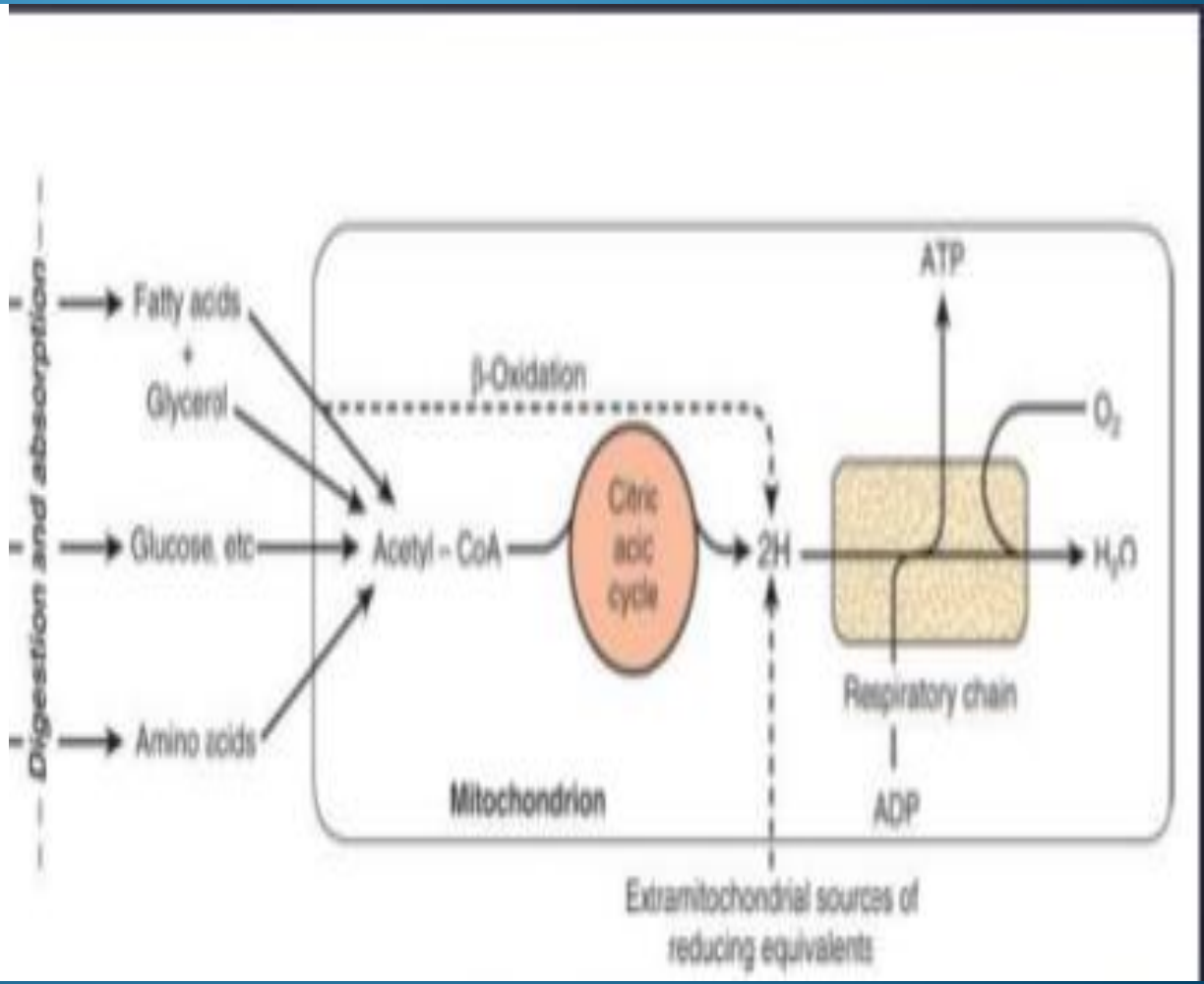
The Respiratory Chain & Oxidative Phosphorylation.



Learning Objectives :

- Define biological oxidation.
- Describe the sources of NADH & $FADH_2$
- Role of Anaerobic Dehydrogenases.
- Enumerate different parts of enzymes & coenzymes that carryout biological oxidation.
- Enlist components of each complex involved in the biological oxidation.
- Describe the transfer of electrons through each complex.

- Describe the transfer of protons from inter mitochondrial membrane to mitochondrial matrix through ATP synthase (Generation of Proton gradient).

- Describe the mechanism of ATP production by ATP synthase (CHEMIOSMOTIC THEORY)

Biological Oxidation and Oxidative Phosphorylation

- The Transfer of Electrons from the reduced Co-enzymes through the respiratory chain to oxygen is known as Biological oxidation.

- Energy released during this process is Trapped as ATP (ADP $\longrightarrow$ ATP)
- This coupling of oxidation with phosphorylation is called oxidative phosphorylation.

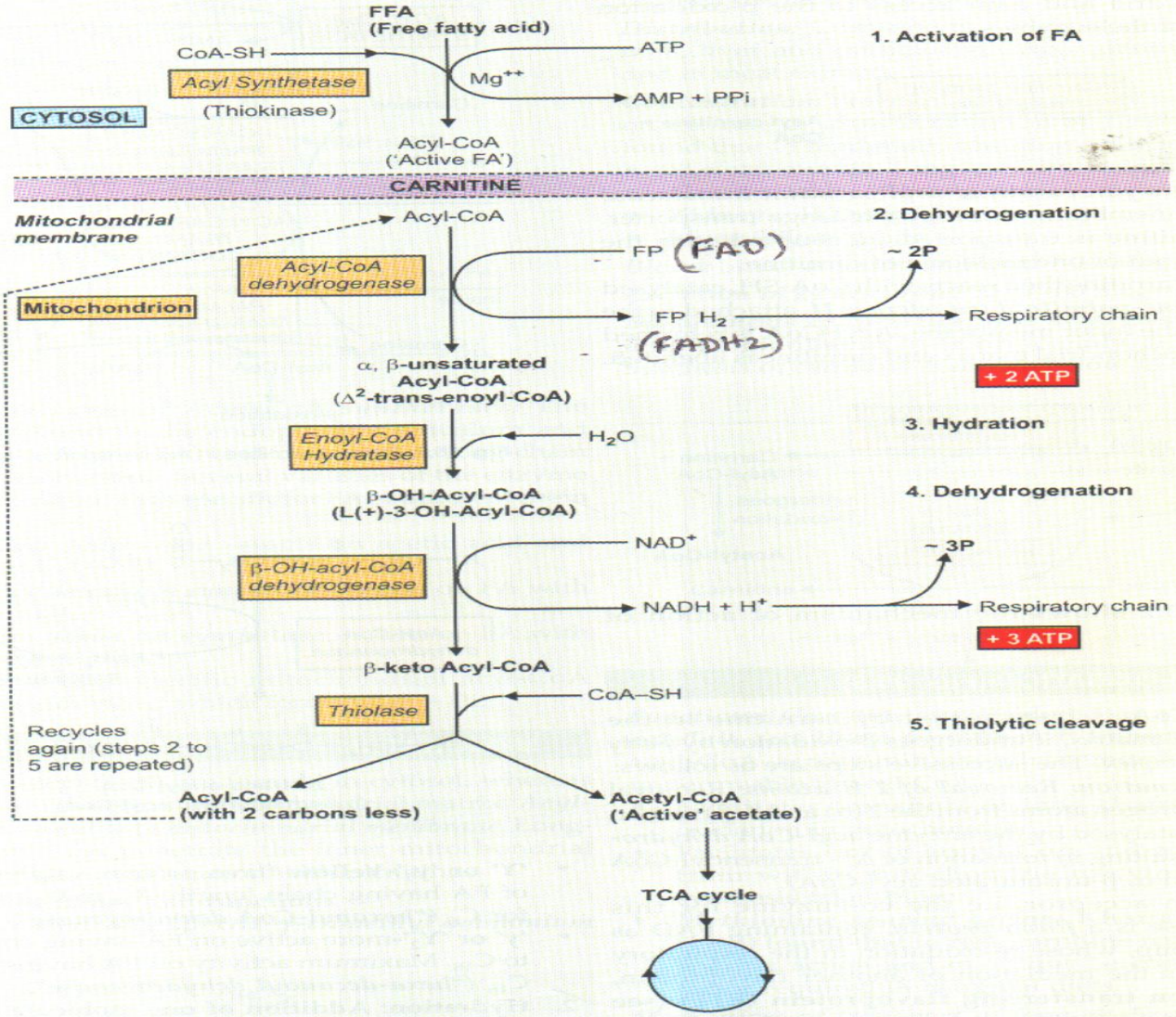


Fig. 25.3: β-oxidation of fatty acids

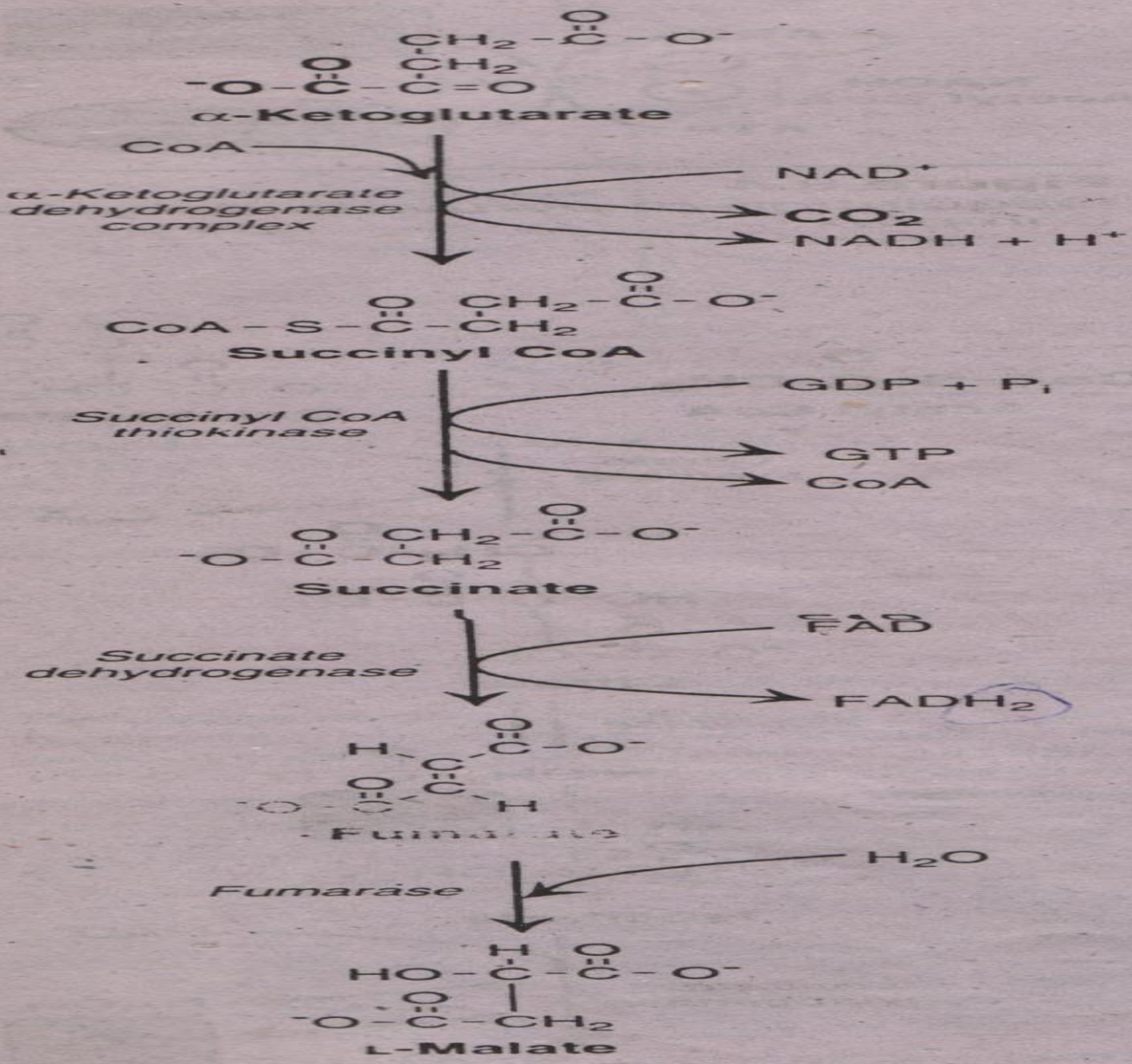
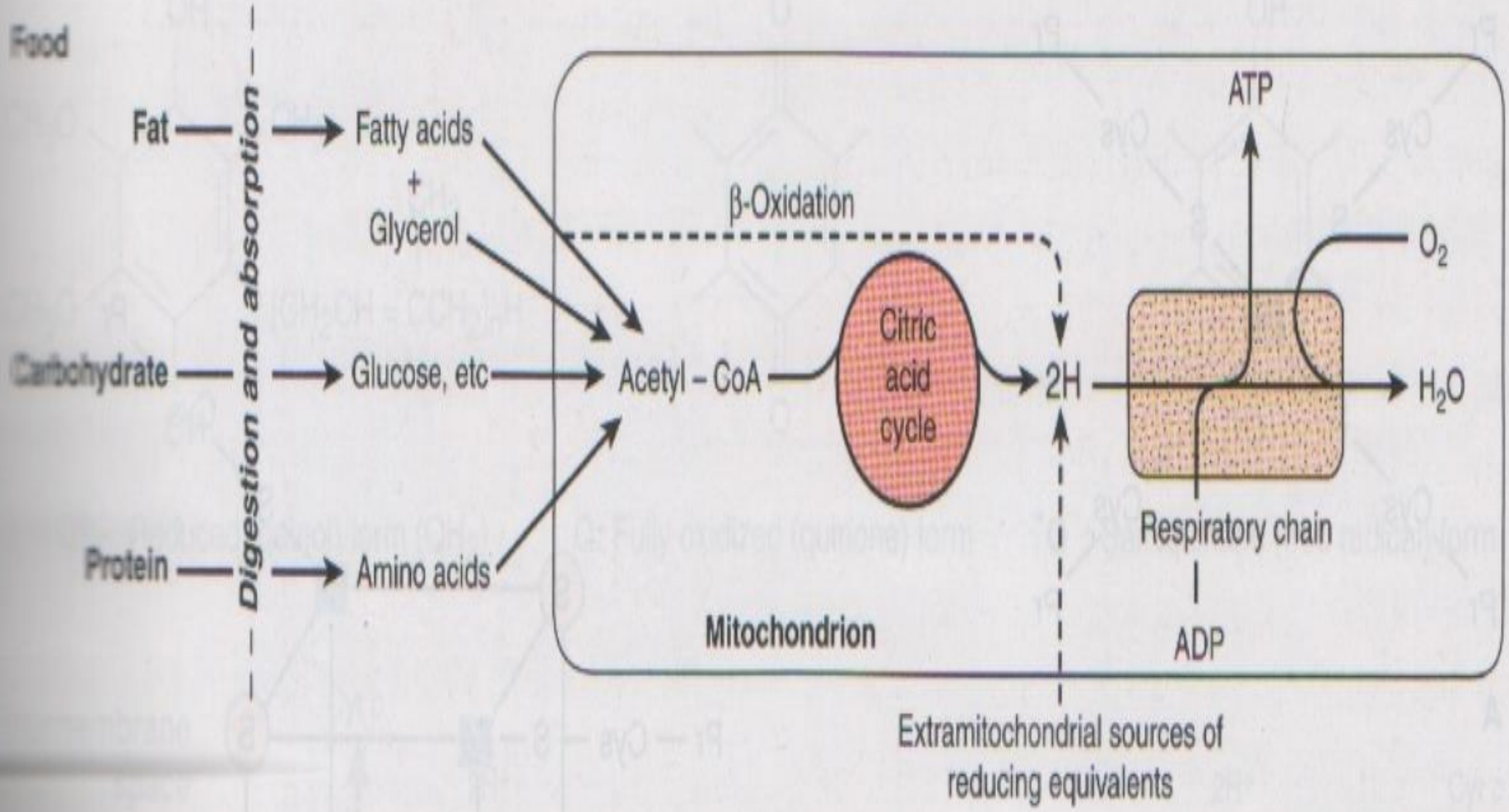


