FREE RADICALS, ANTIOXIDANTS AND CANCER PREVENTION

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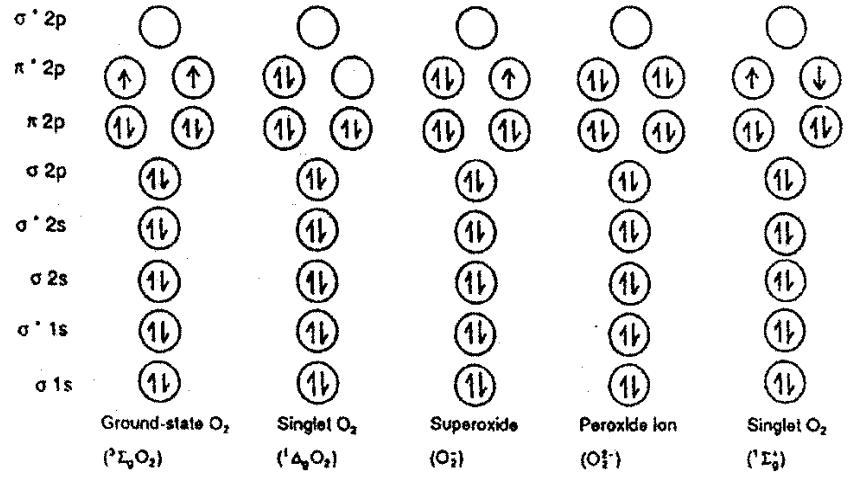
SUMMARY

Evidence is accumulating that most of the diseases that afflict humanity have their origin in deleterious free radical reactions. These diseases include cardiovascular problems, cancer, inflammatory joint disease, senile dementias, and degenerative eye complaints. The basic process of biological aging is also the result of accumulated free radical damage to the organism. The utilization of oxygen in living organisms produces superoxide ($O_2^{\cdot-}$) and hydroxyl ($OH^{\cdot}$) radicals, and the activated oxygen species, singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2). Metabolism of various organic compounds also yields a range of carbon-centered free radicals. These electrophilic species can damage genetic material and oxidize unsaturated fatty acids in cell membranes. Our natural protection against free radicals and activated oxygen compounds involves a sophisticated multi-layered defence screen of enzymes such as catalase and superoxide dismutase, and molecular antioxidants including ascorbic acid (vitamin C) and α-tocopherol (vitamin E). The antioxidant enzymes have, at their active centres, elements such as copper, zinc, iron, manganese and selenium. These, and other trace elements, play a vital role in maintaining our health.

I. THE NATURE OF FREE RADICALS

A free radical is any atom or molecule that contains one or more unpaired electrons; an unpaired electron being one that occupies an atomic or molecular orbital on its own. The Figure shows that π^* 2p (outer) orbitals of some oxygen species (Halliwell and Gutteridge 1984). Even ground state molecular oxygen (the type we breath) is a free radical, which explains its high reactivity (oxidation) with many compounds. However, oxygen cannot normally accept a pair of electrons from a non-radical molecule during oxidation because spin reversal would need to occur before the vacant spaces in the π^* orbital could be filled. Thus oxygen must accept electrons one at a time, which makes the kinetics of many of its reactions slow, unless it gains two single electrons from another free radical (Halliwell and Gutteridge 1984; Proctor and Reynolds 1984). By contrast, singlet oxygen ($^1\Delta_g O_2$) can readily accept a pair of electrons, and its reactions with other molecules are very fast. Note that the peroxide ion is not a radical.



The Table lists some free radicals and activated oxygen species that have been implicated in biological processes, plus their approximate half-lives in a biological system (Pryor 1986). Some free radicals and activated oxygen species, e.g., hydrogen peroxide, lipid peroxide and the semiquinone free radical, are sufficiently stable to diffuse some distance in cells, whereas others, such as the hydroxyl radical ($OH^{\cdot}$), will react with the first organic molecule they encounter (Pryor 1986a).

