Respiratory module

Oxygen Toxicity
Free radicals and antioxidants

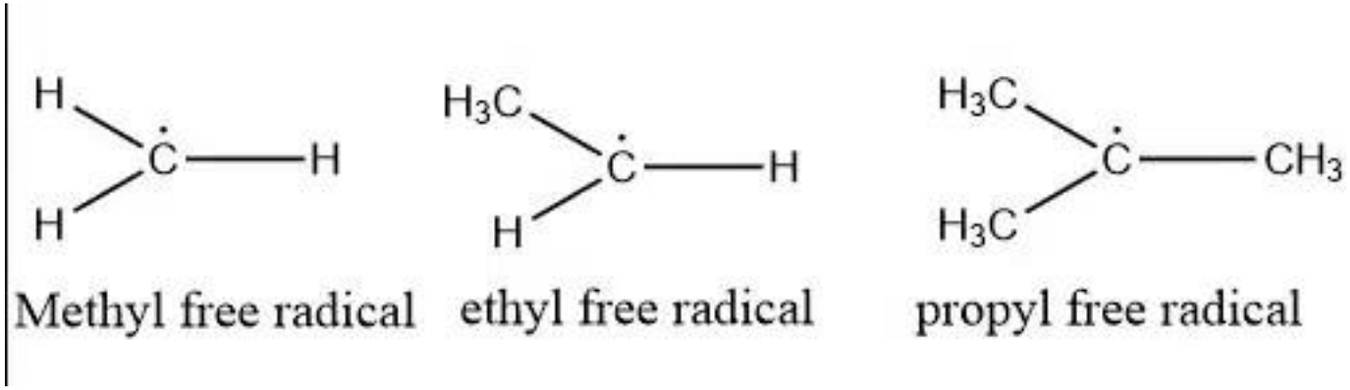
Asst. Prof.Mohammed Hasan Eleyan

Faculty of Medicine

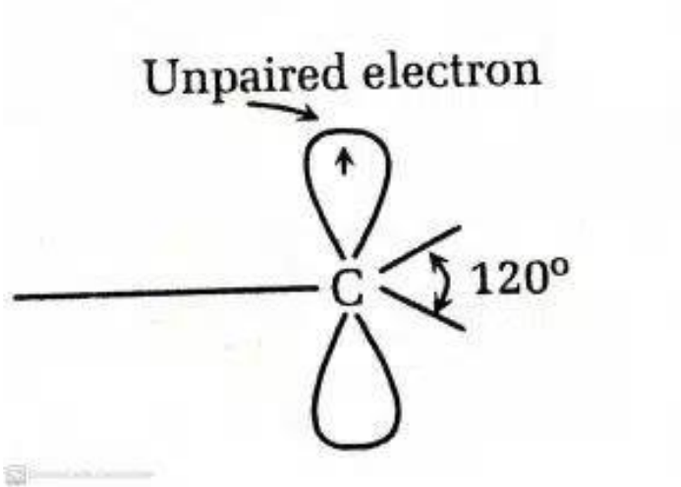
Al-Azhar University-Gaza

Definition of Free Radicals:

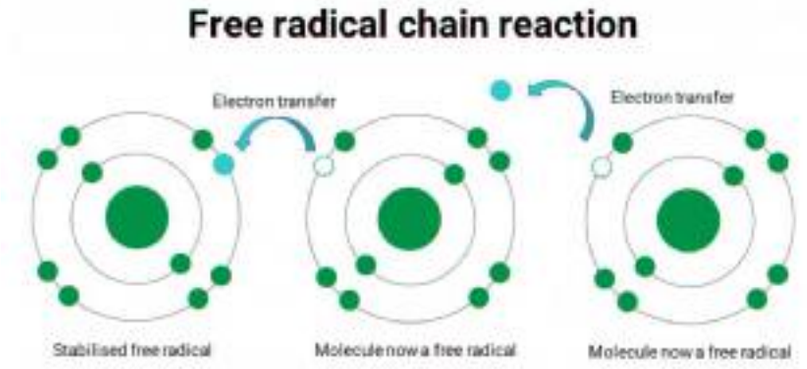
- **Radicals, also known as free radicals in chemistry, are molecules or ions that have unpaired electrons in their atomic orbitals.**
- **Therefore, the free radical will be very reactive and unstable.**



Methyl free radical ethyl free radical propyl free radical



Mechanism of Action:



- **Reactivity and Instability:** Free radicals are unstable because the unpaired electron seeks stability by pairing with another electron. This leads to a chain reaction of electron "theft" (electron transfer), where:

  A. The original free radical becomes stable after gaining an electron.

  B. The atom or molecule that loses the electron becomes a new free radical.

- **Propagation of Chain Reaction:** This chain reaction can lead to significant damage in biological systems, such as cell membranes, proteins, and DNA.

Types of Free Radicals

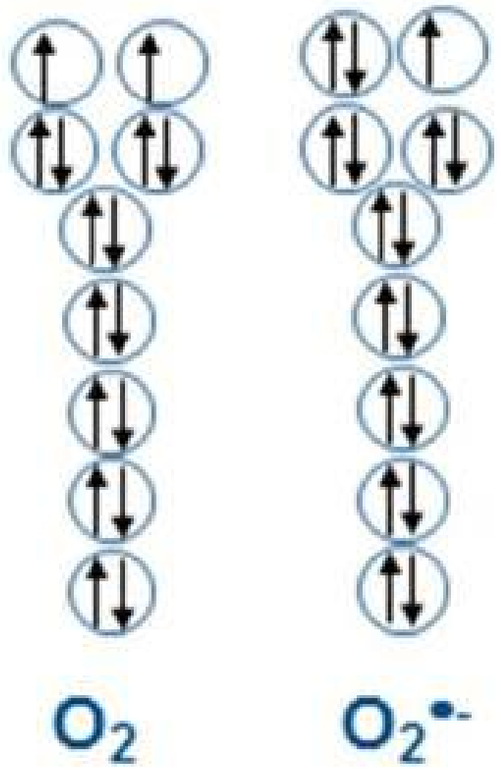
- **Oxygen-Derived Free Radicals**
- **Nitrogen derived free radicals**
- **Lipid derived free radicals**
- **Xenobiotic derived free radicals**

Oxygen-Derived Free Radicals (Reactive Oxygen Species, ROS)

- These are the most common free radicals found in biological systems and are derived from oxygen.
- Examples:

1. Superoxide anion ($O_2^{\bullet -}$)
2. Hydrogen peroxide (H_2O_2) not a free radical but can generate them.
3. Hydroxyl radical ($OH^{\bullet}$)

Simplified version of bonding in diatomic oxygen and superoxide radicals ($O_2^{\bullet -}$)



Partial reduction of molecular oxygen (O_2), forming reactive oxygen species (ROS)

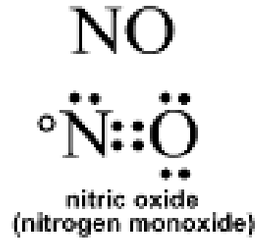
$$O_2 \xrightarrow{+e^-} O_2^- \xrightarrow{+e^-,H^+} H_2O_2 \xrightarrow{\text{Fenton}} OH\cdot$$

Oxygen (O_2) Superoxide anion ($O_2^{\bullet-}$) Hydrogen Peroxide (H_2O_2) Hydroxyl Radical ($OH\cdot$)

