Metabolism

Bioenergetics:

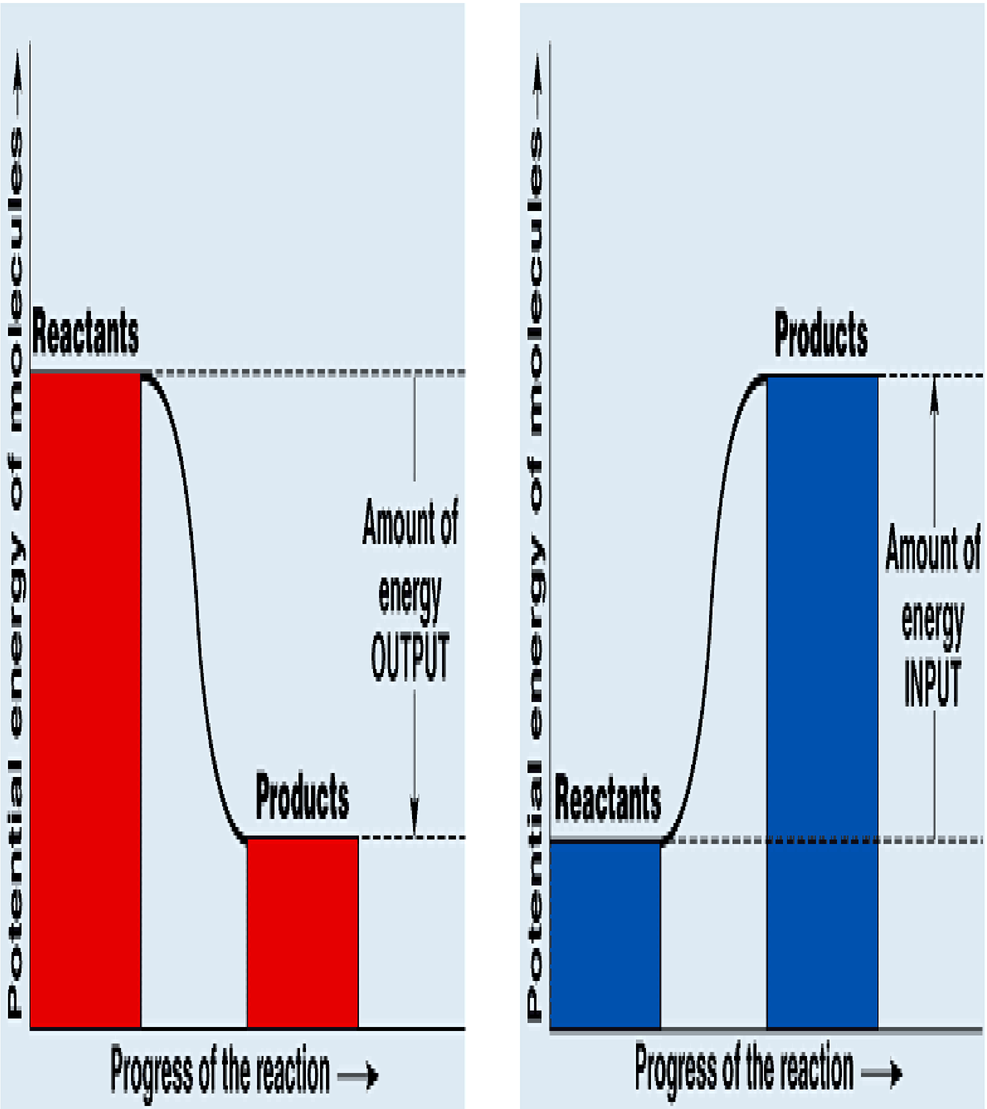
- It is the study of the energy changes accompanying biochemical reactions.
- Biologic systems are essentially isothermic and use chemical energy to power living processes.

Free energy:

- G is energy that can do work when temperature and pressure are uniform, as in a living cell.

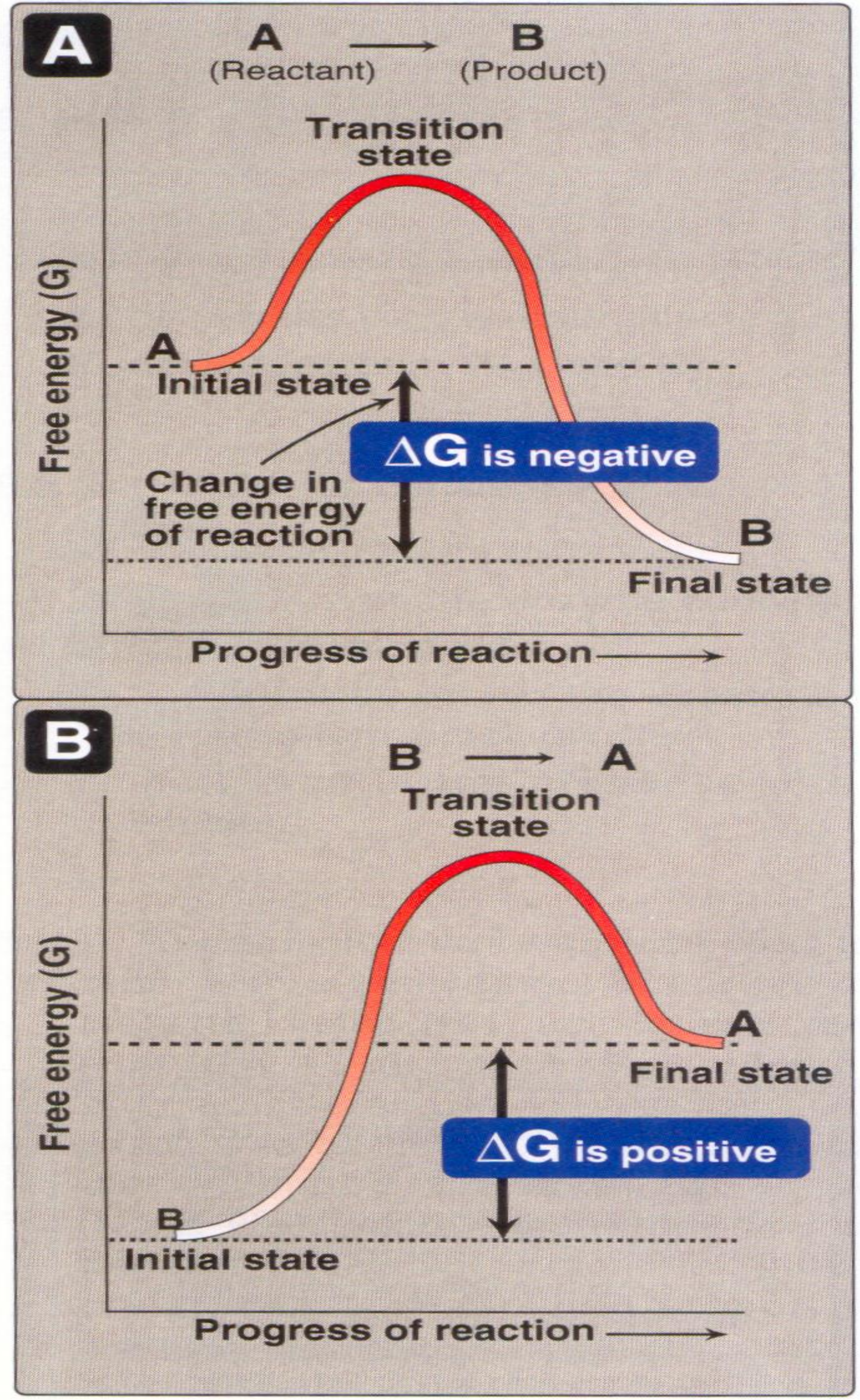
The free energy change (ΔG)

- It is difference between the free-energy content of the products and the free-energy content of the reactants under standard conditions. It depends on the nature and concentration of initial reactants and the final products.



(a) Exergonic reaction (energy released; $\Delta G<0$).

(b) Endergonic reaction (energy required; $\Delta G>0$).



Stages of chemical reactions

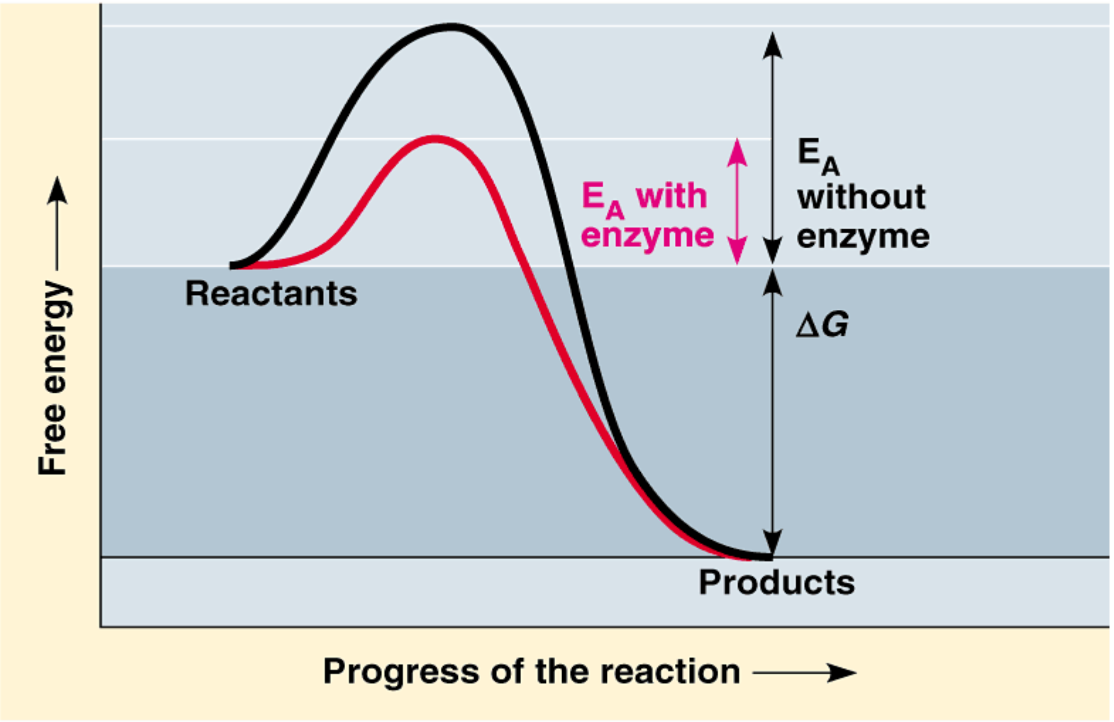
1- Activation Energy:

- Amount of energy that reactant molecules must absorb to start a reaction.
- This energy is usually provided in the form of heat absorbed by the reactant molecules form the surroundings.

2- Transition State:

- Unstable condition of reactant molecules that have absorbed sufficient free energy to react.

3- Products



Metabolic pathways can be grouped into two pathways:

1- Catabolic reactions: degrade molecules to create smaller molecules and energy

2- Anabolic reactions: synthesize molecules for cell maintenance, growth and reproduction

- Catabolism is characterized by oxidation reactions and by release of free energy which is transformed to ATP.

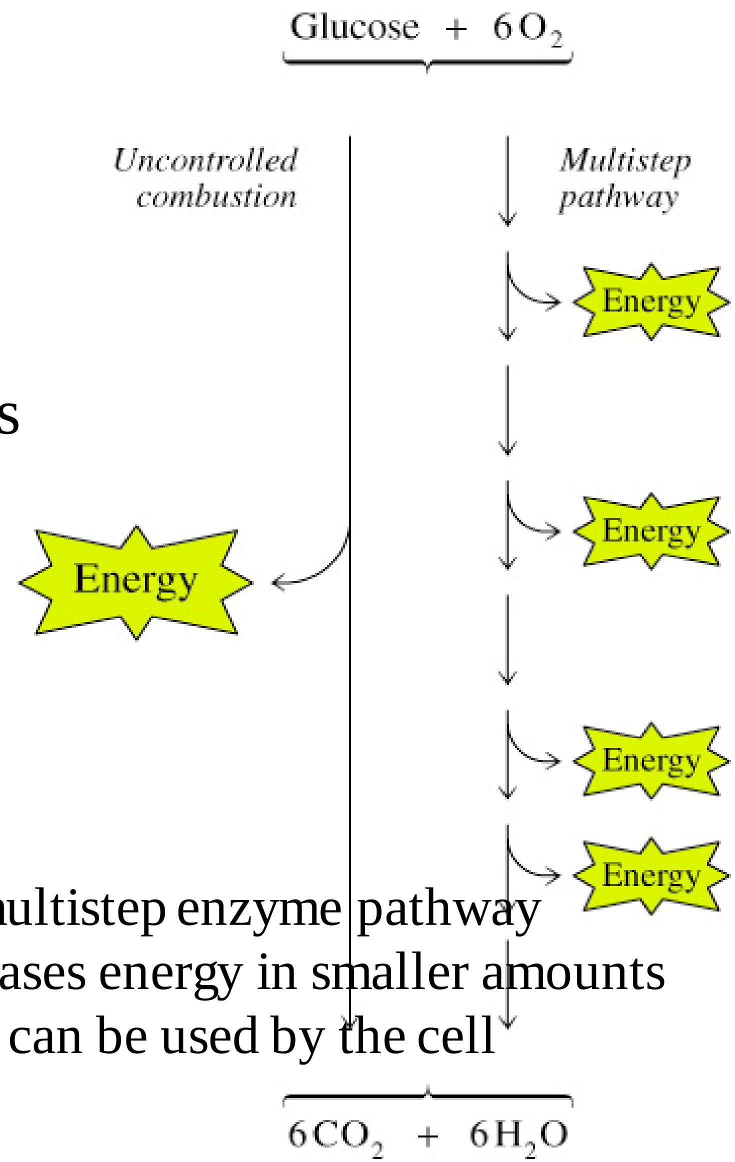
- Anabolism is characterized by reduction reactions and by utilization of energy accumulated in ATP molecules.

- Catabolism and anabolism are tightly linked together by their coordinated energy requirements: catabolic processes release the energy from food and collect it in the ATP; anabolic processes use the free energy stored in ATP to perform work.

Metabolism proceeds by discrete steps

- Multiplestep pathways permit control of energy input and output
- Catabolic multi-step pathways provide energy in smaller stepwise amounts
- Each enzyme in a multi-step pathway usually catalyzes only one single step in the pathway
- Control points occur in multistep pathways
- Metabolic pathways are regulated to permit organisms to respond to changing conditions
- Most pathways are irreversible
- Flux: flow of material through a metabolic pathway which depends upon: 1-Supply of substrates
  2- Removal of products
  3- Pathway enzyme activities

Single-step versus multi-step pathways



A multistep enzyme pathway releases energy in smaller amounts that can be used by the cell

Levels of metabolism regulation

1- Nervous system.

2- Endocrine system.

3- Interaction between organs.

4- Cell (membrane) level.

