Free Rad. Res., Vol. 25, No. 5, pp. 439–454
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COMMENTARY

Vitamin C: Antioxidant or Pro-Oxidant In Vivo?

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Accepted by Prof A. T. Diplock

(Received 13th November 1995; In revised form 3rd March 1996)

Ascorbic acid has a multiplicity of antioxidant properties, but it can exert pro-oxidant effects *in vitro*, usually by interaction with transition metal ions. It is as yet uncertain that these pro-oxidant effects have any biological relevance: some of the available data are summarized.

Keywords: Ascorbate, iron, copper, antioxidant, haemochromatosis

INTRODUCTION

Ascorbic acid is widely-regarded as an important antioxidant *in vivo* and has been called "an outstanding antioxidant in human plasma"[1]. Although vitamin C is known to be essential in the human diet for the action of several hydroxylase enzymes (lysine, proline, and dopamine β-hydroxylases are examples)[2] hard evidence to support the widespread belief in its antioxidant powers is somewhat limited. For example, little *in vivo* data has yet been obtained to support the common view that ascorbate regenerates α-tocopherol from the α-tocopheryl radical[3], although this has been demonstrated multiple times *in vitro* (e.g. references 4–12) including with isolated human LDL[4]. The observation that high dietary vitamin C slightly increased vitamin E levels in rat mutants unable to synthesize ascorbate is consistent with recycling, but the effect was small[13]. In addition, vitamin C can exert pro-oxidant effects *in vitro*, but no one is as yet sure if this is relevant *in vivo*. The purpose of the present article is to review what we know and to suggest how our state of ignorance might be remedied.

BASIC DEFINITIONS: WHAT IS AN ANTIOXIDANT?

"Antioxidant" is a term frequently used but rarely defined. Often, the term is implicitly restricted to chain-breaking antioxidant inhibitors of lipid peroxidation, such as α-tocopherol. However, free radicals generated *in vivo* fre-

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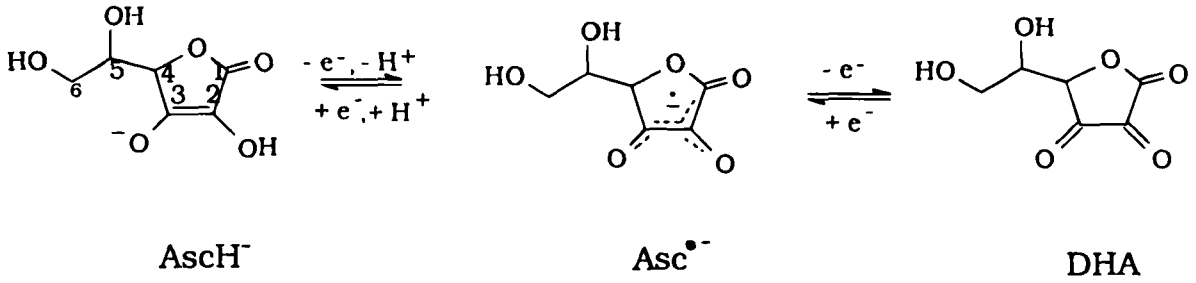

FIGURE 1 Chemical structure of ascorbate and its oxidation products. DHA-dehydroascorbate.

quently damage proteins and DNA as well as lipids, and so a broader definition has been introduced[14,15]—an antioxidant is any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate. The term "oxidizable substrate" covers almost everything found in living cells, including proteins, lipids, carbohydrates and DNA.

When reactive oxygen species (ROS)* and reactive nitrogen species (RNS)* are generated in living systems, a wide variety of antioxidants comes into play. The relative importance of these as protective agents depends upon which ROS/RNS is generated, how it is generated, where it is generated and what target of damage is measured. For example, if human blood plasma is tested for its ability to inhibit iron ion-dependent lipid peroxidation, the proteins transferrin and caeruloplasmin are found to be the most important protective agents[16,17]. When plasma is exposed to nitrogen dioxide, uric acid seems to exert protection against damage to biomolecules by this toxic oxidizing gas[18]. By contrast, when hypochlorous acid (HOCl) is added to plasma, uric acid appears to play little protective role[19]. Similarly, if the oxidative stress is kept the same but a different target of oxidative damage is measured, different answers can result. For example, when plasma is exposed to gas-phase cigarette smoke, lipid peroxidation occurs, an event which can be inhibited by both endogenous and added ascorbate[20]. By contrast, ascorbate has no effect on that oxidative damage to plasma proteins by cigarette smoke measured by the carbonyl assay[21]. Some *known* carcinogens (such as diethylstilboestrol) are powerful inhibitors of lipid peroxidation *in vitro*[22] but may *accelerate* oxidative DNA damage *in vivo*[23]. This is a stark illustration of how careful one has to be in equating "antioxidant" to "safe molecule".

