ANTIOXIDANT ENZYMES

By :-

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During normal metabolic functions, highly reactive compounds called free radicals are generated in the body; however, they may also be introduced from the environment. These molecules are inherently unstable as they possess lone pair of electrons and hence become highly reactive.

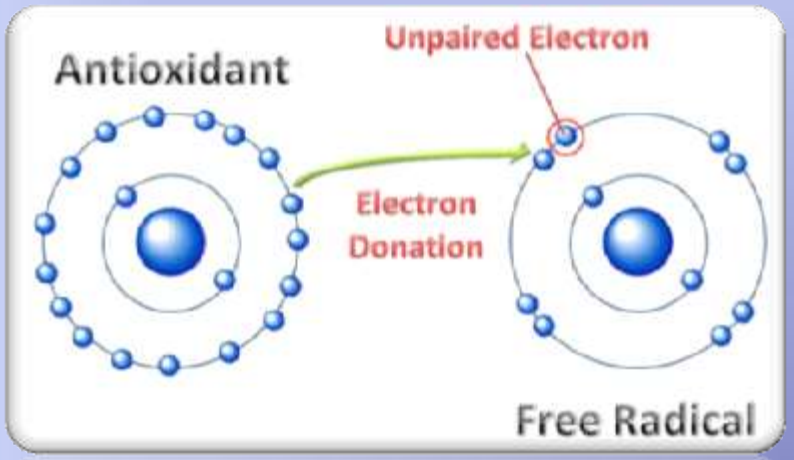
They react with cellular molecules such as proteins, lipids and carbohydrates, and denature them.

Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components. They act by reducing the energy of the free radicals or by giving up some of their electrons for its use, there by causing it to become stable. In addition, they may also interrupt with the oxidizing chain reaction to minimize the damage caused by free radicals .

it has been found that a substantial link exists between free radicals and more than sixty different health conditions, including the aging process, cancer, diabetes, Alzheimer's disease, strokes, heart attacks and atherosclerosis

Free Radicals And Their Scavengers

Free radicals are electrically charged molecules, i.e., they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Although the initial attack causes the free radical to become neutralized another free radical is formed in the process, causing a chain reaction to occur. And until subsequent free radicals are deactivated, thousands of free radical reactions can occur within seconds of the initial reaction.



Reactive Oxygen Species

Reactive oxygen species (ROS) is a term that encompasses all highly reactive, oxygen containing molecules, including free radicals.

Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides.

Antioxidant protection system

A. Endogenous Antioxidants

- Bilirubin
- Thiols, e.g., glutathione, lipoic acid, N-acetyl cysteine
- NADPH and NADH
- Ubiquinone (coenzyme Q10)
- Uric acid
- **Enzymes**

