Oxygen – friend and foe[1]

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As early as 1775, Joseph Priestley suggested that oxygen might not be an entirely unmixed blessing:

'... though pure dephlogisticated air might be very useful as a medicine, it might not be so proper for us in the usual healthy state of the body: for, as a candle burns so much faster in dephlogisticated than in common air, so we might, as may be said, live out too fast, and the animal powers be too soon exhausted in this pure kind of air.'

Although oxygen is by no means unique in combining favourable and unfavourable properties, it is remarkable how, on the one hand, we are totally dependent on oxygen for survival and yet, on the other hand, it has the capacity for inflicting extensive damage on all living tissues.

Oxygen and the evolution of life

The duality of oxygen is apparent in the impact of its evolution on life on the earth. Such a reactive gas could not have been present in the primitive reducing atmosphere evolved from volcanoes and fumaroles, although traces were probably formed by photodissociation of water in the upper atmosphere. Appreciable quantities of oxygen were first formed as a result of photosynthesis, certainly as long as 2000 megayears (Myr) ago (Tyler & Barghoorn 1954) and probably much earlier. This event presupposed the evolution of life based on deoxyribonucleic acid and proteins, now dated as early as 3400–3500 Myr ago (Walter *et al.* 1980). Such systems had evolved in a reducing atmosphere, deriving their energy from anaerobic metabolism of compounds which had been synthesized abiogenetically with solar ultraviolet light as a principal source of energy.

The appearance of oxygen in the biosphere then had two major effects on the evolution of life. First, it made possible aerobic metabolism which greatly increased the supply of high energy phosphates. Not only did this improve the biological energy but it also decreased the level of undesirable products of anaerobic metabolism such as lactic acid. This permitted evolution beyond the most primitive unicellular organisms, and we are now totally dependent on oxygen for survival. Secondly, and no less important, oxygen and its derivative ozone provided screening from solar ultraviolet light. This permitted colonization of the dry land by both plants and animals, an event which the fossil record dates to the mid-Silurian period (440 Myr ago). Advantageous though this development was, it has resulted in the earth being cut off from solar ultraviolet light as an energy source. Thus, if life should be destroyed on earth, it could not repeat its original pattern of evolution until oxygen disappeared from the atmosphere.

Survival of organisms in an oxidizing environment required development of protective mechanisms such as the enzymes superoxide dismutase and catalase, considered below. The mitochondrion may also be seen as a device to detoxify oxygen to water, a harmless end-product.

The oxygen molecule

The destructive character of oxygen derives from its molecular structure and, in particular, the configuration of electrons in the outer 2P shell (Figure 1). Oxygen is unique in having two unpaired electrons with parallel spin (shown by arrows). In a manner of speaking, it is thus a

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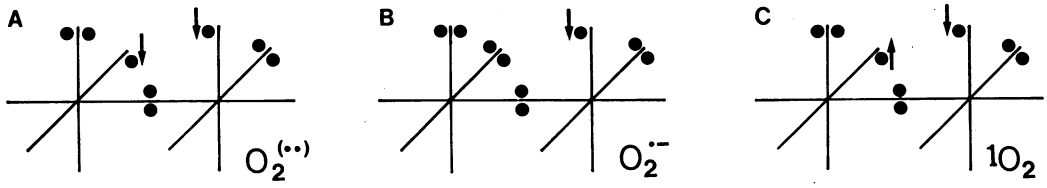

Figure 1. Configuration of the outer shell of electrons in oxygen (A), the superoxide free-radical (B) and singlet oxygen (C)

double free radical but, in fact, the molecule, though reactive, is stable and has an indefinite half-life. The unpaired electrons confer the property of paramagnetism which has been used in gas analysis.

Any change in the configuration of electrons in the 2P shell causes instability and a great increase in reactivity. A single electron may be donated from a wide variety of sources to pair with one of the unpaired electrons (Figure 1). The resultant molecule with one remaining unpaired electron is the superoxide anion radical, which is fundamental to oxygen toxicity. A second possibility is a reversal of the direction of rotation of one of the unpaired electrons resulting in the formation of the species known as singlet oxygen, not a free radical but nonetheless highly reactive. The third possibility is that the two unpaired electrons may each share an electron with a hydrogen atom to form hydrogen peroxide, a powerful and toxic oxidant though not a free radical.

