HOW DO ANTIOXIDANTS WORK IN PLASTICS AND PEOPLE?

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&
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I. Introduction:

A. What is a Free Radical?

"It is an atom or group of atoms possessing one or more unpaired electrons.". The word "free" in front of "radical" is considered unnecessary.

II. Notation

A. Superscript dot to the right, usually

B. Examples (Note: dot, then charge)

$H^{\bullet}$

$Cl^{\bullet}$

$HO^{\bullet}$

$O_2^{\bullet\bullet}$ or $O_2^{2\bullet}$ dioxygen,
the O_2 you are breathing now.

$H_3C^{\bullet}$

$O_2^{\bullet-}$

$CO_2^{\bullet-}$

$Asc^{\bullet-}$

III. Types of radicals we have:

1. Sigma, σ
2. pi - delocalized, π
3. Mixture of sigma and pi
4. carbon-centered $H_3C^{\bullet}$
5. O_2 - centered $H_3COO^{\bullet}$
6. Sulfur - centered $GS^{\bullet}$
7. Nitrogen - centered $R_2{}^{\bullet}NO$
8. Metals: Cu^{2+}, $Fe^{2+,3+}$, Mn^{+2}, Mo, Co^{+2}
9. Reducing radicals, $CO_2^{\bullet -}$, $PQ^{\bullet +}$
10. Oxidizing radicals, $HO^{\bullet}$, $LOO^{\bullet}$
11. ...

IV. Classic Lipid Peroxidation

A. The Basics (one-electron Processes)

Initiation:

$$LH + X^{\bullet} \xrightarrow{R_i} L^{\bullet} + XH$$

Propagation (a cycle of destruction):

$$L^{\bullet} + O_2 \xrightarrow{k_o \cong 3\times10^{8}\ M^{-1}s^{-1}} LOO^{\bullet}$$

$$LOO^{\bullet} + LH \xrightarrow{k_p \cong 10\text{-}50\ M^{-1}s^{-1}} L^{\bullet} + LOOH$$

Termination:

$$2\ LOO^{\bullet} \xrightarrow{k_t \cong 10^{6}\text{-}10^{7}\ M^{-1}s^{-1}} \text{non-radical prod.}$$

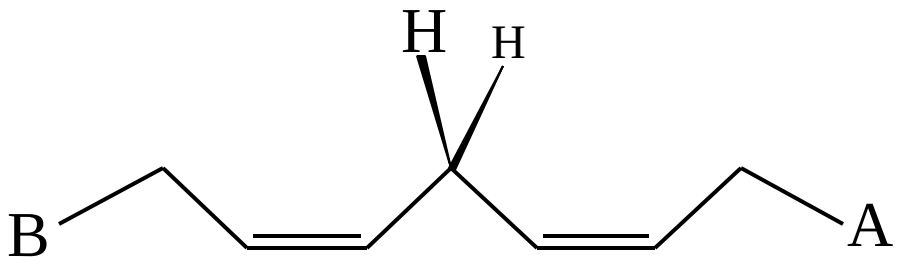
$$L^{\bullet} + LOO^{\bullet} \longrightarrow \text{“ “}$$

$$L^{\bullet} + L^{\bullet} \longrightarrow \text{“ “}$$

B. Oxidizability

of PUFA is dependent on the number

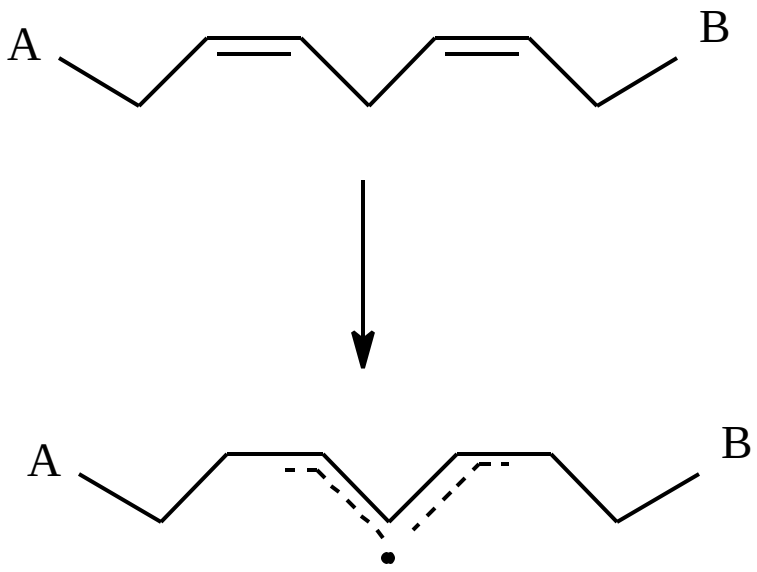
of *bis*-allylic methylene positions



bis-allylic allyl ⟶ C-C=C
bis = double

Bond Dissociation Energy for an alkyl C-H ≈ 104 kcal/mol; for a *bis*-allylic C-H 75 – 80 kcal/mol

C. The Lipid Radical, a pentadienyl-like radical



V. ANTIOXIDANTS

A. Definition

A substance when present in trace (small) amounts inhibits oxidation of the bulk.

