[31] Hess and co-workers have reviewed the role of free radicals in ischemic heart disease,[32] and the thrust of the recent studies appears to justify the model shown in Figure 1.

Figure 1 is designed to show reactions occurring with ischemia as one moves down the ordinate and reactions occurring with reperfusion as one moves later in time to the right along the abscissa. With ischemia, adenosine triphosphate stores are rapidly depleted through inosine to hypoxanthine, which builds to large levels in the ischemic tissue and very likely in its vascular endothelium (Karl E. Arfors, PhD, oral communication, March 1985). Simultaneously, xanthine dehydrogenase is rapidly converted to xanthine oxidase. This sets the stage for enormous generation of free radicals once reperfusion presents large amounts of O_2 to the primed hypoxanthine-xanthine oxidase system, and this quickly generates O_2^- and then $OH^\bullet$ by the Fenton reaction. It thus seems reasonable that if reperfusion were slowly reinstituted, or allopurinol were present to inhibit xanthine oxidase, or scavengers such as DMSO were present, $OH^\bullet$ damage to the endothelium and other tissue, such as myocardial cells, might be ameliorated. Clinical studies to test this thesis are already under way in a number of centers.

Acute Noncardiogenic Pulmonary Edema

The area of free radical research into tissue damage that has been the most widely and aggressively pursued is undoubtedly the lung. It is known that after 72 hours of breathing

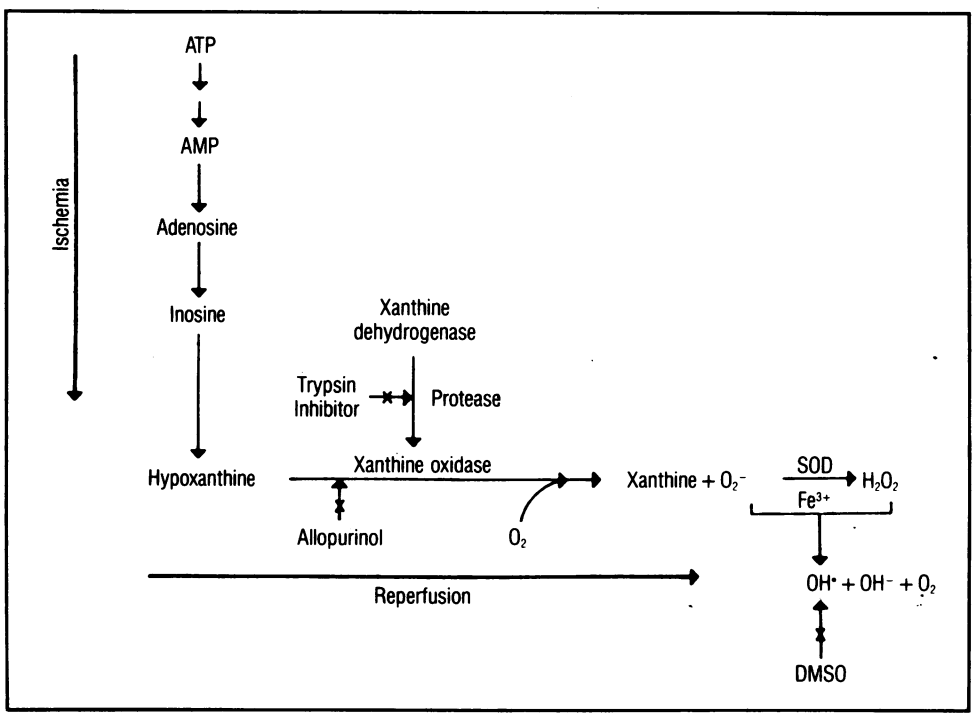

Figure 1.—A scheme compatible with ischemic and reperfusion toxicity by free radicals. As one moves down the ordinate, the vascular endothelium is primed by increased amounts of xanthine and xanthine oxidase. Upon reperfusion occurring along the abscissa, the increased amounts of xanthine and xanthine oxidase produce toxic free radicals. There are many points along the system that are theoretically amenable to therapeutic intervention in humans. (Reprinted from Granger and Parks[33] with permission from the authors and The American Physiological Society.) AMP = adenosine monophosphate, ATP = adenosine triphosphate, DMSO = dimethyl sulfoxide, SOD = superoxide dismutase

100% O_2, increased lung lymph flux of both water and protein and increased lung water develop in sheep lung without a detectable increase in microvascular pressure.[34] In addition, the xanthine-xanthine oxidase system produces similar changes in isolated perfused rabbit lung.[35] Catalase (which degrades H_2O_2) and human erythrocytes (rich in scavengers) can prevent the protein leak in isolated perfused rat lungs.[36]

