Drugs 42 (4): 569-605, 1991
0012-6667/91/0010-0569/$18.50/0

DRU1 61

Drug Antioxidant Effects
A Basis for Drug Selection?

Barry Halliwell
Pulmonary Medicine, UC Davis Medical Center, Sacramento, California, USA

Contents

Summary *A free radical is any species capable of independent existence that contains one or more unpaired electrons. Free radical reactions have been implicated in the pathology of more than 50 human diseases. Radicals and other reactive oxygen species are formed constantly in the human body, both by deliberate synthesis (e.g. by activated phagocytes) and by chemical side-reactions. They are removed by enzymic and nonenzymic antioxidant defence systems. Oxidative stress, occurring when antioxidant defences are inadequate, can damage lipids, proteins, carbohydrates and DNA. A few clinical conditions are caused by oxidative stress, but more often the stress results from the disease. Sometimes it then makes a significant contribution to the disease pathology, and sometimes it does not. Several antioxidants are available for therapeutic use. They include molecules naturally present in the body [superoxide dismutase (SOD), α-tocopherol, glutathione and its precursors, ascorbic acid, adenosine, lactoferrin and carotenoids] as well as synthetic antioxidants [such as thiols, ebselen (PZ51), xanthine oxidase inhibitors, inhibitors of phagocyte function, iron ion chelators and probucol]. The therapeutic efficacy of SOD, α-tocopherol and ascorbic acid in the treatment of human disease is generally unimpressive to date although dietary deficiencies of the last two molecules should certainly be avoided. Xanthine oxidase inhibitors may be of limited relevance as antioxidants for human use. Exciting preliminary results with probucol (antiatherosclerosis), ebselen (anti-inflammatory), and iron ion chelators (in thalassaemia, leukaemia, malaria, stroke, traumatic brain injury and haemorrhagic shock) need to be confirmed by controlled clinical trials. Clinical testing of N-acetylcysteine in HIV-1-positive subjects may also be merited. A few drugs already in clinical use may have some antioxidant properties, but this ability is not widespread and drug-derived radicals may occasionally cause significant damage.*

Free radicals and their relationship to human disease is an important research area at the moment, especially since it has been reported that free radical reactions are implicated in the pathology of over 50 human diseases (listed in Halliwell & Gutteridge 1989).

1. What is a Free Radical?

1.1 Definition

Electrons in atoms occupy regions of space known as orbitals. Each orbital can hold a maximum of 2 electrons, spinning in opposite directions. A free radical can be simply defined as any species capable of independent existence (for however brief a time) that contains one or more unpaired electrons, an unpaired electron being one that is alone in an orbital. Most biological molecules are nonradicals, containing only paired electrons (Halliwell & Gutteridge 1989).

An electron occupying an orbital by itself has 2 possible spin states. Indeed, the technique of electron spin resonance detects radicals because it measures the energy changes that occur as electrons change their spin state. Because electrons are more stable when paired together in orbitals, radicals are in general more reactive than nonradical species, although there is a considerable variation in their individual reactivity (reviewed by Halliwell & Gutteridge 1989).

Radicals can react with other molecules in a number of ways. Thus, if 2 radicals meet, they can combine their unpaired electrons (symbolised by •) and join to form a covalent bond (a shared pair of electrons):

$$A^{\bullet} + A^{\bullet} \rightarrow A\text{–}A \qquad \text{(Eq. 1)}$$

A radical might donate its unpaired electron to another molecule (a reducing radical) or it might take an electron from another molecule in order to pair (an oxidising radical). However, if a radical gives one electron to, takes one electron from or

simply joins on by addition to a nonradical, that nonradical becomes a radical. Thus, a feature of the reactions of free radicals with nonradicals (which includes most biological molecules) is that they tend to proceed as chain reactions: one radical begets another and so on. Only when 2 free radicals meet can these chain reactions be terminated (reaction 1).

1.2 Hydroxyl Radical

The most reactive free radical known is the hydroxyl radical (•OH), whose formation plays a key role in the damage suffered by tissues exposed to high-energy radiation (Von Sonntag 1987). When tissues are exposed to, for example, γ-radiation, most of the energy they take up is absorbed by water, largely because there is more water in tissues than any other molecule. The radiation causes one

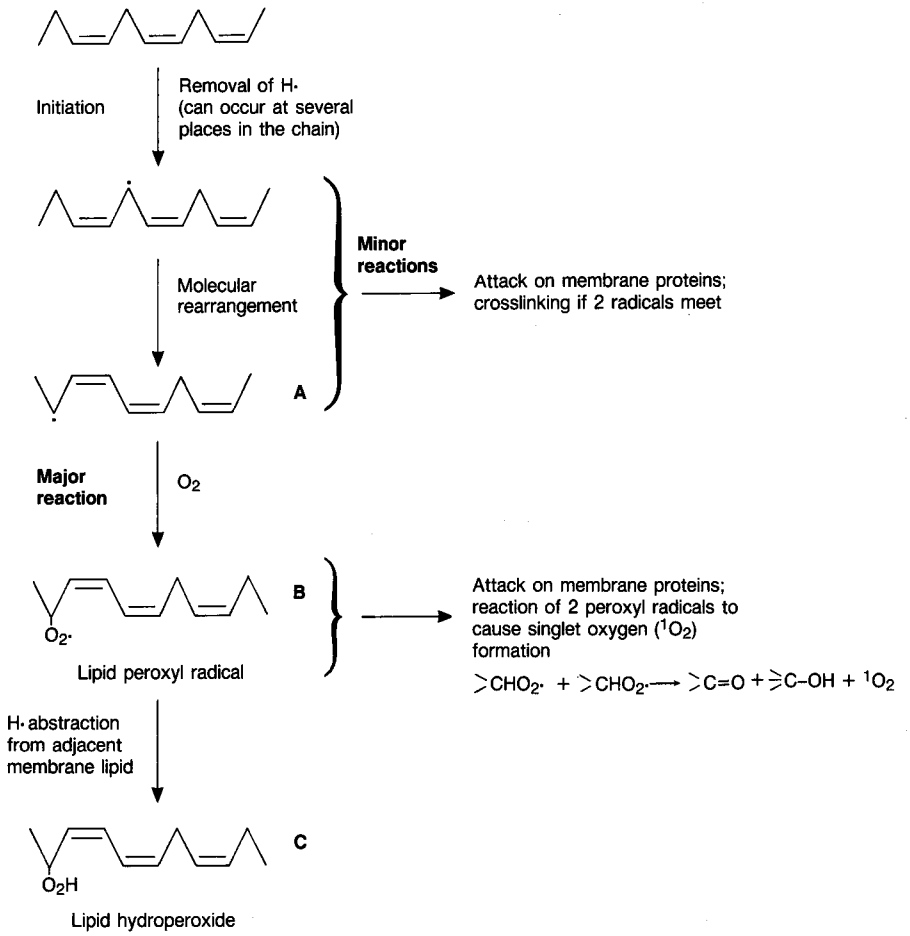
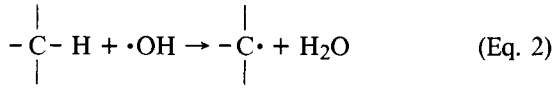


Fig. 1. The free radical chain reaction of lipid peroxidation. Attack of a reactive species such as •OH upon the side-chains of unsaturated fatty acids causes hydrogen atom abstraction. The consequences of such attack upon a fatty acid side-chain containing 3 double bonds are shown. Hydrogen can be abstracted at different points in the side-chain, so that several isomeric lipid hydroperoxides can result. Cyclic peroxides can also be formed. A, B and C have conjugated diene structures (carbon atoms linked by a double, then a single, then another double bond).

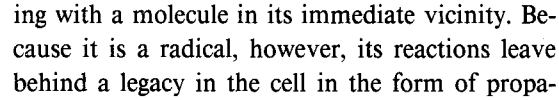
of the oxygen-hydrogen covalent bonds in water to split, leaving a single electron on the hydrogen atom and one on the oxygen atom and creating two radicals, H• and •OH. Since it is so reactive, •OH does not persist for even a microsecond before combining with a molecule in its immediate vicinity. Because it is a radical, however, its reactions leave behind a legacy in the cell in the form of propagating chain reactions. Thus, if •OH attacks DNA, free radical chain reactions occur and cause strand breakage, deoxyribose fragmentation and extensive chemical alteration of the purine and pyrimidine bases (Von Sonntag 1987). The latter pattern of base changes in DNA is apparently diagnostic for attack by •OH (Aruoma et al. 1989a; Halliwell & Aruoma 1991). Imperfect repair of DNA damage caused by •OH can result in proto-oncogene activation and carcinogenesis. Hence, high-energy radiation can lead to cancer, which implies that •OH is a complete carcinogen (reviewed by Breimer 1988).

Perhaps the best characterised biological damage caused by •OH (although not necessarily the most important: see Halliwell 1987) is its ability to initiate the free radical chain reaction known as lipid peroxidation (fig. 1). This occurs when the •OH is generated close to or within membranes and attacks the fatty acid side-chains of the membrane phospholipids. It preferentially attacks side-chains derived from fatty acids with several double bonds, such as arachidonic acid. The •OH abstracts an atom of hydrogen from one of the carbon atoms in the side-chain and combines with it to form water:



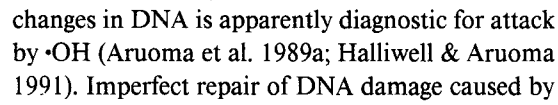
(Eq. 2)

Reaction 2 removes the •OH, but leaves behind a carbon-centered radical (C•) in the membrane. Carbon-centered radicals formed from polyunsaturated fatty acid side-chains usually undergo molecular rearrangement to give conjugated diene structures, which can have various fates. Thus, if 2 such radicals collided in the membrane, cross-linking of fatty acid side-chains could occur as the 2 electrons join to form a covalent bond (fig. 1). Reaction with membrane proteins is also a possibility. However, under physiological conditions, the most likely fate of carbon-centered radicals is to combine with oxygen, creating yet another type of radical, the peroxyl radical (sometimes abbreviated to 'peroxy radical'):



(Eq. 3)

Peroxyl radicals can combine with each other or attack membrane proteins (fig. 1), but they are also reactive enough to attack adjacent fatty acid side-chains, abstracting hydrogen:



lipid hydroperoxide

(Eq. 4)

Another carbon-centered radical is generated, and so the chain reaction (reactions 3 and 4) can continue. Hence, one •OH can result in the conversion of several hundred fatty acid side-chains into lipid hydroperoxides (Halliwell & Gutteridge 1989). Accumulation of lipid hydroperoxides in a membrane disrupts its function and can cause it to collapse. In addition, lipid hydroperoxides can decompose to yield a wide range of highly cytotoxic products, the most studied of which are aldehydes (reviewed by Esterbauer et al. 1988). Most attention has been paid in the literature to malonaldehyde (sometimes called malondialdehyde) but this is relatively unreactive compared with such noxious products as 4-hydroxynonenal (Curzio 1988; Esterbauer et al. 1988). Peroxyl radicals and reactive aldehydes can also cause severe damage to membrane proteins, inactivating receptors and membrane-bound enzymes (e.g. Dean et al. 1986; Roubal & Tappel 1966).

1.3 Formation of Oxygen Radicals *In Vivo*

Interest in the role of free radicals in human metabolism was raised by the discovery in 1968 of an enzyme specific for a free radical substrate, superoxide dismutase (SOD). This enzyme activity is

present in all human cells and functions to remove superoxide radical, the 1-electron reduction product of oxygen:

$$O_2 + e^- \rightarrow O_2^{\cdot -} \quad \text{(Eq. 5)}$$

Superoxide dismutase removes $O_2^{\cdot -}$ by catalysing a dismutation reaction: one $O_2^{\cdot -}$ is oxidised to O_2 and the other reduced to H_2O_2:

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad \text{(Eq. 6)}$$

In the absence of SOD, dismutation of $O_2^{\cdot -}$ occurs nonenzymically but at a rate approximately 4 orders of magnitude less at physiological pH.

The discovery of SOD led to the realisation that $O_2^{\cdot -}$ is formed *in vivo* in living organisms, and SOD functions to remove it. Formation of some $O_2^{\cdot -}$ *in vivo* appears to be a chemical accident (Fridovich 1983; Imlay & Fridovich 1991). For example, when mitochondria are functioning, some of the electrons passing through the respiratory chain leak from the electron carriers and pass directly onto oxygen, reducing it to $O_2^{\cdot -}$ (Fridovich 1983). Many molecules oxidise on contact with oxygen, such as when an adrenalin solution left to stand on a laboratory bench 'goes off' and eventually forms a pink product. The first stage in this oxidation is transfer of an electron from the adrenalin onto O_2, giving $O_2^{\cdot -}$. Such oxidations undoubtedly proceed *in vivo* as well (reviewed in Halliwell & Gutteridge 1989). For example, several sugars, including glucose, react with proteins to produce oxygen radicals. Thus, it has been suggested that years of exposure of body tissues to elevated blood glucose in diabetic patients can result in them suffering an 'oxidative stress' that may contribute to the side effects of hyperglycaemia (discussed by Wolff & Dean 1987). Elevated blood glucose leads to a nonenzymatic glycosylation of proteins, to which free radical generation might contribute (Wolff & Dean 1987).

Although some of the $O_2^{\cdot -}$ production *in vivo* may be accidental, much is functional. Thus, activated phagocytic cells generate $O_2^{\cdot -}$ as has been shown for monocytes, neutrophils, eosinophils and most types of macrophage (Curnutte & Babior 1987). Radical production is important in allowing phagocytes to kill some of the bacterial strains that they can engulf. This can be illustrated by examining patients with chronic granulomatous disease, a collective name given to several conditions in which the membrane-bound NADPH oxidase system in phagocytes that makes the $O_2^{\cdot -}$ does not work (Curnutte & Babior 1987). Such patients have phagocytes that engulf and process bacteria perfectly normally, but several bacterial strains are not killed and are released in viable form when the phagocytes die. Thus, patients suffer severe, persistent, and multiple infections with several organisms, especially *Staphylococcus aureus.*

Another killing mechanism used by neutrophils (but not by macrophages) is the enzyme myeloperoxidase (Weiss 1989). It uses H_2O_2 produced by dismutation of $O_2^{\cdot -}$ to oxidise chloride ions into hypochlorous acid, HOCl, a powerful antibacterial agent (many household bleach products contain the sodium salt of hypochlorous acid):

$$H_2O_2 + Cl^- \rightarrow HOCl + OH^- \quad \text{(Eq. 7)}$$

More recently discovered examples of physiological roles for $O_2^{\cdot -}$ include its possible involvement in fibroblast proliferation (Meier et al. 1990; Murrell et al. 1990) and in vasoregulation (Halliwell 1989a). It is now widely thought (reviewed by Marletta 1989), but not yet completely certain (Myers et al. 1990) that endothelium-derived relaxing factor (EDRF), a humoral agent, produced by vascular endothelium, which is an important mediator of vasodilator responses induced by several pharmacological agents (including acetylcholine and bradykinin), is identical with nitric oxide. Nitric oxide (NO) has one unpaired electron and thus qualifies as a free radical. Vascular endothelium may also produce small amounts of $O_2^{\cdot -}$ which can react with NO. Both NO itself (if generated in excess) and some of the products of its reaction with $O_2^{\cdot -}$ may be highly cytotoxic (Beckman 1990; Beckman et al. 1990; Saran et al. 1990), although others have disputed this and argued that the interaction of NO and $O_2^{\cdot -}$ may have a beneficial role in regulating vascular tone (Halliwell 1989a; Saran et al. 1990).

1.4 Reactive Oxygen Species

Superoxide dismutases remove $O_2^{\cdot -}$ by converting it into $O_2^{\cdot -}$ and H_2O_2. They work in conjunction with two enzymes that remove H_2O_2 in human cells: catalase and glutathione peroxidase (reviewed by Halliwell & Gutteridge 1989). The study of inborn errors of metabolism strongly suggests that glutathione peroxidase is the more important in removing H_2O_2 in human tissues, probably because it is located in the same subcellular compartments (mostly cytosol and some in mitochondria) as SOD. Glutathione peroxidase is the only human enzyme known so far that requires the element selenium for its activity: a selonocysteine residue (side-chain −SeH instead of −SH, as in normal cysteine) is present at the enzyme's active site. It is unlikely, however, that the sole function of selenium in humans is to act as a cofactor for glutathione peroxidase (reviewed by Levander 1987). Glutathione peroxidase removes H_2O_2 by using it to oxidise reduced glutathione (GSH) into oxidised glutathione (GSSG):

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O \quad \text{(Eq. 8)}$$

H_2O_2 is a fairly unreactive molecule that can cross cell membranes easily. Like $O_2^{\cdot -}$, it may perform some physiological roles, e.g. control of platelet adherence (Salvemini & Botting 1990). Since the H_2O_2 molecule has no unpaired electrons, H_2O_2 cannot be described as a radical. Hence, the term 'reactive oxygen species' has been introduced in the biomedical literature to describe collectively not only $O_2^{\cdot -}$ and $^{\cdot}OH$ (radicals) but also H_2O_2 (nonradical). Hypochlorous acid (HOCl) produced by myeloperoxidase can also be included under this heading: it is a powerful oxidising agent but not a radical, having no unpaired electrons. H_2O_2, $O_2^{\cdot -}$, $^{\cdot}OH$ and HOCl are sometimes collectively called 'oxidants'. This is a valid description for H_2O_2, $^{\cdot}OH$ and HOCl, which are oxidising agents. However, $O_2^{\cdot -}$ has both oxidising and reducing properties. Indeed, the latter property is made use of in a popular assay for $O_2^{\cdot -}$, the SOD-inhibitable reduction of cytochrome c, often applied to measure $O_2^{\cdot -}$ production by phagocytes (Curnutte & Babior 1987):

$$\text{cyt c } (Fe^{+++}) + O_2^{\cdot -} \rightarrow O_2 + \text{cyt c } (Fe^{++}) \quad \text{(Eq. 9)}$$

Another reactive oxygen species (but not a radical) is singlet oxygen $^1\Delta_g$, a form of oxygen that has undergone an electronic rearrangement that allows it to react much faster with biological molecules than does 'normal' (ground-state) oxygen. For example, singlet O_2 reacts directly with polyunsaturated fatty acid side-chains in membrane lipids to give lipid peroxides. Singlet O_2 is produced when several pigments are illuminated: light is absorbed by the pigment, which enters a higher electronic excitation state. As this returns to the ground state, energy is transferred onto oxygen, to make singlet O_2. Several compounds found in plants, some cosmetics and some drugs (e.g. tetracyclines) can cause singlet O_2 formation in the presence of light (reviewed by Epstein 1982; Kanofsky 1989).

Usually such 'photosensitisation' reactions are a nuisance. For example, many of the side effects of the nonsteroidal anti-inflammatory drug benoxaprofen could have been related to its ability to act as a photosensitiser (Artuso et al. 1990; Sik et al. 1983), and patients with certain forms of porphyria become light-sensitive because photosensitising porphyrins accumulate in their skin (Mathews-Roth 1987). However, photosensitisation can be made use of in medicine. Components of a porphyrin derivative called haematoporphyrin derivative (HPD) are taken up by tumour tissues. Irradiation of the tumour with light at a wavelength absorbed by HPD can then damage or kill the tumour: this method has been applied in the treatment of some types of lung cancer (reviewed by Ash & Brown 1989), although its real clinical benefit remains to be fully evaluated.

Small amounts of singlet O_2 can be generated in peroxidising lipid systems (fig. 1; Wefers 1987) but, apart from photosensitisation reactions, singlet O_2 does not seem to be a major damaging species in the human body. However, it can be produced by activated eosinophils under certain circumstances (Kanofsky 1989).

1.5 Transition Metal Ions and Free Radical Reactions

Most transition metals have variable oxidation numbers, e.g. iron as Fe^{++} or Fe^{+++} and copper as Cu^{+} or Cu^{++}. Changing between oxidation states involves accepting and donating single electrons, e.g.:

$$Fe^{+++} + e^- \rightleftharpoons Fe^{++} \quad \text{(Eq. 10)}$$

$$Cu^{++} + e^- \rightleftharpoons Cu^{+} \quad \text{(Eq. 11)}$$

Thus, most transition metal ions are remarkably good promoters of free radical reactions (Hill 1981). For example, Fe^{++} ions react with H_2O_2 to form a number of highly-reactive species, one of which is •OH (reviewed by Halliwell & Gutteridge 1990a):

$$Fe^{++} + H_2O_2 \rightarrow \text{intermediate oxo-iron ion complexes (e.g. ferryl)} \rightarrow Fe^{+++} + {\cdot}OH + OH^- \quad \text{(Eq. 12)}$$

Superoxide can re-reduce Fe^{+++} to Fe^{++}:

$$Fe^{+++} + O_2^{\cdot -} \rightarrow \text{intermediate oxo-iron ion complex (perferryl)} \rightarrow Fe^{++} + O_2 \quad \text{(Eq. 13)}$$

Copper ions can also convert H_2O_2 into •OH (Aruoma et al. 1991a). Generation of •OH and other reactive species appears to account for some or all of the toxicity observed when $O_2^{\cdot -}$ and H_2O_2 are generated in excess *in vivo* (reviewed in Halliwell & Aruoma 1991; Halliwell & Gutteridge 1990a; Imlay & Linn 1988). There are very few significant cellular targets in mammals that can be attacked by $O_2^{\cdot -}$ or H_2O_2 generated at physiological rates. It has also been suggested that the peroxynitrite species generated by reaction of $O_2^{\cdot -}$ with NO may decompose to produce •OH under physiological conditions (Beckman 1990) but doubt has been expressed about this (Saran et al. 1990).