There are two broad classes:

1. *Preventative*

2. *Chain-breaking*

B. Preventative Antioxidants reduce the rate of chain initiation

Initiation:

$$L\text{-}H + X^{\bullet} \rightarrow L^{\bullet} + XH$$

or $LOOH + Fe^{2+} \rightarrow LO^{\bullet} + OH^{-} + Fe^{3+}$

or $H_2O_2 + Fe^{2+} \rightarrow HO^{\bullet} + OH^{-} + Fe^{3+}$

or $Fe^{2+} + O_2 \rightarrow (FeO_2)^{2+}$ oxidant

or $^1O_2 + L\text{-}H \rightarrow LOOH \rightarrow LO^{\bullet}$

1. Targets for preventative antioxidants

a. Metals - Fe, Cu

i. Chelates - EDTA
DETAPAC
Desferal® ≡ deferrioximine
Phytate ?

ii. Proteins & metals
Transferrin Fe
Ferritin Fe
Hemes
Hemoglobin
Myoglobin
:
Caeruloplasmin Cu

iii. Key aspect is:

$Fe(III)chelate + O_2^{\bullet -} \rightarrow Fe(II)chelate + O_2$
or $AscH^-$

$Fe(II)chelate + H_2O_2 \rightarrow HO^\bullet + Fe(III)chelate$
or $LOOH \quad LO^\bullet$

iv. Peroxides: H_2O_2, $LOOH$

CAT, GPx, PhGPx, SOD

C. Chain-breaking Antioxidants

Propagation:

$$L^{\bullet} + O_2 \xrightarrow{3\times10^{8} M^{-1}s^{-1}} LOO^{\bullet}$$

$$LOO^{\bullet} + L\text{-}H \xrightarrow{10\ M^{-1}s^{-1}} L^{\bullet} + LOOH \quad [13]^{*}$$

i. Intercept $L^{\bullet}$?

$$L^{\bullet} + \text{Antiox-H} \rightarrow \text{Antiox}^{\bullet} + \text{L-H}$$

Not likely! Why = k's

$$L^{\bullet} + O_2 \xrightarrow{3\times10^{8}\ M^{-1}s^{-1}} LOO^{\bullet}$$

ii. Repair $LOO^{\bullet}$

$$LOO^{\bullet} + \text{Antiox-H} \rightarrow LOOH + \text{Antiox}^{\bullet}$$

$k = 10^4 \rightarrow 10^8\ M^{-1}s^{-1}$

It can be successful.

iii. Good antioxidant (chain-breaking)

a. relatively UNreactive
Antioxidant & $\text{Antiox}^{\bullet}$

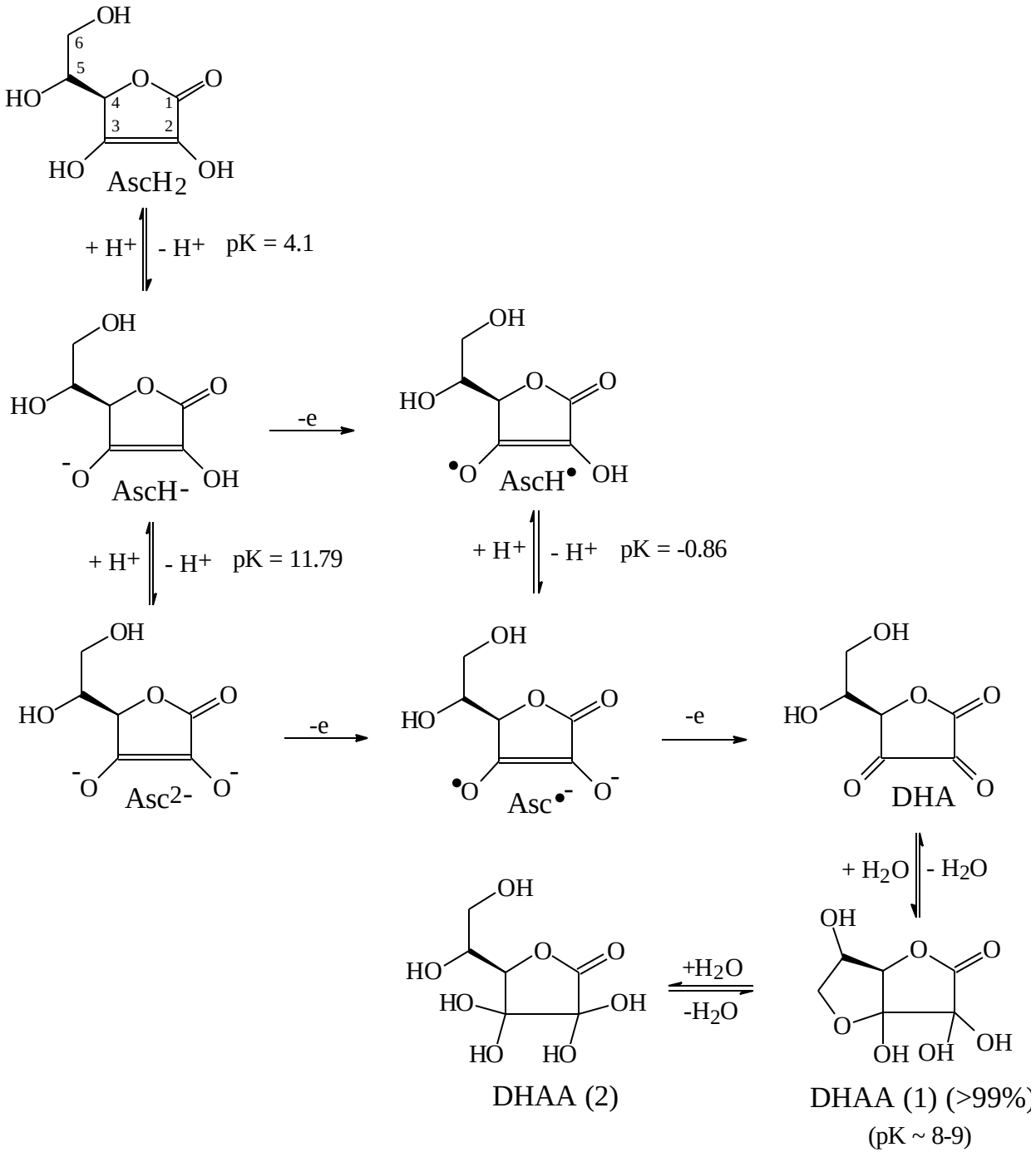
b. $\text{Antiox}^{\bullet}$ - decays to harmless products

c. Does not <u>add</u> O_2.

d. Recycled - somehow.

