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# OXIDATIVE DAMAGE: THE MITOCHONDRIA AS A TARGET

## DAÑO OXIDATIVO: LA MITOCONDRIA COMO BLANCO

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**Abstract**

Reactive oxygen species are constantly produced in aerobic cells as a result of metabolic processes. Oxidative stress can also result from exposure to environmental toxins. Hydrogen peroxide, superoxide anion and hydroxyl radicals are major species produced during metabolism. The hydroxyl radical can react with vital cellular molecules, such as DNA and proteins, leading to oxidative damage. Cells have developed defense systems to survive to chronic exposure to reactive oxygen species.

Chronic exposure to reactive oxygen species is associated with an increasing number of human diseases, neurodegeneration and aging processes. Currently, much attention is focused on the deleterious effects of reactive species on cells but the mechanisms and pathways involved in those effects need to be extensively investigated.

*Key words:* oxidative damage, Fenton reaction, mitochondria, repair.

**Resumen**

Las especies reactivas de oxígeno se producen constantemente en células aeróbicas como resultado de procesos metabólicos. El estrés oxidativo puede resultar también debido a la exposición a tóxicos ambientales. El peróxido de hidrógeno, el anión superóxido y los radicales hidróxilo son las especies más importantes producidas durante el metabolismo. El radical hidróxilo puede reaccionar con moléculas vitales de la célula, tales como ADN y proteínas, produciendo daño oxidativo. Las células han desarrollado sistemas de defensa para sobrevivir a la exposición crónica a las especies reactivas de oxígeno. La exposición crónica a especies reactivas de oxígeno está asociada con enfermedades humanas, neurodegeneración y procesos de envejecimiento. En la actualidad, la atención está enfocada a estudiar los efectos deletéreos de las especies reactivas sobre las células pero los mecanismos involucrados en los mencionados efectos tienen que ser investigados con mucha profundidad.

*Palabras clave:* daño oxidativo, reacción de Fenton, mitocondria, reparación.

## INTRODUCTION

Exposure to reactive oxygen species (ROS) is intrinsic to living aerobic cells. Since Michaelis (1946) and Harman (1956) proposed the theory of free radical formation through two univalent steps from the oxidation of bivalent organic molecules, the issue has become very important to thoroughly understand metabolic pathways and the balance of the equation of oxygen consumption for cells to survive (equation 1):

$$risk \quad \longrightarrow \quad benefit \quad (1)$$

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Both electron transfer and generation of ATP occur on the inner mitochondrial membrane and key metabolic reactions occur within specific submitochondrial compartments. The "*oxygen paradox*" (Fridovich, 1975; Davies and Ursini, 1995; Davies, 1995) refers to the dual effects of oxygen in the sense that if oxygen supports life, it also generates a costly damage to the living cell, but higher eukaryotic aerobic organisms cannot live without oxygen. The four-step electron reduction reaction of the mitochondrial electron transport system is considered to be a relatively safe process (Davies, 1995). However, approximately 2-5% of the total oxygen intake by the mitochondria may leak as superoxide anion (Davies and Ursini, 1995). This can generate unbalance inside the cell, exposing the cell to high risk due to oxidative damage. The electron transport chain (ETC) may be particularly susceptible to higher amounts of reactive oxygen species stimulating the mitochondria to become a self ROS-generator, with catastrophic effects to the cell function (Yakes and Van Houten, 1997) (figure 1).

The overall ROS-cellular interaction, damage, defense and repair mechanisms are very complex. To help to visualize partially the exogenous and endogenous ROS-induced products and cellular oxidative reactions (Henle and Linn, 1997) (figure 2).

Dr. Van Houten's laboratory has been accumulating evidence to support the hypothesis that the mitochondria is a critical target for ROS-induced damage (the basic protocols used are described in Yakes and Van Houten, 1997; Salazar and Van Houten, 1997; Ballinger and Van Houten, 1998; Yakes *et al.*, 1996). The same group is currently performing experiments to analyze oxidative damage in atherioschlerotic patients (Ballinger and Van Houten, 1998) and have plans to apply this technology *in vivo* to test the hypothesis that "oxidative mitochondrial damage is associated to degenerative diseases" (Ritcher, 1992; Miguel, 1992; Ritcher *et al.*, 1988). Damage and perturbation to mitochondria function is biologically significant since, recentely, a number of cancers, neurodegenarative diseases and even the aging process itself has been associated with alterations and accumulation of mtDNA damage (Ritcher, 1992; Miguel, 1992; Simonetti *et al.*, 1992). The increasing observations that mitochondria are more prone to ROS-induced damage leads us to think that mitochondria could be used as a dosimeter for ROS exposure (Yakes and Van Houten, 1997; Salazar and Van Houten, 1997).