These lung injury models, like the venulopathies referred to in the articles by Björk and Arfors and Del Maestro and associates,[26,27] are critically dependent on the presence of granulocytes.[37] These granulocytes appear from the preliminary results by Shasby and Shasby to exert their effects through the arachidonate-cyclooxygenase cascade, since inhibition with aspirin attenuates the lung edema produced by arachidonate and granulocytes and also inhibits the increased permeability effects induced in cultured pulmonary vascular endothelial monolayers.[38] Glutathione peroxidase is an important antioxidant in protecting against these free radicals because selenium-deficient pulmonary epithelial cells more readily lyse when exposed to activated granulocytes than those cells in an adequate selenium medium.[39]

Review of information such as this has led to a well-advanced thesis of free radical damage in lungs subjected to hyperoxia.[40,41] According to Repine and Tate,[41] hyperoxia damages alveolar macrophages that release neutrophil chemoattractants, stimulate granulocyte adherence and also promote granulocytes to release their toxic oxygen free radicals, which are the proximate cause of pulmonary endothelial injury. This mechanism may be critically important even in antialveolar basement membrane disease in the lung because goat-antirabbit alveolar basement membrane antibody does not even bind to rabbit lung in vivo until the rabbits have been subjected to breathing 100% O_2 for 62 to 66 hours, after which time pulmonary edema and hemorrhagic pneumonitis develop.[42]

This thesis of how pulmonary hyperoxic damage occurs already has therapeutic implications, at least for laboratory animals. Hyperoxic rats treated with insufflated erythrocytes have prolonged survival[43]; superoxide dismutase and catalase conjugated to polyethylene glycol decrease pulmonary damage in hyperoxic rats when presented as a lavage.[43] It may be of interest to note that the healing process after O_2^- injury is retarded in hyperoxic dogs treated with 2 mg per kg of body weight per day of dexamethasone.[44]

Gastrointestinal Disease

In much the same way as the developments in ischemic heart disease have shown, workers in gastrointestinal physiology have shown the equivalent effects of ischemia and hypoxanthine-xanthine oxidase, but Granger and Parks have refined the measurements to show that protein solvent drag reflection coefficient of intestinal capillaries falls from .92 to .66 in the presence of free radical excess, thus indicating a widening of pores or pore-equivalents in the blood-tissue barrier.[33] These effects are completely reversed by pretreatment with superoxide dismutase, allopurinol or DMSO. Similar effects of superoxide dismutase or catalase have been found in the isolated perfused canine pancreas model stimulated to inflammation by oleic acid infusion, partial duct obstruction with secretin treatment or two hours of ischemia followed by reperfusion.[45]

Renal Ischemia and Glomerulopathies

Free radicals have been shown to play a role in the ischemia-reperfusion model of acute renal failure in rats.[46] Primary renal glomerular diseases have generally been thought to result either from passive trapping of antigen-antibody complexes with complement activation or from targeted antibodies against glomerular basement membrane. Evidence has been presented that the first type of glomerulonephritis is ameliorated by pretreatment with superoxide dismutase.[47] In more extensive studies, although still preliminary, Rehan and colleagues produced the second type of glomerulonephritis with sheep-anti-rat-glomerular basement membrane antibody in rats that was marked by a sixfold increase in proteinuria within 24 hours. Pretreatment with catalase resulted in a 70% reduction in proteinuria, but superoxide dismutase caused only a 25% reduction.[48] These same authors have produced proteinuria in rats with the neutrophil activator phorbol myristate acetate and were able to prevent the development of proteinuria by coinstilling catalase, but not superoxide dismutase.[49] These studies on free radical participation in glomerular leak of protein emphasize the general relationships between free radicals and the induction of microvasculopathy in a variety of tissues.

Other Neurologic Diseases

Kontos and associates have presented evidence that the free radical cascade produces vascular damage in cats with acute, severe hypertension.[50] Direct free radical generation by injecting a combination of hypoxanthine, xanthine oxidase, adenosine diphosphate and ferric ion into the caudate putamen results in a panoply of pathologic findings, including increased sodium and water content, decreased potassium content, neuronal cytoplasmic vacuolation, granulocyte infiltration, the presence of lipid-laden macrophages, reactive astrocytosis and increased vascular leakage of fluorescent dye.[51]

Some polyphenolic neurotoxins result in $OH^\bullet$ production in which the scavengers appear to be catecholamines thought by some to result in destruction of the parent neurons.[52]

Even in an area seemingly unrelated to free radicals such as spinal cord trauma, cholesterol free radicals have been measured and found to be increased.[53] Thus, a variety of neurologic diseases resulting from numerous proximate causes are at least associated with a local excess of oxygen free radicals.

