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.

* There are 3 conditions under "Bioenergetics" :

1. Isothermic.
2. Exergonic. (Exothermic)
3. Endergonic. (Endothermic)

Free energy:

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

The free energy change (ΔG0)

- It is the 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.

Exergonic:

$\Delta G < 0$

Energy is released to the environment

Tendency to complete it Exergonic reactions is higher than both isothermic & endergonic reactions

So, it's more preferable by our cells.

Endergonic:

$\Delta G > 0$

Energy is absorbed from the enviroment

Tendency to complete it is lower than isothermic & exergonic

it's harder to absorb than to release.

Stages of chemical reactions

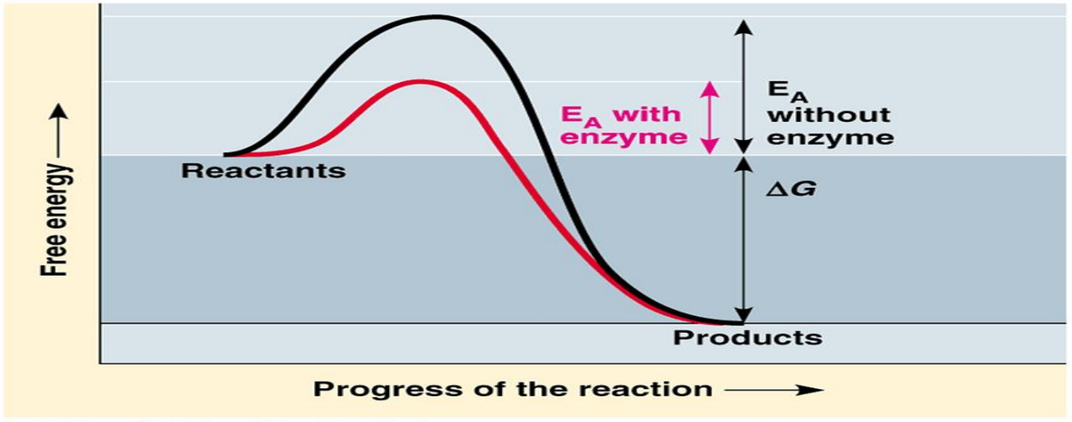
1- Activation Energy:

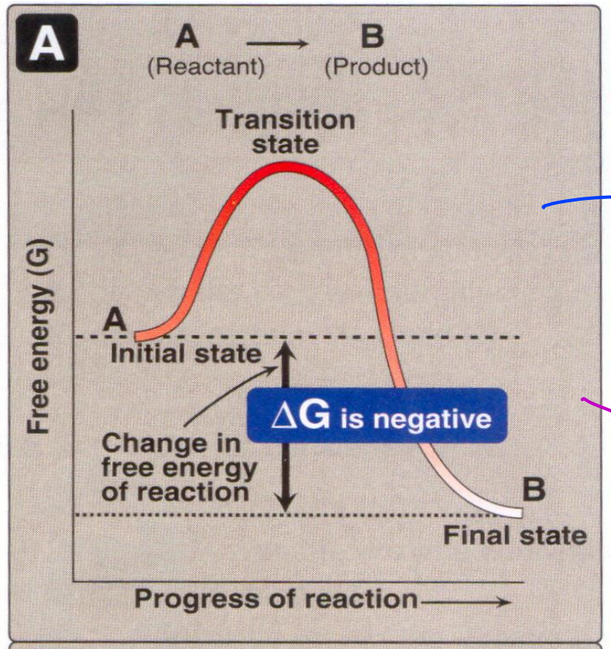
- Amount of energy that reactant molecules must absorb to start a reaction.
- This energy is usually provided in the form of heat absorbed by the reactant molecules form the surroundings.

2- Transition State:

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

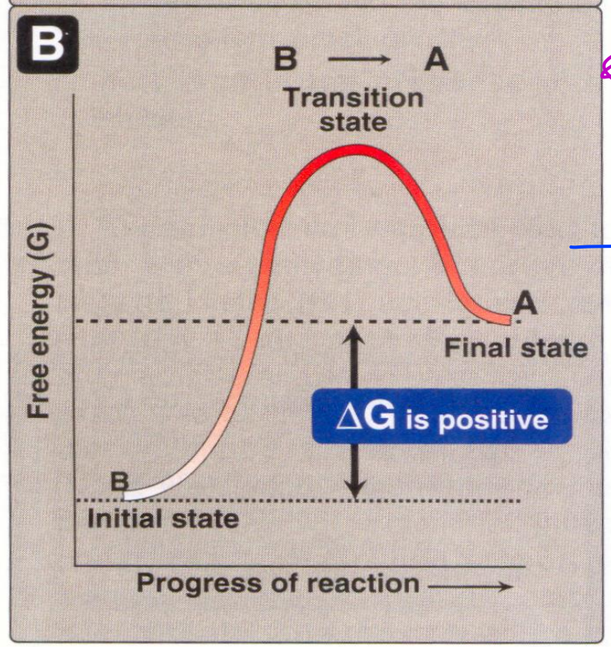
3- Products





→ Exergonic

→ This has higher tendency to be completed than this



→ Endergonic

Metabolic pathways can be grouped into two pathways:

* Release energy, which will be either in the form of ATP or Heat. So, it maintains the body temperature.

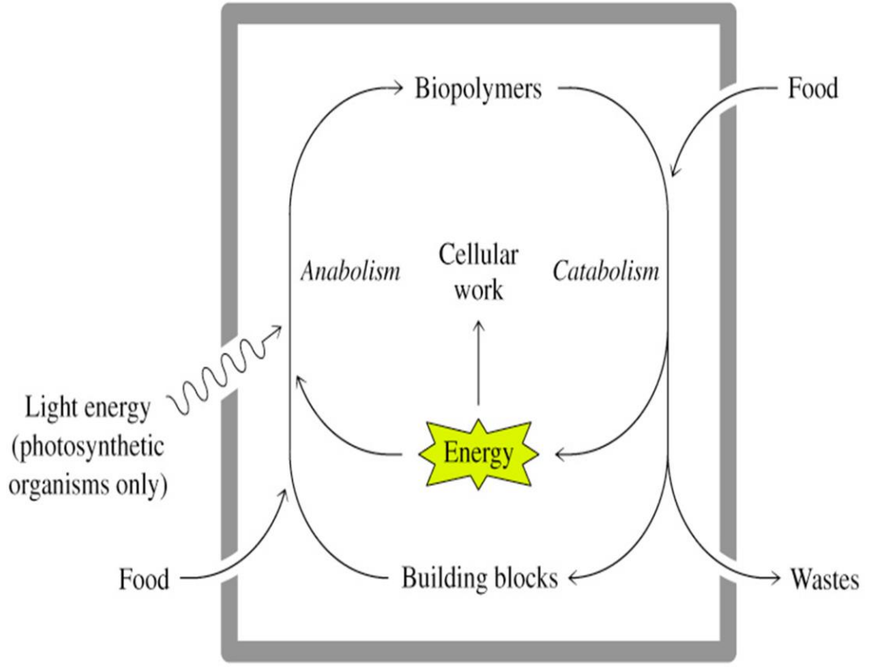
1- Catabolic reactions:

- Degrade molecules to create smaller molecules and energy (oxidation reactions releasing free energy to be transformed to ATP)

2- Anabolic reactions: Synthesis of Macromolecules from micromolecules.

- Synthesize molecules for cell maintenance, growth and reproduction (reduction reactions utilizing energy in ATP molecules)

- Catabolism and anabolism are tightly coupled together by energy.



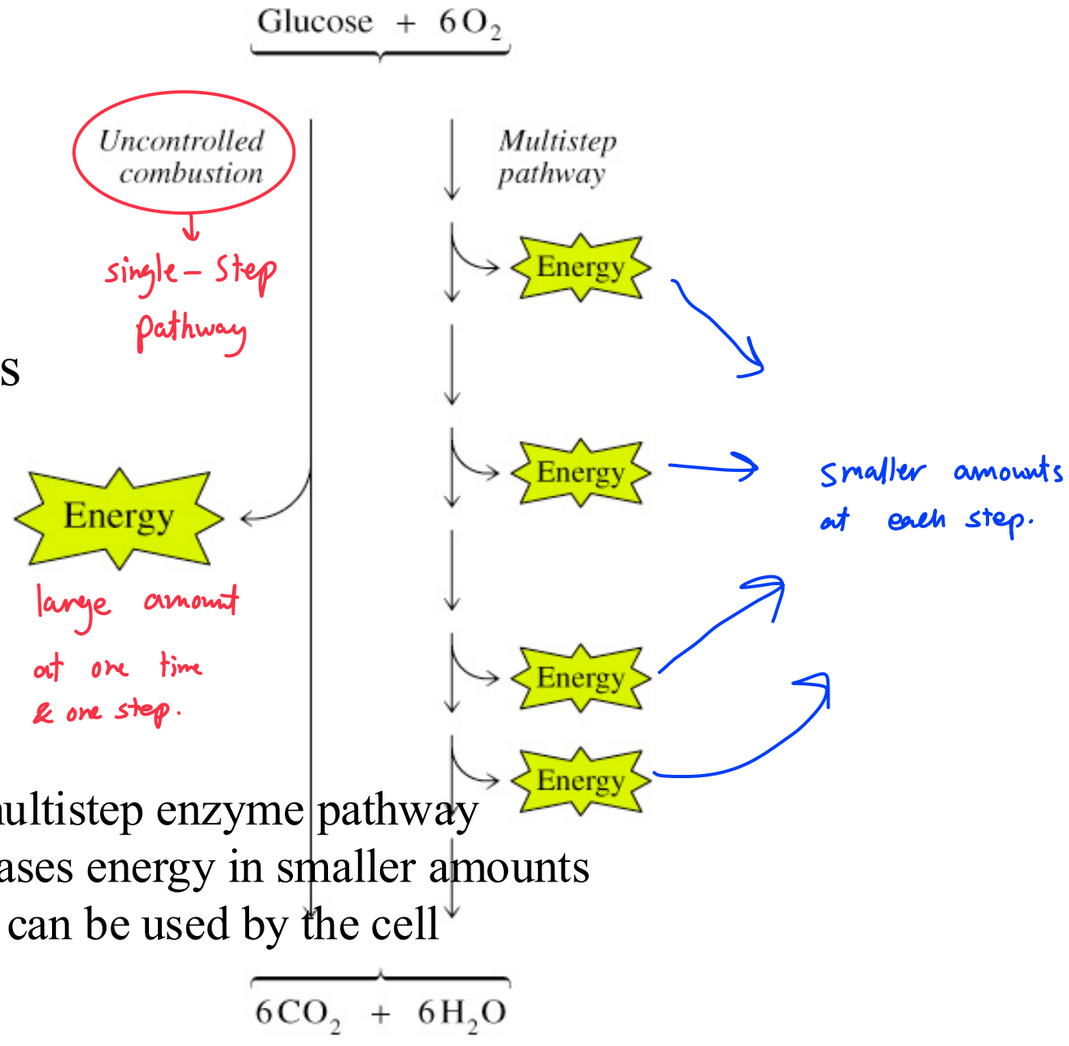
Metabolism proceeds by discrete steps

- 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 reactions are less prefered by cells because higher energy will be released & that means it will produce higher amount of heat which can cause protein denaturation & cellular damage.

