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MATHEMATICS AND INFORMATION TECHNOLOGIES**

**LECTURE No15**

# FREE-RADICAL PROCESSES

Sources of free radical generation in human body. Lipid peroxidation. Basic stages. Antioxidant defense system. Enzymic and nonenzymic antioxidants. Lipid peroxidation and toxicology.

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Free-radical reactions are **ubiquitous** in living organisms. Some are useful; others are unavoidable consequences of the environment in which we live.

When free-radical generation exceeds the capacity of antioxidant defenses, the result is **oxidative stress**.

Oxidative stress occurs in many human diseases and sometimes makes a significant contribution to their pathogenesis. Free-radical reactions have been suggested to be involved in a huge list of diseases, ranging from glomerulonephritis to AIDS .

## SOME DISORDERS REPORTED TO BE ASSOCIATED WITH OXYGEN-DERIVED RADICALS

***Inflammatory-immune injury:*** Glomerulonephritis, vasculitis, autoimmune disease, adult respiratory distress syndrome, rheumatoid arthritis, inflammatory bowel disease, pancreatitis;

***Cancer:*** Radiation-induced cancer, cervical carcinoma, hepatocellular carcinoma, promoters of carcinogenesis, cancer in inflammatory bowel disease;

***Ischemia/reoxygenation:*** Stroke, myocardial infarction, organ transplantation (heart, lung, skin, cornea, kidney), organ preservation, reattachment of severed limbs, frostbite, Dupuytren's contracture, hemorrhagic shock, endotoxic shock, crush injury;

***Metal overload:*** Hemochromatosis, thalassemia, chemotherapy for leukemias, fulminant hepatic failure, Wilson's disease, alcohol-induced iron overload, nickel-induced carcinogenesis, lead poisoning

***Toxins:*** Hemolytic drugs, lead, halogenated hydrocarbons, ozone, oxides of nitrogen, asbestos, other mineral dusts, sulfur dioxide, paraquat, aluminum, cigarette smoke, diabetogenic drugs, fava beans (hemolytic agents), anthracyclines (cardiotoxicity), heavy metals (nephrotoxicity), photosensitizing drugs, contact dermatitis

***Eye disorders:*** Cataract development, deterioration after ocular hemorrhage, photochemical retinal damage, retinopathy of prematurity (retrolental fibroplasia)

***Inborn diseases:*** Porphyrrias, sickle cell anemia, Fanconi anemia, neuronal ceroid lipofuscinoses, thalassemia;

***Insufficient antioxidant protection:*** Keshan disease (severe selenium deficiency), hemolytic disease of prematurity, retinopathy of prematurity, bronchopulmonary dysplasia, intracranial hemorrhage, neurological degeneration due to severe vitamin E deficiency (in inborn errors affecting intestinal fat absorption), acquired immunodeficiency syndrome;

***Brain and central nervous system disorders:*** Stroke, trauma, neurotoxicity (e.g., of aluminum), effects of hyperbaric oxygen, Parkinson's disease, potentiation of traumatic injury, cerebral malaria.

# What is Free Radical ?

- A free radical is defined as any species capable of independent existence that **contains one or more unpaired electrons**. Molecules can be free radicals if one or more of the atoms present have unpaired electrons (e.g. nitric oxide).
- Free radicals of different types vary widely in their chemical reactivity but in general they are more reactive than non-radicals. When two free radicals meet, their unpaired electrons can join to form a pair, and both radicals are lost.
- Since most molecules under physiologic conditions do not have unpaired electrons, free radicals produced *in vivo* will most likely react with non-radicals, generating new free radicals. Hence, free-radical reactions **tend to proceed as chain reactions**.
- The most-studied free-radical chain reaction in living systems is **lipid peroxidation**.

