Free radical

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Case report

- A 2 months old girl, born at a gestational age of 38 weeks was referred for investigations regarding persistent pneumonia. The girl suffered from several episodes of recurrent infections such as neonatal sepsis and gastroenteritis, since the 6^{th} day of her life. At the time of admission, she had received three recent course of antibiotics from the previous hospital including cefepime, clindamycin and imipenem due to persistent pneumonia. On admission, physical examination revealed mild chest retraction and rales in both lung fields. She was alert and responsive, with no neurological deficit. Her height and weight were at the 25^{th}-50^{th} percentile and the 50^{th}-75^{th} percentile for her age respectively.
- A chest X-ray confirmed the presence of bilateral pulmonary consolidation and atelectasis. Diagnostic bronchoalveolar lavage (BAL) was performed to obtain specimens for cytology and culture. A computed tomography (CT) of the thorax showed a left sided consolidation and evidence of hematogenously disseminated multifocal pneumonia.

- She had no family history related to primary immunodeficiency. Her total leucocyte count was 10,500/µL, with 34% neutrophils and 52% lymphocytes. The ESR was 12 mm/hr, and CRP 3.54 mg/dL. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were raised (174 IU/L and 398 IU/L, respectively). Electrolyte levels and the results of renal function tests were normal. Investigations for her immunologic work-up showed Ig G, Ig A, Ig M, Ig E, C3, C4, and CH50 levels to be within her age specific reference range. Lymphocyte subset analysis revealed normal B cells and T cells for her age.
- She **had negative** neutrophil nitroblue tetrazolium (NBT) slide test and **abnormal neutrophil respiratory burst activity test**.

- What could be the possible diagnosis ?

What is its pathophysiology?

Learning objectives

- Definition – free radicals, reactive oxygen species
- Generation
- Damage
- Clinical significance
- Scavenger system

Free radical

- Free radical: is a molecule or an atom with an unpaired electron in its outer most orbital. It is highly reactive and has an extremely short half life of microseconds. It reacts indiscriminately with any molecule that comes in its contact to achieve stability by either accepting an electron or donating an electron.

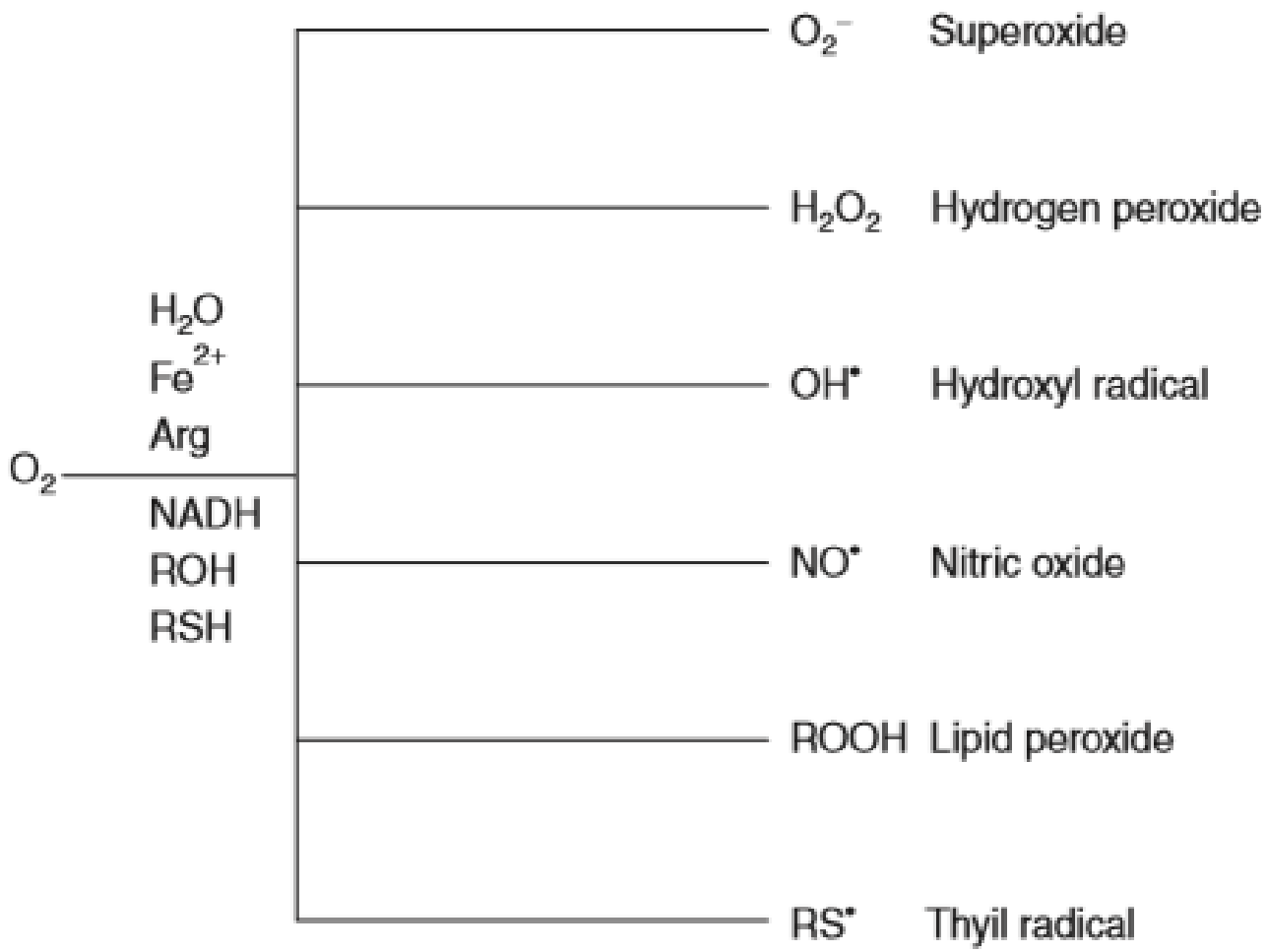
ROS& oxidative damage

- Normally the oxygen is completely reduced to water and successive reduction with four electrons. However incomplete reduction produces ROS such as superoxide, •O2

  −, hydroxyl, •OH, and perhydroxyl, •O2H.

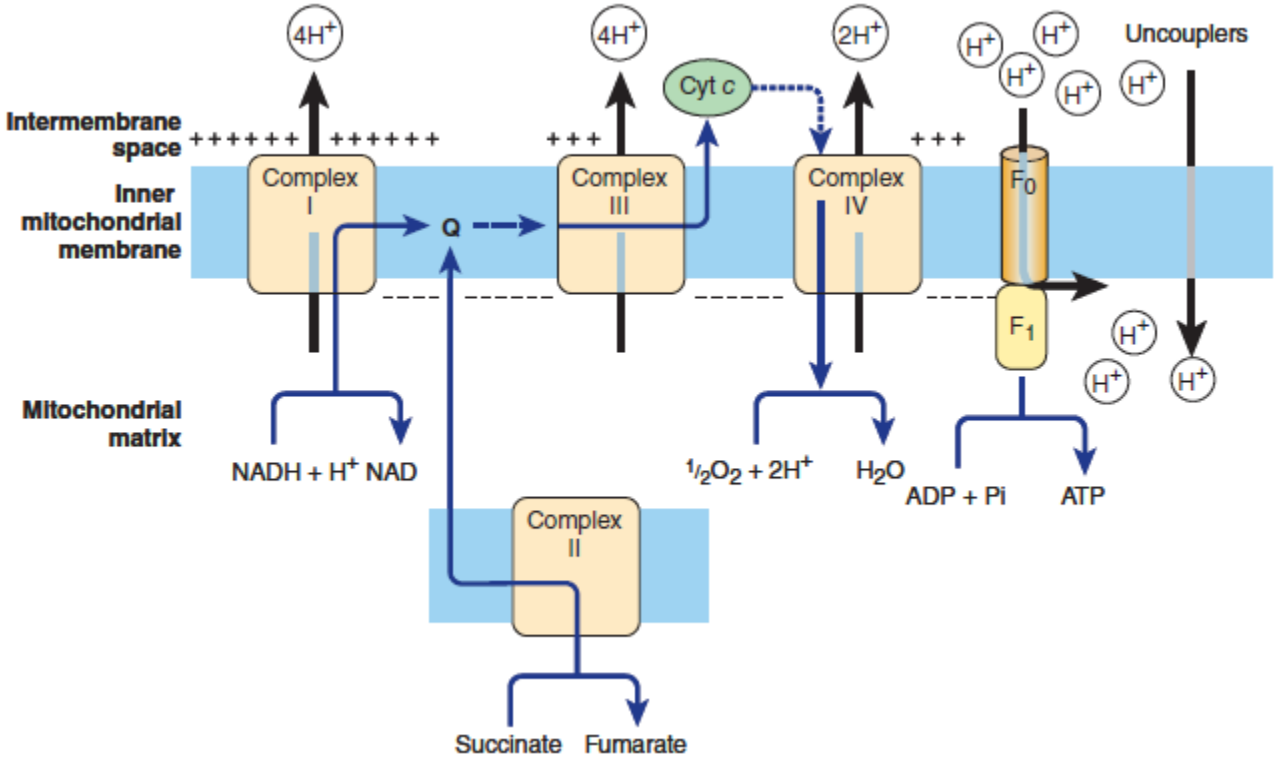
  H2O2 is not a radical by definition but is a highly reactive ROS and produces other species of ROS.
- Tissue damage caused by oxygen radicals is often called **oxidative damage**

