Free Radicals and Antioxidants: Who they are, who generates them and what is the mechanism of action.

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ABSTRACT

Oxidative stress it is a form of "chemical stress" induced in our organism by an imbalance between the production of highly reactive chemical species (free radicals) and the level of physiological defense capabilities (antioxidants).

Free Radicals (ROS) are molecules that, due to their instability, are constantly looking for other molecules to attach to, like molecular sharks.

While the antioxidants are "scavengers", molecular, exogenous scavengers and endogenous.

They “swallow” excess radicals, preventing them from damaging the body.

But sometimes they can be genetically altered (endogenous) or quantitatively scarce (exo-endogenous).

But we ask ourselves what is their list, source and action? The aim of this study is to answer this question.

Keywords : superoxide anion, hydroperoxide radical, resveratrol, 4-hydroxytyrosol and polyunsaturated fatty acids

1. INTRODUCTION

Chemical stress induced by the presence, in a living organism, of an excess of reactive chemical species, generally centered on oxygen (reactive oxygen species, ROS), secondary to an increased production of the same and/or a reduced efficiency of the physiological systems of antioxidant defense (1).

The most accredited theory on aging is Harman's, i.e. the free radical theory.

The mitochondrion is the main creator of the cellular Red-Ox balance, in fact the energy center of the cell.

The mitochondrion is responsible for producing as much ATP as possible consider fuel.
The decrease in the efficiency of production processes ATP are associated with increased ROS generation.

It should be noted that ROS are generated in both intra- and extra-cellular processes and lead to the induction of damage to DNA (but also to all other cellular structures).

1 – Depolarization of the mitochondrial membrane

2 – Reduction of cytosolic and mitochondrial GSH

3 – Reduction of intracellular ATP (2).

1.1 FREE RADICALS (ROS) are molecules that, due to their instability, are constantly looking for other molecules to attach to, like molecular sharks, see Tab. 1.

Tab. 1 The *main* ROS are:

$^{\bullet}O_2^-$ (**superoxide anion**),
$^{\bullet}O_2H$ (**hydroperoxide radical**);
$^{\bullet}OH$ (**hydroxyl radical**);
$NO^{\bullet}$ (**nitrogen monoxide** or **nitric oxide**);
$ONOO^-$ (**peroxynitrite anion**).

Radicals:

$NO_2^{-\bullet}$ (**nitrogen dioxide anion radical**)
$RO^{\bullet}$ (**Alkoxyl radical**)
$ROO^{\bullet}$ (**Peroxyl Radical**)
$R^{\bullet}$ (**Alkyl radical**)
$Q^{\bullet}$ **Semiquinone** (**from Coenz. Q**)
$E\text{-}O^{\bullet}$ (**Phenoxyl** (**from vitamin E**)
$-S^{\bullet}$ (**Tiile**)

Also:

H_2O_2 (**hydrogen peroxide**)
HOCl (**hypochlorous acid**)

Non radicals:

$^{1}O_2$ (**Singlet oxygen**)
O_3 (**Ozone**)
ROOH (**Organic peroxide**)
HOBr (**Hypobromous acid**)
OH^- (**hydroxyl**)
H^- (**hydrogen**)
O^+ (**oxygen)**
HNO_2 (**Nitrous Acid**)
N_2O_4 (**Nitric tetroxide**)
N_2O_3 (**Nitric trioxide**)
ONOOH (**Peroxynitrous acid**)
NO_2^+ (**Nitronium cation**)
ROONO (**(Alkyl)peroxynitrite**)

Superoxide anion: It plays a crucial role in the formation of other radical species. It has low reactivity but high diffusion.

Hydroxyl radical: It has the highest reactivity but has low diffusion. It is capable of attacking all basic biochemical structures: lipids, proteins and DNA bases.

Nitrogen monoxide: Its toxicity is linked to the formation of peroxynitrite. Peroxynitrite causes direct oxidation of proteins and DNA.

Peroxyl radical: It is very reactive and is involved in the propagation phases of radical chain reactions of lipid peroxidation.

Nitrogen dioxide anion radical, can also trigger lipid peroxidation reactions.

Hydrogen peroxide: It is not a radical as such because it does not have unpaired electrons but it is capable of generating radicals and diffuses quickly.

Singlet oxygen: Can react with double bonds, for example fatty acids, producing hydroperoxides (3-4).

Generate Oxidative Stress:

- cigarette smoking
- solar and ionizing radiation
- intense physical effort
- infections and inflammations
- unbalanced diet
- environmental pollution
- heavy metals
- chronic taking or abuse of drugs
- abuse of alcoholic substances.