Figure 9.6
Formation of malate from α-ketoglutarate.


Food
Fat
Fatty acids
+
Glycerol
Carbohydrate
Glucose, etc
Protein
Amino acids
Digestion and absorption
β-Oxidation
Acetyl – CoA
Citric acid cycle
2H
ATP
O_2
H_2O
Respiratory chain
ADP
Mitochondrion
Extramitochondrial sources of reducing equivalents

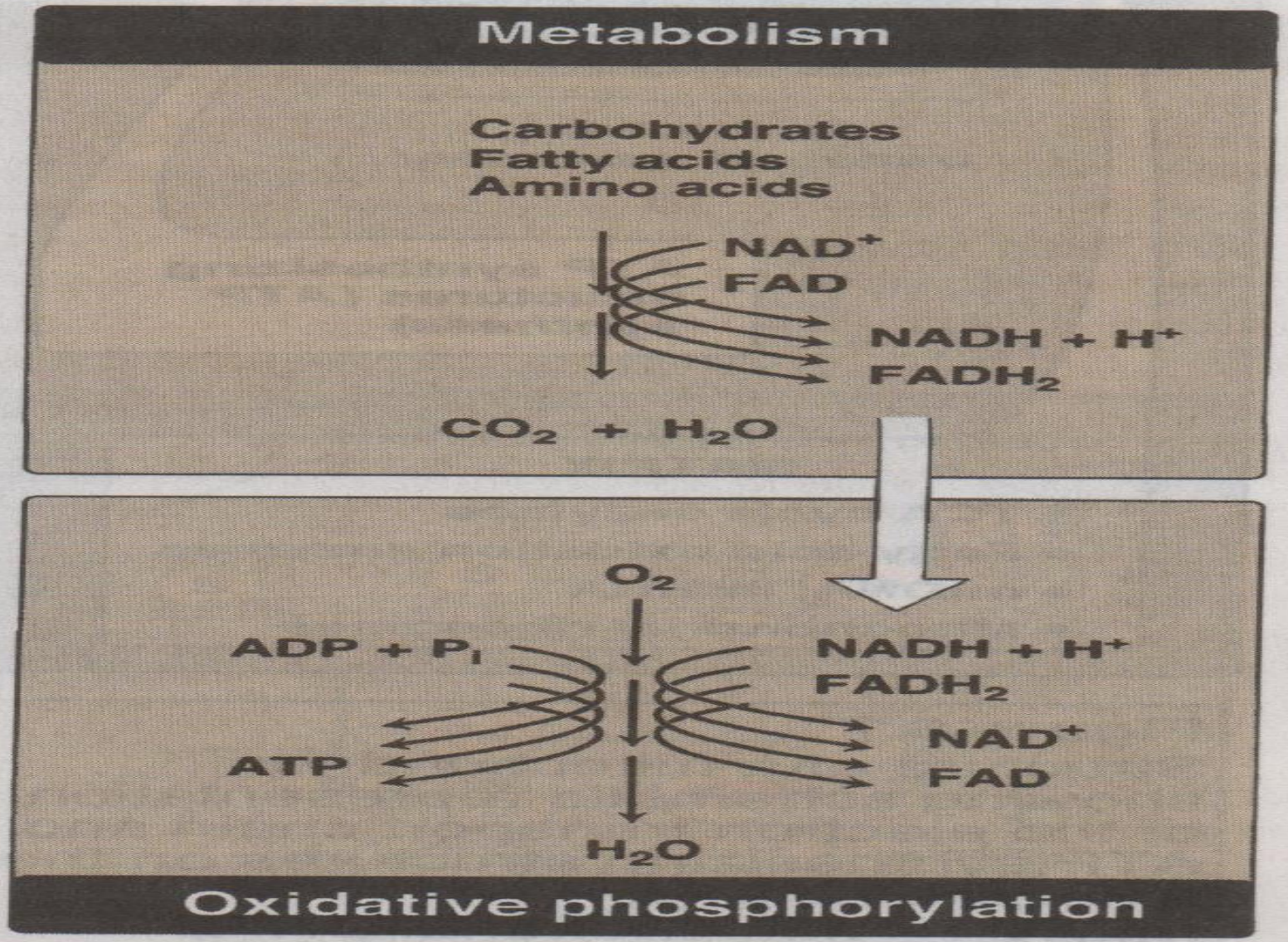
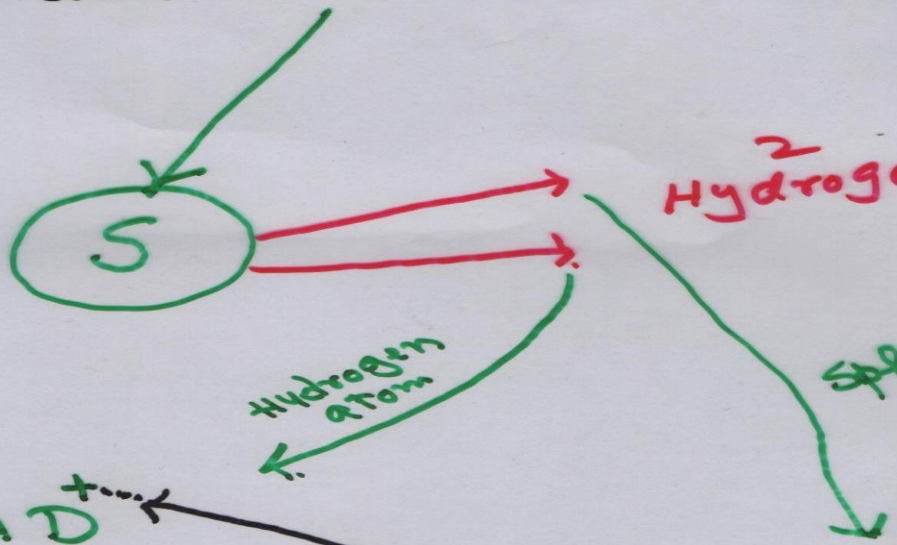


Figure 6.6
The metabolic breakdown of energy-yielding molecules.

Anaerobic Dehydrogenase



NAD^+

One Electron + one Hydrogen ion (H^+)

Released into the surrounding medium.

So $NAD \rightarrow NADH + H^+$

2a

NAD Linked Dehydrogenases:-

Anaerobic Dehydrogenases catalyse removal of hydrogen from substrate.

The coenzymes NAD or FAD act as hydrogen acceptor. when the substrate is oxidised, the co-enzyme is reduced.

$$AH_2 + NAD^+ \longrightarrow A + NADH + H^+$$

FAD — Linked Dehydrogenases :-

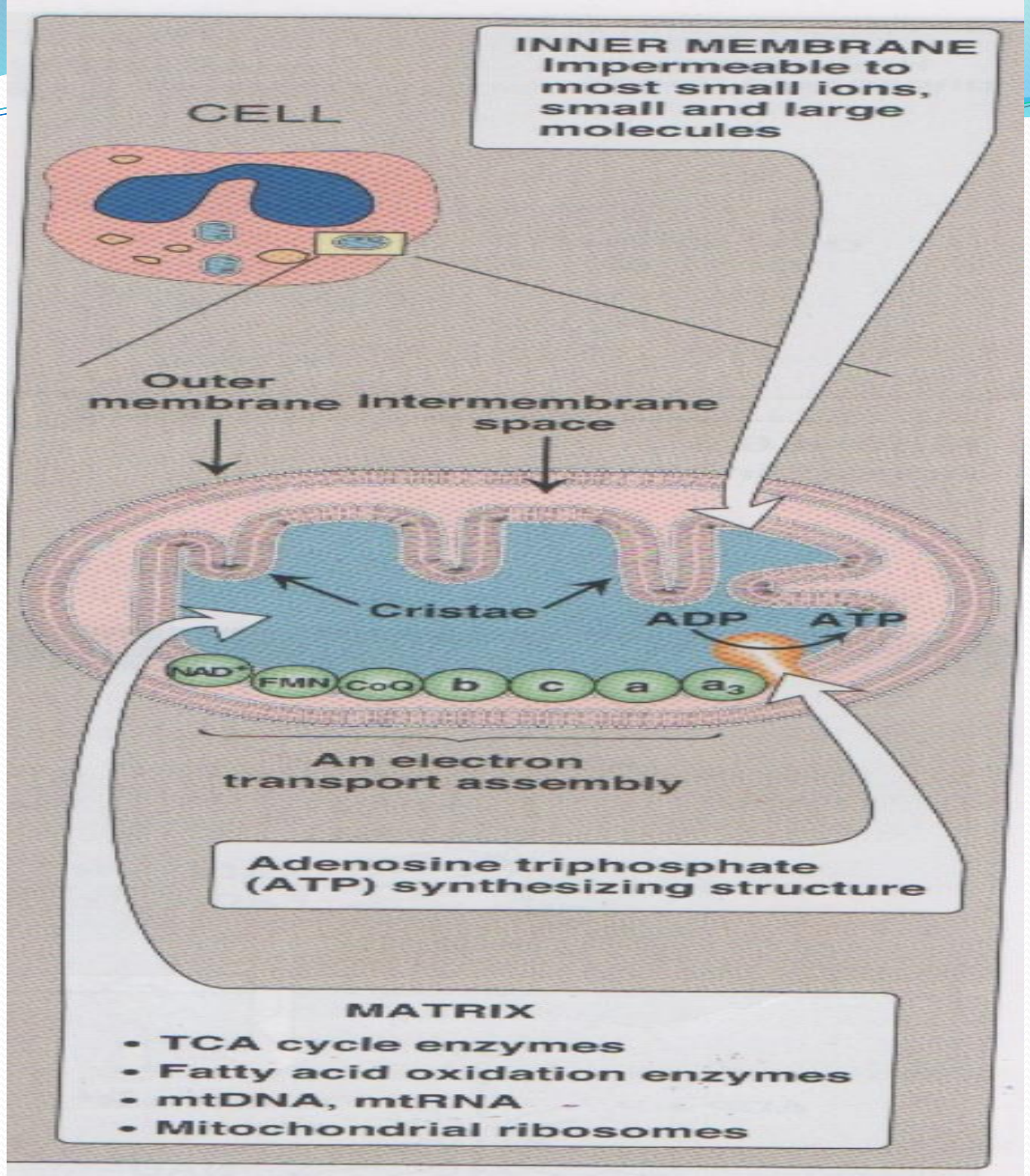
When FAD is the co-enzyme, Both the hydrogen atoms are attached to the Flavin ring.

