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Evolution of antioxidant defence mechanisms

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Summary The metabolic strengths, weaknesses, opportunities and threats of the metabolic ability to split water brought about a proliferation of biological systems, produced a toxic oxygenic environment, and were responsible for the development of antioxidant defence mechanisms. Evolution is driven by heritable adaptations which improve environmental 'fit'. Hence aerobic respiration, using oxygen as a nutrient, came to predominate in biological systems, and antioxidant defence mechanisms which prevent and neutralise toxic oxygen intermediates have become widespread, varied, coordinated and effective. Antioxidant defences are not infallible however. In humans, reactive oxygen species-induced damage is associated with the ageing process, and with chronic diseases including cancer and coronary heart disease. Interestingly, some important antioxidants, including ascorbic acid and the tocopherols, cannot be synthesised by humans and must be taken in the diet. Another antioxidant, uric acid, is found in much higher concentrations in humans than in other mammals, and levels are also affected by diet. In humans, therefore, antioxidant defence against toxic oxygen intermediates is species specific and heavily influenced by nutrition.

In this article, the atmospheric and metabolic changes which produced both the threat and opportunity offered by an oxygenic environment are outlined. An overview of oxygen toxicity, and adaptations to oxidative stress in terms of evolution of antioxidant defences, is presented. Finally, suggested benefits underlying our curious inability to manufacture ascorbic acid, and the possible role of uric acid in human antioxidant defence, are briefly discussed with particular reference to nutrition and toxicology.

Key words aerobic – oxygen – evolution – antioxidants – SOD – ascorbic acid – uric acid

Introduction

The paradox of aerobic life is that the 'vital air' is also a lethal toxin. The cost of fuel-efficient aerobic catabolism is oxidative damage to DNA, protein, lipid and carbohydrate. Antioxidant defences have evolved to protect biological systems against reactive oxygen species (ROS), and a sophisticated, co-operative array of antioxidant defence mechanisms is found in biological systems. Antioxidant defences minimise generation and counteract the damaging effects of reactive oxygen species (ROS) produced within the organism from molecular oxygen. Several antioxidants, such as peroxidases and superoxide dismutases (SOD), are found in most biological systems; however, the same environmental pressure has led to a range of adaptations in other antioxidant mechanisms.

In humans, antioxidant defence is integrated and effective, but not infallible. ROS-induced damage has been im-

plicated in the development of chronic degenerative disease and in the ageing process, and the concept that increased antioxidant defence may lower risk of such disease is supported by biochemical and epidemiological evidence. Interestingly, some important antioxidants, including ascorbic acid and the tocopherols, cannot be synthesised by humans and must be taken in the diet, while another antioxidant, uric acid, is found in much higher concentrations in humans than in other mammals.

In this article, the atmospheric and metabolic changes which produced both the threat and opportunity offered by an oxygenic environment are outlined. An overview of oxygen toxicity, and adaptations to oxidative stress in terms of evolution of antioxidant defences, is presented, and the human antioxidant defence system is described. Finally, suggested benefits underlying our curious inability to manufacture ascorbic acid, and the possible role of uric acid in human antioxidant defence are briefly discussed with particular reference to nutrition and toxicology.

'Evolution' of the atmosphere

The Earth was formed around 4.6 billion (4.6×10^9) years ago as a waterless mass of rock. Earth's primordial atmosphere of hydrogen and helium was stripped by the solar wind during the sun's T Tauri stage, and volatile compounds were blasted into space by the force of the collision between Earth and a Mars-sized object *[1]*. The present atmosphere is entirely secondary, formed firstly by the importation of volatiles, including water, via comets impacting on Earth and by outgassing of the planet, and then by eons of biological processes *[1]*. The early secondary atmosphere consisted of water vapour, carbon dioxide, carbon monoxide, nitrogen, hydrogen chloride and hydrogen. Oxygen freed from water by photodissociation was avidly taken up by atmospheric or surface components, and also formed ozone (O_3), which accumulated in the upper layers of the atmospheric regions, forming a barrier to intense ultraviolet bombardment of the Earth's surface. Free oxygen, therefore, did not exist in any quantity, and a reducing environment was maintained *[1–3]*.

Life on Earth began around 3.5 billion years ago. Evolution of anaerobic fermenters, followed by water-splitting organisms, led to dramatic but gradual changes in the atmosphere *[1–6]*. Procaryotic fermenters used hydrogen and fixed carbon in the form of organic compounds. Cyanobacteria (the blue-greens) used water as a source of the electron donor, hydrogen, and released O_2 as a waste product. Oxygen released was absorbed by the massive oxygen sinks, forming deposits of banded iron (iron oxides) *[1, 2]*. By 2.0 billion years ago, oxygen sinks were approaching saturation, and atmospheric O_2 levels began to rise. Free O_2 levels approached the present atmospheric level (PAL) around 1.6 billion years ago *[4, 7–9]*. Procaryotic life forms predominated initially, but about 1.4 billion years ago the larger, more complex and compartmentalised eucaryotes developed, possibly by serial endosymbiosis of procaryotes *[9–11]*.

