8

◉ Sheet ○ Slides

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We are going to talk in this sheet about tricarboxylic acid cycle (citric acid cycle / kreps cycle).

- It was named originally tricarboxylic acid cycle because the exact intermediate (citrate) wasn't known to be citric acid (tricarboxylic acid : the acid with the 3 carboxylic group ,later on it was recognized as the citric acid) . It is also known as kreps cycle according to the scientist that discovers most of it's reactions and put it in its complete cyclic form

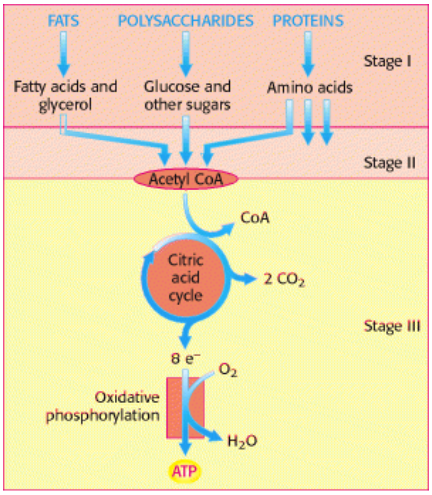
☺ ***We said that the idea of metabolism is to :-***

1- Produce energy
2- Produce building blocks for biosynthesis .

- **So in general** , we start with fats, polysaccharides and proteins, they are all converted and broken down to their building blocks and with several reactions in metabolism the intermediate Acetyl CoA is formed , the acetyl group in the Acetyl CoA then is oxidized in the citric acid cycle to release CO2 to produce "reduced dinucleotides" and these dinucleotides will be oxidized by oxidative phosphorylation to produce ATP .

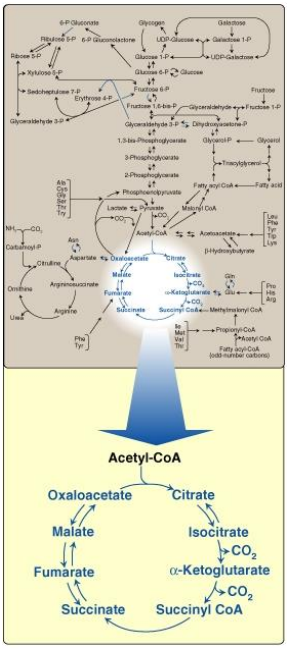
Citric acid cycle

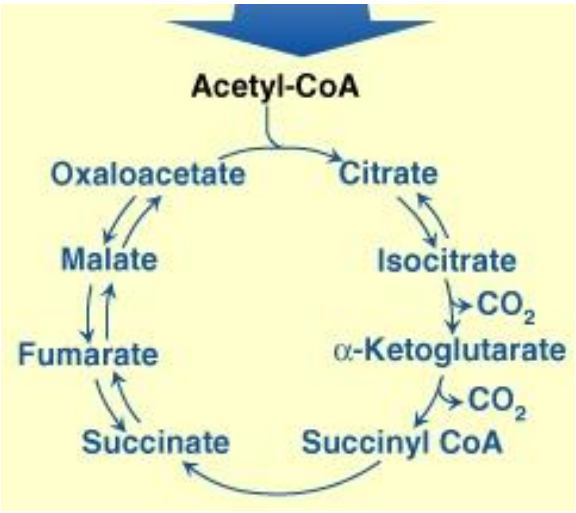
- We can notice that kreps cycle is the final pathway where the oxidative metabolism of carbohydrates, amino acids and fatty acids convert their carbon skeletons to CO2 .
- Most of the body's catabolic pathways converge on the TCA cycle .



- The metabolic pathways are sequence of reaction (where the product of one reaction is the substrate of the second reaction) and converting one substance to another doesn't proceed in one step instead it undergoes several steps which ultimately convert the initial reactant to the final product.

☺ In this picture metabolic reactions (pathways) are shown which includes **citric acid cycle** .