FMN ::

is the component of E.T.C, accepting the hydrogen atoms from NADH2.

- ## $NADP^+$ Linked Dehydrogenase :-

NADPH can't be oxidised for the production of energy, but take part in reductive biosynthetic reactions e.g. Fatty acids and cholesterol synthesis.


CELL
INNER MEMBRANE
Impermeable to most small ions, small and large molecules
Outer membrane
Intermembrane space
Cristae
ADP
ATP
NAD+
FMN
CoQ
b
c
a
a3
An electron transport assembly
Adenosine triphosphate (ATP) synthesizing structure
MATRIX
• TCA cycle enzymes
• Fatty acid oxidation enzymes
• mtDNA, mtRNA
• Mitochondrial ribosomes

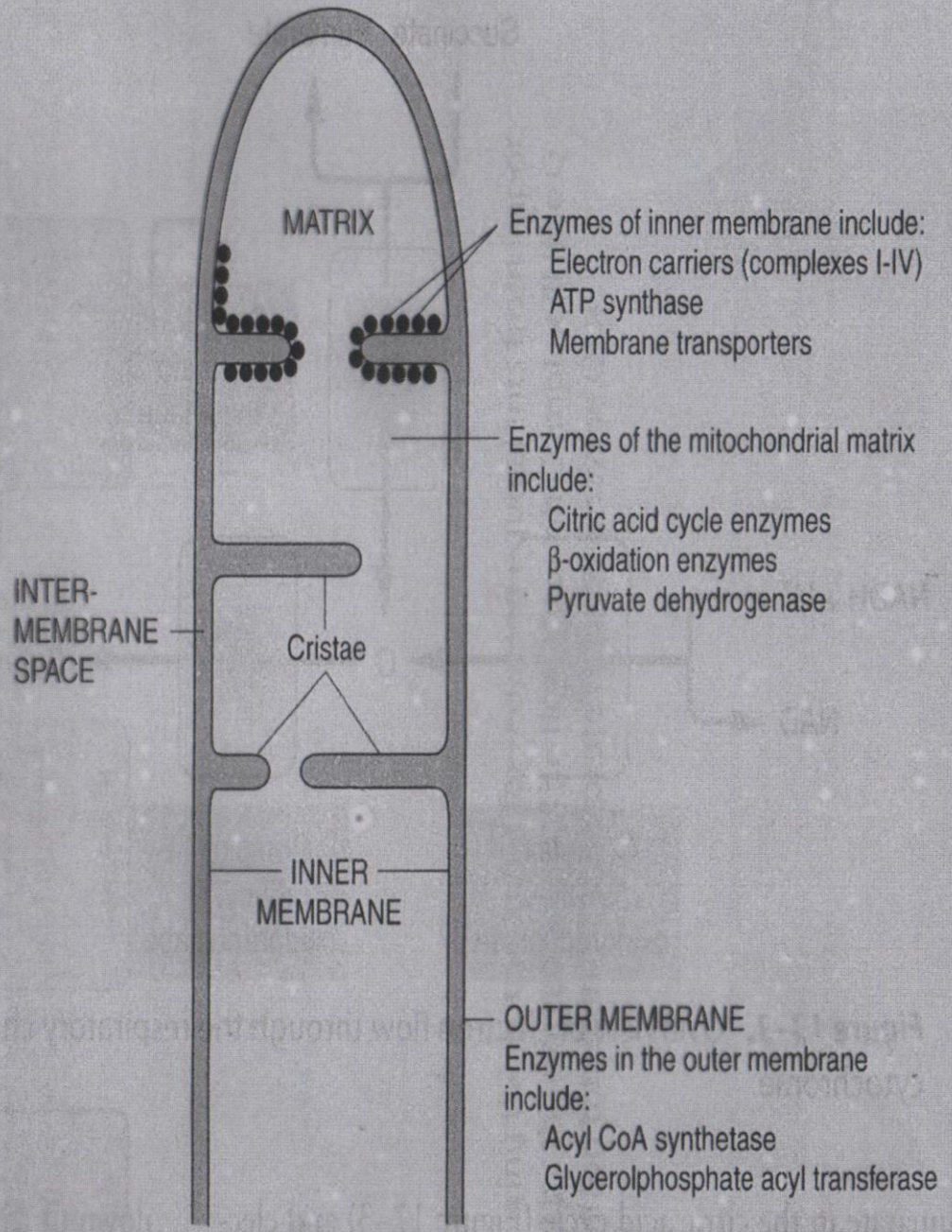


Figure 13–1. Structure of the mitochondrial membranes. Note that the inner membrane contains many folds, or cristae.

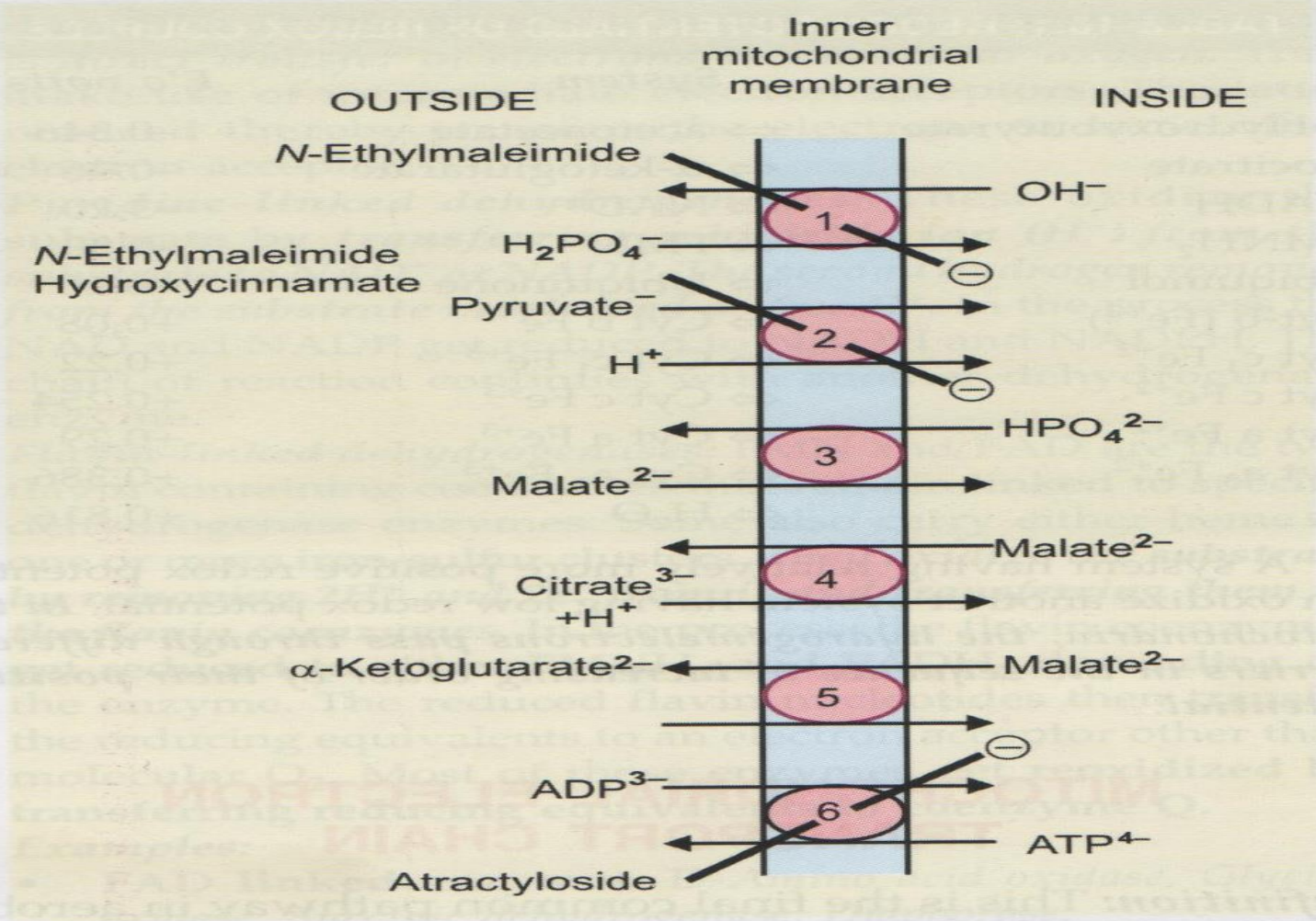


FIG. 10.1: SHOWS SOME OF THE TRANSPORTER SYSTEM IN INNER MITOCHONDRIAL MEMBRANE AND THEIR INHIBITORS. TRANSPORTER SYSTEMS IN THE INNER MITOCHONDRIAL MEMBRANE. (1) PHOSPHATE TRANSPORTER; (2) PYRUVATE SYMPORT; (3) DICARBOXYLATE TRANSPORTER; (4) TRICARBOXYLATE TRANSPORTER; (5) α-KETOGLUTARATE TRANSPORTER; (6) ADENINE NUCLEOTIDE TRANSPORTER. N-ETHYL-MALEIMIDE, HYDROXYCINNAMATE, AND ATRACTYLOSIDE INHIBIT (–) THE INDICATED SYSTEMS

E.T.C

- All the components of E.T.C are located in the inner membrane of mitochondria (except Cyt-C)
- In E.T.C, The electrons are transffered from NADH and FADH to electron carriers.
- `
- Four distinct multi-Protien complexes named as Complex-I,II,III and IV , which are connected by two mobile carriers i.e Co-Q and Cyt-C and
- Complex-V (ATP-Synthase) involved in the production of ATP

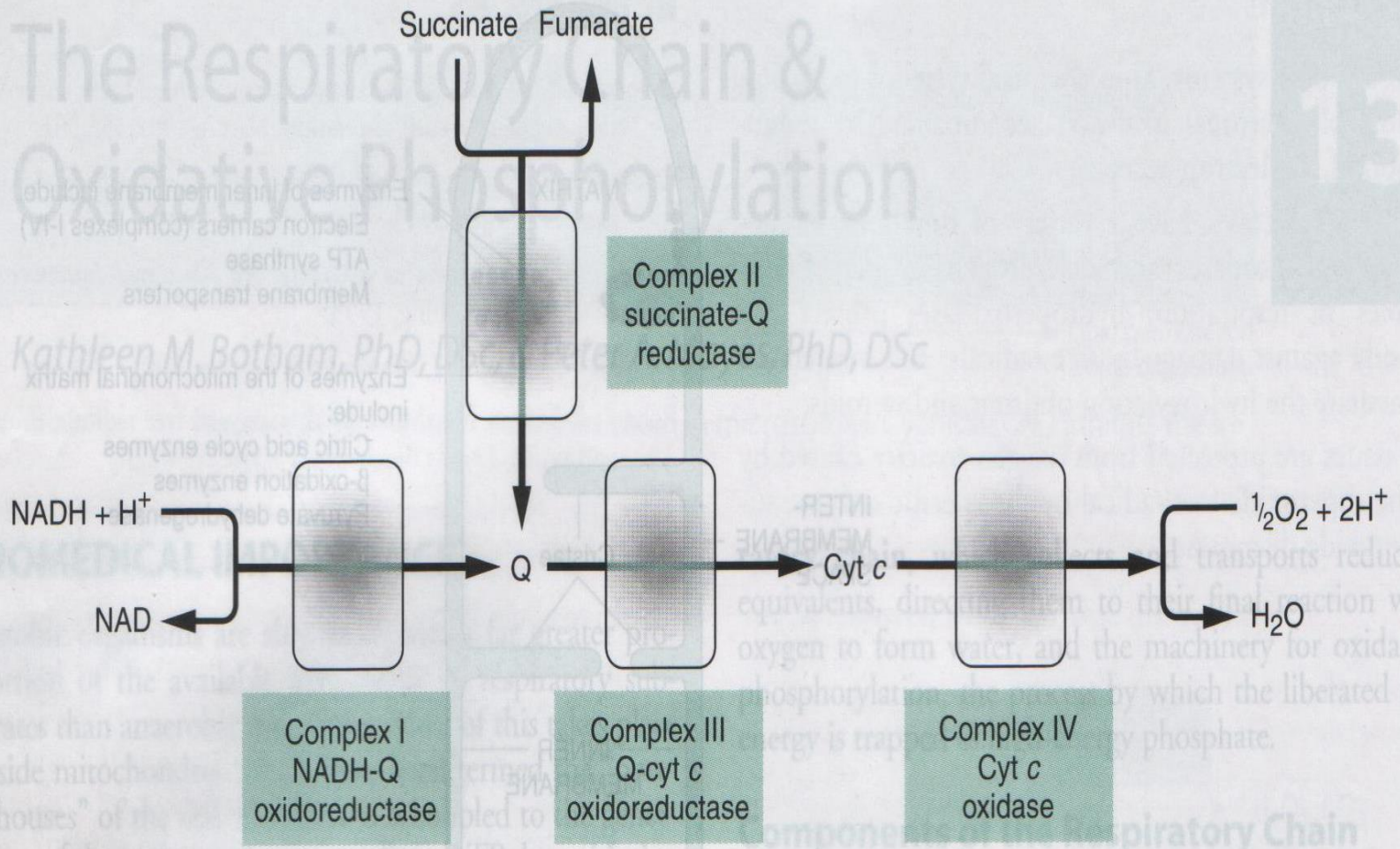


Figure 13–3. Overview of electron flow through the respiratory chain. Q, co-enzyme Q or ubiquinone; cyt, cytochrome.

