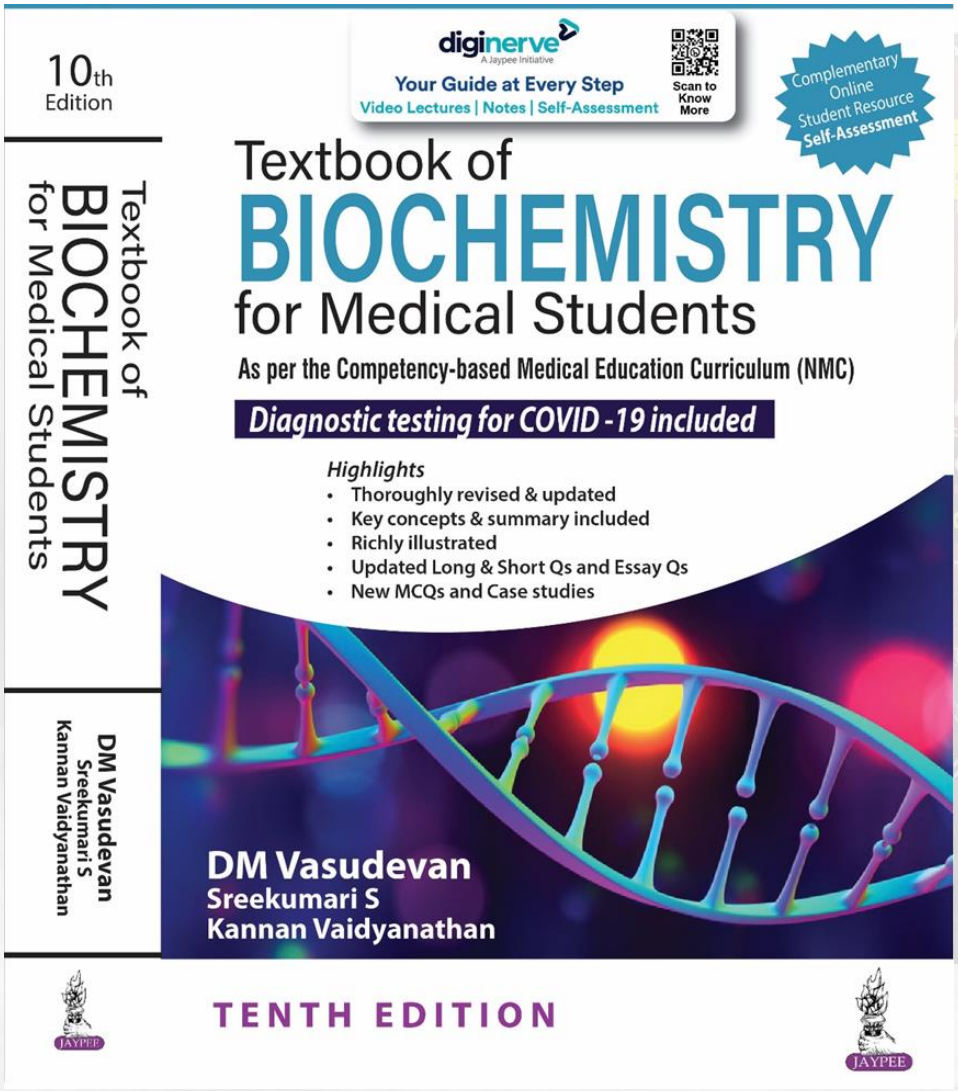


Chapter 26:

Free Radicals and Antioxidants

Textbook of
BIOCHEMISTRY
for Medical Students

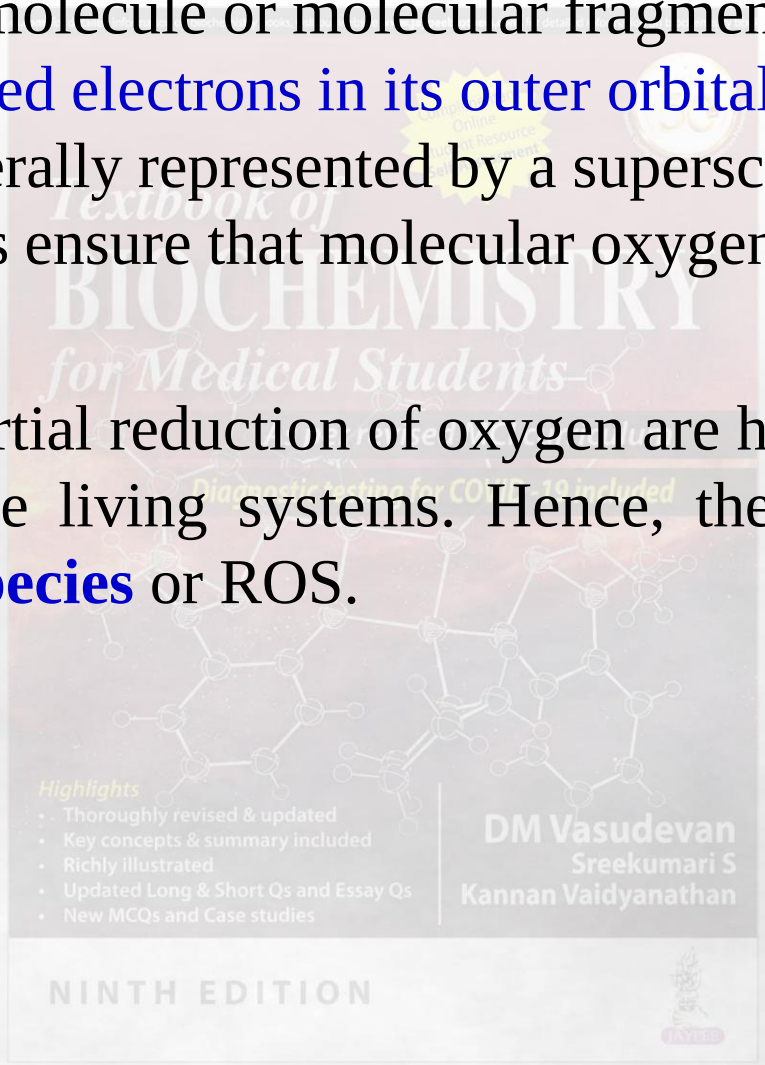
By DM Vasudevan, *et al.*

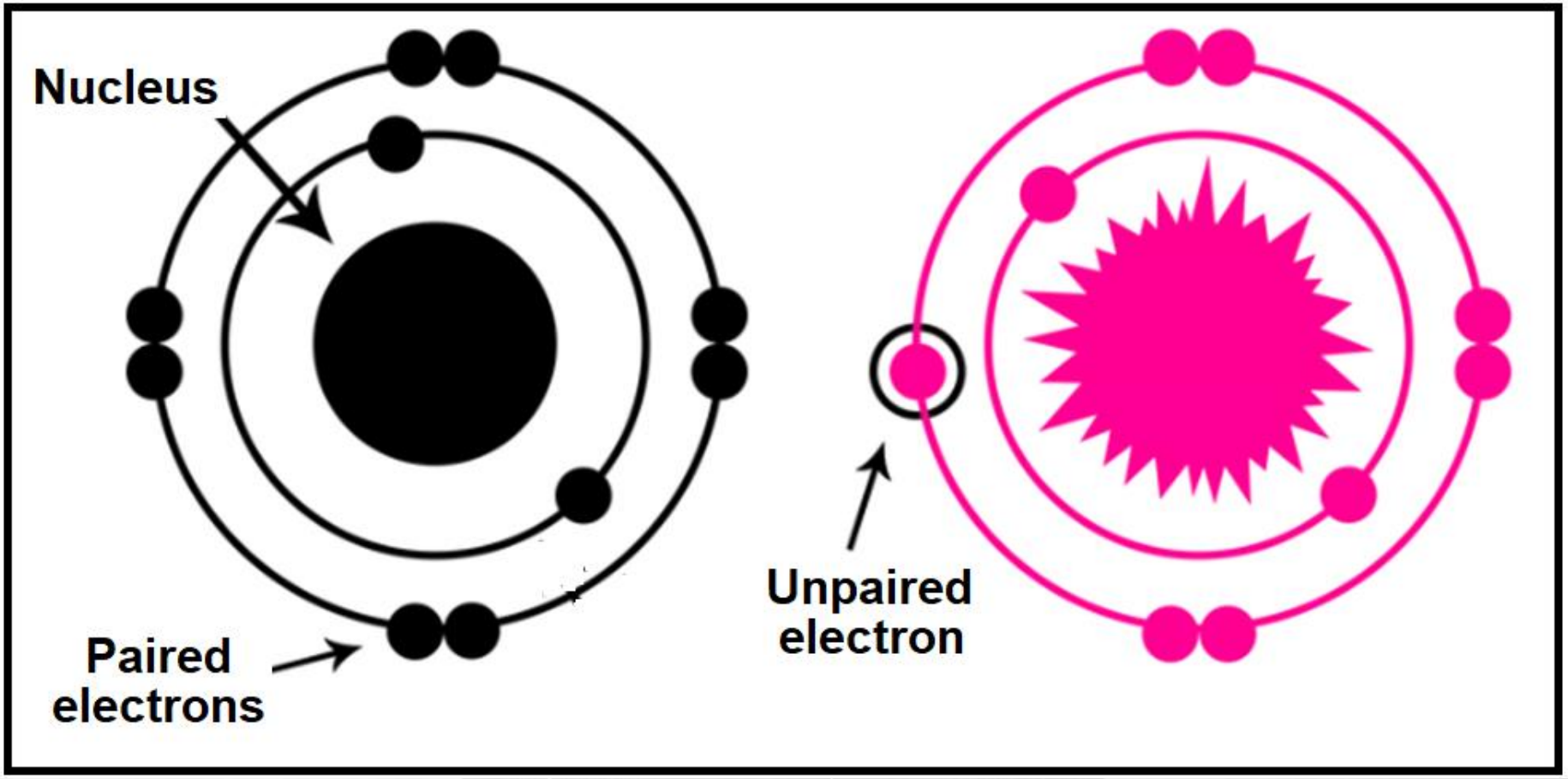
TENTH EDITION

Definition

A free radical is a molecule or molecular fragment that contains one or more unpaired electrons in its outer orbital.
Free radical is generally represented by a superscript dot, **(R•)**.
Oxidation reactions ensure that molecular oxygen is completely reduced to water.