- Citric acid cycle includes 8 different reactions . We start with the compound **oxaloacetate** (the first reaction requires oxaloacetate) and we ended up with **oxaloacetate** (the last reaction produced oxaloacetate) so that's why it is called cycle (we started and ended with the same compound **oxaloacetate).**

  - **The oxaloacetate is very important that you can't start the cycle without it , to start each cycle (for each acetyl CoA to be oxidised) oxaloacetate should be available and at the end it will be released , so it acts as a catalyst without it no reaction can take place .**

- **Before we start talking about citric acid cycle in details we have to understand the reaction that produces the Acetyl CoA (Acetyl CoA conveys**

the carbon atoms within the acetyl group to the citric acid cycle to be oxidized for energy production)

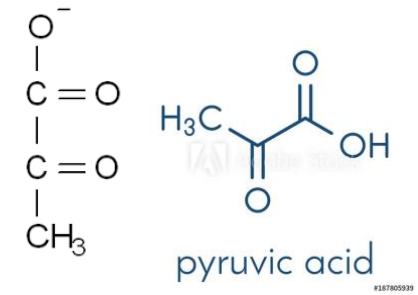
- The first step in providing Acetyl CoA from carbohydrate is to convert pyruvate to AcetyCoA { this reaction takes place in the mitochondrial matrix ; the end product of aerobic glycolysis (pyruvate) is carried to the mitochondrial matrix}

  - This conversion / oxidation of pyruvate to Acetyl CoA is the major source of Acetyl CoA group , we said that Acetyl CoA group can be obtained from fatty acid oxidation or amino acid oxidation but the most common and the major source of pyruvate is the carbohydrate's metabolism , pyruvate will undergo several reactions (decarboxylation +oxidation) to produce the acetyl group of the AcetylCoA.

- **Let's look deeper** :-

pyruvate :

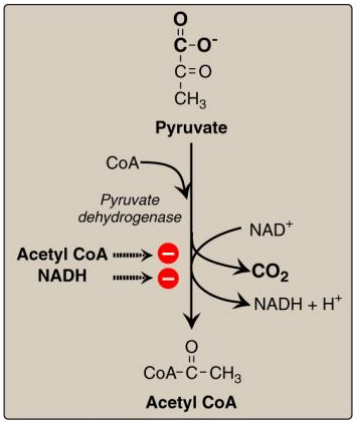
1- It is a 3 carbon carboxylic acid
   A- One of the carbons forms the carboxyl group (the carbon on the top)
   B- The middle carbon (alpha carbon; carbonyl carbon) forms the ketone group ; so we call it alpha keto acid { the carboxylic acid that contains ketone group at alpha carbon
2- We can name it pyruvate (basic form / unprotonated) or pyruvic acid (acidic form / protonated))



Pyruvate

- **So pyruvate will undergo oxidative decarboxylation (by 5 steps) 3 of them are involved in producing AcetylCoA :-**

1- **Pyruvate will undergo decarboxylation (removing of the carboxylic group in the form of CO2)**
2- **The resultant acetaldehyde is oxidized to produce as acetic acid (acetyl group)**
3- **Acetic acid (acetyl group) is directly joined to Coenzyme A producing AcetylCoA**



- The reaction seems to be simple **BUT** it is actually more complex because it involves **decarboxylation** reaction, **oxidation-reduction** reaction and the **addition of the CoA to the acetyl group** .

- This reaction is irreversible with large negative (delta Go) that means once we produce AcetylCoA there is no way we can go back to pyruvate .Why is that or how can you tell that this reaction is irreversible ?

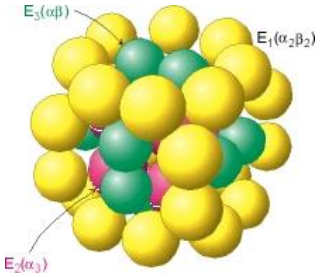
  - This is due to the release of CO2 (decarboxylation) despite that the product has high energy .

☺ The ***enzyme*** catalyzing this reaction can be named :-

1- Pyruvate decarboxylase (due to the decarboxylation reaction)
2- Pyruvate dehydrogenase (due to the oxidation-reduction reaction)

- But it is actually called **pyruvate dehydrogenase** because the oxidation-reduction reaction has more priority and it is more important than decarboxylation reaction .

- **Pyruvate dehydrogenase** is an enzyme complex (it is a protein aggregate of multiple copies of three enzymes : E1,E2,E3 each one catalyzes part of the overall reaction) .



An image showing the enzyme complex , with the enzymes involved in the reaction .

