SHEET NO. 5

METABOLISM

DOCTOR 2019 | MEDICINE | JU

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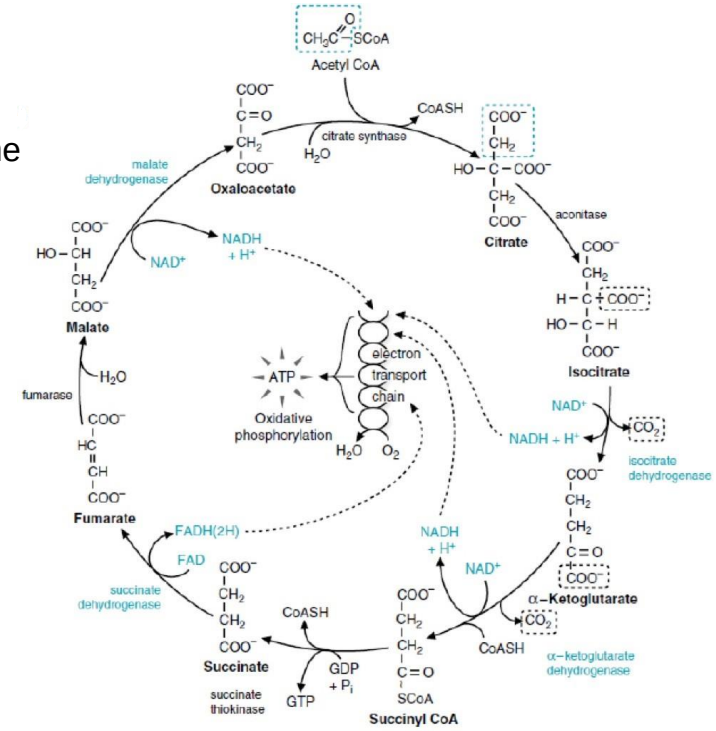
Summary of the 8 steps of citric acid cycle :

Step 1: acetate from the acetyl-CoA joins with a four carbon molecule, **oxaloacetate,** provided by the energy release from the CoA from acetyl-CoA ,so this energy will cause the joining of the acetate (2C molecule) to oxaloacetate (4C molecule) to produce a (6C unit molecule) which is called **citrate** (the first intermediate in the cycle).

Step 2: citrate is converted into it's isomer ,**isocitrate.**
- citrate cannot be oxidised at all, it is in the highest oxidation state with respect to the carboxylic group and with respect to the alcoholic group (it is in the tertiary form), so it has to be isomerized to be converted to isocitrate (where the alcoholic group is in the secondary form)

Step 3 : Isocitrate is oxidized and releases a molecule of **carbon dioxid**e, leaving behind a five- carbon molecule, **α-ketoglutarate**. During this step, **NAD+ is reduced to form NADH**. The enzyme catalyzing this step, **isocitrate dehydrogenase**, is important in regulating the speed of the citric acid cycle.



Step 4 : In this case, **it's α-ketoglutarate that's oxidized**, reducing NAD+ to NADH and releasing a molecule of carbon dioxide in the process. The remaining four-carbon molecule picks up Coenzyme A, forming the unstable compound **succinyl CoA**. The enzyme catalyzing this step is **α-ketoglutarate dehydrogenase.**

Step 5. The CoA of succinyl CoA is replaced by a phosphate group, which is then transferred to GDP **to make GTP.** The four-carbon molecule produced in this step is called **succinate**. Enzyme used is **succinyl CoA synthase.**

Step 6. Succinate is oxidized, forming another four-carbon molecule called **fumarate** (has a double bond). In this reaction, two hydrogen atoms, with their electrons, are transferred to FAD producing FADH2. The enzyme that carries out this step is **succinyl dehydrogenase.**

Step 7. Water is added to the four-carbon molecule fumarate, converting it into another four-carbon molecule called **malate**. The enzyme is **fumarase**.

Step 8. Oxaloacetate, the starting four-carbon compound, **is regenerated** by the oxidation of the alcohol group of malate by **malate dehydrogenase** to a keto group. Another molecule of NAD+ is reduced to NADH in the process.

