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ANTIOXIDANTS IN HUMAN HEALTH AND DISEASE

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ABSTRACT

Free radicals and antioxidants are widely discussed in the clinical and nutritional literature. Antioxidants are needed to prevent the formation and oppose the actions of reactive oxygen and nitrogen species, which are generated in vivo and cause damage to DNA, lipids, proteins, and other biomolecules. Endogenous antioxidant defenses (superoxide dismutases, H_2O_2-removing enzymes, metal binding proteins) are inadequate to prevent damage completely, so diet-derived antioxidants are important in maintaining health. Many dietary compounds have been suggested to be important antioxidants: The evidence for a key role of vitamins E and C is strong, but that for carotenoids and related plant pigments is weaker. Interest is also growing in the role of plant phenolics, especially flavonoids. Some antioxidants can exert prooxidant effects in vitro, but their physiological relevance is uncertain. Experimental approaches to the optimization of antioxidant nutrient intake are proposed.

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INTRODUCTION

Free radicals and antioxidants are widely discussed in the clinical and nutritional literature and lay press. The assumption often is that free radicals are bad and antioxidants are good. By contrast, recent clinical trials suggest that giving the "antioxidant" β-carotene to smokers accelerated the development of lung cancer (1, 99a). This article provides an overview of our current knowledge.

WHY DO WE NEED ANTIOXIDANTS?

The first living organisms appeared on earth when its atmosphere was low in O_2, making them essentially anaerobes. Today, anaerobic microorganisms still survive but their growth is inhibited and they can often be killed by exposure to 21% O_2, the current atmospheric level (6). As the O_2 content of earth's atmosphere rose (from evolution of photosynthetic organisms that used light energy to split water), many species must have died out. Presumably, current-day anaerobes are the descendants of those primitive organisms that coped with rising atmospheric O_2 by restricting themselves to environments O_2 did not penetrate. Other organisms existed, however, that began to evolve antioxidant defense systems to protect against O_2 toxicity, a more fruitful path in retrospect, because organisms that tolerated O_2 could also evolve to use it for metabolic transformations (e.g. oxidase and hydroxylase enzymes, such as cytochromes P450) and for efficient energy production using electron transport chains with O_2 as the terminal electron acceptor. The mitochondrial electron transport chain generates over 80% of the adenosine triphosphate (ATP) needed by aerobic cells, and the lethal effects of such inhibitors as cyanide show how important this activity is to humans.

Aerobes have evolved antioxidant defenses to protect themselves against 21% O_2 only; higher levels injure them (6). For example, the incidence of retinal damage, extending sometimes to blindness, increased abruptly in the early 1940s among premature infants (34). Not until 1954 was it realized that this "retinopathy of prematurity" is associated with the use of high O_2 concentrations in incubators. More careful control of O_2 concentrations (continuous transcutaneous O_2 monitoring, with supplementary O_2 given only as necessary) and administration of the lipid-soluble antioxidant α-tocopherol to babies have decreased the incidence of retinopathy. Unfortunately, the problem has not disappeared, as many premature infants need high levels of O_2 to survive (34).

The earliest suggestion made to explain O_2 toxicity was that O_2 is a direct

inhibitor of metabolically important enzymes (6). However, few targets of direct attack by O_2 in aerobes have been identified. In 1954, Gerschman et al (43) proposed that the damaging effects of O_2 could be attributed to the formation of oxygen radicals. This hypothesis was popularized and converted into the "superoxide theory of O_2 toxicity" after the discovery of a class of enzymes, superoxide dismutases (SODs) (41), that appear specific for catalytic removal of superoxide free radical, $O_2^{\bullet -}$. [A free radical is any species capable of independent existence (hence the term free) that contains one or more unpaired electrons (57), i.e. electrons alone in an atomic or molecular orbital. A superscript dot denotes a free radical.] In its simplest form, the superoxide theory states that O_2 toxicity is due to excess formation of $O_2^{\bullet -}$ and that the SOD enzymes are major antioxidant defenses (41).