VI. Vitamins C & E. (Donor Antioxidants)

A. Vitamin C (ascorbate)



$Asc^{\bullet -}$, H^+ / $AscH^-$ pH 7 $E^{\circ\prime}$ = +282 mV

B. Vitamin E

α, β, γ, δ - Tocopherols

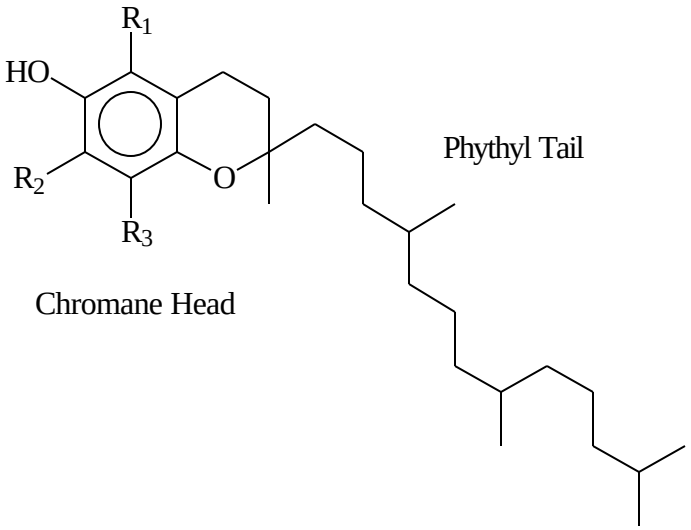


Table 1 Forms of Tocopherol

	R_1	R_2	R_3
α	CH_3	CH_3	CH_3
β	CH_3	H	CH_3
γ	H	CH_3	CH_3
δ	H	H	CH_3

C. The Big Picture: (Buettner, GR. *Arch. Biochem. Biophys.* **300:**535-543,1993)

Figure: Membrane lipid peroxidation. Only one leaflet of the bilayer is represented.

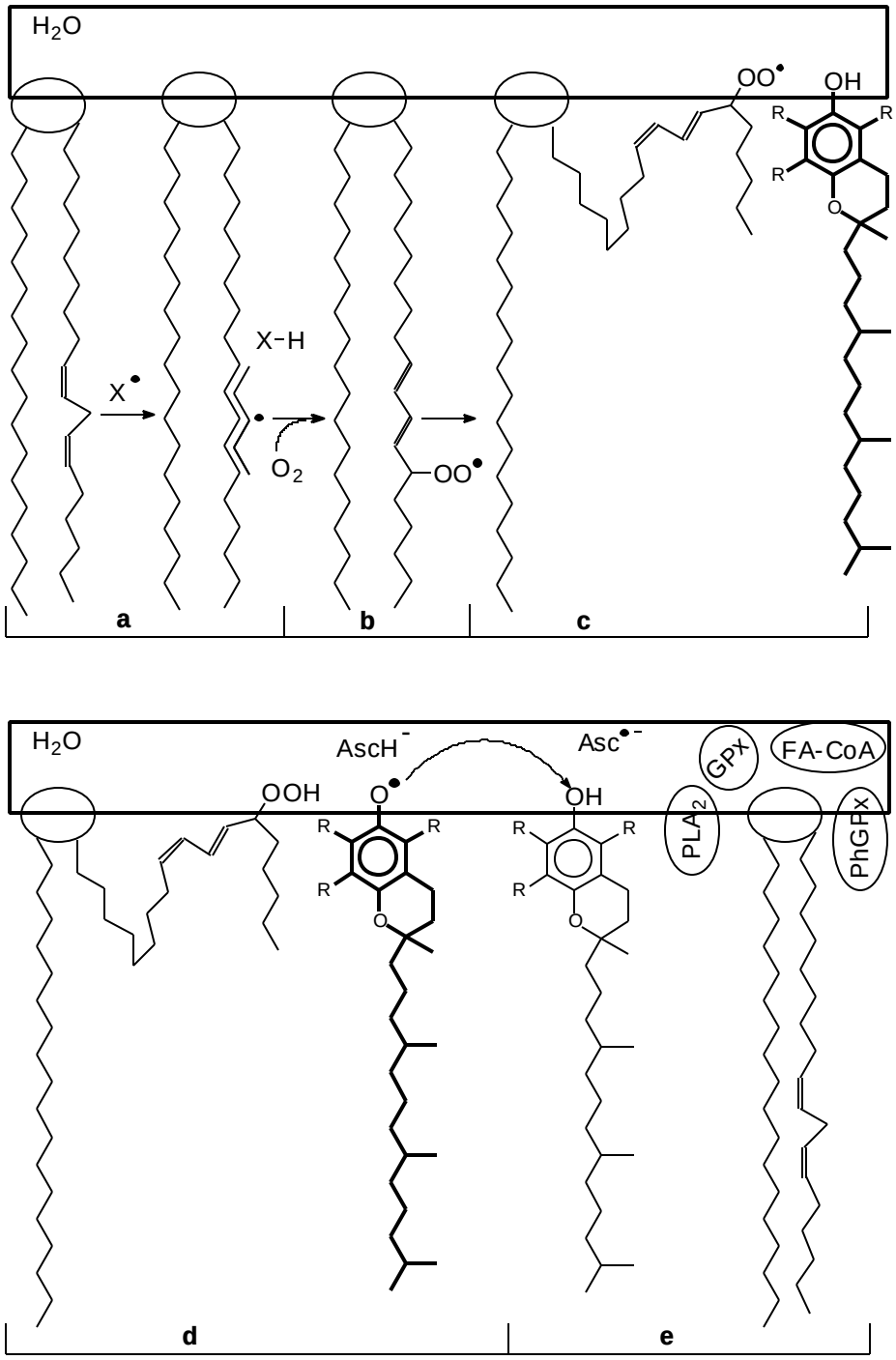
a) Initiation of the peroxidation process by an oxidizing radical, $X^{\bullet}$, by abstraction of a *bis*-allylic hydrogen, thereby forming a pentadienyl radical.

