Introduction

Free radicals are atoms or molecules that contain one or more unpaired electrons. Many radicals are highly reactive and can function as reducing or oxidizing agents by donating electrons to or removing electrons from other molecules. Small amounts of free radicals are constantly being generated in all living organisms. Although free radicals are potentially harmful to cellular components, a substantial body of evidence supports a role for these highly reactive chemical molecules in fundamental cellular reactions and cell cycle regulation. Innate cellular antioxidants provide protection against the noxious effects of chemical reactions involving free radicals. However; occasionally biological processes can result in an increased generation of free radicals, which can exceed the capacity of the cell's antioxidant defense systems and result in oxidative damage to proteins, lipids, and DNA, with possible cell dysfunction or death ***(Caraciolo and Donough, 2004).***

Free radicals have been implicated in the pathogenesis of a wide spectrum of human diseases. Premature infants are probably developmentally unprepared for extrauterine life in an oxygen-rich environment and exhibit a unique sensitivity to oxidant injury. Diseases associated with premature infants, including bronchopulmonary dysplasia, periventricular leukomalacia, intraventricular hemorrhage, retinopathy of prematurity, and necrotizing enterocolitis, have been linked to free radical-mediated cell and tissue injury ***(Donough et al., 2004).***

Furthermore, Newborns and particularly preterm infants are at high risk of oxidative stress and they are very susceptible

to free radical oxidative damage. Indeed, there is evidence of an imbalance between antioxidant- and oxidant-generating systems which causes oxidative damage. The brain may be especially at risk of free radical-mediated injury because neuronal membranes are rich in polyunsaturated fatty acids and because the human newborn has a relative deficiency of brain superoxide dismutase and glutathione peroxidase. The brain of the term fetus is at higher risk of oxidative stress than that of the preterm fetus, as a consequence of its higher concentration of polyunsaturated fatty acids and the maturity of the N-methyl-D-aspartate receptor system at term. There seems to be a maturation-dependent window of vulnerability to free radical attack during oligodendrocyte development. Early in its differentiation, the oligodendrocyte may be vulnerable because of active acquisition of iron for differentiation at a time of relative delay in the development of certain key antioxidant defenses in the brain. Excess free iron and deficient iron-binding and metabolizing capacity are additional features favoring oxidant stress in premature infants. Free radicals may be generated by different mechanisms, such as ischemia-reperfusion, neutrophil and macrophage activation, Fenton chemistry, endothelial cell xanthine oxidase, free fatty acid and prostaglandin metabolism and hypoxia. Reactive oxidant production by these different mechanisms contributes in a piecewise manner to the pathogenesis of perinatal brain injury ***(Karger and Basel, 2001).***

Oxidative stress (OS) results from an imbalance between reducing agents and enzymes involved in the removal of free radicals (FR) and/or reactive oxygen species (ROS). The

consequence of OS on fetal structure involves the activation of a complex array of genes involved in inflammation, coagulation, fibrinolysis, cell cycle and signal transduction ***(Wagenaar et al., 2004)***. It is now recognized that ROS are important for fertilization and developing embryos ***(Dennery, 2004)***. In moderate quantities and in presence of a good antioxidant capacity, FRs are continuously generated in the organism and are essential for cell aerobic metabolism and fetal growth, but they are toxic when overproduced, resulting in an attack of all classes of biological macromolecules, polysaccharides, nucleic acid, lipids and proteins ***(Halliwell, 1994b)***. Hypoxia, hyperoxia, inflammation, Fenton chemistry, endothelial damage, arachidonic acid cascade are other mechanisms that lead to the formation of highly reactive products. FR reactions lead to DNA damage (fragmentation, apoptosis, base modifications and strand breaks), to lipid, protein and polysaccharides oxidation and as a consequence FR reactions may induce a wide range of biological toxic effects ***(Saugstad, 1996a).***

Besides, Radiation-induced carcinogenesis may be initiated by radicals. The signs produced by chronic dietary deficiencies of selenium (Keshan disease) or of vitamin E (neurological disorders seen in patients with inborn errors in the mechanism of intestinal fat absorption) could also be mediated by oxidants. In the premature infant, exposure of the incompletely vascularized retina to elevated concentrations of oxygen can lead to retinopathy of prematurity, which in its worst forms can result in blindness ***(Diplock, 1985).***

Aim of the Work

This work will review current information regarding the status and development of antioxidant systems in the newborn, those conditions in which free radicals have been implicated, and the role of antioxidant therapy in these conditions.

