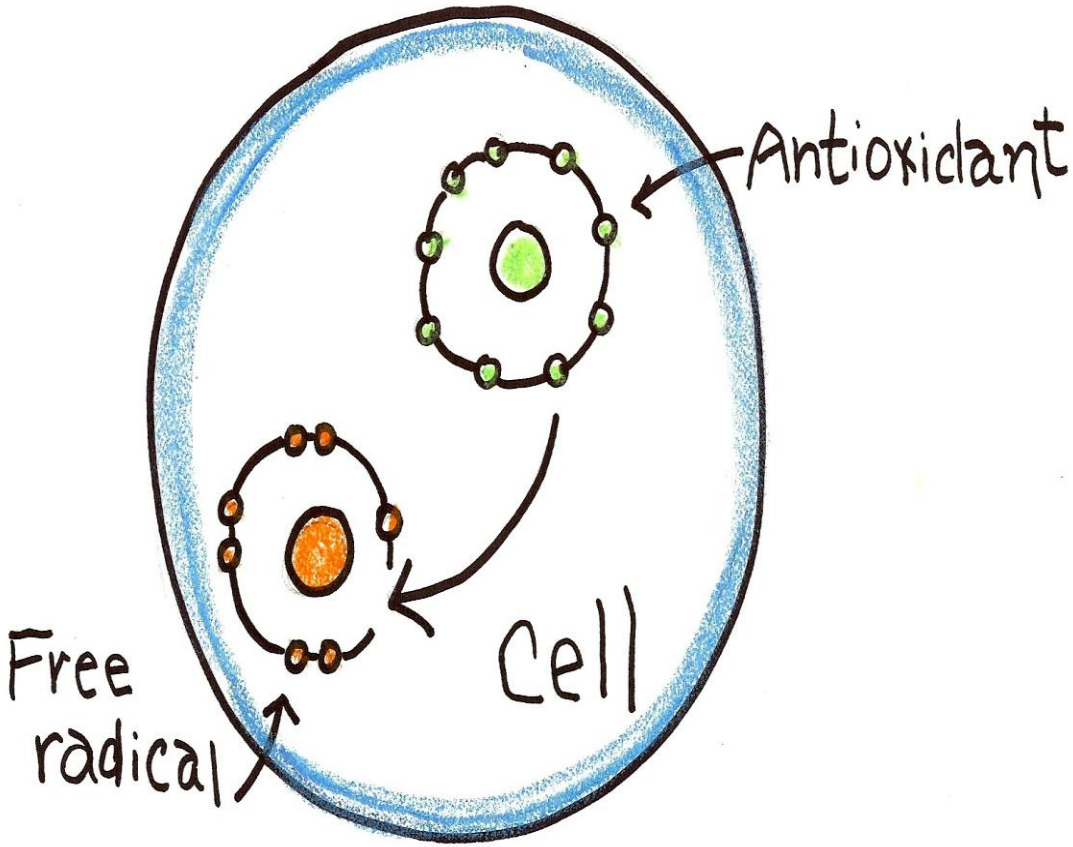
Role of oxygen in the process of oxidation in cells



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Part 1 of the lecture.

1. General characteristics of oxidation processes.
2. Oxidase and peroxidase types of oxidation: schemes, enzymes, biological role.
3. Dioxygenase and monooxygenase types of oxidation: schemes, enzymes, biological role.
4. Microsomal oxidation: scheme, cytochrome P 450 , biological role.
5. Reactive oxygen species: their tissue-damaging effects.
6. Antioxidant systems, role of enzymes and non-enzymatic antioxidants.

Main definitions

- **Oxidation reaction is any process involving the loss of electrons by a molecule, atom or ion, followed by increase in oxidation state of the molecule.**

  Ex: $Fe^{2+} \longrightarrow Fe^{3+} + \bar{e}$

- **In oxidation two or more substances can interact (including oxygen).**
- **The substance that loses electrons is classified as A REDUCTANT or ELECTRON DONOR**

Main definitions

- **Reduction is a gain of electrons by a molecule, atom or ion, followed by decrease in oxidation state of the molecule.**

- **Ex:** $Fe^{3+} + \bar{e} \longrightarrow Fe^{2+}$

- **In other words, reduction is the addition of at least one electron when substances come into contact with each other**
- **The substance that gains electrons is classified as AN OXIDIZING AGENT or ELECTRON ACCEPTOR**

Biological oxidation

- **All oxidation-reduction reactions in the body, catalyzed by the enzymes called**

OXIDOREDUCTASES

(EC: 1st Class)

2 ways how to oxidize something in the cell

I. To remove 2 hydrogen atoms ($2H^+$ and $2\bar{e}$) or only $2\bar{e}$ from a substrate; (dehydrogenases, oxidases)

$$S\bullet H_2 + B = S + B\bullet H_2$$

II. To add oxygen (1 or 2 atoms) to a substrate (oxygenases).

$$S + O_2 = S\bullet O_2$$

Enzymes involved in oxidation and reduction are called OXIDOREDUCTASES and are classified into four groups:

1. Oxidases
2. Dehydrogenases
3. Hydroperoxidases
4. Oxygenases (dioxygenases, monooxygenases)

Types of Oxidation

I. **Oxidase** type
II. **Peroxidation** type
III. **Dioxygenase** type
IV. **Monooxygenase** type

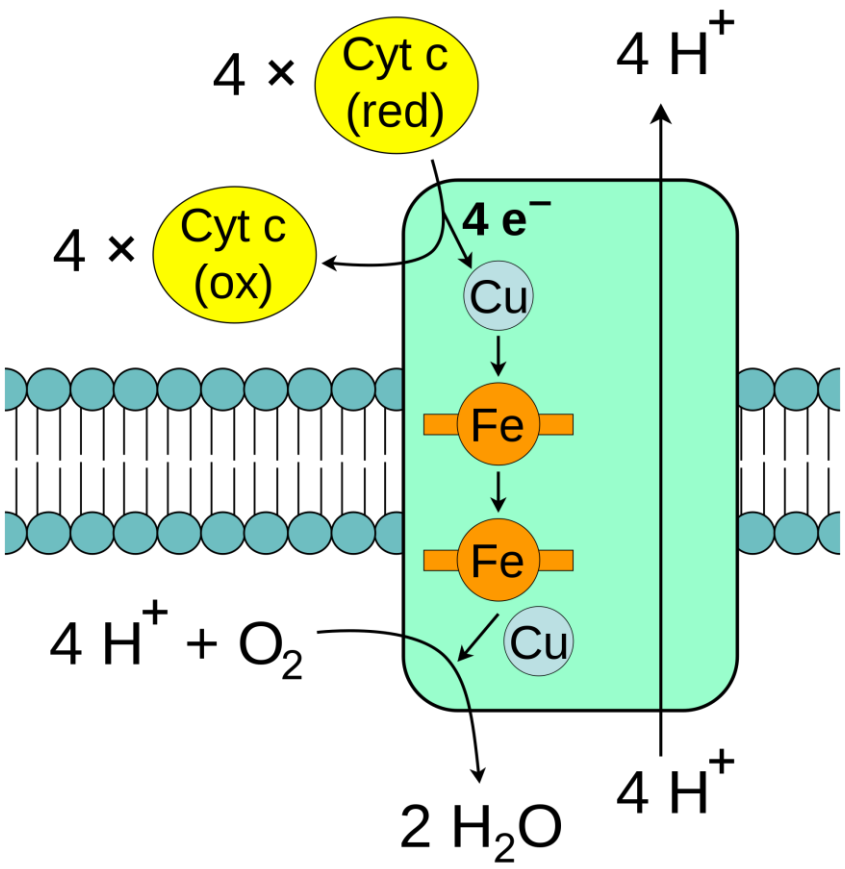
I. Oxidase type

- **OXIDASES catalyze the removal of hydrogen from a substrate using oxygen as a hydrogen acceptor**

$$S\text{-}H_2 + \tfrac{1}{2}O_2 = S + H_2O$$

- **This type of oxidation takes place in the Electron Transport Chain localized on the inner mitochondrial membrane.**
- **The common enzyme is cytochrome-C-oxidase**

Cytochrome-C-oxidase, or cytochrom aa3



- is the last enzyme in the respiratory electron transport chain found in the mitochondrion of eukaryotes.

$$4H^{+}+4\bar{e}+O_2= 2H_20$$

II.Peroxidation type

- In the body these reactions involve donation of hydrogen atoms and reduction of O_2 to hydrogen peroxide (H_2O_2)

- Reactions are catalysed by Flavoproteins *(the enzymes containing FAD or FMN as prosthetic groups)*

$$2\ SH_2 + O_2 + FMN\ (\text{or FAD}) = 2S + FMN{\bullet}H_2\ (\text{or FAD}{\bullet}H_2) + H_2O_2$$

- **Hydrogen peroxide is formed in human and animal organisms as a short-lived product in biochemical processes and is toxic to cells.**

- **Its toxicity is due to oxidation of proteins, membrane lipids and DNA by the peroxide ions.**

- **Hydrogen peroxide is one of the ROS.**

- **Enzymes that degrade hydrogen peroxide are called PEROXIDASES**

Role of peroxidation reactions

- **Peroxidases are located in peroxisomes, that are organelles found in virtually all eukaryotic cells.**

- **Catabolism of very long and branched chain fatty acids, D-amino acids, polyamines, purines, biogenic amines, aldehydes.**

- **“Respiratory burst” in phagocytes.**

III. Dioxygenase type

- **DIOXYGENASES** are oxidoreductase enzymes that incorporate **both atoms of molecular oxygen** into a substrate

$$S + O_2 = SO_2$$

Dioxygenases take part in:

1. **Catabolism of aromatic and aliphatic compounds by cleaving their aromatic rings.**

   Ex.: catabolism of steroid hormones

2. **Synthesis of vitamin PP (niacin) from tryptophan.**
3. **catabolism of phenylalanine and tyrosine (homogentisate 1,2-dioxygenase)**

VI. Monooxygenase type

- **Monooxygenases (or hydroxylases) are enzymes that incorporate one atom O into a substrate forming -OH group.**
- **In this reaction, the two atoms of oxygen are reduced to one hydroxyl group and one H_2O molecule by the concomitant oxidation of a co-substrate ZH_2.**

$$R\text{-}H + ZH_2 + O_2 = R\text{-}OH + Z + H_2O$$

Microsomal oxidation

- occurs in fragments of endoplasmic reticulum (microsomes).
- in MO, one atom of molecular O is incorporated into a substrate liable to oxidation. The other atom of molecular O accepts two hydrogen atoms to form water.

$$R\text{-}H + NADPH(H^+) + O_2 = R\text{-}OH + NADP^+ + H_2O$$

**R-H is a non-polar hydrophobic substrate*

**R-OH is a polar hydrophilic substrate*

Microsomal oxidation involves several components:

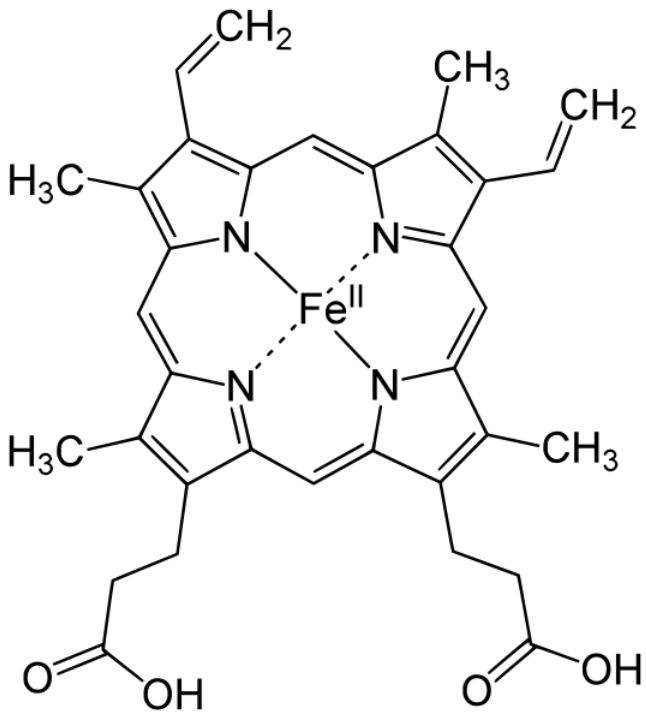
1. **Reduced NADPH(H^+) as a <u>cosubstrate</u>** ***(donor of 2$\bar{e}$ and 2H^+);***

2. **NADPH(H^+)-cytochrome P_{450}-reductase** ***(flavoprotein containing FAD as prosthetic group)***