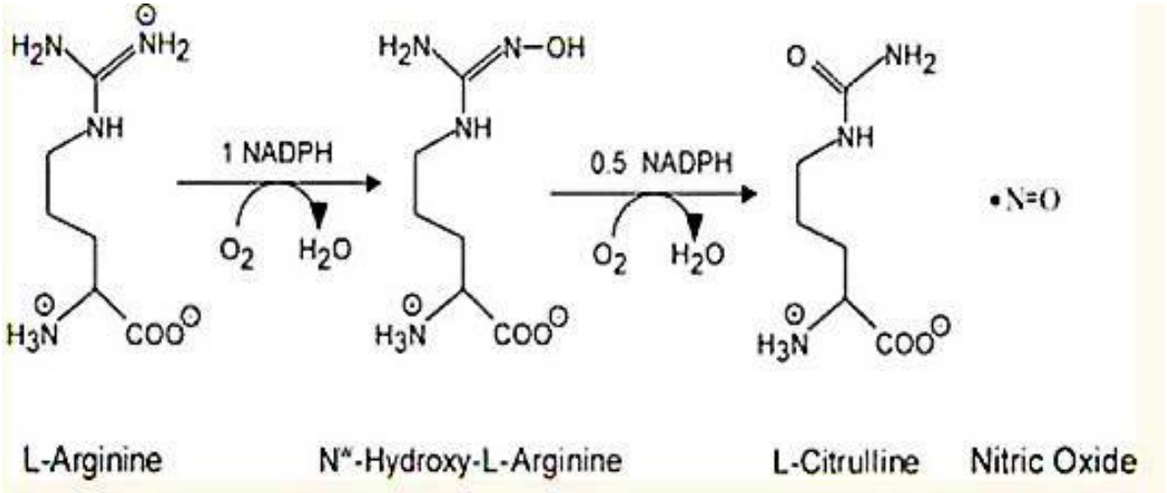
$$H_2O_2 + Fe^{2+} \rightarrow OH\cdot + OH^- + Fe^{3+}$$

Fenton reaction

Nitrogen derived free radicals [Reactive Nitrogen Oxygen species (RNOS)]



- **Nitric oxide (NO•):** **Nitric oxide synthases (NOS) catalyzes the conversion of L-arginine to NO and L-citrulline, using molecular oxygen (O_2) and NADPH.**



- **However, under pathological conditions, excessive NO production contributes to the formation of reactive nitrogen species.**

NO plays roles in:

- **Vasodilation**
- **Neurotransmission in the brain.**
- **Immune defense during infection.**

Nitrogen derived free radicals [Reactive Nitrogen Oxygen species (RNOS)]

- **Peroxynitrite (ONOO–) produced by the reaction of nitric oxide (•NO) with superoxide (O2•–).**
- **Peroxynitrite is a reactive nitrogen species (RNS) but does not have an unpaired electron, so it is not classified as a free radical.**

$$\bullet NO\ (\text{nitric oxide}) + O_2^{\bullet -}\ (\text{superoxide}) \longrightarrow ONOO^-\ (\text{peroxynitrite})$$

Nitrogen derived free radicals [Reactive Nitrogen Oxygen species (RNOS)]

•NO2

- **Peroxynitrite can react with other molecules to form additional types of RNS including nitrogen dioxide (•NO2) and dinitrogen trioxide (N2O3) as well as other types of chemically reactive free radicals. Important reactions involving RNS include:**

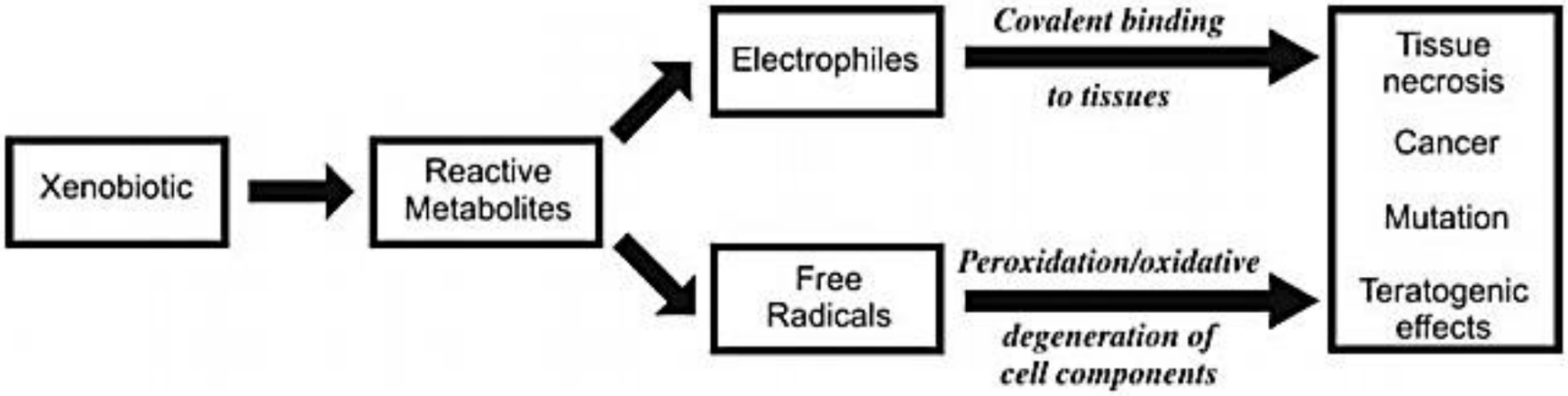
  - $ONOO^- + H^+ \rightarrow ONOOH$ (peroxynitrous acid) $\rightarrow \cdot NO_2$ (nitrogen dioxide) + ·OH (hydroxyl radical)
  - $ONOO^- + CO_2$ (carbon dioxide) $\rightarrow ONOOCO_2^-$ (nitrosoperoxycarbonate)
  - $ONOOCO_2^- \rightarrow \cdot NO_2$ (nitrogen dioxide) + $O{=}C(O\cdot)O^-$ (carbonate radical)
  - $\cdot NO + \cdot NO_2 \rightleftharpoons N_2O_3$ (dinitrogen trioxide)

Lipid derived free radicals:

- They include lipid free radicals (L•), lipid peroxide (LOO•), lipid hyperoxide (LOOH) & Malondialdehyde (MDA)
- Although oxygen free radicals are the most reactive free radicals, their danger is limited because they are short —living, oppositely, Lipid free radicals are more dangerous because they are weaker & long living so; they attack more important molecules particularly DNA.