ROS encountered in living cells

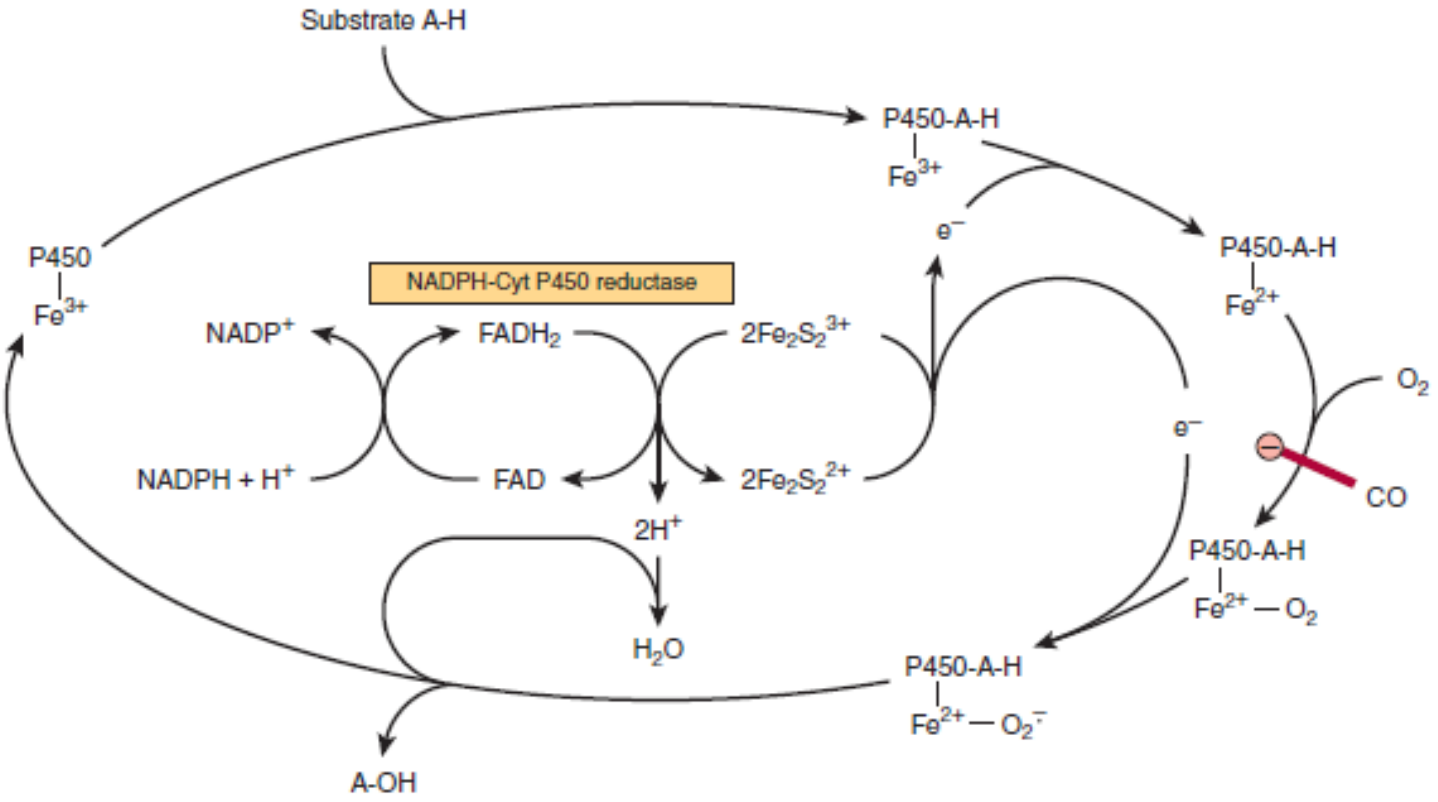


- Sources of ROS in cell

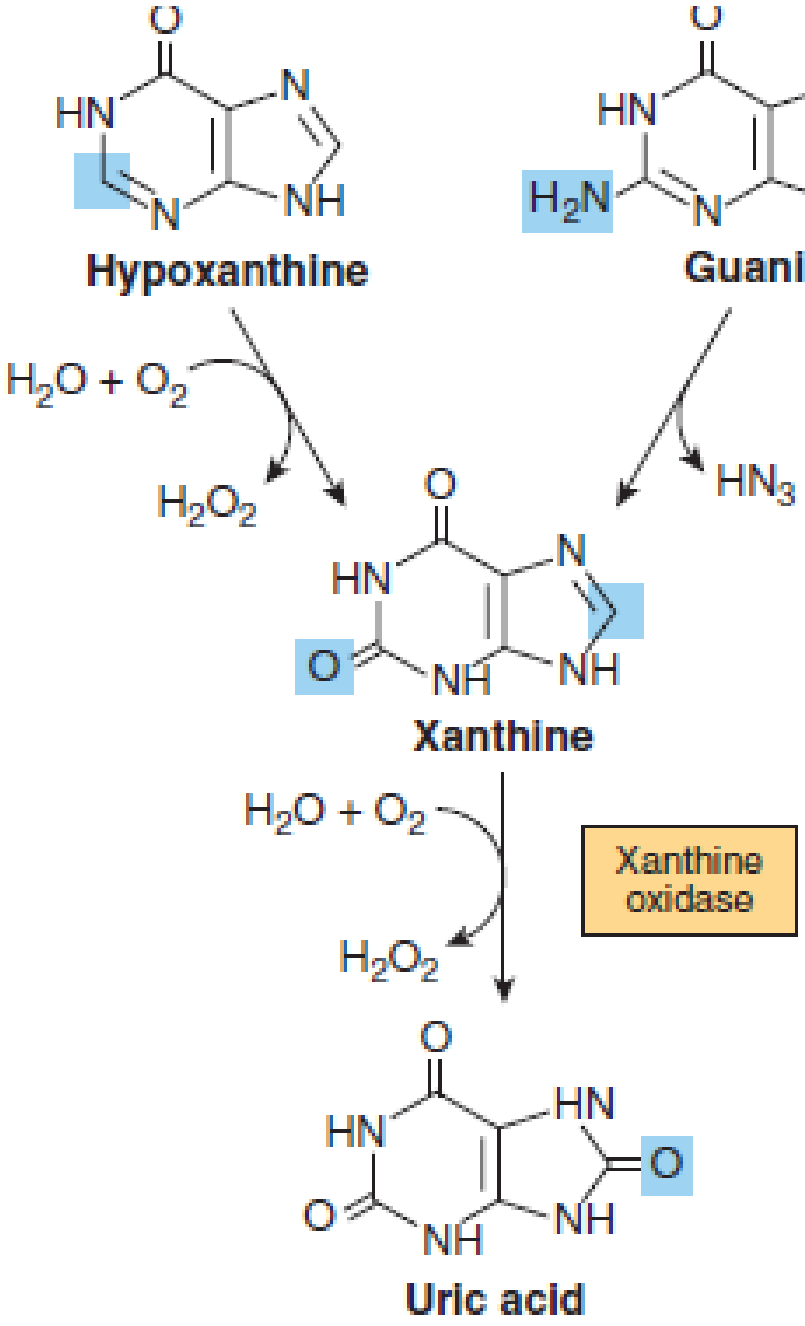
1. Leakage from electron transport chain in mitochondria



2. Cytochrome P450 in Endoplasmic Reticulum



3. Degradation of Purine nucleotides to Uric acid



4. Interaction with metal

Hydroxyl radical

FENTON REACTION

Hydroxyl radical

HABER-WEISS REACTION

5. Peroxisome

- Hydrogen peroxide produced during oxidation of long chain FA in a FAD dependent reaction in peroxisome.