* for Maintainence of a pathway, it needs to maintain 1, 2, 3

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. → "Compartmentation"
5- Molecular level

Stages of metabolism

Catabolism

"Proteins, Carbs & lipids aren't Catabolised but AA, glucose & FA are".

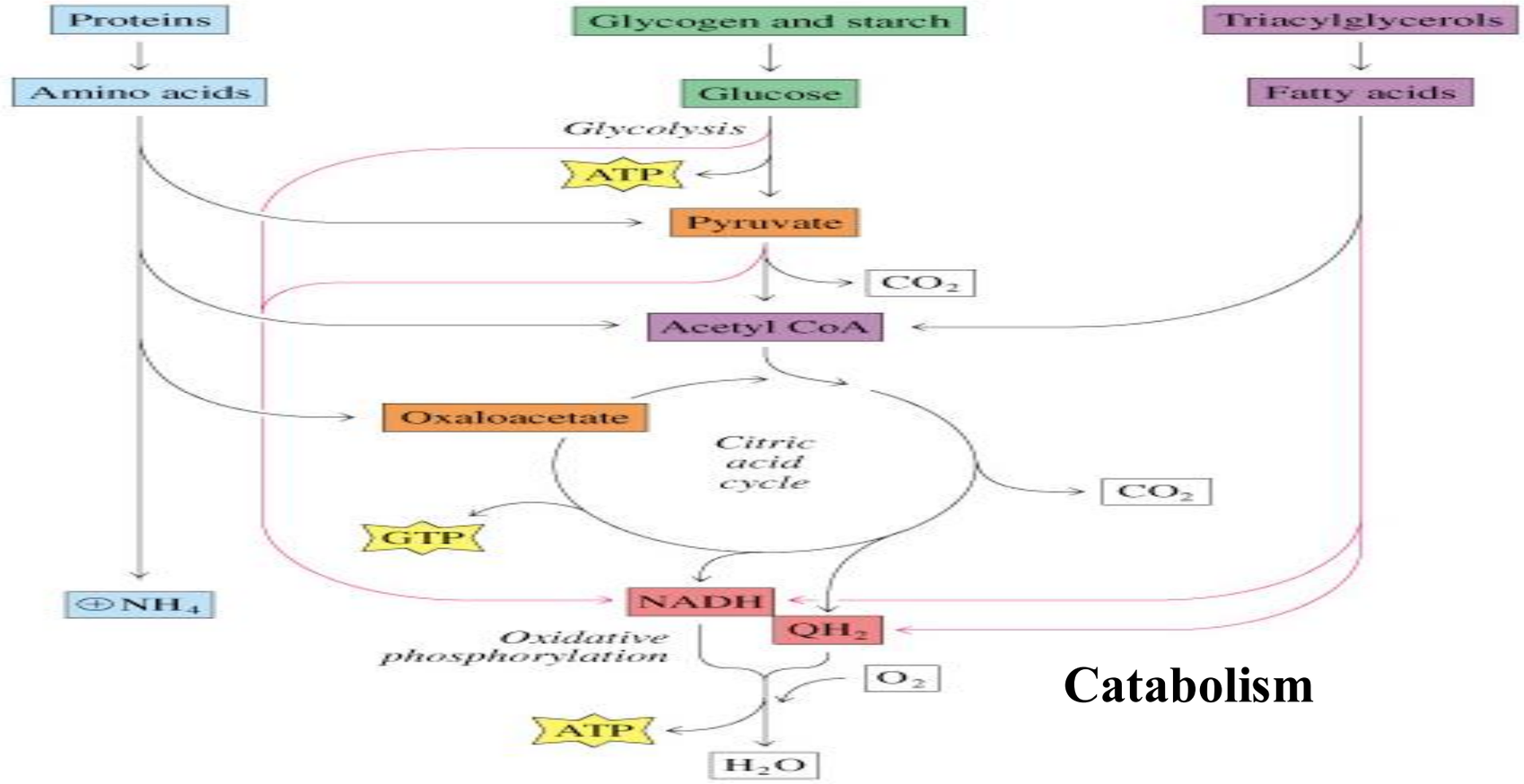
Stage I. Breakdown of macromolecules (proteins, carbohydrates and lipids to respective building blocks. → They're degraded & digested in GIT then absorbed.

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.

* NADH & $FADH_2$ are called: Reducing Equivalents or Hydrogen Carriers.

- 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 be divided into stages

- Anabolic pathways are characterized by divergence.
  - Monosaccharide synthesis begin with CO_2, oxaloacetate, pyruvate or lactate, then, polysaccharides.
  - Amino acids are synthesized from acetyl CoA, pyruvate or keto acids of Krebs cycle, then, proteins
  - Fatty acids are constructed from acetyl CoA, then, fats

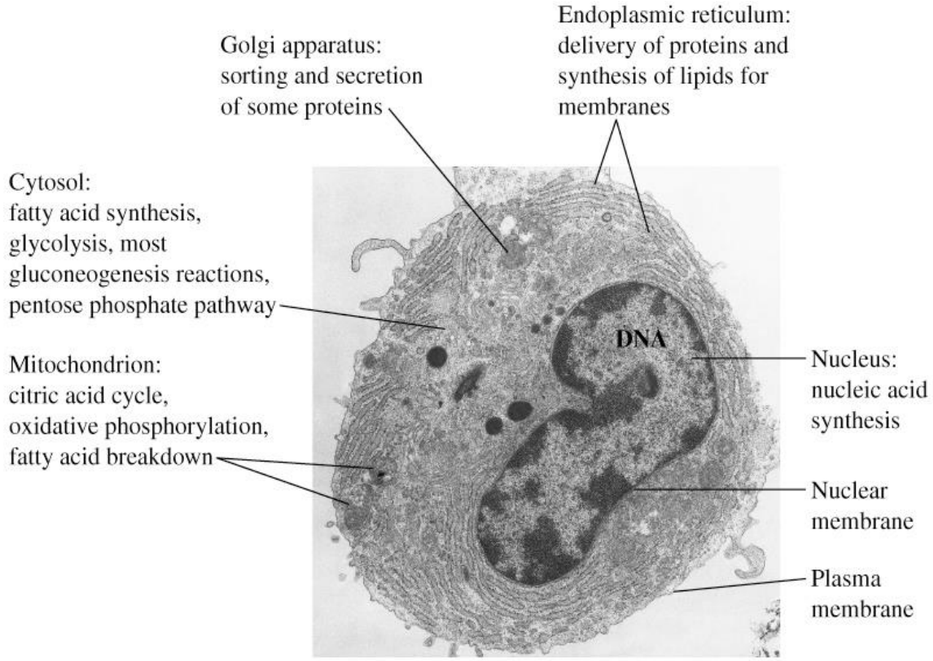
Compartmentation of metabolic processes in cell

- permits:

1- Separate pools of metabolites within a cell

2- Simultaneous operation of opposing metabolic pathways

3- High local concentrations of metabolites such as fatty acid synthesis enzymes (cytosol), fatty acid breakdown enzymes (mitochondria)



The chemistry of metabolism

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:

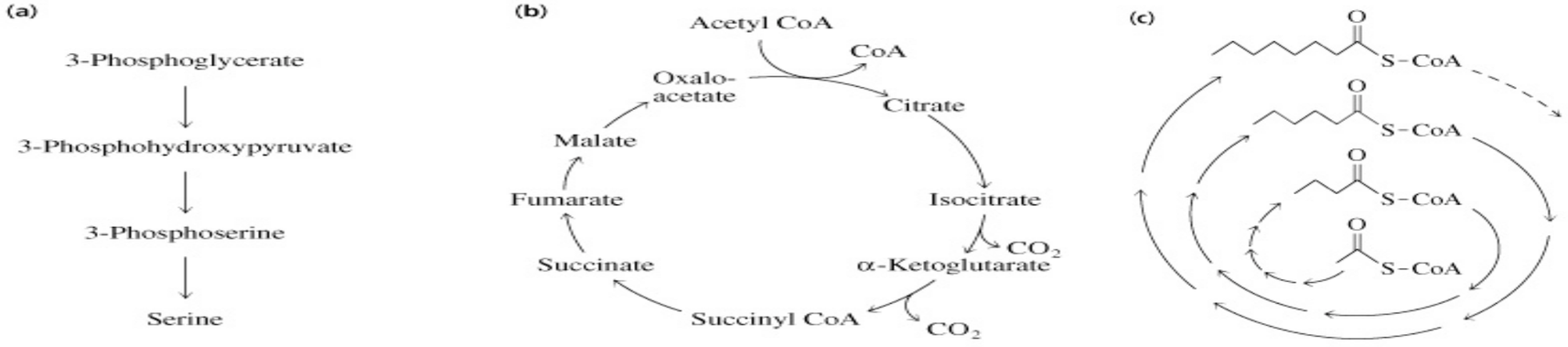
Glycogenesis ← Glycogenolysis ←

(a) Linear (Glycolysis)

Urea Cycle ←

(b) Cyclic (TCA cycle)

(c) Spiral pathway (Fatty acid biosynthesis)