REACTIVE OXYGEN AND NITROGEN SPECIES

Antioxidants are needed to scavenge and prevent the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Table 1). Some of these species are free radicals whereas others are not.

What ROS/RNS Are Made in the Human Body?

Humans are exposed to radiation from the environment, both natural (e.g. radon gas, cosmic radiation) and from man-made sources. Low-wavelength electromagnetic radiation (e.g. gamma rays) can split water in the body to generate hydroxyl radical, $OH^{\bullet}$ (118). Ultraviolet light is insufficiently energetic to split H_2O, but it can cleave the O-O covalent bond in H_2O_2 to give $2OH^{\bullet}$. The viciously reactive $OH^{\bullet}$, once generated, attacks whatever is next to it, i.e. it reacts at its site of formation. Thus, it is difficult to evolve (or design) a scavenger that removes $OH^{\bullet}$ in vivo. Many $OH^{\bullet}$ scavengers described in the literature have high rate constants (often $>10^{10}$ M^{-1} sec^{-1}) for reaction with $OH^{\bullet}$, but most endogenous molecules react equally fast. Examples are albumin [rate constant $> 10^{10}$ M^{-1} sec^{-1} (106)] and glucose (rate constant $\sim 10^9$ M^{-1} sec^{-1}, but present at millimolar concentrations in body fluids). The antioxidant systems that defend against damage by $OH^{\bullet}$ do so by preventing its formation and repairing the damage it causes.

The human body also makes $O_2^{\bullet -}$ (41). Some is made by accident of chemistry. Many molecules in the body can react with O_2 to make $O_2^{\bullet -}$. Examples are adrenaline, dopamine, tetrahydrofolates, and some components of mitochondrial and P450 electron transport chains (41). Such $O_2^{\bullet -}$ generation is the unavoidable consequence of having these molecules in a body that needs oxygen (41, 55). In addition, some $O_2^{\bullet -}$ is made deliberately. For example, the phagocytes (neutrophils, monocytes, macrophages, eosinophils) that de-

Table 1 Antioxidants that affect reactive oxygen species (ROS) and reactive nitrogen species (RNS)[a]

	Radicals	Nonradicals
ROS	Superoxide, $O_2^{\bullet -}$	Hydrogen peroxide, $H2O_2$
	Hydroxyl, $OH^{\bullet}$	Hypochlorous acid, HOCl
	Peroxyl, $RO_2^{\bullet}$	Ozone, O_3
	Alkoxyl, $RO^{\bullet}$	Singlet oxygen $^1\Delta g$
	Hydroperoxyl, $HO_2^{\bullet}$	
RNS	Nitric oxide, $NO^{\bullet}$	Nitrous acid, HNO_2
	Nitrogen dioxide, $NO_2^{\bullet}$	Dinitrogen tetroxide, N_2O_4
		Dinitrogen trioxide, N_2O_3
		Peroxynitrite, $ONOO^-$
		Peroxynitrous acid, ONOOH
		Nitronium cation, NO_2^+
		Alkyl peroxynitrites, ROONO

[a] ROS is a collective term that includes both oxygen radicals and certain nonradicals that are oxidizing agents and/or are easily converted into radicals (HOCl, O_2, $ONOO^-$, $^1O_2$, H_2O_2). RNS is also a collective term that includes nitric oxide and nitrogen dioxide radicals, as well as such nonradicals as HNO_2 and N_2O_3. $ONOO^-$ 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 very few molecules, whereas $OH^{\bullet}$ reacts quickly with almost everything. $RO_2^{\bullet}$ $RO^{\bullet}$ HOCl, $NO_2^{\bullet}$, $ONOO^-$, and O_3 have reactivities intermediate between these extremes.

fend against foreign organisms generate large amounts of $O_2^{\bullet -}$ as part of their killing mechanism (5). This essential defense mechanism can go wrong: Several diseases (such as rheumatoid arthritis and inflammatory bowel disease) are accompanied by excessive phagocyte activation, leading to tissue damage, to which ROS and RNS contribute (5, 57). It has been estimated that 1–3% of the oxygen we breathe in is used to make $O_2^{\bullet -}$ (41). Since humans consume large quantities of O_2, a simple calculation (55) shows that over 2 kg of $O_2^{\bullet -}$ is made in the human body every year—people with chronic inflammations may make much more.