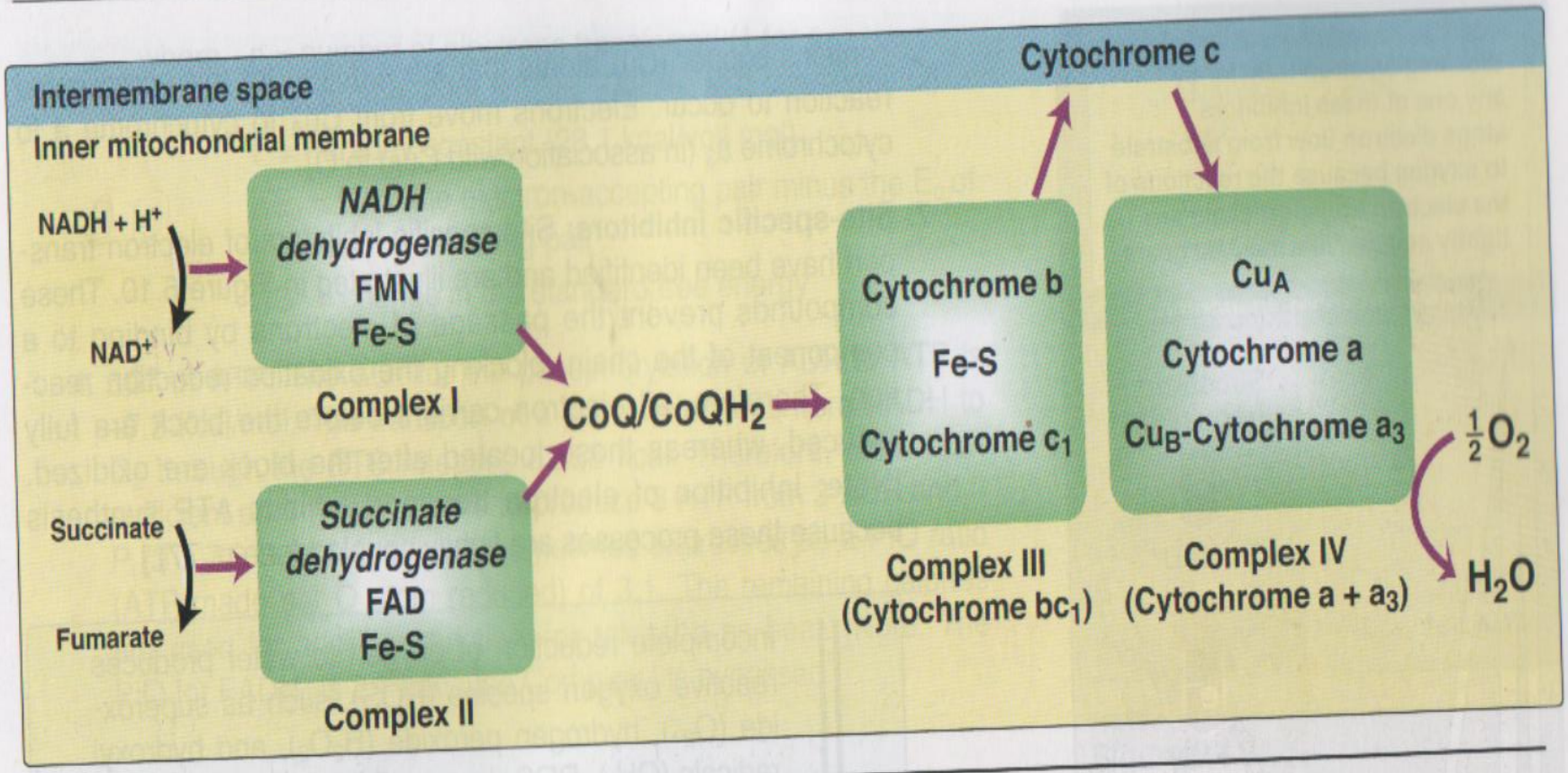


Figure 6.8
Electron transport chain. The flow of electrons is shown by the magenta arrows. NAD(H) = nicotinamide adenine dinucleotide; FAD = flavin adenine dinucleotide; FMN = flavin mononucleotide; Fe-S = iron-sulfur center; CoQ = coenzyme Q.

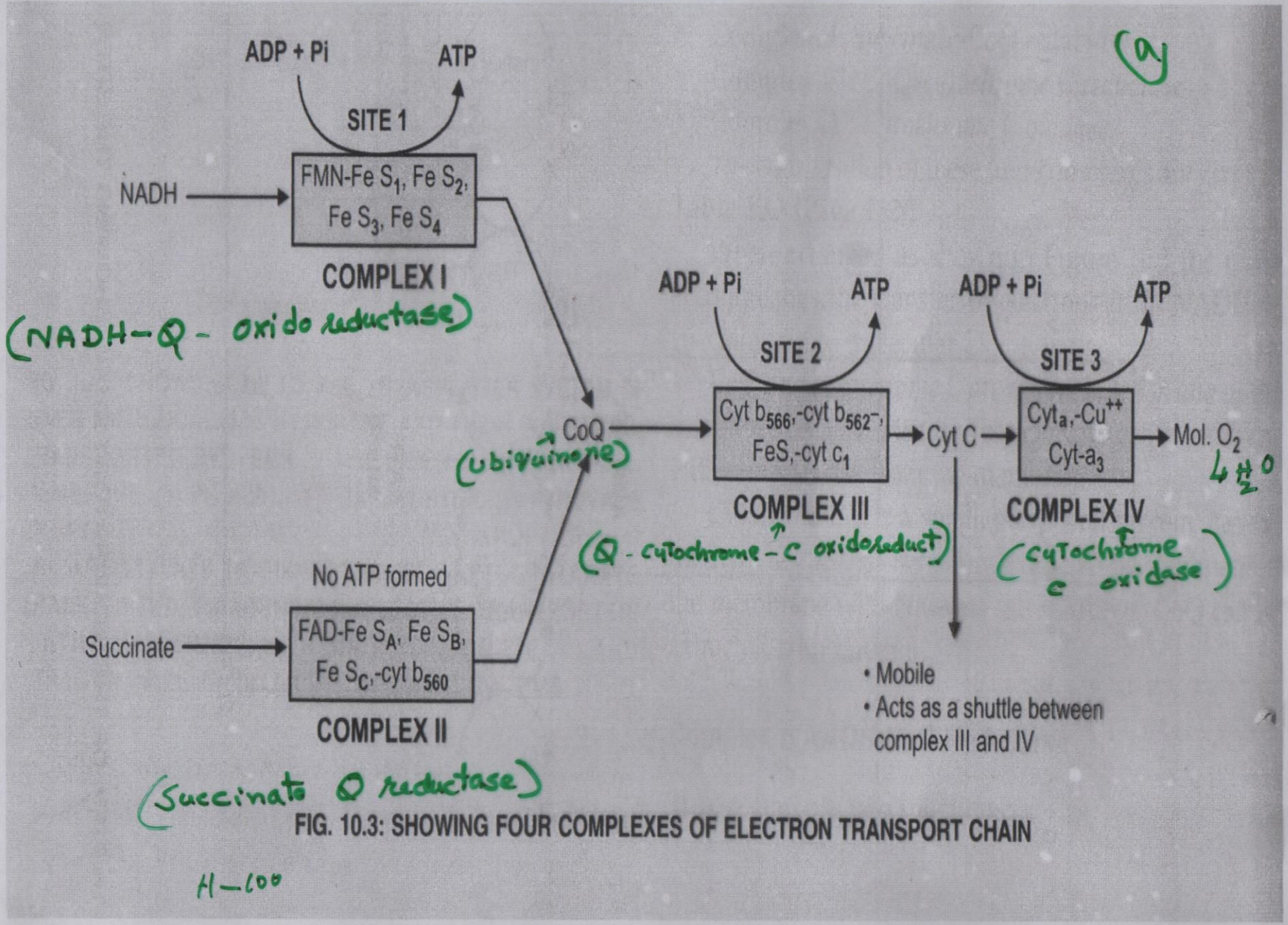


FIG. 10.3: SHOWING FOUR COMPLEXES OF ELECTRON TRANSPORT CHAIN

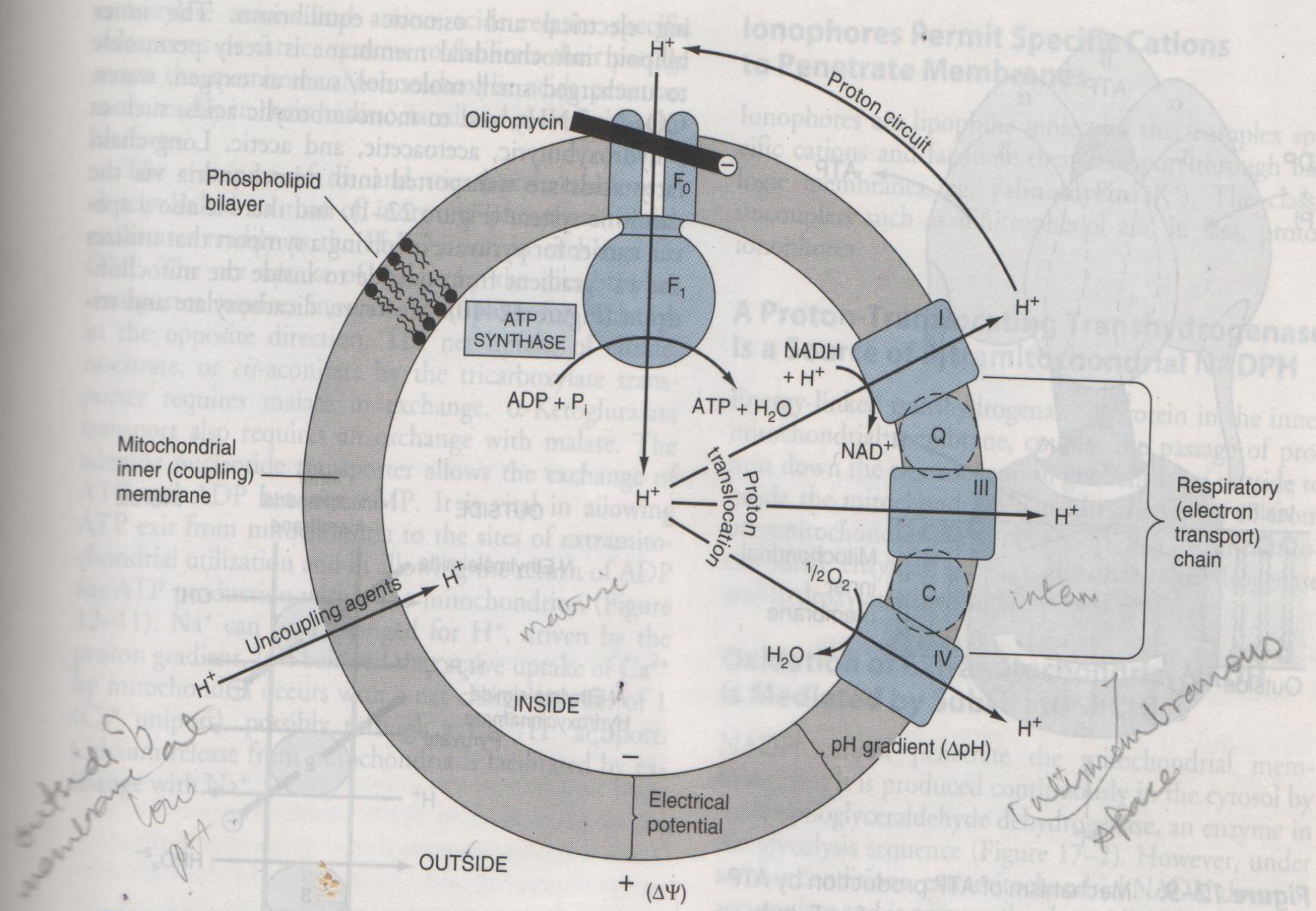


Figure 12–8. Principles of the chemiosmotic theory of oxidative phosphorylation. The main proton circuit is created by the coupling of oxidation in the respiratory chain to proton translocation from the

- The flow of electrons through E.T.C from electron donors e.g NADH to electron acceptor e.g oxygen is

EXERGONIC process i.e it releases energy.

- The synthesis of ATP is ENDERGONIC process ,which requires an input of energy.