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Accelerated lipid peroxidation can result from lack of free radical scavengers in the membrane (e.g., vitamin E) or increased oxidative stress as a result of the intake of xenobiotic drugs (e.g., antimalarial drugs) or breathing air with a higher than normal concentration of oxygen. The aging process almost certainly involves accelerated lipid peroxidation, and lipid peroxides have been called the "ultimate" toxin because of their long biological lifetime and their highly damaging nature (DiGuiseppi and Fridovich 1984).

Lipid peroxidation is a chain reaction. A hydoxyl radical initiates the process by abstracting a hydrogen atom from a polyunsaturated fatty acid (PUFA) side chain (LH) to form water and a carbon-centered free radical (L•). This carbon radical then undergoes an internal rearrangement to yield a conjugated diene, which then reacts with molecular oxygen to give a peroxy radical (LOO•). The peroxy radical then abstracts a hydrogen atom from a second PUFA side chain to yield another L• and continue the chain reaction, forming a lipid peroxide (LOOH) in the process (DiGuiseppi and Fridovich 1984). A single hydroxyl radical can thus cause a calamitous cascade of membrane oxidation. The antioxidant vitamin E (α-tocopherol,EH) terminates this chain reaction by donating a hydrogen atom and trapping peroxy radicals (Wefers and Sies 1988).

$$LOO\bullet + EH \rightarrow LOOH + E\bullet \quad (1)$$

$$LOO\bullet + E\bullet \rightarrow \text{non-reactive product} \quad (2)$$

Vitamin E is the most efficient known terminator of lipid peroxidation; one molecule of α-tocopherol can protect 1,000 lipid molecules (Pauling 1986).

II. FREE RADICAL SCAVENGERS

Utilization of Oxygen

All aerobic organisms have an impressive array of free radical scavengers. Many of these scavengers are designed specifically to protect the organism from oxygen-derived free radicals and other activated oxygen species. Man has the longest maximum lifespan potential of all mammals because he has superb free radical defenses (Cutler 1984).

Life apparently arose spontaneously 3.5 billion years ago from basic chemicals produced from the primitive oxygen-free atmosphere by free radical reactions initiated by ionizing radiation from the sun. About one billion years later, blue-green algae appeared, and some 1.3 billion years ago the concentration of atmospheric oxygen, produced by the photosynthesizing algae, had reached 1% of the present value, the toxic level for the fermentative anaerobes. The anaerobic procaryotes disappeared, except for a few in oxygen-deficient areas, and the sturdier, more complex, and more energy efficient eucaryotes became the dominant cells. Up to 18 times more energy in the form of ATP can be extracted from glucose by oxidizing it to CO_2, compared with anaerobic glycolysis (Greenwood and Hill 1982).

The utilization of oxygen is, however, not without its problems. The <u>in vivo</u> reduction of oxygen produces $O_2^{\bullet-}$, OH• and H_2O_2 (reactions 5-9) which are highly damaging to the cell, and this toxicity increases rapidly if the oxygen concentration becomes much higher than the ambient 20% of the atmosphere. Our margin of safety is narrow. We possess defenses against oxygen toxicity which are sufficient to meet ordinary demands, but which can be easily overwhelmed (Halliwell and Gutteridge 1984).

Enzymatic Defenses

Superoxide dismutase (SOD) catalyses the dismutation of $O_2^{\bullet-}$ to H_2O_2 and $O_2^{\bullet}$. There are three forms of SOD; copper-zinc, manganese and iron. Copper-zinc and manganese SOD are found in eucaryotic cells (including human), while iron-SOD occurs in bacteria.

Copper-zinc SOD consists of two identical sub-units, each having a single intramolecular S-S bond (Florence 1980). These bonds are essential to the stability of the protein, and are unusually resistant to radiation and chemical attack, a property doubtlessly essential to the efficient functioning of SOD as a free radical scavenger (Florence 1980).

Other biologically important free radicals, not listed in Table 1, are nitrogen dioxide and several carbon-centered free radicals. Ozone (O_3), even though it is not a free radical, is a more powerful oxidizing agent than ground state oxygen.