b) Oxygenation to form a peroxyl radical and a conjugated diene.

c) The peroxyl radical moiety partitions to the water-membrane interface where it is poised for repair by tocopherol.

d) The tocopheroxyl radical can be repaired by ascorbate.

e) Tocopherol has been recycled by ascorbate; the resulting ascorbate radical can be recycled by enzyme systems. The enzymes phospholipase A_2 (PLA_2), phospholipid hydroperoxide glutathione peroxidase (PhGPx), glutathione peroxidase (GPx), and fatty acyl-coenzyme A (FA-CoA), cooperate to detoxify and repair the oxidized fatty acid chain of the phospholipid. This cartoon cannot show the dynamic aspects of this process. TOH in the membrane will undoubtedly be bobbing "up and down" so that the position of the "OH" is variable. In addition, TOH and $TO^{\bullet}$ may have somewhat differing positions at the interface. The chemical aspects of this process will undoubtedly also describe the free radical oxidation of LDL.



D. Energetics

Table 1

Redox Couple	$E^{o'}$/mV
$HO^{\bullet}, H^{+}/H_2O$	+ 2310
$RO^{\bullet}, H^{+}$/ROH (aliphatic alkoxyl radical)	+ 1600
$ROO^{\bullet}, H^{+}$/ROOH (alkyl peroxyl radical)	+ 1000
$GS^{\bullet}/GS^{-}$ (glutathione)	+ 920
$PUFA^{\bullet}$, H+/PUFA-H (bis-allylic-H)	+ 600
$TO^{\bullet}, H^{+}$/TOH	+ 480
$H_2O_2, H^{+}/H_2O, HO^{\bullet}$	+ 320
$Ascorbate^{\bullet-}, H^{+}$/Ascorbate monoanion	+ 282
Fe(III) EDTA/ Fe(II) EDTA	+ 120
$O_2/ O_2^{\bullet-}$	- 330
Paraquat/ $Paraquat^{\bullet-}$	- 448
Fe(III) DFO/ Fe(II) DFO	- 450
RSSR/ $RSSR^{\bullet-}$ (GSH)	- 1500
H_2O/ e^{-}_{aq}	- 2870

E. Kinetics

$LOO^{\bullet} + TOH \longrightarrow LOOH + TO^{\bullet}$

$k = 2.5 \times 10^{6}\ M^{-1}s^{-1}$ oleic acid solution

$C1_3COO^{\bullet} + TOH \longrightarrow k = 5 \times 10^{8}\ M^{-1}s^{-1}$ in 2-PrOH / H_2O / acetone

But Larry Patterson Rad. Lab Notre Dame
Linoleate micelles $k = 8 \times 10^{4}\ M^{-1}s^{-1}$

Thus, how does vitamin E protect? Kinetics

$$LOO^{\bullet} + LH \xrightarrow{k \approx (10\text{-}50)\ M^{-1}s^{-1}} LOOH + L^{\bullet}$$

$$LOO^{\bullet} + TOH \xrightarrow{k \approx 8\times10^{4}\ M^{-1}s^{-1}} LOOH + TO^{\bullet}$$

Thus,

$$\frac{\text{Rate } (LOO^{\bullet} + TOH)}{\text{Rate } (LOO^{\bullet} + LH)} = \frac{8\times10^{4} M^{-1}s^{-1}\,[TOH]\,[LOO^{\bullet}]}{10\text{-}50\ M^{-1}s^{-1}\,[LH]\,[LOO^{\bullet}]}$$

$$\frac{R_{inh}}{R_{prop}} = \frac{8 \times 10^3\,[TOH]}{[LH]}$$

1:1 $\Rightarrow$ [TOH]:[LH] = 1:8000

But

9:1 $\Rightarrow$ [TOH]:[LH] = 1:900

99:1 $\Rightarrow$ [TOH]:[LH] = 1:90

How much vit E is there in membranes?
Ratio $\approx$ 1:1000

F. Gey KF. (1998) Vitamins E plus C and interacting conutrients required for optimal health. A critical and constructive review of epidemiology and supplementation data regarding cardiovascular disease and cancer. *Biofactors*. **7(1-2):**113-74, 1998.

Abstract: Antioxidants are crucial components of fruit/vegetable rich diets preventing cardiovascular disease (CVD) and cancer: plasma vitamins C, E, carotenoids from diet correlate prevalence of CVD and cancer inversely, low levels predict an increased risk of individuals which is potentiated by combined inadequacy (e.g., vitamins C + E, C + carotene, A + carotene); self-prescribed rectification of vitamins C and E at adequacy of other micronutrients reduce forthcoming CVD, of vitamins A, C, E, carotene and conutrients also cancer; randomized exclusive supplementation of beta-carotene +/- vitamin A or E lack benefits except prostate cancer reduction by vitamin E, and overall cancer reduction by selenium; randomized intervention with synchronous rectification of vitamins A + C + E + B + minerals reduces CVD and counteracts precancerous lesions; high vitamin E supplements reveal potentials in secondary CVD prevention.