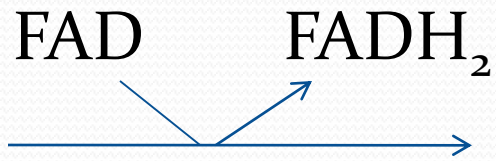
- The Free proton plus hydride ion carried by NADH are transferred to NADH dehyrogenase, embaded in inner mitochondrial membrane.(complex-I)
- Than the FMN accept two hydrogen atoms

  ($2e^- + 2H^+$)becoming $FMNH_2$, then to iron of iron sulphur centre, and then two COQ
- Up to CoQ, Hydrogen atoms are transferred but from CoQ onward , only electrons are transferred.

  $2H^+$ goes in to the medium.

- As electrons flow, they lose energy.
- Part of this energy is used to pump protons across the inner mit membrane in to intermembrane space.
- Rest is used for production of Heat.

- The small energy change (+0.113V) does not allow complex II to pump protons, So does not contribute in ATP formation.



This enzyme system is present in complex II, so this reaction of TCA take place in complex II

- Up to CoQ, H is transferred but from CoQ onward , only electrons are transferred.

$2H^+$ goes in to the medium.

COENZYME Q

- Quinone derivative with isoprenoid tail
- lipid soluble component of ETC
- Mobile carrier
- Can accept hydrogen atoms from

  Complex I

  Complex II and mitochonrial dehydrogenases

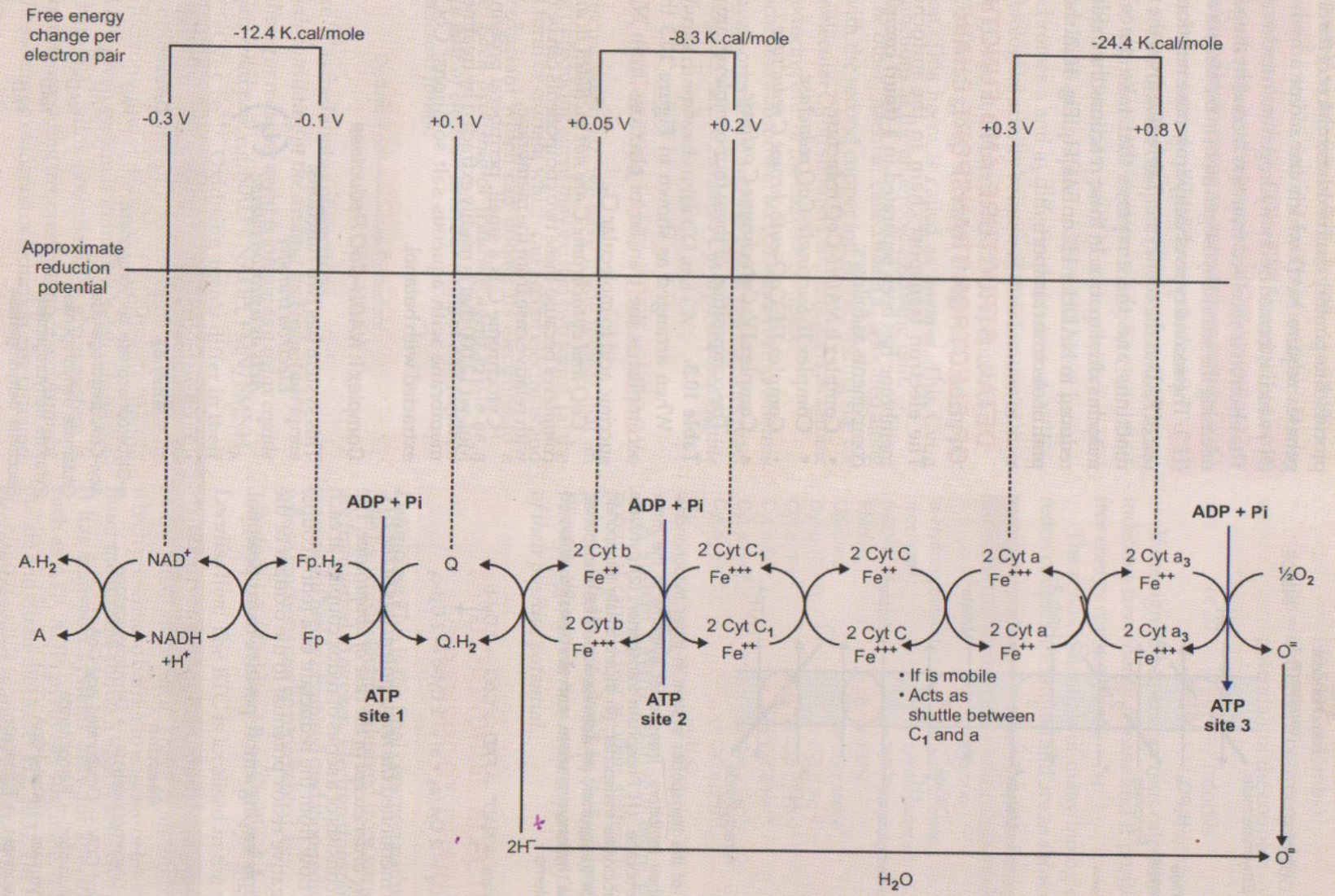


Fig. 10.2: The electron transport system of the respiratory chain showing the sites of formation of 3ATP molecules

RELEASE OF FREE ENERGY DURING ELECTRON TRANSPORT

The free energy released as electrons are transferred along the ETC from an electron donor (reducing agent or reductant) to an electron acceptor (oxidizing agent or oxidant) is used to pump protons at Complexes I, III and IV.

[Note: The electrons can be transferred as hydride ions (:H-) to NAD+ : as hydrogen atoms (**.**H) to FMN, CoQ, and FAD: or as electrons (e-) to cytochromes.]

Incomplete reduction of oxygen to water produces reactive oxygen species (ROS), such as superoxide ($O_2^{-}\cdot$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH\cdot$). ROS damage DNA and proteins and cause lipid peroxidation. Enzymes such as *superoxide dismutase* (*SOD*), *catalase*, and *glutathione peroxidase* are cellular defenses against ROS.

CHEMIOSMOTIC HYPOTHESIS (Mitchell Hypothesis)

- Explains how the free energy generated by the transport of electrons by the ETC is used to produce ATP from ADP+ Pi.

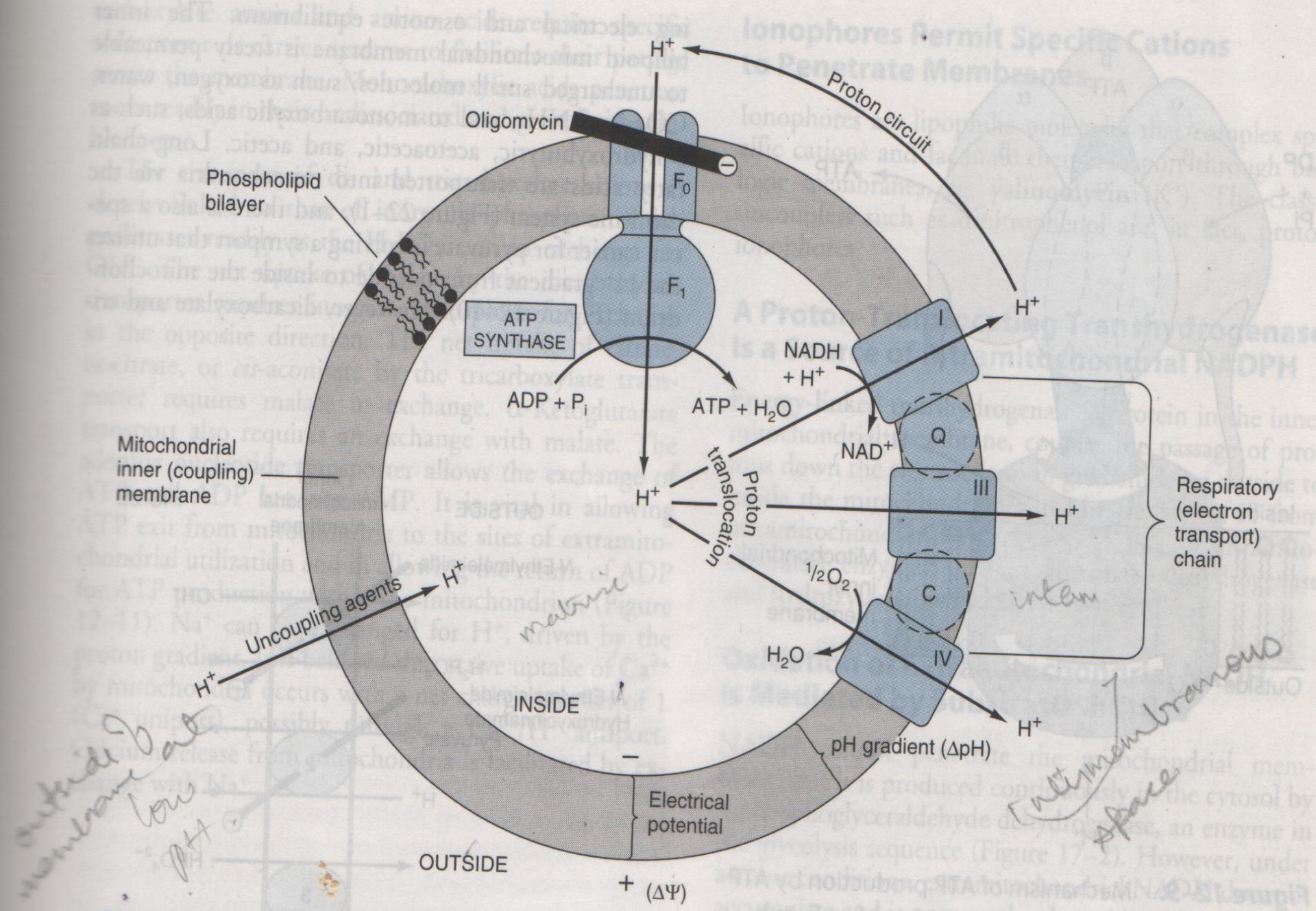


Figure 12–8. Principles of the chemiosmotic theory of oxidative phosphorylation. The main proton circuit is created by the coupling of oxidation in the respiratory chain to proton translocation from the