  - Their physical association links the reaction in proper sequence without the release of the intermediates ; that means each enzyme delivers its product directly to the next enzyme ,so this enzyme complex is more efficient in catalyzing the reaction rather than releasing the product and looking for the next enzyme to bind with .

- **The protein part of the enzyme by itself isn't sufficient to catalyze the reaction so it depends on five coenzymes :-**
  1- Thiamine pyrophosphate (TPP) { thiamine : vitamin B1 }
  2- Lipoic acid
  3- FAD { contains riboflavin which is vitamin B2 }
  4- CoA {contains pantothenic acid which is one of the vitamin B family (B5)
  5- NAD+ { contains niacin or nicotine amide it is a form of vitamin B3 }

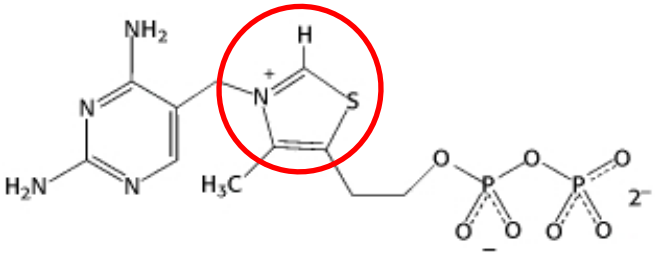
  ☺ these five coenzymes (vitamins) are found **together** in the complex and they all work together as carriers or oxidants of the intermediates ; so for example when you eat plant or animal tissue you will get all of these vitamins together .

  EXTRA !! Deficiencies of thiamine or niacin can cause serious central nervous system problems . This is because brain cells are unable to produce sufficient ATP (via the TCA cycle) if the PDHC (pyruvate dehydrogenase complex) is inactive .

- **Also pyruvate dehydrogenase complex (PDHC) has two additional enzymes that regulate its activity .**

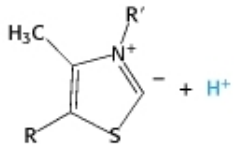
☺ Now, Let's study the structure of these coenzymes :-

1- **Thiamine pyophosphate (TPP)** { **the structure isn't required only recognise the active part }**



Thiamine pyrophosphate (TPP)

☺ The reactive part of this coenzyme is shown by the **red circle** ; this carbon nitrogen double bond with the positive charge makes it easy for the hydrogen connected to the carbon to dissociate (makes it more acidic) whereas the usual hydrogen that is connected to the carbon isn't acidic and can't be released but due to the presence of the double bond and the positive charge it can dissociate producing the anion form of the coenzyme.



Carbanion of TPP

The reactive part of the coenzyme is shown only ; just to make it easy to understand

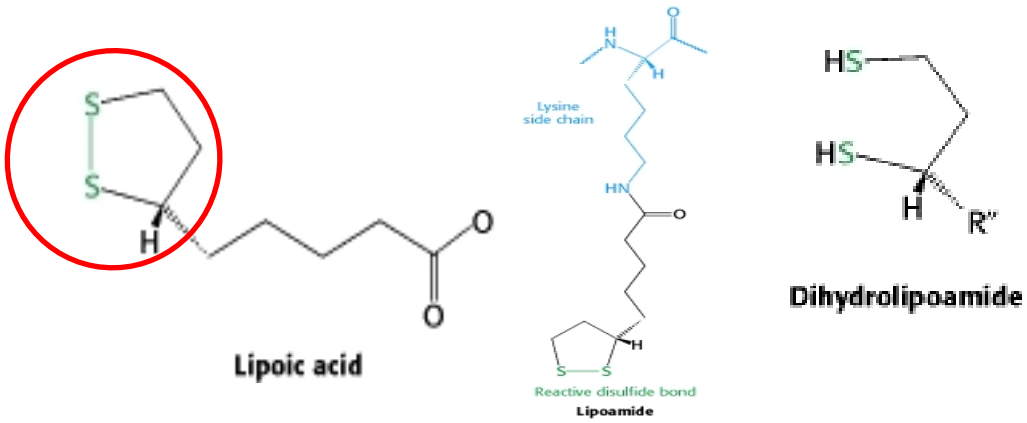
2- **Lipoic acid** { **the structure isn't required only recognise the active part }**
Lipoic acid is connected to the enzyme by amide bond producing lipoamide.

