ELSEVIER

Review / Revue

Oxidative stress: a theoretical model or a biological reality?

Olivier Sorg

Clinique de dermatologie, hôpitaux universitaires de Genève, 24, rue Micheli-du-Crest, 1211 Genève 14, Switzerland

Abstract

Although oxidative stress has been extensively studied the last fifteen years, many physicians and biologists are still sceptical concerning its interest in biology and medicine. This is probably due, in part, to the fact that this subject is a matter of biophysics, and the first studies reported were written using a physical language that inspired these people used to a more concrete problematic very little. Another problem is the difficulty to detect the species mediating oxidative stress, and to determine their role in biological processes. This review is aimed at presenting oxidative stress, as well as reactive oxygen species and free radicals – the molecules that mediate it – in a clear form able to convince all researchers involved in life sciences that these short-lived intermediates are indissociable from any aerobic organism. Moreover, if reactive oxygen species and free radicals are undoubtedly involved in many pathologies, they have physiological functions too. *To cite this article: O. Sorg, C. R. Biologies 327 (2004).*
© 2004 Académie des sciences. Published by Elsevier SAS. All rights reserved.

Résumé

Stress oxydant : un modèle théorique ou une réalité biologique ? Bien que le stress oxydant ait été largement étudié depuis une quinzaine d'années, nombre de médecins et biologistes sont encore sceptiques en ce qui concerne son intérêt en biologie et médecine. Ceci est probablement dû, du moins en partie, au fait que ce sujet relève essentiellement de la biophysique, et que les premières études publiées utilisaient un langage physique qui a peu inspiré ces gens habitués à une problématique plus terre-à-terre. Un autre problème vient de la difficulté qu'il y a à détecter les substances responsables du stress oxydant, ainsi qu'à déterminer leur rôle dans les processus biologiques. L'objectif de cette revue est de présenter le stress oxydant, de même que les molécules qui le génèrent, à savoir les formes réactives de l'oxygène et les radicaux libres, sous une forme simple, apte à convaincre les chercheurs en sciences de la vie du fait que ces intermédiaires labiles sont indissociables de tout organisme aérobie. De plus, si les formes réactives de l'oxygène et les radicaux libres sont incontestablement impliqués dans de nombreuses pathologies, ils ont également des fonctions physiologiques. *Pour citer cet article : O. Sorg, C. R. Biologies 327 (2004).*
© 2004 Académie des sciences. Published by Elsevier SAS. All rights reserved.

E-mail address: olivier.sorg@hcuge.ch (O. Sorg).

Abbreviations

GPX; glutathione peroxidase
ROS; reactive oxygen species
SOD; superoxide dismutase

1. Introduction

Except for anaerobic microorganisms, all living organisms require molecular oxygen as an electron acceptor for efficient production of energy. However, oxygen is a strong oxidant, and it is impossible to avoid secondary oxidations not involved in physiological metabolism. These random oxidations would have deleterious consequences if they were not neutralised by an efficient antioxidant system. From this theoretical evidence, biophysicists introduced the concept of 'oxidative stress' to describe a situation occurring in aerobes when the undesired secondary oxidations induced by oxygen and its derivatives are not efficiently neutralised, and lead to abnormal metabolism, loss of physiological function, disease, and potentially death [1,2]. For many years, biophysicists and theoretical chemists have communicated about oxidative stress and its biological consequences using an abstruse language that did not really appeal to most biologists and physicians. For this reason, the latter did not pay much attention to the growing number of publications focused on this subject, and eventually imagined oxidative stress more as a theoretical model unusable for applied biology and medicine than a biological reality that could have interesting applications. Actually, those who showed the most interest in this concept where people involved in the cosmetic industry. Considering the low level of interest usually shown by the scientific and medical community for cosmetology, it is not surprising that most physicians continued until very recently to perceive oxidative stress as a theoretical model rather than a biological reality that could improve our knowledge on biochemical mechanisms and help finding the search for new treatments for human diseases.

The aim of this review is to convince biochemists, biologists and physicians that oxidative stress is more than a theoretical model or a nice story used by the cosmetic industry to drive business thanks to the fear people have of ageing.

2. What is oxidative stress?

Oxidative stress corresponds to an imbalance between the rate of oxidant production and that of their degradation [2]. Aerobic organisms such as vertebrates and man in particular produce their energy from the oxidation of organic substrates by molecular oxygen. The complete four-electron reduction of molecular oxygen occurs within mitochondria and produces water, at the end of the respiratory chain. Sometimes molecular oxygen is partly reduced instead of the proteins of the respiratory chain, and superoxide and various reactive oxidant intermediates are produced, leading to secondary oxidations [3]. Besides these physiological oxidations and their unavoidable secondary reactions, many substances contained in food are either oxidants by themselves or oxidant precursors [4, 5]. Other oxidants come from the environment: (*i*) air pollutants and oxidant contained in tobacco and fire smoke react with molecules of the skin surface, and some of them penetrate into the skin and reach the circulation [6,7]; (*ii*) UV radiation from the sun are absorbed by cutaneous chromophores and induce the formation of a variety of oxidants [8–10]. Finally, although the organism adapts to any unstable situation by preventing undesirable reactions and repairing damaged molecules and tissues, the very few undesirable reactions that escape the prevention and repair systems accumulate little by little, and will invariably be deleterious after a long period of time [11,12], thus confirming the free radical theory of ageing developed by Denham Harman [13]. All these conditions lead to the production of various oxidants, and if the organism fails to neutralise them, these oxidants accumulate and react with a variety of biomolecules, creating an undesirable situation known as oxidative stress [2].
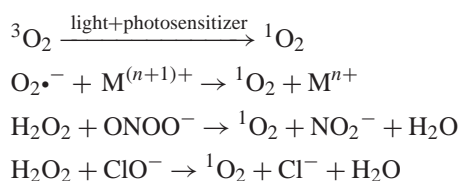
3. Which molecules mediate oxidative stress?

Two classes of molecules mediate most of the reactions leading to oxidative stress: free radicals and reactive oxygen species (ROS) (Table 1, Fig. 1). Free radicals are molecules possessing at least one unpaired electron; for this reason, they are unstable and promote electron transfers, i.e., oxidations and reductions. Lipid radicals are formed by hydrogen abstraction, a process elicited by UV irradiation or the

Table 1
Molecules mediating oxidative stress

Name	Structure	Main reactions
Superoxide	$\bullet O\text{–}O^-$	Catalysis of Haber–Weiß reaction by recycling Fe^{2+} and Cu^+ ions; formation of hydrogen peroxide or peroxynitrite
Hydrogen peroxide	HO–OH	Formation of hydroxyl radical; enzyme inactivation; oxidation of biomolecules
Hydroxyl radical	$\bullet OH$	Hydrogen abstraction; production of free radicals and lipid peroxides; oxidation of thiols
Ozone	$^-O\text{–}O^+{=}O$	Oxidation of all kinds of biomolecules, especially those containing double bonds; formation of ozonides and cytotoxic aldehydes
Singlet oxygen	O=O	Reaction with double bonds, formation of peroxides; decomposition of amino acids and nucleotides
Nitric oxide	$\bullet N{=}O$	Formation of peroxynitrite; reaction with other radicals
Peroxynitrite	$O{=}N\text{–}O\text{–}O^-$	Formation of hydroxyl radical; oxidation of thiols and aromatic groups; conversion of xanthine dehydrogenase to xanthine oxidase; oxidation of biomolecules
Hypochlorite	ClO^-	Oxidation of amino and sulphur-containing groups; formation of chlorine
Radical	$R\bullet$	Hydrogen abstraction; formation of peroxyl radicals and other radicals; decomposition of lipids and other biomolecules
Peroxyl radical	$R\text{–}O\text{–}O\bullet$	Hydrogen abstraction; formation of radicals; decomposition of lipids and other biomolecules
Hydroperoxide	R–O–OH	Oxidation of biomolecules; disruption of biological membranes
Copper and iron ions	Cu^{2+}, Fe^{3+}	Formation of hydroxyl radical by Fenton and Haber–Weiß reactions

hydroxyl radical ($\bullet OH$) [14,15]. The latter, as well as other reactive radicals, belong to the class of ROS. These unstable intermediates derive from molecular oxygen ($\bullet O_2\bullet$ or $^3O_2$), a biradical. In the case of molecular oxygen, the triplet (biradical) state is more stable than the singlet one (non radical, with a double bond); thus singlet oxygen ($^1O_2$) is a ROS. It can be produced by several biochemical oxidations involving peroxidases and lipoxygenases, by reactions between various ROS or in the presence of light, oxygen and a photosensitizer such as porphyrins, as it is the case in congenital erythropoietic porphyria [1,16,17]:
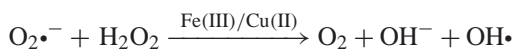
$$^3O_2 \xrightarrow{\text{light+photosensitizer}} {}^1O_2$$