The above definition emphasises the importance of the source of oxidative stress and the target ("oxidisable substrate") measured when defining antioxidants. Change either of these, and the relative protective effectiveness of different antioxidants will change. Hence there is no universal "best" biological antioxidant.

ANTIOXIDANT PROPERTIES OF ASCORBATE *IN VITRO*

Ascorbate readily undergoes oxidation, forming an intermediate radical of low reactivity (Figure 1). The poor reactivity of this radical may account for many of ascorbate's antioxidant effects: a fairly-reactive radical combines with ascorbate and a much less reactive radical (ascorbate radical) is formed[24]. Buettner[25] has summarized the one-electron reduction potentials of various biologically-relevant systems: Table II is a selection from the data he presents. Of course, these are *standard* potentials and the redox behaviour of substances is affected by such factors as concentration and pH. Nevertheless, Table II illustrates

*For definitions of these terms please see Table I.

TABLE I

REACTIVE OXYGEN SPECIES (ROS)	
Radicals	**Non-Radicals**
Superoxide, $O_2^{\bullet -}$	Hydrogen peroxide, H_2O_2
Hydroxyl, $OH^{\bullet}$	Hypochlorous acid, HOCl
Peroxyl, $RO_2^{\bullet}$	Ozone, O_3
Alkoxyl, $RO^{\bullet}$	Singlet oxygen $^1\Delta g$
Hydroperoxyl, $HO_2^{\bullet}$	
REACTIVE NITROGEN SPECIES (RNS)	
Radicals	**Non-Radicals**
	Nitrosyl NO^-
Nitric oxide, $NO^{\bullet}$	Nitrous acid, HNO_2
	Nitroxide NO
Nitrogen dioxide, $NO_2^{\bullet}$	Dinitrogen tetroxide, N_2O_4
	Dinitrogen trioxide, N_2O_3
	Peroxynitrite, $ONOO^-$
	Peroxynitrous acid, ONOOH
	Nitronium cation, (Nitryl), NO_2^+
	Alkyl peroxynitrites, ROONO

ROS is a collective term that includes both oxygen radicals and certain non-radicals that are oxidizing agents and/or are easily converted into radicals (HOCl, O_3, $ONOO^-$, $^1O_2$, H_2O_2). RNS is also a collective term including nitric oxide and nitrogen dioxide radicals, as well as such non-radicals as HNO_2 and N_2O_4. $ONOO^-$ is often included in both categories, and HOCl could equally well be called a "reactive chlorine species". "Reactive" is not always an appropriate term; H_2O_2, $NO^{\bullet}$ and $O_2^{\bullet -}$ react quickly with few molecules whereas $OH^{\bullet}$ reacts quickly with almost everything. $RO_2^{\bullet}$, $RO^{\bullet}$, HOCl, $NO_2^{\bullet}$, $ONOO^-$ and O_3 have intermediate reactivities.

the important point that ascorbate is thermodynamically close to the bottom of the pecking order for oxidizing radicals[25], i.e. it will tend to quench more-reactive species such as $OH^{\bullet}$, $O_2^{\bullet -}$ and urate radical. The ascorbate radical is relatively unreactive, being neither strongly oxidizing nor strongly reducing[24,25]. In particular, it is thermodynamically unlikely[25] that ascorbate radical can reduce O_2 to $O_2^{\bullet -}$, a conclusion consistent with the majority of the experimental data available[24–26].

As can be predicted (Table II), ascorbate has been shown to have a multiplicity of antioxidant properties *in vitro*, some of which are summarized in Table III. Ascorbate may also be an important protective agent against damage by reactive nitrogen species, such as peroxynitrite (Figures 2 and 3) and nitrosating agents formed from nitrite[47]. Ascorbate in respiratory tract lining fluids may be especially important in protecting against damage by inhaled oxidizing air pollutants, such as O_3 and $NO_2^{\bullet}$[43].