Plasma values desirable for primary prevention:

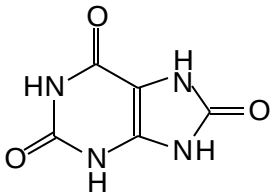
≥ 30 μmol/L lipid-standardized vitamin E, *i.e.*, α-tocopherol/cholesterol ≥ 5.0 μmol/mmol;

≥ 50 μmol/L vitamin C aiming at **vitamin C/vitamin E > 1.3-1.5;**

≥ 0.4 μmol/L beta- (≥ 0.5 μmol/l alpha+ beta-) carotene.

CONCLUSIONS: In CVD vitamin E acts as first risk discriminator, vitamin C as second one; optimal health requires synchronously optimized vitamins C + E, A, carotenoids and vegetable conutrients.

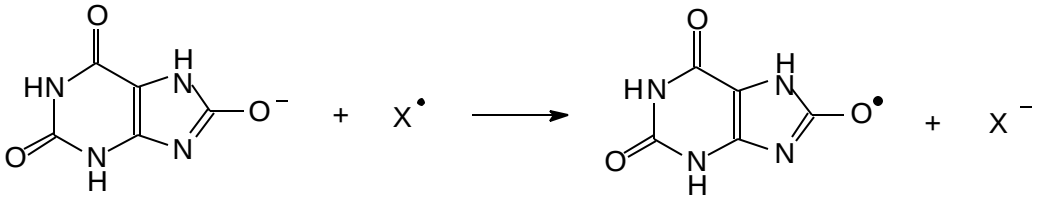
VII. Uric Acid; an antioxidant?



Uric acid

$UH_3 \rightleftharpoons UH_2^- \rightleftharpoons UH^{2-}$

$pK_{a1} = 5.4$ $pK_{a2} = 9.8$



$E^{o\prime}$ = +590 mV for $UH^{\bullet-},H^+/UH^{2-}$; because the reduction potential of the urate radical (+590 mV) is above that of $Asc^{\bullet-}$ (+282 mV) at neutral pH, ascorbate would be expected to repair the urate radical[1].

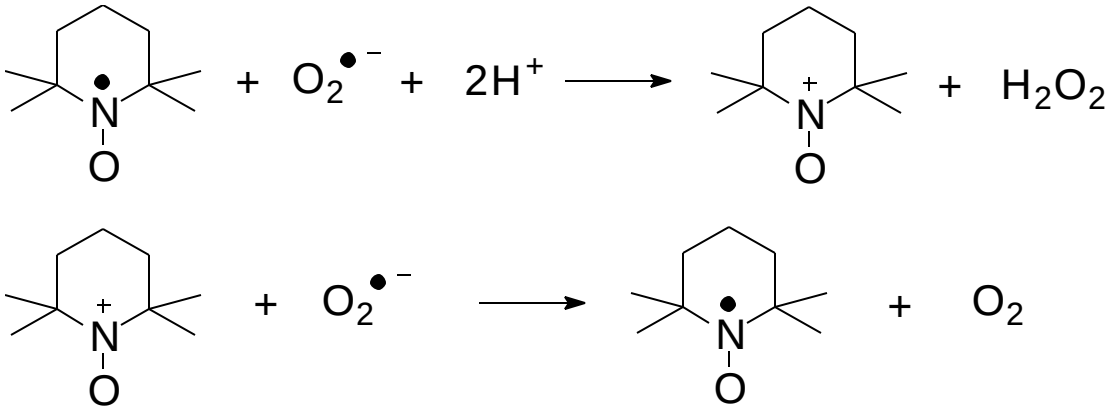
Reaction	rate constant/$M^{-1}s^{-1}$
$UH^{\bullet-} + O_2$	$< 10^{-2}$
$UH^{\bullet-} + AscH^- \rightarrow UH_2^- + Asc^{\bullet-}$	1×10^6
$ROO^\bullet + UH_2^- \rightarrow ROOH + UH^{\bullet-}$	3×10^6
$Cl_3COO^\bullet + UH_2^- \rightarrow Cl_3COOH + UH^{\bullet-}$	3.2×10^8
$^\bullet NO_2 + UH_2^- \rightarrow NO_2^- + UH^{\bullet-} + H^+$	1.8×10^7
$R\text{-}G^\bullet(\text{-H}) + UH_2^- \rightarrow R\text{–}G + UH^{\bullet-}$ (G = guanosine)	1.2×10^9

[1] Simic MG, Jovanovic SV (1989) Antioxidation mechanisms of uric acid. *J Am Chem Soc* **111:** 5778-5782.

VIII. Nitroxides and Hindered Amines

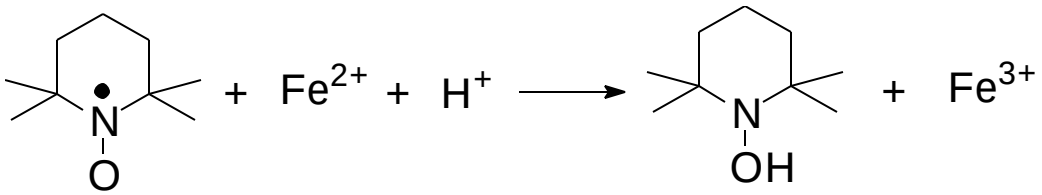
Tempo, car paint, polymerization and radical protection in cancer treatment

A. Nitroxides as SOD mimetics [2]



$k_{cat} = 1.2 \times 10^5\ M^{-1}s^{-1}$ for Tempo

B. Nitroxides as electron sinks [3] [4]

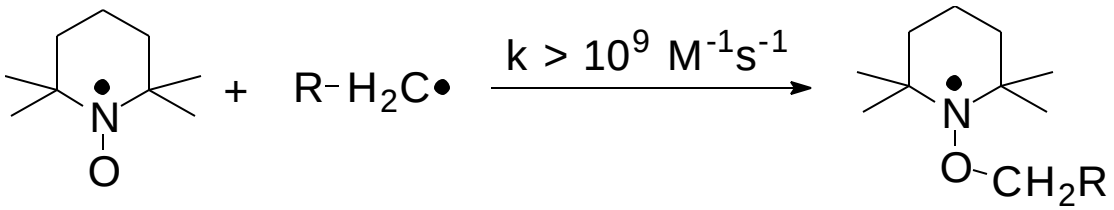


[2] Krishna MC, Grahame DA, Samuni A, Mitchell JB, Russo A. (1992) Oxoammonium cation intermediate in the nitroxide-catalyzed dismutation of superoxide. *Proc Natl Acad Sci* **89:** 5537-5541.