Another physiologic free radical is nitric oxide, $NO^{\bullet}$. It has many useful functions, such as regulation of blood pressure (86), but too much $NO^{\bullet}$ (like too much $O_2^{\bullet -}$) is toxic: Excess $NO^{\bullet}$ production is thought to be an important tissue injury mechanism in such conditions as chronic inflammation, stroke, and septic shock (86).

Hydrogen Peroxide, a Nonradical

Much of the $O_2^{\bullet -}$ generated in vivo undergoes a nonenzymatic or SOD-catalyzed reaction to generate the nonradical H_2O_2 (41):

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2. \quad 1.$$

H_2O_2, like H_2O, easily diffuses within and between cells (57). Indeed, some fish may dispose of H_2O_2 by allowing it to diffuse through the gills into the surrounding water (121). As well as arising from $O_2^{\bullet -}$, H_2O_2 can be directly produced by several oxidase enzymes, including monoamine and amino acid oxidases (22). Xanthine oxidase converts hypoxanthine to xanthine and xanthine to urate, O_2 being simultaneously reduced to both $O_2^{\bullet -}$ and H_2O_2 (49). Levels of xanthine oxidase are normally low in human tissues, but they may increase after injury such as trauma or ischemia (16, 49).

Some metabolic roles for H_2O_2 are known, and others have been proposed. For example, H_2O_2 generated by a Ca^{2+}-dependent reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in the thyroid is used by a peroxidase enzyme to iodinate aromatic rings during thyroid hormone biosynthesis (30). H_2O_2 can regulate gene expression: One mechanism is by (directly or indirectly) displacing an inhibitory subunit from the cytoplasmic gene transcription factor NF-κB (101) [at least in some cell types (15)]. The activated factor then migrates to the nucleus and causes expression of multiple genes. Other peroxides (such as lipid peroxides) can do the same (25). In some HIV-infected cells, H_2O_2 activates NF-κB and induces HIV expression (101).

HOW DO RADICALS REACT?

If two free radicals meet, they can join their unpaired electrons to form a covalent bond; the product is a nonradical. A biologically relevant example is the fast (64) reaction of $NO^{\bullet}$ and $O_2^{\bullet -}$ (7):

$$NO^{\bullet} + O_2^{\bullet -} \rightarrow ONOO^- \text{ (peroxynitrite).} \quad 2.$$

However, when a radical reacts with a nonradical, a new radical results (radicals beget radicals), and a chain reaction can occur (57). Because most biological molecules are nonradicals, the generation of reactive radicals such as $OH^{\bullet}$ in vivo often initiates chain reactions. For example, their attack upon fatty acid side chains in membranes and lipoproteins can initiate the chain reaction of lipid peroxidation (57). Lipid peroxidation contributes to the development of atherosclerosis (109).

If $OH^{\bullet}$ is generated adjacent to DNA, it attacks both the deoxyribose sugar and the purine and pyrimidine bases, forming multiple products (33). This complex "chemical fingerprint" appears diagnostic of an attack by $OH^{\bullet}$ and may be used as a fingerprint to show that DNA has suffered such an attack in vivo (56). Products of an attack by ROS (including $OH^{\bullet}$) and RNS are present at low levels in DNA from healthy human tissues (56), i.e. ROS and

RNS attack DNA in vivo even in healthy people. The $OH^{\bullet}$ must be made in the nucleus because it reacts at its site of formation (56).

