

OXIDATIVE INJURY AND INFLAMMATORY PERIODONTAL DISEASES: THE CHALLENGE OF ANTI-OXIDANTS TO FREE RADICALS AND REACTIVE OXYGEN SPECIES

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ABSTRACT. In recent years, there has been a tremendous expansion in medical and dental research concerned with free radicals, reactive oxygen species, and anti-oxidant defense mechanisms. This review is intended to provide a critical, up-to-date summary of the field, with particular emphasis on its implications for the application of "anti-oxidant therapy" in periodontal disease. We have reviewed the nomenclature, mechanisms of actions, features, and sources of most common free radicals and reactive oxygen species, as well as analyzed the typical biological targets for oxidative damage. Based on a review of direct and indirect anti-oxidant host defenses, particularly in relation to the key role of polymorphonuclear neutrophils in periodontitis, we review current evidence for oxidative damage in chronic inflammatory periodontal disease, and the possible therapeutic effects of anti-oxidants in treating and/or preventing such pathology, with special attention to vitamin E and Co-enzyme Q.

Key words: reactive oxygen species/free radicals, anti-oxidants, polymorphonuclear neutrophils, periodontal disease.

Introduction

Oxygen is required for all mammalian energy needs. Oxygen is used to oxidize molecules rich in carbon and hydrogen (*i.e.*, nutrients) to produce the different forms of energy needed for life. The reduction of molecular oxygen to water is accompanied by a large free energy release that can give rise to Free Radicals (FR) and/or Reactive Oxygen Species (ROS). The most important FR in biological systems are radical derivatives of oxygen. Other highly reactive compounds are known as ROS. ROS include not only oxygen FR but also non-radical oxygen derivatives involved in oxygen radical production (Table 1). The reactivity and associated toxicity of these may be major contributors to the pathogenesis of several chronic degenerative diseases (Halliwell, 1994; Sies, 1997).

Inflammation represents the response of the organism to a noxious stimulus, whether mechanical, chemical, or infectious. It is a localized protective response elicited by injury or destruction of tissues, which serves to destroy, dilute, or wall off both the injurious agent and the injured tissue. Whether acute or chronic, inflammation is dependent upon regulated humoral and cellular responses, and the molecules considered to mediate inflammation at one time or another are legion. However, an event characteristic of mammalian inflammation, tissue infiltration by polymorphonuclear leukocytes and monocytes and subsequent phagocytosis, features a burst of cyanide-insensitive (*i.e.*, non-mitochondrial) O₂ consumption, which may be 10 or 20 times that of resting consumption.

Oxygen uptake in neutrophils and macrophages is due to the action of a plasma-membrane-bound flavoprotein cytochrome b₂₄₅ NADPH oxidase system that increases NADPH production *via* the hexose monophosphate shunt and generates superoxide anion radicals, hydrogen peroxide, hydroxyl radicals, and hypochlorous acid, all capable of damaging either cell membranes or associated biomolecules.

Common periodontal disease (PD) is considered an inflammatory disease, but many aspects of its pathogenesis remain unknown. At present, periodontitis (PE), its destructive phase, is considered to be initiated and perpetuated by a small group of predominantly Gram-negative, anaerobic, or micro-aerophilic bacteria that colonize the subgingival area. Bacteria cause the observed tissue destruction directly by toxic products and indirectly by activating host defense systems, *i.e.*, inflammation (Page and Kornman, 1997). A variety of molecular species appears in the inflamed tissues, among them FR and ROS. The aim of this review is to direct attention to the specific field of FR, ROS, and overall anti-oxidant defense mechanisms, particularly in relation to chronic periodontitis.

FR and ROS: Nomenclature, Features, and Sources

FR DEFINITION AND FORMATION

A FR may be defined as an atomic or molecular species with one or more unpaired electrons in its structure. FR can be positively (NAD^{•+}) or negatively charged (O₂^{•-}) or

TABLE 1

Reactive Oxygen Species

Radicals		Non-radicals	
Superoxide	O ₂ ^{•-}	Singlet Oxygen	¹ O ₂
Hydroxyl	OH [•]	Ozone	O ₃
Hydroperoxyl	HOO [•]	Hypochlorous acid	HOCl
Alkoxyl	RO [•]	Hydrogen peroxide	H ₂ O ₂
Aryloxyl	ArO [•]		
Arylperoxyl	ArOO [•]		
Peroxyl	ROO [•]		
Acyloxyl	RCOO [•]		
Acylperoxyl	RCOOO [•]		

electrically neutral (OH[•]). There are three possible means of FR formation:

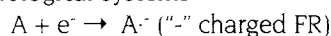
(A) by the homolytic cleavage of the covalent bond of a normal molecule, with each fragment retaining one of the paired electrons (*i.e.*, homolytic fission), requiring a high energy input



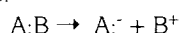
(B) by the loss of a single electron from a normal molecule

$$A + B: \rightarrow A^{\cdot-} + B^{\cdot+} \text{ ("-" and/or "+" charged FR)}$$

(C) by the addition of a single electron to a normal molecule, otherwise called "electron transfer", quite common in biological systems



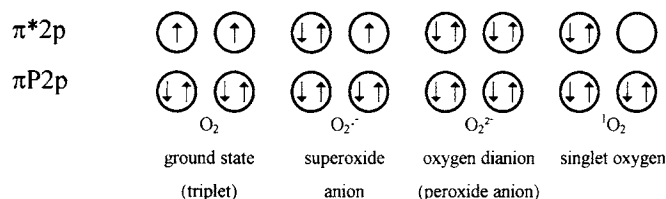
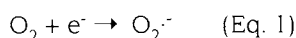
It should be noted that heterolytic fission does not produce FR but only ions, because the electrons of the covalent bond are retained by only one of the fragments of the parent molecule:



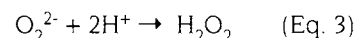
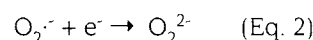
ROS DEFINITION AND FORMATION

Conventionally, molecular oxygen (O₂) is said to be in a triplet ground state (Fig. 1), containing an even number of electrons, two of them unpaired in its molecular orbitals. They have the same spin quantum number, *i.e.*, they display parallel spins, and when O₂ oxidizes another atom (or molecule) by receiving a couple of electrons from it, they both should be of parallel spin to fit into the available spaces of the orbitals. Each of the finally complete orbitals will have a pair of antiparallel spin electrons.

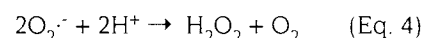
Molecular oxygen usually undergoes one-electron reduction at a time. When oxygen is reduced by a single electron, the species formed is superoxide anion (the "one-electron reduction state"), *i.e.*, a FR:

Figure 1. Electron configuration of O₂ antibonding orbitals.

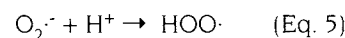
A "two-electron reduction" of oxygen would produce hydrogen peroxide, *i.e.*, a ROS. The electron deficiency of the superoxide radical may be made up by the addition of a second electron to give the peroxy anion; afterward, peroxy anion interacts with two hydrogen ions to form hydrogen peroxide:



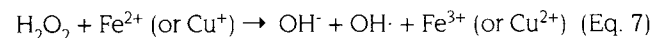
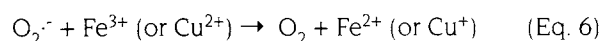
Alternatively, hydrogen peroxide may also be generated in biological systems via the superoxide generating systems (a so-called "dismutation reaction", in which the FR reactants give non-FR products):



Moreover, superoxide can be protonated to form the hydroperoxyl radical:

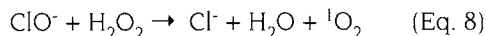


Hydrogen peroxide plays a key role in free radical biochemistry because, in the presence of transition metal ions, it can easily break down to produce the hydroxyl radical (OH[•]), one of the most reactive and damaging FR species. The further reduction of hydrogen peroxide to water involves the addition of two further electrons, producing the harmful hydroxyl radical (the "three-electron reduction state"). This can occur *in vitro* by the so-called superoxide-driven Fenton reaction, or the metal(iron/copper)-catalyzed Haber-Weiss reaction. In this reaction, electrons are supplied by the oxidation of ferrous ions, Fe²⁺ (or cuprous ions, Cu⁺) to ferric ions, Fe³⁺ (or cupric ions, Cu²⁺), respectively (Halliwell and Gutteridge, 1990):



The "movement" of one of the unpaired electrons in a manner that alleviates the spin restriction yields singlet molecular oxygen or ¹O₂ (another ROS) generated in chemical as well as in biological systems, including medical conditions (*e.g.*, the Porphyrrias that are typical diseases in which excessive singlet oxygen is present; on the other hand, singlet oxygen is used in medicine in photo-

dynamic therapy, *e.g.*, to cure Herpes-Simplex-induced sores and psoriasis or to treat the jaundice of newborn babies) (Halliwell and Gutteridge, 1990):



$\text{O}_2^{\cdot-}$ is widely produced by phagocytes during the so-called "respiratory burst", but mitochondria also contribute to its production. Two percent of the O_2 consumed by mitochondria is partially reduced by electrons (Cadenas, 1989) which "leak" from electron carriers in the respiratory chain of healthy, intact mitochondria. Different distinct sites of leakage have been indicated: ubisemiquinone, cyt. b_{566} , and NADH dehydrogenase (Nohl and Jordan, 1986). Mitochondrial uncoupling magnifies this phenomenon. More sophisticated appears to be the xanthine oxidase (XO)/xanthine dehydrogenase (XDH) system. During, or as a consequence of, ischemia, XDH is transformed into XO. XO operates the univalent oxidation of xanthine to uric acid, with concomitant production of $\text{O}_2^{\cdot-}$. Arachidonic acid metabolism via prostaglandin (PGH) synthase and lipoxygenase also yields $\text{O}_2^{\cdot-}$, and it occurs in the auto-oxidation of catecholamines (Halliwell and Gutteridge, 1985). More potential FR/ROS sources derive from the metabolism of different xenobiotics (*e.g.*, adriamycin) that, through oxidoreduction, also generate radicals.

DEFINITION OF OTHER FR/ROS

Oxygen FR are not uniquely important, although they are often the initial species formed. Many other FR/ROS exist. The two nitrogen oxides, NO and NO_2 , are stable FR, nitric dioxide being more reactive than nitric oxide, and can react with heme compounds. NO, relatively unstable in aerobic conditions, is generated in an NADPH-dependent process by NO synthase, which exists as different subfamilies. NO shows important cytotoxic properties and is also a potent vasodilator, originally recognized as endothelium-derived relaxing factor (Ignarro and Murad, 1995). Sulfur atoms can be the center for FR (thiyl radicals, RS^{\cdot}). Thiols also react with NO and NO_2 . Hetero-atom-centered radicals are quite commonly encountered (Pryor, 1986): Hydrogen atom abstraction from thiols is very facile, so that RS^{\cdot} are easily formed by methionine, cysteine, glutathione, etc. Other FR of importance are carbon-centered radicals (R^{\cdot}) that result from the attack of an oxidizing radical (OH^{\cdot}) on compounds such as amino acids, carbohydrates, fatty acids, or DNA bases. R^{\cdot} reaction with oxygen is very rapid, to form the corresponding peroxy radical (alkylperoxy radical) (ROO^{\cdot}) (von Sonntag, 1987). In turn, ROO^{\cdot} can generate the highly unstable alkoxy radicals (RO^{\cdot} or HRO^{\cdot}). Aromatic oxy radicals (ArO^{\cdot}) are a more general form of phenolic radical derivative of the primary phenoxyl radical ($\text{C}_6\text{H}_5\text{O}^{\cdot}$). ArO^{\cdot} arise from heterocyclic physiological anti-oxidants (serotonin, 5-OH-Trp, etc.) and several aromatic hydroxy derivatives (8-hydrox-

yguanosine, uric acid); they decay rapidly and are unreactive toward oxygen (Simic, 1990). Another non-radical but highly reactive compound is hypochlorous acid (HOCl), produced during phagocytosis or as a result of myeloperoxidase activity on H_2O_2 (Winrow *et al.*, 1993). HOCl is a powerful oxidizer of -SH groups on cell surfaces and can inhibit membrane transport systems as well as leading to chlorination of tyrosine residues (Kettle, 1996). Two more radicals involved in biological processes are the semiquinone radical (HQ^{\cdot}) and the semiquinone radical anion ($\text{Q}^{\cdot-}$) (Pryor, 1986). Finally, free or complexed protein-bound transition metals and protein-bound flavin nucleotides are major elements of the enzyme active site involved, and for this reason hemoproteins (*e.g.*, myoglobin) may take an active part in oxygen-dependent injury (McCord, 1993; Roberfroid and Buc Calderon, 1995).

