Biological oxidation

2 nd term

Biochemistry II

3 rd stage

By

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(Objective)

1. To understand what we meant by oxidation & reduction.

2. To know the classification of enzymes involved in oxidation & reduction in human.

3. Describe the action of each group of enzymes involved in oxidation & reduction.

Biological oxidation is the oxidation of energy-rich chemical substances (carbohydrates, lipids, proteins) that occur in biological systems to produce energy.

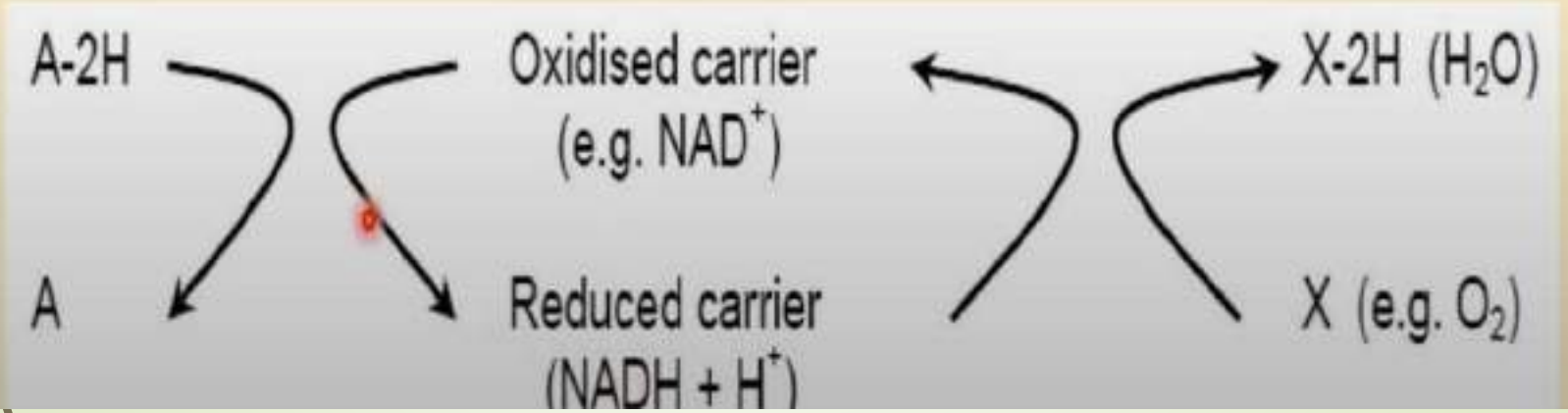
In oxidation processes, oxidizing substances remove protons (H+) and electrons (e-), which are transported to acceptors by special transporters. During transporting H+ and e- to acceptors, energy will be released and accumulate in the ATP molecule.

Biological Oxidation

- Cells release the energy from fuel molecules by Oxidation reactions that involve:-
  - Removal of electrons (e-) OR addition of oxygen OR removal of H-atoms ($H^+ + e^-$).
- All oxidation reactions are accompanied by Reduction reactions Which involve:-
  - The addition of H atoms OR electrons OR the removal of oxygen.
- The H-atoms are transferred initially to carrier molecules that become carrier. The major carrier molecules are:-

Carrier	Oxidized form	Reduced form
Nicotinamide adenine dinucleotide	NAD^+	$NADH+H^+$
Nicotinamide adenine dinucleotide phosphate	$NADP^+$	$NADPH+H^+$
Flavin adenine dinucleotide	FAD	$FADH_2$

- NAD^+, $NADP^+$ and FAD are complex molecules which contain components that cannot be synthesized in the body and have to be supplied in the diet (vitamins).

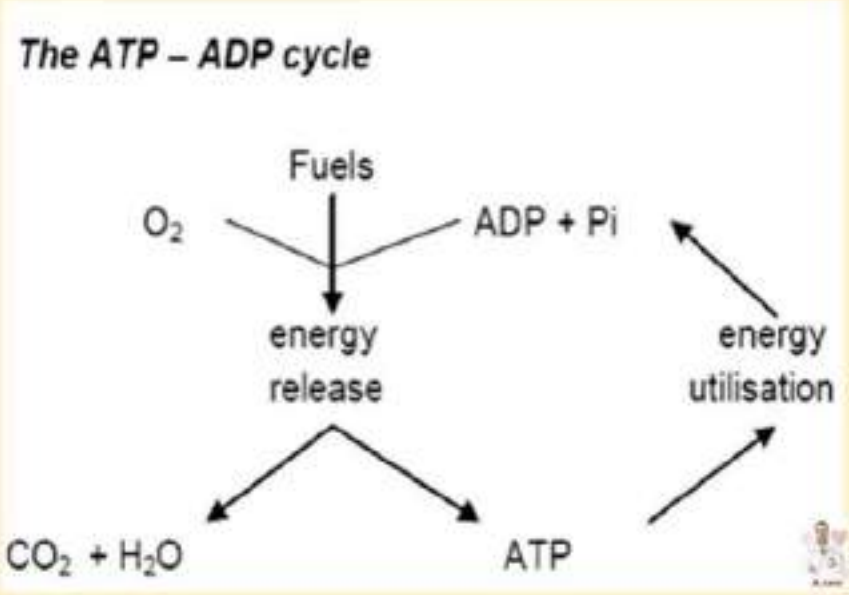


Notes

Endergonic reactions; energy released is greater than the energy input (give energy).

Exergonic reactions; energy input greater than the energy released (take energy).

- **ATP is the direct molecule that can release energy immediately.**



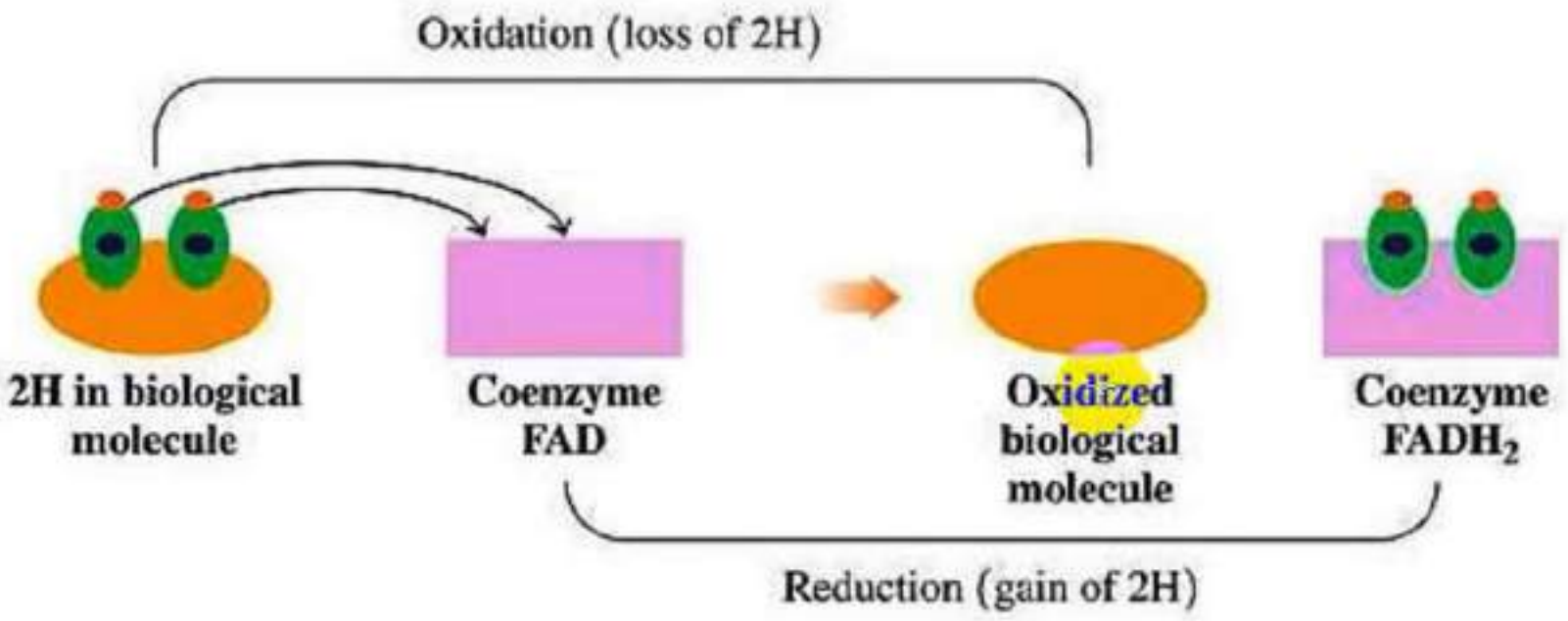
Biological oxidation

- It is an energy-producing reaction in living cells, and it is coupled with a reduction reaction.

Definition	● It is the cellular process in which the organic substances release energy (ATP), produce CO_2 and H_2O through oxidative-reductive reactions which represent the main source of biological energy.

- Oxidation and reduction reactions occur simultaneously, and one does not occur without the other.

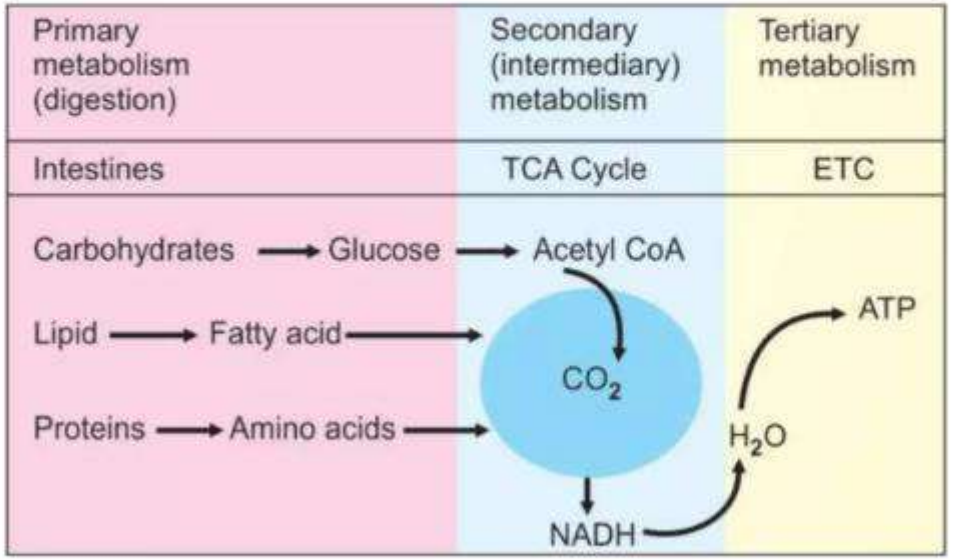
Oxidation reduction in biological systems



- ***Oxidation:*** Is the removal of electrons.
- ***Reduction:*** Is the gain of electrons.
- Oxidation is always accompanied by reduction.
- ***Oxidation-reduction potential or redox potential (E'0):*** Is the free energy change that occur in reactions involving oxidation & reduction.