Oxidative Stress

(Free radicals and Antioxidants)

What is oxidative stress ?

Oxidative stress has been defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses, which may lead to tissue injury ***(Halliwell,1994a)***.

Free Radicals (F.R.):

Electrons in atoms occupy regions of space known as orbitals. Each orbital can hold a maximum of two electrons. ***(Halliwell et al.,1992).***

Free radicals can be defined as any species capable of independent existence (hence the term) free that contains one or more unpaired electrons ***(Gutteridge and Halliwell, 1994).***

Unpaired electrons increase the chemical reactivity of an atom or molecule ***(Betteridge, 2000).*** Each free radical formed by the body can initiate a series of chain reactions, which continue until free radicals are removed. Free radicals disappear from the body following reactions with other free radicals, or more importantly, due to the actions of the antioxidant system ***(McCord, 1985).***

Types of free radicals:

I- Oxygen - centered free radicals:

1) Molecular oxygen either triplet state ($3O_2$) or singlet state ($_1O_2$). O_2 itself is a radical, the diatomic oxygen molecule has two unpaired electrons.
2) Superoxide radical (O_2).
3) Hydroxil radical (OH).
4) Alkoxy radicals (LO) and peroxy radicals (LO_2) which are produced during lipid peroxidation.

II- Non oxygen - centered free radicals:

1) Carbon - centered such as trichloromethyl radical (CCL_3)
2) Hydrogen - centered such as H atom (H).
3) Sulphur-centered such as thyl radical.
4) Nitrogen - centered such as phenyl diazine radical (NO).

III- Non radical toxic oxygen metabolites:

1) Ozone (O_3).
2) Hydroperoxide such as hydrogen peroxide

(Bast et al., 1991)

The hydroxyl radical, the most potent oxidant known, has an extremely short half- life reacting at the site of its formation through its ability to attack most biological molecules resulting

in the propagation of free radical chain reactions ***(Betteridge, 2000).***

Superoxide is formed when oxygen accepts an electron ***(Betteridge, 2000).*** It is much less reactive than OH ***(Halliwell, 1991).*** It can act as a weak oxidizing agent, but is much stronger as a reducing agent of iron complexes such as cytochrome C. It is likely to be more important as a source of hydroxyl radicals and hydrogen peroxide.

Nitric oxide, an example of a physiological radical, is of considerable interest through its role as a mediator of vascular tone ***(Moncada and Orgleuski, 1991).***

Sources of free radicals:

I-Endogenous sources:

(1) Normal aerobic metabolism:

Stepwise reduction of molecular oxygen to water in the mitochondrial electron transport chain generates the superoxide radicals, hydrogen peroxide and the hydroxyl radical ***(Freeman and carpo.,1982).***

(2) Activated phagocytes:

Phagocytic cells destroy bacteria or virus infected cells with an oxidative burst of nitric oxide, superoxide anion, and hydrogen peroxide ***(Ames et al., 1993).*** Their activation imposes oxidative stress on the surrounding tissue as well, and if too many phagocytes are activated or if inflammation goes on for too long,

serious injury can result. Examples of such injury are damage to the joints as a result of chronic joint inflammation in the disease of rheumatoid arthritis, and damage to the large intestine (often leading to cancer) as a result of chronic inflammation of this organ in the inflammatory bowel disease ***(Gutteridge and HalliwelL, 1994).***

(3) Enzymes that produce free radicals:

Xanthine oxidase oxidizes xanthine and hypoxanthine into uric acid, making superoxide radical (O_2) and hydrogen peroxide.

The cytochromes P450 are enzymes found in many body tissues (esp. concentrated in liver). They use O_2 to oxidize a wide range of foreign compounds (drugs, toxins, pesticides, etc.) into products that are usually, less toxic,and cytochromes P450 one of detoxification systems ***(Gutteridge and Halliwell, 1994).***

(4) Heme proteins:

Oxidation of oxyhemoglobin and oxymyoglobin to their met forms is accompanied by the release of heme - bound O_2 as superoxide ***(Halliwell, 1991).***

(5) Metals:

Iron and copper are dangerous catalysts, if free, this can lead to biological damage. Thus free iron or cupper accelerate autoxidation reactions, and both can react with H_2 O_2 to form

highly dangerous OH: The Fenton reaction: Fe II + H_2O_2→OH ± OH. + Fe III ***(Gutteridge and Halliwell, 1994).***

(6) Nitric oxide:

Although NO is biologically essential, production of excess NO in patients with severe infections can do harm e.g. by lowering blood pressure too much and contributing to septic shock ***(Gutteridge and Halliwell, 1994).***

