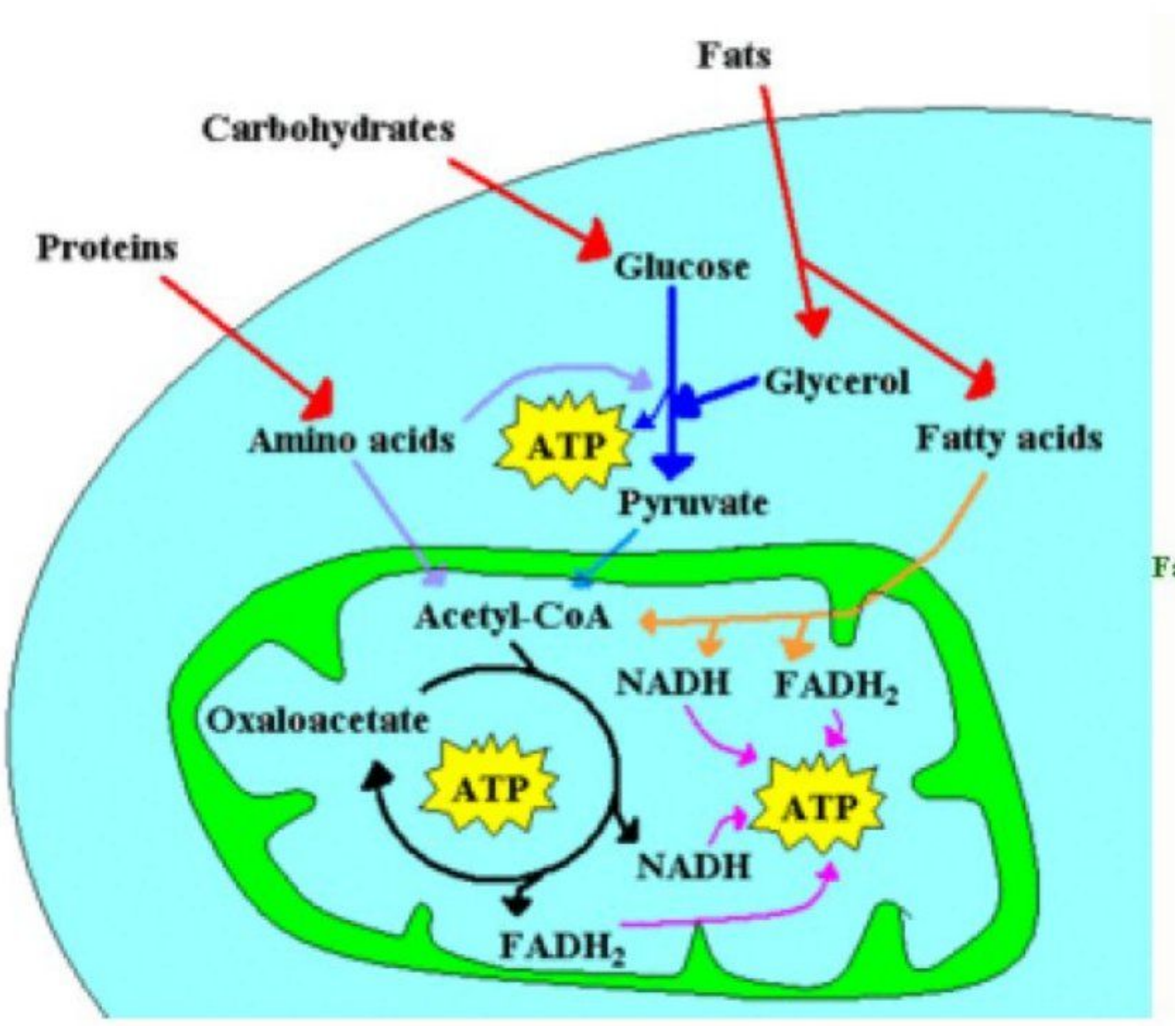
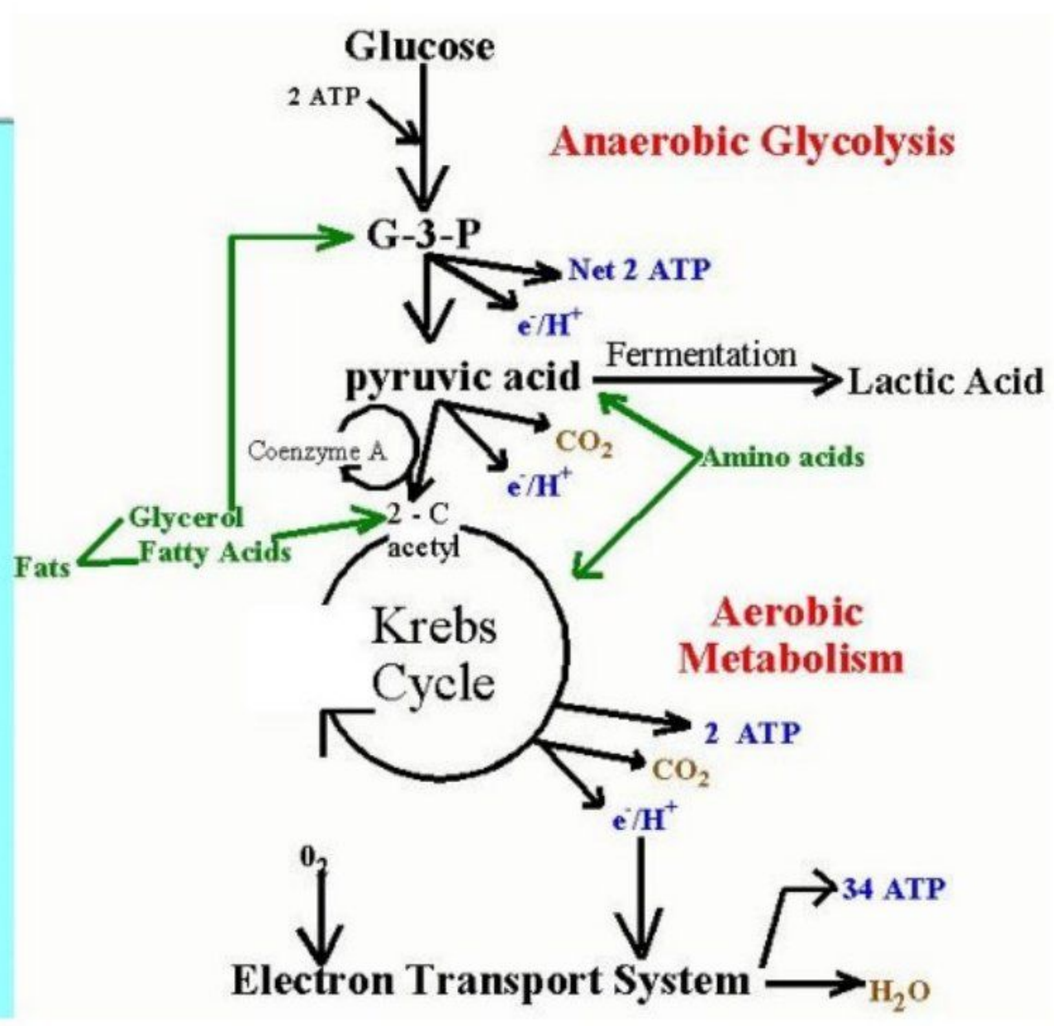
(KREBS, CITRIC ACID OR TCA) CYCLE

HOW DOES IT FIT?