➢ copper/zinc and manganese-dependent superoxide dismutase

➢ iron-dependent catalase

➢ selenium-dependent glutathione peroxidase

Antioxidant protection system

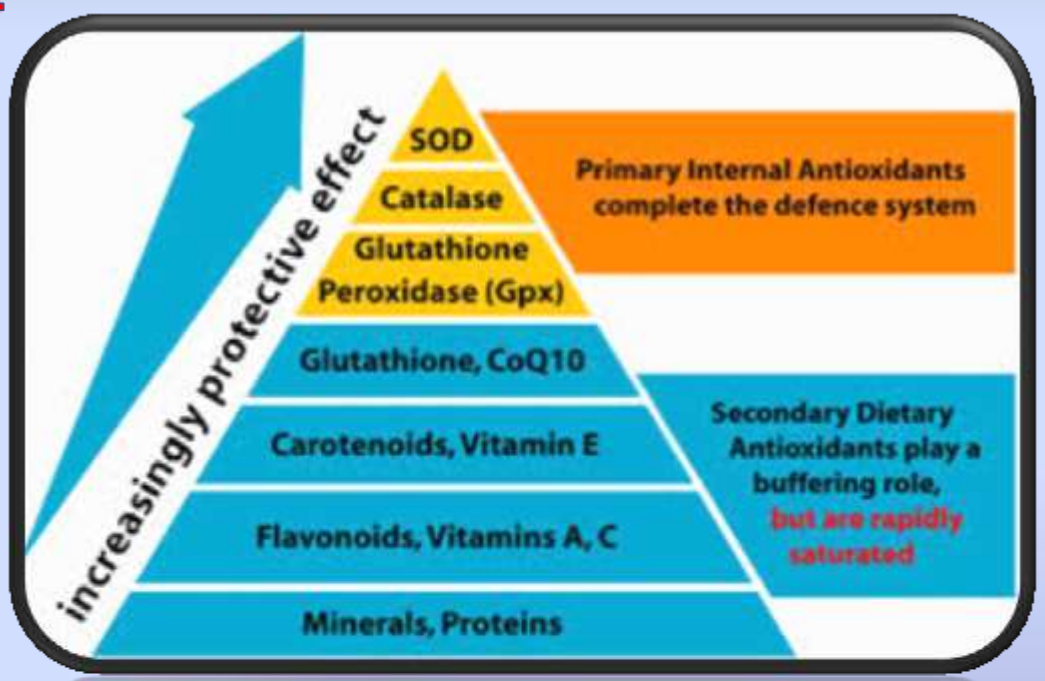
B. Dietary Antioxidants

- Vitamin C
- Vitamin E
- Beta carotene and other carotenoids and oxycarotenoids, e.g., lycopene and lutein
- Polyphenols, e.g., flavonoids, flavones, flavonol's, and Proanthocyanidins

Antioxidant protection system

C. Metal Binding Proteins

- Albumin (copper)
- Ceruloplasmin (copper)
- Metallothionein (copper)
- Ferritin (iron)
- Myoglobin (iron)
- Transferrin (iron)



Whenever the balance between ROS production and antioxidant defense is lost, 'oxidative stress' results which through a series of events deregulates the cellular functions leading to various pathological conditions.

Superoxide Dismutase

SOD is the antioxidant enzyme that catalysed the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2 Peroxide can be destroyed by CAT or GPX reactions.

In living systems, O_2^- is capable of reacting with another molecule of O_2^- (dismutation) or is also able to react with another radical, such as NO. **SOD** reaction has a reaction rate 10 000 times faster than that of spontaneous dismutation, so **SOD** provides the first line of defense against ROS

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

Superoxide Dismutase

SOD destroys O_2^- by successive oxidation and reduction of the transition metal ion at the active site in a Ping Pong type mechanism.

In humans, there are three forms of SOD: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD)

Mn-SOD is a homotetramer containing one manganese atom per subunit those cycles from Mn (III) to Mn (II) and back to Mn (III) during the two step dismutation of superoxide .

Superoxide Dismutase

Mn-SOD has been shown to be greatly induced and depressed by cytokines, but is only moderately influenced by oxidants .

Inactivation of recombinant human mitochondrial Mn- SOD by peroxynitrite is caused by nitration of a specific tyrosine residue

The biological importance of Mn-SOD is demonstrated among others by the following observations:

Superoxide Dismutase

a) inactivation of Mn-SOD genes in Escherichia coli increases mutation frequency when grown under aerobic conditions
b) lack of expression in Mn- SOD knockout mice results in dilated cardiomyopathy and neonatal lethality
c) tumor necrosis factor (TNF) selectively induces Mn-SOD, but not Cu/Zn- SOD, CAT or GPX
d) transection of Mn- SOD cDNA into cultured cells rendered the cells resistant to parquet, TNF and Adriamycin-induced cytotoxicity, and radiation induced-neoplastic transformation
e) expression of human Mn-SOD genes in transgenic mice protects against oxygen induced pulmonary injury and Adriamycin-induced cardiac toxicity

Superoxide Dismutase

Cu/Zn-SOD (SOD-1) is another type of enzyme which is believed to play a major role in the first line of antioxidant defence.