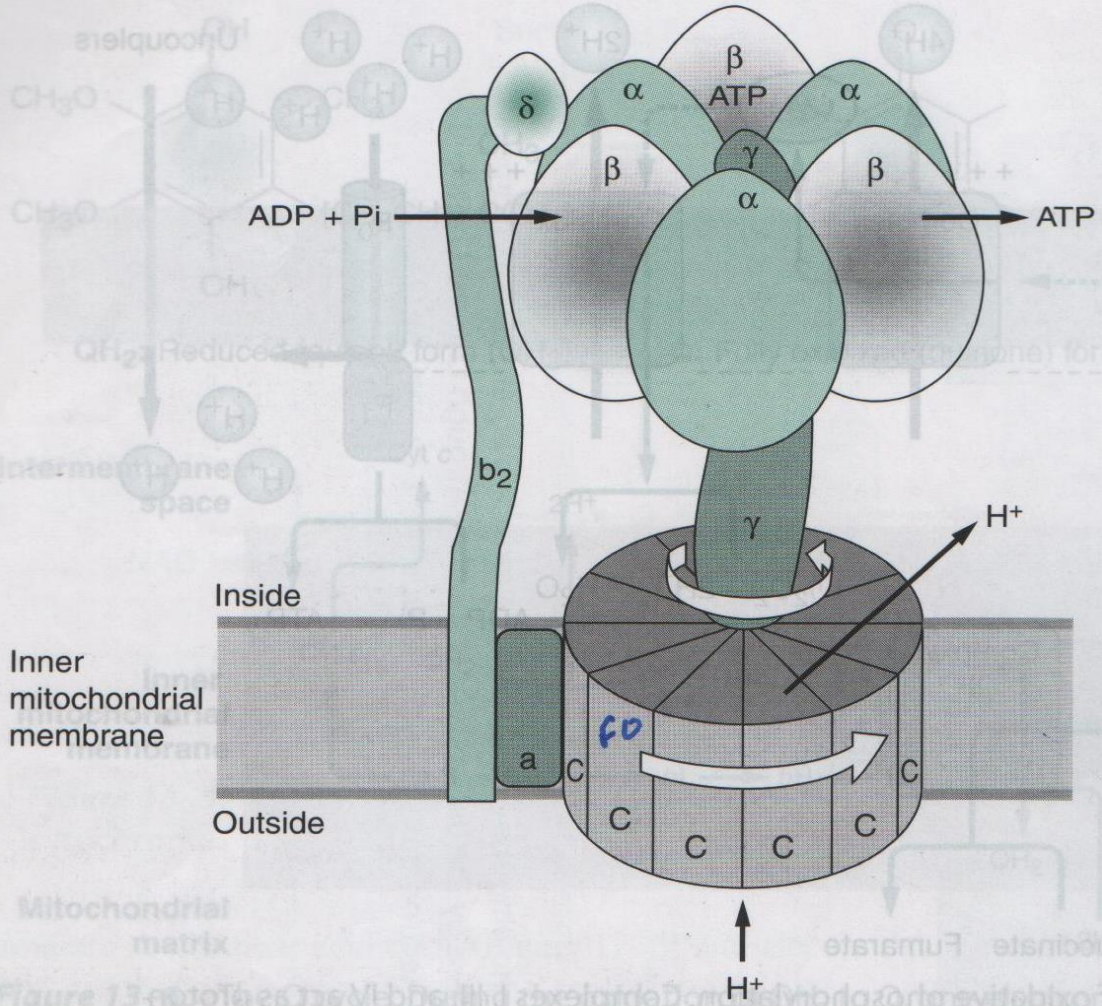


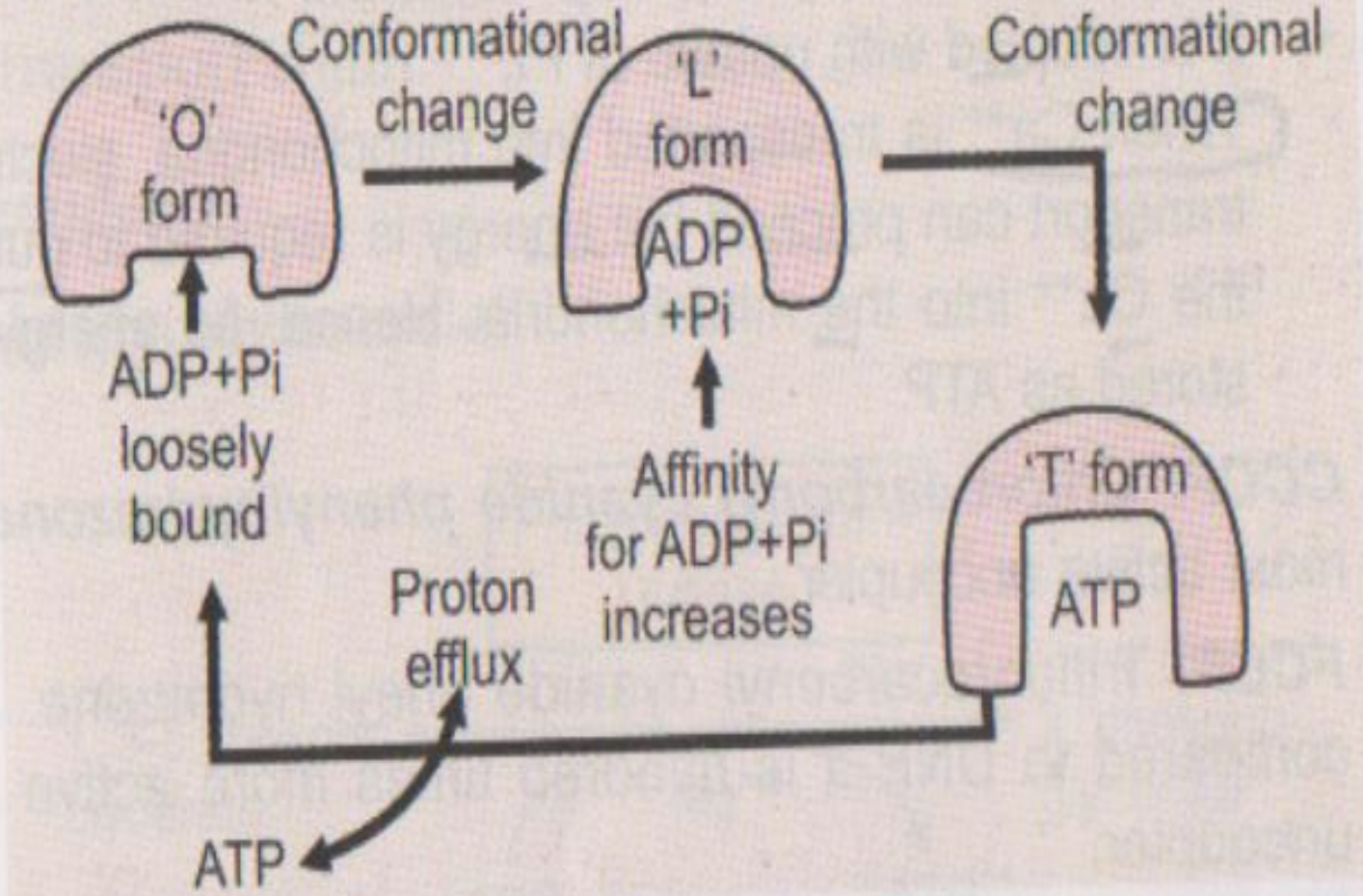
Figure 13–8. Mechanism of ATP production by ATP synthase. The enzyme complex consists of an F_0 subcomplex which is a disk of "C" protein subunits. Attached is a γ subunit in the form of a "bent axle." Protons passing through the disk of "C" units cause it and the attached γ subunit to rotate. The γ subunit fits inside the F_1 subcomplex of three α and three β subunits, which are fixed to the membrane and do not rotate. ADP and P_i are taken up sequentially by the β subunits to form ATP, which is expelled as the rotating γ subunit squeezes each β subunit in turn and changes its conformation. Thus, three ATP molecules are generated per revolution. For clarity, not all the subunits that have been identified are shown—eg, the "axle" also contains an ε subunit.

THE RESPIRATORY CHAIN PROVIDES MOST OF THE ENERGY CAPTURED DURING CATABOLISM

Respiratory Control Ensures a Constant Supply of ATP

The rate of respiration of mitochondria can be controlled

- Boyer’s hypothesis


'O' form
Conformational change
'L' form
ADP +Pi
Conformational change
ADP+Pi loosely bound
Affinity for ADP+Pi increases
'T' form
ATP
Proton efflux
ATP

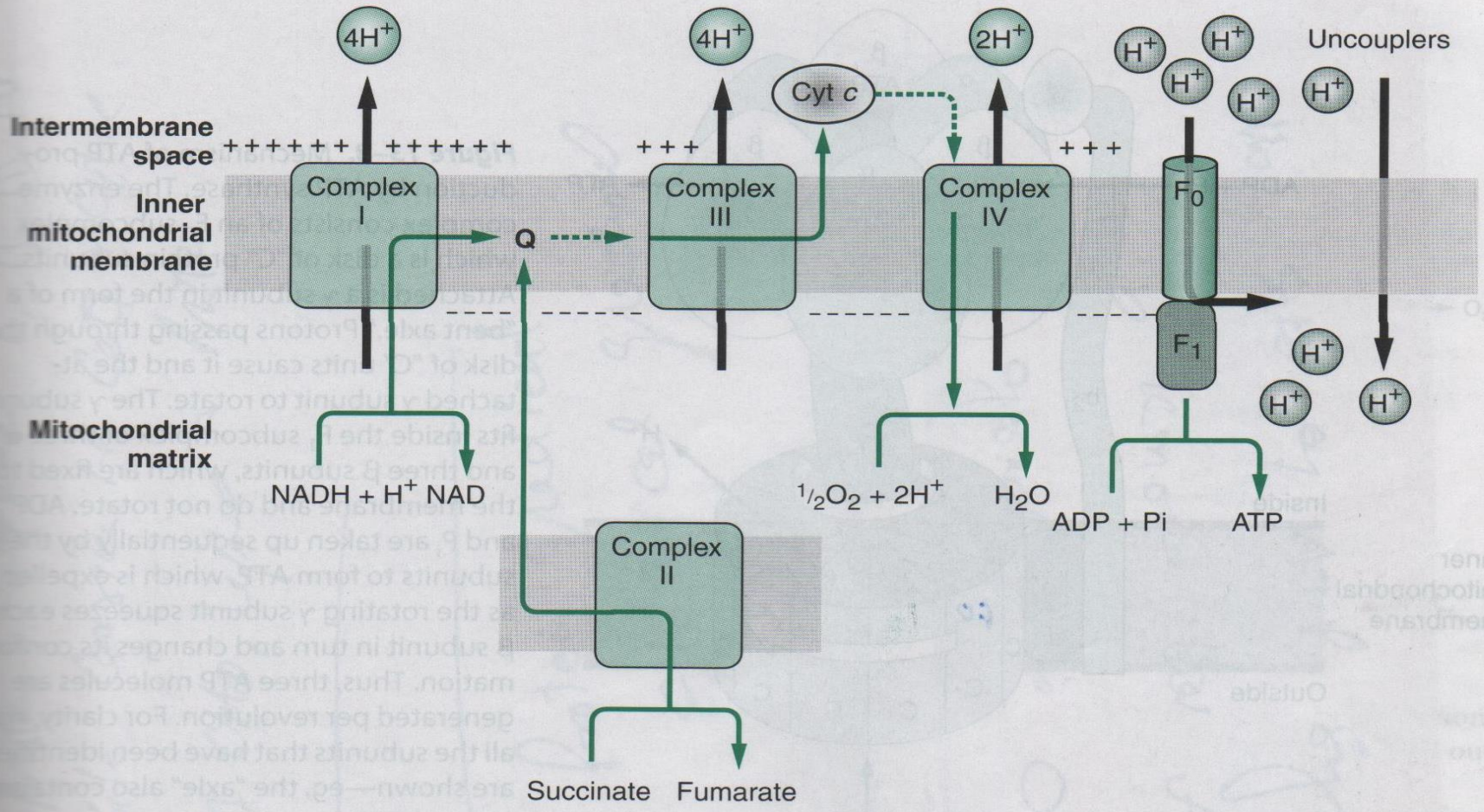


Figure 13–7. The chemiosmotic theory of oxidative phosphorylation. Complexes I, III, and IV act as proton pumps creating a proton gradient across the membrane, which is negative on the matrix side. The proton motive force created drives the synthesis of ATP as the protons flow back into the matrix through the ATP synthase enzyme (see Figure 13–8). Uncouplers increase the permeability of the membrane to ions, collapsing the proton gradient by allowing the H^+ to pass across without going through the ATP synthase and thus uncouple electron flow through the respiratory complexes from ATP synthesis. Q, co-enzyme Q or ubiquinone; cyt, cytochrome.

damaging intermediates such as superoxide anions

A Membrane-Located ATP Synthase

- Molecular oxygen is an ideal terminal electron acceptor and oxidative phosporylation is a vital part of metabolism
- Incomplete reduction of oxygen due to electron leak produces reactive oxygen species such as superoxide and H_2 $O_{2,}$ damaging cells and contributing disease and possibly aging.
- Enzymes such as SOD, catalase, and glutathione per oxydase are cellular defenses against ROS.

Learning Objectives:

- Inhibitors of Respiratory chain
- Inhibitors of Oxidative Phosphorylation
- Uncouplers
- Significance of uncouplers
- Inherited disorders of E.T.C
- Shuttle systems including:
- Glycerolphosphate shuttle system
- Malate shuttle

Inhibitors of E.T.C & oxidative phosphorylation:

- This may be classified as
- 1. Inhibitors of respiratory chain
- 2.Inhibitors of oxidative Phosphorylation
- 3.Uncoupler

1. Inhibitors of respiratory chain

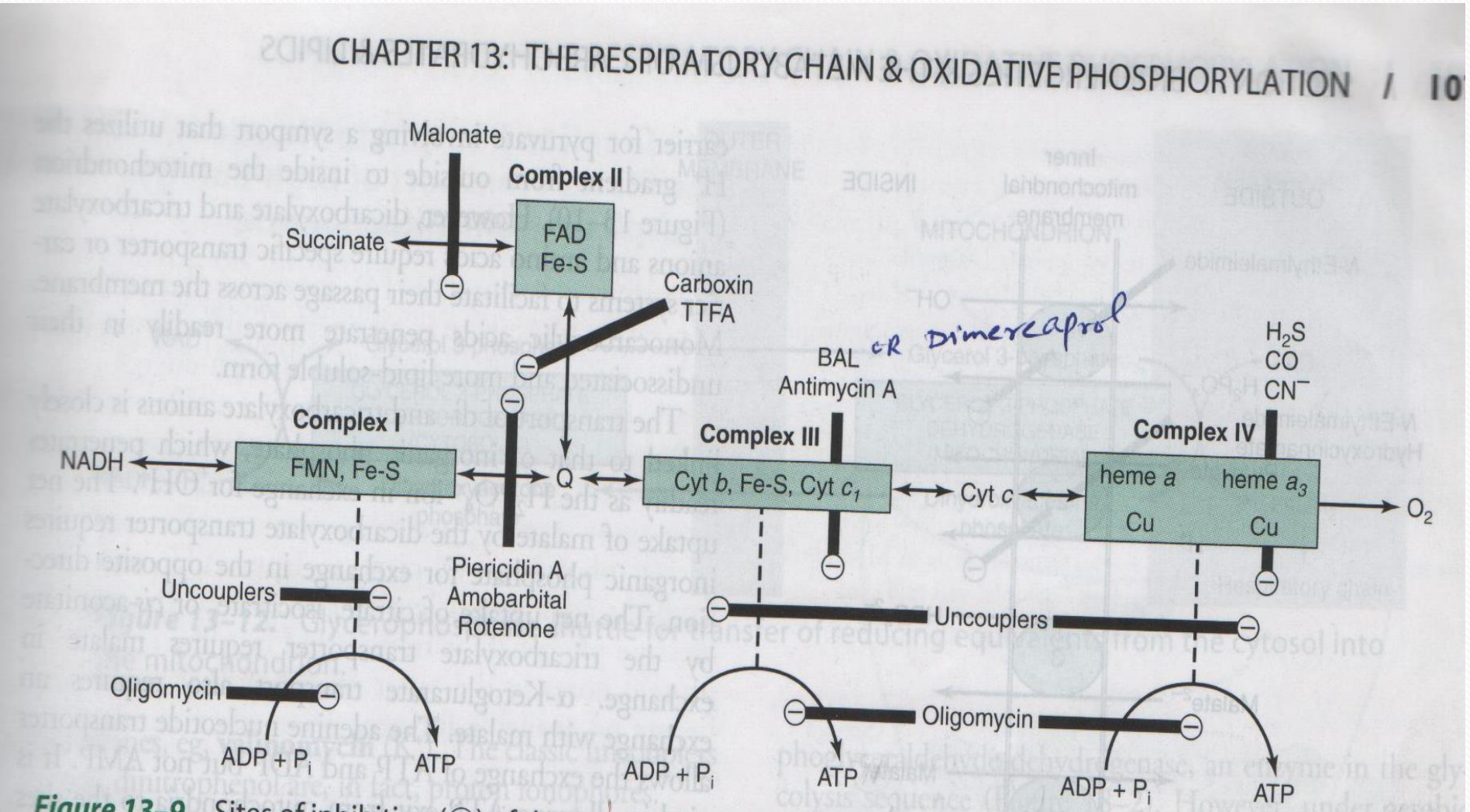


Figure 13–9. Sites of inhibition (⊖) of the respiratory chain by specfic drugs, chemicals, and antibiotics. BAL, dimercaprol. TTFA, an Fe-chelating agent. Other abbreviations as in Figure 13–5.