Relevant Biological Targets for FR and ROS

Because of their high reactivity, several FR and ROS can rapidly modify either small, free biomolecules (*i.e.*, vitamins, amino acids, carbohydrates, lipids) or macromolecules (*i.e.*, proteins, nucleic acids) or even supramolecular structure (*i.e.*, cell membranes, circulating lipoproteins). The type and the extent of damage depend upon the site of generation. For instance, the production of a harmful species, such as OH^{\cdot} , could lead to severe purine or pyrimidine modifications or to strand breakage if it takes place close to DNA, whereas it might have no relevant biological consequences if it occurs close to a generic enzyme molecule whose concentration is not critical in the cell; even if such hypothetical enzyme molecule is inactivated or damaged, there will be several more efficient molecules of the same enzyme (Halliwell and Gutteridge, 1990; Battino *et al.*, 1997a).

There are five principal targets for FR and ROS in living cells: (1) small organic biomolecules, (2) proteins, (3) nucleic acids, (4) gene activation, and (5) unsaturated fatty acids.

(1) SMALL ORGANIC BIOMOLECULES

Such compounds include the following: vitamins (ascorbic acid, carotenoids, α -tocopherol, quinones), carbohydrates (glucose, ribose), amino acids (histidine, tryptophane, cysteine, methionine), uric acid, cholesterol, and small soluble peptides like glutathione. The reaction of FR and ROS with vitamins A, C, or E, quinones, glutathione, and uric acid usually terminates the radical reaction chain.

Carbohydrates are prone to oxidative degradation, and the formation of deoxyribose with a C-4-centered radical causing strand breaks has been demonstrated (Sies, 1986). Sugar radicals can be formed by OH^{\cdot} by hydrogen abstraction at any of the carbon atoms. Further reaction of the sugar radicals formed can lead to DNA strand break-

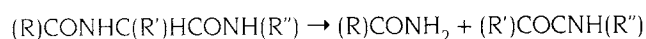
age followed by release of the intact base and of the altered sugar (Diplock, 1994). D-ribose, D-deoxyribose, and D-glucose react with $\text{OH}\cdot$ with known rate constants (in the range of 1 to $3 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$) (Roberfroid and Buc Calderon, 1995). Mucopolysaccharides such as hyaluronic acid can be depolymerized by FR/ROS (Sies, 1997).

Several amino acids undergo direct oxidative modification, which may affect their physiological role, by interaction with FR and ROS. The rate constants regulating the interaction of $\text{OH}\cdot$ with cysteine or methionine (10^9 - $10^{10} \text{ M}^{-1} \cdot \text{s}^{-1}$) are the highest among natural amino acids (Roberfroid and Buc Calderon, 1995). Modifications can be reversible (*e.g.*, the oxidation-reduction of thiol groups) as well as irreversible (*e.g.*, the ring cleavage in histidine) (Sies, 1986; Roberfroid and Buc Calderon, 1995).

Cholesterol oxidation is of special biological interest, yielding cholesterol hydroperoxide and a family of oxysterols oxidized in the sterol B-ring. Some of these cholesterol-derived molecules are present at high concentrations—for instance, in human breast fluid—and may act as mutagens (Sevanian and Peterson, 1984, 1986). Oxidized cholesterol derivatives are also implicated in human atherosclerosis and cardiovascular disease (Sevanian *et al.*, 1995).

(2) PROTEINS

Modification of enzyme structure leading to impaired function and modification of a protein with a key role in cell architecture are the two main sequels of FR/ROS action. The proneness to oxidation of an amino acid residue is different when it is inside a protein chain and is dependent on the protein structure and composition. The sensitivity of a protein to FR/ROS depends on its amino acid composition and on the accessibility of such species to its more susceptible amino acids (Berlett and Stadtman, 1997). The presence, inside the protein domain, of complexed metal ions able to catalyze the decomposition of H_2O_2 usually favors the initiation of a radical reaction chain, causing a site-specific effect (Stadtman, 1992). The result is the conversion of some amino acid residues to carbonyl derivatives related to protein damage. FR/ROS promote considerable protein-protein cross-linking through $\text{OH}\cdot$ -facilitated -S-S- and -Tyr-Tyr- bonding and fragmentation of the polypeptide chain:



Moreover, proteins may also be the target of attack by secondary radicals such as those derived from lipid peroxidation (see below) and/or by degradation products. The latter is the case of malondialdehyde (MDA) or 4-hydroxynonenal (HNE), that may form stable cross-linked products with specific amino acids. The final molecular and biochemical consequences of the interaction of FR/ROS and proteins stem from a chain of events. The next steps

include changes in conformation, enzymatic activity or binding as well as receptor inactivation, increased susceptibility to proteases, and changes in immunogenicity (Roberfroid and Buc Calderon, 1995).

(3) NUCLEIC ACIDS

Both polyribonucleotides and polydesoxyribonucleotides (RNA and DNA) are highly susceptible targets for FR/ROS. It has been claimed (Park *et al.*, 1992), from detection of the *in vivo* production of modified pyrimidine and purine bases presumably proceeding from DNA excision and repair, that FR/ROS are responsible for about 10,000 DNA base modifications *per cell per day*. It is clear, therefore, despite the most efficient and sophisticated cellular mechanisms involved, that a finite fraction of such a massive amount of damage would escape cellular repair, indicating that the potential for damage by FR/ROS that may lead to mutagenesis and carcinogenesis is of major significance (Beckman and Ames, 1997). Primary molecular sites for FR/ROS attack (mainly $\text{OH}\cdot$) are the heterocyclic purinic and pyrimidinic bases or the ribosyl and deoxyribosyl moieties. The effects could be alterations of the (deoxy)ribosyl moiety, of the heterocyclic bases, and even repairable single- and/or double-stranded breaks as well as more drastic and severe modifications, including the addition of chemical groups, opening of the ring(s), molecular rearrangements giving rise to single base changes (G/C to A/T), or, even worse, base deletions, producing abasic sites which, by β elimination, may also cause strand breaking. In addition, the formation of C-centered radicals allows cross-links to occur between bases and amino acid residues in nuclear proteins (*e.g.*, cytidine-tyrosine, thymine-tyrosine) (Henle and Linn, 1997). Reactions at the sugar residue moiety lead to rupture of the phosphoribose backbone, a DNA strand breakage followed by release of both the base and the modified sugar. DNA strand breaks also lead to chromosome damage manifested by breaks and can be assayed by chromosome and chromatid aberrations (Sies, 1986). Finally, as for proteins, nucleic acids can also be affected by interaction with secondary harmful species. For example, the hydroperoxide of linoleic acid (13-hydroperoxylinoleic acid) was found to cause guanine-site-specific double-stranded DNA breakage (Sies, 1986).

(4) GENE ACTIVATION (OF TRANSCRIPTION FACTORS)

FR/ROS have now been recognized to be capable of activating transcription. For instance, ROS rapidly induce *c-fos*, *c-myc*, and *c-jun*, which encode transcription factors participating in the modulation of cell growth, differentiation, and development (Winrow *et al.*, 1993) and can activate apoptosis, a "programmed" form of cell death (Jacobson, 1996). FR/ROS have been implicated in the

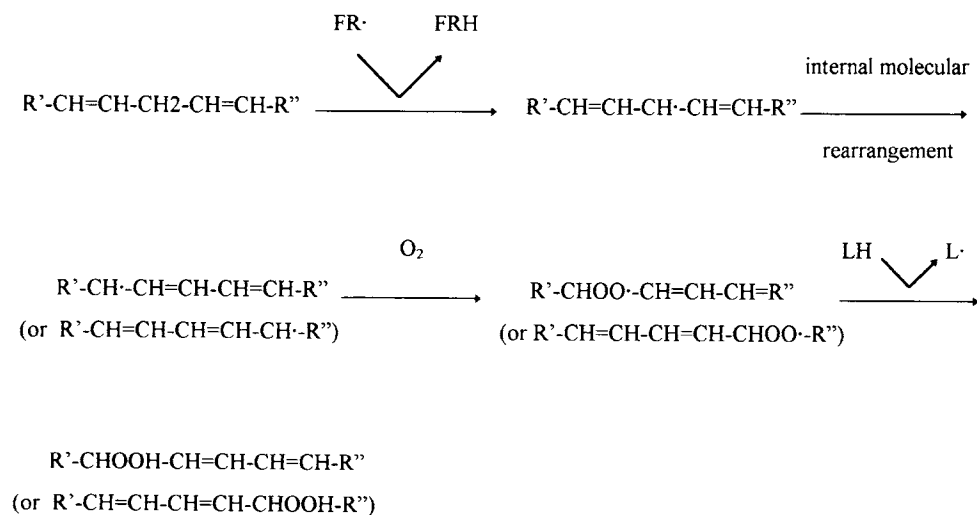


Figure 2. PUFA peroxidation induced by FR.

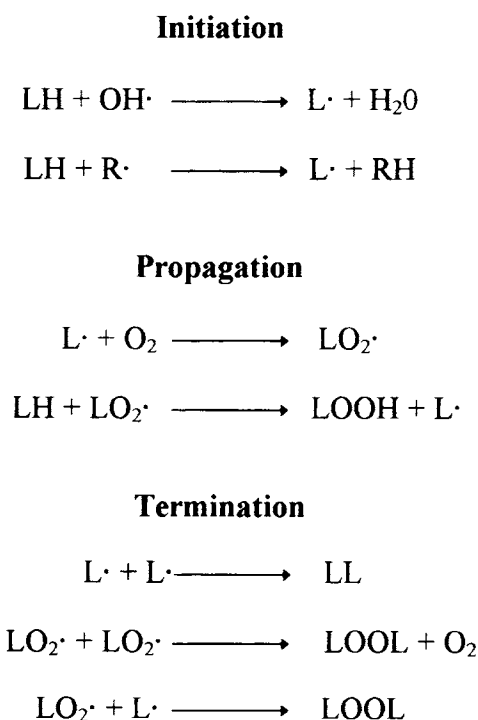


Figure 3. Scheme of initiation, propagation, and termination for a FR chain-reaction.

regulation of mammalian transcription factors such as nuclear factor (NF)- κ B and activator protein-1 (AP-1) (Schreck *et al.*, 1992) and of so-called "heat shock" (or stress protein) transcription factors (HSTF) (Morimoto, 1993). The role played by FR/ROS, in these cases, might be the regulation of genes encoding proteins with potential pro-inflammatory action or, alternatively, proteins potentially acting in a protective fashion. NF- κ B affects

several genes intrinsically linked to the overall inflammatory phenomenon, such as those encoding interleukins (-1, -6, -8), MHC class I antigens, and TNF- α . NF- κ B (a protein heterodimer) exists in an inactive, cytosolic form bound to its inhibitor, I κ B, in non-stimulated cells. Its activators induce the dissociation of I κ B from the NF- κ B-I κ B complex, followed by NF- κ B translocation to the nucleus, which results in gene expression control (Winyard and Blake, 1997). H_2O_2 seems to be an inducer of NF- κ B activity, whereas several anti-oxidants appear to inhibit the activation of NF- κ B. I κ B dis-

sociation from NF- κ B involves its phosphorylation-controlled proteolytic degradation, and the role of ROS could be to control this I κ B phosphorylation, thus clarifying why NF- κ B is blocked by a host of anti-oxidants (Winyard and Blake, 1997). On the other hand, mRNA levels for *c-jun* and *c-fos* are also strongly induced in response to H_2O_2 in this way, increasing AP-1, which is a protein dimer composed of the proto-oncogene products Fos and Jun. Anti-oxidants activate AP-1, suggesting that the AP-1 DNA binding site is an anti-oxidant-responsive element (Meyer *et al.*, 1993).

(5) UNSATURATED FATTY ACIDS

Polyunsaturated fatty acids (PUFAs) exist as free acids (*e.g.*, arachidonic acid), as thioesters (acylSCoA), and as esters (*e.g.*, triglycerides). Each PUFA's double bond is separated from the other by an allylic methylene (CH_2) group. Such a chemical structure makes PUFAs extremely sensitive targets for FR. In fact, a FR with sufficient energy easily abstracts a hydrogen atom from an allylic methylene carbon of a PUFA (LH), thus initiating a chain reaction in bulk lipid. The result is a carbon-centered radical on the lipid itself ($\text{L}\cdot$) which is accompanied by bond rearrangement that results in stabilization by diene conjugate formation (Fig. 1). $\text{L}\cdot$ reacts rapidly ($k > 10^9 \text{ M}^{-1}\text{s}^{-1}$) with O_2 to form a lipid peroxy radical ($\text{LO}_2\cdot$). Such a radical can, in its turn, abstract an H-atom from another PUFA, leaving a carbon-centered radical and a lipid hydroperoxide (LOOH) (Fig. 2) (Diplock, 1994).