Stages of oxidation of food

- First stage
- Second stage
- Third stage



Redox Reactions

- Every oxidation is always accompanied by a reduction process.
- All such reactions are termed as "oxidation-reduction" reactions and shortly referred as "redox".
- These redox reactions are associated with movements of electron.
- The electron donor is called as reductant or reducing agent and
- The electron acceptor, the oxidant or oxidizing agent.
- The system which transfer its electron is changed into oxidant form while the system which accepts electrons gets converted to the reductant form.

- The oxidoreduction reactions occur in living organisms are known as **biological oxidation**.

There are two types of biological oxidation:
1. Anaerobic
2. Aerobic

In **anaerobic** oxidation **H^+ and e^-** transported to another substrates (acceptors) and they are reduced.

Ex: in the anaerobic glycolysis, **H^+ and e^-** transported by NAD to pyruvate and reduce to lactate. An energy which released in this case, accumulated in **2 ATP** molecule. (See anaerobic glycolysis process. Here, NAD transporter of **H^+ and e^-**)

In **aerobic** oxidation **H^+ and e^-** transported to O_2 (acceptor).

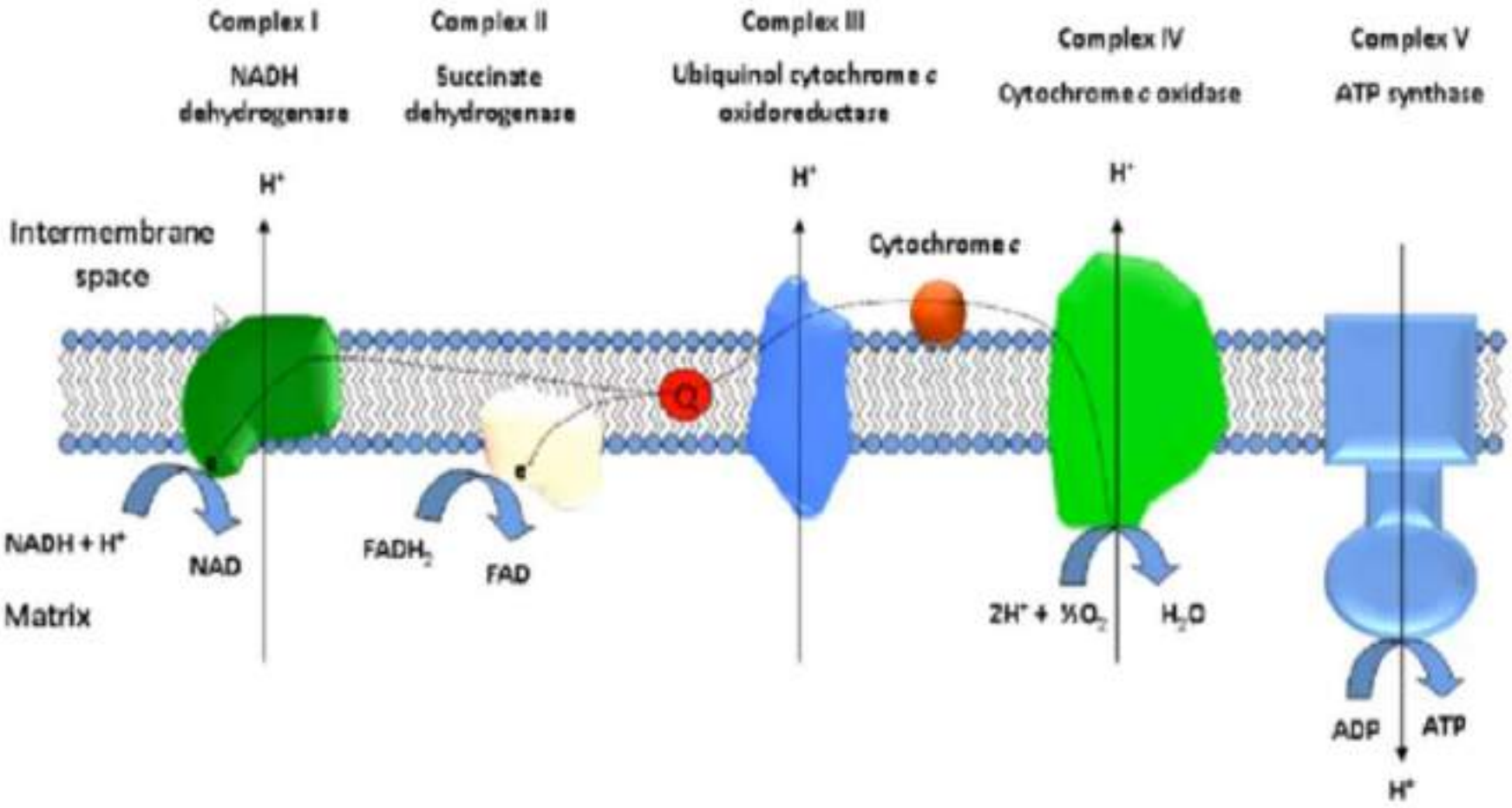
In this case, energy rich chemical substance (carbohydrates, lipids, proteins) oxidized to **CO_2** and **H_2O**.

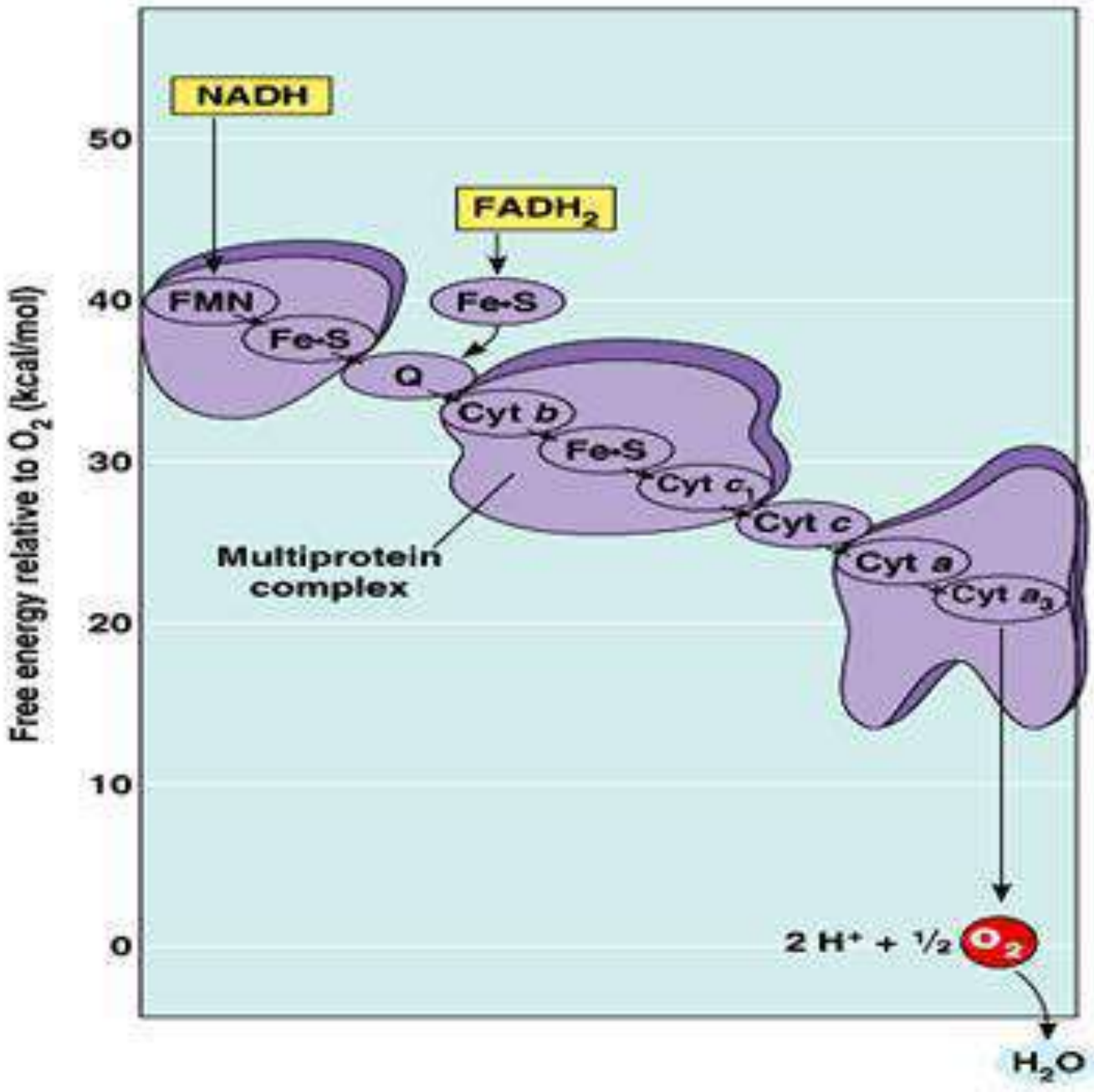
The reducing equivalents **H^+ and e^-** from intermediate substances transferred to **NAD and FAD** to produce **NADH and $FADH_2$** reforms of coenzymes, NADH and FADH2, form of coenzymes **NADH and $FADH_2$** pass through the **electron transport chain(ETC)** or **respiratory chain** and finally reduce **oxygen** to **water.**

Electron transport chain (**ETC**) – a process in which electrons are transported to O_2 by special components: **NAD, FMN, Iron-Sulfur protein, Coenzyme Q, Cytochromes b, c_1, c_2, a, a_3.**

All components of ETC are located on the inner mitochondrial membrane.