Xenobiotic derived free radicals

- **Several toxic, mutagenic, carcinogenic chemical compounds that enter the human body from the environment (exogenous source) are already in a free radical from or are convened by xenobiotic metabolism enzymes into reactive species.**

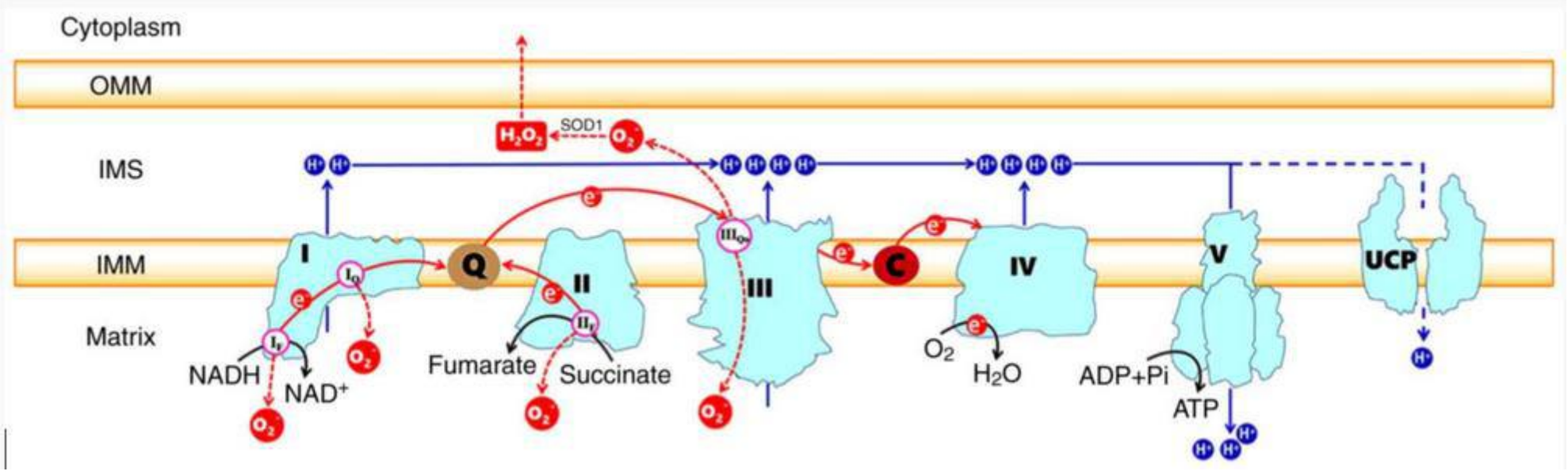


Sources of Free radicals:

- **Endogenous sources**
- **Exogenous sources.**

Endogenous sources:

- **Mitochondria are a main source of cellular ROS. Under physiological conditions, 0.2-2% of the electrons in the ETC do not follow the normal transfer order but instead directly leak out of the ETC and interact with oxygen to produce superoxide or hydrogen peroxide**



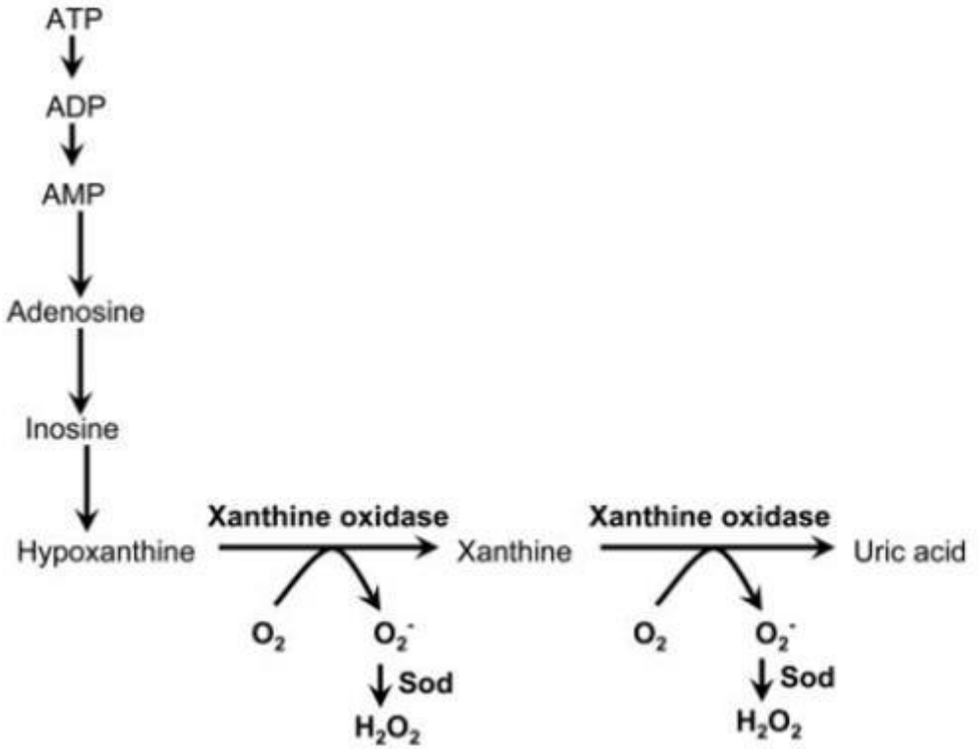
Endogenous sources:

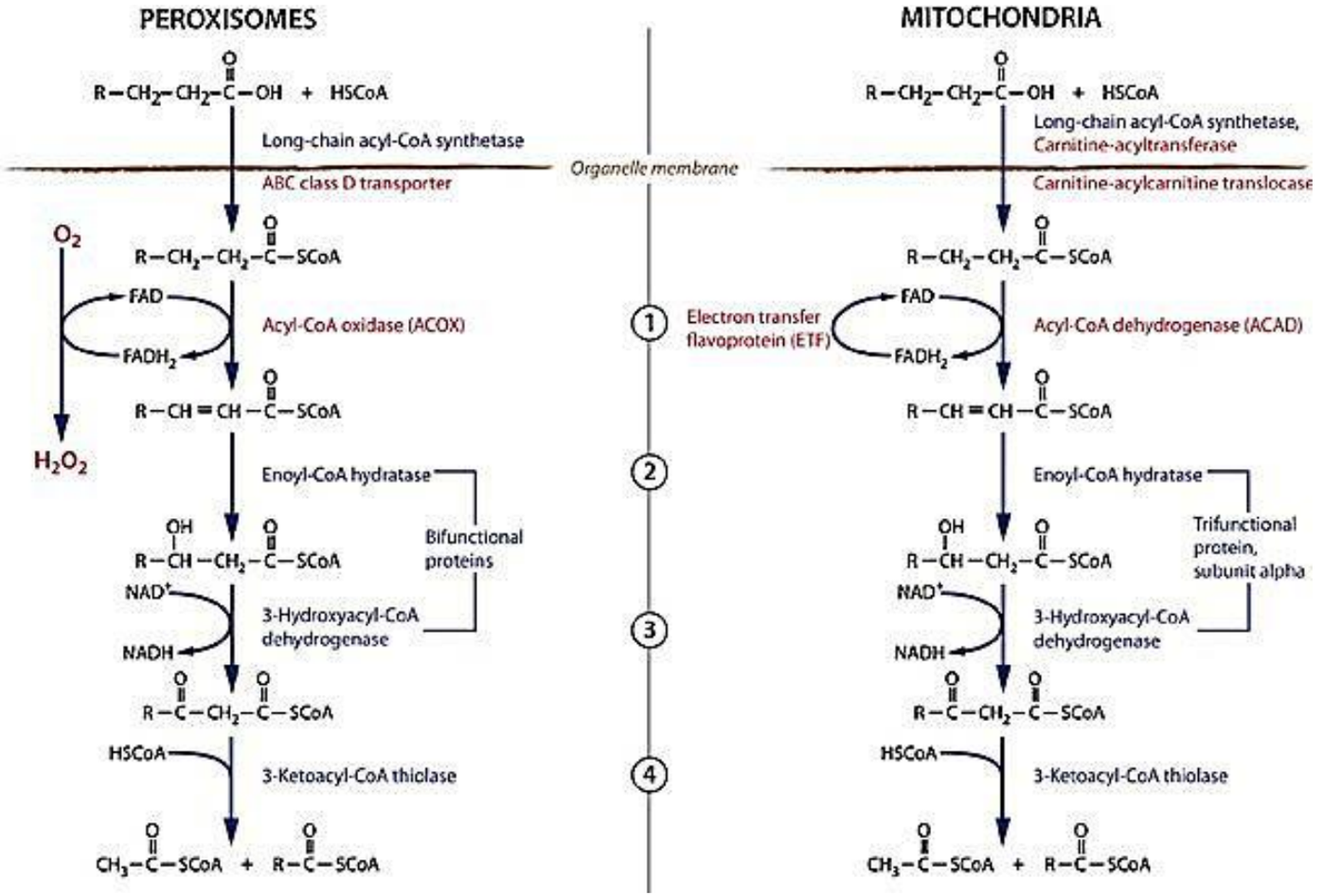
1. **Cytochrome P450-mono-oxygneases: This group of enzymes is involved in the detoxification of various drugs that enter the body. Consumption of alcohol and certain drugs induces expression of theses enzymes. So, patients who abuse such substances are more prone to the deleterious effects of ROS formed by these enzymes.**

$$\mathrm{RH} + O_2 + NADPH + H^+ \rightarrow \mathrm{ROH} + H_2O + NADP^+$$

Endogenous sources:

- **Oxidases and oxygenase enzymes e.g. xanthine oxidase & peroxisomal oxidation of fatty acids produces H2O2.**





Endogenous sources:

- **Auto-oxidation reactions: Transitional metals, Fe+3 & Cu+2 help formation of free radical.**

Iron Auto-Oxidation:

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^- \cdot$$

Ferrous iron (Fe2+) is oxidized to ferric iron (Fe3+), generating **superoxide ($O_2^{-}\bullet$)**.

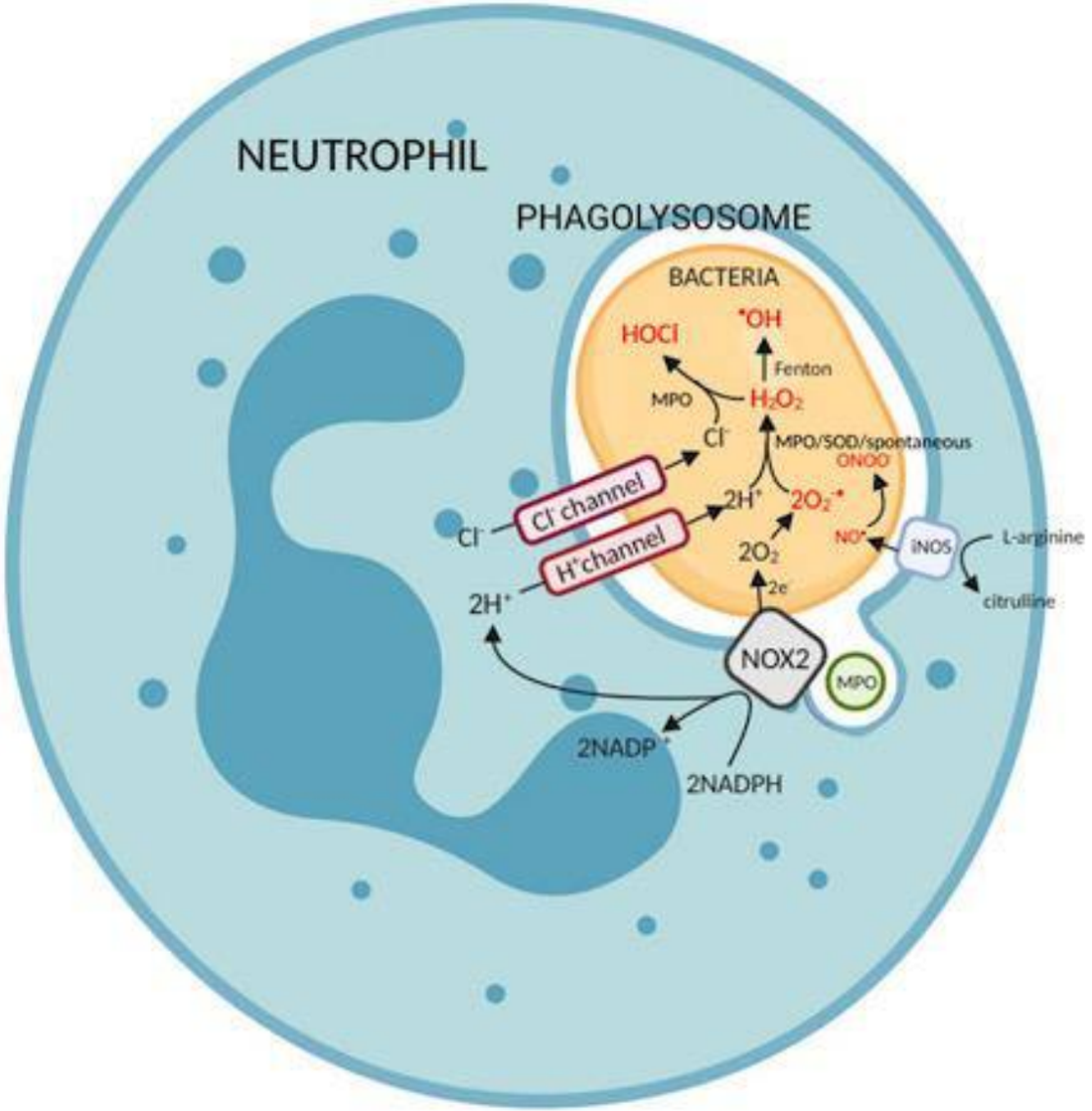
Copper Auto-Oxidation:

$$Cu^{+} + O_2 \rightarrow Cu^{2+} + O_2^- \cdot$$

Cuprous copper (Cu+) is oxidized to cupric copper (Cu2+), generating **superoxide ($O_2^{-}\bullet$)**.

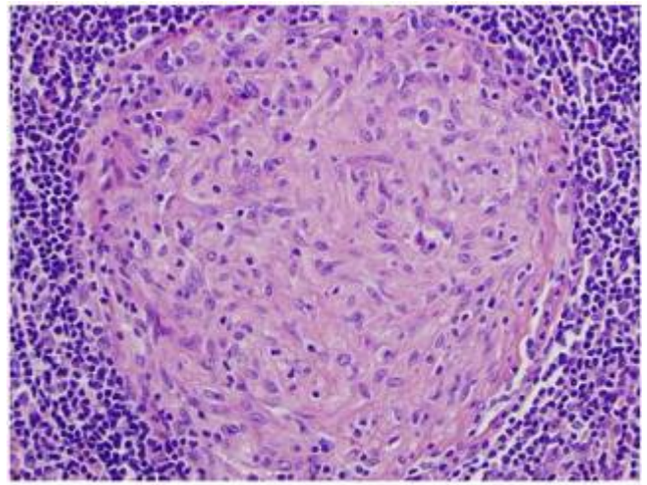
Endogenous sources:

- **Activation of cells of immune system during phagocytosis of invading microorganisms, they utilize NADPH oxidase, myeloperoxidase enzyme & nitric oxide synthase to generate massive amounts of superoxide ($O_2^{\bullet -}$) , hypochlorus acid ($HOCl^{\bullet}$) & Nitric oxide ($NO^{\bullet}$) respectively. These kill phagocytosed microorganism.**



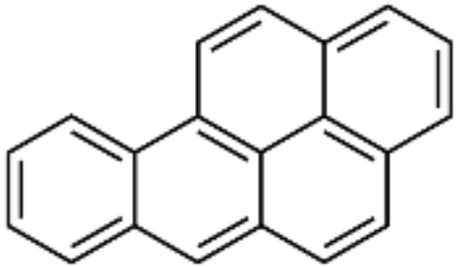
Endogenous sources:

- **Clinical Correlates:**
  - **Chronic granulomatous disease (CGD) results from a deficiency of NADPH oxidase and the inability to effectively kill engulfed microbes, especially bacteria. Patients with CGD present with serious recurrent bacterial infections.**



Exogenous sources.