Iron and copper ions also accelerate lipid peroxidation, for at least 2 reasons. First, they can participate in the formation of species able to abstract hydrogen. There is considerable debate (e.g. see Aruoma et al. 1989b; Minotti & Aust 1987) about which species initiate peroxidation in biological lipid systems (such as lipoproteins and membranes); candidates include ferryl, perferryl, Fe^{++}-Fe^{+++}-O_2 complexes and •OH. However, there is general agreement that metal ions are needed for formation of initiating species, except in organisms exposed to high doses of ionising radiation. Second, transition metal ions decompose lipid hydroperoxides into peroxyl and alkoxyl (lipid-O•) radicals that can abstract hydrogen and perpetuate the chain reaction of lipid peroxidation. This may be represented, in a simplified form, by the equations below, in which L• symbolises a carbon-centered radical (fig. 2):

$$LOOH + Fe^{++}\,(Cu^{+}) \rightarrow \underset{\text{alkoxyl radical}}{LO{\cdot}} + Fe^{+++}\,(Cu^{++}) + OH^- \quad \text{(Eq. 14)}$$

$$LOOH + Fe^{+++} \rightarrow \underset{\text{peroxyl radical}}{LOO{\cdot}} + Fe^{++} + H^+ \quad \text{(Eq. 15)}$$

$$LO{\cdot} + LH \rightarrow LOH + L{\cdot} \quad \text{(Eq. 16)}$$

$$LOO{\cdot} + LH \rightarrow LOOH + L{\cdot} \quad \text{(Eq. 17)}$$

$$L{\cdot} + O_2 \rightarrow LOO{\cdot} \quad \text{(Eq. 18)}$$

Reducing agents, such as ascorbic acid or $O_2^{\cdot -}$, can accelerate these metal ion-dependent peroxidation reactions because Cu^{+} and Fe^{++} ions seem to decompose peroxides faster than do Cu^{++} and Fe^{+++}, respectively. The end-products of these complex metal ion-catalysed breakdowns of lipid hydroperoxides include the cytotoxic aldehydes mentioned previously, as well as hydrocarbon gases such as ethane and pentane (reviewed in Halliwell & Gutteridge 1989).

1.6 Antioxidant Defence *In Vivo:* Intracellular

The word 'antioxidant' can be defined in various ways. Often the term is implicitly restricted to chain-breaking antioxidant inhibitors of lipid per-

oxidation, such as α-tocopherol (see below). However, the author prefers a broader definition – an antioxidant is any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate (Halliwell & Gutteridge 1989). The term 'oxidisable substrate' includes almost everything found in living cells, including proteins, lipids, carbohydrates and DNA.

The major intracellular antioxidants in the human body are probably the enzymes superoxide dismutase, catalase and glutathione peroxidase. Two isoenzymes of SOD exist in human cells: an enzyme containing copper and zinc ions (largely located in the cytosol) and a mitochondrial SOD containing manganese ions. Both catalyse the same reaction (reaction 6; Fridovich 1983). In addition, membranes contain the chain-breaking antioxidant α-tocopherol, a lipid-soluble molecule that is located in the interior of biological membranes. α-Tocopherol is the most important chain-breaking antioxidant present in human membranes (Ingold et al. 1986) but not the only one (Esterbauer et al. 1989). Attached to the hydrophobic structure of α-tocopherol is an -OH group whose hydrogen atom is very easy to remove. Thus, when peroxyl and alkoxyl radicals are generated during lipid peroxidation, they combine preferentially with the antioxidant, e.g.:

$$LOO\cdot + \text{tocopherol-OH} \rightarrow LOOH + \text{tocopherol-O}\cdot \quad \text{(Eq. 19)}$$

instead of with an adjacent fatty acid. This terminates the chain reaction, hence the name chain-breaking antioxidant. It also converts the α-tocopherol into a new radical, tocopherol-O·. This radical is poorly reactive, being unable to attack adjacent fatty acid side-chains, so the chain reaction is stopped. Evidence exists (Esterbauer et al. 1989; McCay 1985) that the tocopherol radical can migrate to the surface of membranes and lipoproteins and be converted back to α-tocopherol by reactions with ascorbic acid (vitamin C) and possibly with GSH, although it is not yet absolutely certain that this happens *in vivo* (Burton et al. 1990). However, it is widely believed that both vitamin C and α-tocopherol help to minimise the consequences of lipid peroxidation in membranes, should this process begin.

The terms 'α-tocopherol' and 'vitamin E' are often used synonymously. This is not quite correct: vitamin E is defined nutritionally as a factor needed in the diet of rats for normal reproduction (Diplock 1985), and compounds other than α-tocopherol (e.g. β-, γ- and δ-tocopherols) have some effect in this assay. However, α-tocopherol is the most effective lipid-soluble antioxidant in animals.

1.7 Antioxidant Defence *In Vivo:* Extracellular

Antioxidant defence in the extracellular compartments of the human body relies largely on different strategies than does intracellular defence. α-Tocopherol is still important. Thus, the content of α-tocopherol in plasma lipoproteins helps to determine their resistance to lipid peroxidation, but it is not the only antioxidant present. This is relevant to the development of atherosclerosis, since peroxidation of low-density lipoproteins appears to contribute to the progression of atherosclerosis (Esterbauer et al. 1989). Indeed, low plasma concentrations of α-tocopherol and vitamin C seem to be associated with an increased incidence of myocardial infarction and of some types of cancer (Gey et al. 1987; Gey 1990). However, it could be argued that these low levels are merely indicative of a poor diet, which predisposes to most diseases. Thus, although one could assume that taking commercial vitamin pills would protect against atherosclerosis, improving one's diet is a more logical recommendation. If low density lipoproteins isolated from human plasma are placed under oxidative stress, peroxidation does not begin until tocopherols, β-carotene, lycopene and phytofluene have been oxidised (Esterbauer et al. 1989).

By contrast with the intracellular environment, enzymic antioxidant defence enzymes are much less prominent in extracellular fluids. Blood plasma, tissue fluid, cerebrospinal fluid, synovial fluid and seminal plasma contain little or no catalase activity, and only low activities of superoxide dismutase and selenium-containing glutathione peroxidase can

be measured. There is also very little reduced glutathione (GSH) in most extracellular fluids – about 2 μmol/L in human plasma (Svardal et al. 1990). The selenium-containing glutathione peroxidase enzyme has been purified from human plasma, and shown to be different from the intracellular enzyme. The functional role of this enzyme is something of an anomaly, since its K_m (substrate concentration for half-maximal velocity) for GSH is in the millimolar range and so it would be unlikely to function effectively at the micromolar levels of GSH found in plasma (reviewed in Halliwell & Gutteridge 1990b). Although much higher (≥ 100 μmol/L) concentrations of GSH are present in human lung lining fluid, its content of glutathione peroxidase is low (Cantin et al, 1987).

'Extracellular' superoxide dismutase (EC-SOD) enzymes have also been described. Although EC-SODs contain copper and zinc, they are very different from intracellular CuZnSOD in that EC-SODs have a much higher relative molecular mass (about 135 000), and possess attached carbohydrate. The biological role of EC-SODs is unclear; such low activities are present in plasma and in other extracellular fluids that bulk-scavenging of $O_2^{\cdot-}$ in extracellular fluids has always seemed (to this author) to be an unlikely physiological function for them. Marklund has shown that some EC-SODs bind to heparin and has proposed that, *in vivo,* they may be associated with endothelial cell surfaces as a protective antioxidant 'layer' over the cells (Marklund, 1990). Trace amounts of EC-SODs are found attached to cells in various tissues, so the term 'extracellular' does not really mean a location within extracellular fluids.

Thus, enzymic removal of $O_2^{\cdot-}$ and H_2O_2 contributes little to the antioxidant activity of extracellular fluids. Indeed, $O_2^{\cdot-}$ and/or H_2O_2 generated extracellularly in small amounts by endothelium (Arroyo et al. 1990), lymphocytes (Maly 1990), platelets (Salvemini & Botting, 1990), fibroblasts (Murrell et al. 1990) and other cells (Burdon & Rice-Evans 1989) may play important physiological roles and thus should not be scavenged with 100% efficiency. Halliwell and Gutteridge (1990a,b) have argued that a major antioxidant defence of human plasma is to prevent $O_2^{\cdot-}$ and H_2O_2 from reacting to form dangerous species such as •OH, by binding transition metal ions in forms that will not stimulate free radical reactions, or by otherwise preventing the metal ions from participating in such reactions. Thus, safe sequestration of iron and copper ions into forms that will not catalyse free radical reactions is an important antioxidant strategy in the human body (Halliwell & Gutteridge 1990a,b).

How is this sequestration achieved? The iron-transport protein transferrin in plasma from healthy humans is only 20 to 30% loaded with iron, so that the content of free ionic iron in plasma is effectively nil. Iron, bound to transferrins will not participate in •OH radical formation or lipid peroxidation (Aruoma & Halliwell 1987; Gutteridge et al. 1981). The physiological importance of this sequestration of iron ions into 'safe' forms is clearly illustrated by an inspection of the multiorgan damage seen in patients with iron-overload disease (McLaren et al. 1983), in whom low-molecular-mass iron ion complexes capable of stimulating lipid peroxidation and •OH generation (Gutteridge et al. 1985) circulate in the plasma.

Haemoglobin can be liberated into plasma from disrupted erythrocytes, and myoglobin can be released from muscles after injury (e.g. myocardial infarction, or skeletal muscle damage during strenuous exercise). Both of these haem-containing proteins are potentially dangerous in that they can accelerate peroxidation of lipids (Galaris et al. 1988; Kanner & Harel 1985), including plasma lipoproteins (Bruckdorfer et al. 1990), in the presence of H_2O_2. They do this by two mechanisms. First, reaction of the protein with equimolar concentrations of H_2O_2 produces an oxo-haem species, probably ferryl, whose formation is associated with production of a reactive oxygen species [possibly a tyrosine peroxyl radical (Davies 1990)] that can stimulate lipid peroxidation and damage other biomolecules (Davies 1990; Galaris et al. 1988; Puppo & Halliwell, 1988a). Second, excess H_2O_2 can cause degradation of the haem rings of myoglobin and haemoglobin, releasing from the proteins iron ions that are capable of stimulating •OH pro-

duction and lipid peroxidation (Gutteridge 1986; Puppo & Halliwell 1988a,b). Haem, released from damaged haem proteins, is also well known to be a powerful stimulator of lipid peroxidation (e.g. Gutteridge & Smith 1988).

Because myoglobin and haemoglobin can be liberated into body fluids after muscle injury (e.g. in the 'crush' syndrome) plasma contains the haemoglobin-binding proteins known as haptoglobins, as well as a haem-binding protein (haemopexin). Binding of haemoglobin to haptoglobins, or of haem to haemopexin, diminishes their effectiveness in stimulating lipid peroxidation (Gutteridge & Smith 1988). The haemoglobin-haptoglobin or haem-haemopexin complexes are rapidly cleared from the circulation.

The plasma copper-containing protein caeruloplasmin is thought to play an essential role in iron metabolism, but it also has antioxidant properties (Gutteridge & Stocks 1981). First, caeruloplasmin has a ferroxidase activity – it oxidises Fe^{++} to Fe^{+++} whilst reducing oxygen to water. However, unlike the nonenzymatic oxidation of Fe^{++} ions by O_2, this caeruloplasmin-catalysed oxidation of Fe^{++} does not release any damaging oxygen radicals: they are kept on the active site of the protein. The ferroxidase activity of caeruloplasmin allows it to inhibit iron ion–dependent lipid peroxidation and $\cdot OH$ formation from H_2O_2 under most conditions, and this is probably the major antioxidant activity of the protein (Halliwell & Gutteridge 1990b).

Albumin can also bind copper ions, and it usually inhibits copper ion–dependent lipid peroxidation and $\cdot OH$ radical formation (reviewed in Halliwell 1988). In fact, the reactions often continue at the sites of metal ion binding and damage the protein, but the high concentration of albumin in plasma and the rapid turnover of this protein mean that such damage is probably biologically insignificant. Thus, binding to albumin of any copper ions released into plasma may lead to damage to the protein if $O_2^{\cdot -}$ and H_2O_2 are generated in plasma. However, albumin may well prevent the copper ions from attaching to more important targets, such as $-SH$ groups essential to membrane function, e.g. on the membrane proteins of endothelial cells or of erythrocytes, where binding of Cu^{++} ions could lead to oxidative damage. Binding to albumin might also help to stop copper ions from accelerating the peroxidation of low-density lipoproteins and promoting atherosclerosis (Esterbauer et al. 1989). The copper ion-albumin complex might be a safe 'transit form' of copper, that can be removed from the circulation by the liver (Halliwell 1988).

Albumin transports fatty acids in the blood, and the bile pigment bilirubin is bound to it. It has been claimed that bilirubin acts as an antioxidant inhibitor of lipid peroxidation *in vitro* (Stocker 1990). Perhaps bilirubin protects albumin-bound fatty acids against peroxidation *in vivo*. Indeed, Stocker (1990) has argued that induction of the enzyme haem oxygenase (shown to occur in cells exposed to oxidative stress) serves not only to remove a pro-oxidant (haem) but also to increase antioxidants (e.g. bilirubin). However, the prevention of copper ion-mediated damage by albumin is probably a property of the protein itself. Albumin is also a powerful scavenger of HOCl in plasma (Wasil et al. 1987).

Ascorbic acid, present in plasma from healthy humans at concentrations of 50 to 200 μmol/L, has multiple antioxidant properties (listed in Halliwell 1990), probably including the ability to regenerate α-tocopherol by reducing α-tocopheryl radicals at the surface of lipoproteins and membranes. High concentrations of ascorbic acid are also present intracellularly, especially in brain and lung cells. Mixtures of ascorbic acid and H_2O_2 with iron or copper ions have powerful pro-oxidant properties, however, because they can form $\cdot OH$ and other reactive species. This emphasises the need for careful sequestration of 'free' transition metal ions in the human body in order for ascorbic acid to exert its antioxidant action. The fact that large amounts of ascorbic acid can safely be given to healthy humans illustrates how effective this sequestration of iron and copper ions is, and means that ascorbic acid is probably an important antioxidant in healthy humans. The administration of ascorbic acid to patients with iron overload can lead to serious con-

sequences, however, unless desferrioxamine is given simultaneously (Nienhuis 1981).

It thus seems that a major antioxidant action of blood plasma is the prevention of transition metal ions from accelerating damaging free-radical reactions such as lipid peroxidation. By contrast, the metal ion-binding antioxidant defences of some other extracellular fluids may be much weaker. Human cerebrospinal fluid (reviewed by Halliwell 1989b) contains little transferrin, albumin or caeruloplasmin, but has high concentrations of ascorbic acid (about 10 times those in plasma) and also contains uric acid, which has some antioxidant properties (Ames et al. 1981). Synovial fluid has lower concentrations of albumin, transferrin and caeruloplasmin than does plasma (Gutteridge 1987), whereas the fluid that lines the alveoli of the lung also has a low protein content but a very high concentration of ascorbic acid (reviewed by Heffner & Repine 1989). It has also been shown that human alveolar lining fluid, unlike other human extracellular fluids, contains significant concentrations of glutathione (Buhl et al. 1989). Seminal plasma may have poor antioxidant capacity, since spermatozoa seem to peroxidise readily (Aitken 1989), but more studies on this fluid are needed.

2. Free Radical Damage to Cells and the Protective Ability of Antioxidants

2.1 Mechanisms of Cell Damage: The Molecular Targets

There is still a general tendency in the literature to equate 'oxidative damage' with 'lipid peroxidation', so that antioxidants are often characterised only by their ability to inhibit lipid peroxidation. However, membrane lipids are only one possible target of oxidative damage (fig. 2). Indeed, very few toxins that injure cells by generating excess reactive oxygen species seem to cause damage by accelerating the bulk peroxidation of membrane lipids (discussed by Kappus 1987 and by Halliwell & Gutteridge 1984, 1990a).

Oxidative stress in cells and tissues results from increased generation of reactive oxygen species and/or from decreases in antioxidant defences. Increased production of $O_2^{\cdot -}$ and H_2O_2 can occur as a result of:

1. Raised O_2 concentrations (sometimes inadvertantly; culture of most mammalian cells under 95% O_2 or even under air exposes them to higher O_2 concentrations than are present *in vivo*).
2. Adding certain toxins (such as alloxan, paraquat or doxorubicin (adriamycin)] that increase intracellular formation of reactive oxygen species.
3. Activating a large number of phagocytes at a localised site ($O_2^{\cdot -}$ and H_2O_2 are produced by activated phagocytes and are essential for the killing of many bacterial strains, but they can do tissue damage when generated in excess).

One early event frequently observed in mammalian cells subjected to oxidative stress is DNA strand breakage, which leads to activation of poly(ADP-ribose) synthetase, an enzyme that cleaves NAD^+ to give ADP-ribose, which is then polymerised. Excess activation of poly(ADP-ribose) synthetase can lead to large falls in cellular NAD^+ concentrations, so interfering with ATP synthesis (Schraufstatter et al. 1987). Oxidative stress can also lead to rises in intracellular 'free' Ca^{++} within the cell by inactivating enzyme systems in the endoplasmic reticulum and mitochondria that sequester free Ca^{++}, and possibly also by damaging transport systems in the plasma membrane that eject Ca^{++} from the cell (discussed by Albano et al. 1991; Orrenius et al. 1989). If free intracellular Ca^{++} becomes too high, proteases can be activated that cleave the cytoskeleton. Calcium could also activate endonucleases that produce DNA strand breaks (Orrenius et al. 1989).