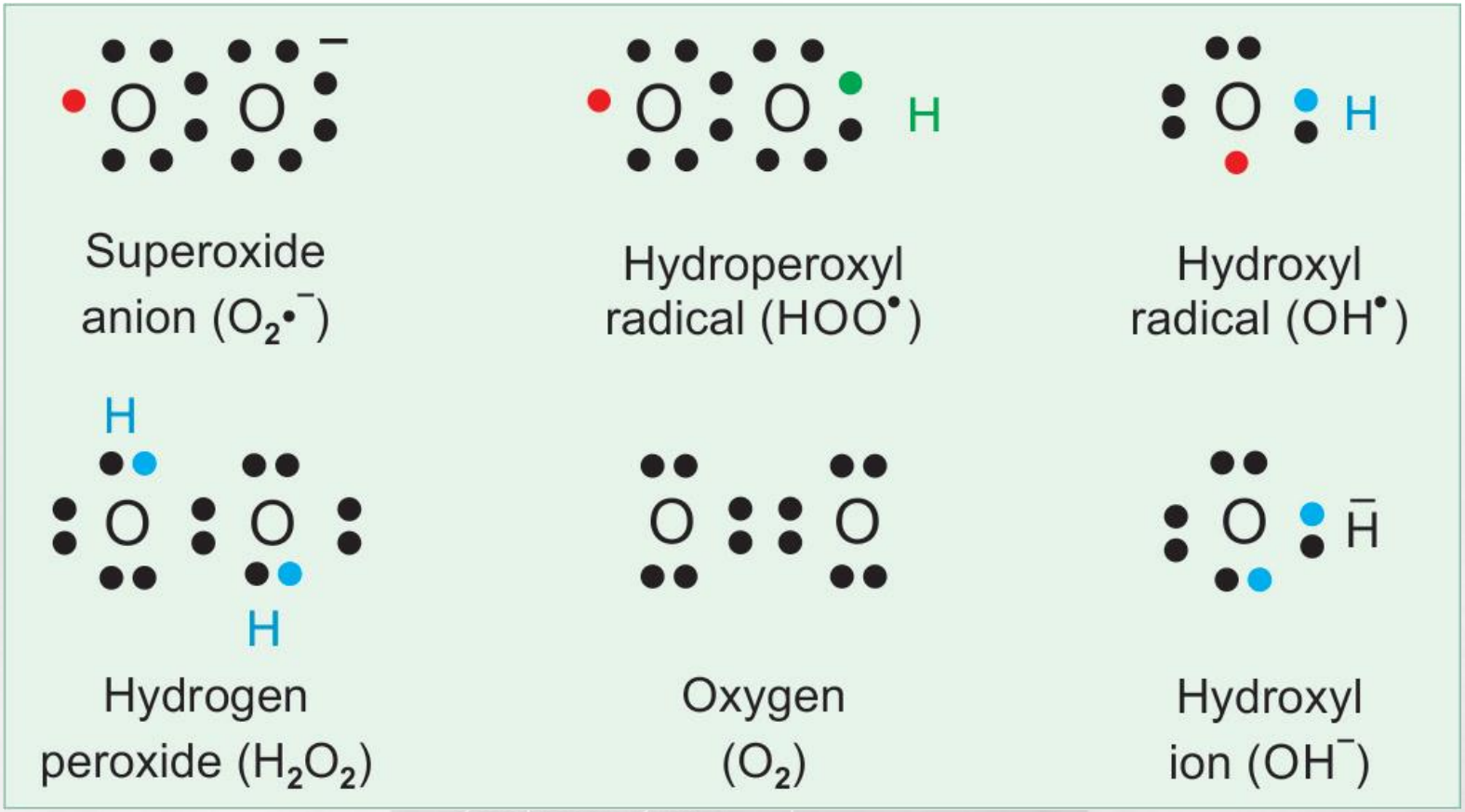
The products of partial reduction of oxygen are highly reactive and create havoc in the living systems. Hence, they are also called **reactive oxygen species** or ROS.





Left side = normal oxygen atom with all paired electrons

Right side = A free radical with an unpaired electron



Some free radicals. Please compare hydroxyl radical (free radical) with hydroxyl ion, which is not a free radical. Also compare oxygen with superoxide anion.

Free Radicals

i) Superoxide anion radical ($O_2^{--\bullet}$)
ii) Hydroperoxyl radical (HOO•)
iii) Hydrogen peroxide (H_2O_2)
iv) Hydroxyl radical (OH•)
v) Lipid peroxide radical (ROO•)
vi) Singlet oxygen ($^1O_2$)
vii) Nitric oxide (NO•)
viii) Peroxy nitrite (ONOO--•)

Hydrogen peroxide and singlet oxygen are not free radicals (they do not have superscript dot). However, because of their extreme reactivity, they are included in the group of reactive oxygen species.

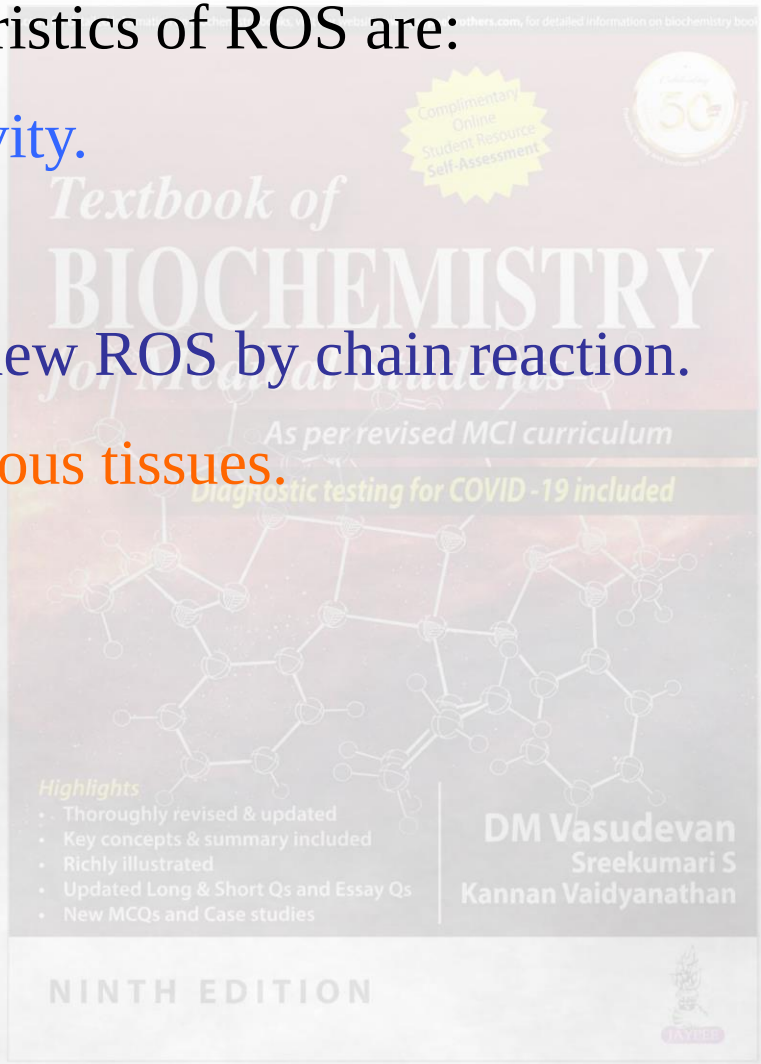
Important characteristics of ROS are:

a) Extreme reactivity.

b) Short life span.

c) Generation of new ROS by chain reaction.

d) Damage to various tissues.

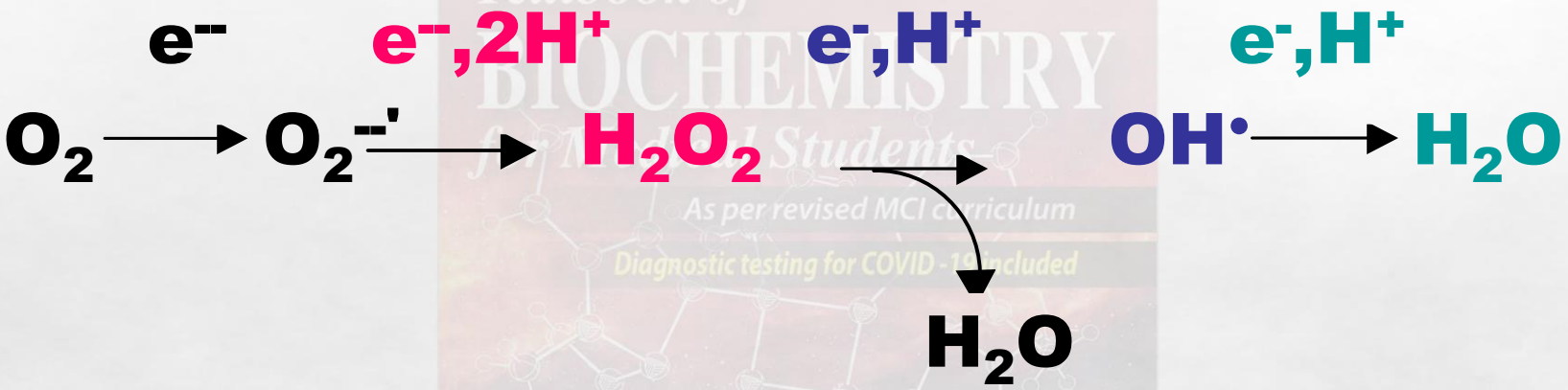


Reactive Oxygen Species (ROS)

Name of ROS	Symbol	Half-life at 37°C
Superoxide	O_2--•	10^{-6} sec
Hydrogen peroxide	H_2O_2	Few minutes
Hydroxyl	OH•	10^{-9} sec
Hydroperoxyl	HOO•	1 sec
Alkoxyl	RO•	10^{-6} sec
Peroxyl	ROO•	1 sec
Singlet oxygen	$1O_2$	10^{-6} sec
Ozone	O_3	10 min
Nitric oxide	NO•	7 min
Peroxynitrite	ONOO --•	2 sec
Nitrogen dioxide	NO_2	Few minutes
Nitronium ion	NO +	Few minutes

