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Biochemistry 2

Doctor 2018 | Medicine | JU

◉ Sheet ○ Slides

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Citric acid cycle:

The cycle is alternatively known as the ***Krebs*** cycle, or the ***Tricarboxylic acid cycle (TCA)***, because citrate contains three carboxylic acid groups.

- The TCA cycle starts with the **condensation** step, combining the two-carbon acetyl group (from acetyl CoA) with a four-carbon oxaloacetate molecule to form a six-carbon molecule of citrate(a tricarboxylic acid), .
- Citrate is **isomerized** to isocitrate.
- Isocitrate undergoes irreversible **oxidative decarboxylation** producing a five-carbon molecule, α-ketoglutarate, together with a molecule of CO_2 and two electrons, which reduce NAD^+ to NADH.
- α-ketoglutarate is **oxidatively decarboxylated** to Succinyl CoA producing CO_2 and NADH , (Steps three and four are both irreversible oxidation and decarboxylation steps, which release electrons that reduce NAD^+ to NADH and release carboxyl groups that form CO_2 molecules).
- Succinyl CoA is **cleaved** producing Succinate and GTP.
- Succinate is **oxidized** to fumarate producing $FADH_2$ (two hydrogens are transferred to FAD, forming $FADH_2$ and oxidizing succinate) .
- **water is added** to fumarate (**hydrating** it), and malate is produced.
- The last step in the citric acid cycle regenerates oxaloacetate by **oxidizing** malate. Another molecule of NADH is produced.

KEY points:

- The four-carbon molecule, oxaloacetate, that began the cycle is regenerated after the eight steps of the citric acid cycle.
- The eight steps of the citric acid cycle are a series of redox, condensation, isomerization, dehydration, hydration, and decarboxylation reactions.

- intermediates of the cycle contain either three or two carboxyl groups (except succinyl-CoA):

  2 intermediates with 3 COO- groups: Citrate and Isocitrate .

  The remaining 5 intermediates contain 2 COO- groups.
- Citrate and Isocitrate are Tricarboxylic intermediates, the rest are dicarboxylic intermediates except succinyl-CoA (contains a single carboxyl group).
- Number of carbon atoms:

  Citrate and Isocitrate (six-carbons)

  α-ketoglutarate(five-carbons)

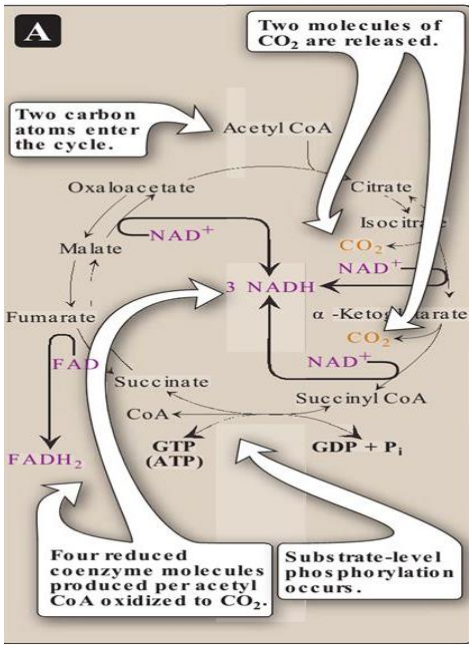
  The remaining intermediates contain 4-carbons.

➢ The primary function of the citric acid cycle is oxidation of acetyl-CoA to carbon dioxide, The energy released from this oxidation is saved as NADH ,FADH2,and GTP , the overall result of the cycle is represented by the following reaction:

- **Net Reaction of TCA Cycle :-**

$$\text{Acetyl CoA} + 3\,\text{NAD}^+ + \text{FAD} + \text{GDP} + \text{Pi} + 2\,\text{H}_2\text{O} \xrightarrow{2\text{H}^+} 2\,\text{CO}_2 + \text{CoA} + 3\,\text{NADH} + \text{FADH}_2 + \text{GTP}$$

NOTICE that none of the intermediates of the TCA cycle appear in this reaction, not as reactants or as products, This emphasizes an important point about the cycle. It does not represent a pathway for the net conversion of acetyl-CoA to any intermediate of the cycle, it does not lead to the net production or consumption of intermediates, each intermediate is produced in one step and used in the subsequent step. The only fate of acetyl CoA in this pathway is its oxidation to CO2.