## CELLULAR SOURCES OF ROS

There are mainly three species involved in ROS damage: hydrogen peroxide ($H_2O_2$), superoxide anion ($O_2^{\cdot -}$) and hydroxyl radical ($OH^{\cdot}$). Cellular sources of $H_2O_2$ are mainly a) mitochondria, b) microsomes, c) peroxisomes, d) cytosolic enzymes, e) the nucleus and f) non-enzymatic sources (Chance *et al.*, 1979). Hydroxyl radicals may be produced from $H_2O_2$ through radiolysis of water by ionizing radiation (γ-rays or X-rays) (Zdzienicka, 1995; Breen and Murphy, 1995) (equation 2):

$$H_2O \xrightarrow{h\nu} H_2O^* \longrightarrow H^{\cdot} + OH^{\cdot} \quad (2)$$

Hydroxyl radicals also may be generated from pure compounds (such as xanthine oxidase, $H_2O_2$) added directly to the experimental medium and it is believed they are responsible for most of the damage caused to the cell *in vitro*. Hydroxyl radicals are produced by the Haber-Weiss reaction, which is a kinetically slow reaction (Haber and Weiss, 1934) (equation 3):

$$H_2O_2 + O_2^{\cdot -} \longrightarrow OH^{\cdot} + OH^{-} + O_2 \quad (3)$$

A modification of the Haber-Weiss reaction is the Fenton reaction, which is a metal bound reaction and also may produce OHi at a much faster rate by reduction of $Fe^{+3}$ to $Fe^{+2}$ by superoxide, then reoxidized to $Fe^{+2}$ by hydrogen peroxide (Luo *et al.*, 1994; Henle and Linn, 1997; Aruoma *et al.*, 1989) (equation 4):

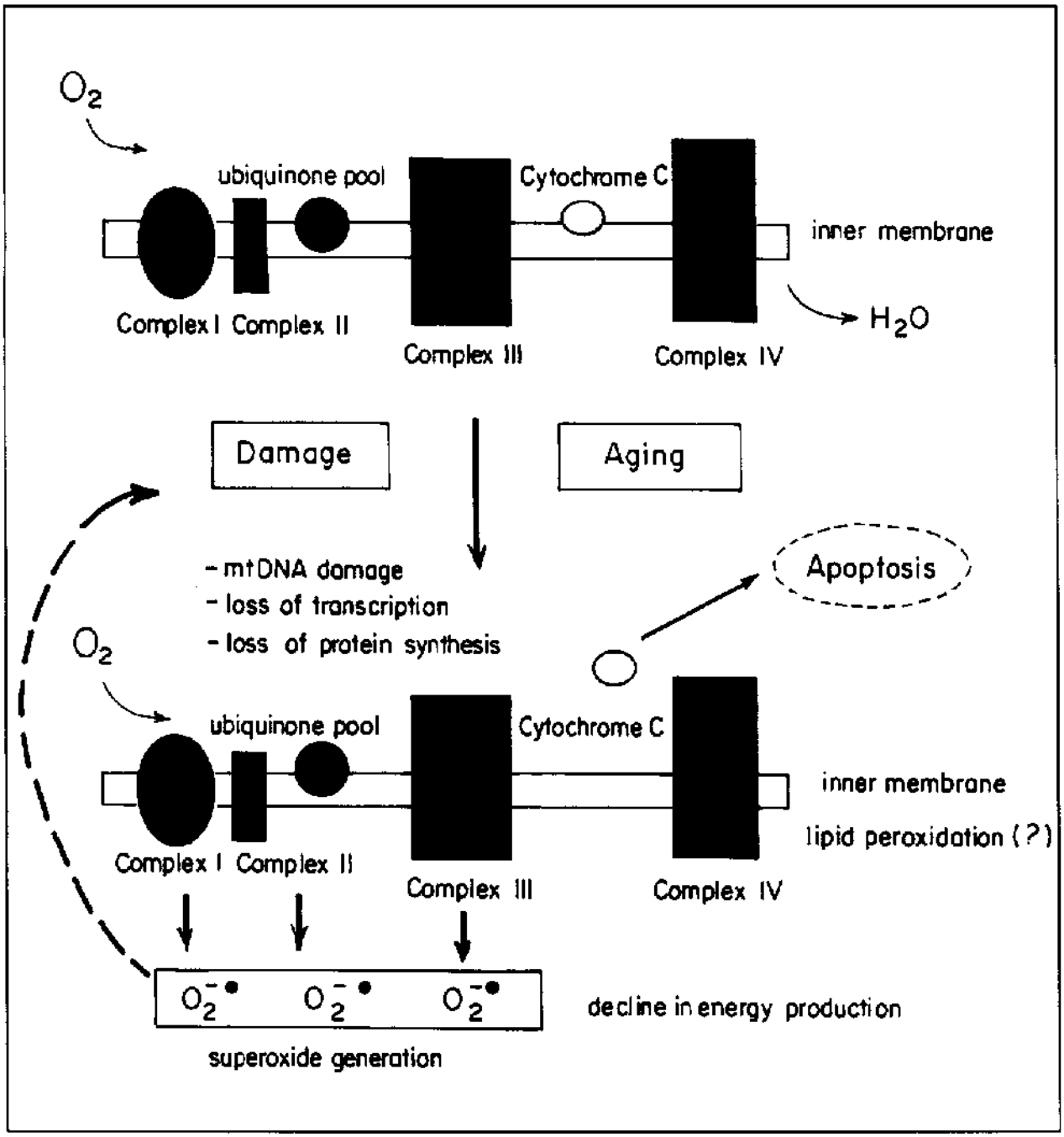


**Figure 1.** Mitochondrial catastrophe model. Under normal conditions oxygen metabolic reactions undergo without any alteration. Under oxidative stress, superoxide leaks and may accumulate, causing mitochondrial DNA damage, loss of transcription, loss of protein synthesis and overproduction of cytochrome *c* leading to apoptosis. Lipid peroxidation and decline in energy production are also consequences of oxidative damage