II- Exogenous sources

(1) Radiation:

Receiving high doses of gamma irradiation mostly occurring either accidentally or due to therapeutic exposure, induces generation of free radicals and increases lipid peroxidation products by damaging to oxygenic molecules and hydrolysis of water ***(Robbins et al., 1996).***

We are exposed to electromagnetic radiation from environment both natural (random and cosmic radiation) and from man - made sources. This radiation can split water in the body to generate OH- and 0_2 radicals which cause damage by reacting with DNA and other macromolecules ***(Halliwell, 1994b).***

(2) Drugs:

"Paracetamol" can deplete antioxidant defenses as its metabolism by liver cytochrome P450 generates a product that reacts with and removes glutathione leading to secondary

oxidative damage which continues to hepatic failure in paracetamol overdose ***(Aust et al., 1993).***

Free radical mediated tissue damage:

Free radicals are inherently unstable molecules because of the presence of unpaired electrons. As a result, they can be highly reactive, reacting locally to accept or donate electrons to other molecules to achieve a more stable state. As most molecules are not free radicals, that majority of reactions will involve non radicals (all biological macromolecules are possible targets) producing free radical chain reaction with the formation of new radicals, which in turn can react with further macromolecules ***(Bettridge, 2000).***

I - Membrane lipid peroxidation:

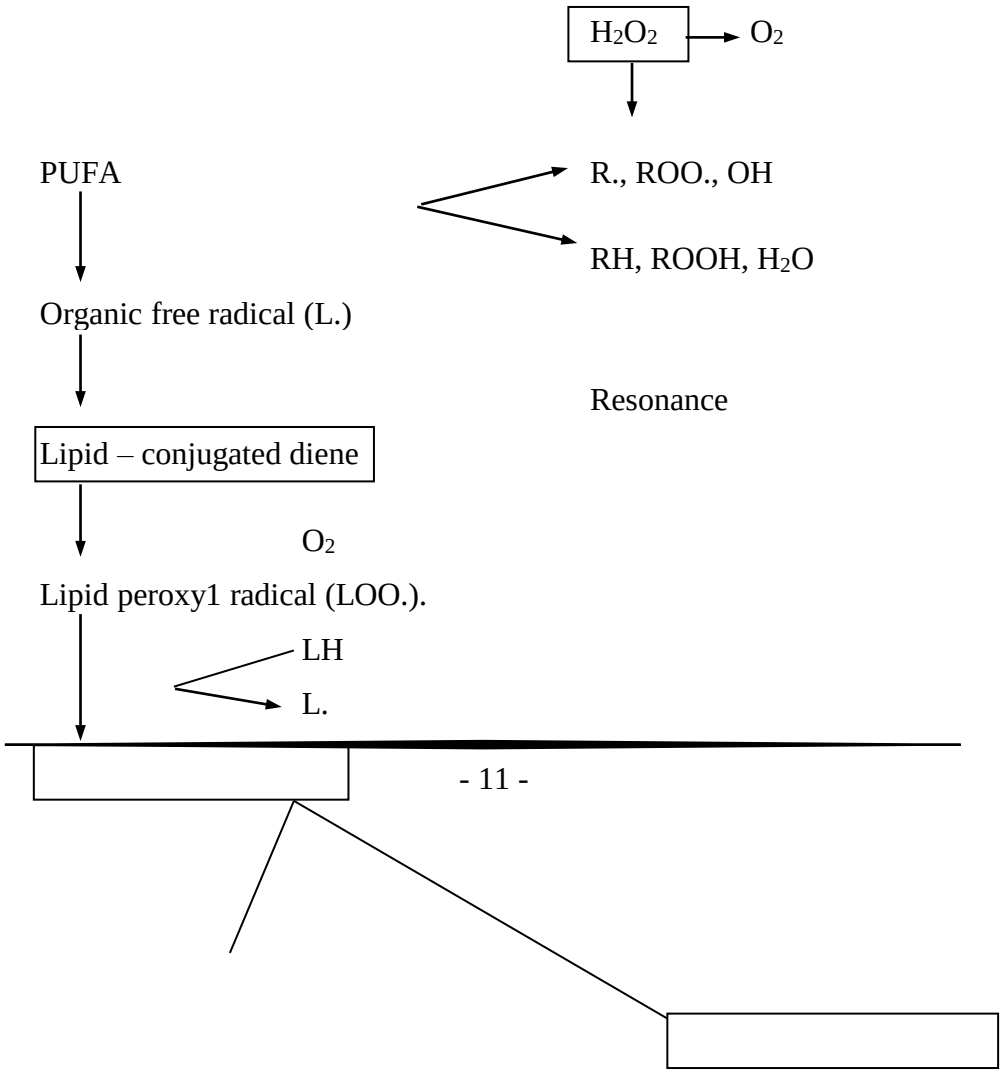
Lipid peroxidation is defined as oxidative deterioration of polyunsaturated lipids. Lipid peroxidation not only damages lipids but also membrane proteins such as receptors and enzymes ***(Gutteridge and Halliuell, 1994).***