The FR chain reaction consists of three essential steps—initiation, propagation, and termination (Fig. 3)—and it propagates until two FR combine with each other to terminate the chain. Hence, a single initiation event can result in the conversion of several PUFA side-chains into lipid hydroperoxides. Thus, an initial event triggering lipid

TABLE 2**Classification of Anti-oxidant Systems Based on Their Mode of Action (Modified from Niki, 1996)**

Type of Defense System	Mode of Action	Examples
Preventive anti-oxidants	Suppress the formation of FR: (a) non-radical decomposition of LOOH and H ₂ O ₂ (b) sequestration of metal by chelation (c) quenching of active O ₂	<ul style="list-style-type: none"> • Catalase, glutathione peroxidase, and -S-transferase • Transferrin, ceruloplasmin, albumin, haptoglobin • superoxide dismutase, carotenoids
Radical-scavenging anti-oxidants	Scavenge radicals to inhibit chain initiation and break chain propagation	<ul style="list-style-type: none"> • Lipophilic: ubiquinol, vit. A, vit. E, carotenoids • Hydrophilic: uric acid, ascorbic acid, albumin, bilirubin
Repair and <i>de novo</i> enzymes	Repair the damage and reconstitute membranes	DNA repair enzymes, protease, transferase, lipase

peroxidation can be amplified as long as O₂ and unoxidized PUFA chains are available (Rice-Evans and Burdon, 1993). In addition, the length and rate constant of a lipid peroxidation chain reaction strongly depend on the degree of lipid unsaturation. In fact, the observed rate constant *per* FR initiation for a series of unsaturated fatty acids ranging from one to six double bonds increases by the order of 0.025:1:2:4:6:8, respectively (Sevanian and Hochstein, 1985).

Lipid peroxidation may give rise to several products (most of them biologically active and cytotoxic) which may be divided into three main categories (Esterbauer, 1993):

- chain-cleavage products;
- products formed by re-arrangement of LOOH or re-arrangement and consecutive oxidation; and
- higher-molecular-weight oxidation products, resulting from di- and polymerization reactions.

Within category (i) we can find substances that result from splitting off the two C-C bonds adjacent to the hydroperoxy group, yielding three main classes of molecules:

- Alkanals, typified by MDA: These are highly reactive compounds that, through reaction with protein thiols and/or cross-linking of amino groups of proteins, can cause considerable intracellular damage.
- Alkenals, typified by HNE. Unlike the radicals, the carbonyl-containing breakdown products are rather long-lived. Alkenals, together with the previously mentioned alkanals, may therefore diffuse from the site of their origin within the membrane or lipoprotein environment affecting their structural and functional integrity, their fluidity, and their permeability (Esterbauer *et al.*, 1991).
- Alkanes, typified by pentane and ethane: These are the end-products of PUFA oxidation.

Anti-oxidants: What They are and How They Act

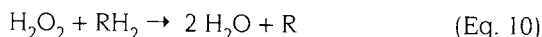
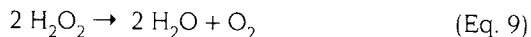
Halliwell (1997) suggested that "an anti-oxidant is any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate." Organism welfare depends on the activity of efficient defense systems against oxidative damage induced by FR/ROS. Different classifications of anti-oxidant defenses have been proposed. A functional classification of anti-oxidant systems based on the way they act (Niki, 1996) appears to be the more useful. On this basis, anti-oxidant defense systems *in vivo* are mainly of three kinds: preventive anti-oxidants, radical scavengers, and, finally, repair and *de novo* enzymes (Table 2).

SUPEROXIDE DISMUTASES (SOD)

Different types of SOD exist: All catalyze the reaction of Eq. 4, accelerating it up to 10,000 times. The different types of SOD are differently distributed among organisms as well as among subcellular compartments, and they contain metals (copper, zinc, manganese, or iron) essential for their catalytic function. Since SOD speed H₂O₂ production, they have to work in conjunction with enzymes that destroy H₂O₂, because the accumulation of such a species that yields OH· (Eq. 7) is even more dangerous than O₂·⁻ (Gutteridge and Halliwell, 1994).

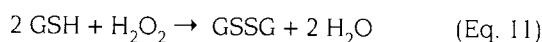
CATALASE

Catalase contains heme-bound iron and is mainly located in peroxisomes; it removes H₂O₂ with great efficacy, its turnover being very high in contrast to its low affinity for H₂O₂ (Gutteridge and Halliwell, 1994). Catalase dismutates the latter to form H₂O and O₂ (Eq. 9) or uses it as an oxidant when working as a peroxidase (Eq. 10) (Roberfroid and Buc Calderon, 1995):

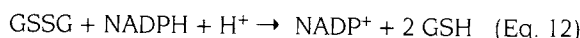


GLUTATHIONE, GLUTATHIONE PEROXIDASE, GLUTATHIONE REDUCTASE, GLUTATHIONE TRANSFERASE

Reduced glutathione (GSH) is a soluble γ -Glu-Cys-Gly tripeptide with a free thiol (-SH) that plays a dual role: It reacts directly with FR, but it also is alternatively a substrate or a co-factor of a transferase (GSH-tr), a peroxidase (GSH-Px), or a reductase (GSH-red). Oxidized glutathione (GSSG) is made by joining two GSH molecules by their -SH groups, losing the two hydrogens, and forming a disulfide bridge. The reaction is catalyzed by a GSH-Px that detoxifies H_2O_2 very effectively (Gutteridge and Halliwell, 1994; Roberfroid and Buc Calderon, 1995):



For its activity, GSH-Px selenium, a chemical element that has some resemblance to sulfur in its properties (Gutteridge and Halliwell, 1994). The efficiency of the latter reaction depends on the availability of intracellular GSH and on the concomitant ability of the cell to reduce GSSG via the NADPH-dependent GSH-red activity:



Moreover, in mammalian cells through a detoxification system, additional to the cytochrome P450, many drugs and/or toxins can be combined with GSH (by GSH-tr) to yield safer products (Gutteridge and Halliwell, 1994).

THIOLS

Thiol groups act as intracellular anti-oxidants by scavenging free radicals and through enzymatic reactions. GSH is the most important cellular thiol, but cysteine (Chapple, 1997), α -lipoic acid, and dihydrolipoic acid (Packer *et al.*, 1996) are also efficient thiol anti-oxidants. Albumin provides thiol groups at high concentrations in human plasma, capable of scavenging a wide range of FR (Gutteridge and Halliwell, 1994).

MYELO-, CHLORO-, AND LACTOPEROXIDASES

These enzymes catalyze halide-mediated hydroperoxide decomposition. During this activity, singlet oxygen is usually generated via a two-stage H_2O_2 disproportionation reaction. The requirement for halide serves as a trigger for H_2O_2 decomposition with formation of a hypohalous acid; finally, a second molecule of H_2O_2 is decomposed by the latter, with generation of singlet oxygen (Cadenas, 1989).

EBSELEN: A SELENO DERIVATIVE WITH GSH LIKE PEROXIDASE ACTIVITY

Ebselen [2-phenyl-1,2-benzisoselenazol-3(2H)-one] is a

seleno-organic compound with GSH-Px-like hydroperoxide-reducing features *in vitro* and oral anti-inflammatory activity *in vivo*. It would inhibit both 5-lipoxygenase and cyclo-oxygenase, thus modulating arachidonic acid metabolism and further eicosanoid synthesis. It would also isomerize the inflammatory mediator leukotriene B_4 to the biologically inactive trans isomer (Roberfroid and Buc Calderon, 1995).

FLAVONOIDS AND POLYPHENOLS

These affect a range of biological functions (*e.g.*, capillary permeability, inhibition of enzymes, carriers, etc.), and they have recently been proposed as radical-scavengers. Quercetin (3,5,7,3',4'-pentahydroxyflavone), probably the most widely known flavonoid, presents more than 140 derivatives. Quercetin functions as an inhibitor of a wide series of enzymes from different classes (*e.g.*, XO, XDH, protein kinase, cyclo-oxygenase, myeloperoxidase, Ca^{2+} -ATPase, etc.), thereby casting doubt as to the likelihood of a single, common mechanism. In fact, a rather confusing picture emerges when one considers the variety of flavonoid functions demonstrated by studies *in vitro* (Bors *et al.*, 1996).

Nevertheless, the cellular mechanisms involved in FR/ROS protection may be related mainly to their direct anti-oxidant properties (*e.g.*, by sparing vitamin E or by regenerating vitamin C) or to their inhibitory activity toward lipoxygenase (Roberfroid and Buc Calderon, 1995).

LAZAROIDS

The 21-aminosteroids—a newly identified family of compounds derived from glucocorticoids, but lacking both glucocorticoid and mineralcorticoid activities—bind iron ions in forms incapable of catalyzing FR reactions (Halliwell and Gutteridge, 1990). The compounds in this family scavenge lipid peroxy radicals and seem to inhibit iron-dependent lipid peroxidation by a mechanism similar to that of vitamin E. An exhaustive recent review covers this extensive field (Villa and Gorini, 1997).

CAROTENOIDS AND VITAMIN A

The anti-oxidant action of carotenoids (α - and β -carotene, lycopene, astaxanthin, canthaxanthin, etc.) is well-documented *in vitro*, and direct evidence of this function *in vivo* has been reported in animal models (Paloza and Krinsky, 1992; Handelman, 1996). The results of bleaching or formation of oxidation products of carotenoids show that they trap O_2 and organic free radical intermediates (Paloza and Krinsky, 1992). Some of the protection by carotenoids can be accounted for on the basis of singlet O_2 and peroxy radicals quenching activities. β -carotene, in the presence of peroxy radicals, produces a carbon-centered carotenyl radical (β -car-) that at low partial O_2 pressures is a very efficient chain terminator. However, the

presence of O_2 allows chain propagation to occur (Roberfroid and Buc Calderon, 1995). In other words, as in the case of other molecules, experimental/physiological conditions determine the anti-oxidant/pro-oxidant behavior of carotenoids. Also, for vitamin A, when it acts as a chain-breaking anti-oxidant, a delicate balance exists between anti- and pro-oxidant activities which depend on the medium, O_2 pressure, and its concentration (Livrea *et al.*, 1996).

URIC ACID

Uric acid is produced by the oxidation of hypoxanthine and xanthine by XO and XDH. In human tissues, because of a lack of urate oxidase, it accumulates as the end-product of purine metabolism. *In vitro*, uric acid could inhibit transition metal-ion-dependent $OH\cdot$ generation, is a powerful quencher and/or scavenger of singlet O_2 , and might trap peroxy radicals in an aqueous phase more effectively than ascorbic acid (Cadenas, 1989; Halliwell, 1996). The role of uric acid as an anti-oxidant *in vivo* seems to be strictly dependent on allantoin levels, one of the products of its oxidation (Halliwell, 1996).

VITAMIN C (ASCORBIC ACID)

Ascorbic acid is the only endogenous anti-oxidant in plasma that can completely protect against peroxidative damage induced by aqueous peroxy radicals and the oxidants released from activated neutrophils (Brown and Jones, 1996). However, ascorbic acid seems to protect against peroxidation indirectly, by interacting with α -tocopherol (Buettner and Jurkiewicz, 1996). There would appear to exist a concerted action of vitamins E and C, in which ascorbic acid regenerates vitamin E, thus maintaining its serum value at a constant level. When ascorbic acid is depleted, no regeneration of vitamin E is possible, and a decrease in its concentration is observed (Gutteridge and Halliwell, 1994; Roberfroid and Buc Calderon, 1995). Chemically, ascorbic acid is an excellent reducing agent (Buettner and Jurkiewicz, 1996), and most of its anti-oxidant properties are ascribed to this feature. Unfortunately, it is also able to reduce iron and copper ions. If H_2O_2 is also present, ascorbate can drastically accelerate $OH\cdot$ formation, according to the mechanism of Eq. 7, thus producing another pro-oxidant. The pro-oxidant effects of ascorbic acid do not usually occur *in vivo*, simply because, in the healthy state, free iron and copper are not available in extracellular fluids (Gutteridge and Halliwell, 1994). Finally, when acting as a pro-oxidant, ascorbic acid can modulate the expression of the procollagen gene through both lipid peroxidation production and stabilization; in this way, it may affect the secretion of collagen, thereby altering fibroblast differentiation through its effects on the extracellular matrix (Brown and Jones, 1996).

