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**The evolution of *Free Radical Biology & Medicine*:
A 20-year history**

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***Free Radical Biology & Medicine*
The last 20 years: The most highly cited papers**

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Editorial

The evolution of *Free Radical Biology & Medicine*: A 20-year history

Free radicals are reactive chemical species that have an odd number of electrons. Because they are so reactive, and because their lifetimes generally are very short, their very existence has often been clouded in acrimonious debate [1]. The role of free radicals in biological systems was, if possible, even more controversial [2]. In the early 1960s, in fact, most biochemists and biologists believed that free radicals were much too short-lived and uncontrollable to play any role in life processes. In that era, a journal devoted to free radical biology would have seemed ludicrous at best.

All of this was dramatically changed when Joe McCord and Irwin Fridovich reported the properties of the enzyme superoxide dismutase (SOD) in 1968. Suddenly a technique was available to probe for the presence of free radicals in living systems. Lo and behold, free radicals proved to be vital in the functioning of every air-living organism! The turnabout could not have been more dramatic. It is striking that the discovery of SOD has not yet resulted in the award of a Noble prize to McCord and Fridovich. We comment on the reasons for this, psychological and scientific, in another place [3].

Hard on the heels of the discovery of SOD came the discovery of the prostaglandin enzyme system, reactions that involve free radical intermediates and produce peroxides and hydroperoxides that play vital roles in many biological processes. The discoveries in that area were followed immediately by a hoard of researchers reporting that radical reactions are involved in many detoxification pathways, and in the reactions of these toxins with their biological targets. At the beginning of the landslide of activity in free radical biology, the President's Symposium of the American Society for Experimental Pathology was held in Atlantic City in April 1970. The group of us involved decided to produce a book on free radical biology [4], which we hoped would be of use in this new and growing field; amazingly, that book grew to become a six-volume set [5].

In 1980 Norman Krinsky chaired the first Gordon Conference titled Oxygen Radicals in Biology, and both of us (separately) have chaired subsequent Gordon Conferences in this series. Next year, Chairs Henry Jay Forman and Rafael Radi and Vice Chairs Stanley L Hazen and Kevin Moore will organize the 25 anniversary 'edition' of this very influential conference. More recently, a new Gordon Conference series titled Oxidative Stress & Disease (to be co-chaired by KJAD in 2009) has been created to complement the continuing Oxygen Radicals in Biology meetings. It is remarkable to consider that the early Gordon Conferences were dominated by heated

discussions of the possible biological significance of the hydroxyl radical, and the meaning (if any) of 'free iron or copper.' It is also sobering to remember that oxidative modification of proteins and DNA was a novel, and controversial, idea just 20 years ago in the early 1980s. Over the years, annual meetings of the Oxygen Society, SFRR Europe, SFRR Asia, SFRR Australasia, and now SFRR Africa and SFRR ASEAN, as well as biennial congresses of SFRR International, have greatly contributed to the development of the free radical field, and FRBM has published meeting abstracts and programs for a good number of such conferences.

In 1985, we began an experiment with Pergamon Press in publishing *Advances in Free Radical Biology & Medicine* (edited by WAP), a review journal, and *Journal of Free Radicals in Biology & Medicine* (edited by KJAD), a forum for original research [6,7]. Over the first two years of each of these publications, we realized that it would be advantageous to both Pergamon and the field to combine our already well-established journals into a single, better vehicle. The marriage of our two publications to form *Free Radical Biology & Medicine* [8] met with a resounding response from the free radical research community, and submissions still continue to increase. In 1985, we began with just six issues per year. By 1988 we had increased to 12 issues annually, which we maintained for several years. Then began our most rapid growth period, mounting to 14 issues in 1996, 18 issues in 1998, and 24 issues in 1999 (not including supplements). We have kept publication to 24 issues annually (every other week), although readers will certainly have noticed that many volumes are almost bursting at the seams!

In 1988 the Oxygen Society (now the Society for Free radical Biology & Medicine) adopted *Free Radical Biology & Medicine* as its official publication. Although there were many initial qualms about the advisability of this move, the outcome has clearly been extremely beneficial for both parties and, more importantly, for our respective readers and members. The Society has provided the journal with a stable and committed audience that we greatly treasure. In return, the journal has provided the Society with a significant and reliable income with which to conduct its affairs. When Elsevier took over *Free Radical Biology & Medicine* in 1995, after the collapse of the Maxwell empire which owned Pergamon Press, we began a process of turning a promising adolescent journal into a mature publication. We hope readers will have noticed the very significant improvements in both the quality and the professional 'feel' of *Free Radical Biology & Medicine* that have taken place

over the past few years. These improvements have combined with the excellent quality of manuscripts submitted, and superb reviewing by free radical scientists around the globe, to raise our impact factor to heights we never expected to achieve.

Towards the end of the 1990s the Oxygen Society and Elsevier concluded a series of negotiations that changed *Free Radical Biology & Medicine* in many ways. First among these changes was the creation of a central editorial office in Newton, Massachusetts, under the excellent direction of Denise Wells. Aided by a very capable staff, Denise helped turn our 'cottage-industry' journal into the professional publication you now enjoy. In the last year, our editorial offices have moved to San Diego, California, in a move so smooth that many readers may not yet even be aware of the change. Second, our 'New Deal' also included the addition of associate editors to be elected in a democratic process, by the Publications Committee of the Oxygen Society. As you will see from our masthead page, *Free Radical Biology & Medicine* now has eight associate editors: Victor M. Darley-USmar, University of Alabama, Birmingham; Phyllis A. Dennery, Children's Hospital of Philadelphia; Henry J. Forman, University of California, Merced; Matthew Grisham, Louisiana State University Health Sciences Center; Harry Ischiropoulos, Children's Hospital of Philadelphia; Balaraman Kalyanaraman, Medical College of Wisconsin; Kevin Moore, Royal Free & University College London Medical School; and L. Jackson Roberts, II, Vanderbilt University. Catherine Rice-Evans of King's College, London University, completed her term as associate editor in December 2004. All these associate editors have greatly contributed to the success of FRBM, and they are major reasons for our survival to age 20. Naturally, the associate editors greatly increase the scope of our expertise, while significantly decreasing manuscript handling times. The third major change, which may be credited to the perseverance of (then Oxygen Society President) Bruce Freeman and subsequently (Publications Committee Chair) Victor Darley Usmar, and the insight of Elsevier executives, was the creation of a new contractual partnership between Elsevier and the Oxygen Society that has proved to be of great value to both parties.

In 1999 Elsevier appointed Anthony Newman, of their Amsterdam headquarters, our new publishing editor. Despite a very long list of Pergamon and Elsevier employees and executives who have exerted significant influence on *Free Radical Biology & Medicine* over the years, it must be said that Anthony Newman has been a peerlessly relentless force for improved quality, decreased manuscript handling and publication times, and faultless integrity. All of us involved with *Free Radical Biology & Medicine* owe Anthony Newman a debt of gratitude for his tireless work on our behalf.

In 2000 one of us (WAP) felt the need to retire from active editing, although you'll still find the occasional book review or editorial with the WAP moniker. This left KJAD as sole editor-in-chief, but also opened the way for more new associate editors to be appointed; a total of eight, as noted above. Naturally, WAP also maintains a strong interest in the health and well-being of his 'child'; some things are for life!

In 2003 the Oxygen Society paid our journal the highest compliment possible, by changing the organization's name to

Society for Free Radical Biology & Medicine. Thus, FRBM is now the official publication of SFRBM. Also in 2003, FRBM became "an affiliate publication of the International Society for Free Radical Research" (SFRR International). These changes are a clear and welcome signal of the cooperation that now exists at all levels within the free radical field, and of the central role played by *Free Radical Biology & Medicine*.

What of the future? Where will *Free Radical Biology & Medicine* be in another 20 years? Frankly, neither of us has a crystal ball, and had we been forced in 1985 to predict the status of FRBM in 2005 we would have been hard pressed to sound confident even of continued existence! Neither of us could have predicted the enormous impact of molecular biology and genetics on our field, although (as the journal title implies) we both were betting on the (then) emerging dominance of biology and medicine in the free radical field. In many ways, new journals are rather like new restaurants—it's much easier to open one than to keep it open for business. Nevertheless, here we are, 20 years later, and we're still serving up an extensive 'menu' of exciting free radical 'dishes.' Thanks to all the contributors; reviewers; editors; SFRBM presidents, officers, and Publications Committee members; Pergamon and Elsevier staff and executives; and (perhaps most of all) you the reader for your support and hard work.

Happy 20th birthday *Free Radical Biology & Medicine*; your 'parents' are very proud!

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Free Radical Biology & Medicine The last 20 years: The most highly cited papers

The relative importance of scientific articles is always of interest. As part of a look back over the last 20 years, the Centre for Science and Technology Studies (CWTS) at the University of Leiden, The Netherlands, was asked to use the Institute for Scientific Information (ISI) information and to provide a list of the most highly cited papers published in *Free Radical Biology & Medicine* since the journal started.

In the period from when the journal started 20 years ago until the end of 2004, a total of 7,354 papers and editorials were published, attracting a total of 82,229 citations.

Below are listed the 100 highest ranked papers over this period, with title, authors, abstracts and keywords for the top 50 papers and title and authors for the remaining 50 papers. There is also a link through into ScienceDirect to access the abstracts of the remaining papers, and also the full papers, if your institute subscribes.

The ranking is based on the total of all of the citations, totalled over the period since publication. The most highly cited paper, by Hermann Esterbauer et al., for example, received almost 1400 citations to date, as per the 2004 data, made available in June 2005.

Members of the Society for Free Radical Biology and Medicine (SFRBM) may also access these past papers plus all of the rest of the entire back-volume collection by using their society username and password within the ScienceDirect Society Service.

The Publisher

Rank: 1. 1387 citations to date
Free Radic. Biol. Med. 13, 341–390 (1992), 10.1016/0891-5849(92)90181-F

Review: The Role of Lipid Peroxidation and Antioxidants in Oxidative Modification of LDL

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Abstract: The purpose of this study is to provide a comprehensive survey on the compositional properties of LDL (e.g., lipid classes, fatty acids, antioxidants) relevant for its susceptibility to oxidation, on the mechanism and kinetics of LDL oxidation, and on the chemical and physico-chemical properties of LDL oxidized by exposure to copper ions. Studies on the occurrence of oxidized LDL in plasma, arteries, and plaques of humans and experimental animals are discussed with particular focus on the

use of poly- and monoclonal antibodies for immunochemical demonstration of apolipoprotein B modifications characteristic for lipid peroxidation. Apart from uptake of oxidized LDL by macrophages, studies describing biological effects of heavily or minimally oxidized LDL are only briefly addressed, since several reviews dealing with this subject were recently published. This article is concluded with a section on the role of natural and synthetic antioxidants in protecting LDL against oxidation, as well as some previously unpublished material from our laboratories.

Keywords: Low density lipoprotein; LDL; Lipid peroxidation; Free radicals; Antioxidants; Vitamin E; Atherosclerosis

Introduction: Atherosclerosis is not a trivial or rare disease: About half of all people enjoying a Western lifestyle are currently dying of myocardial infarcts or strokes caused by sudden damming of arteries narrowed by atherosclerotic plaques. Until recently, only the manifestations of the disease and its consequences have been studied extensively, with the underlying biochemical mechanism of atherogenesis largely unknown. However, a series of separate excellent studies carried out mainly in the last decade (Table 1) have provided the background allowing the formulation of a new reasonable

theory of atherogenesis which has focused much of current research on tests of its validity. The bare bones of this recent postulate is that atherosclerotic plaques form from cells engorged with lipids supplied by blood lipoproteins, modified by a free radical process. Pathological, microscopic, histochemical, and biochemical studies have shown that the occlusions and plaques which form in the intima regions of the major arteries are mainly made up of cells so altered in appearance by internalized lipids that they are known as foam cells. Foam cells were identified as macrophages derived from monocytes circulating in the blood and smooth muscle cells proliferating in the region of the plaque. Their gross alteration is mainly caused by the entry of lipids (e.g., lipoprotein particles modified in or near the artery). These particles bypass the normal tight control exercised by the cells' surface receptors and enter the cells by a different, scavenger pathway, which has no such control. There is much evidence (for review see Ref. 57) that the principal lipoproteins susceptible to the modification leading to foam cell formation are low density lipoproteins (LDL). Since LDL is the main carrier of free and esterified cholesterol in the body, these lipids are the predominant components of the foam cells. This brief summary covers the knowledge of likely atherogenic events derived from studies completed before about 1980.

Concluding paragraph: From all these studies, we conclude that vitamin E has, in addition to its antioxidant function in LDL, a great potential in preventing other deleterious events involved in the pathogenesis of atherosclerosis. The therapeutic potential of vitamin E in various diseases, including those associated with oxidative stress, was recently reviewed by Janero and Chow.

At the time that this paper was published: Hermann Esterbauer, PhD (1936–1997), was a professor of biochemistry at the University of Graz, Austria. He trained in chemistry and biology at the Universities of Vienna and Graz, and graduated with a PhD in 1963. He did postdoctoral work (1973–1974) at the University of Pittsburgh and at the Michigan State University and was visiting professor at the Universities of Turin (1984–1988) and Siena (1989) and at the Brunel University (1987–1991).

Janusz Gebicki, PhD, was associate professor in biology at the Macquarie University, Sydney. He studied chemistry at the University of London, where he gained the BSc and PhD. He subsequently worked at McMaster University, Hamilton, Canada, at the Washington University School of Medicine in St. Louis, Missouri, and at the Brookhaven National University, near New York. He was appointed to Macquarie University, Sydney, after a period as research fellow at the Australian National University.

Herbert Puhl, PhD, was a postdoctoral fellow at the Institute of Biochemistry, University of Graz. He studied biology and chemistry and gained his PhD in 1992 from the University of Graz.