1 Some dysfunctions and pathological states such as cardiovascular diseases, rheumatoid arthritis, inflammatory states in general, trauma to the nervous system, etc.;

2 Diets too rich in proteins and animal fats. A high-calorie diet increases the extent of oxidative stress, while a low-calorie diet reduces it. A 2400 calorie diet requires 660 g of oxygen, of which 90-95% is used for respiration, while the remainder gives rise to reactive forms of oxygen;

3 The presence of an excess of iron which, in the first phase of the transformation, causes the hydroxyl radical to be released from the hydrogen peroxide, which is capable of activating further harmful chemical reactions;

4 Ionizing and solar radiation (excess ozone and UVA and UVB rays). Solar radiation induces photooxidation processes on the skin which degrade the polyunsaturated fatty acids of the cell membranes and consequent formation of free radicals;

5 The action of polluting gases and toxic substances in general (C and PB monoxides produced by engine combustion);

6 Intense physical activity, both resistance and muscular strength, causes a notable increase in reactions that use oxygen and consequent surplus formation of hydrogen peroxide. The biochemical reactions linked to the accumulation and removal of lactic acid from tired muscles contribute to raising the threshold of free radicals (5).

Independent chemical species, characterized by one or more unpaired electrons present in one of its atomic or molecular orbitals.

They can result from the loss or gain of an electron by a non-radical compound, or by the breaking of a covalent bond, with separation of the pair of electrons on different atoms.

Oxidation, or the transfer of one or more electrons, is the chemical basis of oxidative stress.

They are essentially produced during at least four biological processes:

- During the final oxidation of nutritional substrates in the mitochondria;
- In cell-mediated immune reactions; In phase I hepatic detoxification reactions;
- In the reperfusion phases of tissues affected by ischemic phenomena.

Mechanism of action of ROS:

START: formation of free radicals

PROPAGATION: free radicals react with other molecules to produce more free radicals

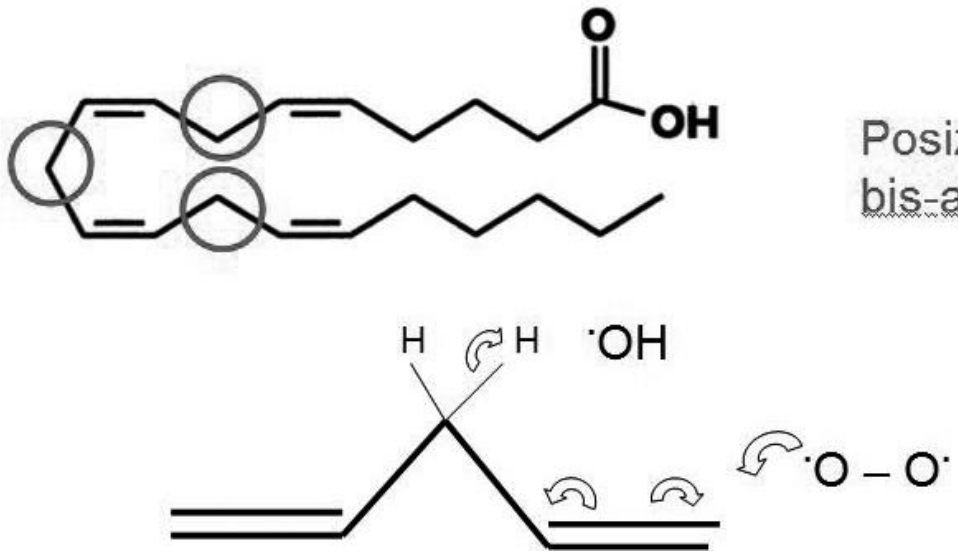
TERMINATION: Free radicals react with each other to form molecules

Oxygen, essential for life, can become, in particular conditions, a killer danger: he has a double face (6).

Thus, if in excess, due to the production or reduced activity of antioxidant defenses, they damage the lipids, proteins and DNA in the cells, contributing to the birth and worsening of various pathologies. If they are in balance with antioxidants, they also play very useful roles for the body.

Lipid peroxidation→Membrane→ Fluidity and permeability

- PUFA (Es. ac. arachidonico)



One of the sites most sensitive to damage caused by ROS is the plasma membrane, in particular the target is at the level of polyunsaturated fatty acids.

