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The Role of Ascorbate in Antioxidant Protection of Biomembranes: Interaction with Vitamin E and Coenzyme Q

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One of the vital roles of ascorbic acid (vitamin C) is to act as an antioxidant to protect cellular components from free radical damage. Ascorbic acid has been shown to scavenge free radicals directly in the aqueous phases of cells and the circulatory system. Ascorbic acid has also been proven to protect membrane and other hydrophobic compartments from such damage by regenerating the antioxidant form of vitamin E. In addition, reduced coenzyme Q, also a resident of hydrophobic compartments, interacts with vitamin E to regenerate its antioxidant form. The mechanism of vitamin C antioxidant function, the myriad of pathologies resulting from its clinical deficiency, and the many health benefits it provides, are reviewed.

KEY WORDS: Vitamin C; ascorbic acid; vitamin E; coenzyme Q; antioxidant; free radicals; membrane; pathology; benefits; review.

"Now I must speak of all kinds of studies of the antiscorbutic factor. It is a thankless task because the number of research studies is so great that it is clearly impossible to review them all; moreover, by failure of agreement, they present such gaps that it is truly difficult to present a consistent thesis."

Mme. L. Randoin, 1923

INTRODUCTION

Ascorbic acid (vitamin C) was selected by biological systems during evolution to perform a large number of vital functions due to its ease of synthesis from D-glucose (Nishikimi and Yagi, 1991) and, based on its redox potential of +0.080, its efficient donation of reducing equivalents to a variety of compounds (Davies *et al.*, 1991). Vitamin C was discovered as an essential nutritional component in humans by its ability to prevent scurvy in individuals deficient in vitamin C (Lind, 1753) due to its function in the hydroxylation reactions of procollagen (Peterkofsky, 1991) and its regulation of collagen synthesis independent of hydroxylation (Houglum *et al.*, 1991). In addition to its vital function in connective tissue protein post-translational hydroxylation and carnitine synthesis (Rebouche, 1991; Ha *et al.*, 1991), vitamin C has been shown to facilitate iron absorption (Siegenberg *et al.*, 1991), regulate cholesterol synthesis (Harwood *et al.*, 1986) and elimination (Ginter, 1978, 1989; Ginter *et al.*, 1981), be involved in or of benefit to xenobiotic detoxification (Ginter, 1989; Gutierrez, 1988; Brodfuehrer and Zannoni, 1986; Matsushita *et al.*, 1993; Nagyova *et al.*, 1994), inhibit mutagenesis and carcinogenesis (Kyrtopoulos *et al.*, 1991; Licht *et al.*, 1988; Fukushima *et al.*, 1990; Mori *et al.*, 1988; Pienkowska *et al.*, 1985; Hasegawa *et al.*, 1990; Hayatsu *et al.*, 1988; Ghaskadbi *et al.*, 1992; Anderson and Theron, 1990; Carpenter, 1991; Krinsky, 1993; Wise *et al.*, 1993; Liehr *et al.*, 1989),