Reduction of oxygen

Most of the oxygen consumed in the body is fully reduced to water in a single stage at the terminal cytochrome of the oxygen transport chain in the mitochrondrion. However, under certain conditions, the reduction of oxygen passes through two intermediate stages (Figure 2). The first stage is the acquisition of a single electron to form the superoxide anion radical. Two molecules of this species then undergo a dismutation in which one electron is transferred, one molecule reverting to oxygen and the other forming a short-lived intermediate which combines with two hydrogen ions to form hydrogen peroxide. This reaction is greatly accelerated by the intracellular enzyme superoxide dismutase which is widely distributed as a defence against

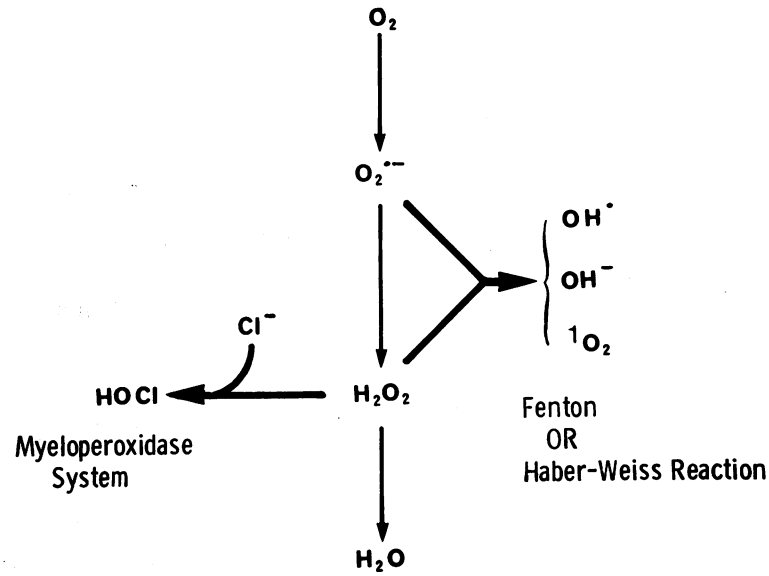

Figure 2. Three-stage reduction of oxygen to water and products derived from the intermediates

oxygen toxicity. Hydrogen peroxide itself is broken down into water and oxygen under the influence of catalase or one of various peroxidases.

The superoxide anion radical and hydrogen peroxide react to form the extremely reactive hydroxyl free radical, hydroxyl ion and singlet oxygen according to the Haber-Weiss or Fenton reactions (Figure 2). On the left of Figure 2 is shown the formation of hypochlorous acid from hydrogen peroxide by the enzyme myeloperoxidase.

This list of oxygen-derived free radicals and other compounds is not complete but probably includes the major substances concerned in the production of tissue damage from oxygen toxicity. Primary targets are sulphydryl-containing proteins, deoxyribonucleic acid and lipids which undergo peroxidation. Extensive chromosome damage and mutagenesis have been demonstrated in cell cultures exposed to high concentrations of oxygen at normal barometric pressure (Sturrock & Nunn 1978).

Biologically advantageous uses of oxygen-derived free radicals

It is characteristic of the duality of the relationship between the animal body and oxygen that the destructive potential outlined above should be turned to uses which are not only biologically advantageous but are essential to the maintenance of normal health. In addition to the well established methods of bacterial killing, neutrophils and macrophages have the capacity to discharge oxygen-derived free radicals into phagocytic vesicles, a process involving a massive increase in their oxygen consumption (Babior *et al.* 1973).

The initial stage is the donation of an electron from NADPH (nicotinamide adenine dinucleotide phosphate, reduced) to molecular oxygen to form the superoxide free radical. NADPH is generated by the hexose monophosphate shunt and the reduction of oxygen is catalysed by the enzyme NADPH oxidase located within the membrane of the phagocytic vesicle. Superoxide anion radical is released into the phagocytic vesicle where it is reduced to hydrogen peroxide which, under the influence of myeloperoxidase, forms hypochlorous acid (Fantone & Ward 1982). The importance of this mechanism is demonstrated by the greatly reduced resistance to infection in chronic granulomatous disease, a condition in which the patient is deficient in NADPH oxidase.

This mechanism is of wider application than just bacterial killing. Oxygen-derived free radicals appear to be concerned in the killing of malarial parasites (Clark & Hunt 1983). It has also been suggested that oxygen may play a role in the destruction of circulating tumour cells, particularly in the pulmonary circulation where the oxygen tension is highest (P Alexander, personal communication).