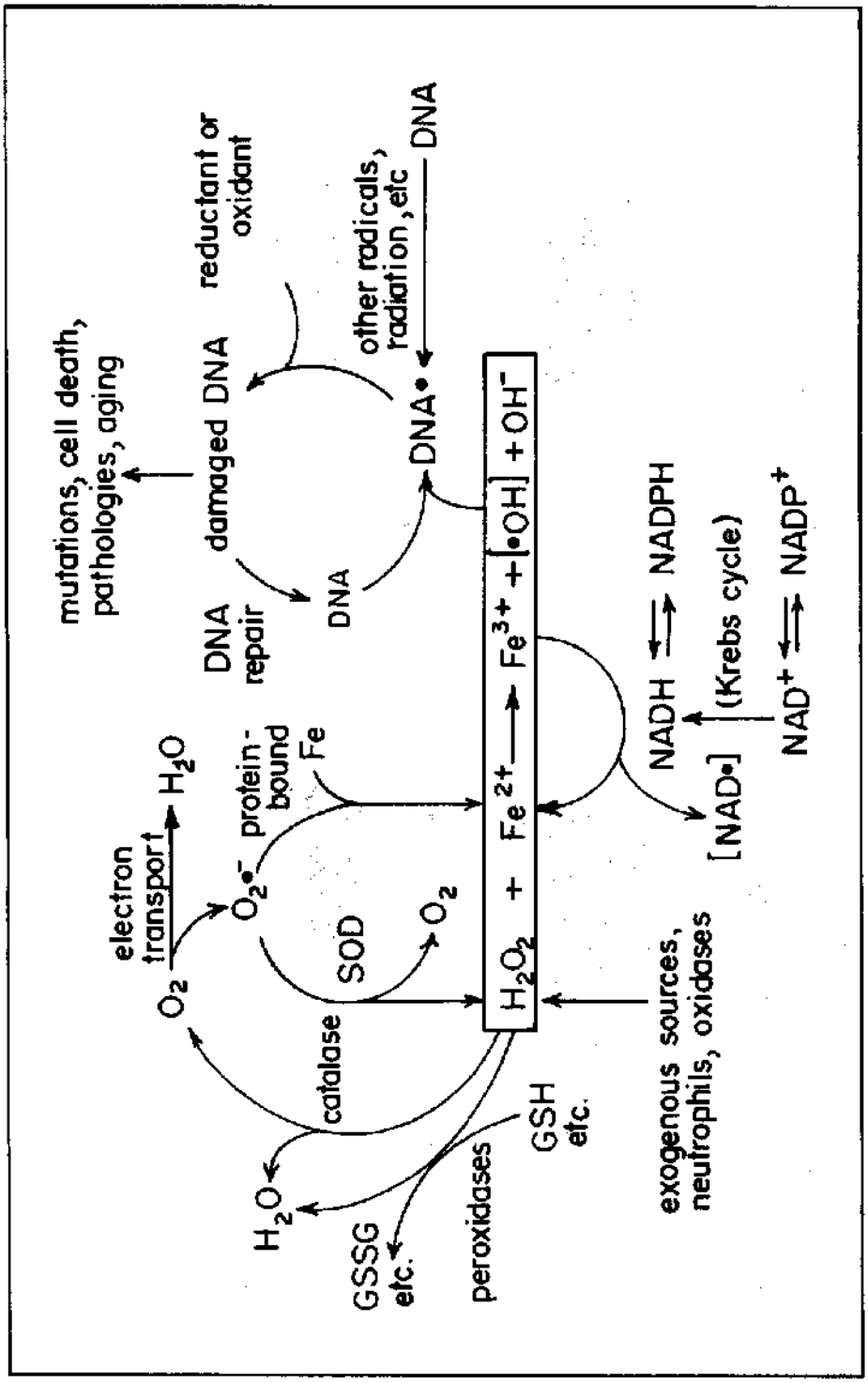


**Figure 2.** Cellular reactions leading to oxidative damage. Hydroxyl groups formed from hydrogen peroxide through the Fenton reaction react with DNA causing cell death and mutations, which may accumulate to accelerate aging or pathologies such as Alzheimer's, Parkinson's, etc. An excess of reactive oxygen species may deplete the antioxidant systems

$$O_2 + Fe^{+3} \longrightarrow Fe^{+2} + O_2$$

$$H_2O_2 + Fe^{+2} \longrightarrow Fe^{+3} + \mathbf{OH^{\bullet}} + OH \quad (4)$$

Superoxide anion is formed by leakage of high-energy electrons along the mitochondrial transport chain and by a variety of cytosolic and membrane-bound enzymes, including xanthine oxidase, the cytochrome p450 complex and phospholipase A2 (Simonian and Coyle, 1996). Under normal metabolic conditions, concentrations of $H_2O_2$ and superoxide in rat liver remains low. The level of $H_2O_2$ is three order of magnitude greater ($10^{-7}$-$10^{-9}$ M) than superoxide anion ($10^{-11}$-$10^{-12}$M) (Chance *et al.*, 1979).