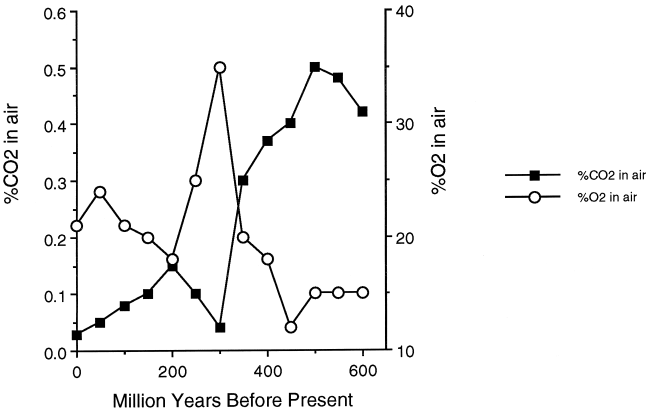

Fig. 1 Changes in atmospheric carbon dioxide and oxygen levels over last 600 million years (from references 1–4).

Over the past 2.5 billion years, therefore, Earth's atmosphere has changed dramatically *[1, 2–4, 8, 9]*. The virtually anaerobic, hypodense, hydrogen-rich atmosphere became hydrogen-depleted but enriched in both CO_2 and O_2, then changed again to become hyperbaric and hyperoxic but with a very limited content of CO_2, before reaching its present day composition and pressure (Fig. 1). This marked change in the atmosphere was caused by degassing of the chemical constituents of Earth, by the importation of volatiles, and by the evolution of biological processes. These processes – the reactions of photosynthesis, and anaerobic and aerobic respiration – depleted atmospheric hydrogen, produced O_2, and fixed carbon from CO_2. Eventually, metabolic processes also evolved to use O_2 as a nutrient, reforming water and releasing CO_2 back into the environment *[5, 6, 12–15]*.

Metabolic evolution – strengths, weaknesses, opportunities and threats

Change is implicit in evolution. Selection pressure results in a continuation and proliferation of those systems which can best deal with change in the environment *[16]*. Those systems which cannot cope with the environmental factors to which they are most frequently exposed decline or cease to exist. Evolution, therefore, results in adaptation to change. This enables survival in spite of, and eventually because of, a changed environment.

The atmospheric environment changed largely as a result of metabolic processes within living systems *[2–4]*. Some of these systems responded to these changes by adapting to the decreased availability of hydrogen, the major electron donor *[6, 12, 17]*. Depletion of atmospheric hydrogen provided a strong selection pressure for those or-

ganisms which could use water as a freely available alternative source of electrons. The strength of this metabolic innovation led to a proliferation of water-splitting cyanobacteria, but introduced a threat in terms of molecular oxygen. There was, therefore, strong selection pressure for biological processes or systems which protected organisms against the toxic effects of oxygen. Consequently, antioxidant defence mechanisms evolved. The increasing availability of oxygen provided the opportunity for fuel-efficient metabolic innovations which used oxygen as a final electron acceptor, and aerobic respiration, therefore, became predominant. The threat of oxidative damage, however, continued to exert selection pressure for metabolic adaptations which conferred protection against the toxic intermediates of oxygen *[17–19].*

There is evidence that cytochrome oxidases, central to aerobic respiration, evolved before oxygenic (water splitting) photosynthetic systems *[15].* Traces of O_2 existed in the early atmosphere, formed by photolysis of water, and these may have been sufficient to activate aerobic enzyme systems *[13, 15].* However, the use of aerobic pathways was restricted by the very low prevailing levels of atmospheric O_2, which were slow to rise owing to the massive O_2 oxygen sinks. Nevertheless, it was inevitable that water-splitting organisms were exposed to a stressful internal oxygenic environment. This radical change in the internal environment imposed strong selection pressure for the continued existence and proliferation of those organisms with some protection against oxidative damage.

Oxygen as a toxin, and the need for antioxidant defences

Antioxidant defences are needed in biological systems because molecular oxygen is an oxidising agent, that is, it can take electrons from another species *[20].* Oxidation of lipid, DNA and protein changes the structure and function of key cellular constituents, resulting in mutation, cell damage and death (Fig. 2) *[20, 21].* Fortunately, the oxidation powers of ground state molecular oxygen are restricted, as electrons can only be absorbed from another species whose electron spin is antiparallel to that of the two unpaired, parallel-spin electrons in diatomic oxygen. This spin restriction means that ground state molecular oxygen is not reactive enough to abstract electrons from other species in general *[20].* Reactivity of molecular oxygen can be increased, however, by removing the spin restriction *[20].* This occurs when a single electron is added, or when energy is transferred to oxygen from a photosensitiser.

Flavin-containing compounds and chlorophyll are photosensitisers, i. e., they are capable of harvesting light and energising molecular oxygen, forming singlet oxygen ($^1\Delta_g O_2$), which has an energy level of 92 kJ above ground state *[18, 20].* Singlet oxygen can interact directly with another molecule, transferring the additional energy and/or