Tissue damage arising from oxygen-derived free radicals

Although there can be no doubt of the beneficial nature of the mechanisms described in the previous section, it is no less clear that oxygen-derived free radicals can, under certain circumstances, result in serious tissue damage. Production of these radicals is directly related to the partial pressure of oxygen and there is good evidence that this mechanism is involved in development of pulmonary oxygen toxicity (Small 1984, Saugstad *et al.* 1984).

Apart from the NADPH oxidase system, there are many substances which can donate an electron from the superoxide free radical and so commence the chain of reactions generating the still more destructive species. Perhaps the clearest example is paraquat, which can insert itself into an electron transport chain, alternating between the single and double ionized state, and so transferring an electron from NADPH to oxygen, thus acting as an analogue of NADPH oxidase. This is the basis of its pulmonary toxicity in which it is synergistic with oxygen itself.

It has long been established that xanthine in the presence of xanthine oxidase and oxygen can generate the superoxide free radical (McCord & Fridovich 1968). This system has been used in laboratory studies to subject organs and cell cultures to free radical attack, but it now appears that it may have direct clinical relevance. Under hypoxic conditions, adenosine triphosphate is progressively degraded through adenosine and inosine to hypoxanthine. Xanthine oxidase converts hypoxanthine to xanthine and, if oxygen is then present, superoxide

free radical will be formed. This mechanism has been proposed as the basis of post-ischaemic shock or reperfusion injury (McCord & Roy 1982). The naturally occurring xanthine dehydrogenase (type D) which does not produce superoxide must be converted into xanthine oxidase (type O), but it is established that allopurinol (a xanthine oxidase inhibitor) as well as free radical scavengers will protect small intestinal mucosa from ischaemic damage (Parks & Granger 1983).

Of particular interest is the potential involvement of oxygen-derived free radicals in the production of adult respiratory distress syndrome (ARDS). The proposed sequence commences with pulmonary margination of neutrophils in response to release of complement 5a, which may result from various predisposing conditions. Although such margination is usually harmless, it is suggested that under certain circumstances the neutrophils initiate the inappropriate production of oxygen-derived free radicals with release, not into a phagocytic vesicle, but extracellularly onto the surface of pulmonary endothelium with resultant damage which becomes the first phase of ARDS (Rinaldo & Rogers 1982). Exposure to high concentrations of oxygen would exacerbate the formation of the free radicals.

These examples of tissue damage by oxygen-derived free radicals are by no means exhaustive but are sufficient to indicate the potential for this type of injury. Neither have I included all known sources of electrons which may trigger the reactions. It should, however, be noted that iron, as haemoglobin and in other forms, may donate an electron by conversion from the ferrous to the ferric state.

Protection from damage by oxygen-derived free radicals

Elucidation of the mechanisms of tissue damage by oxygen-derived free radicals is by no means of purely theoretical interest. There exist a wide range of potential therapeutic measures to limit this type of injury, and their investigation now constitutes a major challenge to the laboratory and clinical investigator.

Firstly, there are the enzymes superoxide dismutase, catalase and the peroxidases. Being mainly intracellular enzymes, their delivery to the site of action presents problems, but induction is possible. For example, 7 days of exposure of rats to 85% oxygen induces superoxide dismutase and results in increased resistance to the toxic effect of 100% oxygen (Crapo & Tierney 1974). Free radical scavengers and antioxidants include the glutathione/glutathione peroxidase system, dimethyl thiourea and dimethyl sulphoxide – already used, so it happens, in the preservation of kidneys for transplantation – ascorbic acid and vitamin E. The role of methyl prednisolone is still uncertain but there is much anecdotal evidence of its efficacy in situations where oxygen-derived free radicals may be implicated. There is firm evidence that it limits damage to chromosomes by oxygen (Sturrock & Hulands 1980). Iron chelation would remove ferrous iron both as an electron donor and as a catalyst in the Fenton reaction. Allopurinol will block the action of xanthine oxidase.

Conclusion

It is probably no more than intuition which led Priestley to suggest that excess oxygen might be harmful. There is a clue in the sentence which follows the quotation at the opening of this paper: 'A moralist, at least, may say, that the air which nature has provided for us is as good as we deserve'.

Whatever the basis for Priestley's prediction, it is now abundantly clear that oxygen has a capacity to harm which rivals in importance its capacity to improve the availability of high-energy phosphate compounds. The discovery of new biological mechanisms often leads us to overemphasize their importance. However, it seems highly likely that elucidation of the full extent of tissue damage by oxygen-derived free radicals will be a fruitful field of research for many years to come.

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