SHEET NO. 4

METABOLISM

DOCTOR 2019 | MEDICINE | JU

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Thermogenesis:

✓ Is the first law of thermodynamics

- It is the energy expended for generating heat (37oC) in addition to that expended for ATP production

✓ it has 2 types:

1. shivering thermogenesis)الارتعاش((ATP utilization) = asynchronous muscle contraction due to sudden change in the body temperature (usually decrease), thus making more ATP and generate heat as a byproduct of making ATP to return the body temp to the normal situation.

(Heat production is a natural consequence of "burning fuels")

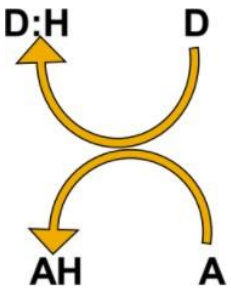
2. Non-shivering thermogenesis (adaptive thermogenesis): the percentage of energy that you are ingesting inside your body to make heat (ATP production efficiency) كمية الطاقة والحرارة اللي ننتجها بشكل عام، وهي مختلفة من شخص آلخر

Oxidation reduction reactions: (we are studying them now because they exist in most of the metabolism of energy reactions.)

✓ Oxidation reduction reactions include moving of electrons without changing the chemical structures

ΔG: the difference in bond energies between materials

redox potential (E) (THE POTENTIAL ENERGY): the driving force of moving the electrons from one atom to another, these electrons are hold on chemical structures which has the ability to donate its electrons or accept its electrons.



✓ redox Potential measures the tendency of oxidant/reductant to gain/lose electrons, to become reduced/oxidized

✓ Electrons move from compounds with lower reduction potential (more negative) to compounds with higher reduction potential (more positive)

ΔE = the difference in electrecal potentials between two points.

or مثال: اللي بخلي الكهربا توصل للبيت هو فرق الجهد بين أعمدة الكهرباء

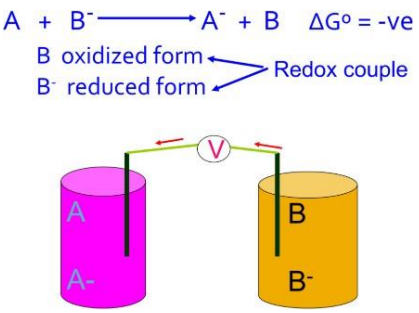
there is a difference in the ability of accepting donating electrons between any 2 chemical materials

✓ The electrons move from the material that has a higher ability to donate electrons to the one which has a lower ability to donate electrons.

✓ **Oxidation and reduction must occur simultaneously**

- **Oxidation:**
  - Gain of Oxygen
  - Loss of Hydrogen
  - Loss of electrons
- **Reduction:**
  - Gain of Hydrogen
  - Gain of electron
  - Loss of Oxygen

Now look at this redox couple: (A) accepts electrons and is converted to the reduced form A- so we have a redox couple (A , A-).



Another redox couple is shown in the illustration.

Now, can we measure redox potential experimentally?

The answer is **yes**. Scientists were able to measure reduction potential for a wide variety of materials with respect to hydrogen electrode (as a standard electrode Eo=0) and they arranged these values from the more negative to the more positive value in a large scale. **The more negative value has high capacity to lose electrons while the more positive value has high tendency to gain electrons.**

For example, if we have 2 reduction potentials: the first equals -600mv while the second equals -500mv then electrons move from the first to the second material.

The importance of this standard electrode is to obtain the exact value of reduction potential because if we used 2 materials of unknown reduction potential, then we will not be able to find the exact value for both since they are different. Another advantage of using hydrogen is that **most materials can gain/lose hydrogen.**

Reduction potential: Ability to accept electrons

Oxidized + e^-	→ Reduced	ΔE^o (V)
Succinate	α ketoglutarate	- 0.67
Acetate	Acetaldehyde	- 0.60
NAD^+	**NADH**	**- 0.32**
Acetaldehyde	Ethanol	- 0.20
Pyruvate	Lactate	- 0.19
Fumarate	Succinate	+ 0.03
Cytochrome^{+3}	Cytochrome^{+2}	+ 0.22
oxygen	**water**	**+ 0.82**

From the table, we notice 2 important points. Firstly, **oxygen** is the final electron acceptor for electrons (electrons from different nutritional materials are trapped by oxygen) thus it has the most positive reduction potential. Secondly, **NADH** has a reduction potential (Eo) of -320 mv thus it gives electrons to oxygen with Eo =+820mv. This direction of electron movements fits the science since we already know that **electron carriers like NADH after produced from Krebs cycle donate their electrons for materials with higher E.**