☺ The reactive part of the coenzyme is the disulfide :- this disulfide can be reduced to form dihydrolipoamide (amide :- because it is part from the amide group) and it can be oxidized .

So, lipoic acid (lipoamide) could exist in two forms :- oxidized and reduced form

Note :- oxidation and reduction happen by adding and removing 2 hydrogen atoms .

☺ lipoic acid is connected to the side chain of the amino acid lysine which contains an amino group , and this side chain is characterized by having a long hydrocarbon chain which allows the coenzyme (lipoic acid) to carry the product from one enzyme to the next enzyme .



- Now lets explain in details the steps of **converting pyruvate to AcetylCoA:-**

Note : please understand the process before you memorise

Note : the red coloured information are details that weren't mentioned in the lecture (they are written only for better understanding the concept)

A- Once pyruvate is formed in glycolysis , it then travels into the mitochondrial matrix by pyruvate mitochondrial carrier of the inner mitochondrial membrane .

☺ remember we have 3 enzymes and 5 coenzymes involved in this reaction:-

A- Pyruvate decarboxylase / dehydrogenase (E1) which requires TPP (thiamine pyrophosphate)

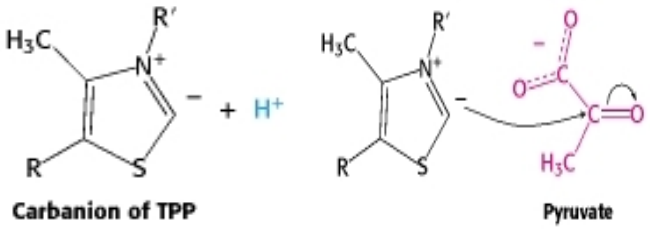
B- Dihydrolipoyl transacetylase (E2) which requires lipoic acid and CoA
C- Dihydrolipoyl dehydrogenase (E3) which requires FAD and NAD+.

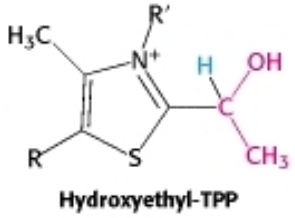
B- In the matrix PDHC (pyruvate dehydrogenase complex) converts pyruvate into AcetylCoA as the following :-

STEP 1 :- Decarboxylation :-

- **This step is catalyzed by pyruvate dehydrogenase which contains a coenzyme (prosthetic group) called TPP (thiamine pyrophosphate)**

1- **What happens is that the negatively charged reactive carbon of the carbanion of thiamine pyrophosphate is attached to the carbonyl carbon of the pyruvate which has a partial positive charge ; once the bond is formed between the two carbons this facilitates the dissociation of the terminal carboxylic group (of the pyruvate) as CO2.**
2- **The remaining two carbon unit from the pyruvate is still attached to the thiamine pyrophosphate (TPP) and we call this structure Hydroxyethel-TPP complex (hydroxyethyl : since it is an ethyl group attached to one hydroxyl group)**