PRO-OXIDANT PROPERTIES OF ASCORBATE

In vitro, however, vitamin C can also exert *pro-oxidant* properties. The classic system of Udenfriend *et al*[48] for making hydroxyl radicals consists of an iron chelate, H_2O_2 and ascorbate. The ascorbate acts as reductant to the iron, easily permitted by the relative reduction potentials (Table II).

$$Fe^{3+} + \text{ascorbate} \rightarrow Fe^{2+} + \text{ascorbate}^{\bullet} \quad (1)$$

$$Fe^{2+} + H_2O_2 \rightarrow OH^{\bullet} + OH^- + Fe^{3+} \quad (2)$$

Iron/ascorbate mixtures have been used for decades to stimulate lipid peroxidation[49]; again, the ascorbate functions mainly by reducing iron ions. Ascorbate can also interact with copper ions (e.g. reference[50]) to generate[51] $OH^{\bullet}$. Instillation of high levels of ascorbate with iron or copper ions into the stomach of animals led to $OH^{\bullet}$ gen-

TABLE II Some Standard Reduction Potentials

Highly Oxidizing	Couple	Standard Reduction Potential (mv)
	$OH^{\bullet}, H^+/H_2O$	2310
	$RO^{\bullet}, H^+/ROH$ (aliphatic alkoxyl)	1600
	$HO_2^{\bullet}, H^+/H_2O_2$	1060
	$O_2^{\bullet-}, 2H^+/H_2O_2$	940
	$RS^{\bullet}/RS^-$ (cysteine)	920
	$HU^{\bullet-}, H^+/UH_2^-$ (urate)	590
	$\alpha T^{\bullet}, H^+/\alpha TH$ (α-tocopherol)	500
	Trolox C ($TO^{\bullet}, H^+/TOH$)	480
	$H_2O_2, H^+/H_2O, OH^{\bullet}$	320
	$ascorbate^{\bullet-}, H^+/ascorbate^-$	282
	Ferricytochrome c/ferrocytochrome c	260
	Ubisemiquinone, H^+/ubiquinol	200
	Fe^{3+}- EDTA/Fe^{2+}- EDTA	120
	Fe^{3+}-citrate/Fe^{2+}-citrate	~100
	Fe^{3+}-ADP/Fe^{2+}-ADP	~100
	Ubiquinone, H^+/ubisemiquinone	−36
	$Dehydroascorbate/ascorbate^{\bullet-}$	−174
	Fe^{3+}-ferritin/ferritin + Fe^{2+}	−190
	$O_2/O_2^{\bullet-}$	−330
	Fe^{3+}-transferrin/Fe^{2+}-transferrin	−400 (pH 7.3)
	Paraquat/paraquat $^{\bullet-}$	−448
	$O_2/H^+, /HO_2^{\bullet}$	−460
Highly reducing	$CO_2/CO_2^{\bullet-}$	−1800
	H_2O/e^-_{aq}	−2840

Data selected from the extensive compilation in [25]. Values refer to pH 7 unless otherwise stated.

eration[52,53] and the mixture of metal ions and ascorbate in some vitamin pills has been claimed to generate $OH^{\bullet}$ once the pills dissolve[54]. A mixture of ascorbate and copper ions (which will generate $OH^{\bullet}$) rapidly inactivates the enzyme catalase[55] and several authors have described cytotoxic and mutagenic effects of ascorbate on isolated cells (for a review of older literature see ref. 56; for other examples see refs. 57–61). The author believes[15] that these effects most likely involve interaction of ascorbate with transition metal ions added to (or contaminating) the cell growth media. Pro-oxidant effects of ascorbate are also well-known to food scientists. For example, Porter[62] referred to the actions of ascorbate in foods in these terms: "of all the paradoxical compounds, ascorbic acid probably tops the list. It is truly a two-headed Janus, a Dr. Jekyll-Mr. Hyde, an oxymoron of antioxidants."