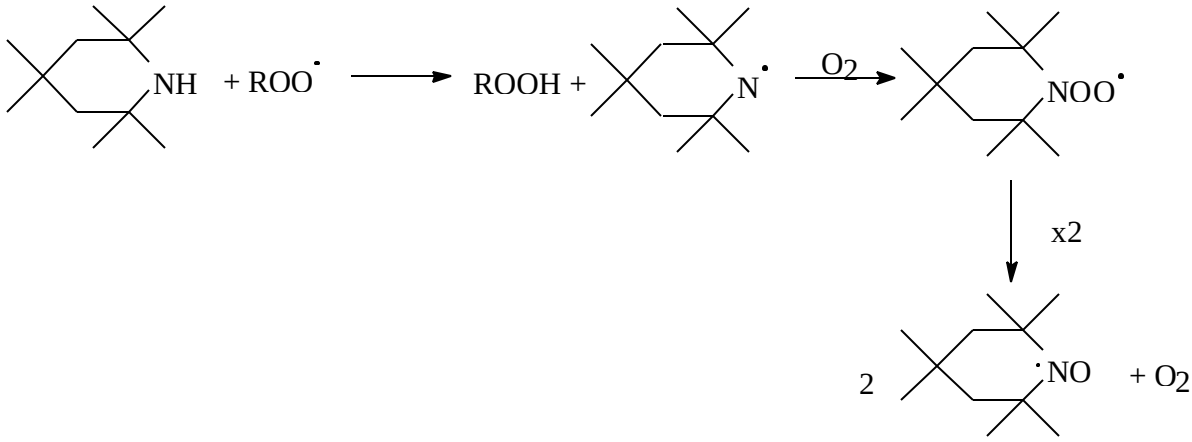
[3] Mitchell JB. DeGraff W. Kaufman D. Krishna MC. Samuni A. Finkelstein E. Ahn MS. Hahn SM. Gamson J. Russo A.. (1991) Inhibition of oxygen-dependent radiation-induced damage by the nitroxide superoxide dismutase mimic, tempol. *Arch Biochem Biophys.* **289:**62-70.

[4] Samuni A. Godinger D. Aronovitch J. Russo A. Mitchell JB. (1991) Nitroxides block DNA scission and protect cells from oxidative damage. *Biochemistry*. **30:**555-61.

Nitroxide reactions with Carbon-centered radicals[5]



C. HALS Hindered Amine Light Stabilizers Syringe nitroxide[6] [7] and other plasiticware[8]



D. Auto paint, UV protection and radiation protection

Tempo, Ford Motor Co. & Topcoat enamels[9]
Protection of skin in radiation therapy[10] [11] [12]

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[5] Moad G, Solomon DH (1995) The Chemistry of Free Radical Polymerization. Ed.1 Pergamon press

[6] Buettner GR, Scott BD, Kerber RE, Mügge A. (1991) Free radicals from plastic syringes. *Free Rad Biol Med* **11:** 69-70.

[7] Buettner GR, Sharma MK. (1993) The syringe nitroxide free radical - Part II. *Free Rad Res Comm* **19:** S227-S230.

[8] Miller CW, Chen GM, Janzen EG. (1999) Detection of free radicals in reperfused dog skin flaps using electron paramagnetic resonance spectroscopy: A pilot study. *Microsurgery*. 1**9(4):**171-175.

[9] Gerlock JL. Bauer DR. Mielewski DF. (1990) Using nitroxide decay to study the photooxidation kinetics of automotive topcoat enamels. *Free Radical Res Comms.* **10:**123-33.

[10] Goffman T, Cuscela D, Glass J, Hahn S, Krishna CM, Lupton G, Mitchell JB. (1992) Topical application of nitroxide protects radiation-induced alopecia in guinea pigs. *Int J Radiation Oncology Biol Phys* **22:** 803-806.

IX. Nitric Oxide:

Pro-oxidant

1. Rxn with superoxide

$t_{1/2}$ ($^{\bullet}NO$) ≈seconds in biological setting i.e. ≈ < 1 s

Rxn with $O_2^{\bullet -}$

$$k = 6x10^9\ M^{-1}s^{-1}$$

$$O_2^{\bullet -} + {}^{\bullet}NO \longrightarrow ONOO^-$$

peroxynitrite

2. Peroxynitrite (Oxoperoxonitrate (1-))

$ONOO^- + H^+ \longrightarrow ONOOH$ $pK_a = 6.5$

$\in_{240}$ (ONOOH) = 770 +/- 50 $M^{-1}cm^{-1}$

ONOOH → HNO_3 proceeds through a first order isomerization.

$O_2^{\bullet -} + {}^{\bullet}NO \longrightarrow ONOO^-$

$ONOO^-$ - powerful one- and two-electron oxidant!

[11] Hahn SM, Tochner Z, Krishna CM, Glass J, Wilson L, Samuni A, Sprague M, Venzon D, Glatstein E, Mitchell JB, Russo A. (1992) Tempol, a stable free radical, is a novel murine radiation protector. *Cancer Research* **52:** 1750-1753.