# What Radicals are Formed in the Human Body?

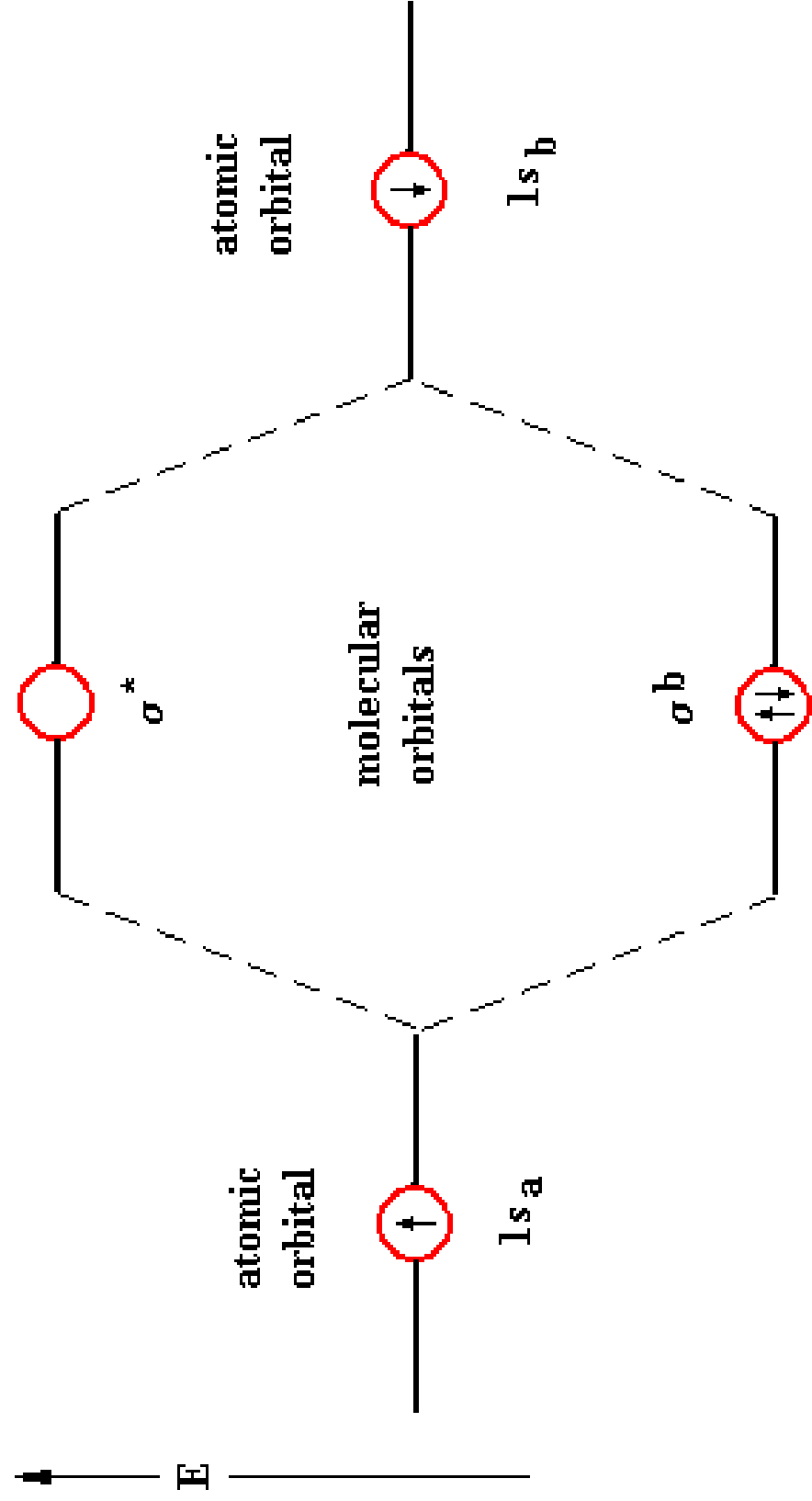
- The body metabolizes **several drugs and poisons** to free radicals.
- The endothelial cells lining blood vessels respond to certain stimuli, such as acetylcholine or bradykinin, by secreting **•NO**. Nitric oxide relaxes the smooth muscle in the vessel wall causing vasodilatation and a drop in blood pressure.
- A much more dangerous free radical than **•NO** is the **hydroxyl radical** (**•OH**). Exposure of water to ionizing radiation, such as  $\gamma$  rays, splits the water molecule to leave an unpaired electron on both hydrogen and oxygen.

The extremely reactive  $\bullet\text{OH}$  can attack almost all molecules present in human cells, starting free-radical chain reactions. Nothing in the cell is safe in its presence:  $\bullet\text{OH}$  fragments DNA (carcinogenesis) and proteins, initiates peroxidation of lipids, and destroys carbohydrates.

Much of the tissue degeneration that occurs after excessive exposure to ionizing radiation is due to free-radical chain reactions started by  $\bullet\text{OH}$ . Background levels of ionizing radiation cause continuous, low-level generation of  $\bullet\text{OH}$  in the human body.

The body produces superoxide radical ( $\text{O}_2^{\bullet-}$ ) by adding one electron to an oxygen molecule.  $\text{O}_2^{\bullet-}$  is much less reactive than  $\bullet\text{OH}$ , but it does react with  $\text{NO}^\bullet$ . Thus, if  $\text{O}_2^{\bullet-}$  is generated by, or close to, vascular endothelial cells, it can oppose the vasodilator action of  $\text{NO}^\bullet$ , that is,  $\text{O}_2^{\bullet-}$  can act as a vasoconstrictor.





Energy level diagram for  $H_2$  molecule formed from  $H_a$  and  $H_b$  atoms.

- $O_2^{\bullet-}$  also serves **useful purposes** in the human body. Lymphocytes and fibroblasts may constantly generate small amounts of  $O_2^{\bullet-}$  as **growth regulators**.
- The human body is equipped with an arsenal of **phagocytic cells** (neutrophils, monocytes, macrophages, eosinophils) that function to recognize, engulf, and destroy foreign material, such as bacteria and viruses. When phagocytes come into contact with unwanted material, an enzyme (NADPH-dependent superoxide synthase) in the cell membrane becomes activated and produces large quantities of  $O_2^{\bullet-}$ . The  $O_2^{\bullet-}$  participates in the mechanism by which engulfed bacteria are killed.
- In **chronic disease**, an inborn defect in one of the components of superoxide synthase renders the enzyme ineffective, so that certain strains of engulfed bacteria are not efficiently killed.