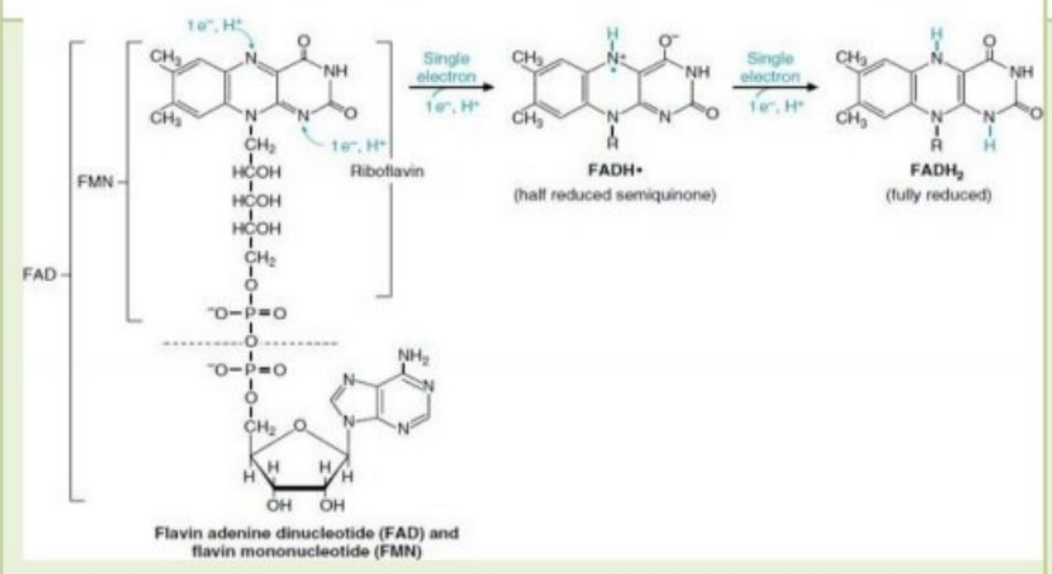




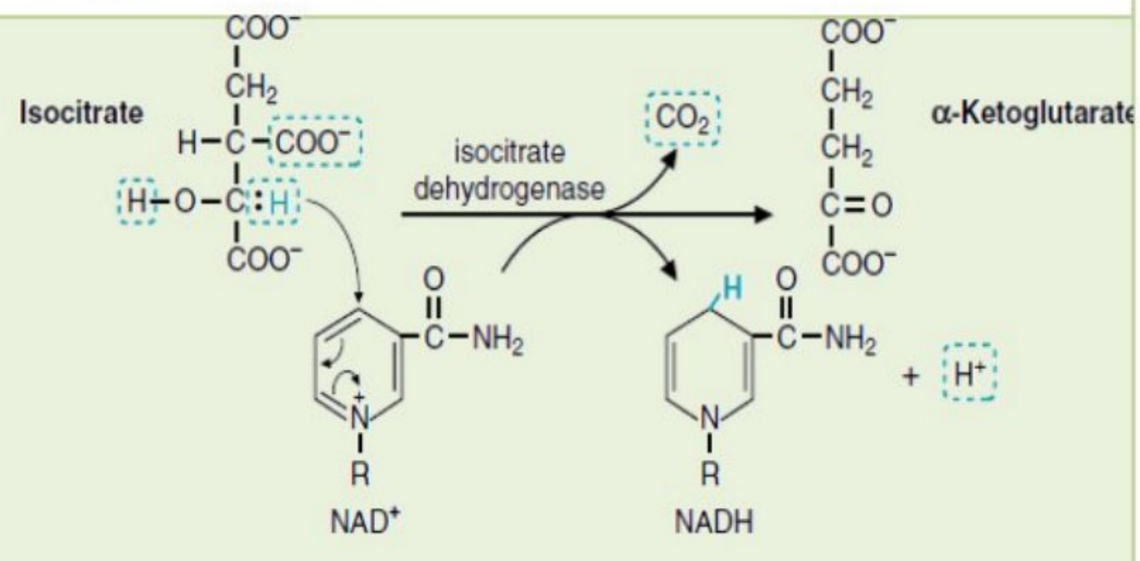
- It's named Krebs for the scientist's name, citric acid cycle because of the intermediate, and TCA (tricarboxylic acid) because citric acid has three carboxyl groups.
- All metabolic processes have in their pathways acetyl CoA, it is a product of fats, carbohydrates, and proteins degradation.
- Acetyl CoA can be used, in addition to Krebs cycle, in making many structures (for e,g. fatty acids, etc..).

ELECTRON (ENERGY) CARRYING MOLECULES (NAD^+ AND FAD)

FAD	NAD^+
Single electrons ($H^\bullet$), different sources	**Pair of electrons (H^-), same source**
Succinate to fumarate, lipoate to lipoate disulfide in α-KG (will be clarified soon).	**Alcohols to ketones by malate dehydrogenase & isocitrate dehydrogenase** (will be clarified soon).
FAD must remain tightly, sometimes covalently, attached to its enzyme.	**NADH plays a regulatory role in balancing**
***E*° for enzyme-bound FAD varies**	**Energy metabolism**



Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)



- **Always involve <u>a pair</u> of chemicals: an electron donor and an electron acceptor (food vs. NAD^+).**
- **NAD^+ vs. FAD**
- **NAD^+ vs. $NADP^+$ (fatty acid synthesis and detoxification reactions).**
- Look at the pictures on the next page, the picture on the right is for NAD^+ and the other one is for FAD.

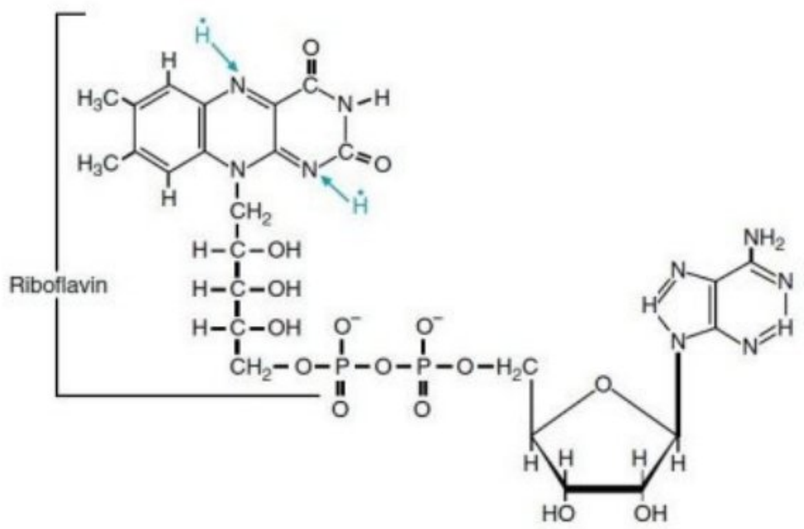
- NAD^+ loses its two electrons at the same time, but FAD can lose them in a sequential manner (one by one), so it passes through free radical state. So, FAD^+ can't exist in solutions by itself, it needs to be covered, and this makes perfect sense, because it can form a free radical which is dangerous. Heme also normally exists inside a protein.

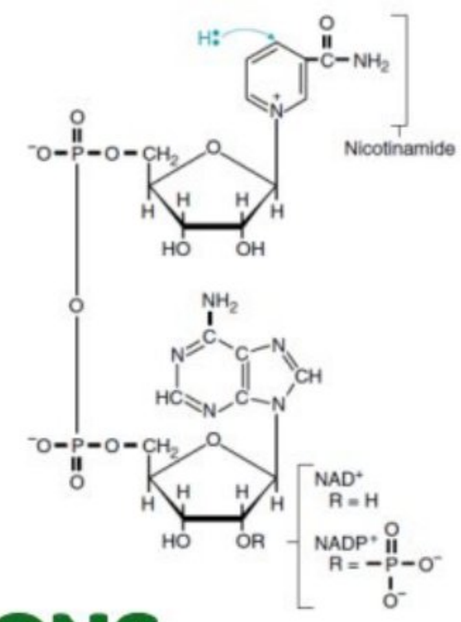
- Both FAD^+ and heme don't have constant reduction potential.

- $NADP^+$ differs from NAD^+ in having an extra phosphate group and regulation (where to use), they can do the same job anyways.

- $\Delta E°$ for NADH and O_2 is more than $\Delta E°$ for $FADH_2$ and O_2. Therefore, $\Delta G°$ and energy from NADH when it loses its two electrons is more than that of $FADH_2$.

- FMN (flavin mononucleotide) differs from FAD only in not having adenine.