VITAMIN E (α -TOCOPHEROL)

Vitamin E has an OH phenolic group which is responsible for its anti-oxidant activity and a phytyl side-chain which favors its insertion into the cell membrane lipid bilayer. However, growing evidence indicates that vitamin E is not always the best lipophilic anti-oxidant (Battino *et al.*, 1991a; Stocker *et al.*, 1991) and, even worse, that vitamin E can also act as a pro-oxidant (Bowry *et al.*, 1992; Bowry and Stocker, 1993; Kontush *et al.*, 1996; Stocker and Bowry, 1996). According to the anti-oxidant view, vitamin E inhibits lipid peroxidation, scavenging peroxy radicals much faster than these radicals can react with adjacent fatty acid side-chains or with membrane proteins (Gutteridge and Halliwell, 1994): The peroxy radical is converted to a lipid peroxide and α -tocopherol to an α -tocopheroxy radical which, in turn, is alternatively regenerated to α -tocopherol by ubiquinol ($CoQH_2$; the reduced form of co-enzyme Q), GSH, or, mainly, by ascorbic acid. The latter interaction generates an ascorbic acid radical (a fairly unreactive species) which, reacting with another ascorbic acid molecule, yields ascorbate and dehydro-ascorbate, or it can be reduced by a CoQ-dependent dehydrogenase (Navarro *et al.*, 1995; Villalba *et al.*, 1995). α -tocopheroxy radical reduction by both ascorbate (Sato *et al.*, 1990; Kagan *et al.*, 1992) and $CoQH_2$ (Frei *et al.*, 1990; Kagan *et al.*, 1990; Yamamoto *et al.*, 1990) has been studied and confirmed *in vitro*. The relative importance of ascorbate and $CoQH_2$ depends on prevailing conditions (Niki, 1996): Ascorbate is consumed first when FR are formed in the aqueous phase, while $CoQH_2$ is consumed first when FR are formed within membranes. On the other hand, Stocker and co-workers (Bowry *et al.*, 1992; Bowry and Stocker, 1993; Stocker and Bowry, 1996) have identified, in isolated LDL, a so-called tocopherol-mediated peroxidation: In this model, the α -tocopherol itself does not act as a chain-breaking anti-oxidant but rather facilitates the transfer of radical reactions from the aqueous phase inside the lipophilic environment, thus mediating radical chain reactions within the lipid moieties. To inactivate this process, suitable reducing agents (called co-anti-oxidants) must be present, or, alternatively, another radical must interact, giving rise to a termination reaction as indicated in Fig. 3. Again, also in this model, the most efficient co-anti-oxidants are ascorbate, in the hydrophilic environment, and $CoQH_2$, in the hydrophobic one (Stocker and Bowry, 1996; Thomas *et al.*, 1997).

CO-ENZYME Q_{10} (CoQ_{10} , OXIDIZED FORM; $CoQ_{10}H_2$, REDUCED FORM)

Considerable evidence has accumulated to indicate the anti-oxidant role of both CoQ_{10} and $CoQ_{10}H_2$ (Frei *et al.*, 1990; Kagan *et al.*, 1990, 1996; Yamamoto *et al.*, 1990;

Stocker *et al.*, 1991; Thomas *et al.*, 1997). The investigations carried out by Battino and co-workers (Battino *et al.*, 1991a,b, 1993, 1995, 1996, 1997a,b; Huertas *et al.*, 1991; Ferri *et al.*, 1994; Quiles *et al.*, 1994; Atleva *et al.*, 1995; Littarru *et al.*, 1996; Armeni *et al.*, 1997; Lodi *et al.*, 1997; Mataix *et al.*, 1997) strongly suggest an essential anti-oxidant role for CoQ₁₀ and CoQ₁₀H₂ on the basis of a series of experiments:

- (a) *In vitro*, (i) with the use of electrochemical techniques in aprotic medium, (ii) during γ -irradiation of mitochondria, (iii) by means of chemiluminescent detection of mitochondrial FR generation, and (iv) by challenging human LDL subfractions with a FR generation compound; and
- (b) *In vivo*, (i) in mitochondria from different brain areas during aging, (ii) in brain mitochondria affected by Parkinson's disease-like syndrome, (iii) in liver mitochondria following different pharmacological and physiological oxidative stresses (*i.e.*, adriamycin administration, variably unsaturated dietary fats, caloric restriction, vitamin E deficiency, exercise), and (iv) in pathological conditions (*i.e.*, abetalipoproteinemia).

Also, CoQ₁₀ can sometimes be considered a pro-oxidant molecule in response to various toxicological and pathophysiological events. These aspects have been clearly reviewed recently (Kagan *et al.*, 1996).

REGULATION OF "ANTI-OXIDANT GENES"

The possibility of gene control of anti-oxidant enzymes has yielded interesting results. The exposure of bacteria to low H₂O₂ concentrations results in the induction of 30 proteins and resistance to killing by higher doses of H₂O₂ (Storz and Tartaglia, 1992). The positive regulator *oxyR* controls the expression of nine of the H₂O₂-inducible proteins, including catalase and GSH-red. On the other hand, strains carrying deletions of the *oxyR* gene are hypersensitive to H₂O₂ and show increased levels of spontaneous mutagenesis, especially during aerobic growth. It is now well-known that anti-oxidant-inducible genes are expressed in most mammalian cells and tissues. Expression of other proteins—such as transcription factors, ferritin, and ribosomal proteins—has been shown to be increased following anti-oxidant administration. Primiano *et al.* (1997) recently exhaustively reviewed these aspects, and Allison (1997) discussed the effects of anti-oxidants on transcription factors (*e.g.*, NF- κ B) as well as on gene expression (*e.g.*, in relation to the production of pro-inflammatory cytokines).

Polymorphonuclear Neutrophils (PMN): A Key Role in Periodontitis

PMN are the predominant leukocytes in blood and consti-

tute the primary cellular host resistance factor against infection. In the oral cavity, following plaque accumulation and the development of clinical inflammation, there is an increase in gingival PMN and in the gingival sulcus. A protective role of PMN in the pathophysiology of PE is suggested by the finding of severe PE in patients with reduced PMN or impaired PMN function. Individuals with early-onset or rapidly progressing forms of PE often exhibit relatively subtle neutrophil defects. However, most studies have not been able to demonstrate PMN defects in patients with various degrees of routine adult chronic PE (Seymour *et al.*, 1986).

PMN possess at least 2 main pathways for controlling micro-organisms (*i.e.*, oxidative and non-oxidative) which either kill bacteria, influence bacterial growth, or modify bacterial colonization in relation to the periodontium (Miyasaki, 1991). Chemotaxis and phagocytosis assessment are the methods traditionally used for measuring PMN functionality. Another PMN function is the well-known "respiratory burst" (RB) that can be measured by different methods.

METHODS FOR EVALUATION OF RB

- (a) Nitroblue tetrazolium (NBT) is a substrate for dehydrogenases and other oxidases. RB activity in stimulated PMN is measured by following spectrophotometrically (at 560 nm) the reduction of NBT (yellow and soluble) to formazan (blue-black and relatively insoluble) by means of O₂⁻ generated in the burst. It is an indicator of the degree of activity in the NADPH oxidase system located in the PMN plasma membrane (Seymour *et al.*, 1986; McCord, 1993).
- (b) Chemiluminescence (CL) can quantitate minor changes in PMN oxidative metabolism (Seymour *et al.*, 1986). The ROS produced by PMN during phagocytosis oxidize the cyclic hydrazine 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol) to an excited 3-aminophthalate anion that relaxes to the ground state with emission of light (Battino *et al.*, 1991a).
- (c) Flow cytometry allows single cells to be characterized. The cells to be analyzed are labeled with fluorescent dyes. The cells are forced through a nozzle in a single-cell stream that passes through a laser beam. The light-scattering is a sign of cell size and granularity. Depending on the numbers and kinds of dye-coupled antibodies used, it is possible to identify different types of cell populations. It also allows one to examine the RB of PMN by evaluating the oxidative product (2', 7'-dichlorofluorescein; DCF) formation of PMN by stimulation with phorbol 12-myristate 13-acetate (PMA). During incubation, a diffusing form of DCF enters into PMN in a non-fluorescent state and is oxidized to highly fluorescent DCF in the PMN stimulated by PMA.
- (d) Finally, O₂⁻ release by PMN during RB can be detected spectrophotometrically as an increasing

absorbance at 550 nm due to cytochrome c reduction. The microplate assay is very advantageous and widely used, since it is sensitive, reproducible, easy, and cheap; it has been recently used for measuring $O_2^{\cdot-}$ release by PMN: there was a two- to three-fold enhancement by amine fluoride and stannous fluoride, in contrast to the reduction achieved with salicylic acid, chlorhexidine (Shapira *et al.*, 1997), or nicotine (Pabst *et al.*, 1995).

ROS PRODUCTION BY PMN: THE ROLE OF PERIODONTOPATHOGENS AND THE "PRIMING" EFFECT

Supragingival plaque induced emission of CL by normal peripheral blood neutrophils (Charon *et al.*, 1987), and at least $O_2^{\cdot-}$ and H_2O_2 are produced by dental-plaque-stimulated neutrophils. The products of some bacteria may lead to altered PMN function, modifying their response to a second stimulus. This action of preparing PMN for stimulation is referred to as "priming". *P. gingivalis* lipopolysaccharide causes a dose-dependent increase in $O_2^{\cdot-}$ production by formylmethionyl-leucyl-phenylalanine (FMLP)-stimulated PMN derived from rapidly progressive periodontitis patients (RPP) (Shapira *et al.*, 1994). The trypsin-like protease from *P. gingivalis* stimulates neutrophils in terms of $O_2^{\cdot-}$ production (Lala *et al.*, 1994). An extract of *A. actinomycetemcomitans* significantly inhibits H_2O_2 production following PMA stimulation, but increases H_2O_2 production following stimulation with FMLP and zymosan (Ashkenazi *et al.*, 1992). Oral treponemes possess factors which inhibit the $O_2^{\cdot-}$ production by PMN (Sela *et al.*, 1988).

ROS PRODUCTION BY PMN: LOCAL AND BLOOD PMN RESPONSES

A spontaneous release of $O_2^{\cdot-}$ by crevicular PMN occurs in healthy individuals and adult periodontitis patients (AP). Circulating PMN of healthy subjects do not show spontaneous $O_2^{\cdot-}$ generation, but this property is evident when PMN are present in the sulcus. On the contrary, the circulating PMN of AP subjects show spontaneous $O_2^{\cdot-}$ formation (Guarnieri *et al.*, 1991). Moreover, cells situated in the local inflammatory sites in AP have a reduced capacity to liberate $O_2^{\cdot-}$ by PMA stimulation (Loesche *et al.*, 1988).

ROS PRODUCTION BY PMN: PATIENTS VS. CONTROL PMN RESPONSES

The production of ROS by PMN isolated from periodontal patients vs. controls remains controversial. PMN from young patients with PE generate increased CL (Asman *et al.*, 1984; Asman, 1988; Shapira *et al.*, 1991; Leino *et al.*, 1994), but PMN from localized juvenile periodontitis patients (LJP) display a normal oxidative capacity (Ellegard *et al.*, 1984; Van Dyke *et al.*, 1986; Zafiropoulos *et al.*, 1991). Release by PMN was low in subjects suffering

from Papillon-Lefèvre syndrome (PLS), as measured by a ferri-cytochrome c reduction assay (Bullon *et al.*, 1993). PMN from both PLS patients and controls produced similar amounts of $O_2^{\cdot-}$ when stimulated with opsonized bacteria or PMA, but both resting PMN and PMN stimulated with polyhistidine from the PLS patients released larger amounts of $O_2^{\cdot-}$ than PMN from the controls (Bimstein *et al.*, 1990). In AP, only a slight increase in CL was observed (Whyte *et al.*, 1989), while there were minor differences (Zafiropoulos *et al.*, 1991), no differences (Mouynet *et al.*, 1994), or a CL decrease (Gomez *et al.*, 1994) among healthy subjects or patients with gingivitis, juvenile periodontitis, or AP. PMN from AP patients have higher CL after Fcg-receptor stimulation than do those from healthy subjects (Gustafsson and Asman, 1996). In RPP patients was found a lowering of CL (Zafiropoulos *et al.*, 1988), and only minor differences in the CL responses between PE patients and healthy controls were found in terms of the oxidative metabolism of PMN (Zafiropoulos *et al.*, 1991). The RB of PMN in the peripheral blood of patients with various types of PE (evaluated by fluorescence/flow cytometry) indicated that all groups contained variable populations of subjects with normal and increased DCF formation, while the control subjects exhibited DCF formation as a single population. The average DCF formation of all groups was significantly higher than that of the control group, if the results were considered as the average of all subjects in each group (Kimura *et al.*, 1993).

FR/ROS-Mediated Damage in Periodontitis

The physiological activity of phagocytosing leukocytes producing a "respiratory burst" can result in mild oxidative damage, usually perfectly controlled by the defense mechanisms of the surrounding tissues. Local factors (plaque micro-organisms, etc.) promoting PE can also unbalance this equilibrium. A massive neutrophil migration to the gingiva and gingival fluid leads to abnormal spreading of FR/ROS produced during the "respiratory explosion".