Ischemic Disease of the Skin

Superoxide dismutase has been found to ameliorate the histologic appearance of skin after prolonged venous hypertensive ischemia in island skin flaps.[54] Although this may suggest a role for oxygen free radical formation in the cutaneous necrosis resulting from ischemia, research in this area is just beginning.

Aging

Allometric data of Tolmasoff and Cutler and co-workers show that superoxide dismutase titer per specific metabolic rate (SMR) is highly positively correlated with the maximum life-span potential (MLP) from shrews to humans.[55,56] Within a species, however, tissue superoxide dismutase decreases with age, as has been shown to occur in the liver of

rats.[57] What role it may have in prolonging the life span is unclear unless most organisms tend to produce excess free radicals. Hence, it might be argued, those with the most antioxidant activity might be expected to survive the longest. One way free radicals might relate to the aging process per se is by promoting cross-linking of collagen throughout life.[58]

The entire contention that superoxide dismutase has anything whatever to do with MLP has been dealt a serious challenge on statistical grounds by Sullivan.[59] He argues that because the reciprocal of the SMR is tightly correlated to the MLP ($r = .952$), then any random variable uncorrelated to the MLP may be highly correlated when divided by the SMR. He notes in particular that Tolmasoff's data on superoxide dismutase do not correlate with the MLP until divided by the SMR, and that even hemoglobin levels divided by the SMR are correlated to the MLP with $r = .940$. One must therefore be extremely cautious about interpreting correlations in which the superoxide dismutase levels are "corrected" by dividing by the SMR.

Nononcogenic Xenobiotics

The toxicity of paraquat is now generally believed to be due to its radicalization by NADPH with donation of its unpaired electron to O_2, thus initiating the cascade resulting in the production of the toxic $OH^{\bullet}$.[60] The mutagenicity of paraquat seems similarly related to the formation of free radicals.

Sickle Cell Disease

Sickle cells have been shown to generate large amounts of O_2^-, H_2O_2 and $OH^{\bullet}$.[61] Hence, the end-organ damage of sickle cell disease may have pathogenetic mechanisms more complex than those due to the mere sludging action of sickle cells, but this possibility has not been widely studied.

Ultrasound and Irradiation

Radiation therapy may owe some of its side effects to the generation of free radicals. In a recent and small double-blind study of irradiated bladder tumors in humans treated either with placebo or bovine superoxide dismutase (orgotein), those receiving the superoxide dismutase had greater maximum voiding volume, less nocturia and less pain documented by reduced usage of analgesia than controls.[62]

There has been a recent unsettling report that ultrasound at even 1 MHz produces cavitation of body fluids that upon collapse results in abundant concentrations of free radicals even when superoxide dismutase is present.[63]

Cancer

At present a great debate exists as to whether free radicals have any role in the pathogenesis of any known cancers despite voluminous contributions of correlative and theoretical studies.[64,65] A recent National Institutes of Health workshop on the subject "was able to reach no clear consensus regarding the mode of involvement of free radicals in tumor promotion."[66] In fact, Drosophila fed carcinogenic or noncarcinogenic diets throughout life show that the free radical content per whole fly (by electron spin resonance technique) was less in the carcinogenic-fed flies than in those fed noncarcinogenic diets.[67]

Preliminary Human Trials—Collagen Diseases

Very limited trials using superoxide dismutase-like substances have shown the following: systemic lupus erythematosus in selected patients may improve immunologically with D-penicillamine.[68] Blood cell chromosome breaks in rheumatoid arthritis, systemic lupus and progressive systemic sclerosis may be improved by in vivo or in vitro treatment with superoxide dismutase.[69] In a 47-patient, double-blind study of orgotein in rheumatoid arthritis, there was substantial subjective, objective and laboratory improvement in the treatment group.[70] In a small number of patients, intraarticular orgotein was thought to have a salutary effect in osteoarthritis.[70] Although these early results are exciting, final conclusions must await larger and more rigorously constructed trials.

Discussion

In the 17 years since the discovery of superoxide dismutase, it has been found that O_2^-, H_2O_2 and $OH^{\bullet}$ are naturally produced in many biologic processes including ischemia, phagocytosis and in the production of xanthine from hypoxanthine and uric acid from xanthine. The hypoxanthine-xanthine-xanthine oxidase system has been a nearly precise analog of ischemia and reperfusion, with tissue damage being the same for both, and with both ameliorated by allopurinol or scavengers. Hallmark diseases of free radical deficiency such as chronic granulocytic disease and possible excess states such as Batten's disease are now becoming more clarified. Nutritional aspects of free radical excess are exemplified by selenium deficiency that depletes the antioxidant, glutathione peroxidase. Treatment trials with superoxide dismutase and scavengers have begun, and some show promising results. The control of oxygen free radical levels in a variety of human diseases is within pharmacologic possibility.

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