In addition, cells contain a small low-molecular-mass iron pool, which supplies iron for the synthesis of ferroproteins. The exact intracellular location of this pool is not clear, but it may be largely compartmentalised into organelles such as mitochondria. Its existence may explain why superoxide dismutase and H_2O_2-removing enzymes are such important intracellular antioxidants; it is vital to remove as much $O_2^{\cdot -}$ and H_2O_2 as possible before they come into contact with this low molecular mass iron pool and generate damaging species such as $^{\cdot}OH$ (Halliwell & Gutteridge 1990b). Thus,

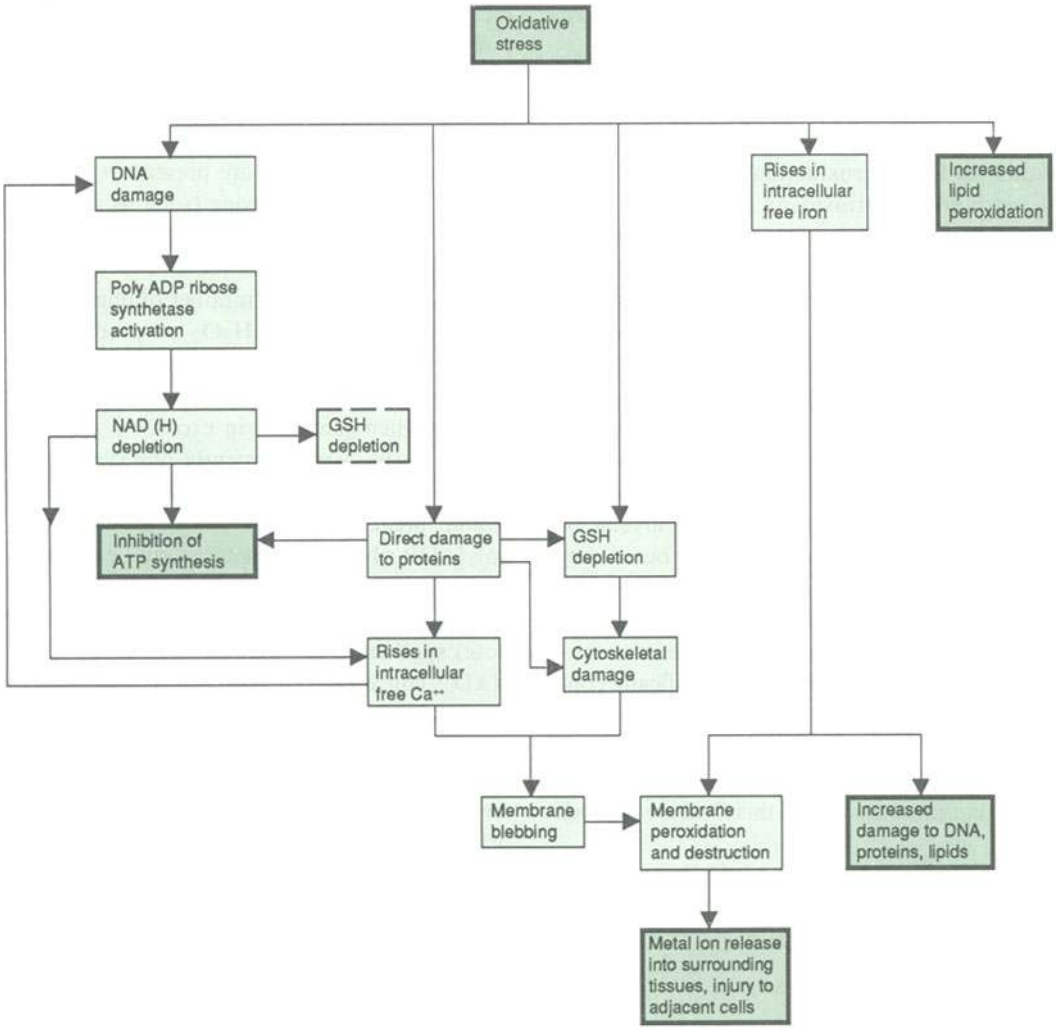


Fig. 2. Schematic illustrating the multiple derangements of cell metabolism that can be brought about by oxidative stress. Direct damage to DNA, proteins and/or lipids is possible. Secondary damage can arise when oxidative stress produces rises in 'free' intracellular metal ions, such as Ca^{++} and possibly Fe^{++}. Ca^{++} can stimulate proteases and nucleases, damaging both DNA and the cytoskeleton. Membrane 'blebs' are 'blow-outs' of the plasma membrane characteristic of cells under stress; the rupture of large blebs may lead to cell death (Orrenius et al. 1989).

damage to proteins and to DNA can occur not only as a result of activation of hydrolytic enzymes, but also by generation of ·OH. The relative significance of these 2 mechanisms is an area of current debate (Halliwell 1987; Halliwell & Aruoma 1991; Imlay & Linn 1988; Orrenius et al. 1989; Schraufstatter et al. 1987; Starke & Farber 1985). In addition, oxidative stress can create more metal ion promoters of free radical reactions. Thus, $O_2^{\cdot -}$ can mobilise a small percentage of the iron ions stored within

the protein ferritin (Biemond et al. 1984; Bolann & Ulvik 1990) and may also be able to release iron ions from the active sites of some iron-sulphur proteins (Flint & Emptage 1991). Similarly, H_2O_2 in excess can degrade haem proteins to liberate iron (Gutteridge 1986; Puppo & Halliwell 1988a,b).

2.2 How to Characterise an Antioxidant

In evaluating the likelihood of a proposal that a compound already present *in vivo* or administered as a drug is acting as an antioxidant *in vivo*, it is important to ask the right questions, as summarised in table I (Halliwell 1990). Antioxidants can act by scavenging reactive oxygen species (e.g. SOD removing $O_2^{\cdot-}$), by inhibiting their formation (e.g. by blocking activation of phagocytes), by binding transition metal ions and preventing formation of ·OH and/or decomposition of lipid hydroperoxides, by repairing damage (e.g. α-tocopherol repairing peroxyl radicals and so terminating the chain reaction of lipid peroxidation) or by any combination of the above.

In testing putative antioxidant activity, it is important to use biologically relevant reactive oxygen species. Fairly simple methods exist for measuring the rate of reaction of a compound with $O_2^{\cdot-}$, H_2O_2, HOCl, ·OH, peroxyl radicals, singlet O_2 and oxidants generated when haem proteins react with H_2O_2. Methods also exist to measure the ability of compounds to suppress metal ion–dependent ·OH generation, production of reactive oxygen species by phagocytes or inhibition of the peroxidation of fatty acids or of membranes (reviewed by Halliwell 1990). In all cases, it is essential to test the compound at the concentrations that are actually present *in vivo*, which are often too low for direct scavenging of reactive oxygen species to be feasible. For example, although many drugs react with ·OH *in vitro*, it is unlikely that this is often a significant mechanism of antioxidant action *in vivo* simply because the drugs are usually present at concentrations that are too low to compete with biological targets for any ·OH generated (explained in table I).

It must also be borne in mind that antioxidants can sometimes do damage in biological systems. This may occasionally be because reactive oxygen species have useful physiological functions and thus too much antioxidant activity can be counter-productive. Hence, intravenous administration of excess α-tocopherol to premature babies has been claimed to lead to an increase in serious infections, presumably by suppression of bacterial killing by phagocytes (discussed by Ehrenkranz 1989). In addition, many lipid-soluble chain-breaking antioxidant inhibitors of lipid peroxidation can have pro-oxidant properties under certain circumstances, often because they can bind Fe^{+++} ions and reduce them to Fe^{++} (Laughton et al. 1989). Even α-tocopherol can be made to exert pro-oxidant effects *in vitro*, although these almost certainly have no physiological relevance (discussed by Halliwell

Table I. Questions to ask when evaluating the proposed role of an 'antioxidant' *in vivo*

1. What biomolecule is the compound supposed to protect? For example, an inhibitor of lipid peroxidation is unlikely to be useful if the oxidative damage is mediated by an attack upon proteins or DNA.
2. Is the compound present *in vivo* at or near that biomolecule at sufficient concentration? For example, many compounds have been suggested to act as ·OH scavengers *in vivo*. In order to compete with biological molecules for ·OH, a scavenger has to be present at at least *millimolar* concentrations *in vivo*. Most drugs never achieve this sort of concentration.
3. How will the compound protect – by scavenging ROS, by preventing their formation or by repairing damage?
4. For naturally occurring antioxidants, is antioxidant protection the primary biological role of the molecule or a secondary one? For example, SOD has probably evolved as an antioxidant enzyme. By contrast, transferrin has probably evolved as an iron transport protein, although the binding of iron ions to transferrin stops them accelerating radical reactions, giving this protein an important secondary role in extracellular antioxidant defence.
5. If the antioxidant acts by scavenging a ROS, can the antioxidant-derived radicals themselves do biological damage?
6. Can the antioxidant cause damage in biological systems different from those in which it exerts protection?

Abbreviation: ROS = reactive oxygen species; SOD = superoxide dismutase.

1990). Propyl gallate, an antioxidant sometimes used in the food industry, has limited solubility in water, but this is enough to allow it to accelerate both •OH formation from H_2O_2 by Fenton chemistry and DNA damage by the antibiotic bleomycin, in both cases by its ability to reduce Fe^{+++} to Fe^{++} (Aruoma et al. 1990; Gutteridge & Xaio-Chang 1981). Many phenolic compounds found in plants (especially flavonoids) have been styled as 'antioxidants' because they inhibit lipid peroxidation – hence the appearance of 'bioflavonoids' (plus or minus vitamin C) on the shelves of health-food stores. However, several plant phenolics can accelerate oxidative damage to nonlipid biomolecules such as DNA (e.g. Laughton et al. 1989). They can do this by reducing Fe^{+++} ions to Fe^{++} and/or by oxidising to produce $O_2^{\cdot -}$ and H_2O_2. Thus, an antioxidant in one system is not an antioxidant in all systems, and this must be borne in mind.

A similar principle might be applicable to ascorbic acid. Tissue injury can result in release of intracellular metal ions and a consequent stimulation of free radical reactions (section 3.2). Whereas ascorbic acid is a good antioxidant in the absence of transition metal ions, in the presence of such ions it can accelerate oxidative damage. It thus follows that the large doses (often > 10 g/day) of ascorbic acid sometimes recommended for consumption might be harmless to healthy subjects, but not necessarily harmless in certain human diseases. In sickle-cell disease (Jain & Williams 1985), rheumatoid arthritis (Lunec & Blake 1985), adult respiratory distress syndrome (ARDS) [Cross et al. 1990] and iron overload disease (Nienhuis 1981), ascorbic acid concentrations in body fluids are markedly subnormal. In rheumatoid arthritis and ARDS, this may be because of the rapid uptake and oxidation of ascorbate by activated neutrophils as well as by the reaction of ascorbate with $O_2^{\cdot -}$ and HOCl. However, replacement of this ascorbate might not necessarily be totally beneficial. It might even be argued that this loss of ascorbic acid is beneficial, if tissue injury is making metal ions available (Halliwell & Gutteridge 1990b).

3. Role of Free Radicals in Human Disease

3.1 Causation of Disease

Does increased formation of free radicals and other reactive oxygen species cause any human disease? Radiation-induced cancer may be initiated by radicals, as discussed previously. The signs produced by chronic dietary deficiencies of selenium (Keshan disease) or of vitamin E (neurological disorders seen in patients with inborn errors in the mechanism of intestinal fat absorption) might also be mediated by reactive oxygen species (reviewed by Levander 1987; Howard 1990). In the premature infant, exposure of the incompletely vascularised retina to elevated concentrations of oxygen can lead to retinopathy of prematurity, which in its most severe forms leads to blindness. Several controlled clinical trials have documented the efficiency of α-tocopherol in minimising the severity of the retinopathy, suggesting (but by no means proving) a role for lipid peroxidation (Howard 1990). Vitamin E supplementation may also diminish the incidence of intraventricular haemorrhage (Chiswick et al. 1991).

3.2 A Significant Consequence of Disease Pathology

For most human diseases, the excess formation of reactive oxygen species is not a cause of the disease, but is secondary to the primary disease process. For example, activated neutrophils produce $O_2^{\cdot -}$, H_2O_2 and HOCl as part of the mechanism of killing bacteria. However, if large numbers of phagocytes become activated in a localised area, they can produce tissue damage. For example, the synovial fluid in the swollen joints of rheumatoid patients contains many activated neutrophils and there is reasonable evidence that reactive oxygen species and other products derived from neutrophils and macrophages are contributing to the joint injury (reviewed by Halliwell et al. 1988a). In some forms of ARDS the lung damage is thought to be mediated by an influx of neutrophils into the lung,

where they become activated to produce prostaglandins, leukotrienes, proteolytic enzymes, such as elastase, and reactive oxygen species (Cross et al. 1990). Among other effects, the reactive oxygen species can inactivate the proteins (such as α_1-antiproteinase) within the lung that normally inhibit the action of serine proteinases, such as elastase, and prevent them from attacking lung elastic fibres. The precise contribution that generation of reactive oxygen species makes to lung damage in ARDS is unknown, but deserves more investigation in view of the high mortality rate of ARDS (Cross et al. 1990).

Another example of secondary oxidative damage is provided by the work of Akaike et al. (1990). They found that superoxide dismutase protects mice against the lethality of an influenza virus infection, apparently by protecting the lungs against excess reactive oxygen species generated by activated phagocytes and by the enzyme xanthine oxidase. Similar oxidative stress may occur in some other viral infections (Peterhans et al. 1988) and during malarial infections (Clark et al. 1986; Hunt & Stocker 1990).

Reactive oxygen species seem also to play a role in the formation of foam cells in atherosclerotic plaques (reviewed by Steinberg et al. 1989). Interest in the role of lipid peroxidation in atherogenesis was raised when it was found that the compound probucol has a marked antiatherogenic effect in hyperlipidaemic rabbits (Carew et al. 1987). Probucol, like α-tocopherol, has easily donatable hydrogen atoms and is a powerful chain-breaking inhibitor of lipid peroxidation. That does not mean, of course, that any protective action of probucol *in vivo* is due to inhibition of lipid peroxidation.

Macrophages possess receptors for low-density lipoprotein (LDL) but if LDL has become peroxidised it is recognised by a separate class of macrophage receptors known as the scavenger receptors. There may be more than one type of scavenger receptor. LDL bound to these receptors is taken up with enhanced efficiency, so that cholesterol rapidly accumulates within the macrophage and may convert it into a foam cell (Mitchinson & Ball 1987). Arterial endothelial cells, smooth muscle cells and macrophages have been shown to be capable of oxidising LDL *in vitro* so that macrophages will internalise it faster. The modification process that allows recognition by the scavenger receptors involves the derivatisation of lysine residues of the apoprotein B moiety of LDL by lipid peroxidation products, such as the cytotoxic aldehyde 4-hydroxynonenal (Steinberg et al. 1989). Human atherosclerotic lesions accumulate the pigment ceroid, which is widely thought to be an end-product of peroxidation (Ball et al. 1987). More rigorous evidence for the presence of peroxidised lipid in atherosclerotic plaques is provided by the use of antibodies. For example, antibodies directed against LDL that has undergone peroxidation or has been treated with 4-hydroxynonenal, bind to rabbit aortic atherosclerotic lesions. In addition, LDL eluted from such lesions can bind to antibody specific for malondialdehyde-treated LDL. Plasma samples from hypercholesterolaemic rabbits and from several human subjects have been found to contain antibodies that will react with peroxidised LDL, suggesting that peroxidation does indeed take place *in vivo* (Steinberg et al. 1989).

Peroxidation of LDL within the vessel wall could have a number of deleterious effects. It has been suggested that products formed in peroxidised LDL, such as lysophosphatidyl choline, might act as chemotactic factors for blood monocytes, encouraging their recruitment into an atherosclerotic lesion and development into macrophages (Steinberg et al. 1989). In addition, low (submicromolar) concentrations of lipid hydroperoxides might accelerate cyclooxygenase- and lipoxygenase-catalysed reactions in endothelium and in any platelets present, leading to enhanced formation of prostaglandins and leukotrienes (Warso & Lands 1983). Oxidised LDL might also stimulate the production of cytokines and growth factors by macrophages, which are complex multifunctional cells (Yokode et al. 1988). There have been several speculations that oxidation products of cholesterol might also be involved in atherogenesis; cholesterol is oxidised into a wide variety of products in peroxidising lipid systems (Sevanian & Peterson 1986; Smith 1987) and oxidised cholesterol has been reported

to be toxic to many cell types, including arterial smooth muscle cells and endothelial cells. It seems likely that cell breakdown in the advanced atherosclerotic plaque can lead not only to activation of lipoxygenases, but also to release of transition metal ions that can stimulate free radical reactions such as lipid peroxidation (Halliwell 1989c).

The clinical consequences of severe atherosclerosis (stroke and myocardial infarction) may also involve reactive oxygen species. One of the most exciting areas of basic research in recent years has been that of ischaemia/reperfusion injury (McCord, 1985), although it has yet to yield clinically useful developments. When tissues are deprived of O_2 they are injured and after a period the injury becomes irreversible and the tissue will die. The length of this critical period depends on the extent of O_2 deprivation (whether it is total ischaemia or merely hypoxia) and the tissue in question: skeletal muscle can be rendered bloodless for hours without much injury, whereas in the brain the period is much shorter. For the heart, up to about 60 minutes seems to be a tolerable period. Thus, ischaemia injures cells and will eventually kill them, and the aim should be to restore blood flow as soon as possible: hence, the use of thrombolytic agents in the treatment of myocardial infarction (reviewed by Bolli 1988).

However, studies on isolated organs and in animals have shown that, provided the period of ischaemia does not itself do irreversible damage, tissue function is better preserved upon reoxygenation if certain antioxidants are included in the reperfusion medium (Bolli 1988; McCord 1985; Simpson & Lucchesi 1987). Hence, restoration of the oxygen supply, although obviously beneficial overall, causes increased formation of reactive oxygen species in the damaged tissue and temporarily worsens the injury. For example, depending on the model system studied, the heart can respond to reperfusion by showing several dysfunctions, such as arrhythmias, delayed return of contractile function ('stunning') and, it has been claimed, an increase in infarct size. Thus, inclusion of antioxidants when blood flow is restored offers protective effects in many animal experiments: antioxidants that have been used include thiol compounds (e.g. mercaptopropionylglycine), catalase, recombinant human superoxide dismutase and desferrioxamine, a chelator that binds iron ions and usually stops them from accelerating free radical reactions (Gutteridge et al. 1979). The use of antioxidants in the treatment of shock and in the preservation of organs for tranplantation is also receiving attention (Bolli 1988; Currin et al. 1990; Fuller et al. 1988; Sanan et al. 1989), as discussed in section 4.

Superoxide dismutase and catalase have been extensively explored as protective agents when added to reperfusates in many types of experiment. In some models significant protection is observed whilst in others the enzymes are without effect. The author's general impression is that SOD is often very protective against myocardial arrhythmias and sometimes protective against stunning, whereas its ability to diminish infarct size is less well established (Bolli 1991; Cohen 1989; Downey et al. 1991; Engler & Gilpin 1989). Protection by some antioxidants is often only observed when the antioxidant is added before ischaemia or at the start of reperfusion: addition after reperfusion has started (even after only 60 seconds) gives no protective effect. This phenomenon has been demonstrated with desferrioxamine and with mercaptopropionylglycine (Bolli et al. 1989) in an open-chest dog model of myocardial stunning after reperfusion.

Why should ischaemia lead to increased generation of reactive oxygen species when tissues are reoxygenated? The answer is not yet clear: possibilities include release of iron ions, catalytic for free radical reactions, from their normal storage sites within the cell (Gower et al. 1989a; Halliwell 1989a; Sanan et al. 1989), disruption of mitochondrial respiratory chains so that more electrons leak to oxygen to form $O_2^{\cdot -}$ and increases in the activities of certain enzymes, such as xanthine oxidase, that generate $O_2^{\cdot -}$ (McCord 1985). However, there is considerable doubt about the role of xanthine oxidase in human myocardial injury (discussed by Kehrer 1989).

3.3 A General Consequence of Tissue Injury

Mechanical (e.g. crushing), chemical or ischaemic injury to tissues may cause cells to rupture and release their contents into the surrounding area

(fig. 3). These contents will include transition metal ions, which are potentially capable of increasing free radical reactions in the surrounding area. For example, administration of cytotoxic drugs to patients with acute myeloid leukaemia has been shown to create a temporary 'iron overload' state, probably due to extensive drug-induced lysis of the leukaemic cells. This increased iron availability could contribute to the side effects of cytotoxic chemotherapy (Halliwell et al. 1988b). Thus, as figure 3 shows, any disease that increases cell death could conceivably result in an increased rate of free radical reactions. This may be completely trivial, i.e. the increased rates of free radical reactions may make no significant contribution to the disease pathology. An example of this may be the increased amounts of lipid hydroperoxides measured in dystrophic muscle, even though there is no evidence that inhibitors of lipid peroxidation or other antioxidants are therapeutically useful in muscular dystrophy (Davison et al. 1988; Stern et al. 1982). Sometimes, however, this injury-related damage may be important: perhaps the greatest interest in this area lies in the sequelae of traumatic or ischaemic injury to the brain. Some areas of the human brain are rich in iron but cerebrospinal fluid has no significant iron-binding capacity, since its content of transferrin is low. Thus, it has been proposed (Halliwell & Gutteridge 1985) that injury to the brain by mechanical means (trauma) or by oxygen deprivation (stroke) can result in release of iron ions into the surrounding area. These ions can facilitate further damage to the surrounding areas by accelerating free radical reactions (fig. 3). This proposal has been given some support from animal studies, using agents (such as aminosteroids) that interfere with iron-dependent radical reactions. One such aminosteroid, U74006F, has been observed, in animal experiments, to decrease reperfusion injury, post-traumatic spinal cord degeneration and neurological damage after head injury (reviewed by Hall & Braughler 1989).