Regulation of the citric acid cycle

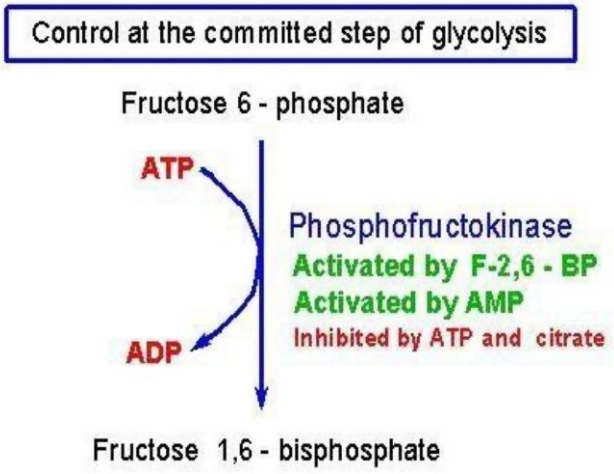
• regulating the glycolysis process:

- the rate limiting step in this pathway is the conversion of **Fructose 6- phosphate** into **Fructose 1,6- bisphosphate** which is catalysed by **phosphofructokinase enzyme**

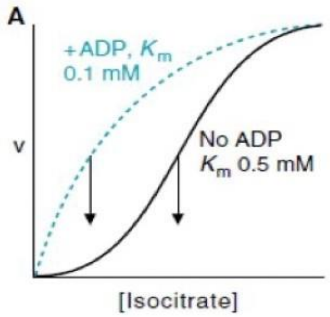
- this the slowest step in glycolysis and the **highest regulation step.**

- this enzyme (phosphofructokinase) is activated by **Fructose 2,6- bisphosphate and by AMP** (it sends a message that we don't have enough ATP)

- it is inhibited by **ATP** and **citrate.**



Regulation of the Citric Acid Cycle

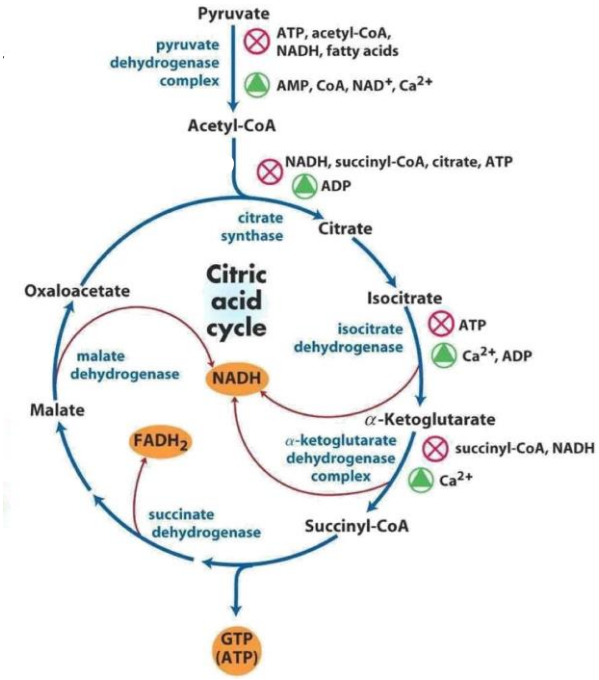
Citrate synthase	-It is the first enzyme in the cycle. It is a simple enzyme (not allosteric). -Excess amounts of citrate will inhibit the activity of this enzyme.
Isocitrate dehydrogenase 	-It facilitates the rate limiting step (isocitrate → alpha ketoglutarate). This step is highly regulated. -It is inhibited by NADH and ATP. Activated by ADP and Ca ions. -It is the only enzyme in the cycle that is activated by ADP. **(ADP is an allosteric activator for isocitrate DH)** **-km** for this enzyme with the presence of ADP decreases. (affinity for substrates increases). -A small change in ADP concentration will affect the enzyme's activity greatly.
α-ketoglutarate dehydrogenase	-Inhibited by its products NADH and succinyl CoA (feedback inhibition). -Activated by Ca ions.