$$O_2\bullet^- + M^{(n+1)+} \rightarrow {}^1O_2 + M^{n+}$$

$$H_2O_2 + ONOO^- \rightarrow {}^1O_2 + NO_2^- + H_2O$$

$$H_2O_2 + ClO^- \rightarrow {}^1O_2 + Cl^- + H_2O$$

Other ROS derive from the secondary reduction of $^3O_2$ in the inner membrane of mitochondria (mainly from ubiquinol oxidation), where oxygen receives one electron in the place of a metalloprotein of the respiratory chain [3,18]. The superoxide radical anion thus formed ($\bullet O_2^-$) can then dismutate into oxygen ($^3O_2$) and hydrogen peroxide (H_2O_2), either spontaneously at acidic pH or in a reaction catalysed by superoxide dismutase (SOD) [1]:

$$2\,O_2\bullet^- + 2\,H^+ \xrightarrow{\text{(SOD)}} O_2 + H_2O_2$$
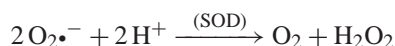
Superoxide and hydrogen peroxide can react with transition metals such as iron or copper to form the strong oxidant hydroxyl radical ($\bullet OH$) [1,19,20]:

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

(Fenton reaction)

$$O_2\bullet^- + H_2O_2 \xrightarrow{\text{Fe(III)/Cu(II)}} O_2 + OH^- + OH\bullet$$

(metal ion-catalysed Haber–Weiß reaction)

Superoxide and hydrogen peroxide are also enzymatically formed by NADPH oxidase in activated neutrophils or macrophages during inflammatory processes [21,22]:

$$NADPH + 2\,O_2 \rightarrow NADP^+ + H^+ + 2\,O_2\bullet^-$$

Generally speaking, all endogenous oxidases – enzymes that transfer two electrons from a substrate (AH_2) to molecular oxygen – produce superoxide and hydrogen peroxide [1]:

$$AH_2 + 2\,O_2 \xrightarrow{\text{oxidase}} A + 2\,H^+ + 2\,O_2\bullet^-$$
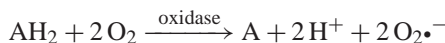
Myeloperoxidase, an enzyme present in neutrophils, but not in macrophages, can produce hypochlorous acid (HOCl) by oxidising chloride ions with hydrogen peroxide [1,23]:

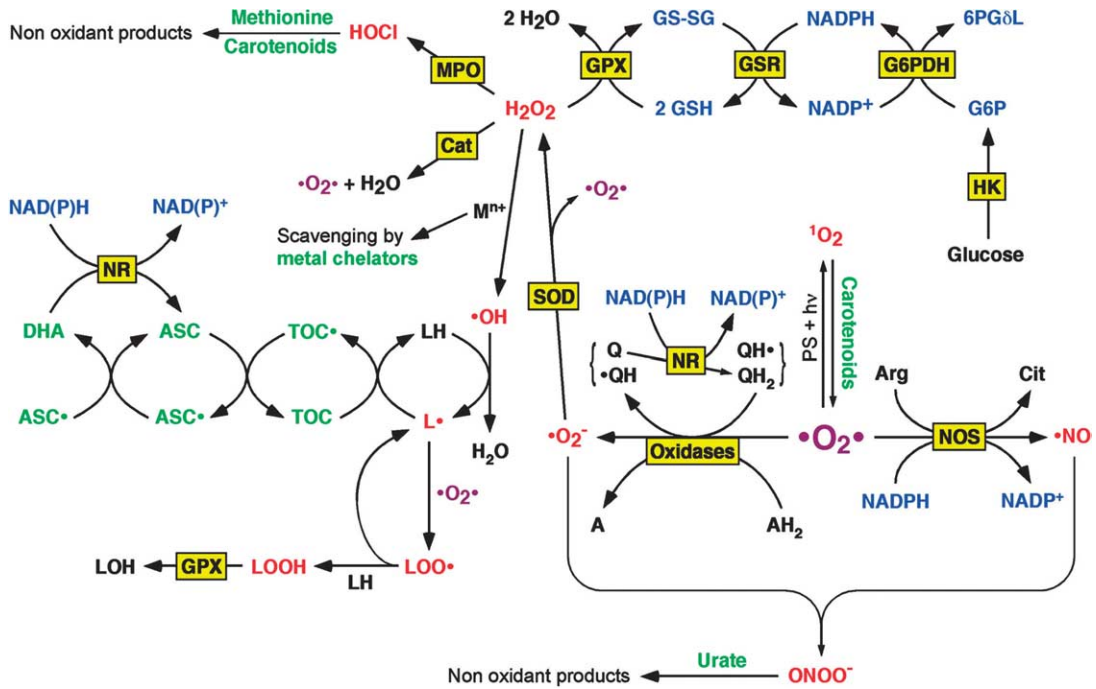$$H_2O_2 + Cl^- \xrightarrow{\text{myeloperoxidase}} HOCl + OH^-$$
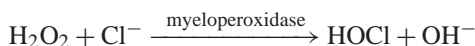
Fig. 1. Overview of free radical and ROS formation and elimination. The main pathway of free radical and ROS formation starts with the partial reduction of molecular oxygen to superoxide by oxidases or the reduced or semiquinone forms of ubiquinol (coenzyme Q). The hydrogen peroxide produced by the dismutation of superoxide can decompose in the presence of transition metals to give rise to hydroxyl radical, an extremely instable oxidant. The latter can initiate lipid peroxidation in the presence of oxygen. Another strong oxidant, peroxynitrite, is formed by reaction between superoxide and nitric oxide, an intercellular messenger produced from arginine, oxygen and NADPH. It thus appears that the more important antioxidants are scavengers of superoxide and hydrogen peroxide. Since the dismutation of superoxide produces hydrogen peroxide, catalase and GPX, the enzymes that remove hydrogen peroxide, should be associated to SOD. Catalase does not require a cofactor, since it catalyses a dismutation ($2 H_2O_2 \rightarrow 2 H_2O + O_2$), but GPX oxidises glutathione, which is then reduced by NADPH, and the latter is finally regenerated by one of the pathways of glucose oxidation; thus glucose appears as a key component of the antioxidant reaction cascade. Abbreviations: 6PGδL, 6-phosphoglucono-δ-lactone; A, an oxidised substrate; AH$_2$, a reduced substrate; Arg, arginine; ASC, ascorbate; ASC$^\bullet$, semi-dehydroascorbate; Cat, catalase; Cit, citrulline; DHA, dehydroascorbate; G6P, glucose 6-phosphate; GSH, reduced glutathione; GSSG, oxidised glutathione; GSR, glutathione reductase; HK, hexokinase; LH, a lipid; L$^\bullet$, a lipid radical; LOH, an hydroxylated lipid; LOO$^\bullet$, a lipid peroxyradical; LOOH, a lipid hydroperoxide; MPO, myeloperoxidase; NR, NAD(P)H reductase; PS, photosensitizer; Q, ubiquinone; QH$^\bullet$, ubisemiquinone; QH$_2$, ubiquinol; TOC, α-tocopherol, TOC$^\bullet$, α-tocopheryl radical.