3.4 What Can We Expect from Antioxidant Therapy?

Tissue destruction and degeneration can result in increased oxidative damage, by such processes as metal ion release, lipoxygenase activation (fig. 3), phagocyte stimulation and disruption of mitochondrial electron transport chains (so that more electrons 'escape' to oxygen to form $O_2^{\cdot-}$). Hence, almost any disease is likely to be accompanied by increased formation of reactive oxygen species, and it is not surprising that the list of diseases in which their formation has been implicated is long and grows longer (Halliwell 1987). For atherosclerosis, rheumatoid arthritis, some forms of ARDS, reoxygenation injury, and traumatic or ischaemic damage to the central nervous system, there is reasonable evidence to suggset that free radical reactions make a significant contribution to the disease path-

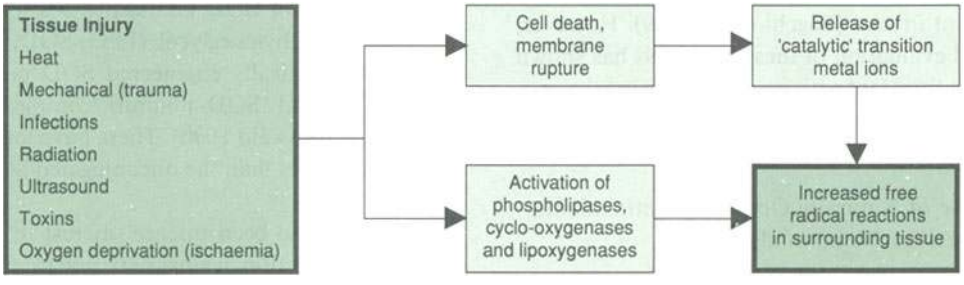


Fig. 3. Diagram showing how cell injury can predispose to increased free radical reactions in the surrounding tissue. Cell injury can activate synthesis of prostaglandins and leukotrienes. It can also release transition metal ions that can accelerate free radical reactions. This stimulation of free radical reactions as a result of injury can sometimes result in a significant spreading of the injury. In other situations, it may be of no biological consequence.

ology. As has been stressed previously, however (Halliwell & Gutteridge 1984), it is equally likely that in some (perhaps most) diseases, the increased formation of reactive oxygen species is an epiphenomenon that is of little clinical relevance and antioxidants are unlikely to be of therapeutic benefit. This is by no means a new situation. Tissue injury usually increases synthesis of prostaglandins, yet cyclooxygenase inhibitors are of little benefit in many human diseases. Thus, in rheumatoid arthritis, they decrease pain and swelling of the joints, but fail to halt the joint destruction processes.

These general principles can help us to interpret the results of antioxidant therapy in various human diseases, and to make proposals for the future.

4. *Antioxidants in Disease Therapy*

Attempts to use antioxidants in the treatment of human disease can be divided into 3 main areas:

1. Administration of antioxidants that occur naturally in the human body, such as α-tocopherol, glutathione or superoxide dismutase (SOD).

2. Administration of synthetic antioxidants, such as probucol or chelating agents that suppress iron ion–dependent free radical reactions.

3. The possibility that drugs developed to protect against other mechanisms of tissue injury might have additional physiological action because they have antioxidant properties; for example, the angiotensin converting enzyme inhibitor captopril has been suggested to function additionally as an antioxidant *in vivo* (Bagchi et al. 1989). However, a detailed evaluation of these proposals has shown that the antioxidant activity of captopril at the concentrations actually achieved *in vivo* is likely to be very limited (Aruoma et al. 1991b).

4.1 Use of Naturally Occurring Antioxidants and Derived Compounds

4.1.1 Superoxide Dismutase

Copper-zinc containing SOD has a well-established anti-inflammatory effect in animal models of acute inflammation, in part because it can decrease the number of neutrophils entering sites of inflammation. It also diminishes their adherence to endothelium (Suzuki et al. 1989). However, SOD enzymes of equal catalytic activity do not suppress acute inflammation to the same extent in rats, and it has been suggested that SOD must bind to specific sites to exert its anti-inflammatory activity. Hence, the surface charge distribution on the SOD protein is an important factor (Jadot et al. 1986; Schalkwijk et al. 1985). Similar results have been obtained in studies of the ability of SOD to suppress adjuvant-induced polyarthritis in rats (Vaille et al. 1990).

There is a considerable amount of older literature claiming that injection of SOD into the knee joints of patients with osteoarthritis or rheumatoid arthritis produces a beneficial effect, but these data have been severely criticised (reviewed by Greenwald 1990). Even the early proponents of the use of SOD in humans now admit that SOD is not, and will probably never become, a drug of choice in the treatment of rheumatoid arthritis (Flohé 1988). The author has been unable to find any report of a well-controlled clinical trial of the effect of SOD in inflammatory joint disease or autoimmune disease in a high-quality journal, although brief reports of uncontrolled studies continue to be published. Now that recombinant human copper-zinc–containing (Ambrosio et al. 1986) and manganese-containing SODs (Beck et al. 1988) are available, such well-controlled studies should be performed to settle the question as to whether SOD will be of any use as an anti-inflammatory agent. A wide variety of SOD conjugates are available, including polyethyleneglycol (PEG)-SOD, cationised SOD, genetically engineered SOD polymers, pyran SOD and SOD-albumin complexes (reviewed by Greenwald 1990). These have longer circulating half-lives than the unconjugated SOD molecules.

There has also been intense interest in the use of SOD in protection against reoxygenation injury. The reported effects of SOD in decreasing infarct size after prolonged myocardial ischaemia seem increasingly questionable (Bolli et al. 1991; Downey 1991; section 3.2) and so hope is being concen-

trated in 2 areas: does a combination of SOD and a thrombolytic agent produce increased functional activity and decreased mortality compared with a thrombolytic agent alone in cases of recent myocardial infarction, and would SOD be helpful in extending the preservation time of organs for transplantation? An answer to the first question must await the results of controlled clinical trials currently in progress, although Petch (1990) and Editorial (1989a) have reported that reperfusion injury does not seem to be a significant problem in the clinical use of thrombolytic agents. One reason for this could be that reperfusion in most animal model systems is sudden, whereas thrombolytic reperfusion in humans is slow, and slow reoxygenation may minimise the injury (e.g. Perry & Wadhwa 1988). Although markers of oxidative reperfusion injury can be detected during postischaemic reperfusion of the hearts of patients after open-heart surgery (del Nido et al. 1987; Ferrari et al. 1990), the clinical significance of this injury may be questioned (Bell et al. 1990; Hermens et al. 1988). Even if human myocardial reoxygenation injury were clinically significant, SOD need not be the ideal protective agent. For example, SOD failed to protect against myocardial stunning in open-chest dogs, although the combination of SOD and catalase was protective (Patel et al. 1990). Further comments about this are made in section 5.

Treatment of human kidney transplant recipients with bovine SOD produced no significant decrease in rates of acute renal failure, but there was a suggestion of benefit that merits further study (Schneeburger et al. 1989). The reported ability of SOD in some animal experiments to offer protection against endotoxic or hypovolaemic shock (Carden et al. 1989; Schneider 1988), pancreatitis (Sanfey et al. 1984), gastrointestinal ischaemia (McCord 1985; Vohra et al. 1989) and preservation of organs other than kidney (Fuller et al. 1988; Huang et al. 1987; Weiss et al. 1989) does not yet appear to have been assessed critically in humans. Even in animal experiments, there is considerable variability in results between different laboratories (e.g. McCord 1985).

The gene encoding human extracellular SOD (EC-SOD; section 1.7) has been cloned and expressed (Tibell et al. 1987). Since reoxygenation injury to tissues probably involves endothelial damage (Forman et al. 1989; Halliwell 1989a; Tsao & Lefer 1990) and some EC-SODs bind to endothelia (Marklund 1990), it has been proposed by Marklund's group that EC-SODs might be especially efficient as protective agents. To the author's knowledge, this proposal has not yet been tested in humans by controlled clinical trial. Inoue et al. (1990) constructed and expressed in yeast a fusion gene consisting of cDNA encoding human Cu-ZnSOD and heparin-binding peptide. The resulting enzyme retained activity and was able to bind to and protect endothelial cells.

4.1.2 Superoxide Dismutase 'Mimics'

Several low-molecular-mass compounds that react with $O_2^{\cdot-}$ have been described. They usually contain transition metal ions. Examples include iron porphyrins (Nagano et al. 1989), a complex of manganese ions with the chelating agent desferrioxamine (Beyer & Fridovich 1989) or copper ions chelated to amino acids or to anti-inflammatory drugs (Sorenson 1984; Weser et al. 1980). Superoxide scavengers that do not involve metal ions, such as nitroxides, have also been described (Mitchell et al. 1990). Apart from the various copper complexes (Sorenson 1984) little information is available on the toxicity of SOD mimics (Steinkuhler et al. 1990). Indeed, the potential use of these compounds in treatment of human disease is uncertain in view of the availability of recombinant human SOD enzymes of very low toxicity.

4.1.3 α-Tocopherol

Prolonged deficiency of α-tocopherol, as in fat malabsorption syndromes, produces severe neurological damage (reviewed by Bieri et al. 1983). The antioxidant action of α-tocopherol in plasma lipoproteins might help to protect against atherosclerosis, as suggested by epidemiological studies (Gey et al. 1987; Gey 1990). Hence, there are good reasons for maintaining an adequate vitamin E intake by eating a healthy diet and for giving supplementary vitamin E to patients with diseases af-

fecting fat absorption. Therapeutic administration of vitamin E to premature babies has been reported to diminish the severity of retrolental fibroplasia (although there is some controversy about this: discussed in Ehrenkranz 1989) and the incidence of both haemolytic syndrome of prematurity and intraventricular haemorrhage (Chiswick et al. 1991; Sinha et al. 1987). Vitamin E supplementation has also been recommended as beneficial in patients suffering from haemolytic syndromes caused by an inborn lack of glutathione synthetase or glucose-6-phosphate dehydrogenase in erythrocytes (e.g. Corash et al. 1980). However, it has been reported that high doses of intravenous vitamin E can produce an increase in the infection rate in premature babies, probably by depressing phagocyte function (Engle et al. 1988). Depression of phagocyte function can also be demonstrated in cells isolated from adults taking oral vitamin E but this effect does not seem to be clinically significant (Bendich & Machlin 1988).

However, attempts to use vitamin E to treat human diseases in which oxidative stress is thought to be important, such as anthracycline-induced cardiac damage (reviewed by Powis 1989), have been largely disappointing. One reason for this could be that it takes a considerable time to raise the vitamin E content of many tissues, especially that of brain, whereas oxidative damage can often be very fast (e.g. within minutes in the reoxygenation injury experiments described by Bolli et al. 1989). In addition, oxidative damage frequently occurs by mechanisms other than lipid peroxidation (Powis 1989; section 2.1).

Pharmacological doses of vitamin E have been claimed to be beneficial in thrombotic vascular disease (reviewed by Bieri et al. 1983), but even if true this might be due to effects other than antioxidant action, such as partial inhibition of lipoxygenase (Reddanna et al. 1985) or a weak suppression of platelet aggregation (Jandak et al. 1989). It is also interesting to note that the reported protective effects of α-tocopheryl succinate against oxidative damage in isolated hepatocytes could not be explained simply by its hydrolysis to free tocopherol and may have involved 'cytoprotective effects' of the succinate ester itself (Carini et al. 1990; Fariss 1990; Fariss et al. 1989).

Several structural analogues of α-tocopherol with improved antioxidant activity (as assayed *in vitro* and/or tested in animals) have been described (Ingold et al. 1986, 1990; Mukai et al. 1989). A water-soluble analogue of α-tocopherol, 'Trolox C', also exists (Thomas & Bielski 1987) but caution should be employed in using it upon humans because it has been shown to be capable of accelerating oxidative damage to substrates other than lipids *in vitro* (Aruoma et al. 1990). In general, the author feels that antioxidant inhibitors of lipid peroxidation are not likely to be of general use in treating human disease, because lipid peroxidation is often a late stage in the oxidative damage mechanism (section 2.1). However, they may have great benefit as protective agents in some cases, e.g. in protecting against the development of atherosclerosis, provided that they can enter and remain in LDL (Esterbauer et al. 1989). The results of a clinical trial (Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism, or DATATOP) in which the combination of vitamin E with an inhibitor of monoamine oxidase B (deprenyl) is being evaluated in the treatment of Parkinson's disease are awaited with interest (Parkinson Study Group 1989). So far, however, the beneficial effect of deprenyl has been much more obvious than that of α-tocopherol.

4.1.4 Ascorbic Acid (Vitamin C)

Vitamin C is essential in the human diet as a cofactor for several hydroxylase enzymes (reviewed by Levine 1986) and deficiency of it should be avoided, especially as deficiency might also predispose to atherosclerosis, as is suggested by epidemiological studies (Gey et al. 1987). It is possible that smokers need a higher vitamin C intake than nonsmokers, since smoking appears to lead to increased vitamin C consumption (e.g. Chow et al. 1986) and vitamin C is thought to be an important antioxidant in the lung (Bendich et al. 1986; Cross et al. 1990). It may also protect against the carcinogenicity of nitrosamines (Shamberger 1984; Tannenbaum et al. 1991). In the absence of metal ions,

ascorbate is a powerful antioxidant *in vitro* (Frei et al. 1989), but in their presence, it can be dangerous, as discussed in section 1.7.

Administration of very high doses (up to 20g daily in some cases) of vitamin C to healthy humans appears to do no significant harm (Bendich et al. 1986) but, to the author's knowledge, no clinical benefit has yet been demonstrated in well-controlled clinical trials in any disease: indeed, high-dose vitamin C appeared to lead to lethal haemorrhagic tumour necrosis in 4 patients with advanced cancer (Cameron & Campbell 1974). Cessation of megadose therapy may lead to rebound low levels of vitamin C in body fluids (e.g. Tsao & Salimi 1984).

A number of lipophilic ascorbate esters (e.g. ascorbyl palmitate) have also been described (e.g. Baader et al. 1988), but reports of properly controlled trials of their use in the treatment of human disease have not yet appeared.

4.1.5 Adenosine

Under certain circumstances, neutrophils can potentiate injury to tissues (section 3.2). For example, antibodies preventing neutrophil adherence decreased infarct size in a dog model of cardiac reoxygenation injury after ischaemia (Werns & Lucchesi 1990). The first step in neutrophil accumulation in several tissues involves adherence to the endothelium, a process that is regulated by many substances produced by endothelial cells. For example, prostacyclin decreases adherence, whereas $O_2^{\cdot -}$ increases it (Suzuki et al. 1989).

The nucleoside adenosine (adenine-ribose) affects the function of both neutrophils (e.g. it decreases $O_2^{\cdot -}$ production) and endothelium and it has been shown to have protective effects in some animal models of reoxygenation injury (Forman et al. 1989; Grisham et al. 1989; Pitarys et al. 1991). Indeed, it has been argued that adenosine is a natural anti-inflammatory agent during transient injury, helping to prevent premature activation of the inflammatory response (Cronstein et al. 1990; Engler 1987). For example, 5-amino-4-imidazole carboxamide-riboside accelerates adenosine release from ischaemic cells, and has been reported to be protective against ischaemic injury (Engler 1987). Compounds acting in a comparable way to adenosine might be very useful as anti-inflammatory agents and in protection against reoxygenation injury (e.g. Kaminski & Proctor 1990), to the extent that this phenomenon is clinically important (section 4.1.1).

4.1.6 Lactoferrin

Lactoferrin is an iron ion-binding protein, similar to transferrin but with a higher affinity for iron ions (especially at acidic pH values). It is present in human secretions (milk, saliva, tear fluid, nasal secretions) and released by activated neutrophils. Iron ions bound to lactoferrin are unable to catalyse free radical reactions (Aruoma & Halliwell 1987; Gutteridge et al. 1981). Indeed, it has been argued that the release of lactoferrin by activated neutrophils is a protective mechanism that helps to diminish iron-dependent free radical reactions in the surrounding area (Halliwell et al. 1981). For example, Ward et al. (1983) found that lactoferrin was able to protect rats against lung injury mediated by reactive oxygen species. Lactoferrin has many other effects on phagocyte function, however (Birgens 1984). Several groups are cloning and expressing the gene for human lactoferrin (e.g. Stowell et al. 1991) and it will be interesting to see if the human protein has any therapeutic uses.

4.1.7 Nicotinamide

Subjecting cells to oxidative stress frequently leads to depletion of intracellular nicotinamide nucleotides by activation of poly(ADP-ribose) synthetase (section 2.1). Several authors have reported protection against cell killing under these circumstances by inhibitors of poly(ADP-ribose) synthetase, such as aminobenzamide, or by supplementation of the cell culture medium with nicotinamide or nicotinic acid (niacin) [e.g. Schraufstatter et al. 1987; Weitberg 1989]. Weitberg (1989) found that the prolonged ingestion of supplementary nicotinic acid by human volunteers led to an increase in the NAD^+ concentrations in their circulating lymphocytes, and that lymphocytes isolated from these volunteers were more resistant to oxidative dam-

age. Wang et al. (1990) found that pretreatment of hamsters with nicotinamide decreased the lung fibrosis observed after subsequent exposure to the antitumour antibiotic bleomycin.

Whether these various observations will turn out to have therapeutic relevance to humans is unclear. A potential problem with the use of ADP-ribose synthetase inhibitors is that blocking the action of this enzyme may decrease DNA repair, so increasing the risk of carcinogenesis: this effect has been demonstrated in mice whose pancreatic β-cells were damaged by treatment with streptozotocin, leading to diabetes. The animals could be protected against the diabetogenic action of streptozotocin by administration of ADP-ribose synthetase inhibitors, but a significant number of animals then developed insulinomas (Okamoto 1986). The results with nicotinamide again illustrate the point that a healthy diet may be a good protection against tissue injury due to oxidative damage.

4.1.8 Glutathione and its Precursors

Glutathione has multiple functions in human metabolism, including the detoxification of xenobiotics. It is also important in antioxidant defence, e.g. by helping to protect DNA against damage by ionising radiation and by acting as a substrate for glutathione peroxidases and transferases (Held 1988; Ross 1988). There have therefore been many suggestions that reduced glutathione (GSH) may be therapeutically useful as an antioxidant and as a general cytoprotective agent, e.g. in the preservation of organs for transplantation and in protecting against tissue damage by cytotoxic drugs such as cyclophosphamide (Currin et al. 1990; Friedman et al. 1990; Menasche et al. 1986). Use of aerosolised GSH solutions has been suggested as a means of diminishing lung damage by reactive oxygen species generated extracellularly from activated phagocytes in such diseases as cystic fibrosis (Buhl et al. 1990). There is also evidence for abnormalities of GSH metabolism in HIV-infected cells and the use of GSH or its precursors in asymptomatic HIV-positive and AIDS patients has been suggested (reviewed by Halliwell & Cross 1991).

GSH itself is not rapidly taken up by cells, but several approaches can be used to overcome this. Methyl and ethyl esters of glutathione have been described, which can apparently cross cell membranes and be hydrolysed to GSH within the cell (Anderson & Meister 1989a). However, there have been some reports of toxicity of GSH esters, possibly due to contaminating compounds arising during their preparation (Thornalley 1991). The compound L-2-oxathiazolidine-4-carboxylate has been reported to be hydrolysed to cysteine *in vivo*, which may lead to an increased synthesis of GSH (Meister 1988; Porta et al. 1991). *N*-Acetylcysteine has been used as an antioxidant in a wide variety of experiments (Moldeus et al. 1986) and is very effective in the treatment of paracetamol (acetaminophen) overdosage in humans (Smilkstein et al. 1988). Paracetamol is metabolised by the hepatic cytochrome P450 system to a reactive quinoneimine that can combine with GSH, so causing severe GSH depletion in the liver. *N*-Acetylcysteine may protect by entering cells and being hydrolysed to cysteine, which stimulates GSH synthesis. Additionally, *N*-acetylcysteine has also been widely used in humans for treatment of various respiratory disorders; it has very limited toxicity but its therapeutic benefits in these conditions have been questioned.

Mixtures of thiols with transition metal ions can be cytotoxic because of reactions that produce both oxygen radicals ($O_2^{\cdot -}$ and $\cdot OH$) and sulphur-containing radicals such as $RS\cdot$, $RSO\cdot$ and $RSO_2\cdot$ (Rowley & Halliwell 1982; Sevilla et al. 1989). GSH is unstable in solution, particularly if iron or copper ions are present, and the oxidation products can cause damage. This must be borne in mind if solutions containing GSH or other thiols are to be stored for any length of time, e.g. in cell culture media or in fluids for organ preservation (e.g. Boudjema et al. 1990; Currin et al. 1990). Cysteine seems to be more toxic than GSH in such systems (Saez et al. 1982), perhaps because it oxidises faster and because the cysteine-S• radical can abstract hydrogen atoms from fatty acids and initiate lipid peroxidation (Schoneich et al. 1989). Thus, cysteine accelerates iron ion–dependent peroxidation

of lipids, whereas GSH and *N*-acetylcysteine do not (Haenen et al. 1989). Some thiols can also mobilise iron ions from ferritin (e.g. Bonomi et al. 1989). Indeed, cysteine is much more toxic when administered to animals that are *N*-acetylcysteine or GSH (Anderson & Meister 1989b). Similarly, high levels of plasma homocysteine in homocystinuric patients predispose to atherosclerosis (Ucland & Refsum 1989).

Sulphur-containing radicals can also be formed when thiols are oxidised by ·OH and possibly by other reactive oxygen species:

$$RSH + \cdot OH \rightarrow RS\cdot + H_2O \quad \text{(Eq. 20)}$$

$$RS\cdot \xrightarrow{O_2} RSO\cdot, RSO_2\cdot \quad \text{(Eq. 21)}$$

As has been stressed previously (Halliwell 1990), when proposing the use of scavengers to remove reactive oxygen species, it is necessary to ask what happens to the radicals derived from the scavenger.