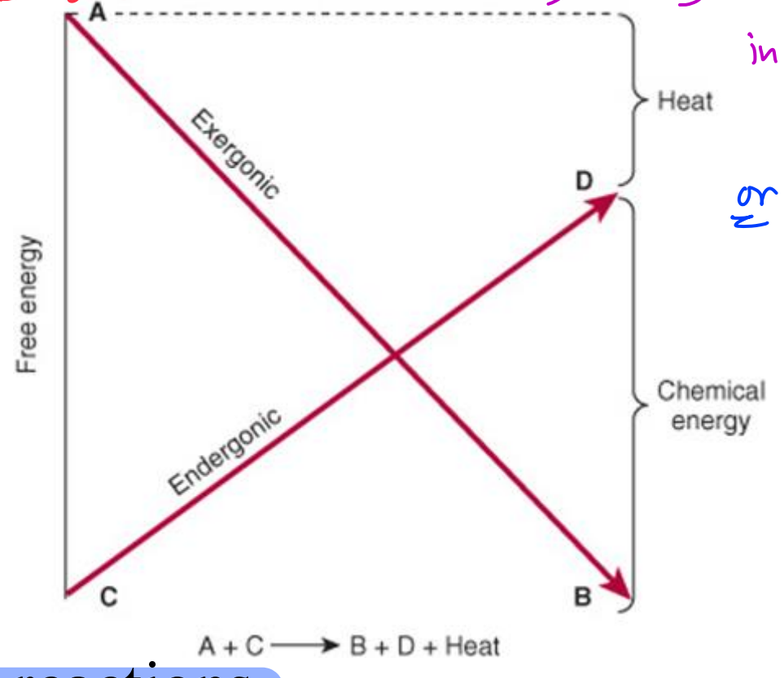
- In practice, an endergonic process cannot exist independently but must be a component of a coupled exergonic- endergonic system. One possible mechanism of coupling could be observed if **common obligatory intermediate** (I) took part in both reactions.

$$A + C \rightarrow I \rightarrow B + D$$

$FADH_2$ & NADH ⟶ They carry Hydrogen & electron in a dissociatable Manner.

or basically carry them temporarily.

* They carry electrons to components that can use electrons for energy production.



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

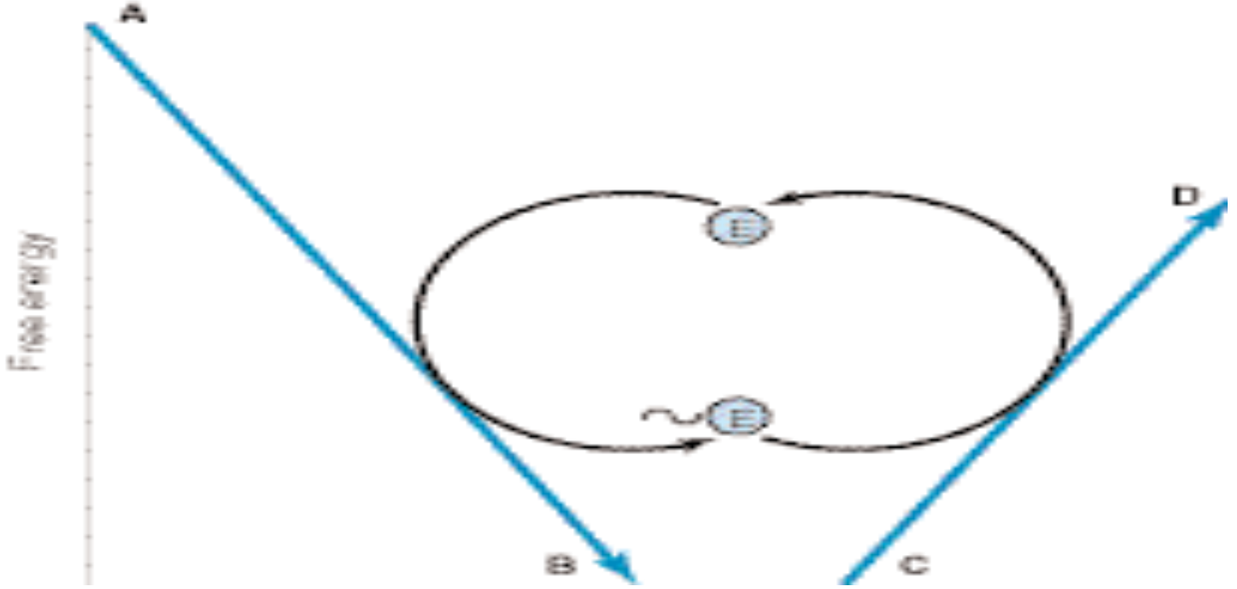
* In which a compound has a high energy bond which will be broken & thus the energy will be released & then an enzyme will incorporate this energy into another compound.

- 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 affecting 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.

* This Alternative Method is known as

" Substrate-level Phosphorylation "



Main Reactions in Coupling Mechanisms	
Common Obligatory Intermediate	The Alternative
Hydrogenation & Dehydrogenation	Transfering energy & Phosphate from one component to the other

The Intermediate value for the free energy of hydrolysis of ATP

- 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.

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

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

* There are high energy compounds but are not phosphate compounds.

- 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).

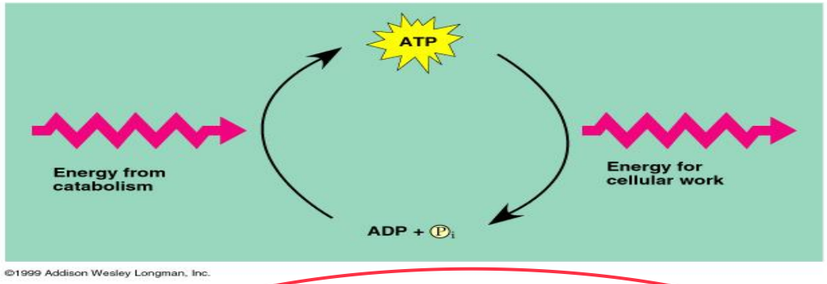
Compound	kJ/mol	kcal/mol
Phosphoenolpyruvate	−61.9	−14.8
Carbamoyl phosphate	−51.4	−12.3
1,3-Bisphosphoglycerate	−49.3	−11.8
Creatine phosphate	−43.1	−10.3
ATP →ADP + Pi	**−30.5**	**−7.3**
ADP →AMP + Pi	−27.6	−6.6
Pyrophosphate	−27.6	−6.6
Glucose 1-phosphate	−20.9	−5.0

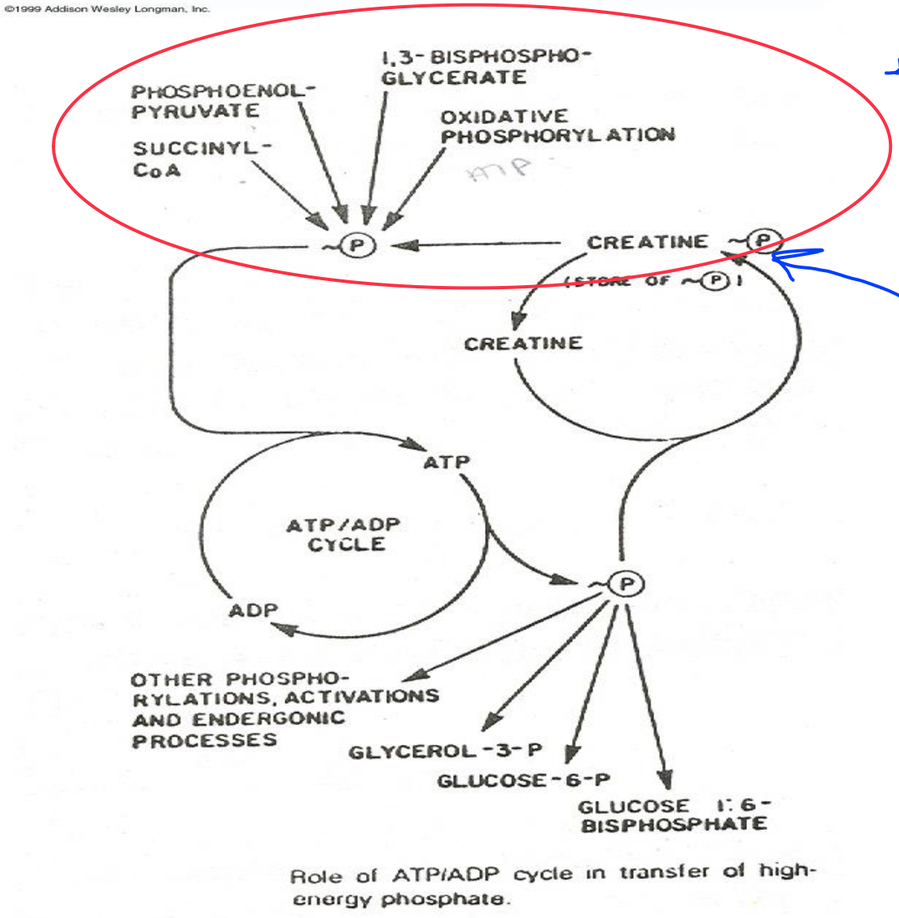
* High-energy phosphate compounds, can be used to produce ATP.

* Low-energy phosphate compounds, can't be used to produce ATP.

* The principle is: to form a certain compound, A compound of higher energy should be used.

- 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.

* Can be used to form ATP

Creatine phosphate

Sources of ATP.

ATP levels are maintained through several processes:

1. Adenylate kinase.

- ATP has two "high-energy" phosphate groups β and γ.
- Splitting off the γ phosphate group of ATP yields ADP and inorganic phosphate. **ATP ⟶ ADP + pi**
- Splitting off both high-energy groups in one step yields AMP and inorganic pyrophosphate (ppi). **ATP ⟶ AMP + 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.

2ADP ⟷ 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.

↳ It means it's involved in breaking Glycogen into Glucose.

* ADP isn't that beneficial, so 2 ADP molecules enter a reaction catalyzed by Adenylate cyclaze, One will act as a donor & become AMP, The other will act as an acceptor & become ATP. Therefore, we formed beneficial compounds, ATP for energy & AMP for producing cAMP 2nd messenger.

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".

