FREE RADICALS IN BIOLOGICAL SYSTEM

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Introduction

Cellular respiration consists of series of oxidation-coupled-reduction reactions (redox reactions) which ultimately yield the highly reduced form of oxygen namely water as one of the end products. Atmospheric air contains 21% oxygen, which sustains the life of aerobic organisms. Higher amounts beyond this optimum level are toxic to all eukaryotic cells. In human beings, 100% oxygen inhalation supplied in the incubators for 6 hrs leads to chest soreness, cough and sore throat and for longer periods, leads to alveolar damage. Such an oxidative stress is normally produced within the biological system during cellular respiration, by the production of powerful reactive oxygen species (ROS) which include super oxide anions ($O_2^{\bullet-}$), hydroxyl radicals ($OH^{\bullet}$), hydrogen peroxide (H_2O_2), peroxyl radicals, nitric oxide ($NO^{\bullet}$), hypocholorous acid (HOCl), peroxides (O_2^{2-}), peroxy nitrite, heme proteins and singlet oxygen.

Free radicals' action is not limited to biological systems alone as, in normal life, there are various instances where the implications of such radicals are encountered such as rapid browning of a cut apple, rusting of a metal (iron, copper) and production of monomers required for polymerization reaction of plastic, polythene and synthetic rubber production. Even paint drying involves free radicals' reactions.

Exposure to ionizing radiations, insecticides, excessive sunlight (UV rays), toxic environmental pollutants in food, water, air and xenobiotics, all lead to the formation of, ROS or free radicals within the body. As metabolic byproducts of fatty acids oxidation, some dangerous free radicals are formed, turning the stored fat rancid.

The body cannot overlook the production of free radicals because they are involved in many essential chemical reactions and if a mismatch arises due to above said reasons and more free radicals are produced than the body needs or if body's capacity of neutralizing free radicals become inadequate, then they will bring about extensive damage to the biological system by way of oxidation/ oxidative stress.

What are free radicals?

.A free radical is any species capable of independent existence that contains one or more unpaired electrons. The unpaired electrons are highly energetic and seek out other electrons to pair, from some another molecule, thereby, changing it or possibly damaging it or causing the second molecule to become a reactive species leading to these chain reactions to continue. In other words, free radicals exist only for a very short period and before being neutralized, they attack other substances turning them into free radicals and set up very damaging chain reactions.

In vivo formation of free radicals

In any biological system, carbon, hydrogen, oxygen and nitrogen are the major constitutional elements, of which oxygen is highly labile to be converted into any of its highly reactive oxygen species like $O_2^{\bullet-}$, $NO^{\bullet}$, $OH^{\bullet}$ and H_2O_2 because of the configuration of electrons in the outermost orbitals in an unpaired fashion. The following three pathways are the methods by which free radicals are produced *in vivo*.

1. Mostly by biochemical redox reactions, which are parts of normal metabolism.

2. Macrophages and neutrophils produce free radicals like HOCl, $O_2^{\bullet-}$ as a part of inflammatory reactions to destroy bacteria and other foreign invaders.

3. Ionizing radiations like X-rays and Gamma rays, UV light, environmental pollutants, automobile exhaust, cigarette smoke, excessive exercise, hyperoxia and ischemia ($O_2^{\bullet-}$, $OH^{\bullet}$ radicals).

Many free radicals and ROS have been implicated in disease development

$OH^{\bullet}$ highly reactive radical which can attack all biological molecules

$O_2^{\bullet-}$ less reactive radical which can travel in the blood and attack a number of biological targets. $O_2^{\bullet-}$ also acts as a vasodilator and may have a role in intracellular signaling and growth regulation.

$NO^{\bullet}$ acts on smooth muscle cells in vessel walls causing relaxation

H_2O_2 crosses cellular membranes easily and may cause

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expression of virus genes. e.g. HIV in infected cells.

Damaging effects of free radicals

Oxygen containing free radicals are highly reactive and cause severe damage by hydrogen abstraction, bond scission and polymerization reaction. The $OH^{\bullet}$ can attack proteins, DNA, carbohydrates and Poly Unsaturated Fatty Acids (PUFA).

(a) Protein oxidation

Free radicals can oxidize aromatic amino acids, cysteine and disulphide (-S-S-) bonds leading to changes in conformational integrity of various extra cellular proteins like immunoglobulins (rheumatoid arthritis), albumin and collagen (cataract).

(b) DNA oxidation

Many of the nuclear enzymes containing metal ions (Fe and Cu) are believed to be the targets for H_2O_2 interaction causing hydroxyl radical production *in situ* which in turn attack DNA of chromosomes causing unraveling of strands and misreading of base pairs. The single and double bond break in DNA leads to mutation, carcinogenesis and cell-death. It has been found that semi-quinones present in cigarette smoke can generate H_2O_2 *in situ* at nuclear level and chelate DNA, leading to DNA strand breaks.

(c) Carbohydrate oxidation

Glucose, mannose and deoxy sugars are auto-oxidised to produce H_2O_2. Oxidation of ribose moiety of DNA leads to genetic damages. Oxygen radicals cause fragmentation of carbohydrate polymers especially hyaluronic acid of synovial fluid and this terminates in rheumatoid arthritis.

(d) PUFA oxidation

The membrane bound lipids containing PUFAs are susceptible to free radicals attack. The –C=C– bond structure of PUFA is susceptible to $OH^{\bullet}$ attack leading to abstraction of hydrogen atom and formation of peroxides products such as diene conjugates. Because of this lipid oxidation, tearing of cell membrane occurs, subjecting the cells to infection and spilling of cytoplasm. Oxygen free radicals react with serum lipoprotein-LDL and such an oxidized LDL can result in the formation of atheromatous plaques in endothelial linings.