N-Acetylcysteine has been reported to have protective effects in many animal models of lung injury. By contrast, it potentiated the lung damage produced by the anticancer drug bleomycin in hamsters (Giri et al. 1988). Bleomycin-dependent lung damage is thought to involve formation of an iron ion–bleomycin complex, which produces strand breakage in DNA in the presence of certain reducing agents (reviewed by Halliwell & Gutteridge 1989). *In vitro, N*-acetylcysteine did not potentiate iron ion-dependent damage to DNA by bleomycin, whereas cysteine did (Evans & Halliwell, unpublished data) and so it is possible that the *N*-acetylcysteine acted as a precursor of intracellular cysteine, which could reduce the iron-bleomycin complex upon the nuclear DNA. Thus, indiscriminate use of thiol compounds as therapeutic agents cannot be recommended.

4.1.9 Carotenoids

Carotenoid pigments, such as β-carotene, are powerful quenchers of singlet O_2 and have found therapeutic use in protecting porphyria patients against photosensitisation damage to the skin, and in protecting animals against damage by photosensitising drugs (Mathews-Roth 1987). They may also have an anticancer effect (Bendich & Olson 1989; Stähelin et al. 1991). It thus seems important to maintain an adequate dietary intake of vegetables. Carotenoids can also act as inhibitors of lipid peroxidation under certain circumstances, but their actions are complex (discussed by Krinsky 1989). Whether they will ever have a major therapeutic use remains unclear as yet.

4.2 Synthetic Antioxidants

4.2.1 Mercaptopropionylglycine and Other Thiols

Many thiols have been tested for their ability to protect cells and small animals against ionizing radiation. They include GSH and its precursors (cysteine, cysteamine), dimercaprol (British Anti-Lewisite) and mercaptopropionylglycine (von Sonntag 1987). In general, the author's impression is that cysteamine (e.g. Issels et al. 1987) and cysteine (section 4.1.8) are more toxic than the others, perhaps because they oxidise faster if transition metal ions are present and/or produce sulphur-containing radicals that are more damaging to biological molecules.

The thiol compound mercaptopropionylglycine (MPG) has been shown to be protective against reperfusion injury after ischaemia in animal models of myocardial stunning and infarction (discussed by Bolli et al. 1989; Puppo et al. 1990). It may act by its ability to scavenge reactive oxygen species, but which species it acts upon is as yet uncertain (Puppo et al. 1990). Several clinical studies have claimed that MPG is beneficial in the treatment of various diseases (listed in Bolli et al. 1989) but well-controlled trials of its effects against reperfusion injury do not appear to have been published.

4.2.2 Ebselen: A Glutathione Peroxidase Mimic?

Ebselen (PZ51; 2-phenyl-1,2-benzisoselenazolin-3(2H)-one) is an organoselenium compound that catalyses removal of peroxides by reaction with GSH, and was developed as a glutathione peroxidase 'mimic'. It has been shown to have anti-in-

flammatory effects in a number of small mammals (Parnham et al. 1991). However, it is uncertain whether these effects can be attributed to removal of H_2O_2, since ebselen also inhibits cyclooxygenase, lipoxygenase, the production of reactive oxygen species by activated neutrophils and lipid peroxidation in membranes (Cotgreave et al. 1989; Hayaishi & Slater 1986; Leurs et al. 1989; Parnham et al. 1991). Ebselen does not appear to have been effectively tested in controlled clinical trials for beneficial effects in humans to date, although its metabolism in the human body has been described (Fischer et al. 1988). One potentially worrying observation is that ebselen also inhibits elongation and desaturation of long-chain fatty acids in rat liver microsomes (Laguna et al. 1989), although the toxicity of the drug to animals has been reported as very low (Parnham et al. 1991).

4.2.3 Allopurinol, Oxypurinol and Other Xanthine Oxidase Inhibitors

Allopurinol inhibits the production of $O_2^{\cdot-}$, H_2O_2 and uric acid by the enzyme xanthine oxidase. It inhibits by first acting as a substrate for the enzyme, so producing the true inhibitor, oxypurinol (Spector 1988). Oxypurinol is also the major metabolite formed when allopurinol is adminstered to humans (Reiter et al. 1983). When it was proposed that xanthine oxidase is the major source of the reactive oxygen species generated when ischaemic tissues are reoxygenated (McCord 1985), interest naturally arose in the use of allopurinol or oxypurinol as protectors against tissue damage. Indeed, some animal experiments found that allopurinol has protective effects against cardiac, cerebral or gastrointestinal reoxygenation injury (Itoh et al. 1986; McCord 1985; Parks 1989) as well as in some animal models of haemorrhagic shock (Allan et al. 1986). It might be thought more logical to use oxypurinol as a protective agent, since it is the true inhibitor of xanthine oxidase (Spector 1988). However, allopurinol was reported to be more effective than oxypurinol in reducing urinary uric acid excretion in humans (Chalmers et al. 1968). Similarly B103U, an analogue of oxypurinol in which the oxygen at position 6 is replaced by sulphur, did not inhibit the enzyme any better *in vivo* than did oxypurinol (Spector et al. 1989), although it was a more powerful inhibitor *in vitro*.

Since allopurinol is widely used to treat hyperuricaemia in humans (Chalmers et al. 1968), its toxicological profile is well known and it might therefore seem to be the agent of choice in testing for beneficial effects in diseases to which ischaemia/reperfusion is thought to contribute: it has always seemed to the author that preventing the formation of excess reactive oxygen species is more likely to be effective than attempting to scavenge them once they have been formed. Unfortunately the evidence that xanthine oxidase makes any significant contribution to the generation of reactive oxygen species in human heart and brain is very weak (section 3.2; Wajner & Harkness 1989; Werns & Lucchesi 1990). In addition, many studies in which protection has been shown have required pretreatment of animals with the drug, which is not usually possible in clinical cases of human ischaemia/reperfusion. However, xanthine oxidase activity has been detected in human gut, in the palmar fascia of patients with Dupuytren's contracture (Murrell et al. 1987), in the circulation of patients after reperfusion of a tourniqued limb (Friedl et al. 1990) and in the synovia of patients with rheumatoid arthritis (Merry et al. 1989). The therapeutic possibilities of treatment with allopurinol in these diseases have been discussed, but not yet evaluated clinically to date. Allopurinol and oxypurinol could also be beneficial in enhanced preservation of organs for transplantation (Chambers et al. 1987). Salim (1990) has recently reported that allopurinol was as effective as cimetidine in preventing the recurrence of duodenal ulcer in humans.

As well as inhibiting xanthine oxidase, oxypurinol can also scavenge HOCl (Grootveld et al. 1987), which might contribute to its protective effects under certain circumstances. *In vitro,* both allopurinol and oxypurinol can react with $\cdot OH$, but the concentrations achieved *in vivo* are probably too low for this to be a significant mechanism of protection (discussed by Halliwell 1990). An additional mechanism by which allopurinol or oxy-

purinol could protect tissues is by preventing oxidation of hypoxanthine, so enhancing its salvage for reincorporation into adenine nucleotides when the tissue is reoxygenated (Lasley et al. 1988).

Other inhibitors of xanthine oxidase that have been described in the literature include lodoxamide (White 1981), pterin-6-aldehyde (Nishino & Tsushima 1986) and amflutizole (Werns & Lucceshi 1990).

4.2.4 Inhibitors of the Generation of Reactive Oxygen Species by Phagocytes

Generation of reactive oxygen species by activated phagocytes may contribute to inflammatory and reoxygenation injury (section 3.2). Thus, it is possible that inhibitors of phagocyte recruitment, adherence to endothelium, or activation of the respiratory burst oxidase complex could be therapeutically useful, as has already been discussed in the case of adenosine (section 4.1.5). Several anti-inflammatory drugs have been suggested to suppress $O_2^{\cdot -}$ formation by phagocytes, but convincing evidence for this mechanism of action *in vivo* has been obtained only in a few cases (discussed by Halliwell et al. 1988a). Cross (1990) has reviewed the compounds that have been shown to inhibit phagocyte $O_2^{\cdot -}$ generation *in vitro:* none of them appears suitable for therapeutic use as yet. Hart et al. (1990) reported that apocynin, a phenolic compound of plant origin, is oxidised to a toxic product by phagocytes containing peroxidase (such as neutrophils, which possess myeloperoxidase) and thus exerted an anti-inflammatory effect in rats. Only activated phagocytes (producing the H_2O_2 necessary for peroxidase action) would be affected.

4.2.5 Chelating Agents: Deferoxamine

Chelating agents have been successfully used in the treatment of metal ion poisoning for many years, e.g. dimercaprol to treat arsenic poisoning, penicillamine and trientine to treat Wilson's disease and diethylenetriaminepentaacetic acid to remove lead or plutonium (Jones 1991; Jones & Pratt 1976). Graf and Eaton (1990) have pointed out that phytic acid (inositol hexaphosphate), an insoluble constituent of the human diet, is also a powerful chelator of iron ions that suppresses iron-dependent free radical reactions. Based on this observation, the potential therapeutic use of inositol 1,2,6-triphosphate in human disease is being evaluated (Claxson et al. 1990).

The only reasonably specific iron ion chelator approved for routine clinical use in humans is deferoxamine (sometimes called desferrioxamine: commercial preparations of the drug are usually deferoxamine B methanesulphonate). Deferoxamine, which is produced by *Streptomyces pilosus,* is widely and effectively used for the prevention and treatment of iron overload in patients who have ingested toxic oral doses of iron salts, or who require multiple blood transfusions, e.g. for the treatment of thalassaemia (Modell et al. 1982). Deferoxamine also binds Al^{+++} ions, although with a stability constant 6 orders of magnitude less than for its binding of Fe^{+++} ions, and has been used to decrease body aluminium loads in renal dialysis patients (McCarthy et al. 1990). It has recently been claimed that deferoxamine has beneficial effects in the treatment of patients with Alzheimer's disease (McLachlan et al. 1991).

Deferoxamine is a powerful chelator of Fe^{+++}. In the presence of physiological buffer systems, deferoxamine inhibits iron ion–dependent lipid peroxidation and the generation of highly-reactive oxidising species such as $^{\cdot}OH$ from $O_2^{\cdot -}$ and H_2O_2 in the presence of iron ions (reviewed by Halliwell 1989d). Large doses of deferoxamine can be injected into animals or humans: at least 50 mg/kg bodyweight per day appears fairly safe in thalassaemic patients (Davis et al. 1983) provided that they do not become iron-deficient (Porter et al. 1989a). Hence, deferoxamine has been proposed for use as a 'probe' to study the importance of iron-dependent free radical reactions as mediators of tissue injury (Gutteridge et al. 1979). Indeed, deferoxamine has been used in this way in many animal model systems (reviewed by Halliwell 1989d). It is generally observed to be protective, probably by its iron-chelating actions in most cases, although it has also been shown to interfere with the uptake of paraquat by isolated lung cells (van der Wal et al. 1990), which partially explains its pro-

tective effect against cytotoxicity of this herbicide. Deferoxamine can also react directly with several reactive oxygen species, including $RO_2^{\bullet}$, $O_2^{\cdot -}$, $^{\bullet}OH$, HOCl, oxidants produced by mixing haem proteins with H_2O_2, and peroxynitrite formed by reaction of NO with $O_2^{\cdot -}$. Most of these reactions are of little or no significance *in vivo*, simply because the concentration of deferoxamine achieved during normal therapeutic use is too low for scavenging of reactive oxygen species to be a feasible mechanism of action (Halliwell 1989d). However, the ability of deferoxamine to scavenge peroxynitrite has been suggested to account for its ability to protect against reoxygenation injury in some myocardial systems (Beckman et al. 1990), a proposal which merits further investigation. It has also been shown (at high concentrations) to block the formation of xanthine oxidase activity in cultured bovine pulmonary endothelial cells (Rinaldo & Gorry 1990). Deferoxamine is very poor at removing iron ions bound to transferrin or lactoferrin, which may be advantageous since iron bound to these proteins does not stimulate free radical reactions (Aruoma & Halliwell 1987; Gutteridge et al. 1981).

Deferoxamine, at therapeutically relevant concentrations of 20 to 100 μmol/L, can inhibit cell proliferation *in vitro*, and *in vivo* (Estrov et al. 1988). It may do so by depriving the cells of iron and inhibiting the enzyme ribonucleoside diphosphate reductase, but there is considerable debate as to whether or not this is the only cytostatic mechanism. Thus, when deferoxamine is administered by continuous infusion over several days, as in some studies of its effect upon chronic inflammation or autoimmune disease in animals, it is possible (but remains to be proved) that inhibition of the proliferation of inflammatory cells such as lymphocytes could contribute to the protective effects observed. The cytostatic action of deferoxamine or other iron ion–chelating agents might conceivably be of therapeutic use in suppressing lympocyte-mediated reactions (Bierer & Nathan 1990; Bradley et al. 1986), e.g. graft rejection or the replication of HIV (Baruchel et al. 1991; Tabor et al. 1991).

Because iron is essential for normal cell function, prolonged administration of any powerful iron-chelating agent to humans (other than patients suffering from iron overload) will probably cause serious side effects. Hence, proposals to use long term deferoxamine administration in the treatment of chronic disease in humans, such as Alzheimer's disease or rheumatoid arthritis, must be approached with caution, as must prolonged treatment of aluminium-overloaded patients with this drug (Blake et al. 1985; McCarthy et al. 1990). However, the side effects of chronic deferoxamine administration should not preclude its acute use, e.g. in combination with thrombolytic agents in the treatment of myocardial reperfusion injury, in haemorrhagic shock (Sanan et al. 1989) or in ameliorating cell damage by toxins such as the anthracycline antitumour antibiotics (Halliwell et al. 1988b). Deferoxamine might be especially useful in myocardial reperfusion systems (Menasche et al. 1990). For example, it can sometimes protect against myocardial stunning much more effectively than does SOD (Bolli et al. 1990). Thus, if clinical trials (section 4.1.1) show that recombinant human SOD has a beneficial effect when combined with thrombolytic agents in the treatment of recent myocardial infarction, then the possibility of using deferoxamine as a much cheaper alternative is well worth exploring. Deferoxamine might also have applications in the preservation of organs for transplantation (Gower et al. 1989b; Yoon et al. 1989). It is interesting to note that, despite the putative iron ion dependency of the lung damage induced by the anticancer drug bleomycin in animals (section 4.1.8) neither deferoxamine nor other iron chelators so far tested appear to offer any protective effects (Tryka 1989; Ward et al. 1988).

Hallaway et al. (1989) have described high-molecular-mass derivatives of deferoxamine, made by attaching it to polymers such as dextran or hydroxyethyl starch. Conjugation to these polymers was reported not to diminish Fe^{+++}-binding activity, but to markedly raise the LD_{50} and the circulating plasma lifetime in animals. Whether these high-molecular-mass forms of deferoxamine will be therapeutically useful remains to be seen.

4.2.6 Other Iron Chelators: Hydroxypyridones

A major problem with deferoxamine is that it is not absorbed through the gut and has to be administered to thalassaemic patients by intravenous or subcutaneous infusion, leading to poor patient compliance. The drug is also expensive. There has therefore been a search for orally active chelating agents that are, hopefully, cheaper (Jones 1982). Some work has been reported with pyridoxal isonicotinoyl hydrazone (discussed by Hershko 1988), deferrithiocin (Biener & Nathan 1990; Wolfe 1990) and 2,3-dihydroxybenzoate (Grady et al. 1978), but most attention is currently concentrated on the 3-hydroxypyrid-4-ones. For example, 1,2-dimethyl-3-hydroxypyridin-4-one (LI) has undergone preliminary tests in humans. It appears to be more toxic than deferoxamine (Editorial 1989b), and other hydroxypyridones (such as the 1,2-diethyl compound) are now undergoing evaluation (Porter et al. 1989b,c, 1990; Dobbin & Hider 1990; Hershko et al. 1991).

The increased toxicity of hydroxypyridones, when compared with deferoxamine, might be due to features of their chemistry (Halliwell & Grootveld 1987). Three moles of chelator are needed to complex 1 Fe^{+++} ion completely, whereas deferoxamine chelates Fe^{+++} at a 1 : 1 molar ratio: the deferoxamine molecule 'wraps around' the iron ion, encasing it in an envelope of organic material. Upon dilution, 3 : 1 hydroxypyridone : iron ion complexes tend to dissociate into 2 : 1 and 1 : 1 complexes, leaving available coordination sites on the iron that could allow the complexes to catalyse free radical reactions. Indeed, Kontoghiorghes et al. (1986) found that 3 : 1 chelator-iron complexes did not stimulate iron-dependent free radical reactions, whereas 2 : 1 or 1 : 1 complexes seemed able to do so. In addition, hydroxypyridones can mobilise iron ions from transferrin (Kontoghiorghes 1986a) or lactoferrin (Kontoghiorghes 1986b). Thus, one can envisage a scenario in which iron ions safely protein-bound (i.e. unable to stimulate free radical reactions) are mobilised and transferrred to other sites in the body at which damage can be caused (Halliwell & Grootveld 1987). Caution therefore should be employed in proposing long term administration of these compounds to humans, especially those not suffering from iron overload, without close medical supervision (Hershko 1988; Nathan & Piomelli 1990).

One aspect of the use of iron-chelating drugs that deserves further attention is their antimalarial action. Deferoxamine (Hershko & Peto 1988; Pollack et al. 1987) inhibits the growth of malarial parasites in culture and in infected animals. Hydroxypyridones are also effective against parasites *in vitro* (Heppner et al. 1988; Hershko et al. 1991). Similar suggestions have been made for use of chelators against trypanosomal infections (Shapiro et al. 1982). Clark et al. (1986) have proposed that reactive oxygen species are involved in the pathology of malarial infections, and various antioxidants have been found to be beneficial in malaria-infected animals (e.g. Thumwood et al. 1989). By contrast, several antiparasitic drugs have been claimed to exert their action by increased generation of reactive oxygen species *in vivo* (reviewed by Docampo 1990).

4.2.7 Other Iron Chelators: ICRF-187 and the 'Lazaroids'

Another compound whose action *in vivo* may relate to iron ion chelation is ICRF-187, which was originally synthesised as an anti-tumour agent and extensively tested in animals and humans, but with disappointing results. ICRF-187 is hydrolysed *in vivo* to produce a metal ion chelator structurally similar to edetic acid (EDTA). ICRF-187 has been reported to ameliorate the cardiotoxicity of anthracyclines in human breast cancer patients, while not affecting the therapeutic efficiency of these drugs (Speyer et al. 1988). It has therefore been suggested (Halliwell et al. 1988b; Halliwell & Bomford 1989) that careful use of low concentrations of chelating agents may help to minimise anthracycline-dependent cardiotoxicity in other cancers, particularly the leukaemias, in which chemotherapy can cause transient iron overload (section 3.3). ICRF-187 is the (+)-isomer of the racemic mixture first described as ICRF-159 [1,2-*bis*-(3,5-dioxopiperazine-1-yl)propane]. ICRF-187 is preferred for use because of its increased solubility in water. It

has also been reported to protect against the diabetogenic action of alloxan in mice, which is thought to be due to increased free radical formation in the pancreatic islets (El-Hage et al. 1981).

Since iron-dependent free radical reactions appear to make significant contributions to injury to the CNS after stroke or trauma (section 3.3), several antioxidants (e.g. U74006F) have been developed that can cross the blood-brain barrier (Hall & Braughler 1989). These compounds are 21-aminosteroids ('lazaroids') without glucocorticoid activity and they are powerful inhibitors of iron ion-dependent lipid peroxidation *in vitro,* although whether they act *in vivo* as iron ion chelators and/or as chain-breaking antioxidants is unclear as yet. They have been reported to diminish brain or spinal cord injury resulting from stroke or trauma in animal experiments (Hall & Braughler 1989; Jacobsen et al. 1990; Steinke et al. 1989). Results of clinical trials in humans are awaited with interest.

4.2.8 Probucol and Other Chain-Breaking Antioxidants

Several phenolic chain-breaking antioxidants have been proposed for use in humans. They include MK-447 (2-aminomethyl-4-*tert*-butyl-6-iodophenol), ONO-3144 (2-aminomethyl-4-*tert*-butyl-6-propionylphenol) and R-830 (2,6-di-*tert*-butyl-4-[2′-thenoyl]phenol). All have shown anti-inflammatory effects in animal models of acute inflammation (Swingle et al. 1987). R-830 is reported to be in early clinical trials as a topical preparation for skin inflammation, but little information on human use of the other compounds has appeared. It must be remembered that chain-breaking antioxidants can often show pro-oxidant properties towards nonlipid biomolecules (section 2.2), that any anti-inflammatory action they have could be related to interference with the synthesis of prostaglandins and/or leukotrienes (e.g. by inhibiting lipoxygenase and/or cyclooxygenase) rather than by inhibiting lipid peroxidation, and that lipid peroxidation is often not a major mechanism of cell injury by oxidative stress (section 2.1). These reasons may account for the observation that the anti-inflammatory activity of commercially available phenolic antioxidants has been described as 'not impressive' (Swingle et al. 1987).