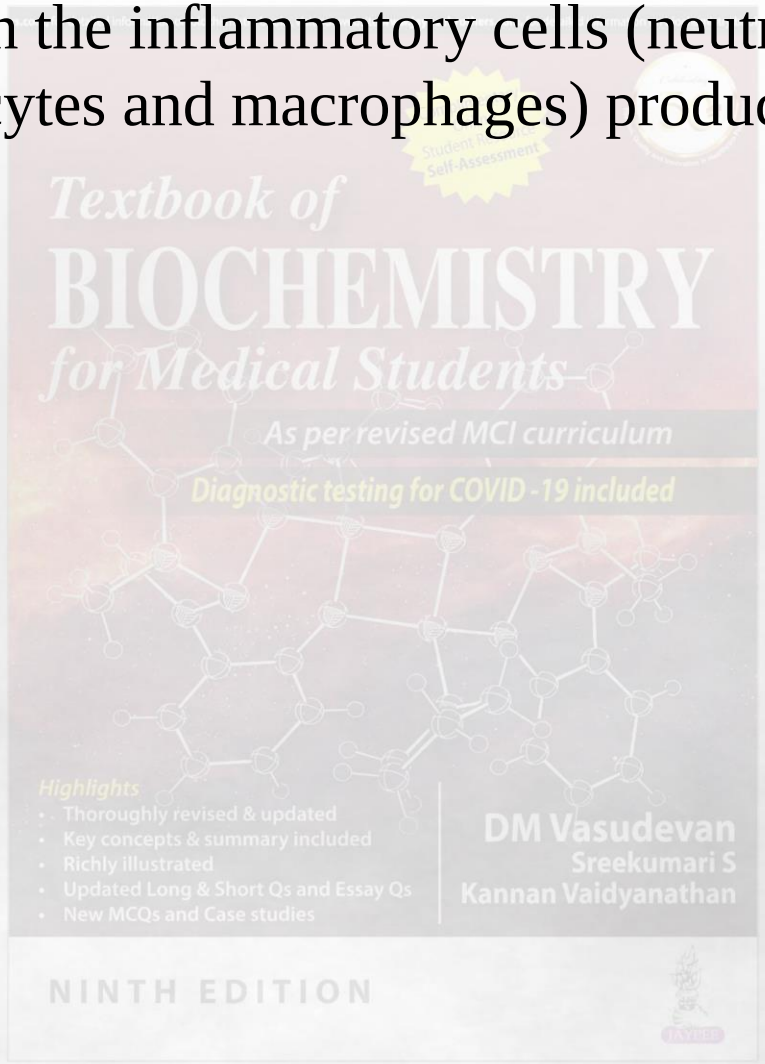
Generation of Free Radicals

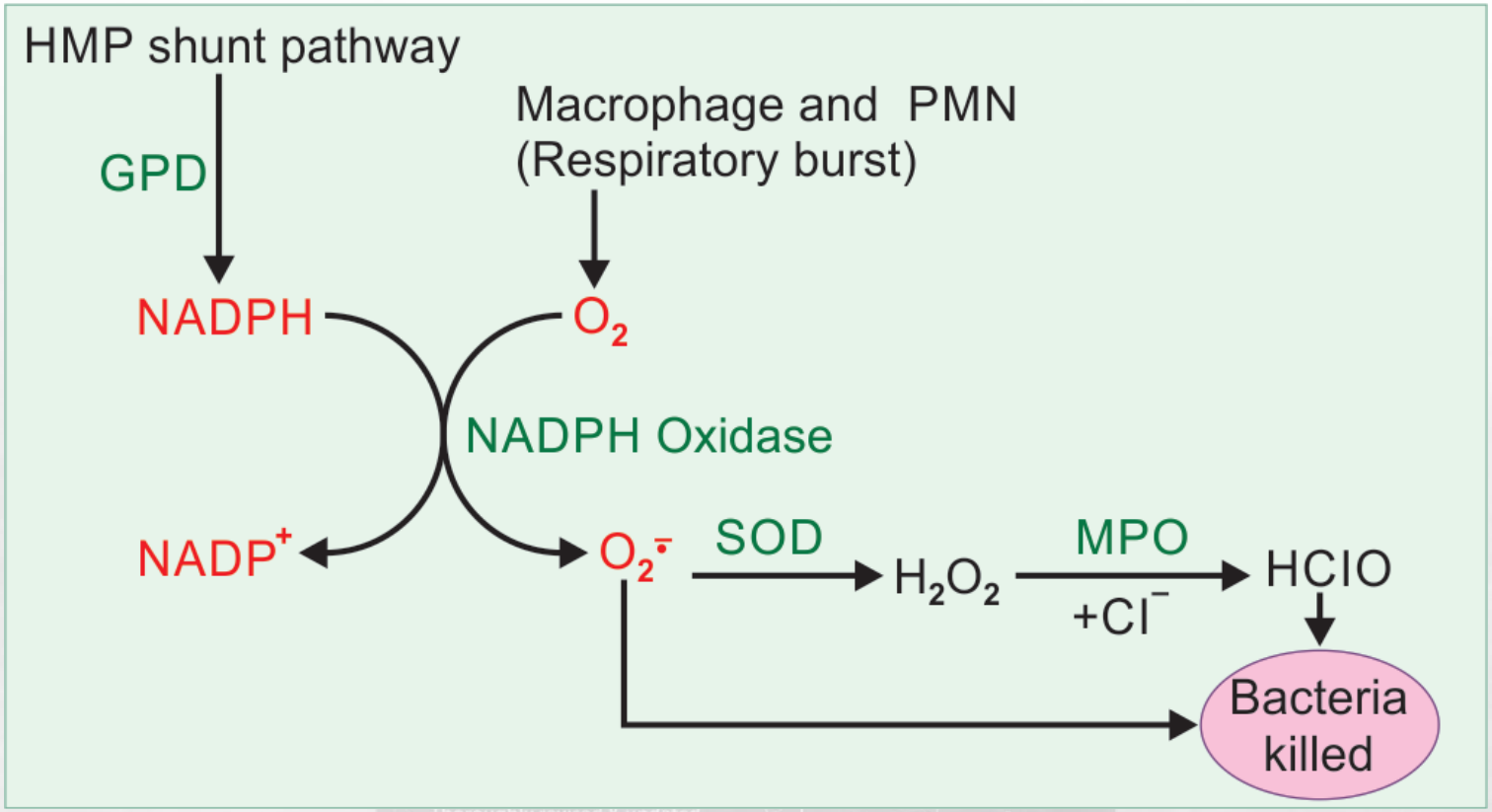
The sequential univalent reduction steps of oxygen may be represented as:



Generation of Free Radicals

NADPH oxidase in the inflammatory cells (neutrophils, eosinophils, monocytes and macrophages) produce superoxide anion





Generation of reactive oxygen species in macrophages. (GDP: glucose-6-phosphate dehydrogenase; SOD: superoxide dismutase; MPO: myeloperoxidase.)

Superoxide dismutase (SOD)

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

Catalase

$$H_2O_2 \longrightarrow 2H_2O + O_2$$

Glutathione peroxidase

$$2GSH + R{-}O{-}OH \longrightarrow GSSG + H_2O + ROH$$

Myeloperoxidase

$$H_2O_2 + X^- + H^+ \longrightarrow HOX + H_2O$$

Where, X = Cl^-, Br^- or SCN^-

Reactions involved in reactive oxygen species.

Iron catalyzed Haber-Weiss reaction

$$O_2^{\bullet -} + H_2O_2 \longrightarrow O_2 + OH + OH^-$$

Glutathione reductase

$$GSSG + NADPH + H^+ \longrightarrow 2GSH + NADP$$

NADPH oxidase

$$2O_2 + NADPH \longrightarrow 2O_2^{\bullet -} + NADP + H^+$$

Fenton reaction

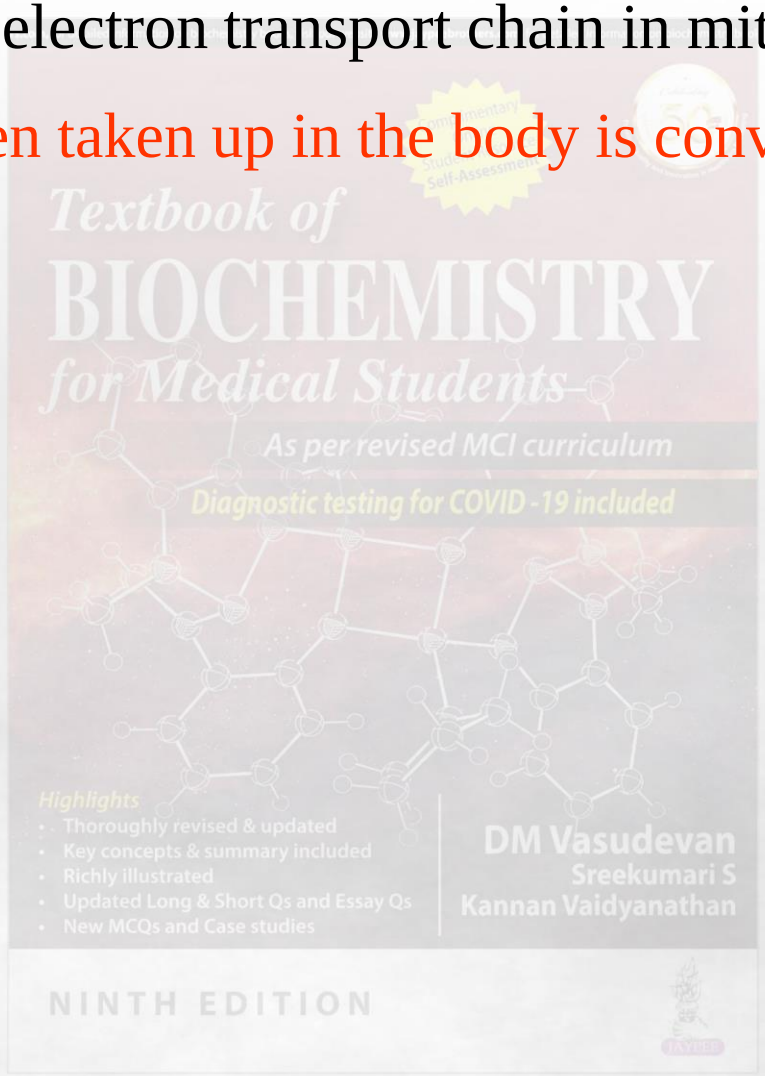
$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\cdot} + OH^-$$

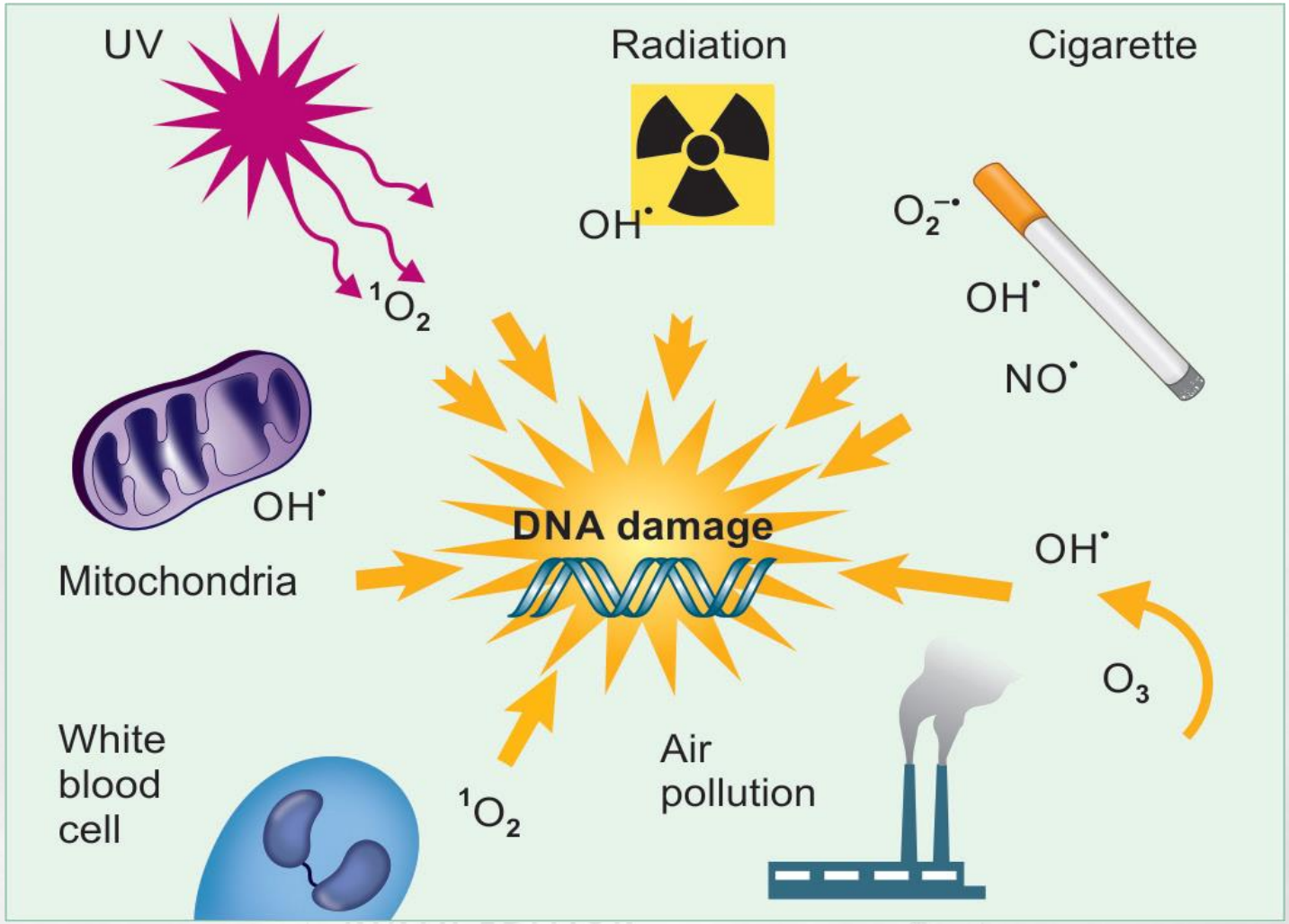
Reactions involved in reactive oxygen species. Note: Fenton reaction and Haber-Weiss reactions are dependent on iron.