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strengthen immune system function (Block *et al.*, 1991; Dobias *et al.*, 1986; Leibovitz and Siegal, 1981; Johnston *et al.*, 1987; Meydani, 1993; Haskell and Johnston, 1991; Jacob *et al.*, 1991; Bendich, 1988, 1992; Chandra, 1992), have a role in prevention and treatment of cancer (Cameron and Pauling, 1976, 1980; Richards, 1988, 1991; Pavelic *et al.*, 1989; Henson *et al.*, 1991; Block, 1992, 1993; Eichholzer *et al.*, 1992; Gey, 1992, 1993; De Loecker *et al.*, 1993; Huber *et al.*, 1993; Kaugers *et al.*, 1993), cellular differentiation (Franceschi, 1992), cataract and inflammatory eye episode prevention (Bensch *et al.*, 1985; Williams and Paterson, 1986; Garland, 1991; Organisciak *et al.*, 1992; Penn *et al.*, 1992; Taylor, 1992, 1993; Ames *et al.*, 1993; Bunce, 1993; Eye Disease Case-Control Study Group, 1993), regulation of aging and inhibition of aging-related pathologies (Beyer and Starnes, 1985; Newton *et al.*, 1985; Cutler, 1986; Sram *et al.*, 1990; Garland, 1991; Deucher, 1992; Goode *et al.*, 1992; Ames *et al.*, 1993; Block, 1993; Fernandez-Calle *et al.*, 1993; Lopez-Torres *et al.*, 1993; Meydani, 1993), exercise injury prevention (Goldfarb, 1993; Jakeman and Maxwell, 1993; Peters *et al.*, 1993; Viguie *et al.*, 1993), cardiovascular disease prevention and hypertension treatment (Gerster, 1991; Eichholzer *et al.*, 1992; Gey, 1992, 1993; Jacques, 1992a, b; Simon, 1992; Gey *et al.*, 1993; Kaul *et al.*, 1993; Jackques *et al.*, 1994), lowering of ischemia-reperfusion injury and improvement of organ transplantation success (Sakamoto *et al.*, 1992; Doppelfeld and Parnham, 1992; Lee *et al.*, 1992; Rabl *et al.*, 1993; Rice-Evans and Diplock, 1993; Sciamanna and Lee, 1993), treatment of HIV infection (Harakeh *et al.*, 1990; Baker, 1991; Harakeh and Jariwalla, 1991; Baruchel and Wainberg, 1992), lowering of side-effect toxicity of cancer treatment (Fujita *et al.*, 1982; Shimpo *et al.*, 1991; Rivas-Olmedo *et al.*, 1992), prevention and treatment of stroke (Eichholzer *et al.*, 1992), inhibition of LDL oxidation (Jailal and Grundy, 1993), protection of brain (Makar *et al.*, 1994) and pulmonary function (Schwartz and Weiss, 1994), and a number of other processes.

Because of the dire consequence and obvious association of scurvy resulting from ascorbic acid deficiency, it has long been assumed that scurvy is the only pathology related to vitamin C deficiency and that an intake of vitamin C adequate to prevent scurvy is sufficient in all respects. A large number of observations have provided strong support for the concept that the suggested minimum daily requirement of ascorbic acid (ca. 60 mg/day) is insufficient and results in subclinical vitamin C deficiency associated with a number of clinical entities. The pathological consequences of subclinical vitamin C deficiency have been detailed in several volumes and articles (Stone, 1972; Basu and Schorah, 1982; Clemetson, 1989; Davies *et al.*, 1991; Gaby *et al.*, 1991; Stähelin *et al.*, 1991; Eichholzer *et al.*, 1992) and the ethical-political aspects of medical use of vitamin C have been reviewed (Richards, 1988, 1991). Because of the proliferation of publications over the past several years regarding the beneficial effects of so-called "megadoses" of vitamin C in cancer prevention and a large number of other pathologies, the adequacy of the RDA of 60 mg/day (100 mg/day for smokers) (Schectman *et al.*, 1991; Schectman, 1993) has been questioned and an increased daily consumption implied or suggested (Bieri, 1987; Block *et al.*, 1991; Block, 1991a, b, 1993a, b; Bates, 1993). As many of the pathologies prevented or remedied by vitamin C are related to free radical damage to various cellular components including membranes (Hayaishi *et al.*, 1988; Halliwell and Gutteridge, 1989; Beyer, 1990; Ernster and Beyer, 1991; Pryor, 1991; Wolff, 1993) and, as vitamin C has been shown to act as an efficient antioxidant (Bendich *et al.*, 1986; Bieri, 1987; Wefers and Sies, 1988; Basaga, 1990; Chow, 1991; Niki, 1991; Stocker and Frei, 1991; Byers and Perry, 1992; Meister, 1992; Sies *et al.*, 1992; Mulholland and Strain, 1993; Nordmann, 1993; Rice-Evans and Diplock, 1993; Rose and Bode, 1993), it is appropriate that this article reviews the recent literature with respect to the antioxidant function of vitamin C in the protection of membranes from free radical damage.