Peroxynitrite ($ONOO^-$), formed by the reaction of nitric oxide ($NO^{\bullet}$) with superoxide anion ($O_2^{\bullet-}$), is a highly reactive molecule that also breaks down to form $OH^{\bullet}$ (Simonian and Coyle, 1996). Nitric oxide is formed from the conversion of L-arginine to L-citrulline by nitric oxide synthetase (NOS). The presence of $NO^{\bullet}$-related compounds in the cell as a byproduct of normal mammalian metabolism has imprinted the term for "*nitrosative stress*". Nitric oxide is a messenger molecule that regulates macrophage killing of tumor cells and bacteria, blood vessel relaxation and also is a neurotransmitter. S-nitrosothiols (RSNOs) impair cellular functions by nitrosation of cellular targets (Hausladen *et al.*, 1996). Among the mechanisms proposed for $NO^{\bullet}$ neurotoxicity and bactericidal actions involve monoADP-rybosylation and S-nitrosylation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), inhibition of mitochondrial enzymes such as *cis*-aconitase, inhibitation of the ETC, inhibition of ribonucleotide reductase and DNA damage. DNA damage activates poly-ADP-ribose polymerase which leads to energy depletion and subsequent neurotoxicity in rat brain (Zhang *et al.*, 1994).

When $NO^{\bullet}$ was added to intact human cells, relative survival decreased in a dose dependent manner and the levels of mutations increased 15-18-fold above the background in both the hypoxanthine-guanine phosphoribosyl transferase (*HPRT*) and thymidine kinase (*TK*) gene loci. Single-stranded DNA breaks also were induced in a time response manner (Nguyen *et al.*, 1992). The role of exogenous N-nitroso compounds in gastric carcinogenesis also has been studied in a southern Colombian population at high risk for gastric cancer (Mannick *et al.*, 1996). The hypothesis tested in the study sought to prove that *Helicobacter pylori* infection would lead to the production of reactive nitrogen species, $NO^{\bullet}$ and $ONOO^-$, as part of the host immune response increasing DNA damage and apoptosis in gastric epithelial cells. The same study concluded that dietary supplementation with β-carotene and ascorbic acid may prevent the formation of these potential carcinogens. On the other hand, a wide variety of environmental chemicals such as paraquat, cigarette smoke and other environmental toxicants induce the production of ROS (Meneghini, 1988; Wang *et al.*, 1992).

## TARGETS OF REACTIVE OXIGEN SPECIES

The ROS can react with vital cellular molecules such as proteins (Davies, 1993), membrane lipids (Hruszkewycz, 1988; Bindoli, 1988), and DNA. Reactions of ROS with DNA result in single-strand breaks (Breen and Murphy, 1995; Ward, 1988; Czene and Ringdahl, 1995; Halliwell and Aruoma, 1991), abasic sites, and damaged bases such as thymidine glycol and 8-hydroxy-deoxyguanosine (8-OH-G) (Cheng *et al.*, 1992). The spectrum of adducts in mammalian chromatin oxidized *in vitro* and *in vivo* includes more than twenty known products, including damage to all four bases and thymine-tyrosine cross-links (Halliwell and Aruoma, 1991), carbonylation and loss of sulfhydryls in proteins (Stadtman, 1992). The guanine analogue, 8-OH-G, represents only 10% of the total observed oxidative damage (Ward, 1988), causing G ——> T and A——> C substitutions. This is a biologically relevant base modification in mammalian DNA whose levels increase with oxidative stress, being a specific

marker for oxidative damage. Animals with a high basal metabolic rate show higher amounts of 8-OH-G in cellular DNA (Shigenaga *et al.*, 1989), and amounts of this modified base are higher in mtDNA than in nuclear DNA (Ritcher *et al.*, 1988).

Two possible mechanisms to explain ROS-induced damage are: 1) the most reactive radical responsible for the damage is $OH^{\bullet}$, which is produced from $H_2O_2$. Hydrogen peroxide, which readily crosses biological membranes, can penetrate the nucleus and react with iron or copper to form $OH^{\bullet}$. Because of the high reactivity of $OH^{\bullet}$ and its incapacity to diffuse significant distances within the cell, this mechanism is only possible if the $OH^{\bullet}$ is generated from $H_2O_2$ by reaction with metal ions bound upon or very close to the DNA. Since oxidative phosphorylation (OXPHOS)-proteins contain iron-cooper clusters, 1) mitochondria might be more prone to attack by ROS, and 2) ROS may trigger off a series of metabolic events within the cell, leading to activation of nuclease enzyme, which cleave the DNA backbone. The possibility that oxidative stress causes increased levels in intracellular free $Ca^{+2}$ has been suggested. For example, increased $Ca^{+2}$ levels might fragment DNA by activating $Ca^{+2}$-dependent endonucleases in an apoptosis-like mechanism (Halliwell and Aruoma, 1991). The role of ROS-induced and spontaneous damage at the cellular level and prevention and the role of mitochondria in apoptosis as well as in human diseases is an area of growing interest (Henle and Linn, 1997). Bcl-2 is an integral outer mitochondrial membrane protein. Its overexpression prevents cells from undergoing apoptosis when cytochrome *c* is released by the mitochondria. Therefore, a possible role of Bcl-2 is to prevent apoptosis by blocking cytochrome *c* release from mitochondria (Yang *et al.*, 1997; Kluck *et al.*, 1997).