3. **Cytochrome P_{450} (Fe^{3+})**

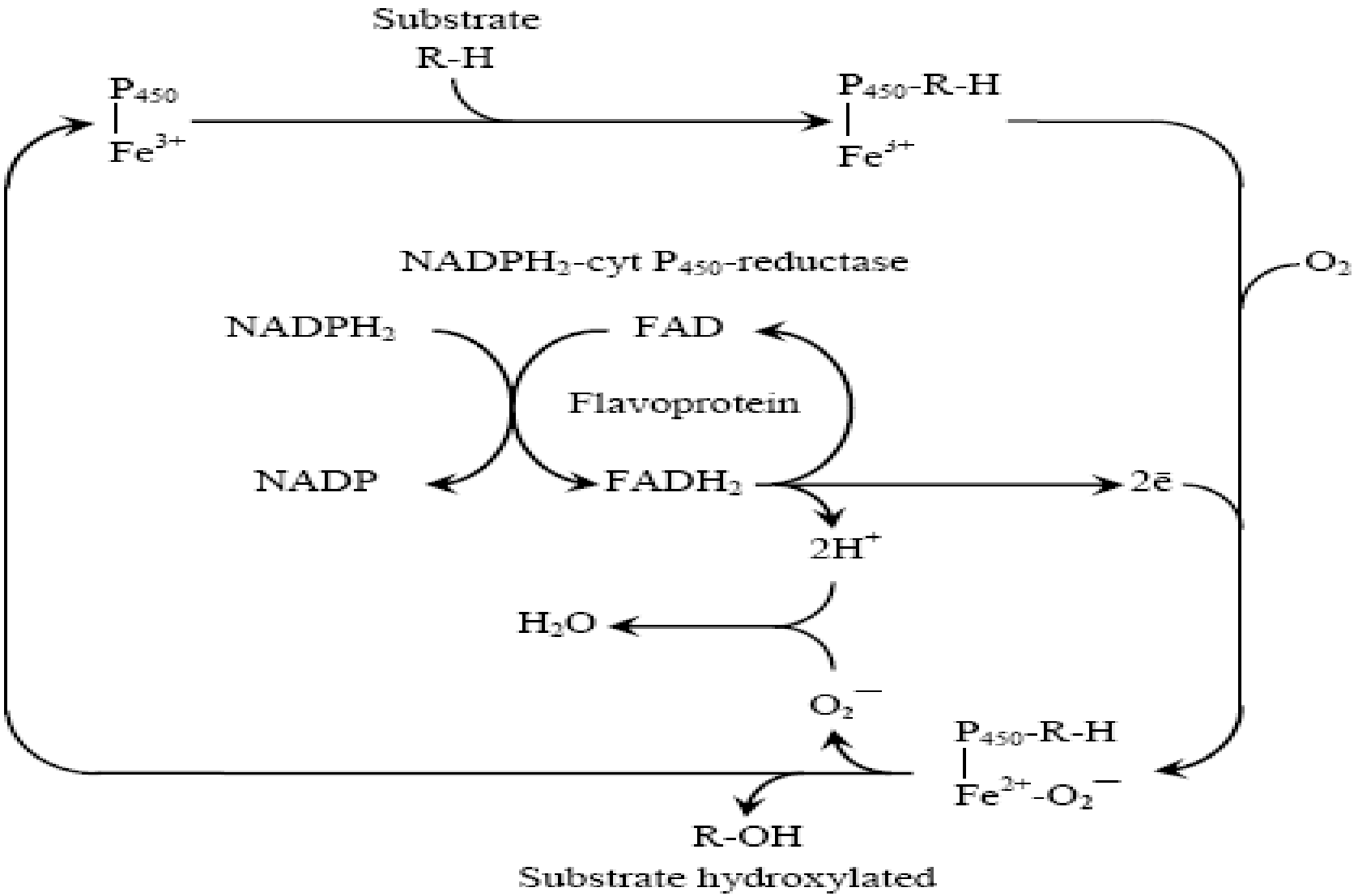
Cytochromes P450 (CYPs)

- are **hemoproteins** or conjugated proteins containing heme as a cofactor, and Fe^{3+} or Fe^{2+}
- They are the terminal oxidase enzymes in electron transfer chain, broadly categorized as **P450-containing systems.**
- The term **"P450"** is derived from the spectrophotometric peak at the wavelenth of the absorption maximum of the enzyme (450 nm) when it is in the reduced state.



Scheme of Microsomal Oxidation

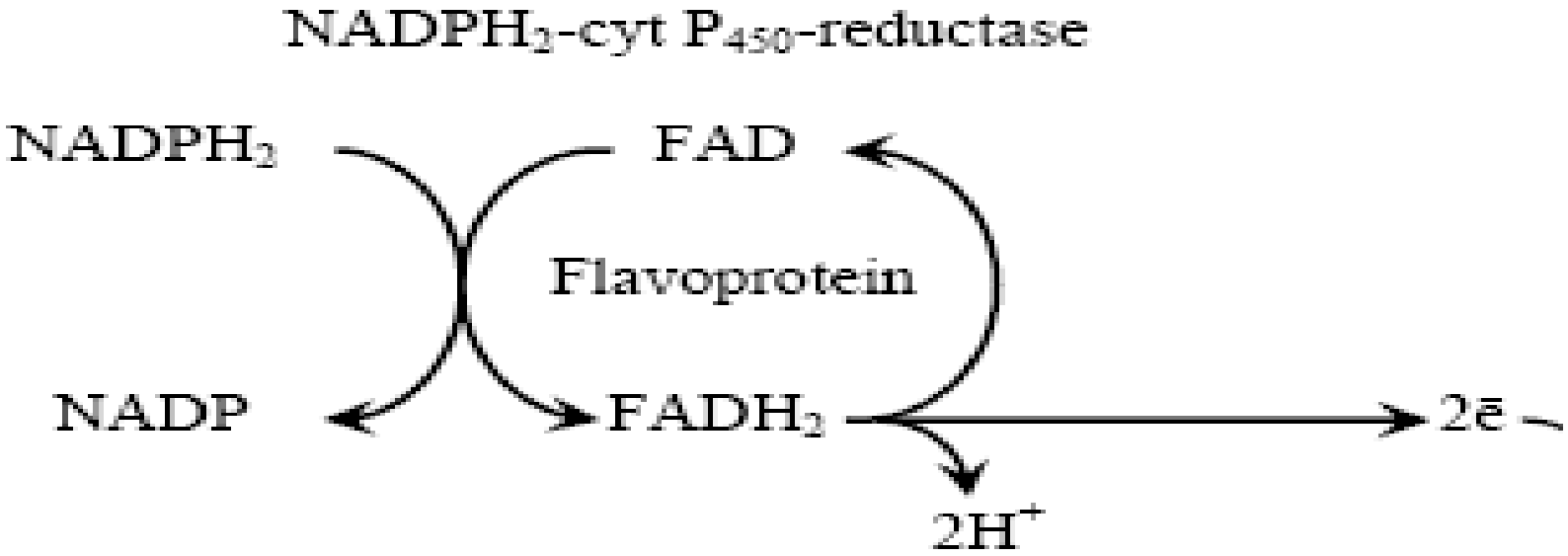
(Biochemistry: manual.. /ed. Petushok NE, et al – 2014. - P. 92.)



