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Bio chemistry

Doctor 2017 | Medicine | JU

Sheet ○ Slides

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DOCTOR

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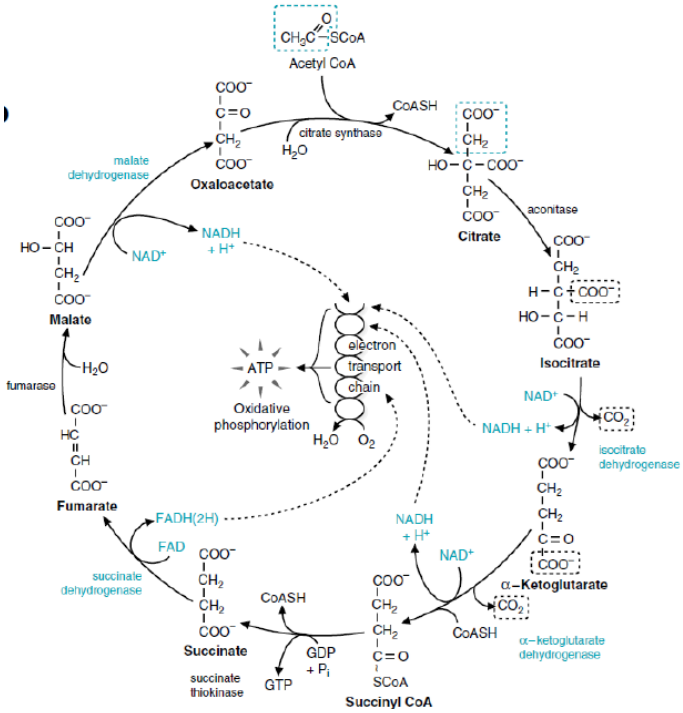
Summary of the 8 steps of citric acid cycle

Step 1. Acetyl CoA joins with a four-carbon molecule, **oxaloacetate**, releasing the CoA group and forming a **six-carbon** molecule called **citrate.**

Step 2. Citrate is converted into its isomer, **isocitrate.**

Step 3. Isocitrate is oxidized and releases a molecule of **carbon dioxide**, 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**.

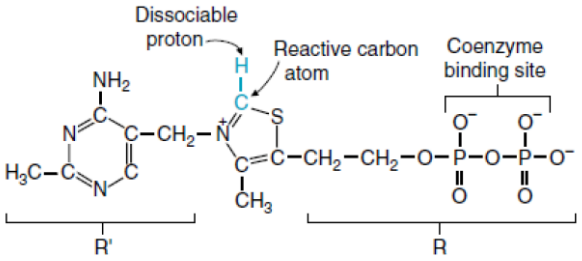
Step 8. Oxaloacetate, the starting **four**-carbon compound, is regenerated by the **oxidation** of the **alcohol** group of malate by **malate dehydrogenase** to a **keto** group. Another molecule of NAD+ is reduced to NADH in the process.

-Out of the four dehydrogenases, only succinyl dehydrogenase reduces FAD instead of NAD+.

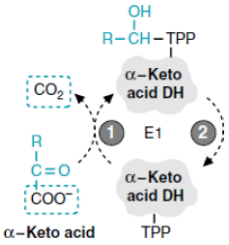
Thiamine pyrophosphate (B1 derivative)

-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.



Thiamine pyrophosphate (TPP)



Oxidative decarboxylation of pyruvate

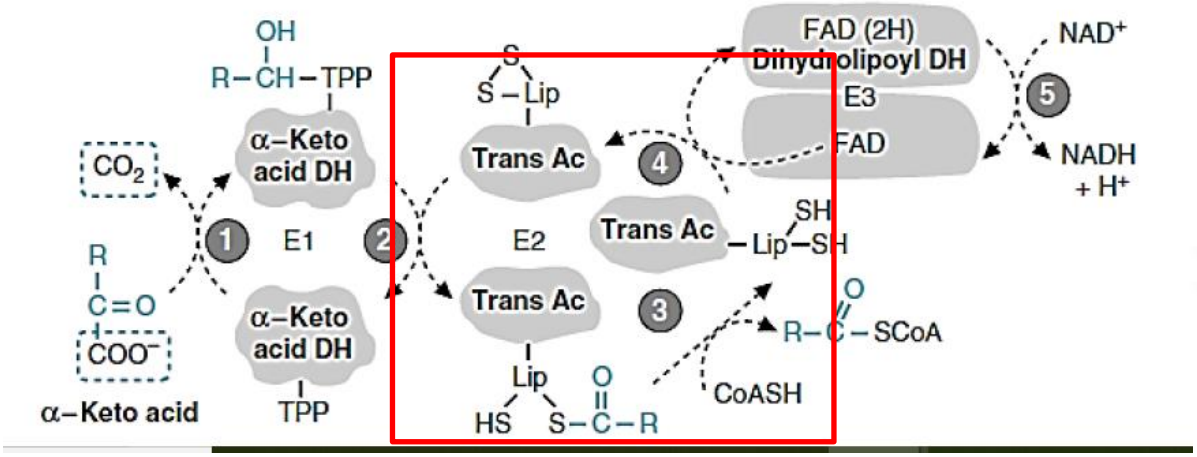
When pyruvate is in the mitochondrial matrix, it is converted to acetyl CoA by the pyruvate dehydrogenase complex (PDH complex), which is a multienzyme complex.