Hypochlorous acid (or its conjugated base hypochlorite, depending of the pH), is a powerful oxidant towards various biomolecules, especially amino groups; furthermore, in acidic pH it readily decomposes to liberate the highly toxic chlorine gas Cl$_2$ [1,24]:

$$HOCl + H^+ + Cl^- \rightarrow H_2O + Cl_2$$

As mentioned above, hydrogen peroxide can react with hypochlorite to form singlet oxygen. Under physiological conditions, 1–2% of the consumed oxygen is converted to ROS. Nitric oxide ($\bullet$NO), an intercellular messenger, is produced from oxygen by various nitric

oxide synthases [1,25]:

$$O_2 + arginine + NADPH$$
$$\xrightarrow{\text{nitric oxide syntase}} \bullet NO + citrulline + H_2O + NADP^+$$
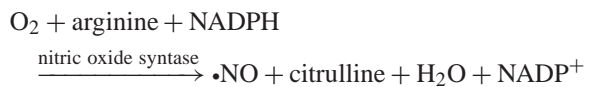
this free radical, by reacting with superoxide – another radical – produces the strong oxidant peroxynitrite (ONOO$^-$) [1,26,27]:

$$O_2\bullet^- + \bullet NO \rightarrow ONOO^-$$

The main biological targets of free radicals and ROS are proteins, lipids and DNA. Protein oxidation leads to loss of function or premature degradation in pro-

Table 2
Endogenous antioxidants

Antioxidant	Phase	Action
Superoxide dismutases (SOD)	Hydrophilic	Dismutation of $O_2{}^-$ into H_2O_2 and O_2
Catalase	Hydrophilic	Dismutation of H_2O_2 into H_2O and O_2
Glutathione peroxidases (GPX)	Hydrophilic or lipophilic	Reduction of R–OOH into R–OH
Glutathione reductase (GSR)	Hydrophilic	Reduction of oxidised glutathione
Glutathione-S-transferases (GST)	Hydrophilic	Conjugation of R–OOH to GSH ($\rightarrow$ GS–OR)
Metallothioneins	Hydrophilic	Binding to transition metals (= neutralisation)
Thioredoxins	Hydrophilic	Reduction of R–S–S–R into R–SH
Glutathione	Hydrophilic	Reduction of R–S–S–R into R–SH
		Free radical scavenger
		Cofactor of GPX and GST
Ubiquinol	Lipophilic	Free radical scavenger (prevents LPO)
(Dihydro)lipoic acid	Amphiphilic	ROS scavenger
		Increases antioxidant and phase II enzymes
Ascorbic acid (vitamin C)	Hydrophilic	Free radical scavenger
		Recycles tocopherols (vitamin E)
		Maintains enzymes in their reduced state
Retinoids (vit. A) and carotenoids	Lipophilic	Free radical scavengers
		Singlet oxygen ($^1O_2$) quencher
Tocopherols (vitamin E)	Lipophilic	Free radical scavenger (prevents LPO)
		Increases selenium absorption
Selenium	Amphiphilic	Constituent of GPX and thioredoxins

Abbreviations: GPX, glutathione peroxidase; GSR, glutathione reductase; GST, gluthahione-S-transferase; LPO, lipid peroxidation; SOD, superoxide dismutase.

teasomes. Lipid peroxidation, occurring mostly in the plasma membrane or the stratum corneum – the most superficial layer of the epidermis – alters the physical properties of cell membranes or the epidermis, and thereby their biological function. Oxidation of DNA can lead to gene mutation, and thus abnormal protein synthesis, alteration in gene expression, apoptosis and cell death [28–30].

4. Endogenous defences against oxidative stress

Strict aerobic organisms cannot live in the presence of oxygen, because they have insufficient defences against the multiple secondary reactions induced by oxygen. All other organisms which can or must live in the presence of oxygen possess an efficient battery of antioxidant defences able to trap reactive intermediates before they have time to oxidise biomolecules or reduce those which have been oxidised (Table 2, Fig. 1) [31–33]. Due to the great variety of reactive intermediates that must be neutralised, as well as the variety of oxidised biomolecules which must be reduced, there are a lot of different antioxidants;

moreover, there is a need for antioxidants for both hydrophilic (cytosol, extracellular fluids) and lipophilic (membranes, lipids) phases [1,31–36].

The most efficient antioxidants are enzymes that catalyse the reduction of ROS: superoxide dismutase (SOD) catalyses the dismutation of superoxide ($\cdot O_2{}^-$) into hydrogen peroxide (H_2O_2) and oxygen ($^3O_2$), catalase catalyses hydrogen peroxide (H_2O_2) dismutation into water (H_2O) and oxygen ($^3O_2$), glutathione peroxidases (GPX) reduce both hydrogen (H_2O_2) and organic (R-OOH) hydroperoxides. The oxidised glutathione cofactor (GS–SG) is then reduced (2 GSH) by glutathione reductase (GSR). Various GPX isoforms exist, which are specific for hydrophilic or lipophilic phases. Metallothioneins are small proteins with several cysteine residues which bind transition metal ions: this can both detoxify metals and avoid them catalysing the Haber–Weiß and Fenton reactions that lead to the production of the hydroxyl radical ($\cdot$OH). The tripeptide glutathione and the selenoprotein thioredoxin are efficient in reducing disulphur bridges (R–S–S–R') into thiols (R–SH + R'–SH), as well as reducing thiyl radicals (R–S$\cdot$); the whole sys-

tem involving thioredoxin, thioredoxin peroxidase and thioredoxin reductase is known as peroxiredoxin. Besides being an antioxidant by itself, glutathione is also the cofactor for GPX and GSR. Ubiquinol (coenzyme Q, QH$_2$), plays an essential role in the mitochondrial electron-transport chain in creating a proton gradient between both sides of inner mitochondrial membrane. Due to its two-step oxidation via a radical intermediate:

$$QH_2 \xrightarrow{-1e^-} QH\bullet \xrightarrow{-1e^-} Q$$
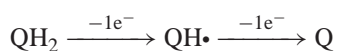
ubiquinol is both a promoter of superoxide formation and an efficient free radical scavenger, especially in lipid phase. Lipoic acid, a component of pyruvate dehydrogenase complex, and its reduced form dihydrolipoic acid, are both metal chelators and ROS scavengers; moreover, dihydrolipoic acid recycles vitamins C and E [37]. Thus, besides their central role in energy metabolism, lipoic and dihydrolipoic acids prevent oxidative stress at various stages [38,39]. L-Ascorbic acid (vitamin C) is a water-soluble low molecular weight antioxidant required for collagen synthesis, iron absorption and maintenance of the redox status of cells [40]. It recycles vitamin E, the predominant membrane antioxidant, as well as many other oxidised biomolecules, and scavenges free radicals [41]. Carotenoids (terpenoid molecules synthesised by plants, among which vitamin A precursors such as β-carotene) are free radical scavengers, and most importantly, singlet oxygen quenchers. This is particularly important for the retina, where singlet oxygen is produced following interactions between visible light, oxygen (triplet) and various photosensitizers [42–45]. Tocopherols (vitamin E) are the main antioxidants in the lipophilic phase; once oxidised, they become radicals, then they are converted to their functional reduced state by ascorbic acid [31–33,41, 46]. Selenium is a trace element present in the food as selenites, selenates or selenomethionine, which are precursors for selenocysteine (Table 2, Fig. 1). The latter is directly incorporated into glutathione peroxidases and thioredoxins from the UGA codon.

5. Oxidative stress is involved in many pathologies

An oxidative stress is often associated to all kinds of diseases, although it is not always easy to determine whether it is a cause or a consequence of the observed condition. A selection of pathologies for which the mechanism of oxidative stress is well documented is described below.

5.1. Ischaemia-reperfusion

The mechanism of ischaemia and tissue injury during reperfusion has been extensively studied, especially in the heart [47]; it is thus a good example of the involvement of oxidative stress in a frequent pathology.

During ischaemia, blood glucose falls, glycolysis and oxidative phosphorylation rates decrease, and subsequently ATP stores are consumed. The two consequences of ATP depletion are (i) accumulation of AMP [48] due to the following reactions:

$$ATP + ROH \rightarrow ADP + ROPO_3^{2-}$$

$$2\,ADP \rightleftharpoons ATP + AMP$$

where ROH and ROPO$_3^{2-}$ are a hydroxyl-containing substrate and its phosphate ester, respectively, as well as (ii) a decrease in the activity of Na$^+$/K$^+$-ATPase, the ATP-dependent pump that maintains the physiological gradients of sodium and potassium ions between both sides of cellular membranes [49]. AMP is metabolised to hypoxanthine; thus ATP depletion leads to hypoxanthine accumulation [49]. On the other hand, the decrease of the activity of ATP-dependent ionic pumps induces a depolarisation of the plasmic membrane, promoting a massive influx of calcium ions within cells [50]. High cytoplasmic Ca^{2+} has two consequences: one is the production of ROS in mitochondria due to the disruption of the mitochondrial proton gradient, which also impairs ATP production, and the second one is the activation of calcium-dependent proteases such as calpains [51]. Calpains and ROS can convert the enzyme xanthine dehydrogenase to xanthine oxidase, due to limited proteolysis and cysteine oxidation [52]. This alteration does not change anything for its substrates hypoxanthine and xanthine, which are converted to xanthine and uric acid, respectively. The difference concerns the electron acceptor: the dehydrogenase form has a binding site for NAD$^+$, and substrate oxidation regenerates NADH, whereas the oxidase form cannot bind NAD$^+$, and the enzyme transfers the electrons com-

ing from the substrates to oxygen, leading to the formation of superoxide, and then hydrogen peroxide. Furthermore, due to hypoxanthine accumulation in response to ischaemia, xanthine oxidase is activated and gives rise to a dramatic production of superoxide ions as soon as oxygen is reperfused [53–55]. Finally, the activity of antioxidant enzymes such as catalase and SOD, two of the enzymes that play a central role in ROS scavenging, are decreased following ischaemia [56].