• **Important notes :**

- Calcium ions activate many enzymes involved in metabolism since it causes muscle contraction. This means that more energy is needed so more ATP is produced.

- ADP/ATP and NAD+/NADH ratios control the rate of Krebs cycle.

- High levels of ADP and NAD+ activate the cycle. On the other hand, high levels of ATP and NADH inhibit the cycle.



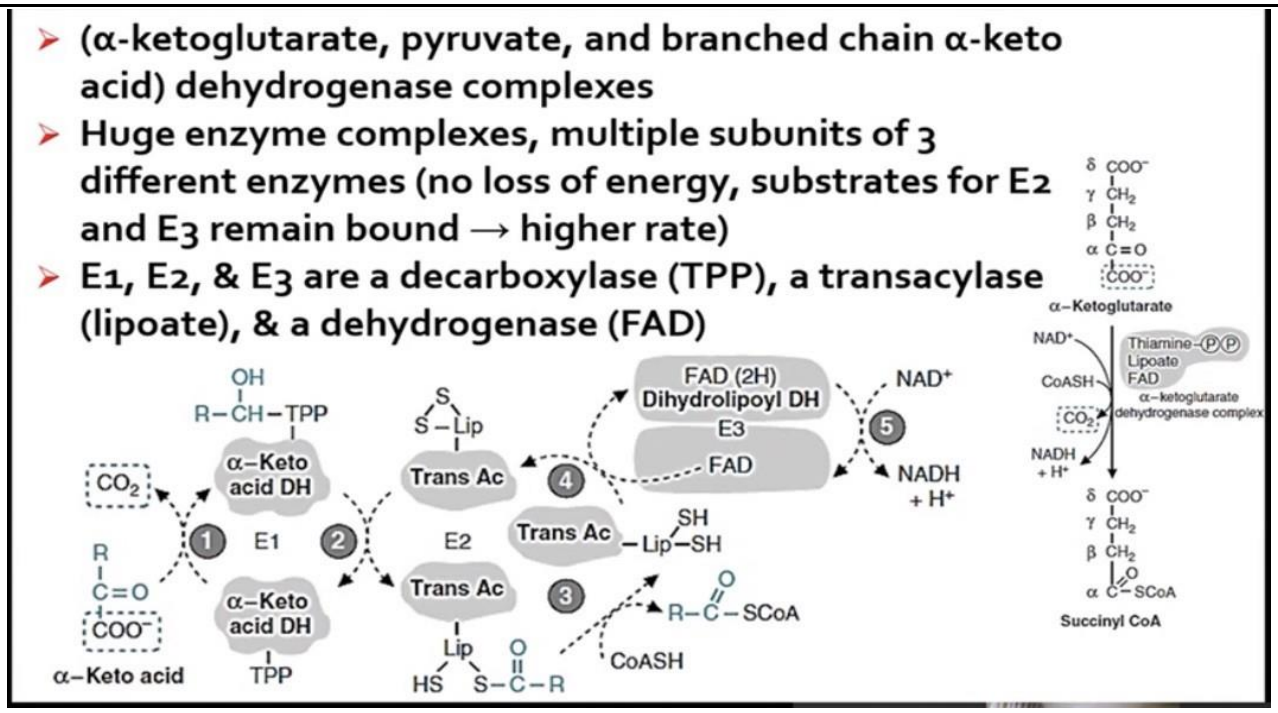
a- ketoacid dehydrogenase complexes

- now will discuss the conversion of a-ketoglutarate to succinyl CoA
- a-ketoglutarate is **oxidatively decarboxylated** to form succinyl CoA by the enzyme **a-ketoglutarate dehydrogenase.**
- a CO2 molecule is removed in this step.
- the enzyme **a-ketoglutarate dehydrogenase** is a **multienzyme complex** having **3 enzyme proteins and 5 co-enzymes.**
- it is called **a-ketoglutarate dehydrogenase complex .**
- we have similar complexes work in the same mechanism(pyruvate dehydrogenase complex and a-keto acid dehydrogenase complex)

