In this section of *Resonance,* we invite readers to pose questions likely to be raised in a classroom situation. We may suggest strategies for dealing with them, or invite responses, or both. “Classroom” is equally a forum for raising broader issues and sharing personal experiences and viewpoints on matters related to teaching and learning science.

An Appreciation of Free Radical Chemistry
3. Free Radicals in Diseases and Health

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In the last 20-25 years, considerable insight has been gained regarding the pivotal role played by free radicals in controlling and directing biological processes that cause diseases, as well as defend against them to maintain health. The manifestation of several hereditary diseases has been traced to the eventual tissue injury/ DNA damage through free radical reactions taking place at various levels in the biological system. A large body of experimental evidence indicates that the chemical transformations that induce cancer, stimulate atherosclerosis, lead to Parkinson's and Alzheimer's diseases, cause ischemia, and impel ageing process are basically free radical in nature. Ironically, free radicals are also part of the body's defence mechanism. If genetic predisposition and the life style favour the former, then health becomes the casualty.

Oxygen – a Double-edged Sword

As mentioned in Part 1[1], we live in the midst of an ocean of natural as well as unnatural free radicals. The diradical oxygen is essential for our life and we cannot completely avoid the other free radicals, even when our life style is moderate. The cells have

[1] Part 1. Introduction, *Resonance*, Vol.10, No.2, pp.72-78, 2005.
Part 2. Free Radical Reactions in Industry, *Resonance*, Vol.10, No.3, pp.71-79, 2005.

Keywords
Tissue injury, DNA damage, lipid peroxidation, free radical oxidants, reactive oxygen species (ROS), oxidative stress, antioxidants, ageing, phenolics, food preservatives, vitamins.

$$C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,CO_2 + 6\,H_2O \qquad (1)$$

glucose

$$C_{57}H_{104}O_6 + 80\,O_2 \longrightarrow 57\,CO_2 + 52\,H_2O \qquad (2)$$

glyceryl trioleate (a lipid)

Scheme 1.

to deal with about 0.2 mM concentration of dissolved oxygen in cytosol. The concentration of other free radicals obviously depends on the environmental pollution level, and habits such as smoking. In addition, free radicals are generated in all cells endogenously as by-products of the oxygen metabolism.

Energy required for life is derived by the oxidative metabolism of foods we eat. Carbon and hydrogen in carbohydrates, lipids, etc. are oxidised (metabolised) to CO_2 and H_2O, e.g., glucose and glyceryl trioleate molecules in *Scheme* 1.

Made in Mitochondria

The oxidising reactions, including the incomplete oxidation steps, generate by-products of the oxygen metabolism, with the inhaled oxygen undergoing the transformations as shown in *Scheme* 2.

Molecular oxygen (O_2), superoxide ($O2^{\cdot -}$), hydroperoxy radical ($H\text{-}O\text{-}O^{\cdot}$), and hydroxy radical ($HO^{\cdot}$) are all free radicals and are very reactive oxidants. $HO^{\cdot}$ is the most reactive and is capable of causing the most damage to biological systems. Hydrogen peroxide, though not a radical, is a reactive oxidant by itself as

$$O_2 + e^- \xrightarrow[\text{or mitochondria}]{\text{flavin-containing enzymes in cytosol}} O_2^{\cdot -} \qquad (3)$$

$$O_2^{\cdot -} + H^+ \longrightarrow H\text{-}O\text{-}O^{\cdot} \qquad (4)$$

$$O_2^{\cdot -} \xrightarrow[2\,H^+ + e^-]{\text{superoxide dismutase (Cu / Zn-SOD; Mn-SOD)}} H_2O_2 \qquad (5)$$

$$H_2O_2 \xrightarrow[\text{Prx}]{\text{GPx / GSH catalase}} H_2O + O_2 \qquad (6)$$

GPx / GSH catalase = glutathione peroxidase

Prx = peroxiredoxin

$$H_2O_2 + Fe^{2+} / Cu^{+} \longrightarrow HO^{\cdot} + HO^{-} \qquad (7)$$

(Fenton's reaction)

Scheme 2.

well as by producing the HO· radicals. These are collectively called reactive oxygen species (ROS) and are a significant challenge for the integrity of the genome. External source of radiation can also generate ROS within the cell. Other endogenous free radicals result from the metabolism of drugs, pollutants, chemicals, etc., together called xenobiotics.