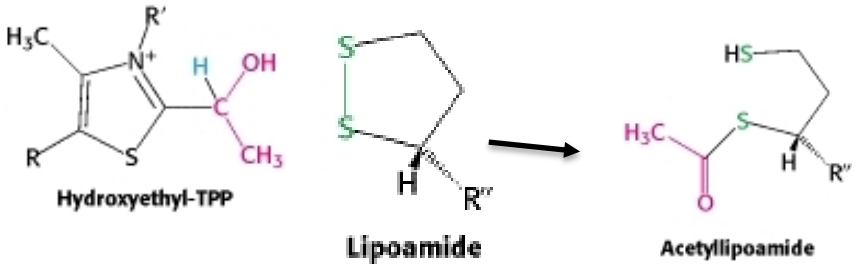




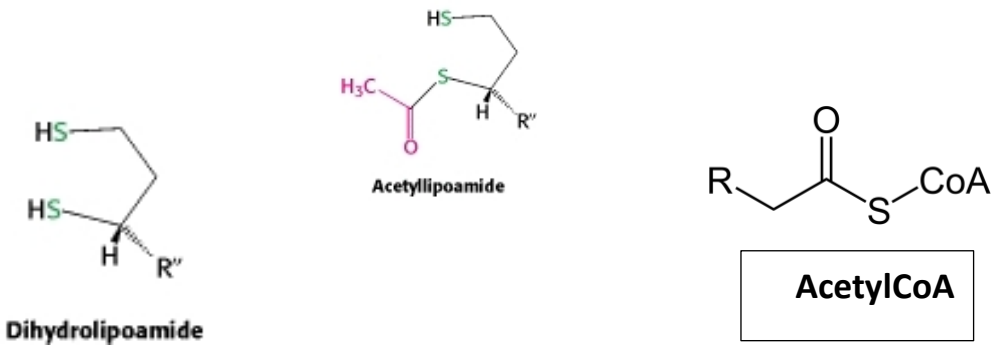
STEP 2 + STEP 3:- OXIDATION-REDUCTION AND TRANSFER OF Acetyl GROUP TO CoA FORMING AcetylCoA :-

- **This step is catalyzed by the enzyme dihydrolipoyl transacetylase which contains a coenzyme (lipoic acid and CoA)**

1- What happens is that the oxidized form of the lipoic acid (lipoamide) gets closer to the Hydroxyethyl-TPP complex and the hydroxyethyl intermediate is oxidized (to acetic acid / acetyl) by transfer to the disulfide form of lipoic acid



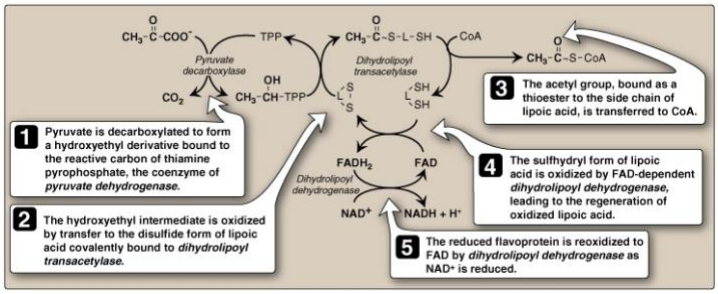
2- The Acetyl group , bound as a thioester (thioester bond is a high energy bond) to the side chain of lipoic acid (forming Acetyllipoamide) , is transferred to CoA producing Acetyl CoA and the reduced form of the lipoic acid (Dihydrolipoamide) .



STEP : 4 :-

- **This step is catalyzed by the enzyme dihydrolipoyl dehydrogenase which contains the coenzymes FAD and NAD+ .**
  1- **What happens is that the sulfhydryl form (reduced form) of lipoic acid is oxidized by FAD-dependent dihydrolipoyl dehydrogenase , leading to the generation of oxidized lipoic acid and reducing FAD to FADH2**
  2- **The reduced flavoprotein (FADH2) on the enzyme is reoxidized to FAD by the same enzyme as NAD+ is reduced to NADH**

- **YOU CAN HAVE A LOOK AT THE OVERALL REACTION TO SUMMARIZE THE WHOLE CONCEPT !!**



- **Always in metabolism there is probability to have deficiency in certain enzymes and this makes the enzyme un functional .**

- **Example :1:- clinical hint : pyruvate dehydrogenase deficiency (E1 deficiency) .**

A deficiency in the E1 component of the PDHC (pyruvate dehydrogenase complex (AcetylCoA won't be produced)

☺ Although it is rare, yet it is the most common biochemical cause of congenital lactic acidosis .

The deficiency results in a decreased ability to convert pyruvate to AcetylCoA , causing pyruvate to be shunted to lactate , and because lactic acid isn't metabolized it will accumulate causing Lactic Acidosis . This creates particular problems of the brain , which relies only on the carbohydrates metabolism in the TCA cycle for most of its energy (other tissues can use fatty acid metabolism for energy source but the brain can't) and AcetylCoA is also important in generating the neurotransmitter acetylcholine in the brain also, the brain is particularly sensitive to acidosis .

☺ symptoms are variable and include (the doctor said you are not required to memorize the symptoms what you have to know is that the brain is the most affected in this condition) :-

- neurodegeneration, muscle spasticity and in the neonatal-onset form , early death .

☺ although there is no proven treatment for PDHC deficiency , dietary restriction of carbohydrate and supplementation with thiamine pyrophosphate TPP may reduce the symptoms in selected patients .