Hence, when metal ions are present, ascorbate can often *stimulate* free radical damage *in vitro*. For example, a copper ion/ascorbate/H_2O_2 mixture causes severe oxidative damage to the bases of DNA by generating $OH^{\bullet}$[51]. An interesting apparent exception is LDL: even in the presence of copper ions, ascorbate delays oxidation of LDL, by recycling α-tocopherol and by other mechanisms[4,63,64]. However, once LDL oxidation is well under way, and presumably all the α-tocopherol has been oxidized, vitamin C can accelerate LDL oxidation, i.e. it can become pro-oxidant[65]. The physiological relevance of the complex interactions between LDL antioxidants and the transition metal ions that may

TABLE III Ascorbic Acid as an Antioxidant *In Vitro*

Scavenges O_2^- and $HO_2^\bullet$ (overall rate constant $>10^5 M^{-1}s^{-1}$ at pH 7.4).[27-29]
Scavenges water-soluble peroxyl ($RO_2^\bullet$) radicals.[30-32] Lipophilic ascorbate esters can also scavenge lipid-soluble $RO_2^\bullet$ radicals.[33]
Scavenges thiyl and sulphenyl radicals.[34,35]
Prevents damage by radicals arising by attack of $OH^\bullet$ or $RO_2^\bullet$ upon uric acid, probably by reacting with urate radicals.[36]
Powerful scavenger of hypochlorous acid.[37]
Inhibits damage by peroxynitrite (Figs. 1 and 2).
Inhibits lipid peroxidation by haemoglobin- or myoglobin-H_2O_2 mixtures and prevents peroxide-dependent haem breakdown and release of "catalytic" iron ions.[39]
Powerful scavenger and quencher of singlet O_2.[40]
May regenerate α-tocopherol from α-tocopheryl radicals in membranes and lipoproteins.[4-13]
Scavenges nitroxide radicals (JMC Gutteridge, personal communication).
Scavenges $OH^\bullet$ radicals (rate constant $> 10^9 M^{-1}s^{-1}$).[41]
Protects plasma lipids against peroxidation induced by activated neutrophils.[1]
May protect membranes and lipoproteins against lipid peroxidation induced by species present in cigarette smoke.[20,42]
Powerful scavenger of O_3 and $NO_2^\bullet$ in human body fluids, probably protects lung lining fluids against inhaled oxidizing air pollutants.[43]
Inhibits oxidative damage by radicals generated from certain drugs (e.g. phenylbutazone).[44,45]
Protects against oxidized LDL-induced phagocyte adhesion to endothelium in a hamster dorsal skin-fold chamber model.[46]

be present in atherosclerotic lesions[66,67] or released by vessel wall injury[68] are by no means understood, so it is impossible to say which is the most biologically-relevant scenario. Patients with iron overload disease do not appear to suffer greatly-enhanced rates of atherosclerosis development[69], although iron overload has been reported to augment the development of atherosclerosis in hypocholesterolaemic rabbits[70]. This perhaps suggests that ascorbate interactions with "catalytic" iron ions present in iron-overloaded patients (see below) do not, in general, facilitate LDL oxidation in the arterial wall to an extent that aggravates atherosclerosis, although it is possible that iron overload predisposes to myocardial infarction by other mechanisms such as direct free radical-mediated toxicity to the heart[71-74].

PHYSIOLOGICAL RELEVANCE OF THE ANTIOXIDANT EFFECTS OF ASCORBATE: WHAT *IN VIVO* DATA DO WE HAVE?

We know that vitamin C is essential in the human diet; there is an established deficiency disease (scurvy) and the role of ascorbate as a cofactor for several enzymes is well established (reviewed in ref. 2). Ascorbate plays key roles in the regulation of cellular iron metabolism[75,76]. It is also thought to aid the absorption of inorganic iron from the gut by reducing Fe(III) to the more easily-absorbable Fe^{2+} (discussed in ref. 77). Ascorbate is present in gastric juice, and may aid in eliminating nitrosamine carcinogens from the diet or formed in the stomach, hence helping to protect against one *cause* of stomach cancer[78,79,181]. The RDA for vitamin C (40 mg/day in the UK, 60 mg/day in the USA, higher for smokers, may be sufficient to do all these things[80,81]. However, the strong epidemiological evidence for the protective effect of ascorbate against certain forms of cancer[78,81] is not evidence that this anti-cancer action is exerted by an antioxidant mechanism. A similar comment may be made about the reported effect of ascorbate on haemostatic factors[82].

Nevertheless, it seems chemically very likely that ascorbate does exert some antioxidant properties *in vivo*. It scavenges many ROS/RNS (Table III; Figures 2 and 3) and it is widely distributed in cells and extracellular fluids at concentrations that would be capable of scavenging ROS/RNS[25,83], e.g. millimolar concentrations in human neutrophils[83] and lymphocytes[181]. But how can in vivo antioxidant action be proved?