5- Molecular level

Stages of metabolism

Catabolism

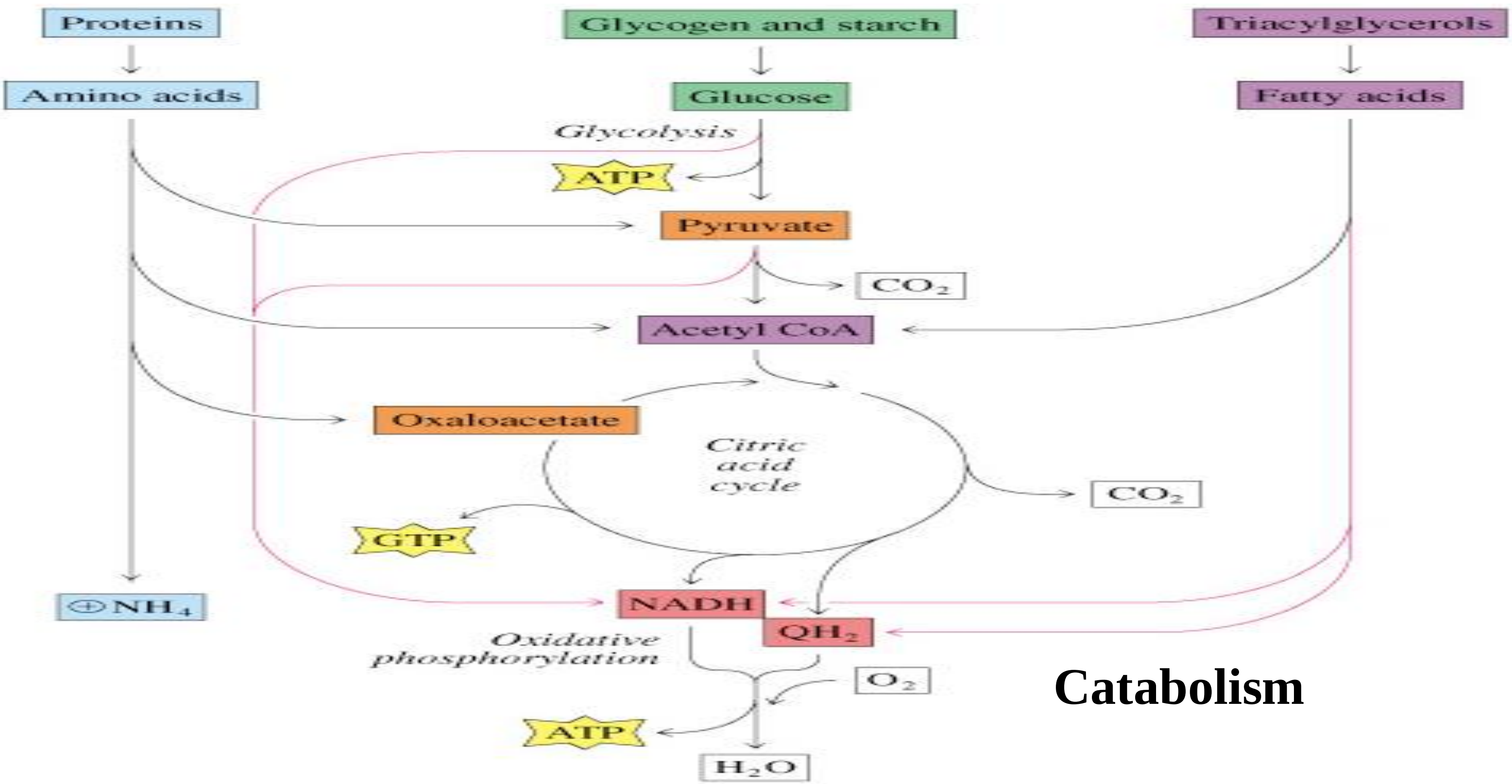
Stage I. Breakdown of macromolecules (proteins, carbohydrates and lipids to respective building blocks.

Stage II. Amino acids, fatty acids and glucose are oxidized to common metabolite (acetyl CoA)

Stage III. Acetyl CoA is oxidized in citric acid cycle to CO_2 and water. As result reduced cofactor, $NADH_2$ and $FADH_2$, are formed which give up their electrons. Electrons are transported via the tissue respiration chain and released energy is coupled directly to ATP synthesis.

Catabolism is characterized by convergence of three major routs toward a final common pathway.

Different proteins, fats and carbohydrates enter the same pathway TCA cycle.



Catabolism

Anabolism can also be divided into stages, however the anabolic pathways are characterized by divergence.
Monosaccharide synthesis begin with CO_2, oxaloacetate, pyruvate or lactate.
Amino acids are synthesized from acetyl CoA, pyruvate or keto acids of Krebs cycle.

Fatty acids are constructed from acetyl CoA.

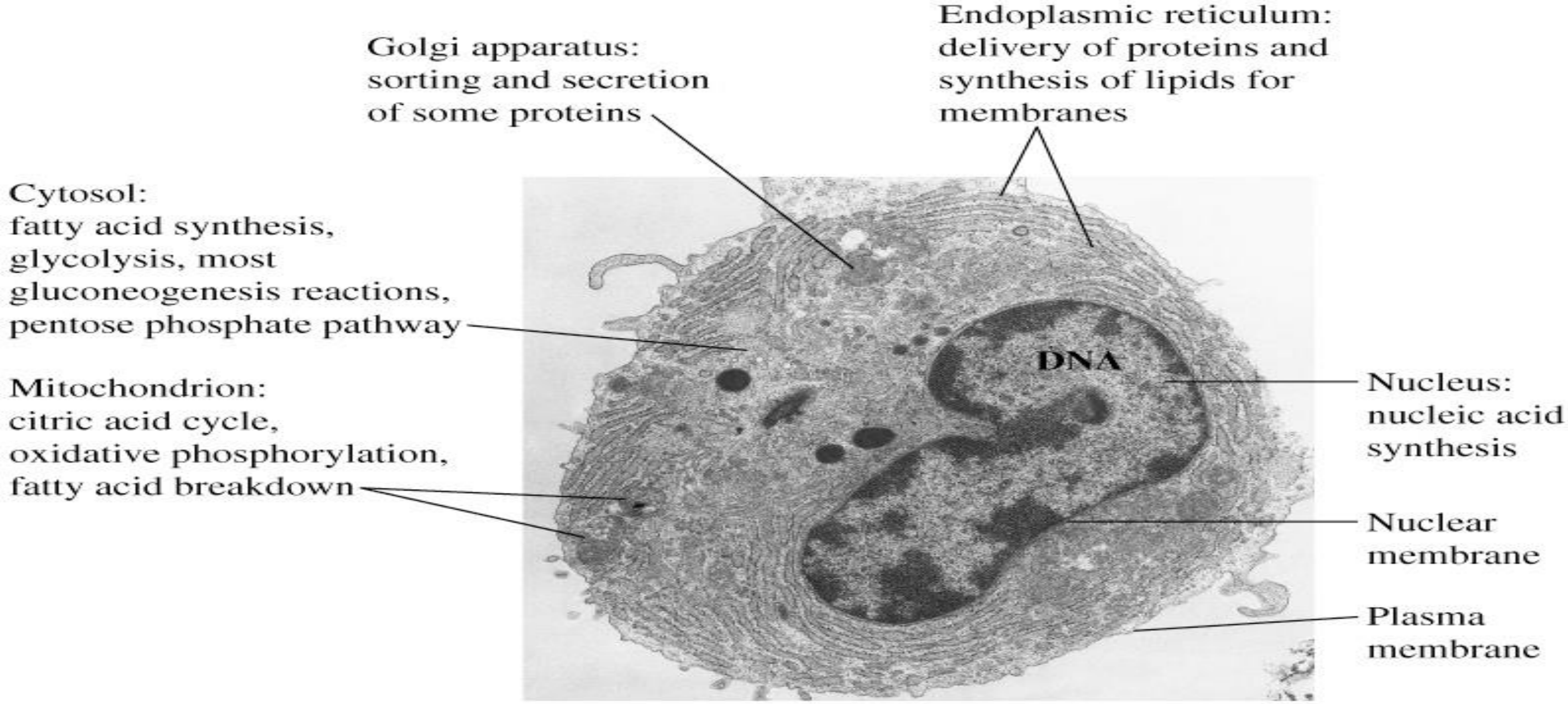
On the next stage monosaccharides, amino acids and fatty acids are used for the synthesis of polysaccharides, proteins and fats.

Compartmentation of metabolic processes in cell

- permits:

1- Separate pools of metabolites within a cell
2- Simultaneous operation of opposing metabolic paths
3- High local concentrations of metabolites

- Example: fatty acid synthesis enzymes (cytosol), fatty acid breakdown enzymes (mitochondria)



<u>The chemistry of metabolism</u>

There are about 3000 reactions in human cell.

All these reactions are divided into six categories:

1- Oxidation-reduction reactions

2- Group transfer reactions

3- Hydrolysis reactions

4- Nonhydrolytic cleavage reactions

5- Isomerization and rearrangement reactions

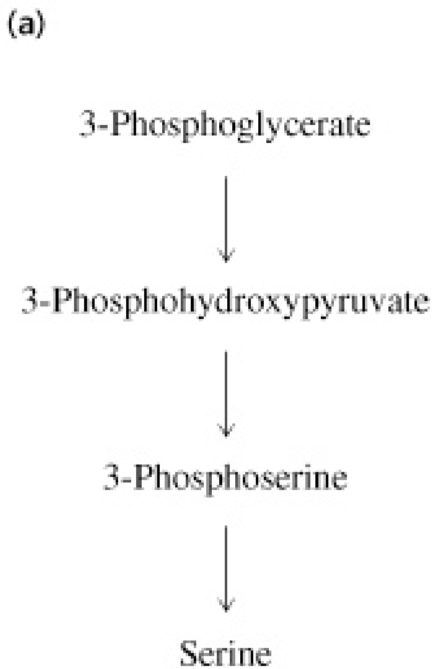
6- Bond formation reactions using energy from ATP

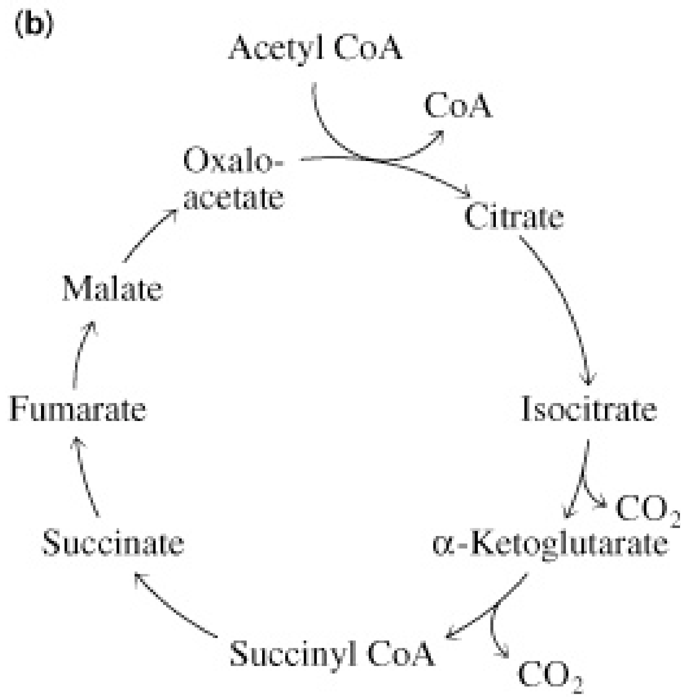
Metabolic pathway may be:

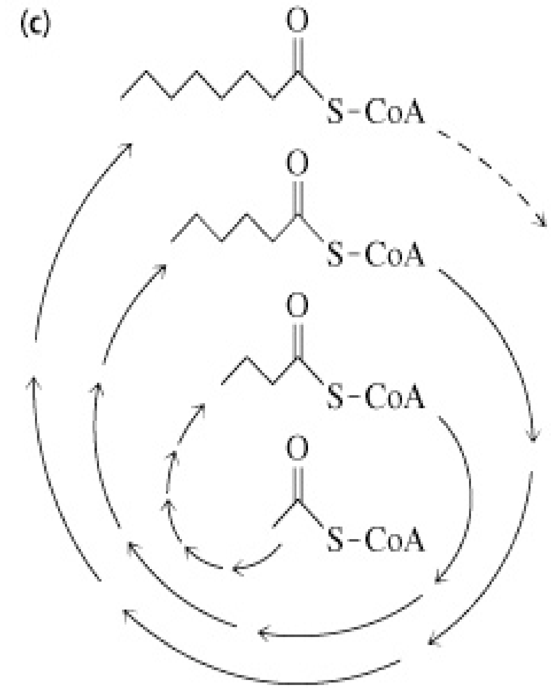
(a) Linear (Glycolysis)

(b) Cyclic (TCA cycle)

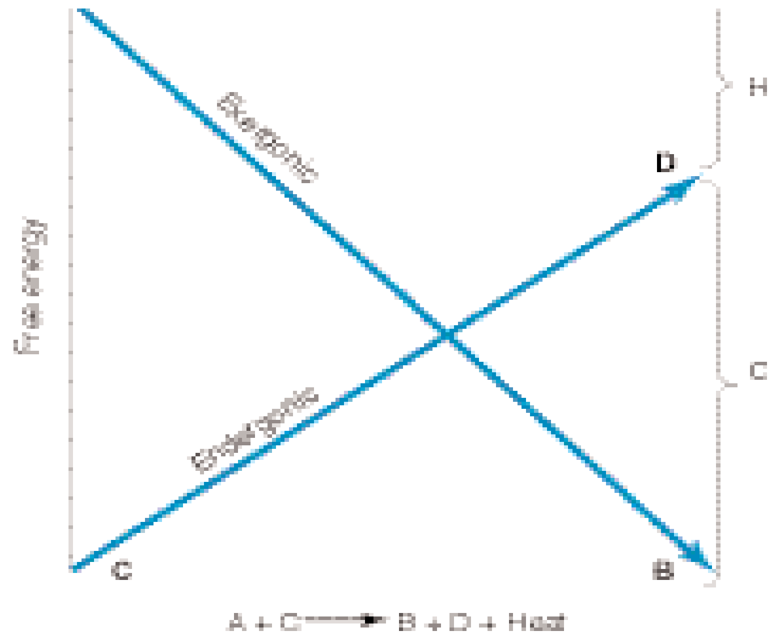
(c) Spiral pathway (Fatty acid biosynthesis)





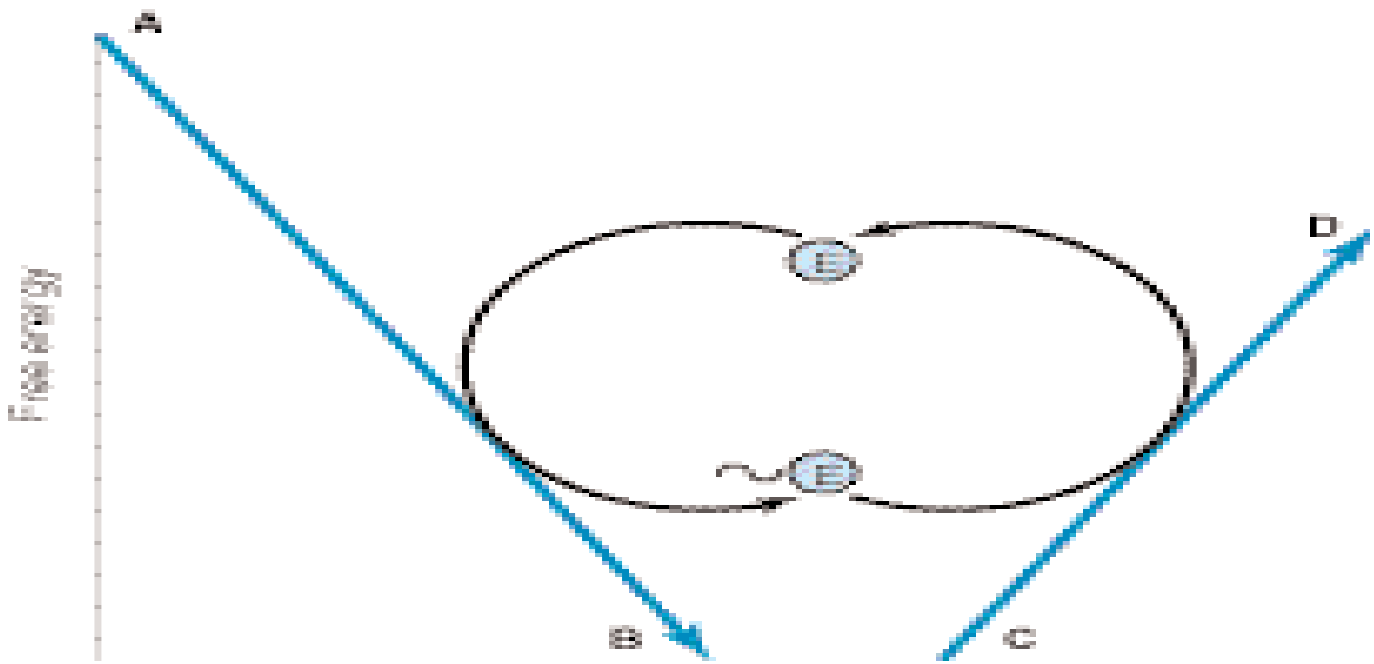


- In practice, an endergonic process cannot exist independently but must be a component of a coupled exergonic- endergonic system where the overall net change is exergonic.
- One possible mechanism of coupling could be observed if common obligatory intermediate (I) took part in both reactions. $A + C \rightarrow I \rightarrow B + D$
- Some exergonic and endergonic reactions in biologic systems are coupled in this way.
- Indeed, these relationships supply a basis for the concept of respiratory control, the process that prevents an organism from burning out of control.