As we talked before about ΔG and its relation to bond energy, we can say the difference in energy caused by reduction potential is another diameter of what ΔG measures. So, ΔG is not only concerned with bond energy. The reduction potential, not bond energy, is the driving force for electrons movement. Therefore, **if we inverted the sign of reduction potential value then electrons will move in the backward direction**. There must be a mathematic relation that governs the direction of electrons movement. Moreover, it should not contain any variable other than ΔG and ΔE.

$$\Delta G^o = - n f \Delta E^o$$

- F = Farady constant = 23.06 kcal/*Volt*

(n) constant: the number of electrons moving

Also, the following relation can be used:

$$\Delta G = - n f \Delta E$$

For a reaction to be favorable, spontaneous and exergonic (-ve ΔG) then ΔE must have a +ve value. The following example supports the previous statement.

NADH has a reduction potential Eo= -320 mv thus it gives electrons to oxygen with Eo = +820mv.
ΔEo = Eo (final oxygen) – Eo (initial NADH) = +820 – (-320) = +1140 mv (positive value and spontaneous reaction)

As we seen before, the sign of ΔE o is +ve thus when scientists wrote equation, they inserted the -ve sign to fit the real situation.

Question:

Calculate ΔG^o of the following reaction

$$NADH + 1/2O_2 \longrightarrow NAD^+ + H_2O$$

$$NADH \longrightarrow NAD+ + 2e^- \quad \Delta E^o = +0.32\ V$$
$$O + 2e^- \longrightarrow O^{2-} \quad \Delta E^o = +0.82\ V$$

Solution: ΔE o =1140 mv
= 1.14 volt
ΔG o = -nf ΔE o
= -(2) (23.06)(1.14) ➔ $\Delta G^o = - 52.6$ kcal/mol

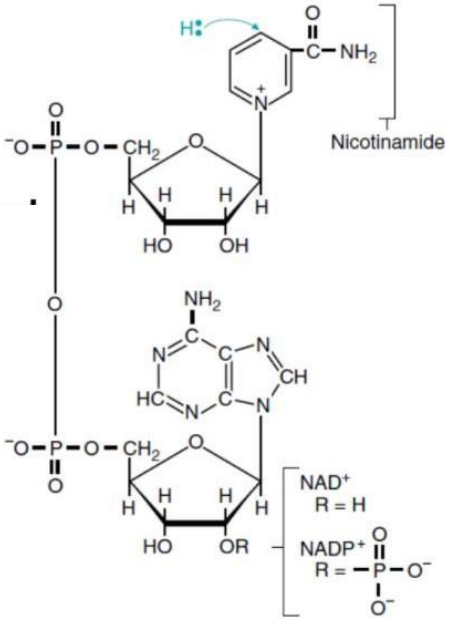
Now, let us talk about **electron carriers** (that transports electrons to ETC).

There are 2 main electron carriers:

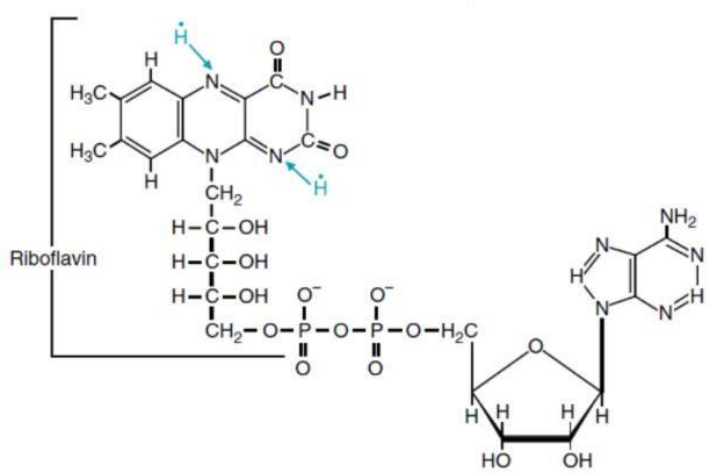
- ✓ NAD+ (niacin, B3) & FAD (riboflavin, B2).

  NAD+ accepts a single hydride ion H - (2 electrons) on nicotinic ring with one step, so it does not form a radical (will not be harmful) and thus can be found free as both NAD+/NADH in mitochondria/cytosol, as a result, it has a fixed reduction potential.