Steps of Microsomal Oxidation

I. **$NADPH_2$-cyt P_{450}-reductase takes up 2 hydrogens from $NADPH_2$ and divides them to $2H^+$ and *$2\bar{e}$.***

- **$2H^+$ are released to form H_20**
- ***$2\bar{e}$ are transferred to cyt P_{450}***



Steps of Microsomal Oxidation (cont.-ed)

II. Hydrophobic non-polar substrate RH forms complex with cyt P_{450}

$cyt\ P_{450}(Fe^{3+})\text{-}RH$

III. This complex is attacked with O_2 and $2\bar{e}$ from the I step to form peroxicomplex

$cyt\ P_{450}\ (Fe^{2+})\text{-}RH\text{-}O_2^-$

Steps of Microsomal Oxidation (cont.-ed)

IV. The complex [cyt P_{450} (Fe^{2+})-RH-O_2^-] release R-OH by incorporating of one of the oxygen atoms into the RH.

V. One $\bar{e}$ *(from* Fe^{2+}) goes to O^- forming O^{2-}

VI. Resulted O^{2-} attaches $2H^+$ from the I step to form one molecule of H_2O.

VII. Regeneration of cytochrom P_{450}(Fe^{3+}).

Biological role of microsomal oxidation

- In humans CYPs metabolize thousands of endogenous and exogenous chemicals.
- Synthesis of cholesterol, bile acids, prostaglandins
- Metabolism of steroid hormones (sex hormones)
- Metabolism (detoxification) of toxic non-polar substances (XENOBIOTICS) in the liver.
- Synthesis of carcinogens (benzopyrene to hydroxybenzopyrene).

Reactive Oxygen Species (ROS)

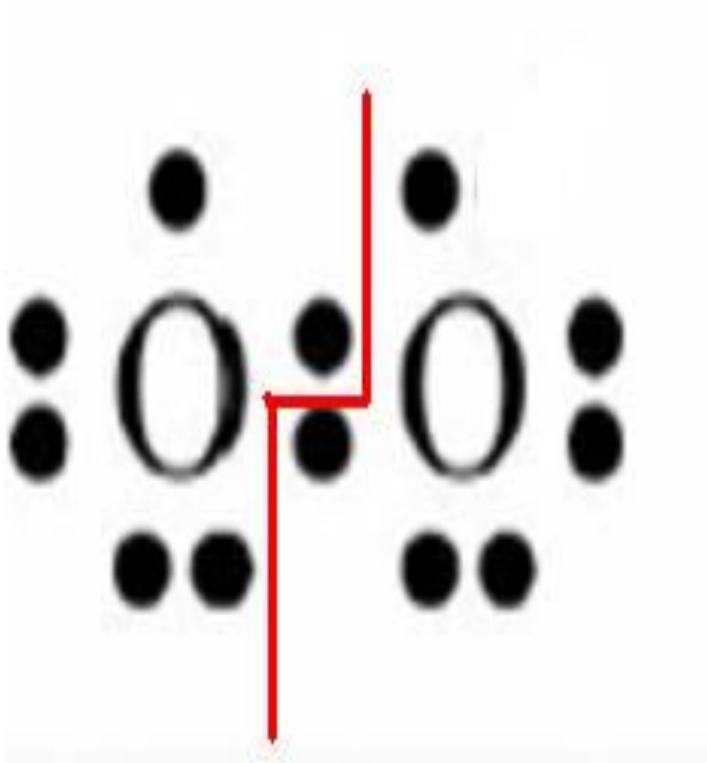
Free radical

- is an atom, molecule, or ion with unpaired valence electrons in outer shell configuration, and therefore may be seen as having one or more "dagling" covalent bonds

- Free radical is denoted by superscript dot

$$R^{\bullet}$$

- Electrons in an atom or molecule occupy orbits
- The outer orbital of the oxygen atom contains 6 electrons: (4 paired electrons, spinning in opposite directions, and 2 unpaired electrons)
- The chemical covalent bond in molecular oxygen consists of a pair of electrons, each component of the bond donating one electron.



O_2

Reactive oxygen species (ROS)

- Aerobic organisms produce a number of reactive free radicals from oxygen continuously in cells during respiration, metabolism and phagocytosis.
- Majority of Reactive Oxygen Species (ROS) are produced in Mitochondria
- Non-mitochondrial generation of ROS:
  - Hydrogen peroxide in peroxisomes
  - Lipid radicals (in cell membranes, EPR)

ROS are mainly harmful byproducts of partial reduction of oxygen in the mitochondrial Electron Transport Chain.

- In Complex IV up to 98% of O_2 is converted to harmless water when all of the electrons are transferred to it.

$$2H^{+}+2\bar{e}+ \tfrac{1}{2}O_2 = H_20$$

- BUT ~ 1 to 2% oxygen can gain one or two electrons to produce superoxide radicals

$$O_2 + \bar{e} = \bullet O_2^{-} + \bar{e} = \bullet O_2^{2-}$$

Members of ROS group

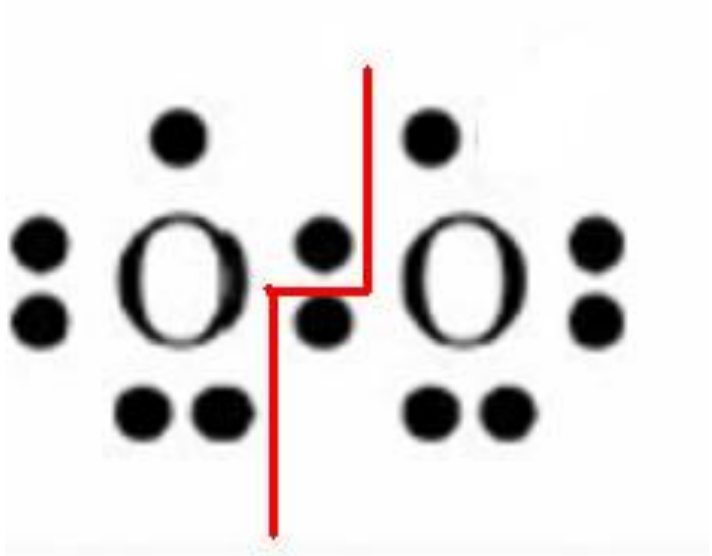
- Superoxide anion radical - $^{\bullet}O_2^-$
- Hydrogen peroxide - H_2O_2
- Hydroxyl radical - $^{\bullet}OH$
- Perhydroxyl radical - $^{\bullet}O_2H$
- Lipid peroxide radical - $ROO^{\bullet}$
- Singlet oxygen - $^1O_2$
- Nitric oxide - $NO^{\bullet}$