Production of free radicals by phagocytes is useful in killing foreign organisms, but it also **can do harm to the phagocyte** and to the surrounding tissues. In some human diseases **the harm outweighs the good**.

E.g., the inflamed joints of patients with **rheumatoid arthritis** contain many activated phagocytes. Overproduction of  $O_2^{\bullet-}$  and other ROS contributes to tissue injury in the inflamed joint.

In patients with **ulcerative colitis**, the excess free-radical production by phagocytic cells in the chronically inflamed bowel may contribute to the increased risk of colon cancer.

In some forms of **adult respiratory distress syndrome**, excessive infiltration and activation of neutrophils may contribute to severe lung injury.

Human cells also form hydrogen peroxide by additional mechanisms such as **oxidation of certain amino acids in peroxisomes**.

Like  $\text{O}_2^{\bullet-}$ ,  $\text{H}_2\text{O}_2$  is fairly unreactive. The danger comes when both  $\text{O}_2^{\bullet-}$  and  $\text{H}_2\text{O}_2$  interact with iron or copper ions, producing both  $\bullet\text{OH}$  and other dangerous species.

Comparable reactions can be written in which  $\text{H}_2\text{O}_2$  reacts with cuprous ( $\text{Cu}^+$ ) ion to give  $\bullet\text{OH}$ , and  $\text{O}_2^{\bullet-}$  reduces cupric ( $\text{Cu}^{2+}$ ) ion to  $\text{Cu}^+$ .

# Lipid Peroxidation

One of the targets of free-radical attack in human cells are the lipids of cell membranes. Both **free PUFAs** and those **incorporated into lipids** are readily attacked by free radicals, becoming oxidized to lipid peroxides.

By contrast, both monounsaturated and saturated fatty acids are much more resistant to free-radical attack.

**Lipid peroxides are toxic and damage most body cells.** At high temperatures, they decompose to produce a range of unpleasant-tasting and foul-smelling products such as epoxides, ketones, acids, and aldehydes.

Unfortunately, lipid peroxides that come into contact with iron or copper ions, even at body temperature, decompose to noxious products similar to those generated by heating.

Two of the many toxic aldehydes that can be produced by peroxide decomposition are **malondialdehyde** (MDA) and the even more noxious **4-hydroxynonenal**. These can attack proteins, especially thiol (-SH) and amino (-NH<sub>2</sub>) groups.

The structure of MDA enables it to form both intramolecular cross-links and cross-links between different protein molecules.

Proteins damage occurs from reactions of hydroxynonenal with thiol groups (-SH) on proteins.

The fluorescent granules of age pigment that accumulate in old cells contain products of the reaction of proteins with such aldehydes and other end-products of peroxide decomposition. Hydroxynonenal can injure human cells even when **it is present at only micromolar concentrations**.

# Initiation of Lipid Peroxidation

Many different mechanisms can **initiate** peroxidation of PUFA's - e. g.  $\bullet\text{OH}$  formed by ionizing radiation, mixtures of iron and copper ions with  $\text{O}_2^{\bullet-}$  and  $\text{H}_2\text{O}_2$ .

Both  $\bullet\text{OH}$  and metal-ion free-radical complexes probably strip hydrogen atoms from the hydrocarbon chains of the fatty acids. The **greater** the number of **double bonds**, the **easier the removal of hydrogen**, which is why PUFA's are particularly susceptible to attack.

Since a hydrogen atom contains only one electron, its removal from the carbon to which it is attached leaves behind an unpaired electron on that carbon. The resulting carbon-centered radical can undergo different chemical reactions but the most likely one in the human body is a rearrangement of its structure, followed by reaction with oxygen to form a **peroxyl radical**.

When generated in membranes, peroxy radicals can do several things:

1. **They can abstract hydrogen atoms** from adjacent PUFA side chains, thus propagating the free-radical chain reaction of lipid peroxidation. **Hence, a single initiating event can result in conversion of hundreds of fatty acid side chains into lipid peroxides.** The length of the propagation stage depends on many factors, including the relative amounts of lipid and protein. **The chances that a peroxy radical will react with a membrane protein will increase as the amount of protein in the membrane rises.**
2. They can react with **amino acid residues** on membrane proteins, impairing the functions of proteins as enzymes or receptors.



3. They can **oxidize cholesterol** in membranes to a variety of products. The literature contains considerable speculation that oxidation products of cholesterol, rather than cholesterol itself, are important in promoting atherosclerosis.
4. Two peroxy radicals can react with each other if they happen to collide within the membrane.
5. They can react with antioxidants.

### **Propagation and decomposition stages of lipid peroxidation. Metal Ions and the Peroxidation Process**

The potential therapeutic use of metal-ion chelators as antioxidants makes it worthwhile to emphasize the key role that iron and copper ions play in accelerating lipid peroxidation.