Vitamin C has been shown to be able to function as a chain-breaking antioxidant of lipid peroxidation in an *in vitro* cell-free system (Doba *et al.*, 1985; Niki *et al.*, 1985) and to protect various foods from oxidative deterioration (Cort, 1982; Bendich *et al.*, 1986; Frankel, 1989; Niki, 1991). Vitamin C is able to perform as an antioxidant either by reacting directly with a number of oxy- or peroxy-radicals in the aqueous phase. Since ascorbic acid is a weak, dibasic acid which is water soluble at physiological pH, it has long been assumed that its antioxidant function is confined to the aqueous phase of the cell and circulatory system. However, the demonstration that vitamin C may interact with the alpha-tocopheroxyl radical, regenerating the antioxidant form of vitamin E, alpha-tocopherol (Packer *et al.*, 1979; Niki *et al.*, 1982; McCay, 1985), provided evidence that

vitamin C also serves indirectly in the protection of membrane components susceptible to free radical damage. In addition to vitamin E, coenzyme Q in the hydroquinone state ($CoQH_2$) has been shown to function as an efficient antioxidant in the many cellular membranes in which it resides (for reviews see Beyer *et al.*, 1986, 1987; Beyer, 1989, 1990, 1992, 1994; Beyer and Ernster, 1990; Ernster and Beyer, 1991; Ernster and Forsmark-Andrée, 1993). This review, then, will report some of the relatively recent findings demonstrating the antioxidant role of vitamin C in the protection of membranes, and other hydrophobic compartments, from oxidative free radical damage.

ANTIOXIDANT CHEMISTRY AND MECHANISMS

Ascorbic acid (AH_2) has a dissociation constant in water of 7.94×10^{-5} ($pK_a = 4.10$) (Handbook, 1992–93), resulting in the dominant form at pH 7 being the ascorbate anion (AH^-). Dehydroascorbic acid (A) can also be formed via a two-step reversible oxidation process involving the formation of the intermediate ascorbyl radical ($A^{-\bullet}$) (Laroff *et al.*, 1972). The interconversions of these three forms of vitamin C are shown in Fig. 1. The dehydroascorbate component may exist in several states, the hydrated hemiketal being the preferred state under aqueous conditions (Tolbert and Ward, 1982). It is important to note that delocalization of the unpaired electron in the ascorbyl radical ($A^{-\bullet}$) renders it rather unreactive. Its fate is to disproportionate according to the sequence

$$2A^{-\bullet} + H^+ \rightarrow AH^- + A \qquad (1)$$

or it may react with other free radicals and in so doing it terminates the propagation of free radical reactions in which it may be involved (Bielski, 1982).

Ascorbic acid is able to react with the superoxide radical as shown in reaction (2):

$$O_2^{-\bullet} + AH^- \rightarrow HO_2^- + A^{-\bullet} \qquad (2)$$

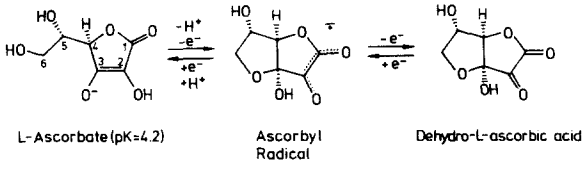

Fig. 1. Molecular interconversion of vitamin C.

and is also able to scavenge the hydroxyl (or perhydroxyl) radical:

$$HO_2^{\bullet} + AH^- \rightarrow H_2O_2 + A^{-\bullet} \qquad (3)$$

Formation of the superoxide and hydroperoxyl radicals are considered the most common under physiological conditions (Fridovich, 1976) and their reactions with ascorbate have been studied with flash photolysis and pulse radiolysis (Bielski, 1982; Cabelli and Bielski, 1983; Nadezhdin and Dunford, 1979). In addition, ascorbate is able to react efficiently and directly with the following radical species with k values between 2×10^{-6} and $2 \times 10^{-8}\ M^{-1}\ s^{-1}$: $Cl_3COO^{\bullet}$, $Cl_2CHOO^{\bullet}$, $ClCH_2OO^{\bullet}$, $^{\bullet}OOCCl_2CO_2^-$, $^{\bullet}OOCHClCO_2^-$, $CH_3OO^{\bullet}$, and $(CH_3)_2C(OH)\cdot CH_2OO^{\bullet}$ (Niki, 1991). Ascorbate has also been shown to be an effective scavenger of singlet oxygen (Bodannes and Chan, 1979) as well as the highly damaging hydroxyl radical ($OH^{\bullet}$) (Rose, 1990).