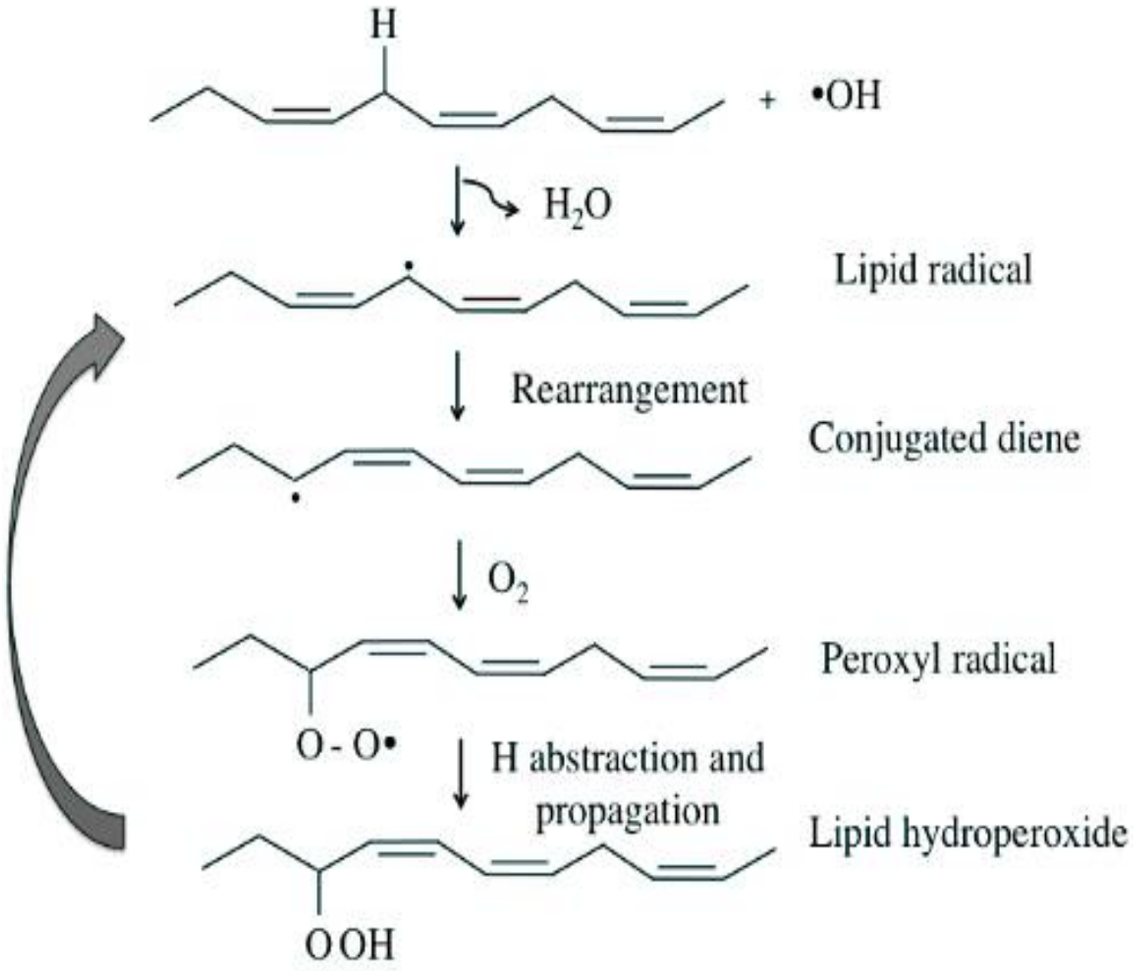
- Cigarette smoke → all types of free radicals.
- Ozone (0_3) → major pollutant of heavily polluted cities.
- Ionizing radiation → X and γ-rays → (•OH)
- Non-ionizing radiation → UV rays.
- Toxins, e.g. benzopyrene and aniline dye.
- Air Pollution by Incomplete Combustion of Fuel



Benzo-a-pyrene

Damaging Effects of Free radical

- **Damage to lipid (lipid peroxidation)**
- **Protein damage**
- **DNA damage**

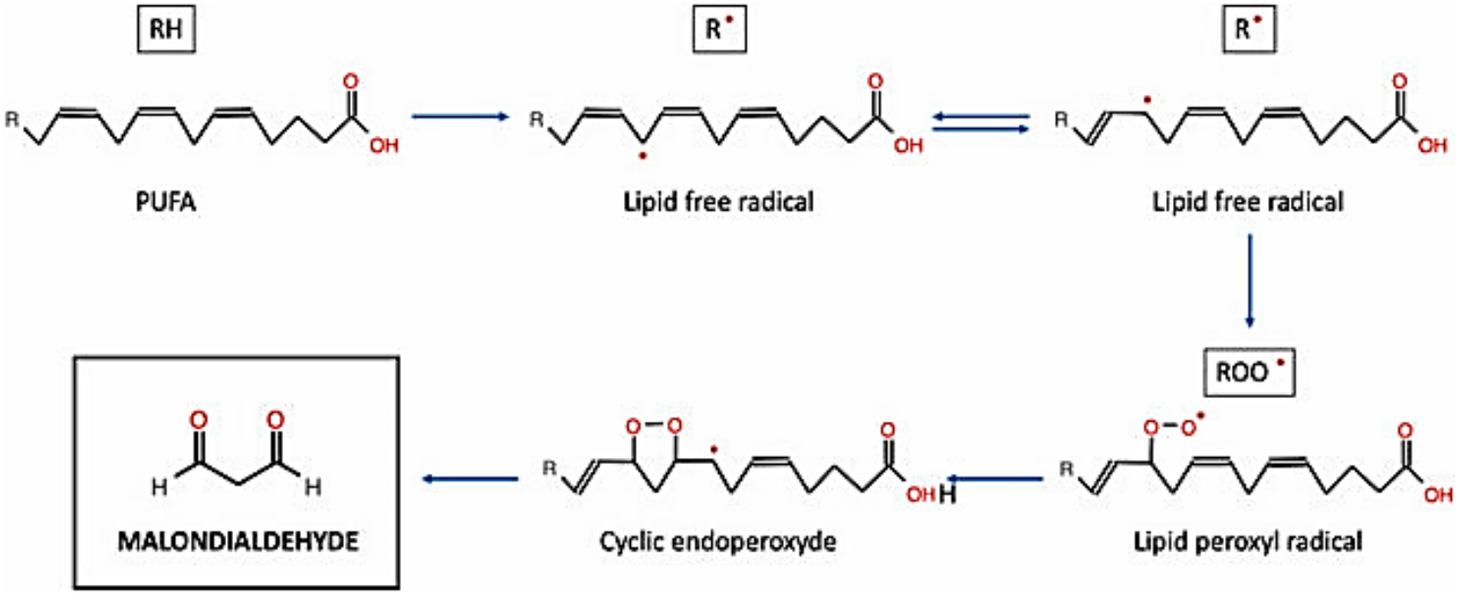


Damage to lipid (lipid peroxidation):