Mn- SOD is essential for life whereas Cu/Zn-SOD is not

Extracellular superoxide dismutase (EC-SOD) is a secretory, tetrameric, copper and zinc containing glycoprotein; with a high affinity for certain glycosaminoglycans such as heparin and heparin sulphate.

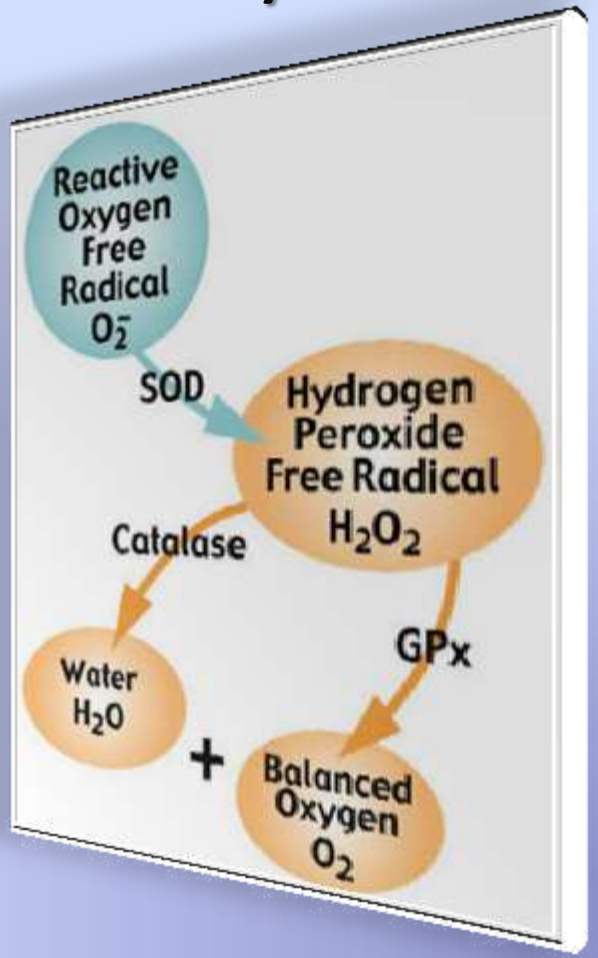
EC-SOD was found in the interstitial spaces of tissues and also in extracellular fluids, in plasma, lymph, and synovial fluid.

Superoxide Dismutase

EC-SOD is not induced by its substrate or by other oxidants and its coordinated by cytokines

FeSOD isozymes, often detected in plants, are usually associated with the chloroplast compartment

NiSOD is the most recent class of SOD, which was discovered in Streptomyces and cyanobacteria



Application

Superoxide Dismutase

- **SOD is found in our skin and it is essential to generate adequate amounts of skin-building cells called fibroblasts.**
- **SOD plays a significant role in preventing the development of the Amyotrophic Lateral Sclerosis (ALS). This kind of illness can lead to death because it affects the nerve cells in the spinal cord and the brain.**
- **this enzyme is also used for treatment of inflammatory diseases, burn injuries, prostate problems, arthritis, corneal ulcer, and reversing the long term effects of radiation and smoke exposure.**

- Additionally, if superoxide dismutase is made into a lotion and applied to the skin, it will prevent the formation of wrinkles. It will also heal wounds, reduce the appearance of scars, and lighten skin pigmentation that has been caused by UV rays.

- SOD is also known to help carry nitric oxide into our hair follicles. This is beneficial for people who are experiencing premature hair loss due to a genetic predisposition or free radicals. Because this enzyme is a very potent antioxidant, Since nitric oxide relaxes the blood vessels and allows more blood to circulate to the hair follicles.