* Creatine + ATP ⇌ phosphocreatin + ADP

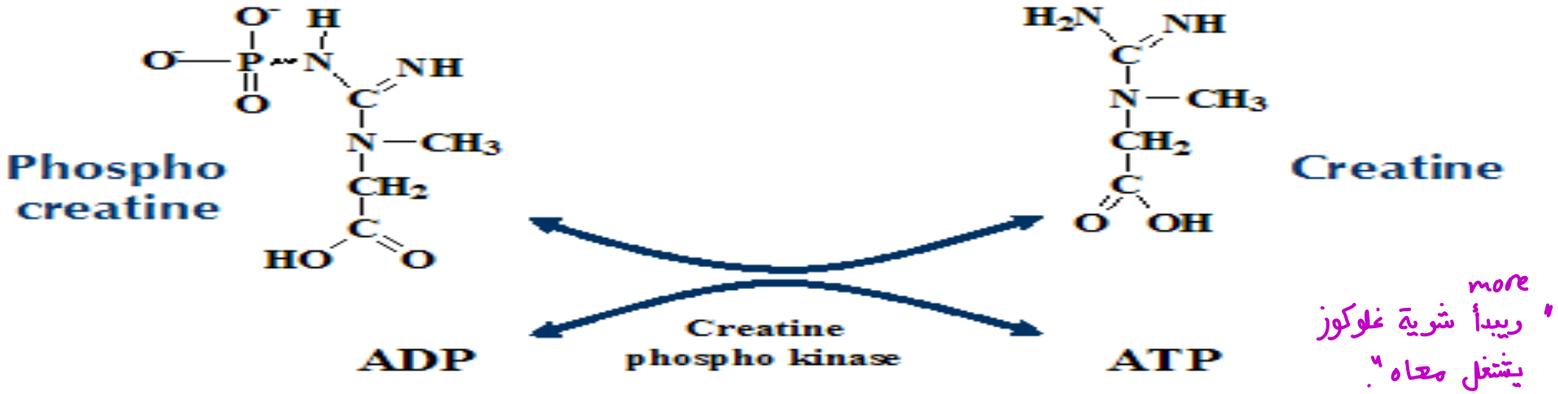
Why does our body produce creatine phosphate?

* because the half life of ATP is so short, so we need Creatine phosphate as it has a longer half life. However, it isn't that long, it just extends the presence of energy for more seconds.

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



more ريبدأ شوية غلوكوز يشتغل معاه

*Q: An athlete runs a distance of 200 m in 20 seconds:

a) What's the main source of energy during the first 10 seconds?

phosphocreatine system.

b) What's the main source of energy between 10 & 20 seconds?

← phosphocreatine system & Glycolic system

* One Reaction, one enzyme, one product.

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.

* The difference between Aerobic & Anaerobic is Oxygen & Mitochondria.

3. Anaerobic Metabolism.

- This is a rapid 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.

- 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 disadvantage is that a lot of lactic acid is produced and accumulates in the working muscle and lipids cannot be used as substrates for anaerobic metabolism.

- Muscles exhaust their stored glycogen and take up so much glucose from the blood resulting in hypoglycemia and CNS malfunction.

4. Aerobic Metabolism.

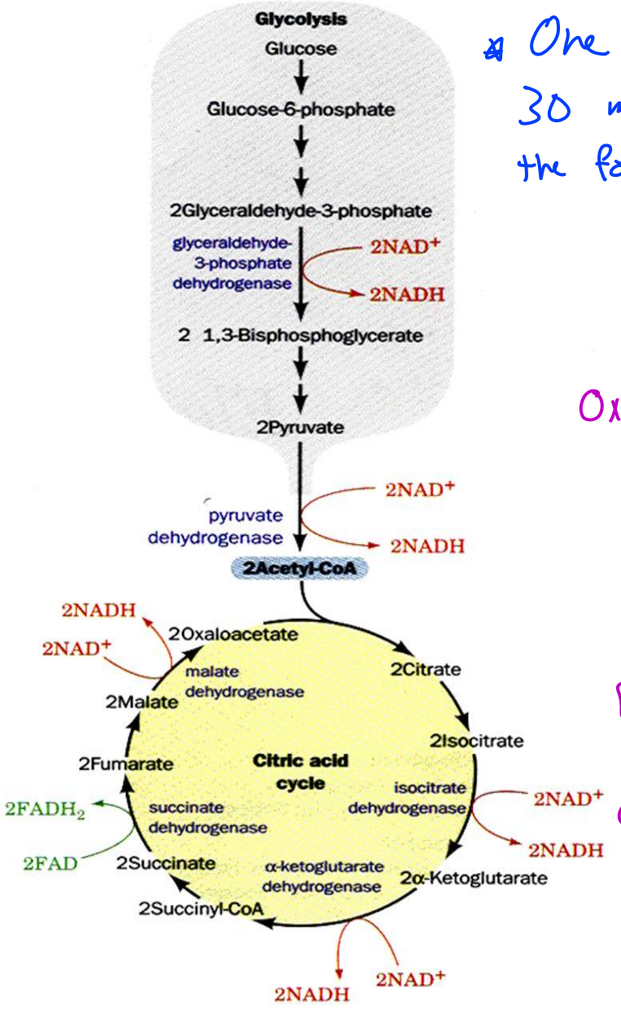
- All of our cells, with the important exception of blood cells, contain mitochondria which use oxygen and form water while oxidizing our "food".

- Mitochondrial substrate is acetyl-CoA coming from all types of nutrients

- Approximately 40% 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 to maintain our temperature

- It is a slow process, produces 10 moles of ATP for each mole of acetyl-CoA.



* One Glucose produces 30 moles of ATP in the following pathways:

Glycolysis
↓
Oxidative decarboxylation of pyruvate
↓
Kreds cycle
↓
Electron transport chain
&
Oxidative Phosphorylation

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).

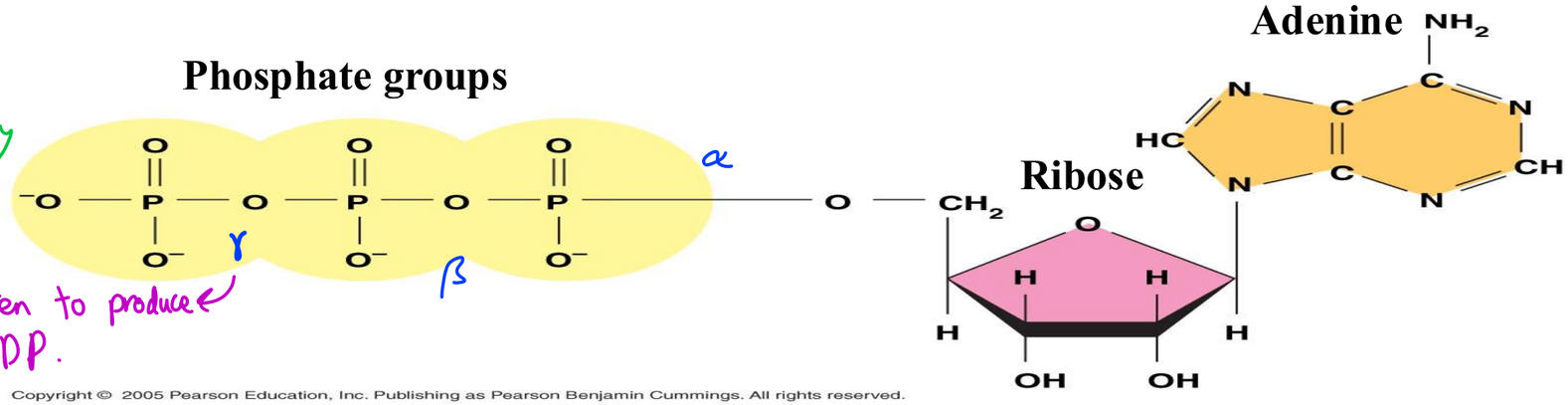
Importance of ATP as a source of energy :

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.

* ATP contains 3 phosphate bonds :

α, β, γ → high-energy bonds

between the phosphate & Ribose, it's a low-energy bond



Electron transport chain (ETC)

A Multi-step pathway

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

So, it's a multi-step pathway

Redox Potential (electron affinity)

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

Redox chain: → Ends with oxygen.

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

A → B → C → D…..

- Each component 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.

Under Aerobic Conditions

✡ Brief Reminder :

— Three processes occur so NADH & $FADH_2$ enter the Electron transport chain :

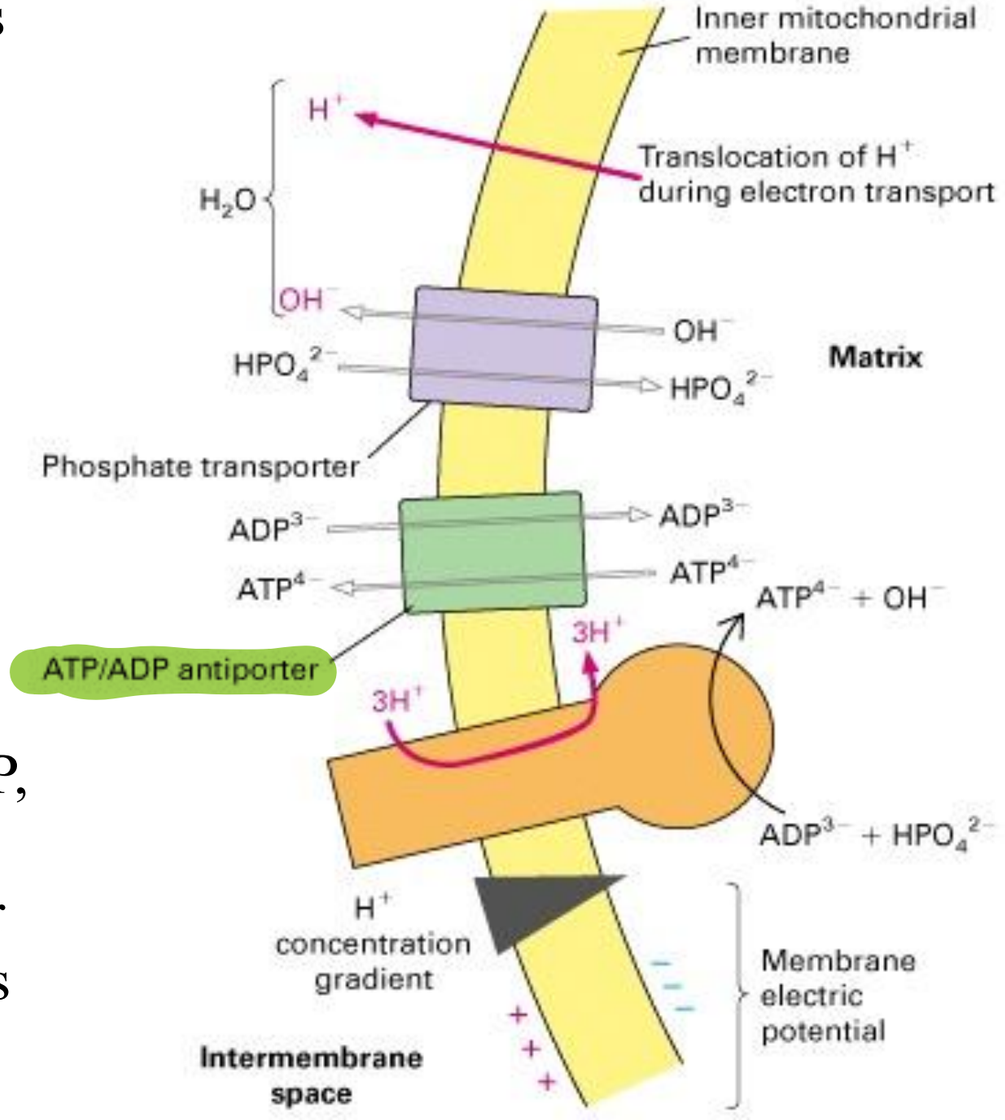
Glycolysis
↓
Oxidative decarboxylation of pyruvate
↓
Krebs cycle