Günther Jürgens, PhD, was associate professor of biochemistry at the Institute of Medical Biochemistry, Medical School, University of Graz. He studied chemistry, performed his thesis in physical chemistry and biochemistry, and graduated with a PhD at the University of Graz in 1974.

Rank: 2. 968 citations to date

Free Radic. Biol. Med. 20, 933–956 (1996), 10.1016/0891-5849(95)02227-9

Review: Structure–Antioxidant Activity Relationships of Flavonoids and Phenolic Acids

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Abstract: The recent explosion of interest in the bioactivity of the the flavonoids of higher plants is due, at least in part, to the potential health benefits of these polyphenolic components of major dietary constituents. This review article discusses the biological properties of the flavonoids and focuses on the relationship between their antioxidant activity, as hydrogen donating free radical scavengers, and their chemical structures. This culminates in a proposed hierarchy of antioxidant activity in the aqueous phase. The cumulative findings concerning structure–antioxidant activity relationships in the lipophilic phase derive from studies on fatty acids, liposomes, and low-density lipoproteins; the factors underlying the influence of the different classes of polyphenols in enhancing their resistance to oxidation are discussed and support the contention that the partition coefficients of the flavonoids as well as their rates of reaction with the relevant radicals define the antioxidant activities in the lipophilic phase.

Keywords: Flavonoid; Antioxidant; Total antioxidant activity; Catechin; Low-density lipoprotein; Anthocyanidin; ABTS; Tea; Wine

Rank: 3. 603 citations to date

Free Radic. Biol. Med. 22, 269–285 (1997), 10.1016/S0891-5849(96)00275-4

Review: Oxidants as Stimulators of Signal Transduction

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Abstract: Redox (oxidation-reduction) reactions regulate signal transduction. Oxidants such as superoxide, hydrogen peroxide, hydroxyl radicals, and lipid hydroperoxides (i.e. reactive oxygen species) are now realized as signaling molecules under subtoxic conditions. Nitric oxide is also an example of a redox mediator. Reactive oxygen species induce various biological processes such as gene expression by stimulating signal transduction components such as Ca^{2+} -signaling and protein phosphorylation. Various oxidants increase cytosolic Ca^{2+} ; however, the exact origin of Ca^{2+} is controversial. Ca^{2+} may be released from the endoplasmic reticulum, extracellular space, or mitochondria in response to oxidant-influence on Ca^{2+} pumps, channels, and transporters. Alternatively, oxidants may release Ca^{2+} from Ca^{2+} binding proteins. Various oxidants stimulate tyrosine as well as serine/threonine phosphorylation, and direct stimulation of protein kinases and inhibition of protein phosphatases by oxidants have been proposed as mechanisms. The oxidant-stimulation of the effector molecules such as phospholipase A_2 as well as the activation of oxidative stress-responsive transcription factors may also depend on the oxidant-mediated activation of Ca^{2+} -signalling and/or protein phosphorylation. In addition to the stimulation of signal transduction by oxidants, the observations that ligand–receptor interactions produce reactive oxygen species and that antioxidants block receptor-mediated signal transduction led to a proposal that reactive oxygen species may be second messengers for transcription factor activation, apoptosis, bone resorption, cell growth, and chemotaxis. Physiological significance of the role of biological oxidants in the regulation of signal transduction as well as the mechanisms of the oxidant-stimulation of signal transduction are discussed.

Keywords: Calcium; Free radical; Peroxide; Phospholipase; Phosphorylation; Second messenger; Superoxide; Transcription factor

Rank: 4. 570 citations to date
Free Radic. Biol. Med. 20, 707–727 (1996), 10.1016/0891-5849(95)02173-6

Review: The Role of Oxidized Lipoproteins in Atherogenesis

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Abstract: This article reviews our current understanding of the mechanisms of low-density lipoprotein (LDL) oxidation and the potential role of oxidized lipoproteins in atherosclerosis.

Studies in hypercholesterolemic animal models indicate that oxidation of LDL is likely to play an important role in atherogenesis. Epidemiological investigations further suggest that the dietary intake of antioxidants is inversely associated with the risk of vascular disease, suggesting that oxidized LDL may be important in human atherosclerosis. By activating inflammatory events, oxidized lipoproteins may contribute to all stages of the atherosclerotic process. Lipoprotein oxidation is promoted by several different systems in vitro, including free and protein-bound metal ions, thiols, reactive oxygen intermediates, lipoxygenase, peroxynitrite, and myeloperoxidase. Intracellular proteins that bind iron or regulate iron metabolism might also play an important role. The physiologically relevant pathways have yet to be identified, however. We assess recent findings on the effects of antioxidants in vivo and suggest potential strategies for inhibiting oxidation in the vessel wall.

Keywords: Atherosclerosis; Lipoproteins; Antioxidant; Lipid peroxidation; Free radical; Lipoxygenase; Peroxynitrite; Myeloperoxidase; Monocytes; Endothelium; Nitric oxide

Rank: 5. 562 citations to date
Free Radic. Biol. Med. 18, 321–336 (1995), 10.1016/0891-5849(95)02173-6

Review: Oxidative Mechanisms in the Toxicity of Metal Ions

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Abstract: The role of reactive oxygen species, with the subsequent oxidative deterioration of biological macromolecules in the toxicities associated with transition metal ions, is reviewed. Recent studies have shown that metals, including iron, copper, chromium, and vanadium undergo redox cycling, while cadmium, mercury, and nickel, as well as lead, deplete glutathione and protein-bound sulfhydryl groups, resulting in the production of reactive oxygen species as superoxide ion, hydrogen peroxide, and hydroxyl radical. As a consequence, enhanced lipid peroxidation, DNA damage, and altered calcium and sulfhydryl homeostasis occur. Fenton-like reactions may be commonly associated with most membranous fractions including mitochondria, microsomes, and peroxisomes. Phagocytic cells may be another important source of reactive oxygen species in response to metal ions. Furthermore, various studies have suggested that the ability to generate reactive oxygen species by redox cycling quinones and related compounds may require metal ions. Recent studies have suggested that metal ions may enhance the production of tumor necrosis factor alpha (TNF α) and activate protein kinase C, as well as induce the production of stress proteins. Thus, some mechanisms associated with the toxicities of metal ions are very similar to the effects produced

by many organic xenobiotics. Specific differences in the toxicities of metal ions may be related to differences in solubilities, absorbability, transport, chemical reactivity, and the complexes that are formed within the body. This review summarizes current studies that have been conducted with transition metal ions as well as lead, regarding the production of reactive oxygen species and oxidative tissue damage.

Keywords: Iron; Copper; Cadmium; Chromium; Mercury; Nickel; Vanadium; Lead; Zinc; Free radicals; Oxidative stress; Redox cycling; Glutathione depletion; Lipid peroxidation; DNA damage; Stress proteins

Rank: 6. 520 citations to date

Free Radic. Biol. Med. 23, 134–147 (1997), 10.1016/S0891-5849(96)00629-6

Review: Oxidative Stress Hypothesis in Alzheimer's Disease

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Abstract: The major hurdle in understanding Alzheimer's disease (AD) is a lack of knowledge about the etiology and pathogenesis of selective neuron death. In recent years, considerable data have accrued indicating that the brain in AD is under increased oxidative stress and this may have a role in the pathogenesis of neuron degeneration and death in this disorder. The direct evidence supporting increased oxidative stress in AD is: (1) increased brain Fe, Al, and Hg in AD, capable of stimulating free radical generation; (2) increased lipid peroxidation and decreased polyunsaturated fatty acids in the AD brain, and increased 4-hydroxynonenal, an aldehyde product of lipid peroxidation in AD ventricular fluid; (3) increased protein and DNA oxidation in the AD brain; (4) diminished energy metabolism and decreased cytochrome c oxidase in the brain in AD; (5) advanced glycation end products (AGE), malondialdehyde, carbonyls, peroxynitrite, heme oxygenase-1 and SOD-1 in neurofibrillary tangles and AGE, heme oxygenase-1, SOD-1 in senile plaques; and (6) studies showing that amyloid beta peptide is capable of generating free radicals. Supporting indirect evidence comes from a variety of in vitro studies showing that free radicals are capable of mediating neuron degeneration and death. Overall, these studies indicate that free radicals are possibly involved in the pathogenesis of neuron death in AD. Because tissue injury itself can induce reactive oxygen species (ROS) generation, it is not known whether this is a primary or secondary event. Even if free radical generation is secondary to other initiating causes, they are deleterious and part of a cascade of events that can lead to neuron death, suggesting that therapeutic efforts aimed at removal of ROS or prevention of their formation may be beneficial in AD.

Keywords: Alzheimer's disease; Oxidative stress; Antioxidants; Lipid peroxidation; Amyloid beta peptide; Protein and DNA oxidation; 4-Hydroxynonenal; Brain iron; Aluminum, Mercury

Rank: 7. 413 citations to date

Free Radic. Biol. Med. 14, 325–337 (1993), 10.1016/0891-5849(93)90029-T

Review: Oxygen Free Radicals and Metallothionein

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Abstract: It is generally accepted that the principal roles of metallothionein lie in the detoxification of heavy metals and regulation of the metabolism of essential trace metals. However, there is increasing evidence that it can act as a free radical scavenger. This article reviews the evidence supporting such a physiological role and describes induction of metallothionein synthesis by oxidative stress, possible mediators for this induction, and the radical scavenging capability of metallothionein in tissues and cells. The relationship between metallothionein and other antioxidant defense systems and the medical implications of the free radical scavenging properties of metallothionein are also discussed.

Keywords: Metallothionein; Radical scavenger; Lipid peroxidation; Paraquat; Vitamin E; Carbon tetrachloride; Zinc; Oxidative stress; Free radicals

Rank: 8. 374 citations to date

Free Radic. Biol. Med. 18, 775–794 (1995), 10.1016/0891-5849(94)00198-S

Review: Superoxide and Hydrogen Peroxide in Relation to Mammalian Cell Proliferation

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Abstract: A wide variety of normal and malignant cell types generate and release superoxide or hydrogen peroxide in vitro either in response to specific cytokine/growth factor stimulus or constitutively in the case of tumour cells. These species at submicromolar levels appear to act as novel intra and intercellular "messengers" capable of promoting growth responses in culture. The mechanisms may involve direct interaction with specific receptors or oxidation of growth signal

transduction molecules such as protein kinases, protein phosphatases, transcription factors, or transcription factor inhibitors. It is also possible that hydrogen peroxide may modulate the redox state and activity of these important signal transduction proteins indirectly through changes in cellular levels of GSH and GSSG. Critical balances appear to exist in relation to cell proliferation on one hand and lipid peroxidation and cell death on the other. Progression to a more prooxidant state whilst initially leading to enhanced proliferative responses results subsequently in increased cell death.

Keywords: Cell proliferation; Cell death; Apoptosis; Superoxide; Hydrogen peroxide; Cellular redox state; Glutathione; Protein kinases; Transcription factors; Life signals; Free radicals

Rank: 9. 373 citations to date

Free Radic. Biol. Med. 22, 749–760 (1997), 10.1016/S0891-5849(96)00351-6

Antioxidant and Prooxidant Behavior of Flavonoids: Structure-Activity Relationships

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Abstract: The antioxidant and prooxidant behavior of flavonoids and the related activity-structure relationships were investigated in this study using the oxygen radical absorbance capacity assay. Three different reactive species were used in the assay: 2,2'-azobis(2-amidino-propane) dihydrochloride, a peroxy radical generator; Cu²⁺/H₂O₂, mainly a hydroxyl radical generator; and Cu²⁺, a transition metal. Flavonoids including flavones, isoflavones, and flavanones acted as antioxidants against peroxy and hydroxyl radicals and served as prooxidants in the presence of Cu²⁺. Both the antioxidant and the copper-initiated prooxidant activities of a flavonoid depend upon the number of hydroxyl substitutions in its backbone structure, which has neither antioxidant nor prooxidant action. In general, the more hydroxyl substitutions, the stronger the antioxidant and prooxidant activities. The flavonoids that contain multiple hydroxyl substitutions showed antiperoxy radical activities several times stronger than Trolox, an α -tocopherol analogue. The single hydroxyl substitution at position 5 provides no activity, whereas the di-OH substitution at 3' and 4' is particularly important to the peroxy radical absorbing activity of a flavonoid. The conjugation between rings A and B does not affect the antioxidant activity but is very important for the copper-initiated prooxidant action of a flavonoid. The *O*-methylation of the hydroxyl substitutions inactivates both the antioxidant and the prooxidant activities of the flavonoids.

Keywords: Flavonoid; Isoflavone; Flavanone; Flavone; Antioxidant; Prooxidant; Free radicals

Rank: 10. 360 citations to date

Free Radic. Biol. Med. 19, 227–250 (1995), 10.1016/0891-5849(95)00017-R

Review: Alpha-Lipoic Acid as a Biological Antioxidant

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Abstract: α -Lipoic acid, which plays an essential role in mitochondrial dehydrogenase reactions, has recently gained considerable attention as an antioxidant. Lipoate, or its reduced form, dihydrolipoate, reacts with reactive oxygen species such as superoxide radicals, hydroxyl radicals, hypochlorous acid, peroxy radicals, and singlet oxygen. It also protects membranes by interacting with vitamin C and glutathione, which may in turn recycle vitamin E. In addition to its antioxidant activities, dihydrolipoate may exert prooxidant actions through reduction of iron. α -Lipoic acid administration has been shown to be beneficial in a number of oxidative stress models such as ischemia-reperfusion injury, diabetes (both α -lipoic acid and dihydrolipoic acid exhibit hydrophobic binding to proteins such as albumin, which can prevent glycation reactions), cataract formation, HIV activation, neurodegeneration, and radiation injury. Furthermore, lipoate can function as a redox regulator of proteins such as myoglobin, prolactin, thioredoxin and NF- κ B transcription factor. We review the properties of lipoate in terms of (1) reactions with reactive oxygen species; (2) interactions with other antioxidants; (3) beneficial effects in oxidative stress models or clinical conditions.