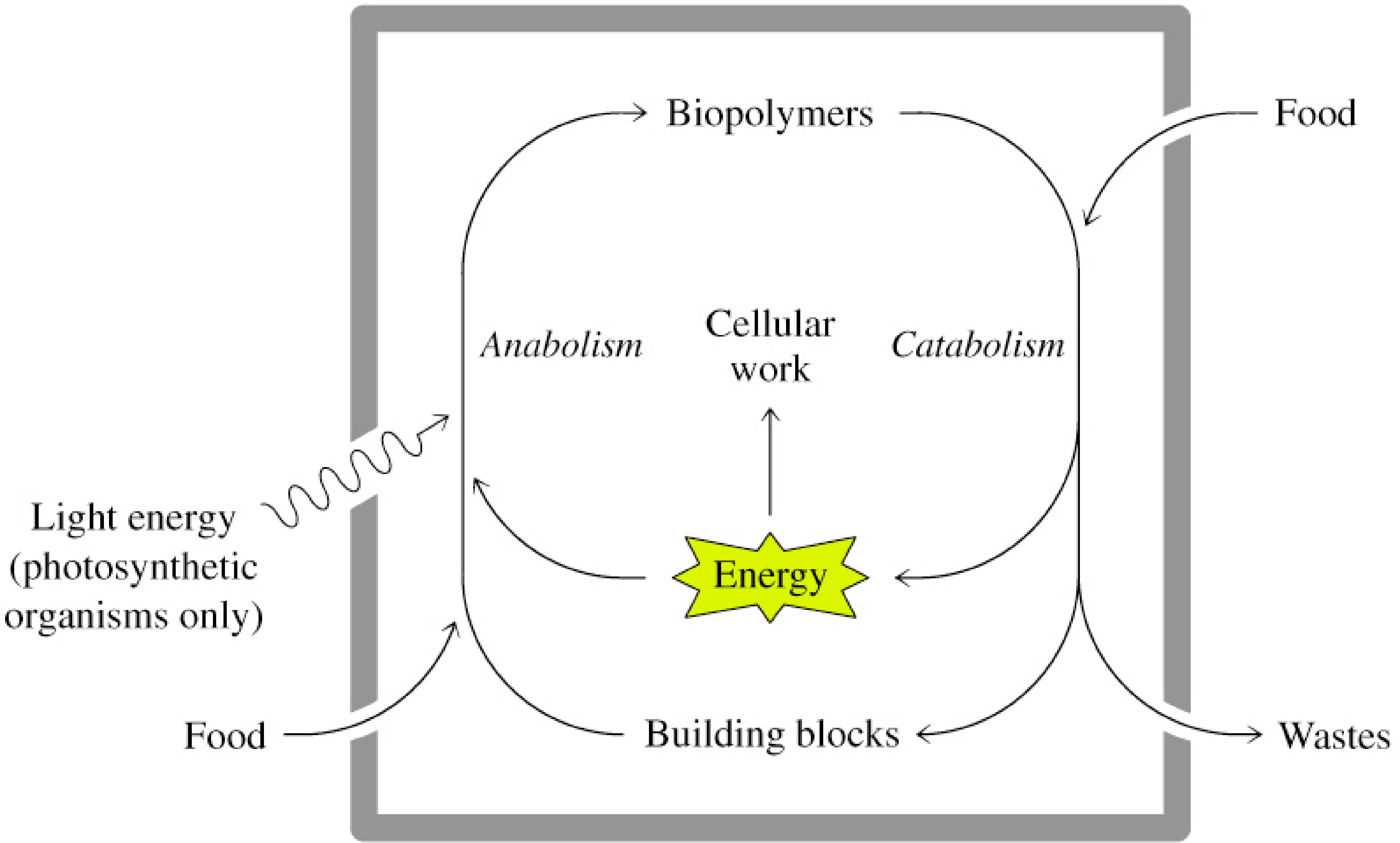
- The coupling concept is provided by forming an intermediate carrier through dehydrogenation/hydrogenations reactions

- An alternative method of coupling an exergonic to an endergonic process is to synthesize a compound of high-energy potential in the exergonic reaction and to incorporate this new compound into the endergonic reaction, thus effecting a transference of free energy from the exergonic to the endergonic pathway.
- The biologic advantage of this mechanism is that the compound of high potential energy, (~E) , unlike I in the previous system.



- Transfer of free energy from an exergonic to an endergonic reaction via a high-energy intermediate compound (~E)

Anabolism and catabolism are coupled by energy



The Intermediate value for the free energy of hydrolysis of ATP

-The standard free energy of hydrolysis of a number of biochemically important phosphates.

- The tendency of each of the phosphate groups to transfer to a suitable acceptor may be obtained from the $\Delta G0'$ of hydrolysis at 37 °C.

-The value for the hydrolysis of the terminal phosphate of ATP divides the list of energy compounds into two groups:

- Low-energy phosphates, exemplified by the ester phosphates found in the intermediates of glycolysis, have $\Delta G0$ values smaller than that of ATP, while in high-energy phosphates the value is higher than that of ATP.

-The components of the high-energy phosphates compounds , including ATP, are usually anhydrides (1-phosphate of 1,3-biphosphoglycerate), enolphosphates (phosphoenolpyruvate), and phosphoguanidines (creatine phosphate, arginine phosphate).

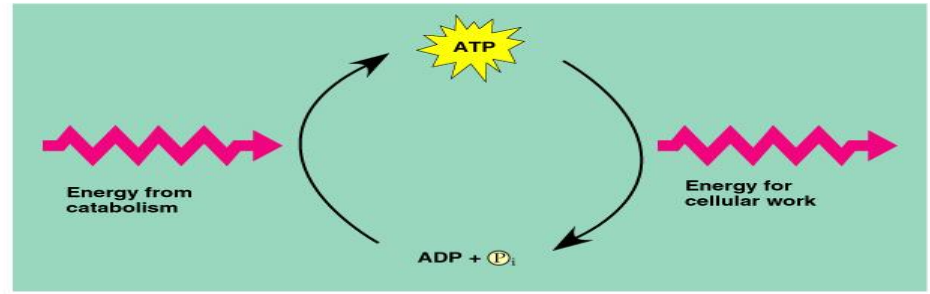
- The intermediate position of ATP allows it to play an important role in energy transfer.

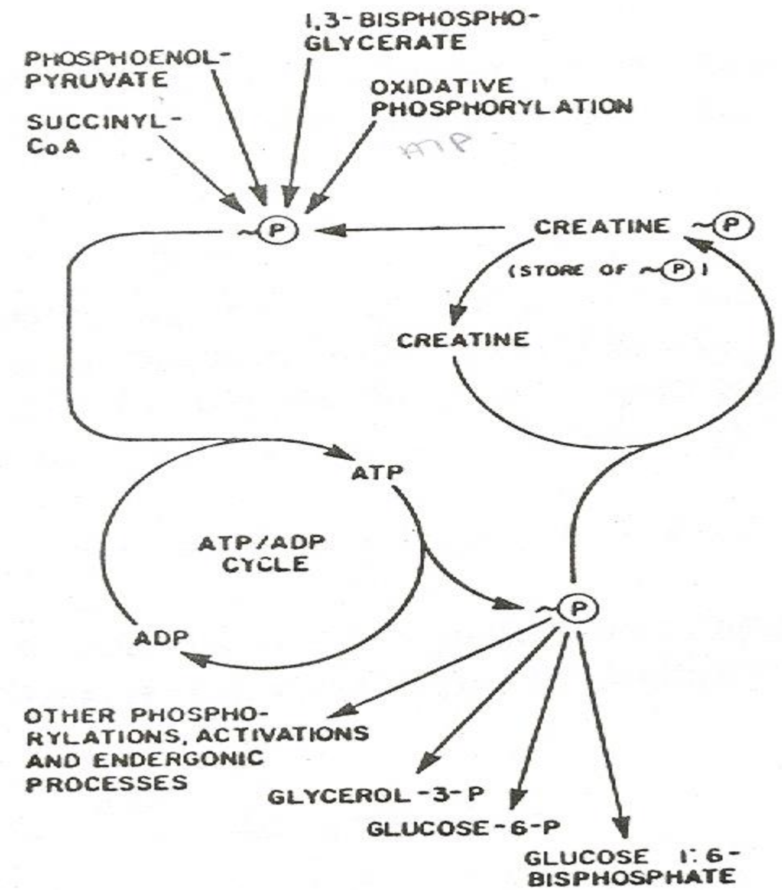
-The high free energy change on hydrolysis of ATP is due to relief of charge repulsion of adjacent negatively charged oxygen atoms.

- Other “high-energy compounds” are thiol esters as coenzyme A (acetyl-CoA), acyl carrier protein, amino acid esters involved in protein synthesis, S- adenosylmethionine (SAM), UDPGlc (uridine diphosphate glucose), and PRPP (5-phosphoribosyl-1-pyrophosphate).

Compound	**kJ/mol**	**kcal/mol**
Phosphoenolpyruvate	−61.9	−14.8
Carbamoyl phosphate	−51.4	−12.3
1,3-Bisphosphoglycerate	−49.3	−11.8
Creatine phosphate	−43.1	−10.3
ATP →ADP + Pi	**−30.5**	**−7.3**
ADP →AMP + Pi	−27.6	−6.6
Pyrophosphate	−27.6	−6.6
Glucose 1-phosphate	−20.9	−5.0

- ATP powers cellular work by coupling exergonic reactions to endergonic reactions
- A cell does three main kinds of work: Mechanical, Transport and Chemical
- To do work, cells manage energy resources by energy coupling, the use of an exergonic process to drive an endergonic one
- ATP is the cell's energy shuttle providing energy for cellular functions





Role of ATP/ADP cycle in transfer of high-energy phosphate.

Sources of ATP.

ATP levels are maintained through several processes:

1. Adenylate kinase.

- ATP has two "high-energy" phosphate groups. Splitting off the gamma phosphate group of ATP yields ADP and inorganic phosphate.
- Splitting off both high-energy groups in one step yields AMP and inorganic pyrophosphate (ppi).
- Adenylate kinase, an enzyme found in all tissues, catalyzes a transfer of the energy-rich phosphate bond from one ADP molecule to another, giving ATP and AMP.
- The conversion is very rapid in muscle and liver.

$$\mathbf{2ADP \rightleftharpoons ATP + AMP}$$

-AMP levels are crucial in adjusting the balance between carbohydrate and fatty acid metabolism in varying physiological situations.

- AMP is an active intracellular signal substance.

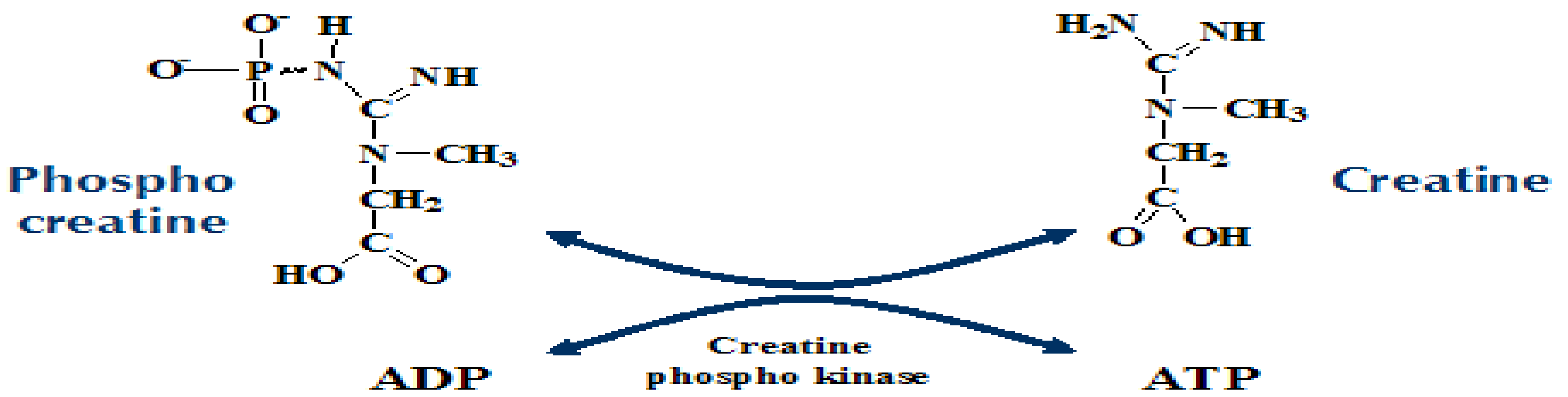
-AMP is also an activator of glycogen mobilization and, therefore, sugar metabolism.

2. **Creatine Phosphokinase / Phosphocreatine**.

- Most of our body tissues contain phosphocreatine at concentrations approximately three times that of ATP.
- Phosphocreatine is a reserve source of high-energy phosphate.
- This reserve can be transferred to ADP, thus forming ATP to replace that used by working muscle.
- While the creatine phosphokinase reaction is the most rapid ATP-yielding reaction we possess, the amount of ATP which is produced is quite small.
- Muscle tissues have about 5 mmol/l ATP and approximately 17-20 mmol/l of creatine phosphate.
- Under extreme work (sprinting, for example) the phosphocreatine reserves are used up in about 30-40 seconds.
- However, "seconds do count" in sport. During those few seconds muscles can and do work with "explosive force".

TABLE 1.1	THE ENERGY SYSTEMS AND THEIR APPROXIMATE CONTRIBUTIONS TO VARIOUS DURATIONS OF EXERCISE *AT MAXIMAL INTENSITY* (1)
ENERGY SYSTEM	**DURATION**
Phosphocreatine system	0–10 seconds
Phosphocreatine system and glycolytic system (slow)	10–30 seconds
Glycolytic system (fast)	30 seconds–2 minutes
Glycolytic system (fast) and oxidative system	2–3 minutes
Oxidative system	< 3 minutes and rest

Creatine Phosphate-ATP interaction



1.3 CHARACTERISTICS OF THE PHOSPHOCREATINE SYSTEM

1. It involves only one chemical step.
2. It is catalyzed by the enzyme creatine kinase (CK).
3. Its chemical reaction is very fast.
4. One ATP is generated per phosphocreatine molecule.
5. The reaction lasts for 5 to 10 seconds at maximal intensity.
6. It is anaerobic.
7. Fatigue is associated with the depletion of phosphocreatine.
8. It is the dominant energy system in speed and explosive power events.