  -NADP+ is different from NAD+ only by **a ssphosphate group** instead of **a hydrogen atom** as shown in the previous figure. Both of them carries 2 electrons but NAD+ participates in **catabolism** while NADP+ participates in **anabolism**. So, different structures that do the same function for better organization and regulation.



- ✓ FAD accepts 2 protons (2 electrons) sequentially since there are 2 H atoms thus it forms a radical intermediate and passes through (one electron/free radical state) that is harmful. Therefore**, it cannot be found free in the cytosol** and is always bound to proteins. Also, its reduction potential depends on the protein it is bound to. **FMN** also carries 2 electrons sequentially, but for better organization: one works in anabolic reactions while the other in catabolic reactions.



Kreb's, Citric Acid, TCA

- **To begin with, the previous topic "Bioenergetics" aim is to be able to understand Kreb's cycle and oxidative phosphorylation.**

- **Kreb's was named after the German scientist Hans Krebs**

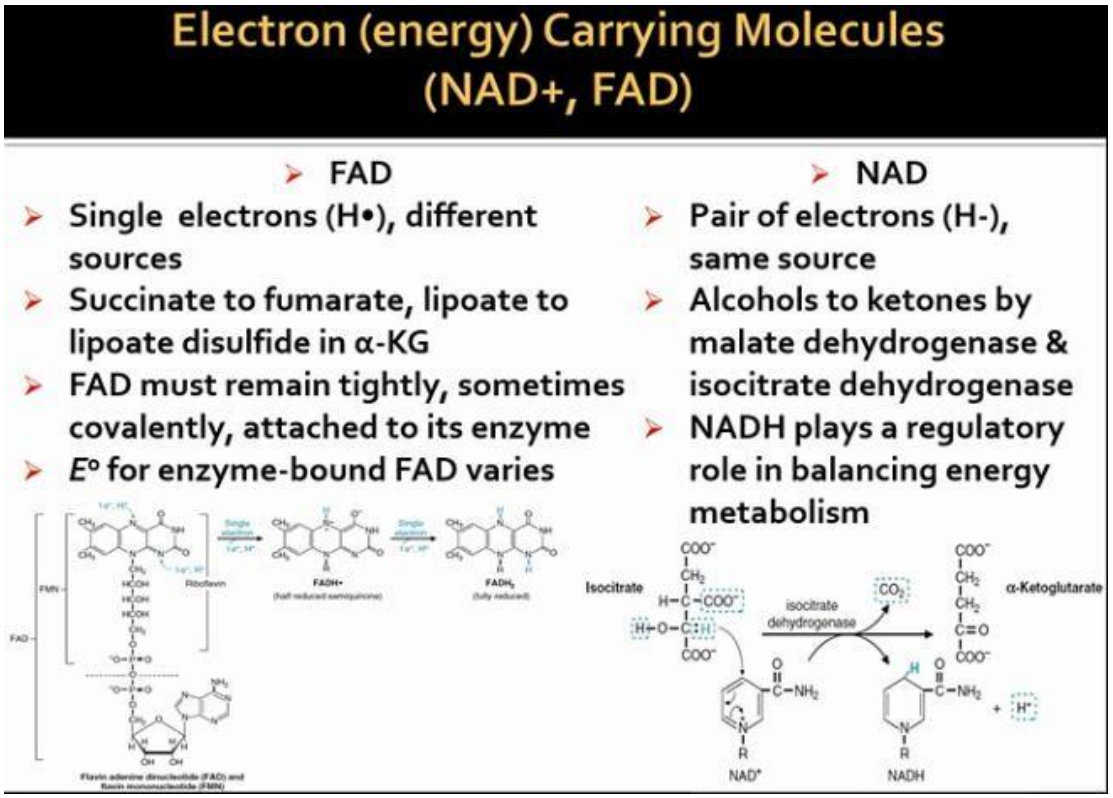
- **It is also named as Citric acid cycle due to the presence of an acid in the steps . Additionally, a cycle generally could be also named by the first/last component (usually) of it or even named as an intermediate compound in the middle of the cycle (should have a huge value).**

TCA cycle- Tricarboxylic Acid cycle, thus this implies that Citric acid has 3 carboxylic acid groups in it