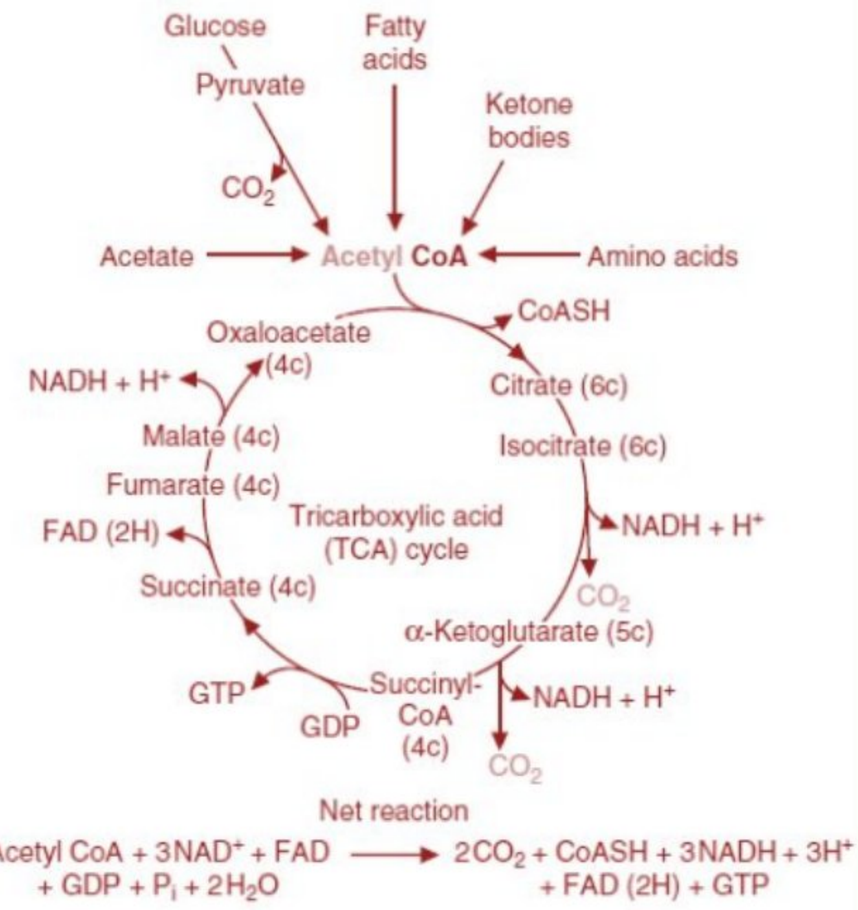


COMPONENTS & STEPWISE REACTIONS

- **No introduced O_2, two CO_2 exit.**

- It has 8 reactions,
  8 intermediates and 8 enzymes. All of them are for memorizing.

- Citric acid (the first molecule in the cycle) has 6 carbons, the last molecule in the cycle is oxaloacetate which has 4 carbons. So, obviously, there are two carbons lost in the cycle as two CO_2 molecules.



- The cycle has two parts:
  - The first part (from citrate to succinyl CoA): responsible for getting rid of the two carbons as CO_2.
  - The second part (from succinyl CoA to oxaloacetate): responsible for regeneration of oxaloacetate.

- **CIA Sent Soldiers For My Office (Citrate, isocitrate, α-KG, succinyl CoA, succinate, fumarate, malate, and oxaloacetate). You can use this mnemonic to memorize the structures in it.**

- Side note: don't forget to look at the picture in the previous page when reading each step.

STEPS OF KREBS CYCLE

1. Acetyl CoA (2C) + oxaloacetate (4C) → citrate (6C)

- There is bond formation here, so the reaction needs energy that comes from breaking acetyl CoA down.
- Enzyme: citrate synthase.
- Remember, citrate has three carboxyl groups.

2. Citrate (6C) → isocitrate (6C)

- **Why to make Isocitrate from citrate?**

The goal of this cycle is to produce energy, and as we said before, oxidation of food produces energy. Now, because citrate has a tertiary alcohol group, it can't be oxidized unless it's converted to its secondary form (isocitrate).

- **3° to 2° alcohol.**
- Enzyme: aconitase.

3. isocitrate (6C) → α-ketoglutarate (5C)

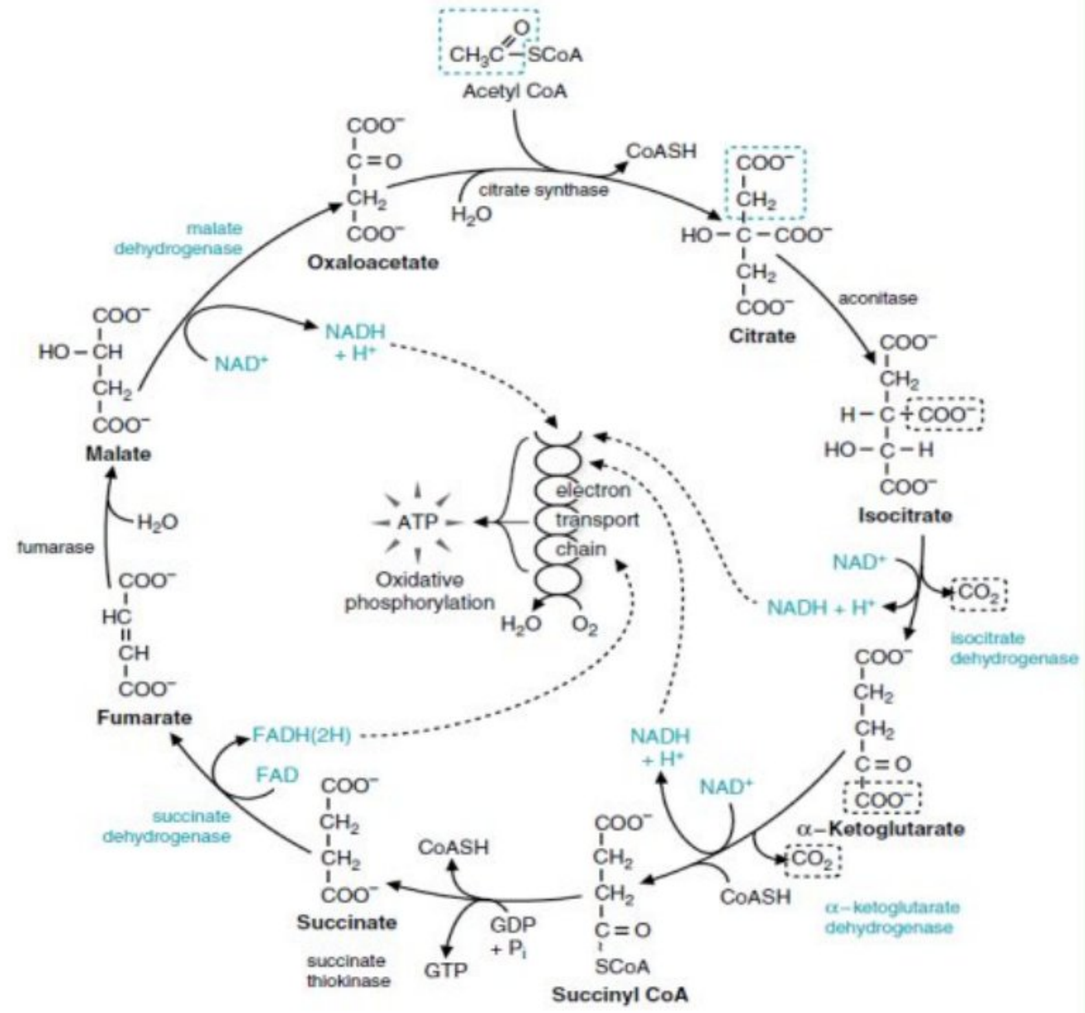
- **Oxidative decarboxylation, CO_2.**
- **Where does the CO_2 exit?**

The doctor didn't answer this question but google says "waste gas".