ROS cause Injury to Biomolecules

The result of oxidative changes that the ROS bring about in cellular biomolecules is known as oxidative stress. A brief flow chart is shown in *Scheme* 3.

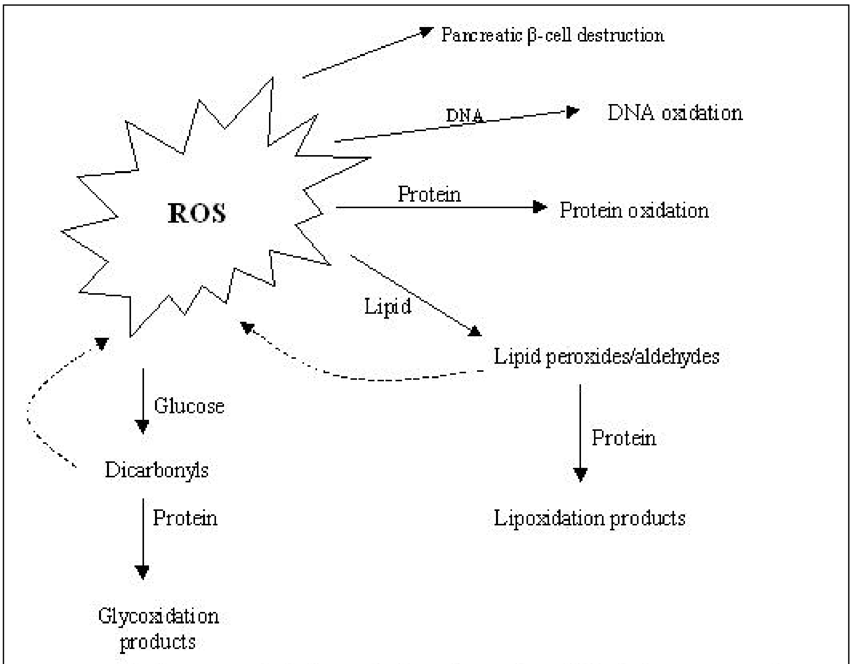
$$HO^{\bullet} / HOO^{\bullet} + RH \longrightarrow R^{\bullet} + H_2O \;/\; H_2O_2$$

$R^{\bullet}$ reacts with O_2, $HO^{\bullet}$, $HOO^{\bullet}$, $NO^{\bullet}$, etc

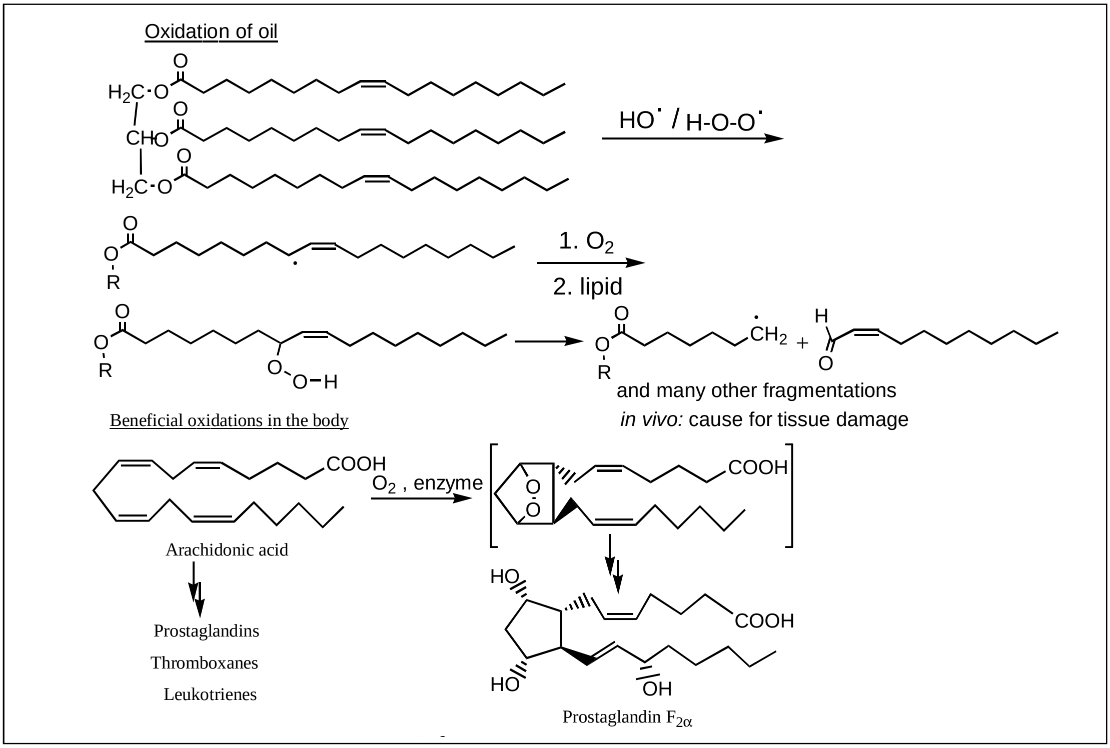
(RH = lipid, DNA, protein or inhibitor)