Keywords: Antioxidant; Dihydrolipoate; Dihydrolipoic acid; α -Lipoate; α -Lipoic acid; Oxidative stress; Redox regulation; Review; Thioctic acid; Free radicals

Rank: 11. 350 citations to date

Free Radic. Biol. Med. 18, 1033–1077 (1995), 10.1016/0891-5849(94)00209-3

Review: Reactions of Oxyl Radicals with DNA

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Abstract: The importance of radical-induced damage to DNA is apparent from the ever-increasing number of publications in this area. This review focuses on the damage caused to DNA by reactive oxygen-centred radicals, however formed. These may be hydroxyl radicals, which arise either from the radiolysis of water by ionizing radiation (γ -rays or X-rays), or from a purely chemical source. Alternatively, metal-bound oxyl radicals (M-O \cdot) are also active intermediates in DNA-cleaving reactions and may be formed from synthetic compounds or from natural products such as bleomycin (BLM). Chemical mechanisms leading to the observed degradation products are covered in detail. The biological effects of some of the DNA base lesions formed are touched upon, concentrating on the molecular mechanisms behind the initial events that lead to mutagenesis.

Keywords: Free radical; DNA; Ionizing radiation; Hydroxyl radical; Bleomycin; Nucleic acid

Rank: 12. 336 citations to date

Free Radic. Biol. Med. 25, 434–456 (1998), 10.1016/S0891-5849(98)00092-6

Forum: Chemical Biology of Nitric Oxide: Insights into Regulatory, Cytotoxic, and Cytoprotective Mechanisms of Nitric Oxide

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Abstract: There has been confusion as to what role(s) nitric oxide (NO) has in different physiological and pathophysiological mechanisms. Some studies imply that NO has cytotoxic properties and is the genesis of numerous diseases and degenerative states, whereas other reports suggest that NO prevents injurious conditions from developing and promotes events which return tissue to homeostasis. The primary determinant(s) of how NO affects biological systems centers on its chemistry. The chemistry of NO in biological systems is extensive and complex. To simplify this discussion, we have formulated the “chemical biology of NO” to describe the pertinent chemical reactions under specific biological conditions. The chemical biology of NO is divided into two major categories, direct and indirect. Direct effects are defined as those reactions fast enough to occur between NO and specific biological molecules. Indirect effects do not involve NO, but rather are mediated by reactive nitrogen oxide species (RNOS) formed from the reaction of NO either with oxygen or superoxide. RNOS formed from NO can mediate either nitrosative or oxidative stress. This report discusses various aspects of the chemical biology of NO relating to biological molecules such as guanylate cyclase, cytochrome P450, nitric oxide synthase, catalase, and DNA and explores the potential roles of NO in different biological events. Also, the implications of different chemical reactions of NO with cellular

processes such as mitochondrial respiration, metal homeostasis, and lipid metabolism are discussed. Finally, a discussion of the chemical biology of NO in different cytotoxic mechanisms is presented.

Keywords: Nitric oxide; Reactive nitrogen oxide species; Superoxide; Mitochondrial respiration; Metal homeostasis; Lipid metabolism

Rank: 13. 305 citations to date

Free Radic. Biol. Med. 10, 225–242 (1991), 10.1016/0891-5849(91)90080-M

Review: Chemical Determination of Free Radical-Induced Damage to DNA

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Abstract: Free radical-induced damage to DNA in vivo can result in deleterious biological consequences such as the initiation and promotion of cancer. Chemical characterization and quantitation of such DNA damage is essential for an understanding of its biological consequences and cellular repair. Methodologies incorporating the technique of gas chromatography/mass spectrometry (GC/MS) have been developed in recent years for measurement of free radical-induced DNA damage. The use of GC/MS with selected-ion monitoring (SIM) facilitates unequivocal identification and quantitation of a large number of products of all four DNA bases produced in DNA by reactions with hydroxyl radical, hydrated electron, and H atom. Hydroxyl radical-induced DNA-protein cross-links in mammalian chromatin, and products of the sugar moiety in DNA are also unequivocally identified and quantitated. The sensitivity and selectivity of the GC/MS-SIM technique enables the measurement of DNA base products even in isolated mammalian chromatin without the necessity of first isolating DNA, and despite the presence of histones. Recent results reviewed in this article demonstrate the usefulness of the GC/MS technique for chemical determination of free radical-induced DNA damage in DNA as well as in mammalian chromatin under a vast variety of conditions of free radical production.

Keywords: Hydroxyl radical; Gas chromatography/mass spectrometry; Mammalian chromatin; DNA base products; DNA-protein cross-links; Hydrogen peroxide; Ionizing radiation; Metal ions; Superoxide dismutase; Free radical

Rank: 14. 302 citations to date

Free Radic. Biol. Med. 21, 335–348 (1996), 10.1016/0891-5849(96)00109-8

Review: Redox Regulation of Transcriptional Activators

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Abstract: Transcription factors/activators are a group of proteins that bind to specific consensus sequences (cis elements) in the promoter regions of downstream target/effector genes and transactivate or repress effector gene expression. The up- or downregulation of effector genes will ultimately lead to many biological changes such as proliferation, growth suppression, differentiation, or senescence. Transcription factors are subject to transcriptional and post-translational regulation. This review will focus on the redox (reduction/oxidation) regulation of transcription factors/activators with emphasis on p53, AP-1, and NF- κ B. The redox regulation of transcriptional activators occurs through highly conserved cysteine residues in the DNA binding domains of these proteins. In vitro studies have shown that reducing environments increase, while oxidizing conditions inhibit sequence-specific DNA binding of these transcriptional activators. When intact cells have been used for study, a more complex regulation has been observed. Reduction/oxidation can either up- or downregulate DNA binding and/or transactivation activities in transcriptional activator-dependent as well as cell type-dependent manners. In general, reductants decrease p53 and NF- κ B activities but dramatically activate AP-1 activity. Oxidants, on the other hand, greatly activate NF- κ B activity. Furthermore, redox-induced biochemical alterations sometimes lead to change in the biological functions of these proteins. Therefore, differential regulation of these transcriptional activators, which in turn, regulate many target/effector genes, may provide an additional mechanism by which small antioxidant molecules play protective roles in anticancer and antiaging processes. Better understanding of the mechanism of redox regulation, particularly in vivo, will have an important impact on drug discovery for chemoprevention and therapy of human diseases such as cancer.

Keywords: Free radicals; Redox regulation; p53; AP-1; NF- κ B; Transcription factors/activators

Rank: 15. 298 citations to date

Free Radic. Biol. Med. 12, 219–240 (1992), 10.1016/0891-5849(92)90030-K

Review: Implication of Free Radical Mechanisms in Ethanol-Induced Cellular Injury

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Abstract: Numerous experimental data reviewed in the present article indicate that free radical mechanisms contribute to ethanol-induced liver injury. Increased generation of oxygen- and ethanol-derived free radicals has been observed at the microsomal level, especially through the intervention of the ethanol-inducible cytochrome P450 isoform (CYP2E 1). Furthermore, an ethanol-linked enhancement in free radical generation can occur through the cytosolic xanthine and/or aldehyde oxidases, as well as through the mitochondrial respiratory chain. Ethanol administration also elicits hepatic disturbances in the availability of non-safely-sequestered iron derivatives and in the antioxidant defense. The resulting oxidative stress leads, in some experimental conditions, to enhanced lipid peroxidation and can also affect other important cellular components, such as proteins or DNA. The reported production of a chemoattractant for human neutrophils may be of special importance in the pathogenesis of alcoholic hepatitis. Free radical mechanisms also appear to be implicated in the toxicity of ethanol on various extrahepatic tissues. Most of the experimental data available concern the gastric mucosa, the central nervous system, the heart, and the testes. Clinical studies have not yet demonstrated the role of free radical mechanisms in the pathogenesis of ethanol-induced cellular injury in alcoholics. However, many data support the involvement of such mechanisms and suggest that dietary and/or pharmacological agents able to prevent an ethanol-induced oxidative stress may reduce the incidence of ethanol toxicity in humans.

Keywords: Free radicals; Ethanol; Alcoholism; Antioxidants; Lipid peroxidation; Oxidative stress; Liver; Extrahepatic tissues

Rank: 16. 284 citations to date

Free Radic. Biol. Med. 16, 149–156 (1994), 10.1016/0891-5849(94)90138-4

Peroxynitrite-Mediated Oxidation of Dihydrorhodamine 123

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Abstract: Nitric oxide reacts with superoxide to form peroxynitrite, which may be an important mediator of free radical-induced cellular injury. Oxidation of dihydrorhodamine to fluorescent rhodamine is a marker of cellular oxidant production. We investigated the mechanisms of peroxynitrite-mediated formation of rhodamine from dihydrorhodamine. Peroxynitrite at low levels (0–1000 nM) induced a linear, concentration-

dependent, oxidation of dihydrorhodamine. Hydroxyl radical scavengers mannitol and dimethylsulfoxide had minimal effect (<10%) on rhodamine production. Peroxynitrite-mediated formation of rhodamine was not dependent on metal ion catalyzed reactions because studies were performed in metal ion-free buffer and rhodamine formation was not enhanced in the presence of Fe^{3+} ethylenediaminetetraacetic acid (EDTA). Thus, rhodamine formation appears to be mediated directly by peroxynitrite. Superoxide dismutase slightly enhanced rhodamine production. L-cysteine was an efficient inhibitor ($K_I \sim 25 \mu\text{M}$) of dihydrorhodamine oxidation through competitive oxidation of free sulfhydryls. Urate was also an efficient inhibitor ($K_I \sim 2.5 \mu\text{M}$), possibly by reduction of an intermediate dihydrorhodamine radical and recycling of dihydrorhodamine. Under anaerobic conditions, nitric oxide did not oxidize dihydrorhodamine and inhibited spontaneous oxidation of dihydrorhodamine. In the presence of oxygen, nitric oxide induces a relatively slow oxidation of dihydrorhodamine due to the formation of nitrogen dioxide. We conclude that dihydrorhodamine is a sensitive and efficient trap for peroxynitrite and may serve as a probe for peroxynitrite production.

Keywords: Peroxynitrite; Nitric oxide; Dihydrorhodamine; Rhodamine; Free radicals

Rank: 17. 283 citations to date

Free Radic. Biol. Med. 22, 1115–1126 (1997), 10.1016/S0891-5849(96)00501-1

Hypothesis: Redox Regulation of NF-Kappa B Activation

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Abstract: Cytosolic reactions of the nuclear factor kappa B/inhibitor (NF- κ B/I κ B) complex leading to its activation, NF- κ B translocation into the nucleus, DNA binding, and transactivation have been described with some degree of clarity, but the upstream processes that stimulate those cytosolic reactions remain obscure. These processes definitely involve multiple protein serine/threonine kinases, as proximal modifiers of I κ B, as well as the corresponding phosphatases, upstream kinases, and phosphatases, including those acting on tyrosine residues. This complex cascade of phosphorylation and dephosphorylation is modulated by redox reactions of unknown nature in the sense that the oxidant status of the cytosol increases the phosphorylation and degradation of I κ B. NF- κ B action, however, requires a thioredoxin-dependent reduced status in the nucleus. Upstream kinase(s) and/or phosphatase(s) prone to

thiolation or oxidation of vicinal SH groups are at present considered the best candidates mediating the redox regulation of NF- κ B.

Keywords: NF- κ B, I κ B; Rel proteins; H_2O_2 ; Lipid hydroperoxides; Antioxidants; Protein kinases; Protein phosphatases; Thioredoxin, Glutathione peroxidases; Selenium

Rank: 18. 268 citations to date

Free Radic. Biol. Med. 14, 303–311 (1993), 10.1016/0891-5849(93)90027-R

Oxygen-Radical Absorbance Capacity Assay for Antioxidants

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Abstract: A relatively simple but sensitive and reliable method of quantitating the oxygen-radical absorbing capacity (ORAC) of antioxidants in serum using a few μl is described. In this assay system, β -phycoerythrin (β -PE) is used as an indicator protein, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) as a peroxy radical generator, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, a water-soluble vitamin E analogue) as a control standard. Results are expressed as ORAC units, where 1 ORAC unit equals the net protection produced by 1 μM Trolox. The uniqueness of this assay is that total antioxidant capacity of a sample is estimated by taking the oxidation reaction to completion. At this point all of the nonprotein antioxidants (which include α -tocopherol, vitamin C, β -carotene, uric acid, and bilirubin) and most of the albumin in the sample are oxidized by the peroxy radical. Results are quantified by measuring the protection produced by antioxidants. This solves many problems associated with kinetics or lag-time measurements. A linear correlation of ORAC value with concentration of serum, Trolox, vitamin C, uric acid, and bovine albumin is demonstrated. The coefficient of variation within a run is found to be about 2% and from run to run about 5%. Trolox, α -tocopherol, vitamin C, β -carotene, uric acid, and bilirubin completely protect β -PE from oxidation, while bovine albumin protects β -PE only partially. On a molar basis, the relative peroxy radical absorbance capacity of Trolox, α -tocopherol acid succinate, uric acid, bilirubin, and vitamin C is 1 : 1 : 0.92 : 0.84 : 0.52. Bovine albumin per unit weight has a lower peroxy radical absorbing capacity than these antioxidants. However, the serum protein fraction, containing some lipid-soluble antioxidants, represents the major contributor to the ORAC value found in whole serum. The minimum amount of vitamin C and uric acid which could still be detectable when added to a

serum supernatant fraction is 1.5 μg and 0.59 μg , respectively, which account for about 1% of the total ORAC value of the serum supernatant fraction.