- Gluta means five.
- Enzyme: isocitrate dehydrogenase.
- Side note from me: wherever there is a dehydrogenase enzyme in this cycle, there will be NADH or $FADH_2$ produced, because there is a hydrogen that is removed from the structure.
- Side note from me: isocitrate dehydrogenase is a complex enzyme that has two functions; decarboxylation and dehydrogenation.
- CO_2 and NADH are produced

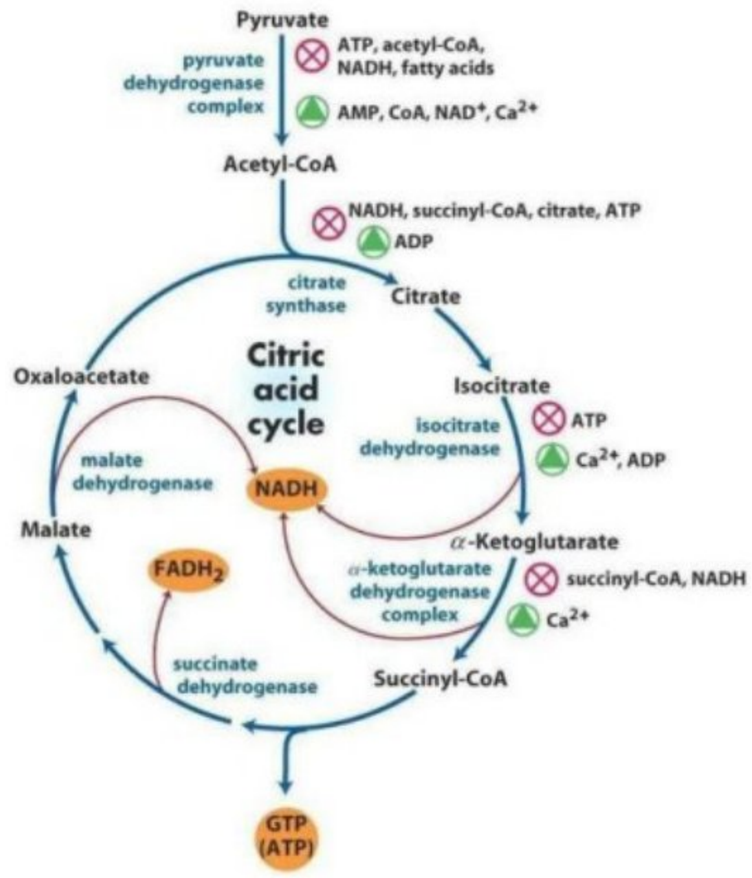
4. α-ketoglutarate (5C) → succinyl CoA (4C)

- **Oxidative decarboxylation.**
- **Thiamine pyrophosphate, lipoic acid, and FAD.**
- **Keto group oxidized to acid, CoA-SH, succinyl CoA.**
- **Energy conserved as NADH, thioester bond.**
- **The only irreversible step in the whole reaction cycle.**



- The mechanism of this reaction will be totally clarified in the next lecture.
- Enzyme: α-ketoglutarate dehydrogenase (a complex enzyme too).
- The lost carboxyl group here is terminal, the compound will have a free terminal carbonyl group, so the molecule is unstable. That's why it binds to CoA.

- This picture is in the slides, the doctor didn't talk about it, it is about regulation of the cycle's enzymes.



أعيذُها نظراتٍ منكَ صادقة أن تَحْسبَ الشَّحمَ فيمَن شَحْمُهُ وَرَمُ

سيَعلَمُ الجمعُ ممَّن ضمَّ مَجلسُنا بأنَّني خيرُ مَن تسعى بهِ قَدَمُ

CONTINUING WITH THE REACTIONS:

The second half of the cycle is engaged in rearranging the 4C molecule to make it mimics what we started with (Oxaloacetate).

(recap the first four reactions from the previous sheet)

5- Succinyl coA → succinate (4C):

The high-energy thioester bond of succinyl coA is cleaved by the enzyme succinate thiokinase (also called succinyl coA synthetase). The reaction produces energy and is coupled to phosphorylation of GDP to GTP (the reaction is called substrate-level phosphorylation). GTP and ATP are in equilibrium (another example of substrate-level phosphorylation).

6- Succinate → Fumarate (oxidized):

The major difference between Succinate and Oxaloacetate is the keto group (we can go from alkane to ketone by converting the alkane into alkene→ addition of water (forming a secondary alcohol) → oxidation of the secondary alcohol, which is basically the remaining steps of the cycle). Succinate is oxidized to fumarate by succinate dehydrogenase (also called succinase) (the reaction is removing hydrogen, so we need a dehydrogenase). The two hydrogens are removed from two neighbor carbons, so the coenzyme (electron acceptor) should be FAD producing $FADH_2$, the only $FADH_2$ produced from the cycle.

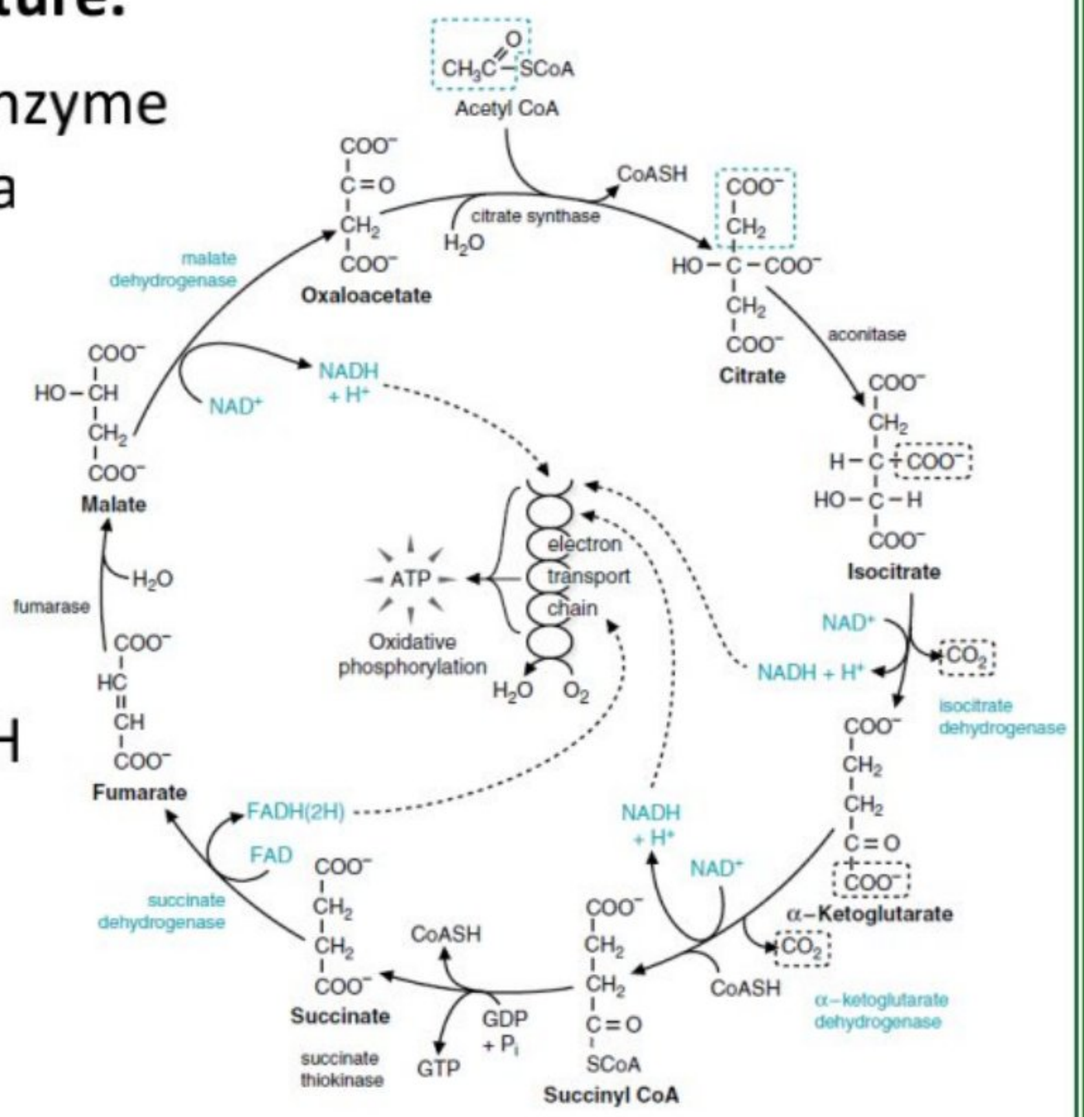
7- Fumarate → malate (hydration):

Fumarate has a double bond in its structure.

Fumarate is hydrated to malate by the enzyme fumarase (fumarate hydratase) forming a secondary alcohol.