The amounts of end products of $OH^{\bullet}$ attack on DNA are greater in DNA isolated from cancerous tumors (81, 91), perhaps because of increased $OH^{\bullet}$ formation. In healthy cells, repair enzymes remove damaged bases from DNA constantly (31) and an alternative possibility is that repair is less efficient in cancer cells. The existence of these important repair systems is further evidence that DNA is assaulted by ROS/RNS in vivo.

TOXICITY OF SUPEROXIDE, HYDROGEN PEROXIDE, AND NITRIC OXIDE

Removal of $O_2^{\bullet-}$ and other ROS by antioxidant defenses appears essential for aerobic life (22, 41, 57, 112). Why? In organic media $O_2^{\bullet-}$ can be very reactive, but in aqueous media it is not (41, 57). However, $O_2^{\bullet-}$ does react rapidly with a few molecules, including bacterial iron-sulfur proteins, such as the enzyme aconitase (40). Whether or not similar $O_2^{\bullet-}$-sensitive targets exist in human cells remains to be established, although $O_2^{\bullet-}$ may be able to attack the reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase complex of the mitochondrial electron transport chain (124).

Another important exception to the unreactivity of $O_2^{\bullet-}$ is its very fast combination with $NO^{\bullet}$. In general, $NO^{\bullet}$ is poorly reactive with nonradicals (86), but it reacts quickly with $O_2^{\bullet-}$ and several other radicals (28, 64, 78). Excess $NO^{\bullet}$ is cytotoxic, both directly, e.g. by combining with a tyrosine radical essential for the catalytic function of the enzyme ribonucleoside diphosphate reductase (78), and indirectly, by forming $ONOO^-$ (28, 64, 86, 100). Since $NO^{\bullet}$ relaxes smooth muscle in blood vessel walls to lower blood pressure, then $O_2^{\bullet-}$, by removing $NO^{\bullet}$, can be a vasoconstrictor. Thus, excess vascular $O_2^{\bullet-}$ production could contribute to hypertension and vasospasm (77, 89). Peroxynitrite formed in blood vessel walls (8) may aggravate atherosclerosis by depleting antioxidants (7, 116) and causing peroxidation of low-density lipoproteins (LDL) (48). Nitration of aromatic amino acids (especially tyrosine) by $ONOO^-$ can interfere with cell signal transduction (8, 28, 64). Both thiols and ascorbate can protect against damage by peroxynitrite (7, 116).

H_2O_2 is also poorly reactive, but high (>50 μM) levels can attack certain cellular targets (65). For example, H_2O_2 interferes with ATP production by several mechanisms, including oxidizing an essential –SH group on the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase.

Much of the toxicity of $O_2^{\bullet-}$ and H_2O_2 involves formation of $OH^{\bullet}$ (41, 57, 58). For example, addition of excess H_2O_2 to mammalian cells causes DNA strand breakage and base modification (56, 65); the pattern of the latter is characteristic of $OH^{\bullet}$ attack (33). Three mechanisms have been proposed to

explain $OH^{\bullet}$ formation from $O_2^{\bullet-}$ and H_2O_2. One is via $ONOO^-$, which can decompose to generate a species that resembles $OH^{\bullet}$ (7, 115). An earlier proposal was the superoxide-driven Fenton reaction

$$O_2^{\bullet-} + Fe(III) \rightarrow Fe(II) + O_2, \quad 3.$$
$$Fe(II) + H_2O_2 \rightarrow OH^{\bullet} + OH^- + Fe(III). \quad 4.$$

Copper ions also catalyze formation of $OH^{\bullet}$ from $O_2^{\bullet-}$ and H_2O_2 (58). A third mechanism is the reaction of $O_2^{\bullet-}$ with hypochlorous acid (21), an antibacterial ROS generated by activated neutrophils (5),

$$O_2^{\bullet-} + HOCl \rightarrow OH^{\bullet} + OH^- + O_2. \quad 5.$$

Iron and copper ions are not only damaging because they catalyze $OH^{\bullet}$ formation, but also because they accelerate autoxidation reactions and lipid peroxidation (58). They decompose lipid hydroperoxide into peroxyl and alkoxyl radicals that can efficiently recruit new fatty acid side chains into the peroxidation cycle (58). Copper is especially effective in promoting LDL oxidation (37).