How could TPP reduce the symptoms ??
Sometimes the enzyme isn't totally absent it may bind to TTP (thiamine pyrophosphate) which may reduce the symptoms .

• Example :2:- clinical hint : Arsenic poisoning :-

Arsenic causes inhibition of enzymes that require lipoic acid as a coenzyme such as E2 of the PDH complex .

-Arsenite (trivalent form of arsenic) forms a stable complex with the –SH groups of lipoic acid which makes it unavailable to serve as a coenzyme.

-Arsenite binding to lipoic acid in the PDH complex, pyruvate and consequently lactate will accumulate .

-Brain is particularly affected, causing neurologic disturbances and death.

Citric acid cycle :-

Step :1:- citrate synthesis

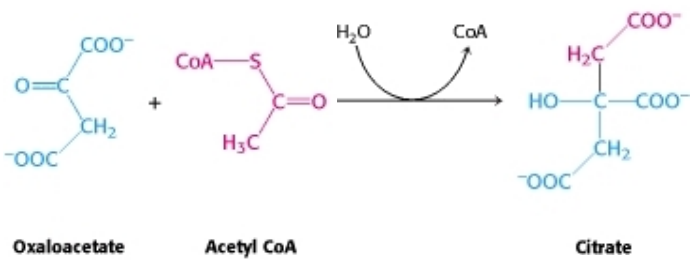
☺ An irreversible condensation of AcetylCoA and Oxaloacetate to form citrate and it is catalyzed by citrate synthase .

A- We have AcetylCoA which an Acetyl group attached by thioester bond to CoA

B- Oxaloacetate :- it is a 4 carbon compound (oxalo=4) having two carboxylic groups (two of the carbons are carboxylic groups) and one ketone group on alpha-carbon ; so it is a alpha keto acid with two carboxylic groups and CH2.

- Oxaloacetate is formed from oxalic acid (2 carbon atoms) and acetate also with two carbon atoms .

C- Citrate :- it is citric acid that is found in the commercial drinks .
Citric acid is a 6 carbon compound composed of three carboxylic groups that's why it is called tricarboxylic acid .



- This reaction has highly negative change in standard free energy which strongly favors citrate formation .
  - But how can we tell that this reaction is highly exergonic and produces high amount of energy (we don't have ATP production) ??

  Because of the AcetylCoA HYDROLYSIS and we said that hydrolysis is an exergonic reactions
- Since the reaction is irreversible this means it needs to be regulated

Step:2:-citrate isomerization

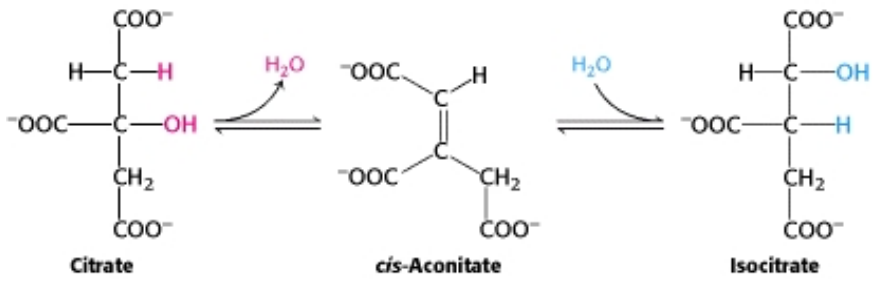
☺ Citrate is isomerized into isocitrate through hydroxyl group migration (dehydration step followed by a hydration step) catalyzed by aconitase .

- So, the difference between the two isomers (citrate and isocitrate)is the location of the hydroxyl group [OH on carbon 2 in isocitrate and on carbon 3 in citrate]
- The reaction is reversible since the (deltaGo) for the reaction is almost zero .

Note :- aconitase is inhibited by fluoroacetate a plant toxin that is used as a rat poison . HOW ??

Fluoroacetate is converted to fluoroacetylCoA that condenses with oxaloacetate to form fluorocitrate , a potent inhibitor of aconitase .