Mechanism of action

• Initially α-ketoglutarate and thiamine pyrophosphate (TPP or vitamin B1) are bound by α-ketoglutarate acid dehydrogenase subunits, then α-ketoglutarate is decarboxylated by α-ketoglutarate acid dehydrogenase with help from TPP.
E2 (transacetylase with cofactor lipoate) transfers substrate to CoA, forming succinyl CoA. Lipoate has 2 sulfur atoms connected by a disulfide bond, E1 donates the decarboxylated α-KG to one of the sulfur atoms causing the breaking of the disulfide bridge. The other sulfur atom converts to thiol group by combining with H+ from the solution. (succinate combines with CoA because when a carboxylic group is being released as a CO2 molecule the adjacent carbon becomes terminal, thus succinate becomes reactive and combines with anything to hold it and the best acyl carrier molecule is CoenzymeA. Then E2 (transacetylase) becomes oxidized and the electrons are being loaded to FAD molecule producing FADH2.

- (α-ketoglutarate, pyruvate, and branched chain α-keto acid) dehydrogenase complexes
- Huge enzyme complexes, multiple subunits of 3 different enzymes (no loss of energy, substrates for E2 and E3 remain bound → higher rate)
- E1, E2, & E3 are a decarboxylase (TPP), a transacylase (lipoate), & a dehydrogenase (FAD)



•The co-enzymes needed are:

1-Thiamine pyrophosphate (TPP)
2-Co-enzyme A (CoA)
3-FAD
4-NAD+
5-Lipoamide

• the three enzymes are:

E1: decarboxylase
E2: acylase
E3: dehydrogenase

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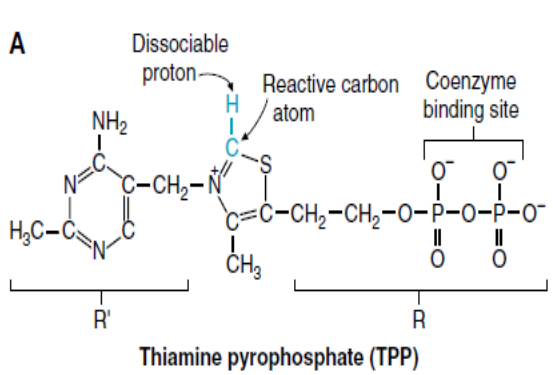
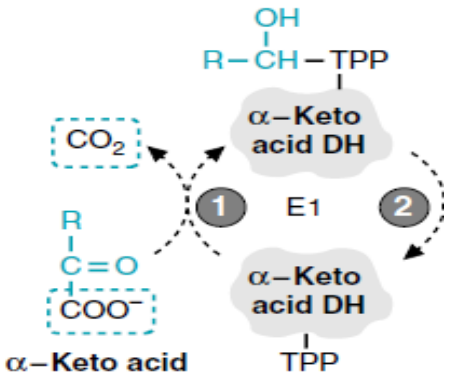
Thiamine pyrophosphate (B1 derivative)

-Thiamine pyrophosphate is a coenzyme for dehydrogenase complexes. In vitamin B1 deficiency, decarboxylation reactions will stop. This leads to the accumulation of the substrates (alpha ketoglutarate, pyruvate and alpha keto acids) of the E1 component in the blood.

-Deficiency of vitamins B2, B3, B5 will also lead to substrate accumulation in the blood.





Oxidative decarboxylation of pyruvate

- When pyruvate is in the mitochondrial matrix, it is converted to acetyl CoA by the pyruvate dehydrogenase complex (PDH complex), which is a multienzyme complex.