ROS, especially $OH^{\bullet}$ and $ONOO^{-}$, can produce functional alterations in lipids, proteins and DNA (Davies, 1993; Hruszkewycz, 1988; Bindoli, 1988; Stadtman, 1992). The incorporation of molecular oxygen into polyunsaturated fatty acids initiates a chain reaction in which ROS, including $OH^{\bullet}$, $H_2O_2$ and peroxyl and alkoxyl radicals, are formed. Oxidative lipid damage, termed *lipid peroxidation*, produces a progressive loss of membrane fluidity, reduces membrane potential, and increases permeability to ions such as $Ca^{+2}$ (Simonian and Coyle, 1996; Stadtman, 1992).

## CELLULAR DEFENSES AND DETOXIFICATION SYSTEMS

Cells have developed enzymes to deal with ROS-generated damage by increasing levels of enzymes that metabolize oxygen-reduction products. Mainly, these enzymes belong into three categories: *i*) superoxide dismutase (SOD), *ii*) catalase and *iii*) gluthatione peroxidase. *i*) SOD dismutates superoxide radical anion to hydrogen peroxide (Meneghini, 1988) (equation 5):

$$2H^{+} + 2O_2^{\bullet-} \xrightarrow{SOD} H_2O_2 + O_2 \quad (5)$$

The reaction occurs spontaneously or is catalyzed by SOD, which appears to be the major source of protection. Superoxide dismutase is a widely distributed enzyme existing in a variety of forms. This copper and zinc-containing enzyme is present in cytoplasm, nuclear matrix, peroxisomes, erythrocytes and in the mitochondrial intermembrane space as a manganese containing form. An extracellular (EC-SOD) form also is found in extracellular endothelial interstitial space. Prokaryotic plants contain a Fe-SOD form. *ii*) Catalase, virtually present in all mammalian cells types in subcellular organelles as peroxisomes. It is more substrate-specific and mainly reacts with peroxides type as hydrogen peroxide and methyl or ethyl hydroperoxides to produce water as a final product (Meneghini, 1988; Wang *et al.*, 1992) (equation 6):

$$2H_2O_2 \xrightarrow{catalase} 2H_2O + O_2 \quad (6)$$

If iron is present, a second oxygen electron reduction may lead to the formation of $OH^{\bullet}$.

Physiological variations of catalase content in the tissues may lead to different rates of $H_2O_2$ content. To illustrate this, tissues containing high levels of catalase (liver and kidney) would present low levels of $H_2O_2$; on the other hand, tissues containing low levels of catalase (heart and brain) would present high levels of $H_2O_2$. The enzyme activity increases linearly, with the presence of $H_2O_2$ maintaining a controlled intracellular level of $H_2O_2$. *iii*) Glutathione peroxidase (GPX) catalyzes the reaction of hydroperoxides with reduced glutathione (GSH) to form oxidized glutathione disulfide (GSSG) and the reduction product of the hydroperoxide (Chance *et al.*, 1979) (equation 7):

$$H_2O_2 + 2\,GSH \xrightarrow{GPX} GSSG + 2H_2O \quad (7)$$

This enzyme contains selenium as part of the active center and may detoxify low levels of $H_2O_2$ and lipid peroxides. Glutathione activity is high in liver and erythrocytes, moderate in heart and lung and low in muscle (Chance *et al.*, 1979). Other small molecule antioxidants derived from dietary intake of fruit and vegetables play an important role in helping the cellular detoxification processes (Ames *et al.*, 1993; Beckman and Ames, 1997).

## CONSEQUENCES OF UNREPAIRED AND ACCUMULATED MITOCHONDRIAL ROS-INDUCED DNA DAMAGE

Mitochondria are semi-autonomous organelles whose main function is to generate ATP and water during OXPHOS through a 4 step electron reduction reaction summarized as follows (Chance *et al.*, 1979) (equation 8):

$$O_2 \xrightarrow{e^-} O_2^{\bullet-} \xrightarrow[2H^+]{e^-} H_2O_2 \xrightarrow[H^+]{e^-} OH^{\bullet} \xrightarrow{e^-} H_2O \quad (8)$$