Respiratory chain

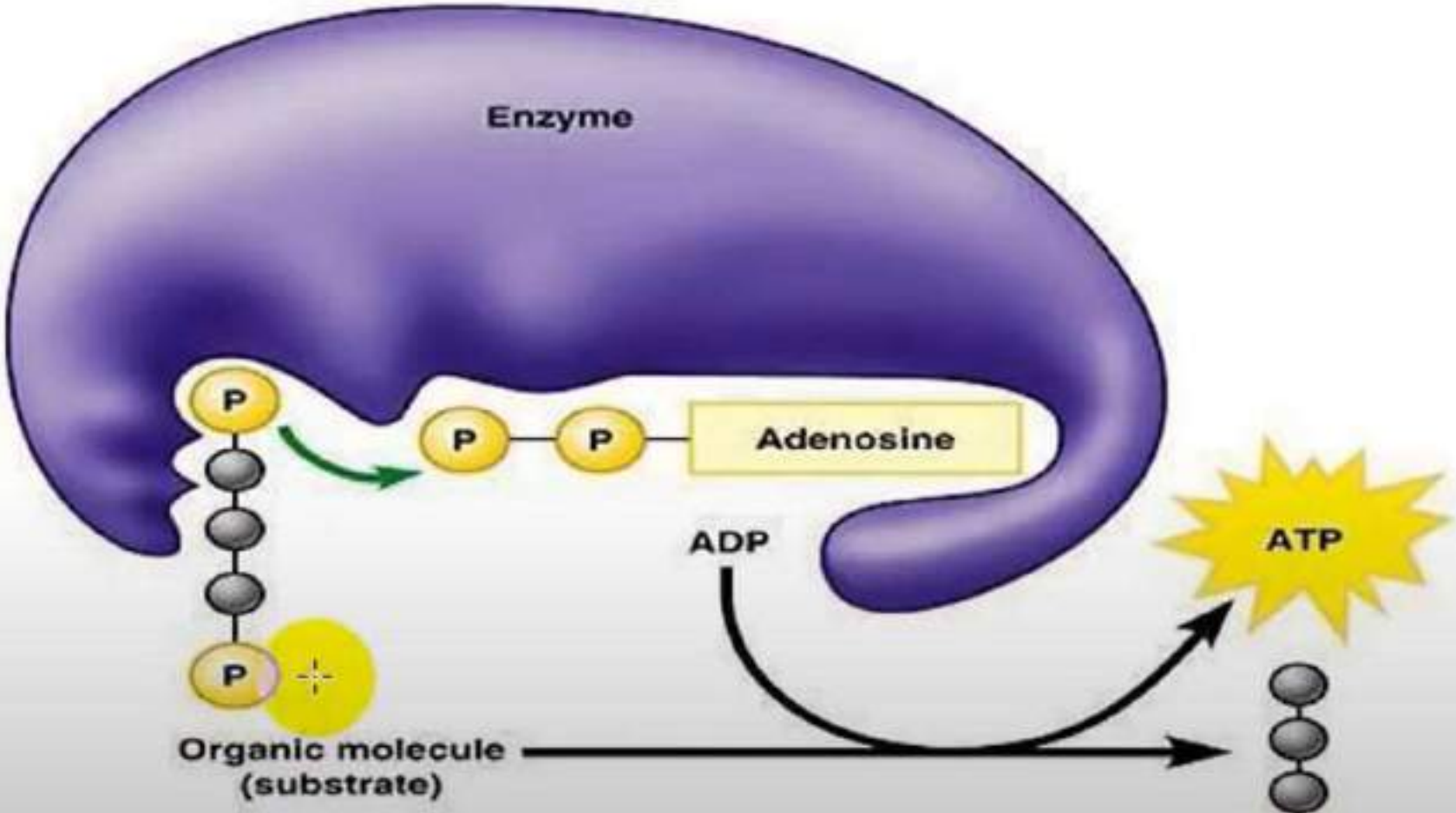



NADH
FADH2
FMN
Fe-S
Fe-S
Q
Cyt b
Fe-S
Cyt c1
Cyt c
Cyt a
Cyt a3
Multiprotein complex
2 H+ + ½ O2
H2O
Free energy relative to O2 (kcal/mol)
50
40
30
20
10
0

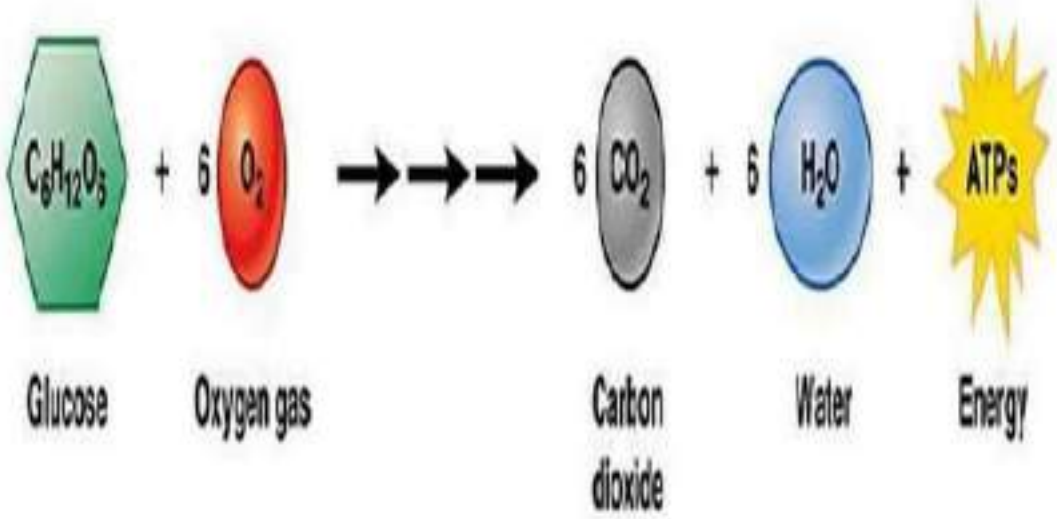
How energy is extracted from food molecules and used to synthesize ATP?

Imp.

1. Substrate-level phosphorylation	• Transferring a phosphate directly from substrate molecules to ADP • A small amount of ATP is formed in glycolysis and the citric acid cycle (Krebs' cycle).



2. Oxidative phosphorylation	• Use of ATP synthase and energy derived from a proton (H^+) gradient to make ATP. • Occurs only in O_2 presence. • It accounts for almost 90% of the ATP generated by cellular respiration.



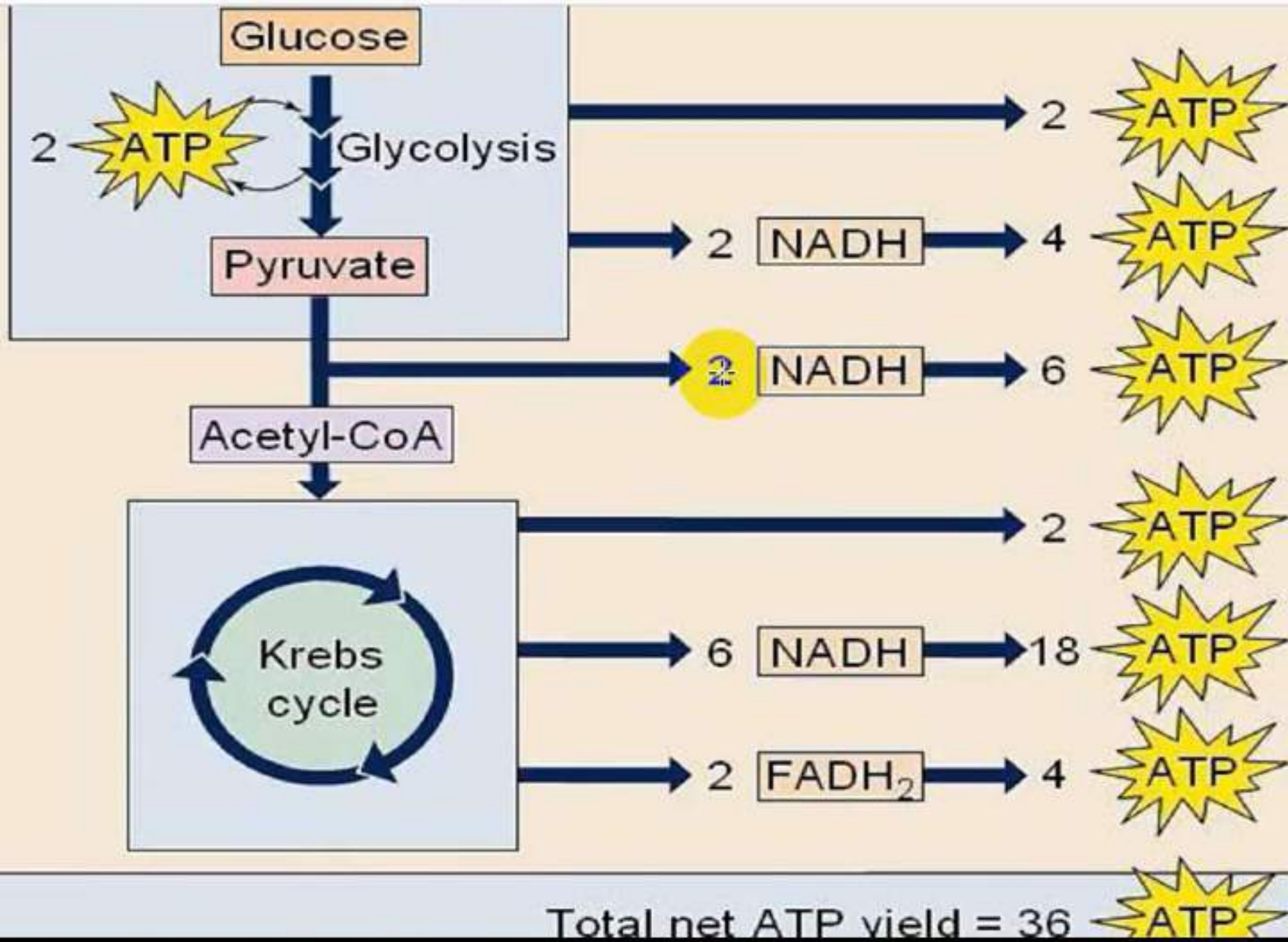
Example: Oxidation of glucose

The oxidation of glucose proceeds in stages:

O_2 present (36-38 ATP)	O_2 absent (2 ATP)
1. Glycolysis 2. Pyruvate oxidation 3. Krebs cycle 4. Electron transport chain and chemiosmosis	1. Glycolysis 2. Fermentation

◎ ***NADH and $FADH_2$***

- Collected during cellular respiration
- Act like shopping carts to collect energy that will be turned into ATP during the ETC
  - ✓ NADH = 3 ATP
  - ✓ $FADH_2$ = 2 ATP


Glucose
2 ATP
Glycolysis
Pyruvate
Acetyl-CoA
Krebs cycle
2 ATP
2 NADH 4 ATP
2 NADH 6 ATP
2 ATP
6 NADH 18 ATP
2 FADH2 4 ATP
Total net ATP yield = 36 ATP

The fate of pyruvate depends on O_2 availability

- When oxygen is present pyruvate is oxidized to acetyl CoA which enters the Krebs cycle.
- Without oxygen, pyruvate is reduced in order to oxidize NADH back to NAD^+

For glycolysis to continue, NADH must be recycled to NAD^+ by

1. **Fermentation:** occurs when oxygen is not available; an organic molecule is the final electron acceptor.
2. **Aerobic respiration:** occurs when oxygen is available as the final electron acceptor.