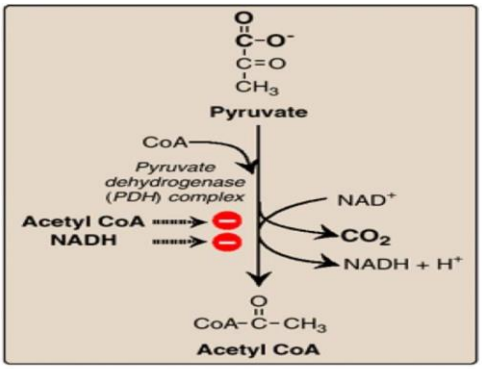
-Component enzymes: The PDH complex is a protein aggregate of multiple copies of three enzymes, E1, E2, E3.

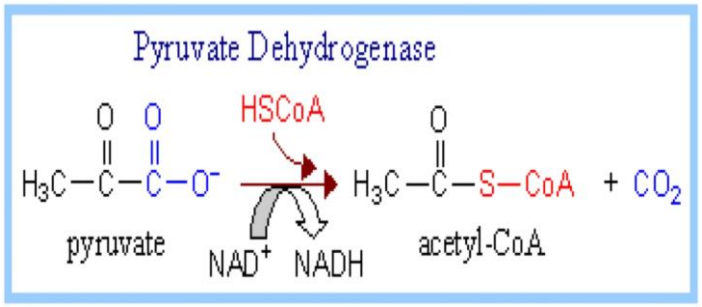
-Coenzymes: E1 requires thiamine pyrophosphate (TPP), E2 requires lipoic acid and CoA, and E3 requires FAD and NAD+.

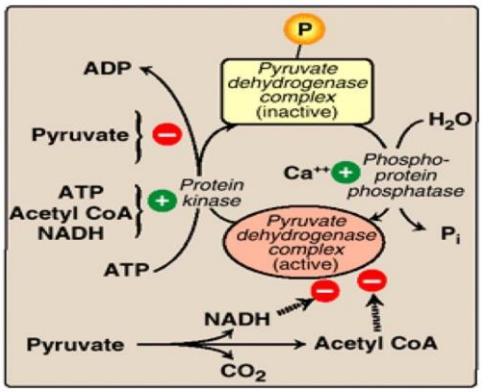
-**Pyruvate dehydrogenase complex deficiency**: A deficiency in the E1 component of the PDH complex, although rare, is the most common biochemical cause of **congenital lactic acidosis. This enzyme deficiency results in an inability to convert pyruvate to acetyl CoA, causing pyruvate to be shunted to lactate.**

-The gene for the E1 component is X linked, and, because both males and females may be affected, the deficiency is classified as X-linked dominant.

-Although there is no proven treatment for PDH complex deficiency, dietary restriction of carbohydrate and supplementation with thiamine may reduce symptoms in select patients.







Arsenic poisoning: is due to inhibition of enzymes that require lipoic acid as a coenzyme, including E2 of the PDH complex, α-ketoglutarate dehydrogenase and branched-chain α-keto acid dehydrogenase. **Arsenic attacks the disulfide bond in lipoic acid forms a stable complex with the 2 sulfur atoms of lipoic acid, making that compound unavailable to serve as a coenzyme (because the sulfurs are now unable to form any bonds with the carbons).** When it binds to lipoic acid in the PDH complex, **pyruvate accumulates.** Depending on the levels of toxicity and the conc. of As present it might prevent the entire reaction from proceeding and the entire cycle will be stopped which is why **it can be fatal** (no energy metabolism).