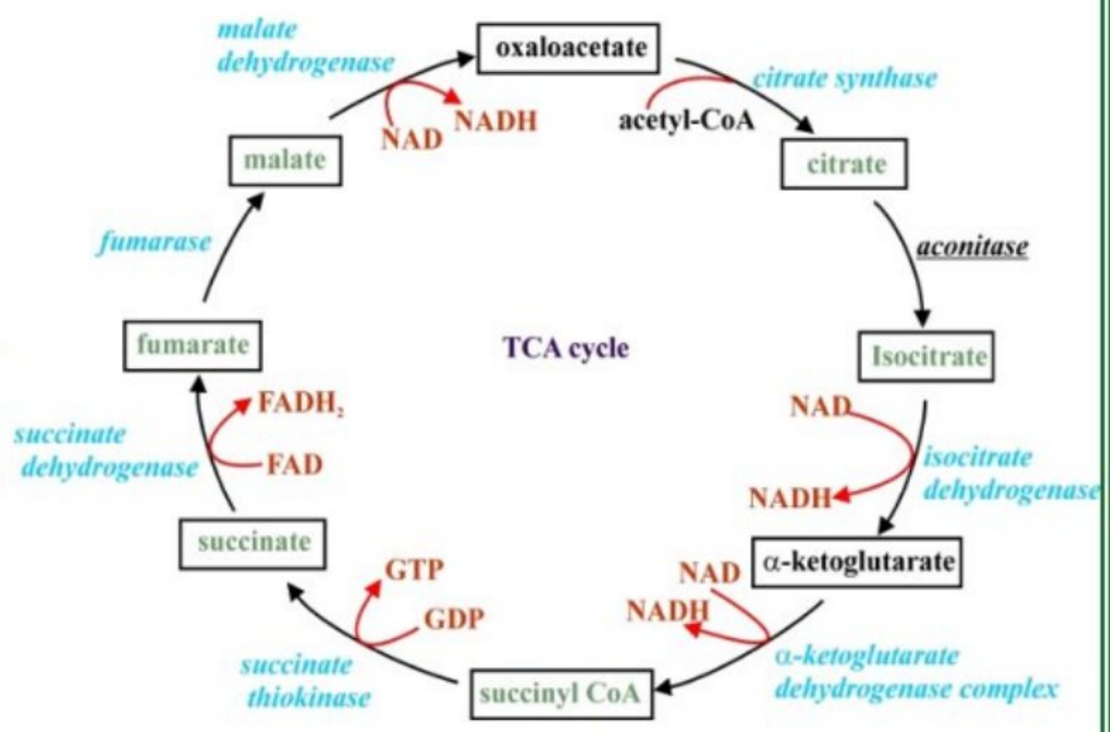
8- Malate → oxaloacetate (oxidized):

The secondary alcohol of malate is oxidized forming a ketone (oxaloacetate) by malate dehydrogenase. The third and final NADH is produced from this reaction.



ENZYMES OF THE TCA CYCLE:

1. Citrate synthase.
2. Acotinase.
3. Isocitrate dehydrogenase.
4. α -ketoglutarate dehydrogenase.
5. Succinate thiokinase.
6. Succinate dehydrogenase.
7. Fumarase.
8. Malate dehydrogenase



❖ **What drives the reaction forward?**

FORMATION OF CITRATE

➢ **What drives the reaction forward?**

Hydrolysis of acetyl CoA.

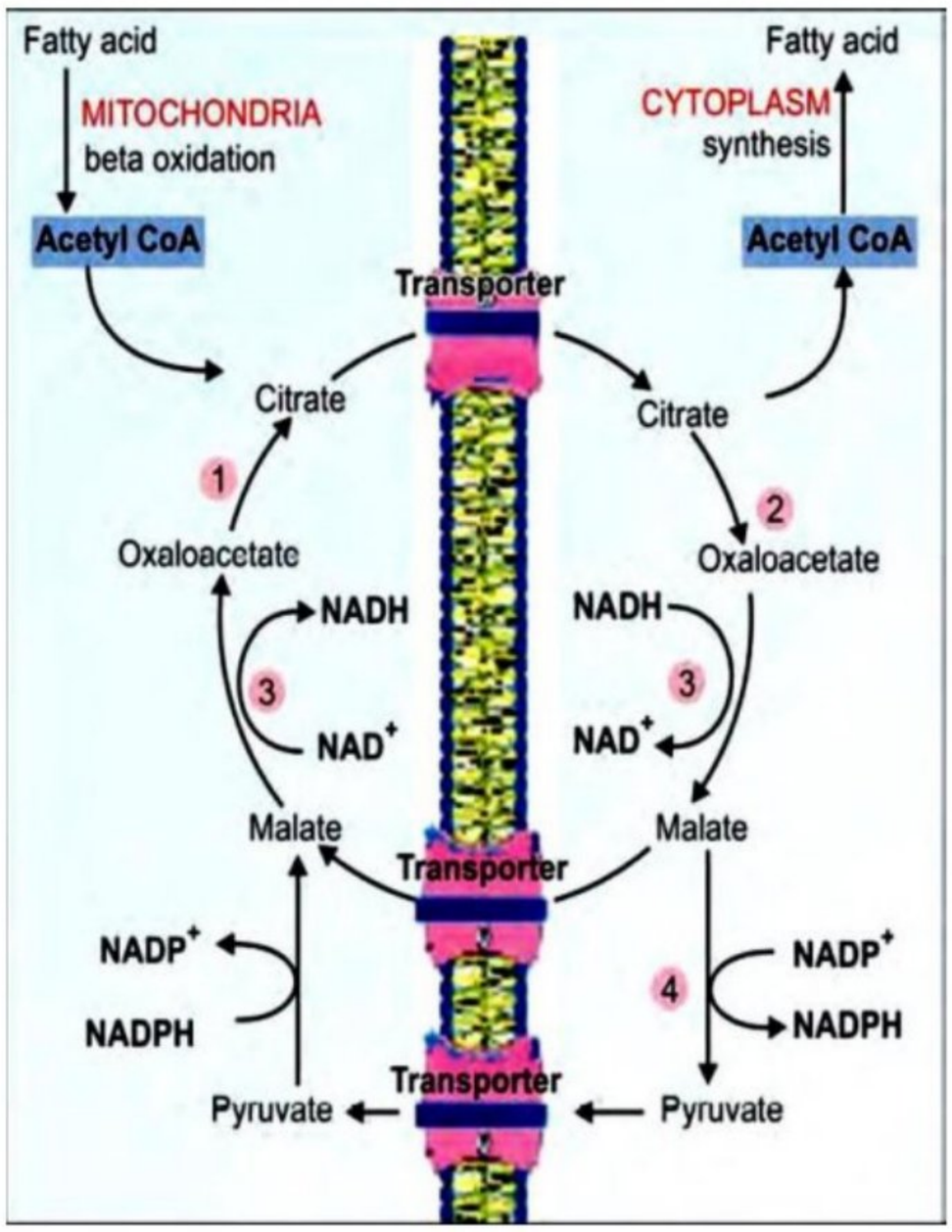
➢ **Is it reversible or irreversible?**

Irreversible.

➢ **Can it be reversed?**

Yes, by ATP-Citrate lyase or ATP-Citratase outside Krebs cycle (it cannot be reversed inside the cycle itself, why?

→hydrolysis of coenzyme A gives a huge amount of energy (approximately 13kcal/mol) with comparison to ATP, so it can't be reversed within the cycle).