Keywords: Oxygen radicals; Antioxidant; α -tocopherol; β -carotene; Vitamin C; Uric acid; Bilirubin; Free radical

Rank: 18. 268 citations to date

Free Radic. Biol. Med. 17, 235–248 (1994), 10.1016/0891-5849(94)90079-5

Review: Importance of Se-Glutathione Peroxidase, Catalase, and Cu/Zn-SOD for Cell Survival Against Oxidative Stress

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Abstract: Eukaryotic cells have to constantly cope with highly reactive oxygen-derived free radicals. Their defense against these free radicals is achieved by natural antioxidant molecules but also by antioxidant enzymes. In this paper, we review some of the data comparing the efficiency of three different antioxidant enzymes: Cu/Zn-superoxide dismutase (Cu/Zn-SOD), catalase, and selenium-glutathione peroxidase. We perform our comparison on one experimental model (human fibroblasts) where the activities of these three antioxidant enzymes have been modulated inside the cells, and the repercussion of these changes was investigated in different conditions. We also focus our attention on the protecting role of selenium-glutathione peroxidase, because this enzyme is very rarely studied due to the difficulties linked to its biochemical properties. These studies evidenced that all three antioxidant enzymes give protection for the cells. They show a high efficiency for selenium-glutathione peroxidase and emphasize the fact that each enzyme has a specific as well as an irreplaceable function. They are all necessary, for the survival of the cell even in normal conditions. In addition, these three enzymes act in a cooperative or synergistic way to ensure a global cell protection. However, optimal protection is achieved only when an appropriate balance between the activities of these enzymes is maintained. Interpretation of the deleterious effects of free radicals has to be analyzed not only as a function of the amount of free radicals produced but also relative to the efficiency and to the activities of these enzymatic and chemical antioxidant systems. The threshold of protection can indeed vary dramatically as a function of the level of activity of these enzymes.

Keywords: Selenium-glutathione peroxidase; Cu/Zn-SOD; Catalase; Oxidative stress; Microinjection; Free radicals

Rank: 20. 256 citations to date

Free Radic. Biol. Med. 20, 331–342 (1996), 10.1016/0891-5849(95)02047-0

Structural Aspects of Antioxidant Activity of Flavonoids

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Abstract: Flavonoids, a group of naturally occurring antioxidants and iron chelators, might be used as cardioprotective agents in doxorubicin-induced cardiotoxicity, which is believed to be caused by the formation of oxygen free radicals. To investigate the underlying molecular mechanism, we tested a large group of flavonoids from all major structural subclasses on their ability to inhibit doxorubicin (enzymatically)-induced and Fe^{2+} /ascorbate (nonenzymatically)-induced microsomal lipid peroxidation (LPO) and to chelate Fe^{2+} . In addition, we measured half peak oxidation potentials (Ep/2). LPO inhibition data gave a good qualitative correlation with the oxidation potentials. Most flavonoids tested chelated Fe^{2+} , but there were large differences in the chelating capacity. For good scavenging activity, a catechol moiety on ring B is required. The 3-OH moiety can function as a chelation site and can also be oxidized. The 3-OH group in combination with a C2 C3 double bond, increases the scavenging activity. Fe^{2+} chelation only plays a role in the LPO inhibition by less active scavengers. Chelation can then raise the activity to the level of the most active scavengers, possibly by site-specific scavenging. It can be concluded that Ep/2 values and iron chelating activity can almost completely describe the LPO inhibiting behaviour of the flavonoids.

Keywords: Free radicals; Antioxidant; Flavonoid; Oxidation potential; Lipid peroxidation

Rank: 21. 233 citations to date

Free Radic. Biol. Med. 25, 392–403 (1998), 10.1016/S0891-5849(98)00095-1

Forum: Oxidative Chemistry of Nitric Oxide: The Roles of Superoxide, Peroxynitrite, and Carbon Dioxide

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Abstract: The roles of superoxide ($O_2^{\cdot-}$), peroxynitrite, and carbon dioxide in the oxidative chemistry of nitric oxide ($\cdot NO$) are reviewed. The formation of peroxynitrite from $\cdot NO$ and $O_2^{\cdot-}$ is controlled by superoxide dismutase (SOD), which can lower the concentration of superoxide ions. The concentration of CO_2 in vivo is high (ca. 1 mM), and the rate constant for reaction of CO_2 with $^{\cdot}OONO$ is large (pH-independent $k = 5.8 \times 10^4 M^{-1}s^{-1}$). Consequently, the rate of reaction of peroxynitrite with CO_2 is so fast that most commonly used scavengers would need to be present at very high, near toxic levels in order to compete with peroxynitrite for CO_2 . Therefore, in the presence of physiological levels of bicarbonate, only a limited number of biotargets react *directly* with peroxynitrite. These include heme-containing proteins such as hemoglobin, peroxidases such as myeloperoxidase, selenoproteins such as glutathione peroxidase, proteins containing zinc-thiolate centers such as the DNA-binding transcription factors, and the synthetic antioxidant ebselen. The mechanism of the reaction of CO_2 with $^{\cdot}OONO$ produces metastable nitrating, nitrosating, and oxidizing species as intermediates. An analysis of the lifetimes of the possible intermediates and of the catalysis of peroxynitrite decompositions suggests that the reactive intermediates responsible for reactions with a variety of substrates may be the free radicals $\cdot NO_2$ and $CO_3^{\cdot-}$. Biologically important reactions of these free radicals are, for example, the nitration of tyrosine residues. These nitrations can be pathological, but they also may play a signal transduction role, because nitration of tyrosine can modulate phosphorylation and thus control enzymatic activity. In principle, it might be possible to block the biological effects of peroxynitrite by scavenging the free radicals $\cdot NO_2$ and $CO_3^{\cdot-}$. Because it is difficult to directly scavenge peroxynitrite because of its fast reaction with CO_2 , scavenging of intermediates from the peroxynitrite/ CO_2 reaction would provide an additional way of preventing peroxynitrite-mediated cellular effects. The biological effects of peroxynitrite also can be prevented by limiting the formation of peroxynitrite from $\cdot NO$ by lowering the concentration of $O_2^{\cdot-}$ using SOD or SOD mimics. Increased formation of peroxynitrite has been linked to Alzheimer's disease, rheumatoid arthritis, atherosclerosis, lung injury, amyotrophic lateral sclerosis, and other diseases.

Keywords: Nitric oxide; Superoxide; Peroxynitrite; Carbon dioxide; Superoxide dismutase; Oxidative biology; Radical; Nitric oxide synthase

Rank: 22. 223 citations to date
Free Radic. Biol. Med. 29, 222–230 (2000), 10.1016/S0891-5849(00)00317-8

Review: Mitochondrial Free Radical Generation, Oxidative Stress, and Aging

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Abstract: Mitochondria have been described as “the powerhouses of the cell” because they link the energy-releasing activities of electron transport and proton pumping with the energy conserving process of oxidative phosphorylation, to harness the value of foods in the form of ATP. Such energetic processes are not without dangers, however, and the electron transport chain has proved to be somewhat “leaky.” Such side reactions of the mitochondrial electron transport chain with molecular oxygen directly generate the superoxide anion radical ($O_2^{\cdot-}$), which dismutates to form hydrogen peroxide (H_2O_2), which can further react to form the hydroxyl radical (HO^{\cdot}). In addition to these toxic electron transport chain reactions of the inner mitochondrial membrane, the mitochondrial outer membrane enzyme monoamine oxidase catalyzes the oxidative deamination of biogenic amines and is a quantitatively large source of H_2O_2 that contributes to an increase in the steady state concentrations of reactive species within both the mitochondrial matrix and cytosol. In this article we review the mitochondrial rates of production and steady state levels of these reactive oxygen species. Reactive oxygen species generated by mitochondria, or from other sites within or outside the cell, cause damage to mitochondrial components and initiate degradative processes. Such toxic reactions contribute significantly to the aging process and form the central dogma of “The Free Radical Theory of Aging.” In this article we review current understandings of mitochondrial DNA, RNA, and protein modifications by oxidative stress and the enzymatic removal of oxidatively damaged products by nucleases and proteases. The possible contributions of mitochondrial oxidative polynucleotide and protein turnover to apoptosis and aging are explored.

Keywords: Mitochondria; Electron transport; Free radicals; Oxidative stress; Aging; Apoptosis; Nucleases; Proteases; Proteasome; DNA/RNA damage; Monoamine oxidase

Rank: 22. 223 citations to date
Free Radic. Biol. Med. 30, 1191–1212 (2001), 10.1016/S0891-5849(01)00480-4

Review: Redox Environment of the Cell as Viewed Through the Redox State of the Glutathione Disulfide/Glutathione Couple

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Abstract: Redox state is a term used widely in the research field of free radicals and oxidative stress. Unfortunately, it is used as a general term referring to relative changes that are not well defined or quantitated. In this review we provide a definition for the redox environment of biological fluids, cell organelles,

cells, or tissue. We illustrate how the reduction potential of various redox couples can be estimated with the Nernst equation and show how pH and the concentrations of the species comprising different redox couples influence the reduction potential. We discuss how the redox state of the glutathione disulfide-glutathione couple (GSSG/2GSH) can serve as an important indicator of redox environment. There are many redox couples in a cell that work together to maintain the redox environment; the GSSG/2GSH couple is the most abundant redox couple in a cell. Changes of the half-cell reduction potential (E_{hc}) of the GSSG/2GSH couple appear to correlate with the biological status of the cell: proliferation $E_{hc} \approx -240$ mV; differentiation $E_{hc} \approx -200$ mV; or apoptosis $E_{hc} \approx -170$ mV. These estimates can be used to more fully understand the redox biochemistry that results from oxidative stress. These are the first steps toward a new quantitative biology, which hopefully will provide a rationale and understanding of the cellular mechanisms associated with cell growth and development, signaling, and reductive or oxidative stress.

Keywords: Glutathione; NADPH; Nernst equation; Reduction potentials; Free radicals

Rank: 24. 222 citations to date

Free Radic. Biol. Med. 16, 845–850 (1994), 10.1016/0891-5849(94)90202-X

The Correlation between Active Oxygen Scavenging and Antioxidative Effects of Flavonoids

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Abstract: The abilities of 15 flavonoids as a scavenger of active oxygens (hydroxyl radical and superoxide anion) were studied. Hydroxyl radical ($\cdot\text{OH}$) was generated by the Fenton system, and assayed by the determination of methanesulfinic acid (MSA) formed from the reaction of dimethyl sulfoxide (DMSO) with $\cdot\text{OH}$. (+)-Catechin, (–)-epicatechin, 7,8-dihydroxy flavone, and rutin showed the $\cdot\text{OH}$ scavenging effect 100–300 times superior to that of mannitol, a typical $\cdot\text{OH}$ scavenger. The other flavonoids showed no $\cdot\text{OH}$ scavenging effect at their concentrations up to 50 μM . Baicalein, quercetin, morin, and myricetin unexpectedly increased the $\cdot\text{OH}$ production in the Fenton system. The flavonoids tested now, except monohydroxy flavones, were more or less inhibitive to the superoxide anion (O_2^-) generation in the hypoxanthine-xanthine oxidase system. A great part of this inhibitory effect was likely owing to suppression of xanthine oxidase activity by the flavonoids. The flavonoids, which scavenged $\cdot\text{OH}$ or O_2^- , were necessarily antioxidants to the peroxidation of methyl linoleate. However, there was a type of flavonoid such as

morin, which have neither $\cdot\text{OH}$ nor O_2^- scavenging effect, but was a strong antioxidant.

Keywords: Flavonoids; Hydroxyl radical; Scavenger; Lipid peroxidation; Free radicals

Rank: 25. 213 citations to date

Free Radic. Biol. Med. 12, 293–315 (1992), 10.1016/0891-5849(92)90117-Y

Review: The Role of Free Radicals in Asbestos-Induced Diseases

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Abstract: Asbestos exposure causes pulmonary fibrosis and malignant neoplasms by mechanisms that remain uncertain. In this review, we explore the evidence supporting the hypothesis that free radicals and other reactive oxygen species (ROS) are an important mechanism by which asbestos mediates tissue damage. There appears to be at least two principal mechanisms by which asbestos can induce ROS production; one operates in cell-free systems and the other involves mediation by phagocytic cells. Asbestos and other synthetic mineral fibers can generate free radicals in cell-free systems containing atmospheric oxygen. In particular, the hydroxyl radical often appears to be involved, and the iron content of the fibers has an important role in the generation of this reactive radical. However, asbestos also appears to catalyze electron transfer reactions that do not require iron. Iron chelators either inhibit or augment asbestos-catalyzed generation of the hydroxyl radical and/or pathological changes, depending on the chelator and the nature of the asbestos sample used. The second principal mechanism for asbestos-induced ROS generation involves the activation of phagocytic cells. A variety of mineral fibers have been shown to augment the release of reactive oxygen intermediates from phagocytic cells such as neutrophils and alveolar macrophages. The molecular mechanisms involved are unclear but may involve incomplete phagocytosis with subsequent oxidant release, stimulation of the phospholipase C pathway, and/or IgG-fragment receptor activation. Reactive oxygen species are important mediators of asbestos-induced toxicity to a number of pulmonary cells including alveolar macrophages, epithelial cells, mesothelial cells, and endothelial cells. Reactive oxygen species may contribute to the well-known synergistic effects of asbestos and cigarette smoke on the lung, and the reasons for this synergy are discussed. We conclude that there is strong evidence supporting the premise that reactive oxygen species and/or free radicals contribute to asbestos-induced and cigarette smoke/asbestos-induced lung injury and that

strategies aimed at reducing the oxidant stress on pulmonary cells may attenuate the deleterious effects of asbestos.