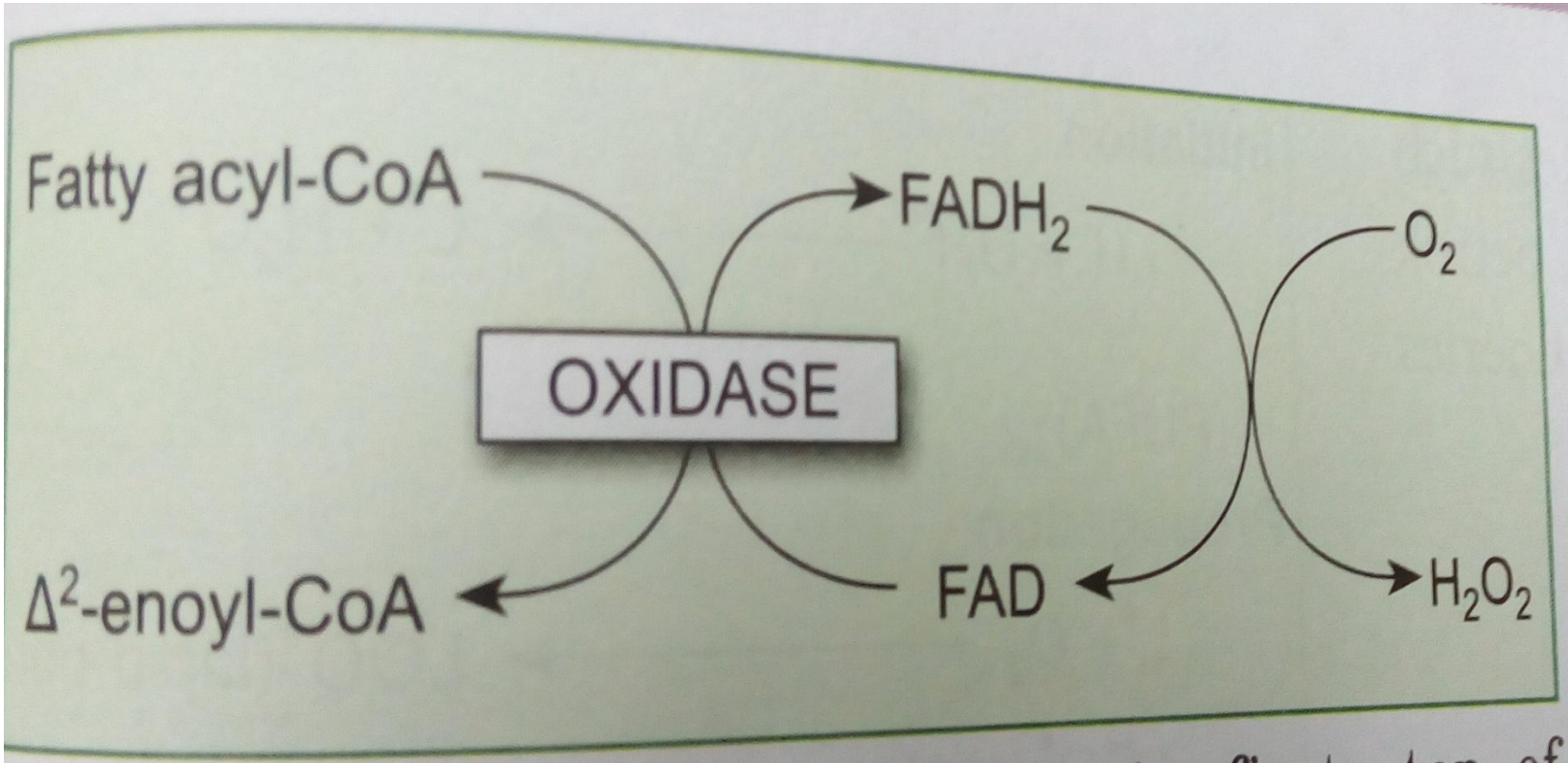
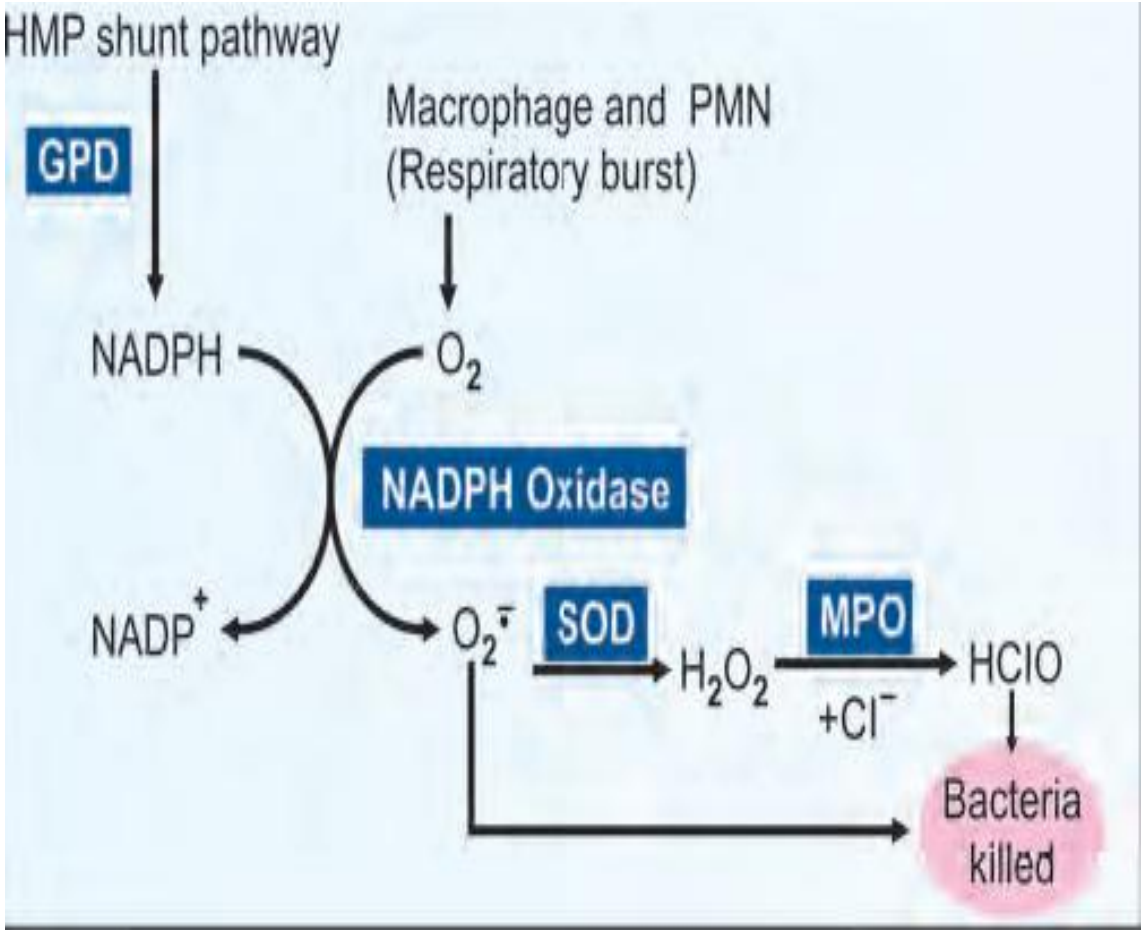


Fig. 60.3: Production of H_2O_2 in first step of peroxisomal oxidation of fatty acids

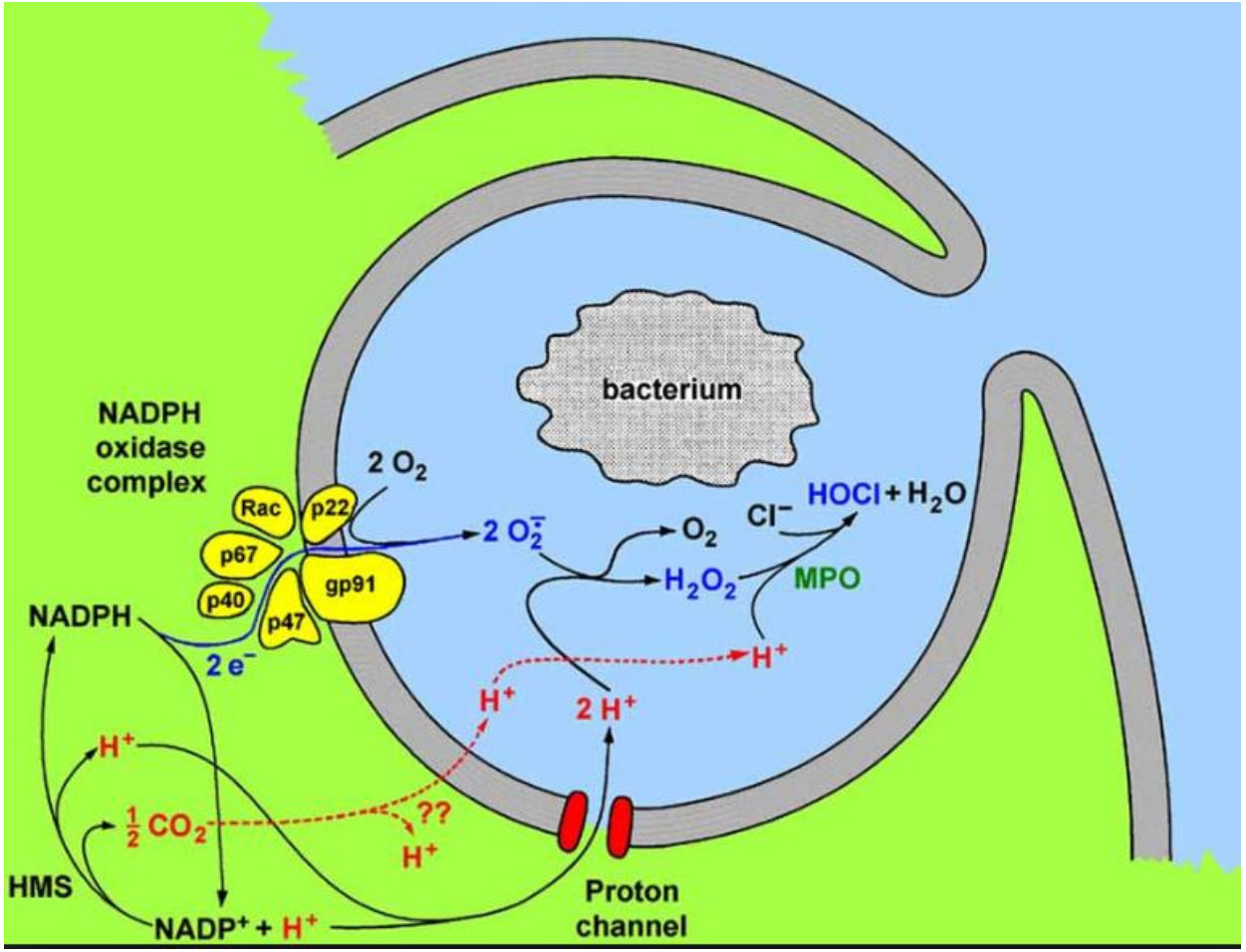
6. Respiratory burst

- Neutrophils, eosinophils, monocytes and macrophages produces superoxide anion by respiratory burst during phagocytosis by NADPH oxidase.