In some cases involving naturally-occurring putative antioxidants, it has been possible to remove the compound in question and look for evidence of increased oxidative damage. For example, mutants of *E. coli* genetically-engineered to lack both MnSOD and FeSOD show

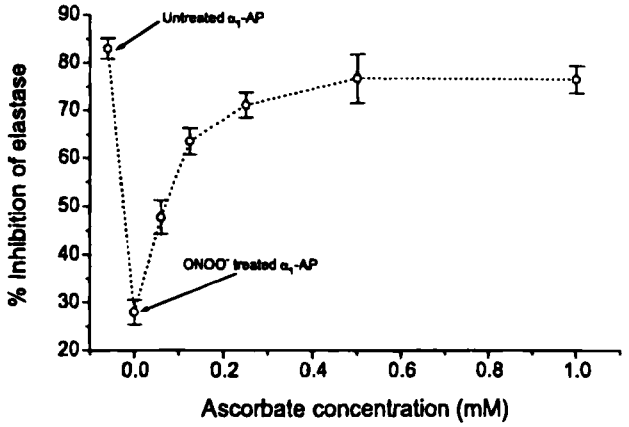

FIGURE 2 Protection by ascorbate against inactivation of α_1-antiproteinase by peroxynitrite. For details of experimental conditions see.[38] Ascorbate was present at the final concentrations stated, peroxynitrite was 0.5 mM.

severe damage when grown aerobically[84] and damage can be minimized by introducing a gene coding for SOD, even mammalian CuZnSOD[85]. These and other experiments illustrate the physiological antioxidant role of SOD. For ascorbate, the effects of dietary depletion can be studied in experimental animals such as the guinea-pig, or a mutant rat strain (ODS rats) that is unable to synthesize ascorbate[86,87]. Surprisingly little work has been reported in which "state of the art" parameters of oxidative damage[88] were measured in such animals in relation to ascorbate intake, although studies of ascorbate-vitamin E interactions have been carried out in guinea pigs[3]. An early study on guinea pigs showed that a vitamin C-deficient diet led to increased exhalation of pentane and ethane, suggestive of increased lipid peroxidation *in vivo*[89]. Unfortunately, the validity of such hydrocarbon measurements as an index of lipid peroxidation has repeatedly been questioned[90,91]. Vitamin C intake (in the range 150–900 mg/kg diet) did not appear to affect lipid peroxidation in ODS rats, as measured by a specific assay[13].

If ascorbate is really acting as an antioxidant *in vivo* is it depleted under conditions of oxidative

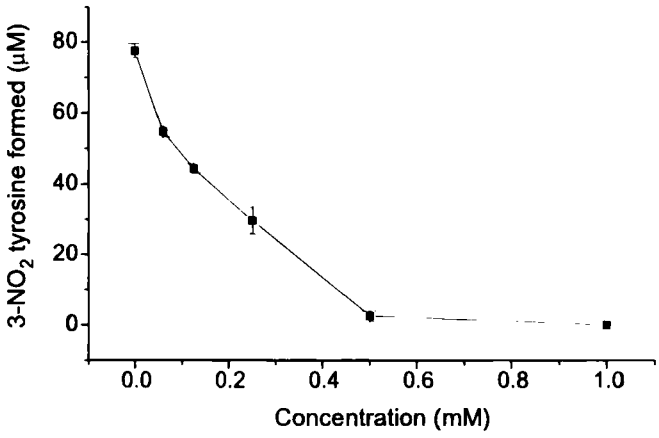

FIGURE 3 Protection by ascorbate against nitration of tyrosine by peroxynitrite. For details of experimental conditions see.[38] Peroxynitrite and tyrosine were 1.0 mM and ascorbate was present at the final concentrations stated.

stress? The answer seems to be "yes". Thus ascorbic acid becomes oxidized to dehydroascorbate in synovial fluid in the knee-joints of patients with active rheumatoid arthritis[92,93]. Presumably ascorbate is acting to scavenge ROS/RNS (Table III, Figures 2 and 3) derived from the many activated phagocytes present. Ascorbate is also oxidized in the body fluids of patients with adult respiratory distress syndrome, in whom there is often massive infiltration and activation of neutrophils in the lung[94]. This loss of ascorbate would at first sight seem unexpected, since enzymic systems exist *in vivo* to reduce ascorbate radical back to ascorbate at the expense of NADH (the NADH-semidehydroascorbate reductase enzyme) or of GSH (the dehydroascorbate reductase enzyme) (reviewed in refs. 2,95). However, these enzymes seem to be largely intracellular and so ascorbic acid is rapidly depleted in human extracellular fluids under conditions of oxidative stress, presumably by the reactions