Do Metal Ion Catalysts of Free Radical Reactions Exist In Vivo?

Fortunately, iron and copper ions in chemical forms that can do these nasty things are in short supply in vivo (59). The body contains a system of transport and storage proteins that ensures that as much iron and copper as possible is safely sequestered in noncatalytic forms (58).

Escherichia coli mutants lacking SOD are hypersensitive to damage by H_2O_2 (112), and intracellular SOD protects hepatocytes against damage by H_2O_2 (74). These and much other data are consistent with a role of $O_2^{\bullet-}$ in facilitating damage by H_2O_2, and Reactions 3 and 4 provide one explanation why. Additionally, $O_2^{\bullet-}$ may help to provide the catalytic metal ions. Thus, $O_2^{\bullet-}$ can release iron ions from ferritin (12). Because the amount of $O_2^{\bullet-}$-releasable iron is small (14), ferritin-bound iron is still much safer than an equivalent amount of iron ions. Superoxide might also release iron during its attack on iron-sulfur proteins (40). Hydrogen peroxide releases iron from the heme rings of myoglobin, cytochrome *c*, and hemoglobin (52, 61), and $ONOO^-$ displaces copper from the major copper-containing plasma protein, ceruloplasmin (110). Perhaps this is a source of copper for LDL oxidation in human atherosclerotic lesions (105).

ANTIOXIDANT DEFENSES

All organisms suffer some exposure to $OH^{\bullet}$ because it is generated during the splitting of water, driven by environmental radiation (118). Once $OH^{\bullet}$ forms,

damage is probably unavoidable and must be dealt with by repair systems, such as DNA repair enzymes (31) and proteases that degrade abnormal (including free radical-damaged) proteins (50). Many antioxidant defenses serve to minimize production of $OH^{\bullet}$ by other mechanisms in vivo.

ANTIOXIDANTS SYNTHESIZED IN THE HUMAN BODY

Enzymes and Their Substrates

Superoxide dismutases remove $O_2^{\bullet -}$ by accelerating the rate of its dismutation (Equation 1) by about four orders of magnitude (41) at pH 7.4. Mammalian cells have a SOD enzyme containing active site manganese (MnSOD) in the mitochondria. A SOD with active site copper and zinc (CuZnSOD) is also present largely in the cytosol (41). The familial dominant form of amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease), a fatal degenerative disorder of motor neurons in the brain and spinal cord, is somehow related to mutations affecting CuZnSOD (98). These mutations usually decrease activity somewhat (98), but they may also somehow cause this normally protective protein to become toxic (51). One possibility is that the mutant enzymes are unstable and readily release copper, a powerful prooxidant (107).

SOD enzymes must collaborate with H_2O_2-removing enzymes. Catalases convert H_2O_2 to water and O_2 and are located in the peroxisomes of most mammalian cells, probably serving to destroy H_2O_2 generated by oxidases located within these organelles (22). However, it is likely that the major H_2O_2-removing enzymes in mammalian cells are the glutathione peroxidase (GSHPX) enzymes, which contain active site selenium and are involved not only in H_2O_2 removal (22, 114), but also in the metabolism of lipid peroxides (80, 114). Reduced glutathione, the substrate of GSHPX, may additionally exert direct antioxidant effects. Its reactions with $O_2^{\bullet -}$ and H_2O_2 are slow, but it reacts fairly fast with peroxyl radicals (118) and is a powerful scavenger of certain RNS, such as $ONOO^-$ (7, 28, 36).