- **Lipid peroxidation is initiated by the hydroxyl radical abstracting a hydrogen from the methylene group adjacent to a double bond of fatty acids.**
- **The fatty acid may also be part of the phospholipid or esterified to the –OH group of cholesterol in a lipoprotein. This process gives rise to an unstable lipid radical, which undergoes rearrangement of double bonds and addition of oxygen to form a peroxyl radical.**
- **The lipid radical or the peroxyl radical could react with a neighboring fatty acyl chain as well, thereby propagating the peroxidation process.**
- **The chain reaction may be terminated by eventual formation of a lipid hydroperoxide.**

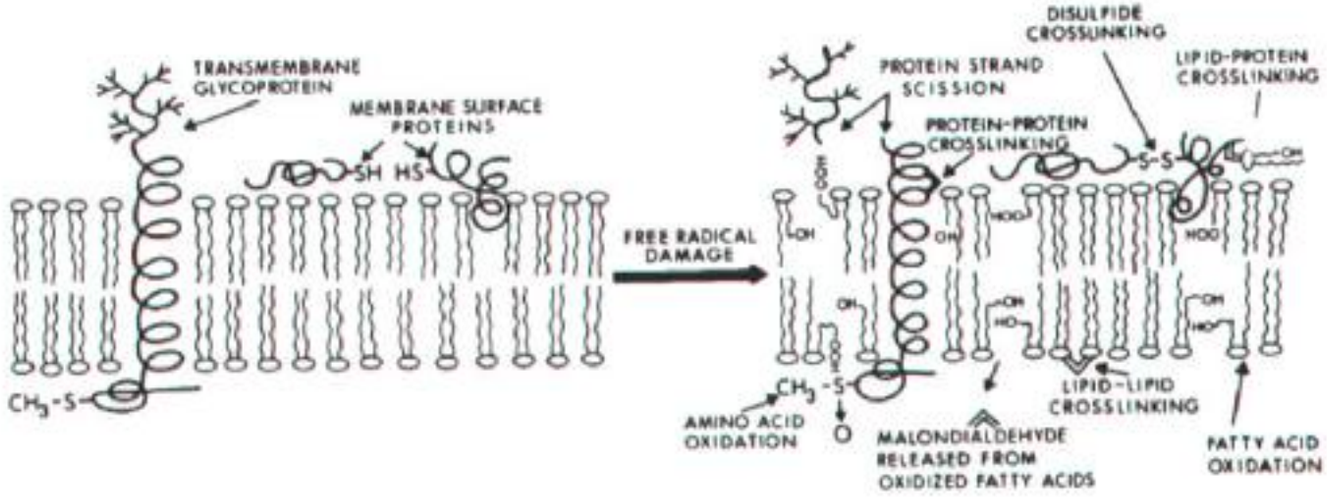
Malondialdehyde (MDA)

- **Malondialdehyde (MDA) formation through lipid peroxidation in mitochondria. Lipids are very sensitive to ROS attack and MDA is a biomarker for lipid peroxidation assessment.**

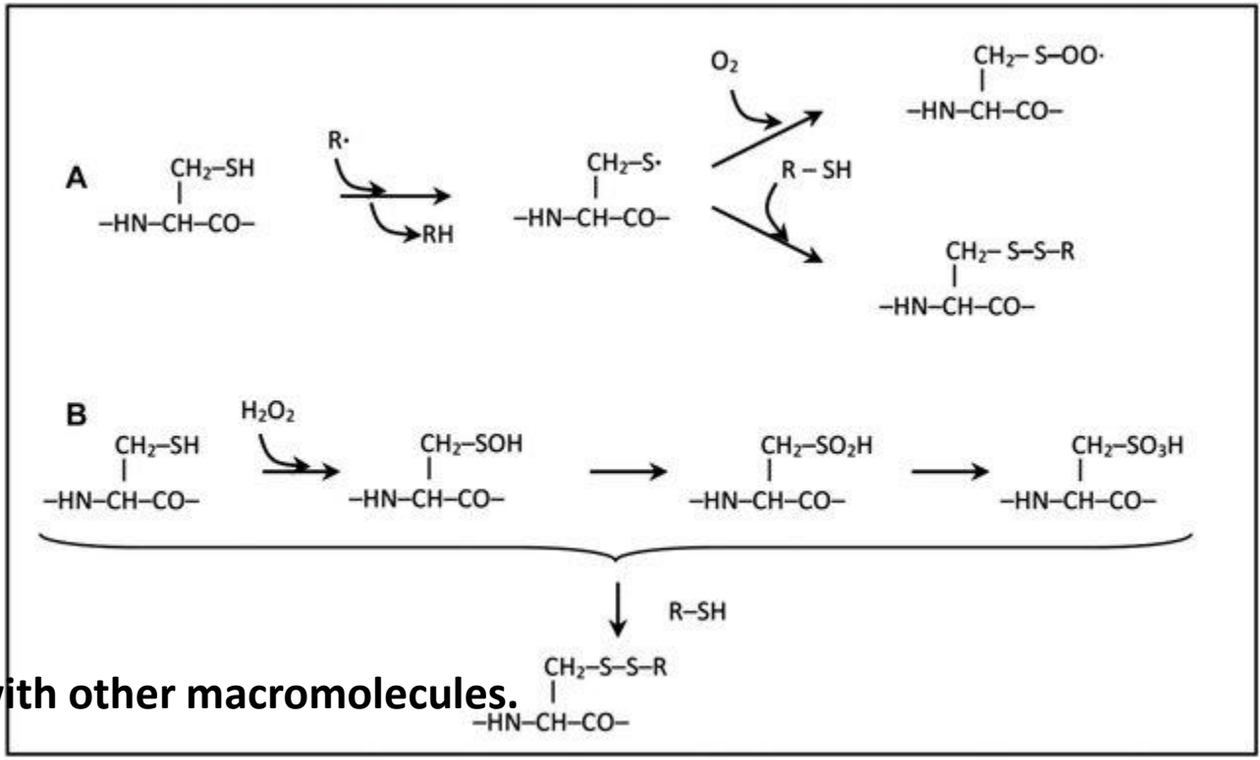


Damage to lipid (lipid peroxidation):

- This peroxidation affects polyunsaturated fatty acids (PUFAs), So, it affects unsaturated phospholipids, glycolipids and cholesterol in cell membranes.
- Peroxidation of these lipids disturbs membrane structure and functions which results in increase membrane fluidity, change transport mechanisms and inactivation of membrane bound receptors and enzymes.