Keywords: Free radicals; Reactive oxygen species; Oxidants; Asbestos; Lung injury; Iron chelators; Phagocytic cells; Hydrogen peroxide; Hydroxyl radical; Cigarette smoke and tar; Antioxidants, Cancer

Rank: 26. 209 citations to date

Free Radic. Biol. Med. 12, 417–427 (1992), 10.1016/0891-5849(92)90091-T

Review: Ferritin as a Source of Iron for Oxidative Damage

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Abstract: The generation of deleterious activated oxygen species capable of damaging DNA, lipids, and proteins requires a catalyst such as iron. Once released, ferritin iron is capable of catalyzing these reactions. Thus, agents that promote iron release may lead to increased oxidative damage. The superoxide anion formed enzymatically, radiolytically, via metal-catalyzed oxidations, or by redox cycling xenobiotics reductively mobilizes ferritin iron and promotes oxidative damage. In addition, a growing list of compounds capable of undergoing single electron oxidation/reduction reactions exemplified by paraquat, adriamycin, and alloxan have been reported to release iron from ferritin. Because the rapid removal of iron from ferritin requires reduction of the iron core, it is not surprising that the reduction potential of a compound is a primary factor that determines whether a compound will mobilize ferritin iron. The reduction potential does not, however, predict the rate of iron release. Therefore, ferritin-dependent oxidative damage may be involved in the pathogenesis of diseases where increased superoxide formation occurs and the toxicity of chemicals that increase superoxide production or have an adequate reduction potential to mobilize ferritin iron.

Keywords: Free radicals; Superoxide; Ferritin; Iron; Lipid peroxidation; Iron release

Rank: 27. 207 citations to date

Free Radic. Biol. Med. 15, 77–96 (1993), 10.1016/0891-5849(93)90127-G

Review: Current Status of Antioxidant Therapy

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Abstract: There is evidence that free radical damage contributes to the aetiology of many chronic health problems such as emphysema, cardiovascular and inflammatory diseases, cataracts, and cancer. In this review we are not concerned with tissue damage in vivo induced directly by radicals from exogenous sources, such as air pollutants and tobacco smoke, high-pressure oxygen, irradiation, or through the metabolism of certain solvents, drugs, and pesticides. Rather, we address some of the disease states associated with increased oxidative stress from endogenous sources and the possible therapeutic advantage of the antioxidant treatment. This raises the question of the antioxidant status of individuals and its role in protection against amplification of certain disease processes. We have chosen to concentrate mainly on coronary heart disease, reperfusion injury, and organ storage for transplantation.

Keywords: Free radical; Antioxidant; α -Tocopherol; Thiol drugs; Hydroxamates; Desferrioxamine; Atherosclerosis; Reperfusion injury; Oxidised LDL

Rank: 28. 207 citations to date

Free Radic. Biol. Med. 16, 383–391 (1994), 10.1016/0891-5849(94)90040-X

Review: Free Radicals in Diabetic Endothelial Cell Dysfunction

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Abstract: Several studies have shown impairment of endothelium-dependent relaxations as well as increased release of vasoconstrictor prostanoids in arteries from diabetic animals and humans. This impairment is restored towards normal by prostaglandin (PG) H_2 /thromboxane A_2 receptor blockade or superoxide dismutase, indicating that the PGH_2 and/or superoxide anion ($O_2^{\cdot-}$) generated contributes to the abnormality. Of particular note is that PGH_2 impairs endothelium-dependent relaxations and causes contractions by a mechanism that involves generation of $O_2^{\cdot-}$ in the endothelium. The effects of elevated glucose are exacerbated by increased aldose reductase activity leading to depletion of NADPH and generation of reactive oxidants. Because NADPH is required for generation of nitric oxide from L-arginine, the depletion of NADPH leads to reduced nitric oxide formation. In a manner similar to that observed with elevated glucose, oxygen-derived free radicals or activation of protein kinase C also cause impairment of endothelium-dependent relaxations, smooth muscle contractions, and release constrictor prostanoids, indicating that a common mechanism for the impairment of endothelial cell function may be operative in diabetes. In this review the cumulative effects of oxidative stress on diabetic endothelial cell dysfunction, together with the complex

interrelationship of cyclooxygenase catalysis, protein kinase C activity, and flux through the polyol pathway, are considered.

Keywords: Diabetes; Nitric oxide; Polyols; Free radical; Protein kinase C; Prostaglandin H₂

Rank: 29. 207 citations to date

Free Radic. Biol. Med. 28, 463–499 (2000), 10.1016/S0891-5849(99)00242-7

Review: Oxidative Stress and Gene Regulation

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Abstract: Reactive oxygen species are produced by all aerobic cells and are widely believed to play a pivotal role in aging as well as a number of degenerative diseases. The consequences of the generation of oxidants in cells does not appear to be limited to promotion of deleterious effects. Alterations in oxidative metabolism have long been known to occur during differentiation and development. Experimental perturbations in cellular redox state have been shown to exert a strong impact on these processes. The discovery of specific genes and pathways affected by oxidants led to the hypothesis that reactive oxygen species serve as subcellular messengers in gene regulatory and signal transduction pathways. Additionally, antioxidants can activate numerous genes and pathways. The burgeoning growth in the number of pathways shown to be dependent on oxidation or antioxidation has accelerated during the last decade. In the discussion presented here, we provide a tabular summary of many of the redox effects on gene expression and signaling pathways that are currently known to exist.

Keywords: Free radical; Signal transduction; Oxidative stress; Antioxidants; MAP kinase; NF- κ B

Rank: 30. 204 citations to date

Free Radic. Biol. Med. 18, 93–105 (1995), 10.1016/0891-5849(94)00158-G

Review: Oxidation of Methionyl Residues in Proteins: Tools, Targets, and Reversal

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Abstract: Methionine (Met) is one of the most readily oxidized amino acid constituents of proteins. It is attacked

by H₂O₂, hydroxyl radicals, hypochlorite, chloramines, and peroxyxynitrite, all these oxidants being produced in biological systems. The oxidation product, Met sulfoxide, can be reduced back to Met by Met sulfoxide reductase. Numerous proteins lose functional activity by Met oxidation. However, functional activation of proteins by Met oxidation has also been observed. Functional changes by Met oxidation in a given protein appear to have pathophysiological significance in some cases. Considering the reversibility of Met oxidation and the functional changes associated with the oxidation, it seems possible that Met oxidation/reduction in proteins may be one means to control homeostasis in biological systems.

Keywords: Methionine oxidation; Methionine sulfoxide reduction; Protein; Activation/inactivation by Met oxidation; Free radicals

Rank: 31. 203 citations to date

Free Radic. Biol. Med. 17, 333–349 (1994), 10.1016/0891-5849(94)90019-1

Review: The Redox Couple between Glutathione and Ascorbic Acid: A Chemical and Physiological Perspective

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Abstract: This article provides a comprehensive analysis of the redox reaction between glutathione/glutathione disulfide and ascorbic acid/dehydroascorbic acid. It includes an historical perspective of the progression of the experiments, first begun more than 60 years ago and continuing today with heightened importance. Indeed, the antioxidant capacity of glutathione and ascorbic acid, whether singly or in combination, linked via the redox couple, is a subject of intense interest for studies by bench scientists and clinicians, particularly because a growing body of evidence suggests that free radicals may be involved in a variety of diseases. The authors begin with a detailed summary of “test tube” experiments (the chemical perspective) that have revealed the conditions that regulate the rate of the redox coupling between glutathione and dehydroascorbic acid and that promote or inhibit the decomposition of dehydroascorbic acid in ordinary, buffered aqueous media; results obtained in the authors’ laboratory are used for illustration purposes and uniformity of presentation. The authors then proceed to a critical examination of the extent to which the redox couple between glutathione and ascorbic acid operates in a cell, using the often published antioxidant cascade (See Fig. 1) as the model for the analysis (the physiological perspective). The evidence for and the evidence against the presence of the enzyme dehydroascorbate reductase in animal cells is outlined in a balanced way in an attempt to make sense of this continuing controversy. Next, the authors carefully document the many studies showing that

exogenous dehydroascorbic acid is transported into cells where it is reduced to ascorbic acid by glutathione. Finally, they probe the functional significance and efficiency of the redox couple in monolayer cultures of human retinal pigment epithelial (RPE) cells, as a prototypical cellular model. The authors include the results of new experiments showing that incubation of RPE cells with a nitroxide, TEMPOL, leads to the selective oxidation of intracellular ascorbic acid. This approach is desirable because it dissects the cascade at a specific site and permits measurements of the levels of ascorbic acid and glutathione in the cells before, during, and after oxidation. The results show that only partial regeneration of ascorbic acid is obtained when control conditions are restored. However, if either ascorbic acid or dehydroascorbic acid is added to the media during the recovery period following treatment of cells with TEMPOL, then full recovery of ascorbic acid is observed. These results raise certain concerns whether the activity of the redox couple between glutathione and dehydroascorbic acid is sufficient to restore the level of ascorbic acid in oxidatively challenged cells, when exogenous dehydroascorbic acid is unavailable. This leads to the suggestion that the transmembrane uptake of ascorbic acid and dehydroascorbic acid (with subsequent redox reduction to ascorbic acid) is an important component in the overall cellular machinery that regulates the intracellular concentration of ascorbic acid.

Keywords: Glutathione; Ascorbic acid; Redox couples; Free radicals; Oxidative stress; Antioxidants

Rank: 32. 200 citations to date

Free Radic. Biol. Med. 26, 1231–1237 (1999), 10.1016/S0891-5849(98)00315-3

Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay

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Abstract: A method for the screening of antioxidant activity is reported as a decolorization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, carotenoids, and plasma antioxidants. The pre-formed radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants. The influences of both the concentration of antioxidant and duration of reaction on the inhibition of the radical cation absorption are taken into account when determining the antioxidant activity. This assay clearly improves the original TEAC assay (the ferryl myoglobin/ABTS assay) for the

determination of antioxidant activity in a number of ways. First, the chemistry involves the direct generation of the ABTS radical monocation with no involvement of an intermediary radical. Second, it is a decolorization assay; thus the radical cation is pre-formed prior to addition of antioxidant test systems, rather than the generation of the radical taking place continually in the presence of the antioxidant. Hence the results obtained with the improved system may not always be directly comparable with those obtained using the original TEAC assay. Third, it is applicable to both aqueous and lipophilic systems.

Keywords: ABTS radical cation; Antioxidant activity; Polyphenol; Flavonoid; Hydroxycinnamate; Free radical; Oxidation; TEAC

Rank: 33. 191 citations to date

Free Radic. Biol. Med. 14, 615–631 (1993), 10.1016/0891-5849(93)90143-I

Review: Towards the Physiological Function of Uric Acid

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Abstract: Uric acid, or more correctly (at physiological pH values), its monoanion urate, is traditionally considered to be a metabolically inert end-product of purine metabolism in man, without any physiological value. However, this ubiquitous compound has proven to be a selective antioxidant, capable especially of reaction with hydroxyl radicals and hypochlorous acid, itself being converted to innocuous products (allantoin, allantoate, glyoxylate, urea, oxalate). There is now evidence for such processes not only in vitro and in isolated organs, but also in the human lung in vivo. Urate may also serve as an oxidisable cosubstrate for the enzyme cyclooxygenase. As shown for the coronary system, a major site of production of urate is the microvascular endothelium, and there is generally a net release of urate from the human myocardium in vivo. In isolated organ preparations, urate protects against reperfusion damage induced by activated granulocytes, cells known to produce a variety of radicals and oxidants. Intriguingly, urate prevents oxidative inactivation of endothelial enzymes (cyclooxygenase, angiotensin converting enzyme) and preserves the ability of the endothelium to mediate vascular dilatation in the face of oxidative stress, suggesting a particular relationship between the site of urate formation and the need for a biologically potent radical scavenger and antioxidant.

Keywords: Allantoin; Angiotensin converting enzyme; Coronary endothelium; Cyclooxygenase; Free radical; Hypochlorite; Neutrophils; Oxalate; Oxidant; Xanthine oxidase

Rank: 33. 191 citations to date
Free Radic. Biol. Med. 10, 161–169 (1991), 10.1016/0891-5849(91)90009-R

Review: Oxygen Free Radicals and Parkinson's Disease

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Abstract: The involvement of oxygen radicals in the pathogenesis of Parkinson's disease has been suggested for some time. This article reviews the evidence supporting the involvement of oxygen radicals in the disease process in the brain. This includes a discussion of iron, lipid peroxidation, peroxidase, catalase, superoxide dismutase, and glutathione levels in the brain. In addition, various theories of induction of Parkinson's disease are discussed in relation to the possible involvement of oxygen radicals. These theories include the environmental toxin theory, the dopamine turnover theory, and the cerebral blood flow theory.

Keywords: Parkinson's disease; Superoxide radical anion; Dopaminergic neurons; Substantia nigra; Caudate nucleus; Iron; Glutathione; Defense mechanisms

Rank: 35. 190 citations to date
Free Radic. Biol. Med. 15, 353–363 (1993), 10.1016/0891-5849(93)90035-S

A Comparative Evaluation of Thiobarbituric Acid Methods for the Determination of Malondialdehyde in Biological Materials

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Abstract: A comparative evaluation was made of the conventional spectrophotometric procedure and three published high performance liquid chromatographic (HPLC) procedures for the determination of malondialdehyde (MDA) as the thiobarbituric acid (TBA) derivative when applied to liver, fish meal, serum, and urine. Except for urine, spectrophotometric analysis overestimated MDA content. Purification of the TBA–MDA complex obtained from liver and fish meal on reverse phase cartridges was found to entail a loss of complex bound to residual peptides in the trichloroacetic acid (TCA) extract. Mincing as opposed to homogenizing liver samples led to a doubling of values for MDA content. Hexanal was a major TBA reactant, in addition to MDA, in all the samples. Acid hydrolysis

and heat were necessary for the release of MDA bound to the amino groups of proteins and other amino compounds. Methods for free MDA have limited application to biological materials except short term in vitro preparations such as peroxidizing microsomes, in which free MDA accumulates. On the basis of these and other observations, a modified HPLC procedure for the determination of MDA as the TBA–MDA complex is proposed.