Metal Ion Sequestration

An additional important antioxidant defense is the presence of metal ion storage and transport proteins (59), including metallothioneins (24). For example, iron in the two iron-binding sites of transferrin or lactoferrin will not catalyze free radical reactions (3). Sometimes sequestration is compromised. In a high percentage of preterm babies, and in a lower percentage of apparently normal full-term babies, transferrin is iron saturated and plasma contains iron that can catalyze damaging free radical reactions, such as lipid peroxidation and $OH^{\bullet}$ formation (38, 72, 85). Such babies could be at high risk of free radical damage if exposed to elevated O_2, to toxins generating $O_2^{\bullet -}$ and H_2O_2, or to infection (raising endogenous production of $O_2^{\bullet -}$ and H_2O_2).

DIETARY ANTIOXIDANTS

Established Antioxidants

VITAMIN E α-Tocopherol, the major constituent of the fat-soluble vitamin known as vitamin E, is the most important (18), but by no means the only (37, 70), chain-breaking antioxidant within membranes and lipoproteins. α-Tocopherol inhibits lipid peroxidation by scavenging peroxyl radical intermediates in the chain reaction (70):

$$\alpha TH + LOO^{\bullet} \rightarrow \alpha T^{\bullet} + LOOH \qquad 6.$$

The resulting α-tocopherol radical ($\alpha T^{\bullet}$), although not completely unreactive (88), is much less efficient at attacking fatty acid side chains than are peroxyl ($LOO^{\bullet}$) radicals, so the overall effect of α-tocopherol under physiologic conditions is usually to slow the chain reaction of lipid peroxidation (88). Several mechanisms may convert $\alpha T^{\bullet}$ back to α-tocopherol, none yet rigorously proved to operate in vivo. The most likely is the reaction of $\alpha T^{\bullet}$ with ascorbate (vitamin C) at the surface of membranes and lipoproteins (37, 88). Dietary vitamin E is important in slowing the development of atherosclerosis (reviewed in 19). In premature babies, vitamin E supplementation diminishes the risk and/or severity of retinopathy of prematurity and intracranial hemorrhage (34, 39). Severe deprivation of vitamin E, e.g. in patients with defective intestinal fat absorption, produces neurodegeneration (113).

VITAMIN C Ascorbate may have several antioxidant actions in vivo (9) in addition to its putative ability to regenerate α-tocopherol. In the respiratory tract, it may react rapidly with such air pollutants as O_3, cigarette smoke, and $NO_2^{\bullet}$ (27). Unlike vitamin E, vitamin C has several well-established other metabolic roles (e.g. as a cofactor for such enzymes as proline, lysine, and dopamine-β-hydroxylases).

Less Well-Established Dietary Antioxidants

Many other dietary constituents have been proposed to be important free radical scavengers in vivo, but the evidence is not conclusive as yet (Table 2).

Despite all these endogenous and dietary antioxidants, some ROS/RNS escape to do damage in the human body. Thus, DNA undergoes constant oxidative damage and must be repaired (31). Free radicals attack proteins, and the damaged products are degraded (50). End products of lipid peroxidation, e.g. isoprostanes (87), are measurable in human body fluids, in atherosclerotic lesions (109), and in "age pigments" (57). End products of DNA damage are

Table 2 Dietary antioxidants, fact and fiction[a]

Putative antioxidant	Status
Vitamin E	Essential antioxidant in humans (19, 44)
Vitamin C	Multiple metabolic roles, antioxidant action only one of its effects
β-Carotene, other carotenoids, related plant pigments	Epidemiologic evidence that high body levels are associated with diminished risk of cancer and cardiovascular disease, particularly in smokers (e.g. 73, 96). Often simplistically grouped with vitamins E and C as antioxidant nutrients. Although many carotenoids have been claimed to exert antioxidant events in vivo under certain conditions (32, 47, 73, 82, 84) [although sometimes questionable assays have been used such as plasma TBARS (57) and pentane exhalation (20)], it is not yet proved that any protective effects they exert against human disease are due to antioxidant action (42). Conversion to retinoids and/or effects on cell communication may be equally or more important (11, 90, 119). Many of the apparent protective effects of carotenoids (e.g. 97), as well as reports of deleterious effects (1, 99a), involve smokers, but β–carotene did not decrease the elevated urinary excretion of 8-hydroxydeoxyguanosine, a putative index of oxidative DNA damage, in smokers (117).
Flavonoids, other plant phenolics, wine phenolics	Many plant phenols inhibit lipid peroxidation and lipoxygenase enzymes in vitro, e.g. flavonoids (71, 76, 108), and may be important dietary antioxidants (29, 62). It has been speculated that flavonoids in red wine could explain the "French paradox" (71). Some phenolics are pro-oxidant in vitro if mixed with copper or iron ions (63, 76, 95). More data are needed on absorption and bioavailability of phenolics, but there is evidence that wine and tea phenolics can be absorbed (23, 29, 45, 62, 95, 120). Plant phenols might scavenge RNS, e.g. preventing tyrosine nitration by $ONOO^-$, but the biological properties of any resulting nitroso/nitro phenolics must be considered (75).