- ✓ **TCA is the 3rd Stage of energy metabolism**

<u>Regarding NAD+ and FAD this slide is a recap of what We took during the last course (Biochemistry-1) it was re-mentioned in this Sheet page 4 too</u>

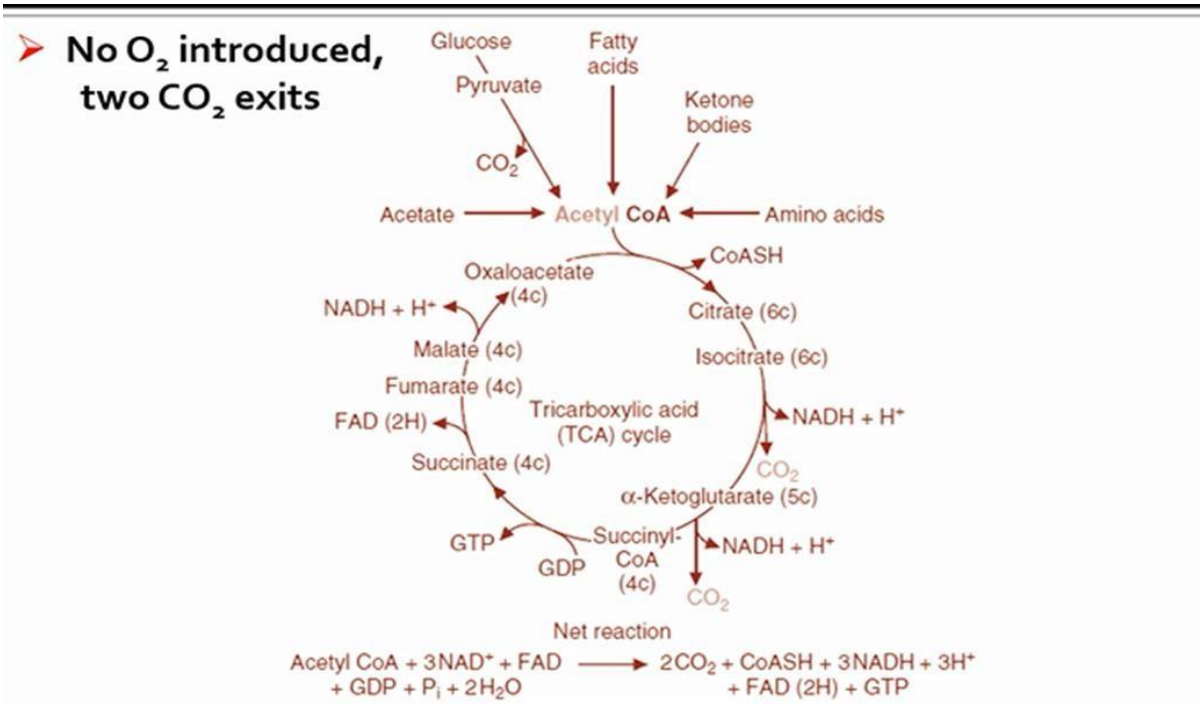
<u>Thereby Kindly refer to Sheet 27 pages 3 & 4</u>



The doctor expects us to know the following for examination as well as for our own knowledge:-

ALL the 8 steps of this cycle.
ALL the 8 enzymes of this cycle.
All the 8 intermediates of this cycle structure wise along with their naming.
Most importantly the sequence of the cycle.

First of all, Acetyl CoA enters from outside and starts the Krebs cycle "Not an intermediate of the cycle itself". Acetyl (2C) only enters the cycle while CoA leaves as CoA-SH. However, the Acetyl group (2C) should leave the cycle eventually as CO2 otherwise It'd get inflated. When the Acetyl group (2C) enters the cycle it joins with Oxaloacetate (4C) forming Citrate (2+4=6C) which is then isomerized to Isocitrate (6C the no. of C atoms should not be different), then converted to Alpha-Ketoglutarate (5C) then to Succinyl-CoA (4C) then to Succinate (4C), Fumarate (4C) and Malate (4C) eventually reaching to Oxaloacetate(4C).