Generation of Free Radicals

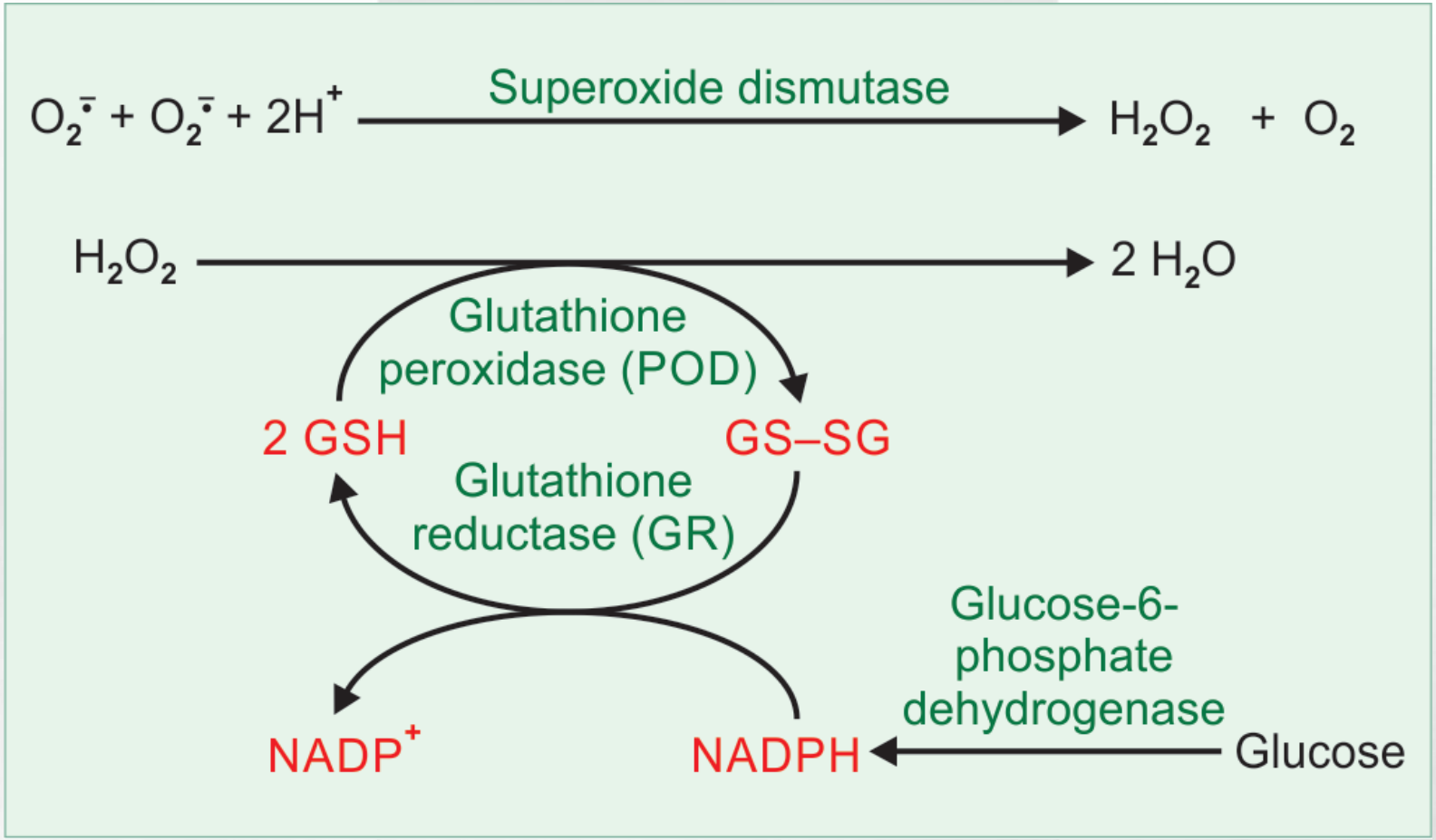
Due to leaks in the electron transport chain in mitochondria.

About 4% of oxygen taken up in the body is converted to free radicals.





Formation of free radicals.



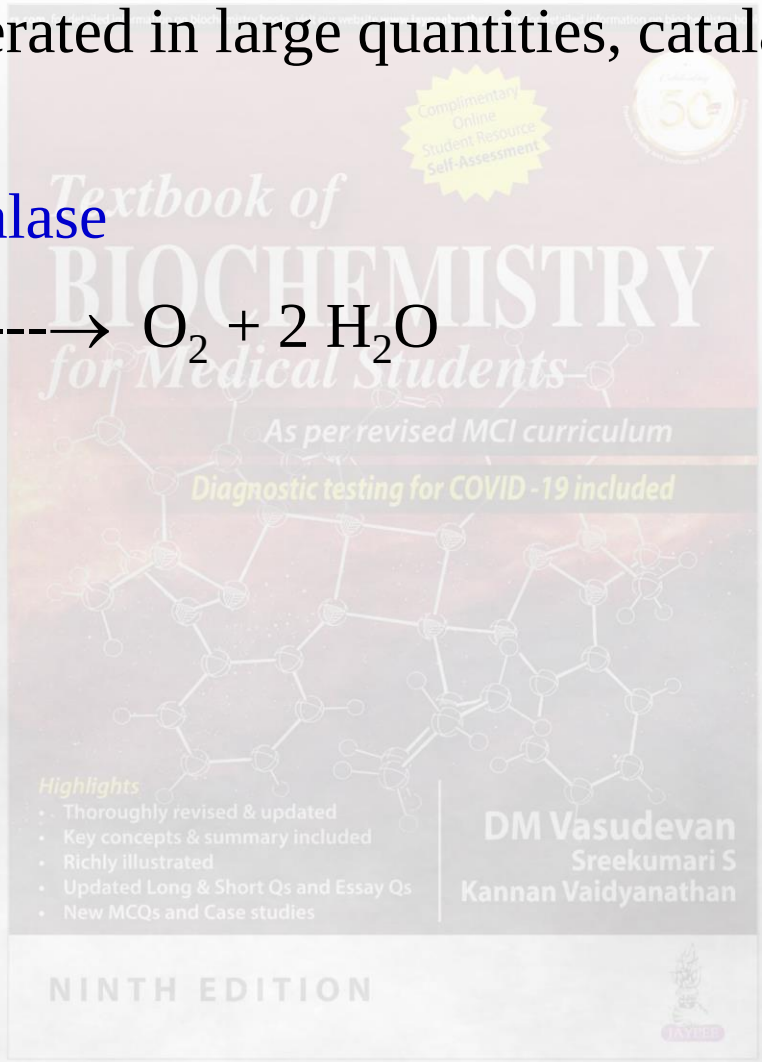
Free radical scavenging enzymes

Catalase

When H_2O_2 is generated in large quantities, catalase is also used for its removal.

Catalase

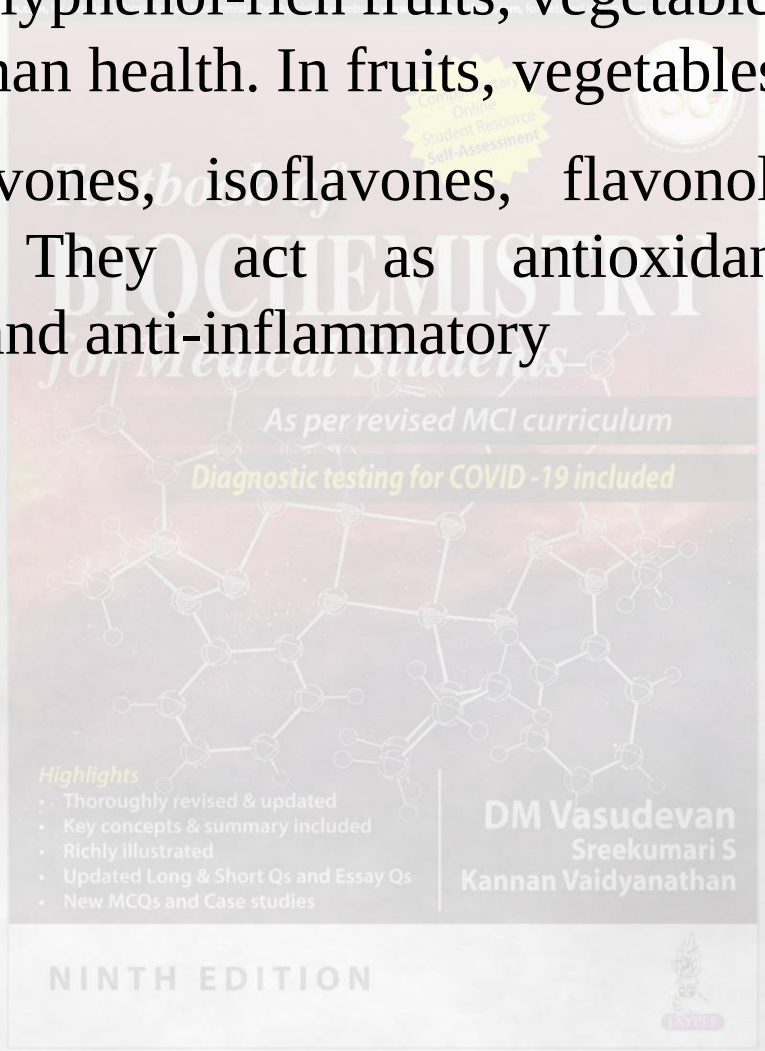
$$2\ H_2O_2 \xrightarrow{\text{Catalase}} O_2 + 2\ H_2O$$

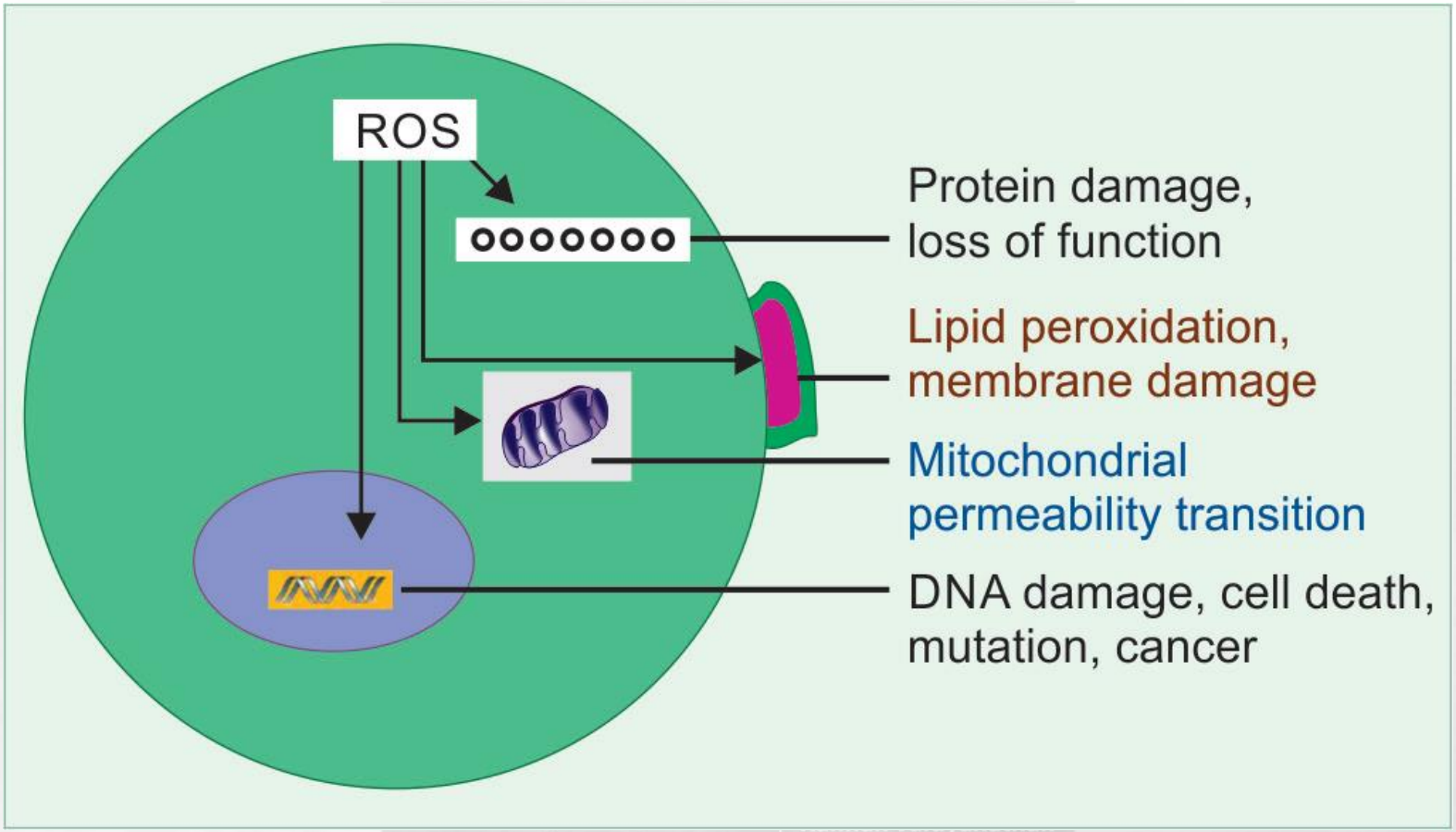


Polyphenols

Consumption of polyphenol-rich fruits, vegetables, and beverages is beneficial to human health. In fruits, vegetables, tea.

They contain flavones, isoflavones, flavonols, catechins and phenolic acids. They act as antioxidant, antiapoptosis, anticarcinogenic, and anti-inflammatory





Damage produced by reactive oxygen species

Clinical Significance

1. Chronic Inflammation

Rheumatoid arthritis chronic ulcerative colitis, **chronic glomerulonephritis** etc.