In summary, during ischaemia, (*i*) the tissues accumulate a substrate for xanthine oxidase, (*ii*) the latter is formed by the alteration of xanthine dehydrogenase, and (*iii*) the tissue defences against oxidative stress are decreased. Thus, as soon as oxygen is reperfused, all these conditions act synergistically to produce high levels of ROS in the tissues that have undergone ischaemia (Fig. 2).

5.2. Atherosclerosis

Many cases of myocardial infarctions and localised cerebral ischaemia are the consequence of atherosclerosis, a disease of arteries characterised by a local thickening of the inner coat of the vessels. It is now widely accepted that ROS play an important role in the initiation and the development of atherosclerosis. Many genetic and environmental conditions (hypercholesterolemia, diabetes, hypertension, smoking, ageing...) can lead to the peroxidation of LDL lipids. These oxidised LDL bind to special LDL receptors, called scavenger receptors, expressed by macrophages; the latter become then foam cells, i.e. lipid-laden distorted cells from macrophage or vascular origin that contribute to the formation of atheroma plaques. Activated enzymes such as xanthine oxidase, NADPH oxidase or nitric oxide synthase then produce more ROS, leading to the formation of fibrous plaques; the latter consist of fibrous cap, composed mostly of smooth muscle cells and dense connective tissue surrounding by macrophages, T lymphocytes and other smooth muscle cells [47,57–59].

Thus the initiation process can be due either to excess of fat, a defective metabolism of lipids, an infection, the presence of toxins or toxic metabolites, or an oxidative stress, then oxidative stress promotes the development of the disease.
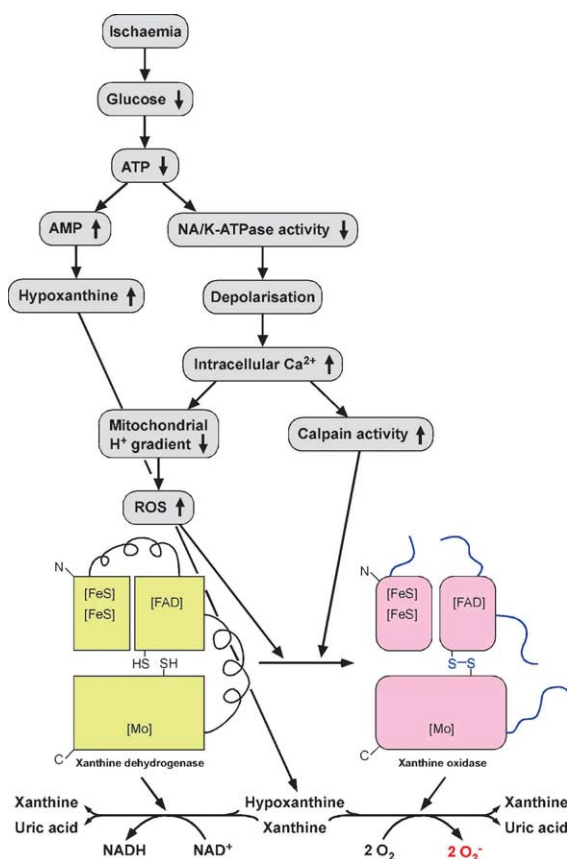

Fig. 2. Mechanism of ROS production during reperfusion after ischaemia. During ischaemia, blood glucose falls down, leading to ATP depletion. As a consequence, hypoxanthine, a substrate for xanthine dehydrogenase and xanthine oxidase, accumulates in the cells. Due to the lack of ATP, the activity of ATP-dependent pumps such as Na^+/K^+-ATPase decreases, which leads to depolarisation and a massive influx of Ca^{2+} ions, promoting the loss of mitochondrial proton gradient and then the production of superoxide and other ROS, as well as the induction of calcium-dependent proteases such as calpains. The latter, as well as ROS, convert xanthine dehydrogenase to its oxidase form, and when oxygen is reperfusated, the high hypoxanthine concentration activates xanthine oxidase, which produces high levels of ROS.

5.3. Neurodegenerative diseases

The brain accounts for only $\approx 2\%$ of body mass, but it processes 20% of basal oxygen consumption in order to support the neuronal electric activity. On the other hand, nitric oxide plays a crucial role as a biological messenger, and is produced by three kinds of nitric oxide synthases (neuronal, endothelial and inducible) [25,60]. Thus the brain produces high levels of super-

oxide and nitric oxide, and these molecules can react to form peroxynitrite, one of the most reactive oxygen species [61]. For these reasons, oxidative stress plays a pivotal role in neurodegenerative disorders, and we discuss below the three major neurodegenerative diseases, in which the link with oxidative stress has been extensively studied [62,63].

5.3.1. Excitotoxicity

A major consequence of ROS production in the brain is the inhibition by various ROS of glutamate uptake by astrocytes [64–66]. The latter constitute most of the cortical mass of the brain, and one of their vital roles is the efficient uptake of glutamate that is released into the extracellular space by neurones during their electric activity. A decrease in glutamate uptake by astrocytes leads to a depolarisation of neuronal plasma membranes due to excess activation on NMDA receptors; this induces a high influx of calcium ions within neurones, a condition that activates calcium-dependent enzymes such as calpains and phospholipase A_2, as well as a decrease of mitochondrial activity. As discussed above, calpains convert xanthine dehydrogenase to its oxidase form, giving rise to superoxide and hydrogen peroxide, whereas activation of phospholipase A_2 releases arachidonate, thus producing ROS via its metabolism to bioactive lipids, as well as a direct inhibition of glutamate uptake [67]. On the other hand, a high level of glutamate or other excitatory amino acids inhibits the uptake of cystine by brain cells; since cystine transport into brain cells is essential to supply cysteine for synthesis of the antioxidant and GPX-cofactor glutathione, this decreases the antioxidant defences of brain cells against oxidative stress; this cytotoxic mechanism elicited by excitatory amino acids is called excitotoxicity [68]. Taken together, these conditions act synergistically in different cycles to induce cell death due to the generation of an oxidative stress and intracellular calcium overload [63,69] (Fig. 3).

5.3.2. Alzheimer's disease

Alzheimer's disease (AD), one of several disorders that cause the gradual loss of brain cells, is the leading cause of dementia. Its neuropathological hallmarks are neurofibrillary tangles and senile plaques, two forms of protein aggregation, i.e., β-amyloid and hyperphosphorylated tau, respectively. Although the participation of oxidative stress as a cause of the disease is still a matter of debate, the brain of Alzheimer's patients is associated with many markers of oxidative stress, and the latter increases the severity of symptoms [62,70–72].

5.3.3. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is caused by the degeneration of both upper and lower motor neurons, resulting in skeletal muscle atrophy and weakness, and culminating in respiratory insufficiency. Although the cause of ALS is still unknown, several mechanisms are implicated in the pathogenesis of motor neuron degeneration, including excitotoxicity, immune activation, mitochondrial dysfunction, protein aggregation, altered proteosomal function, and apoptosis [73–75]. These mechanisms are not mutually exclusive, but rather act in synergy to worsen the resulting cytotoxicity. 10–15% of ALS cases are inherited, among them 25–30% are due to mutations of SOD. These mutations seem to be associated with a toxic gain of function rather than a loss of function; these abnormal forms of SOD are thus believed to play a role in the generation of an oxidative stress by a mechanism that remains to be elucidated [76–78]. As for AD, several markers of oxidative damage are found in the brain of all ALS patients, confirming the role of oxidative stress in the pathology of ALS, although it is not clear whether it participates as an initiator or an amplifier of the symptoms, or both.