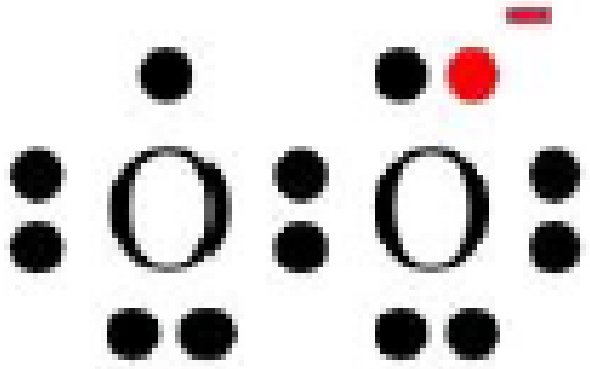
Molecular oxygen vs. Superoxide anion

O_2

$\bullet O_2^-$

- **Dot-O-two-minus**

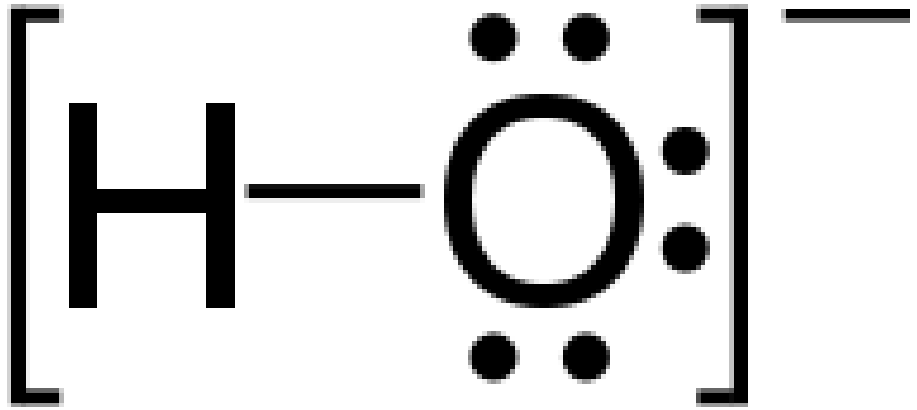




The 6 of outer shell electrons of each O atom are shown in black. One electron pair is shared in middle, the unpaired electron is labeled by a red dot. The additional electron (red) conferring a negative charge.

Hydroxyl anion vs. Hydroxyl radical

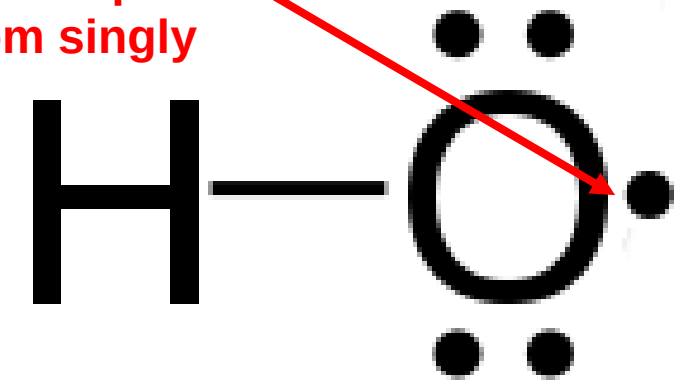
Hydroxide ion



OH^-

Unpaired electron occupies outer orbital of atom singly

Hydroxyl radical



$\bullet OH$

Properties of ROS

- **Extreme reactivity**
- **Short life span (10^{-9} to 10^{-12} sec)**
- **Generation of new ROS by chain reaction**
- **Damage to various tissues.**

Damage produced by ROS

- Almost all biological macromolecules are damaged by the free radicals
- Oxidation of polyunsaturated fatty acids (PUFA) in plasma membrane leads to loss of membrane functions (Lipid peroxidation)
- Oxidation of sulfhydryl groups in enzymes [-S-S-], modification of amino acids, loss of function and fragmentation of proteins.

Damage produced by ROS *(cont-ed)*

- **Polysaccharides undergo degradation**
- **DNA is damaged by strand breaks, depletions of nucleotides (mutations in the cell genome)**
- **The DNA damage may directly cause inhibition of protein and enzyme synthesis and indirectly cause cell death or mutation and carcinogenesis.**

"Respiratory burst" in phagocytes.

- **is the rapid release of H_2O_2 and other ROS (superoxide radical) from different types of immune cells (e.g., neutrophils, monocytes), as they come into contact with different bacteria or fungi.**

- **Respiratory burst plays an important role in the immune system. It is a crucial reaction that occurs in phagocytes to degrade internalized particles and bacteria.**

Environmental (external) factors in formation of free radicals

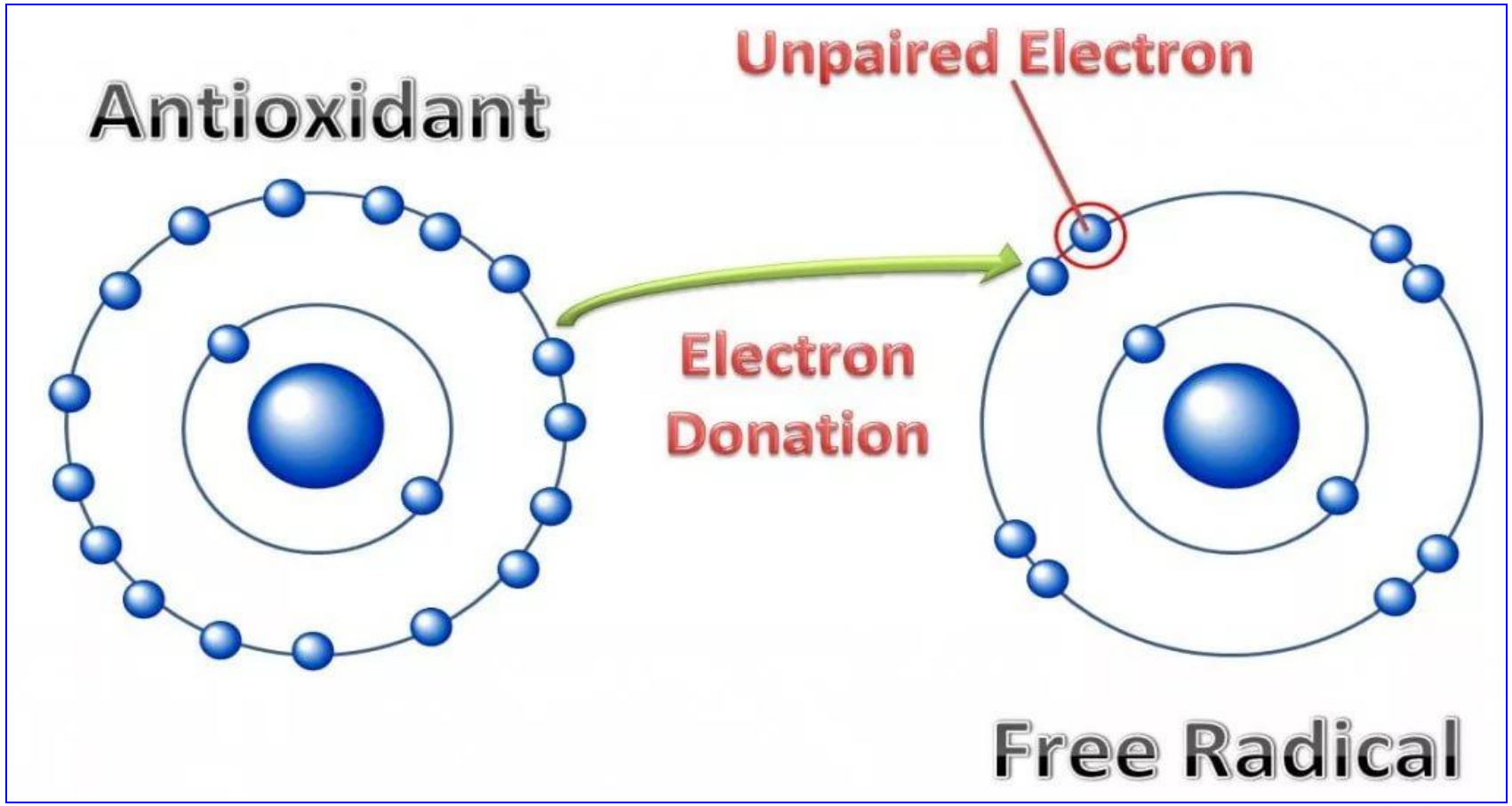
ROS can be induced through external sources such as