2. Acute Inflammation

Activated macrophages

3. Diseases of the Eye

Retrolental fibroplasia

(retinopathy of prematurity)

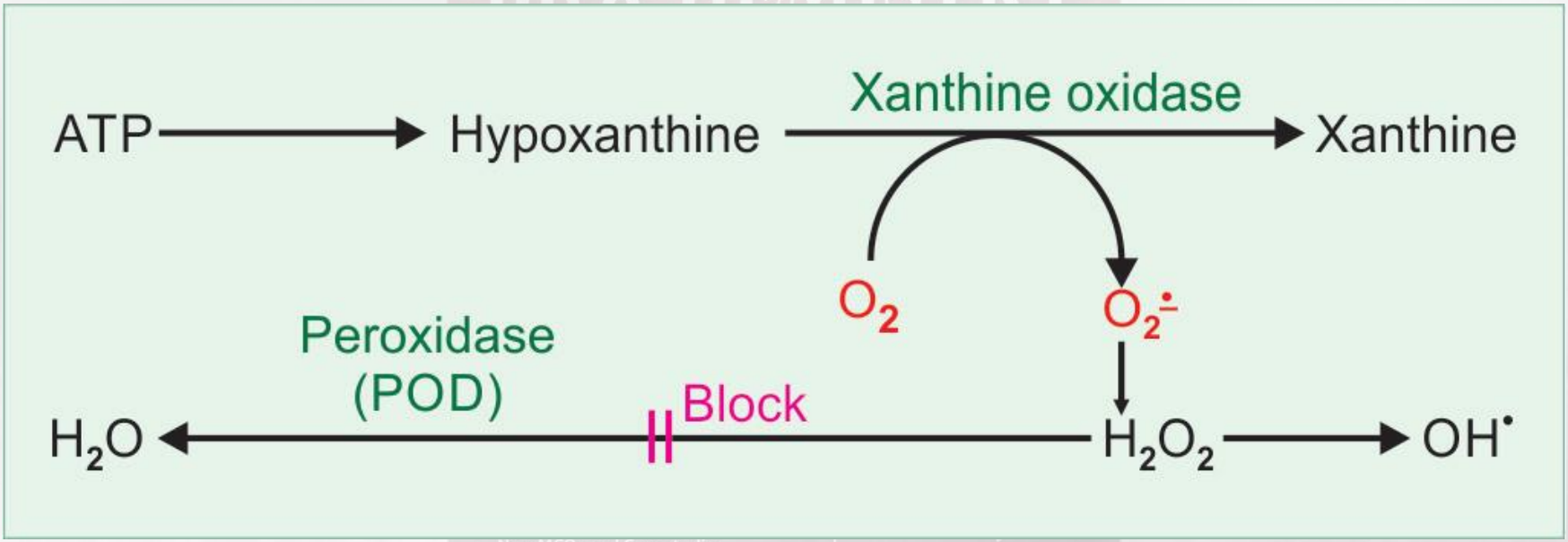
Cataract formation

is partly due to photochemical generation of free radicals. Tissues of the eye, including the lens, has high concentration of free radical scavenging enzymes.

Clinical Significance

4. Reperfusion Injury

Reperfusion injury after myocardial ischemia is caused by free radicals.



Clinical Significance

5. Atherosclerosis and Myocardial Infarction

Low density lipoproteins (LDL) are deposited under the ndothelial cells, which undergo oxidation by free radicals. This attracts macrophages. Macrophages are then converted into **foam cells**. This initiates the atherosclerotic plaque formation.

Anti-oxidants offer some protective effect.

6. Carcinogenesis

Free radicals produce DNA damage, and accumulated damages lead to somatic mutations and malignancy.

Clinical Significance

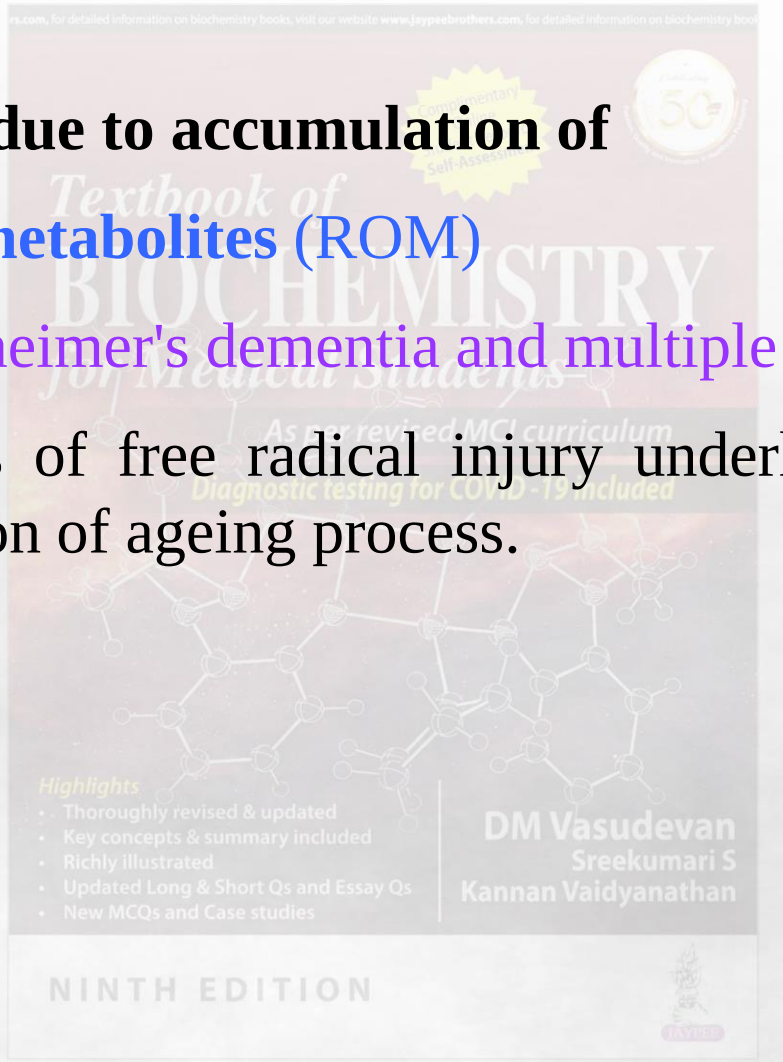
7. Ageing process

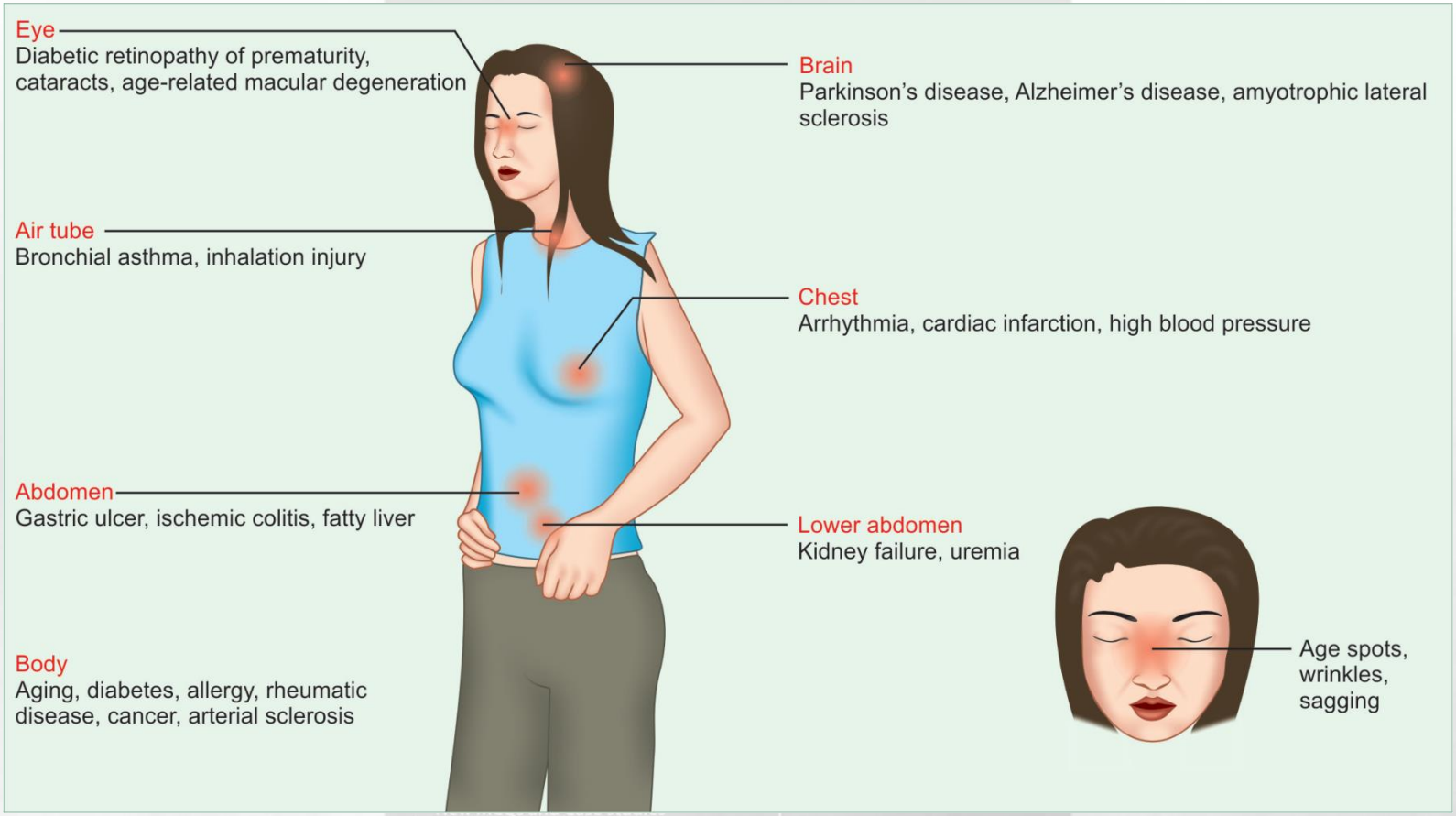
Biological ageing due to accumulation of

Reactive oxygen metabolites (ROM)

Parkinsonism, Alzheimer's dementia and multiple sclerosis.

Cumulative effects of free radical injury underline the cause for gradual deterioration of ageing process.