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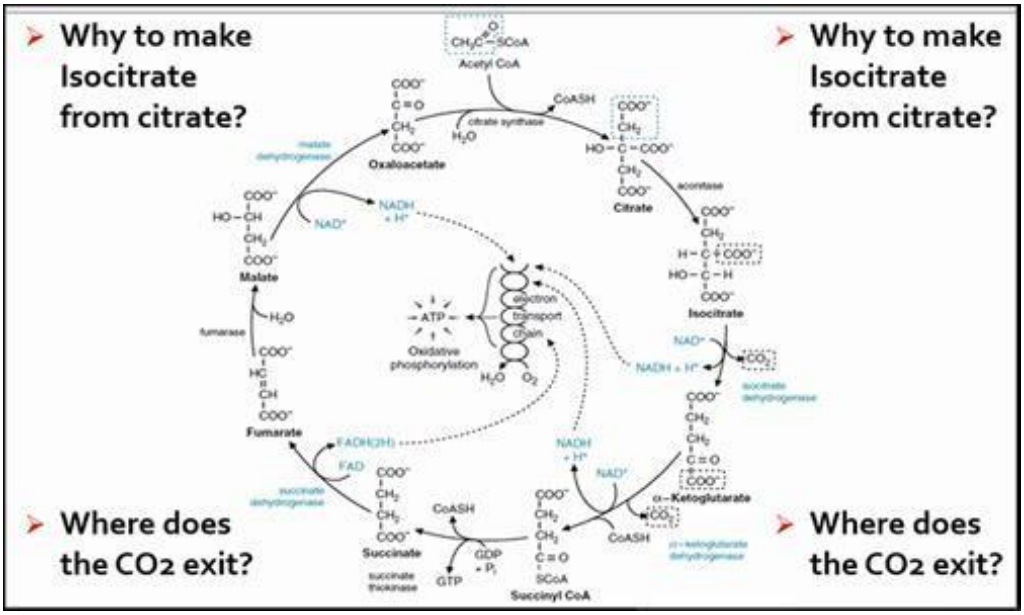
A mnemonic to memorize the intermediate compounds of the cycle each red colored letter represents the first letter an intermediate in the cycle

In order to understand the cycle let's split it into 2 halves

1) The cycle engages itself by returning the 6C unit molecule (Citrate/Isocitrate) to the a 4C unit molecule.
2) The cycle engages itself by converting that 4C unit molecule to resemble the first compound in the cycle which is the 4C unit molecule (Oxaloacetate).

PS: Each half consists of 4 reactions



How does this occur?

As you can see in the cycle above the Acetyl group (2C) that joins Oxaloacetate (4C) by citrate synthase is within a blue-dotted box which doesn't get eliminated immediately as CO2 from this cycle. However, these 2C would be removed eventually during the following cycles. The citrate (6C) which forms occurs by a reduction process which acquires energy from CoA which leaves Acetyl CoA as CoASH. Since the hydroxyl group of Citrate (6C) is tertiary it cannot be oxidized, hence it should be isomerized to Isocitrate by the enzyme an Isomerase (called aconitase due to the intermediate "aconitate"). The Isocitrate produced could be oxidized due to the presence of a secondary hydroxyl group in it (the main aim behind this isomerization reaction). In the last 2 steps of the first half **(1st step)** Isocitrate gets oxidized to Alpha-ketoglutarate (5C) by the loss of 1C atom as CO2 by Isocitrate dehydrogenase complex (has 2 actions; decarboxylation and dehydrogenation), it removes 1 C atom (Decarboxylation) and removes 2H atoms(producing NADH) from the secondary C atom of Isocitrate (Dehydrogenation). Then Alpha-ketolglutarate would get further decarboxylated and dehydrogenated **(2nd Step)** by the enzyme Alpha-ketoglutarate dehydrogenase complex resulting in the removal of the peripheral Carboxylic acid group (as CO2) and releases NADH leaving a

highly reactive carbonyl group on the side which binds to CoA forming Succinyl CoA (4C).

In the second half of the cycle the aim is to convert succinyl CoA (4C) to Oxaloacetate (4C). To begin with, CoA should leave peripherally releasing energy which will be used to produce GTP (energy produced by metabolism of ATP=GTP) catalyzed by succinate thiokinase, hence forming succinate (4C) which is converted to an alkene (C=C) by succinate dehydrogenase called fumarate (4C) releasing FADH2 . By the action of the fumarase enzyme a water molecule is added to fumarate (hydration of alkenes) forming Malate (4C) which contains a secondary hydroxyl group that gets oxidized to ketone group by the action of malate dehydrogenase forming Oxaloacetate (4C) giving off NADH.