Keywords: Malondialdehyde; Thiobarbituric acid; Lipid peroxidation; Free radicals

Rank: 36. 189 citations to date
Free Radic. Biol. Med. 16, 29–33 (1994), 10.1016/0891-5849(94)90239-9

The Role of O₂^{•-} in the Production of HO[•]: In Vitro and in Vivo

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Abstract: In vitro O₂^{•-} reduces Fe(III) to Fe(II), which, in turn, reduces the H₂O₂, yielding Fe(II)O or HO[•]. In vivo O₂^{•-} increases the supply of free iron by oxidatively attacking the [4Fe-4S] clusters of dehydratases such that they release Fe(II), which can then reduce H₂O₂. In vivo, O₂^{•-} also increases the production of H₂O₂ by acting as an oxidant toward the dehydratases and toward other cellular reductants.

Keywords: Hydroxyl radical; Dehydratases; Aconitase; Superoxide; Free radicals

Rank: 37. 182 citations to date
Free Radic. Biol. Med. 13, 435–448 (1992), 10.1016/0891-5849(92)90184-I

Antioxidant Potential of Ferulic Acid

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Abstract: Ferulic acid is a ubiquitous plant constituent that arises from the metabolism of phenylalanine and tyrosine. It occurs primarily in seeds and leaves both in its free form and covalently linked to lignin and other biopolymers. Due to its phenolic nucleus and an extended side chain conjugation, it readily forms a resonance stabilized phenoxy radical which accounts for its potent antioxidant potential. UV absorption by ferulic acid catalyzes stable phenoxy radical formation and thereby potentiates its ability to terminate free radical chain reactions. By virtue of effectively scavenging deleterious

radicals and suppressing radiation-induced oxidative reactions, ferulic acid may serve an important antioxidant function in preserving physiological integrity of cells exposed to both air and impinging UV radiation. Similar photoprotection is afforded to skin by ferulic acid dissolved in cosmetic lotions. Its addition to foods inhibits lipid peroxidation and subsequent oxidative spoilage. By the same mechanism ferulic acid may protect against various inflammatory diseases. A number of other industrial applications are based on the antioxidant potential of ferulic acid.

Keywords: Coniferic acid; Ferulic acid; Food preservative; Free radical; Iron chelation; γ -Oryzanol; Phenolic antioxidant; UV absorber

Rank: 38. 179 citations to date

Free Radic. Biol. Med. 13, 581–583 (1992), 10.1016/0891-5849(92)90151-6

Intracranial Microdialysis of Salicylic Acid to Detect Hydroxyl Radical Generation Through Dopamine Autooxidation in the Caudate Nucleus: Effects of MPP⁺

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Abstract: Ringer's solution containing salicylic acid (5 nmol/ μ l/min) was infused directly through an intracranial microdialysis probe to detect the generation of hydroxyl radicals (\cdot OH) reflected by the formation of dihydroxybenzoic acids (DHBA) in the caudate nucleus of anesthetized rats. Brain dialysate was assayed for dopamine, 2,3-, and 2,5-DHBA by a high-pressure liquid chromatography-electrochemical (HPLC-EC) procedure. 1-Methyl-4-phenylpyridinium ions (MPP⁺, 0 to 150 nmol) increased dose-dependently the release of dopamine and the formation of DHBA. A positive linear correlation between the release of dopamine and the formation of 2,3- or 2,5-DHBA was observed ($R^2 = .98$). The present results demonstrate the validity of the use of not only 2,3-DHBA but also 2,5-DHBA as an in vivo index of oxidative damage generated by reactive \cdot OH radicals. In conclusion, the present study demonstrates a novel use of intracranial microdialysis of salicylic acid to assess the oxidative damage elicited by \cdot OH in living brain.

Keywords: Hydroxyl radical; MPTP; MPP⁺; Salicylic acid; Dopamine; Parkinson's disease, Free radicals

Rank: 38. 179 citations to date

Free Radic. Biol. Med. 29, 323–333 (2000), 10.1016/S0891-5849(00)00302-6

Triggering and Modulation of Apoptosis by Oxidative Stress

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Abstract: Cell survival requires multiple factors, including appropriate proportions of molecular oxygen and various antioxidants. Although most oxidative insults can be overcome by the cell's natural defenses, sustained perturbation of this balance may result in either apoptotic or necrotic cell death. Numerous, recent studies have shown that the mode of cell death that occurs depends on the severity of the insult. Oxidants and antioxidants can not only determine cell fate, but can also modulate the mode of cell death. Effects of oxidative stress on components of the apoptotic machinery may mediate this modulation. This review will address some of the current paradigms for oxidative stress and apoptosis, and discuss the potential mechanisms by which oxidants can modulate the apoptotic pathway.

Keywords: Caspases; Glutathione; Hydrogen peroxide; Dithiocarbamates; Necrosis; Free radicals

Rank: 40. 177 citations to date

Free Radic. Biol. Med. 19, 481–486 (1995), 10.1016/0891-5849(94)00240-K

Flavonoids as Antioxidant Agents: Importance of Their Interaction with Biomembranes

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Abstract: Flavonoids, a group of phenolic compounds widely occurring in the plant kingdom, have been reported to possess strong antioxidant activity. In the present study, four flavonoids (quercetin, hesperetin, naringenin, rutin), chosen according to their structural characteristics, were tested in two different in vitro experimental models: (1) Fe²⁺-induced linoleate peroxidation (Fe²⁺-ILP), by detection of conjugated dienes; and (2) autooxidation of rat cerebral membranes (ARCM), by using thiobarbituric acid for assay of free malondialdehyde production. The results obtained were also interpreted in the light of flavonoid interactions, studied by differential scanning calorimetry, with dipalmitoylphosphatidylcholine (DPPC) vesicles as a biological membrane model. The antilipoperoxidant activity of the flavonoids tested can be classified as follows: rutin > hesperetin > quercetin >>

naringenin in the Fe^{2+} -ILP test; quercetin > rutin >> hesperetin > naringenin in the ARCM test. Quercetin, hesperetin, and naringenin interacted with DPPC liposomes causing different shifts, toward lower values, of the main transition peak temperature (T_m) typical for DPPC liposomes; however, no change in T_m of DPPC dispersion was observed in the presence of rutin. The hypothesis will be discussed that flavonoid capacity to modify membrane-dependent processes, such as free-radical-induced membrane lipoperoxidation, is related not only to their structural characteristics but also to their ability to interact with and penetrate the lipid bilayers.

Keywords: Flavonoids; Lipoperoxidation; Free radicals; Antioxidant activity; Model membranes

Rank: 41. 175 citations to date
Free Radic. Biol. Med. 21, 307–315 (1996), 10.1016/S0891-5849(96)00046-9

Evaluation of the Antioxidant Activity of Melatonin in Vitro

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Abstract: Melatonin is being increasingly promoted as a treatment for “jet lag” and insomnia and has been suggested to act as an antioxidant in vivo. The antioxidant and potential pro-oxidant activities of melatonin were investigated in vitro. Melatonin was able to scavenge hypochlorous acid (HOCl) at a rate sufficient to protect catalase against inactivation by this molecule. Melatonin could also prevent the oxidation of 5-thio-2-nitrobenzoic acid by HOCl.

Melatonin decreased the peroxidation of ox-brain phospholipids with a calculated IC_{50} of $(210 \pm 2.3) \mu\text{M}$. In contrast, serotonin which also scavenged HOCl, was much more effective in decreasing phospholipid peroxidation ($\text{IC}_{50} 15 \pm 5 \mu\text{M}$). Both compounds reacted with trichloromethylperoxy radical ($\text{CCl}_3\text{O}_2^\bullet$) with rate constants of $(2.7 \pm 0.2) \times 10^8$ and $(1.2 \pm 0.1) \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ respectively. Melatonin did not scavenge superoxide radical and weakly protected DNA against damage by the ferric bleomycin system. By contrast serotonin was weakly pro-oxidant in the ferric-bleomycin system and strongly pro-oxidant in the Fe^{3+} -EDTA/ H_2O_2 -deoxyribose system. Solubility restrictions precluded examination of melatonin in this system. Our data show that melatonin exerts only limited direct antioxidant activities.

Keywords: Melatonin; Free radicals; Antioxidants; Peroxyl radicals; Lipid peroxidation

Rank: 41. 175 citations to date
Free Radic. Biol. Med. 27, 916–921 (1999), 10.1016/S0891-5849(99)00177-X

Forum: Glutathione and Its Role in Cellular Functions

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Abstract: Glutathione (GSH) is the major cellular thiol participating in cellular redox reactions and thioether formation. This article serves as introduction to the FRBM Forum on glutathione and emphasizes cellular functions: What is GSH? Where does it come from? Where does it go? What does it do? What is new and noteworthy? Research tools, historical remarks, and links to current trends.

Keywords: Glutathione; Free radicals; Glutathionylation; Redox state; Thiols; Glutathione conjugation; Subcellular compartmentation; Apoptosis; Gene expression

Rank: 43. 172 citations to date
Free Radic. Biol. Med. 27, 951–965 (1999), 10.1016/S0891-5849(99)00173-2

Forum: Tissue-Specific Functions of Individual Glutathione Peroxidases

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Abstract: The family of glutathione peroxidases comprises four distinct mammalian selenoproteins. The classical enzyme (cGPx) is ubiquitously distributed. According to animal, cell culture and inverse genetic studies, its primary function is to counteract oxidative attack. It is dispensable in unstressed animals, and accordingly ranks low in the hierarchy of glutathione peroxidases. The gastrointestinal isoenzyme (GI-GPx) is most related to cGPx and is exclusively expressed in the gastrointestinal tract. It might provide a barrier against hydroperoxides derived from the diet or from metabolism of ingested xenobiotics. The extreme stability in selenium deficiency ranks this glutathione peroxidase highest in the hierarchy of selenoproteins and points to a more vital function than that of cGPx. Plasma GPx (pGPx) behaves similar to cGPx in selenium deficiency. It is directed to extracellular compartments and is expressed in various tissues in contact with body fluids, e.g., kidney, ciliary body, and maternal/fetal interfaces. It has to be

rated as an efficient extracellular antioxidant device, though with low capacity because of the limited extracellular content of potential thiol substrates. Phospholipid hydroperoxide glutathione peroxidase (PHGPx), originally presumed to be a universal antioxidant enzyme protecting membrane lipids, appears to have adopted a variety of specific roles like silencing lipoxygenases and becoming an enzymatically inactive structural component of the mitochondrial capsule during sperm maturation. Thus, all individual isoenzymes are efficient peroxidases in principle, but beyond their mere antioxidant potential may exert cell- and tissue-specific roles in metabolic regulation, as is evident for PHGPx and may be expected for others.

Keywords: Glutathione peroxidases; Tissue distribution; Free radicals; Redoxregulation; Cellular signaling; Spermatogenesis; Lipoxygenase; Apoptosis

Rank: 44. 170 citations to date

Free Radic. Biol. Med. 15, 447–451 (1993), 10.1016/0891-5849(93)90044-U

Review: Luminol and Lucigenin as Detectors of $O_2^{\bullet-}$

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Abstract: Univalent oxidation of luminol and univalent reduction of lucigenin must precede reaction with $O_2^{\bullet-}$ if that reaction is to lead to luminescence. The assumption that luminol or lucigenin, per se, reacts with $O_2^{\bullet-}$ in a way leading to luminescence is incorrect, and leads to misinterpretation of results. The chemical reactions leading to the $O_2^{\bullet-}$ -dependent luminescences of luminol and of lucigenin are discussed.

Keywords: Luminol; $O_2^{\bullet-}$ -dependent luminescence of Lucigenin; $O_2^{\bullet-}$ -dependent luminescence of Luminescence; $O_2^{\bullet-}$ -dependent; Superoxide-dependent luminescence; Superoxide dismutase-inhibitable luminescence; Free radicals

Rank: 44. 170 citations to date

Free Radic. Biol. Med. 20, 553–566 (1996), 10.1016/0891-5849(95)02111-6

Iron-Induced Carcinogenesis: The Role of Redox Regulation

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Abstract: Redox cycling is a characteristic of transition metals such as iron. Iron is hypothesized to have been actively involved in the birth of primitive life on earth through the generation of reducing equivalents in the

presence of UV light. Iron is an essential metal in mammals for oxygen transport by hemoglobin and for the function of many enzymes including catalase and cytochromes. However, the “free” or “catalytic” form of iron mediates the production of reactive oxygen species via the Fenton reaction and induces oxidative stress. Serum “free” iron is observed in rare situations such as in severe hemochromatosis in which serum transferrin is saturated. However, it is known that superoxide can release “free” iron from ferritin and hemosiderin in the cell. “Free” iron is quite cytotoxic as well as mutagenic and carcinogenic. Iron compounds were first reported to induce sarcomas in rats by Richmond in 1959. Thereafter, several iron-induced carcinogenesis models were established, including the ferric nitrilotriacetate model by Okada and colleagues. Iron may have a role in the carcinogenic process of other transition metals such as copper and nickel, or other kinds of carcinogens such as nitrosamine and even virus-induced carcinogenesis. In humans, genetic hemochromatosis and asbestosis are two major diseases associated with iron-induced carcinogenesis. There is an increasing number of reports of an association between increased body iron stores and increased risk of cancer. Iron-induced oxidative stress results in two possible consequences: (1) redox regulation failure that leads to lipid peroxidation and oxidative DNA and protein damage; (2) redox regulation that activates a variety of reducing and oxystress-protective mechanisms via signal transduction. Both consequences appear to play a role in iron-induced carcinogenesis.