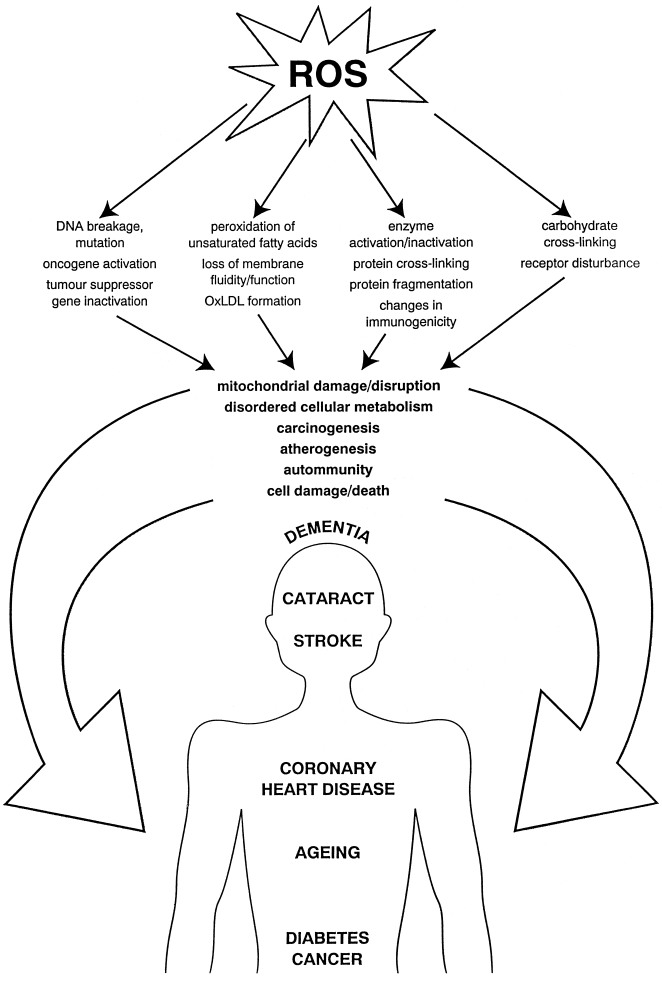

Fig. 2 Reactive oxygen species increase risk of disease through damage to key biological structures (reproduced with permission from [21].

changing the structure of the target molecule. Important target molecules include the essential amino acids methionine, cysteine, tryptophan and histidine, and lipids containing carbon-to-carbon double (C=C) covalent bonds *[20].*

The one electron reduction of ground state molecular oxygen produces superoxide ($O_2^{\cdot-}$), and a further single electron reduction produces hydrogen peroxide (H_2O_2) *[20].* Unlike $O_2^{\cdot-}$, the uncharged H_2O_2 can readily diffuse across cell membranes. These two ROS together can form the fiercely reactive hydroxyl radical ($\cdot OH$) via the Haber-Weiss reaction:

$$H_2O_2 + O_2^{\cdot-} \rightarrow \cdot OH + OH^- + O_2$$

This reaction is very slow in aqueous solutions unless free transition metal ions, such as Fe^{2+} or Cu^+, are present, in which case the reaction (known as the Fenton reaction when iron-catalysed), proceeds very quickly *[20].*

$Metal(_{ox}) + O_2^{\cdot-} \rightarrow Metal(_{red}) + O_2$

$Metal(_{red}) + H_2O_2 \rightarrow Metal(_{ox}) + \cdot OH + OH^-$

giving an overall reaction of:

$$H_2O_2 + O_2^{\cdot-} \xrightarrow{\text{metal catalyst}} \cdot OH + OH^- + O_2$$

The hydroxyl radical (·OH) reacts with anything it contacts, wreaking indiscriminate but extensive intracellular damage. However, before the development of an oxygenic atmosphere, iron and copper were likely to have been in the form of insoluble complexes, and so effectively unavailable for the intracellular catalysis of ·OH production from H_2O_2 and $O_2^{\cdot-}$ *[7, 12, 19, 22]*. Therefore, while some ·OH may have formed within or on the surface of cells from Haber-Weiss chemistry and from photodissociation of water, it is likely that oxidative damage within cells was initially limited to that caused by $^1\Delta_gO_2$ and $O_2^{\cdot-}$ formed *in situ*. Consequently, those organisms which had some defence against these ROS were better equipped to thrive.

Early developments in antioxidant defence

In general terms, an antioxidant is anything which can prevent or inhibit oxidation *[20, 21]*. This can be achieved by preventing the generation of ROS, or by inactivating ROS. Several types of antioxidant mechanisms exist, and these are outlined in Table 1.

The first antioxidant mechanisms were probably simple physical barriers *[14]*. Intracellular sequestration of photosensitising pigments, such as chlorophyll, development of UV screens, and compartmentalisation of vulnerable cellular components, would serve to protect against the production and action of ROS.

Superoxide can form H_2O_2 and this can, in turn, form the highly reactive.OH. Removal of $O_2^{\cdot-}$, therefore, is a key antioxidant defence mechanism *[18]*. Superoxide can spontaneously dismutate to form H_2O_2. This reaction is more rapid in mildly acidic conditions when there is protonation of some $O_2^{\cdot-}$ to the more reactive hydroperoxyl ($HO_2^{\cdot-}$) radical *[18, 20]*. It is likely that the relatively high CO_2 content of the atmosphere (see Fig. 1) would have caused acidification of the surface layers of the oceans and tidal pools and, possibly, early intracellular fluids, promoting H_2O_2 formation from $O_2^{\cdot-}$. The release of molecular oxygen formed partially oxidised iron salts, which are soluble. The concentration of Fe^{2+} in the oceans and tidal pools, therefore, increased. H_2O_2 produced within, diffusing from and accumulating around cells would have reacted with traces of Fe^{2+} in the immediate vicinity. In this way the very reactive ·OH could have formed on or near the highly susceptible, polyunsaturated fatty acid-rich membrane of eucaryotic cells. Natural selection, therefore, would have favoured those organisms which could produce envelopes around, or expendable radical traps on the surface of, the membrane *[14]*. These would have 'buffered' ·OH action and prevented oxidative damage to essential membrane constituents.

The first membrane defence is likely to have been a coating on the exterior surface *[14, 18]*. A replaceable anionic coating of a simple polymer would have bound extracellular metals ions, such as Fe^{2+}, effectively 'absorbing' the subsequent ROS-action and protecting the cell membrane. As the concentration of partially oxidised, soluble forms of cationic transition metal ions increased in the aqueous environment surrounding cells *[19, 22]*, membranes hindering their access to the interior of the cell were required to prevent intracellular Fenton chemistry *[14]*. As oxidised ions, e. g. Fe^{3+}, accumulated on the progressively more damaged coating, some are likely to have become internalised.