The mitochondrial genome encodes thirteen polypeptides involved in OXPHOS, twenty two tRNA's and two ribosomal RNA's. Damage to this genome could result in loss of mitochondrial function. In mammals, the accumulation rate of mutations in mitochondrial DNA (mtDNA) is much higher than in nuclear DNA (Ritcher *et al.*, 1988). Moreover, it has been suggested that the steady-state level of oxidized bases in mtDNA is ten to fifteen times higher than in nuclear DNA (Ritcher, 1992; Shigenaga *et al.*, 1994). Mitochondrial energy production may be diminished by presence of ROS. For example, $H_2O_2$ reduced ATPase activity in bovine heart submitochondrial particles (Zhang *et al.*, 1990). Oxidant injury can be mediated through depletion of nicotinamide adenine dinucleotide. Total intracellular $NAD^+$ levels are decreased with $H_2O_2$ concentrations of 40 μM or higher. The production of ATP is reversible using doses of $H_2O_2$ of 250 μM and the depletion of NAD and ATP is irreversible when doses are higher, causing lysis of the cells and cell death (Schraufstatter *et al.*, 1986). In addition, mitochondrial breakage was induced by the herbicide paraquat in human cells (Wang *et al.*, 1992). Treacy *et al.* (1994) suggested that pyrrol-type pesticides cause cell death by inhibiting OXPHOS, thereby disrupting the proton gradient across mitochondrial membranes and subsequently inhibiting ATP production. Furthermore, a number of studies have linked mitochondrial damage to degenerative human diseases, cancer and aging (Ritcher, 1992; Miguel, 1992; Simonetti *et al.*, 1992; Tritschler and Medori, 1993; Ritcher *et al.*, 1988; Shigenaga *et al.*, 1994; Baggetto, 1993). Using other eukaryotic systems, Agarwal and Sohal (1991) and Sohal *et al.* (1995) showed that aged insects produce more ROS than young ones. Anther type of mitochondrial damage associated to a certain extent with the presence of ROS is DNA fragmentation and DNA strand breaks which are produced after exposure to $H_2O_2$ in different cell systems (Schraufstatter *et al.*, 1986; Spragg, 1991).

## ROS, AGING AND NEURODEGENARATIVE DISEASES

Cells are chronically exposed to low levels of ROS due to exogenous and endogenous sources (Chance *et al.*, 1979; Meneghini, 1988). The importance of radical-induced DNA damage has become more apparent during the last decade. It is suggested that long term ROS-cell exposure is associated with human diseases such as chronic inflammation, certain types of cancer, neurodegenerative disease (Alzheimer's, Parkinson's) and even aging processes (Miguel, 1992; Tritschler and Medori, 1993; Shigenaga *et al.*, 1994; Koller, 1997; Mizuno *et al.*, 1993; Schapira, 1993; Niwa *et al.*, 1985). As mentioned before, under normal physiological conditions, potentially deleterious reactive oxygen metabolites are generated through the normal metabolism of aerobic cells. Cells are exposed to a chronic state of oxidative stress due to an imbalance between antioxidants and prooxidants. The hypothesis that the amount of oxidative damage increases as organisms age and that accumulation of oxidative damage as a major cause of senescence is supported by the following observations (Mizuno *et al.*, 1993; Sohal and Weindruch, 1996): a) overexpression of antioxidative enzymes retards the age-related accumulation of oxidative damage and extends the maximum life-span of transgenic *Drosophila melanogaster*, b) rates of $H_2O_2$ and $O_2^{\bullet-}$ production by the mitochondria inversely correlates with variation in longevity among different species, c) restriction of caloric intake helps relieve the effects of oxidative stress by lowering steady-state levels of oxidative stress and damage, retarding changes associated with age and extending the maximum life-span in mammals.

The mechanisms and pathophysiology of Parkinson's disease are unknown. ATP production in the mitochondria is decreased by reducing activity of complex I (Schapira, 1993). This mitochondrial energy crisis has been associated with Parkinson's disease, opening the possibility that mitochondrial damage might be linked to Parkinson's disease. Other causes of possible pathogenesis include excitotoxicity, disturbances of calcium homeostasis, immunological mechanisms, and infectious etiologies (Koller, 1997). It is possible that the generation of free radicals leads to neuronal cell death (Simonian and Coyle, 1996; Naoi *et al.*, 1993). The observation that the protoxin 1-methyl -4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP) can cause Parkinson's-like symptoms (Singer *et al.*, 1993) in both animals and humans (Fallon *et al.*,1997) suggest the possibility that exposure to some exogenous agents may cause Parkinson's disease. The lipophilic molecule readily crosses the blood-brain barrier and is rapidly converted by monoamine oxidase B (MAO-B) to 1-methyl-4-phenylpyridium ($MPP^+$), which is the toxic metabolite. The dopamine transporter protein mediates the uptake of $MPP^+$ into the dopaminergic terminal where it concentrates in the mitochondrial matrix causing inhibition of NADH $CoQ_1$ reductase (complex I) and depletion of ATP, resulting in cell death (Koller, 1997).

Other human organ and tissue-specific diseases such as rheumatoid arthritis (joints), degenerative retinal damage, cataractogenesis (eye), Parkinson's, Alzheimer's (brain), dermatitis (skin), asthma (lung), malaria, Fanconi anemia (erythrocytes), atherosclerosis (vessels) and aging, cancer, amyloid diseases, inflammatory-immune injury (multiorgan) have been associated with increased levels of ROS (Favier *et al.*, 1995) (figure 3).