NADPH oxidase



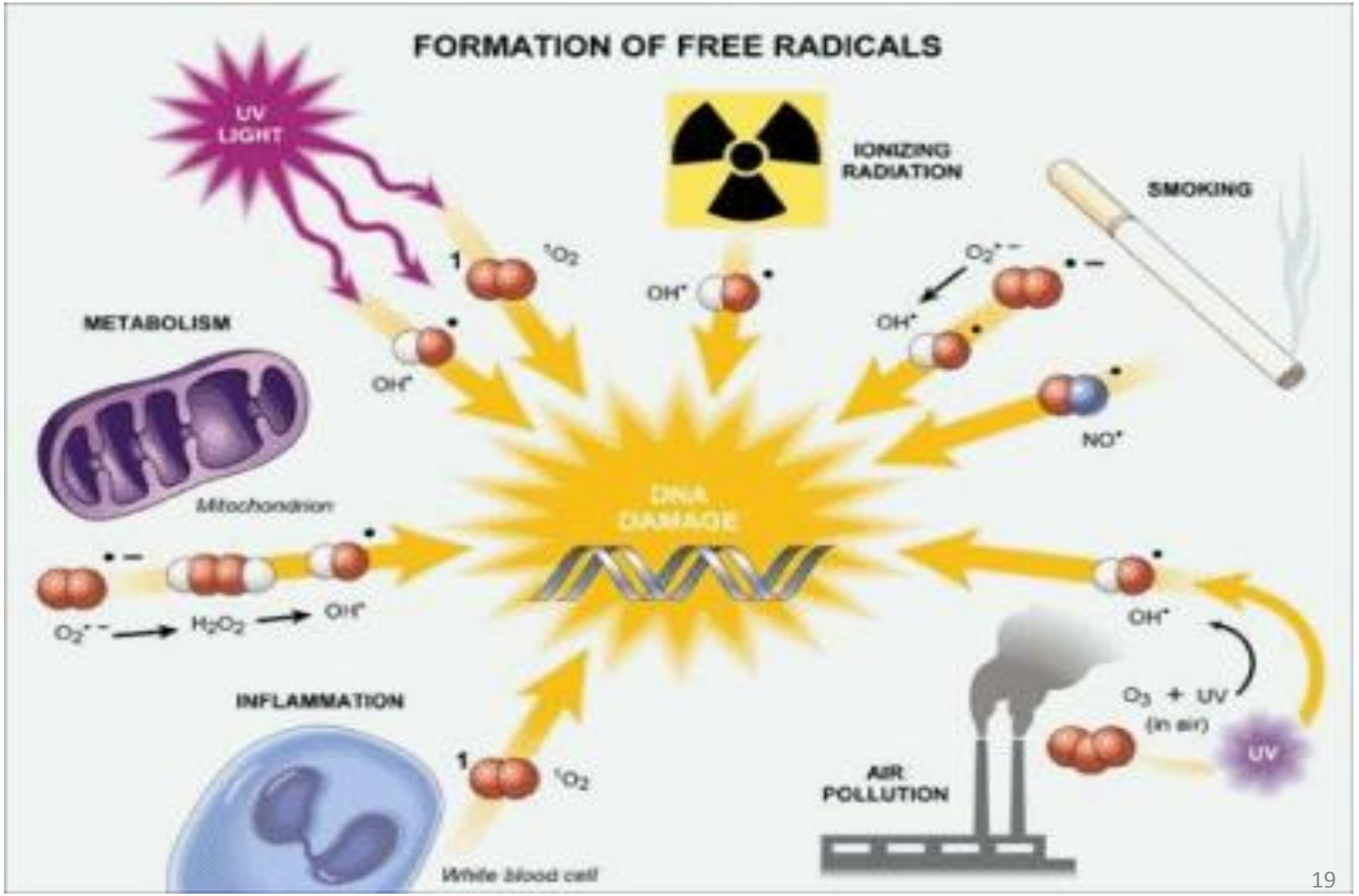
Activation of macrophages and consumption of oxygen by the cell is increased drastically called as respiratory burst


bacterium
NADPH oxidase complex
Rac
p22
p67
p40
p47
gp91
2 O2
2 O2−
O2
H2O2
Cl−
HOCl + H2O
MPO
NADPH
2 e−
H+
2 H+
½ CO2
??
HMS
NADP+ + H+
Proton channel

Other exogenous sources

- Drugs and chemicals: Acetaminophen : Hgh dose cause liver damage
- Peroxidation catalyzed by lipo-oxygenase in platelets and leukocytes.
- H_2O -------(gamma, UV radiation)----→ e^- + OH•
- Oxygen photolysis by light produced singlet oxygen
- Cigarate smoking
- Environmental pollution

FORMATION OF FREE RADICALS



Damage caused by free radicals

1. Proteins
2. Lipid peroxidation
3. Nucleic acids

Cellular damage caused by ROS

- Protein
  - Aminoacids like proline, histidine, arginine, methionine are particularly susceptible to oxidative damage .
    - Protein fragmentation
    - Protein aggregation
    - Protein-protein cross linking

Lipid Peroxidation

- In vitro, peroxidation would lead to rancidity of fats and oils.

- In vivo, the membrane lipids are more liable to attack by free radicals and produce damage to integrity of the membrane.

Lipid peroxidation

1. Initiation:

$$ROOH + Metal^{(n)+} \rightarrow ROO^{\bullet} + Metal^{(n-1)+} + H^{+}$$

$$X^{\bullet} + RH \rightarrow R^{\bullet} + XH$$

2. Propagation:

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$

$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}, \text{etc}$$

3. Termination:

$$ROO^{\bullet} + ROO^{\bullet} \rightarrow ROOR + O_2$$

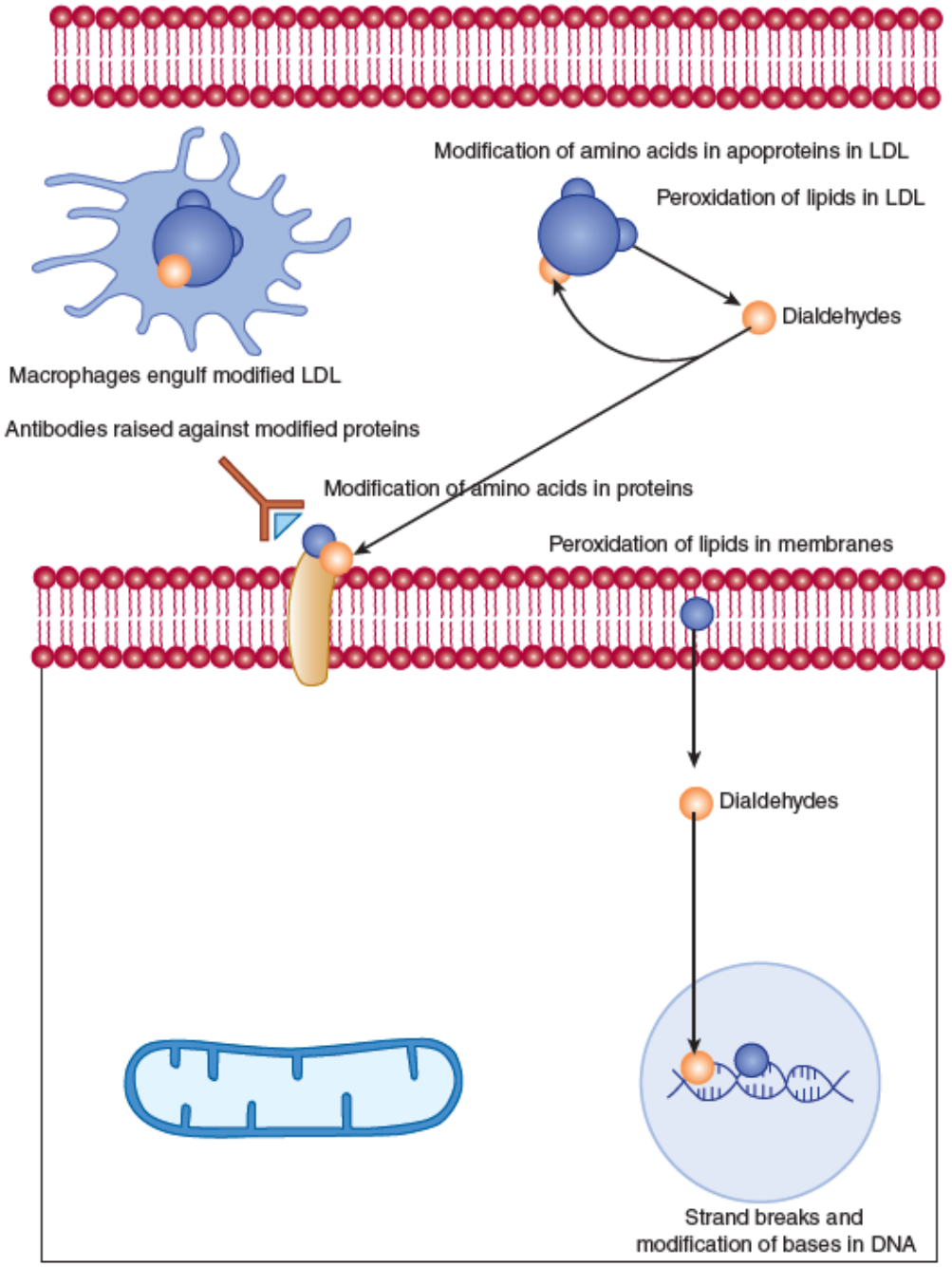
$$ROO^{\bullet} + R^{\bullet} \rightarrow ROOR$$