$$\text{ascorbate} \xrightarrow{\text{radical attack}} \text{ascorbate radical} \quad (3)$$

$$2\ \text{ascorbate radical} \xrightarrow{\text{disproportionation}} \text{ascorbate} + \text{dehydroascorbate} \quad (4)$$

$$\text{dehydroascorbate} \xrightarrow[\text{breakdown}]{\text{rapid non-enzymic}} \text{oxalate, threonate, other oxidation products} \quad (5)$$

Glutathione deficiency in newborn rats and in guinea pigs is lethal, but death can be prevented by high doses of ascorbate. The onset of scurvy in guinea pigs fed a diet low in ascorbate is delayed substantially by GSH precursors. Hence there is evidence for interactions between GSH and ascorbate *in vivo*[96].

Can oxidation products of ascorbate be measured under conditions of oxidative stress? Direct epr measurement of ascorbate radical has given promising results which are, in general, consistent with oxidation of ascorbate at sites of oxidative stress[25,97,98].

RELEVANCE OF PRO-OXIDANT EFFECTS: IS ASCORBATE TOXIC TO HUMANS?

It should perhaps first be noted that *in vitro* pro-oxidant effects are not unique to ascorbate; they can be demonstrated with many reducing agents, including GSH and NAD(P)H[99–102]. In the context of dietary antioxidants, there is considerable current interest in the antioxidant effects of plant phenolics (e.g. in wine[103]), such as the flavonoids[103–105]. However, several plant phenolics can be made to exert pro-oxidant effects *in vitro*. Often, they inhibit lipid peroxidation but, when mixed with iron or copper ions, they can damage other biological molecules, including DNA and proteins, *in vitro* (e.g. refs. 105–113). So if ascorbate's pro-oxidant effects are relevant *in vivo*, the pro-oxidant effects of these other reductants might also be expected to occur.

Medical and lay interest in "optimal" ascorbate intakes was raised by claims that that megadoses (10g per day or more) can protect against the common cold and can be used in the treatment of advanced human cancer (reviewed in ref. 114). The anti-cancer effect[114,115] was never independently confirmed[116]. Indeed, perusal of one of the original papers[115] reveals the worrying observation that four cancer patients died of haemorrhagic tumour necrosis soon after vitamin C treatment was started. Vitamin C does not cure the common cold or prevent its occurrence; debate continues as to whether there is a small effect on duration of cold episodes[117–119].

Several reports have appeared about alleged toxic effects of repeated high dose vitamin C (Table IV). Even if these are correct, they are not necessarily related to pro-oxidant effects. In any case, data are limited and documentation is often inadequate (many letters and case reports, no full papers). For example, it is often said that increased vitamin C intake predisposes to kidney stones, and that people who stop taking large doses of ascorbate become scorbutic, but I have been unable to find detailed literature documentations of either phenomenon. Ascorbate can

TABLE IV Toxic Effects of Vitamin C

Stomach cramps, nausea, diarrhoea (only with multi-gram doses) (reference[118] and many personal communications).
Possibility of sodium overload (if large amounts of sodium salt taken) or acidosis (if large amounts of free acid taken) - no clear literature documentations of such effects.
Increased risk of oxalosis, perhaps leading to kidney stones[120].
"Rebound effect", cessation of mega-doses leading to very low ascorbate levels[121].
Serious cardiovascular disturbances when excess ascorbic acid given to iron-overloaded patients[122,123].
Ability to glycate proteins, e.g. lens crystallins[124-126].
Various anecdotal reports of haemolytic events (e.g. in paroxysmal nocturnal haemoglobinuria[127] and glucose-6-phosphate dehydrogenase deficiency[128,129]. These studies used iv infusion of high levels of ascorbate[128], or implicated ascorbate in foods/drinks without evidence that ascorbate was responsible for the observed effect[129].

modify proteins *in vitro* by glycation-type reactions, which probably involve transition metal ions, especially copper (S. P. Wolff, personal communication). Such reactions have been suggested to contribute to the modification of lens proteins and development of cataract[125,126], but epidemiological evidence suggests that, if anything, ascorbate is protective against cataract development[130,131].