✓ General summary for the products of TCA cycle:
It produces 3 NADH molecules
It produces 1 FADH2 molecules
It produces 1 GTP (same as ATP)

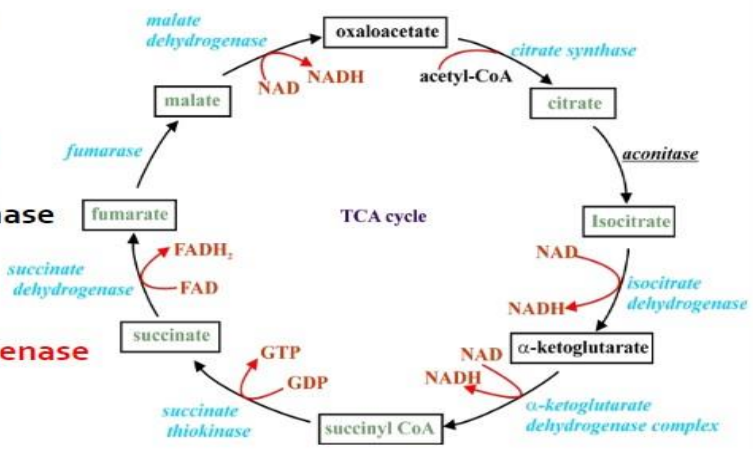
Structure of Oxaloacetate: It is a keto-acid with 1 ketone group and 2 carboxylic acid groups.
Structure of Citrate: It contains 3 Carboxylic acid groups with a tertiary hydroxyl group on the 3rd C atom which cannot get oxidized.
Structure of Isocitrate: It contains 3 Carboxylic acid groups with a secondary hydroxyl group on the 2nd C atom which could be oxidized.

PS: The oxidation of food releases energy as ATP (Breaking down of molecules)
The reduction of molecules which requires energy (Buildup of molecules)
The function of CoA is to transfer a group of carbons from a compound to another

- ✓ When an enzyme name is accompanied by "complex" this means that it performs more than 1 reaction. Example: Alpha-ketoglutarate dehydrogenase complex does (1) decarboxylation and (2) dehydrogenation.

Enzymes of the TCA Cycle

- Citrate cynthase
- Aconitase
- Isocitrate dehydrogenase
- α-ketoglutarate dehydrogenase
- Succinate thiokinase
- Succinate dehydrogenase
- Fumarase
- Malate dehydrogenase



Formation and Oxidation of Isocitrate

- Oxidative decarboxylation, CO_2
- 3° to 2° alcohol

Control at the committed step of glycolysis

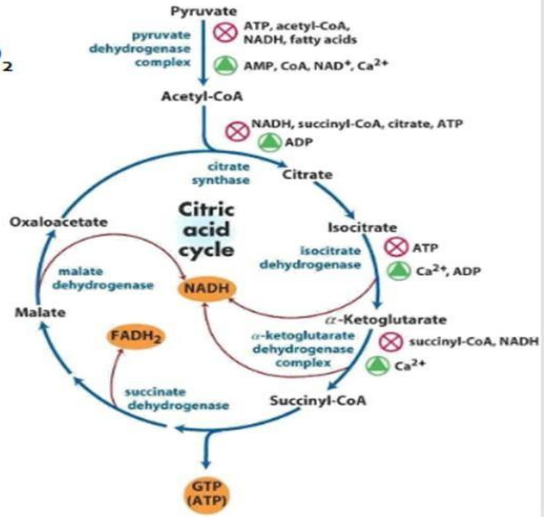
Fructose 6 - phosphate

ATP

Phosphofructokinase
Activated by F-2,6 - BP
Activated by AMP
Inhibited by ATP and citrate

ADP

Fructose 1,6 - bisphosphate



In this slide the importance of isocitrate is mentioned, since it has a secondary alcohol group which could be oxidized into ketone group.
Additionally, in the cycle above there are regulators of some steps.

⊗ Such groups are inhibitory (Decrease certain steps).

△ Such groups are promoters (Increase certain steps).