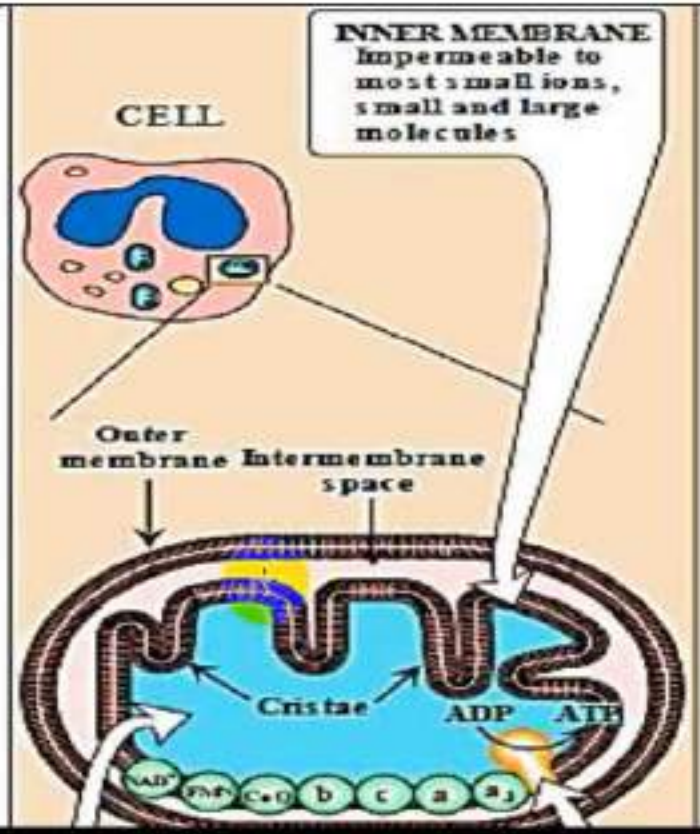
Mitochondria

- Mitochondria is the power house of the cell

Mitochondria-Structure

1. **Outer membrane:** freely permeable to most ions and small molecules.
2. **Inter-membrane space:** separates outer and inner mitochondrial membranes.
3. **Inner membrane**

- It is **impermeable** to most small ions , including protons and small molecules such as ATP, ADP, pyruvate
- Specialized carriers or transport systems are required to move ions or molecules across this membrane



- **The components of the ETC** (except for cytochrome c which is found in the inter-membrane space) are located in the inner mitochondrial membrane.
- It also is highly convoluted. The convolutions, called cristae, serve to greatly increase the surface area of the inner membrane.

4. Matrix:

- It contains **NAD+ and FAD (the oxidized forms of the two coenzymes that are required as hydrogen acceptors)**
- **It contains protons** that can be pumped across the inner mitochondrial membrane to create a gradient that drives ATP synthesis.
- ADP and Pi that are used to produce ATP.

Electron transport chain (ETC)

Site	Occur in mitochondria (امتحـــــان) Across inner mitochondrial membrane

Organization of ETC:

- The inner mitochondrial membrane can disrupted into 5 separate protein complexes

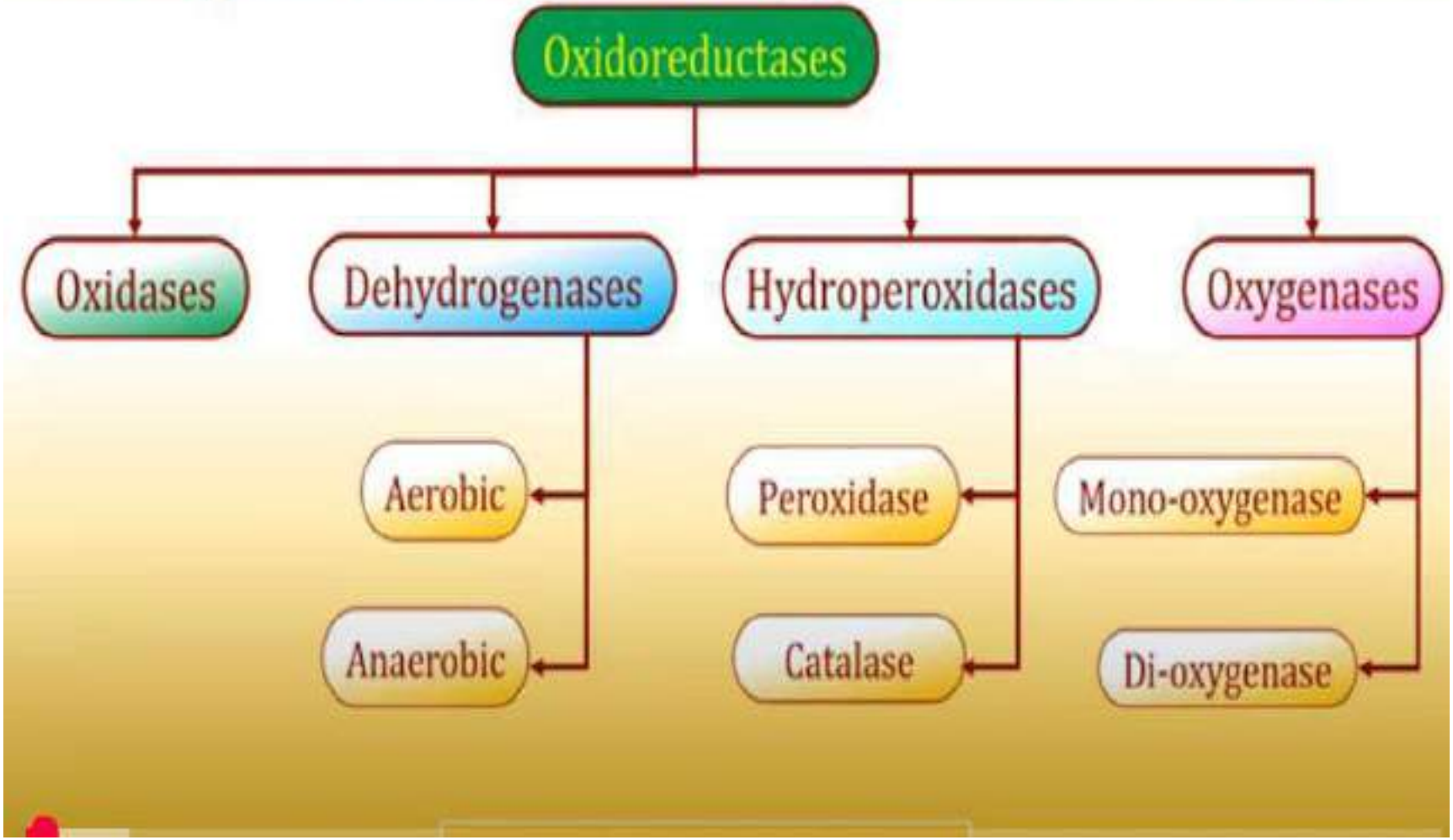
ETC complexes: [redacted]

Complex I	NADH dehydrogenase
Complex II	Succinate dehydrogenase
Complex III	Cytochrome b-c complex
Complex IV	Cytochrome oxidase (cytochrome $a+a_3$)
Complex V	ATP synthase

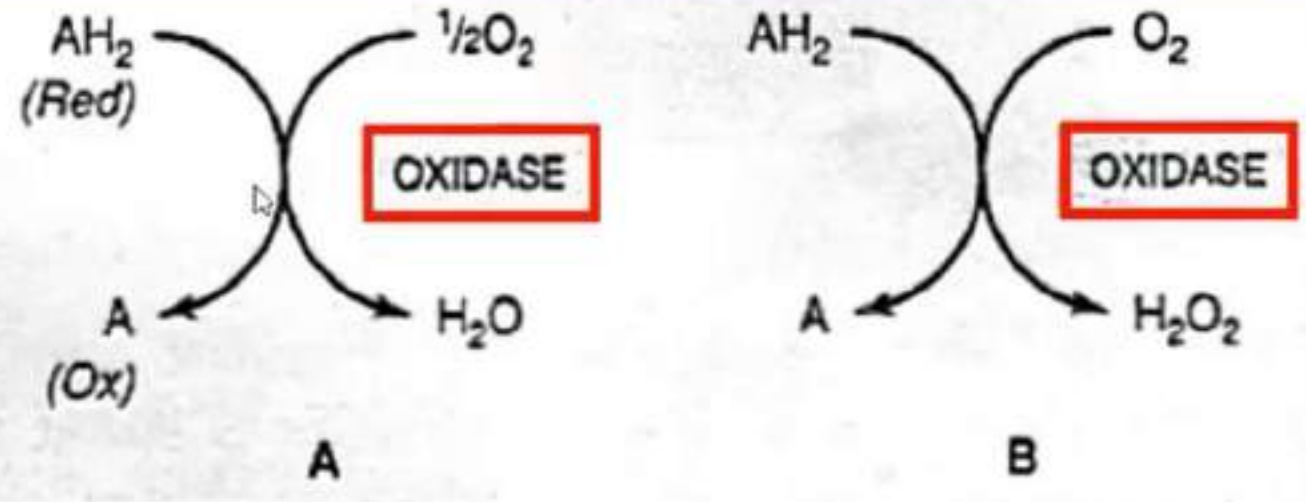
Mobile electron carriers: [redacted]

- Coenzyme Q
- Cytochrome c

Enzymes of Biological Oxidation



Oxidases



Oxidation of a metabolite catalyzed by an oxidase (A) forming H_2O, (B) forming H_2O_2.

A-Cytochrome oxidase (Cytochrome aa_3):

Hemoprotein widely distributed in many tissues & it is one of the components of respiratory chain.

Inhibited by CO , cyanide & hydrogen sulfide.

Contains two molecules of heme as prosthetic group.

B-Flavoprotein enzymes:

Contain FMN or FAD as prosthetic groups *metalloflavoproteins* ((Flavoprotein containing one or more metals as essential cofactors)).

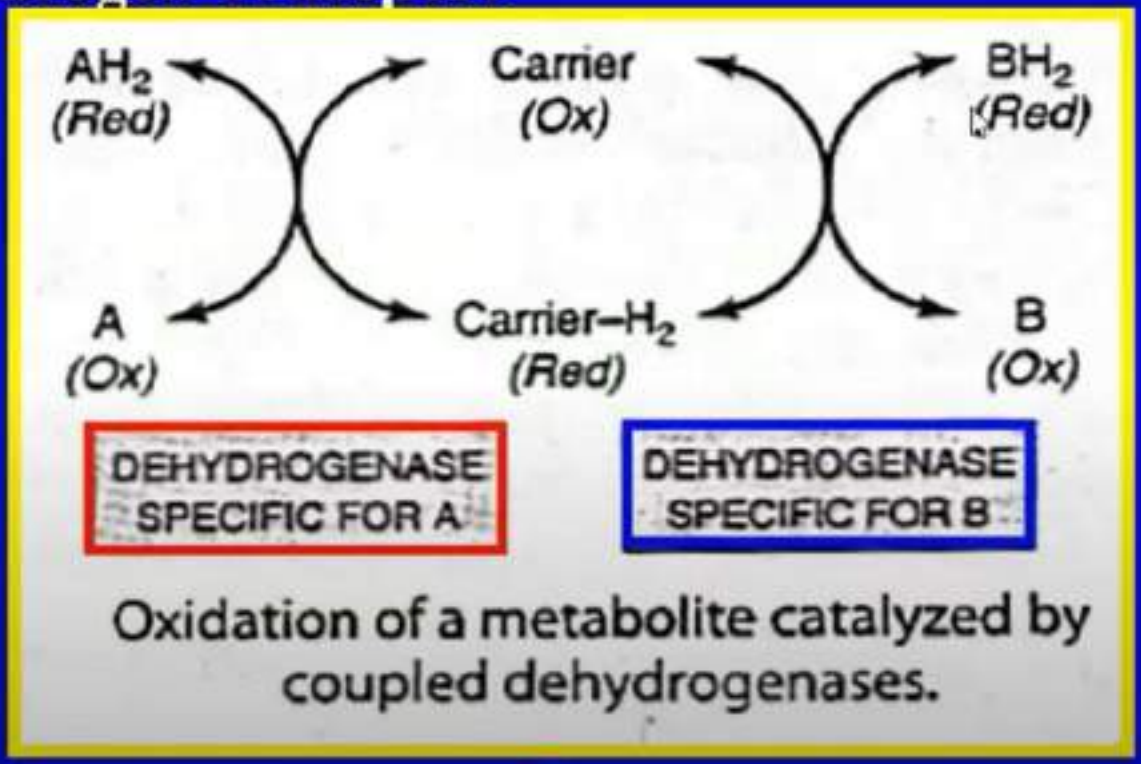
Examples of *Flavoprotein* enzymes:

1-L-amino acid oxidase: Kidney.