- **Energy produced by the cycle** :

Oxidation of one NADH by the electron transport chain leads to formation of 2.5 or approximately three ATP, whereas oxidation of FADH2 yields approximately two ATP.

One more molecule of ATP is produced for each molecule of acetyl-CoA processed. Actually, ATP is not directly produced by the cycle, the released energy directly links inorganic phosphate to GDP (a high-energy molecule similar to ATP,the energy from GTP is then transferred to ATP as follows):

$$GTP + ADP \rightleftharpoons ATP + GDP$$

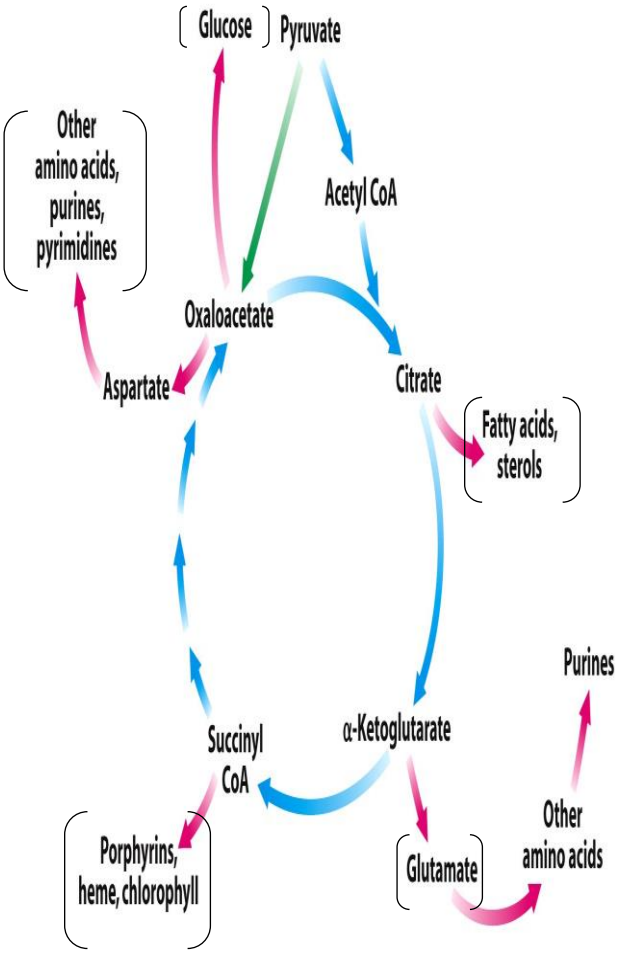
- Number of ATP molecules produced from the oxidation of one molecule of acetyl-CoA (shown in the figure).:

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

- **KEY POINT:**
  Although oxygen is not directly required in the cycle, the pathway will **NOT** occur anaerobically because NADH and FADH2 will accumulate (in their reduced form) if oxygen is not available for the electron transport chain, Oxygen is required for the citric acid cycle indirectly as an electron acceptor at the end of the electron-transport chain, necessary to regenerate NAD+ and FAD.