EFFECTS OF PMN PRODUCTS ON CELLS

Gingival epithelial cells are highly susceptible to attack by PMN-derived oxidants (Altman *et al.*, 1992); in fact, human PMN, *in vitro*, produce desquamation and lysis of gingival epithelial cells. The desquamation is likely a consequence of the digestion of extracellular matrix constituents by PMN neutral proteases, and the lysis is probably caused by PMN oxidants generated by myeloperoxidase. PMN-mediated damage to human periodontal ligament-derived fibroblasts was obtained when PMN and fibroblasts were co-cultured *in vitro* and the PMN stimulated with both FMLP and endotoxin. Endotoxin promoted adhesion of PMN to fibroblasts, which is a necessary, but by itself insufficient, first step in causing damage. It is speculated that a second stimulus, such as FMLP, then promotes

PMN protease or FR/ROS release into the sequestered micro-environment between the PMN and adherent fibroblasts, leading to cell damage (Deguchi *et al.*, 1990).

IN VIVO MODELS: ROLES OF LIPID PEROXIDATION AND DRUGS IN PE

Two reviews concerning the possible role of lipid peroxidation in PE pathogenesis (Voskresenskii and Tkachenko, 1991) and the evidence for a therapeutic use of anti-oxidants in PE treatment and prevention (Bobyrev *et al.*, 1994a) exist in recent databases. Rats with spontaneous and experimental PE displayed higher levels of lipid peroxidation, measured as MDA and/or hydroperoxides (HP), and lower levels of SOD and catalase activities in both blood and periodontal tissues than did control animals. Cytomedin (an alkaline polypeptide) administration would be expected to have been beneficial by enhancing SOD and catalase activities (Mishchenko *et al.*, 1991; Silenko *et al.*, 1991, 1994a,b). Similar damage can be observed in the periodontal tissues of rats subjected to NaF intoxication (10 mg/kg/day *per* 30 days) (Silenko *et al.*, 1992). The possibility of provoking PE together with similar biochemical changes in the periodontium by means of pro-oxidant xenobiotics was confirmed (Bobyrev *et al.*, 1994b). Long-term administration of diphenin and delagil (up to 150 days) resulted in PE pathology, in highly increased levels of lipid peroxidation (MDA, HP) and percentage hemolysis, as well as in decreased anti-oxidant enzyme activities (SOD and catalase) and ceruloplasmin. Nevertheless, some data disagree: In fact, increased diene-conjugated contents and peroxidation rates in both gingiva and alveolar bone in (cat) PE were confirmed, but, on the other hand, a concomitant enhanced GSH-red activity and α -tocopherol content were also reported (Levitskii *et al.*, 1987). However, it must be stated that data in this paper do not completely fit the abstract in its English version.

IN VIVO MODELS: ROLE OF IRON IN PE

Iron levels are higher in the gingival fluid of human subjects affected by PE (Petrovich *et al.*, 1996). This could have unfavorable consequences due to the ability of iron to catalyze FR/ROS reactions (Eqs. 6 and 7), thus enhancing the growth of some periodontitis-related organisms; but, according to the authors, it seems that the free iron-binding capacity of saliva is also improved.

MALNUTRITION:

A MAIN CAUSE OF PE DEVELOPMENT

Pain stress and an anti-oxidant-deficient diet can provoke a "deterioration" of the periodontium (Tarasenko and Voskresenskii, 1986; Deviatkina *et al.*, 1989). The interrelationships among poor sanitation (including poor oral hygiene), economic poverty, malnutrition, impaired immune function, infections, and many diseases with an

inflammatory component, with particular attention to PE, have been recently discussed (Enwonwu, 1994, 1995). The activity of bacteria-agglutinating glycoprotein in saliva is decreased in malnutrition, and this may promote enhanced formation of dental plaque; moreover, a still-unexplained overgrowth of potential periodontal pathogens in protein-energy malnutrition (PEM), together with changes in metabolism of the salivary glands (*e.g.*, higher arginine levels would favor elevation of plaque pH), may contribute to easier development of PE. PMN usually elaborate destructive oxidants, proteinases, and other factors in response to periodontal pathogens, but PEM may unbalance such a steady-state; thus, PEM is characterized by marked tissue depletion of key anti-oxidant nutrients, and the response to infections is impaired. Since PEM also affects the production and cellular activation of cytokines, inverts the helper-suppressor T-cell ratio, and provokes histaminemia and hormonal imbalance, it has been suggested that the concomitant deficiencies of several essential macro- and micronutrients would adversely influence periodontal infections.

PATHOLOGICAL STATUS INDUCING PE

A possible relationship between PE and Advanced Glycation End-products (AGEs) responsible for inducing oxidative stress in tissues of diabetics has been suggested (Schmidt *et al.*, 1996). The hypothesis that AGEs present in diabetic gingiva might be directly related to arising and worsening PE in diabetics is novel, but, unfortunately, the conclusion was based on data derived from only 3 mice and four human diabetics.

Anti-oxidants in Periodontitis

RELATIONSHIP BETWEEN PEROXIDATION PRODUCTS AND ANTI-OXIDANT MOLECULES

An inverse relationship between peroxidation products and anti-oxidant molecules or enzymes in spontaneous or in experimental PE has been stressed (Mishchenko *et al.*, 1991; Silenko *et al.*, 1991, 1992, 1994a,b; Voskresenskii, 1991; Bobyrev, 1994a,b). Such an association may similarly be evident in the pathogenesis of human chronic apical PE (Marton *et al.*, 1993). Only one study (Levitskii *et al.*, 1987) indicated an unexplained, and never confirmed, concomitant increment in peroxidation parameters and some anti-oxidant mechanisms (*i.e.*, GSH-red activity and vitamin E content). The only data, from Khmelevskii *et al.* (1985), would solely confirm higher levels of GSH-red activity and -SH group in PE than in controls, but without any comment as to the relative possible FR/ROS-induced periodontal tissue damage. The recent detection of high levels of metallothionein in the gingiva of smokers with advanced PE would indicate an attempt to defend against FR in the gingiva of the smokers (Katsuragi *et al.*, 1997).

TOTAL ANTI-OXIDANT ACTIVITIES OF SALIVA IN RELATION TO PE

Part of oral anti-oxidant protection is attributable to uric acid and, to a lesser extent, to ascorbic acid and albumin (Moore *et al.*, 1994; Chapple, 1997). FR production in PE is counteracted by increased secretion of gingival crevicular fluid without depletion of anti-oxidants (Moore *et al.*, 1994).

CHEMOTHERAPEUTICS' ANTI-OXIDANT ACTIVITY

Another promising approach would seem to be the study of the possible anti-oxidant activities of some chemotherapeutics used to treat PD. For example, some papers claimed, for chlorhexidine, an anti-oxidant but still partially unclear role in its molecular mechanism (Goultschin and Levy, 1986; Firatli *et al.*, 1994); however, chlorhexidine and salicylic acid reduce $O_2^{\cdot -}$ produced by PMN (Shapira *et al.*, 1997).

DIETARY INTAKE OF ANTI-OXIDANTS

Possible relationships between anti-oxidant dietary deficiency and resulting peroxidative damage at the tissue level, with possible development even of severe PE, have been widely discussed (Waerhaug, 1967; Tarasenko and Voskresenskii, 1986; Deviatkina *et al.*, 1989; Speirs and Beeley, 1992; Enwonwu, 1994, 1995). The lowered GSH levels found in PE do not necessarily imply its involvement in anti-oxidant activity, since its -SH may simply have been used by oral bacteria to form hydrogen sulfide (Carlsson *et al.*, 1993; Tang-Larsen *et al.*, 1995). However, whatever the reason for -SH reduction, the result is a lowered overall anti-oxidant potential of the periodontium. Other data suggest that collagen degradation during PE could be limited by vitamin E and selenium (Asman *et al.*, 1994), since they are FR/ROS inactivators, or even better by supplementary ascorbate (Suomalainen *et al.*, 1991), which is an inhibitor of human neutrophil collagenase. Ascorbic acid has been suggested to influence early stages of gingival inflammation and crevicular bleeding (Jacob *et al.*, 1987), since it was found that deficient intake (< 5 mg/day) resulted in inflamed, bleeding gingivae.

VITAMIN A AND INFLAMMATORY PERIODONTAL DISEASE

Up to the present, no clear evidence has emerged in relation to the possible anti-oxidant activity of vitamin A in PE. In 1967, an epidemiological study by Waerhaug in Ceylon (now Sri Lanka) on more than 8000 inhabitants indicated no such relationship. The following year, an investigation on vitamin-A-deficient rats before and after diet supplementation (Schneider and Pose, 1968) indicated that vitamin A increased leukocyte infiltration and epithelial hyperkeratosis and reversed or slowed the bone resorption that was accelerated by vitamin A deficiency.

Blood vitamin A is not affected by zinc sulfate treatment in PE patients (Cerna *et al.*, 1988). Vitamin A increases during pregnancy concomitantly with periodontal inflammation (Cerna *et al.*, 1990), but it does not happen in pregnant diabetics (Cerna *et al.*, 1992). It has been suggested that vitamin A in toothpaste would be useful in treating PE, since fewer deep pockets and reduced gingival bleeding were found following its use (Trykowski *et al.*, 1994).

VITAMIN E AND INFLAMMATORY PERIODONTAL DISEASE

A vitamin-E-deficient diet as well as a vitamin E supplement can affect several periodontal parameters (in the rat) (Schneider and Pose, 1969). Vitamin E deficiency does not increase PE (in rats) (Parrish *et al.*, 1977), and no significant difference in vitamin E levels in PD patients was found (Slade *et al.*, 1976). The authors agreed that their respective data did not provide any support for the treatment of PD with vitamin E. No variations in vitamin A or E levels in PD patients after zinc sulfate treatment, during pregnancy and during pregnancy in diabetic women, were found (Cerna *et al.*, 1988, 1990, 1992). Gingival vitamin E concentrations decrease in males and females in proportion to the severity of pocket depth and gingival and bleeding indices, and in older subjects more affected by PE, vitamin E tended to increase (Fujikawa, 1983). Some positive effects after vitamin E administration on rat bone loss induced by stress have been observed, and vitamin E efficacy was detected only when the stress was introduced abruptly (Cohen and Meyer, 1993). However, other studies found no effects of vitamin E on PD (Li *et al.*, 1996; Cohen *et al.*, 1991).

CoQ IN INFLAMMATORY PERIODONTAL DISEASE

The effects of CoQ administration on hypercitricemia in both human patients and rats with PE (Tsunemitsu and Matsumura, 1967; Tsunemitsu *et al.*, 1968; Matsumura *et al.*, 1969a,b) were studied by the homologue CoQ_7 , and prolonged administration of CoQ_7 alleviated the histopathological alterations found in citrated rats (*i.e.*, marked osteoporosis of the alveolar bone accompanied by myelofibrosis and atrophy of the periodontal ligament). The reason for this effect remained unclear.

Evidence emerged for a CoQ deficiency in the gingiva of patients with PE, on the basis (Littarru *et al.*, 1971) of the exogenous-CoQ-mediated stimulation of succinate dehydrogenase-CoQ (SDH) in periodontal tissues from humans with PE. But it was not clear why PE subjects had basal SDH-specific activities two- to three-fold higher than those of control subjects. Later, it was found that another CoQ-dependent enzyme was not affected at all in PE (Nakamura *et al.*, 1973), so that CoQ deficiency would exist only on the basis of the first enzyme studied. A study involving CoQ administration in PE confirmed that no

CoQ₁₀ deficiency existed at SDH sites in either the CoQ or the placebo group (Iwamoto *et al.*, 1981). Moreover, SDH from dog gingiva could be similarly stimulated in control and PE animals, but with no direct evidence for such CoQ deficiency (Hsieh *et al.*, 1983). Another study demonstrated identical CoQ₁₀ levels in gingiva (whole homogenate) in dogs both with and without experimental PE (induced by the placement of cotton floss) (Shizukuishi *et al.*, 1984). However, in the Nakamura *et al.* (1973) study, the control group was represented by non-inflamed sites in the mouths of patients with PE, without comment as to the possibility that both control and experimental sites may have had bone destruction (*i.e.*, there was no evaluation of PE clinical status).