[12] DeGraff WG, Krishna MC, Kaufman D, Mitchell JB. (1992) Nitroxide-mediated protection against x-ray- and neocarzinostatin-induced DNA damage. *Free Rad Biol Med* **13:** 479-487.

3. Reactions of $^{\bullet}NO$ with O_2

$$2\ ^{\bullet}NO + O_2 \longrightarrow 2\ ^{\bullet}NO_2$$

$$^{\bullet}NO + {}^{\bullet}NO_2 \longleftrightarrow N_2O_3$$

$$N_2O_3 + H_2O \longleftrightarrow 2\ H^+ + 2\ NO_2^-$$

The net reaction is:

$$4\ ^{\bullet}NO + O_2 + 2H_2O \longrightarrow 4\ H^+ + 4\ NO_2^-$$

The rate of this process is governed by a rare, third-order rate constant where $k = 2.4 \times 10^6\ M^{-2}\ s^{-1}$ at 37°C [13]. This k is the same at pH 4.9 and 7.4. Nitrate is not formed. The activation enery is 2 kcal/mol.[14]

$$-d[^{\bullet}NO]/dt = +d[NO_2^-]/dt = 4k[^{\bullet}NO]^2[O_2]$$

$t_{1/2}(^{\bullet}NO) \approx$ seconds, but in a biological setting $\approx < 1$ s

Nitric oxide can serve as a chain-terminating antioxidant during lipid peroxidation. Lipid peroxidation occurs in a three-step process: initiation, propagation and termination.

LH + oxidant$^{\bullet}$ ® L$^{\bullet}$ + oxidant-H	*(initiation)*
L$^{\bullet}$ + O_2 ® LOO$^{\bullet}$	*(propagation cycle)*
LOO$^{\bullet}$ + LH ® L$^{\bullet}$ + LOOH	*(propagation cycle)*
L$^{\bullet}$ + L$^{\bullet}$ ® non-radical products	*(termination)*
L$^{\bullet}$ + LOO$^{\bullet}$ ® non-radical products	*(termination)*
LOO$^{\bullet}$ + Antioxidant-H → LOOH + Antioxidant$^{\bullet}$	*(chain-termination)*

[13] Ford *et al. FEBS Lett* **326:**1-3, 199
[14] Lewis & Deen, *Chem. Res. Toxicol.* **7**:568-574, 1994.

It is in the termination process that •NO may play a crucial role; •NO could serve as an antioxidant *via* the following chain-termination reactions:

$LOO^{\bullet} + {}^{\bullet}NO \rightarrow LOON{=}O$ *(chain-termination)*

$LO^{\bullet} + {}^{\bullet}NO \rightarrow LON{=}O$ *(chain-termination)*

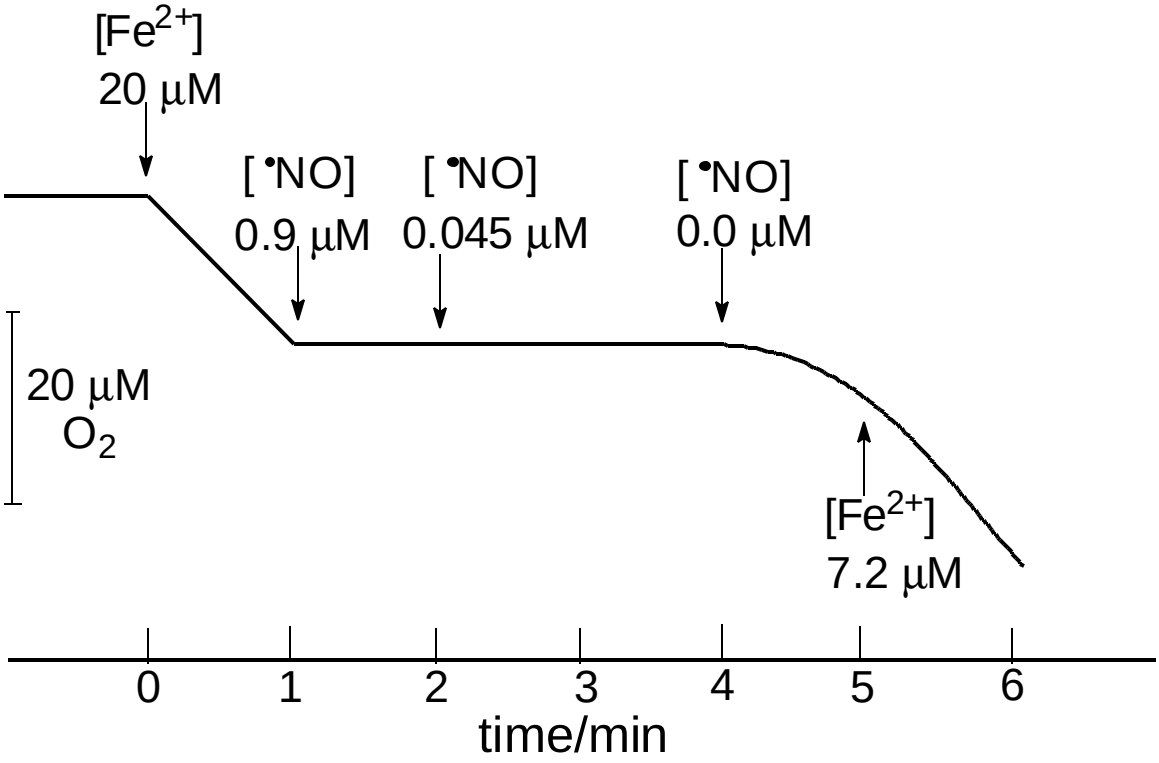

Figure: Changes in concentration of •NO and Fe^{2+} during cellular lipid peroxidation and its inhibition by •NO. Shown are the concentrations of •NO and Fe^{2+} at key time points. Cellular (HL-60) lipid peroxidation was initiated with 20 μM Fe^{2+}. At 1 min after the addition of Fe^{2+}, 0.9 μM •NO was introduced. •NO was rapidly depleted and is below the limit of detection at about 4 min. At the time of •NO depletion, rapid O_2 uptake resumes. This reinitiation of O_2 consumption is due to Fe^{2+} that is still present at 7.2 μM or about 36% of its original value.[15]

[15] Kelley EE, Wagner BA, Buettner GR, Burns CP. (1999) Nitric oxide inhibits iron-induced lipid peroxidation in HL-60 cells. *Arch Biochem Biophys* **370:** 97-104.