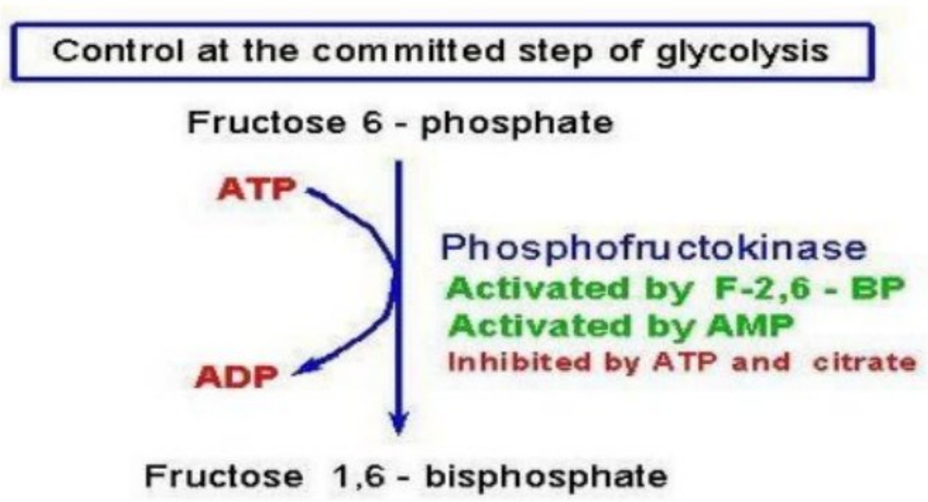


FORMATION AND OXIDATION OF ISOCITRATE

➢ **Oxidative decarboxylation.**

➢ **3^0 alcohol to 2^0 alcohol.**

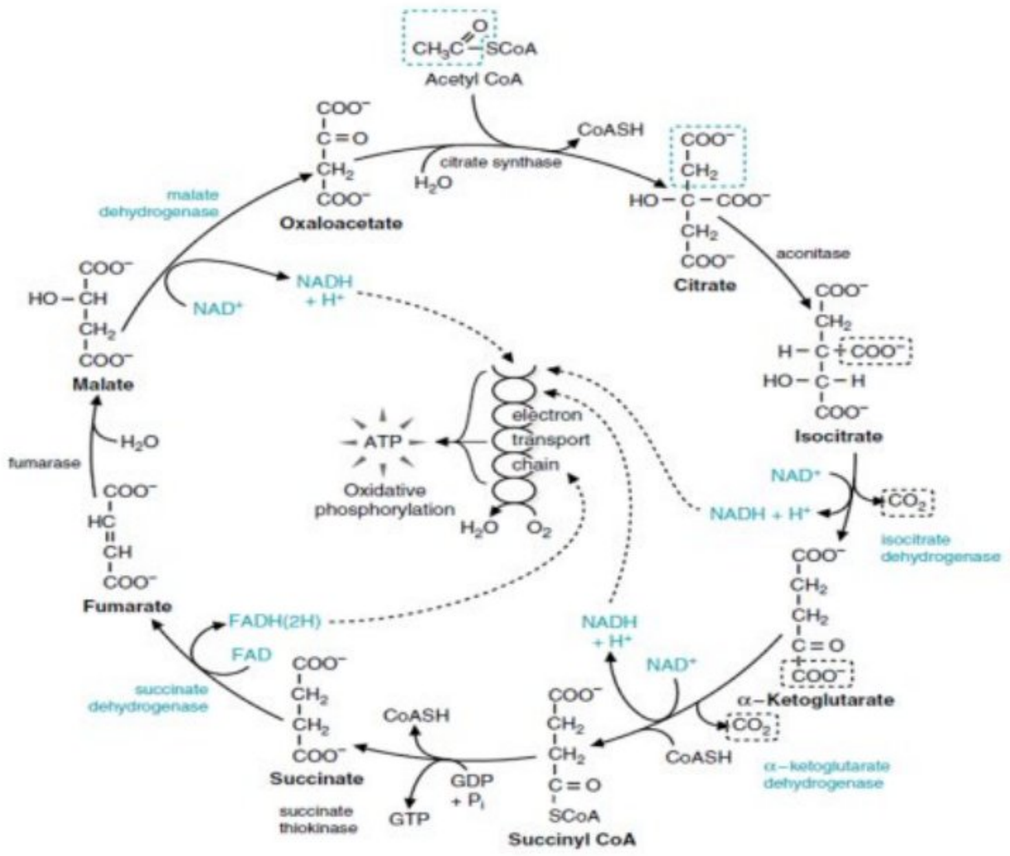
➢ Citrate can exit the mitochondrial matrix to the cytosol and control the rate-limiting step of glycolysis.



- The rate-limiting step of glycolysis is the one that converts Fructose-6-phosphate into fructose-1,6-biphosphate.
- This step is inhibited by the high concentrations of ATP and citrate, which makes a lot of sense!
  High ATP and citrate concentrations indicates that we don't need more glycolysis to happen and produce pyruvate and citrate.

ALPHA-KETOGLUTURATE TO SUCCINYL COA

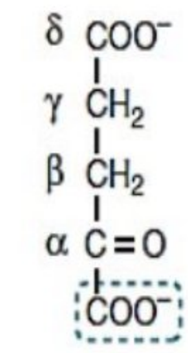
- **Oxidative decarboxylation.**
- **Thiamine pyrophosphate, lipoic acid, and FAD.**
- **Keto group oxidized to acid, CoASH, succinyl CoA.**
- **Energy conserved as NADH, thioester bond.**
- **The only irreversible step in the whole reaction cycle (there is none enzymes that can reverse this reaction inside the body).**



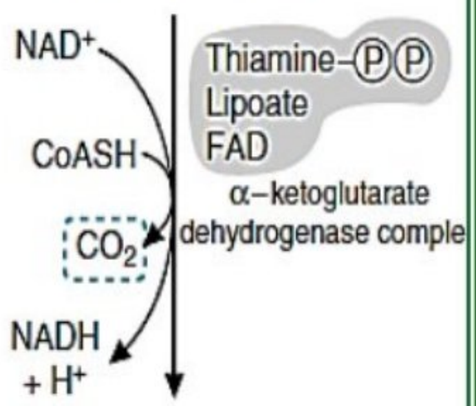
A-KETOACID DEHYDROGENASE COMPLEXES (TLFCN; COENZYMES)

- **(α-ketoglutarate, pyruvate, and branched chain α-ketoacid) dehydrogenase complexes (a complex enzyme: contains more than one enzyme in its structure).**

  Remember: one way of enzyme regulation is to complex one enzyme whose product is the substrate of the second one.

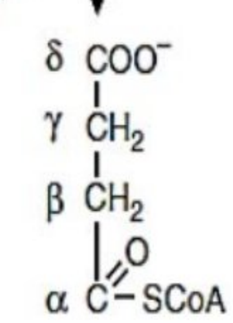


α–Ketoglutarate



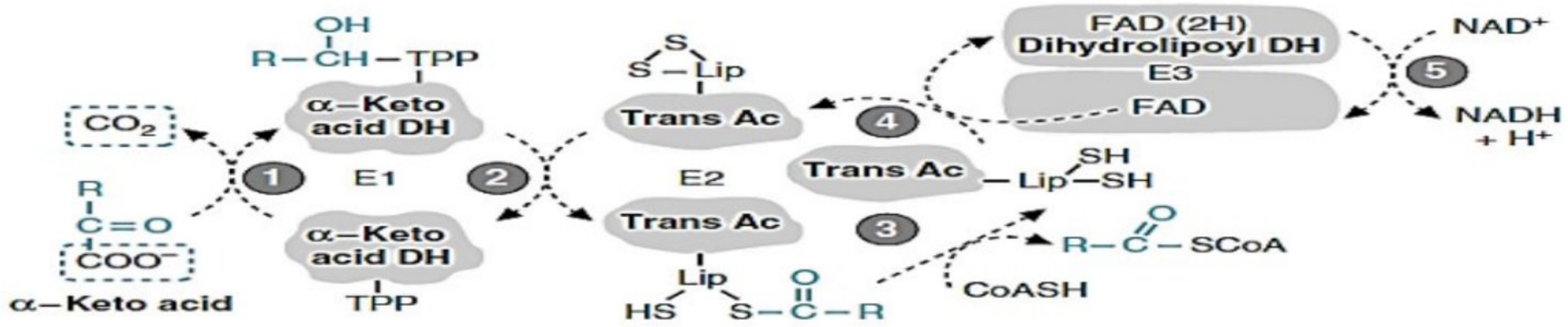
- **Huge enzyme complexes, multiple subunits of 3 different enzymes (no loss of energy, substrates for E2 and E3 remain bound→ higher rate).**

  The dehydrogenase converted α-ketoglutarate into succinyl coA, which includes: decarboxylation (lyase reaction), addition of coA which is an acyl carrier group (transacylase), production of NADH (dehydrogenase reaction).



Succinyl CoA

- **E1, E2, & E3 are a decarboxylase (coenzyme: TPP), a transacylase (coenzyme: lipoate), & a dehydrogenase (coenzyme: FAD).**



E1: α-ketoglutarate

The enzyme is a decarboxylase which needs TPP (thiamine(B1) +PP) as a coenzyme. TPP attacks the carbonyl group releasing the terminal carboxylic group. The carbons are now loaded on TPP, enzyme must return to its original structure, so it hands the carbons to the next enzyme (transacylase). CO_2 is released.

E2:

Transacylase needs lipoate (contain 2 sulfur atoms that are joined by a disulfide bond) as a coenzyme. The disulfide bond is broken down forming a thiol group and the other sulfur is bound to the carbons. Again, it is not the original form of the enzyme, so, the carbons are handled to coA leaving the sulfur atom unbound and reactive (where an H^+ binds and forms another thiol). The FAD accepts the two hydrogens to reform the disulfide bond (FAD is used because the 2H come from different atoms).