DNA damage occurs by ROS mediated hydroxylation of its bases, which leads to impairment of its function. In the case of

Scheme 3.



(Broken arrows indicate production of more free radicals in these processes)



Scheme 4.

proteins, ROS can oxidise amino acid side chain, oxidise protein backbone causing its fragmentation, or induce fragmentation of protein-protein linking. The attack of ROS on lipids leads to preoxidation and further to aldehydes, damaging the cell membrane; in the process, more ROS are formed (*Scheme* 4).

Oxidation of glucose produces glycoxidation products and more ROS. This establishes a self-perpetuating vicious and interactive cycle of molecular damage. If the damage controlling mechanism is not effective, diseases set in and progress. In any case, with advancing age some free radical damage keeps accumulating, which leads to diseases in the old age.

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Nitric Oxide and other Free Radicals

Nitric oxide (nitrosyl radical, NO·) is another free radical that has an important biological role. NO· is produced in the body. It relaxes muscles in blood vessels, and lowers blood pressure. (Many blood pressure-lowering drugs, e.g. nitroglycerine, amyl

$$NO^{\cdot -} + O_2^{\cdot -} \longrightarrow OONO^- \quad (8)$$
$$OONO^- + CO_2 \longrightarrow ONOOCO_2^- \quad (9)$$
$$OONO^- + H^+ \longrightarrow HOONO \quad (10)$$
$$HOONO \longrightarrow HO^{\cdot} + NO_2^{\cdot} \quad (11)$$
$$HOONO \longrightarrow HNO_3 \quad (12)$$

Scheme 5.

nitrite work on this principle). But, excess NO· produced in cases of severe infection can be harmful. Unlike HO· or $O_2^{\cdot -}$, NO· is a much slower reacting radical (see Part 2), and it combines with other free radicals and inhibits further reaction (e.g., with alkyl radicals in lipid perxidation) or generate more reactive products as given in *Scheme* 5.

Peroxynitrite, $OONO^-$, is highly cytotoxic, and the other products from it also damage biomolecules. Studies have shown that NOCl, HOCl, and NO_2· are also generated in vivo, and are sources of HO·, NO·, Cl· and NO_2· radicals.

Antioxidants Rein in ROS

If the activity of ROS, other cellular free radical by-products, and free radicals from exogeneous sources is not contained, they can cause certain cancers, cataracts, neural diseases, diabetes, cardiovascular diseases, and weaken immune system. Accumulation of cellular damage due to free radicals is considered to be one of the main causes of ageing. Fortunately, there are several damage control mechanisms. Enzymes such as superoxide dismutase (SOD), methionine reductase, catalase, and glutathione peroxidase destroy most of the free radicals. There are other chemicals we obtain from foods, particularly fruits and vegetables, which effectively inhibit the harmful free radical activity. They are all collectively called antioxidants.

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How do Antioxidants Function?