Many flavonoids and other phenols of plant origin have been reported to have anti-inflammatory effects in some animal model systems (Lewis 1989; Villar et al. 1984) and to inhibit lipid peroxidation *in vitro* (reviewed by Larson 1988), but similar cautions apply (e.g. Donatus et al. 1990; Hodnick et al. 1986; Laughton et al. 1989). Flavonoids are often powerful inhibitors of lipoxygenase and cyclooxygenase (e.g. Moroney et al. 1988) and they exert a wide range of other biochemical effects, so that it is usually difficult to attribute any biological actions that they have to antioxidant activity. Several plant phenolics can be oxidised by myeloperoxidase to give toxic products (Simons et al. 1990), which may explain some of their reported effects in suppressing $O_2^{\cdot-}$ generation by activated neutrophils (Hart et al. 1990). Some phenols can also exert pro-oxidant effects *in vitro* (Laughton et al. 1989).

Interest in the role of lipid peroxidation in atherosclerosis (section 3.2) was raised when it was found that the phenolic compound probucol (4,4′-[isopropylidenedithio]*bis* [2,6-di-*tert*-butylphenol]) has a marked antiatherogenic effect in hyperlipidaemic rabbits (Carew et al. 1987). Probucol was introduced as an agent that lowers blood cholesterol concentrations, but it is also a powerful chain-breaking inhibitor of lipid peroxidation (Parthasarathy et al. 1986) that does not appear to have pro-oxidant activity to other biomolecules (Aruoma et al. 1990). The potential therapeutic use of probucol to diminish the development of atherosclerotic lesions in coronary arteries after bypass surgery is currently being evaluated. There is no direct proof as yet that any antiatherogenic effect of probucol is actually related to an inhibition of lipid peroxidation and not to other effects on monocytes and macrophages within atherosclerotic lesions.

5. Antioxidant Properties of Drugs Developed for Other Purposes

Many drugs already used in humans have been discovered to be capable of acting as antioxidants *in vitro* in some assay systems. They include non-

steroidal anti-inflammatory drugs (discussed by Halliwell et al. 1988a), the angiotensin converting enzyme inhibitor captopril (Bagchi et al. 1989), the sulphasalazine metabolite 5-aminosalicylate (Aruoma et al. 1987) and several compounds acting as Ca^{++} antagonists (e.g. Janero et al. 1988). However, very few drugs are present *in vivo* at concentrations that would allow them to compete with biological molecules for reaction with •OH or HOCl (table I): possible exceptions include salicylate, penicillamine and gold thiols as used in the treatment of rheumatoid arthritis (reviewed by Halliwell et al. 1988a) and sulfasalazine or aminosalicylates as used in the treatment of inflammatory bowel disease (Ahnfelt-Ronne et al. 1990). For drugs that can act as radical scavengers *in vivo*, it is important to ensure that toxic drug-derived radicals are not produced during the reaction. For example, sulphur-containing radicals derived from penicillamine are capable of inactivating α_1-antiproteinase, the major inhibitor of serine proteases in human extracellular fluids (Aruoma et al. 1989c).

6. Conclusion

Tissue injury mechanisms in human disease are very complex. Taking ischaemia/reperfusion injury as an example, the extent of protection by antioxidants in different animal model systems can vary enormously. Just by slight changes in experimental conditions, a mechanism of tissue injury that is largely free radical–dependent can be easily changed to something that is scarcely affected by antioxidants. For example, patients with myocardial infarction are all different. They have had different periods of ischaemia. Some of them may have been treated with thrombolytic agents, some not, and so on. Thus, calcium blockers or free radical scavengers alone may not afford satisfactory protection against ischaemia/reperfusion after thrombolysis. To overcome this we might attempt to protect against all potential mechanisms of cell injury simultaneously. Polypharmacy is not encouraged, but in fact quite often a single drug may act by multiple mechanisms. Many of the drugs in clinical use may well be doing several different things.

Drugs with multiple mechanisms of protective action, including antioxidant properties, may be one way forward to minimising tissue injury in human disease. Certainly, the potential therapeutic effects of *N*-acetylcysteine in HIV-I infection (Halliwell & Cross 1991), probucol in atherosclerosis, ebselen in chronic inflammation and iron chelators in Alzheimer's disease, leukaemia, organ preservation and malaria merit urgent evaluation. Probably less attention should be paid to SOD and vitamin E, which have been therapeutically disappointing (except for vitamin E in ameliorating the consequences of prematurity and fat malabsorption syndromes).

Acknowledgements

The author thanks Dr JMC Gutteridge for his helpful comments. Research funding from the British Heart Foundation and the Arthritis and Rheumatism Council (UK) is gratefully acknowledged.

References

Ahnfelt-Ronne I, Nielsen OH, Christensen A, Longholz E, Binder V, et al. Clinical evidence supporting the radical scavenger mechanism of 5-aminosalicylic acid. Gastroenterology 98: 1162-1169, 1990

Aitken RJ. The role of free oxygen radicals and sperm function. International Journal of Andrology 12: 95-97, 1989

Akaike T, Ando M, Oda T, Doi T, Ijiri S, et al. Dependence on O_2^- generation by xanthine oxidase of pathogenesis of influenza virus infection in mice. Journal of Clinical Investigation 85: 739-745, 1990

Albano E, Bellomo G, Paiola M, Carini R, Dianzani MU. Stimulation of lipid peroxidation increases the intracellular calcium content of isolated hepatocytes. Biochimica et Biophysica Acta 1091: 310-316, 1991

Allan G, Cambridge D, Lee-Tsang-Tan L, Van Way CW, Whiting MV. The protective action of allopurinol in an experimental model of haemorrhagic shock and reperfusion. British Journal of Pharmacology 89: 149-155, 1986

Ambrosio G, Becker LC, Hutchins GM, Weisman HF, Weisfeldt ML. Reduction in experimental infarct size by recombinant human superoxide dismutase: insights into the pathophysiology of reperfusion injury. Circulation 74: 1424-1433, 1986

Ames BN, Cathcart R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant- and radical-causing aging and cancer: a hypothesis. Proceedings of the National Academy of Sciences of the USA 78: 6858-6862, 1981

Anderson ME, Meister A. Glutathione monoesters. Analytical Biochemistry 183: 16-20, 1989a

Anderson ME, Meister A. Marked increase of cysteine levels in

many regions of the brain after administration of 2-oxothiazolidine-4-carboxylate. FASEB Journal 3: 1632-1636, 1989b
Arroyo CM, Carmichael AJ, Bouscarel B, Liang JH, Weglicki WB. Endothelial cells as a source of oxygen free radicals: an ESR study. Free Radical Research Communications 9: 287-296, 1990
Artuso T, Bernadon J, Meunier B, Paillous N. DNA strand breaks photosensitized by benoxaprofen and other non-steroidal anti-inflammatory agents. Biochemical Pharmacology 39: 407-413, 1990
Aruoma CI, Akanmu D, Cecchini R, Halliwell B. Evaluation of the ability of the angiotensin-converting enzyme inhibitor captopril to scavenge reactive oxygen species. Chemico-Biological Interactions 77: 363-314, 1991b
Aruoma OI, Halliwell B. Superoxide-dependent and ascorbate-dependent formation of hydroxyl radicals from hydrogen peroxide in the presence of iron. Biochemical Journal 241: 273-278, 1987
Aruoma OI, Wasil M, Halliwell B, Hoey BM, Butler J. The scavenging of oxidants by sulphasalazine and its metabolites. Biochemical Pharmacology 36: 3739-3742, 1987
Aruoma OI, Halliwell B, Dizdaroglu M. Iron ion-dependent modification of bases in DNA by the superoxide radical-generating system hypoxanthine/xanthine oxidase. Journal of Biological Chemistry 264: 13024-13028, 1989a
Aruoma OI, Halliwell B, Laughton MJ, Quinlan GJ, Gutteridge JMC. The mechanism of initiation of lipid peroxidation. Biochemical Journal 258: 617-620, 1989b
Aruoma OI, Halliwell B, Butler J, Hoey BM. Apparent inactivation of α_1-antiproteinase by sulphur-containing radicals derived from penicillamine. Biochemical Pharmacology 38: 4353-4357, 1989c
Aruoma OI, Evans PJ, Kaur H, Sutcliffe L, Halliwell B. An evaluation of the antioxidant and potential pro-oxidant properties of food additives and of Trolox C, vitamin E and probucol. Free Radical Research Communications 10: 143-157, 1990
Aruoma OI, Halliwell B, Gajewski E, Dizdaroglu M. Copper-ion-dependent damage to the bases in DNA in the presence of hydrogen peroxide. Biochemical Journal, in press, 1991a
Ash D, Brown SB. Photodynamic therapy-achievements and prospects. British Journal of Cancer 60: 151-152, 1989
Baader WJ, Hatzelmann A, Ullrich V. The suppression of granulocyte functions by lipophilic antioxidants. Biochemical Pharmacology 37: 1089-1098, 1988
Bagchi D, Iyengar J, Stockwell P, Das DK. Enhanced prostaglandin production in the ischemic-reperfused myocardium by captopril linked with its free radical scavenging action. Prostaglandins, Leukotrienes and Essential Fatty Acids 38: 145-150, 1989
Ball RY, Carpenter KLH, Mitchinson MJ. What is the significance of ceroid in human atherosclerosis? Archives of Pathology and Laboratory Medicine 111: 1134-1140, 1987
Baruchel S, Gao Q, Wainberg MA. Desferrioxamine and HIV. Lancet 337: 1356, 1991
Beck Y, Bartfield D, Yavin Z, Levanon A, Gorecki M, et al. Efficient production of active human manganese superoxide dismutase in *Escherichia coli*. Biotechnology 6: 930-935, 1988
Beckman JS. Ischaemic injury mediator. Nature 345: 27-28, 1990
Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proceedings of the National Academy of Sciences of the USA 87: 1620-1624, 1990
Bell D, Jackson M, Nicoll JJ, Millar A, Dawes J, et al. Inflammatory response, neutrophil activation, and free radical production after acute myocardial infarction: effect of thrombolytic treatment. British Heart Journal 63: 82-87, 1990
Bendich A, Olson JA. Biological actions of carotenoids. FASEB Journal 3: 1927-1932, 1989
Bendich A, Machlin LJ, Scandurra O, Burton GW, Wayner DDM. The antioxidant role of vitamin C. Advances in Free Radical Biology and Medicine 2: 419-444, 1986
Bendich A, Machlin LJ. Safety of oral intake of vitamin E. American Journal of Clinical Nutrition 48: 612-619, 1988
Beyer Jr WF, Fridovich I. Characterization of a superoxide dismutase mimic prepared from desferrioxamine and MnO_2. Archives of Biochemistry and Biophysics 271: 149-156, 1989
Biemond P, Van Eijk HG, Swaak AJG, Koster JF. Iron mobilization from ferritin by superoxide derived from stimulated polymorphonuclear leukocytes: possible mechanism in inflammation diseases. Journal of Clinical Investigation 73: 1576-1579, 1984
Bieri JG, Corash L, Van Hubbard S. Medical uses of vitamin E. New England Journal of Medicine 308: 1063-1071, 1983
Bierer BE, Nathan DG. The effect of desferrithiocin, an oral iron chelator, on T-cell function. Blood 76: 2052-2059, 1990
Birgens HS. The biological significance of lactoferrin in haematology. Scandinavian Journal of Haematology 33: 225-230, 1984
Blake DR, Winyard P, Lunec J, Williams A, Good P, et al. Cerebral and ocular toxicity induced by desferrioxamine. Quarterly Journal of Medicine 219: 345-355, 1985
Bolann BJ, Ulvik RJ. On the limited ability of superoxide to release iron from ferritin. European Journal of Biochemistry 193: 899-904, 1990
Bolli R. Oxygen-derived free radicals and postischemic myocardial dysfunction ('stunned myocardium'). Journal of the American College of Cardiology 12: 239-249, 1988
Bolli R. Superoxide dismutase ten years later: a drug in search of a use. Journal of the American College of Cardiology 18: 231-233, 1991
Bolli R, Jerudi MO, Patel BS, Aruoma OI, Halliwell B, et al. Marked reduction of free radical generation and contractile dysfunction by antioxidant therapy begun at the time of reperfusion. Circulation Research 65: 607-622, 1989
Bolli R, Patel BS, Jeroudi MO, Li XY, Triana JF, et al. Iron-mediated radical reactions upon reperfusion contribute to myocardial stunning. American Journal of Physiology 259: H1901-H1911, 1990
Bonomi F, Cerioli A, Pagani S. Molecular aspects of the removal of ferritin-bound iron by DL-dihydrolipoate. Biochimica et Biophysica Acta 994: 180-186, 1989
Boudjema K, Van Gulik TM, Lindell SL, Vieugdenhil PS, Southard JH, et al. Effect of oxidized and reduced glutathione in liver preservation. Transplantation 50: 948-951, 1990
Bradley B, Prowse SJ, Bauling P, Lafferty EJ. Desferrioxamine treatment prevents chronic islet allograft damage. Diabetes 35: 550-555, 1986
Breimer LH. Ionizing radiation-induced mutagenesis. British Journal of Cancer 57: 6-18, 1988
Bruckdorfer KR, Jacobs M, Rice-Evans C. Endothelium-derived relaxing factor (nitric oxide) lipoprotein oxidation and atherosclerosis. Biochemical Society Transactions 18: 1061-1063, 1990
Buhl R, Jaffe HA, Holroyd KJ, Wells FB, Mastrangeli A, et al. Systemic glutathione deficiency in symptom-free HIV-seropositive patients. Lancet 2: 1294-1296, 1989
Buhl R, Vogelmeier C, Critenden M, Hubbard RC, Hoyt Jr RF, et al. Augmentation of glutathione in the fluid lining the epithelium of the lower respiratory tract by directly administering glutathione aerosol. Proceedings of the National Academy of Sciences of the USA 87: 4063-4067, 1990
Burdon RH, Rice-Evans CRE. Free radicals and the regulation of mammalian cell proliferation. Free Radical Research Communications 6: 345-358, 1989
Burton GW, Wronska U, Stone L, Foster DO, Ingold KU. Biokinetics of dietary RRR-α-tocopherol in the male guinea pig at three dietary levels of vitamin C and two levels of vitamin E:

evidence that vitamin C does not "spare" vitamin E *in vivo*. Lipids 25: 199-210, 1990
Cameron E, Campbell A. The orthomolecular treatment of cancer. Chemico-Biological Interactions 9: 285-315, 1974
Cantin AM, North SL, Hubbard RC, Crystal RG. Normal alveolar epithelial lining fluid contains high levels of glutathione. Journal of Applied Physiology 63: 152-157, 1987
Carden DL, Smith JK, Zimmerman BJ, Korthuis RJ, Granger DN. Reperfusion injury following circulatory collapse: the role of reactive oxygen metabolites. Journal of Critical Care 4: 294-307, 1989
Carew TE, Schwenke DC, Steinberg D. Antiatherogenic effect of probucol unrelated to its hypocholesterolaemic effect: evidence that antioxidants *in vivo* can selectively inhibit LDL degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. Proceedings of the National Academy of Sciences of the USA 84: 7725-7729, 1987
Carini R, Poli G, Dianzani MU, Maddix SP, Slater TF, et al. Comparative evaluation of the antioxidant properties of α-tocopherol, α-tocopherol polyethylene glycol 1000 succinate and α-tocopherol succinate in isolated hepatocytes and liver microsomal suspensions. Biochemical Pharmacology 39: 1597-1601, 1990
Chalmers RA, Kromer H, Scott JT, Walt RWE. A comparative study of the xanthine oxidase inhibitors allopurinol and oxypurinol in man. Clinical Science 35: 353-362, 1968
Chambers DJ, Braimbridge MV, Hearse DJ. Free radicals and cardioplegia: allopurinol and oxypurinol reduce myocardial injury following ischemic arrest. Annals of Thoracic Surgery 44: 291-297, 1987
Chiswick M, Gladman G, Sinha S, Toner N, Davies J. Vitamin E supplementation and periventricular hemorrhage in the newborn. American Journal of Clinical Nutrition 53: 370S-372S, 1991
Chow CK, Thacker RR, Changchit C, Bridges RB, Rehm SR, et al. Lower levels of vitamin C and carotenes in plasma of cigarette smokers. Journal of the American College of Nutrition 5: 305-312, 1986
Clark IA, Hunt WH, Cowden WB. Oxygen-derived free radicals in the pathogenesis of parasitic disease. Advances in Parasitology 25: 1-44, 1986
Claxson A, Morris C, Blake D, Siren M, Halliwell B, et al. The anti-inflammatory effects of D-myo-inositol-1,2,6-triphosphate (PP56) on animal models of inflammation. Agents and Actions 29: 67-70, 1990
Cohen MV. Free radicals in ischemic and reperfusion myocardial injury: is this the time for clinical trials? Annals of Internal Medicine 111: 918-931, 1989
Corash L, Spielberg S, Bartsocas C, Boxer L, Steinherz R, et al. Reduced chronic hemolysis during high-dose vitamin E administration in Mediterranean-type glucose-6-phosphate dehydrogenase deficiency. New England Journal of Medicine 303: 416-420, 1980
Cotgreave IA, Duddy SK, Kass GEN, Thompson D, Moldeus P. Studies on the anti-inflammatory activity of Ebselen. Biochemical Pharmacology 38: 649-656, 1989
Cronstein BN, Daguma L, Nichols D, Hutchison AJ, Williams M. The adenosine/neutrophil paradox resolved: human neutrophils possess both A_1 and A_2 receptors that promote chemotaxis and inhibit O_2^- generation, respectively. Journal of Clinical Investigation 85: 1150-1157, 1990
Cross AR. Inhibitors of the leukocyte superoxide generating oxidase. Free Radical Biology and Medicine 8: 71-93, 1990
Cross CE, Forte T, Stocker R, Louie S, Yamamoto Y, et al. Oxidative stress and abnormal cholesterol metabolism in patients with adult respiratory distress syndrome. Journal of Laboratory and Clinical Medicine 115: 396-404, 1990
Curnutte JT, Babior BM. Chronic granulomatous disease. Advances in Human Genetics 16: 229-245, 1987
Currin RT, Toole JG, Thurman RG, Lemasters JJ. Evidence that Carolina rinse solution protects sinusoidal endothelial cells against reperfusion injury after cold ischemic storage of rat liver. Transplantation 50: 1076-1078, 1990
Curzio M. Interaction between neutrophils and 4-hydroxyalkenals and consequences on neutrophil mobility. Free Radical Research Communications 5: 55-66, 1988
Davies MJ. Detection of myoglobin-derived radicals on reaction of metmyoglobin with hydrogen peroxide and other peroxidic compounds. Free Radical Research Communications 10: 361-370, 1990
Davis SC, Marcus RE, Hungerford JL, Miller MH, Arden GB, et al. Ocular toxicity of high-dose intravenous desferrioxamine. Lancet 1: 181-184, 1983
Davison A, Tibbits G, Shi Z, Moon J. Active oxygen in neuromuscular disorders. Molecular and Cellular Biochemistry 84: 199-216, 1988
Dean RT, Thomas SM, Garner A. Free radical-mediated fragmentation of monoamine oxidase in the mitochondrial membrane: role of lipid radicals. Biochemical Journal 240: 489-494, 1986
Del Nido PJ, Mickle DAG, Wilson GJ, Benson LN, Coles JG, et al. Evidence of myocardial free radical injury during elective repair of tetralogy of Fallot. Circulation 76 (Suppl. V): V-174-V-179, 1987
Diplock AT. Fat-soluble vitamins, their biochemistry and applications, pp. 154-224, Heinemann, London, 1985
Dobbin PS, Hider RC. Iron chelation therapy. Chemistry in Britain 565-568, 1990
Docampo R. Sensitivity of parasites to free radical damage by antiparasitic drugs. Chemico-Biological Interactions 73: 1-27, 1990
Donatus IA, Sardjoko, Vermeulen NPE. Cytotoxic and cytoprotective activities of curcumin. Biochemical Pharmacology 39: 1869-1875, 1990
Downey JM, Omar B, Ooiwa H, McCord J. Superoxide dismutase therapy for myocardial ischaemia. Free Radical Research Communications 12: 703-720, 1991
Editorial. Reperfusion injury after thrombolytic therapy for acute myocardial infarction. Lancet 2: 655-657, 1989a
Editorial. Oral iron chelators. Lancet 2: 1016-1017, 1989b
Ehrenkranz RA. Vitamin E and retinopathy of prematurity: still controversial. Journal of Pediatrics 114: 801-803, 1989
El-Hage AH, Herman EH, Ferrans VJ. Reduction in the diabetogenic effect of alloxan in mice by treatment with the antineoplastic agent ICRF-187. Research Communications in Chemical Pathology and Pharmacology 33: 509-523, 1981
Engle WA, Yoder MC, Baurley JL, Yu PL. Vitamin E decreases superoxide anion production by polymorphonuclear leukocytes. Pediatric Research 23: 245-248, 1988
Engler R. Consequences of activation and adenosine-mediated inhibition of granulocytes during myocardial ischemia. Federation Proceedings 46: 2407-2412, 1987
Engler R, Gilpin E. Can superoxide dismutase alter myocardial infarct size? Circulation 79: 1137-1142, 1989
Epstein JH. Chemical phototoxicity in humans. Journal of the National Cancer Institute 69: 265-268, 1982
Esterbauer H, Zollner H, Schaur RJ. Hydroxyalkenals: cytotoxic products of lipid peroxidation. ISI Atlas of Science, Biochemistry 1: 311-315, 1988
Esterbauer H, Striegl G, Puhl H, Rotheneder M. Continuous monitoring of *in vitro* oxidation of human low density lipoprotein. Free Radical Research Communications 6: 67-75, 1989
Estrov Z, Cohen A, Gelfand EW, Freedman MH. *In vitro* cytotoxicity of deferoxamine on human bone marrow haematopoietic progenitors. Toxicology In Vitro 2: 131-134, 1988