1. Reaction of these ions with  $\text{O}_2^{\bullet-}$  and  $\text{H}_2\text{O}_2$  generates noxious species that can initiate peroxidation.

2. Lipid peroxides are fairly stable at 37°C, but **iron or copper ions can readily decompose them**. Decomposition produces a wide range of products, including epoxides, hydrocarbon gases, and cytotoxic aldehydes. In addition, peroxide decomposition by metal ions generates peroxy radicals and other free radicals that can abstract hydrogen atoms from PUFA side chains and propagate the chain reaction.

### **Singlet Oxygen**

Another way of starting lipid peroxidation is the direct combination of PUFAs or their side chains with an **exceptionally reactive form of oxygen known as singlet oxygen**, produced when energy absorbed by the oxygen molecule rearranges its electrons.

During lipid peroxidation, peroxy radicals that collide with each other can react to **form a small amount of singlet oxygen**, which then can generate more peroxides.

However, singlet oxygen formation appears to be a minor reaction pathway under most circumstances.

**Singlet oxygen is produced also when certain compounds are illuminated in the presence of oxygen.** They absorb light, enter a higher electronic excitation state, and transfer the excess energy to oxygen, converting it to the singlet state.

Such photosensitizing agents include dyes (e.g., eosin), certain classes of drugs (e.g., tetracyclines), and several substances found in the human body (e.g., porphyrins, the vitamin riboflavin, and the bile pigment bilirubin).

E.g. **Accumulation of porphyrins** in the skin of patients with certain forms of porphyria can lead to **skin damage** when sunlight and porphyrins interact to generate singlet oxygen.

Drug- and disease-induced **photosensitization** reactions are unpleasant, but some photosensitization reactions are **therapeutically useful** in clinical medicine.

E.g. Hematoporphyrin derivative is a mixture of porphyrins that can be taken up by certain cancer cells, especially some types of lung cancer. Since porphyrins are fluorescent, their presence **can identify the site of the tumor**. Illumination of the tumor with light of the correct  $\lambda$  from a laser through a fiber optic cable attached to a bronchoscope can generate singlet oxygen within the tumor, helping to destroy it.

## Consequences of Free-Radical Processes

If free radicals are continuously generated in the human body, and if PUFAs are sensitive to free-radical attack, then **why does the body use PUFAs at all?**

The answer is : They perform essential functions.

It is known their role in maintaining **the correct degree of fluidity in membranes.**

The PUFA **arachidonic acid** acts as a **starting point for the synthesis of prostaglandins and leukotrienes.** Activation of **phospholipase enzymes** in membranes in response to cell injury or hormonal stimuli can split off arachidonic acid from membrane phospholipids. **Cyclooxygenase** then acts on arachidonate, initiating formation of prostacyclins, thromboxanes and prostaglandins.

Prostaglandins have a multiplicity of actions in the human body, including in gastric and renal function. **Prostacyclin** (or prostaglandin I<sub>2</sub>) prevents **platelets from adhering to the vascular endothelium** (thus regulating coagulation) and dilates blood vessels.

By contrast, **thromboxane A<sub>2</sub>** promotes platelet aggregation and constricts blood vessels.

At sites of inflammation, **prostaglandins** contribute to **pain and swelling**: many patients with rheumatoid arthritis are given drugs that inhibit cyclooxygenase to dampen the excessive pain and swelling in their joints.

**Lipoxygenases** found in several human tissues (including phagocytes, skin, platelets) convert arachidonic acid into **leukotrienes**.

**Leukotriene B<sub>4</sub>** is a chemotactic agent that **attracts phagocytes** to a site of inflammation. Like prostaglandins (and oxygen radicals), leukotrienes perform essential roles in the body but can be a nuisance if generated in excess. The excessive production of leukotrienes in the respiratory tract in response to inhaled allergens is one of the factors causing **asthma**; inhibitors of lipoxygenase are being evaluated for the treatment of asthma.

The **initial products** of cyclooxygenase and lipoxygenase action are **peroxides**. These enzymes **catalyze a controlled and specific peroxidation of PUFAs** to yield well-defined peroxide products, i.e., prostaglandin G<sub>2</sub>.

By contrast, the peroxidation induced by exposure of lipids to oxygen radicals produces a complex mixture of many different products **with no specificity**.



However, **enzymic** and **nonenzymic** peroxidation are intimately related in at least two ways:

**First**, injury to tissues can activate phospholipases, cyclooxygenases, and lipoxygenases to yield peroxides. Furthermore, injury can release intracellular iron and copper ions into surrounding tissue, where they can decompose the peroxides to peroxy radicals, which can attack and peroxidize adjacent lipids.