Antioxidants defend against the free radical menace in three different ways. They may (i) bind to free radicals and deactivate them, (ii) convert reactive free radicals into non-damaging species, Indian curries, sambar, rasam, etc., prepared using a variety of spices, with turmeric as the main ingredient, also supply antioxidants in good quantities.

or (iii) repair the cellular damage.

A variety of foods that we consume constitute a number of important antioxidants. They are, for example, vitamins A, C and E, carotenoids, phenolic compounds like curcumin in turmeric, resveratrol in red wine, flavonoids, etc. In addition, sulphur compounds like allicin in garlic and selenium compounds are also good antioxidants. Strong and bright coloured vegetables and fruits particularly are rich sources of antioxidants. Indian curries, sambar, rasam, etc., prepared using a variety of spices, with turmeric as the main ingredient, also supply them in good quantities.

The French Paradox and the Curative Curry

Red wine has drawn much attention of researchers because the southern French people who consume red wine regularly show the least incidence of heart diseases. This benign outcome of drinking an alcoholic beverage, popularly called "French Paradox", is attributed to the radical scavenging property of resveratrol present in red wine in high concentration.

Likewise, the curry eating Indians show a much lower prevalence (almost one-tenth) of Alzheimer's disease than the Americans. The credit for this is given to curcumin in turmeric, the main ingredient of all curries.

It is also found that the individual antioxidants in isolation, given as food supplements, do not work as effectively.

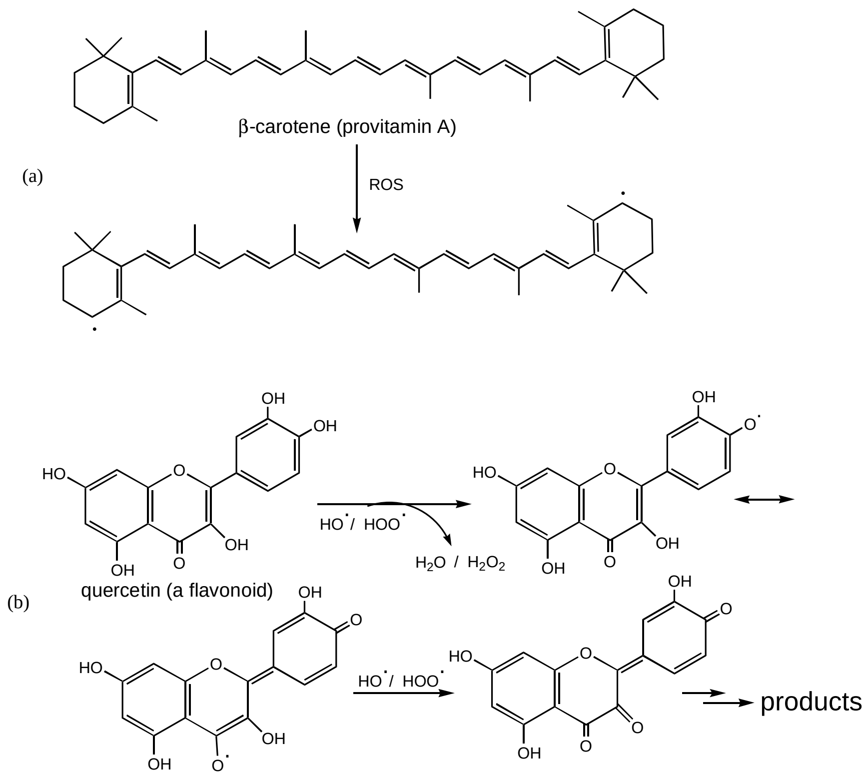
It is generally agreed by scientists that fruits and vegetables eaten in adequate amount are effective in lowering the incidence of cardiovascular diseases, and certain cancers. Unfortunately, it is also found that the individual antioxidants in isolation, given as food supplements, do not work as effectively. An antioxidant identified as a therapeutic against one disease may not be effective against another. For example, curcumin, believed to control Alzheimer's disease, does not seem to work against heart diseases, and vice versa in the case of resveratrol, though both work by free radical inhibition mechanism. These observations indicate that certain synergy is necessary for the effective functioning of the antioxidants in the cellular environment.