Inhibitors of ETC

Site-I (Complex-I)

- ***Rotenone:*** A fish poison and also insecticide. Inhibits transfer of electrons through complex-I-*NADH-Q-reductase*.
- ***Amobarbital (Amytal) and Secobarbital:*** Inhibits electron transfer through NADH-Q reductase.
- ***Piericidin A:*** An antibiotic. Blocks electron transfer by competing with CoQ.
- ***Drugs:*** Chlorpromazine and hypotensive drug like guanethidine.

Site-II (Complex III)

- ***Antimycin A***
- ***BAL (Dimer-Caprol)***
- ***Hypoglycaemic drugs: like Phenformin***

} Blocks electron transfer from cyt b to c_1

Site-III (Complex IV)

- ***Cyanide***
- ***H_2S***
- ***Azide***

} Inhibits terminal transfer of electrons to molecular O_2

- ***Co (Carbon monoxide):*** Inhibits Cyt. oxidase by combining with O_2 binding site. It can be reversed by illumination with light.

Complex II: *Succinate dehydrogenase FAD*

- ***Carboxin***
- ***TTFA***

} Specifically inhibit transfer of reducing equivalent from *succinate dehydrogenase*

- ***Malonate:*** A competitive inhibitor of succinate dehydrogenase.

2.Inhibitors of oxidative Phosphorylation

- 1.Oligomycin:

It binds with the enzyme ATP synthase and blocks the proton channels

- Atrctyloside:

It is a glycoside, it blocks the translocase that is responsible for movement of ATP and ADP

- Bongregate:

Toxin produced by Pseudomonas.It acts similarly to atractyloside .

3. Uncoupler

Inhibit the coupling between the electron transport and phosphorylation and thus inhibit ATP synthesis without affecting the respiratory chain and ATP synthase (Complex V) by

increasing the permeability of inner mitochondrial membrane to protons

2,4,Dinitrophenol,

- dinitrocresol
- Toxic doses of Aspirin
- valinomycin
- Germmicidin A.
- FCCP

2,4 Dinitrophenol was used for weight reduction, but now banned.

- FCCP is 100 times more effective as un coupler then DNP.

- CCCP
- VALINOMYCIN
- DICOUMAROL(Vitamin K analogue)
- CALCIUM

Physiological Uncouplers ARE

- Thermogenin, Excessive Thyroxine, Long chain Free Fatty acids, Un conjugated hyperbilirubinaemia and EFA dificiency.

:Thermogenin: is Present in Brown Adipose tissues

:Much in infants, but also in upper back and Neck of adult and in some animals.

SINGFICANCE OF UNCOUPLING

- Maintenance of body Temperature in infants, hairless animals, hibernating animals
- Thermogenin blocks the formation of ATP and generates heat.

Coupler

The E.T.C and Phosphorylation are coupled

by Proton gradient.

The Electrochemical Potential difference across the membrane, once established as a result of Proton Translocation, inhibits further transport of reducing equivalents through the E.T.C unless this proton gradient is dissipated by entry of Proton, through A.T.P synthase.

IONOPHORES

They complex the specific cations and facilitate their transport through biological membranes, due to their Lipophilic property.

e.g. Valinomycin, helps in penentration of K+ through mitochondrial membrane

The classical uncoupler e.g D.N.P is infact Proton ionophore.

Oxidative phosphorylation

Oxidative processes, such as the TCA cycle and the β-oxidation of fatty acids

produce

NADH and $FADH_2$

donate electrons to

Electron transport chain

comprised of

- FMN, FAD-containing *dehydrogenases*
- CoQ (coenzyme Q)
- Cytochrome bc_1
- Cytochrome c
- Cytochrome a + a_3 (*cytochrome oxidase*)

involved in → Apoptosis

→ Only component that can react directly with oxygen

leads to

Electron flow

coupled with

Transport of protons (H^+)

from

The matrix to the intermembrane space

creating

An electrical and a pH gradient

across

The inner mitochondrial membrane

Notable because

- Rich in protein
- Impermeable to most small molecules
- Contains trans-porters for specific compounds

allowing

Protons to reenter the mitochondrial matrix

by

Passing through F_o channel in the *ATP synthase* complex (Complex V)

Notable because

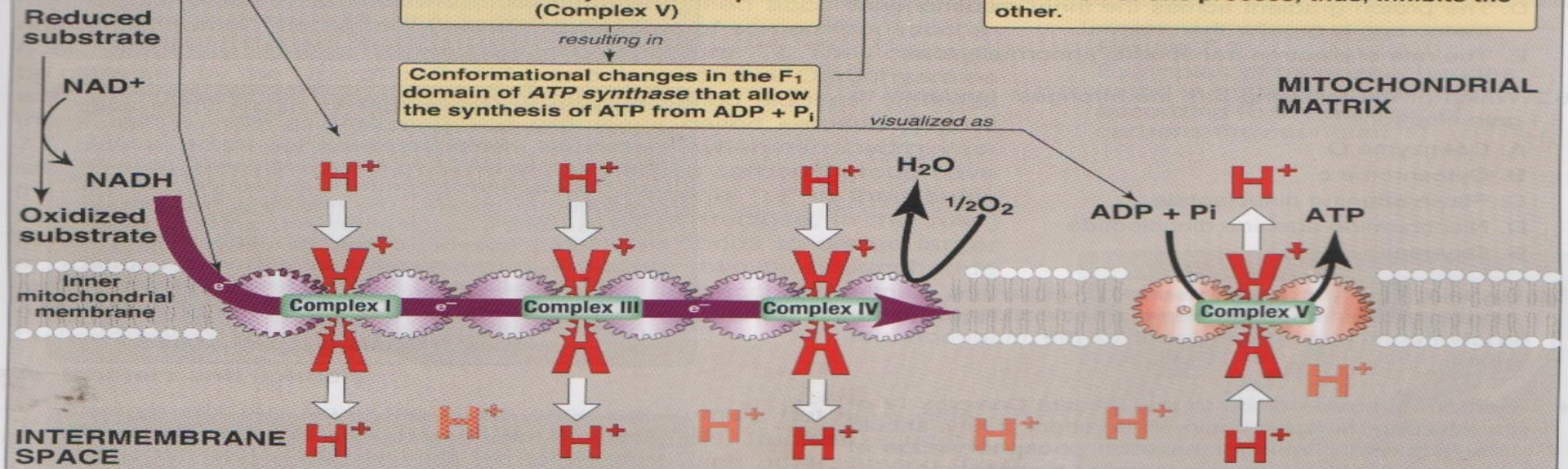
Electron transport and phosphorylation are tightly coupled processes, and the proton gradient is the common intermediate. Inhibition of one process, thus, inhibits the other.

resulting in

Conformational changes in the F_1 domain of *ATP synthase* that allow the synthesis of ATP from ADP + P_i

visualized as

visualized as



Inherited Disorders

1.Infentile Mitochondrial myopathy

-Deficiency of most of oxidoreductases of E.T.C

2. MELAS:

-NADH-Ubiquinone oxido reductase deficiency (complex-I)

-Deficiency of cytochrome oxidase

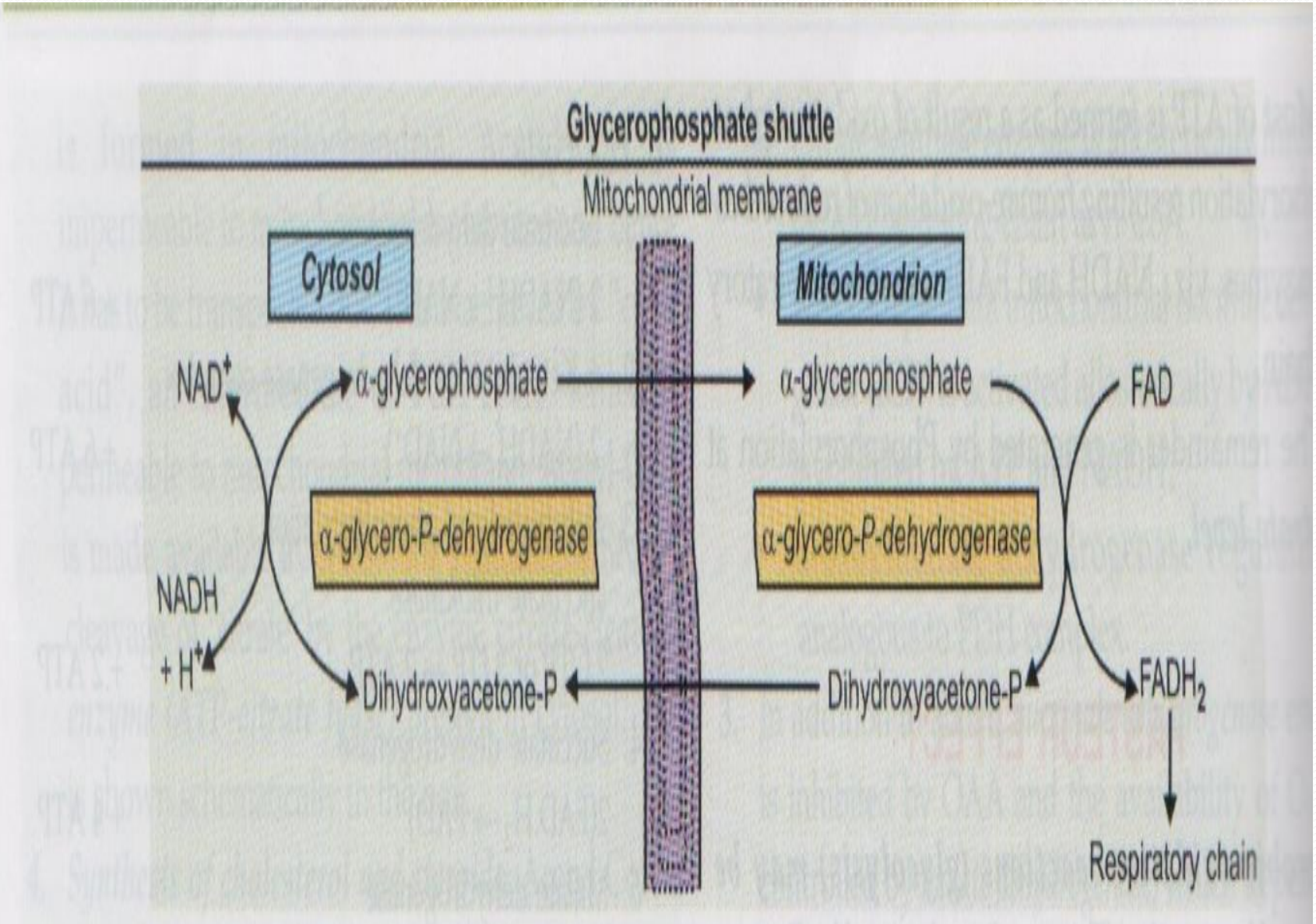
SHUTTLE SYSTEMS

- In Glycolysis NADH is produced in cytosole.
- Inner mitochondrial membrane is impermeable to NADH.
- Specific Dehydrogenases Act as shuttle to transport two electrons (reducing equivalent) of NADH from cytosol in to matrix.

