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Oxygen Toxicity

Introduction to a Protective Enzyme: Superoxide Dismutase

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Hyperbaric chamber

THE PRESSURE OF OXYGEN in inspired air at sea level (0.21 atmospheres absolute, or Ata) is not overtly toxic to man. Inhalation of pure oxygen (one Ata) for longer than one day will produce discernible manifestations of toxicity in man, however, and death will ensue from irreversible pulmonary damage if this exposure is continued, uninterrupted, for several days. The latent period of exposure before toxic manifestations become readily discernible is even shorter when individuals are exposed to inspired pressures of oxygen greater than can be attained at sea level. At inspired pressures greater than 2.5 Ata in a hyperbaric chamber, the initial overt manifestation of toxicity is likely to be neurologic rather than pulmonary.¹ Convulsions occur abruptly, leading to coma and death in experimental animals if the hyperoxic exposure is continued. Fortunately, the clinically discernible manifestations of oxygen toxicity can be reversed if the hyperoxic exposure is terminated promptly. This is obviously so for neurologic manifestations, since the end point occurs acutely and can be identified promptly. In practice, the more important pulmonary manifestations of toxicity (the only ones encountered by physicians without access to hyperbaric chambers) develop insidiously, and the hyperoxic exposure may not be terminated until irreversible damage has occurred. The prudent physician is aware of these risks and attempts to limit exposure of his patients to dosages that are not associated with overt toxicity. The concentration of oxygen in inspired gas is kept below 40–50% when long-term therapy is anticipated. Higher doses of oxygen are used in an interrupted manner, if possible, thereby extending tolerance greatly. Finally, these guidelines are exceeded and the likelihood of overt toxicity is accepted reluctant-

ly when high doses of oxygen are the only means to maintain levels of oxygen in arterial blood adequate to sustain life.

Investigations of oxygen toxicity have included studies *in vitro* of diverse forms of life ranging from microorganisms to simians and scattered observations in man. Paul Bert observed that oxygen pressures greater than 2.5 Ata, if continued in an uninterrupted manner, would lead to convulsions, coma, hypothermia, a decreased oxygen uptake, and death of laboratory animals.² J. L. Smith demonstrated that an uninterrupted exposure to approximately one Ata of oxygen would after several days lead to respiratory distress, gross manifestations of hypoxia, and death in experimental animals.³ Pulmonary tissue in these animals exhibited generalized severe damage at necropsy.

The pathology of pulmonary oxygen toxicity has been studied repeatedly and is now known to include abnormalities of pulmonary capillary endothelium, proliferation of pulmonary capillaries, transudation of fluid and formed elements in alveolar air spaces, formation of hyaline membranes within alveoli, patches of pneumonia, and hemorrhage.^{4,5} Intermittent exposures and prolonged inhalation of intermediate concentrations of oxygen delay the onset of overt toxicity.^{6,7} Metabolic and biochemical studies of the effects of oxygen toxicity reveal decreased oxygen uptake by cell fractions, a reduction in the intracellular level of high energy phosphates, apparent inactivation of some enzymes containing sulfhydryl groups, and release into cytoplasm of enzymes normally confined to lysosomes.^{8,9,10} Investigations have failed to distinguish whether any of these abnormalities is a cause or a result of the primary intracellular events that culminate in oxygen toxicity. A variety of pharmacologic protectants have been identified that might permit safe use of otherwise toxic dosages of oxygen. These in general delay but do not prevent the occurrence of oxygen toxicity. Among the protectants elucidated in various experimental models are vitamin E, succinate, gamma amino butyric acid, glutathione, disulfiram, and certain

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monamine oxidase inhibitors.¹¹ None of these agents has yet come into general clinical use.

To find a way of preventing oxygen toxicity we need to know the chemical basis of the toxicity and what natural protective mechanisms may have already evolved. We might begin by exploring the reasons for the contrasting abilities of aerobes and of obligate anaerobes to tolerate oxygen. Early explanations were based upon hydrogen peroxide and upon those enzymes such as catalases which consume hydrogen peroxide. Thus, many oxidative reactions, both enzymatic and nonenzymatic, are known to cause the divalent reduction of oxygen to hydrogen peroxide. Hydrogen peroxide is a reactive species and its accumulation inside cells would certainly lead to the death of these cells. Hydrogen peroxide was thus considered to be the agent of oxygen toxicity,¹³ and catalases and peroxidases, the defenses that permitted aerobic life by preventing the accumulation of this reactive compound. This theory, though meritorious, is not entirely satisfactory because aerobes have been described which lack catalase and anaerobes have been described which contain this enzyme.¹⁴⁻¹⁶ In addition, the hydrogen peroxide theory of oxygen toxicity is incomplete in that it fails to consider reduction products of oxygen, which are even more reactive and more inimical to life than is hydrogen peroxide.

Oxygen exhibits a distinct preference for reactions in which one electron is transferred at a time. The quantum-mechanical reasoning which underlies this assertion need not concern us here. However, the facts are that the reduction of oxygen often proceeds in univalent steps so that the first reduction product of oxygen, at neutral pH, will be O_2^- . This is called the superoxide radical and because of its reactivity and fleeting lifetime it was, for too long, of concern only to radiation chemists, who generated it with bursts of ionizing radiation and who explored its properties during the milliseconds of its existence.

In recent years it has become clear that the superoxide radical needs to concern biologists because this reactive radical is generated by a wide variety of biochemical events.^{17, 18} Thus, there are oxidative enzymes which, in their normal functioning, generate substantial amounts of superoxide radical and there are numerous nonenzymatic oxidations of materials, found inside of cells, which also produce superoxide radical. We must anticipate, therefore, that oxygen-metabolizing cells will be exposed to a flux of superoxide radical the

reactivity of which, if unopposed, would destroy these cells. What then may be said about a defense against the superoxide radical?

The primary defense appears to be an enzyme which catalyzes the reaction $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$ and which has been named superoxide dismutase.¹⁹ This enzyme is ubiquitous among oxygen-metabolizing cells but is lacking in obligate anaerobes.²⁰ Superoxide dismutase is an enormously efficient catalyst which operates at rates approaching the theoretical limit set by rates of diffusion.^{21, 22} Raising the concentration of oxygen induces increased accumulation of this enzyme in microorganisms and furthermore cells which contain high concentration of superoxide dismutase are more resistant to the lethality of hyperbaric oxygen than comparable cells which contain lower levels of this enzyme.²³ In addition, it has been possible to prepare mutants of *Escherichia coli* which exhibited a temperature-dependent defect in their ability to accumulate superoxide dismutase. These mutants had a parallel, temperature-dependent inability to grow in the presence of oxygen.¹⁸ Work currently underway suggests that mammalian cells are just as dependent upon superoxide dismutase for their ability to tolerate exposure to oxygen as are the microorganisms thus far investigated.

All of this evidence supports the hypothesis that the superoxide radical is an important agent of oxygen toxicity and that the enzyme superoxide dismutase, which so efficiently eliminates this radical from aqueous solutions, is an essential component of the defenses which have evolved to deal with it. With this knowledge, we may now attempt to devise means of minimizing the production of superoxide radicals in cells exposed to hyperoxia, or failing in that, to increase intracellular levels of superoxide dismutase. At any rate we now appear to be in a position to rationally attack a major component of oxygen toxicity; we know the identity of both the culprit and of the intracellular guardian.

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Circulation, Volume XLVIII, November 1973

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