Biochemical and physiological mitochondrial dysfunction might be increased by accumulation of dysfunctional proteins. Therefore, accumulation of oxidized proteins may lead to loss of energy production and an increased production of oxidants in the aged cells (Stadtman, 1992).

## REPAIR OF ROS-INDUCED DAMAGE

Although the cellular machinery for repairing oxidative damage remains relatively unexplored,

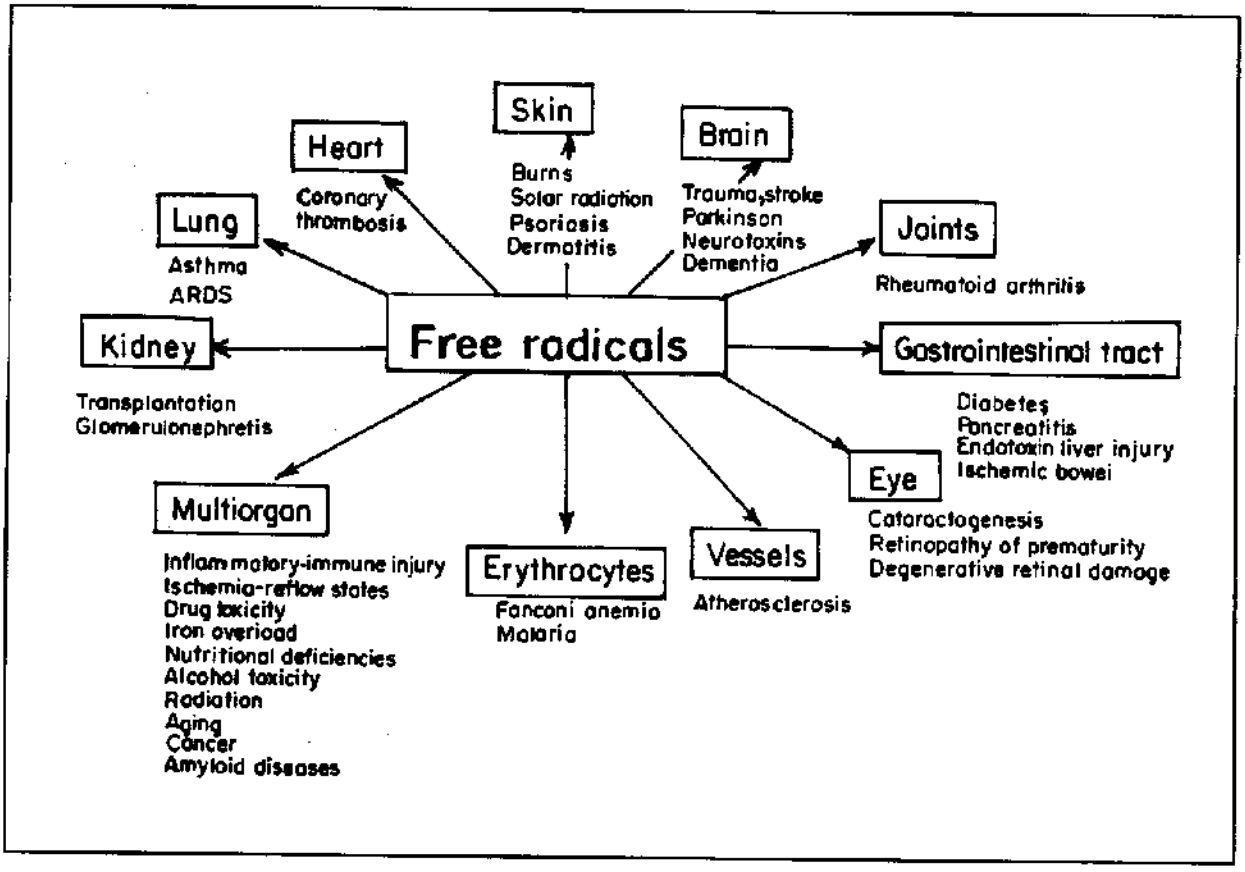


**Figure 3.** Spectrum of human diseases associated with oxidative damage. Many human diseases have been associated with an excessive free radical production, which may play a significant role in tissue injury

some knowledge has been generated. Cells are able to repair oxidized lipids (phospholipase $A_2$ cleaves lipid peroxides from phospholipids) (Pacifici and Davies, 1991). There are proteases that act specifically on oxidized proteins (Rivett, 1990) and oxidized nucleic acids (glycosylases specifically recognize oxidized bases from DNA (Tchou *et al.*, 1991).