Species	Symbol	Half-Life(s) at 37°C[a]
Molecular Oxygen	O_2	$>10^2$
Hydroxyl radical	$OH\cdot$	$1x10^{-9}$
Superoxide radical	$O_2^{-\cdot}$	$1x10^{-6}$
Singlet oxygen	$^1O_2$	$1x10^{-6}$
Hydrogen peroxide	H_2O_2	10^b
Lipid peroxide	ROOH	$>10^2$
Alkoxyl radical	$RO\cdot$	$1x10^{-6}$
Peroxyl radical	$ROO\cdot$	$1x10^{-2}$
Semiquinone radical	$Q^{-\cdot}$	$>10^2$

a In biological system.
b Short lifetime in presence of catalase or glutathione peroxidase.

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However, like many highly reactive non-radical species, most of the reactions of ozone with organic compounds involve free radical production (Pryor 1986; 1986a).

Photochemical smog contains ozone and NOx ($NO+NO_2$). Both NO and NO_2 are stable free radicals that react rapidly with biological compounds such as thiols and hemoglobin. Whereas smog usually contains less than 1ppm of NOx, undiluted cigarette smoke has several hundred ppm; in fact gas-phase tobacco smoke contains 10^{17} reactive oxyradicals per puff (Cross 1987; Hoffmann 1987)!

Carbon-centered free radicals are also common in biological systems. Here the odd electron is located on a carbon, rather than an oxygen, atom. Similarly, nitrogen-(e.g., amines) and sulfur-e.g., thiols) centered free radicals are commonly encountered.

Lipid peroxidation

Lipid peroxidation is one of the most important free radical-mediated biological processes, and involves both oxygen - and carbon - centered free radicals (Gutteridge et al., 1986; Halliwell 1987). Lipid peroxidation involves attack on a polyunsaturated fatty acid molecule (e.g., linoleic acid) in a biological membrane, leading to decreased membrane fluidity, increased non-specific membrane permeability ("leaky" membranes) and inactivation of some membrane-bound enzymes.

Two enzymes, glutathione peroxidase and catalase, are used to catalyze the decomposition of H_2O_2. Glutathione peroxidase has four atoms of selenium/mole, and uses glutathione as substrate to reduce H_2O_2 and lipid peroxides. Catalase is a hemoprotein, and has the advantage that it does not require an auxiliary reductant to destroy H_2O_2. These two enzymes, together with SOD, work in a synergistic fashion to protect lipid membranes and protein sulfhydryl groups, especially in the mitochondrion, from attack by $O_2^{\cdot-}$ and H_2O_2. Glutathione peroxidase also removes lipid peroxides, and thus inhibits the chain reaction of lipid peroxidation.

Non-Enzymatic Defenses

The free radical dissociating enzyme defense system is backed up by an array of non-enzymatic defenses (a "strategic reserve"), consisting of small nucleophilic molecules that constitute a multi-layered defense array against activated oxygen species, and which also scavenge carbon-centered free radicals. Some of these antioxidants, with approximate average concentrations in human blood plasma (mgL^{-1}) are: ascorbic acid (vitamin C), 10; reduced glutathione, 400 (whole blood); α-tocopherol (vitamin E), 10; uric acid, 50; β-carotene, 2; and ceruloplasmin, 340. Albumin and glucose also have free radical scavenging properties (Halliwell and Gutteridge 1986).

Vitamin E, like β-carotene, is lipid soluble and occurs where it is most needed, in the cell membrane, where it performs the critical task of terminating the potentially calamitous chain reaction of lipid peroxidation. Ascorbic acid is not lipid soluble, but is a versatile reductant, and reacts synergistically with vitamin E and reduced glutathione (GSH) to produce a powerful antioxidant system (Wefers and Sies 1988; Florence 1983; Lathia et al. 1988).