- ***The Citric Acid Cycle Is a Source of Biosynthetic Precursors:***

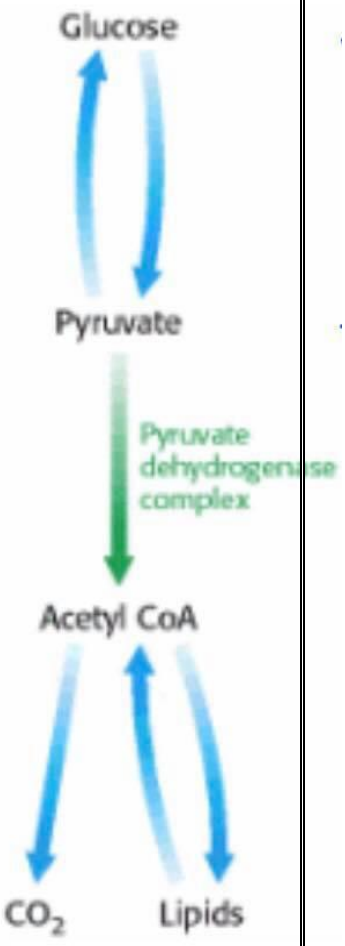
Intermediates of the TCA cycle serve as precursors for biosynthesis of biomolecules :



- Citrate may leave the mitochondria to deliver acetyl-CoA into the cytoplasm for fatty acids synthesis.
- Succinyl CoA is an intermediate that can be used for Porphyrins synthesis like heme(One of the best-known families of porphyrin complexes) .
- Oxaloacetate has a role in ***Gluconeogenesis*** (generation of glucose from non-carbohydrate substrates)
- Some amino acids like Aspartate and Glutamate are synthesized by transfer of an "amino group" to the α-keto acids Oxaloacetate and α-ketoglutarate, respectively (Transamination reactions).
- When intermediates are drawn out of the citric acid cycle, the cycle slows. Therefore when intermediates leave the cycle they must be replaced to ensure sufficient energy for the cell.
- Anaplerotic reactions ('to fill') *are chemical reactions that form intermediates of a metabolic pathway*. Since the TCA cycle intermediates are used for anabolism, their concentration varies according to the needs of the cell.
- Reactions that replenish the TCA cycle intermediates are called anaplerotic reactions.
- Oxaloacetate can be considered as a primary substrate of the TCA cycle. It is replenished from pyruvate by the enzyme pyruvate carboxylase
- *Carboxylation of Pyruvate produces Oxaloacetate (*anaplerotic reaction):

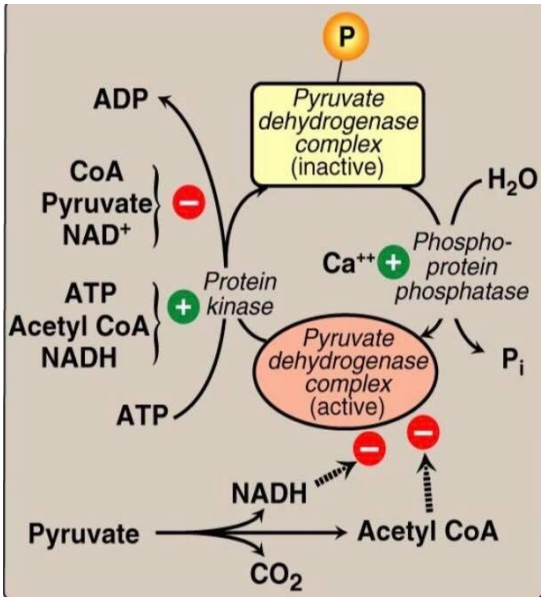
Pyruvate + CO_2 or HCO_3^- + ATP ⟶ Oxaloacetate + ADP + Pi