- **Ultraviolet rays from the sun**
- **Ionizing radiation**
- **Atmospheric and chemical pollution**
- **Alcohol abuse**
- **Cigarette smoke**
- **Unhealthy (poor) food.**
- **Stress.**

Internal sources of ROS

- Formation of ROS occurs during a variety of pathological responses, including
  - Inflammation
  - Aging
  - Respiratory diseases
  - Skin diseases
  - Atherosclerosis and myocardial infarction
  - Shock related injury
  - Carcinogenesis
  - Apoptosis.

Antioxidant systems



Our cells regulate ROS in one of two manners:

- **Complex IV in the ETC holds the oxygen and its derivatives very tightly; it only releases it upon the conversion into safe molecule of water.**

- **For the few ROS that are released, cells use ANTIOXIDANTS, that are**

  protective substances and enzymes to locate and convert ROS into safe products

Antioxidants can be…….

I. Enzymatic or non-enzymatic.

II. Preventive or chain breaking.

- Preventive antioxidants inhibit the initial production of free radicals. They are catalase, glutathione peroxidase.
- Chain breaking antioxidants - once the peroxyl radicals are generated, the chain breaking antioxidants can inhibit the propagation phase. They include superoxide dismutase, uric acid, and vitamin E.

Enzymatic antioxidants

1. Superoxide dismutase
2. Catalase
3. Glutathione peroxidase
4. Glutathione reductase

Superoxide dismutases (SOD)

- Class of enzymes, that catalyzed dismutation of two superoxide anions into molecular oxygen and hydrogen peroxide.

$$\bullet O_2^- + \bullet O_2^- + 2H^+ = H_2O_2 + O_2$$

3 isoenzymes of SOD are described

SOD1 – in the cytoplasm (copper-zinc dependent)

SOD2- in the mitochondria (manganese dependent)

SOD3- in the extracellur space

Catalase

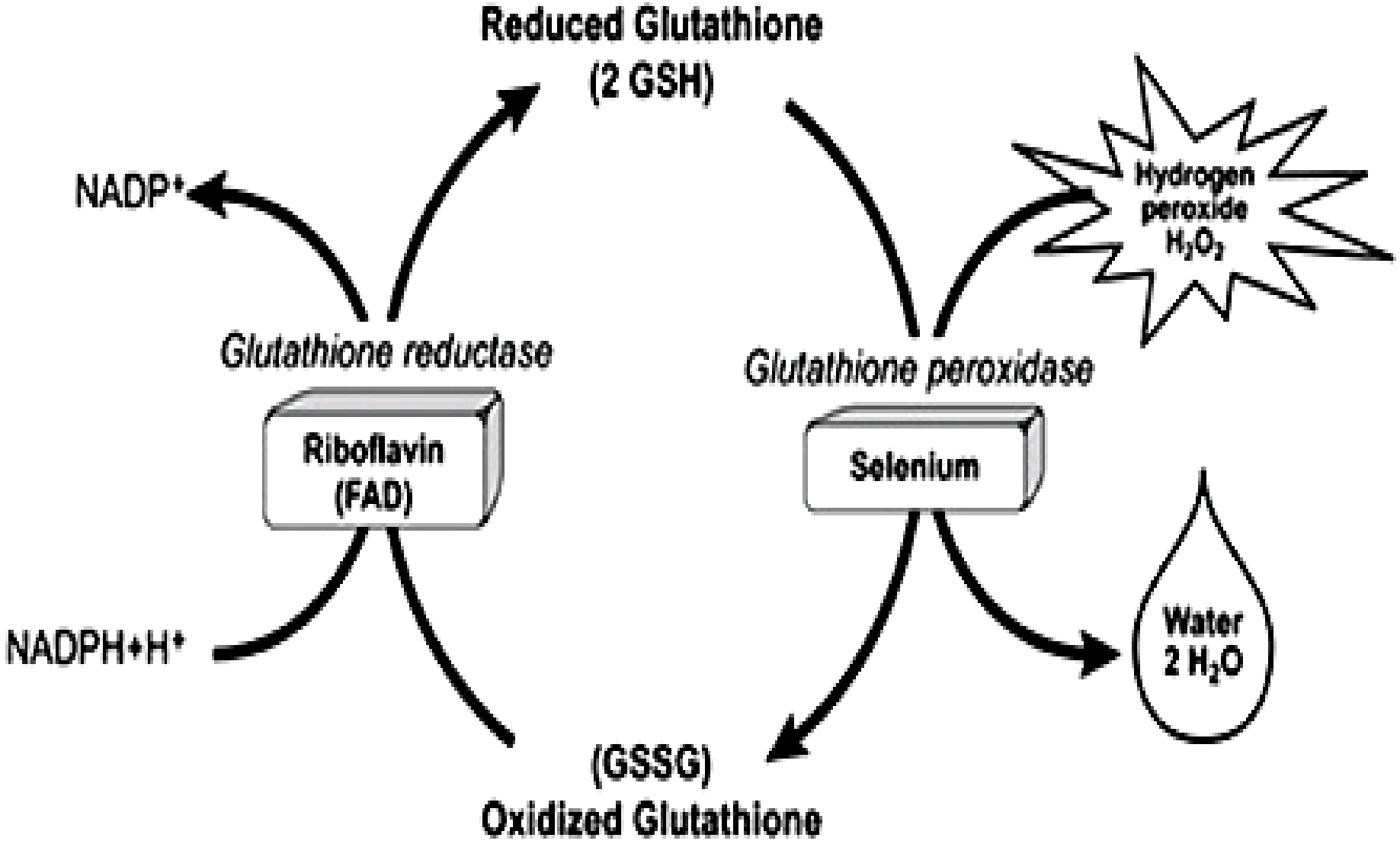
- This is a heme protein.
- Catalase is concentrated in peroxisomes located next to mitochondria
- The enzyme locates and converts 2 molecules of hydrogen peroxide into two water molecules and molecular oxygen