In the presence of transition metals, and at high concentrations of ascorbate, hydroperoxyl and superoxide radicals may be formed *in vitro* (Halliwell and Gutteridge, 1989; Cheeseman *et al.*, 1984). The autooxidation of ascorbate proceeds with the formation of an intermediate superoxide anion:

$$AH^- + O_2 \rightarrow A^{-\bullet} + O_2^{-\bullet} + H^+ \qquad (4)$$

a process which is inhibited in the presence of the enzyme superoxide dismutase (Puget and Michaelson, 1974; Scarpa *et al.*, 1983).

Although the ability of ascorbic acid, at high concentrations, to form free radicals and initiate the process of lipid peroxidation has been demonstrated *in vitro* (Girotti, 1985), leading to rumors that high intakes of vitamin C results in damage to the recipient, there is little objective evidence supporting significant prooxidant activity of ascorbic acid *in vivo* (Bendich *et al.*, 1986).

ANTIOXIDANT HYDROPHOBIC PHASE MEMBRANE PROTECTION

A number of hydrophobic phase and membrane components including polyunsaturated fatty acids and esters, lipoproteins, and phospholipids are oxidized by free radicals in a three-stage chain oxidation process of lipid peroxidation. This process, and the antioxidant effects of ascorbic acid, has been studied *in vitro* using azo initiators (A–N=N–A), such as 2,2′-azobis(2-amidinopropane)dihydrochloride,

which are able to generate free radicals (Yamamoto *et al.*, 1984; Barclay *et al.*, 1984). The reactions of the three stages of azo dye-initiated lipid peroxidation are shown in reaction (5)–(10), where A–N=N=A is the azo initiator, LH is a lipid, $L^{\bullet}$ a lipid radical, and $LO_2^{\bullet}$ a lipid peroxyl radical.

Initiation:

$$A\text{–}N{=}N\text{–}A \rightarrow A^{\bullet} + N_2 + {}^{\bullet}A \quad (5)$$

$$A^{\bullet} + O_2 \rightarrow AO_2^{\bullet} \quad (6)$$

$$AO_2^{\bullet} + LH + O_2 \rightarrow AOOH + LO_2^{\bullet} \quad (7)$$

Propagation:

$$LO_2^{\bullet} + LH \rightarrow LOOH + L^{\bullet} \quad (8)$$

$$L^{\bullet} + O_2 \rightarrow LO_2^{\bullet} \quad (9)$$

Termination:

$$2LO_2^{\bullet} \rightarrow \text{Inert products} \quad (10)$$

Chain-breaking antioxidants, including ascorbic acid, inhibit the peroxidation process by scavenging $AO_2^{\bullet}$ or $LO_2^{\bullet}$ radicals, thereby inhibiting the initiation stage. As Niki *et al.* (1984) have demonstrated, the length of the induction period of the initiation stage of azo initiator-induced oxidation of methyl linoleate in organic solvent is directly proportional to ascorbic acid concentration. Ascorbic acid has also been shown to inhibit the oxidation of aqueous phase-dispersed phosphatidylcholine liposomes and methyl linoleate micelles under conditions of free radical generation in the aqueous phase (Yamamoto *et al.*, 1984; Niki *et al.*, 1985; Doba *et al.*, 1985). Ascorbic acid has also been shown to prevent free radical damage to membranes of erythrocytes in aqueous suspension via oxidation of membrane proteins and lipids resulting in hemolysis (Yamamoto *et al.*, 1986; Miki *et al.*, 1987; Niki, 1987). On the basis of such observations, it has been concluded that ascorbic acid scavenges oxygen radicals formed in the aqueous phase before the radicals attack erythrocyte membranes, and thus it protects membranes from oxidative damage (Niki, 1991).

Biological systems possess a large variety and concentration of radical-trapping antioxidants (Halliwell and Gutteridge, 1989), some of which are primary (major or only function) and some of which are secondary or incidental (Beyer, 1994). Measurement of total radical-trapping antioxidant potential (TRAP) in blood plasma indicates that vitamin C, vitamin E, urate, protein (Wayner *et al.*, 1985, 1987), and coenzyme Q (Beyer, 1992) are the major functional antioxidants. Niki *et al.* (1988) have demonstrated that, during free radical generation in the aqueous phase of whole blood suspensions, a number of antioxidants in both the plasma and the erythrocytes interact with the radicals and decrease oxidative damage. The primary antioxidant, which is the most efficient and is consumed prior to utilization of the other antioxidants, is ascorbic acid, followed by bilirubin, uric acid, coenzyme Q, and vitamin E in plasma. After most of the plasma antioxidant capacity is depleted, erythrocyte membrane vitamin E and –SH groups commence function. Niki (1991) has offered the logical explanation that vitamin C is the "primary and the most important defence against the radicals in the aqueous phase."