Two such shuttle systems are

1. Glycerophosphate Shuttle.
2. Malate shuttle

Glycerolphosphate shuttle system



Glycerolphosphate shuttle system

-Glycero-P-dehydrogenase Enzyme in Cytosole is NAD-Linked

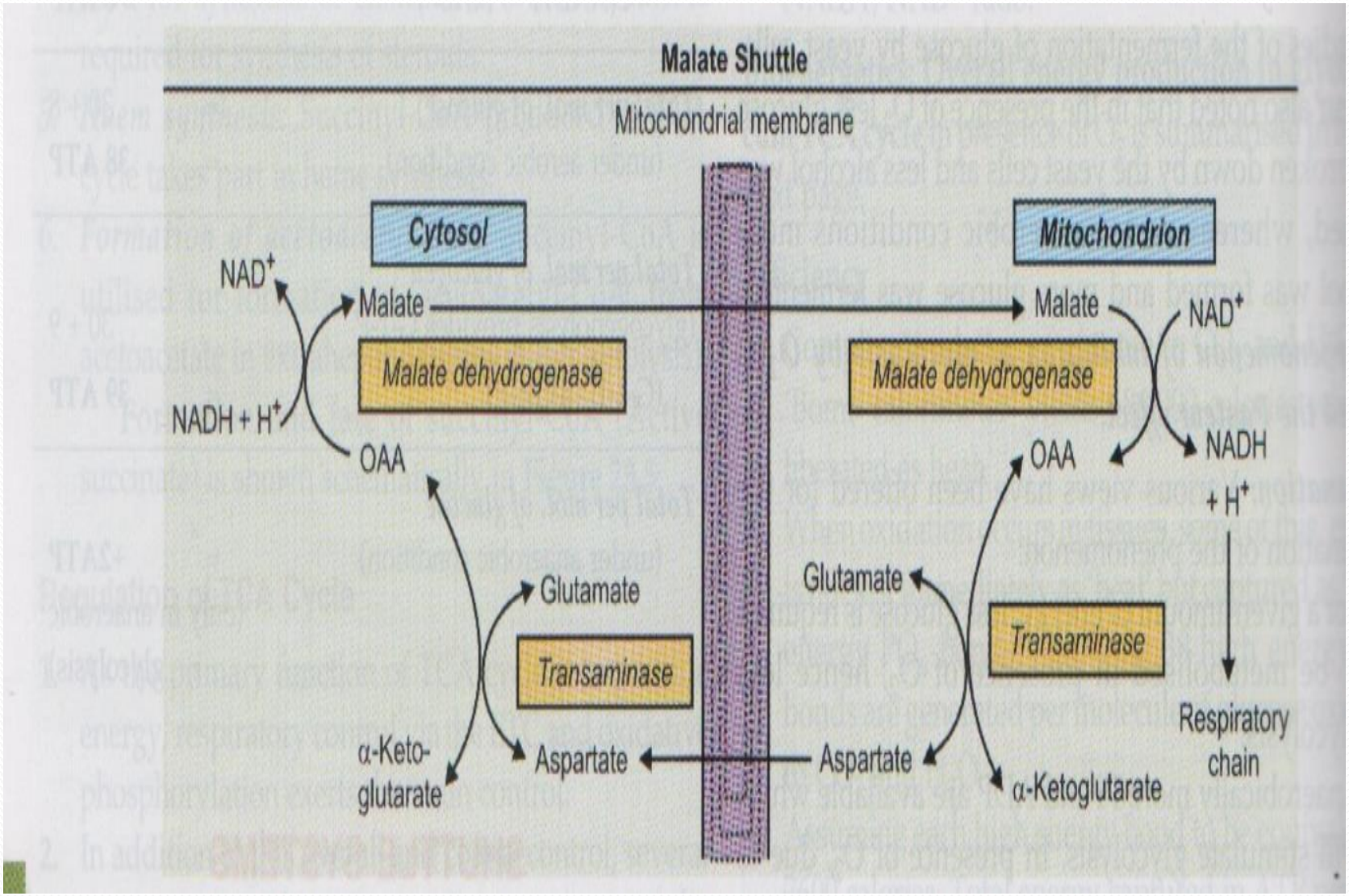
But in mitochondria, it is FP-Dependant(i.e FAD)

-Might be important in liver, and heat muscle,but mitochondrial FP-dependant G-P-D is deficient in other tissue.

-FAD linked mitochondrial G.P.D decreases after thyroidectomy and increases after thyroxine Administration

-Cytosolic NADH will give 2ATP by this shuttle.

Malate Shuttle



Malate Shuttle

-This shuttle system is more common and universe
-Reduced NADH is reformed in mitochondria.
-Rather complex as OAA is impermeable to mitochondrial membrane .
:Cytosolic NADH will give 3ATP by this shuttle.

-So by Glycrophosphate shuttle
Net ATP by Glycolysis
TCA cycle per molecule of Glucose will be 36ATP (2 ATP Less)
-By Malate shuttle it will be 38ATP (More universe)

3 High Energy Compounds:

These Compounds when hydrolysed, release a large quantity of Energy. The high Energy bond in these compound is indicated by Squiggle (~) bond.

The free energy of hydrolysis is indicated by $\Delta G^{\circ\prime}$

Energy Rich Comp	$\Delta G^{\circ\prime}$ in K cal/mole
Phosphate Compounds	
1- Nucleotides ATP, GTP, UTP	− 7.3 K Cal
2- Creatine − P	− 10.5 K Cal
3- 1,3, bis-P. glycerate	− 10.1 K Cal.
4- Phospho enol Pyruvate	− 14.8 K Cal.
Sulfur Compounds	
5- Acetyl-CoA, Succinyl CoA, HMG CoA	− 7.5 K Cal
6- SAM	− 7.0 K Cal

4-a

ATP

. Universal Currency of Energy

- Hydrolysis of ATP to ADP release −7.3 K Cal/mole.
- At rest $Na^+ - K^+$ ATPase uses up one-Third of all ATP formed.
- Other Energy requiring process ar biosynt of macromolecule musle contr, etc.

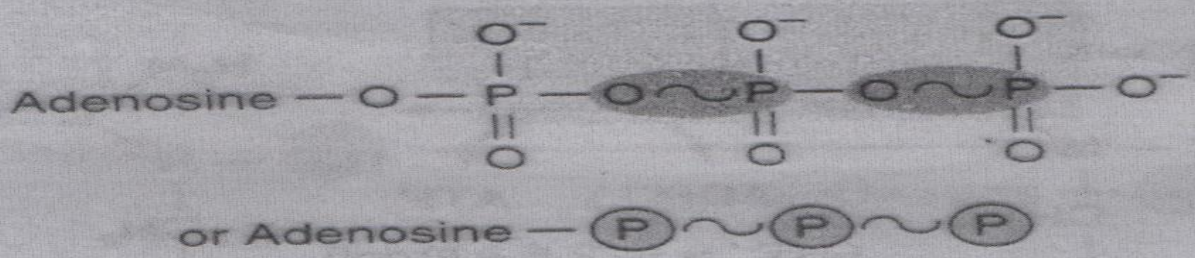
Diff types of reactions performed by ATP

1- Glucose + ATP ⟶ G.6.P + ADP

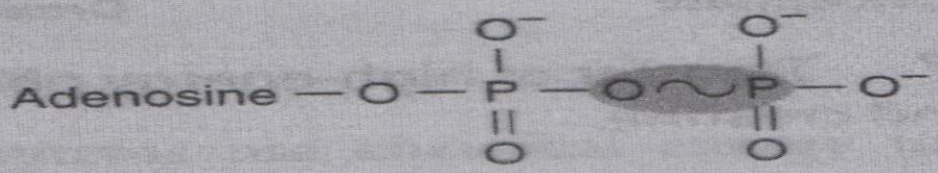
2. F.A + CoASH —(ATP, Mg^{++})→ AMP + PPi → Fatty-acyl-CoA

3: Ribose-5-P —(ATP, Mg^{++})→ AMP → 5-Phosphoribosyl-P-P

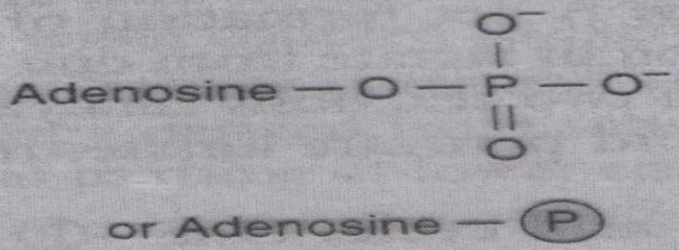
4.. Methionine + ATP ⟶ SAM + PPi + Pi



Adenosine triphosphate (ATP)



Adenosine diphosphate (ADP)



Adenosine monophosphate (AMP)

Figure 10–5. Structure of ATP, ADP, and AMP showing the position and the number of high-energy phosphates (~Ⓟ).

Various Mechanisms of ATP formation

1. Electron Transport Chain and oxid-Phosphorylation
2. Substrate level Phosphorylation

a. Two reactions in glucolysis i.e ATP

i. 1,3,bisphosphoglycerate → 3Phosphoglycerate
(ADP → ATP)

ii. Phosphoenol pyruvate → Enol Pyruvate
(ADP → ATP)

b. One reaction in T.C.A cycle i.e

Succinyl-CoA + ADP → ATP + Succinic Acid

3. In Lohman reaction :

ATP can be formed from creatine-P in muscles

4. Myoknase reaction

In Muscles Two ADP molecules can react to produce one molecule of ATP and AMP.

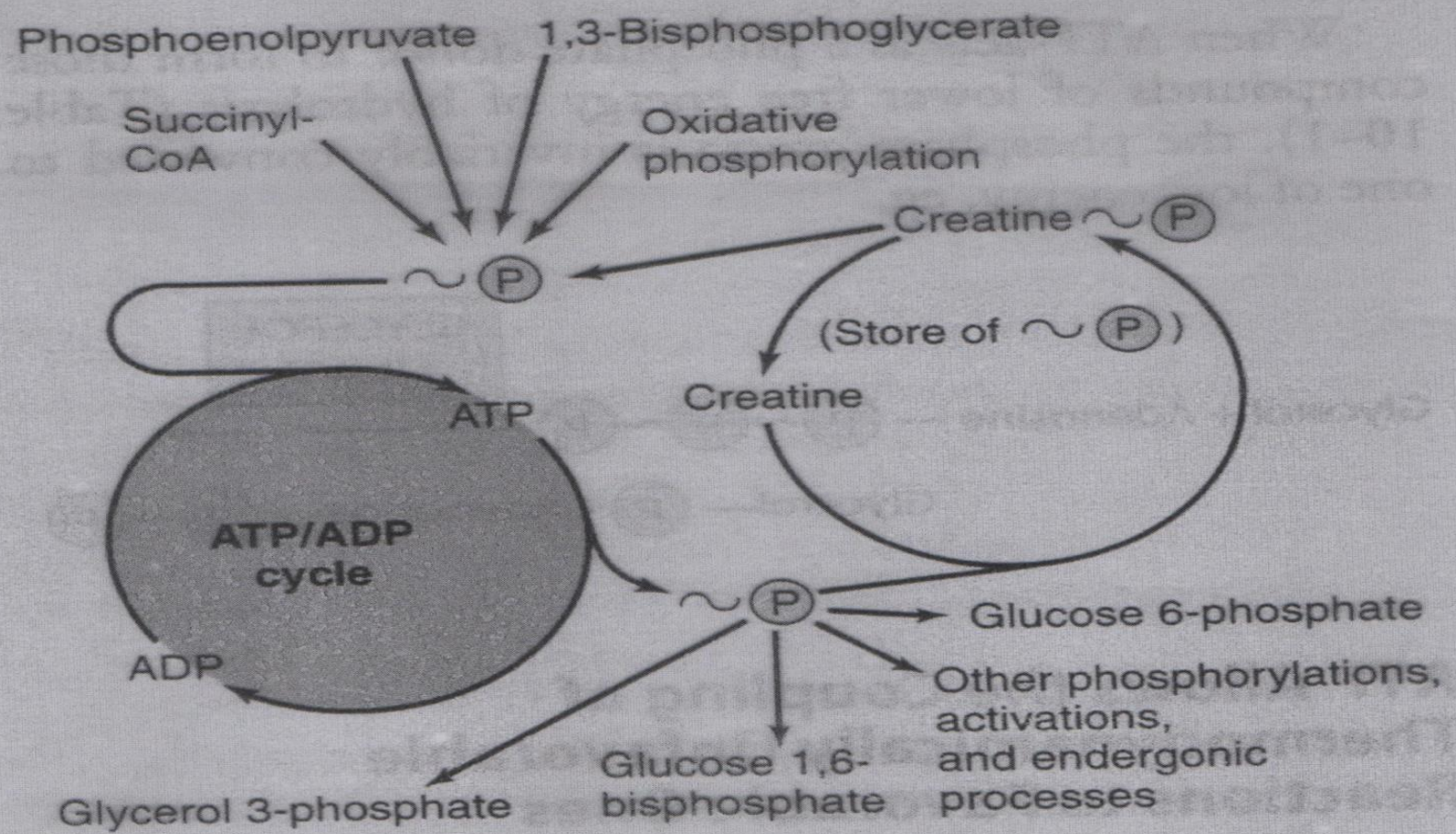


Figure 10–6. Role of ATP/ADP cycle in transfer of high-energy phosphate.

Reducing equivalents for E.T.C

Two electron of NADH and $FADH_2$ which enter the electron transport chain are called Reducing equivalent.