Keywords: Iron; Carcinogenesis; Neoplasm; Free radicals; Reactive oxygen species; Redox; Lipid peroxidation; Oxidative DNA damage; Hemochromatosis; Asbestosis

Rank: 46. 169 citations to date

Free Radic. Biol. Med. 14, 389–395 (1993), 10.1016/0891-5849(93)90088-C

Superoxide and Nitric Oxide Cooperation in Hypoxia/Reoxygenation-Induced Neuron Injury

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Abstract: Oxygen-derived free radicals are implicated in hypoxia- and reoxygenation-related brain injury. In addition, excitatory amino acid neurotransmitters seem to be involved in this neurotoxicity and could act through the L-arginine/nitric oxide (NO) synthase pathway. In the present study we have used rat forebrain neurons in culture submitted to hypoxia/reoxygenation to investigate the relative role of free radicals, glutamate, and nitric oxide in hypoxic neuronal injury. Hypoxia (5 h) followed by reoxygenation (0–24 h) induced cell damage assessed by lactic dehydrogenase

release into culture medium. Superoxide dismutase (SOD, 500 U/mL), D-L-2-amino-5-phosphonovaleric acid (100 μ M), a glutamate receptor antagonist, and N^G-nitro-L-arginine (100 μ M), an NO synthase inhibitor, protected the neurons. The effect of N^G-nitro-L-arginine was reversed by adding L-arginine (10 mM) in the culture medium, and hemoglobin, which scavenges NO, also afforded protection. Hypoxia (5 h) provoked glutamate release from neurons, and this effect was inhibited by SOD. Exogenous glutamate (1–100 μ M) induced lactic dehydrogenase release, and this effect was inhibited by glutamate antagonism, NO synthase inhibition, or superoxide radical scavenging. These data are consistent with the following sequence of events in hypoxia-related neurotoxicity: free radical formation, glutamate release, and activation of NO synthase leading to superoxide and NO cooperative toxicity.

Keywords: Superoxide; Nitric oxide; Neuron culture; Hypoxia; Glutamate; Excitotoxic amino acids; Ischemia; Free radicals

Rank: 46. 169 citations to date

Free Radic. Biol. Med. 26, 463–471 (1999), 10.1016/S0891-5849(98)00216-0

Review: Mitochondrial Damage Induced by Conditions of Oxidative Stress

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Abstract: Up to 2% of the oxygen consumed by the mitochondrial respiratory chain undergoes one electron reduction, typically by the semiquinone form of coenzyme Q, to generate the superoxide radical, and subsequently other reactive oxygen species such as hydrogen peroxide and the hydroxyl radical. Under conditions in which mitochondrial generation of reactive oxygen species is increased (such as in the presence of Ca²⁺ ions or when the mitochondrial antioxidant defense mechanisms are compromised), these reactive oxygen species may lead to irreversible damage of mitochondrial DNA, membrane lipids and proteins, resulting in mitochondrial dysfunction and ultimately cell death. The nature of this damage and the cellular conditions in which it occurs are discussed in this review article.

Keywords: Mitochondria; Oxidative stress; Reactive oxygen species; Free radicals; Lipid peroxidation; Mitochondrial permeability transition; Necrosis; Apoptosis

Rank: 48. 168 citations to date

Free Radic. Biol. Med. 15, 621–627 (1993), 10.1016/0891-5849(93)90165-Q

Relationship between Mitochondrial Superoxide and Hydrogen Peroxide Production and Longevity of Mammalian Species

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Abstract: The objective of this study was to examine the possible involvement of oxygen free radicals in the aging process. Rates of mitochondrial O₂^{•-} and H₂O₂ production and oxygen consumption in the kidney and the heart were compared among seven different mammalian species namely, mouse, hamster, rat, guinea pig, rabbit, pig, and cow, whose maximum life span potential (MLSP) varies from 3.5 to 30 years. The rates of mitochondrial O₂^{•-} and H₂O₂ generation were inversely correlated to MLSP, and directly related to specific metabolic rate and state 4 mitochondrial respiration. Results of this study indicate that under identical conditions, mitochondria from shorter-lived species produce relatively higher amounts of reactive oxygen species than those from the longer-lived species, and, thus, support the free radical hypothesis of aging.

Keywords: Oxy-radicals; Aging; Free radicals; Mitochondria; Life span; Oxidative stress; Oxygen

Rank: 49. 167 citations to date

Free Radic. Biol. Med. 16, 331–338 (1994), 10.1016/0891-5849(94)90034-5

On the pH-Dependent Yield of Hydroxyl Radical Products from Peroxynitrite

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Abstract: Nitric oxide reacts rapidly with superoxide to give the strongly oxidizing peroxynitrite anion (ONOO⁻), which undergoes spontaneous first-order decomposition when protonated. The oxidative chemistry of peroxynitrite (ONOO⁻) is highly pH-dependent. At acidic pH, peroxynitrous acid (ONOOH) oxidizes dimethylsulfoxide to formaldehyde and 2,2'-azino-bis-(3-ethyl-1,2-dihydrobenzothiazoline 6-sulfonate) (ABTS) to the greenish-colored ABTS^{•+} radical cation. The product yield from dimethyl-

sulfoxide and ABTS decreased at more alkaline pH with apparent pK_a s of 7.9 and 8.2, respectively. Decreasing yield with increasing pH could not be explained by the oxidation of either formaldehyde or $ABTS^{\bullet+}$ by peroxynitrite. In the presence of 50 mM dimethylsulfoxide, nitrogen dioxide was formed in approximately equimolar amounts to the other reaction product, formaldehyde. The yield of nitrogen dioxide also decreased with an apparent pK_a of 8.0. We propose that the complex oxidative chemistry of peroxynitrite is controlled by the pH-dependent isomerization of the relatively stable *cis*-configuration (predominant at high pH) to the *trans*-configuration. *Trans*-peroxynitrous acid can form a vibrationally excited intermediate capable of reacting like hydroxyl radical. The vibrationally excited intermediate can also directly rearrange to nitric acid, reducing the apparent hydroxyl radical yield to less than 30%. The loss of hydroxyl radical-like reactivity can be explained on the basis of ionization of *trans*-peroxynitrous acid to the *trans*-anion, which in turn undergoes internal rearrangement to nitrate without forming a strong oxidant. We propose that the pK_a of 6.8 measured by absorbance at 302 nm in phosphate buffer corresponds to protonation of *cis*-peroxynitrite anion, whereas the loss of hydroxyl radical-like reactivity with a pK_a of 8 corresponds to that of the *trans*-peroxynitrite anion.

Keywords: Superoxide; Nitric oxide; EDRF; Peroxynitrous acid; Free radicals

Rank: 49. 167 citations to date
Free Radic. Biol. Med. 20, 35–43 (1996), 10.1016/0891-5849(95)02014-4

Antioxidant Properties of Hydroxy-Flavones

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Abstract: The antioxidant properties of 24 hydroxy-flavones were evaluated. Results show that 2',3',4'-OH substitution on the B ring plays a crucial role in radical scavenger activity in the DPPH assay and in the inhibitory effect on peroxydation of tissue lipids in the MDA test. The formation of stable radicals for this type of compounds has been studied by ESR. In addition, it has been found that 7-hydroxy-flavones are potent competitive inhibitors of xanthine oxidase. It is proposed that the C-7 OH of flavones may take the place of the C-2 or C-6 OH of xanthine in the active site of the enzyme. A C-4' OH or C-4' OMe substitution on the 7-hydroxy flavones is not favourable to a fit in the active site. The 2',3',4'-trihydroxy-flavones inhibited XO by another process, which remains to be determined. In summary, this study provides evidence that hydroxy-flavones exhibit interesting antioxidant properties expressed either by the capacity to scavenge free radicals (for 2',3',4'-trihydroxy-flavones) or to

competitively inhibit xanthine oxidase (for 7-hydroxy-flavones). These compounds may be drug candidates for treating pathologies related to free radical oxidation.

Keywords: Flavones; Radical scavengers; Xanthine oxidase inhibitors; ESR; Free radicals

Rank: 51. 163 citations to date
Free Radic. Biol. Med. 15, 435–445 (1993), 10.1016/0891-5849(93)90043-T

Review: The Fenton Reagents

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^aDepartment of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem; and ^bDepartment of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva, Israel

Rank: 52. 161 citations to date
Free Radic. Biol. Med. 19, 505–510 (1995), 10.1016/0891-5849(95)00034-U

The Reaction of NO[•] with O₂^{•-} and HO₂: A Pulse Radiolysis Study

Sara Goldstein and Gidon Czapski

Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem

Rank: 53. 159 citations to date
Free Radic. Biol. Med. 28, 505–513 (2000), 10.1016/S0891-5849(99)00264-6

Forum: Measurement of F₂-Isoprostanes as an Index of Oxidative Stress in Vivo

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Departments of Pharmacology and Medicine, Vanderbilt University, Nashville, TN, USA

Rank: 54. 156 citations to date
Free Radic. Biol. Med. 28, 1317–1327 (2000), 10.1016/S0891-5849(00)00218-5

Forum: Recent Advances Towards Understanding Redox Mechanisms in the Activation of Nuclear Factor κ b

Yvonne M. W. Janssen-Heininger^a, Matthew E. Poynter^a, and Patrick A. Baeuerle^b

^aDepartment of Pathology, University of Vermont, Burlington, VT, USA; and ^bMicromet GmbH, Martinsried, Germany

Rank: 55. 155 citations to date
Free Radic. Biol. Med. 14, 313–323 (1993), 10.1016/0891-5849(93)90028-S

Review: Ebselen, a Selenoorganic Compound as Glutathione Peroxidase Mimic

Helmut Sies

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Rank: 56. 152 citations to date
Free Radic. Biol. Med. 14, 85–90 (1993), 10.1016/0891-5849(93)90512-S

Hypothesis: Superoxide as an Intracellular Radical Sink

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Department of Pathology, Christchurch School of Medicine, PO Box 4345, Christchurch, New Zealand

Rank: 57. 151 citations to date
Free Radic. Biol. Med. 14, 37–47 (1993), 10.1016/0891-5849(93)90507-Q

Biological Effects of Diesel Exhaust Particles. I. In Vitro Production of Superoxide and in Vivo Toxicity in Mouse

Masaru Sagai^a, Hiroki Saito^b, Takamichi Ichinose^a, Masahiko Kodama^c, and Yoki Mori^b

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Rank: 57. 151 citations to date
Free Radic. Biol. Med. 14, 49–55 (1993), 10.1016/0891-5849(93)90508-R

Ascorbate Free Radical as a Marker of Oxidative Stress: An EPR Study

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Rank: 59. 150 citations to date
Free Radic. Biol. Med. 21, 703–707 (1996), 10.1016/0891-5849(96)00129-3

Absorption and Disposition Kinetics of the Dietary Antioxidant Quercetin in Man

Peter C.H. Hollman^a, Martijn van der Gaag^b, Marcel J.B. Mengelers^a, John M.P. van Trijp^a, Jeanne H. M. de Vries^b, and Martijn B. Katan^b

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Rank: 60. 148 citations to date
Free Radic. Biol. Med. 13, 247–270 (1992), 10.1016/0891-5849(92)90021-8

Free Radical Formation Induced by Ultrasound and Its Biological Implications

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Rank: 60. 148 citations to date
Free Radic. Biol. Med. 16, 675–684 (1994), 10.1016/0891-5849(94)90182-1

Oxidative Stress-Induced Apoptosis Prevented by Trolox

Virginia J. Forrest^a, Yuan-Hsu Kang^a, David E. McClain^b, Douglas H. Robinson^a, and Narayani Ramakrishnan^b

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Rank: 62. 147 citations to date
Free Radic. Biol. Med. 27, 936–944 (1999), 10.1016/S0891-5849(99)00175-6

Forum: Gene Expression and the Thiol Redox State

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Rank: 63. 143 citations to date
Free Radic. Biol. Med. 22, 287–305 (1997), 10.1016/S0891-5849(96)00327-9

Review: Oxidative Damage and Fibrogenesis

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Department of Experimental Medicine and Oncology, Centro Interuniversitario di Fisiopatologia Epatica and C.N.R. Center for Immunogenetics and Experimental Oncology, University of Torino, Torino, Italy

Rank: 64. 142 citations to date

Free Radic. Biol. Med. 12, 83–88 (1992), 10.1016/0891-5849(92)90060-T

Hypothesis: How Far Does Ozone Penetrate into the Pulmonary Air/Tissue Boundary Before It Reacts?