Electron transport 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 O_2 (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 respiratory chain components.

- 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.

So, They need channels for their transport.



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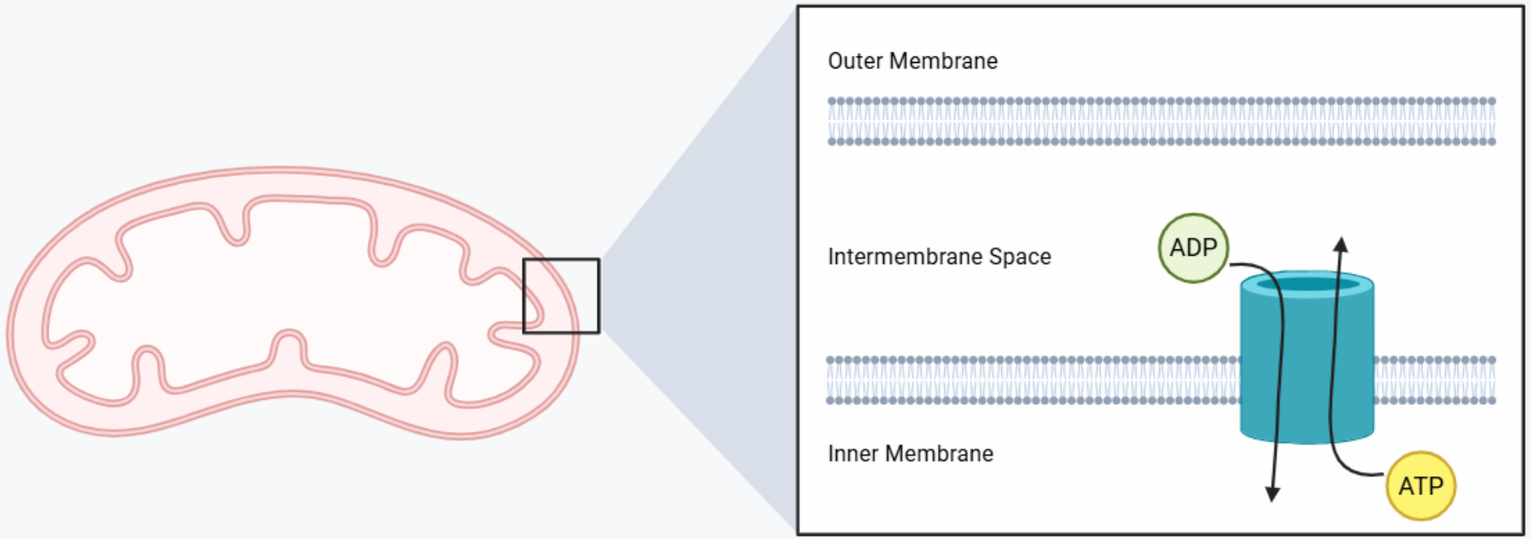


A figure of ATP-ADP Translocase.

ATP-ADP Antiporters are needed to prevent accumulation of ATP inside Mitochondria because it leads to negative feedback on Electron Transport Chain.

* Complex II isn't a component of the inner mitochondrial membrane, & isn't on the same line but is protruding into the Mitchondrial Matrix.

Organization of Electron transport 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.

* Each one of the complexes contains an enzyme, a coenzyme & a cofactor.

Complex I

- Contains an enzyme called NADH dehydrogenase.
- Its coenzyme is FMN (can accept two hydrogen atoms to become $FMNH_2$)

Cofactor ← - It contains several iron and sulfur atoms (iron sulfur protein). → 9 Iron & Sulfur clusters

- NAD+ is reduced to $NADH+H^+$ by dehydrogenases that remove hydrogen atoms from their substrates.

Complex II

has a role in Krebs cycle.

- 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).

9 Iron & Sulfur clusters

Complex III

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

* 2 Iron & Sulfur clusters

Cofactor

- Coenzyme : Copper.

Complex IV

Cofactor

- 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.

* It doesn't contain Iron & Sulfur clusters.

* Iron is the Coenzyme.

Complex	Enzyme	Coenzyme	Cofactor	Iron-Sulfur Clusters
Complex I	NAD Dehydrogenase	FMN	Iron & Sulfur	9 Clusters
Complex II	flavo-protein Dehydrogenase e.g. Succinate Dehydrogenase	FAD	Iron & Sulfur	9 Clusters
Complex III	Cytochrome Reductase	Copper	Iron & Sulfur	2 Clusters
Complex IV	Cytochrome a & a3	Iron	Copper	None

Ubiquinone "Coenzyme Q"

The most collecting point in ETC.

- 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.

* There're 2 entry sites for Hydrogen into ETC:
  - Complex I
  - Complex II

Cytochromes ⟹ It moves between Complex III & IV

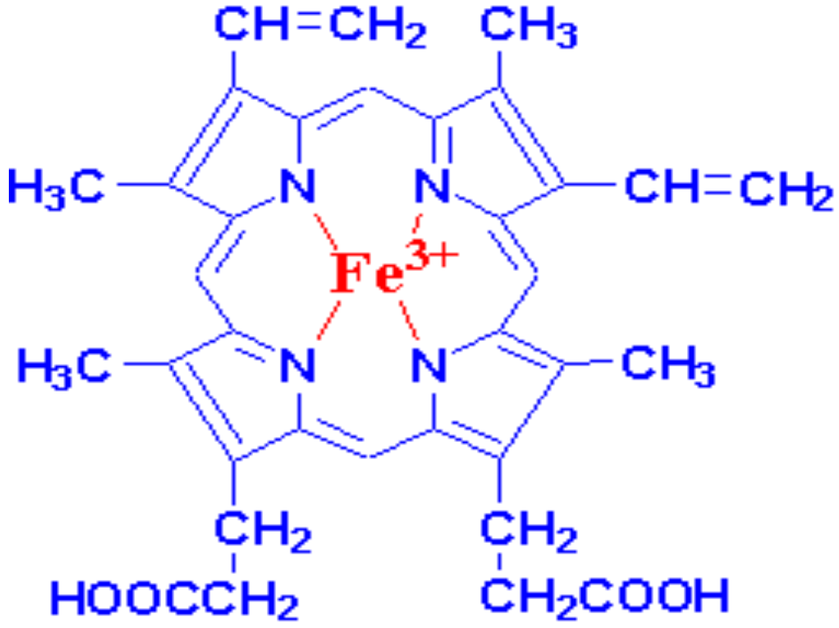
- Cytochromes are proteins that contain an iron-containing heme group. This iron oscillates between ferric form (Fe^{+++}) when it losses an electron, and ferrous form (Fe^{++}) when it accepts an electron.
- All are integral membrane proteins with the exception of cytochrome C, a soluble free protein.

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.