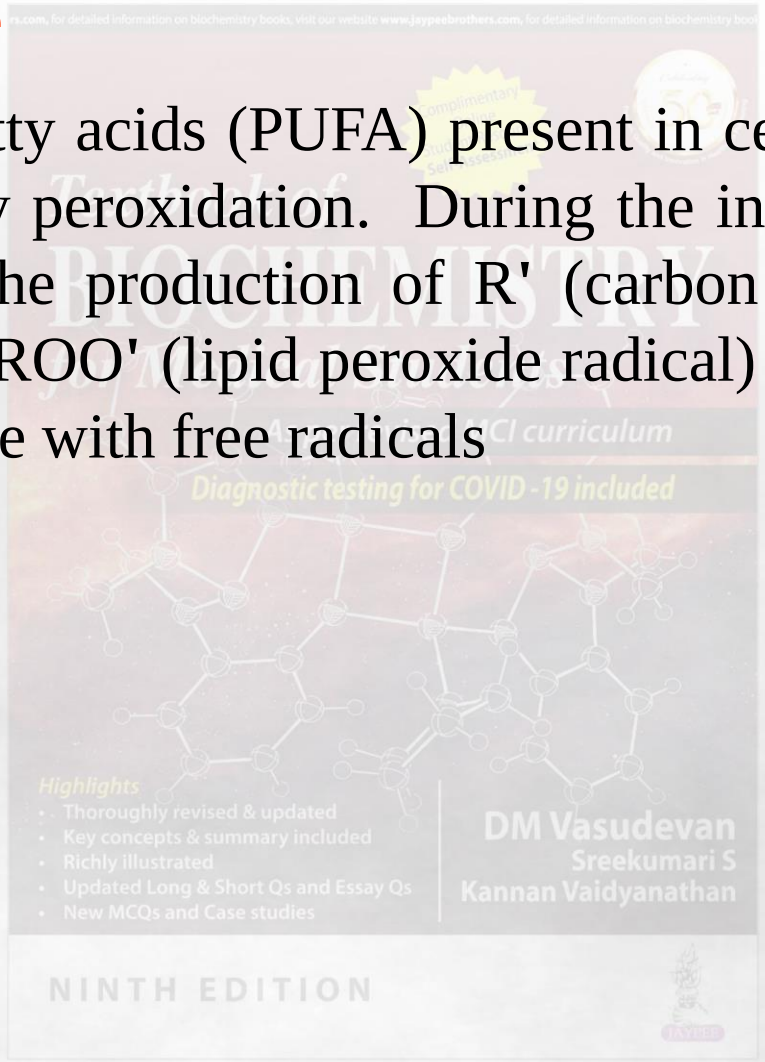


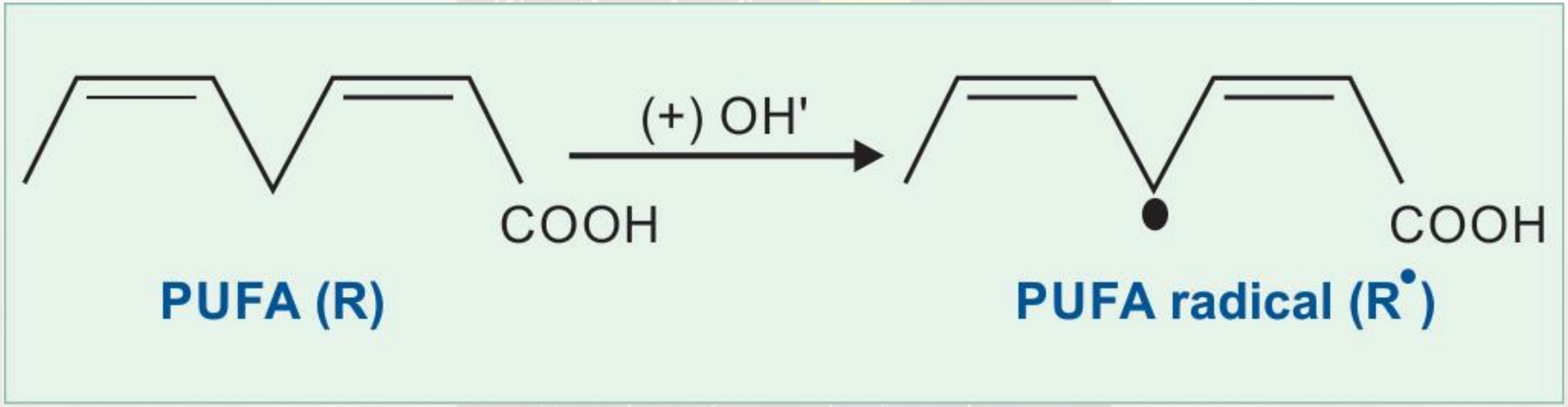
Free radical toxicity.

Lipid Peroxidation

1. Initiation Phase

Polyunsaturated fatty acids (PUFA) present in cell membranes are easily destroyed by peroxidation. During the initiation phase, the primary event is the production of R' (carbon centered radical) (PUFA radical) or ROO' (lipid peroxide radical) by the interaction of a PUFA molecule with free radicals



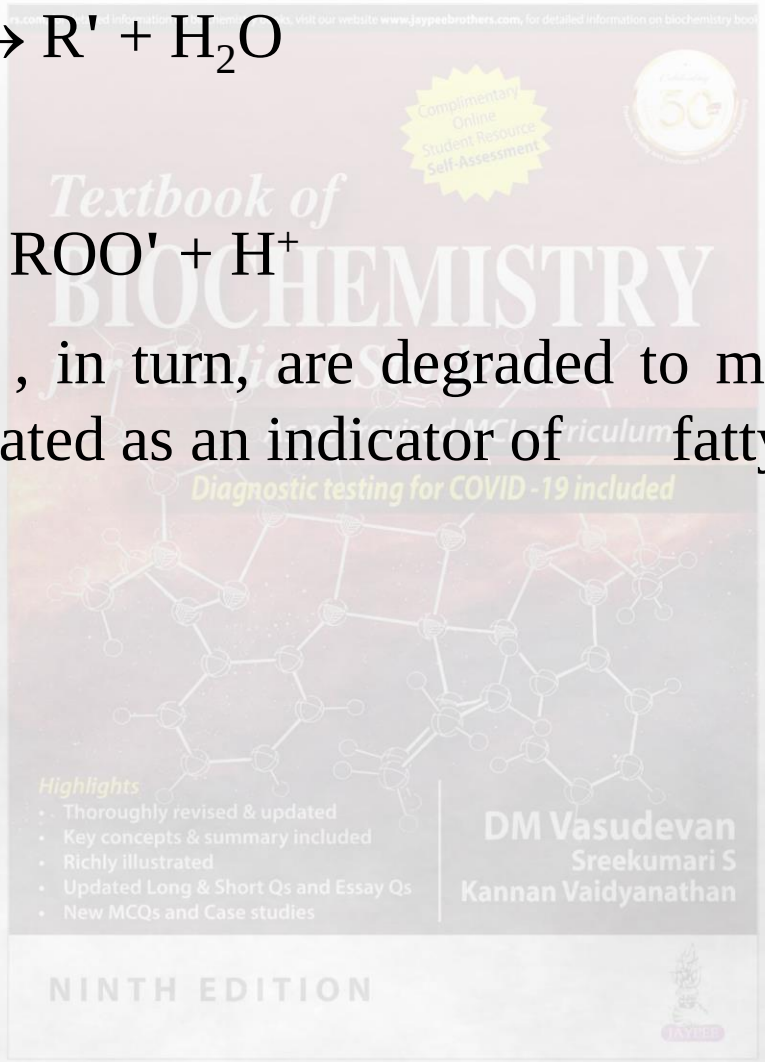


Peroxidation of polyunsaturated fatty acids (PUFA).

$RH + OH' \xrightarrow{\text{metal ion}} R' + H_2O$

$ROOH \longrightarrow ROO' + H^+$

The R' and ROO' , in turn, are degraded to malon dialdehyde (3 carbon). It is estimated as an indicator of fatty acid breakdown by free radicals.

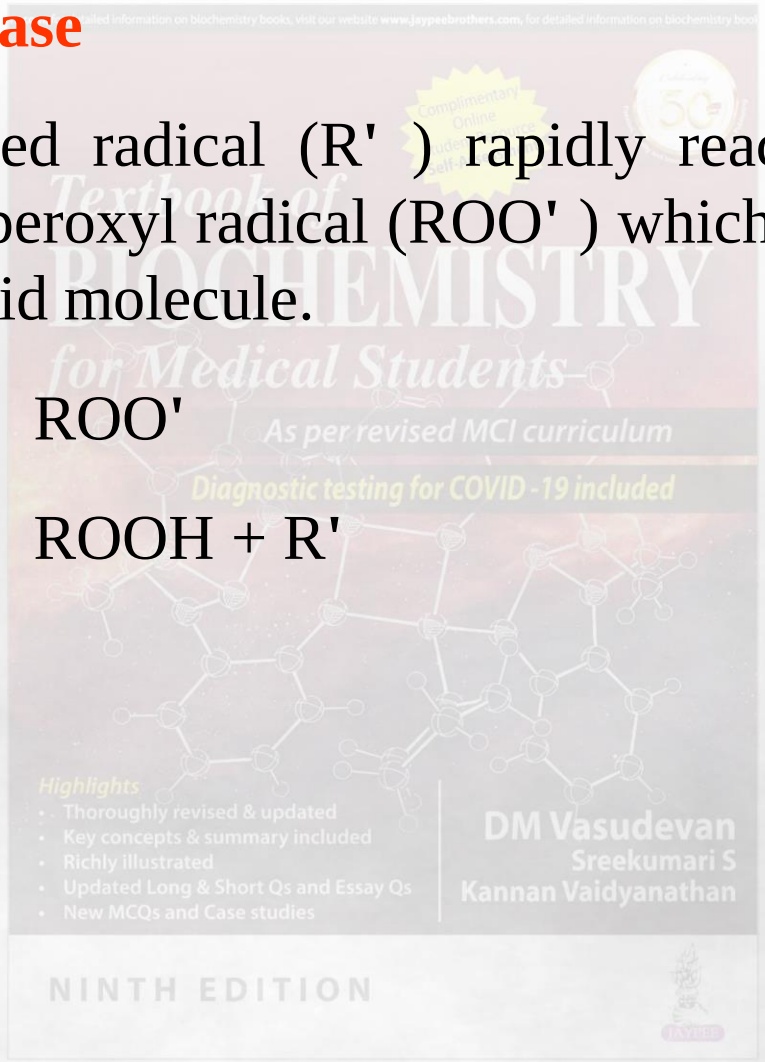


2. Propagation Phase

The carbon centered radical (R') rapidly reacts with molecular oxygen forming a peroxyl radical (ROO') which can attack another polyunsaturated lipid molecule.

$$R' + O2 \rightarrow ROO'$$

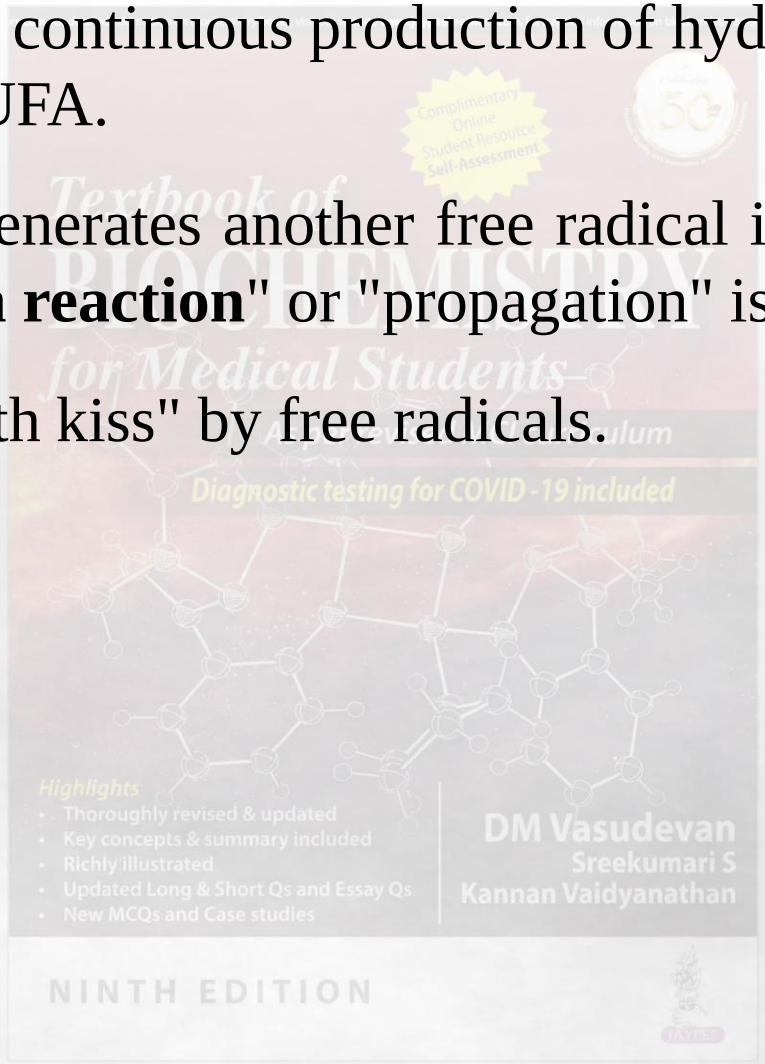
$$ROO' + RH \rightarrow ROOH + R'$$



This would lead to continuous production of hydroperoxide with consumption of PUFA.

One free radical generates another free radical in the neighbouring molecule; a "**chain reaction**" or "propagation" is initiated.

This is called "death kiss" by free radicals.