Bioenergitics of the TCA cycle

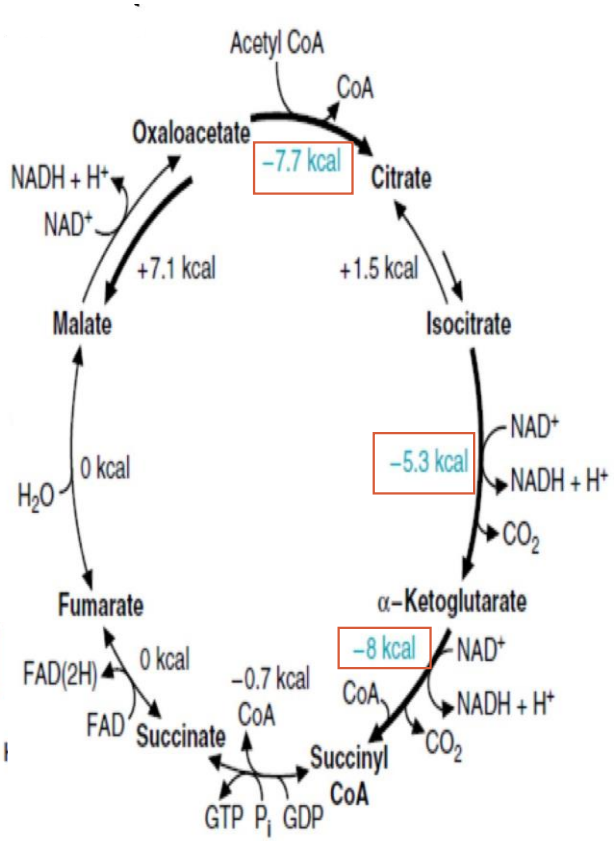
Energy Yield: (actual yield/theoretical yield) X 100%

-Theoretical yield of CAC: 228 kcal/mole of **acetate**

-Yield: (207/228) X 100% = 90%. This process is highly efficient.

-Three reactions have large (-ve)values.

-The cycle goes in one direction due to the overall negative ΔG.

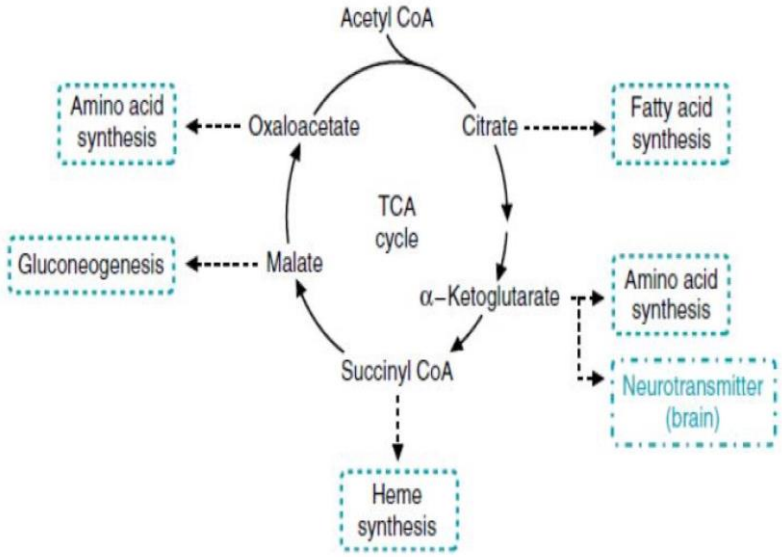


kcal/mole	
3 NADH: 3 × 53	= 159
1 FAD(2H)	= 41
1 GTP	= 7
Sum	= 207

Citric Acid Cycle Intermediates

- **The citric acid cycle provides precursors for many biosynthetic pathways.**

Oxaloacetate (and other keto acids)	-Can be used in amino acid synthesis. -Example: oxaloacetate →aspartate
Citrate	Can leave the mitochondria and be used in **fatty acid** synthesis.
α-Ketoglutarate	-Can be turned to the amino acid glutamate. -Glutamate can function as a **neurotransmitter.** -The inhibitory neurotransmitter **GABA** can be synthesized using glutamate. **-**Glutamine is used to synthesize amino acids. It is synthesized in skeletal muscles then transported to other tissues, so they can synthesize amino acids and proteins.
Succinyl CoA	Used in **heme** biosynthesis in bone marrow.
Malate	-A key molecule in gluconeogenesis. -**Gluconeogenesis** is the process of generating glucose from **non-carbohydrate** sources when the person is fasting. It mostly occurs in the liver and kidneys.



Anaplerotic reactions

Reactions that replenish the intermediates of the citric acid cycle.