Antioxidants Break the Radical Chain

Let us now consider some antioxidants and try to understand how they are likely to act in damage control roles. Vitamins A, C and E, flavonoids, phenolic compounds, carotenoids that are common constituents of vegetables and fruits, are the natural antioxidants able to check and balance the harmful effects of the reactive free radicals.

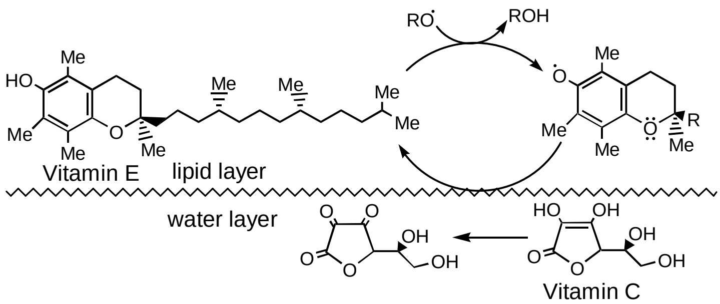
A glance at the structures of these molecules (*Scheme* 6) reveals that each one of them has one or more easily detachable hydrogen atoms. When a highly reactive free radical, say an ROS, takes away one such hydrogen atom, it loses its free radical character and damage causing reactivity, and the antioxidant molecule is converted into a free radical, but of a far greater stability rendered

Scheme 6.

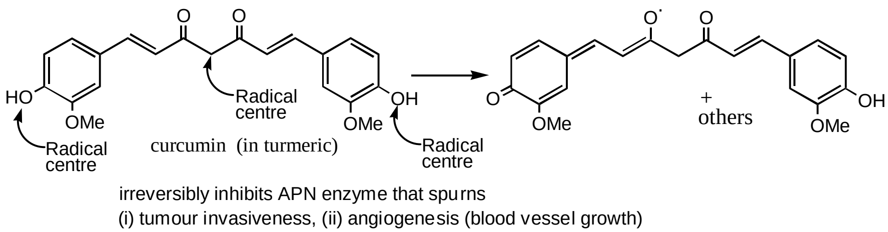


Scheme 6 continued...

(c)



(d)



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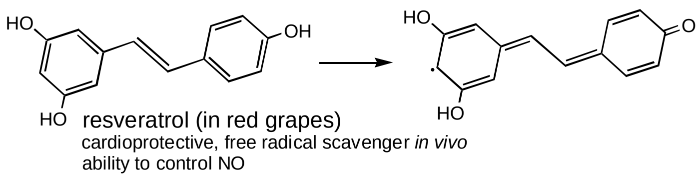

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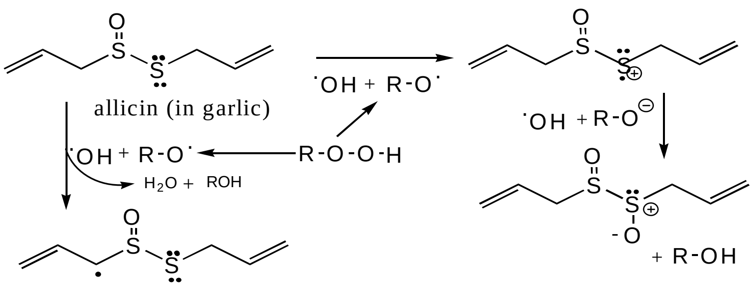


stable by resonance and steric effects. A stable radical means it is a non-reactive radical and it can cause practically no harm to biomolecules. This explanation is of course a much-simplified mechanistic picture of what is actually happening in the cellular environment, yet one can gain some rough image of how the antioxidants probably function.

Antioxidants in Food Preservation

Phenolic compounds are used as food preservatives, which quench the reactive free radicals. Some examples are butylated hydroxyanisole (BHA) and butylated hydroxy toluene (BHT), L-ascorbic acid (vitamin C) and its sodium and calcium salts and Tocopherols (vitamin E and derivatives).

Table 1. Sources and effects of free radicals and antioxidants.