3. **Anaerobic Metabolism.**

- In "second place" in the ATP-synthesis race (after phosphocreatine) comes ATP synthesis coupled to anaerobic metabolism.
-This is the cytosolic formation of ATP driven by oxidation of glucose (or glucosyl groups from glycogen) to pyruvate and lactate.
- ATP formation through cytosolic glycolysis proceeds with a speed equal to about 50% of that we see using creatine phosphate and creatine phosphokinase.
- Rapid, yes, but how much ATP can we make when the oxidation process is limited to formation of pyruvate and lactate from glucose or glycogen?
- Only two ATP molecules result for each glucose molecule that is processed.
-Three ATPs are formed for each glucosyl group that derived from glycogen.
-The anaerobic glycolysis is very rapid. While it is relatively ineffective measured by energy production per glucose molecule consumed, glycolysis does turn out a lot of ATP in a short time.

-The big (and painful) disadvantage is that a lot of lactic acid is produced and accumulates in the working muscle.

- Furthermore, lipids cannot be used as substrates for anaerobic metabolism.
- Only glucose or glycogen work here.
- If we press anaerobic glycolysis to the limits, muscles exhaust their stored glycogen and take up so much glucose from the blood that hypoglycemia and CNS malfunction result.

4. <u>Aerobic Metabolism.</u>

- ATP-balancing
- ATP synthesis
- All of our cells, with the important exception of blood cells, contain Mitochondria which use oxygen and form water while oxidizing our "food".
- Mitochondrial substrate is acetyl-CoA.
- All food that can be reduced to 2-carbon fragments can serve as a substrate for mitochondrial ATP production.

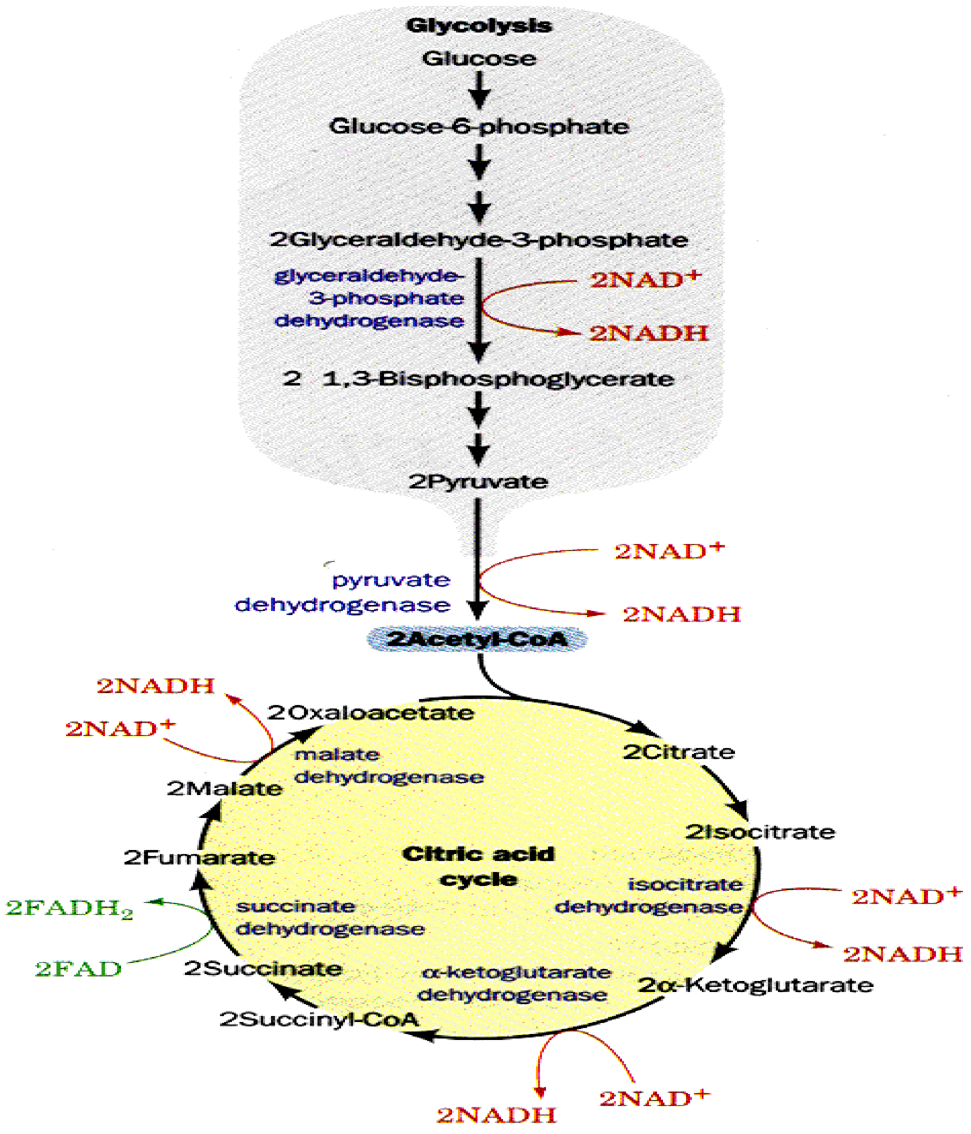
-The combustion process is coupled to reduction of oxygen giving water as a product.

- Approximately 30% of the energy released in this process is trapped in the terminal phosphate group in ATP.

-The rest of the energy in acetyl-CoA escapes as heat, keeping us nice and warm!

-We produce around 10 moles of ATP for each mole of acetyl-CoA that is processed.

-While aerobic synthesis of ATP is the most effective way to produce "useable energy", it is a relatively slow process.

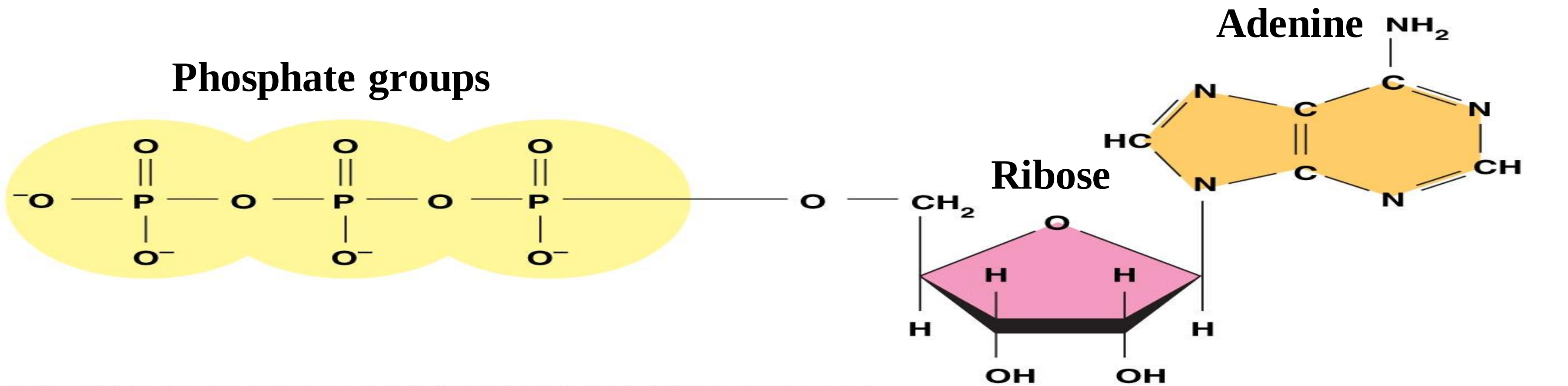


ATP (adenosine triphosphate)

- Compounds with high energy bonds release large quantities of free energy on hydrolysis.
- The most important parts of the ATP molecule are its two phosphodiester bonds (high energy bonds).
- Breaking down either of these bonds is accompanied by the release of energy (7.3 Kcal/mol for each bond).

Importance of ATP as a source of energy :

1- Synthesis of macromolecules: such as DNA and RNA, protein, ….etc
2- Support the endergonic reaction in metabolic pathways.
3- Important for active transport across membranes.
4- Important for muscle contraction…etc.
5- transmission of impulses along neurons.



Electron transport chain (ETC)

Oxidation reduction reactions (Redox reactions)

- Commonly the oxidation reactions are accompanied by reduction reactions and they are called redox reaction.
- Redox reactions are accompanied by energy liberation, necessary for the cells.

- In the redox reaction. H_2 is oxidized while, O_2 is reduced, and if occurs it will be accompanied by a massive energy explosion.

$$2H_2 + O_2 \longrightarrow 2H_2O + \text{energy}$$

- Instead of massive energy is liberated, hydrogen must be transferred to oxygen in gradual steps. Thus, small fractions of energy are liberated and stored for further use

Electrons are transferred from one molecule to another in one of four different ways

- They may be transferred directly as electrons as the Fe 2+ / Fe 3+ redox pair.
- Electrons may be transferred as incorporated in hydrogen atoms as in case of FAD

$$AH_2 \longrightarrow A$$

$$FAD \longrightarrow FADH_2$$

- Electrons may be transferred as hydride ions (H+)

$$AH_2 \longrightarrow A$$

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

- Electron may be transferred as a direct combination of an organic reductant with oxygen

$$R\text{-}CH_2 + \tfrac{1}{2}\ O_2 \longrightarrow R\text{-}CH_2OH$$

<u>Redox Potential (electron affinity)</u>

- Oxygen has the highest electron affinity i.e. highest redox potential.
- Hydrogen has the lowest electron affinity i.e. lowest redox potential.

<u>Redox chain</u>:

- It is a chain of different compounds of increasing redox potentials between hydrogen and oxygen.

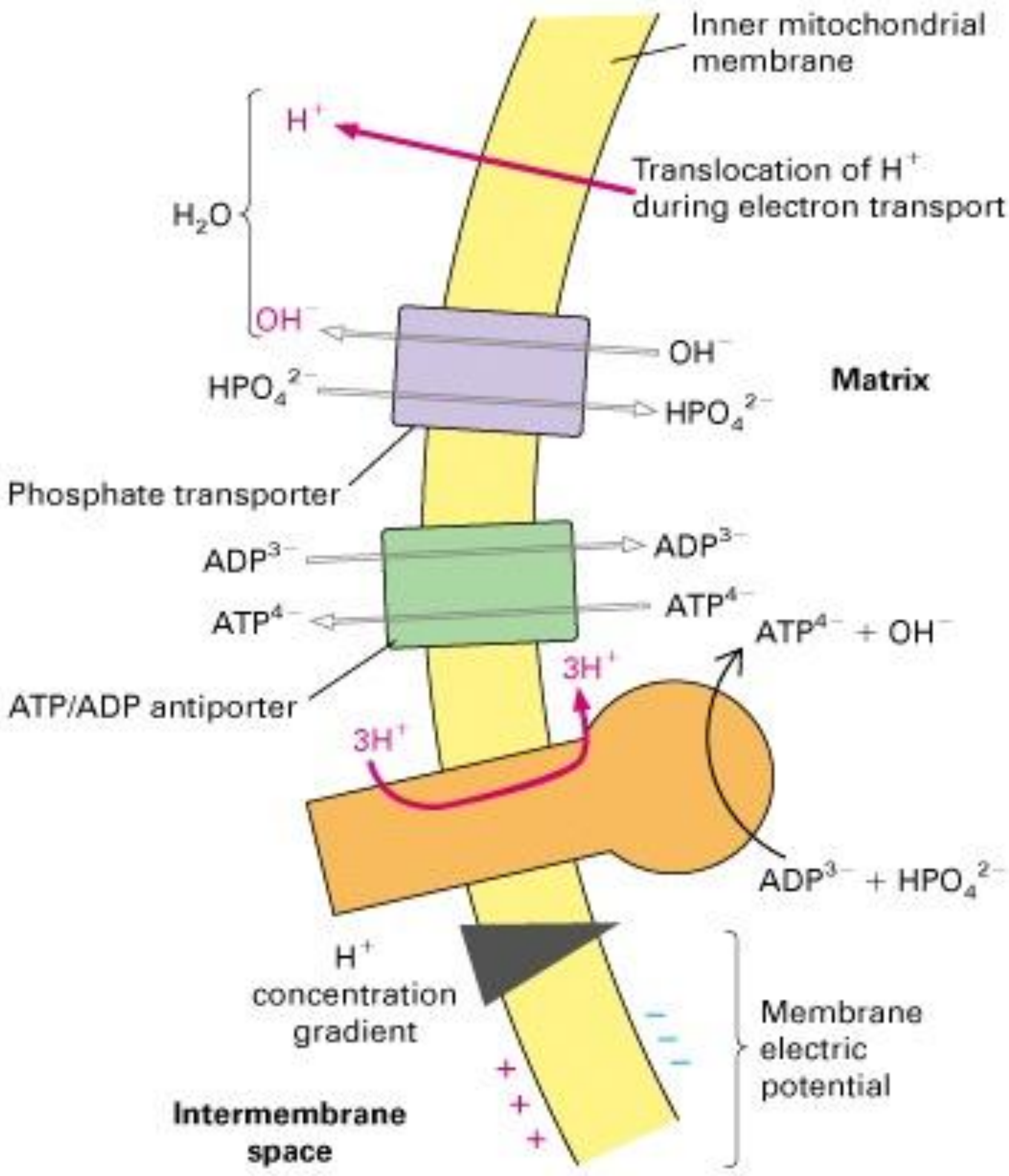
$$\mathbf{A \rightarrow B \rightarrow C \rightarrow D.....}$$

- Each components of redox chain has a redox potential higher than hydrogen and lower than oxygen.
- During hydrogen (H^+ and electron) transfer through different components of the redox chain, energy is liberated in steps and in small amounts to be utilized.