With the increase in intracellular iron came the threat of intracellular Fenton chemistry, and simple barriers and non-specific chemical traps were no longer sufficient. More specialised antioxidant mechanisms were needed to prevent ROS formation and to neutralise those ROS which were increasingly and unavoidably produced. The metabolic opportunity arose, however, for newly 'bioavailable' transition metal ions to be used as components of more specialised antioxidant defence mechanisms using catalytic redox reaction centres *[7, 18, 20, 22]*.

Table 1 Types of antioxidant action

	Action	Example
Prevention	Protein binding/ inactivation of metal ions	transferrin, ferritin, caeruloplasmin, albumin
Enzymatic diversion/ neutralisation	Specific channelling of ROS[1] into harmless products	superoxidase dismutase, catalase glutathione peroxidase
Scavenging	Sacrificial interaction with ROS by expendable (replaceable or recyclable) substrates	ascorbic acid, alpha tocopherol, uric acid, bilirubin, glutathione
Quenching	Absorption of electrons and/or energy	alpha tocopherol, beta carotene

[1] ROS, reactive oxygen species

Specialised antioxidant mechanisms: prevention, diversion, dismutation, scavenging and quenching

The most reactive ROS is ·OH, which reacts at diffusion limited rates with virtually anything it contacts *[20]*. Owing to this very high reactivity, no specific antioxidant mechanism against ·OH is feasible, and antioxidant strategies which prevented ·OH production were favoured. Other evolutionary developments involved diversion of H_2O_2 to water, dismutation of $O_2^{\cdot-}$, scavenging of ROS, and

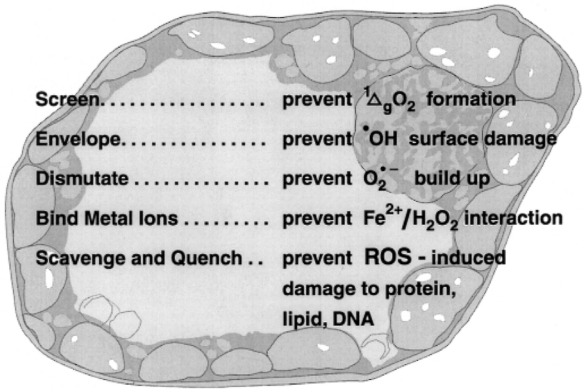

Fig. 3 Strategies for antioxidant defence.

quenching of excess energy. These antioxidant mechanisms are outlined in Fig. 3.

Prevention and diversion

Binding iron and copper in an inactive form prevents metal ion catalysed degradation of peroxides and, thereby, prevents ·OH production *[20].* Removal of H_2O_2 (and other peroxides) by a strategy of diversion into water production also prevents ·OH production *[20].* These more specialised preventive strategies are protein based. The first metal ion-binding mechanisms were probably unlikely to have inactivated the redox action of iron or copper, but acted by diverting ·OH formation away from key biological sites, targeting damage onto themselves and sacrificially 'buffering' the action of ROS. This type of antioxidant mechanism is effective, and is still used in biological systems, for example, albumin acts as a sacrificial antioxidant *[20].* A more efficient strategy, however, involves specific binding, with concomitant inactivation of the metal ion and prevention of ROS generation. This requires specialised structures, and has resulted in the evolution of specific proteins, including transferrin, ferritin and caeruloplasmin *[20–22].* Transferrin and ferritin bind iron *in vivo* in the physiologically more safe ferric (Fe^{3+}) form; copper (Cu^{2+}) is carried mainly in caeruloplasmin. The combination of specific and non-specific protein binding mechanisms normally keeps the concentration of free iron and copper in human plasma at extremely low levels, estimated to be 10^{-23} and 10^{-18} mol/l respectively, thereby minimising metal-ion catalysed production of ·OH *[23].*

Peroxide is also required for production of ·OH. Prevention of H_2O_2 generation does not appear to be possible, and indeed dismutation of $O_2^{\cdot -}$ to H_2O_2 appears to be a key antioxidant strategy *[18, 24–26]*, and so facilitated removal of H_2O_2 is needed. In procaryotes and single celled eucaryotes, H_2O_2 build-up could have been prevented by the simple diffusion of H_2O_2 out of the cell. As cells became more structured and organisms became multi-cellular, however, a mechanism to promote removal of H_2O_2 was needed. Diversion of H_2O_2 into water is a safe and effective mechanism, but requires a biological catalyst.

Enzymes which catalyse removal of H_2O_2 include the catalases and peroxidases. These antioxidants are found in almost all aerobic organisms, and promote the following reactions:

catalases: $2H_2O_2 \rightarrow 2H_2O + O_2$

peroxidases: $SH_2 + H_2O_2 \rightarrow S + 2H_2O$

Catalase and most of the peroxidases contain an Fe^{3+} protoporphyrin group at the active site *[20].* This reflects the evolutionary age of this defence mechanism, as iron became metabolically available before copper, which is the other major transition metal ion used in redox catalysis *[18].* Catalase activity is restricted largely, if not exclusively, to within peroxisomes. Other organelles, such as mitochondria and chloroplasts, use peroxidases to remove H_2O_2. In animals, glutathione peroxidase (GPx) acts in co-operation with catalase in removal of H_2O_2 *[20, 27, 28].* GPx contains selenium at the active site, and uses the endogenous tripeptide glutathione (GSH) as a specific co-factor for hydrogen donation in the following reaction:

glutathione peroxidase: $2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$

The oxidised form of glutathione (GSSG) is reduced, and so recycled, by glutathione reductase enzymes *[20, 27]:*

glutathione reductase:

$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$$

An efficient, effective and co-operative enzymatic system, therefore, has evolved to divert the potentially toxic H_2O_2 to water. This, in combination with metal ion binding, normally prevents ·OH generation *in vivo [20, 28]*

Dismutation

Superoxide is only moderately reactive, but can cause damage and, owing to its charged nature, cannot leave cells by simple diffusion. More importantly perhaps, $O_2^{\cdot -}$ can promote Fenton chemistry, as it can redox recycle transition metal ions. Superoxide can be removed by dismutation into H_2O_2; however, spontaneous dismutation of $O_2^{\cdot -}$ is slow at neutral (physiological) pH. While strategic channelling of $O_2^{\cdot -}$ into H_2O_2 would benefit the cell, owing to loss of H_2O_2 by diffusion, H_2O_2 is potentially highly toxic if free metal ions are present. Therefore, unless H_2O_2 had a specific purpose, as an intracellular cell signalling mechanism for example *[20, 28, 29]* the benefits of dismutation may have been realised only after the development of an effective system of H_2O_2 removal.

Manganese (Mn) has superoxide dismutase (SOD)-like activity when it accumulates inside cells *[14, 30],* and the

European Journal of Nutrition, Vol. 39, Number 2 (2000)

first SOD may have simply used this property. However, eucaryotes have developed specific enzymes containing Fe, Mn, or Cu, and with these the dismutation of $O_2^{\cdot -}$ proceeds at a virtually diffusion-limited rate:

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$$

SODs are widely distributed in living systems; however, different types exist *[18, 20, 24–26]*. The familiar blue-green copper-containing SOD, which is found within the cytoplasm, is likely to be the most recently evolved form, and is distinctly different in structure from the Fe and Mn forms *[18]*. Iron and Mn predated Cu as metabolic components *[7, 19, 22]*. SODs containing Fe and Mn are structurally homologous, and were innovative metabolic adaptations at a time in evolutionary history when soluble forms of iron and manganese, but not yet copper, were bioavailable *[7, 19, 22, 26]*. It is thought that mitochondria may have originally been independent organisms that were incorporated into eucaryotes by a process of symbiotic fusion *[18, 19, 25]*. This would account for an earlier evolutionary form of SOD, the manganese containing form, being found in an apparently more recent biological setting – the mitochondria.

Scavenging and quenching

Prevention, diversion and dismutation strategies effectively and harmlessly channel $O_2^{\cdot -}$ and H_2O_2, into water, but they are not infallible. Some ROS escape to initiate peroxidation of, for example, polyunsaturated fatty acids (PUFA). Evolutionary developments in eucaryotes included PUFA-rich structures such as membranes and lipoproteins *[14, 20, 27]*. Peroxidation of PUFA affect the fluidity and function of these structures, and protection is essential *[20, 21, 31]*. This type of antioxidant protection required the development of scavengers – small molecules which interact with primary ROS, such as $O_2^{\cdot -}$ or with secondary reactive species such as carbon-centred lipid radicals *[20, 21, 28]*. The common selection pressure of the need for scavenging produced multiple forms of the same type of defence, and many scavenging antioxidants exist in biological systems. Scavengers include the water-soluble ascorbic acid (vitamin C) and the lipid-soluble and membrane-bound tocopherols and tocotrienols ('vitamin E') *[20, 21, 32–37]*. Scavengers are expendable in a sense, and must be either replaced by *de novo* synthesis or recycled, but their antioxidant action is indispensable.

In addition to the problem of escape of ROS from enzymatic inactivation, the problem associated with $^1\Delta_gO_2$ remained unaddressed. Singlet oxygen is formed in chloroplasts and other organelles containing photosensitisers *[20]*. To prevent formation of $^1\Delta_gO_2$, and to protect these organelles from any formed, an antioxidant mechanism to absorb, or quench, energy was needed. Carotenoids are very effective quenching antioxidants, and are vital components of membranes surrounding chloroplasts *[20, 38]*.

Various types of antioxidant, therefore, have developed and these reflect different selection pressures over time (Table 1 and Fig. 4). Different forms have developed for the same purpose, and some species- or phyla- specific configurations are found. For example, SODs and peroxidases are widespread, but in animals GPx is also an important member of the antioxidant enzyme group. Quenching antioxidants are restricted to light-harvesting plants. Tocopherols are also manufactured only in plants, but are needed by animals. Ascorbic acid is an essential antioxidant, but interestingly cannot be synthesised in a few species, including *Homo Sapiens [20, 21, 24–28, 32–35]*.

The puzzle of ascorbic acid – the missing antioxidant

Ascorbic acid is an important scavenging antioxidant *[20, 21, 32–35]* and contributes up to 30 % of the 'total antioxidant power' of plasma *[38]*. Most species of plants and animals synthesise ascorbic acid from glucose, but humans and a few other animals and birds cannot, even though they have retained an absolute requirement for it. This makes ascorbic acid an essential component of the human diet *[32–35]*. Our inability to make ascorbic acid is due to a lack of L-gulono-lactone oxidase (GLO), which catalyses the final step in the biosynthetic pathway *[32, 40–42]*. The gene for GLO is highly mutated and inactive in humans; mutations are likely to have accumulated since the cessation of transcription, as there is no selection pressure against ineffective mutations. The puzzle lies in finding the evolutionary advantage in a mutation which prevented synthesis of ascorbic acid in the first instance.