$$R^{\bullet} + R^{\bullet} \rightarrow RR$$

Damages to DNA

- Damage to DNA
  - in germ line cells in ovaries and testes – heritable mutation
  - in somatic cells – cancers.
- dialdehydes formed as a result of radical-induced lipid peroxidation in cell membranes can also modify bases in DNA
- Chain break

Tissue damage by radicals



Role of free radicals in autoimmune disease

- Modification of amino acids in the protein – by direct radical action or by reaction with products of lipid peroxidation.
- Protein recognized as non-self by the immune system.
- Production of antibodies – cross react with normal tissue proteins – initiating autoimmune disease.

Total body radical burden can be estimated by measuring products of lipid peroxidation:-

- FOX (Ferrous Oxidation in Xylenol) Assay
- Estimation of dialdehydes
- Measurement of Pentane and Methane in Exhaled air

FOX assay

- Lipid peroxides can be measured by the ferrous oxidation in xylenol orange (FOX) assay.
- Acidic conditions fe^{++} ⟶ fe^{+++}
- Which form chromophore with xylenol orange.

TBARS assay

- The dialdehydes (malondialdehyde) formed from lipid peroxides can be measured by reaction with thiobarbituric acid, they form red fluorescent product - generally reported as TBARS (thiobarbuturic acid reactive substances).

Pentane and Ethane assay

- Peroxidation of n-6 PUFA form – pentane
- Peroxidation of n-3 PUFA form – ethane
- Both can be measured in exhaled air.

Clinical significance of free radicals

- Chronic inflammation – rheumatoid arthritis due to free radicals released by neutrophils
  - ROS induced tissue damage – ulcerative colitis, chronic glomerulonephritis
- Acute inflammation – macrophages produce free radicals at inflammatory site
  - Respiratory burst – increase activity of NADPH oxidase in macrophages and neutrophils

- **In chronic granulomatous disease (CGD),** NADPH oxidase is absent.
- So, bacteria are ingested normally but they cannot be destroyed.

Respiratory disease

- Breathing of 100% oxygen for more than 24 hr produce destruction of endothelium and lung edema – due to free radicals release
- Premature newborn – high oxygen exposure for prolonged period causes – bronchopulmonary dysplasia
- Adult – ARDS due to pulmonary edema caused by free radicals

Disease of eyes

- ROP (Retrolental fibroplasia) –
  - Premature newborn treated with pure oxygen for long time.
  - Free radicals causing thromboxane release, sustained vascular contracture and cellular injury.

- Cataract - related with aging and photochemical generation of free radicals

Atherosclerosis and MI

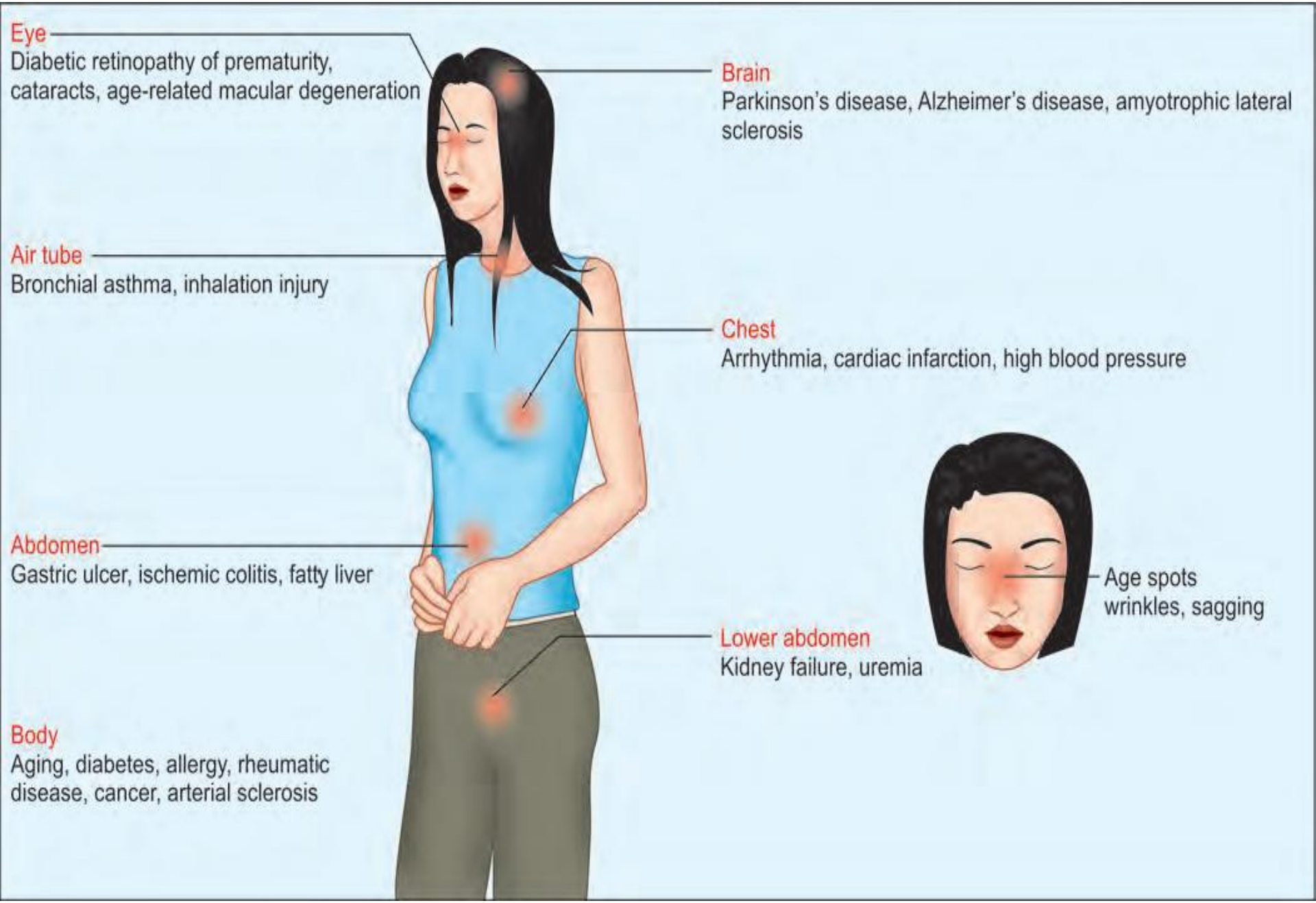
- LDL deposited in the endothelial cells, undergo oxidation by free radicals.
- Attracts macrophages – form foam cells – formation of plaque – atherosclerosis.

Skin disease

- Plant products like psoralens are administered in the treatment of psoriasis and leucoderma.
- Drug applied to affected skin and irradiated by UV light
- Singlet oxygen is produced with clinical benefit

Carcinogenesis and treatment

- Cancer is treated by radiotherapy
- Irradiation produced reactive oxygen species in the cells which trigger the cell death


Eye
Diabetic retinopathy of prematurity, cataracts, age-related macular degeneration
Brain
Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis
Air tube
Bronchial asthma, inhalation injury
Chest
Arrhythmia, cardiac infarction, high blood pressure
Abdomen
Gastric ulcer, ischemic colitis, fatty liver
Age spots
wrinkles, sagging
Lower abdomen
Kidney failure, uremia
Body
Aging, diabetes, allergy, rheumatic disease, cancer, arterial sclerosis

- **Antioxidant**

Antioxidants

- Substances which protect against cellular injury by ROS or other oxidants by scavenging them are called antioxidants
- In normal healthy state a balance is maintained between FRs & antioxidants.
- Moreover we can as well supplement these from outside (in vitro Antioxidants).