William A. Pryor

Biodynamics Institute, Louisiana State University, Baton Rouge, LA 70803-1800, USA

Rank: 64. 142 citations to date

Free Radic. Biol. Med. 18, 75–83 (1995), 10.1016/0891-5849(94)00105-S

A Practical Method for Preparing Peroxynitrite Solutions of Low Ionic Strength and Free of Hydrogen Peroxide

William A. Pryor, Rafael Cueto, Xia Jin, Willem H. Koppenol, Maria Ngu-Schwemlein, Giuseppe L. Squadrito, Prasanna L. Uppu, and Rao M. Uppu

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Rank: 64. 142 citations to date

Free Radic. Biol. Med. 27, 922–935 (1999), 10.1016/S0891-5849(99)00176-8

Forum: Biologic and Pharmacologic Regulation of Mammalian Glutathione

Owen W. Griffith

Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI, USA

Rank: 67. 141 citations to date

Free Radic. Biol. Med. 17, 429–437 (1994), 10.1016/0891-5849(94)90169-4

Differential Susceptibility of Plasma Proteins to Oxidative Modification: Examination by Western Blot ImmunoassayEmily Shacter^a, Joy A. Williams^a, Michael Lim^b, and Rodney L. Levine^b

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Rank: 68. 140 citations to date

Free Radic. Biol. Med. 21, 855–869 (1996), 10.1016/0891-5849(96)00170-0

Review: DNA Strand Breakage and Activation of Poly-ADP Ribosyltransferase: A Cytotoxic Pathway Triggered by Peroxynitrite

Csaba Szabó

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Rank: 68. 140 citations to date

Free Radic. Biol. Med. 22, 359–378 (1997), 10.1016/S0891-5849(96)00269-9

Hypothesis: Neuroprotection by the Metabolic Antioxidant α -Lipoic AcidLester Packer^a, Hans J. Tritschler^b, and Klaus Wessel^b

^aDepartment of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3200, USA and ^bASTA Medica AG, 45 Weismullerstrasse, D-60314, Frankfurt am Main, Germany

Rank: 68. 140 citations to date

Free Radic. Biol. Med. 30, 463–488 (2001), 10.1016/S0891-5849(00)00373-7

Forum: Unraveling Peroxynitrite Formation in Biological Systems

Rafael Radi^a, Gonzalo Peluffo^a, María Noel Alvarez^a, Mercedes Naviliat^b and Alfonso Cayota^c

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Rank: 71. 136 citations to date

Free Radic. Biol. Med. 12, 245–250 (1992), 10.1016/0891-5849(92)90111-S

Lipid Peroxidation Products Mediate the Formation of 8-Hydroxydeoxyguanosine in DNA

Jeen-Woo Park and Robert A. Floyd

Molecular Toxicology Research Program, Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, OK 73104, USA

Rank: 71. 136 citations to date
Free Radic. Biol. Med. 22, 57–71 (1997), 10.1016/S0891-5849(96)00224-9

Requirement for, Promotion, or Inhibition by α -Tocopherol of Radical-Induced Initiation of Plasma Lipoprotein Lipid Peroxidation

Jiří Neuzil, Shane R. Thomas, and Roland Stocker

Biochemistry Group, The Heart Research Institute, 145 Missenden Road, Camperdown, Sydney, NSW 2050, Australia

Rank: 71. 136 citations to date
Free Radic. Biol. Med. 25, 385–391 (1998), 10.1016/S0891-5849(98)00093-8

Forum: The Basic Chemistry of Nitrogen Monoxide and Peroxynitrite

W. H. Koppenol

Laboratorium für Anorganische Chemie, Eidgenössische Technische Hochschule, Zürich, Switzerland

Rank: 71. 136 citations to date
Free Radic. Biol. Med. 28, 1815–1826 (2000), 10.1016/S0891-5849(00)00344-0

Forum: The Oxidative Modification Hypothesis of Atherogenesis: An Overview

Guy M. Chisolm^a and Daniel Steinberg^b

^aDepartment of Cell Biology, Cleveland Clinic Foundation, Cleveland, OH, USA and ^bUniversity of California, San Diego, La Jolla, CA, USA

Rank: 75. 135 citations to date
Free Radic. Biol. Med. 26, 202–226 (1999), 10.1016/S0891-5849(98)00196-8

Review: Biomarkers of Free Radical Damage: Applications in Experimental Animals and in Humans

Loeckie L. de Zwart, John H. N. Meerman, Jan N. M. Commandeur, and Nico P. E. Vermeulen

Leiden-Amsterdam Center for Drug Research (LACDR), Division of Molecular Toxicology, Department of Pharmacology, Vrije Universiteit, Amsterdam, The Netherlands

Rank: 76. 134 citations to date
Free Radic. Biol. Med. 31, 1287–1312 (2001), 10.1016/S0891-5849(01)00724-9

Review: Reactive Oxygen Species, Antioxidants, and the Mammalian Thioredoxin System

Jonas Nordberg and Elias S. J. Arnér

Medical Nobel Institute for Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden

Rank: 77. 133 citations to date
Free Radic. Biol. Med. 23, 367–372 (1997), 10.1016/S0891-5849(96)00614-4

Reaction of Melatonin and Related Indoles with Hydroxyl Radicals: EPR and Spin Trapping Investigations

Zenon Matuszak, Krzysztof J. Reszka, and Colin F. Chignell

Laboratory of Molecular Biophysics, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC 27709, USA

Rank: 77. 133 citations to date
Free Radic. Biol. Med. 28, 1456–1462 (2000), 10.1016/S0891-5849(00)00252-5

Forum: Reactive Oxygen Species, Cell Signaling, and Cell Injury

Kenneth Hensley, Kent A. Robinson, S. Prasad Gabbita, Scott Salsman, and Robert A. Floyd

Free Radical Biology and Aging Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA

Rank: 79. 131 citations to date
Free Radic. Biol. Med. 17, 45–64 (1994), 10.1016/0891-5849(94)90007-8

Review: On the Nature of Selenium Toxicity and Carcinostatic Activity

Julian E. Spallholz

Food and Nutrition, Texas Tech University, Lubbock, TX, USA

Rank: 79. 131 citations to date
Free Radic. Biol. Med. 23, 783–792 (1997), 10.1016/S0891-5849(97)00016-6

Review: Iron Homeostasis, Oxidative Stress, and DNA Damage

Rogério Meneghini

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, SP, Brasil

Rank: 79. 131 citations to date
Free Radic. Biol. Med. 28, 289–309 (2000), 10.1016/S0891-5849(99)00223-3

Hypothesis: The Heme Synthesis and Degradation Pathways: Role in Oxidant Sensitivity: Heme Oxygenase Has Both Pro- and Antioxidant Properties

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Department of Internal Medicine, Division of Endocrinology, Metabolism, and Molecular Medicine, Southern Illinois University School of Medicine, Springfield, IL, USA; and Department of Pharmacy and Pharmacology, University of Bath, Bath, United Kingdom

Rank: 82. 130 citations to date
Free Radic. Biol. Med. 23, 361–366 (1997), 10.1016/S0891-5849(97)00104-4

Protein Carbonyl Measurement by a Sensitive ELISA Method

Hendrikje Buss, Timothy P. Chan, Karl B. Sluis, Neil M. Domigan, and Christine C. Winterbourn

Department of Pathology, Christchurch School of Medicine, Christchurch, New Zealand

Rank: 82. 130 citations to date
Free Radic. Biol. Med. 28, 141–164 (2000), 10.1016/S0891-5849(99)00224-5

Review: Vitamin E and Heart Disease: Basic Science to Clinical Intervention Trials

William A. Pryor

The Biodynamics Institute, Louisiana State University, Baton Rouge, LA, USA

Rank: 84. 129 citations to date
Free Radic. Biol. Med. 18, 29–36 (1995), 10.1016/0891-5849(94)00102-P

A Fluorescence-Based Method for Measuring Total Plasma Antioxidant Capability

Andrea Ghiselli, Mauro Serafini, Giuseppe Maiani, Elena Azzini, and Anna Ferro-Luzzi

National Institute of Nutrition, Rome, Italy

Rank: 84. 129 citations to date
Free Radic. Biol. Med. 21, 651–668 (1996), 10.1016/0891-5849(96)00162-1

Review: Aging and Oxidative Stress: Modulation by Dietary Restriction

Byung Pal Yu

Department of Physiology, The University of Texas Health Science Center, San Antonio, Texas, USA

Rank: 84. 129 citations to date
Free Radic. Biol. Med. 21, 323–333 (1996), 10.1016/0891-5849(96)00051-2

Review: Redox Modulation of Tyrosine Phosphorylation-Dependent Signal Transduction Pathways

Hugo P. Monteiro^a and Arnold Stern^b

^aFundação Pró-Sangue Hemocentro S. Paulo, S. Paulo, Brazil, and ^bDepartment of Pharmacology, New York University Medical Center, New York, NY, USA

Rank: 84. 129 citations to date
Free Radic. Biol. Med. 28, 1349–1361 (2000), 10.1016/S0891-5849(00)00221-5

Forum: Protein Kinase C Signaling and Oxidative Stress

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Rank: 88. 127 citations to date
Free Radic. Biol. Med. 19, 77–101 (1995), 10.1016/0891-5849(94)00244-E

Review: Age- and Peroxidative Stress-Related Modifications of the Cerebral Enzymatic Activities Linked to Mitochondria and the Glutathione System

Gianni Benzi and Antonio Moretti

Institute of Pharmacology, Faculty of Science, University of Pavia, Italy

Rank: 89. 125 citations to date
Free Radic. Biol. Med. 16, 99–109 (1994), 10.1016/0891-5849(94)90246-1

Review: Reactive Oxygen Species, Chromosome Mutation, and Cancer: Possible Role of Clastogenic Factors in Carcinogenesis

Ingrid Emerit

Centre National de la Recherche Scientifique and University of Paris VI, Paris, France

Rank: 89. 125 citations to date
Free Radic. Biol. Med. 19, 541–552 (1995), 10.1016/0891-5849(95)00052-Y

Inhibition of Human Low-Density Lipoprotein Oxidation by Caffeic Acid and Other Hydroxycinnamic Acid Derivatives

Mirella Nardini, Massimo D'Aquino, Gianni Tomassi^a, Vincenzo Gentili, Maurizio Di Felice, and Cristina Scaccini

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Rank: 91. 124 citations to date
Free Radic. Biol. Med. 13, 557–580 (1992), 10.1016/0891-5849(92)90150-F

Review: Metabolism of Oxygen Radicals in Peroxisomes and Cellular Implications

Luis A. del Río, Luisa M. Sandalio, José M. Palma^a, Pablo Bueno, and Francisco J. Corpas

Unidad de Bioquímica Vegetal, Estación Experimental del Zaidín, CSIC, Apdo. 419, E-18080 Granada, Spain; and ^aUnidad de Fisiología Vegetal, Instituto de Investigaciones Agrobiológicas de Galicia, CSIC, Apdo. 122, E-15780 Santiago de Compostela, Spain

Rank: 92. 123 citations to date
Free Radic. Biol. Med. 20, 463–466 (1996), 10.1016/0891-5849(96)02051-5

Low Antioxidant Enzyme Gene Expression in Pancreatic Islets Compared with Various Other Mouse Tissues

Sigurd Lenzen, Jens Drinkgern, and Markus Tiedge

Institute of Clinical Biochemistry, Hannover Medical School, Hannover, Germany

Rank: 93. 122 citations to date
Free Radic. Biol. Med. 21, 995–1001 (1996), 10.1016/S0891-5849(96)00240-7

Nitric Oxide-Mediated Mitochondrial Damage: A Potential Neuroprotective Role for Glutathione

Juan P. Bolaños, Simon J. R. Heales, Stefan Peuchen, Jane E. Barker, John M. Land, and John B. Clark

Department of Neurochemistry, Institute of Neurology, London, UK

Rank: 94. 121 citations to date
Free Radic. Biol. Med. 14, 351–360 (1993), 10.1016/0891-5849(93)90084-8

Reactive Nitrogen Intermediates and Antimicrobial Activity: Role of Nitrite

Seymour J. Klebanoff

Department of Medicine, S J-10, University of Washington, Seattle, WA 98195, USA

Rank: 94. 121 citations to date
Free Radic. Biol. Med. 18, 85–92 (1995), 10.1016/0891-5849(94)E0133-4

The Kinetics of the Oxidation of L-Ascorbic Acid by Peroxynitrite

Delland Bartlett^a, Daniel F. Church^a, Patricia L. Bounds^d, and W. H. Koppenol^{b,c}

^aDepartments of Chemistry and ^bBiochemistry and ^cBiodynamics Institute, Louisiana State University, Baton Rouge, LA, USA; and ^dDepartment of Chemistry and Physics, Southeastern Louisiana State University, Hammond, LA, USA

Rank: 96. 120 citations to date
Free Radic. Biol. Med. 21, 771–781 (1996), 10.1016/0891-5849(96)00176-1

Differential Activation of ERK, JNK/SAPK and P3/CSBP/RK Map Kinase Family Members during the Cellular Response to Arsenite

Yusen Liu, Kathryn Z. Guyton, Myriam Gorospe, Qingbo Xu, John C. Lee^a, and Nikki J. Holbrook

^aGene Expression and Aging Section, Gerontology Research Center, National Institute on Aging, Baltimore, Maryland, USA; and ^aDepartment of Cellular Biochemistry, Smithkline Beecham Pharmaceuticals, King of Prussia, Pennsylvania, USA

Rank: 96. 120 citations to date
Free Radic. Biol. Med. 27, 612–616 (1999), 10.1016/S0891-5849(99)00107-0

Quantifying Cellular Oxidative Stress by Dichlorofluorescein Assay Using Microplate Reader

Hong Wang and James A. Joseph

Neuroscience Laboratory, USDA-ARS, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA

Rank: 98. 119 citations to date

Free Radic. Biol. Med. 15, 311–328 (1993), 10.1016/0891-5849(93)90078-9

Review: Molecular Pharmacology of Vitamin E: Structural Aspects of Antioxidant Activity

Saskia A. B. E. van Acker, Luc M. H. Koymans, and Aalt Bast

Department of Pharmacochemistry, Faculty of Chemistry, Vrije Universiteit, Amsterdam, The Netherlands

Rank: 98. 119 citations to date

Free Radic. Biol. Med. 22, 73–83 (1997), 10.1016/S0891-5849(96)00235-3

Apoptotic vs. Nonapoptotic Cytotoxicity Induced by Hydrogen Peroxide

Agnes M. Gardner, Feng-hao Xu, Catherine Fady, Fred J. Jacoby, Dianne C. Duffey^a, Yiping Tu, and Alan Lichtenstein

Departments of Medicine and ^aHead and Neck Surgery, VA Wadsworth-UCLA Medical Center, Los Angeles, CA 90073, USA

Rank: 100. 116 citations to date

Free Radic. Biol. Med. 12, 101–106 (1992), 10.1016/0891-5849(92)90002-X

Quercetin Prevents the Cytotoxicity of Oxidized LDL on Lymphoid Cell Lines

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