A Sources of free radicals

1. Automobile exhaust fumes
2. Ultraviolet radiation - interaction with chemicals
3. Smoking of cigarettes, cigars, beedis, etc. (one puff of cigarette smoke is estimated to contain 10^{14} free radicals with >4000 compounds, including $NO^{\cdot}$ and $NO_2^{\cdot}$)
4. Burning of organic matter - during cooking, forest fires, etc.
5. Volcanic activities
6. Radioactive decay – a, b and g radiation
7. Lightning - particularly oxides of nitrogen
8. By-products of oxygen metabolism. (Illness causes the body to produce greater amounts of harmful radicals than in healthy conditions)
9. Industrial effluents
10. Chemicals – excess alcohol intake, certain drugs, asbestos, certain pesticides and herbicides, some metal ions, fungal toxins, etc. inflict oxidative stress

B Diseases caused by free radicals

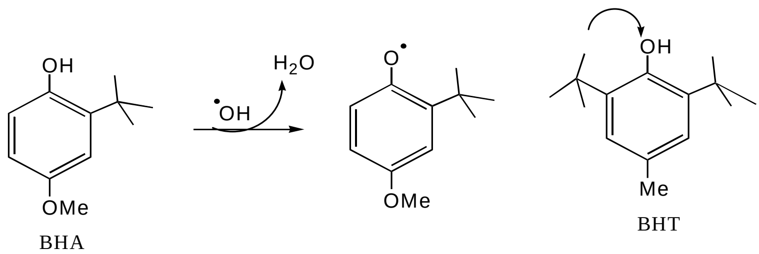
1.	Cancer and other malignancies	4.	Ischemia/reperfusion injury
2.	Atherosclerosis	5.	Radiation injury
3.	Degenerative neurological diseases	6.	Ageing

C Antioxidant radical inhibitors

1. Enzymes (endogenous) - Super oxide dismutase, Methionine reductase, Glutathione peroxidase, Catalase
2. From external sources - vitamins, flavonoids and other phenolic compounds; carotenoids; sulphur and selenium compounds

D Sources of natural antioxidants

1.	Fruits	4.	Grains
2.	Nuts	5.	Leaves
3.	Vegetables	6.	Spices



Postscript

When, in 1969, Joe McCord and Irwin Fridovich of Duke University, USA, discovered that the common intracellular protein superoxide dismutase functions as free radical scavenger, which suggested the SOD's defence mechanism against the endogenously generated ROS, their idea was considered absurd. However, the newer discoveries of pathology of free radical diseases and the role of SODs and antioxidants in controlling them, gradually established the importance and wide scope of their seminal contribution to the field of free radical biology.

These days, much of medical research is devoted to investigations for knowing more about the part played by free radicals in most diseases and about antioxidants in preventing or even reversing them. At the other end, for some people, the faith in the healing power of antioxidants has reached the level of superstition, which is evident from the publicity given in women's magazines, health magazines, pamphlets and even books, to antioxidant creams, antioxidants and dietary supplements that are supposed to prolong life, stop ageing or prevent diseases. The hype apart, free radicals in biological systems are indeed extremely important. A better understanding of their functioning and proper control would greatly help in leading a long and healthy life.

In the next part, we shall see what the free radicals do in the atmosphere and how they punch hole in the ozone blanket.

Suggested Reading

[1] Proceedings: International Conference on 'Antioxidants and Free Radicals in Health-Nutrition and Radio-Protectors'; IV Annual Conference of the Society for Free Radical Research in India, 8-10 January, 2005, Bangalore; http://www. sjmc-sfrr.org

[2] Antioxidants and Free Radicals, http://www.rice.edu

[3] Free Radicals: The cause of virtually all disease, http://www.oralchelation.com

[4] P Renaud and M P Sibi (Editors), Radicals in Bio-materials, *Radicals in Organic Synthesis*, Wiley-VCH, pp. 505 - 577, 2001.

[5] M M Cayuela, Oxygen Free Radicals and Human Disease, *Biochemie.*, Vol. 77, pp. 147-161, 1995.

[6] T K Basu (Editor), N J Temple and M L Garg, *Antioxidants in Human Health*, CAB International, 1999.

[7] R S Sohal, R J Mockett and W C Orr, Mechanism of Ageing: An Appraisal of the Oxidative Stress Hypothesis, *Free Radical Biology and Medicine*, Vol. 33, pp. 575-586, 2002.