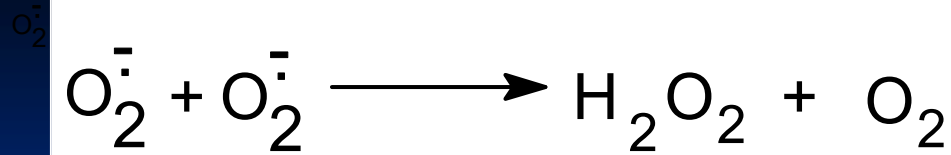
**Second**, peroxides generated by nonenzymic peroxidation of lipids can stimulate the action of cyclooxygenases and lipoxygenases, speeding up production of prostaglandins and leukotrienes (provided that the arachidonic acid substrate is available). This activation can turn into inhibition if lipid peroxide concentrations rise above micromolar ranges.



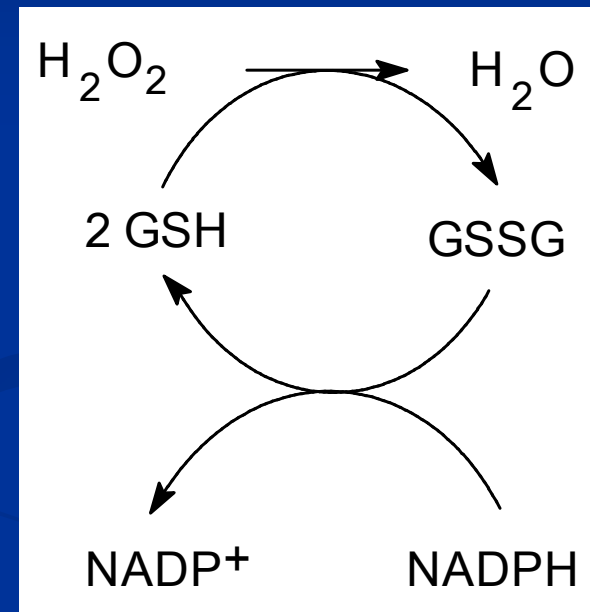
## ANTIOXIDANT DEFENSE SYSTEM

If peroxidation of food lipids leads to rancidity, what stops the lipids in the human body from going rancid ?  
The answer is that we have a complex series of antioxidant defenses.

The mitochondria and cytosol of human cells contain superoxide dismutase (SOD) enzymes, which, by converting  $\text{O}_2^{\bullet-}$  to  $\text{H}_2\text{O}_2$ , greatly accelerate the rate at which  $\text{O}_2^{\bullet-}$  is removed.



$\text{H}_2\text{O}_2$  resulting from the actions of **SOD** and some other enzymes (such as amino acid oxidases in peroxisomes) is decomposed by two other enzymes, glutathione peroxidase and catalase.



Superoxide dismutases convert superoxide (produced in cytosol and mitochondria) to  $\text{H}_2\text{O}_2$ .  $\text{O}_2^{\cdot-} + \text{O}_2^{\cdot-} \longrightarrow \text{H}_2\text{O}_2 + \text{O}_2$  Glutathione peroxidase (GSH-Px) uses  $\text{H}_2\text{O}_2$  in these subcellular compartments to convert reduced (GSH) to oxidized glutathione (GSSG).

In humans, GSH-Px is **the only enzyme** known to require **selenium** for its activity. Glutathione reductase (GR) reduces GSSG back to GSH at the expense of NADPH oxidation.

**GSH-Px**, which acts on **both PUFA peroxides and  $\text{H}_2\text{O}_2$**  metabolizes the released peroxides.

Mammalian cells may also contain a phospholipid hydroperoxide glutathione peroxidase, which can directly act on hydroperoxides in membranes.

**Most enzymes that directly produce  $\text{H}_2\text{O}_2$  are present in peroxisomes.** Catalase (CTS), on the other hand, decomposes  $\text{H}_2\text{O}_2$  to water and oxygen.

It is known that iron and copper ions can greatly potentiate the toxicity of  $O_2^{\bullet-}$  and  $H_2O_2$  by causing formation of  $\bullet OH$  and other ROS.

An important antioxidant defense strategy is to keep the amount of free metal ions in the body to the absolute minimum. **Iron** is transported in plasma **bounded to transferrin**. Thus it can not stimulate lipid peroxidation. Similarly, most **copper** ions in plasma are **bound to ceruloplasmin**, thus being unable to stimulate lipid peroxidation, as well.

However, **the body cells need metal ions** to synthesize intracellular **metalloproteins** such as cytochromes in mitochondria.

Between transportation and final location in the cell, iron and copper ions appear to exist in a "free" form which could accelerate free-radical reactions.

The amounts of these "low-molecular" intracellular ions are kept as small as possible by their binding to some intracellular storage proteins, such as ferritin. When cells are ruptured, intracellular metal ions are released into the surroundings where they accelerate free-radical reactions.