2-Xanthine oxidase: Contains molybdenum, important in uric acid synthesis.

Dehydrogenases

1-Hydrogen Transport



Oxidation of a metabolite catalyzed by coupled dehydrogenases.

Coenzymes (hydrogen carriers)

A)-Nicotinamides (NAD^+ & $NADP^+$):

NAD^+:

Glycolysis, citric acid cycle & respiratory chain.

$NADP^+$:

Fatty acid synthesis, steroid synthesis & in pentose phosphate pathway).

B)-Riboflavin (FMN & FAD):

Concerned with respiratory chain .

Oxidative decarboxylation of pyruvate & α-ketoglutarate.

Note:-Cytochromes except cytochrome oxidase classified as dehydrogenases.

Hydroperoxidases

Protect body against the harmful effect of peroxides .

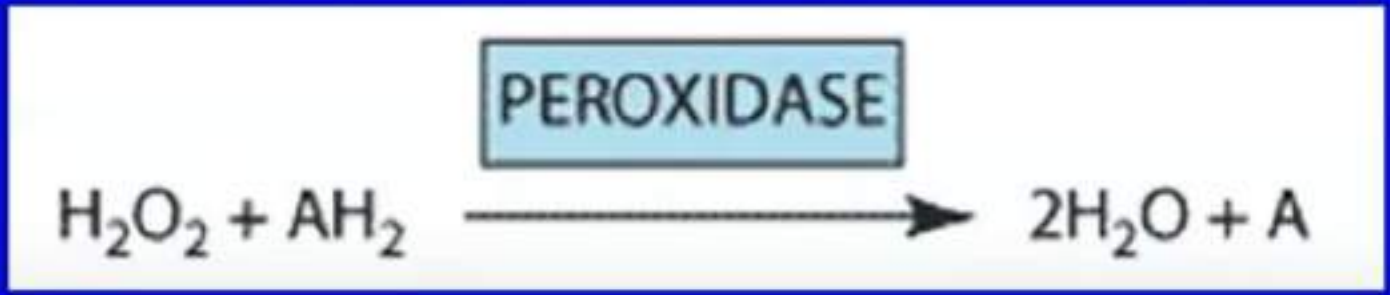
Hydroperoxidases include:-

1-Peroxidases.

2-Catalase.

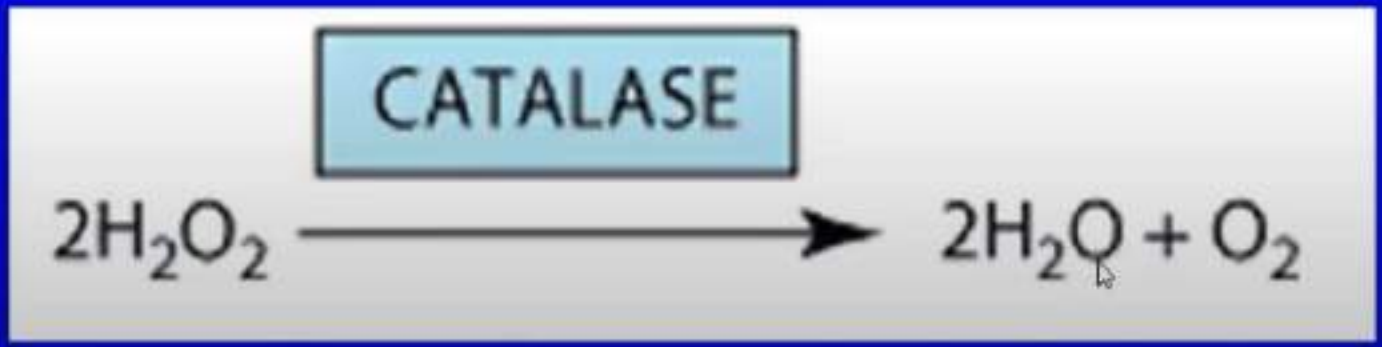
1-Peroxidases

Present in milk & in various tissues as leukocytes & platelets.



2-Catalase

Hemoprotein containing four heme groups.



Oxygenases

Catalyze the direct transfer & incorporation of oxygen into a substrate molecule.

Two groups:-

A)-Dioxygenases.

B)-Monooxygenases

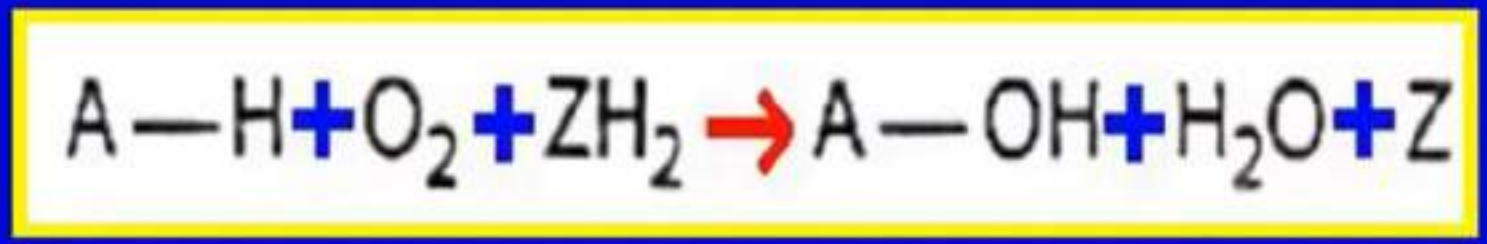
A)-Dioxygenases.

*Homogentisate dioxygenase.

*3-hydroxyanthranilate dioxygenase.

*L-tryptophan dioxygenase (tryptophan pyrrolase)

B)-Monooxygenases



Cytochromes P450

Cytochromes P450

Heme-containing monooxygenases , ↑1000, located mainly in the endoplasmic reticulum of liver & intestine, also f in the mitochondria.

Uses of cytochrome p450

1)-Detoxification of drugs in the liver microsomes.

2)-Mitochondrial cytochrome P450 found in steroidogenic tissues share in steroid hormone biosynthesis.

3)-In kidney; 25-hydroxycholecalciferol hydroxylation

4)- In liver ; bile acid biosynthesis.

Superoxide Dismutase

Transfer of a single electron to O_2 generates the potentially damaging superoxide anion free radical ($O_2^{\cdot -}$) .

STEP 1:

Superoxide Anion is converted into Hydrogen Peroxide

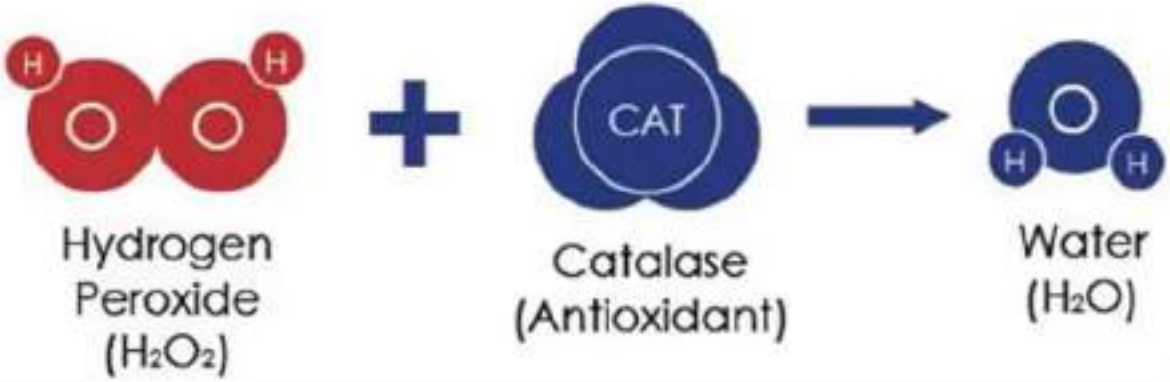
Superoxide Anion ($-O_2$)

Superoxide Dismutase (Antioxidant)

Hydrogen Peroxide (H_2O_2)

STEP 2:

Hydrogen Peroxide is converted into water



ATP: The basic transporter of cellular energy !!!

- 1 molecule ATP energy: -7.3 kcal/mol

- **Functions of ATP**

-Mobility

-Membrane transport

-Signal transduction

--Synthesis of nucleotides

Oxidative Phosphorylation

Oxidative phosphorylation is defined as "ATP synthesis by the transport of electron to the molecular oxygen".

The oxidative phosphorylation enables the aerobic living organisms to capture a far greater proportion of available free energy of the oxidizing substrates in the form of ATP !!!

Members of the electron transport chain

- **Complex I** - NADH dehydrogenase, also called NADH coenzyme Q reductase located in the inner mitochondrial membrane and also contains non heme iron atoms.
- These dehydrogenase enzyme does not react with oxygen instead an electron carrier is interposed between the metabolite and next member in the chain.
- These enzymes consist of a protein part and a non protein part which is a coenzyme. The co enzyme NAD+ or NADP+ are utilized as the prime carriers of hydrogen.

Members of the electron transport chain

- **Complex II** - Coenzyme Q (Q for Quinone) or cytochrome c reductase is a Ubiquinone.
- It is in the inner membrane in the free form or protein bound form.
- Coenzyme Q occupies the position between metalloflavoproteins and cytochrome in the chain.
- At the point of coenzyme the H+ ion dissociate and go into solution, leaving the electrons to the cytochromes .

Members of the electron transport chain

- **Complex III** -Cytochrome c oxidase.
- Cytochromes are very similar to the structure of myoglobin or hemoglobin.
- The significant feature is the heme structure containing the iron (Fe) ions, initially in the +3 state and changed to the +2 state by the addition of an electron.