- OH (The hydroxyl radical) removes a hydrogen atom from a polyunsaturated fatty acid, thus starting a chain of lipid peroxidation reactions (Fig. 1) (7-8).

Table 2 **Clinical conditions in which it was suggested the involvement of oxidative damage**
Immune-inflammatory diseases AIDS Rheumatoid arthritis
Degenerative syndromes Aging Alzheimer's disease Parkinson's disease Atherosclerosis Cataractogenesis
Diseases caused by toxic environmental factors Pulmonary emphysema Ethylism Acute hepatitis endotoxin Transfusion syndrome Cancer, Chemicals Hemolytic syndromes (favism)
Diseases caused by physical factors Exposure to ionizing radiation
Ischemia-reperfusion syndromes Heart attack, Cerebral infarction Transplantation of organs

CANCER

- Stress facilitates early tumor development experimentally induced and its metastasis.
- Stress abolishes natural genetic resistance to experimental tumors.
- Stress reduces survival after tumor implantation.
- Stress causes chromosomal aberrations, alterations of DNA repair mechanisms (9) see Tab. 2.

1.2 ANTIOXIDANTS

They are "scavengers", molecular, exogenous scavengers and endogenous.

They “swallow” excess radicals, preventing them from damaging the body.

But sometimes they can be genetically altered (endogenous) or quantitatively scarce (exo-endogenous).

Imbalance of antioxidant processes:

- **Reduced intake**
- and/or **decreased synthesis**
- and/or **reduced capacity of use**
- and/or **increased consumption of antioxidants**.

The most common **mechanism of action** is the donation of an electron by the antioxidant.

Antioxidants preventatives: They block the formation of radicals

Antioxidants scavenger: They block the initiation reaction and propagation

Antioxidants of repair and de novo: They repair the damage and reconstitute the membranes

Also

PRIMARY: They prevent the formation of radical species or sequester transition metals.

SECONDARY: They react with formed radicals and convert them into less reactive forms, interrupting the chain reaction.

Tab. 3 Main ANTIOXIDANTS

Endogenous	Exogenous **(Diet)**
INTRA or ENZYMES	Phytochemicals
SOD (Mn e Cu/Zn),CAT, GPX, GRD, GST, NOS, Desaturates and Lipoic acid	Phenols, polyphenols and **Carotenoids** (Wine, Beer, Tea, Coffee, Pomegranate and Onion) [Resveratrol], {flavonoids, phenolic acids, stilbenes or lignans} **Flavonoids:** anthocyanins, flavones, flavanones, isoflavones, and flavanols (Quercetin and Chlorophyll) ***Flavanols:*** Catechins, Epicatechins, and Proanthocyanidins
EXTRA or Metal chelating molecules	Phytosterols
Albumin, Ferritin, Transferrin, Ceruloplasmin, Lactoferrin	Saponins
Vitamins	Hydroxytyrosol and Oleuropein
A [**Carotenoids**] (β-Carotene), C, E, Lycopene and *bio*flavonoids	PUFAs (Fatty acids, Omega 3-6)
(Vitamin similar)	Amino acids
Coenzyme Q_{10} (Ubiquinone), Lipoic acid	Methionine, Arginine, Histidine, Cysteine, lysine and Tryptophan
Minerals/salts/ions	Proteins
Se, Zn, Mg^{2+}, Cr, Cu^{2+}, Ge, Mn, Mo and K	Proteins-SH, Metal binders (Fe, Cu)
Thiols	Other molecules
GSH and Thioredoxin	Uric acid, Creatine and bilirubin

***** Oxycarotenoids (Zeaxanthin and lutein)
** 2-deoxy-D-glucose
*** Melatonin

SOD: Converts superoxide anion and hydrogen peroxide.

Localized in the mitochondria (Mn-dip) and in the cytosol (Cu/Zn-dip) see Tab. 3.

Primary cellular defense against superoxide anion.

CAT: They reduce hydrogen peroxide into water and oxygen.

Primary cellular defense against hydrogen peroxide.

Located in mitochondria and peroxisomes (Fe-dip).

GPX: They reduce peroxides in the presence of glutathione.

Different substrates (hydrogen peroxide, organic peroxides).

Requires selenium (cofactor) and glutathione (reducer).

Different localizations (cells, extracellular fluids).

GSH: Low MW endogenous hydrophilic tripeptide (GLU-CYS-GLY).

Glutathione peroxidase (GPX) cofactor.

Allows GPX to perform its antioxidant role.

Vit. C: Low molecular weight, hydrophilic, exogenous substance.