Fariss MW. Oxygen toxicity: unique cytoprotective properties of vitamin E succinate in hepatocytes. Free Radical Biology and Medicine 9: 333-343, 1990

Fariss MW, Merson MH, O'Hara TM. α-Tocopheryl succinate protects hepatocytes from chemical-induced toxicity under physiological calcium conditions. Toxicology Letters 47: 61-75, 1989

Fischer H, Terlinden R, Löhr JP, Römer A. A novel biologically active selenoorganic compound VIII: biotransformation of Ebselen. Xenobiotica 18: 1347-1359, 1988

Ferrari R, Alfieri O, Curello S, Ceconi C, Cargnoni A, et al. Occurrence of oxidative stress during reperfusion of the human heart. Circulation 81: 201-211, 1990

Flint DH, Emptage MH. A new mode of action for superoxide: inactivation of enzymes with [Fe-S] clusters. Free Radical Research Communications, in press, 1991

Flohé L. Superoxide dismutase for therapeutic use: clinical experience, dead ends and hopes. Molecular and Cellular Biochemistry 84: 123-131, 1988

Forman MB, Puett DW, Virmiani R. Endothelial and myocardial injury during ischemia and reperfusion: pathogenesis and therapeutic implications. Journal of the American College of Cardiology 13: 450-459, 1989

Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. Proceedings of the National Academy of Sciences of the USA 86: 6377-6381, 1989

Fridovich I. Superoxide radical, an endogenous toxicant. Annual Review of Pharmacology and Toxicology 23: 239-257, 1983

Friedl HP, Smith DJ, Till GO, Thomson PD, Louis DS, et al. Ischemia-reperfusion in humans. Appearance of xanthine oxidase activity. American Journal of Pathology 136: 491-495, 1990

Friedman HS, Colvin OM, Aisaka K, Popp J, Bossen EH, et al. Glutathione protects cardiac and skeletal muscle from cyclophosphamide-induced injury. Cancer Research 50: 2455-2462, 1990

Fuller BJ, Gower JD, Green CJ. Free radical damage and organ preservation: fact or fiction? Cryobiology 25: 377-393, 1988

Galaris D, Mira D, Sevanian A, Cadenas E, Hochstein P. Co-oxidation of salicylate and cholesterol during the oxidation of metmyoglobin by H_2O_2. Archives of Biochemistry and Biophysics 262: 221-231, 1988

Gey KF. The antioxidant hypothesis of cardiovascular disease: epidemiology and mechanisms. Biochemical Society Transactions 18: 1041-1045, 1990

Gey KF, Brubacher GB, Stahelin HB. Plasma levels of antioxidant vitamins in relation to ischaemic heart disease and cancer. American Journal of Clinical Nutrition 45: 1368-1377, 1987

Giri SN, Hyde DM, Schiedt MJ. Effects of repeated administration of *N*-acetyl-*L*-cysteine on sulfhydryl levels of different tissues and bleomycin-induced lung fibrosis in hamsters. Journal of Laboratory and Clinical Medicine 111: 715-724, 1988

Gower JD, Healing G, Fuller BJ, Simpkin S, Green CJ. Protection against oxidative damage in cold-stored rabbit kidneys by desferrioxamine and indomethacin. Cryobiology 26: 309-317, 1989b

Gower J, Healing G, Green C. Measurement by HPLC of desferrioxamine-available iron in rabbit kidneys to assess the effect of ischaemia on the distribution of iron within the total pool. Free Radical Research Communications 5: 291-299, 1989a

Grady RW, Graziano JH, White GP, Jacobs A, Cerami A. The development of new iron-chelating drugs II. Journal of Pharmacology and Experimental Therapeutics 205: 757-765, 1978

Graf E, Eaton JW. Antioxidant functions of phytic acid. Free Radical Biology and Medicine 8: 61-69, 1990

Greenwald RA. Superoxide dismutase and catalase as therapeutic agents for human diseases: a critical review. Free Radical Biology and Medicine 8: 201-209, 1990

Greenwald RA. Therapeutic usages of oxygen radical scavengers in human diseases: myths and realities. Free Radical Research Communications 12: 531-538, 1991

Grisham MB, Hernandez LA, Granger DN. Adenosine inhibits ischemia-reperfusion-induced leukocyte adherence and extravasation. American Journal of Physiology 257: H1334-H1339, 1989

Grootveld M, Halliwell B, Moorhouse CP. Action of uric acid, allopurinol and oxypurinol on the myeloperoxidase-derived oxidant hypochlorous acid. Free Radical Research Communications 4: 69-76, 1987

Gutteridge JMC. Iron promoters of the Fenton reaction and lipid peroxidation can be released from haemoglobin by peroxides. FEBS Letters 201: 291-295, 1986

Gutteridge JMC. Bleomycin-detectable iron in knee-joint synovial fluid from arthritic patients and its relationship to the extracellular antioxidant activities of caeruloplasmin, transferrin and lactoferrin. Biochemical Journal 245: 415-421, 1987

Gutteridge JMC, Smith A. Antioxidant protection by haemopexin of haem-stimulated lipid peroxidation. Biochemical Journal 256: 861-865, 1988

Gutteridge JMC, Stocks J. Ceruloplasmin: physiological and pathological perspectives. CRC Critical Reviews in Clinical Laboratory Science 14: 257-329, 1981

Gutteridge JMC, Xaio-Chang F. Enhancement of bleomycin-iron free radical damage to DNA by antioxidants and their inhibition of lipid peroxidation. FEBS Letters 123: 71-74, 1981

Gutteridge JMC, Richmond R, Halliwell B. Inhibition of the iron-catalyzed formation of hydroxyl radicals from superoxide and of lipid peroxidation by desferrioxamine. Biochemical Journal 184: 469-472, 1979

Gutteridge JMC, Paterson SK, Segal AW, Halliwell B. Inhibition of lipid peroxidation by the iron-binding protein lactoferrin. Biochemical Journal 199: 259-261, 1981

Gutteridge JMC, Rowley DA, Griffiths E, Halliwell B. Low-molecular-weight iron complexes and oxygen radical reactions in idiopathic haemochromatosis. Clinical Science 68: 463-467, 1985

Haenen GRMM, Vermeulen NPE, Timmerman H, Bast A. Effect of thiols on lipid peroxidation in rat liver microsomes. Chemico-Biological Interactions 71: 201-212, 1989

Hall ED, Braughler JM. Central nervous system trauma and stroke. Free Radical Biology and Medicine 6: 303-313, 1989

Hallaway PE, Eaton JW, Panter SS, Hedlund BE. Modulation of deferoxamine toxicity and clearance by covalent attachment to biocompatible polymers. Proceedings of the National Academy of Sciences of the USA 86: 10108-10112, 1989

Halliwell B. Oxidants and human disease: some new concepts. FASEB Journal 1: 358-364, 1987

Halliwell B. Albumin, an important extracellular antioxidant? Biochemical Pharmacology 37: 569-571, 1988

Halliwell B. Superoxide, iron, vascular endothelium and reperfusion injury. Free Radical Research Communications 5: 315-318, 1989a

Halliwell B. Oxidants and the central nervous system: some fundamental questions. Acta Neurologica Scandinavica 126: 23-33, 1989b

Halliwell B. Free radicals, reactive oxygen species and human disease: a critical evaluation with special reference to atherosclerosis. British Journal of Experimental Pathology 70: 737-757, 1989c

Halliwell B. Protection against tissue damage in vivo by desferrioxamine: what is its mechanism of action? Free Radical Biology and Medicine 7: 645-651, 1989d

Halliwell B. How to characterize a biological antioxidant. Free Radical Research Communications 9: 1-36, 1990

Halliwell B, Aruoma OI. DNA damage by oxygen-derived species: Its mechanism and measurement in mammalian systems. FEBS Letters 281: 9-19, 1991

Halliwell B, Bomford A. ICRF-187 and doxorubicin-induced cardiac toxicity. New England Journal of Medicine 320: 399-400, 1989

Halliwell B, Cross CE. Reactive oxygen species, antioxidants and acquired immunodeficiency syndrome. Sense or speculation? Archives of Internal Medicine 151: 29-31, 1991

Halliwell B, Grootveld M. The measurement of free radical reactions in humans. FEBS Letters 213: 9-14, 1987

Halliwell B, Gutteridge JMC. Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. Lancet 1: 1396-1398, 1984

Halliwell B, Gutteridge JMC. Oxygen radicals and the nervous system. Trends in Neuroscience 8: 22-26, 1985

Halliwell B, Gutteridge JMC. Free radicals in biology and medicine, 2nd ed., Clarendon Press, Oxford, 1989

Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: an overview. Methods in Enzymology 186: 1-86, 1990a

Halliwell B, Gutteridge JMC. The antioxidants of extracellular fluids. Archives of Biochemistry and Biophysics, 280: 1-8, 1990b

Halliwell B, Gutteridge JMC, Blake D. Metal ions and oxygen radical reactions in human inflammatory joint disease. Philosophical Transactions of the Royal Society, series B 311: 659-671, 1985

Halliwell B, Hoult JRS, Blake DR. Oxidants, inflammation and anti-inflammatory drugs. FASEB Journal 2: 501-514, 1988a

Halliwell B, Aruoma OI, Mufti G, Bomford A. Bleomycin-detectable iron in serum from leukaemic patients before and after chemotherapy. FEBS Letters 241: 202-204, 1988b

Hart BAT, Simons JM, Knaan-Shanzer S, Bakker NPM, Labadie RP. Antiarthritic activity of the newly developed neutrophil oxidative burst antagonist apocynin. Free Radical Biology and Medicine 9: 127-131, 1990

Hayashi M, Slater TF. Inhibitory effects of Ebselen on lipid peroxidation in rat liver microsomes. Free Radical Research Communications 2: 179-185, 1986

Heffner JE, Repine JE. Pulmonary strategies of antioxidant defense. American Review of Respiratory Disease 140: 531-554, 1989

Held KD. Models for thiol protection of DNA in cells. Pharmacology and Therapeutics 39: 123-131, 1988

Heppner DG, Hallaway PE, Kontoghiorghes GJ, Eaton JW. Antimalarial properties of orally active iron chelators. Blood 72: 358-361, 1988

Hermens WT, Willems GM, van der Vusse GJ. Minimal myocardial injury after uncomplicated coronary bypass surgery: various sources of overestimation. Clinica Chimica Acta 173: 243-250, 1988

Hershko C. Oral iron chelating drugs: coming but not yet ready for clinical use. British Medical Journal 296: 1081, 1988

Hershko C, Peto TEA. Deferoxamine inhibition of malaria is independent of host iron status. Journal of Experimental Medicine 168: 375-387, 1988

Hershko C, Theanacho EN, Spira DT, Peter HH, Dobbin P, et al. The effect of N-alkyl modification on the antimalarial activity of 5-hydroxypyridin-4-one oral iron chelators. Blood 77: 637-643, 1991

Hill HAO. Oxygen, oxidases and the essential trace metals. Philosophical Transactions of the Royal Society of London B294: 119-128, 1981

Hodnick WF, Kung FS, Roettger WJ, Bohmont CW, Pardini RS. Inhibition of mitochondrial respiration and production of toxic oxygen radicals by flavonoids. Biochemical Pharmacology 35: 2345-2357, 1986

Howard LJ. The neurologic syndrome of vitamin E deficiency: laboratory and electrophysiological assessment. Nutrition Reviews 48: 169-177, 1990

Huang L, Privalle CT, Serafin D, Klitzman B. Increased survival of skin flaps by scavengers of superoxide radical. FASEB Journal 1: 129-132, 1987

Hunt WH, Stocker R. Oxidative stress and the redox status of malaria-infected erythrocytes. Blood Cells 16: 499-526, 1990

Imlay JA. Fridovich I. Assays of metabolic superoxide production in *Escherichia coli*. Journal of Biological Chemistry 266: 6957-6965, 1991

Imlay JA, Linn S. DNA damage and oxygen radical toxicity. Science 240: 1302-1309, 1988

Ingold KU, Burton GW, Foster DO, Zucker M, Hughes L, et al. A new vitamin E analogue more active than α-tocopherol in the rat curative myopathy bioassay. FEBS Letters 205: 117-120, 1986

Ingold KU, Burton GW, Foster DO, Hughes L. Further studies of a new vitamin E analogue more active than α-tocopherol in the rat curative myopathy bioassay. FEBS Letters 267: 63-65, 1990

Inoue M, Watanabe N, Morino Y, Tanaka Y, Amachi T, et al. Inhibition of oxygen toxicity by targeting superoxide dismutase to endothelial cell surface. FEBS Letters 269: 89-92, 1990

Issels RD, Bourier S, Boning B, Li GC, Mak JJ, et al. Influence of oxidative stress induced by cysteamine upon the induction and development of thermotolerance in Chinese Hamster ovary cells. Cancer Research 47: 2268-2274, 1987

Itoh T, Kawamaki M, Yamauchi Y, Shimizu S, Nakamura M. Effect of allopurinol on ischemia and reperfusion-induced cerebral injury in spontaneously hypertensive rats. Stroke 17: 1284-1287, 1986

Jacobsen EJ, McCall JM, Ayer DE, Van Doornik FJ, Palmer JR, et al. Novel 21-aminosteroids that inhibit iron-dependent lipid peroxidation and protect against central nervous system trauma. Journal of Medicinal Chemistry 33: 1145-1151, 1990

Jadot G, Michelson AM, Puget K, Baret A. Antiinflammatory activity of superoxide dismutases: inhibition of carrageenan-induced edema in rats. Free Radical Research Communications 1: 395-403, 1986

Jain SK, Williams DM. Reduced levels of plasma ascorbic acid (vitamin C) in sickle cell disease patients: its possible role in the oxidant damage to sickle cells in vivo. Clinica Chimica Acta 149: 257-261, 1985

Jandak J, Steiner M, Richardson PD. α-Tocopherol, an effective inhibitor of platelet adhesion. Blood 73: 141-149, 1989

Janero DR, Burghardt B, Lopez R. Protection of cardiac membrane phospholipid against oxidative injury by calcium antagonists. Biochemical Pharmacology 37: 4197-4203, 1988

Jones MM. Newer trends in therapeutic chelating agents. Trends in Pharmacological Sciences 3: 335-337, 1982

Jones MM. New developments in therapeutic chelating agents as antidotes for metal poisoning. Critical Reviews in Toxicology 21: 209-233, 1991

Jones MH, Pratt TH. Therapeutic chelating agents. Journal of Chemical Education 53: 342-347, 1976

Kaminski PM, Proctor KG. Actions of adenosine on nitroblue tetrazolium deposition and surface pH during intestinal reperfusion injury. Circulation Research 66: 1713-1719, 1990

Kanner J, Harel S. Initiation of membranal lipid peroxidation by activated metmyoglobin and methaemoglobin. Archives of Biochemistry and Biophysics 237: 314-321, 1985

Kanofsky JR. Singlet oxygen production by biological systems. Chemico-Biological Interactions 70: 1-28, 1989

Kappus H. A survey of chemicals inducing lipid peroxidation in biological systems. Chemistry and Physics of Lipids 45: 105-115, 1987

Kehrer JP. Concepts related to the study of reactive oxygen and

cardiac reperfusion injury. Free Radical Research Communications 5: 305-314, 1989
Kontoghiorghes GJ. The study of iron mobilisation from transferrin using α-ketohydroxy heteroaromatic chelators. Biochimica et Biophysica Acta 869: 141-146, 1986a
Kontoghiorghes GJ. Iron mobilisation from lactoferrin by chelators at physiological pH. Biochimica et Biophysica Acta 882: 267-270, 1986b
Kontoghiorghes GJ, Jackson MJ, Lunec J. In vitro screening of iron chelators using models of free radical damage. Free Radical Research Communications 2: 115-124, 1986
Krinsky NI. Antioxidant functions of carotenoids. Free Radical Biology and Medicine 7: 617-635. 1989
Laguna JC, Nagi MN, Cook L, Cinti DL. Action of Ebselen on rat hepatic microsomal enzyme-catalyzed fatty acid chain elongation, desaturation and drug biotransformation. Archives of Biochemistry and Biophysics 269: 272-283, 1989
Larson RA. The antioxidants of higher plants. Phytochemistry 27: 969-978, 1988
Lasley RD, Ely SW, Berne RM, Mentzer RM Jr. Allopurinol enhanced adenine nucleotide repletion after myocardial ischemia in the isolated rat heart. Journal of Clinical Investigation 81: 16-20, 1988
Laughton MJ, Halliwell B, Evans PJ, Hoult JRS. Antioxidant and pro-oxidant actions of the plant phenolics quercetin, gossypol and myricetin. Biochemical Pharmacology 38: 2859-2865, 1989
Leurs H, Timmerman H, Bast A. Inhibition of superoxide anion radical production by Ebselen (PZ51) and its sulfur analogue (PZ25) in guinea pig alveolar macrophages. Biochemistry International 18: 295-299, 1989
Levander OA. A global view of human selenium nutrition. Annual Review of Nutrition 7: 227-250, 1987
Levine M. New concepts in the biology and biochemistry of ascorbic acid. New England Journal of Medicine 314: 892-902, 1986
Lewis DAL. Anti-inflammatory drugs from plant and marine sources. Agents and Actions 27 (Suppl.): 1-373, 1989
Lunec J, Blake DR. The determination of dehydroascorbic acid and ascorbic acid in the serum and synovial fluid of patients with rheumatoid arthritis. Free Radical Research Communications 1: 31-39, 1985
Maly FE. The B lymphocyte: a newly recognized source of reactive oxygen species with immunoregulatory potential. Free Radical Research Communications 8: 143-148, 1990
Marklund SL. Expression of extracellular superoxide dismutase by human cell lines. Biochemical Journal 266: 213-219, 1990
Marletta MA. Nitric oxide: biosynthesis and biological significance. Trends in Biochemical Sciences 14: 488-492, 1989
Mathews-Roth MM. Photoprotection by carotenoids. Federation Proceedings 46: 1890-1893, 1987
McCarthy JT, Milliner DS, Johnson WJ. Clinical experience with desferrioxamine in dialysis patients with aluminium toxicity. Quarterly Journal of Medicine 74: 257-276, 1990
McCay PB. Vitamin E: interactions with free radicals and ascorbate. Annual Review of Nutrition 5: 323-340, 1985
McCord JM. Oxygen-derived free radicals in post ischemic tissue injury. New England Journal of Medicine 312: 159-163, 1985
McLachlan DRC, Dalton HJ, Kruch TPA, Bell MY, Smith WL, et al. Intramuscular desferrioxamine in patients with Alzheimer's disease. Lancet 337: 1304-1308, 1991
McLaren GD, Muir WA, Kellermeyer RW. Iron overload disorders: natural history, pathogenesis, diagnosis and therapy. CRC Critical Reviews in Clinical Laboratory Science 19: 205-266, 1983
Meier B, Radeke HH, Selle S, Raspe HH, Sies H, et al. Human fibroblasts release reactive oxygen species in response to treatment with synovial fluids from patients suffering from arthritis. Free Radical Research Communications 8: 149-160, 1990
Meister A. Glutathione metabolism and its selective modification. Journal of Biological Chemistry 263: 17205-17208, 1988
Menasche P, Grousset C, Gauduel Y, Piwnica A. A comparative study of free radical scavengers in cardioplegic solutions. Journal of Thoracic and Cardiovascular Surgery 92: 264-271, 1986
Menasche P, Pasquier C, Belluci S, Lorente P, Jaillon P, et al. Deferoxamine reduces neutrophil-mediated free radical production during cardiopulmonary bypass in man. Journal of Thoracic and Cardiovascular Surgery 96: 582-589, 1990
Merry P, Winyard PG, Morris CJ, Grootveld M, Blake DR. Oxygen free radicals, inflammation and synovitis: the current status. Annals of the Rheumatic Diseases 48: 864-870, 1989
Miners JO, Drew R, Birkett DJ. Mechanism of action of paracetamol protective agents in mice *in vivo*. Biochemical Pharmacology 33: 2995-3000, 1984
Minotti G, Aust SD. The role of iron in the initiation of lipid peroxidation. Chemistry and Physics of Lipids 44; 191-208, 1987
Mitchell JB, Samuni A, Krishna MC, de Graff WG, Ahm MS, et al. Biologically active metal-independent superoxide dismutase mimics. Biochemistry 29: 2802-2807, 1990
Mitchinson MJ, Ball RY. Macrophages and atherogenesis. Lancet 2: 146-148, 1987
Modell B, Letsky EA, Flynn DM, Peto R, Weatherall DJ. Survival and desferrioxamine in thalassaemia major. British Medical Journal 284: 1081-1084, 1982
Moldeus P, Cotgreave IA, Berggren M. Lung protection by a thiol-containing antioxidant: N-acetylcysteine. Respiration 50 (Suppl. 1): 31-42, 1986
Moroney MA, Alcaraz MJ, Forder RA, Carey F, Hoult JRS. Selectivity of neutrophil 5-lipoxygenase and cyclooxygenase inhibition by an anti-inflammatory flavonoid glycoside and related aglycone flavonoids. Journal of Pharmacy and Pharmacology 40: 787-792, 1988
Mukai K, Okabe K, Hosose H. Synthesis and stopped-flow investigation of antioxidant activity of tocopherols. Finding of new tocopherol derivatives having the highest antioxidant activity among phenolic antioxidants. Journal of Organic Chemistry 54: 557-560, 1989
Murrell GAC, Francis MJO, Bromley L. Free radicals and Dupuytren's contracture. British Medical Journal 295: 1373-1375, 1987
Murrell GAC, Francis MJO, Bromley L. Modulation of fibroblast proliferation by oxygen free radicals. Biochemical Journal 265: 659-665, 1990
Myers PR, Minor Jr RL, Guerra Jr R, Bates JN, Harrison DG. Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble 5-nitrosocysteine than nitric oxide. Nature 345: 161-163, 1990
Nagano T, Hirano T, Hirobe M. Superoxide dismutase mimics based on iron *in vivo*. Journal of Biological Chemistry 264: 9243-9249, 1989
Nathan DG, Piomelli S. Introduction: oral iron chelators. Seminars in Hematology 27: 83-85, 1990
Nienhuis AW. Vitamin C and iron. New England Journal of Medicine 304: 170-171, 1981
Nishino T, Tsushima K. Interaction of milk xanthine oxidase with folic acid. Journal of Biological Chemistry 261: 11242-11246, 1986
Okamoto H. Molecular basis of experimental diabetes: degeneration, oncogenesis and regeneration of pancreatic β-cells of islets of Langerhans. Bioessays 2: 15-21, 1986
Orrenius S, McConkey DJ, Bellomo G, Nicotera P. Role of Ca^{2+} in toxic cell killing. Trends in Pharmacological Sciences 10: 281-285, 1989
Parkinson Study Group. Effect of deprenyl on the progression of disability in early Parkinson's disease. New England Journal of Medicine 321: 1364-1371, 1989