Complex IV (cytochrome oxidase)

- **Complex IV**-(cytochrome oxidase), catalyses the transport of electrons from cytochrome c to molecular O_2 and thus the reduction of O_2 to H_2O.
- It contains cytochrome a and a.3

Members of the electron transport chain

- **Complex V** - ATP synthase, also known as the F0 F1 particle has two components F0 andF1
- (F - indicates the factor). F1 protruding into matrix from the inner membrane and F0 embedded and extend across the inner membrane.
- The protruding F1 is essential for the energy coupling to ATP molecule.
- Careful removal of this component (experimentally) leads to impairment in ATP production though the intact respiratory chain is present.

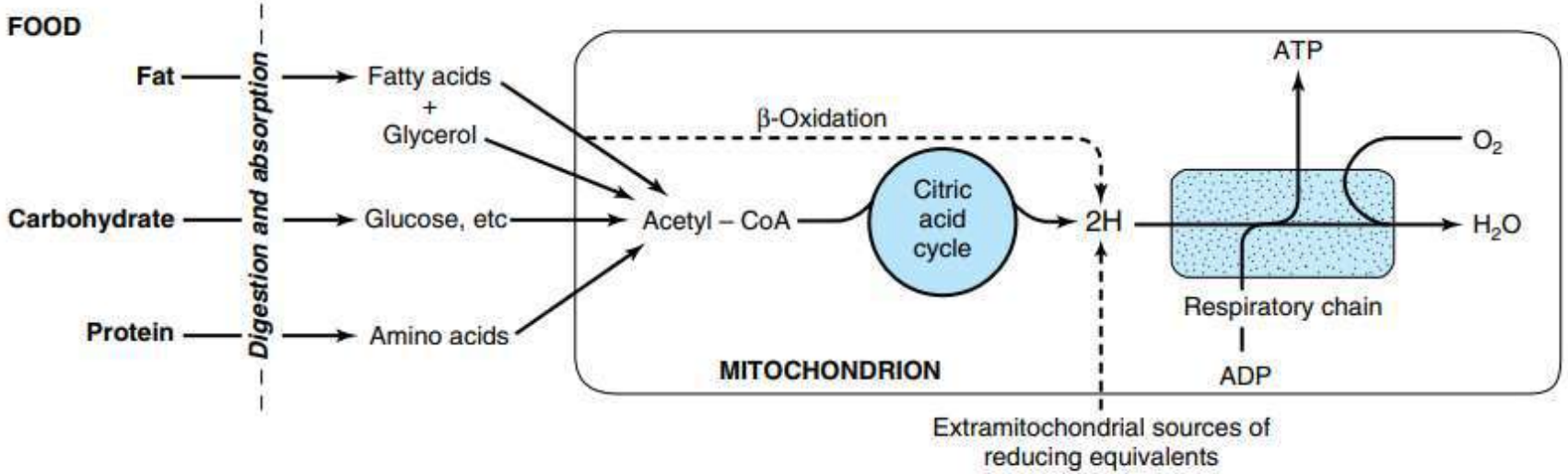


Figure 12–2. Role of the respiratory chain of mitochondria in the conversion of food energy to ATP. Oxidation of the major foodstuffs leads to the generation of reducing equivalents (2H) that are collected by the respiratory chain for oxidation and coupled generation of ATP.

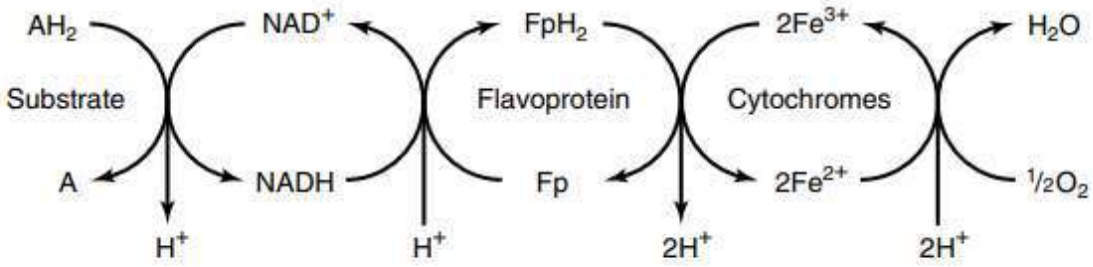


Figure 12–3. Transport of reducing equivalents through the respiratory chain.

MECHANISM OF OXIDATIVE PHOSPHORYLATION

- The most important among them-namely, **chemical coupling, and chemiosmotic-**are discussed below

Chemical coupling hypothesis

- This hypothesis was put forth by **Edward Slater** (1953).
- According to this hypothesis, **during the course of electron transfer** in the respiratory chain, **a series of phosphorylated high-energy intermediates is first produced** which are utilized for the synthesis of ATP.
- These reactions are believed to be analogous to the substrate-level phosphorylation that occurs in glycolysis or citric acid cycle.
- However, this hypothesis lacks experimental evidence, since all attempts, so far, to isolate any one of the high-energy intermediates have not been successful.

hemiosmotic hypothesis

This mechanism, was originally proposed by **Peter Mitchell**. (1961), is now widely accepted.

It explains **how the transport of electrons through the respiratory chain is effectively utilized to produce ATP from ADP + Pi.**

The concept of the chemiosmotic hypothesis is comparable with **energy stored in a battery separated by positive and negative charges.**

Inhibitors of Oxidative Phosphorylation

- The use of inhibitors gives much information about the electron transport chain.
- They are classified as

A. Inhibitors of respiratory chain,

B. Inhibitors of oxidative phosphorylation,

C. Uncouplers of phosphorylation.

INHIBITORS OF THE ELECTRON TRANSPORT CHAIN

- The inhibitors bind to one of the components of ETC and block the transport of electrons. (No ATP)
- The **synthesis of ATP** (phosphorylation) is dependent on **electron transport**. Hence, all the **site-specific inhibitors** of ETC also **inhibit ATP formation**.
- Three **possible sites of action for the inhibitors** of ETC are identified.

Inhibition

1_ Inhibitors of electron transport chain

2_ Inhibitors of oxidative phosphorylation

1_ Inhibitors of complex 1

2_ Inhibitors of complex 2

3_ Inhibitors of complex 3

4_ Inhibitors of complex 4

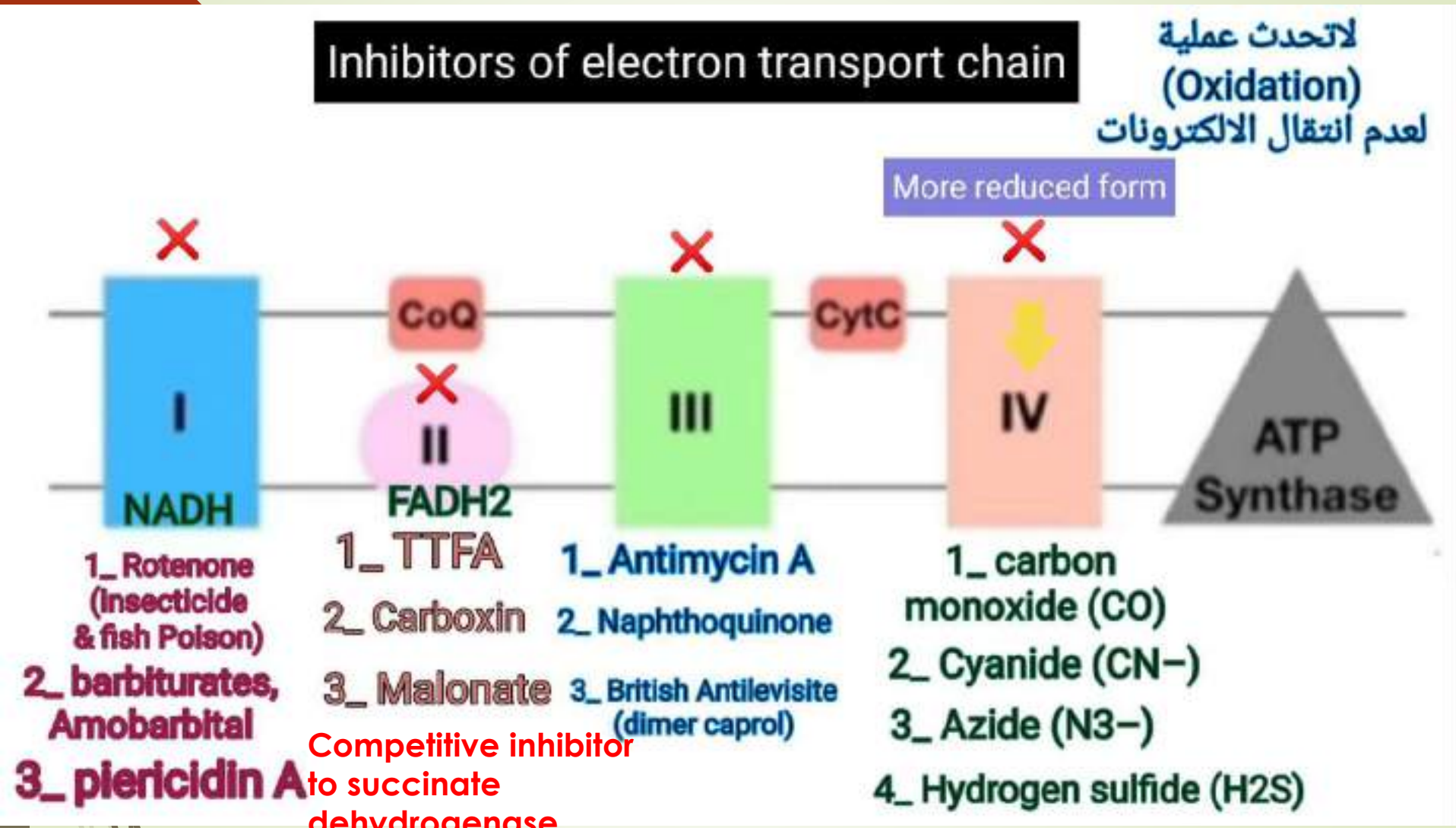
Inhibitors of complex 5 (Inhibitors of ATP Synthase)

Inhibitors of Oxidative Phosphorylation

A. **Inhibitors of Respiratory Chain**

- ✓ Inhibitors that arrest respiration are barbiturates like amobarbital, antibiotic like piericidin A , antimycin A and fish poison retinone.
- ✓ The carbon monoxide (CO) and cyanide (CN) inhibit cytochrome oxidase so that it cannot transport electrons to oxygen.
- ✓ This blocks the further passage of electrons through the chain, halting ATP production and life.