5.3.4. Parkinson's disease

Parkinson's disease (PD) is the most common neurodegenerative movement disorder; it is due to the degeneration of dopaminergic neurones in *substantia nigra*. As for ALS and AD, there are both sporadic and familial forms of the disease [62,79]. Autoxidation of dopamine, as well as its catabolism by monoamine oxidase, produce superoxide and hydrogen peroxide [80,81]. This led to the concept that the metabolism of dopamine might be responsible for the high basal levels of oxidative stress in *substantia nigra*. On the other hand, since the story of a student in chemistry who unwittingly produced the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) for his own consumption, this substance has been extensively used in animal models and was shown to be taken up by astrocytes, and then oxidised by monoamine oxidase in another compound, 1-methyl-4-phenyl-pyridinium ion
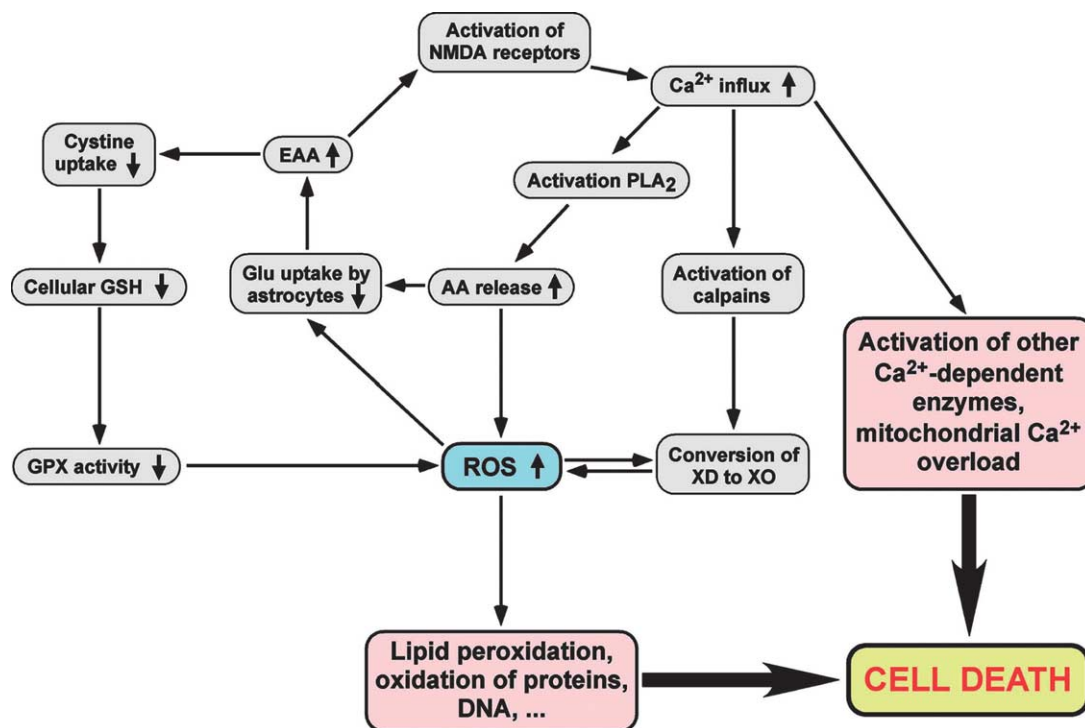
Fig. 3. Relationships between oxidative stress, neuronal activity and cytotoxicity. The release of EAA such as Glu, excessive Ca^{2+} ions influx and ROS production are linked together in various cycles, leading to cell death. If Glu uptake by astrocytes is inhibited by ROS or excessive AA release, this increases the extracellular EAA concentration, which in turn decreases antioxidant defences, increases ROS production, induces the depolarisation of cellular membranes, and finally provokes cell death. Abbreviations: AA, arachidonate; EAA, excitatory amino acids; Glu, glutamate; PLA_2, phospholipase A_2; XD, xanthine dehydrogenase; XO, xanthine oxidase.

(MPP^+), which cannot diffuse through cellular membranes, but is specifically taken up by dopaminergic neurones and produces symptoms very similar to those of PD; once in the cytoplasm, MPP^+ kills the cells by interfering with the mitochondrial respiratory chain by a mechanism that increases superoxide production [82,83]. Thus dopaminergic neurones are submitted to a chronic oxidative stress and can degenerate when ROS and free radicals are not efficiently neutralised by their antioxidant defences.

For the three major neurodegenerative diseases mentioned above, a familial form has been found and could explain a part of the prevalence of the disease, although the relationships between the known genetic mutations and the development of the disease is still not clear. However, for a large part of patients suffering from one of these diseases, an important question concerns the possible involvement of environmental factors. As discussed above, the neurotoxin MPTP is an example of an exogenous substance that induces

similar pathological alterations to those found in idiopathic PD, with similar clinical symptoms.

5.3.5. Parkinsonism–dementia complex

In this context, it is interesting to report a surprising observation made in Marianne Islands, especially Guam and Rota. Investigators found a possible link between an ALS-like disease and a toxic substance produced by cyanobacteria. In these islands, the diet of the indigenous Chamorro people may account for the high incidence of a neurological disease called amyotrophic lateral sclerosis/parkinsonism–dementia complex (ALS–PDC), which has symptoms of ALS, Parkinson's, or Alzheimer's disease [84,85]. The bats forage on seeds from cycad trees, which contain cyanobacteria in their roots that produce the neurotoxin β-methylamino-L-alanine (BMAA). BMMA has a similar structure to that of the excitatory aminoacid glutamate, and is an excitotoxin, causing a persistent excitation of neurons that ultimately exhausts the cells

until they can no longer function. This substance was shown to be concentrated 100 000 times by the food chain from bacteria to flying foxes. Chamorro people who died of ALS–PDC had high levels of BMAA in their brain, while healthy brain tissue does not have the toxin. Thus a neurotoxin that induces excitotoxicity and oxidative stress could be responsible, at least in part, for the very high incidence of a neurodegenerative syndrome observed in a limited region under special circumstances, and similar to that of the three major neurodegenerative diseases found in the world.

ROS, as short-lived and reactive molecules, are good candidates for intracellular signalling [86–88].

5.4. Modulation of signal transduction by ROS

If the role of nitric oxide as a cellular messenger is well known, such a role for the other ROS arose only recently. Although the precise mechanism linking the binding of extracellular ligands to the intracellular generation of ROS remains to be established, the latter have been shown to modulate gene expression in response to extracellular messengers. Thus cellular transcriptional signalling by ROS in response to cytotoxic injury and inflammation is mediated mainly by activation of MAP kinases, the Rho family of small G proteins, the Src family of tyrosine kinases, Ras, and cytokines; the modulation of gene expression is then mediated by the transcription factors AP-1, ATF, and NF-kappaB [88,89]. On the other hand, specific genes are also induced during hypoxia, i.e., under conditions opposite to oxidative stress. Cellular transcriptional response on hypoxia was shown to be mediated by activation of the transcription factors HIF-1 and AP-1 [89].

5.5. Ageing

Two general theories explain intrinsic ageing: genetic theories and theories about the accumulation of cellular damages. According to the latter, the natural metabolic activity produces "metabolic waste" that the organism cannot always eliminate properly. These catabolites are oxidised molecules that can become oxidants for other ones if they are not eliminated. Indeed, many markers of oxidative stress are increased in the tissues of aged individuals [90–93]. With the wire of time, these cellular damages accumulate in the organism and become cytotoxic. This concept, already mentioned by Denham Harman in 1956 as 'free radical theory of ageing' [94] was confirmed by many studies which reported the involvement of oxidative stress in ageing processes [1,13,92,95,96].

6. Are reactive oxygen species always deleterious?

In the previous sections, we have discussed the inevitable formation of ROS and free radicals in normal metabolism of aerobes, the defences of the organism against them, as well as the possible deleterious consequences when the rate of their formation exceeds that of their neutralisation. But do ROS and free radicals always represent an undesirable consequence of oxygen metabolism, or do they play a beneficial role too?

For instance, the reactive oxygen or nitrogen species nitric oxide, besides being an intermediate in the formation of the strong oxidant peroxynitrite, as mentioned above, has several physiological functions: in the nervous system, it acts as a neuromodulator and plays a role in synaptic plasticity and long-term memory, whereas in the vascular system, it controls blood pressure, inhibits platelet aggregation and kills certain pathogenic microorganisms [97,98].

Many physiological reactions are oxidoreduction reactions, i.e., electrons are transferred from one molecule to another, giving rise to free radical intermediates; furthermore, many enzyme-catalysed reactions produce free radicals as intermediates, which allows decreasing the activation energy of the general reaction.