Several more papers (Matsumura *et al.*, 1973; Iwamoto *et al.*, 1975; Wilkinson *et al.*, 1975, 1976; Hansen *et al.*, 1976; Folkers and Watanabe, 1977) have limited actual relevance because CoQ deficiency in PE was not shown. The papers dealing with CoQ₁₀ administration in PE (Wilkinson *et al.*, 1977; Iwamoto *et al.*, 1981; Shizukuishi *et al.*, 1984, 1986) are also lacking for similar reasons (*i.e.*, inflamed gingiva does not imply PE). However, it was found that:

- (i) the specific activity and % deficiency of three mitochondrial respiratory chain enzymes in normal gingiva were not affected by oral administration of CoQ₁₀ (Shizukuishi *et al.*, 1984);
- (ii) PE did not affect actual CoQ₁₀ levels in gingiva (Shizukuishi *et al.*, 1984);
- (iii) CoQ₁₀ deficiency at SDH sites was not found in either CoQ or placebo groups (Iwamoto *et al.*, 1981);
- (iv) CoQ administration resulted in limited efficacy in ameliorating gingival index, plaque index, and pocket depth (Shizukuishi *et al.*, 1984, 1986); and
- (v) when compared with a placebo group, CoQ administration was ineffective on the PE indices tested (Iwamoto *et al.*, 1981).

The effects of CoQ₁₀ on the immune system of patients with PE were also studied: The T₄/T₈ ratio increased after 2 mos of CoQ₁₀ administration, while IgG levels increased significantly after 6 mos (Hanioka *et al.*, 1993). The following two studies suddenly moved from systemic to topical administration of CoQ₁₀ for improving PE (McRee *et al.*, 1993; Hanioka *et al.*, 1994). The results of the former suggested a very limited effect after CoQ₁₀ administration, while the results of the latter were inconclusive. Many of the methodological defects outlined in this discussion have been considered in a recent debate in the *British Dental Journal* about the possible beneficial effects of CoQ₁₀ in PE (Lister, 1995; Watts, 1995). Watts commented on the very limited data in the peer-reviewed dental literature, and he also remarked that several of the papers suffered from inadequate statistical analysis.

Conclusions

FR/ROS are essential to many normal biological processes, and low doses of certain radicals or radical-derived species can stimulate the growth of fibroblasts and epithelial cells in culture. When anti-oxidant systems are unable to counteract their action efficiently, tissue damage can result. A low FR/ROS often behaves as an inductive stimulus, whereas higher levels may result in injury (Battino *et al.*, 1995).

Physiological alterations and pathological status, directly or indirectly produced by FR/ROS actions, depend on a disequilibrium between increased FR/ROS production and decreased (or insufficiently increased) anti-oxidant levels or activities. Such conditions usually lead to impairment of part or all of the anti-oxidant defense system and to the appearance of biological damage. There is evidence that something similar may take place in PE as in other inflammatory diseases.

This concept has led to a search for appropriate "anti-oxidant therapy" in inflammatory disease. The successful therapeutic manipulation of the cellular response by anti-oxidant molecules might necessitate the maintenance of the critical balance between FR/ROS and anti-oxidant defense systems (Winyard and Blake, 1997). It may be necessary to deliver anti-oxidants selectively to specific cell types and to define the concentrations suitable for blocking inappropriate cell responses but leaving the unimpaired physiological levels of FR/ROS activity necessary for normal cell function.

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REFERENCES

- Allison AC (1997). Antioxidant drug targeting. In: Antioxidants in disease mechanisms and therapy. Sies H, editor. Advances in pharmacology. Vol. 38. San Diego: Academic Press, pp. 273-291.
- Altman LC, Baker C, Fleckman P, Luchtel D, Oda D (1992). Neutrophil-mediated damage to human gingival epithelial cells. *J Periodont Res* 27:70-79.
- Armeni T, Tomasetti M, Svegliati Baroni S, Saccucci F, Marra M, Pieri C, *et al.* (1997). Dietary restriction affects antioxidant levels in rat liver mitochondria during ageing. *Molec Aspects Med* 18(Suppl):241-245.

- Ashkenazi M, White RR, Dennison DK (1992). Neutrophil modulation by *A. actinomycetemcomitans*. II. Phagocytosis and development of respiratory burst. *J Periodont Res* 27:457-465.
- Asman B (1988). Peripheral PMN cells in juvenile periodontitis. Increased release of elastase and oxygen radicals after stimulation with opsonized bacteria. *J Clin Periodontol* 153:360-364.
- Asman B, Engstrom PE, Olsson T, Bergstrom K (1984). Increased luminol enhanced chemiluminescence from peripheral granulocytes in juvenile periodontitis. *Scand J Dent Res* 92:218-233.
- Asman B, Wijkander P, Hjerpe A (1994). Reduction of collagen degradation in experimental granulation tissue by vitamin E and selenium. *J Clin Periodontol* 21:45-47.
- Atleva R, Tomasetti M, Battino M, Curatola G, Littarru GP, Folkers K (1995). The roles of CoQ₁₀ in preventing peroxidation of LDL subfractions. *Proc Natl Acad Sci USA* 92:9388-9391.
- Battino M, Ferri E, Girotti S, Lenaz G (1991a). Free radical scavenging activity of coenzyme Q measured by a chemiluminescent assay. *Anal Chim Acta* 255:367-371.
- Battino M, Ferri E, Gattavecchia E, Sassi S, Lenaz G (1991b). Coenzyme Q as a possible membrane protecting agent against γ -irradiation damages. In: Biomedical and clinical aspects of Coenzyme Q. Vol. 6. Folkers K, Littarru GP, Yamagami T, editors. Amsterdam: Elsevier, pp. 181-190.
- Battino M, Lenaz G, Littarru GP (1993). Evidences for long chain CoQ homologs involvement in free radical scavenging activity. In: Free radicals and antioxidants in nutrition. Corongiu F, Banni S, Dessì MA, Rice-Evans C, editors. London: Richelieu Press, pp. 37-49.
- Battino M, Gorini A, Villa RF, Genova ML, Bovina C, Sassi S, et al. (1995). Coenzyme Q content in synaptic and non-synaptic mitochondria from different brain regions in the ageing rat. *Mech Ageing Dev* 78:173-187.
- Battino M, Littarru GP, Gorini A, Villa RF (1996). Coenzyme Q, peroxidation and cytochrome oxidase features after Parkinson's-like disease by MPTP toxicity in intrasynaptic and non-synaptic mitochondria from *Macaca fascicularis* cerebral cortex and hippocampus: action of dihydroergocriptine. *Neurochem Res* 21:1505-1514.
- Battino M, Ferri E, Gattavecchia E, Breccia A, Genova ML, Littarru GP, et al. (1997a). Mitochondrial respiratory chain features after γ -irradiation. *Free Rad Res* 26:431-438.
- Battino M, Svegliati Baroni S, Littarru GP, Bompadre S, Leone L, Gorini A, et al. (1997b). Coenzyme Q homologs and vitamin E in synaptic and non-synaptic occipital cerebral cortex mitochondria in the ageing rat. *Molec Aspects Med* 18(Suppl):279-282.
- Beckman KB, Ames BN (1997). Oxidative decay of DNA. *J Biol Chem* 272:19633-19636.
- Berlett BS, Stadtman ER (1997). Protein oxidation in aging, disease and oxidative stress. *J Biol Chem* 272:20313-20316.
- Bimstein E, Lustmann J, Sela MN, Ben Neriah Z, Soskolne WA (1990). Periodontitis associated with Papillon-Lefèvre syndrome. *J Periodontol* 61:373-377.
- Bobyrev VN, Rozkolupa NV, Skripnikova TP (1994a). Experimental and clinical bases for the use of antioxidants as agents for treating and preventing periodontitis. *Stomatologiya* 73(3):11-18.
- Bobyrev VN, Kovalev EV, Rozkolupa NV, Eremina NF, Voskresenskii ON (1994b). Biochemical and ultrastructural changes in the periodontium during the chronic administration of pro-oxidant xenobiotics. *Stomatologiya* 73(4):57-61.
- Bors W, Heller W, Michel C, Stettmaier K (1996). Flavonoids and polyphenols: chemistry and biology. In: Handbook of antioxidants. Cadenas E, Packer L, editors. New York: Marcel Dekker, Inc., pp. 409-466.
- Bowry VW, Stocker R (1993). Tocopherol-mediated peroxidation. The prooxidant effect of vitamin E on the radical initiated oxidation of human low density lipoprotein. *J Am Chem Soc* 115:6029-6040.
- Bowry VW, Ingold KU, Stocker R (1992). Vitamin E in human low density lipoprotein. When and how this antioxidant becomes pro-oxidant. *Biochem J* 288:341-344.
- Brown LAS, Jones DP (1996). The biology of ascorbic acid. In: Handbook of antioxidants. Cadenas E, Packer L, editors. New York: Marcel Dekker, Inc., pp. 117-154.
- Buettner GR, Jurkiewicz BA (1996). Chemistry and biochemistry of ascorbic acid. In: Handbook of antioxidants. Cadenas E, Packer L, editors. New York: Marcel Dekker, Inc., pp. 91-115.
- Bullon P, Pascual A, Fernandez-Novoa MC, Borobio MV, Muniain MA, Camacho F (1993). Late onset Papillon-Lefèvre syndrome? A chromosomal, neutrophil function and microbiological study. *J Clin Periodontol* 20:662-667.
- Cadenas E (1989). Biochemistry of oxygen toxicity. *Ann Rev Biochem* 58:79-110.
- Carlsson J, Larsen JT, Edlund MB (1993). *Peptostreptococcus micros* has a uniquely high capacity to form hydrogen sulfide from glutathione. *Oral Microbiol Immunol* 8:42-45.
- Cerna H, Vesely J, Fingerova H, Pohanska J (1988). Blood serum vitamin E and A levels during peroral therapy of periodontal disease with zinc sulphate. *Acta Univ Palacki Olomuc Fac Med* 119:187-193.
- Cerna H, Vesely J, Nastoupilova E, Lechner J, Fingerova H, Pohanska J (1990). Periodontium and vitamin E and A in pregnancy. *Acta Univ Palacki Olomuc Fac Med* 125:173-179.
- Cerna H, Vesely J, Krikal Z (1992). Vitamin E and A and periodontium in pregnant diabetics. *Acta Univ Palacki Olomuc Fac Med* 134:93-95.
- Chapple IL (1997). Reactive oxygen species and antioxi-