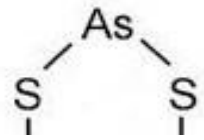
-**Component enzymes**: The PDH complex is a protein aggregate of multiple copies of three enzymes, E1, E2, E3.

-**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).





Bioenergetics of citric acid cycle

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

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

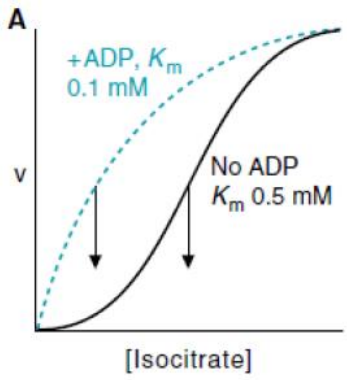
-Actual yield:

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

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

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

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.

-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.

-**Citrate ←→ isocitrate** is a **reversible** reaction.

-The equilibrium **favors citrate**. Citrate concentration is much higher than that of isocitrate (20:1) in equilibrium.

-To shift the reaction to isocitrate's side you need to increase the concentration of citrate so the ratio becomes more than 20:1.

-The body doesn't want reactions that produce energy to proceed easily because that would result in the loss of a lot of energy, so the reaction will proceed faster when energy is needed (increased concentration of citrate).

-The same happens with **malate ←→ oxaloacetate**. Malate concentrations must be kept higher than oxaloacetate concentrations to make the reaction go forward.

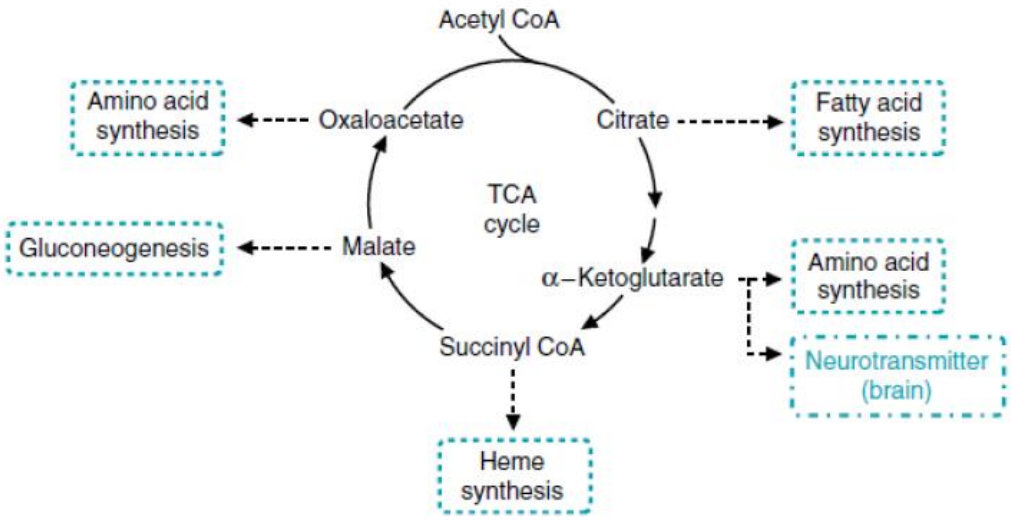
-This is controlled by the *km values* for the enzymes, the enzymes have high km values (low affinity for substrates) so the concentrations need to be high to push the reaction forward.

Example: **malate DH** has a **low affinity** (high km) for malate. So, malate must be in high concentrations to increase the activity of the enzyme.

Citric Acid Cycle Intermediates

The citric acid cycle provides precursors for many biosynthetic pathways.

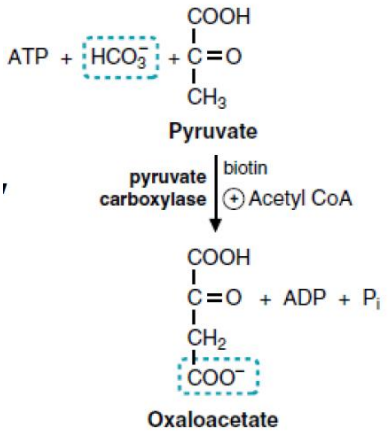
Oxaloacetate (and other keto acids)	-Can be used in amino acid synthesis. -Example: oxaloacetate →aspartate
Citrate	Can leave the mitochondria and be used in **fatty acid** synthesis.
α-Ketoglutarate	-Can be turned to the amino acid glutamate. -Glutamate can function as a **neurotransmitter.** -The inhibitory 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.