NOTE ;- THE INTERMEDIATE (CIS-ACONITATE) ISN'T REQUIRED WITH US



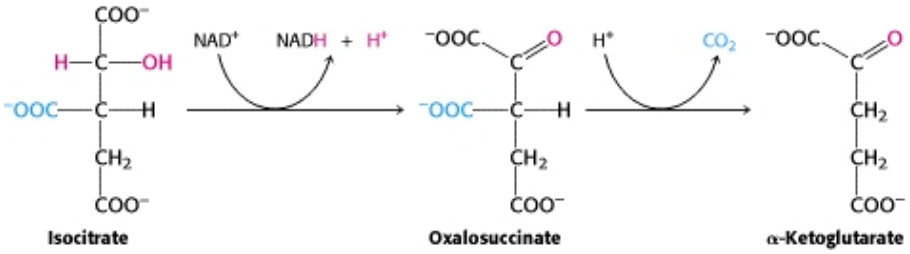
Step :3 :-oxidative decarboxylation of isocitrate

☺ Isocitrate dehydrogenase (we have two reactions: oxidation and decarboxylation but the priority for the oxidation reaction as we said so that's why it is called

dehydrogenase) catalyzes the irreversible oxidative decarboxylation of isocitrate to alpha-ketoglutarate

- irreversible : because of the releasing of CO2

- **Oxidation** happens to the secondary alcohol producing ketone group . As a result, the presence of this ketone group at carbon 2 makes it very easy for the adjacent carboxylic group to be released (**Decarboxylation**)



Step: 4:-oxidative decarboxylation of alpha-ketoglutarate

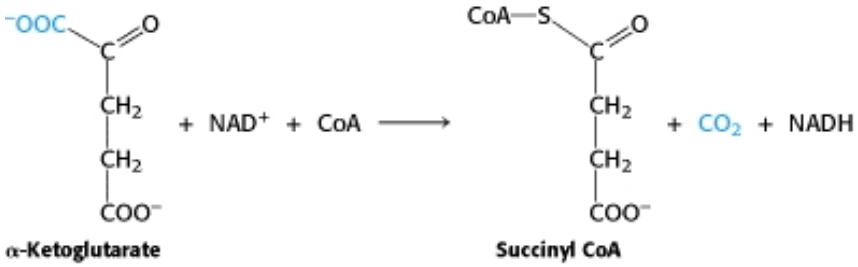
☺ The irreversible conversion of alpha-ketoglutarate to succinyl CoA is catalyzed by the alpha-ketoglutarate dehydrogenase complex .

- Describe **alpha-ketoglutarate** :- a 5 carbon compound containing two carboxylic group and ketone group at carbon alpha

The mechanism of this oxidative decarboxylation is very similar to that used for the conversion of pyruvate to acetylCoA by the PDHC (pyruvate dehydrogenase complex) .

And the complex also requires the five coenzymes (TPP [thiamine pyrophosphate], lipoic acid , FAD, NAD+ and CoA)

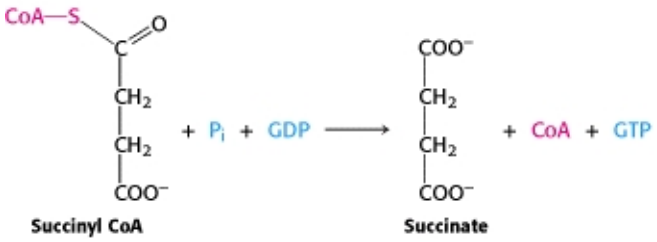
The large negative (delta Go) of the reaction favors formation of succinyl CoA, a high-energy thioester similar to acetyl CoA (so even though the reaction is irreversible and releases high energy , high amount of this energy is captured in this bond in the product) .



Step : 5:- succinyl coenzyme A cleavage

☺ Succinate thiokinase cleaves the high-energy thioester bond of succinyl CoA .

This reaction is coupled to phosphorylation of guanosine diphosphate (GDP)to guanosine triphosphate (GTP) [the energy produced due to the cleavage is 7.5 kcal which is sufficient to produce GTP] . ATP is produced from GTP since both of them are energetically interconvertible by the reaction :- (the generation of GTP by succinate thiokinase is an example of substrate-level phosphorylation [that means phosphorylation without oxidation ; not oxidative phosphorylation])



Step:6:- succinate oxidation :-

☺ Succinate is oxidized to fumarate by succinate dehydrogenase , as its coenzyme FAD is reduced to FADH2 .

- Describe **succinate** :- a 4 carbon compound with two carboxylic groups.