Catalase

- Animal CAT are heme-containing enzymes that convert hydrogen peroxide (H_2O_2) to water and O_2
- they are largely localized in subcellular organelles such as peroxisomes Mitochondria and the endoplasmic reticulum contain little CAT. Thus, intracellular H_2O_2 cannot be eliminated unless it diffuses to the peroxisomes

$$2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$$

- Catalase also binds NADPH as a reducing equivalent to prevent oxidative inactivation of the enzyme by H_2O_2

Application

Catalase

-CAT is used in the food industry for removing hydrogen peroxide from milk prior to cheese production.

-Another use is in food wrappers where it prevents food from oxidizing

-CAT is also used in the textile industry, removing hydrogen peroxide from fabrics to make sure the material is peroxide-free

- A minor use is in contact lens hygiene - a few lens-cleaning products disinfect the lens using a hydrogen peroxide solution; a solution containing CAT is then used to decompose the hydrogen peroxide before the lens is used again.

- Recently, CAT has also begun to be used in the aesthetics industry.
-Several mask treatments combine the enzyme with hydrogen peroxide on the face with the intent of increasing cellular oxygenation in the upper layers of the epidermis

Glutathione peroxidase

- Glutathione peroxidase (GPx) is an enzyme that is responsible for protecting cells from damage due to free radicals like hydrogen and lipid peroxides.
- GPX catalyzes the reduction of hydro peroxides using GSH, thereby protecting mammalian cells against oxidative damage. In fact, glutathione metabolism is one of the most essential ant oxidative defence mechanisms.

There are five GPX isoenzymes found in mammal, the levels of each isoform vary depending on the tissue type

GPX1 is predominantly present in erythrocytes, kidney, and liver

Cytosolic and mitochondria glutathione peroxidase (cGPX or GPX1) reduces fatty acid hydro peroxides and H_2O_2 at the expense of glutathione

. GPX1 and the phospholipid hydro peroxide glutathione peroxidase (PHGPX or GPX4) are found in most tissues.

GPX4 is located in both the cytosol and the membrane fraction

. Cytosolic GPX2 or GPX-G1, and extracellular GPX3 or GPX-P is poorly detected in most tissues except for the gastrointestinal tract and kidney,

. Recently, a new member, GPX5 expressed specifically in mouse epididymis,

Application

Glutathione peroxidase

. Levels of GPX in the body are closely linked with that of glutathione, the master antioxidant.

The role as antioxidant is particularly important for brain as it is very sensitive to presence of free radicals.

Combination of certain

antioxidants like glutathione, vitamin C and E, selenium and glutathione peroxidase are very powerful in helping the body fight against the free radicals.

GSH ensures that the red blood cells remain intact and protect the white blood cells (which are responsible forimmunity)

Glutathione is found in vegetables and fruit, but cooking will significantly reduce its potency.

Superoxide Reductase (SOR)

the enzyme responsible for the detoxification of O_2^- ,

found only in prokaryotic cells, allowing them to survive

in the presence of O_2

Thiol-containing Enzymes:-

- They are responsible for disposal of H_2O_2 .
- Ex:- thioredoxin reductases (TRRs), thioredoxin peroxidases (PRXs), and glutaredoxins.
- they are existing in both cytosol and mitochondria.
- they are expressed in bronchial and alveolar epithelium and they have potential protective effect in the development of ROS-mediated lung injury

Glutathione tr es (GSTs):-

- Glutathione transferases (GSTs), another antioxidant enzyme family, inactivate secondary metabolites, such as unsaturated aldehydes, epoxides, and hydroperoxides.
- Three major families of GSTs have been described: cytosolic GST, mitochondrial GST and membrane-associated microsomal GST that has a role in eicosanoid and GSH metabolism

Indole-2,3-dioxygenase (IDO)

-IOD uses O_2^- as cofactor in the initial step during the degradation of the indole ring of Trp to form kynurenine.

- The enzyme scavenges O_2^- , which increases only when SOD is inhibited

-IDO is stimulated by pro-inflammatory cytokines, especially IFN-virus infection and the administration of bacterial endotoxin .

