

## Role of Reactive Oxygen Species in Biological Processes

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**Summary.** The steady-state formation of prooxidants in cells and organs is balanced by a similar rate of their consumption by antioxidants that are enzymatic and/or nonenzymatic. "Oxidative stress" results from imbalance in this prooxidant-antioxidant equilibrium in favor of the prooxidants. A number of diseases are associated with oxidative stress, being the basis of a potential antioxidant therapy. However, current evidence in clinical research does not show unequivocal distinction between causal or associative relationships of prooxidants to the disease process.

**Key words:** Oxidative stress – Oxidants – Antioxidants – Nitric oxide – Free radicals

It is now well-accepted that exposure of living organisms to reactive oxygen species, notably oxygen free radicals and hydrogen peroxide, is associated with the very fact of aerobic life (for reviews, see [4, 9, 31, 33]). Notions that the challenge comes from external noxious sources such as ionizing radiation, toxins, drugs, chemicals, environmental pollutants are correct, but it is equally true that every cell in the living organism can generate reactive oxygen species as well, and some cell-types are even specialized to do so continually or in the form of an 'oxidative burst'. Our own metabolism produces and needs free radicals even under healthy conditions, and one may be surprised to find that these attacking species have a diversity of useful effects. An interesting result of recent studies has been that oxygen free radicals can be

generated under the control of stimuli and signal molecules; a veritable network of functions is being uncovered here. One recent highlight has been the elucidation of the biology of the nitric oxide radical.

### Processes and Cell Responses

Numerous molecular and cellular processes involve reactive radical intermediates. While the role of free radicals in carcinogen activation and the neutrophil respiratory burst have been studied for some time, the role of oxygen radicals in vasodilation has been one of the more recent additions to this field. Starting out from the discovery by Furchgott and Zawadzki [10] that endothelium produced a "relaxing factor" (EDRF, endothelium-derived relaxing factor) of short biological half-life, and the assignment to NO, nitric oxide, by Palmer et al. [25] to this function, there has been a veritable explosion of knowledge in this area, unraveling a novel biochemistry of arginine, and explaining the long-known pharmacological effects of nitrovasodilators [22].

Our own recent work in this area was on nitric oxide production by cultured aortic endothelial cells in response to thiol depletion and replenishment [21], arguing against hypotheses suggesting that cellular thiols are either substrates or necessary cofactors in the pathway of NO synthesis in these cells. Phorbol-ester-activated Kupffer cells generate luminol-dependent chemiluminescence, and it was demonstrated that this photoemission depends largely on L-arginine metabolism by NO synthase, requiring the concurrent formation of NO and superoxide/hydrogen peroxide [38]. Further, we have found that superoxide dismutase is capable of reversible conversion of the nitroxyl anion to nitric oxide [20], so that it is conceivable

*Abbreviations:* ARDS = adult respiratory distress syndrome; EDRF = endothelium-derived relaxing factor; MOF = multi-organ failure

that SOD may protect NO and endothelium-derived relaxing factor by a mechanism in addition to superoxide scavenging [17] and that the nitroxyl anion may be a physiologically important form of endothelium-derived relaxing factor.

### Towards Clinical Medicine

Assertions have been made that reactive oxygen species may be critically involved in human health and disease (see [11, 12]). However, the numerous attempts in recent years to successfully apply antioxidant therapy have generated less than satisfactory results. One area that was greeted enthusiastically in clinical medicine is that of reoxygenation injury, or 'ischemia-reperfusion', and the role of xanthine oxidase. The initial euphoria has been dampened, but in certain organs and clinical settings the ischemia-reperfusion problem has potential [23, 27, 28]. One basic problem in the transposition of biochemistry and pharmacology of reactive oxygen to clinical medicine is the fact that diseases under scrutiny are multifactorial. For example, the adult respiratory distress syndrome (ARDS), multi-organ failure (MOF), and traumatic or hemorrhagic shock include components amenable to antioxidant therapy and protection, but other factors such as proteinase activation are of cardinal importance as well (see [34]). Even an activation of HIV-1 by oxidative stress has been reported [15], and thiols, low in plasma of HIV-1-infected patients [8], regulate activation of nuclear factor kappa-B and transcription of human immunodeficiency virus [29, 36]. Interleukins, tumor necrosis factor, and other cytokines are key players in this field.