-One important example is the conversion of carbon dioxide and pyruvate to **oxaloacetate** which is catalyzed by Pyruvate carboxylase.

- Pyruvate carboxylase is a mitochondrial matrix protein (that requires biotin).

-The pyruvate carboxylase reaction occurs in the mitochondria of liver, kidney, brain, fibroblasts and adipocyte cells and has two purposes:
1- To provide an important substrate for gluconeogenesis.
2- And to provide oxaloacetate that can replenish the TCA cycle intermediates that may become depleted.

-Pyruvate carboxylase is activated by acetyl CoA. That makes sense because acetyl CoA enters the cycle by reacting with oxaloacetate.

-High levels of acetyl CoA in mitochondria signal a metabolic state in which the increased synthesis of oxaloacetate is required.

-Gluconeogenesis is very active in the liver and kidneys. This process consumes malate. This decreases the concentration of malate and oxaloacetate.

-Oxaloacetate has the lowest concentration in kidneys and liver (where the concentration of pyruvate carboxylase is highest).

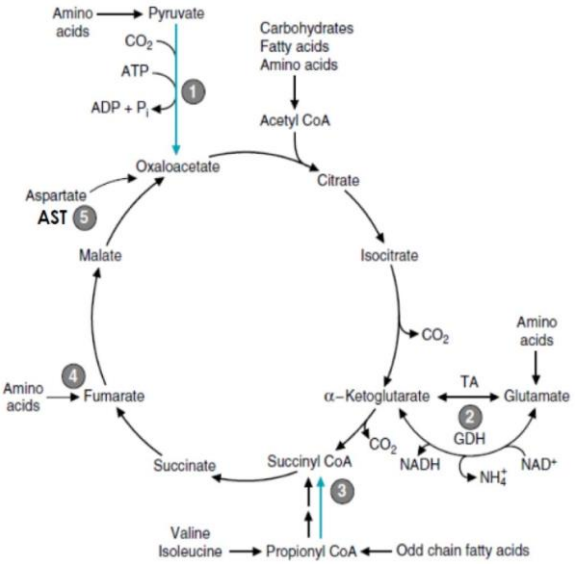
Other anaplerotic pathways
-Amino acid degradation:

-Aspartate can provide oxaloacetate

-Glutamate provides α-Ketoglutarate

-Propionyl CoA provides succinyl CoA

-Many amino acids can provide fumarate.



صلوا على حبيب الله محمد عليه الصلاة والسلام

Oxidative Phosphorylation

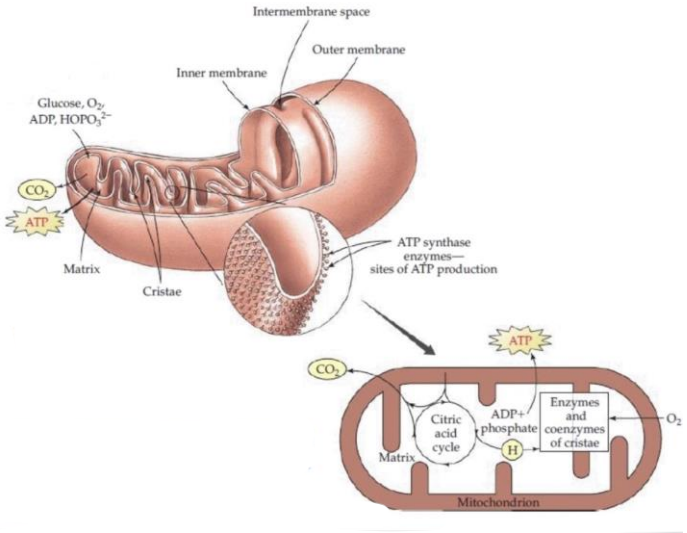
- **the mitochondria**

- The mitochondrion contains an outer and an inner membrane separated by the intermembrane space.
- the outer membrane is permeable to small molecules (MW<5000), however, the inner membrane is **impermeable** to any thing , even to H+ .
- any thing that should cross the inner membrane should have a specific transporter .