Free radical scavenging system

- Preventive antioxidant: reduce the rate of chain initiation
  - Glutathione peroxidase
  - Catalase

- Chain breaking antioxidant: Interfere with chain propagation
  - SOD
  - Uric acid
  - Vit E

- Enzymes
  - SOD
  - Catalase
  - Glutathione peroxidase
- Non enzymes
  - Transferrin and ferritin
  - Cerruloplasmin
  - Albumin
  - Glutathione
  - Uric acid
  - Bilirubin
  - Ubiquinone
  - Vit E
  - Vit C
  - Beta carotene
  - Selenium

Superoxide dismutase

$$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$$

- SOD - is present in all oxygen-metabolizing cells, different cofactors (metals)
- SOD contain Mn, Cu and Zn as co-factor.
- mitochondrial SOD is Mn dependent.=SOD2=Chr 6= idiopathic cardiomyopathy, premature aging
- Cytoplasmic SOD is Cu and Zn dependent= SOD1= Chr 21.
- Defect in SOD1 – amyotrophic lateral sclerosis(Lou Gehring's
- SOD 3= extracellular SOD [Cu-Zn]=Chr 4= disease associated Asbestosis

SOD

- Increase concentration of SOD during exercise
- Because aerobic metabolism - increases ROS generation during exercise
- This is turn stimulates the cell to synthesize more enzymes.
- The net effect is increase protection during the post-exercise or rest periods.

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Catalase

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

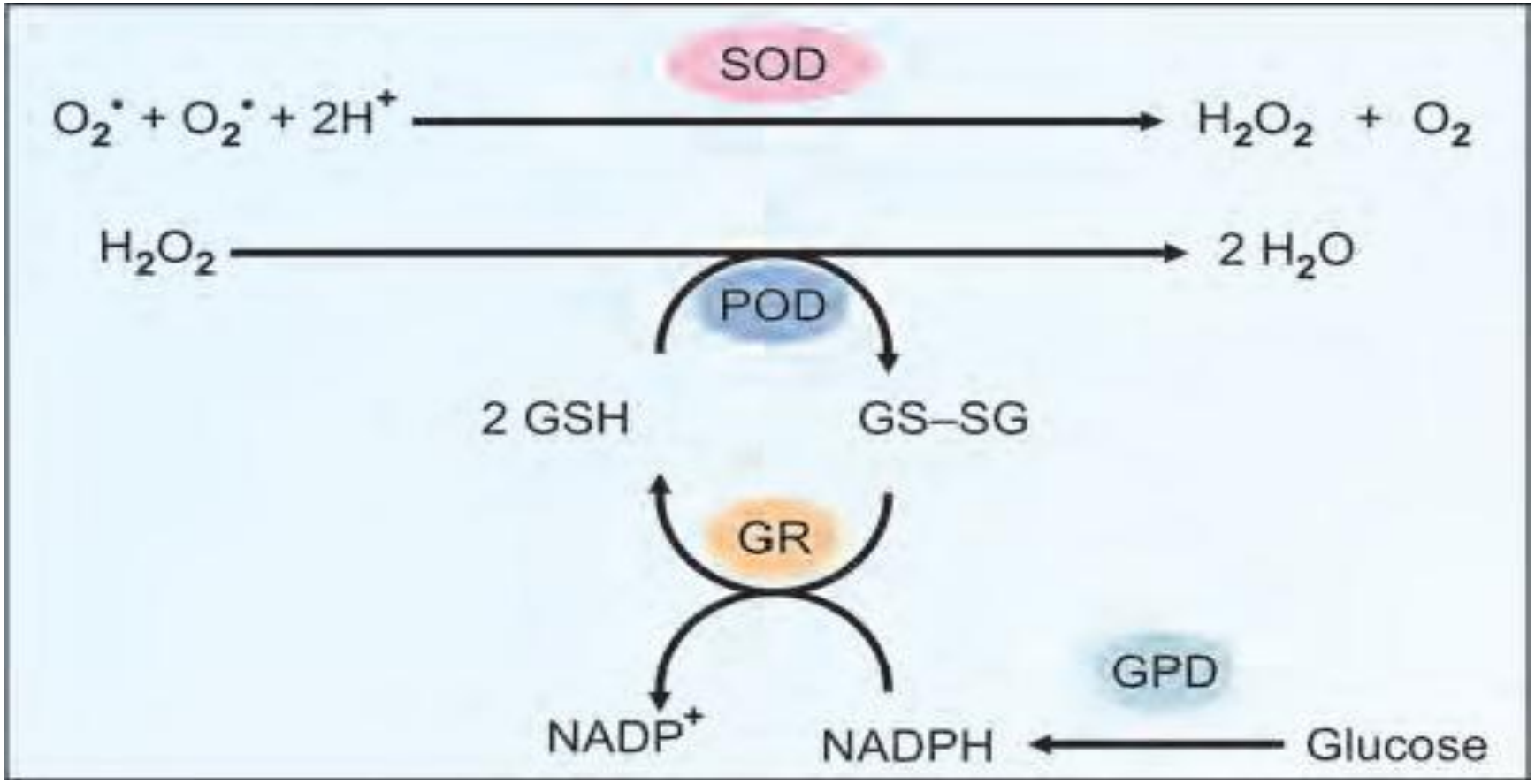
- High affinity to H_2O_2 : peroxisomes, mitochondria, cytoplasm of erytrocytes

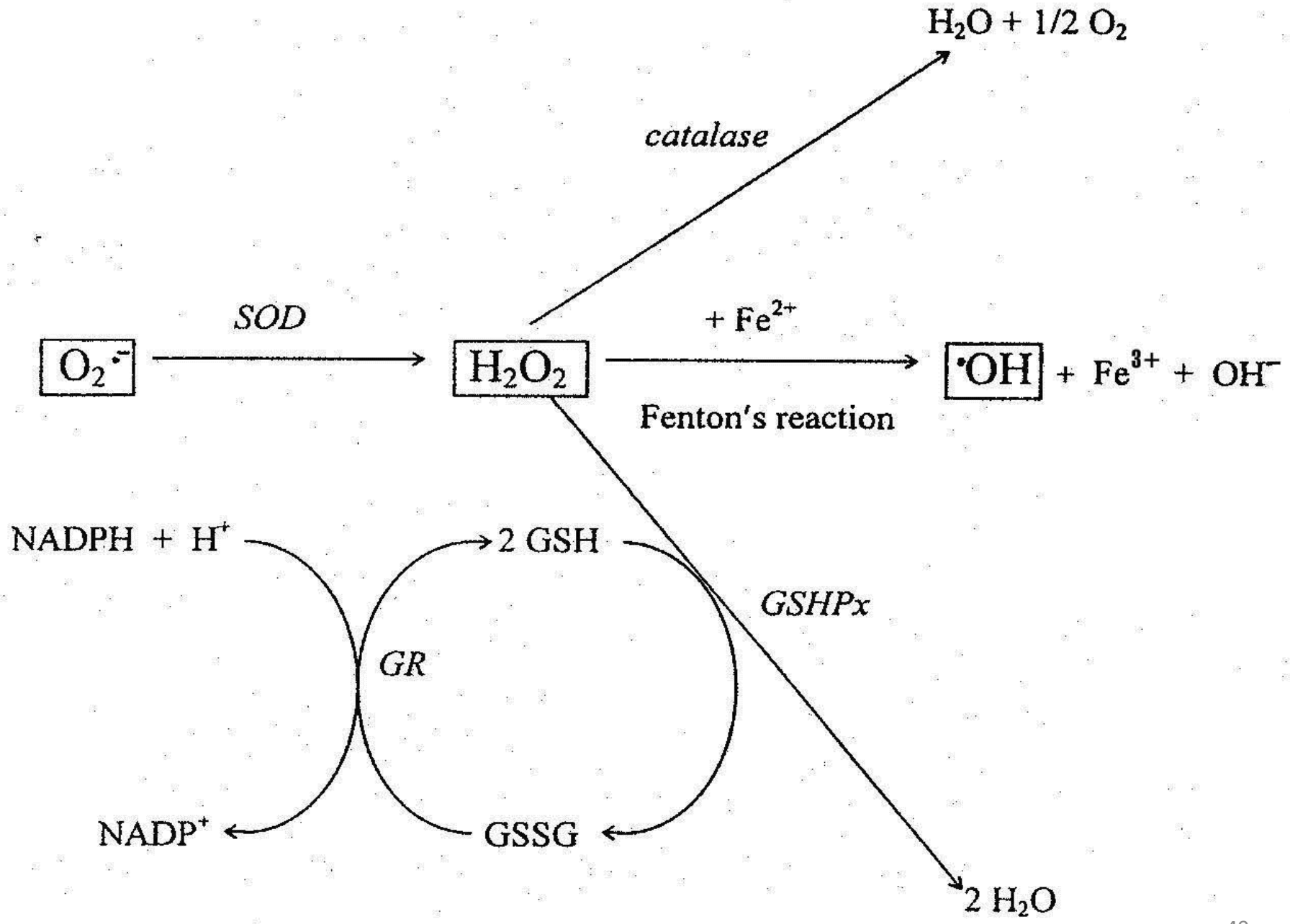
Glutathione peroxidases

- It is selenium dependant enzyme
- It catalyses reduction of hydrogen peroxide and lipid peroxide by glutathione
- Sulfhydryl groups of reduced glutathione (GSH) serves as a electron donor.
- It is oxidized to the disulphide form (GS-SG) which is reduced back by reductase enzyme using NADPH

Glutathione reductase

- Is flavo-protein enzyme using FAD as a co-factor to reform the reduced glutathione from oxidized glutathione
- The reducing equivalent donor is NADPH
- NADPH comes from HMP shunt.