- dants in inflammatory diseases. *J Clin Periodont* 24:287-296.
- Charon JA, Joachim F, Champagne C, Torpier G, Capron A (1987). Effect of dental plaque on the oxidative metabolism of normal neutrophils. *Oral Microbiol Immunol* 2:92-96.
- Cohen ME, Meyer DM (1993). Effect of dietary vitamin E supplementation and rotational stress on alveolar bone loss in rice rats. *Arch Oral Biol* 38:601-606.
- Cohen RE, Ciancio SG, Mather ML, Curro FA (1991). Effect of vitamin E gel, placebo gel and chlorhexidine on periodontal disease. *Clin Prev Dent* 13:20-24.
- Deguchi S, Hori T, Creamer H, Gabler W (1990). Neutrophil-mediated damage to human periodontal ligament-derived fibroblast: role of lipopolysaccharide. *J Periodont Res* 25:293-299.
- Deviatkina TA, Tarasenko LM, Kovalenko EG (1989). Antioxidant deficiency and reaction of tissues on the acute emotional-pain stress. *Vopr Med Khim* 35:45-49.
- Diplock AT (1994). Antioxidants and disease prevention. *Molec Aspects Med* 15:293-376.
- Ellegaard B, Borregaard N, Ellegaard J (1984). Neutrophil chemotaxis and phagocytosis in juvenile periodontitis. *J Periodont Res* 19:261-268.
- Enwonwu CO (1994). Cellular and molecular effects of malnutrition and their relevance to periodontal disease. *J Clin Periodontol* 21:643-657.
- Enwonwu CO (1995). Interface of malnutrition and periodontal disease. *Am J Clin Nutr* 61(Suppl):430-436.
- Esterbauer H (1993). Cytotoxicity and genotoxicity of lipid-oxidation products. *Am J Clin Nutr* 57(Suppl):779-786.
- Esterbauer H, Schaur RJ, Zollner H (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Rad Biol Med* 11:81-128.
- Ferri E, Gattavecchia E, Feroci G, Battino M (1994). Interaction between reactive oxygen species and coenzyme Q_{10} in an aprotic medium: a cyclic voltammetry study. *Molec Aspects Med* 15(Suppl):83-88.
- Firatli E, Unal T, Onan U, Sandalli P (1994). Anti-oxidative activities of some chemotherapeutics. A possible mechanism in reducing gingival inflammation. *J Clin Periodontol* 21:680-683.
- Folkers K, Watanabe T (1977). Bioenergetics in clinical medicine. X. Survey of the adjunctive use of Coenzyme Q with oral therapy in treating periodontal disease. *J Med* 8:333-348.
- Frei B, Kim MC, Ames BN (1990). Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. *Proc Natl Acad Sci USA* 87:4879-4883.
- Fujikawa K (1983). Alpha-tocopherol content in the human gingiva in chronic marginal periodontitis. *Nippon Shishubyo Gakkai Kaishi* 25:178-184 [in Japanese].
- Gomez RS, Da Costa JE, Lorentz TM, Almeida Garrocho A, Nogueira-Machado JA (1994). Chemoluminescence generation and MTT dye reduction by polymorphonuclear leukocytes from periodontal disease patients. *J Periodont Res* 29:109-112.
- Goultschin J, Levy H (1986). Inhibition of superoxide generation by human polymorphonuclear leukocytes with chlorhexidine. Its possible relation to periodontal disease. *J Periodontol* 57:422-425.
- Guarnieri C, Zucchelli G, Bernardi F, Scheda M, Valentini AF, Calandriello M (1991). Enhanced superoxide production with no change of the antioxidant activity in gingival fluid of patients with chronic adult periodontitis. *Free Rad Res Commun* 15:11-16.
- Gustafsson A, Asman B (1996). Increased release of free oxygen radicals from peripheral neutrophils in adult periodontitis after Fc γ -receptor stimulation. *J Clin Periodontol* 23:38-44.
- Gutteridge JMC, Halliwell B (1994). Antioxidants in nutrition, health, and disease. New York: Oxford University Press.
- Halliwell B (1994). Free radicals, antioxidants and human disease: curiosity, cause or consequence. *Lancet* 344:721-724.
- Halliwell B (1996). Uric acid: an example of antioxidant evaluation. In: Handbook of antioxidants. Cadenas E, Packer L, editors. New York: Marcel Dekker, Inc., pp. 243-256.
- Halliwell B (1997). Antioxidants: the basics—what they are and how to evaluate them. In: Antioxidants in disease mechanisms and therapy. Advances in pharmacology. Vol. 38. San Diego: Academic Press, pp. 3-20.
- Halliwell B, Gutteridge JMC (1985). Free radicals in biology and medicine. New York: Oxford University Press.
- Halliwell B, Gutteridge JM (1990). Role of free radicals and catalytic metal ions in human disease: an overview. *Meth Enzymol* 186:1-85.
- Handelman GJ (1996). Carotenoids as scavengers of active oxygen species. In: Handbook of antioxidants. Cadenas E, Packer L, editors. New York: Marcel Dekker, Inc., pp. 259-314.
- Hanioka T, McRee JT, Xia L-J, Shizukuishi S, Folkers K (1993). Therapy with Coenzyme Q_{10} for patients with periodontal disease. 2. Effect of Coenzyme Q_{10} on the immune system. *J Dent Health* 43:667-672.
- Hanioka T, Tanaka M, Ojima M, Shizukuishi S, Folkers K (1994). Effect of topical application of Coenzyme Q_{10} on adult periodontitis. *Molec Aspects Med* 15(Suppl):241-248.
- Hansen IL, Iwamoto Y, Kishi T, Folkers K, Thompson LE (1976). Bioenergetics in clinical medicine. IX. Gingival and leucocytic deficiencies of Coenzyme Q_{10} in patients with periodontal disease. *Res Commun Chem Pathol Pharmacol* 14:729-738.
- Henle ES, Linn S (1997). Formation, prevention and repair of DNA damage by iron/hydrogen peroxide. *J Biol Chem* 272:19095-19098.

- Hsieh C, Inoshita E, Hanioka T, Tamagawa H, Takagaki M, Shizukuishi S (1983). Changes in coenzyme Q-dependent enzyme activities in the inflamed gingiva of dogs. *J Osaka Univ Dent Sch* 23:93-98.
- Huertas JR, Battino M, Lenaz G, Mataix FJ (1991). Changes in mitochondrial and microsomal rat liver coenzyme Q₉ and Q₁₀ content induced by dietary fat and endogenous lipid peroxidation. *FEBS Lett* 287:89-92.
- Ignarro L, Murad F, editors (1995). Nitric oxide. Biochemistry, molecular biology, and therapeutic implications. In: *Advances in pharmacology*. Vol. 34. San Diego: Academic Press.
- Iwamoto Y, Nakamura R, Folkers K, Morrison RF (1975). Study of periodontal disease and Coenzyme Q. *Res Commun Chem Pathol Pharmacol* 11:265-271.
- Iwamoto Y, Watanabe T, Okamoto H, Ohata N, Folkers K (1981). Clinical effect of Coenzyme Q₁₀ on periodontal disease. In: *Biomedical and clinical aspects of Coenzyme Q*. Vol. 3. Folkers K, Yamamura Y, editors. Amsterdam: Elsevier, pp. 109-119.
- Jacob RA, Omaye ST, Skala JH, Leggott PJ, Rothman DL, Murray PA (1987). Experimental vitamin C depletion and supplementation in young men. Nutrient interactions and dental health effects. *Ann NY Acad Sci* 498:333-346.
- Jacobson MD (1996). Reactive oxygen species and programmed cell death. *TIBS* 21:83-86.
- Kagan VE, Serbinova EA, Packer L (1990). Antioxidant effects of ubiquinones in microsomes and mitochondria are mediated by tocopherol recycling. *Biochem Biophys Res Commun* 169:851-857.
- Kagan VE, Serbinova EA, Fore T, Scita G, Packer L (1992). Recycling of vitamin E in human low density lipoprotein. *J Lipid Res* 33:385-397.
- Kagan VE, Nohl H, Quinn PJ (1996). Coenzyme Q: its role in scavenging and generation of radicals in membranes. In: *Handbook of antioxidants*. Cadenas E, Packer L, editors. New York: Marcel Dekker, Inc., pp. 157-201.
- Katsuragi H, Hasegawa A, Saito K (1997). Distribution of metallothionein in cigarette smokers and non smokers in advanced periodontitis patients. *J Periodontol* 68:1005-1009.
- Kettle AJ (1996). Neutrophils convert tyrosyl residues in albumin to chlorotyrosine. *FEBS Lett* 379:103-106.
- Khmelevskii IuV, Danilevskii NF, Borisenko AV, Poberezkina NV (1985). Effect of vitamin A, E and K on the indices of the glutathione antiperoxide system in gingival tissues in periodontitis. *Vopr Pitani* 4:54-56.
- Kimura S, Yonemura T, Kaya H (1993). Increased oxidative product formation by peripheral blood polymorphonuclear leukocytes in human periodontal disease. *J Periodont Res* 28:197-203.
- Kontush A, Meyer S, Finckh B, Kohlschütter A, Beisiegel U (1996). α -tocopherol as a reductant for Cu(II) in human lipoproteins. *J Biol Chem* 271:11106-11112.
- Lala A, Amano A, Sojar HT, Radel SJ, De Nardin E (1994). P. *gingivalis* trypsin-like protease: a possible natural ligand for the neutrophil formyl peptide receptor. *Biochem Biophys Res Commun* 199:1489-1496.
- Leino L, Hurttia HM, Sorvajarvi K, Sewon LA (1994). Increased respiratory burst activity is associated with normal expression of IgG-Fc-receptors and complement receptors in peripheral neutrophils from patients with juvenile periodontitis. *J Periodont Res* 29:179-184.
- Levitskii AP, Kozlianina NP, Skliar VE (1987). Lipid peroxidation and antioxidant systems in cat periodontal tissue. *Vopr Med Khim* 33:107-111.
- Li KL, Vogel R, Jeffcoat MK, Alfano MC, Smith MA, Collins JG, et al. (1996). The effect of ketoprofen creams on periodontal disease in rhesus monkey. *J Periodont Res* 31:525-532.
- Lister RE (1995). Coenzyme Q₁₀ and periodontal disease (letter). *Br Dent J* 179:200-201.
- Littarru GP, Nakamura R, Ho L, Folkers K, Kuzell WC (1971). Deficiency of coenzyme Q₁₀ in gingival tissue from patients with periodontal disease. *Proc Natl Acad Sci USA* 68:2332-2335.
- Littarru GP, Battino M, Folkers K (1996). Clinical aspects of coenzyme Q: improvement of cellular bioenergetics or antioxidant protection? In: *Handbook of antioxidants*. Cadenas E, Packer L, editors. New York: Marcel Dekker, Inc., pp. 203-239.
- Livrea MA, Tesoriere L, Freisleben HJ (1996). Vitamin A as an antioxidant. In: *Handbook of antioxidants*. Cadenas E, Packer L, editors. New York: Marcel Dekker, Inc., pp. 371-405.
- Lodi R, Rinaldi R, Gaddi A, Iotti S, D'Alessandro R, Scoz N, et al. (1997). Brain and skeletal muscle bioenergetics failure in familial hypobetalipoproteinemia. *J Neurol Neurosurg Psychiatry* 62:574-580.
- Loesche WJ, Robinson JP, Flynn M, Hudson JL, Duque RE (1988). Reduced oxidative function in gingival crevicular neutrophils in periodontal diseases. *Infect Immun* 56:156-160.
- Marton IJ, Balla G, Hegedus C, Redi P, Szilagyi Z, Karmazsin L, et al. (1993). The role of reactive oxygen intermediates in the pathogenesis of chronic apical periodontitis. *Oral Microbiol Immunol* 8:254-257.
- Mataix J, Mañas M, Quiles JL, Battino M, Cassinello M, Lopez-Frias M, et al. (1997). Coenzyme Q content depends upon oxidative stress and dietary fat unsaturation. *Molec Aspects Med* 18(Suppl):129-135.
- Matsumura T, Kimata M, Tsunemitsu A, Akiyoshi M (1969a). Effect of coenzyme Q administration on the periodontal lesions in citrated rats. *Koku Eisei Gakkai Zasshi* 19:134-142.
- Matsumura T, Kimata M, Tsunemitsu A, Akiyoshi M (1969b). Effect of coenzyme Q on blood-citrate and calcium lev-