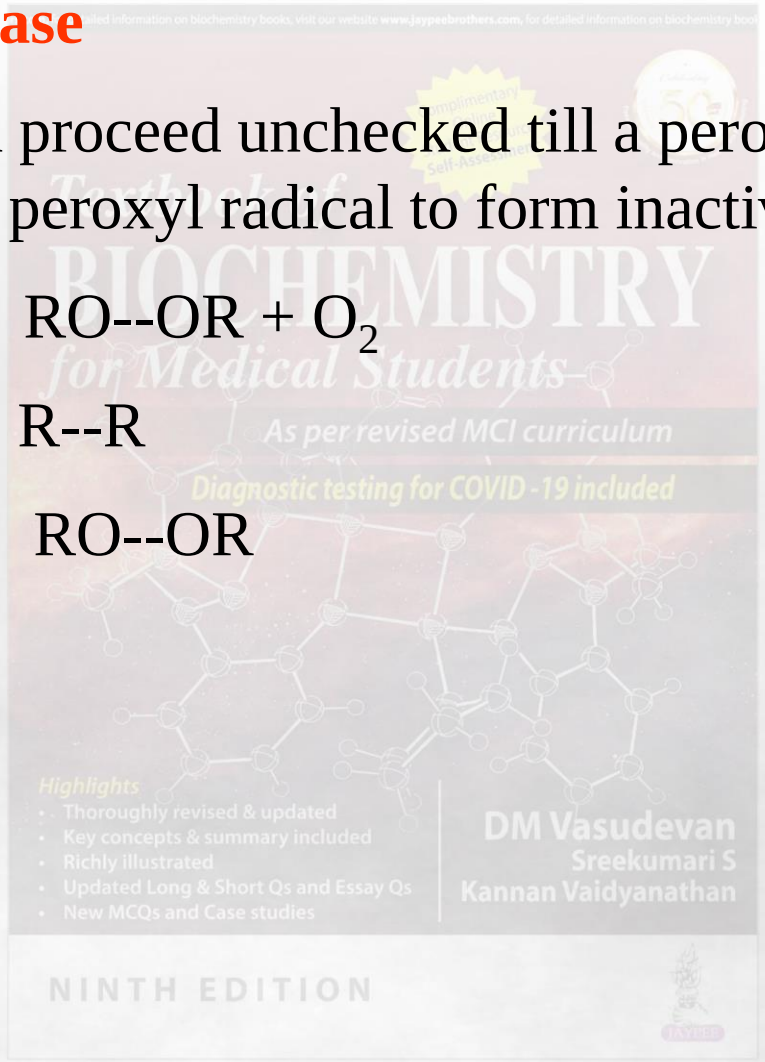
3. Termination Phase

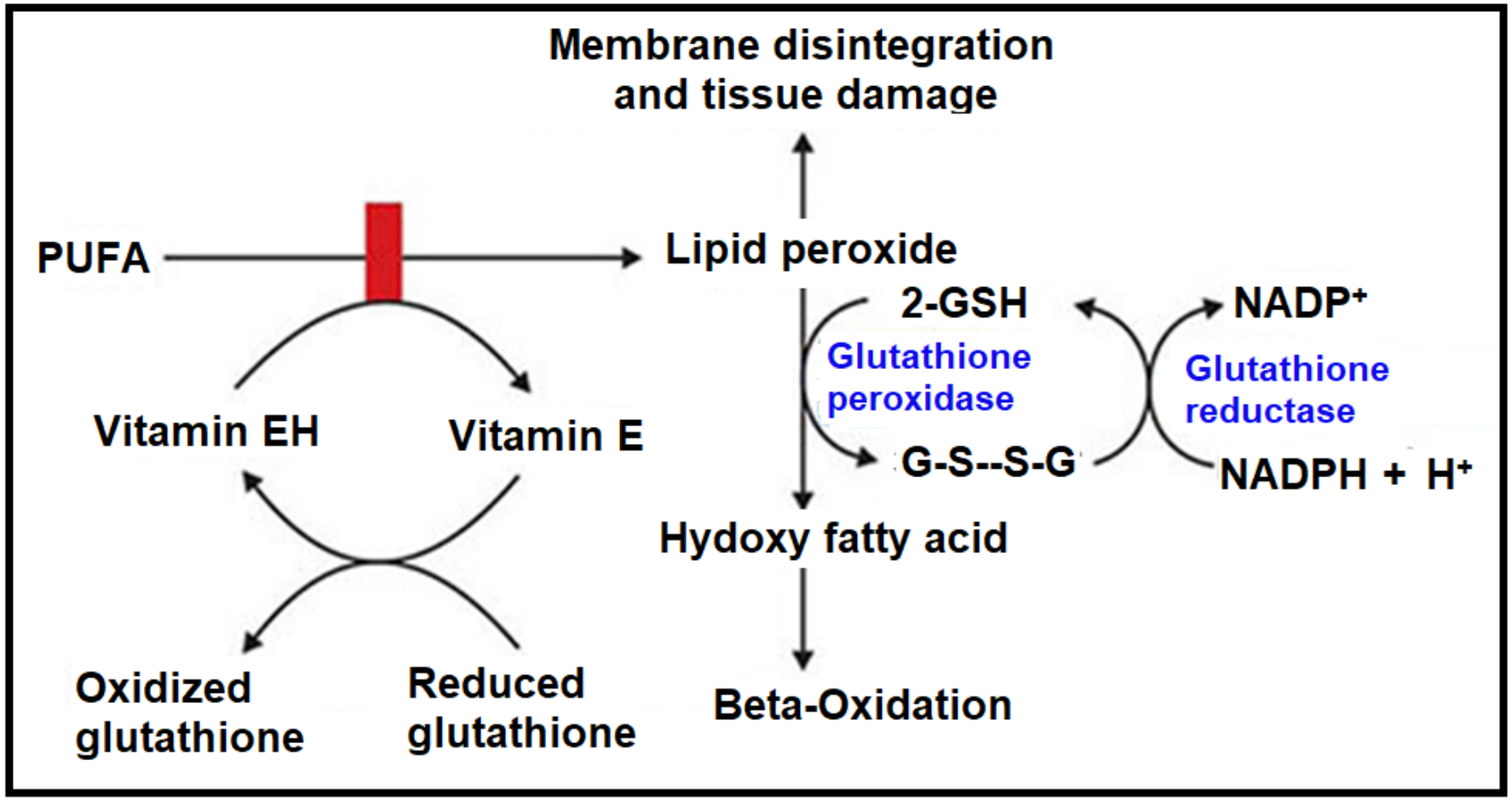
The reaction would proceed unchecked till a peroxyl radical reacts with another peroxyl radical to form inactive products.

$$ROO' + ROO' \rightarrow RO\text{--}OR + O_2$$

$$R' + R' \rightarrow R\text{--}R$$

$$ROO' + R' \rightarrow RO\text{--}OR$$



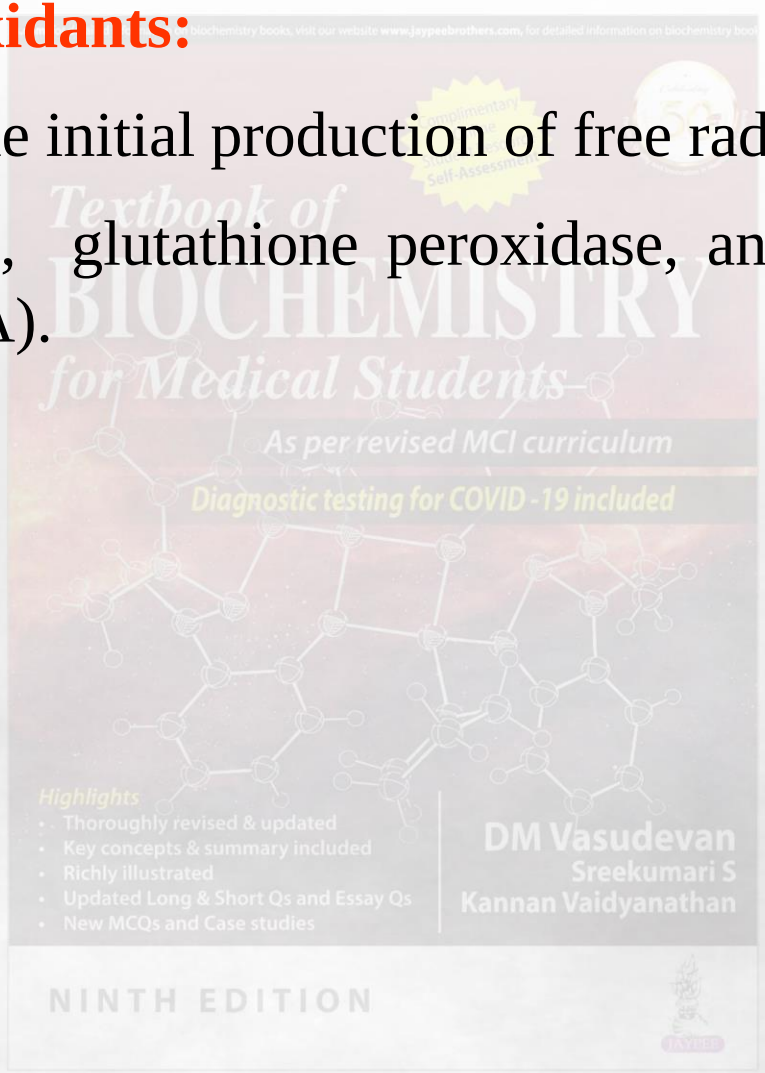


Role of antioxidants in lipid peroxidation.

Preventive anti-oxidants:

They will inhibit the initial production of free radicals.

They are catalase, glutathione peroxidase, and ethylene diamine tetra acetate (EDTA).



Chain breaking anti-oxidants:

They can inhibit propagative phase. They include superoxide dismutase, uric acid and vitamin E.

Alpha tocopherol (T-OH) (vitamin E) would intercept the peroxyl free radical and inactivate it before a PUFA can be attacked.

$$T\text{-}OH + ROO' \rightarrow TO' + ROOH$$

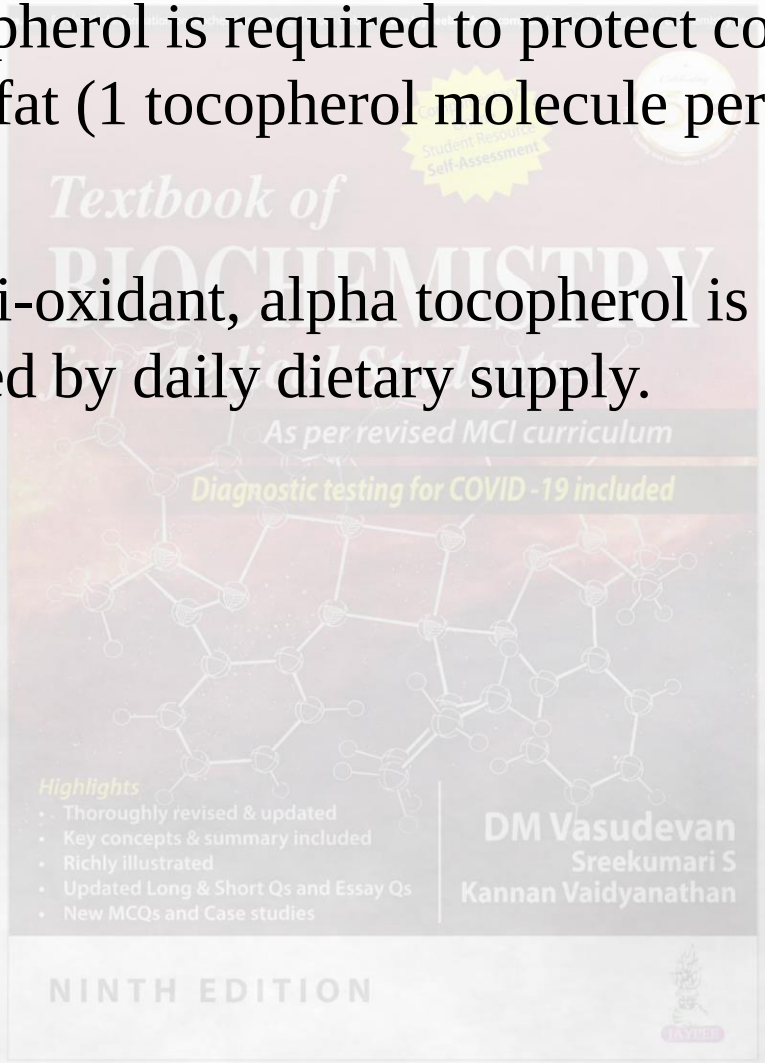
The tocoperoxyl radical can react with another peroxyl radical getting converted to inactive products.

$$TO' + ROO' \rightarrow \text{inactive products}$$

Vitamin E (Alpha tocopherol) acts as the **most effective** naturally occurring chain breaking **anti-oxidant** in tissues.