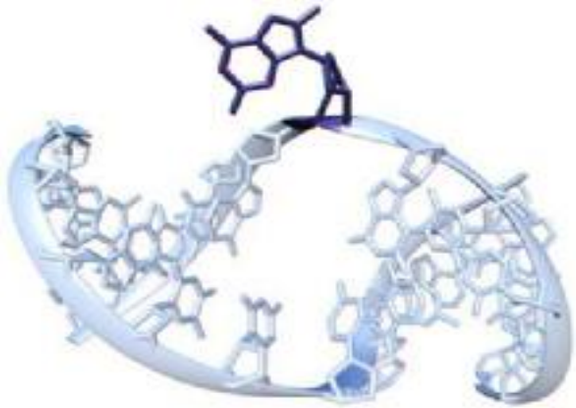
Protein damage:



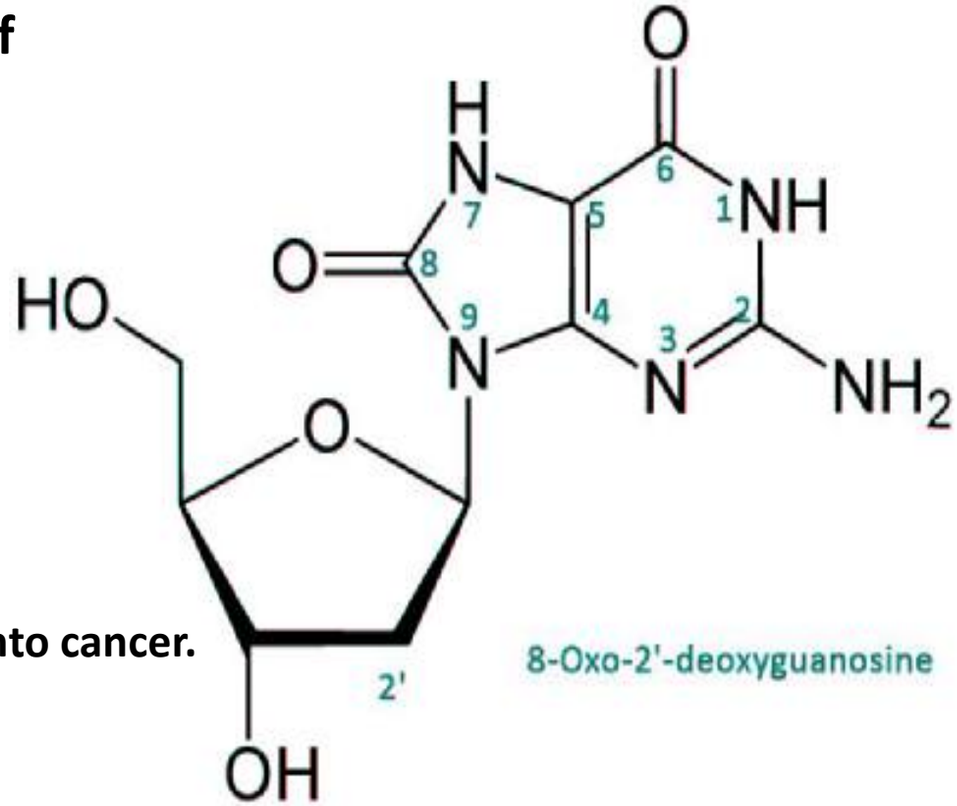
- **This includes:**
  - **Oxidation of—SH groups in proteins**
  - **Breakage of the protein backbone**
  - **Cross linkage of proteins with each other and with other macromolecules.**
- **This leads to:**
  - **Loss of biological activity of proteins i.e. enzymes.**
  - **Denaturation of structural protein leading destruction of architecture of the cells and basement membrane & finally cell death.**

Note: Sulfenic acid R–S–OH
Sulfinic acids (R–S(=O)OH)
Sulfonic acids ($R–S(=O)_2OH$),

DNA damage:



- **Oxidation & Damage of Bases & sugars: 8-Oxo-2'-deoxyguanosine (8-oxo-dG) is an oxidized derivative of deoxyguanosine.**
- **Breakage of one or two strands of DNA.**
- **Cross linking between DNA & DNA or DNA &protein.**
- **This leads to:**
  - **Mutations followed by cell death or cellular transformation into cancer.**



8-Oxo-2'-deoxyguanosine

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Antioxidants

- **Antioxidants are molecules, enzymes, or mechanisms that prevents generation of free radicals (Primary/Preventive), scavenges free radicals (Secondary/Scavenging) or repairs damage induced by free radicals (Tertiary/Reparative).**

Primary Antioxidants (Preventive Antioxidants)

- **Primary antioxidants prevent the generation of free radicals by targeting the processes or factors that lead to their formation.**

1. **Chelating proteins for transition metals**
2. **Enzyme antioxidants**

Chelating proteins for transition metals

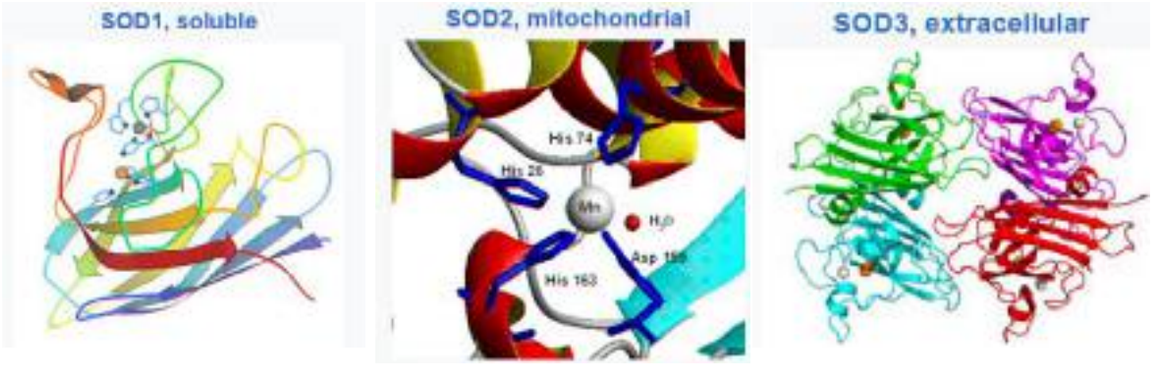
- They prevent the existence of free iron or copper and thus prevents the iron or copper catalyzed free radical formation:
- Transferrin, Ferritin, Lactoferrin: Iron-binding proteins that sequester free iron.
- Ceruloplasmin: Copper-binding protein.

Enzyme antioxidants

- **Enzyme antioxidants are critical components of the body's defense system against free radicals. They neutralize reactive oxygen species (ROS) and reactive nitrogen species (RNS) through enzymatic reactions, preventing oxidative damage to biomolecules like DNA, proteins, and lipids.**

1. **Superoxide Dismutase (SOD)**
2. **Catalase**
3. **Glutathione Peroxidase (GPX)**

Superoxide Dismutase (SOD)



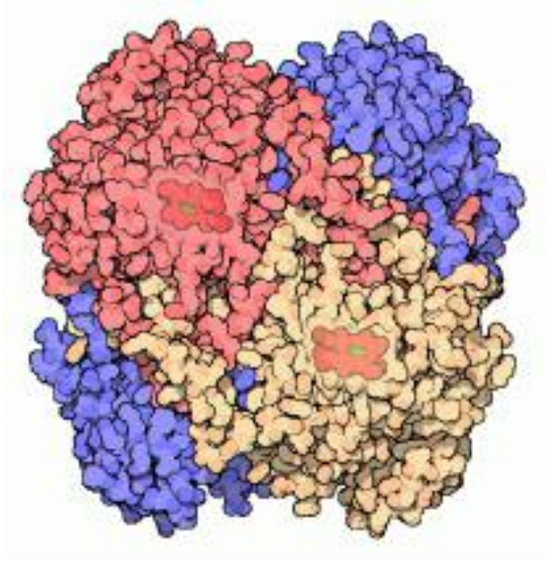
- **Function:**
  - **Converts superoxide anion (O2−) into hydrogen peroxide (H2O2) and oxygen (O2) through a dismutation reaction.**

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

- **Types of SOD:**
  1. **Cu/Zn-SOD: Found in the cytoplasm; contains copper (Cu2+) and zinc (Zn2+) ions as cofactors.**
  2. **Mn-SOD: Found in the mitochondria; uses manganese (Mn2+) as a cofactor.**
  3. **Extracellular-SOD (EC-SOD): Found in the extracellular space; primarily involves Cu2+ and Zn2+.**