Several advantages to the loss of ascorbic acid synthesis in humans have been suggested *[40–45]*. These include: lowering the mean age of the population, thereby enhancing fertility; providing protection against the haemolytic effects of glucose-6-phosphate dehydrogenase deficiency, a trait selected for in malarial belts; accelerated evolution owing to increased ROS effects on the genome; enhanced effectiveness of the early immune system. None of these arguments is convincing, given that ascorbic acid is still a vital component of human metabolism. A more feasible argument involves improved metabolic efficiency *[14]*. In situations where ascorbic acid was in plentiful supply from dietary sources – as was likely in the early hominid diet – there may have been positive selection pressure for a mutation which initially downregulated, and then lost, transcription of GLO. In addition, if a different antioxidant could be made at less metabolic cost than the exogenously plentiful ascorbic acid, then a mutation involving loss of GLO would have conferred the necessary biological advantage needed for selection. It is interesting to note that H_2O_2 is produced during synthesis of ascorbic acid *[35]*. Obtaining ascorbic acid exclusively from exogenous

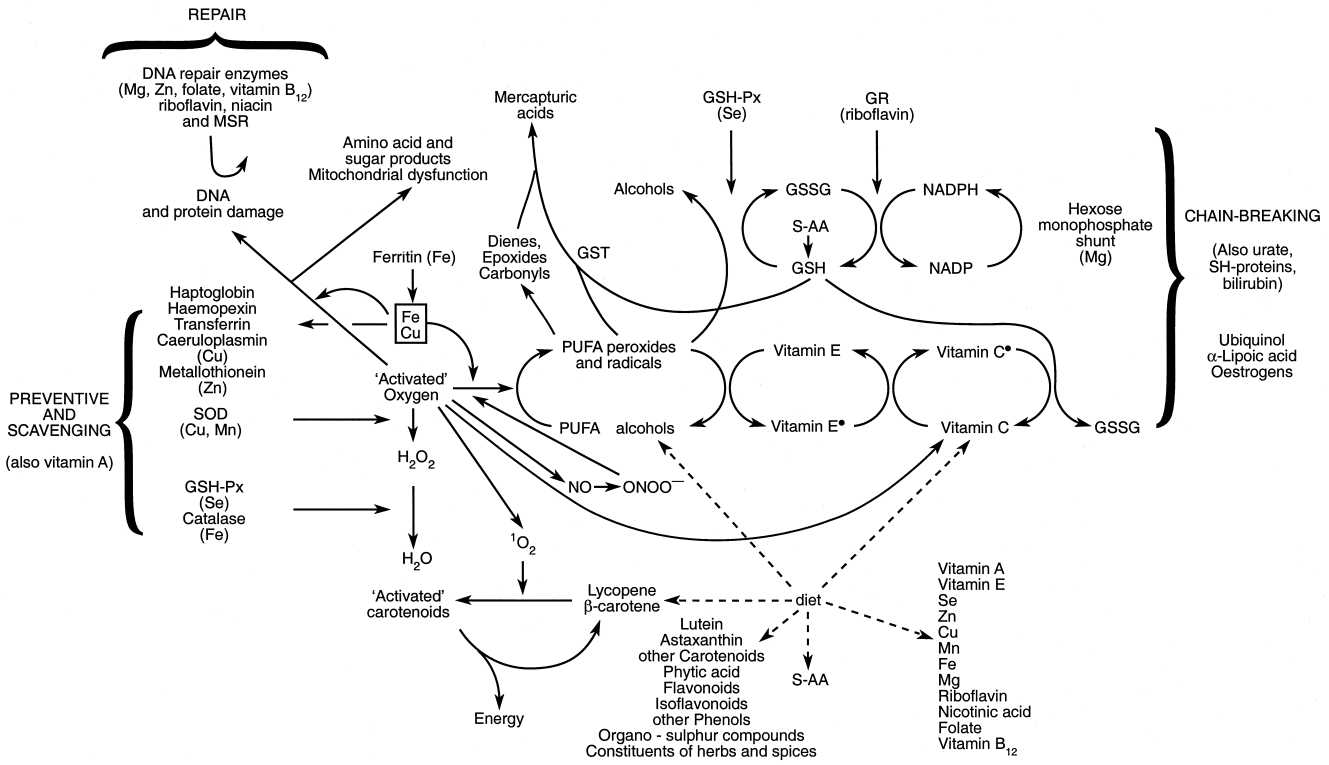

Fig. 4. Antioxidant defence system (after Strain 1991). GR, glutathione reductase (EC1.6.4.2); GSH, reduced glutathione; GSH-Px glutathione peroxidase (EC1.11.1.9); GSSG, oxidized glutathione; GST, glutathione-S-transferase (EC 2.5.1.18); MSR, methionine sulphoxide reductase (EC1.8.4.5); PUFA, polyunsaturated fatty acids; S-AA, sulphur amino acids; SH-proteins, sulphydryl proteins; SOD, superoxide dismutase (EC1.15.1.1); Fe Cu, transition metal-catalysed oxidant damage to biomolecules.