Role of free radicals in diseases

Free radicals have been implicated in over 100 human and animal diseases, ranging from cardiovascular to Parkinson's disease. Some of the disorders include Alzheimer's disease, arthritis, hemorrhoids, Parkinson's disease, rheumatism, heart attack, AIDS, cataract, stroke, stress, jet lag, senility, varicose veins, phlebitis, immune system disorders, cancer and a long list of degenerative diseases including aging. Free radicals are also thought to contribute to heart disease.

Body's antioxidant system

The biological system possesses a number of mechanisms to control the production of the ROS and to limit or repair the damaged tissue. The antioxidant system of the body got three important components.

Anti-oxidant enzymes

The antioxidant enzymes mainly function to break down the dangerous superoxide free radicals and convert them to less dangerous H_2O_2.

1. Superoxide Dismutase (SOD)

In cytosol, it is a zinc/ copper containing enzyme and in mitochondira, a manganese containing enzyme. It removes the superoxide radical by combining it with protons to form hydrogen peroxide and oxygen.

$$2O_2^{\bullet -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

2. Catalase

Sl.No.	Type of antioxidants	Action	Examples
1.	Preventive antioxidants	Prevent formation of new ROS	Ceruloplasmin (Cu), metallothionine (Cu), albumin (Cu), transferring (Fe), ferritin (Fe) and myoglobin (Fe).
2.	Scavenging antioxidants	Remove ROS once formed, thereby, preventing free radicals to do their reactions	(a) Enzymes like Superoxide Dismutase (SOD), Glutathione peroxidase (GPx), Glutathione reductase (GR), Catalase and Metalloenzymes. (b) Small molecules like glutathione, ascorbate (Vit. C), tocopherol (Vit. E), bilirubin, uric acid, carotenoids and flavanoids.
3.	Repair enzymes	Repair or remove ROS damaged biomolecules	DNA repair enzymes and methionine sulphoxide reductase.

An iron containing enzyme. converts hydrogen peroxide to water and oxygen.

$$2O_2^{\cdot -} + 2H^+ \xrightarrow{\text{Catalase}} H_2O + \tfrac{1}{2} O_2$$

3. Glutathione Peroxidase (GPx)

Selenium containing enzyme, reduces hydrogen peroxide to water by converting reduced glutathione (GSH) into oxidized glutathione (GSSG).

$$H_2O_2 + 2GSH \xrightarrow{GPx} 2H_2O + GSSG$$

Reduced glutathione Oxidised glutathione

For the regeneration of reduced glutathione, the NADPH dependent glutathione reductase (GR) is very essential.

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2GSH + NADP^+$$

Every cell in the body contains the instructions for making these three enzymes.

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MPI TO INCREASE MEAT PRODUCTION

The state owned meat products of India will increase its annual meat production form 300 tones to 600 tones as efforts are on to modernize existing facilities in its plant at Koothatukulam . Managing Director Dr. Mohanan P.V. said that the annual production at its pig and rabbit farms would also be increased as part of augmenting meat production. The price of meat products of the MPI had been slashed by 25 per cent as a Christmas gif and this has resulted in a boom in sale.

Future Plans

1. The annual production of MPI will be increased from 300 MT to 600 MT. The modernization works in the plant to increase the production will be commissioned in January 2007.
2. The present production from the pig and rabbit farm will be increased. The annual production of pig lings will be increased from 2400 to 4800. Also the production of rabbit kits will be increased from 5000 to 15000 per year. In addition to this under the technical guidance of MPI, rabbit and pig breeding unit will be started on a franchisee basis in selected districts of Kerala in collaboration with Kudumbasree /Self Help Groups/Voluntary organizations. It is estimated to produce and distribute 20,000 pig lings and 35,000 rabbit kits through out the state.
3. MPI will provide technical guidance to local self government institutions for constructing slaughter houses. MPI is the nodal agency for constructing slaughter houses. The meat produced in such slaughter houses will be marketed in the label of MPI if it satisfies the specified quality standards. If there is sufficient production, the meat produced in such slaughter house will be converted to products in the factory of MPI. Central Government assistance will be made available for the construction of slaughter houses. The local self Governments may contact MPI in this regard.
4. There is increased foreign and domestic demand for organic meat. To utilize this MPI will promote organic rabbit and duck farming. MPI will start production and export of organic meat certified by INDOCERT (India's Organic Certifying Agency). Organic duck farming already started in Adat panchayath, Trichur dist. On a trial basis
5. As more of Kudumbasree and SHG start Quail, Poultry, Rabbit, Goat, Pig rearing in Kerala, it is estimated to produce 140 MT rabbit meat, 70 MT Quail Meat 200 MT Mutton, 300 MT Pork more per year. MPI had sought permission from the Government to purchase these raw materials for meat production from these registered agencies without tenders and also avoiding exploitation of middle men. The meat so produced will be procured and marketed with the help of Uvasree and the discussions are going on in this regard.
6. MPI will export meat and meat products from next year onwards. MPI will organize new projects to produce products according to taste of Europeans and will market these products in collaboration with international marketing agencies.
7. MPI will expand its meat marketing and steps have already been taken to appoint agencies to market meat in all districts. MPI will market its meat through agencies like Civil Supplies, Horticorp, Matsyafed and Kudumbasree.
8. MPI will organize awareness programmes to educate people about the harmful effects of unscientific slaughter.
9. MPI will take initiative to develop a meat policy for the state in association with AHD.
10. MPI is planning to conduct MPI products Expo in the districts in Kerala and Metropolitan cities in India where MPI products are not available.