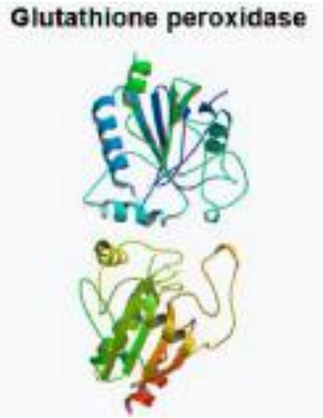
Catalase

- **Function:**
  - **Catalyzes the decomposition of hydrogen peroxide (H2O2H) into water (H2O) and oxygen (O2).**
- **Heme-containing enzyme: Contains an iron (Fe) atom in its active site.**



$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Glutathione Peroxidase (GPX)



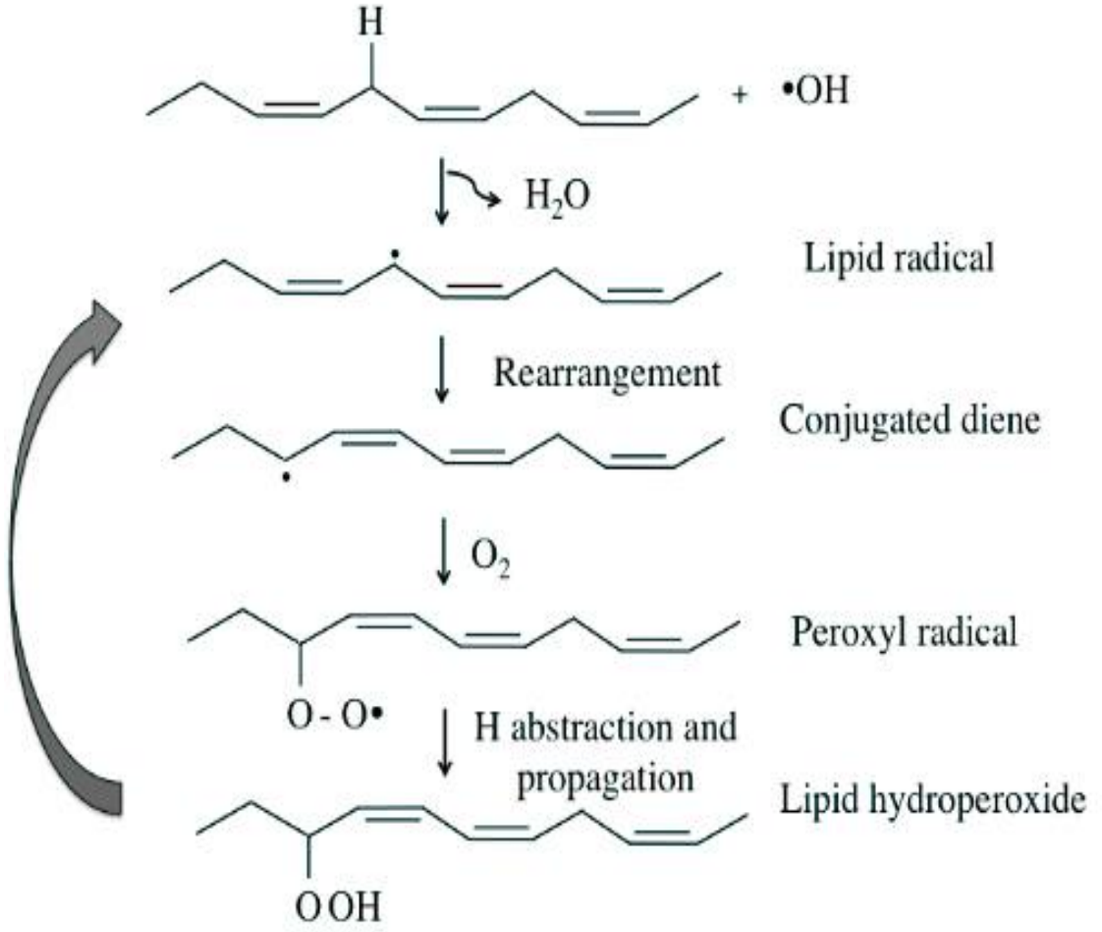
- **Function:**
  - **Reduces hydrogen peroxide (H2O2) or organic hydroperoxides (ROOH) to water (H2O) or the corresponding alcohol (ROH), using reduced glutathione (GSH) as a coenzyme.**

$$H_2O_2 + 2GSH \xrightarrow{\text{GPX}} 2H_2O + GSSG$$

$$ROOH + 2GSH \xrightarrow{\text{GPX}} ROH + H_2O + GSSG$$

Secondary antioxidants

- **Secondary antioxidants, also called chain-breaking antioxidants, play a vital role in minimizing damage caused by free radicals, especially during lipid peroxidation. They act by scavenging lipid free radicals, thereby halting the chain reaction and preventing further oxidative damage to cell membranes and other structures.**



Secondary antioxidants

- Vitamins, e.g. Vit. E, A, and C which is the most potent
- Non — Vitamins: include uric acid , bilirubin, glucose and proteins as albumin.

Tertiary Antioxidants (Reparative Antioxidants)

- Tertiary antioxidants, also called reparative antioxidants, are involved in repairing or replacing damaged molecules that have been affected by free radicals and reactive species. Instead of preventing or scavenging free radicals like primary and secondary antioxidants, tertiary antioxidants focus on restoring the structural and functional integrity of biomolecules.
- Examples:
  - DNA Repair Enzymes
  - Lysosomal Enzymes

DNA Repair Enzymes

- **Function:** Repair oxidative damage to DNA bases, breaks in DNA strands, or cross-linking between DNA and proteins.
- **Examples:**
  - **Base Excision Repair (BER):** Repairs oxidized DNA bases such as 8-oxo-deoxyguanosine.
  - **Nucleotide Excision Repair (NER):** Removes bulky DNA lesions caused by oxidative damage or UV radiation.
  - **Double-Strand Break Repair (DSBR):** Repairs breaks in both DNA strands caused by oxidative stress.

Lysosomal Enzymes

- Function: These enzymes degrade and recycle damaged biomolecules (proteins, lipids, and nucleic acids) that cannot be repaired.
- Examples:
- Proteases: Break down damaged or oxidized proteins.
- Lipases: Degrade oxidized lipids.
- Nucleases: Hydrolyze damaged DNA or RNA.

Oxidative stress

- **The generation of oxidant free radicals and the level of antioxidant defense systems are approximately balanced, the imbalance of oxidant free radicals/antioxidant systems is called oxidative stress. This imbalance is due either to:**
- **Increase formation of reactive species. and/ or,**
- **Decrease body-scavenging ability for these free radicals. and/ or**
- **Increase the availability of transition metal ions.**

Diseases related to the free radicals

Ischemic Reperfusion Injury.	Diabetes Mellitus and its complications.
Atherosclerosis.	Eye injury: e.g. cataract and retinopathy.
Cancers: due to DNA mutations.	Neurological diseases: e.g. Alzheimer's disease.
Aging: Neurodegenerative diseases, cancers and health diseases	Renal failure.
Prematurity diseases; Premature babies.	Male infertility: decreased motility & abnormalities of spermatozoa.
Immunological diseases: e.g. rheumatoid arthritis.	GIT diseases: e.g. Inflammatory bowel diseases.
Diabetes Mellitus and its complications.	Hematological diseases: e.g. favism and anemia.
Respiratory diseases: e.g. emphysema.	

Thanks