Respiratory chain (ETC)

- It is a system of electron carriers located in the inner-mitochondrial membrane, oxidizes the reduced cofactors by transferring electrons in a series of steps to O2 (the terminal electron acceptor).
- Free energy released by these oxidation reactions is used to drive the synthesis of ATP.
- Each component of the chain can accept electrons from the preceding carrier and transfer them to the following one.
- A variety of substances (carbohydrates, fatty acids and amino acids) can use respiratory chain as a final pathway as they give electrons to the oxidized NAD^+ and FAD^+ to form the energy rich reduced coenzymes $NADH+H^+$, $FADH_2$.
- $NADH+H^+$ and $FADH_2$ give hydrogen and a pair of electrons to electron carriers collectively, called the <u>respiratory chain components.</u>

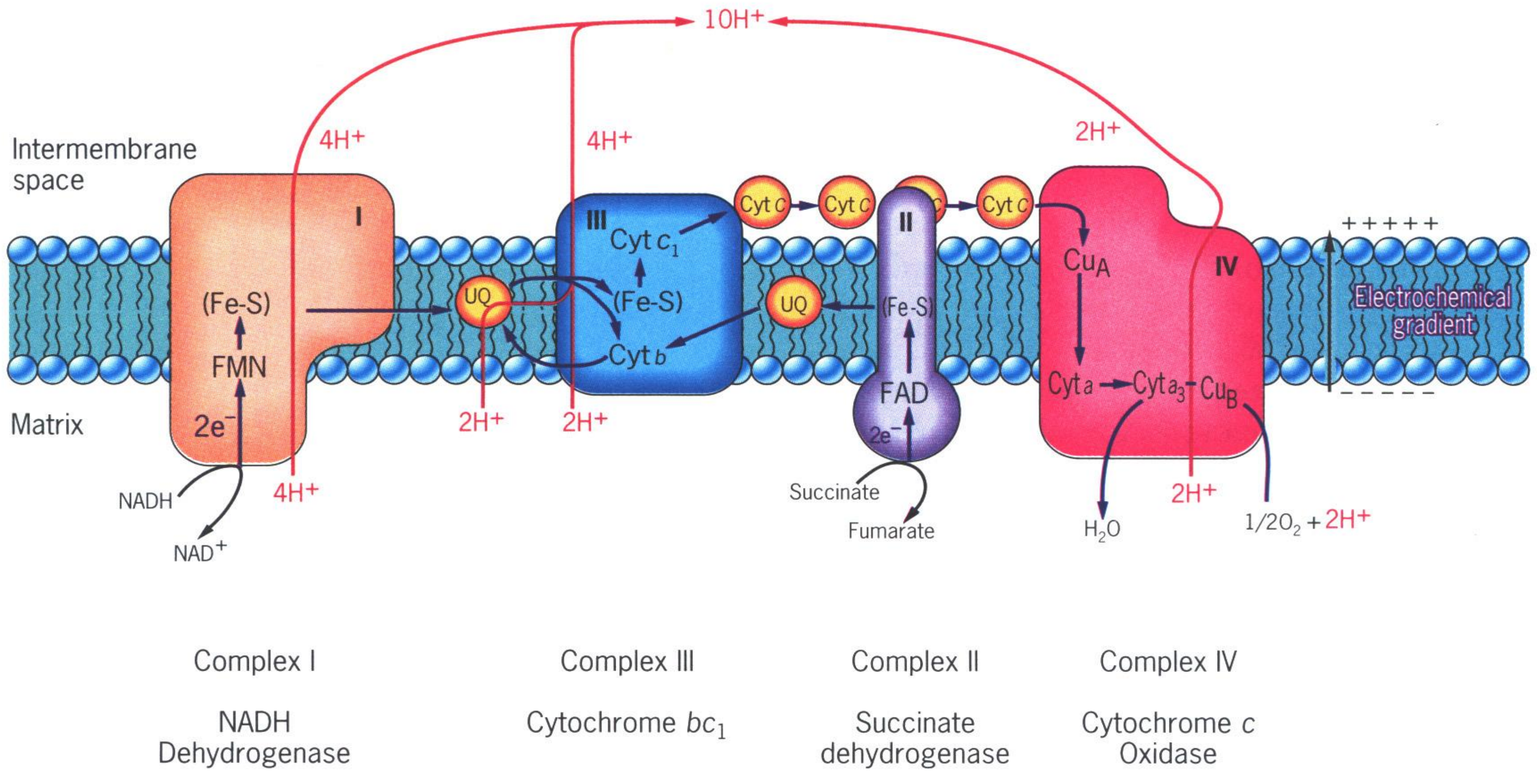
- Outer mitochondrial membrane is permeable to most ions as O_2, CO_2, NH_3 and monocarboxylic acids.
- Di- and tricarboxylic acids need special transporters.
- ATP and ADP need special transporter to allow ADP in and ATP out of mitochondria.
- Inner membrane is impermeable to most ions & molecules: H^+, Na^+, K^+, ATP, ADP, pyruvate.
- Matrix contains enzymes for oxidation of pyruvate., A.A.s , F.A.s and TCA.



Organization of respiratory chain

- The inner mitochondrial membrane contains four enzymatic complexes (I, II, III, IV) and complex V catalyses ATP synthesis, arranged in order of increasing electronegativity (weakest to strongest)
- Each complex accepts or donates electrons to relatively mobile electron carriers as coenzyme Q and cytochrome C.
- Oxidative phosphorylation starts by entry of electrons into the respiratory chain.
- Most of these electrons arise by the action of dehydrogenases that collect electrons from catabolic pathways and pass them to the electron acceptors NAD and FAD.
- As electrons are passed down the respiratory chain, they lose much of their free energy.

- Part of this energy can be captured and stored by the production of ATP from ADP and inorganic phosphate (Pi).
- The process is called oxidative phosphorylation.
- The remainder of the free energy not trapped as ATP is released as heat.



Components of the respiratory chain

-With the exception of coenzyme Q, all members of this chain are proteins.

- All are embedded in the inner mitochondrial membrane.

Complex I:

- Contains an enzyme called NADH dehydrogenase.
- Its coenzyme is FMN (can accept two hydrogen atoms to become $FMNH_2$)
- It contains several iron and sulfur atoms (iron sulfur protein).
- NAD+ is reduced to $NADH+H^+$ by dehydrogenases that remove hydrogen atoms from their substrates.

Complex II:

- The entry point of $FADH_2$ (its coenzyme is FAD).
- Contains an enzyme called: flavo - protein dehydrogenase e.g. succinate dehydrogenase of TCA and acyl CoA dehydrogenase of β oxidation of fatty acids.
- It contains iron and sulfur atoms (iron sulfur protein).

Complex III:

- It is cytochrome reductase complex, or cytochrome bc1 complex”
- Transfers electron from QH_2 to cytochrome C.
- Contains an enzyme cytochrome b.

Complex IV:

- This complex contains cytochrome a, a3 and 2 copper atoms.
- Complex IV catalyzes the transfer of electrons from reduced cytochrome C to molecular oxygen.
- The copper atoms are crucial for such a transfer.

Ubiquinone “Coenzyme Q”

- It is a lipid soluble vitamin K derivative
- Coenzyme Q can accept hydrogen ions both from $FMNH_2$, produced by NADH dehydrogenase (complex I) and from $FADH_2$ which is produced by (complex II).
- It is freely diffusible between the lipid bilayer of inner mitochondrial membrane.

Cytochromes

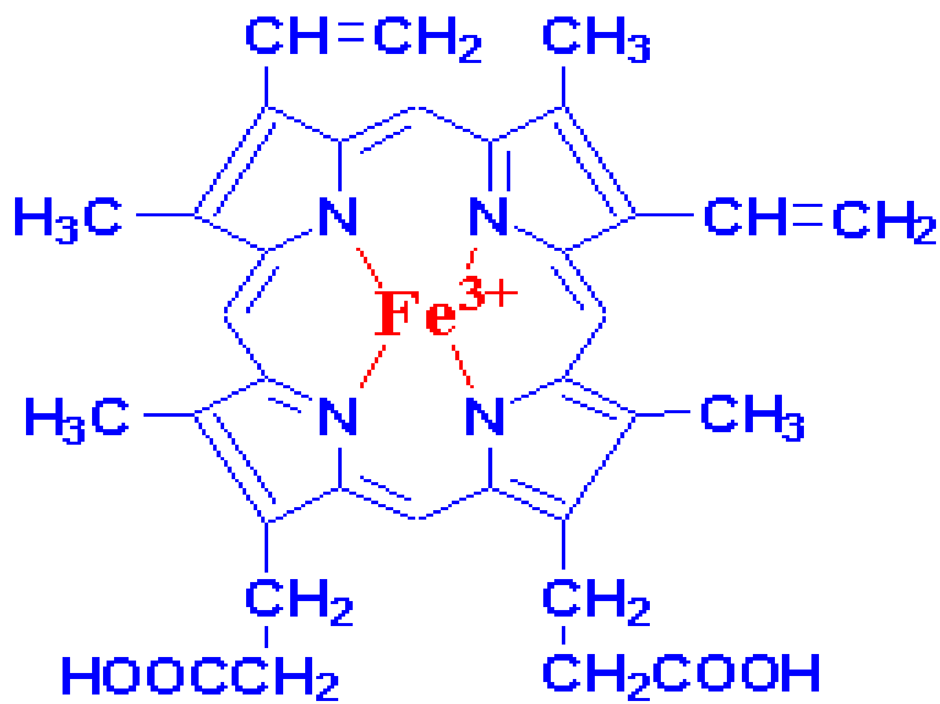
- Cytochromes are proteins that contain an iron-containing heme group. This iron oscillates between ferric form (Fe^{+++}) when it losses an electron, and ferrous form (Fe^{++}) when it accepts an electron.
- All are integral membrane proteins with the exception of cytochrome C, a soluble free protein.

TABLE 19–3 The Protein Components of the Mitochondrial Electron-Transfer Chain

Enzyme complex/protein	Mass (kDa)	Number of subunits*	Prosthetic group(s)
I NADH dehydrogenase	850	43 (14)	FMN, Fe-S
II Succinate dehydrogenase	140	4	FAD, Fe-S
III Ubiquinone cytochrome *c* oxidoreductase	250	11	Hemes, Fe-S
Cytochrome *c*†	13	1	Heme
IV Cytochrome oxidase	160	13 (3–4)	Hemes; Cu_A, Cu_B

*Numbers of subunits in the bacterial equivalents in parentheses.
†Cytochrome *c* is not part of an enzyme complex; it moves between Complexes III and IV as a freely soluble protein.

- Cytochrome a3 contains copper in addition to iron and called cytochrome oxidase, it is the terminal component of the ETC.



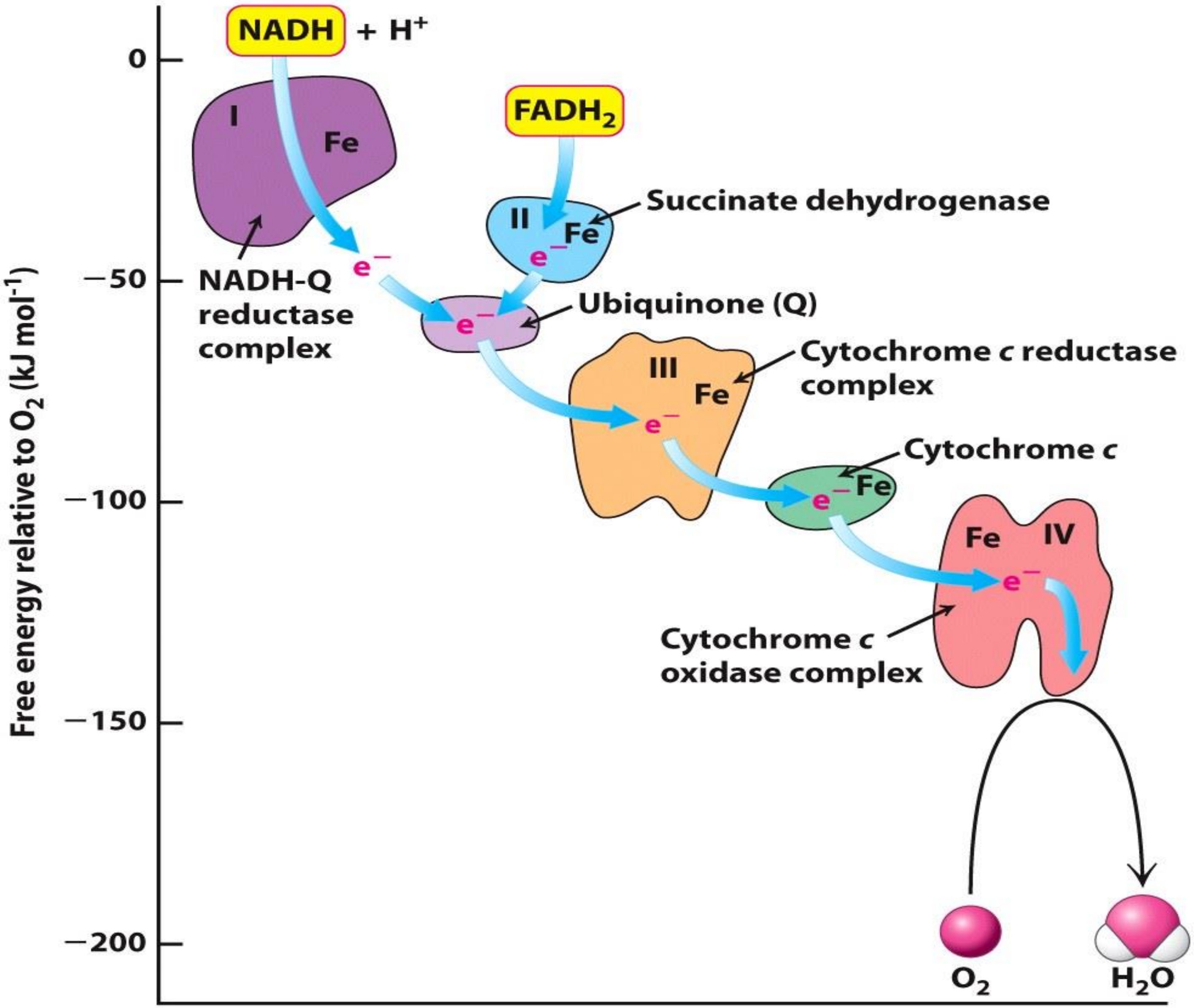
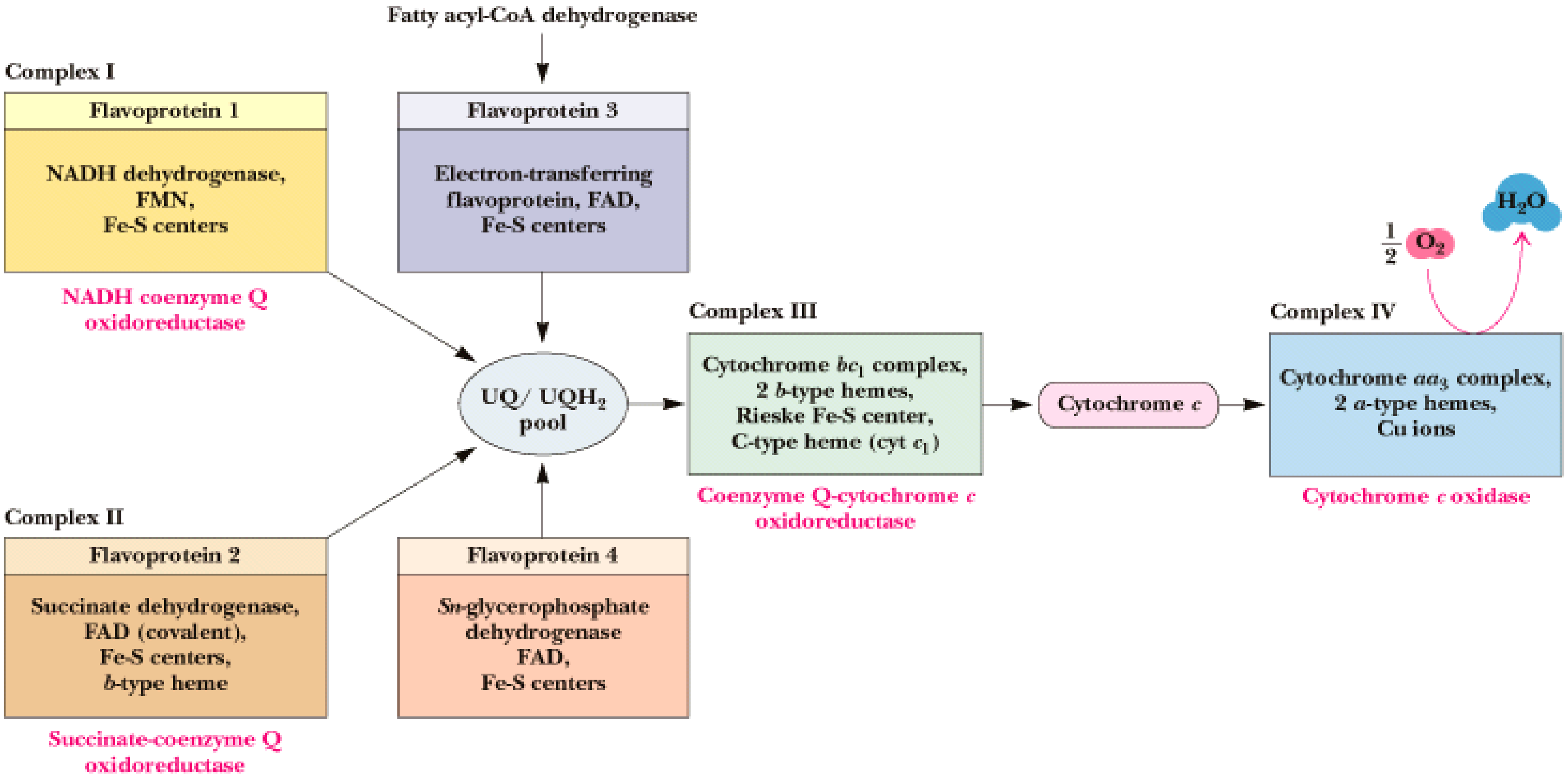