- Succinate dehydrogenase is the only enzyme of the TCA cycle that is embedded in the inner mitochondrial membrane .As such it functions as complex 2 in ETC (electron transport chain) .

☺ **Extra ! Someone might ask why did we use FAD instead of NAD + ?? That is because the reducing power of succinate isn't sufficient to reduce NAD+ .**

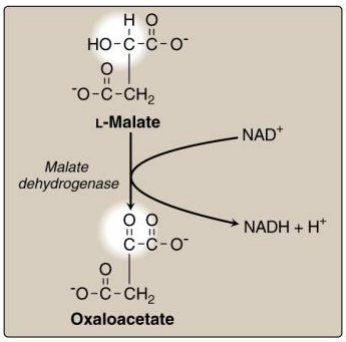
Step :7 : Fumarate hydration :-

☺ Fumarate is hydrated to malate in a freely reversible reaction catalyzed by fumarase .

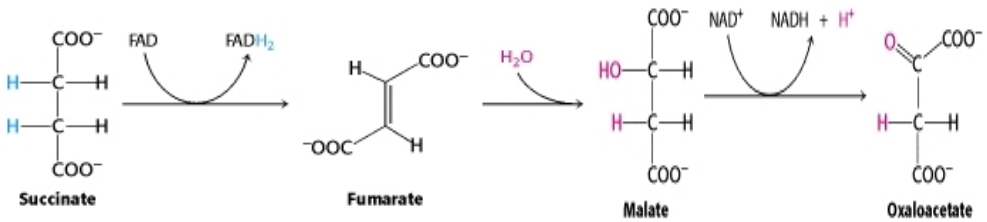
Step:8: malate oxidation :-

☺ Malate is oxidized to Oxaloacetate by malate dehydrogenase .

- describe malate (حمض التفاح) : it is a 4 carbon compound with two carboxylic groups .

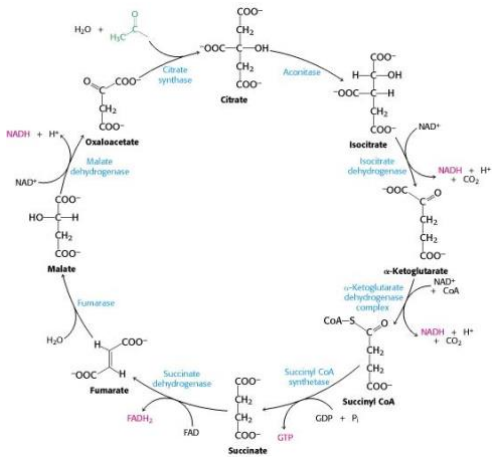


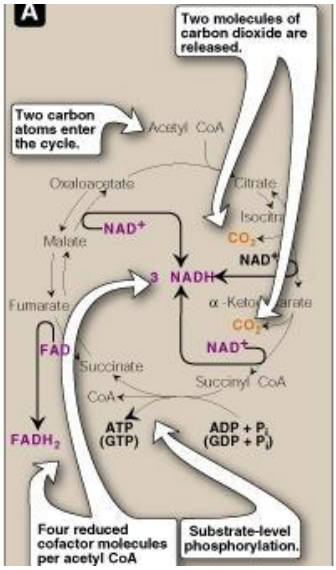
Step 6+7+8 :-



☺ so for every cycle AcetylCoA is oxidized and Oxaloacetate is used and produces so its level won't decrease nor increase and this is true for all the intermediates

So this is the net reaction :-





Energy producing reaction	Number of ATP produced
3 NADH ⟶ 3 NAD^+	9
$FADH_2$ ⟶ FAD	2
GDP + P_i ⟶ GTP	1
	12 ATP/acetyl CoA oxidized

Figure 9.7
Number of ATP molecules produced from the oxidation of one molecule of acetyl CoA (using both substrate-level and oxidative phosphorylation).

$$\text{Acetyl CoA} + 3\,NAD^+ + FAD + GDP + P_i + 2\,H_2O \longrightarrow 2\,CO_2 + 3\,NADH + FADH_2 + GTP + 2\,H^+ + CoA$$

Some mnemonics

CIA Sends Soldiers From My Office

Citrate

Isocitrate

Alfa Keto Glutarate

Succinyl CoA

Succinate

Fumarate

Malate

Oxaloacetate