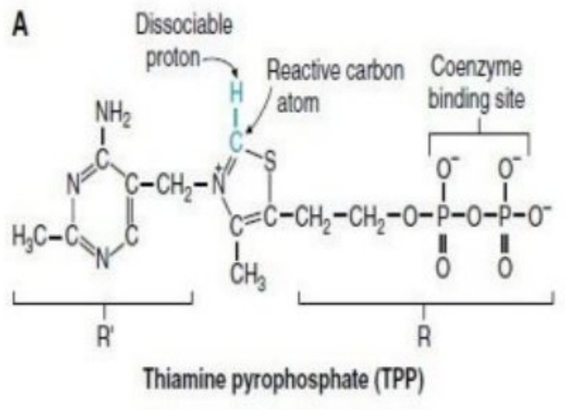
E3:

Dihydrolipoyl DH with its coenzyme $FADH_2$ (that accepted the 2H) transfers hydride ion to NAD^+ and H^+ to the solution, consequently, reattains its original form.

→this mechanism applies to α-ketoacids (the same exact reaction happens in pyruvate conversion with minor differences).
→5 coenzymes are involved in this conversion, 3 of them are complexed with the enzyme complex.

THIAMINE PYROPHOSPHATE

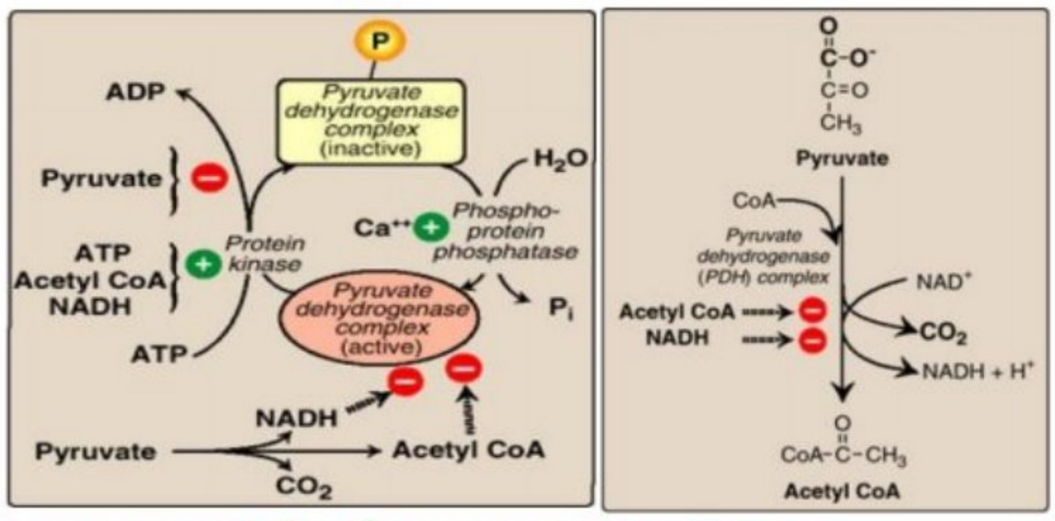
- Thiamine (vitamin B1) is important for the action of α-ketoacid dehydrogenases as TPP.
- **Thiamine deficiency, α-ketoglutarate, pyruvate, & branched chain α-keto acids accumulate in the blood.**



Thiamine pyrophosphate (TPP)

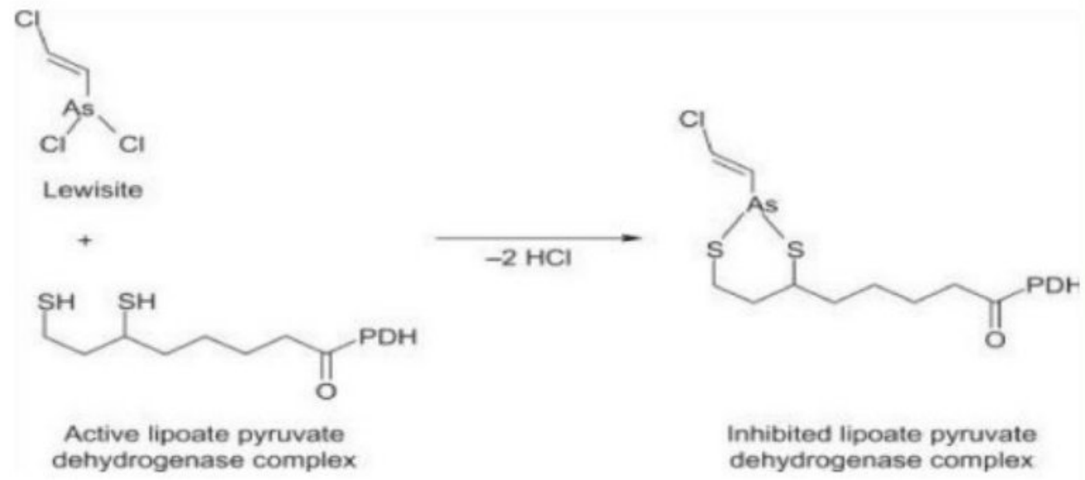
OXIDATIVE DECARBOXYLATION OF PYRUVATE

- Component enzymes.
- Coenzymes.
- Regulation of the pyruvate dehydrogenase complex.
- Pyruvate dehydrogenase deficiency: A deficiency in E1 component is the most common biochemical cause of congenital lactic acidosis (X-linked, no treatment).



- Mechanism of arsenic poisoning.

Arsenic binds the 2 sulfurs of lipoic acid, breaks the disulfide bond and inhibits its action.



GENERATION OF GTP

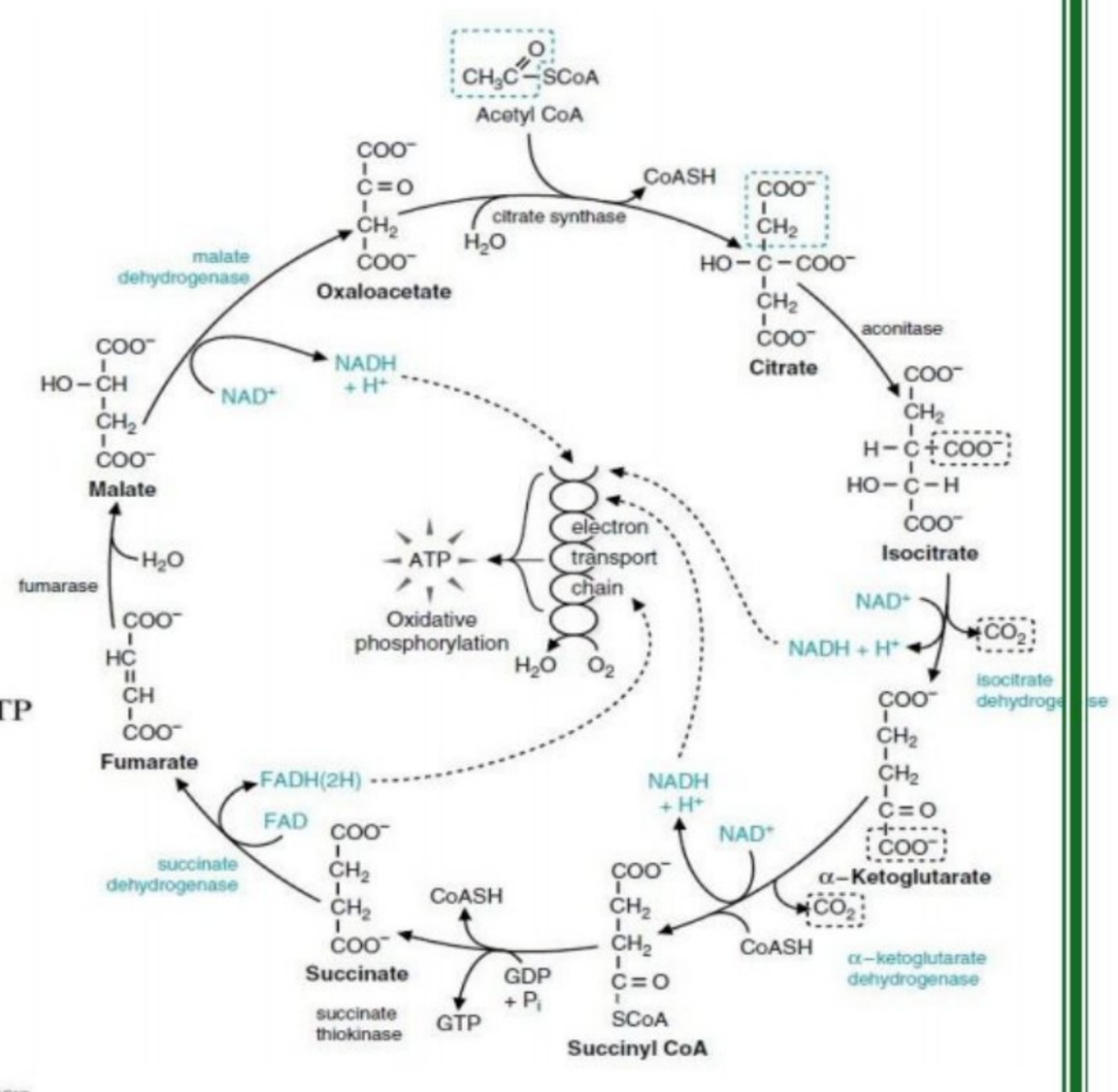
- Succinyl CoA thioester bond, succinate thiokinase, substrate level phosphorylation.
- GTP +ADP ↔ GDP + ATP

Side note: what applies to ATP applies to GTP.