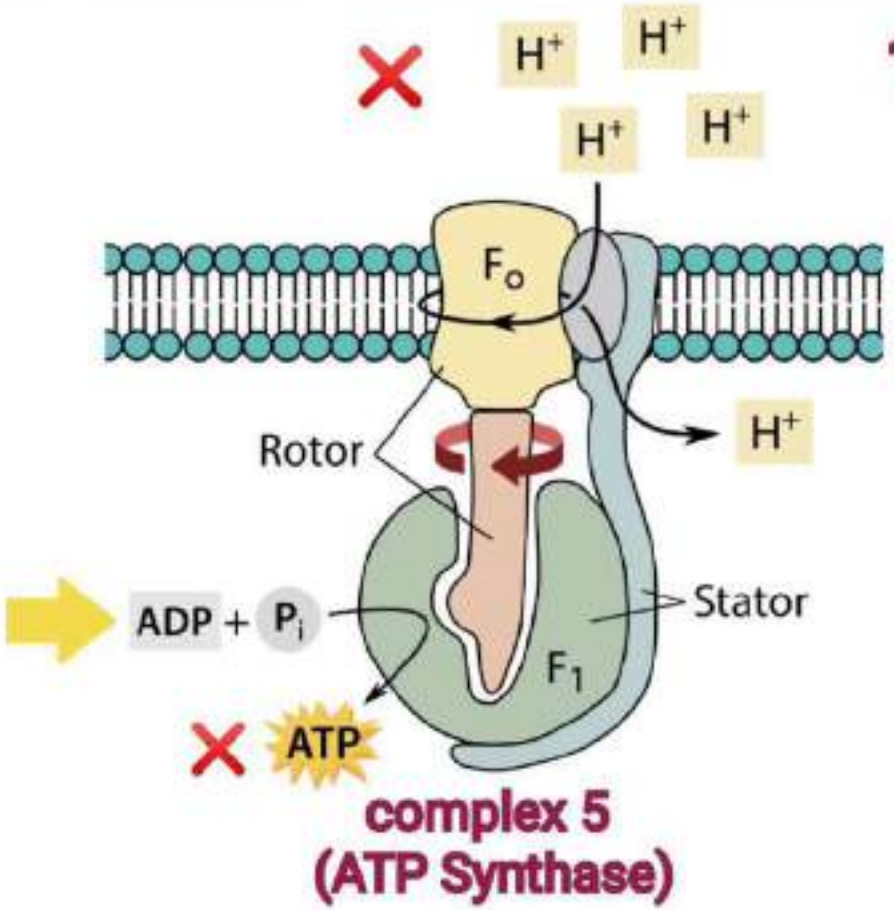
1. **NADH and coenzyme Q:** Fish poison rotenone, barbiturate drug amytal, and antibiotic piercidin A inhibit this site.
2. **Between cytochrome b and c1:** Antimycin A -an antibiotic, and British antilewisite (**BAL**)-an antidote used against war-gas-are the two important inhibitors of the site between cytochrome b and c1.
3. **Inhibitors of cytochrome oxidase:** Carbon monoxide, cyanide, hydrogen sulfide, and azide effectively inhibit cytochrome oxidase. **Carbon monoxide** reacts with the reduced form of the cytochrome while cyanide and azide react with the oxidized form.



Competitive inhibitor to succinate dehydrogenase

TTFA : Trienoyl Tri Fluoro Acetone

Inhibitors of Oxidative phosphorylation



1_ Antibiotics

oligomycin

Inhibits F0 & F1

2_ Aurovertin

inhibits F1

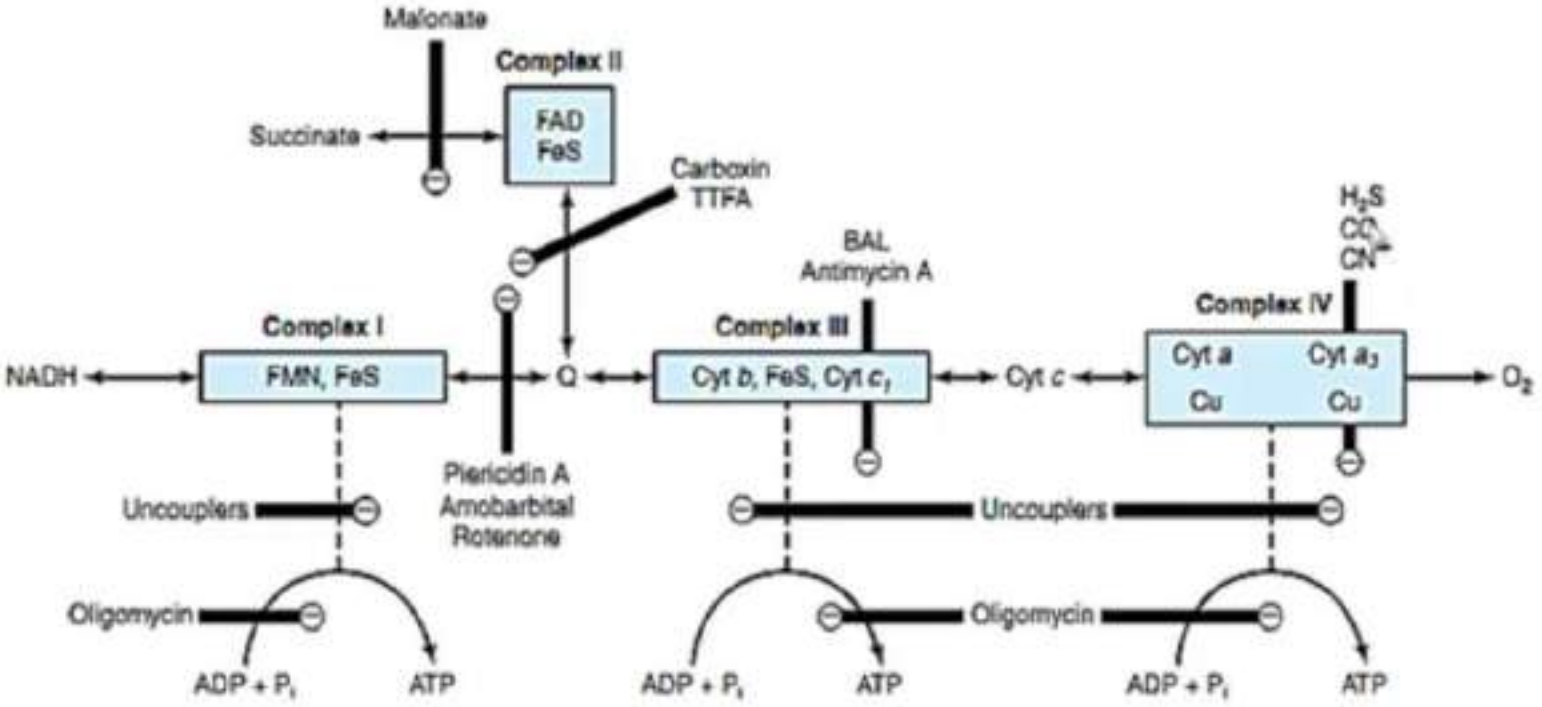
3_ Venturicidin

inhibits F0

4_ Atractyloside

inhibits ADP in & ATP out

Inhibitors of Respiratory Chain



Inhibitors of Oxidative Phosphorylation

B. Inhibitors of oxidative phosphorylation

- ✓ Inhibitors of oxidative phosphorylation are oligomycin and atrctyloside.
- ✓ Inhibitors of ATP synthase

Oligomycin, Venturicidin — Inhibition of Fo and CFo units

- ✓ Dicyclohexylcarbodiimide (DCCD) : Inhibition of proton efflux from Fo and Cfo