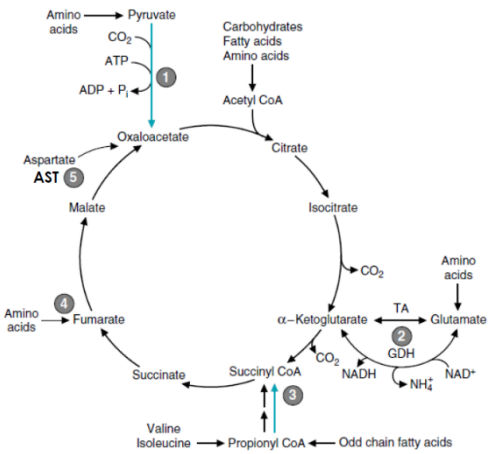
-Pyruvate carboxylase is **activated by acetyl CoA**. That makes sense because acetyl CoA enters the cycle by reacting with oxaloacetate.
High levels of acetyl CoA in mitochondria signal a metabolic state in which the **increased synthesis of oxaloacetate is required.**

-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.

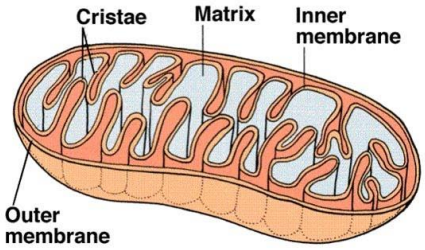


Oxidative Phosphorylation

-Oxidation and phosphorylation are coupled to each other. Phosphorylation is always preceded by oxidation.

The electron transport chain of the mitochondrion

-The mitochondrion contains **an outer and an inner membrane** separated by the **intermembrane space**.



-The ETC is located in the **inner** mitochondrial membrane.

-The outer membrane contains special channels that make it **freely permeable** to most ions and small molecules (up to 5 kDa).

-**The inner membrane** is a specialized structure that is **impermeable** to most small ions, **including protons.** It is **very rich in protein**. **Specialized carriers or transport systems** are required to move ions or molecules across this membrane.

-NAD+ and FAD are reduced in the citric acid cycle and glycolysis to NADH and FADH2. These reduced coenzymes can, in turn, each **donate a pair of electrons** to the electron transport chain.

-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.

-Free energy is linked to reduction potential through this equation $\Delta G^o = -nF\Delta E^o$. **Whenever there is a change in reduction potential energy there will be a free energy change.**

-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.**

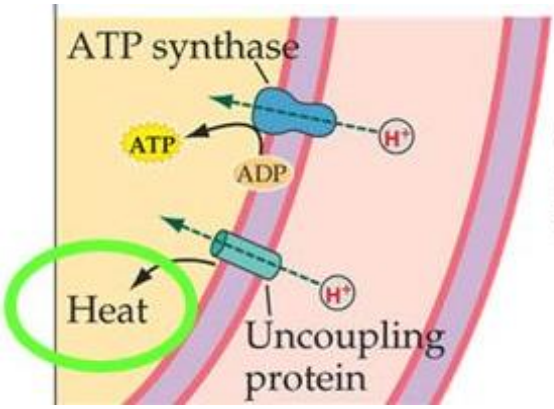
- This process creates an **electrochemical gradient** (with more positive charges on the outside of the membrane than on the inside).
The energy generated by this **proton gradient** is sufficient to **drive ATP synthesis**. Thus, the proton gradient serves as the common intermediate that **couples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

-Pumping of protons stops when the gradient is too large. The gradient will create pressure on the membrane and there will not be sufficient energy to pump any more protons out.

- After protons have been pumped to the intermembrane space, they reenter the matrix (according to the gradient) through an enzyme called **ATP synthase.** This enzyme drives the synthesis of ATP by binding ADP with a phosphate group.

Uncoupling proteins

As their name suggests, they uncouple oxidation and phosphorylation.
Uncoupling proteins occur in the **inner** mitochondrial membrane. These proteins form **channels** that allow protons to reenter the mitochondrial matrix **without energy being captured as ATP**. The energy is released as **heat.**



(4) Back-diffusion of protons in a specialized cell with UCP1

-When the concentration of uncoupling proteins increases, more heat is generated. Uncoupling proteins are found in large concentrations of mitochondria of **brown adipose tissue.**

-Genetic defects in uncoupling proteins lead to more ATP being available. This causes **obesity** due to decreased rates of metabolism.

Good Luck