SOD
$O_2^{\bullet} + O_2^{\bullet} + 2H^+$
$H_2O_2 + O_2$
H_2O_2
$2\ H_2O$
POD
2 GSH
GS–SG
GR
GPD
$NADP^+$
NADPH
Glucose


$H_2O + 1/2\ O_2$
catalase
SOD
$+ Fe^{2+}$
$O_2^{\bullet-}$
H_2O_2
$^{\bullet}OH + Fe^{3+} + OH^-$
Fenton's reaction
NADPH + H^+
2 GSH
GSHPx
GR
$NADP^+$
GSSG
$2\ H_2O$

Non-enzymatic antioxidants

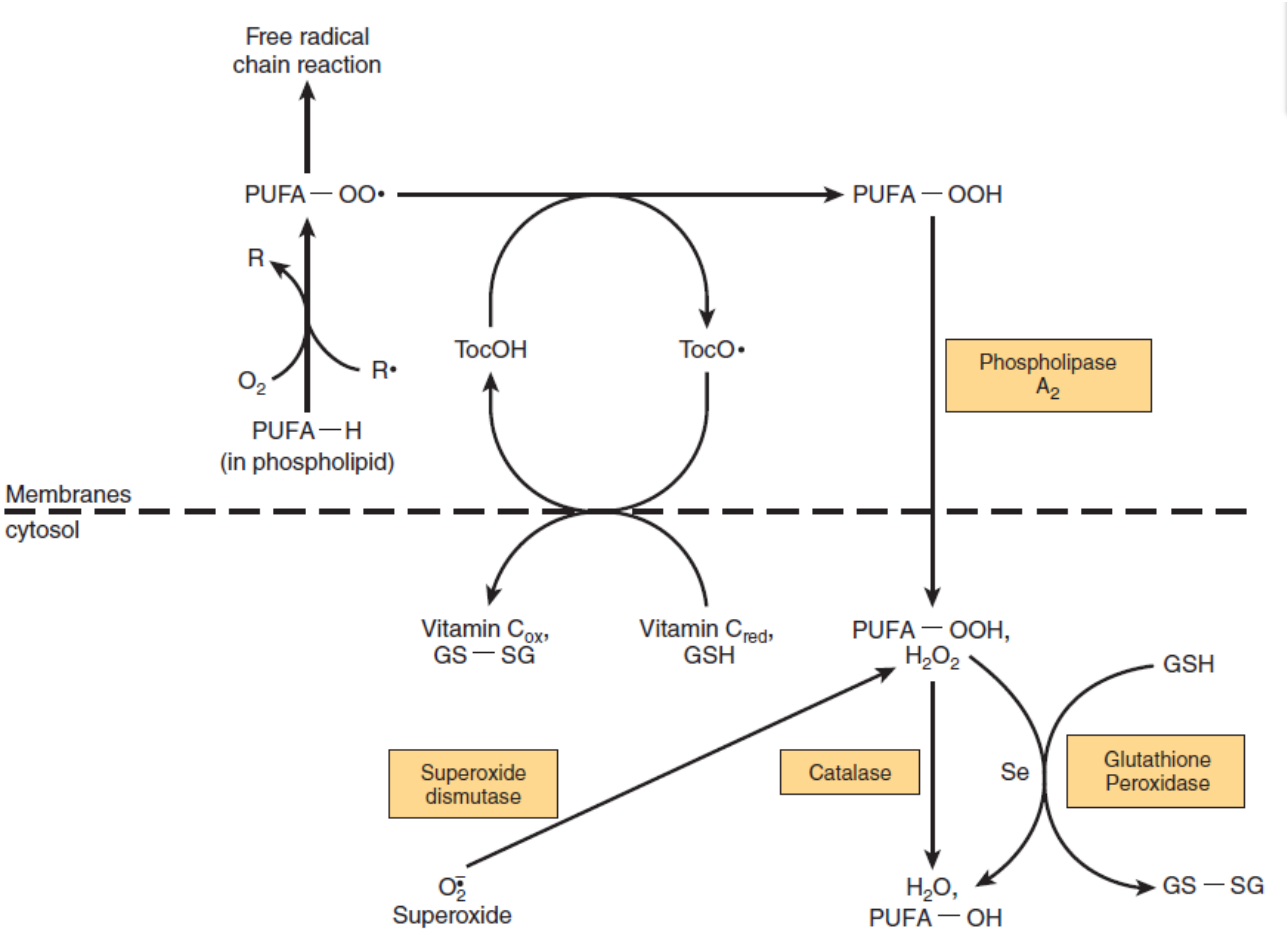
- Metal binding proteins – Transferrin, Ferritin, Ceruloplasmin.
- Albumin – bind with various oxidizing substances.
- Glutathione
- Bilirubin – principal antioxidants in plasma. One molecule of bilirubin scavenges two hydroperoxy radicals and gets oxidized to biliverdin.

- Uric acid
  - Capability of scavenging free electron and prevents the propagation of FR damage in plasma.

Vitamin E

- Fat-soluble antioxidant
- Absorbed in Small Intestines
- Primary defender against effects of free radicals in the body
- Stored in liver and fat cells.
- Protects components of the cell and their membrane from destruction

Vitamin E Is the Major Lipid-Soluble Antioxidant in Cell Membranes & Plasma Lipoproteins



Vitamin E

- Lipid peroxides are neutralized by glutathione peroxidase
- Thus vitamin E acts synergistically with glutathione peroxidase enzyme which contains selenium.
- So sparing effect on dietary requirement of selenium by supplementing the activity of this enzyme.
- Organelles in the cells exposed to the highest amounts of oxygen like mitochondria seem to have the highest amount of Vitamin E.
- Evidence suggests that it protects LDL against oxidation, which in turn protects us against heart disease.

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Vitamin C

- Gives up electrons very easily when they are needed.
- Helps to reactivate Vitamin E, glutathione, urate and beta carotene.
- Having the ability to recycle themselves over and over again.
- Protects oxygen and iron from oxidation.
- Helps protect arteries against oxidative damage.
- It works in aqueose environment by breaking chain reaction and can scavenge physiologically important reactive oxygen and nitrogen species

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Vitamin A and Beta carotene

• Beta Carotene is a water soluble precursor of Vitamin A, and is an antioxidant in itself;

• It is chain breaking antioxidant by trapping peroxy radicals in tissues at low partial pressure of oxygen.

Found In:

Corn, squash and carrots, egg yolk, and other pigmented fruits and vegetables. This is what helps give them their **yellow** color.

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Selenium

- An essential trace mineral,
  RDA of 70 ug/day.

- Found in Glutathion peroxidase which is a free radical scavenging enzyme that contains selenium.It destroys peroxides and thus protects lipid membranes as does Vitamin E.

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BHA (Butylated Hydroxyanisole)

- Generally made in the lab as an antioxidant
- Used as a food preservative.
- Protects the oxidation of fats or oils.
- Naturally found in Rosemary
- Encourages the development of tumors
- May enlarge liver and bladder

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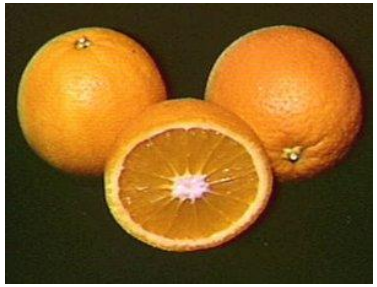
Phytochemicals

•Compounds found in plant-derived foods that have biological activity in the body.

•Contribute to food taste, aromas, colors and other characteristics.

•Act as antioxidants, suppressing the development of diseases.

•Work better when combined with other phytochemicals.



•May help keep cholesterol in check

Phytochemicals

Most Commonly Studied Phytochemicals

There are also hundreds more phytochemicals existing and in need of discovery!

Food	Phytochemical(s)
Allium vegetables (garlic, onions, chives, leeks)	Allyl sulfides
Cruciferous vegetables (broccoli, cauliflower, cabbage, Brussels sprouts, kale, turnips, bok choy, kohlrabi)	Indoles/glucosinolates Sulfaforaphane Isothiocyanates/thiocyanates Thiols
Solanaceous vegetables (tomatoes, peppers)	Lycopene
Umbelliferous vegetables (carrots, celery, cilantro, parsley, parsnips)	Carotenoids Phthalides Polyacetylenes
Compositae plants (artichoke)	Silymarin
Citrus fruits (oranges, lemons, grapefruit) Glucarates	Monoterpenes (limonene) Carotenoids
Other fruits (grapes, berries, cherries, apples, cantaloupe, watermelon, pomegranate)	Ellagic acid Phenols Flavonoids (quercetin)
Beans, grains, seeds (soybeans, oats, barley, brown rice, whole wheat, flax seed) Protease inhibitors	Flavonoids (isoflavones) Phytic acid Saponins
Herbs, spices (ginger, mint, rosemary, thyme, oregano, sage, basil, tumeric, caraway, fennel)	Gingerols Flavonoids Monoterpenes (limonene)
Licorice root Green tea Polyphenols	Glycyrrhizin Catechins

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Flavanoids

• Sometimes referred to as “Super Antioxidants.”