One question concerning the antioxidant function of vitamin C which must be addressed is the relevance of mechanistic and *in vitro* studies to the role of vitamin C *in vivo*. Using a vitamin C-deficient rat model, Kunert and Tappel (1983) have demonstrated an increase in whole-body lipid peroxidation while Kato *et al.* (1981) have reported inhibition by ascorbic acid of lipid peroxidation in rat liver.

Although vitamin C has been shown conclusively to be an efficient antioxidant in the aqueous phase, its lack of solubility in the membrane hydrophobic phase restricts its ability to inactivate free radicals formed within membranes or block their damaging effects (Niki *et al.*, 1985; Doba *et al.*, 1985).

ROLE OF VITAMIN C IN ANTIOXIDANT PROTECTION OF MEMBRANES

As mentioned above, although ascorbic acid is able to protect membranes from free radicals generated in the aqueous phase, it is not an efficient scavenger of free radicals generated within the hydrophobic membrane phase. As first suggested by Tappel (1962, 1968), aerobic organisms have selected a mechanism to protect membranes from free radical damage utilizing a chemical interaction between ascorbic acid in the aqueous phase and regeneration of alpha-tocopherol in the membrane hydrophobic phase. The literature regarding the role of ascorbic acid in the generation of the active antioxidant form of vitamin E, alpha-tocopherol, up to 1988 has been reviewed (Niki, 1991). Recent information relevant to the role of antioxidants functional in the membrane phase (vitamin E and coenzyme Q) and the regeneration of their active

antioxidant forms will now be addressed. In general, recent literature on the interaction between vitamin C and vitamin E has provided strong support for the nonenzymatic regeneration of alpha-tocopherol (TCOH) from the alpha-tocopheroxyl radical ($TCO^{\bullet}$), formed when alpha-tocopherol scavenges a peroxyl radical ($ROO^{\bullet}$), by ascorbate (AH^{-}) as shown in reactions (11) and (12):

$$ROO^{\bullet} + TCOH \rightarrow ROOH + TCO^{\bullet} \quad (11)$$

$$TCO^{\bullet} + AH^{-} \rightarrow TCOH + A^{-\bullet} \quad (12)$$

Ascorbate is regenerated either by GSH-dependent dehydroascorbate reductase (Halliwell and Gutteridge, 1989) or nonenzymatically by dihydrolipoic acid (Constantinescu *et al.*, 1993). Because free radicals generated in biological systems at physiological pH, and their reduced forms, are uncharged, it has been hypothesized (Njus and Kelly, 1991) that both vitamins C and E donate single hydrogen atoms *in vivo* instead of separate electron transfer and proton equilibrium steps. The dependence of ascorbate and glutathione upon vitamin E in protection against microsomal lipid peroxidation has been reported by Wefers and Sies (1988a), and the same authors have reviewed the roles of vitamins E and C, beta-carotene, and other carotenoids in antioxidant defense (Wefers and Sies, 1988b; Sies *et al.*, 1992). A significant contribution to the understanding of the functional significance of the interactive antioxidant roles of vitamins C and E has been provided by the demonstration of this process *in vivo* utilizing a mutant rat strain unable to synthesize ascorbic acid (Igarashi *et al.*, 1991). The antilipoperoxidant activities of alpha-tocopherol and ascorbic acid, protecting erythrocyte membranes, have been shown to be potentiated by rutin (Nègre-Salvayre *et al.*, 1991), a water-soluble flavenoid, secondary antioxidant (Beyer, 1994).