Scavenger against various radicals (HO·, ROO· and $^{\bullet}O_2^-$).

By reducing the tocopheryl radical it regenerates vitamin E.

Uric acid: Low molecular weight, hydrophilic substance.

Powerful scavenger against various oxidants (HO·, $^{*}O_2$, O_3, HCLO).

Lipoic acid: Low molecular weight, relatively hydrophilic substance.

Scavenger against various oxidants (HO·, O_2*, HCIO).

Allows the regeneration of vitamins C and E.

Coenzyme Q_{10}: Low molecular weight lipophilic substance.

Scavenger against peroxyl radicals (ROO·).

Allows the regeneration of tocopherols.

Polyphenols and flavonoids: Large class of naturally occurring substances.

They include anthocyanins and anthoxanthins (fruits and vegetables).

Scavenger action against HO· and $^{\bullet}O_2^-$ radicals.

Possible anti-atherogenic action.

Enzymes: The activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) constitute a first line antioxidant defence system.

Regarding vitamins in general**:** A, in synergy with E, selenium and glutathione peroxidase, prevent lipid peroxidation.

C reduces vitamin E and in synergy with it protects the skin from UVA and UVB.

C is the most important antioxidant and a very capable scanvenger.

A prevents the oxidation of C and acts in synergy with the B Complex. E, Ca and P.

Regarding minerals in general**:** Some play a crucial role in some enzymatic systems (CuZnSOD, MnSOD, seleno-dependent GSH peroxidase).

Others have their own antioxidant activity.

Still others are heavy metal antagonists.

As for polyphenols: Large class of compounds derived from secondary metabolism of plants.

Chemically they are cyclic derivatives of benzene substituted with hydroxyl groups.

They include either simple molecules such as phenolic acids or highly polymerized ones such as tannins.

Scavenger against HO• and $\bullet O_2^-$ radicals (10,11).

A correct diet suited to the needs of the individual could be sufficient to inactivate the radical species without using supplementation with antioxidants.

But food intake is often variable; therefore, supplementation with appropriate antioxidant molecules may be necessary, always keeping at least the level of free radicals and the antioxidant capacity in the blood under control.

The protection necessary for an organism, to be effective, MUST be: specific, balanced and complete.

2. CONCLUSIONS

In reality, Harman's theory of aging does not work completely.

He is unable to explain many things and above all he has so far failed in the use of the "Antidote" to ROS: Antioxidants.

Anyway although many age-associated degenerative processes can
be explained through Harman's theory, or similar theories,
it must be recognized that a series of processes appear codified:

Aging and death are programmed.

REFERENCES

1. D J Betteridge **What is oxidative stress?** Metabolism 2000 Feb;49(2 Suppl 1):3-8.
2. D Harman **Free radical theory of aging: history** EXS 1992:62:1-10.
3. Alugoju Phaniendra Dinesh Babu Jestadi Latha Periyasamy **Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases** Ind J Clin Biochem (Jan-Mar 2015) 30(1):11–26.
4. Eva Tvrdá, Filip Benko **Free radicals: what they are and what they do** Pathology Oxidative Stress and Dietary Antioxidants 2020, Pages 3-13.
5. E Cadenas, K J Davies **Mitochondrial free radical generation, oxidative stress, and aging** Free Radic Biol Med 2000 Aug;29(3-4):222-30.
6. Saverio Russo **Initiation, propagation and termination in free radical copolymerization** Volume10-11, Issue1 October 1987 Pages 395-414.
7. Ayesha Rahman Ahmed, Fred F. Farris, Sidhartha D. Ray **Lipid peroxidation** Reference Module in Biomedical Sciences 2023
8. Jonas Van der Paal, Erik C. Neyts, Christof C. W. Verlackt, and Annemie Bogaerts **Effect of lipid peroxidation on membrane permeability of cancer and normal cells subjected to oxidative stress** Chem Sci. 2016 Jan 1; 7(1): 489–498.
9. John D Hayes , Albena T Dinkova-Kostova, Kenneth D Tew **Oxidative Stress in Cancer Cancer Cell** 2020 Aug 10;38(2):167-197.
10. Barry Halliwell **Antioxidants: The Basics-what they are and how to Evaluate them** Advances in Pharmacology Volume 38, 1996, Pages 3-20.
11. Attila Hunyad **The mechanism(s) of action of antioxidants: From scavenging reactive oxygen/nitrogen species to redox signaling and the generation of bioactive secondary metabolites** November 2019 Volume 39, Issue 6 Pages 2505-2533.