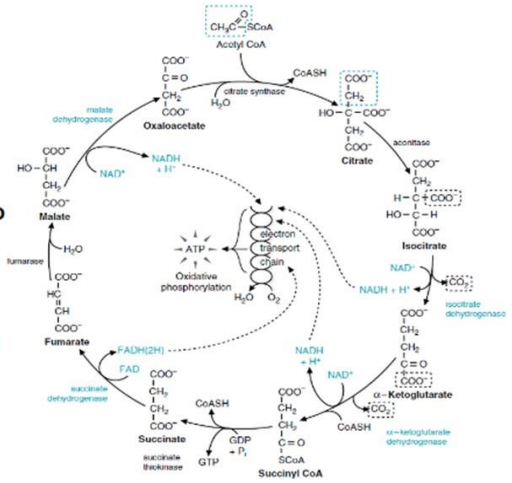
There is an example where Fructose-2,6-bisphosphate (F-2,6-BP) and AMP upregulate the conversion of Fructose 6-phosphate to Fructose 1,6-bisphosphate.
Whereas ATP and Citrate downregulate the conversion of Fructose 6-phosphate to Fructose 1,6-bisphosphate

This reaction was mentioned in the "How does this occur?" paragraph

In the second point of this slide Thiamine pyrophosphate, lipoic acid and FAD.
are all co-factors which participate in the formation of the Alpha-ketoglutarate dehydrogenase complex.

α-Ketoglutarate to Succinyl CoA

- Oxidative decarboxylation
- Thiamine pyrophosphate, lipoic acid, and FAD
- Keto group oxidized to acid, CoA-SH, succinyl CoA
- Energy conserved as NADH, thioester bond

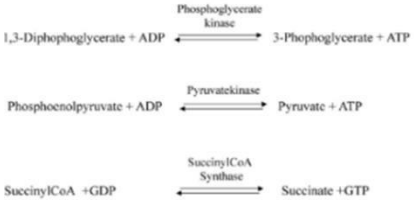


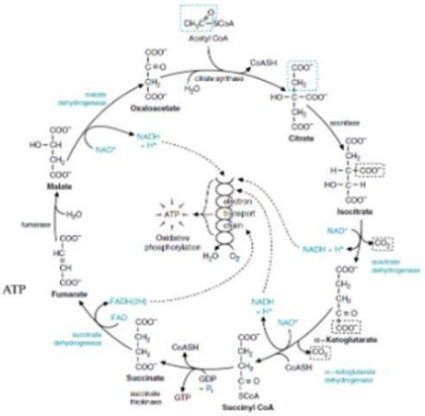
- This slide shows the way by which GTP (same as ATP) is produced by the energy released when Succinyl CoA thioester bond was cleaved, shown by the last equation in this slide.

- The last 3 reactions in this slide show the substrate level phosphorylation which yields in ATP and GTP

Generation of GTP

- Succinyl CoA thioester bond, succinate thiokinase, substrate level phosphorylation

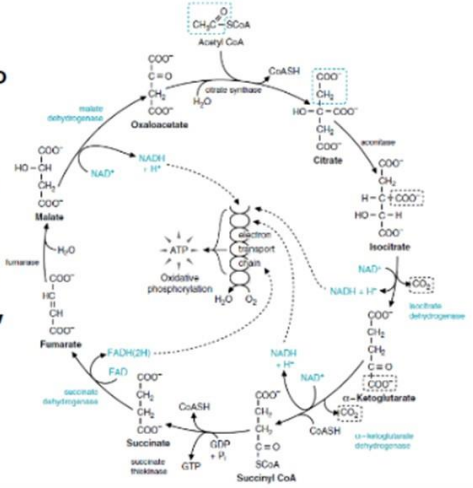




- This slide is basically the second half of the TCA cycle that was explained in detail in the paragraph "How does this occur?"

Oxidation of Succinate to Oxaloacetate

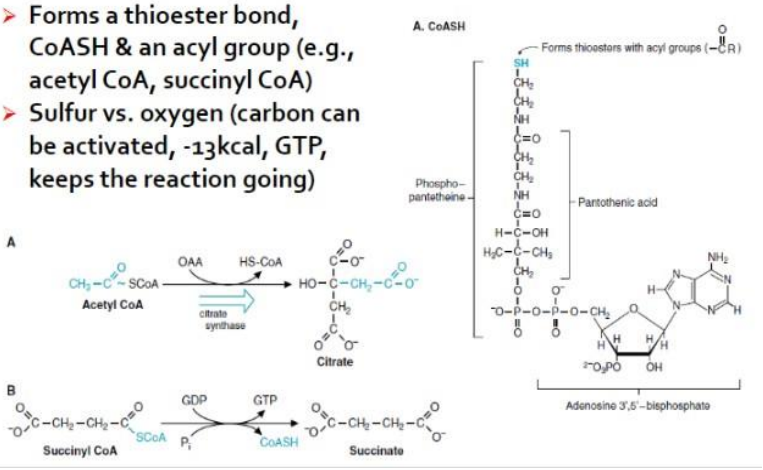
- Oxidation of succinate to fumarate, succinate dehydrogenase, FAD
- Fumarase, OH + H^+ from water, fumarate to malate
- Alcohol group of malate oxidized to a keto group, NADH



- This slide talks about the reactions of CoA and its role in GTP production.
- In reaction A it shows how CoA-HS leaves the reaction when Acetyl CoA and Oxaloacetate combine together forming citrate.
- In reaction B it shows how GTP is produced by the release of CoA-SH when Succinyl CoA is converted to Succinate.