- pyruvate carboxylase is a Biotin-Containing Enzyme.
- The reaction requires energy to occur. The production of oxaloacetate from pyruvate is considered the most important anaplerotic reaction .
- ***Regulation of Citric Acid Cycle:***
- It is the final common pathway for the aerobic oxidation of fuel molecules
- It is an important source of building blocks for a number of biomolecules.
- Entry into the cycle and the rate of the cycle itself are controlled at several stages.
- Although PDH complex is not part of the TCA cycle but it supplies substrate for the cycle thus it is a highly regulated enzyme complex.
- Pyruvate dehydrogenase reaction is irreversible.
- Glucose can be formed from pyruvate. However, the formation of acetyl CoA from pyruvate is an irreversible step in animals. They are unable to convert acetyl CoA back into glucose.
- **EXTRA INFO**: 2C Acetyl CoA enters the TCA , 2 molecules of CO2 are released & the oxaloacetate is regenerated. There is **no NET** production of oxaloacetate therefore the cycle does not represent a pathway by which there can be net synthesis of glucose from acetyl-CoA.



- ***Fat can be produced from glucose, But fat can not be converted to glucose.***
- ***Regulation of the pyruvate dehydrogenase complex (covalent modification):***
- Allosteric regulation and reversible phosphorylation by two regulatory enzymes alternately activate and inactivate the complex.

- **PDH kinase** phosphorylates and **inactivates** the enzyme, whereas **PDH phosphatase** or **phosphoprotein phosphatase** dephosphorylates (hydrolysis) and **activates** the enzyme.
- In the presence of high energy signals the PDH complex is turned off ,
  The rise in ATP/ADP, NADH/NAD+, or Acetyl-CoA/CoA ratios inhibits the enzyme.

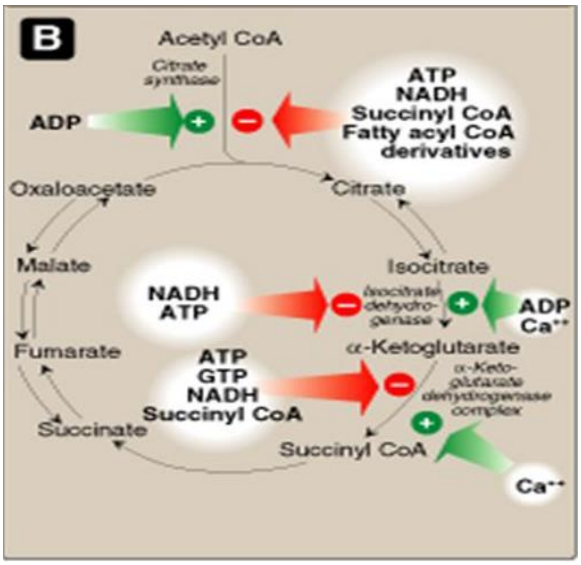


DIRECT REGULATION	*INDIRECT REGULATION*
Activators :NONE	*Activators:* *Activators of PDH phosphatase (Ca+2 ,insulin)* *Inhibitors of PDH kinase:* *(Pyruvate,ADP,CoA,NAD+)*
Inhibitors: *High concentrations of reaction products (Acetyl-CoA and NADH) inhibit the complex (product or feedback inhibition).*	*Inhibitors:* *Activators of PDH kinase:* *(ATP. Acetyl-CoA, NADH)*

➢ *Phosphatase Deficiency (congenital disease):*

- Pyruvate dehydrogenase is always phosphorylated. and thus inactive.
- glucose is processed to lactic acid⟶lactic acidosis
- Malfunctioning of many tissues, most notably the central nervous system.
- Inability of producing acetyl-CoA, an important component in the biogenic synthesis of acetylcholine (neurotransmitter used at the neuromuscular junction).

➢ ***The TCA cycle is regulated allosterically at the three irreversible steps***, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase(citrate synthase is not mentioned in the slides but is also regulated since it catalyzes a crucial irreversible step in the cycle).