-The induction of IDO causes a marked increase in Trp catabolism

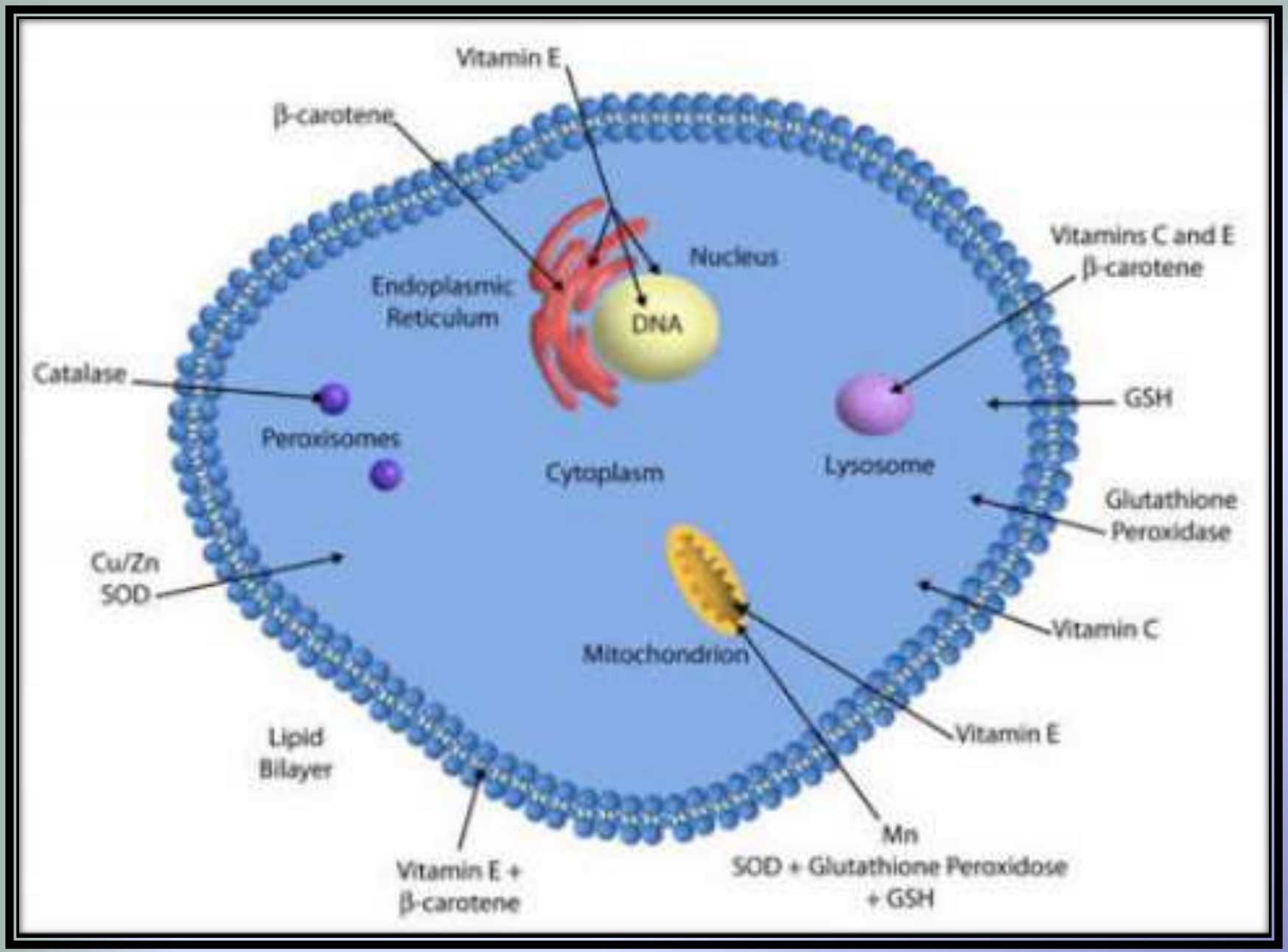
-Trp is essential for the growth of bacteria and the growth of bacteria is suppressed by actively depleting Trp within infected cells by IDO