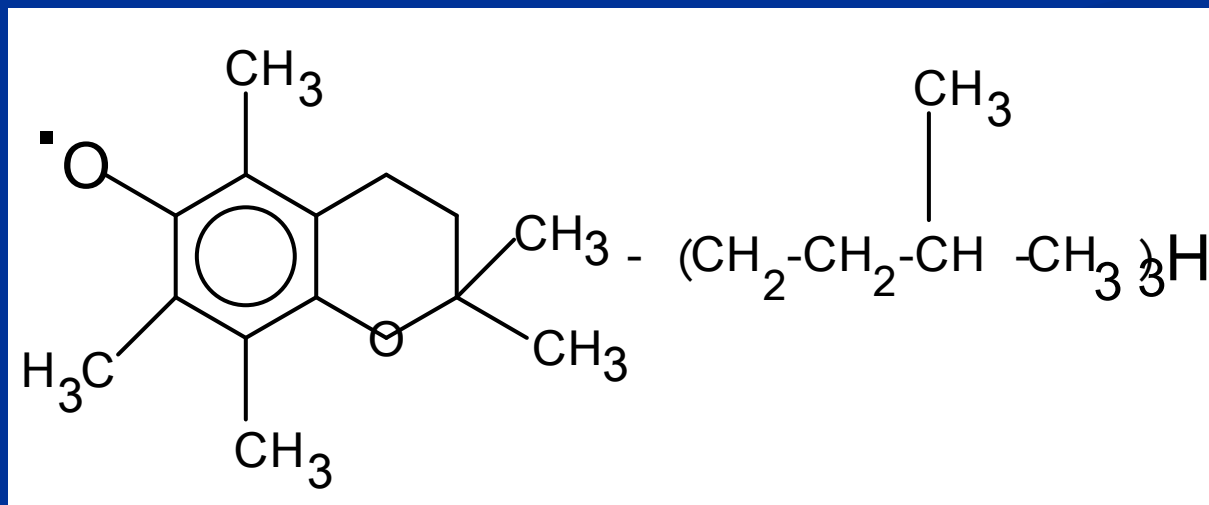
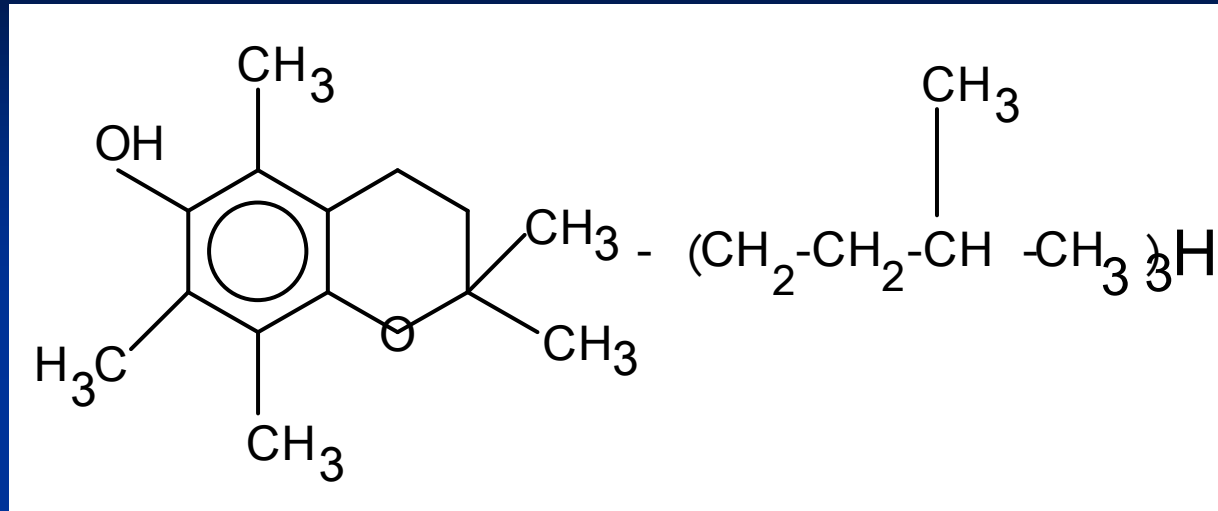
A study of patients with traumatic brain injury shows that the "free" iron contents of cerebrospinal fluid rise in parallel to the severity of the brain injury.

Alpha-tocopherol is an important lipid-soluble antioxidant. It is often called vitamin E, but the term is not quite correct. **"Vitamin E" refers to different tocopherols.**

The other antioxidants, such as SOD, CTS, GSH-Px, transferrin and ceruloplasmin operate in the aqueous phase of cells or body fluids. Being a hydrophobic molecule,  $\alpha$ -tocopherol localizes into the hydrophobic interior of biological membranes or the phospholipid "coat" of plasma lipoproteins. It has a hydroxyl group whose hydrogen atom can be easily removed.

Peroxyl radicals ( $\text{ROO}^\bullet$ ) generated in membranes during lipid peroxidation interact much faster with  $\alpha$ -tocopherol than with the adjacent PUFA side chains of membrane phospholipids or with membrane proteins.

The result is the production of a tocopherol radical (Tocopherol-O•).



Although  $\alpha$ -tocopherol acts largely as a chain-breaking antioxidant, it **can also destroy singlet oxygen**.