Figure 20.6
Biochemistry: A Short Course, Second Edition

Garrett & Grisham: Biochemistry, 2/e
Figure 21.4



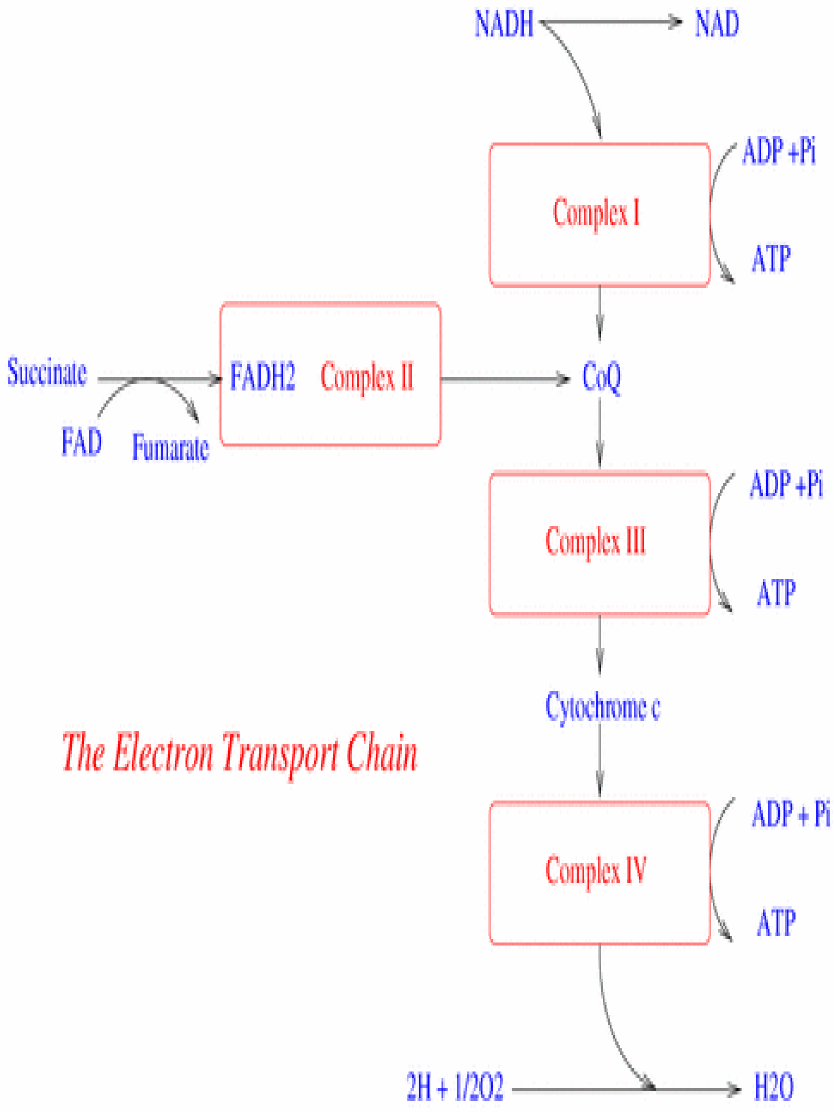
Saunders College Publishing

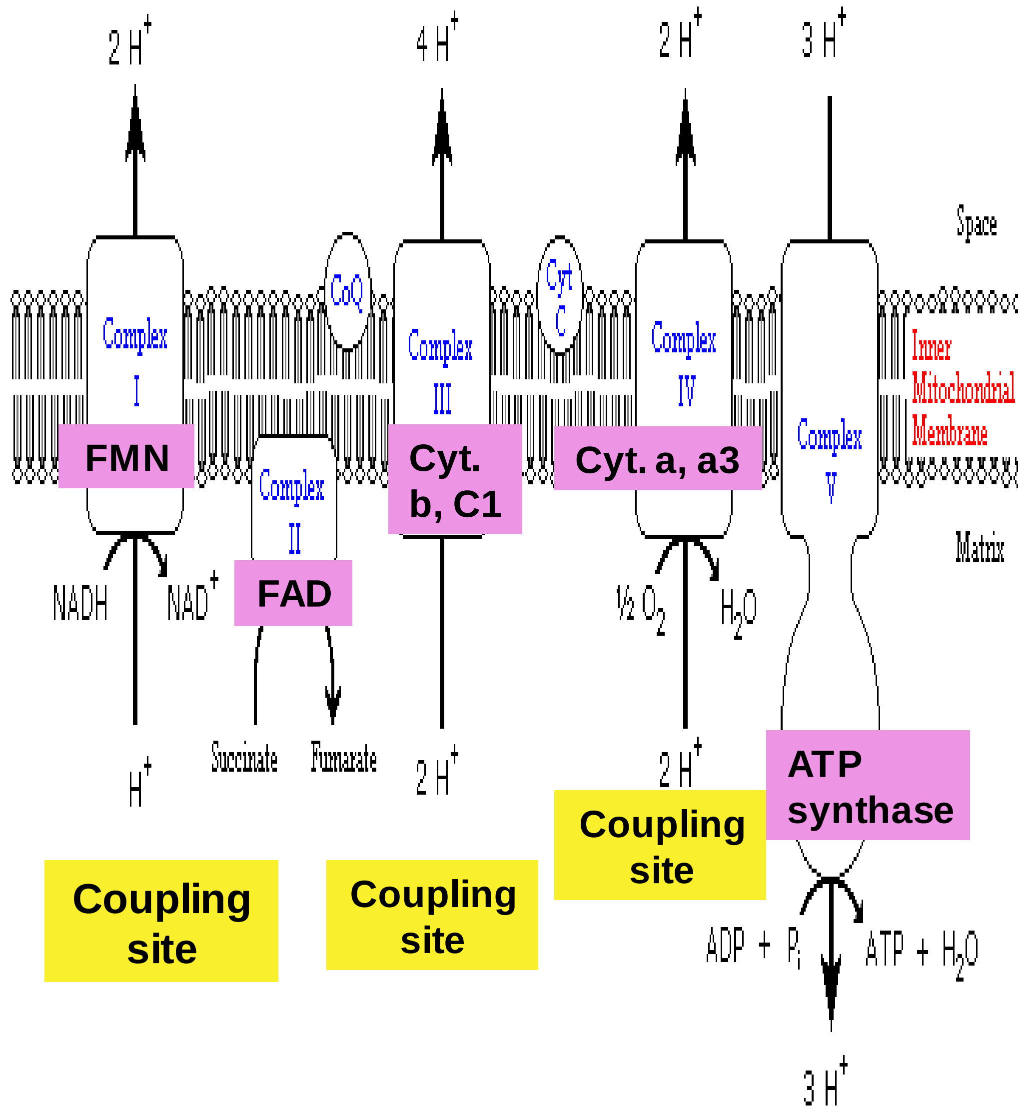
- As electrons pass down the respiratory chain, they lose much of their free energy.
- Part of this energy can be captured and stored as ATP from {ADP and inorganic phosphate (Pi)}.
-The process is called oxidative phosphorylation.
-The non trapped free energy as ATP so, released as heat.

Oxidative phosphorylation

- Oxidative phosphorylation is a coupling process of oxidation and phosphorylation.
-The flow of electrons from NADH to oxygen (oxidation) results in ATP synthesis by phosphorylation of ADP with inorganic phosphate (phosphorylation), therefore, there is coupling between oxidation and phosphorylation.
-Two theories explain the ATP synthesis; chemiosmotic hypothesis and membrane transport system.

Respiratory chain





Inhibitors of respiratory chain:

- Are compounds prevent the passage of electrons to bind a component of the chain (the three sites responsible for electrochemical potential difference), blocking the oxidation reduction reaction.
- There are specific sites for binding inhibitors:

Site I: binding with complex I as barbiturates, rotenone (an insecticide) and piercidin A (an antibiotic).

Site II: binding with complex III as antimycin A and dimercaprol.

Site III: binding with complex IV as H_2S, cyanide (CN), carbon monoxide (CO) and sodium azide.

- Because electron transport and oxidative phosphorylation are tightly coupled, inhibition of the respiratory chain also inhibits ATP synthesis.

4- ADP/ATP transporter inhibitors as atractyloside.

N.B. Malonate which acts as competitive inhibitor of succinate dehydrogenase inhibits ETC through complex II.

Cyanide poisoning

- Cyanide is one of the most potent and rapidly acting poisons. Cyanide binds to cytochrome aa3 so, inhibits the oxidative phosphorylation at level of cytochrome oxidase complex (complex IV).
- The energy production of cells will be blocked resulting in tissue asphyxia especially of central nervous system leading to death.

Chemiosmotic hypothesis:

-This hypothesis postulates that the transfer of electrons along the respiratory chain is accompanied by outward pumping of protons across the inner mitochondrial membrane.

Proton pump

-The transport of electrons down the respiratory chain creates an energy which is used to transport H+ from mitochondrial matrix across inner mitochondrial membrane → inner mitochondrial space.

-This process is carried out by complexes I, III, IV to create across the inner mitochondrial membrane:

- An electrical gradient with more positive changes on the outside of the membrane than on the inside.
- A pH gradient as the outside of the membrane is at lower pH than the inside.

- The energy generated is sufficient for ATP production.

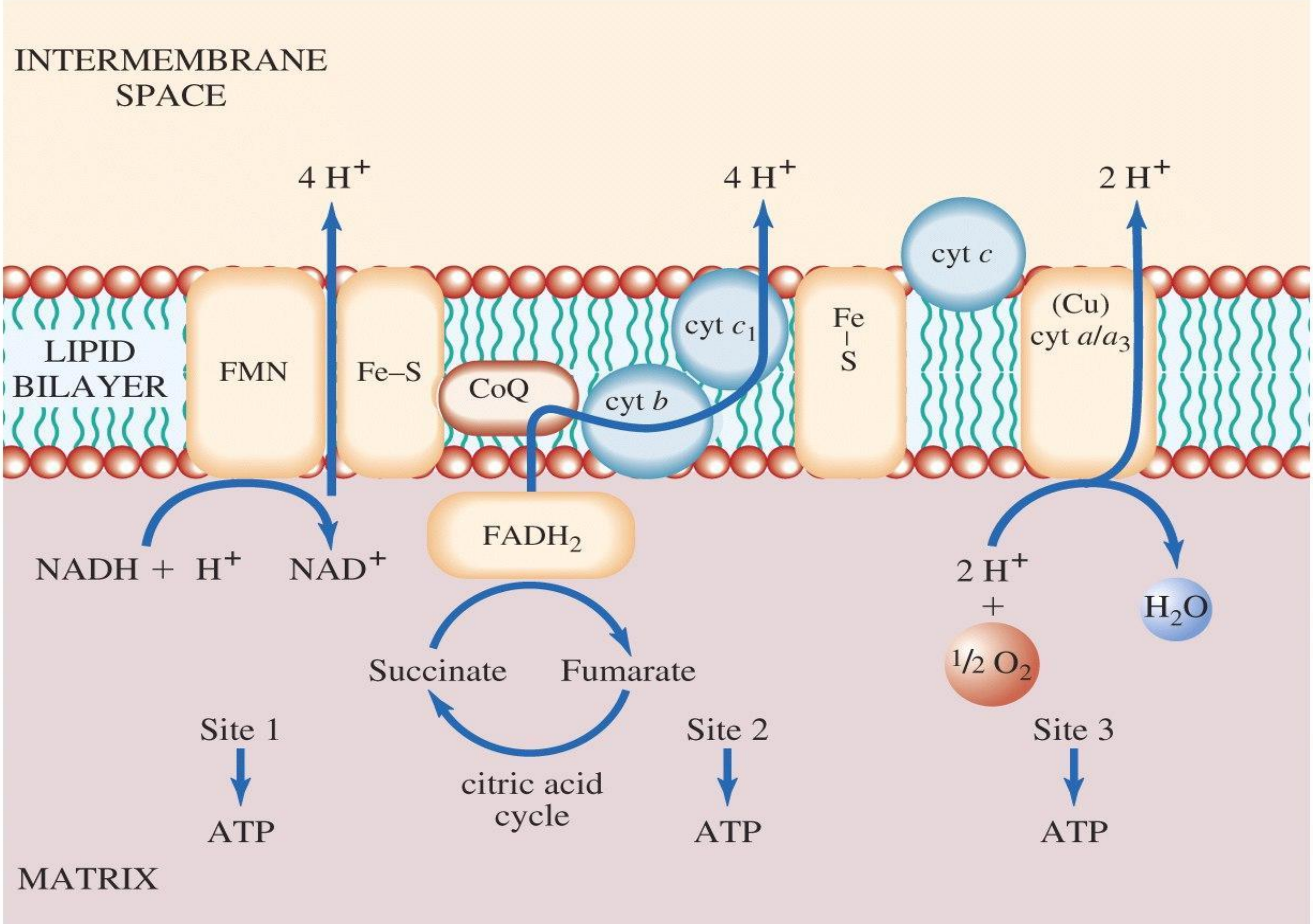
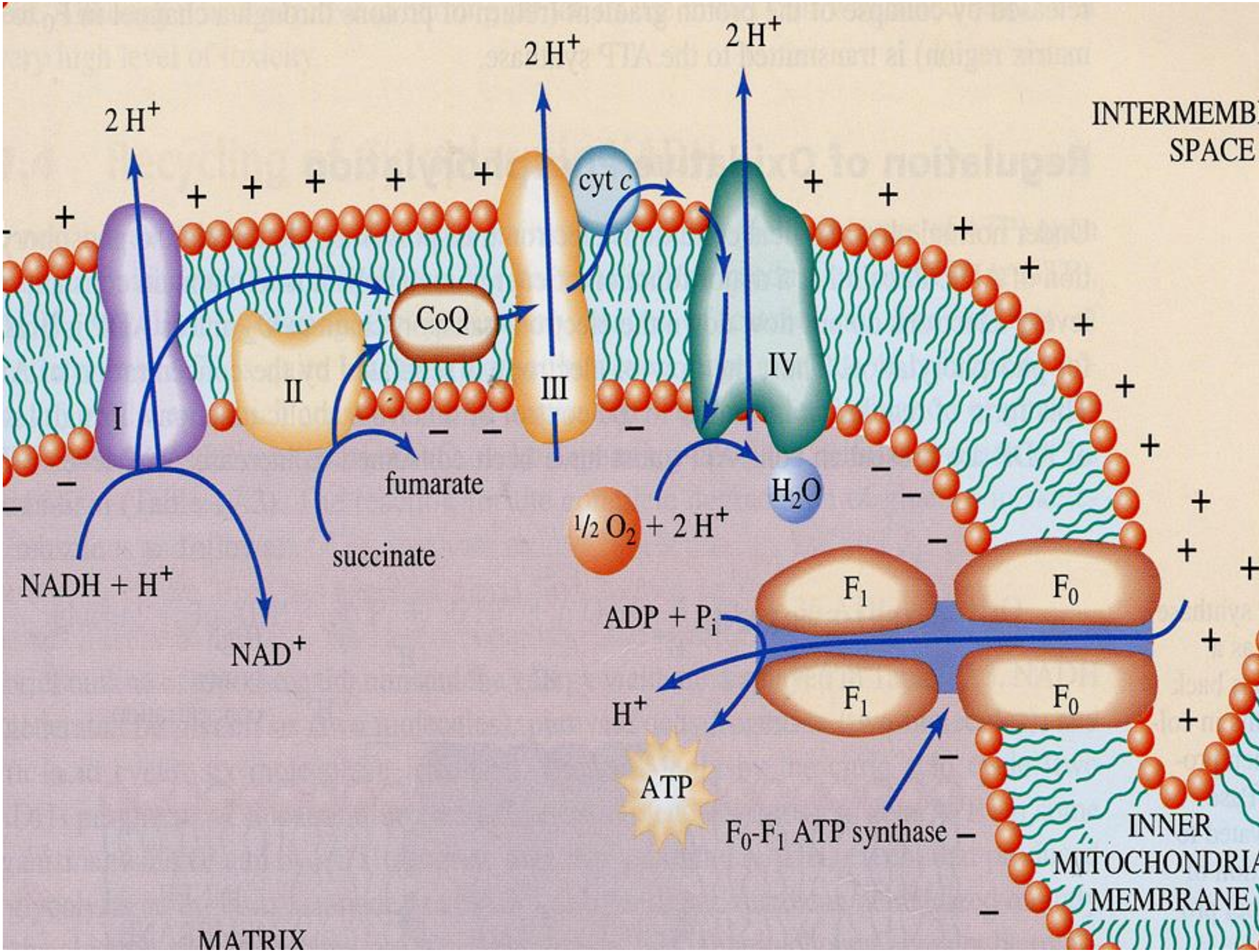