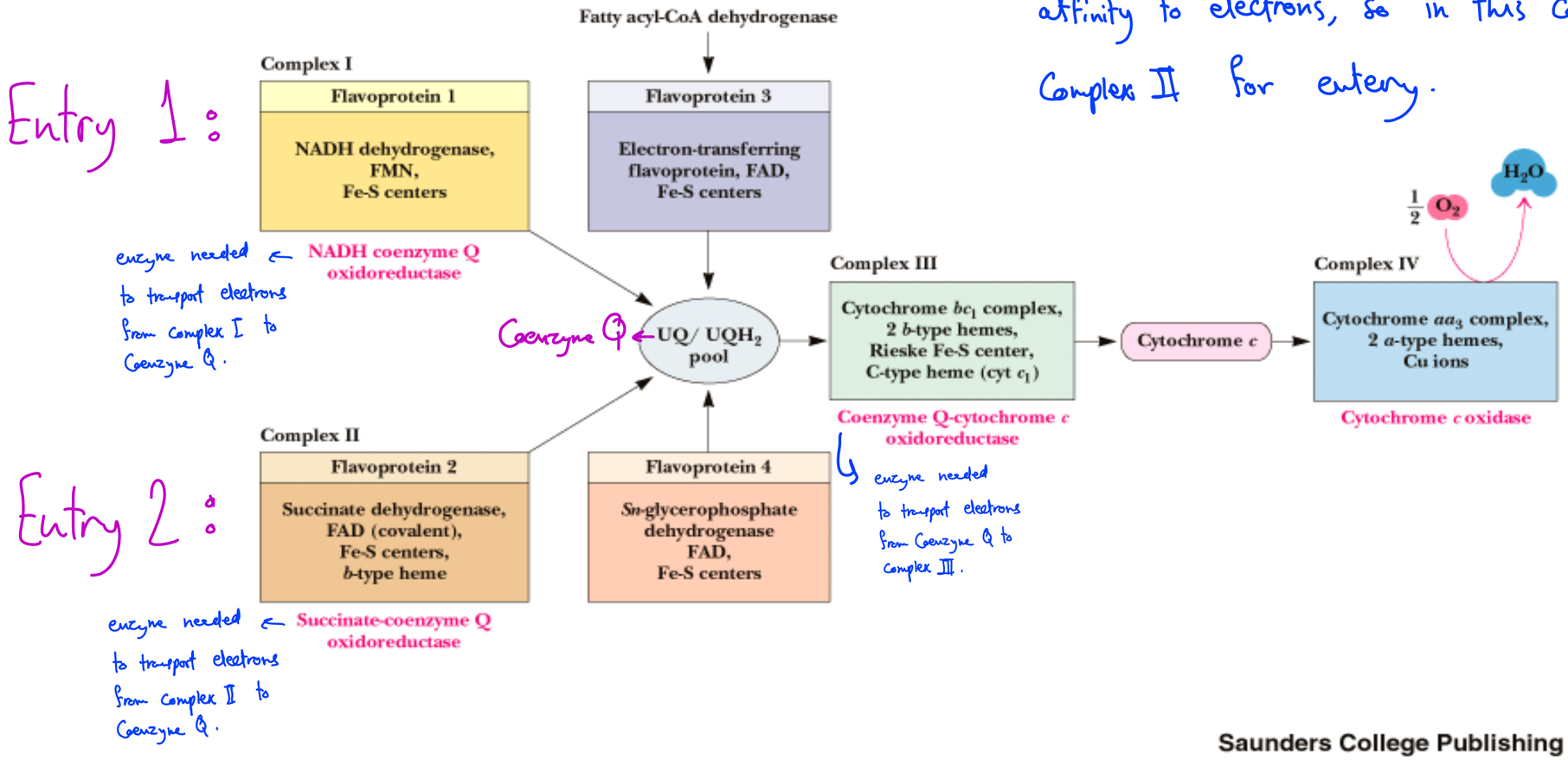
- Cytochrome a3 contains copper in addition to iron and called cytochrome oxidase, it is the terminal component of the ETC.



* Q: Why are there 2 entry site for Hydrogen?

– A compound can have a Redox potential higher than Complex I Redox potential or affinity to electrons, so in this case we need Complex II for entery.

Garrett & Grisham: Biochemistry, 2/e
Figure 21.4



Entry 1:

enzyme needed to transport electrons from complex I to Coenzyme Q.

Entry 2:

enzyme needed to transport electrons from complex II to Coenzyme Q.

enzyme needed to transport electrons from Coenzyme Q to complex III.

- 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. → "Coupled"
-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.
- Chemiosmotic hypothesis and membrane transport system can explain synthesis of ATP.

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.

* Complex II doesn't act as a proton pump or participate in the production of ATP, so it isn't considered as a coupling site.

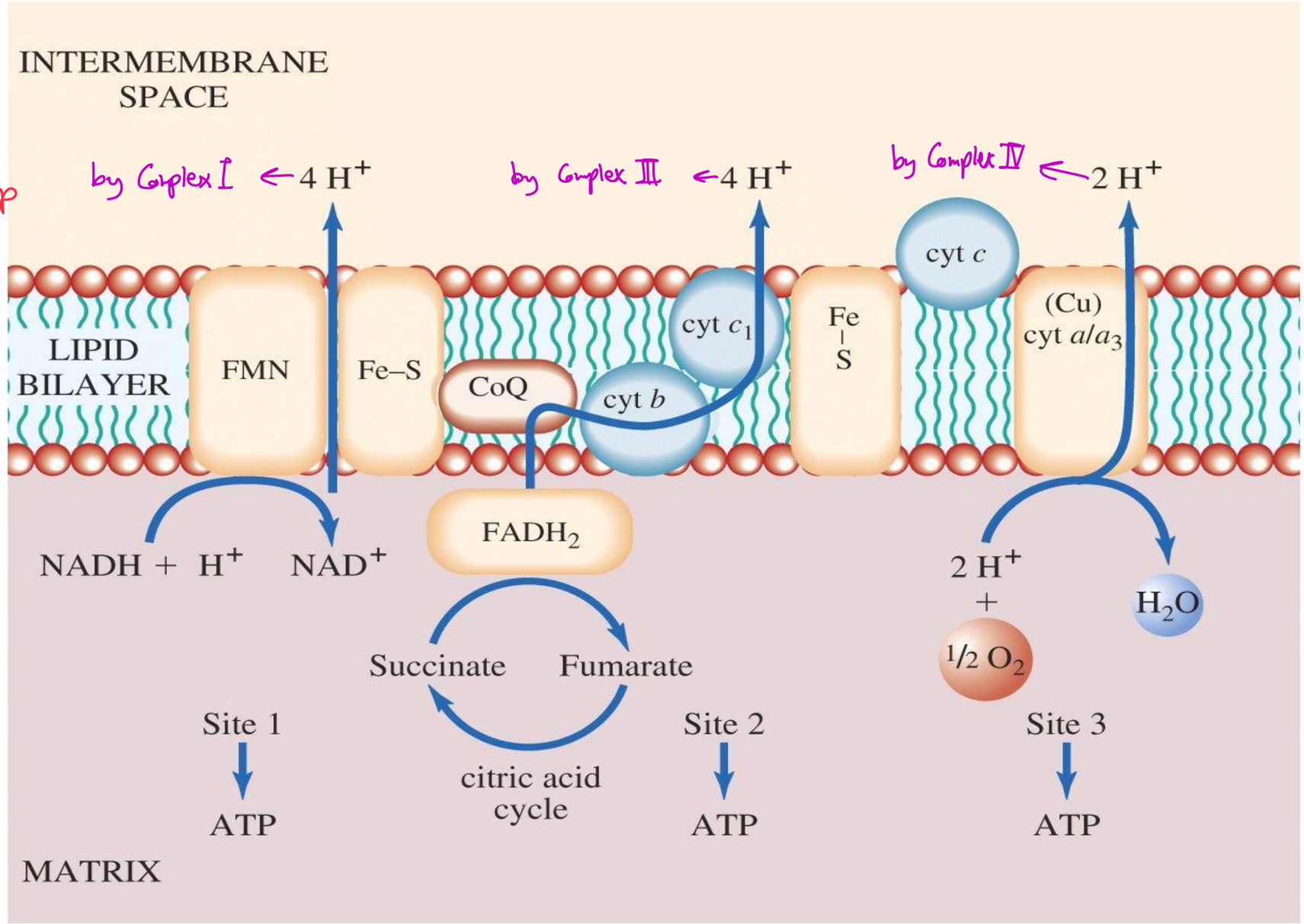


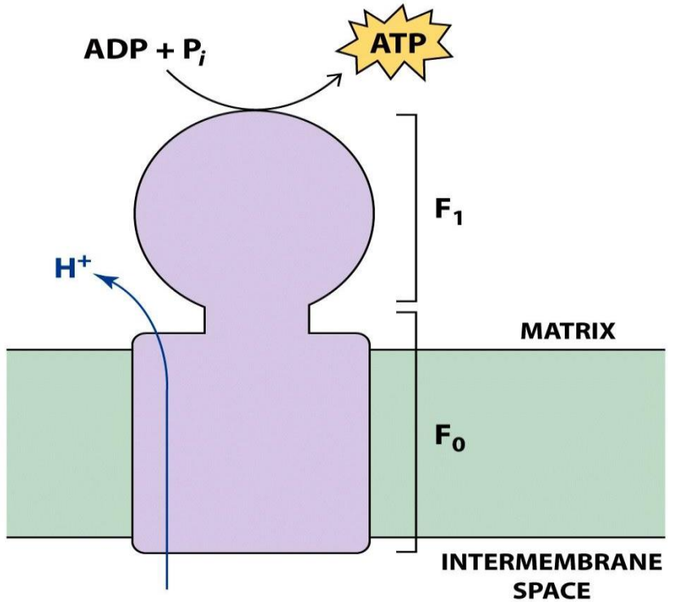
Figure 17-12 Concepts in Biochemistry, 3/e

ATP synthase (complex V)

- 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 proton channel 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)