$\alpha$ -tocopherol is the most important lipid-soluble chain-breaking antioxidant in the human body, but it is not the only one. The peroxidation of low-density lipoproteins isolated from human plasma reaches its maximum rate only when  $\alpha$ -tocopherol, all the other tocopherols,  $\beta$ -carotene, lycopene, and phytofluene are oxidized.

The last three compounds (like tocopherols) are products of plant origin; their real **importance** as antioxidants in humans **remains to be established**, but there is good epidemiologic evidence that a high intake of green leafy vegetables helps protect against heart disease and some forms of cancer.



The chain-breaking antioxidant action of tocopherols converts them into tocopherol-O $\cdot$  radicals.

These radicals can migrate to membrane surfaces or lipoprotein particle surfaces and be reduced back to tocopherol by reaction with ascorbic acid in the aqueous phase. Thus, both **vit C and E may cooperate** in minimizing the rate of lipid peroxidation.

However, vit C can stimulate peroxidation in the presence of iron, it can reduce Fe<sup>3+</sup> ions to Fe<sup>2+</sup>, thereby accelerating the formation of reactive oxygen radicals such as  $\cdot\text{OH}$ .

The chain-breaking antioxidants cannot neutralize all the lipid peroxides produced in membrane. However, the body has **a mechanism for repairing peroxidative damage** to membrane lipids. Phospholipases cleave oxidized PUFA side chains from membrane lipids.

# Lipid Peroxidation and Toxicology

The key question on oxidative stress is:

"in which diseases is it important?" or

"in which diseases does it significantly contribute to the tissue injury taking place?" Until very recently, the lack of suitable assays for measuring free radicals and free-radical damage in the human body precluded precise answers to this question.

**Oxidative stress apparently does contribute significantly to the pathogenesis of atherosclerosis and CNS injury.**

## **Atherosclerosis**

Atherosclerosis is narrowing of the arterial lumen consequent to localized thickening of the intima. Myocardial and cerebral infarcts resulting from tissue ischemia are frequent consequences of atherosclerosis and are major causes of death in industrialized countries.

The initiating factor in atherosclerosis is unknown but may involve injury to the endothelial cells lining the vessel wall, perhaps as a result of **turbulent blood flow**.

Monocytes adhere to functionally deranged endothelium and then enter the intima, where they develop into macrophages. Both monocytes and macrophages produce superoxide when activated. Smooth muscle cells and lymphocytes also proliferate in this early atherosclerotic lesion, probably in response to stimuli produced by macrophages.

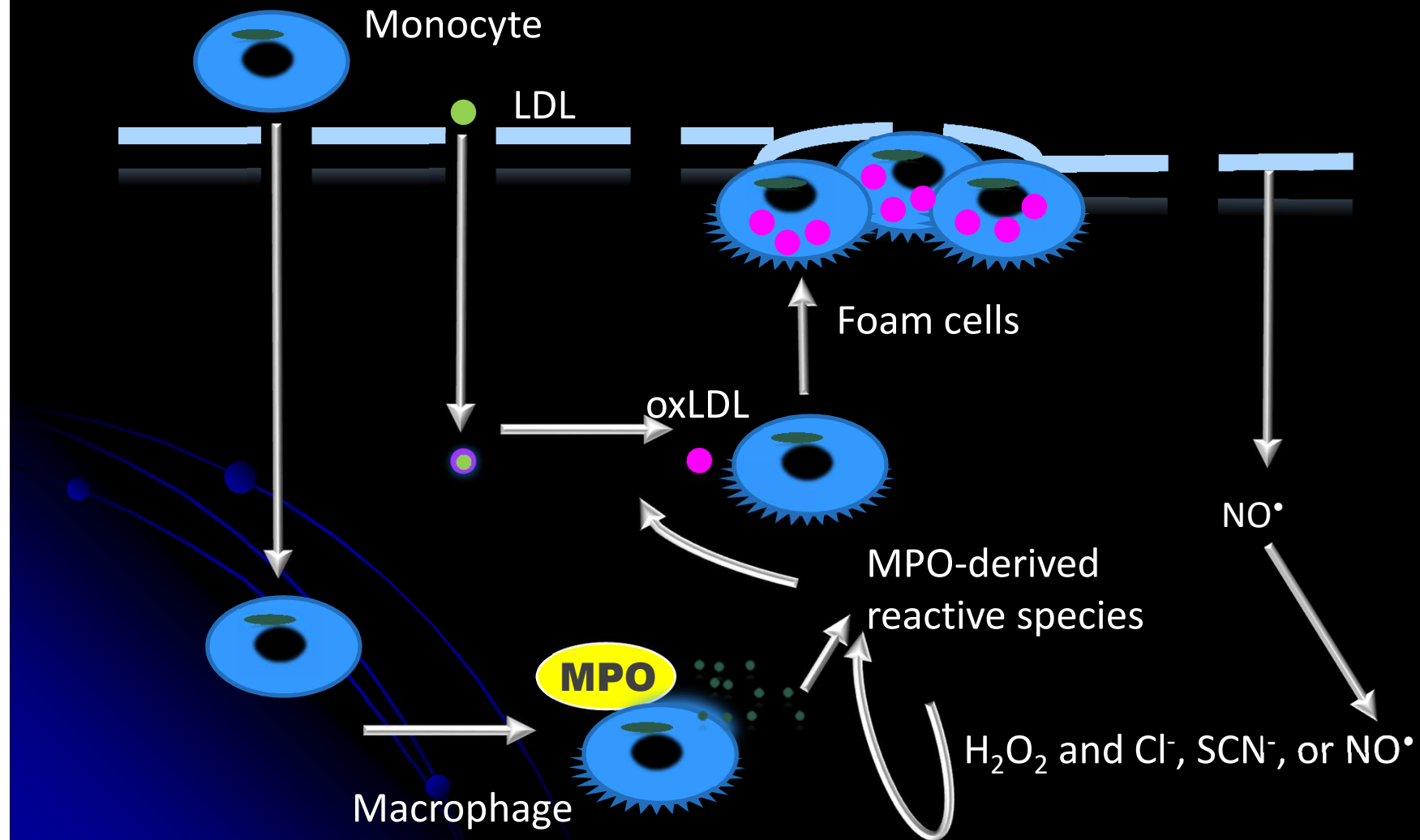
A characteristic cell type found in both **early and advanced** atherosclerotic lesions is **the lipid-laden foam cell**. It is believed that LDL from plasma enter the vessel wall and undergo peroxidation within atherosclerotic lesions.