Traditionally vitamin E (TOH) has been thought to be the principal chain-breaking antioxidant in blood and in lipid structures of cells.[16] However, the experiments done that lead to this conclusion were done in the absence of •NO.

From the data presented above in conjunction with kinetic information from the literature, it is possible to make a kinetic estimate of the importance of TOH *vs* •NO as a chain-breaking antioxidant in our experiments with cells. A typical TOH level in cells is 1000-2000 PUFA/1 TOH.[17] If PUFA constitutes about 22% of the lipids in a cell membrane,[18],[19] then there are about 5,000-10,000 total fatty acid chains/1 TOH. As a first approximation, the mole fraction of TOH in lipid regions of membranes will be

$$\textbf{Mole fraction (TOH)} \approx 1/(5{,}000 \text{ to } 10{,}000) \approx 1/7{,}500 \approx 1.3 \times 10^{-4}.$$

Because TOH and the various fatty acids have similar properties, we assume, for simplicity, that they have the same partial molar volume in the lipid regions of cell membranes. Using a density of ≈ 0.9 g/mL and an average molecular weight of 300 g/mole for the fatty acyl chains of the lipids in a cell membrane, then the effective molarity of the fatty acyl chains in the lipid regions of membranes will be ≈ 3 M. The molarity of TOH will then be about

$$\textbf{M(TOH) will be } 3 \text{ M} \times 1.3 \times 10^{-4} \approx 400 \text{ mM}.$$

[16] Burton GW, Joyce A, Ingold KU. (1983) Is vitamin E the only lipid soluable, chain breaking antioxidant in human blood plasma and erythrocyte membranes? *Arch Biochem Biophys* **221**: 281-290.

[17] Kelley EE, Buettner GR, Burns CP. (1995) Relative α-tocopherol deficiency in cultured tumor cells: Free radical-mediated lipid peroxidation, lipid oxidizability, and cellular polyunsaturated fatty acid content. *Arch Biochem Biophys* **319**: 102-109.

[18] Wagner BA, Buettner G R, Oberley LW, Burns CP. (1998) Sensitivity of K562 and HL-60 cells to edelfosin, an ether lipid drug, correlates with production of active oxygen species. *Cancer Res* **58**: 2809-2816.

[19] Kelley EE, Buettner GR, Burns CP. (1995) Relative α-tocopherol deficiency in cultured tumor cells: Free radical-mediated lipid peroxidation, lipid oxidizability, and cellular polyunsaturated fatty acid content. *Arch Biochem Biophys* **319**: 102-109.

The rate at which •NO would terminate the chain propagation reactions in lipid peroxidation by reacting with the chain-carrying peroxyl radical, LOO•, will be:

$$\textbf{rate inhibition (•NO)} = k_{NO}\,[\text{•NO}]\,[\text{LOO•}]$$

where $k_{NO} = 2 \times 10^9$ $M^{-1}s^{-1}$ [20] and [•NO] = 8 × 45 nM = 360 nM. Here 8 is the estimated membrane/water partition coefficient for •NO;[21] [22] 45 nM is the measured •NO concentration in the aqueous phase of our cell suspension during the inhibition phase of lipid peroxidation. Thus

$$\textbf{rate inhibition (•NO)} = 2 \times 10^9\ M^{-1}s^{-1} \times 360 \times 10^{-9}\ M\,[\text{LOO•}]$$

The rate at which TOH would terminate these reactions would be:

$$\textbf{rate inhibition (TOH)} = k_{TOH}\,[\text{TOH}]\,[\text{LOO•}]$$

where $k_{TOH} = 8 \times 10^4$ $M^{-1}s^{-1}$ [23] and [TOH] = 400 μM. Thus, rate inhibition (TOH) $= 8 \times 10^4\ M^{-1}s^{-1} \times 400 \times 10^{-6}$ M [LOO•] The ratio of these rates is

$$\textbf{rate (•NO)/rate (TOH)} = \textbf{20/1}$$

in these experiments.

[20] Padmaja S, Huie RE. (1993) The reaction of nitric oxide with organic peroxyl radicals. *Biochem Biophys Res Commun* **195**: 539-544.

[21] Rubbo H, Radi R, Trujillo M, Telleri R, Kalyanaraman B, Barnes S, Kirk M, Freeman BA. (1994) Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. *J Biol Chem* **269**: 26066-26075.

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Comparing rate constants for the reaction of .NO and TOH with lipid peroxyl radicals we have:

In fact, if the concentration and kinetic parameters used to make this estimate were to accurately represent these processes in cells, then an aqueous concentration of $^{\bullet}$NO of only 2 nM would provide antioxidant protection for membrane lipids equal to that of vitamin E. This is a remarkably low concentration of $^{\bullet}$NO and is easily achievable in many cells and tissues.

When comparing estimated rate constants

$$k_{NO} = 2 \times 10^{9}\ M^{-1}S^{-1}$$
$$k_{TOH} = 8 \times 10^{4}\ M^{-1}S^{-1}$$

$$k_{NO} / k_{TOH} = 2.5 \times 10^{4}$$

No matter how you slice it, $^{\bullet}$NO appears to be a great antioxidant.