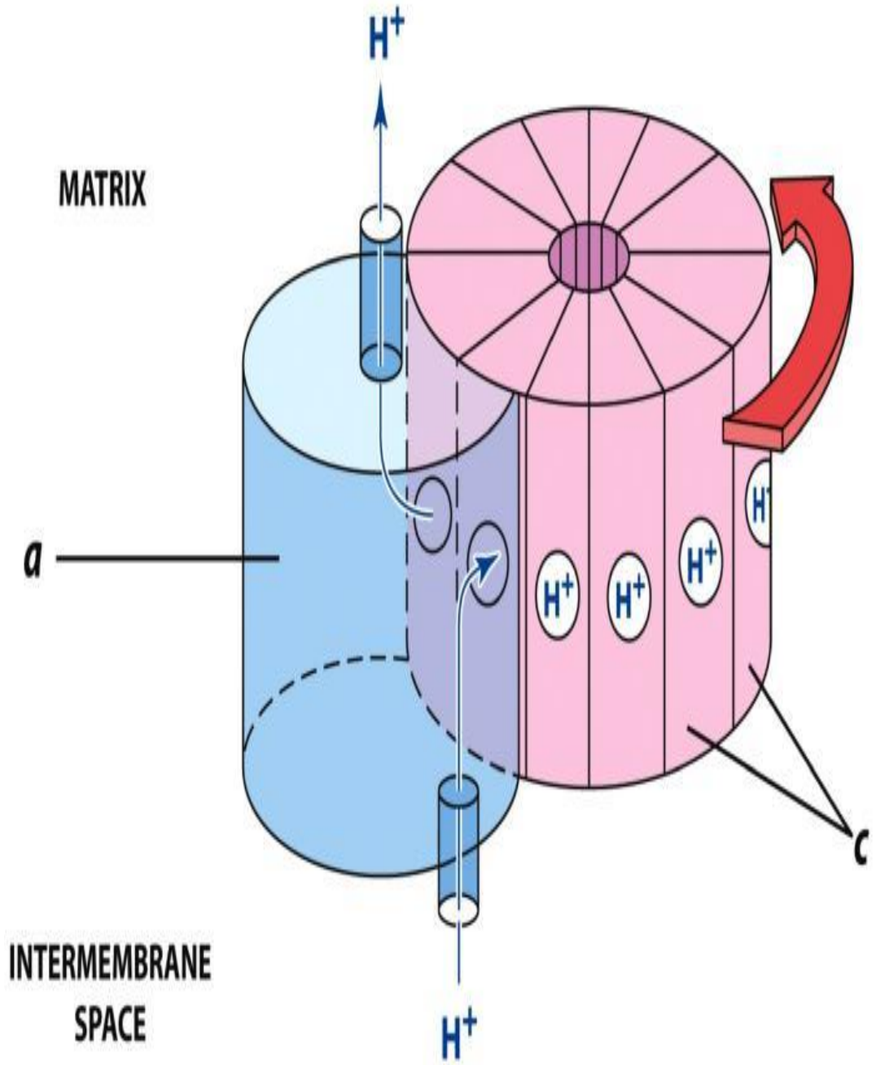


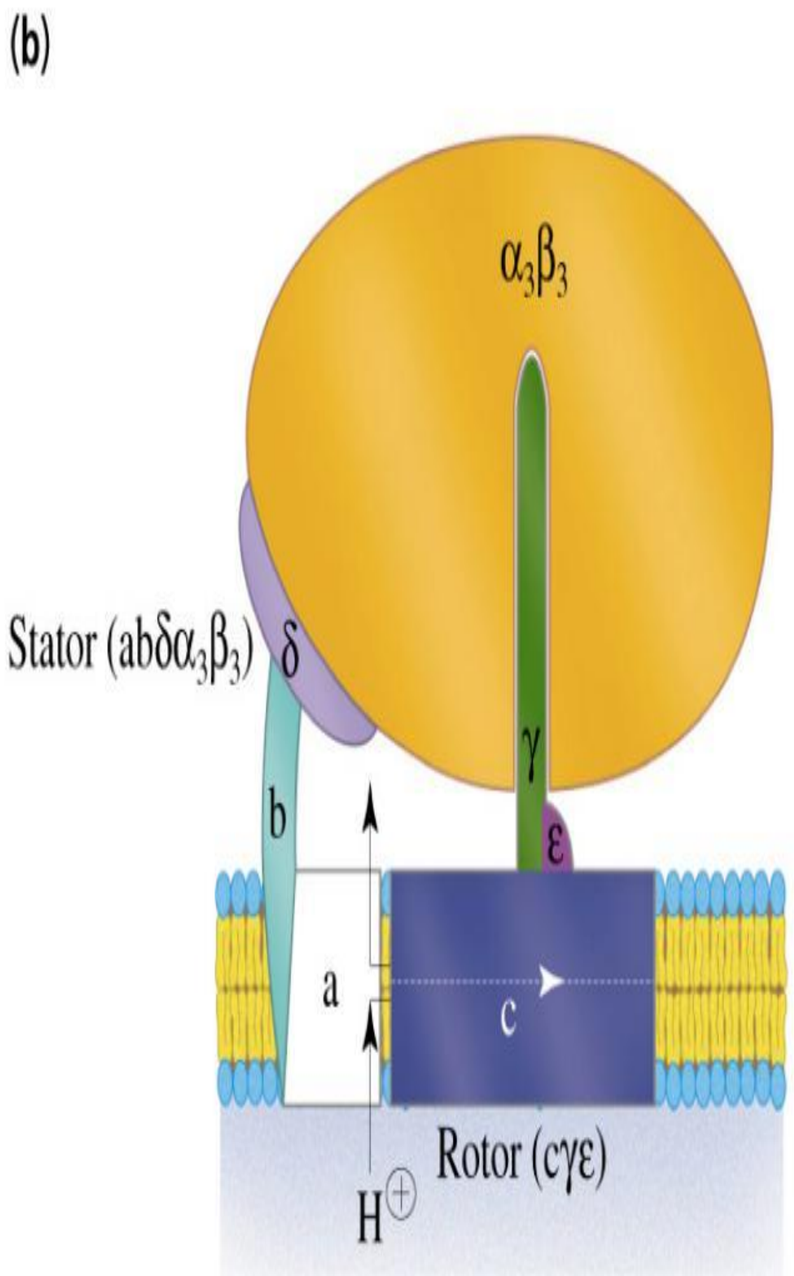
- **F_1 (knob) contains the <u>catalytic (ATP-synthesizing) subunits</u>**
    - Where ATP synthesis takes place
    - F_1 knobs: inner face of the inner mitochondrial membrane
        - (subunit composition: **$\alpha 3\beta_3\gamma\delta\varepsilon$**)
        - $\alpha_3\beta_3$ oligomer of F_1 is 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

Rotation of subunits in Opposite directions activates ATP Synthase.



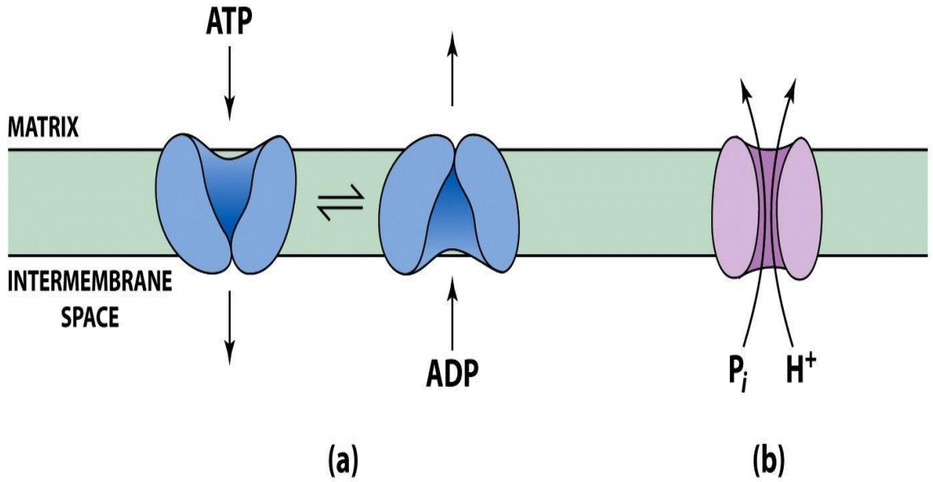


Regulation: → basically affect levels of Glucose or Activity of Glycolysis & Krebs Cycle & you will regulate ETC.

- 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 ← * It's responsible for Glycogen degradation in liver & Muscle.
    - liver → degrades it into Glucose → Can be used for Blood Glucose Regulation. → "Liver is Glucostat".
    - Muscle → degrades it into Glucose-6-Phosphate → Enters Glycolysis, not used in Blood Glucose Regulation.
  - PFK-1 ← Important for Glycolysis
  - Citrate synthase → Important for Krebs Cycle.

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 → Into the Matrix.

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

Thus when hydrogens enter the ETC through Complex I, we produce 2.5 ATP

Thus when hydrogens enter the ETC through Complex II, we produce 1.5 ATP

- It equals zero in presence of uncouplers.

* See Slides 37 & 40 to understand

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).

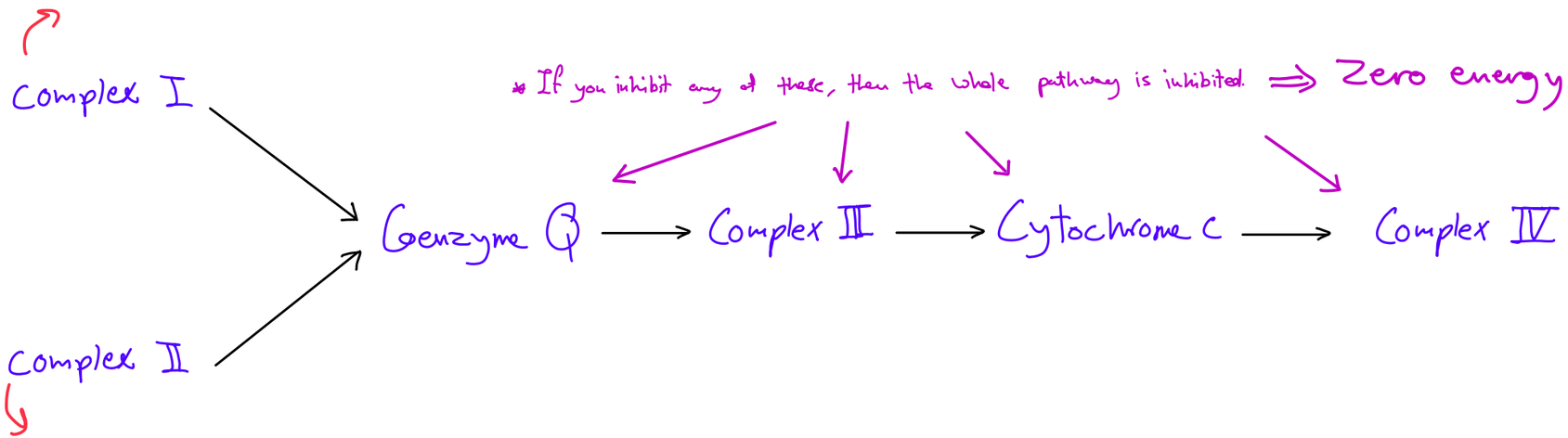
Hypnotic

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.

If you inhibit it, there's still complex II to enter the pathway ⇒ Energy is produced by $FADH_2$ only in this case.



If you inhibit it, there's still complex I to enter the pathway ⇒ Energy is produced by NADH only in this case.

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.

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.

F_0

* proton will get back, that prevents F_0 proton channel activation because it decreases the gradient.

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 of pH gradient.

* They decrease the electrical gradient but not the chemical gradient, however it is not sufficient.