Inhibitors of Oxidative Phosphorylation

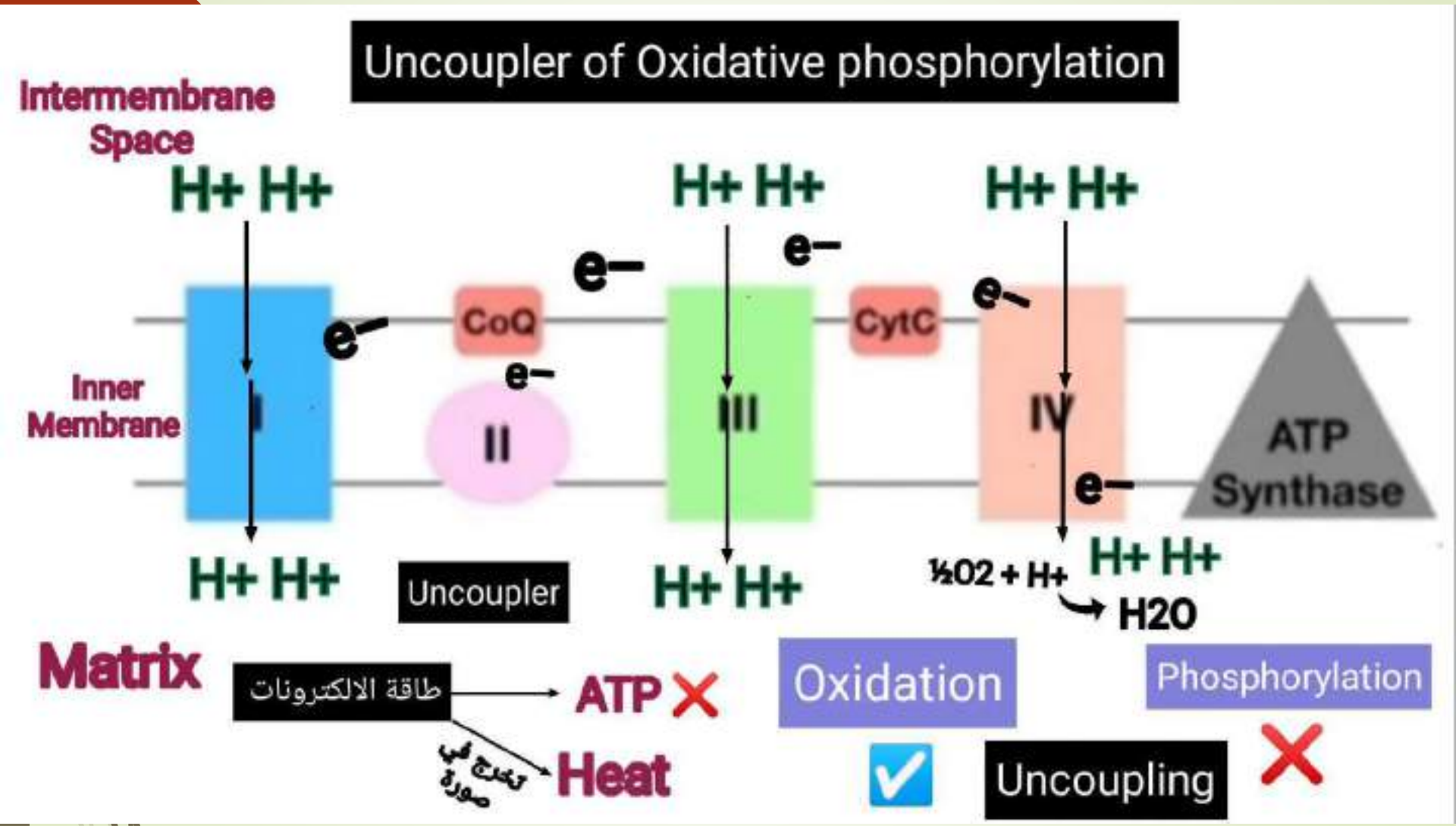
C. Uncouplers of phosphorylation

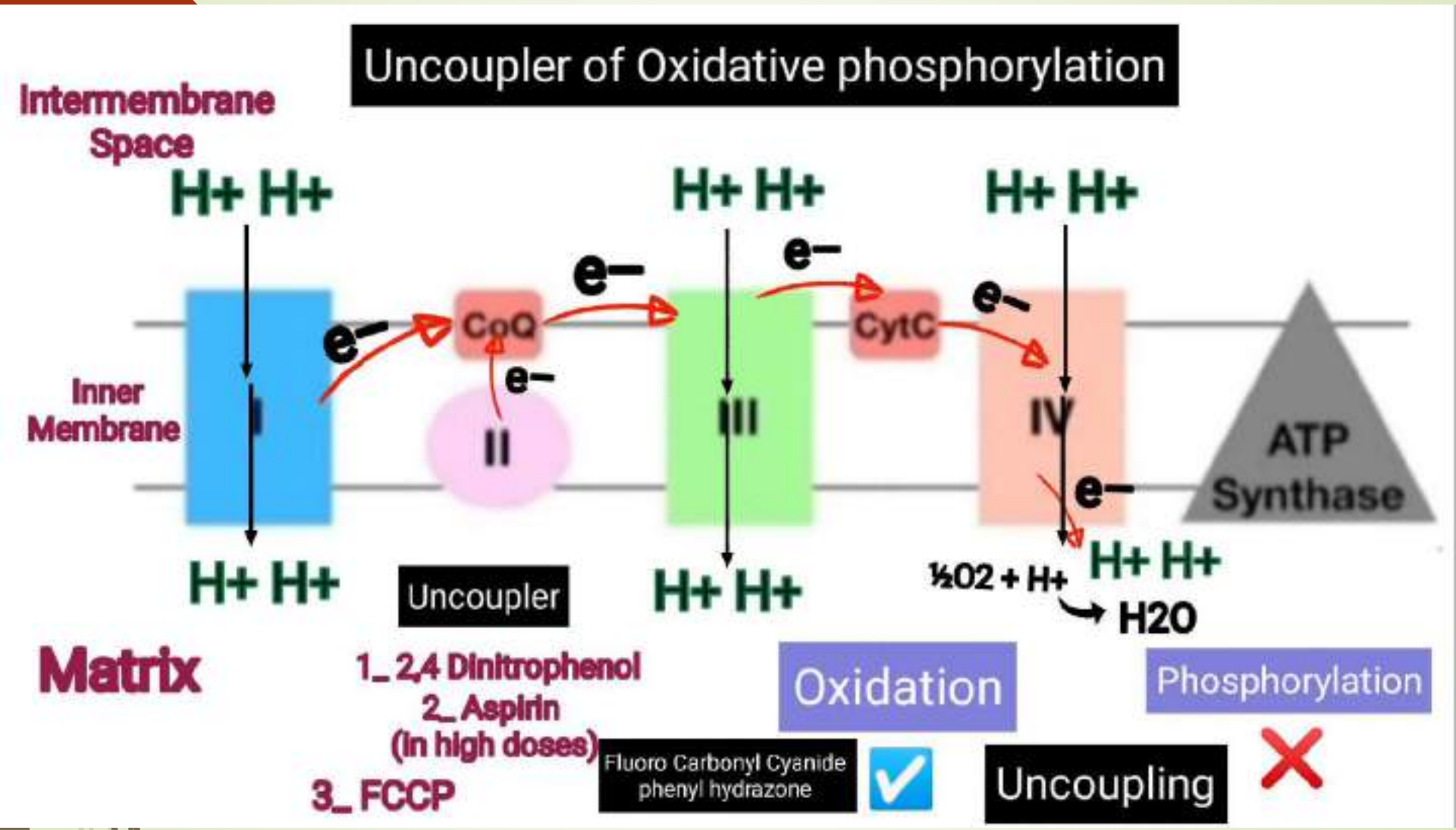
- Uncouplers dissolve in the membrane, and function as carriers for H^+ or it can be an ionophores.
- Uncouplers block oxidative phosphorylation by dissipating the H^+ electrochemical gradient by an uncoupling the essential linkage between electron transport and ATP synthesis.
- Uncouplers are 2,4 dinitro phenol, dinitrocresol, pentacholorophenol.

Inhibitors of Oxidative Phosphorylation

C. Uncouplers of phosphorylation

- ✓ Ionophores (ion carriers) are lipid soluble substances capable of carrying specific ions through the membrane.
- ✓ They slightly differ in their action from the uncouplers as they also transport cations other than H^+ through the membrane.
- ✓ Valiomycin forms a lipid complex through which the K^+ ion readily pass through.
- ✓ The ionophore gramicidin induces penetration to H^+, K^+ or Na^+ and uncouples the oxidative phosphorylation.


Uncoupler of Oxidative phosphorylation
Intermembrane Space
H+ H+
e–
CoQ
CytC
Inner Membrane
II
III
IV
ATP Synthase
½02 + H+
H2O
Uncoupler
Matrix
طاقة الالكترونات
ATP
تخرج في صورة
Heat
Oxidation
Phosphorylation
Uncoupling


Uncoupler of Oxidative phosphorylation
Intermembrane Space
H+ H+
H+ H+
H+ H+
e–
e–
e–
e–
e–
e–
CoQ
CytC
Inner Membrane
I
II
III
IV
ATP Synthase
H+ H+
H+ H+
½O2 + H+
H+ H+
H2O
Uncoupler
Matrix
1_ 2,4 Dinitrophenol
2_ Aspirin
(In high doses)
3_ FCCP
Oxidation
Phosphorylation
Fluoro Carbonyl Cyanide phenyl hydrazone
Uncoupling
4-CCCP
Chlorocarbonyl Cyanide phenyl hydrazone

INHIBITORS OF OXIDATIVE PHOSPHORYLATION

(1. **Uncouplers**- 2,4-dinitrophenol (DNP), dinitro cresol, pentachlorophenol, trifluoro carbonyl cyanide, and phenylhydrazone (FCCP))

2. **Physiological uncouplers** (thermogenin, thyroxine, and long-chain free fatty acids)

EXPLANATIONS-HOMEWORK

Physiological Uncouplers

1_ Thyroxine (thyroid hormone)

2_ Thermogenin

in brown Adipose tissues

brown Fat

Newborn

White Fat

prevent production ATP

energy in electrons → Converts to → **Heat**

Make baby feels warmth

brown adipose tissues زيادة

زيادة معدلات الحرق

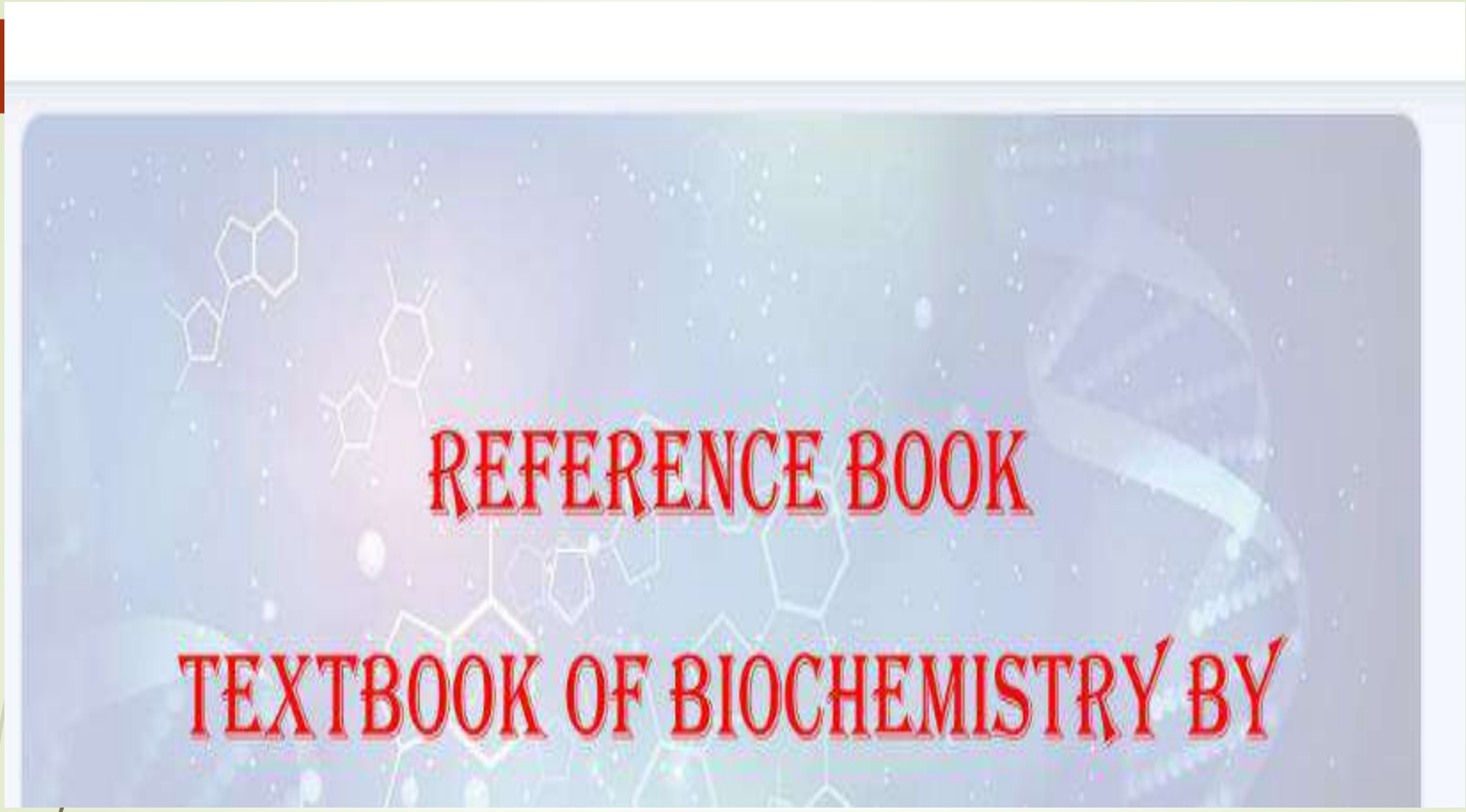
Mitochondria Nucleus Lipid droplet

SUMMARY

- **In biologic systems, as in chemical systems, oxidation (loss of electrons) is always accompanied by reduction of an electron acceptor.**

- **Oxidoreductases have a variety of functions in metabolism; oxidases and dehydrogenases play major roles in respiration; hydroperoxidases protect the body against damage by free radicals; and oxygenases mediate the hydroxylation of drugs and steroids.**

- **Tissues are protected from oxygen toxicity caused by the superoxide free radical by the specific enzyme superoxide dismutase.**



Harper's
Illustrated
Biochemistry
a LANGE medical book
twenty-sixth edition

THANKS


من ظن بالله خيراً ، فلن يُخيّب الله
ظنّه، ومن شكر الله على نعمة ،
زادهُ الله من فضله ، ومن توكل
على الله فهو حسبه"