Figure 17-12 Concepts in Biochemistry, 3/e

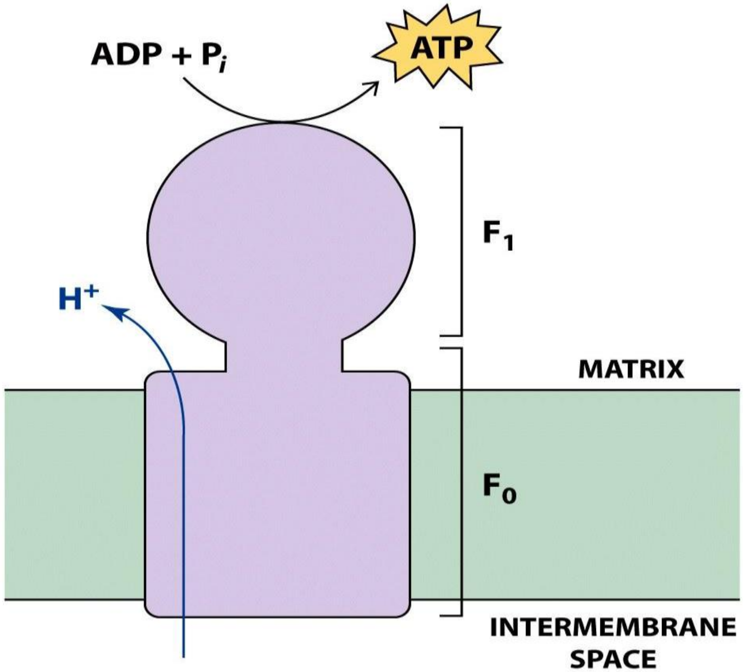

2 H+
2 H+
2 H+
INTERMEMBR
SPACE
cyt c
CoQ
I
II
III
IV
fumarate
succinate
NADH + H+
NAD+
1/2 O2 + 2 H+
H2O
F1
F0
F1
F0
ADP + Pi
H+
ATP
F0-F1 ATP synthase
INNER
MITOCHONDRIA
MEMBRANE
MATRIX

<u>ATP synthase (complex V)</u>

- ATP synthase enzyme presents in the inner mitochondrial membrane, it is a phosphorylating enzyme complex and it is formed of 2 subunits:
    - **F_1** subunit which protrudes into matrix**.**
    - **F_0** subunit which presents in the membrane.
- The energy stored in the electrochemical gradient is used to drive the synthesis of ATP by the movement of protons down the electrochemical gradient using **ATP synthase**.
- The protons outside the inner mitochondrial membrane can re-enter the mitochondrial matrix by passing through channel (**F0- F1** complex) to pass by ATP synthase enzyme which is present in F1 subunit.
- This results in the synthesis of ATP from ADP + Pi.
- At the same time decreases the pH and electrical gradients.

ATP Synthase

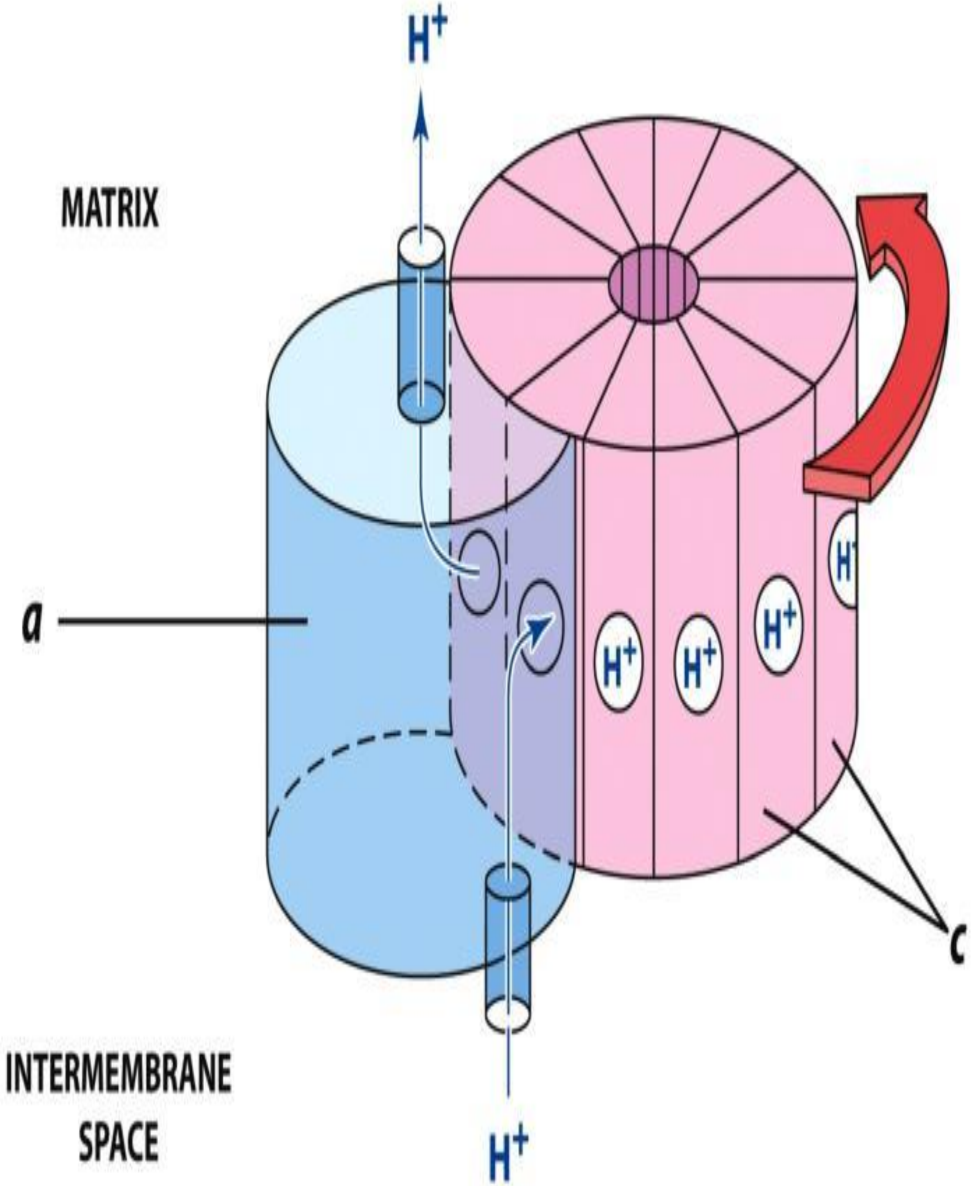
- **F_0F_1 ATP Synthase** uses the proton gradient energy for the synthesis of ATP
- Large transmembrane protein complex
- Faces into the mitochondrial matrix – spans the IMM
- Composed of a "knob-and-stalk" structure
- **F_0 (stalk) has a <u>proton channel</u> which spans the membrane.**
    - Forms a proton pore
    - Membrane-spanning portion – integral membrane protein
    - Made up of 4 different subunits
    - F_0 subunit composition: $a_1b_2c_{9\text{-}12}$ (c subunits form cylindrical, membrane-bound base)



- **F_1 (knob) contains the <u>catalytic (ATP-synthesizing) subunits</u>**
    - Where ATP synthesis takes place
    - F_1 knobs: inner face of the inner mitochondrial membrane
        - (subunit composition: **$\alpha 3\beta_3\gamma\delta\varepsilon$**)
        - $\alpha_3\beta_3$ oligomer of F_1 is connected to catalytic (C) subunits by a multisubunit stalk of γ and ε chains
- Protons passage through F_0 into the matrix is coupled to ATP formation
- Estimated passage of **3 H+ / ATP** synthesized
- F_0 is sensitive to **oligomycin**, it binds in the channel and blocks H^+ passage, thereby inhibiting ATP synthesis

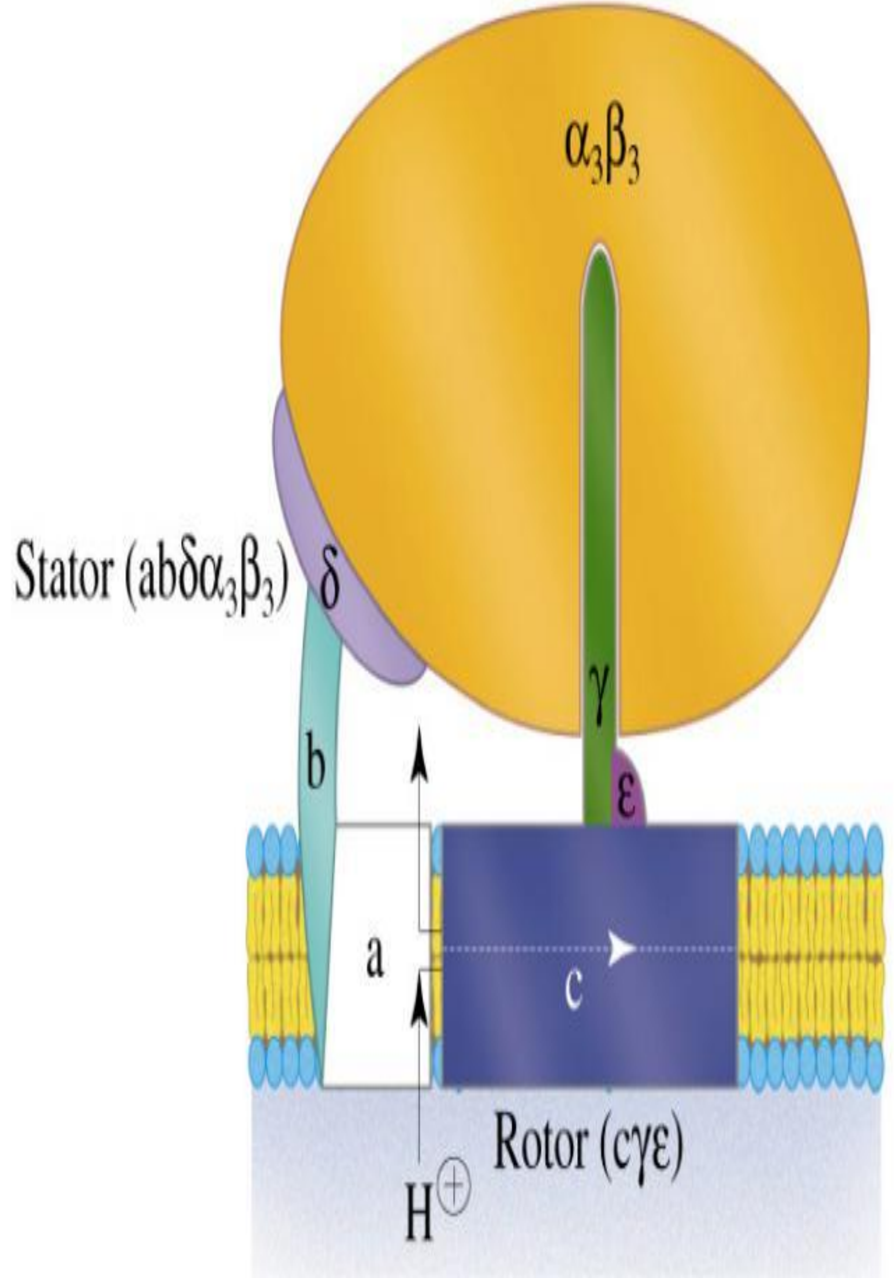
Mechanism of ATP Synthase

- F_1-F_0 complex serves as the molecular apparatus for coupling H+ movement to ATP synthase.
- There are 3 active sites, one in each β subunit
- Passage of protons through the F_0 channel causes the rotor to spin in one direction and the stator to spin in the opposite direction
- Proton flow → C unit rotates → γ rotates → conformation changes → ATP synthesized



H^+
MATRIX
H^+
H^+
H^+
a
c
INTERMEMBRANE SPACE
H^+

(b)



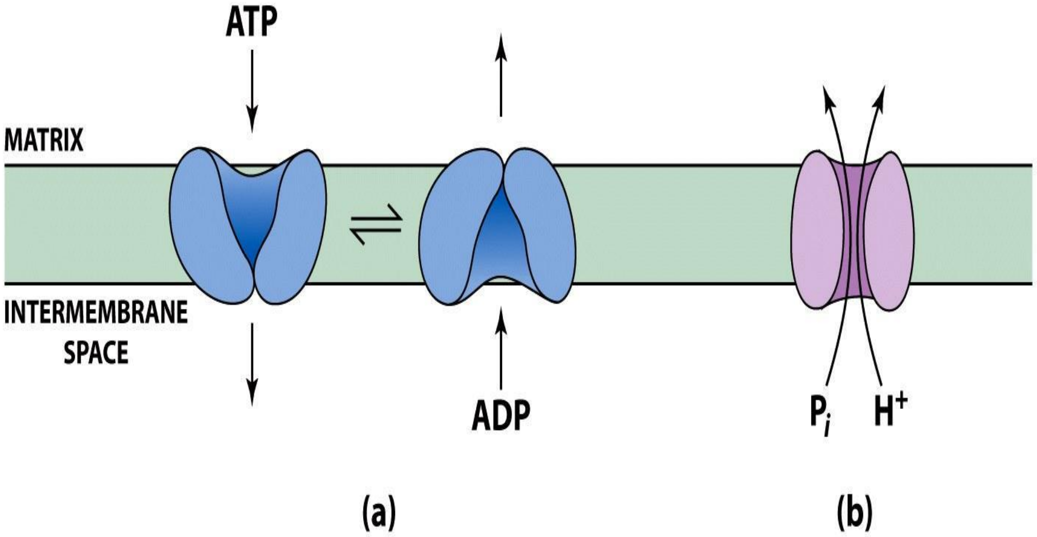
$\alpha_3\beta_3$
Stator ($ab\delta\alpha_3\beta_3$)
δ
γ
b
ε
a
c
Rotor (cγε)
$H^{\oplus}$

Regulation:

- Electrons do not flow unless ADP is present for phosphorylation
- Increased ADP levels cause an increase in catabolic reactions of various enzymes including:
    - Glycogen phosphorylase
    - PFK-1
    - Citrate synthase

ATP, ADP and Pi active transport across the inner mitochondrial membrane

- ATP is synthesized in the mitochondrial matrix
- ATP must be transported to the cytosol in exchange with ADP and Pi
- ADP/ATP carrier exchanges mitochondrial ATP^{4-} for cytosolic ADP^{3-}
- The exchange causes a net loss of -1 in the matrix (draws some energy from the H+ gradient)
- Adenine nucleotide translocase: unidirectional exchange of ATP for ADP (antiport)
- Symport of Pi and H^+ is electroneutral



The P:O Ratio

$$\text{P:O ratio} = \frac{\text{molecules of ADP phosphorylated}}{\text{atoms of oxygen reduced}}$$

- Translocation of $3H^+$ required by ATP synthase for each ATP produced
- 1 H^+ needed for transport of P_i, ADP and ATP
- Net: 4 H^+ transported for each ATP synthesized

Calculation of the P:O ratio

Complex	I	III	IV
$\#H^+$ translocated/$2e^-$	4	4	2

- Since 4 H^+ are required for each ATP synthesized:

For NADH: 10 H^+ translocated / O ($2e^-$)

So, P/O = (10 H^+/ 4 H^+) = 2.5 ATP/O

For succinate substrate = 6 H^+/ O ($2e^-$)

So, P/O = (6 H^+/ 4 H^+) = 1.5 ATP/O

- It equals zero in presence of uncouplers.