[a] A diet rich in fruits, nuts, grains and vegetables is protective against several human diseases. This may be due to the antioxidants they contain and/or to the many other compounds present (13, 68, 122, 123).

present at low steady state levels in DNA from human tissues and are excreted in urine (2, 56, 79, 117).

OXIDATIVE STRESS

Because production of ROS and antioxidant defenses are approximately balanced in vivo, it is easy to tip this balance in favor of the ROS and create the situation of oxidative stress (103). Oxidative stress may occur in several ways. (*a*) Inadequate diet-derived antioxidants is one possibility. Malnutrition may lead to inadequate dietary intake of α-tocopherol, ascorbic acid, sulfur-containing amino acids [needed for reduced glutathione (GSH) synthesis], or riboflavin

[needed to make the flavin adenine dinucleotide (FAD) cofactor of glutathione reductase] (46). Lack of dietary protein may lead to inadequate synthesis of metal ion binding proteins (46). (*b*) Another possibility is excess production of $O_2^{\bullet-}$ and H_2O_2, e.g. by exposure to drugs or toxins that are metabolized to produce free radicals, or by excessive activation of "natural" radical-producing systems (e.g. phagocytes in chronic inflammatory diseases) (57).

Cells often tolerate mild oxidative stress by up-regulating synthesis of antioxidant defense systems in an attempt to restore the balance (e.g. 66). However, severe oxidative stress produces DNA damage, rises in intracellular free Ca^{2+} and iron, damage to proteins (including membrane ion transporters), and lipid peroxidation. Cell injury and death may result (57, 60, 92).

OXIDATIVE STRESS AND HUMAN DISEASE—PREVENTION BY DIET?

Damaged tissues undergo more free radical reactions than healthy ones (53, 60). In most human diseases, oxidative stress is a secondary phenomenon, not the primary cause of the disease (53). This does not mean that oxidative stress is unimportant: For example, secondary oxidative damage to lipids in blood vessel walls is a significant contributor to the development of atherosclerosis, and low dietary vitamin E intake is a risk factor. Dietary vitamin E requirement is probably raised if the percentage of polyunsaturated fatty acids in the diet is increased, a phenomenon well-known in animals but not yet fully explored in humans. DNA damage by ROS and RNS probably contributes to the age-related development of cancer (2, 111). Oxidative stress contributes to tissue damage in rheumatoid arthritis (57), inflammatory bowel diseases (57), and Parkinson's disease (67).

Evidence is growing that the major killers, cardiovascular disease and cancer, can be prevented or delayed to some extent by dietary changes, such as reduction in fat intake and increased consumption of fruits, grains, and vegetables (13, 122). We obtain several compounds from a healthy diet that may act to diminish oxidative damage in vivo (Table 2). Since our endogenous antioxidant defenses are not 100% efficient, it is reasonable to propose that dietary antioxidants are important in diminishing the cumulative effects of oxidative damage over the long human lifespan, and that they account for some of the beneficial effects of fruits, grains, and vegetables. For example, if continuous free radical damage to DNA, perhaps not always efficiently repaired, is involved in the development of spontaneous cancers, then a good intake of dietary antioxidants should be preventative (2, 13). One should be careful about overemphasizing the role of antioxidants; plants contain a wide range of putative anticancer agents (e.g. 68, 123).