CoA

- Forms a thioester bond, CoASH & an acyl group (e.g., acetyl CoA, succinyl CoA)
- Sulfur vs. oxygen (carbon can be activated, -13kcal, GTP, keeps the reaction going)



Note: Sheet 7 of Doctor 2017 could be helpful!

TCA cycle practice questions

1) If the Krebs cycle is overstimulated, the body will produce too much of which of the following molecules?

A) Oxygen
B) Glucose
C) Carbon dioxide
D) Acetyl CoA
E) Pyruvate

2) MO'TASEM ABU JABER took a neural sample and separated the cell body from the axon. He noticed that when he placed both parts on a pyruvate plate, the cell body began releasing carbon dioxide. What could explain the result?

A) The cell body contains mitochondria
B) The carbon dioxide is used as a messenger to communicate with the axon
C) The cell body is degrading
D) The carbon dioxide came from the plate
E) None of these

3) In the citric acid cycle, a flavin coenzyme is required for?

A) condensation of acetyl-CoA and oxaloacetate
B) oxidation of fumarate
C) oxidation of isocitrate
D) oxidation of malate
E) oxidation of succinate

4) All of the oxidative steps of the citric acid cycle are linked to the reduction of NAD+ except:

A) isocitrate dehydrogenase
B) α-KG dehydrogenase
C) pyruvate dehydrogenase
D) succinate dehydrogenase

5) For the following reaction, Maltate + NAD+ Oxaloacetate + NADH , ΔG°= +29.7 kj/mol. The reaction:

A) can never occur in a cell
B) can only occur in a cell if it is coupled to another reaction for which ΔG° is positive
C) can only occur in a cell in which NADH is converted to NAD+ by electron transport
D) may occur in cells at certain concentrations of substrate and product
E) would always proceed at a very slow rate

6) The reaction of the citric acid cycle that produces an ATP equivalent (in the form of GTP) by substrate-level phosphorylation is the conversion of?
A) citrate to isocitrate
B) fumarate to malate
C) malate to oxaloacetate
D) succinate to fumarate
E) succinyl-CoA to succinate

7) Which one of the following enzymatic activities would be decreased by thiamine deficiency?
A) Fumarase
B) Isocitrate dehydrogenase
C) Malate dehydrogenase
D) Succinate dehydrogenase
E) α-Ketoglutarate dehydrogenase complex

8) The two moles of CO_2 produced in the first turn of the citric acid cycle have their origin in the?
A) carboxyl and methylene carbons of oxaloacetate
B) carboxyl group of acetate and a carboxyl group of oxaloacetate
C) carboxyl group of acetate and the keto group of oxaloacetate
D) two carbon atoms of acetate
E) two carboxyl groups derived from oxaloacetate

9) Oxaloacetate uniformly labeled with 14C (i.e., with equal amounts of 14C in each of its carbon atoms) is condensed with unlabeled acetyl-CoA. After a single pass through the citric acid cycle back to oxaloacetate, what fraction of the original radioactivity will be found in the oxaloacetate:

A) 1/4
B) 1/2
C) 3/4
D) all
E) 0

10) Malonate is a competitive inhibitor of succinate dehydrogenase. If malonate is added to a mitochondrial preparation that is oxidizing pyruvate as a substrate, which of the following compounds would you expect to decrease in concentration

A) Citrate
B) Fumarate
C) Isocitrate
D) Pyruvate
E) Succinate

11) In mammals, each of the following occurs during the citric acid cycle except

: A) formation of α-ketoglutarate
B) generation of NADH and FADH
C) metabolism of acetate to carbon dioxide and water
D) net synthesis of oxaloacetate from acetyl-CoA
E) oxidation of acetyl-CoA

Answers: 1) C
2) A
3) E
4) D
5) D
6) E
7) E
8) E
9) B
10) B
11) D