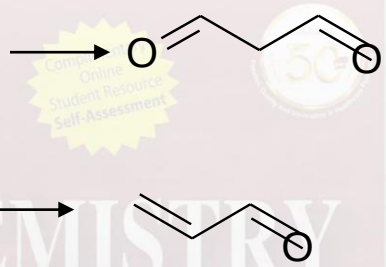
Only traces of tocopherol is required to protect considerable amounts of polyunsaturated fat (1 tocopherol molecule per 1000 lipid molecules).

While acting as anti-oxidant, alpha tocopherol is consumed. Hence it has to be replenished by daily dietary supply.



Biomarkers of Oxidative Stress

1. Lipid peroxidation generates
   Malondialdehyde (MDA)
   Hydroxy nonenal (HNE)
   Acrolein (2-propenal)



2. Protein cleavage, crosslinking inhibition of enzyme activity altered immunogenicity

3. 8-iso-PGF_2a or F_2-isoP It is most reliable assessment for oxidative stress status New risk factor for coronary disease

Biomarkers of Oxidative Stress

4. Glutathione

   Reduced glutathione (GSH)

   Glutathione disulphide(GSSG)

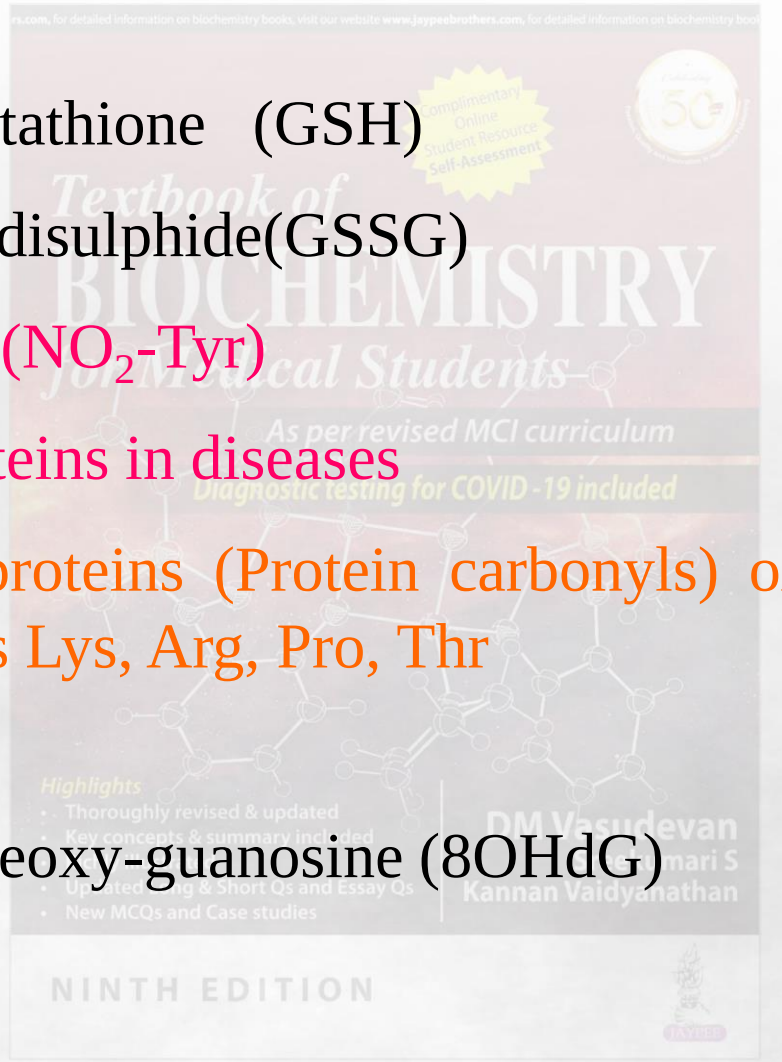
5. 3-nitrotyrosine (NO_2-Tyr)

   Nitrated proteins in diseases

6. Carbonylated proteins (Protein carbonyls) oxidation of amino acid side chains Lys, Arg, Pro, Thr

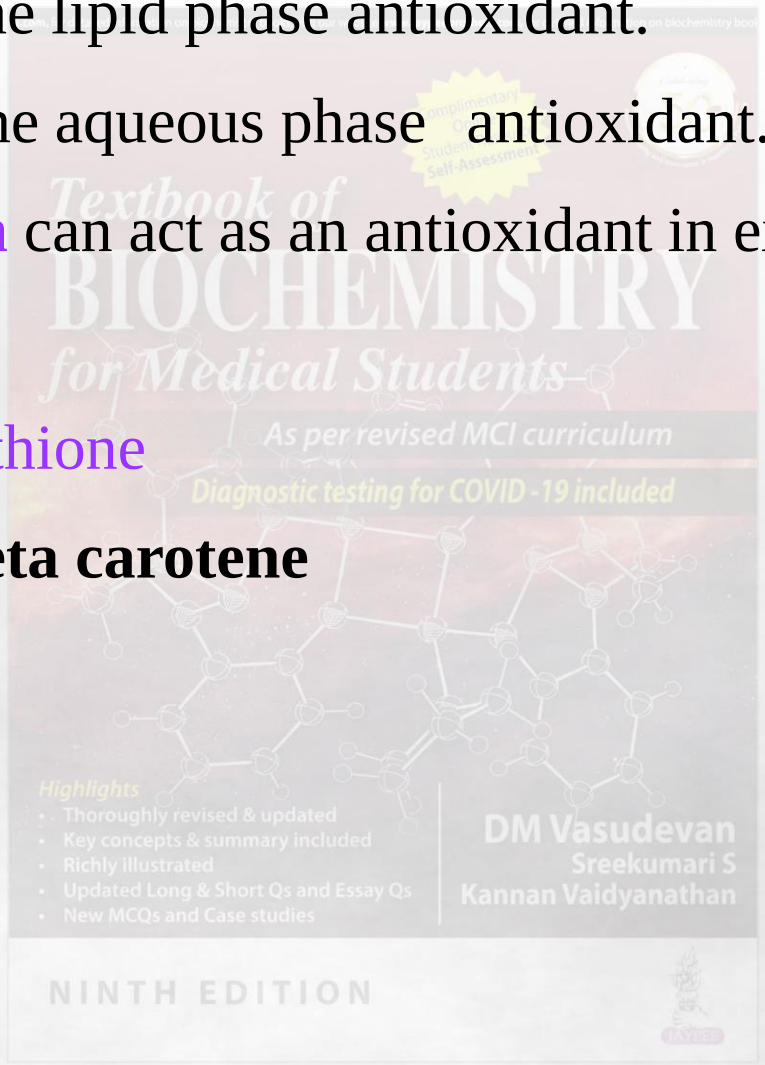
7. DNA breaks

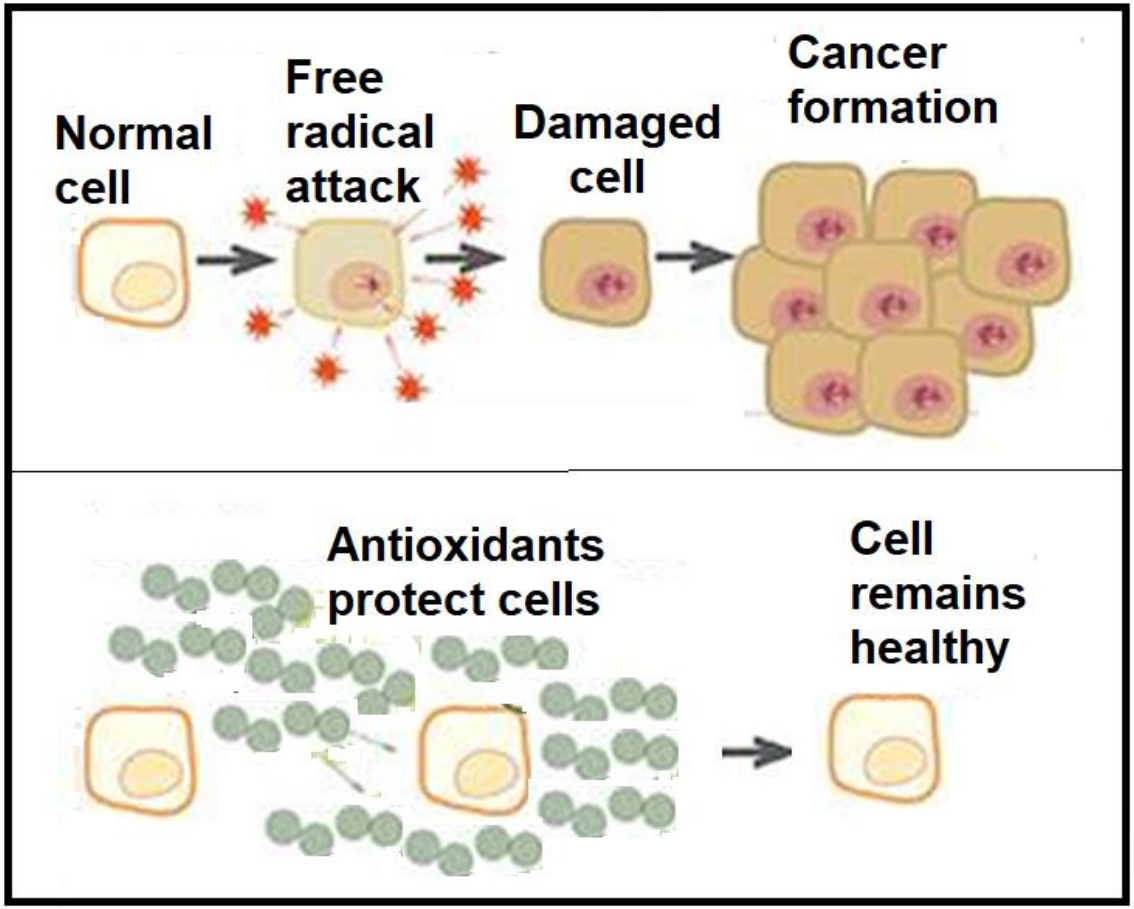
   8-hydroxy-deoxy-guanosine (8OHdG)



Anti-oxidants

1. **Vitamin E** is the lipid phase antioxidant.
2. **Vitamin C** is the aqueous phase antioxidant.
3. **Ceruloplasmin** can act as an antioxidant in extracellular fluid.
4. **Caffeine**
5. Cysteine, glutathione
6. **vitamin A , Beta carotene**





The upper part, free radicals attack normal cells to produce cancer. The lower part, theoretically, antioxidants can protect cells from transforming into cancer cells.

Phytonutrients

Phytonutrients are a group of plant products present in natural food that have antioxidant activity. The role of phytonutrients in facilitating the inactivation and removal of unwanted compounds from the body is being increasingly recognized. Some of the well-known phytonutrients are cruciferous **vegetables** which include cabbage, cauliflower, broccoli, brussels sprouts etc. They help in the detoxification of procarcinogens like polycyclic hydrocarbons, heterocyclic aromatic amines and amides and polychlorinated biphenyls. **Resveratrol**, present in grapes and berries have antioxidant action and helps in the detoxification of procarcinogens. **Curcumin** found in turmeric is well known for its modulatory effect on detoxification in a dose dependent manner.

Polyphenols

Consumption of polyphenol rich fruits, vegetables, and beverages is beneficial to human health. Dietary polyphenols represent a wide variety of compounds that occur in fruits, vegetables, wine, tea and chocolate. They contain flavones, isoflavones, flavonols, catechins and phenolic acids. They act as agents having antioxidant, antiapoptotic, anti-aging, anticarcinogenic, anti-inflammatory, and anti-atherosclerotic effect. They are protective against cardiovascular diseases. Work from the author's lab suggests that grape polyphenols can prevent brain damage due to alcohol. Oral administration of grape polyphenol extract ameliorates cerebral ischemia induced neuronal damages. Grape seed procyanidins prevent low grade inflammation by modulating cytokine expression in rats.