The author feels that there is no convincing evidence for toxic effects of ascorbate in healthy people. However, the possibility of pro-oxidant effects should not be dismissed lightly. There is good evidence for an ongoing background level of oxidative damage to DNA, lipids and proteins in the human body (reviewed in[88]), and the pattern of damage to DNA bears the hallmark of attack by $OH^{\bullet}$[132]. Stadtman *et al*[133,134] have argued that much *in vivo* oxidative protein damage involves metal ion-dependent $OH^{\bullet}$ generation, a process which can be accelerated by ascorbate *in vitro*. Ames *et al*[135] and Totter[136] propose that oxidative damage to DNA is a major contributor to the age-related increase in the development of human cancer. If they are right, even a small rise in $OH^{\bullet}$ generation over a lifetime could increase the incidence of cancer. So we must be very sure that ascorbic acid is really safe before proposing large intakes on a regular basis. Indeed, patients with iron overload consequent upon idiopathic haemochromatosis show increased incidences of hepatoma, as well as diabetes and chronic joint inflammation[137].

The Key Question: Availability of Transition Metal Ions

Are the pro-oxidant effects of ascorbate (and of other reducing agents such as plant phenolics) relevant *in vivo*? In the authors's opinion the key factor is the availability of "catalytic" transition metal ions. This relates to another important nutritional question: what is the optimal intake of iron and copper? Iron is essential for human health, especially in children and pregnant women, but could too much iron intake cause harm, either in the body or in the colon (where unabsorbed excess dietary iron/copper will end up)? Considerable research in recent years has shown that, in the healthy human body, iron and copper ions appear to be largely sequestered in forms unable to catalyse free radical reactions (reviewed in refs. 17,138). Hence, for example, human plasma appears to have no non-protein-bound transition metal ions catalytic for free radical reactions[17,138]. Thus the pro-oxidant properties of ascorbate (and any dietary plant phenolics that are absorbed through the gut) would not be expected to be biologically significant in plasma at least.

But is it possible that pro-oxidant effects do occur in cells and are simply masked by the dominant antioxidant effects of ascorbate? If so, how would this balance be affected by raising ascorbate levels? Metals cannot always be kept sequestered: within cells they must leave ferritin and travel to the proteins that require them. Our ignorance of the chemical nature of the "low molecular mass intracellular iron pool" and its putative ability to catalyze free radical damage is profound[138]. We do know that free radical damage to lipids, proteins and DNA can be demonstrated to occur in the human body (reviewed in ref. 88).

We might perhaps learn something by looking at pathological situations. It has been said that twice as many adult men in the USA have haemochromatosis as have real iron-deficiency anaemia[139,140] and the prevalence of iron overload due to homozygous haemochromatosis in apparently-healthy Australians was about 1 in 300[141]. Values for homozygous haemochromatosis in populations of Northern European origin now living in different countries range from 1 in 86 to 1 in 1351, with an average of 1 in 200 to 1 in 400 (reviewed in[142]). Patients with iron overload resulting from haemochromatosis or other disorders have non-transferrin-bound iron in their plasma and tissues that appears capable of catalyzing free radical reactions[143–148] and parameters consistent with oxidative damage are increased in these patients[146,149,150]. There are several published "short reports" that giving vitamin C to iron-overloaded subjects without administration of an iron chelating agent (such as desferal) can produce deleterious clinical effects[122,123,151]. Iron-overloaded patients have sub-normal plasma ascorbate levels[149,152,180]; one should probably not even try to "correct" this without bringing the iron overload under control. The 1992 management protocol for the treatment of thalassaemia patients published by the Cooley's Anemia Foundation (New York) states "Vitamin C increases the availability of iron and so may increase its toxicity if large doses are taken without simultaneous Desferal® infusion. Therefore the following precautions are recommended

a. Start treatment with vitamin C only after an initial month of treatment with Desferal®.
b. Give vitamin C supplements only if the patient is receiving Desferal® regularly.
c. Do not exceed a daily dose of 200 mg.

However, should the prevalence of haemochromatosis be an argument for minimizing the ascorbate intake of the general population? A simple solution to the problem of haemochromatosis would be if screening for the haemochromatosis gene (or checking of blood iron status, e.g. by measurement of serum ferritin levels or percentage transferrin saturation) became a part of routine medical examinations. Such screening may be justified by the devastating consequences of prolonged iron overload (e.g. hepatoma) and the ease with which it can be prevented or treated if caught early[137,142,153].