• Shown to have: antiviral, antiallergic, anti-inflammatory, antithrombogenic and anticarcinogenic effects.

• Over 4000 flavanoids have been found, fall in four different groups: flavones, flavanones, catechins, and anthocyanins.

•Found in: certain fruits, flowers, roots, stems, tea, wine, grains and vegetables.

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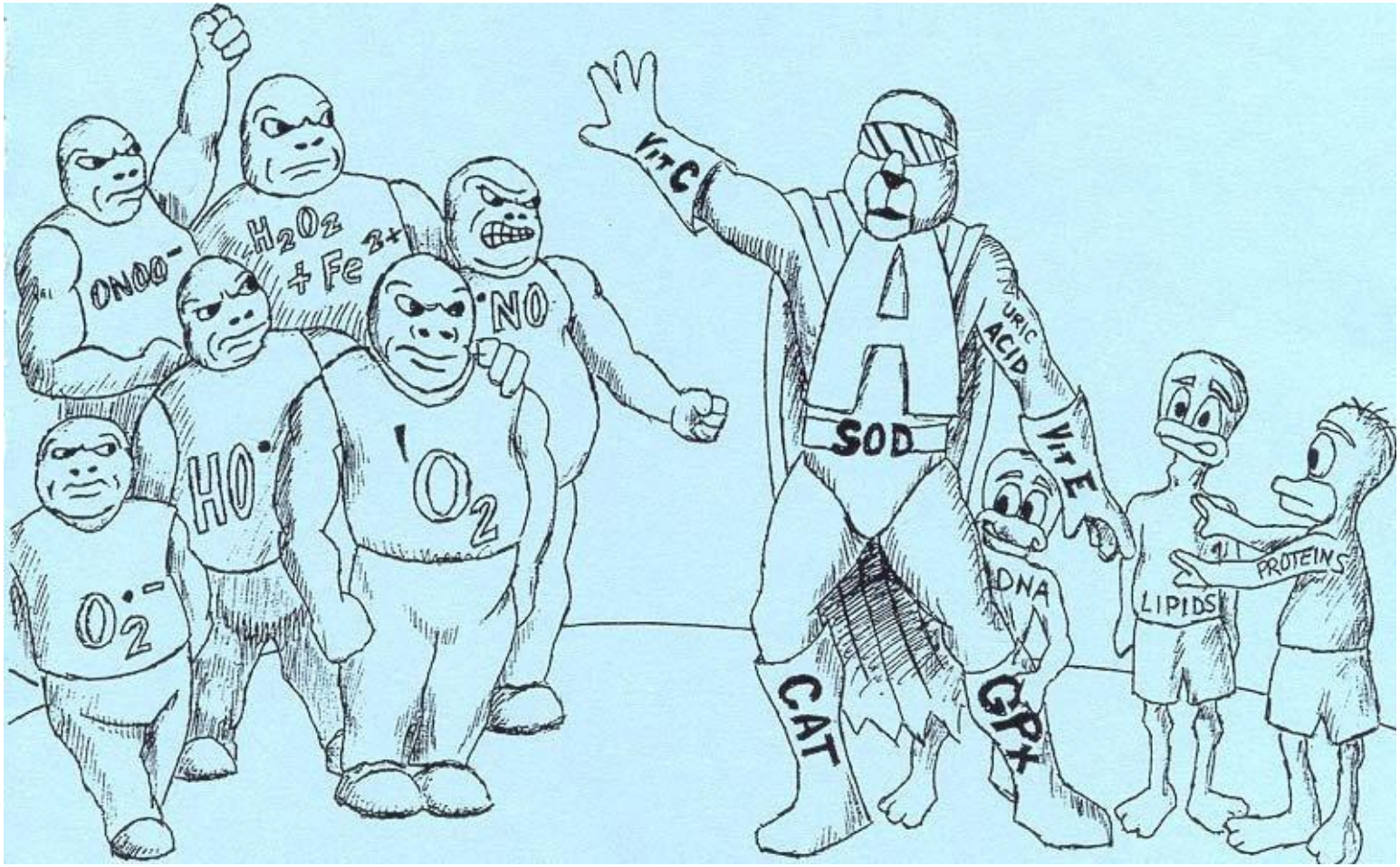
Flavanoids

- 20 times stronger antioxidant then Vitamin C and 50 times stronger then Vitamin E.
- Water soluble
- Ability to attach to cells and their proteins for up to 72 hours protecting them from oxidation and free radical damage.
- Able to cross the blood-brain barrier

FREE RADICALS AND ANTI-OXIDANTS



- **The Antioxidant Paradox- Prooxidant**

The Antioxidant Paradox—Antioxidants Can Also Be Pro-Oxidants

Antioxidant roles:
Ascorbate + $^{\cdot}O_2^-$ → H_2O_2 + monodehydroascorbate; catalase and peroxidases catalyze the reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$
Ascorbate + $^{\cdot}OH \rightarrow H_2O$ + monodehydroascorbate
Pro-oxidant roles:
Ascorbate + $O_2 \rightarrow {}^{\cdot}O_2^-$ + monodehydroascoebate
Ascorbate + $Cu^{2+} \rightarrow Cu^{+}$ + monodehydroasacorbate;
$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^- + {}^{\cdot}OH$

Vitamin C

- these pro-oxidant actions require relatively high concentrations of **ascorbate**, which are unlikely to be reached in tissues
- once the plasma concentration of ascorbate reaches about 30 mmol/L, the renal threshold is reached,
- at intakes above about 100 to 120 mg/d the vitamin is excreted in the urine quantitatively with intake.

β-carotene.

- **carotene** is protective against lung and other cancers.
- two major intervention trials in the 1990s showed an increase in death from lung (and other) cancer
- β-carotene is a radical-trapping antioxidant:

  under conditions of low partial pressure of oxygen,
- Under high partial pressures of oxygen (as in the lungs)

  and especially in high concentrations, β-carotene is an autocatalytic pro-oxidant,
- can initiate radical damage to lipids and proteins.

vitamin E

- vitamin E is protective against atherosclerosis and cardiovascular disease.
- meta-analysis of intervention trials with vitamin E shows increased mortality among those taking (high dose) supplements.
- vitamin E acts as an antioxidant by forming a stable radical that persists long enough to undergo metabolism to nonradical products.
- radical also persists long enough to penetrate deeper in to the lipoprotein, causing further radical damage,

Summary

- Free radicals are highly reactive molecular specie
- They can modify, proteins, nucleic acids and fatty acids in cell membranes and plasma lipoproteins.
- Radical damage to lipids and proteins in plasma lipoproteins is a factor in the development of atherosclerosis and coronary artery disease;
- radical damage to nucleic acids may induce heritable mutations and cance
- radical damage to proteins may lead to the development of autoimmune diseases.

- Oxygen radicals arise as a result of exposure to ionizing radiation, nonenzymic reactions of transition metal ions, the respiratory burst of activated macrophages
- Protection against radical damage is afforded by enzymes that remove superoxide ions and hydrogen peroxide, enzymic reduction of lipid peroxides linked to oxidation of glutathione, nonenzymic reaction of lipid peroxides with vitamin E
- Except in people who were initially deficient, intervention trials of vitamin E and β-carotene have generally shown increased mortality among those taking the supplements

- at higher concentrations of oxygen β carotin is an autocatalytic pro-oxidant
- Vitamin E forms a stable radical penetrating further into lipoproteins and tissues, so increasing radical damage.
- Radicals are important in cell signaling for apoptosis of DNA damaged cells
- high concentrations of antioxidants, may quench the signaling radicals, so increasing, the risk of cancer development.

MCQ 1

- Which one of the following is NOT a source of oxygen radicals?

A. Action of superoxide dismutase

B. Activation of macrophages

C. Nonenzymic reactions of transition metal ions

D. Reaction of β-carotene with oxygen

E. Ultraviolet radiation

MCQ 2

- Which one of the following best explains the antioxidant action of vitamin E?

A. It forms a stable radical that can be reduced back to active vitamin E by reaction with vitamin C.

B. It is a radical, so that when it reacts with another radical a nonradical product is formed.

C. It is converted to a stable radical by reaction with vitamin C.

D. It is lipid soluble and can react with free radicals in the blood plasma resulting from nitric oxide (NO) formation by vascular endothelium.

E. Oxidized vitamin E can be reduced back to active vitamin E by reaction with glutathione and glutathione peroxidase.

References

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3. SK Gupta, textbook of medical biochemistry Free Radicals and Antioxidants, 1st edition, pg no 843-52.