Reactive free radicals, e.g., the hydroxyl radical, have the capacity to abstract a hydrogen atom (H) from a methelene group (CH_2) of fatty acids leaving behind an unpaired electron on the carbon (CH).

Polyunsaturated fatty acids are particularly prone to free radical attack because of the presence of a double bond, weakens the carbon-hydrogen bond at the adjacent carbon atom. The remaining carbon-centered radical undergoes molecular rearrangement resulting in a conjugated diene.

Conjugated dienes can combine with oxygen forming a peroxyl radical. This in itself is able to abstract hydrogen atom and begin a chain reaction that continues either until the substrate is consumed or the reaction is terminated by a chain-breaking antioxidant such as vitamin E. The resulting lipid peroxides can be catalyzed by transition metals producing alkoxyl and peroxyl radicals, which can stimulate further lipid peroxidation ***(Betlridge, 2000).***

Lipid peroxidation generates hydroperoxides, endoperoxides, aldehydes, and the end product malondialdehyde, ethane and pentane ***(Fardy and Silverman, 1995)***. Extensive peroxidation in cell membranes will result in changes in fluidity, increased permeability, a decrease in membrane potential and eventually membrane rupture ***(Bettridge, 2000).***



Lipid – hydroperoxide (LOOH)

Fe^{++}

Peroxyl, alkoxy1 Decomposition

Free radicals Malondialdehyde, etc.

Figure (1): Pathway for lipid peroxidation induced by oxygen and other free radicals ***(Ronald et al., 1996).***

II- Protein damage:

Free radicals oxidize sulphydryl-containing proteins (e.g., glutathione and cytoskeleton) thus inactivating enzymes and altering structural protein ***(Ronald et al., 1996).***

Oxidative damage to proteins results in the formation of protein carbonyls ***(Fardy and Silverman, 1995).***

III- DNA damage:

Cell mutation and death from ionizing radiation is primarily due to free radical reactions with DNA.

Cytotoxicity is a consequence of chromosomal aberrations arising from either nucleic acid base modification or DNA strand scission ***(Ward, 1981).*** Oxidative damage to DNA results in the formation of 8-hydroxydeoxyguanosine ***(Fardy and Silvernan, 1995).***

IV- Disturbance of Ca^{++} handling:

Oxygen free radical has been suggested to be involved in inhibitions or progression of excessive Ca^{++} influx ***(Garlick et al., 1987)*** because of these reactive metabolites occurred immediately upon reintroduction of O_2 followed by massive accumulation of Ca^{++} ***(Ronald et al.,1996).***

If the Ca^{++} level rises too high, it can activate enzymes that attack DNA (nucleases) and so fragmenting the DNA. High Ca^{++} can also activate enzymes that cleave structural proteins within the cell causing the membrane to bleb out, the bulge may rupture, producing a hole in the membrane that kills the cell ***(Gutteridge and Halliwell, 1994).***

Table (1): Clinical conditions in which the involvement of oxygen radicals has been suggested.

Inflammatory — immune injury	**Alcoholism**
• Glomerulonephritis (idiopathic, membranous). • Vasculitis (hepatitis B virus, drugs). • Rheumatoid arthritis. • Inflammatory bowel disease. **Ischeamia - reflow states** • Stroke/myocardial infarction/ arrhythmia. • Organ transplantation. • Inflamed rheumatoid joint. • Frostbite. • Dupuytren's contracture. **Drug and toxin induced reactions** **Iron overload** • Idiopathic hemochromatosis. • Dietary iron overloads (Bantu). • Thalassaemia and other chronic anemias treated with multiple blood transfusions.	Including alcohol- induced iron overload and alcoholic myopathy. **Radiation injury** • Nuclear explosions. • Accidental exposure. • Radiotherapy. **Aging** Disorders of premature aging. **Red Blood Cell** • Phenyl hydrazine effect. • Primaqiune. • Lead poisoning. • Protoporphyrin photooxidation. • Malaria • Sickle cell anemia. • Favism • Fanconi's anemia • Hemolytic anemia of prematurity • Oral iron poisoning.