The beneficial effect of an increase in the total radical-trapping antioxidant potential (TRAP) of plasma of humans, stimulated by oral supplementation with large doses of vitamins C and E, has been reported (Mulholland and Strain, 1993). Using an ESR steady-state method, Roginsky and Stegmann (1993) have studied the kinetics of the reaction between ascorbate and the phenoxyl radical from alpha-tocopherol. In a recent publication on "the pecking order of free radicals and antioxidants," Buettner (1993) has reviewed the thermodynamics of free radical reactions of interest to health, the fundamental thermodynamics and kinetic properties associated with chain-breaking antioxidants, and the "interfacial" nature of the reaction between the tocopheroxyl free radical and ascorbate. In a review appropriately entitled "Partners in Defense, Vitamin E and Vitamin C," Chan (1993) has reviewed the concept of nonenzymic regeneration of vitamin E by vitamin C and the enzymatic regeneration of hydrophobic-phase vitamin E by glutathione. Contributions to understanding the interactive roles of vitamins C and E in the prevention of free radical oxidation of plasma LDL, an essential event in cholesterol plaque formation, have been published recently (Frei, 1991; Mackness *et al.*, 1993) and reviewed (Jackson *et al.*, 1993).

In addition to vitamin C, reduced coenzyme Q ($CoQH_2$) has been shown to protect the many cellular membranes and plasma lipoproteins in which it resides from free radical damage (see Beyer, 1992, 1994 for recent reviews). In addition to direct interaction of $CoQH_2$ with lipid peroxyl radicals (Beyer, 1988; Beyer *et al.*, 1987), $CoQH_2$ has been shown to be an efficient regenerator of alpha-tocopherol from the tocopheroxyl radical (Frei *et al.*, 1990; Mukai *et al.*, 1990, 1992; Packer *et al.*, 1991; Ernster *et al.*, 1992; Freisleben and Packer, 1993). The possible sequence of events depicting the interaction of $CoQH_2$ with the superoxide radical ($O_2^{-\bullet}$) and the perferryl radical (Fe^{3+}–$O_2^{-\bullet}$) (cf. Beyer and Ernster, 1990; Beyer, 1992) are shown in reactions (13) and (14):

$$CoQH_2 + O_2^{-\bullet} \rightarrow CoQ^{-\bullet} + H_2O_2 \quad (13)$$

$$CoQH_2 + Fe^{3+}\text{–}O_2^{-\bullet} \rightarrow CoQ^{-\bullet} + Fe^{3+} + H_2O_2 \quad (14)$$

The H_2O_2 generated in reactions (13) and (14) would be removed by catalase, peroxidase, or GSH-peroxidase (Beyer, 1992). The quenching of carbon-centered lipid radicals ($CL^{\bullet}$) and/or lipid peroxyl radicals ($LOO^{\bullet}$) by $CoQH_2$ has also received consideration (Beyer and Ernster, 1990; Forsmark *et al.*, 1991) and is depicted in reactions (15) and (16):

$$CoQH_2 + L^{\bullet} \rightarrow CoQ^{-\bullet} + LH + H^{+} \quad (15)$$

$$CoQH_2 + LOO^{\bullet} \rightarrow CoQ^{-\bullet} + LOOH + H^{+} \quad (16)$$

As mentioned above, reduced coenzyme Q is able to regenerate the active form of vitamin E from the alpha-tocopheroxyl radical ($TCO^{\bullet}$) according to

reaction (17):

$$CoQH_2 + TCO^{\bullet} \rightarrow CoQ^{-\bullet} + TCOH + H^+ \quad (17)$$

Another possible mechanism accounting for the antioxidant activity of $CoQH_2$ is derived from observations of Cadenas *et al.* (1988) who have observed the interaction of superoxide dismutase (SOD) with hydroquinones and, in conjunction with the two-electron quinone reductase DT-diaphorase (Ernster *et al.*, 1987), inhibits autooxidation of hydroquinones. Cadenas *et al.* (1988) have proposed a new function for SOD, a superoxide : semiquinone oxidoreductase activity, in which SOD–Cu^{2+} is reduced by the superoxide radical:

$$SOD{-}Cu^{2+} + O^{-\bullet} \rightarrow SOD{-}Cu^{+} + O_2 \quad (18)$$

followed by oxidation of SOD–Cu^+ by a semiquinone intermediate:

$$SOD{-}Cu^{+} + Q^{-\bullet} \rightarrow SOD{-}Cu^{2+} + Q^{2-} \quad (19)$$

If, in fact, DT-diaphorase were able to interact with coenzyme Q to form $CoQH_2$, indirect evidence for which has been presented (Beyer, 1994), the sum of reactions (18) and (19) is shown in reaction (20):

$$O_2^{-\bullet} + CoQ^{-\bullet} \rightarrow O_2 + CoQ^{2-} \text{ (or } CoQH_2) \quad (20)$$

The reaction between DT-diaphorase and coenzyme Q would dismutate the CoQ semiquinone and thus prevent the transfer of the unpaired electron to oxygen and formation of the superoxide radical.