$$2H_2O_2 = 2H_2O + O_2$$

"Glutathione system"

- consists of
  - NADPH
  - reduced glutathione (GSH),
  - oxidized glutathione (GSSG),
  - glutathione peroxidase,
  - glutathione reductase,
- Glutathione peroxidase reduces lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water.
- Glutathione reductase restores GSH from GSSG

Glutathione Oxidation Reduction (Redox) Cycle



Water soluble non-enzymatic antioxidants

- Glutathione
- Ascorbic acid (vit. C)
- Uric acid
- Thiols (taurine, cysteine)
- Coenzyme A

- Metals binding proteins:
  - Ceruloplasmin
  - Ferritin
  - Lactoferrin
  - Metallotheinein
  - Transferrin
  - Hemoglobin
  - Myoglobin

Lipid soluble non-enzymatic antioxidants

- Vitamin E (alpha-tocopherol)
- Provitamin A (beta-carotene)
- Vitamin K
- Coenzyme Q

Part 2 of the lecture.

1. Metabolism and metabolic pathways. Interrelations between anabolism and catabolism.

2. The specific and common pathways of catabolism.

3. Experimental study of metabolism, the use of radioisotope tracers.

Metabolism

- Is the set of life-sustaining chemical and physical transformations within the cells of living organisms.

- represents all the enzymatic reactions and physical processes in the body.

The main purposes of metabolism

1. **Uptake of substances from the outside *(nutrition, respiration).***
2. **Conversion of food/fuel to energy to run cellular processes.**
3. **Conversion of food/fuel to building blocks for proteins, lipids, nucleic acids, and some carbohydrates.**
4. **Excretion of end products from the body.**

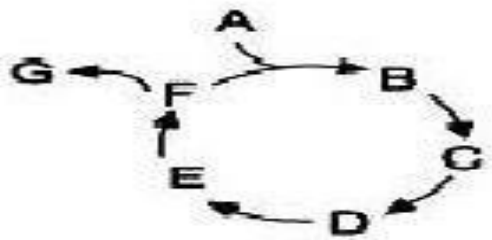
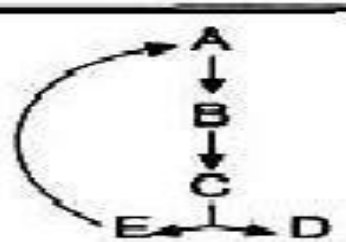
Metabolic pathways :

- are linked series of enzymatic reactions that begin with a particular reactant and terminate with an end product.

- The reactants, products, and intermediate substrates of enzymatic reactions are known as **metabolites** or **intermediates**.

- The product of one enzymatic reaction acts as the substrate for the next reaction in the pathway.

4 types of metabolic pathways

Scheme	Types of metabolic pathways	Examples
A → B → C → D → E	Linear	Glycolysis
A → B → C → D → E; C → F → G	Branched	Synthesis of purine nucleotides
	Cyclic	Tricarboxylic acid cycle
		Urea cycle
	Spiral	Beta-oxidation of fatty acids

1. **Linear metabolic pathway represents the series of reactions when substrate A is converted by the enzyme E_1 to produce product B. In turn B is a substrate for E_2 to produce C. This process continues until the final product P. (E.g.: *Glycolysis*).**

2. **In cyclic metabolic pathway the starting molecule is regenerated during one run of the cycle *(E.g.: Tricarboxylic acid cycle, urea cycle).***

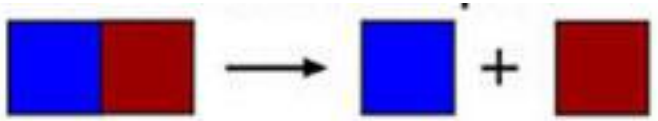
3. **In branched metabolic pathway the intermediate substrate C can be converted either into the substrate D by the enzyme E3, or the substrate F by the enzyme E4. Each of these intermediates serves as the beginning of new pathway *(E.g.: Synthesis of purine nucleotides).***

4. **In spiral metabolic pathway the enzyme E3 cleaves substance C into D and F. After that intermediate F serves as the beginning of new cycle *(E.g.: β-oxidation of fatty acids).***

CATABOLIC PATHWAY (CATABOLISM)

breaking down of complex molecules to simpler compounds with release of energy

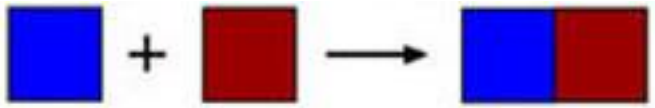
EX: digestive enzymes break down food



ANABOLIC PATHWAY (ANABOLISM)

synthesis of complex molecules from simpler components with use of energy.

EX: linking amino acids to form proteins



Interrelations between catabolism and anabolism

- **Catabolism includes breaking down and oxidizing food molecules with release of energy.**

- **Anabolism is the set of constructive metabolic processes where the energy released by catabolism is used to synthesize complex molecules.**