Organs such as the eye lens or skin and the lung apparently have direct relations to oxygen species, but certainly other organs in the body are open to oxygen radical attack, given the activity of the neutrophils and other cell-types, e.g. macrophages. Even tissue cells have recently been shown to give off superoxide in response to cytokines and other types of stimuli, potentially as a metabolic regulatory signal [18, 19].

### Antioxidant Defense

The genetic control of antioxidant defense has been elucidated with microorganisms, and the striking relationships to other adaptive responses such as the heat shock response underline the central functions in evolution and development. There is evidence for induction of transcription of the protooncogenes, *c-fos* and *c-myc*, in mouse epidermis

cells by oxidative stress [5] and for redox regulations of Fos and Jun proteins on DNA-binding activity [1]. Thus, oxidants are implicated in alterations in gene expression and have potential significance in differentiation and development [2].

Another fascinating aspect of antioxidant defense is related to a number of antioxidant vitamins and micronutrients [16]. The tocopherols in the lipid phase protect membranes and lipoproteins [6, 35, 37]. Other nutritive antioxidants are the carotenoids [7, 14], a topic that deserves attention because of epidemiological evidence in cancer prevention. Thus, leafy green and yellow vegetables are of interest in this respect, but also in plant physiology itself, the carotenoids fulfill essential roles in preventing harmful photooxidation reactions: what keeps plants from getting sunburnt? There would be no photosynthesis were it not for the presence of carotenoids in the photosynthetic reaction center. Also, we should not forget the other side of the coin: plants contain carcinogens, and dietary pesticides are predominantly of natural origin [3].

The realm of food science and technology encompasses the use of antioxidants. The metabolism of some of the phenolic antioxidants generates potentially harmful reactive species, so that currently there is an increased search for the employment of natural antioxidants in food processing, food preservation and food coloring. Health food, to a large extent, focuses on protective functions by biological antioxidants. An adequate assessment of the daily needs, the definition of a "prudent diet" and the discussion on the benefits and risks of dietary supplements all underline the need for appropriate basic research in this area.

Much research and development of compounds exerting antioxidant effects has been carried out in the hope of controlling oxidative stress in a number of human diseases. An area of particular interest emerged in the prevention of arteriosclerosis, as studied by the effects of probucol, a phenolic antioxidant [26]. Long-term protection is required in modulating degenerative diseases (arthritis, cataract, diabetes, arteriosclerosis, cancer, etc) as well as the aging process itself.

### Oxidative Stress

"Oxidative stress" was defined as a disturbance in the prooxidant-antioxidant balance in favor of the former [30]. As there has been a proliferation of publications using this term, a few cautionary words may be appropriate. How can it be defined operationally? The answer to this question is some-

what arbitrary. Considering the normal (healthy) state, oxidative challenge occurs in many cell-types, but this alone does not constitute an oxidative stress. Likewise, a simple loss of antioxidant as resulting from limited nutritional supply is not sufficient. However, when there is an increased formation of prooxidants such as hydrogen peroxide, which is accompanied by a loss of glutathione caused by the formation of glutathione disulphide, we approach a definition. Even a severe loss of antioxidant may, however, mean that there is no resulting damage. Useful definition of "oxidative stress" therefore would be: a disturbance in the prooxidant-antioxidant balance in favor of the former, leading to potential damage. Such a definition would incorporate damage products as indicators of oxidative stress [31, 32], and accordingly this area has been the subject of much research with damaged DNA bases, protein oxidation products and products of lipid peroxidation being examined as indicators of oxidative stress [24].

An adequate assessment of the occurrence and activity of free radicals in biological systems is difficult. In particular, the widespread use of measurements of malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury requires caution and correlative data [13]: in fact, it is scientifically unsound to equate increased plasma or serum levels of thiobarbituric acid-reactive material alone with the occurrence of a "free radical disease". The diversity of reaction products in oxidative stress is illustrated by the results obtained by employing novel techniques [24].

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