Although it may be argued that, based upon the high degree of hydrophilicity of ascorbic acid and hydrophobicity of alpha-tocopherol, requiring residence in two distinct cellular compartments, the chemical interaction of the two molecules is unlikely *in vivo*, a number of studies clearly indicate just such synergistic interaction in liposomal and membrane systems (Niki *et al.*, 1985; Doba *et al.*, 1985; Chan, 1993; Buettner, 1993; Roginsky and Stegmann, 1993; Sies *et al.*, 1992; Nègre-Salvayre *et al.*, 1991). Recent studies have also demonstrated synergism between vitamins C and E *in vivo* (Anderson and Theron, 1990; Igarashi *et al.*, 1991; Mulholland and Strain, 1993).

CONCLUSIONS

Very convincing evidence exists providing strong support for an antioxidant role for ascorbic acid, protecting not only the aqueous compartments of the cell, extracellular matrix, and circulatory system in which it resides but, in addition, hydrophobic phases such as circulatory system lipoproteins and membrane systems in general. Ascorbic acid may perform its antioxidant function either by quenching various free radical species directly or by reducing membrane-bound oxidized vitamin E at the membrane surface. The ability of reduced coenzyme Q, also a membrane-bound component, to also regenerate the antioxidant form of vitamin E has also been demonstrated. Further experiments should be designed to investigate in detail the interaction of vitamins C and E, and coenzyme Q, all powerful antioxidants, in the vital protection of membrane components against injury from damaging free radicals.

NOTE ADDED IN PROOF

Subsequent to submission of the manuscript, a number of articles relevant to issues treated above have been published. In addition to the impressive number of functions listed in the Introduction section, vitamin C has been reported to be of benefit to patients with macular degeneration (Seddon and Hunnekens, 1994) and HTLV-I-associated myelopathy (Kataoka *et al.*, 1993) and may exert a protective effect against the development of gastric cancer in humans (Dyke *et al.*, 1994). In addition, vitamin C has been shown to protect cells from free radical damaging effects of gamma-radiation in embryos (Yoshimura *et al.*, 1993) and testes (Nara *et al.*, 1994) and to function as a vital antioxidant in the brain (Reiber *et al.*, 1994). Ascorbic acid treatment has also been shown to protect against the free radical-associated toxic effects of cadmium (Shiraishi *et al.*, 1993), trimethyltin (Bannon *et al.*, 1993) and endotoxin-induced lung injury (Dwengen *et al.*, 1994). Recent evidence also indicates that vitamin C decreases endogenous lipid peroxidation (Chakraborty *et al.*, 1994, Garg and Mahajan, 1993; Barja *et al.*, 1994), protein oxidative damage (Barja *et al.*, 1994), affects longevity (Garg and Mahajam, 1993; Yu, 1994), inhibits skin tumor promotion (Battalora *et al.*, 1993), protects the liver from oxidative damage (Chen and Tappel, 1994) and reduces erythrocyte membrane nitroxide radicals (Zhang and Fung, 1994). Synergism between vitamins C and E in membrane protection has been confirmed (Lambelet *et al.*, 1994) and reviewed (Reed, 1993). The protective role

of coenzyme Q_{10} in low density lipiprotein oxidizability by copper has also been demonstrated (Kontash *et al.*, 1994). Current controversies regarding use of vitamin supplements have been reviewed (Reynolds, 1994) and the conclusion reached that high-dose nutritional vitamin supplementation provides major benefits with respect to prevention of cancer, cardiovascular disease, carpal tunnel syndrome, and neural tube defects, "to name a few." In addition, review articles, regarding nutrient antioxidants and cellular defense mechanisms against damage from free radicals have appeared (Yu, 1994; Bonorden and Pariza, 1994).

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