When considering a 'useful role' for ROS, we usually design the killing mechanism of microorganisms by neutrophils and other phagocytic cells. Phagocytosis is a complex process, requiring the activation of neutrophils or macrophages [24,99]. One of its many steps involves the production in the extracellular space of superoxide and hydrogen peroxide (see Section 3). Hydrogen peroxide crosses biological membranes easily, and is toxic to microorganisms either directly by promoting several oxidations, or indirectly by forming a more reactive species such as the hydroxyl radical or hypochlorite. The highly reactive hydroxyl radical is produced from superoxide and/or hydrogen peroxide by Haber–Weiß or Fenton reactions, whereas hypochlorite is produced by myeloperoxidase [1,23, 24,100]. Thus the production of several ROS by activated neutrophils or macrophages is a defence mecha-

nism against invading microorganisms. Since the latter are much more sensitive to ROS than human tissue, there is a 'bactericidal window' for which the ROS concentration is sufficient to kill bacteria without causing damage to host tissue (in normal conditions).

Eicosanoids constitute an important family of bioactive lipids, comprising prostaglandins, thromboxanes and leukotrienes. They are derived from arachidonate (C20:4) under the action of prostaglandin H synthase (cyclooxygenase) or lipoxygenases. Eicosanoids locally exert various biological roles such as the mediation of inflammation, the regulation of blood flow, ion transport across membranes, the modulation of synaptic activity or the induction of sleep [101,102].

An interesting example of the involvement of ROS in signal transduction for a physiological process is the capacitation of spermatozoa by superoxide. ROS have beneficial or detrimental effects on sperm functions depending on the nature and the concentration of the ROS involved, as well as the moment and the location of exposure. Sperm capacitation and the acrosome reaction are complex processes required for ovule fertilisation; they are regulated by signal transduction mechanisms involving G proteins, calcium ions and ROS [103,104]. In particular, low concentrations of superoxide trigger this phenomenon, whereas excessive generation of hydrogen peroxide in semen could be a cause for infertility. Conversely, removal of superoxide by superoxide dismutase prevents sperm hyperactivation and capacitation induced by various biological fluids [103].

Besides the participation of ROS in physiological and defence processes, ROS are used as therapeutic agents too. The best example is probably photodynamic therapy (PDT). It requires a photosensitiser, visible light, and molecular oxygen to selectively kill cells. When localised in the target tissue, the photosensitiser is activated by light to produce oxygen intermediates (e.g., singlet oxygen) that destroy target tissue cells (see Section 3). The easy access of skin to visible light and molecular oxygen has led dermatologists to apply PDT to cutaneous disorders. In particular, PDT was shown to be successful in treating actinic keratoses (precancerous skin lesions), basal cell carcinoma, and Bowen's disease. The most popular photosensitiser is δ-aminolevulinic acid, the endogenous precursor of porphyrins, which does not make patients susceptible to phototoxicity for extended periods. Thus besides serious conditions such as non-melanoma skin cancers and premalignant lesions, PDT is also used for the treatment of acne and as an adjuvant to photorejuvenation procedures [105,106].

7. Discussion

There are so many publications reporting the involvement of ROS and free radicals in biological processes that it is impossible to ignore them. A problem encountered with these molecules is the difficulty to detect them, due to their short half-life. Thus a complex physical instrumentation (and knowledge!) is required to analyse them directly, precluding their use in many laboratories equipped for standard biochemical research. For this reason, indirect analytical methods were developed, but the interpretation of the results, and their relationships to biological phenomena is often difficult. This explains in part the scepticism of many biologists and physicians towards this exciting subject.

In this review I discussed the ineluctable production of ROS and secondary oxidations in any aerobic organism. The nature of the ROS involved is well defined, and superoxide anion plays a pivotal role in the formation of other ROS, since it represents the required intermediate in any reduction of molecular oxygen. Another important point which one should bear in mind, is the biradical nature of molecular oxygen: this explains its moderate reactivity towards most (non-radical) biological molecules, but its high reactivity in the presence of free radicals. The detrimental role of ROS in frequent pathologies such as ischaemia and neurodegenerative disorders has been extensively studied, and the knowledge of the biochemical mechanisms involving ROS should allow developing new strategies to prevent and care these diseases [58]. Due to the unavoidable presence of ROS and free radicals in all aerobic organisms, the latter possess efficient antioxidant systems that prevent an excessive concentration of these molecules in biological tissues in normal conditions. However, various genetic or environmental conditions sometimes lead to an imbalance between the production and the decomposition of these reactive intermediates: this condition is called oxidative stress, and this balance disruption can have deleterious consequences. Conversely, an adequate level of certain ROS can have a physiological role, as for instance the

catalysis of many biochemical reactions, the defence against invading pathogens or the capacitation of spermatozoa.

As a conclusion, the generation of ROS and free radicals is inevitable and useful in any aerobic organism, whereas oxidative stress is an undesirable condition that interferes with normal metabolism. A better understanding of this field, which is at the border of physics and life sciences, by all scientists involved in biomedical research, is desired and should improve our knowledge in biology and medicine.

Acknowledgements

References

[1] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, third ed., Oxford University Press, Oxford, 1999.

[2] H. Sies, Oxidative stress. II. Oxidants and Antioxidants, Academic Press, London, 1991.

[3] S.S. Liu, Generating, partitioning, targeting and functioning of superoxide in mitochondria, Biosci. Rep. 17 (3) (1997) 259–272.

[4] S.C. Sahu, Dual role of organosulfur compounds in foods: a review, J. Environ. Sci. Health Part C Environ. Carcinog. Ecotoxicol. Rev. 20 (1) (2002) 61–76.

[5] M. Chevion, T. Navok, G. Glaser, J. Mager, The chemistry of favism-inducing compounds. The properties of isouramil and divicine and their reaction with glutathione, Eur. J. Biochem. 127 (2) (1982) 405–409.

[6] P. Boffetta, F. Nyberg, Contribution of environmental factors to cancer risk, Br. Med. Bull. 68 (1) (2003) 71–94.

[7] Y.K. Youn, C. Lalonde, R. Demling, Oxidants and the pathophysiology of burn and smoke inhalation injury, Free Radic. Biol. Med. 12 (5) (1992) 409–415.

[8] H.S. Black, Potential involvement of free radical reaction in ultraviolet light mediated cutaneous damage, Photochem. Photobiol. 46 (1987) 213–221.

[9] L.L. Hruza, A.P. Pentland, Mechanisms of UV-induced inflammation, J. Invest. Dermatol. 100 (1) (1993) 3S–4S.

[10] M. Ichihashi, M. Ueda, A. Budiyanto, T. Bito, M. Oka, M. Fukunaga, K. Tsuru, T. Horikawa, UV-induced skin damage, Toxicology 189 (1–2) (2003) 21–39.

[11] S. Tahara, M. Matsuo, T. Kaneko, Age-related changes in oxidative damage to lipids and DNA in rat skin, Mech. Ageing Dev. 122 (4) (2001) 415–426.

[12] M. Kuro-o, Disease model: human aging, Trends Mol. Med. 7 (4) (2001) 179–181.

[13] D. Harman, Aging: overview, Ann. N.Y. Acad. Sci. 928 (2001) 1–21.

[14] R.M. Tyrrell, Ultraviolet radiation and free radical damage to skin, Biochem. Soc. Symp. 61 (1995) 47–53.

[15] J. Taira, K. Mimura, T. Yoneya, A. Hagi, A. Murakami, K. Makino, Hydroxyl radical formation by UV-irradiated epidermal cells, J. Biochem. (Tokyo) 111 (6) (1992) 693–695.

[16] M.M. Mathews-Roth, Erythropoietic protoporphyria: treatment with antioxidants and potential cure with gene therapy, Methods Enzymol. 319 (2000) 479–484.

[17] J.R. Kanofsky, Singlet oxygen production by biological systems, Chem. Biol. Interact. 70 (1–2) (1989) 1–28.

[18] S. Raha, B.H. Robinson, Mitochondria, oxygen free radicals, disease and ageing, Trends Biochem. Sci. 25 (10) (2000) 502–508.

[19] C.C. Winterbourn, Toxicity of iron and hydrogen peroxide: the Fenton reaction, Toxicol. Lett. 82–83 (1995) 969–974.

[20] G.R. Buettner, B.A. Jurkiewicz, Catalytic metals, ascorbate and free radicals: combinations to avoid, Radiat. Res. 145 (1996) 532–541.

[21] B.M. Babior, J.D. Lambeth, W. Nauseef, The neutrophil NADPH oxidase, Arch. Biochem. Biophys. 397 (2) (2002) 342–344.

[22] A.W. Segal, A. Abo, The biochemical basis of the NADPH oxidase of phagocytes, Trends Biochem. Sci. 18 (1993) 43–47.

[23] C.C. Winterbourn, M.C. Vissers, A.J. Kettle, Myeloperoxidase, Curr. Opin. Hematol. 7 (1) (2000) 53–58.