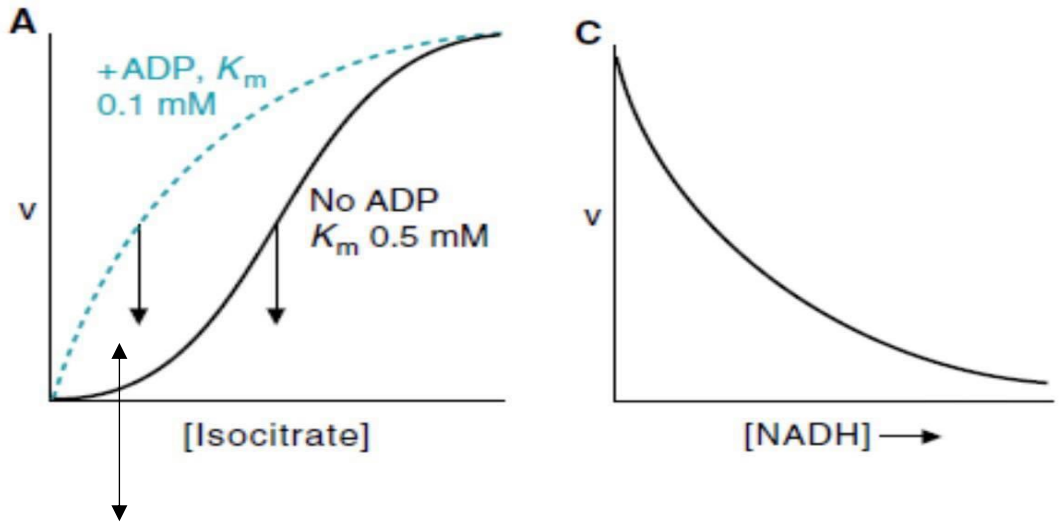


➢ The citric acid cycle is regulated primarily by [ATP] and [NADH].

➢ a-ketoglutarate dehydrogenase complex is similar to PDH complex.

Enzyme	Activators	Inhibitors
Isocitrate dehydrogenase (IDH)	ADP,Ca+2	ATP, NADH
α-Ketoglutarate dehydrogenase	Ca+2	succinyl CoA and NADH, high energy charge ATP/ADP
Extra: Citrate synthase	ADP	ATP, NADH, Citrate, Succinyl CoA

- **The effect of ADP and NADH on the activity of isocitrate Dehydrogenase (Allosteric enzyme):**
- Isocitrate dehydrogenase is the major control enzyme, it is inhibited by NADH and ATP and activated by ADP.
- The following figure shows a sigmoidal curve when reaction velocity (Vo) is plotted against substrate concentration [S] (isocitrate),this enzyme exhibits cooperativity (the presence of a substrate molecule at one site on the enzyme enhances the catalytic properties of the other substrate-binding sites).
- Effectors can move the plot left (rate increase) or right (rate decrease) by shifting the Km for substrate.
- **When [ADP] (+ve effector) is elevated, the plot shifts to the left and shows more hyperbolic curve similar to enzymes following Michaelis-Menten kinetics** (at the same [S],increased enzymatic activity is noticed (increased rate)).
- -ve effectors inhibit enzyme's activity, velocity drops sharply at high [NADH] this represents a feedback inhibition.



LOWER Km value → higher affinity.

- **Dietary deficiency of thiamine (vitamin B1)**
- Thiamine deficiency is a medical condition of low levels of thiamine (vitamin B1), A serious health problem in communities where rice is the major food.

> ***Refined foods are highly processed foods that have been stripped of their original* nutrient content and fiber.**
>
> **Examples: White bread and white rice**

- Thiamine is naturally present in some foods, including whole grain products such as cereals. That's why People who consume diets consisting of primarily refined grains are at risk for thiamine deficiency.

- Vitamin B1 deficiency occurs frequently in South-East Asia, a serious health problem in communities where refined rice is the major food.

- Thiamine deficiency is often related to chronic alcoholism.

- Thiamine pyrophosphate is cofactor of: pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and transketolase.

- ↑↑pyruvate and α-ketoglutarate in the blood (The concentrations of blood pyruvic and α-ketoglutaric acids are elevated, in relation to the function of thiamine as part of a coenzyme for the decarboxylation of both keto acids).