Fig. 4 An overview of antioxidant defence. Reproduced with permission from: [23]

sources, therefore, may have helped conserve endogenous antioxidants, such as glutathione. It is interesting also that an inverse relationship across different phyla has been reported for SOD and GLO activities *[42],* and that our inability to manufacture ascorbic acid may have coincided, in evolutionary terms, with our inability to change uric acid into allantoin, perhaps implying a possible partial replacement of one antioxidant by another *[46, 47].*

Uric acid is a product of purine catabolism and is produced in relatively high concentrations in humans owing to the lack of the enzyme uricase which, in other animals, oxidises uric acid to allantoin. Uric acid levels are also influenced by diet, and increase with intake of meat or alcohol *[46–48].* Uric acid has antioxidant properties and, like ascorbic acid, is water soluble *[47].* Additional uric acid and/or SOD, therefore, may have been cost-effective metabolic innovations which, provided dietary input of ascorbic acid remained adequate, more than compensated for the lack of endogenous ascorbic acid. A diametrical view, however, is that a less reducing and more pro-oxidant intracellular environment may have been beneficial. For example, if ROS were used in cell signalling, or in an evolving redox controlled gene transcription system *[25, 28, 29, 49],* then selection pressure would have been against an overly efficient antioxidant scavenging system. In evolution, immediate physiological benefit may well be selected over long-term benefits, particularly if long-term benefits are realised in the post-reproductive years. The possibility exists, therefore, that we cannot make ascorbic acid because, at some point in our evolutionary development at least, less ascorbic acid improved our biological fit.

Nutrition and toxicology

The metabolic strengths, weaknesses, opportunities and threats of water-splitting brought about a proliferation of biological systems, and produced a toxic oxygenic environment. This environment was responsible for the development of antioxidant defence mechanisms. Evolution is driven by heritable adaptations which improve environmental fit. Hence, varied and efficient antioxidant defence mechanisms became established, and the ability to use oxygen in fuel-efficient aerobic respiration evolved and came to predominate.

Evolutionary adaptation, however, stops at the 'break even' point. There is no selection pressure for adaptations which do not in some way confer additional physiological

benefit at a metabolically acceptable cost. Oxygen is now an essential nutrient for most living systems, but remains potentially toxic. Antioxidant defence mechanisms which prevent and neutralise toxic oxygen intermediates are widespread, diverse, co-ordinated and effective. They are not, however, infallible. Moreover, in humans, antioxidant defence against reactive oxygen intermediates is heavily influenced by nutrition, owing to our lack of endogenous ascorbic acid and tocopherol production, and our inability to produce uricase.

In humans, ROS-induced damage is associated with the ageing process, and with chronic diseases including cancer and coronary heart disease *[20, 21, 28, 31, 46, 47]*. Nutritional strategies to improve *in vivo* antioxidant status may be effective in lowering risk of the toxic effect of ROS *[50, 51]*. Such strategies could take antioxidant defence beyond the simple 'break even' point; however, the effectiveness of such strategies remains to be confirmed. Furthermore, ROS may have important roles in cell signalling and transcriptional control *[28, 49]*. It is possible, therefore, that excessive intake of antioxidant micronutrients could have toxic effects. In biological systems, toxins may become nutrients, and nutrients may be toxic in certain situations *[16]*. The term 'nutritional toxicology', therefore, is not the paradox it may at first appear.