[24] S.J. Klebanoff, Myeloperoxidase, Proc. Assoc. Am. Physicians 111 (5) (1999) 383–389.

[25] T.M. Dawson, V.L. Dawson, Nitric oxide synthase: role as a transmitter/mediator in the brain and endocrine system, Annu. Rev. Med. 47 (1996) 219–227.

[26] G.L. Squadrito, W.A. Pryor, The formation of peroxynitrite in vivo from nitric oxide and superoxide, Chem.-Biol. Interact. 96 (1995) 203–206.

[27] W.A. Pryor, G.L. Squadrito, The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide, Am. J. Physiol. 268 (1995) L699–L722.

[28] S. Toyokuni, Reactive oxygen species-induced molecular damage and its application in pathology, Pathol. Int. 49 (2) (1999) 91–102.

[29] M. Amici, G. Lupidi, M. Angeletti, E. Fioretti, A.M. Eleuteri, Peroxynitrite-induced oxidation and its effects on isolated proteasomal systems, Free Radic. Biol. Med. 34 (8) (2003) 987–996.

[30] R. Kohen, A. Nyska, Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification, Toxicol. Pathol. 30 (6) (2002) 620–650.

[31] O. Blokhina, E. Virolainen, K.V. Fagerstedt, Antioxidants, oxidative damage and oxygen deprivation stress: a review, Ann. Bot. (Lond.) (special issue) 91 (2003) 179–194.

[32] S.R. Pinnell, Cutaneous photodamage, oxidative stress, and topical antioxidant protection, J. Am. Acad. Dermatol. 48 (1) (2003) 1–19, quiz 20–22.

[33] D.P. Steenvoorden, G.M. van Henegouwen, The use of endogenous antioxidants to improve photoprotection, J. Photochem. Photobiol. B 41 (1997) 1–10.

[34] O. Sorg, C. Antille, J.-H. Saurat, Retinoids, other topical vitamins, and antioxidants, in: D.S. Rigel, R.S. Weiss, H.W. Lim, J.S. Dover (Eds.), Photoaging, Marcel Dekker, New York, 2004, pp. 89–115.

[35] J.J. Thiele, C. Schroeter, S.N. Hsieh, M. Podda, L. Packer, The antioxidant network of the stratum corneum, Curr. Probl. Dermatol. 29 (2001) 26–42.

[36] Z.A. Wood, E. Schroder, J. Robin Harris, L.B. Poole, Structure, mechanism and regulation of peroxiredoxins, Trends Biochem. Sci. 28 (1) (2003) 32–40.

[37] G.P. Biewenga, G.R. Haenen, A. Bast, The pharmacology of the antioxidant lipoic acid, Gen. Pharmacol. 29 (3) (1997) 315–331.

[38] G. Chidlow, K.G. Schmidt, J.P. Wood, J. Melena, N.N. Osborne, alpha-lipoic acid protects the retina against ischemia-reperfusion, Neuropharmacology 43 (6) (2002) 1015–1025.

[39] M.A. Lynch, Lipoic acid confers protection against oxidative injury in non-neuronal and neuronal tissue, Nutr. Neurosci. 4 (6) (2001) 419–438.

[40] S. Englard, S. Seifter, The biochemical functions of ascorbic acid, Annu. Rev. Nutr. 6 (1986) 365–406.

[41] A.C. Chan, Partners in defense, vitamin E and vitamin C, Can. J. Physiol. Pharmacol. 71 (9) (1993) 725–731.

[42] G.J. Handelman, The evolving role of carotenoids in human biochemistry, Nutrition 17 (10) (2001) 818–822.

[43] F. Böhm, R. Edge, E.J. Land, D.J. McGarvey, T.G. Truscott, Carotenoids enhance vitamin E antioxidant efficiency, J. Am. Chem. Soc. 119 (1997) 621–622.

[44] T. Iyama, A. Takasuga, M. Azuma, beta-Carotene accumulation in mouse tissues and a protective role against lipid peroxidation, Int. J. Vitam. Nutr. Res. 66 (1996) 301–305.

[45] C.F. Rosen, Topical and systemic photoprotection, Dermatol. Ther. 16 (1) (2003) 8–15.

[46] K. Pehr, R.R. Forsey, Why don't we use vitamin E in dermatology?, Can. Med. Assoc. J. 149 (9) (1993) 1247–1253.

[47] C. Ceconi, A. Boraso, A. Cargnoni, R. Ferrari, Oxidative stress in cardiovascular disease: myth or fact?, Arch. Biochem. Biophys. 420 (2) (2003) 217–221.

[48] M. Van Bilsen, G.J. van der Vusse, W.A. Coumans, M.J. de Groot, P.H. Willemsen, R.S. Reneman, Degradation of adenine nucleotides in ischemic and reperfused rat heart, Am. J. Physiol. 257 (1 Pt 2) (1989) H47–H54.

[49] R.S. Vander Heide, M.L. Hill, K.A. Reimer, R.B. Jennings, Effect of reversible ischemia on the activity of the mitochondrial ATPase: relationship to ischemic preconditioning, J. Mol. Cell. Cardiol. 28 (1) (1996) 103–112.

[50] K. Katsura, E.B. Rodriguez de Turco, J. Folbergrova, N.G. Bazan, B.K. Siesjo, Coupling among energy failure, loss of ion homeostasis, and phospholipase A2 and C activation during ischemia, J. Neurochem. 61 (5) (1993) 1677–1684.

[51] S.L. Budd, Mechanisms of neuronal damage in brain hypoxia/ischemia: focus on the role of mitochondrial calcium accumulation, Pharmacol. Ther. 80 (2) (1998) 203–229.

[52] Y. Cheng, P. Wixom, M.R. James-Kracke, A.Y. Sun, Effects of extracellular ATP on Fe^{2+}-induced cytotoxicity in PC-12 cells, J. Neurochem. 63 (3) (1994) 895–902.

[53] R. Rees, D. Smith, T.D. Li, B. Cashmer, W. Garner, J. Punch, D.J. Smith Jr., The role of xanthine oxidase and xanthine dehydrogenase in skin ischemia, J. Surg. Res. 56 (1994) 162–167.

[54] T. Nishino, S. Nakanishi, K. Okamoto, J. Mizushima, H. Hori, T. Iwasaki, K. Ichimori, H. Nakazawa, Conversion of xanthine dehydrogenase into oxidase and its role in reperfusion injury, Biochem. Soc. Trans. 25 (3) (1997) 783–786.

[55] T. Nishino, The conversion of xanthine dehydrogenase to xanthine oxidase and the role of the enzyme in reperfusion injury, J. Biochem. 116 (1994) 1–6.

[56] H.M. Homi, J.J. Freitas, R. Curi, I.T. Velasco, B.A. Junior, Changes in superoxide dismutase and catalase activities of rat brain regions during early global transient ischemia/reperfusion, Neurosci. Lett. 333 (1) (2002) 37–40.

[57] D. Harrison, K.K. Griendling, U. Landmesser, B. Hornig, H. Drexler, Role of oxidative stress in atherosclerosis, Am. J. Cardiol. 91 (A) (2003) A–1A.

[58] C.A. Hamilton, W.H. Miller, S. Al-Benna, M.J. Brosnan, R.D. Drummond, M.W. McBride, A.F. Dominiczak, Strategies to reduce oxidative stress in cardiovascular disease, Clin. Sci. 106 (3) (2004) 219–234.

[59] N.S. Dhalla, R.M. Temsah, T. Netticadan, Role of oxidative stress in cardiovascular diseases, J. Hypertens. 18 (6) (2000) 655–673.

[60] C. Szabó, Physiological and pathophysiological roles of nitric oxide in the central nervous system, Brain Res. Bull. 41 (1996) 131–141.

[61] S. Porasuphatana, P. Tsai, G.M. Rosen, The generation of free radicals by nitric oxide synthase, Comp. Biochem. Physiol. C Toxicol. Pharmacol. 134 (3) (2003) 281–289.

[62] A.V. Rao, B. Balachandran, Role of oxidative stress and antioxidants in neurodegenerative diseases, Nutr. Neurosci. 5 (5) (2002) 291–309.

[63] J. Emerit, M. Edeas, F. Bricaire, Neurodegenerative diseases and oxidative stress, Biomed. Pharmacother. 58 (1) (2004) 39–46.

[64] A. Volterra, D. Trotti, C. Tromba, S. Floridi, G. Racagni, Glutamate uptake inhibition by oxygen free radicals in rat cortical astrocytes, J. Neurosci. 14 (1994) 2924–2932.