Parks DA. Oxygen radicals: mediators of gastrointestinal pathophysiology. Gut 30: 293-298, 1989
Parnham MJ, Leyck S, Graf E, Dowling EJ, Blake DR. The pharmacology of ebselen. Agents and Actions 32: 4-9, 1991
Parthasarathy S, Young SG, Witztum JL, Pittman RC, Steinberg D. Probucol inhibits oxidative modification of low density lipoprotein. Journal of Clinical Investigation 77: 641-644, 1986
Patel BS, Jeroudi MO, O'Neill PG, Roberts R, Bolli R. Effect of human recombinant superoxide dismutase on canine myocardial infarction. American Journal of Physiology 258: H369-H380, 1990
Perry MA, Wadhwa SS. Gradual reintroduction of oxygen reduces reperfusion injury in cat stomach. American Journal of Physiology 254: G366-G372, 1988
Petch MC. Dangers of thrombolysis. British Medical Journal 300: 483, 1990
Peterhans E, Jungi TW, Stocker R. Autotoxicity and reactive oxygen in viral disease. In Cerutti PA et al. (Eds) Oxy-radicals in molecular biology and pathology, pp. 543-562, AR Liss Inc., New York, 1988
Pitarys CJ, Virmani R, Vildibill HD, Jackson EK, Forman MB. Reduction of myocardial reperfusion injury by intravenous adenosine administered during the early reperfusion period. Circulation 83: 237-247, 1991
Pollack S, Rossan RN, Davidson DE, Escajadillo A. Desferrioxamine suppresses *Plasmodium falciparum* in Astus monkeys. Proceedings of the Society for Experimental Biology and Medicine 184: 162-164, 1987
Porta P, Aebi S, Summer K, Lauterburg BH. L-2-oxothiazolidine-4-carboxylic acid, a cysteine prodrug: pharmacokinetics and effects on thiols in plasma and lymphocytes in human. Journal of Pharmacology and Experimental Therapeutics 257: 331-334, 1991
Porter JB, Hoyes KP, Abeysinghe R, Huehns ER, Hider RC. Animal toxicology of iron chelator L1. Lancet ii: 156, 1989b
Porter JB, Jaswon MS, Huehns ER, East CA, Hazell JWP. Desferrioxamine ototoxicity: evaluation of risk factors in thalassaemic patients and guidelines for safe dosage. British Journal of Haematology 73: 403-409, 1989a
Porter JB, Morgan J, Hoyes EP, Burke LC, Huehns ER, Hider RC. Relative oral efficacy and acute toxicity of hydroxypyrid-4-one iron chelators in mice. Blood 76: 2389-2396, 1990
Powis G. Free radical formation by antitumor quinones. Free Radical Biology and Medicine 6: 63-101, 1989
Puppo A, Halliwell B. Formation of hydroxyl radicals from hydrogen peroxide in the presence of iron: is haemoglobin a biological Fenton reagent? Biochemical Journal 249: 185-190, 1988a
Puppo A, Halliwell B. Formation of hydroxyl radical in biological systems. Does myoglobin stimulate hydroxyl radical formation from hydrogen peroxide? Free Radical Research Communications 4: 415-422, 1988b
Puppo A, Cecchini R, Aruoma OI, Bolli R, Halliwell B. Scavenging of hypochlorous acid and of myoglobin-derived oxidants by the cardioprotective agent mercaptopropionylglycine. Free Radical Research Communications 10: 371-381, 1990
Reddanna P, Rao MK, Reddy CC. Inhibition of 5-lipoxygenase by vitamin E. FEBS Letters 193: 39-43, 1985
Reiter S, Simmonds HA, Webster DR, Watson AR. On the metabolism of allopurinol. Biochemical Pharmacology 32: 2167-2174, 1983
Rinaldo JE, Gorry M. Protection by deferoxamine from endothelial injury: a possible link with inhibition of intracellular xanthine oxidase. American Journal of Respiratory Cell and Molecular Biology 3: 525-533, 1990
Ross D. Glutathione, free radicals and chemotherapeutic agents. Pharmacology and Therapeutics 37: 231-249, 1988
Roubal WT, Tappel AL. Damage to proteins, enzymes and amino acids by peroxidizing lipids. Archives of Biochemistry and Biophysics 113: 5-8, 1966
Rowley DA, Halliwell B. Superoxide-dependent formation of hydroxyl radicals in the presence of thiol compounds. FEBS Letters 138: 33-36, 1982
Saez G, Thornalley PJ, Hill HAO, Hems R, Bannister JV. The production of free radicals during the autoxidation of cysteine and their effect on isolated rat hepatocytes. Biochimica et Biophysica Acta 719: 24-31, 1982
Salim AS. Oxygen-derived free radicals and the prevention of duodenal ulcer relapse: a new approach. American Journal of Medical Sciences 300: 1-6, 1990
Salvemini D, Botting R. The effect of free radical scavengers on platelet adhesion and aggregation. Drug News and Perspectives 3: 202-212, 1990
Sanan S, Sharma G, Malhotra R, Sanan DP, Jain P, et al. Protection by desferrioxamine against histopathological changes of the liver in the post-oligaemic phase of clinical haemorrhagic shock in dogs: correlation with improved survival rate and recovery. Free Radical Research Communications 6: 29-38, 1989
Sanfey H, Bulkley GB, Cameron JL. The role of oxygen-derived free radicals in the pathogenesis of acute pancreatitis. Annals of Surgery 200: 405-413, 1984
Saran M, Michel C, Bors W. Reaction of NO with superoxide. Free Radical Research Communications 10: 221-226, 1990
Schalkwijk J, van den Berg WB, van de Putte LBA, Joosten LAB, van den Bersselaar L. Cationization of catalase, peroxidase and superoxide dismutase. Journal of Clinical Investigation 76: 198-205, 1985
Schneeburger H, Illner WD, Abendroth D, Bulkley G, Rutili F, et al. First clinical experiences with superoxide dismutase in kidney transplantation – results of a double-blind randomized study. Transplantation Proceedings 21: 1245-1246, 1989
Schneider J. Prophylactic and curative effects of recombinant human superoxide dismutase in lethal rat endotoxemia. Arzneimittel Forschung Drug Research 38: 1381-1386, 1988
Schöneich C, Asmus KD, Dillinger U, von Bruchhausen F. Thiyl radical attack on polyunsaturated fatty acids: a possible route to lipid peroxidation. Biochemical and Biophysical Research Communications 161: 113-120, 1989
Schraufstatter IU, Hyslop PA, Jackson J, Cochrane CG. Oxidant injury of cells. International Journal of Tissue Reactions IX: 317-324, 1987
Sevanian A, Peterson AR. The cytotoxic and mutagenic properties of cholesterol oxidation products. Food Chemistry and Toxicology 24: 1103-1110, 1986
Sevilla MD, Yan M, Becker D, Gillich S. ESR investigations of the reactions of radiation-produced thiyl and DNA peroxyl radicals: formation of sulfoxyl radicals. Free Radical Research Communications 6: 21-24, 1989
Shamberger RJ. Genetic toxicology of ascorbic acid. Mutation Research 133; 135-159, 1984
Shapiro A, Nathan HC, Hutner SH, Garofalo J, McLaughlin SD, et al. In vivo and in vitro activity by diverse chelators against *Trypanosoma brucei brucei*. Journal of Protozoology 29: 85-90, 1982
Sik RH, Paschall CS, Chignell CF. The phototoxic effect of benoxaprofen and its analogs on human erythrocytes and rat peritoneal mast cells. Photochemistry and Photobiology 38: 411-415, 1983
Simons JM, Hart BA, Ching TRIV, Van Dijk HV, Labadie RP. Metabolic activation of natural phenols into selective oxidative burst agonists by activated human neutrophils. Free Radical Biology and Medicine 8: 251-258, 1990
Simpson PJ, Lucchesi BR. Free radicals and myocardial ischaemia and reperfusion injury. Journal of Laboratory and Clinical Medicine 110: 13-30, 1987

Sinha S, Davies J, Toner N, Bogle S, Chiswick M. Vitamin E supplementation reduces frequency of periventricular haemorrhage in very preterm babies. Lancet 1: 466-470, 1987

Smilkstein MJ, Knapp GL, Kulig KW, Rumack BH. Efficacy of oral N-acetylcysteine in the treatment of acetaminophen overdose. New England Journal of Medicine 319: 1557-1562, 1988

Smith LL. Cholesterol autoxidation 1981-1986. Chemistry and Physics of Lipids 44; 87-100, 1987

Sorenson JRJ. Bis(3,5-diisopropylsalicylato)copper(II), a potent radioprotectant with superoxide dismutase mimetic activity. Journal of Medicinal Chemistry 27: 1747-1749, 1984

Spector T. Oxypurinol as an inhibitor of xanthine oxidase-catalyzed production of superoxide radical. Biochemical Pharmacology 37: 349-352, 1988

Spector T, Hall WW, Porter DJT, Lambe CU, Nelson DJ, et al. Inhibition of xanthine oxidase by 4-hydroxy-6-mercaptopyrazolo[3,4-d]pyrimidine. Biochemical Pharmacology 38: 4315-4320, 1989

Speyer JL, Green MD, Kramer E, Rey M, Sanger J, et al. Protective effect of the bispiperazinedione ICRF-187 against doxorubicin-induced cardiac toxicity in women with advanced breast cancer. New England Journal of Medicine 319: 745-752, 1988

Stähelin HB, Gey L-F, Eichholzer M, Lüdin E. β-carotene and cancer prevention: the Basel study. American Journal of Clinical Nutrition 53: 2655-2695, 1991

Starke PE, Farber JL. Ferric iron and superoxide ions are required for the killing of cultured hepatocytes by hydrogen peroxide. Journal of Biological Chemistry 260: 10099-10104, 1985

Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. New England Journal of Medicine 320: 915-924, 1989

Steinke DE, Weir BKA, Findlay JM, Tanabe T, Grace M, et al. A trial of the 21-aminosteroid U74006F in a primate model of chronic cerebral vasospasm. Neurosurgery 24: 179-186, 1989

Steinkuhler C, Mavelli I, Rossi L, Pedersen JZ, Melino G, et al. Cytotoxicity of a low molecular weight Cu_2Zn_2 superoxide dismutase active center analog in human erythroleukemia cells. Biochemical Pharmacology 39: 1473-1479, 1990

Stern LZ, Ringel SP, Ziter FA, Menander-Huber KB, Ionasescu V, et al. Drug trial of superoxide dismutase in Duchenne's muscular dystrophy. Archives of Neurology 39: 342-344, 1982

Stocker R. Induction of haem oxygenase as a defence against oxidative stress. Free Radical Research Communications 9: 101-112, 1990

Stowell KM, Rado TA, Funk WD, Tweedie JW. Expression of cloned human lactoferrin in baby-hamster kidney cells. Biochemical Journal 276: 349-355, 1991

Suzuki M, Inauen W, Kvietys PR, Grisham MB, Meininger C, et al. Superoxide mediates reperfusion-induced leukocyte-endothelial cell interactions. American Journal of Physiology 257: H1740-H1745, 1989

Svardal AM, Mansoor MA, Ucland PM. Determination of reduced, oxidized, and protein-bound glutathione in human plasma with precolumn derivatization with monobromobimane and liquid chromatography. Analytical Biochemistry 184: 338-346, 1990

Swingle KF, Bell RL, Moore GGI. Anti-inflammatory activity of antioxidants. In Rainsford KD (Ed.) Anti-inflammatory and anti-rheumatic drugs, Vol. III, CRC Press, Boca Raton, 1987

Tabor E, Epstein JS, Hewlett JK, Lee SF. Inhibition by desferrioxamine of in-vitro replication of HIV-I. Lancet 337: 795, 1991

Tannenbaum SR, Wishnock JS, Leaf CD. Inhibition of nitrosamine formation by ascorbic acid. American Journal of Clinical Nutrition 53: 247S-250S, 1991

Thomas MJ, Bielski BHJ. Oxidation and reaction of Trolox C, a tocopherol analogue, in aqueous solution: a pulse-radiolysis study. Journal of the American Chemical Society 111: 3315-3319, 1989

Thornalley PK. Esterification of reduced glutathione. Biochemical Journal 275: 535-537, 1991

Thumwood CM, Hunt NH, Cowden WB, Clark IA. Antioxidants can prevent cerebral malaria in *Plasmodium berghei*-infected mice. British Journal of Experimental Pathology 70: 293-303, 1989

Tibell L, Hjalmarsson K, Edlund T, Skogman G, Engstrom A, et al. Expression of human extracellular superoxide dismutase in chinese hamster ovary cells and characterization of the product. Proceedings of the National Academy of Sciences of the USA 84: 6634-6638, 1987

Tryka AF. ICRF-187 and polyhydroxyphenyl derivatives fail to protect against bleomycin induced lung injury. Toxicology 59: 127-138, 1989

Tsao CS, Salimi SL. Evidence of rebound effect with ascorbic acid. Medical Hypotheses 13: 303-310, 1984

Tsao PS, Lefer AM. Time course and mechanism of endothelial dysfunction in isolated ischemic- and hypoxic-perfused rat hearts. American Journal of Physiology 259: H1660-H1666, 1990

Ucland PM, Refsum H. Plasma homocysteine, a risk factor for vascular disease: plasma levels in health, disease and drug therapy. Journal of Laboratory and Clinical Medicine 114: 473-501, 1989

Vaille A, Jadot G, Elizagaray A. Anti-inflammatory activity of various superoxide dismutases on polyarthritis in the Lewis rat. Biochemical Pharmacology 39: 247-255, 1990

Van der Wal NAA, Van Oirschot JFLM, Van Dijk A, Verhoef J, Sweder Van Asbeck B. Mechanism of protection of alveolar type II cells against paraquat-induced cytotoxicity by deferoxamine. Biochemical Pharmacology 39: 1665-1671, 1990

Villar A, Gasco MA, Alcaraz MJ. Anti-inflammatory and anti-ulcer properties of hypolactin-8-glucoside, a novel plant flavonoid. Journal of Pharmacy and Pharmacology 36: 820-823, 1984

Vohra K, Rosenfeld W, Singh I, Anderson V. Ischemic injury to newborn rabbit ileum: protective role of human superoxide dismutase. Journal of Pediatric Surgery 24: 893-898, 1989

Von Sonntag C. The chemical basis of radiation biology, Taylor and Francis, London, 1987

Wajner M, Harkness RA. Distribution of xanthine dehydrogenase and oxidase activities in human and rabbit tissues. Biochimica et Biophysica Acta 991: 79-84, 1989

Wang Q, Giri SN, Hyde DM, Nakashima JM, Javadi I. Niacin attenuates bleomycin-induced lung fibrosis in the hamster. Journal of Biochemical Toxicology 5: 13-22, 1990

Ward HE, Hicks M, Nicholson A, Berend N. Deferoxamine infusion does not inhibit bleomycin-induced lung damage in the rat. American Review of Respiratory Disease 137: 1356-1359, 1988

Ward PA, Till GO, Kunkel R, Beauchamp C. Evidence of role of hydroxyl radical in complement and neutrophil-mediated tissue injury. Journal of Clinical Investigation 72: 789-801, 1983

Warso MA, Lands WEM. Lipid peroxidation in relation to prostacyclin and thromboxane physiology and pathophysiology. British Medical Bulletin 39: 277-280, 1983

Wasil M, Halliwell B, Hutchison DCS, Baum H. The antioxidant action of extracellular fluids. Biochemical Journal 243: 219-223, 1987

Wefers H. Singlet oxygen in biological systems. Bioelectrochemistry and Bioenergetics 18: 91-104, 1987

Weiss APC, Carey LA, Randolph MA, Moore JR, Weiland AJ. Oxygen radical scavengers improve vascular potency and bone-muscle cell survival in an ischemic extremity replant model. Plastic and Reconstructive Surgery 84: 117-123, 1989

Weiss SJ. Tissue destruction by neutrophils. New England Journal of Medicine 320: 365-376, 1989

Weitberg AB. Effect of nicotinic acid supplementation in vivo on oxygen radical-induced genetic damage in human lymphocytes. Mutation Research 216: 197-201, 1989

Werns SW, Lucchesi BR. Free radicals and ischemic tissue injury. Trends in Pharmacological Sciences 11: 161-166, 1990

Weser U, Sellinger KH, Lengfelder E, Werner W, Strahle J. Structure of Cu_2 (indomethacin)$_4$ and the reaction with superoxide in aprotic systems. Biochimica et Biophysica Acta 631: 232-245, 1980

White GJ. Inhibition of oxidation enzymes by anti-allergy drugs. Agents and Actions 11: 503-509, 1981

Wolfe LC. Desferrithiocin. Seminars in Hematology 27: 117-120, 1990

Wolff SP, Dean RT. Glucose autoxidation and protein modification. Biochemical Journal 245: 243-250, 1987

Yokode M, Kita T, Kikawa Y, Ogorochi T, Narumiya S, et al. Stimulated arachidonate metabolism during foam cell transformation of mouse peritoneal macrophages with oxidized low density lipoprotein. Journal of Clinical Investigation 81; 720-729, 1988

Yoon JO, Im MJ, Manson PN, Bulkley GB, Hoopes JE. The role of metal ions in ischemia/reperfusion injury in skin flaps. Journal of Surgical Research 46: 163-165, 1989

Correspondence and reprints: Professor *Barry Halliwell,* Pulmonary Medicine, UC Davis Medical Center, 4301 X Street, Sacramento, CA 95817, USA.