Diabetes mellitus and its complications. **Lung** • Cigarette smoking effects. • Emphysema. • Hyperoxia. • Bronchopuimonary displasia. • Oxidants pollutants (03,ab2). • Mineral dust pneumoconiosis asbestos carcinogenicity bleomycin toxicity SO2 toxicity. • Parquet toxicity. **Skin** • Solar radiation. • Thermal injury. • Porphyria. • Hypericin, other photosensitizes contact dermatitis.	**Brain / nervous system, neuromuscular Disorders** • Hyperbarric oxygen toxicity. • Vit E deficiency. • Neurotoxins. • Parkinson's disease. • Hypertensive cerebrovascular injury potentiation of traumatic injury. • Muscular dystrophy. • Multiple sclerosis. **Carcinogenesis** **Eye** • Cataractogenesis. • Ocular hemorrhage. • Degenerative retinal damage. • Retinopathy of prematurity. • (retrolental filroplasia) phobic retinopathy.

(Haliwell and Gutteridge, 1991)

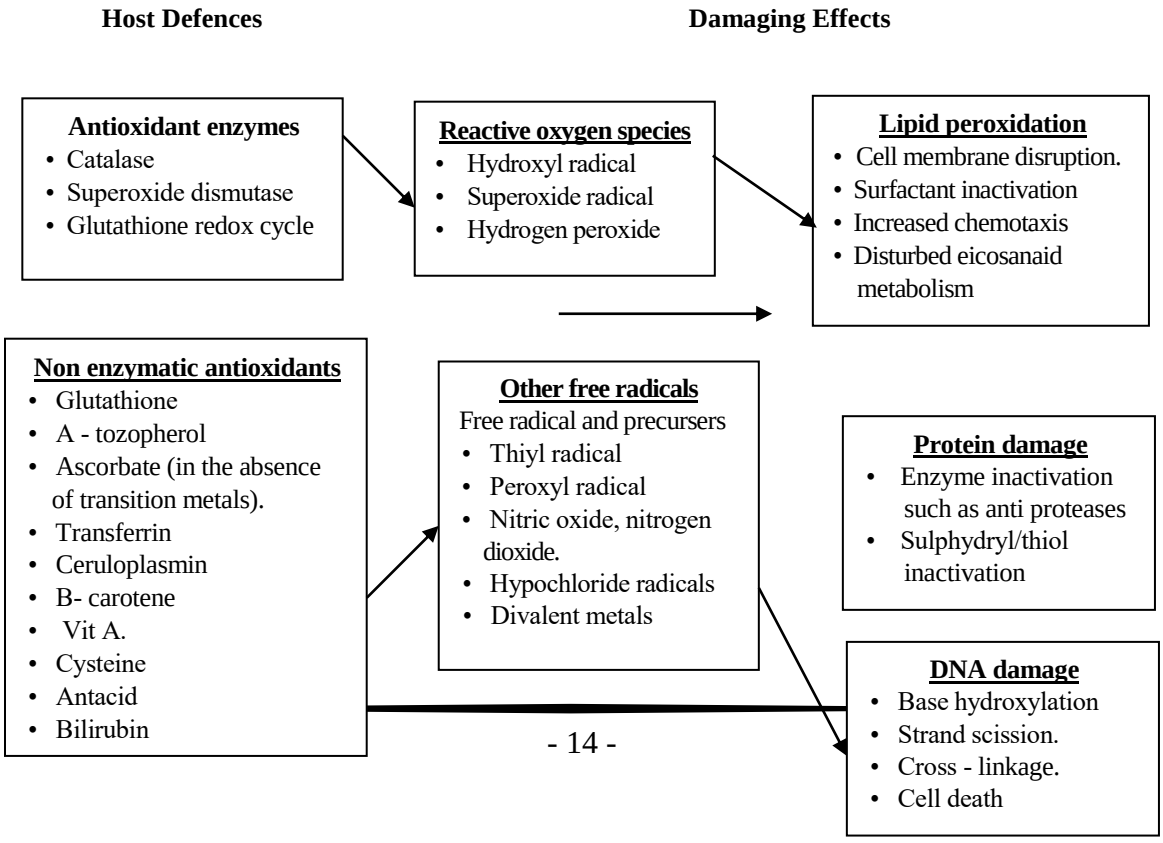


Figure (2): Reactive oxygen species and other free radicals: Some defects and host defenses ***(Fardy and Silverman, 1995).***