Repair of DNA ensures stability and genetic continuity in proliferating cells and multicellular organisms. Repair is also an important line of cellular defense against agents that might cause cancer, aging and neurodegenarative diseases. In human, there are various repair systems protecting the integrity of the genome by repairing modified bases, DNA adducts, cross-links and strand breaks. Three basic mechanisms are responsible for repairing the damage: base excision, nucleotide excision and recombination. In base excision repair, the modified base(s) in the DNA is (are) removed by a glycosylase that hydrolyzes the glycosylic bond. The resulting abasic site (AP site) is removed by AP-lyase/endonucleases and then the base (s) is (are) replaced (Friedberg *et al.*, 1995) (figure 4). Base excision repair eliminates lesions that do not deform the DNA helix. These lesions are mainly generated during ROS-induced damage. In nucleotide excision repair, double incisions are made at either side of the modification and, the damaged nucleotide is released in an oligomer. The resulting gap is filled in and sealed. DNA bulky adducts are efficiently repaired by nucleotide excision repair. Double strand breaks also arise as normal intermediates in various cellular processes such as recombination and rearrangement of immunoglobulin and T-cell receptor genes during

lymphocyte development. Double strand breaks repair is accomplished by recombinational repair. The mechanisms of this type of repair remains to be elucidated. Basically, the ends of the strands are brought together, arrayed, and filled in to generate 3'-OH and 5'-P termini; finally, the two pieces are ligated (Aziz Sancar, 1995).

Some evidence that mitochondria are able to repair some forms of DNA damage is the presence of glycosylases (Driggers *et al.*, 1993; LeDoux *et al.*, 1992; 1993; Shen *et al.*, 1995), γ-polymerase and β-polymerases, which have been found in mitochondrion of the trypanosomatid *Crithidia fasciculata* (Torri and Englund, 1995). Repair

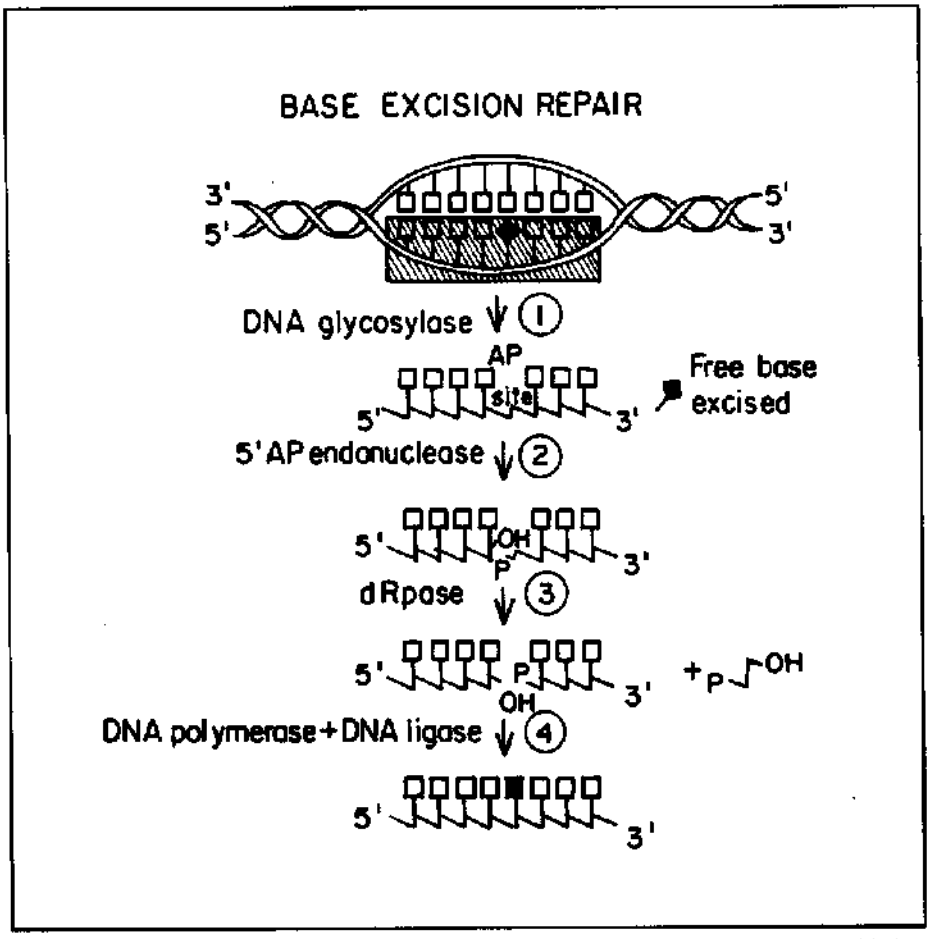


**Figure 4**. Repair of damaged bases. Base excision repair is the main pathway removing oxidative DNA lesions

experiments performed in Dr. Van Houten's laboratory (Yakes and Van Houten, 1997; Salazar and Van Houten, 1997) indicate that mtDNA is able to efficiently recover template amplification. Activity of the mitochondrial enzyme succinate dehydrogenase is also recuperated after exposure to acute or chronic exposure to $H_2O_2$ when allow to recovery during 1-3 hr.

## Abbreviations

ROS, reactive oxygen species; ETC, electron transport chain; $H_2O_2$, hydrogen peroxide; $OH^{\bullet}$, hydroxyl radical; OXPHOS, oxidative phosphorylation; $ONOO^{-}$, peroxynitrite; $NO^{\bullet}$, nitric oxide; $O2^{-}$, superoxide anion; NOS, nitric oxide synthetase; SOD, superoxide dismutase;

GPX, glutathione peroxidase; mtDNA, mitochondrial DNA; HGPRT, hypoxanthine-guanine phosphoribosyl transferase; TK, thymidine kinase.

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