-The inner mitochondrial membrane bears the components of the respiratory chain and the ATP synthase

-Matrix: contains pyruvate dehydrogenase complex & TCA cycle enzymes, fatty acid β-oxidation pathway, and the pathways of amino acid oxidation.

-In other words: matrix contains all pathways of fuel oxidation except glycolysis (cytosol)



Oxidative phosphorylation

Oxidative phosphorylation have 3 major aspects:
(1) It involves flow of electrons through a chain of membrane- bound carriers (prosthetic groups)
(2) The free energy available (exergonic) is coupled to transport protons across a proton-impermeable membrane
(3) The transmembrane flow of protons down their concentration gradient provides the free energy for synthesis of ATP (ATP synthase)

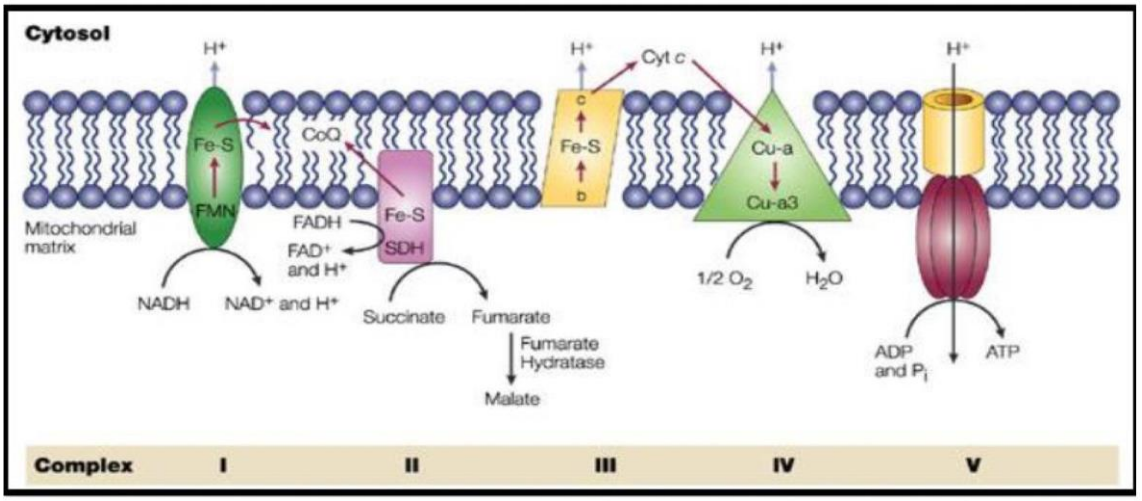
-Electrons move according to **reduction potential difference.** They move from compounds with lower reduction potential with compounds with higher reduction potential. Oxygen has the highest (most positive) reduction potential in the ETC, it turns into water as it accepts the last electrons in the chain.
-As electrons are passed down the ETC, they lose much of their free energy. The free energy released as electrons are transferred along the ETC from an electron donor to an electron acceptor is used to pump protons from the matrix to the intermembrane space **against their electrochemical gradient.**

-This process creates an **electrochemical gradient** (with more positive charges on the outside of the membrane than on the inside).
-The energy generated by this proton gradient is sufficient to drive ATP synthesis. Thus, the proton gradient serves as the common intermediate that couples oxidation to phosphorylation.

Electron transport chain

- oxidation reduction reaction occurs in a pathway called **electron transport chain.**
- electron transport chain doesn't produce ATP by it self , what produces ATP is the oxidative phosphorylation
- what are the substrates for the electron transport chain?

1- NADH which is generated from TCA and Glycolysis and Pyruvate dehydrogenase. NADH is found free in the solution of the matrix and carries two electrons in the form of hydride ions.
2- FADH2 which is generated from TCA. It is bound to succinate dehydrogenase (enzyme in the TCA that is embedded in the inner mitochondrial membrane and it also forms complex 2 of the ETC) and carries two electrons in the form of two hydrogen.



"Wherever the art of Medicine is loved, there is also a love of Humanity. "