- els and on the periodontium of citrated rats. *J Dent Res* 48:1264-1267.
- Matsumura T, Saji S, Nakamura R, Folkers K (1973). Evidence for enhanced treatment of periodontal disease by therapy with Coenzyme Q. *Int J Vitam Nutr Res* 43:537-538.
- McCord JM (1993). Human disease, free radicals, and the oxidant/antioxidant balance. *Clin Biochem* 26:351-357.
- McRee JT, Hanioka T, Shizukuishi S, Folkers K (1993). Therapy with Coenzyme Q₁₀ for patients with periodontal disease. 1. Effect of Coenzyme Q₁₀ on subgingival micro-organisms. *J Dent Health* 43:659-666.
- Meyer MR, Schreck R, Baeuerle PA (1993). Hydrogen peroxide and antioxidants have opposite effects on activation of NF- κ B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. *EMBO J* 12:2005-2015.
- Mishchenko VP, Silenko Iul, Khavinson VKh, Tokar' DL (1991). The effect of periodontal cytomedin on lipid peroxidation and hemostasis in spontaneous periodontitis in rats. *Stomatologiia* 70(5):12-14.
- Miyasaki KT (1991). The neutrophil: mechanisms of controlling periodontal bacteria. *J Periodontol* 62:761-774.
- Moore S, Calder KA, Miller NJ, Rice-Evans CA (1994). Antioxidant activity of saliva and periodontal disease. *Free Rad Res* 21:417-425.
- Morimoto RI (1993). Cells in stress: transcriptional activation of heat shock genes. *Science* 259:1409-1410.
- Mouynet P, Delamaire M, Legoff MC, Genetet B, Yardin M, Michel JF (1994). Ex vivo studies of polymorphonuclear neutrophils from patients with early onset periodontitis. (II). Chemiluminescence response analysis. *J Clin Periodontol* 21:533-539.
- Nakamura R, Littarru GP, Folkers K, Wilkinson EG (1973). Deficiency of coenzyme Q in gingiva of patients with periodontal disease. *Int J Vitam Nutr Res* 43:84-92.
- Niki E (1996). α -Tocopherol. In: Handbook of antioxidants. Cadenas E, Packer L, editors. New York: Marcel Dekker, Inc., pp. 3-25.
- Nohl H, Jordan W (1986). The mitochondrial site of superoxide formation. *Biochem Biophys Res Commun* 138:533-539.
- Pabst MJ, Pabst KM, Collier JA, Coleman TC, Lemons-Prince ML, Godat MS, *et al.* (1995). Inhibition of neutrophil and monocyte defensive functions by nicotine. *J Periodontol* 66:1047-1055.
- Packer L, Witt EH, Tritschler HJ (1996). Antioxidant properties and clinical applications of α -lipoic acid and dihydrolipoic acid. In: Handbook of antioxidants. Cadenas E, Packer L, editors. New York: Marcel Dekker, Inc., pp. 545-591.
- Page RC, Kornman KS, editors (1997). The pathogenesis of human periodontitis: an introduction. *Periodontol* 2000 14:9-11.
- Palozza P, Krinsky NI (1992). Antioxidant effects of carotenoids *in vivo* and *in vitro*: an overview. *Meth Enzymol* 213:403-420.
- Park EM, Shigenaga MK, Degan P, Korn TS, Kitzler JW, Wehr CM, *et al.* (1992). Assay of excised oxidative DNA lesions: isolation of 8-oxoguanine and its nucleoside derivatives from biological fluids with a monoclonal antibody column. *Proc Natl Acad Sci USA* 89:3375-3379.
- Parrish JH Jr, De Marco TJ, Bissada NF (1977). Vitamin E and periodontitis in the rat. *Oral Surg Oral Med Oral Pathol* 44:210-218.
- Petrovich IuA, Podorozhanaia RP, Genesina TI, Beloklitskaia GF (1996). Iron in oral cavity fluid in gingival inflammation. *Patol Fiziol Eksp Ter* 3:22-24.
- Primiano T, Sutter TR, Kensler TW (1997). Antioxidant-inducible genes. In: Antioxidants in disease mechanisms and therapy. Sies H, editor. Advances in pharmacology. Vol. 38. San Diego: Academic Press, pp. 293-328.
- Pryor WA (1986). Oxy-radicals and related species: their formation, lifetimes and reactions. *Ann Rev Physiol* 48:657-667.
- Quiles JL, Huertas JR, Mañas M, Battino M, Cassinello M, Littarru GP, *et al.* (1994). Peroxidative extent and coenzyme Q levels in the rat: influence of physical training and dietary fats. *Molec Aspects Med* 15(Suppl):89-95.
- Roberfroid M, Buc Calderon P (1995). Free radicals and oxidation phenomena in biological systems. New York: Marcel Dekker, Inc.
- Rice-Evans C, Burdon R (1993). Free radical-lipid interactions and their pathological consequences. *Prog Lipid Res* 32:71-110.
- Sato K, Niki E, Shimasaki H (1990). Free radical mediated chain oxidation of low density lipoprotein and its synergistic inhibition by vitamin E and C. *Arch Biochem Biophys* 279:402-405.
- Schmidt AM, Weidman E, Lalla E, Yan SD, Hori O, Cao R, *et al.* (1996). Advanced glycation endproducts (AGEs) induce oxidant stress in the gingiva: a potential mechanism underlying accelerated periodontal disease associated with diabetes. *J Periodont Res* 31:508-515.
- Schneider HG, Pose G (1968). The effect of oral vitamin A supply on periodontal alterations due to prolonged hypovitaminosis A. *Dtsch Stomatol* 18:408-423.
- Schneider HG, Pose G (1969). Influence of tocopherol on the periodontium of molars in rats fed a diet lacking in vitamin E. *Arch Oral Biol* 14:431-433.
- Schreck R, Alberman K, Bauerle PA (1992). Nuclear factor KB: an oxidative stress-responsive transcription factor of eucaryotic cells. *Free Rad Res Commun* 17:221-237.
- Sela MN, Weinberg A, Borinsky R, Holt SC, Dishon T (1988). Inhibition of superoxide production in human polymorphonuclear leukocytes by oral treponemal factors. *Infect Immun* 56:589-594.

- Sevanian A, Hochstein P (1985). Mechanisms and consequences of lipid peroxidation in biological systems. *Ann Rev Nutr* 5:365-390.
- Sevanian A, Peterson AR (1984). Cholesterol epoxide is a direct-acting mutagen. *Proc Natl Acad Sci USA* 81:4198-4202.
- Sevanian A, Peterson AR (1986). The cytotoxic and mutagenic properties of cholesterol oxidation products. *Food Chem Toxicol* 24:1103-1010.
- Sevanian A, Hodis HN, Hwang J, Peterson H (1995). Involvement of cholesterol oxides in the atherogenic action of oxidised LDL. In: Free radicals, lipoprotein oxidation and atherosclerosis. Bellomo G, Finardi G, Maggi E, Rice-Evans C, editors. London: Richelieu Press, pp. 139-161.
- Seymour GJ, Whyte GJ, Powell RN (1986). Chemiluminescence in the assessment of polymorphonuclear leukocyte function in chronic inflammatory periodontal disease. *J Oral Pathol* 15:125-131.
- Shapira L, Borinski R, Sela MN, Soskolne A (1991). Superoxide formation and chemiluminescence of peripheral polymorphonuclear leukocytes in rapidly progressive periodontitis. *J Clin Periodontol* 18:44-48.
- Shapira L, Gordon B, Warbington M, Van Dyke TE (1994). Priming effect of *P. gingivalis* lipopolysaccharide on superoxide production by neutrophils from healthy and rapidly progressive periodontitis subjects. *J Periodontol* 65:129-133.
- Shapira L, Schatzker Y, Gedalia I, Borinski R, Sela MN (1997). Effects of amine and stannous fluoride on human neutrophil functions *in vitro*. *J Dent Res* 76:1381-1386.
- Shizukuishi S, Inoshita E, Tsunemitsu A, Takahashi K, Kishi T, Folkers K (1984). Therapy by Coenzyme Q₁₀ of experimental periodontitis in a dog-model supports results of human periodontitis therapy. In: Biomedical and clinical aspects of Coenzyme Q. Vol. 4. Folkers K, Yamamura Y, editors. Amsterdam: Elsevier, pp. 153-162.
- Shizukuishi S, Hanioka T, Tsunemitsu A, Fukunaga Y, Kishi T, Sato N (1986). Clinical effect of Coenzyme Q₁₀ on periodontal disease; evaluation of oxygen utilisation in gingiva by tissue reflectance spectrophotometry. In: Biomedical and clinical aspects of Coenzyme Q. Vol. 5. Folkers K, Yamamura Y, editors. Amsterdam: Elsevier, pp. 359-368.
- Sies H (1986). Biochemistry of oxidative stress. *Angew Chem Int Ed Engl* 25:1058-1071.
- Sies H, editor (1997). Antioxidants in disease mechanisms and therapy. In: Advances in pharmacology. Vol. 38. San Diego: Academic Press.
- Silenko Iul, Mishchenko VP, Tokar' DL, Khavinson VKh, Zhukova Mu (1991). The mechanism of the therapeutic effect of periodontal cytomedin on the course of experimental periodontitis. *Stomatologiia* 70(4):13-15.
- Silenko Iul, Tsebrzhinskii OI, Mishchenko VP (1992). The mechanisms of the action of fluorine on periodontal tissue. *Fiziol Zh* 38:85-90.
- Silenko Iul, Vesnina LE, Mishchenko VP (1994a). Effect of parodontium polypeptide on the lipid peroxidation of the membranes and coagulation of erythrocytes in rats with spontaneous parodontitis. *Fiziol Zh* 40:88-91.
- Silenko Iul, Mishchenko VP, Tokar' DL, Khavinson VKh, Popsuiko GI (1994b). The effect of periodontal cytomedin on free-radical lipid oxidation and on anti-aggregation activity in the periodontium in chronic stress. *Stomatologiia* 73(4):6-8.
- Simic MG (1990). Pulse radiolysis in study of oxygen radicals. *Meth Enzymol* 186:89-100.
- Slade EW Jr, Bartuska D, Rose LF, Cohen DW (1976). Vitamin E and periodontal disease. *J Periodontol* 47:352-354.
- Speirs RL, Beeley JA (1992). Food and oral health: 2. Periodontium and oral mucosa. *Dent Update* 19:161-167.
- Stadtman ER (1992). Protein oxidation and ageing. *Science* 257:1220-1224.
- Stocker R, Bowry VW (1996). Tocopherol-mediated peroxidation of lipoprotein lipids and its inhibition by co-antioxidants. In: Handbook of antioxidants. Cadenas E, Packer L, editors. New York: Marcel Dekker, Inc., pp. 27-41.
- Stocker R, Bowry VW, Frei B (1991). Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does α -tocopherol. *Proc Natl Acad Sci USA* 88:1646-1650.
- Storz G, Tartaglia LA (1992). OxyR: a regulator of antioxidant genes. *J Nutr* 122:627-630.
- Suomalainen K, Sorsa T, Lindy O, Saari H, Konttinen YT, Uitto VJ (1991). Hypochlorous acid induced activation of human neutrophil and gingival crevicular fluid collagenase can be inhibited by ascorbate. *Scand J Dent Res* 99:397-405.
- Tang-Larsen J, Claesson R, Edlund MB, Carlsson J (1995). Competition for peptides and amino acids among periodontal bacteria. *J Periodont Res* 30:390-395.
- Tarasenko LM, Voskresenskii ON (1986). Role of lipid peroxidation in the pathogenesis of periodontal damage in stress. *Patol Fiziol Eksp Ter* 6:12-14.
- Thomas SR, Neuzil J, Stocker R (1997). Inhibition of LDL oxidation by ubiquinol-10. A protective mechanism for coenzyme Q in atherogenesis? *Molec Aspects Med* 18(Suppl):85-103.
- Trykowski J, Patalias B, Senator M (1994). The use of tooth-paste with vitamin A in treating periodontitis. *Stomatologiia* 73(1):11-13.
- Tsunemitsu A, Matsumura T (1967). Effect of Coenzyme Q administration on hypercitricemia of patients with periodontal disease. *J Dent Res* 46:1382-1384.
- Tsunemitsu A, Honjo K, Nakamura R, Kani M, Matsumura T (1968). Effect of ubiquinone 35 on hypercitricemia. *J Periodontol* 39:215-218.

- Van Dyke TE, Zinney W, Winkel K, Taufiq A, Offenbacher S, Arnold RR (1986). Neutrophil function in localized juvenile periodontitis: phagocytosis, superoxide production and specific granule release. *J Periodontol* 57:703-708.
- Villa RF, Gorini A (1997). Pharmacology of lazaroids and brain energy metabolism: a review. *Pharmacol Rev* 49:99-136.
- von Sonntag C (1987). The chemical basis of radiation biology. London: Taylor & Francis.
- Voskresenskii ON, Tkachenko EK (1991). The role of lipid peroxidation in the pathogenesis of periodontitis. *Stomatologiya* 70(4):5-10.
- Waerhaug J (1967). Prevalence of periodontal disease in Ceylon. Association with age, sex, oral hygiene, socioeconomic factors, vitamin deficiencies, malnutrition, betel and tobacco consumption and ethnic group. Final report. *Acta Odontol Scand* 25:205-231.
- Watts TL (1995). Coenzyme Q₁₀ and periodontal treatment: is there any beneficial effect? *Br Dent J* 178:209-213.
- Whyte GJ, Seymour GJ, Cheung K, Robinson MF (1989). Chemiluminescence of peripheral polymorphonuclear leukocytes from adult periodontitis. *J Clin Periodontol* 16:69-74.
- Wilkinson EG, Arnold RM, Folkers K, Hansen I, Kishi H (1975). Bioenergetics in clinical medicine. II. Adjunctive treatment with Coenzyme Q in periodontal disease. *Res Commun Chem Pathol Pharmacol* 12:111-123.
- Wilkinson EG, Arnold RM, Folkers K (1976). Bioenergetics in clinical medicine. VI. Adjunctive treatment of periodontal disease with Coenzyme Q₁₀. *Res Commun Chem Pathol Pharmacol* 14:715-719.
- Wilkinson EG, Arnold RM, Folkers K (1977). Treatment of periodontal and other soft tissue diseases of the oral cavity with Coenzyme Q. In: Biomedical and clinical aspects of Coenzyme Q. Vol. 1. Folkers K, Yamamura Y, editors. Amsterdam: Elsevier, pp. 251-265.
- Winrow VR, Winyard PG, Morris CJ, Blake DR (1993). Free radicals in inflammation: second messengers and mediators of tissue destruction. *Br Med Bull* 49:506-522.
- Winyard PG, Blake DR (1997). Antioxidants, redox-regulated transcription factors, and inflammation. In: Antioxidants in disease mechanisms and therapy. Sies H, editor. Advances in pharmacology. Vol. 38. San Diego: Academic Press, pp. 403-421.
- Yamamoto Y, Komuro E, Niki E (1990). Antioxidant activity of ubiquinol in solution and phosphatidylcholine liposome. *J Nutr Sci Vitaminol* 36:505-511.
- Zafiropoulos GG, Flores-De-Jacoby L, Czerch W, Kolb G, Markitzu A, Havemann K (1988). Neutrophil function in patients with localised juvenile periodontitis and with rapidly progressive periodontitis. *J Biol Buccale* 16:151-156.
- Zafiropoulos GG, Flores-de-Jacoby L, Plate VM, Eckle I, Kolb G (1991). Polymorphonuclear neutrophil chemiluminescence in periodontal disease. *J Clin Periodontol* 18:634-639.