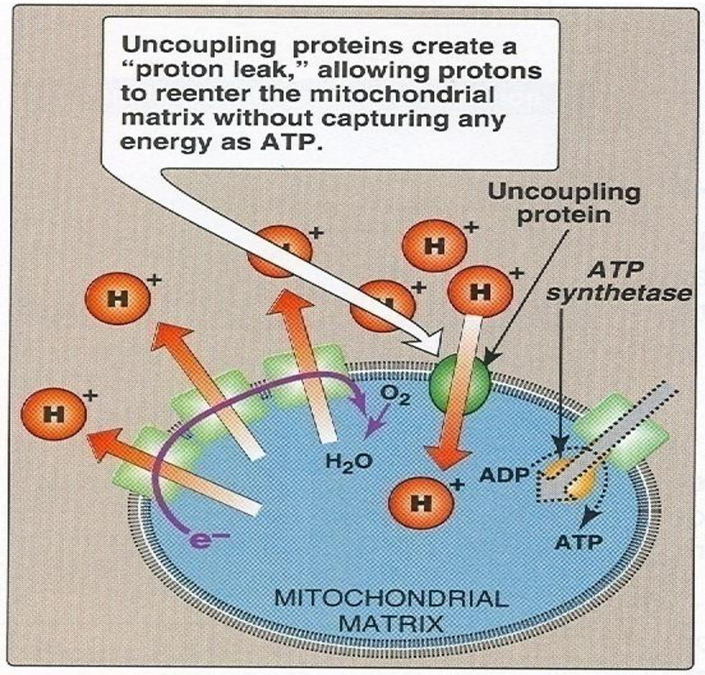


Figure 6.14
Transport of H^+ across mitochondrial membrane by 2,4-dinitrophenol.

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

6- Snake venoms. → These contain phospholipases that damage membranes, like IMM, & thus prevent the process from happening.

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 tissues) "Eating Too much, gaining no weight".

-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.

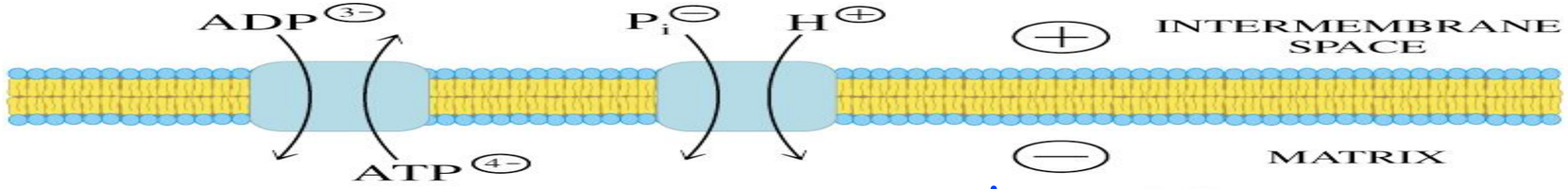
-The molecular mechanism of UCP1-mediated uncoupling is reasonably well understood; UCP1 allows protons to reenter the mitochondrial matrix without passing through F0-F1 complex (ATP synthase), allowing respiration (and hence heat production) to proceed in the absence of ATP synthesis.

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

<u>Membrane transport chain</u>

-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

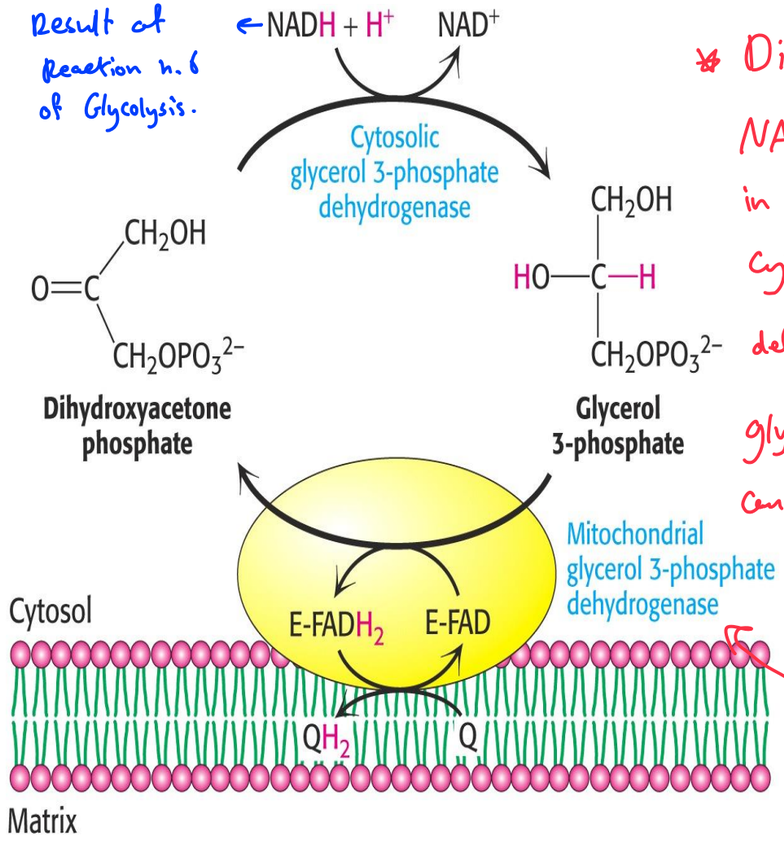
By 2 mechanisms:

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

Result of Reaction n.6 of Glycolysis.

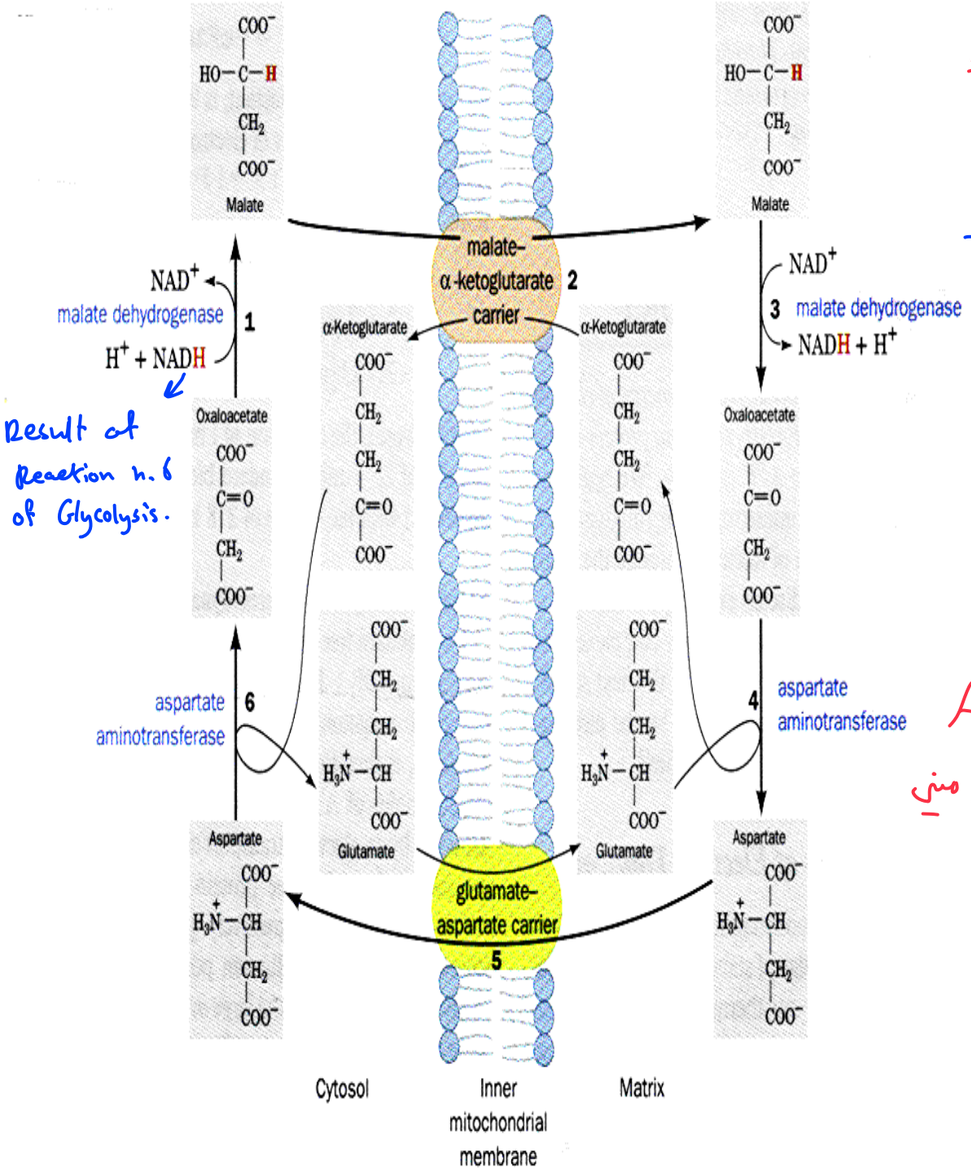


* Dihydroxyacetone takes $NADH + H^+$ & their electrons in a reaction catalyzed by cytosolic glycerol 3-phosphate dehydrogenase & forms glycerol 3-phosphate which can enter into Mitochondria & Reduce FAD into $FADH_2$ by ——.

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

* Then Oxaloacetate reacts with glutamate by A.A
↓
Oxaloacetate becomes Aspartate & Glutamate becomes α-ketoglutarate
↓
Aspartate gets out of the Matrix through its carrier. α-ketoglutarate as well.
↓
They react in cytosol & form Oxaloacetate & Glutamate
↓
Cycle Continues



* Q: What happens if this shunt fails ?

- NADH will separate from H^+ & set it free, that decreases pH which disrupt enzymatic activity in the Cytosol.

A.A اختصار مني

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: See slide 12.

- 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.

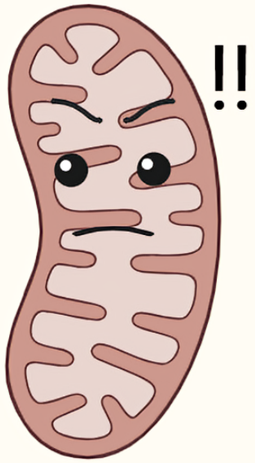
- For this reason, there must be an efficient and controlled way for the production of ATP. → So that ATP production continues.

1- Availability of ADP (ATP/ADP transporter may rate limiting at certain times.

2- Availability of electrons (↑ NADH/NAD and/or ↑ FADH2/FAD).

3- Availability of O2.

4- Insulin ⟶ It's an Anabolic hormone & is important for producing components of membranes in general, Thus it's important for ETC.



So it finished?!
Never thought it would.

لا تنسونا من صالح دعائكم.