Uncouplers of oxidative phosphorylation

-Uncouplers are a group of substances that interrupt (uncouple) oxidation and phosphorylation i.e. oxidation will proceed building proton gradients but will not result in ATP synthesis ,so, energy released by electron transport will be lost in the form of heat.

-This explains the hotness sensation after these substances intake.

1- **Oligomycin:** This drug binds to the stalk of ATP synthase , closes the H channel and prevents re-entry of protons to the mitochondrial matrix.

2- **2,4 dinitrophenol :** it increases the permeability of the inner mitochondrial membrane to proton causing decrease in the proton gradient.

3- **Calcium and high doses of aspirin** : this explains the fever that accompanies toxic overdoses of these drugs.

4- Ionophores : e.g. Valinomycin and Nigericin.

-They are lipophilic substances and they have the ability to make a complex with cations as potassium "K" and facilitate their transport into mitochondria and other biological membranes.

-They inhibit phosphorylation because both electrical and pH gradient.

5- High level of Thyroxine: as in thyrotoxicosis and bilirubin.

6- Snake venoms.

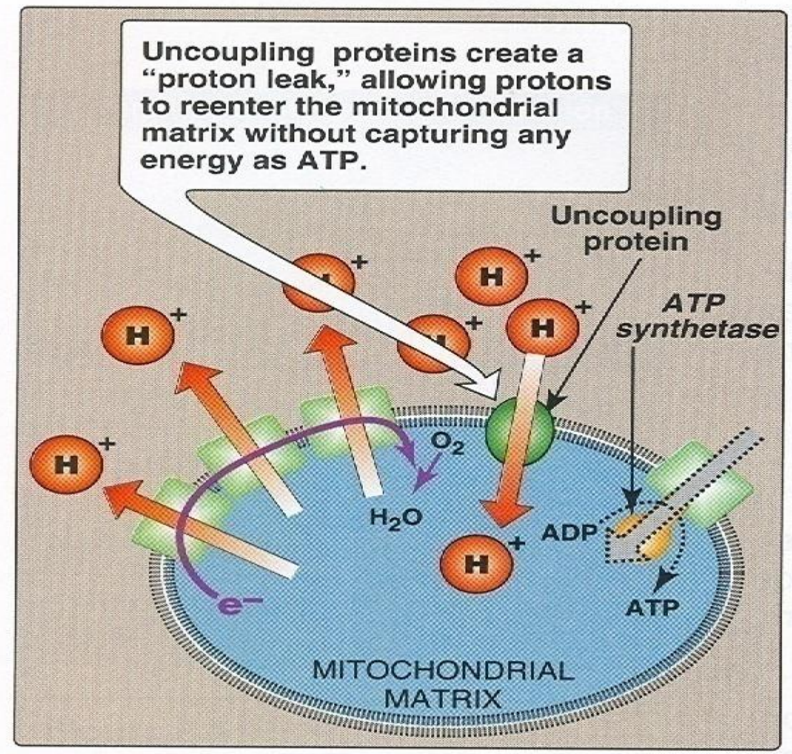


Figure 6.14
Transport of H^+ across mitochondrial membrane by 2,4-dinitrophenol.

N.B. Uncoupling proteins (UCP) = separate oxidation from ATP synthesis (the synthesis is interrupted) → energy from H+ gradient is released as a **heat**

7- Thermogenin: (brown adipose tissue)

-Thermogenin also called uncoupling protein 1, or UCP1 is an uncoupling protein found in the mitochondria of brown adipose tissue.

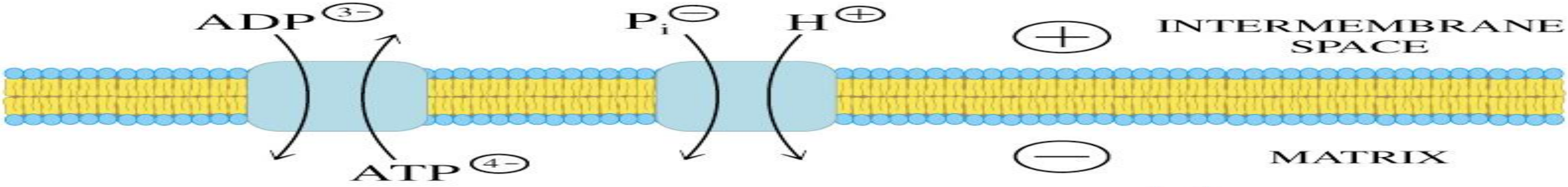
- It is used to generate heat by non-shivering thermogenesis.
- Non-shivering thermogenesis is the primary means of heat generation in hibernating mammals and in human infants.

-The molecular mechanism of UCP1-mediated uncoupling is reasonably well understood; UCP1 allows protons to reenter the mitochondrial matrix without passing through F0-F1 complex (ATP synthase), allowing respiration (and hence heat production) to proceed in the absence of ATP synthesis.

- UCP1 is restricted to brown fat, where it provides a mechanism for the enormous heat-generating capacity of the tissue.

Membrane transport chain

-The inner mitochondrial membrane contains numerous transport proteins (carriers) that permit passage of specific molecules from the cytosol to the mitochondrial matrix e.g. ADP-ATP carrier (adenine nucleotide translocase) which carries ADP from cytosol into mitochondria, while, carrying ATP from the matrix back to cytosol.



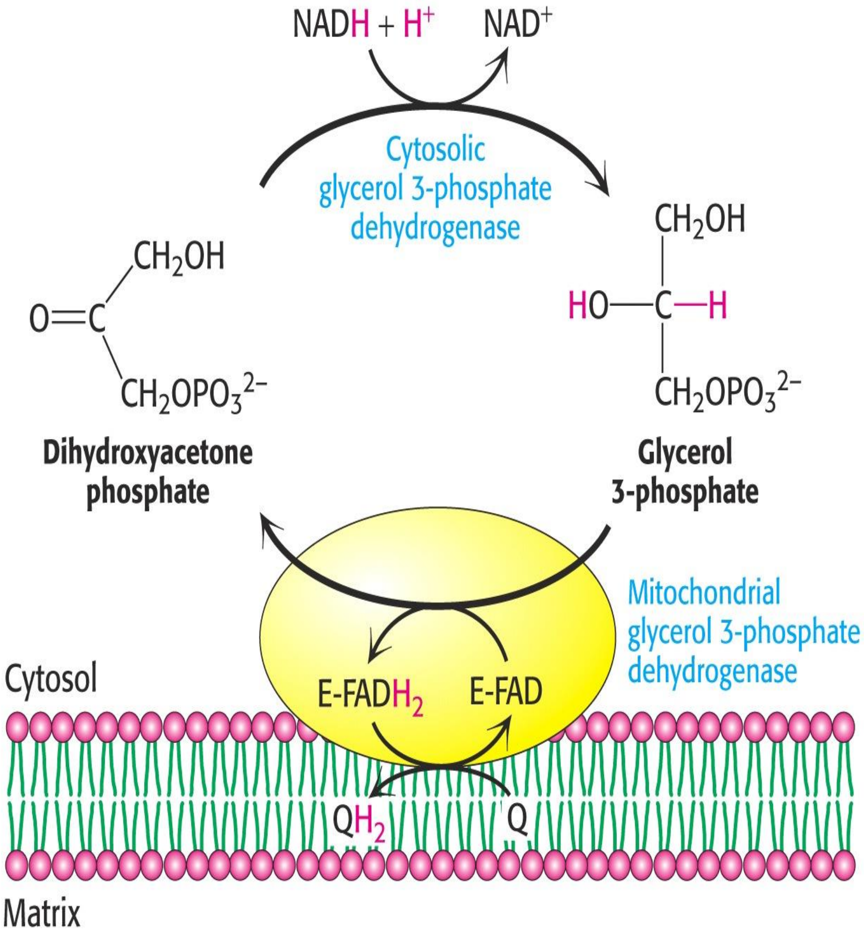
What about NADH from glycolysis?

- NADH made in cytosol
- Can't get into mitochondrial matrix

By 2 mechanisms:

A- In muscle and brain (Glycerol phosphate shuttle)

- Each NADH converted to $FADH_2$ inside mitochondrion
- $FADH_2$ enters later in the electron transport chain
- Produces 2 ATP



B- In liver and heart (Malate / aspartate shuttle)

-NADH oxidized while reducing oxaloacetate to malate by malate dehydrogenase
- Malate crosses membrane
- Malate reoxidized to oxaloacetate
- Malate dehydrogenase
- NAD+ reduced to NADH
- NADH via electron transport yields 3ATP

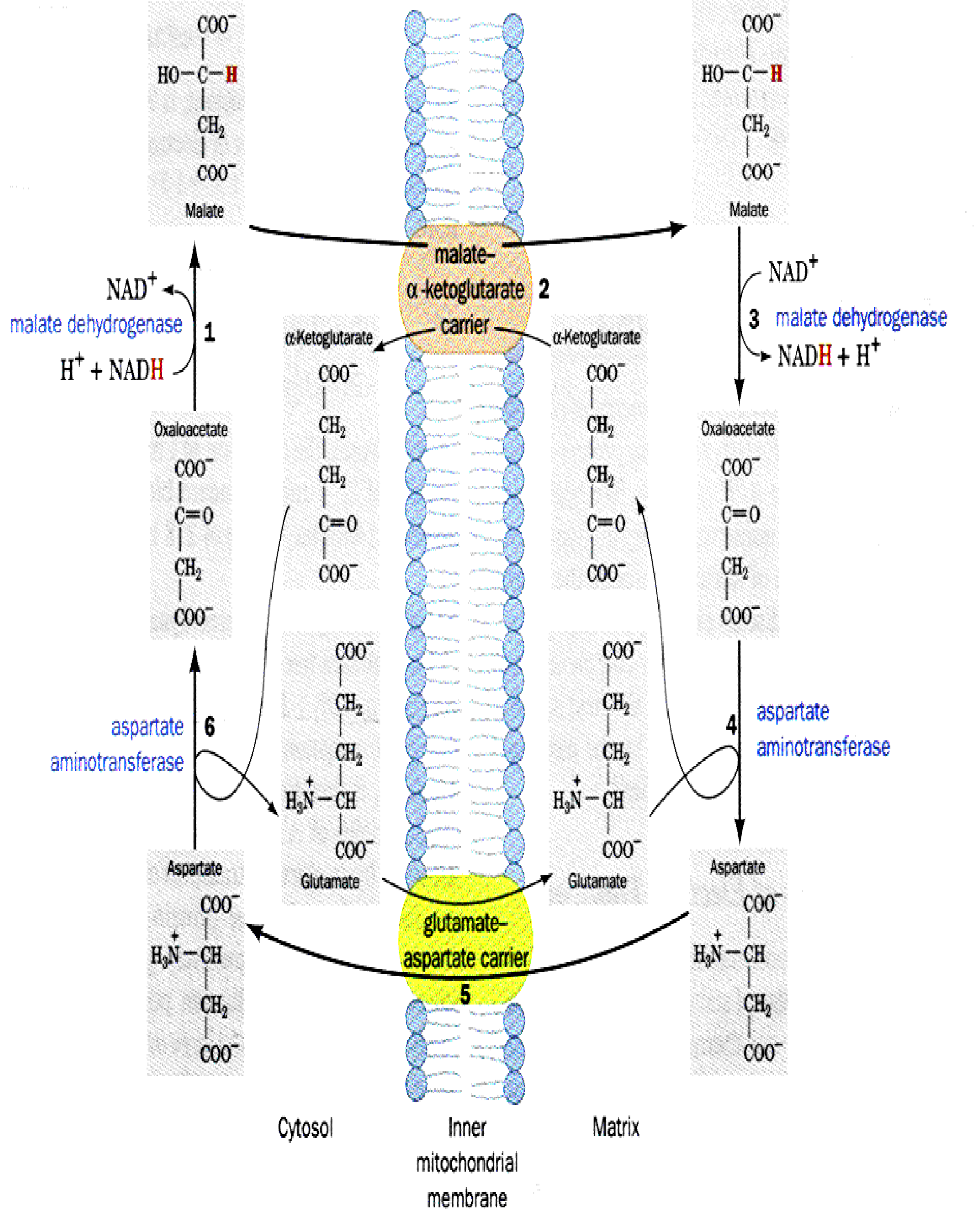


FIGURE 20-7. The malate–aspartate shuttle. The electrons of cytosolic NADH are transported to mitochondrial NADH (shown in red as hydride transfers) in Steps 1 to 3. Steps 4 to 6 then serve to regenerate cytosolic oxaloacetate.

Inherited defects in oxidative phosphorylation

- **Mitochondrial DNA (mtDNA) (37 genes)** is maternally inherited as mitochondria of sperm cell do not enter the fertilized ova.
- Mitochondrial DNA (mtDNA) codes for **13 polypeptide** (of total 120) required for oxidative phosphorylation, 22 tRNA and 2 rRNA. (while the remaining are synthesized in the cytosol & are transported into the mitochondria).
- Defects of oxidative phosphorylation usually results from **alteration in mtDNA** (mutation rate 10 times more than that of nuclear DNA).
- **Tissues with greater ATP requirement** (as CNS, skeletal muscles. & cardiac muscle, kidney & liver) are most affected by defects in oxidative phosphorylation.

Examples for diseases caused by mutations in mtDNA:

1- Mitochondrial myopathies (defective energy production → muscle cramping, weakness and severe fatigue).

2- Leber hereditary optic neuropathy (bilateral loss of vision due to optic nerve damage).

Substrate Level Phosphorylation:

- Very small amount of ATP molecules are produced
- Few reactions can form ATP at substrate level: e.g.

1.Glycolysis (phosphoglycerate kinase and pyruvate kinase)
2.TCA cycle (succinate thiokinase)

Respiratory control:

-There is no mechanism for storage of ATP and ATP present at any moment is only enough to meet the need of our cell for only few seconds.

- For this reason, there must be an efficient and controlled way for the production of ATP.

1- Availability of ADP (ATP/ADP transporter may rate limiting at certain times.
2- Availability of electrons (↑ NADH/NAD and/or ↑ $FADH_2$/FAD).
3- Availability of O_2.
4- Insulin