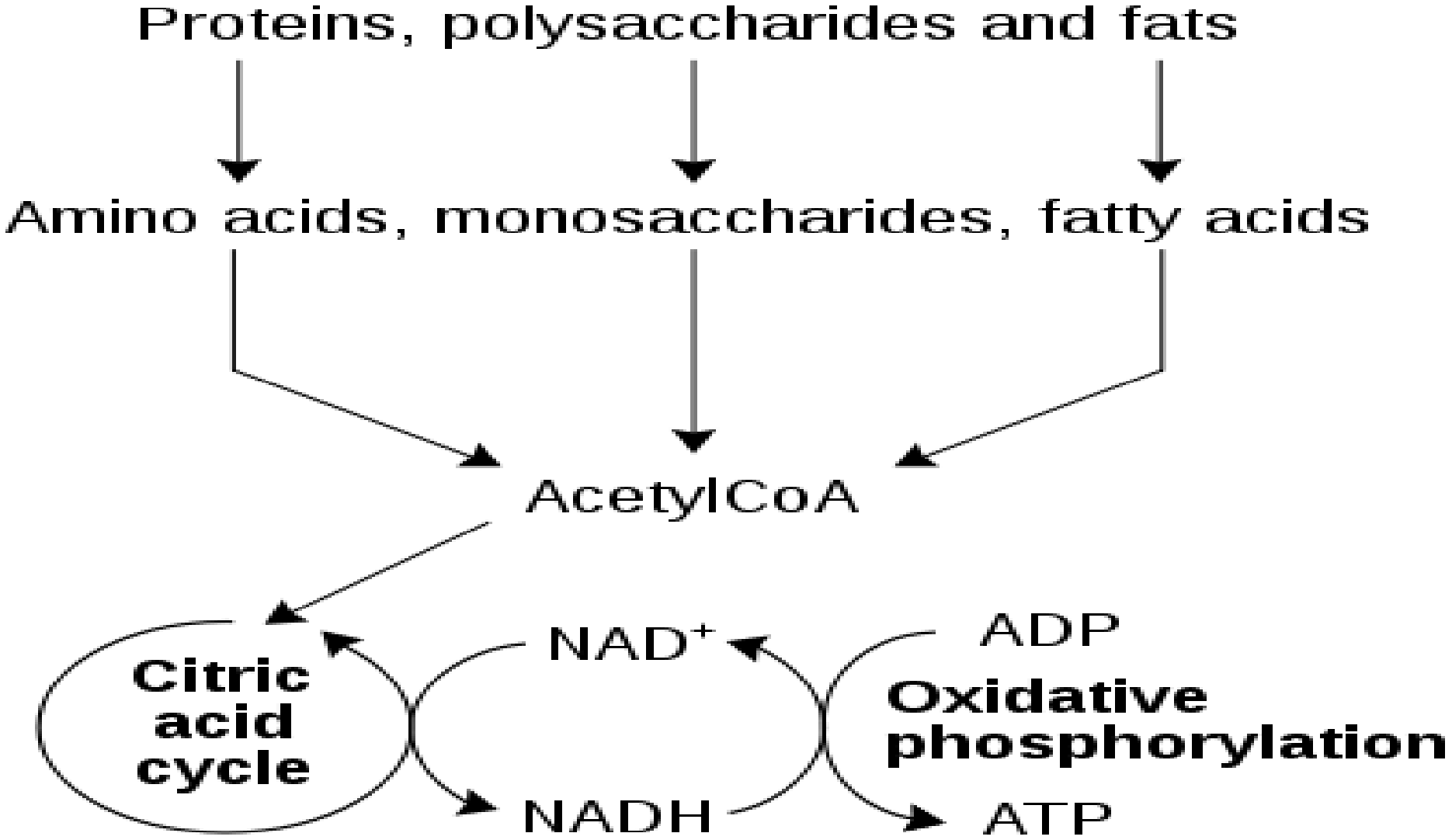
- **Anabolism involves three basic stages.**
  - **the production of precursors such as amino acids, monosaccharides, and nucleotides,**
  - **Activation of precursor molecules into reactive forms using energy from ATP**
  - **the assembly of these precursors into complex molecules such as proteins, polysaccharides, lipids, and nucleic acids.**

The specific and common pathways for the catabolism of dietary proteins, carbohydrates and lipids

I stage - specific

II stage - specific

III stage - common



I. **Stage I (specific) - digestion.** Large molecules are digested into relatively small, simple ones components in the digestive tract or inside the cells.

II. **Stage II (specific) – production of acetyl CoA.** The smaller molecules from food are taken up by cells and degraded to even simpler units, primarily two-carbon acetyl portion of acetyl coenzyme A (acetyl CoA).

III. **Stage III (common catabolic pathway).**

It consists of the citric acid cycle followed by the electron transport chain (ETC) and oxidative phosphorylation (OPh). Acetyl CoA undergoes utilization in the citric acid cycle to produce two molecules of CO_2, and reduced cofactors – NADH and $FADH_2$. After that NADH and $FADH_2$ produced in the citric acid cycle are oxidized in the ETC. The energy liberated from their oxidation appears in the form of energy-rich molecules of ATP in the process called OPh.

Dietary molecules	End products of metabolism
Carbohydrates	CO_2, H_2O
Lipids	CO_2, H_2O
Proteins, amino acids	CO_2, H_2O, NH_3, urea, creatinine, indican
Nucleic acids, nitrogenous bases	H_2O, NH_3, uric acid

EXPERIMENTAL STUDY OF METABOLISM

- **Metabolic pathways can be studied at different levels of organization:**

1. **Whole organism.**
2. **Isolated organs.**
3. **Organ slices.**
4. **Cell cultures (including microorganisms).**
5. **Homogenates of tissues.**
6. **Separated subcellular organelles.**
7. **Purified molecules of enzymes to study their kinetics and responses to inhibitors, other purified molecules, and their fragments.**

- **Homogenate is a homogenized sample is equal in composition. Homogenate usually lacks cell structures and contains mixture of cell organelles and membrane fragments.**
- **Homogenization is a process whereby a biological sample is brought to a state such that all fractions of the sample are equal in composition. Homogenizer is equipment used for homogenization.**
- **Subcellular fractionation is the process used to separate various cellular components *(nuclei, mitochondria, microsomes)***

In vivo studies ***Latin "within the living"***	**Living organism, including animal testing and clinical trials *(development of new drugs, new surgical procedures)***
In vitro studies ***Latin "within the glass"***	**Isolated organ**
	Organ slices
	Cell cultures
	Homogenates of tissues
	Separated subcellular fractions
	Purified molecules of proteins, etc.

Methods used for the study of metabolic pathways

- Homogenization,
- Dialysis,
- Electrophoresis,
- Determination of the enzymatic activity using colorimetric (spectrophotometric) techniques.
- Chromatography
- Gas Chromatography-Mass Spectrometry
- In vivo nucleic magnetic resonance spectroscopy (NMR)
- Metabolomics.

Use of isotope tracers in medicine and research

(^{3}H , ^{32}P, ^{13}C,^{14}C , ^{35}S, ^{131}I)

- Radiopharmaceuticals are group of drugs that are used as diagnostic and therapeutic agents.
- Allow doctors and researchers to obtain biochemical information about the tissue of the human body in a non-invasive way
- Allow to study conversions of different substances in metabolic pathways.

Ex.: use of ^{3}H , ^{32}P, and ^{14}C for labeling of glucose and amino acids to study their metabolism.