Tissue Injury and Metal Ion Release

A second caveat is that injury to human tissues, by any one of a number of causes, causes increased availability of "catalytic" transition metal ions (reviewed in ref. 154). This has been widely demonstrated, e.g. in humans suffering from brain injury (reviewed in ref. 154), subjected to cardiopulmonary by-pass[155], in liver failure[156], suffering from rheumatoid arthritis[157], in cancer patients given chemotherapy[158–160] and in premature babies[161–164]. It is interesting that the first trial claiming to rebut Pauling's early work on anti-cancer effects of mega-dose vitamin C involved patients who had received chemotherapy; it is not impossible that iron overload could have negated any benefits of the administered ascorbate. However, a later trial avoided this problem (reviewed in ref. 116) and the result was still negative.

As we all get older, we get sicker. In advanced human atherosclerotic lesions, metal ions catalytic for free radical reactions can be measured[66,67]: indeed, the lesion contents will *stimulate* $OH^{\bullet}$ formation in the presence of H_2O_2 and ascorbate *in vitro*[66]. There are repeated (but controversial) *suggestions*[74,165–168] that high body iron and/or copper[169] stores are associated with increased risk of cancer and cardiovascular disease. Could this be because the more iron or copper is in a tissue, the more is potentially mobilizable to catalyze free radical reactions after an injury[154,170]? If this is so, then the pro-oxidant effects of ascorbate might conceivably be aggravated. Indeed, it has been argued that the decline in ascorbate at the onset of many oxidative stresses is beneficial[17,138,154], first because the

ascorbate is helping to scavenge radicals and recycle α-tocopherol, and second that ascorbate removal minimizes its potential pro-oxidant interactions with metal ions released by tissue damage. Thus it is *possible* that giving lots of ascorbate to sick people may not be a good thing. This is pure speculation however: there is no *in vivo* evidence as yet. Perhaps any pro-oxidant properties of ascorbate under these circumstances are still outweighed by its antioxidant effects.

Another question often asked is whether ascorbate could favour excessive uptake of iron into the human body, since the reduction of ferric ions to Fe^{2+} by ascorbate is believed to facilitate iron uptake in the gut. There is no evidence to support this view in healthy subjects[171–173]; iron uptake appears tightly regulated whatever the ascorbate intake. However, the issue needs to be addressed in relation to haemochromatosis.

CONCLUSION

Ascorbate is essential in the human diet, but many unanswered questions remain. In healthy subjects, the RDA for ascorbate probably helps protect against various diseases, including stomach cancer, probably by protecting against nitrosamines. Gey *et al*[80,174] carefully reviewed several epidemiological studies and concluded that plasma concentrations of ~50 μM ascorbate are associated with decreased risk of cardiovascular disease. Such levels are easily achievable by diet alone. The studies of Fraga *et al*[175,176] showed that 60 mg/day of ascorbate seemed to be enough to normalize levels of oxidative DNA damage in sperm in previously-scorbutic human subjects. In sperm collected from human volunteers, only very low seminal fluid ascorbate levels were associated with elevated DNA damage. A high dietary intake of vitamin C appears not to be protective against breast cancer[177] and, apart from stomach cancer, many other studies are equivocal about the protective effects of ascorbate[177,178,181]. In the Linxian study[179], supplementation of a Chinese population with molybdenum plus vitamin C at doses about twice the USA RDA (120 mg, 30 μg) showed no evidence of a reduction in cancer incidence or mortality.

In summary, there is evidence that oxidative damage to DNA, proteins and lipids occurs in the human body[88], but no evidence that pro-oxidant effects of ascorbate are responsible (although we cannot prove that they are not). Much more research is needed in which "state of the art" parameters of oxidative damage are measured in animals and humans in relation to intake of ascorbate and other antioxidants. No clearly-documented toxicities of even mega-dose ascorbate seem to exist, except perhaps in iron-overload diseases (even there the evidence is limited)[180]. What data are available on optimal ascorbate intakes suggest, however, that there may be no extra benefit from large intakes. The author recommends a diet with plenty of fruits and vegetables and avoiding smoking (which is known to deplete ascorbate). Dietary supplementation with ascorbate (if any) should use only amounts close to the RDA.

Acknowledgements

I am very grateful to MAFF (UK) for research support.

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