Selenium

Selenium may be the most important antioxidant element in the human body (Shamberger 1984; Dreosti 1986). In addition to being essential to the functioning of glutathione peroxidase, it appears to have other more subtle roles, such as enhancing DNA repair mechanisms while delaying cell mitosis (Ip 1985). This role may allow initiated cancer cells to repair themselves before division, so that the progeny are not malignant. An international study of the selenium content of the diet showed an excellent inverse correlation with cancer incidence (Clark 1985), as did blood selenium and cancer incidence in Provinces across China (Yu et al. 1985). China is an ideal country to study the epidemiology of cancer and selenium, because the soil (and hence crops) in some areas of China are so low in selenium that a specific type of cardiomyopathy (Keshan Disease) occurs, while soil selenium is so high in other Provinces that chronic seleniosis sometimes occurs. New Zealanders are particularly low in selenium, and suffer high rates of cancer, cardiovascular disease, asthma, and sudden infant death syndrome (SIDS). Low dietary selenium has also been linked (Robinson 1988) with inflammatory diseases such as rheumatism, arthritis, and repetitive strain injury, a not unlikely situation considering the free radical scavenging properties of selenium. A study has been initiated in Christchurch Hospital on the relationship between dietary selenium and SIDS (C.C. Winterbourn, private communication).

Selenium yeast is a cheap and convenient dietary selenium supplement for those who are not yeast sensitive.

III. PROTECTION AGAINST CANCER AND AGING BY DIETARY CONTROL OF FREE RADICAL FORMATION

The possibility that cancer can be minimized and, the rate of aging reduced by the intake of antioxidants (free radical scavengers) has intrigued nutritionists for the past 20 years (Florence 1983; Gey et al. 1987). If cancer and aging are indeed caused by oxidizing free radicals, an increase in the concentration of anti-oxidant in tissues and the circulatory system may offer some protection. Certainly, many serum antioxidants show a decline with age (Young 1984), and an increase in dietary antioxidants usually brings about an increase in mean lifetime (but not

maximum lifespan) in experimental animals (Harman 1984). Prospective human studies have shown that lower rates of cardiovascular disease and cancer are associated with a high status of serum vitamins A and E, β-carotene and selenium. Several epidemiological studies have indicated that the cruciferous vegetables, cabbage, broccoli, brussel sprouts and cauliflower, protect against cancer (Ansher et al. 1986). The active compound in these vegetables is believed to be a dithiolthione.

Nitrosamines, formed from dietary amines and nitrite (present in preserved food or produced naturally from nitrate), are perhaps the most universal and potent class of carcinogen (Hoffmann 1987; Lathia et al. 1988; Shamberger 1984). We are exposed to them continuously, and they are probably the specific cause of gastric (stomach) cancer (Shamberger 1984). Their formation is catalyzed by dietary compounds such as thiocyanate, iodide and polyphenols, and several nitrosamines are present in tobacco and tobacco smoke (Hoffman 1987). Ascorbic acid destroys nitrosamines rapidly and completely, and a combination of vitamins C and E is even more effective. A high concentration of free vitamin C in the stomach, intestines, bladder and tissues would seem to be desirable for protection against nitrosamines.

The argument is often put forward that intakes of vitamin C greater than about 150 mg per day are unnecessary because, when more than this is taken, vitamin C often appears in the urine. However, despite its appearance in the urine, much more than 150 mg per day is usually needed to ensure tissue saturation of the vitamin (Pauling 1986). There are no known ill effects of vitamin C when taken at the rate of 1 to 3 grams per day (Pauling 1986). The frequently-raised connection between oxalate renal stones and vitamin C is false, and arose from inadequate analytical methods, where urinary vitamin C interfered in the determination of oxalate (Pauling 1986; Cathcart 1985).

Australia is one of the few countries in the world where the over-the-counter sale of dietary supplements containing selenium is prohibited. In excess, selenium, like many freely available drugs such as aspirin, is toxic (Shamberger 1984). But this is not sufficient reason for making it unavailable to the public. The Chinese cancer study (Yu et al. 1985) suggests that the optimum serum concentration of selenium is 250-300 μg/L, compared with an average of 90 μg Se/L for adult Australians. In most countries, supplementation, e.g., by low-cost selenium yeast tablets, would be necessary to achieve this serum concentration. If the tablets were prepared to contain, say, 50μg selenium per tablet, a huge excess over the recommended dosage would be needed to cause even low-grade chronic selenium toxicity (Shamberger 1984).

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