- A positive diagnosis test for thiamine deficiency involves measuring the activity of the enzyme transketolase in erythrocytes, which is easily measured (reliable diagnostic indicator of the disease).

- Deficiency is characterized by neurologic and cardiac symptoms.

(The previous details are not fully required, just focus on the general idea).

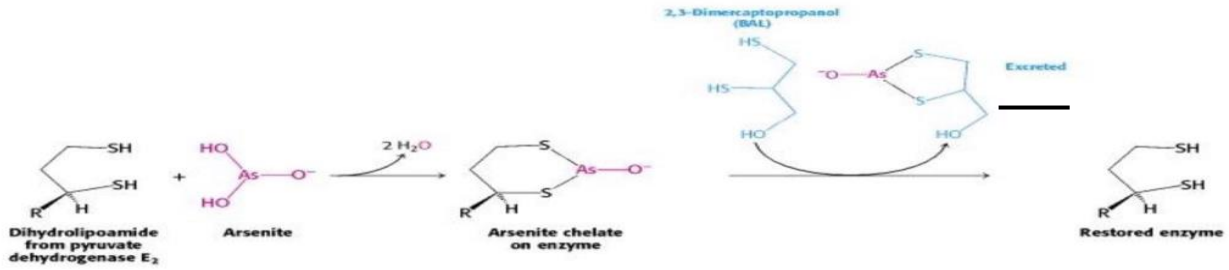
- **Mercury or Arsenite Poisoning:**
- Both elements have a high affinity for neighboring sulfhydryls, (dihydrolipoyl groups) .The binding of mercury or arsenite to the dihydrolipoyl groups inhibits the complex⟶central nervous system pathologies

- **Treatment:**

- Administration of sulfhydryl reagents with adjacent sulfhydryl groups.
- **Dimercaprol** also called British anti-Lewisite (BAL) is used to treat arsenic or mercury poisoning. (used as an antidote ⟶ a substance that can counteract a form of poisoning).

- **HOW SO? EXTRA**

- Dimercaprol is a molecule with two sulfhydryl groups that acts by binding heavy metals, and thus competing and blocking the binding of the toxic metals to sulfhydryl containing metabolic enzymes.
- Dimercaprol competes with the thiol groups for binding the metal ion, which is then excreted in the urine.



QUIZ:

1.Anaplerotic reactions are those that result in replenishing intermediates in the TCA cycle. Which of the following enzymes catalyzes an anaplerotic reaction?

a) Malate dehydrogenase

b) Pyruvate carboxylase

c) Pyruvate kinase

d) Citrate synthase

e) Succinyl-co A Thiokinase

2.A 2-year-old child was brought to pediatric emergency with convulsions. The child was diagnosed with ammonia intoxication due to some urea cycle disorder. Reduced formation of GABA is considered to be the most important cause of convulsion due to depletion of glutamate from where it is produced by decarboxylation. Which of the following intermediates of TCA cycle is involved in the formation of Glutamate ?

a) Succinate

b) Malate

c) α-Ketoglutarate

d) Isocitrate

e) Pyruvate.

3.Thiamine deficiency will not only lead to the accumulation of pyruvate and lactate, but also the accumulation of :

a) Succinyl-CoA.

b) Oxaloacetate.

c) Malate.

d) Succinate.

e) alpha-ketoglutarate.

4.How would adding NADH affect Isocitrate dehydrogenase plot?

a) Makes it more hyperbolic.

b) Shifts it to the left.

c) Shifts it to the right.

d)More than one of the above.

5.Which of the following increases the oxidation of Acetyl-CoA by TCA cycle?

a) Low acetyl-CoA concentration.

b) High ATP/ADP ratio.

c) High NAD+/NADH ratio

d) None of the above.

ANSWERS:

1. **(B)**
2. **(C)**
3. **(E)**
4. **(C)**
5. **(C)**

Best of luck ♥