-IDO is down-regulated by NO

Heme Oxygenase

- ✓ Heme oxygenase(HO) is the rate limiting enzyme in the degradation of heme and results in the release of biliverdin, iron and carbon monoxide , Biliverdin reductase subsequently converts biliverdin to bilirubin .
- ✓ there is 3isoforms of heme oxygenase .
- ✓ The protective effects resulting from HO-1 activity are due to its inducibility by a variety of stimuli including heme, nitric oxide (NO), cadmium, growth factors, hyperoxia

- ✓ **HO-1 plays a cytoprotective role in modulating tissue responses to injury in several pathophysiological states. It has antioxidant, anti-inflammatory and anti-apoptotic properties HO-1 has been implicated in protection against ischemia-reperfusion injury, inflammation/immune dysfunction**
- ✓ **HO-2, act as a physiological regulator of cellular function**


Vitamin E
β-carotene
Nucleus
Endoplasmic Reticulum
DNA
Vitamins C and E
β-carotene
Catalase
GSH
Peroxisomes
Cytoplasm
Lysosome
Glutathione Peroxidase
Cu/Zn SOD
Vitamin C
Mitochondrion
Vitamin E
Lipid Bilayer
Mn SOD + Glutathione Peroxidose + GSH
Vitamin E + β-carotene

Clinical Applications Of Antioxidant Enzymes

1. Chronic Inflammation:

Chronic inflammatory diseases such as rheumatoid arthritis are self-perpetuated by the free radicals released by neutrophils. Both corticosteroids and non-steroids anti inflammatory drugs interfere with formation of free radicals and interrupt the disease process.

2. Acute Inflammation:

At the inflammatory site, activated macrophages produce free Radicals .

3. Respiratory Diseases:

- Breathing of 100 % oxygen for more than 24 hr produces destruction of endothelium and lung edema. This is due to the release of free radicals by activated neutrophils.
- In premature newborn infants, prolonged exposure to high oxygen concentration is responsible for bronchopulmonary dysplasia.

- **Adult respiratory distress syndrome (ARDS) is characterized by pulmonary edema. ARDS is produced when neutrophils are recruited to lungs which subsequently release free radicals.**

- **Cigarette smoke contains free radicals. It attracts neutrophils to the site which releases more free radicals. leading to lung damage.**

4. Diseases Of The Eye:

- Retrolental fibroplasia or retinopathy of prematurity is a condition seen in premature infants treated with pure oxygen for a long time. It is caused by free radicals, causing thromboxane release, sustained vascular contracture and cellular injury.

- Cataract formation is related with ageing process. Cataract is partly due to photochemical generation of free radicals.

5. Shock Related Injury:

Release of free radicals from phagocytes damage membranes by lipid peroxidation. They release leucotrienes from platelets and proteases from macrophages. All these factors cause increased vascular permeability, resulting in tissue edema. Anti-oxidants have a protective effect.

6. Arthrosclerosis And Myocardial Infraction:

Low density lipoproteins (LDL) promote atherosclerosis. They are deposited under the endothelial cells, which undergo oxidation by free radicals released from endothelial cells. This attracts macrophages.

Macrophages are them converted into foam cells. This initiates the atherosclerotic plaque formation.

7. Peptic Ulcer:

Peptic ulcer is produced by erosion of gastric mucosa by hydrochloric acid. It is shown that superoxide anions are involved in the formation of ulcer. This infection potentiates the macrophage oxidative burst leading to tissue destruction.

8. Cancer Treatment :

Free radicals contribute to cancer development because of their mutagenic property. Free radicals produce DNA damage, and accumulated damages lead to somatic mutations and malignancy