CAN ANTIOXIDANTS BE PRO-OXIDANT?

Although ascorbic acid has multiple antioxidant properties, it can be pro-oxidant in vitro in the presence of transition metal ions; mixing ascorbate with iron ions can cause $OH^{\bullet}$ generation and lipid peroxidation (57). Instillation of ascorbate with iron or copper ions into the stomach of animals led to $OH^{\bullet}$ generation (69, 104). A mixture of ascorbate and copper rapidly oxidizes DNA bases (4); cytotoxic and mutagenic effects of ascorbate on isolated cells have been described (102), probably involving its interaction with iron and/or copper ions added to (or contaminating) the culture media (57). This pro-oxidant effect of ascorbate is also well-known to food scientists (93). Several plant phenolics exert similar pro-oxidant effects in vitro (Table 2). Often they inhibit lipid peroxidation, but when mixed with iron or copper ions they can damage other biological molecules, including DNA and proteins (76, 106).

Are these pro-oxidant effects relevant in vivo? It presumably depends on the availability of catalytic metal ions. This relates to another important nutritional question: What is the optimal intake of iron? Iron is essential for human health, especially in children and pregnant women, but could too much iron intake cause harm (17)? In the healthy human body, metal ions appear largely sequestered in forms unable to catalyze free radical reactions. Hence, the antioxidant properties of ascorbate (and any plant phenolics that are absorbed) probably predominate over pro-oxidant effects.

There are two caveats. First, some apparently healthy people are not. Twice as many adult men in the United States have the inborn disease idiopathic hemochromatosis as have real iron-deficiency anemia (26, 35). Idiopathic hemochromatosis leads to iron overload, with iron catalytic for free radical reactions present in plasma (54). Giving vitamin C to iron-overloaded patients without an iron chelating agent (such as desferal) has produced serious side effects (83, 99). Similarly, there is considerable debate about the possible pro-oxidant effect of ascorbate in iron-overloaded premature babies (10, 94).

The second caveat is that injury to tissues can release iron and copper ions. For example, metal ions catalytic for free radical reactions have been measured in advanced human atherosclerotic lesions (105). There are repeated (although controversial) suggestions that high body iron and/or copper stores are associated with increased risk of cancer and cardiovascular disease (reviewed in 17). Could this be because the more iron or copper is in a tissue, the more could be liberated to catalyze free radical reactions after injury? If so, then the in vitro pro-oxidant effects of ascorbate and flavonoids might become physiologically (or pathologically) relevant.

CONCLUSION

Many unanswered questions remain (Table 2). Vitamin E seems protective against neurodegeneration, cardiovascular disease, retinopathy of prematurity, and possibly intra-cranial hemorrhage (in premature babies), but what is the optimal dietary intake? Does it depend on the polyunsaturated fatty acid content of the diet? Carotenoids may be important anticancer agents, but is this a result of antioxidant properties? Vitamin C may help protect against cardiovascular disease and some forms of cancer (e.g. stomach cancer), but could very high intakes do good or harm? Are the in vitro pro-oxidant effects of vitamin C and plant phenolics biologically relevant? Is ascorbate good for most healthy adults but bad for sick people? Would a high tissue level of antioxidants enable one to recover better from such traumas as stroke and open-heart surgery? Is there too much iron supplementation? Fortunately, experimental tools to answer these questions are now becoming available. Thus, methods exist for measuring both ongoing and steady-state (i.e. the balance between damage and repair) oxidative damage to DNA, proteins, and lipids in the human body (55). These methods may help us to gain information about optimal nutritional intakes. If the major killers, cancer and vascular disease, could be delayed for even a few years by dietary changes, the social and economic benefits would be enormous. The increasing evidence for involvement of oxidative stress in the pathology of neurodegenerative disease (67) also has exciting nutritional implications.

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