How LDL peroxidation is initiated is unclear. Lipoxygenases in injured endothelial cells may be responsible. In addition, metal ions released from damaged cells in the lesion may interact with superoxide from activated monocytes and macrophages to yield ROS.

Peroxidized LDL are recognized by special receptors ("scavenger receptors") on macrophages and taken up at a high rate. Continued LDL uptake converts macrophages into foam cells.

The ability of LDL to resist peroxidation depends on their contents of chain-breaking antioxidants, which have to be depleted before peroxidation has accelerated. That may be why, high plasma concentrations of  $\alpha$ -tocopherol and vitamin C seem to correlate with low rates of cardiovascular diseases.

## ❖ Cardiovascular Disease



# Damage to Brain and Nervous System

The consequences of ischemic damage to the brain (stroke) and traumatic injury to the brain or spinal cord are major clinical problems. Free-radical reactions are considered to enhance the effects of the initial tissue insult.

It was shown many years ago that when brain tissue is ground up in a buffer solution, the resulting homogenate undergoes peroxidation at an exceptionally rapid rate. Trauma or ischemia might be thought of as essentially causing a partial "**homogenization**" of the tissue by destroying cellular integrity.

The brain and spinal cord may be prone to oxidative stress for several reasons:

- The membrane lipids are rich in PUFA side chains.
- Activities of antioxidant defense systems in the brain are only moderate.

- Several areas of the brain are rich in intracellular iron, which is easily released by cell injury.

Peroxidation of brain homogenates is almost completely inhibited by chelating agents, such as desferrioxamine, that bind iron ions and prevent them from accelerating free-radical reactions.

- Cerebrospinal fluid, unlike plasma, contains very little transferrin and therefore cannot easily bind released iron.
- The nervous system is rich in epinephrine, norepinephrine, and dopamine, all of which react with oxygen to form superoxide, and iron ions accelerate these oxidations.

Thus, ischemic or traumatic injury to the brain and spinal cord may lead to an acceleration of free-radical reactions that could spread the damage into surrounding areas and thereby worsen its consequences.



E.g., peroxidation of brain lipids can destroy the function of receptors.

Pretreatment of animals with  $\alpha$ -tocopherol to raise tissue levels of this antioxidant has been reported to lessen secondary damage after ischemic or traumatic injury to the brain and spinal cord. However, since it takes weeks to raise levels of  $\alpha$ -tocopherol in the nervous system, giving it after the injury has taken place, is useless.

Perhaps other chain-breaking antioxidants could be used, **if they could cross the blood-brain barrier.**

Iron-chelating agents might prevent iron-dependent free-radical reactions, but again they would need to cross the blood-brain barrier. For example, desferrioxamine has shown protective effects against tissue injury in several animal models of human disease, but it does not cross the blood-brain barrier.

Some antioxidants are currently available for potential use in the treatment of human diseases.



The design of antioxidants for safe use in humans is not simple. Some of the questions that have to be considered when evaluating a proposed antioxidant are:

**1. What biomolecule is the compound supposed to protect?**

An inhibitor of lipid peroxidation is unlikely to be useful if the oxidative damage is mediated by an attack on proteins or DNA.

**2. Will the compound be present *in vivo* at or near that biomolecule in a sufficient concentration?**

**3. How does the compound protect** - by scavenging radicals, by preventing their formation, or by repairing damage? If the antioxidant acts by scavenging radicals, can the resulting antioxidant-derived radicals produce tissue damage?

**4. Can the antioxidant cause damage** in biological systems different from those in which it exerts protection?

Careful testing of antioxidant level *in vivo* by appropriate methods may prove valuable for prophylaxis and treatment.