References

1. Thompson GR, Turk J (1999) Earth Science and the Environment. Harcourt Brace, Orlando
2. Berner RA (1997) The rise of plants and their effect on weathering and atmospheric CO_2. Science 249: 56–91
3. Berner RA, Canfield DE (1989) A new model for atmospheric oxygen over Phanerozoic time. Am J Sci 289: 333–361
4. Cloud PE (1968) Atmospheric and hydrospheric evolution on the primitive Earth. Both secular accretion and biological and geochemical processes have affected Earth's volatile envelope. Science 160: 729–736
5. Blankenship RE, Hartman H (1998) The origin and evolution of oxygenic photosynthesis. Trends in Biochemical Science 23: 94–97
6. Broda E (1975) The beginning of photosynthesis. Origins of Life 6: 247–251
7. Frieden E (1974) The evolution of metals as essential elements (with special reference to iron and copper). Adv Exp Med Biol 48: 1–31
8. Graham JB, Dudley R, Aguilar NM, Gans C (1995) Implications of the late Palaezoic oxygen pulse for physiology and evolution. Nature 375: 117–120
9. Dudley R (1998) Atmospheric oxygen, giant palaeozoic insects and the evolution of aerial locomototor performance. J Exper Biol 201: 1043–1050
10. Schopf JW, Oehler DZ (1978) The evolution of the earliest cells. Scientific American 239: 110–120
11. Margulis L (1981) Symbiosis in Cell Evolution. WH Freeman, San Francisco
12. Barnabas J, Schwartz RM, Dayhoff MO (1982) Evolution of the major metabolic innovations in the Precambrian. Origins of Life 12: 81–91
13. Jahnke L, Klein HP (1979) Oxygen as a factor in eukaroyte evolution: some effects of low levels of oxygen on *Saccharomyces cerevisiae*. Origins of Life 9: 329–334
14. Bilinski T (1991) Oxygen toxicity and microbial evolution. BioSystems 24: 305–312
15. Castresana J, Saraste M (1995) Evolution of energetic metabolism: the respiration early hypothesis. Trends Biochem Sci 20: 443–448
16. Bickham JW, Smolen MJ (1994) Somatic and heritable effects of environmental genotoxins and the emergence of evolutionary toxicology. Environ Health Perspect 102: 25–28
17. Walker JCG (1980) Atmospheric constraints on the evolution of metabolism. Origins of Life 10: 93–104
18. Fridovich I (1974) Superoxide and evolution. Horizons Biochem Biophys 1: 1–37
19. Kamaluddin, SM, Deopujari SW (1986) Chemical evolution of iron containing enzymes: mixed ligand complexes of iron as intermediary steps. Origins of Life 17: 59–68
20. Halliwell B, Gutteridge JMC (1999) Free Radicals in Biology and Medicine. Clarendon Press, Oxford
21. Strain JJ, Benzie IFF (1999) Antioxidants: diet and antioxidant defence. In: Sadler M, Strain JJ, Cabellero B (eds) The Encyclopedia of Human Nutrition. Academic Press, London, pp 95–106
22. Ochiai EI (1983) Copper and the biological evolution. BioSystems 16: 81–86
23. May PM, Linder PW, Williams DR (1977) Computer simulations of metal-ion equilibria in biofluids: models for the low-molecular-weight complex distribution of calcium (II), magnesium (II), manganese (II), iron (III), copper (II), zinc (II) and lead (II) ions in blood plasma. J. Chem. Soc. Dalton Trans. 588–595
24. Lumsden J, Hall DO (1975) Superoxide dismutase in photosynthetic organisms provides an evolutionary hypothesis. Nature 257: 670–671
25. Fridovich I (1997) Superoxide anion radical ($O_2^{\cdot-}$), superoxide dismutases and related matters. J Biol Chem 272: 18515–18517
26. Bannister WH, Bannister JV, Barra D, Bond J, Bossa F (1991) Evolutionary aspects of superoxide dismutase: the copper/zinc enzyme. Free Rad Res Comm 12–13: 349–361
27. Sundquist AR, Fahey RC (1989) Evolution of antioxidant mechanisms: thiol dependent peroxidases and thioltransferase among procaryotes. J Mol Evol 29: 429–435
28. Fridovich I (1998) Oxygen toxicity: a radical explanation. J Exp Biol 210: 1203–1209
29. Barja G (1993) Oxygen radicals, a failure or a success of evolution? Free Rad Res Comm 18: 63–70
30. Archibald FS, Fridovich I (1981) Manganese and defense against oxygen toxicity in *Lactobacillus plantarum*. J Bacteriol 145: 442–451
31. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witzum JL (1989) Beyond cholesterol; modifications of low-density lipoprotein that increase its atherogenicity. New Engl J Med 320: 915–924
32. Bender DA (1999) Ascorbic acid: physiology, dietary sources and requirements. In: Sadler M, Strain JJ, Cabellero B (eds) The Encyclopedia of Human Nutrition, Academic Press, London, pp 144–150
33. Nishikimi M, Yagi K (1996) Biochemistry and molecular biology of ascorbic acid biosynthesis. In: Harris JR (ed) Subcellular Biochemistry: Ascorbic Acid: Biochemistry and Biomedical Cell Biology, Vol 25, Plenum Press, New York, pp 17–39
34. Barja G (1996) Ascorbic acid and aging. In: Harris JR (ed) Subcellular Biochemistry: Ascorbic Acid: Biochemistry and Biomedical Cell Biology, Vol 25. Plenum Press, New York, pp 157–188
35. Banhegyi G, Braun L, Csala M, Puska F, Mandl J (1997) Ascorbate metabolism and its regulation in animals. Free Rad Biol Med 23: 793–803

36. Kamal-Eldin A, Appleqvist A (1996) The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids, 31: 671–701
37. Sheehy T, Morrissey PA (1999) Tocopherols: physiology. In: Sadler M, Strain JJ, Cabellero B (eds) The Encyclopedia of Human Nutrition, Academic Press, London. pp 1878–1884
38. Krinsky NI, Rock CL (1998) Carotenoids: chemistry sources and physiology. In: Sadler M, Strain JJ, Cabellero B (eds) The Encyclopedia of Human Nutrition, Academic Press, London, pp 304–305
39. Benzie IFF, Strain JJ (1999) Ferric reducing (antioxidant) power as a measure of antioxidant capacity: the FRAP assay. In: Packer L (ed) Oxidants and Antioxidants, Vol 299 of Methods in Enzymology. Academic Press,Orlando, pp 15–27
40. Chatterjee IB (1973) Evolution and biosynthesis of ascorbic acid. Science 182: 1271–1272
41. Pauling L (1970) Evolution and the need for ascorbic acid. Proc Natl Acad Sci 67: 1643–1648
42. Nandi A, Mukhopadhyay CK, Ghosh MK, Chattopadhyay DJ, Chatterjee IB (1997) Evolutionary significance of vitamin C biosynthesis in terrestrial vertebrates. Free Rad Biol Med 22: 1047–1054
43. Millar J (1992) Vitamin C – the primate fertility factor? Med Hypotheses 38: 292–295
44. Calabrese EJ (1982) Evolutionary loss of ascorbic acid synthesis: how it may have enhanced the survival interests of man. Med Hypotheses 8: 173–175
45. Challem JJ (1997) Did the loss of endogenous ascorbate propel the evolution of Anthropoidea and *Homo sapiens*? Med Hypotheses 48: 387–392
46. Cutler RG (1991) Antioxidants and aging. Am J Clin Nutr 53: 373S–379S
47. Ames BN, Cathcart R, Schwiers E, Hochstein P (1981) Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. Proc Natl Acad Sci 78: 6858–6862
48. Benzie IFF, Strain JJ (1996) Uric acid – friend or foe. Redox Report 2: 231–234
49. Jackson MJ, McArdle A, McArdle F (1998) Antioxidant micronutrients and gene expression. Proc Nutr Soc 57: 310–315
50. Halliwell B (1996) Oxidative stress, nutrition and health. Experimental strategies for optimisation of nutritional antioxidant intake in humans. Free Rad Res 25: 57–74
51. Benzie IFF (1999) Antioxidants: observational epidemiology. In: Sadler M, Strain JJ, Cabellero B (eds) The Encyclopedia of Human Nutrition, Academic Press, London, pp 106–115