[65] O. Sorg, T.F.W. Horn, N. Yu, D.L. Gruol, F.E. Bloom, Inhibition of astrocyte glutamate uptake by reactive oxygen species: role of antioxidant enzymes, Mol. Med. 3 (1997) 431–440.

[66] J. Raber, O. Sorg, T.F. Horn, N. Yu, G.F. Koob, I.L. Campbell, F.E. Bloom, Inflammatory cytokines: putative regulators of neuronal and neuro-endocrine function, Brain Res. Brain Res. Rev. 26 (2–3) (1998) 320–326.

[67] C. Pompeia, T. Lima, R. Curi, Arachidonic acid cytotoxicity: can arachidonic acid be a physiological mediator of cell death?, Cell Biochem. Funct. 21 (2) (2003) 97–104.

[68] G.J. McBean, J. Flynn, Molecular mechanisms of cystine transport, Biochem. Soc. Trans. 29 (Pt 6) (2001) 717–722.

[69] S.C. Bondy, C.P. LeBel, The relationship between excitotoxicity and oxidative stress in the central nervous system, Free Radic. Biol. Med. 14 (1993) 633–642.

[70] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297 (5580) (2002) 353–356.

[71] W. Gsell, G. Jungkunz, P. Riederer, Functional neurochemistry of Alzheimer's disease, Curr. Pharm. Des. 10 (3) (2004) 265–293.

[72] M.P. Mattson, Oxidative stress, perturbed calcium homeostasis, and immune dysfunction in Alzheimer's disease, J. Neurovirol. 8 (6) (2002) 539–550.

[73] E.P. Simpson, A.A. Yen, S.H. Appel, Oxidative stress: a common denominator in the pathogenesis of amyotrophic lateral sclerosis, Curr. Opin. Rheumatol. 15 (6) (2003) 730–736.

[74] D. Abele, Toxic oxygen: the radical life-giver, Nature 420 (6911) (2002) 27.

[75] M. Strong, J. Rosenfeld, Amyotrophic lateral sclerosis: a review of current concepts, Amyotroph. Lateral Scler. Other Motor Neuron Disord. 4 (3) (2003) 136–143.

[76] J.S. Valentine, P.J. Hart, E.B. Gralla, Copper-zinc superoxide dismutase and ALS, Adv. Exp. Med. Biol. 448 (1999) 193–203.

[77] R.H. Brown Jr., Superoxide dismutase in familial amyotrophic lateral sclerosis: Models for gain of function, Curr. Opin. Neurobiol. 5 (1995) 841–846.

[78] A. Radunovic, P.N. Leigh, Cu/Zn superoxide dismutase gene mutations in amyotrophic lateral sclerosis: correlation between genotype and clinical features, J. Neurol. Neurosurg. Psychiatr. 61 (1996) 565–572.

[79] C.W. Olanow, W.G. Tatton, Etiology and pathogenesis of Parkinson's disease, Annu. Rev. Neurosci. 22 (1999) 123–144.

[80] L. Galzigna, A. De Iuliis, L. Zanatta, Enzymatic dopamine peroxidation in substantia nigra of human brain, Clin. Chim. Acta 300 (1–2) (2000) 131–138.

[81] J. Haavik, B. Almås, T. Flatmark, Generation of reactive oxygen species by tyrosine hydroxylase: a possible contribution to the degeneration of dopaminergic neurons?, J. Neurochem. 68 (1997) 328–332.

[82] T.P. Singer, A.J. Trevor, N. Castagnoli Jr., Biochemistry of the neurotoxic action of MPTP: or how a faulty batch of 'designer drug' led to parkinsonism in drug abusers, Trends Biochem. Sci. 12 (1987) 266–270.

[83] S.G. Speciale, MPTP: insights into parkinsonian neurodegeneration, Neurotoxicol. Teratol. 24 (5) (2002) 607–620.

[84] P.A. Cox, S.A. Banack, S.J. Murch, Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam, Proc. Natl Acad. Sci. USA 100 (23) (2003) 13380–13383.

[85] H.R. Morris, S. Al-Sarraj, C. Schwab, K. Gwinn-Hardy, J. Perez-Tur, N.W. Wood, J. Hardy, A.J. Lees, P.L. McGeer, S.E. Daniel, J.C. Steele, A clinical and pathological study of motor neurone disease on Guam, Brain 124 (Pt 11) (2001) 2215–2222.

[86] K. Schulze-Osthoff, M.K. Bauer, M. Vogt, S. Wesselborg, Oxidative stress and signal transduction, Int. J. Vitam. Nutr. Res. 67 (5) (1997) 336–342.

[87] K. Chen, S.R. Thomas, J.F. Keaney Jr., Beyond LDL oxidation: ROS in vascular signal transduction, Free Radic. Biol. Med. 35 (2) (2003) 117–132.

[88] H. Takano, Y. Zou, H. Hasegawa, H. Akazawa, T. Nagai, I. Komuro, Oxidative stress-induced signal transduction pathways in cardiac myocytes: involvement of ROS in heart diseases, Antioxid. Redox Signal 5 (6) (2003) 789–794.

[89] K.T. Turpaev, Reactive oxygen species and regulation of gene expression, Biochemistry (Mosc) 67 (3) (2002) 281–292.

[90] H.K. Biesalski, M. Berneburg, T. Grune, M. Kerscher, J. Krutmann, B. Raab, J. Reimann, T. Reuther, L. Robert, T. Schwarz, Oxidative and premature skin ageing, Exp. Dermatol. (Suppl.) 12 (3) (2003) 3–15.

[91] C. Nishigori, Y. Hattori, Y. Arima, Y. Miyachi, Photoaging and oxidative stress, Exp. Dermatol. 12 (S2) (2003) 18–21.

[92] R.S. Sohal, R. Weindruch, Oxidative stress, caloric restriction, and aging, Science 273 (5271) (1996) 59–63.

[93] T. Finkel, N.J. Holbrook, Oxidants, oxidative stress and the biology of ageing, Nature 408 (2000) 239–247.

[94] D. Harman, Aging: a theory based on free radical and radiation chemistry, J. Gerontol. 11 (1956) 298–300.

[95] A.S. Polla, L.L. Polla, B.S. Polla, Iron as the malignant spirit in successful ageing, Ageing Res. Rev. 2 (1) (2003) 25–37.

[96] H.K. Biesalski, Free radical theory of aging, Curr. Opin. Clin. Nutr. Metab. Care 5 (1) (2002) 5–10.

[97] F.C. Fang, Perspectives series: host/pathogen interactions. Mechanisms of nitric oxide-related antimicrobial activity, J. Clin. Invest. 99 (12) (1997) 2818–2825.

[98] J.P. Eiserich, S. Baldus, M.L. Brennan, W. Ma, C. Zhang, A. Tousson, L. Castro, A.J. Lusis, W.M. Nauseef, C.R. White, B.A. Freeman, Myeloperoxidase, a leukocyte-derived vascular NO oxidase, Science 296 (5577) (2002) 2391–2394.

[99] W.L. Lee, R.E. Harrison, S. Grinstein, Phagocytosis by neutrophils, Microbes Infect. 5 (14) (2003) 1299–1306.

[100] S.L. Hazen, F.F. Hsu, K. Duffin, J.W. Heinecke, Molecular chlorine generated by the myeloperoxidase hydrogen peroxide chloride system of phagocytes converts low-density lipoprotein cholesterol into a family of chlorinated sterols, J. Biol. Chem. 271 (1996) 23080–23088.

[101] C.D. Funk, Prostaglandins and leukotrienes: advances in eicosanoid biology, Science 294 (2001) 1871–1875.

[102] J. Lieb, Eicosanoids: the molecules of evolution, Med. Hypotheses 56 (6) (2001) 686–693.

[103] E. de Lamirande, C. Gagnon, Impact of reactive oxygen species on spermatozoa: a balancing act between beneficial and detrimental effects, Hum. Reprod. (Suppl.) 10 (1) (1995) 15–21.

[104] C. O'Flaherty, N. Beorlegui, M.T. Beconi, Participation of superoxide anion in the capacitation of cryopreserved bovine sperm, Int. J. Androl. 26 (2) (2003) 109–114.

[105] A.F. Taub, Photodynamic therapy in dermatology: history and horizons, J. Drugs Dermatol. (Suppl.) 3 (1) (2004) S8–S25.

[106] R. Bissonette, A. Bergeron, Y. Liu, Large-surface photodynamic therapy with aminolevulinic acid: treatment of actinic keratoses and beyond, J. Drugs Dermatol. (Suppl.) 3 (1) (2004) S26–S31.