1,3-Diphoglycerate + ADP ⇌ (Phosphoglycerate kinase) 3-Phophoglycerate + ATP

Phosphoenolpyruvate + ADP ⇌ (Pyruvatekinase) Pyruvate + ATP

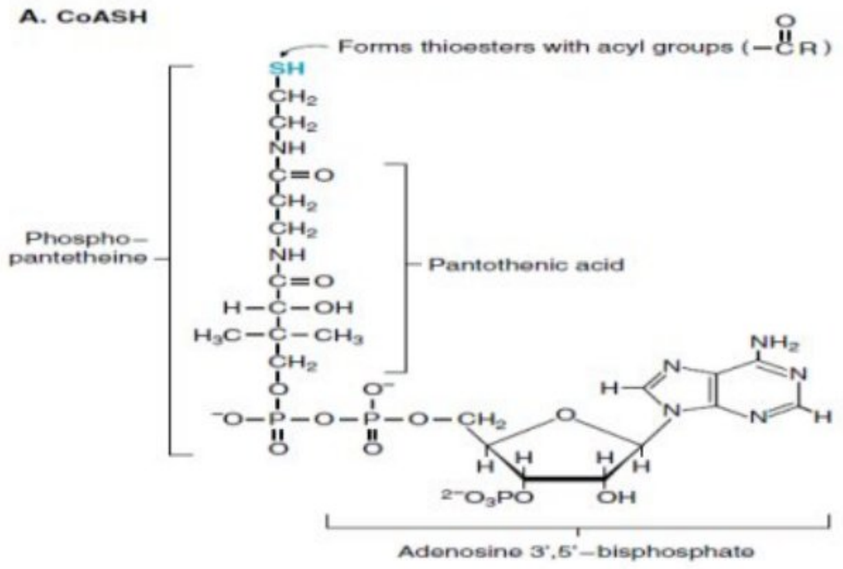
SuccinylCoA +GDP ⇌ (SuccinylCoA Synthase) Succinate +GTP

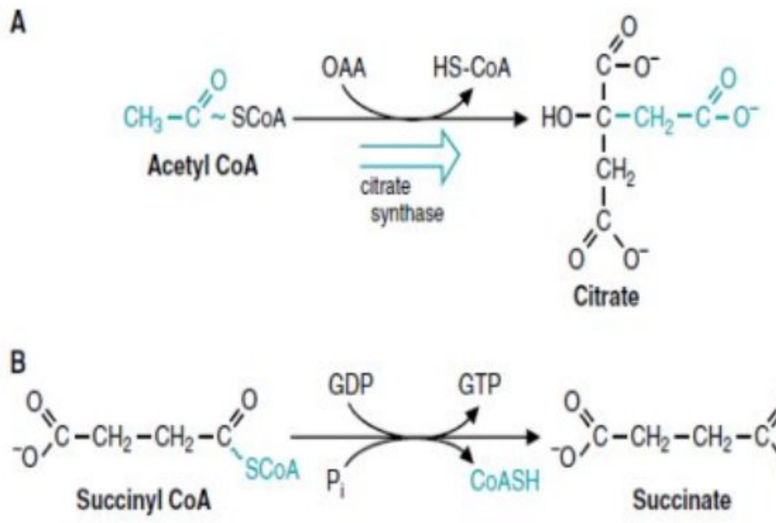


COA

- 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).

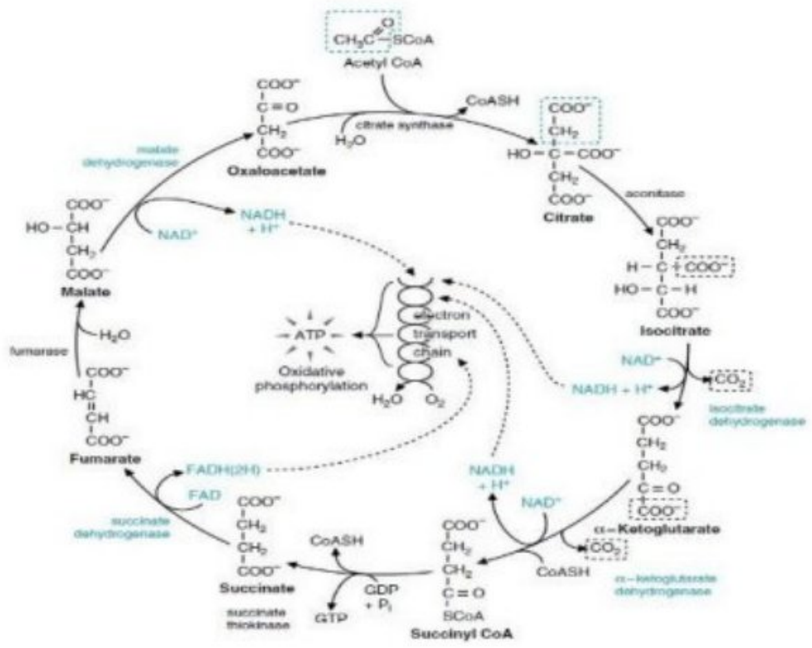
It is known for having a high energy value.





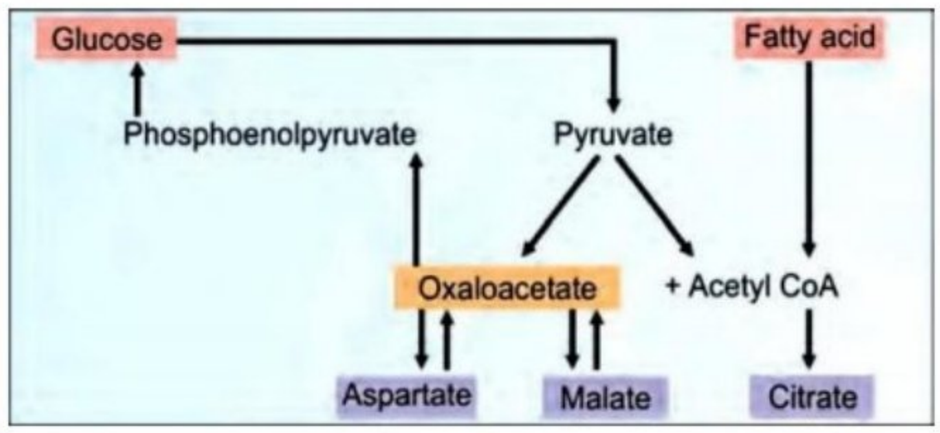
OXIDATION OF SUCCINATE TO OXALOACETATE

- 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.



OXALOACETATE AS A JUNCTION POINT

- Viewed as a <u>catalyst.</u>
- An important junction point in metabolism.



Oxaloacetate is an important molecule in metabolic pathways, follow up:

*It binds with the acetate part of acetyl CoA and finally gets it out of the cycle as CO2 and gets regenerated, wait a minute, binds, works then being regenerated?

That is what an enzyme does, even though oxaloacetate is certainly not an enzyme, it is viewed as a catalyst.

*As you can see in the figure, you can synthesize or break down glucose building on oxaloacetate, that's why we call it a junction point.
*And in the biochemistry course, which I can assure you enjoyed, we discussed that we can get Aspartate from oxaloacetate through transamination.
*Reduction of oxaloacetate results in malate.

BIOENERGETICS OF TCA CYCLE

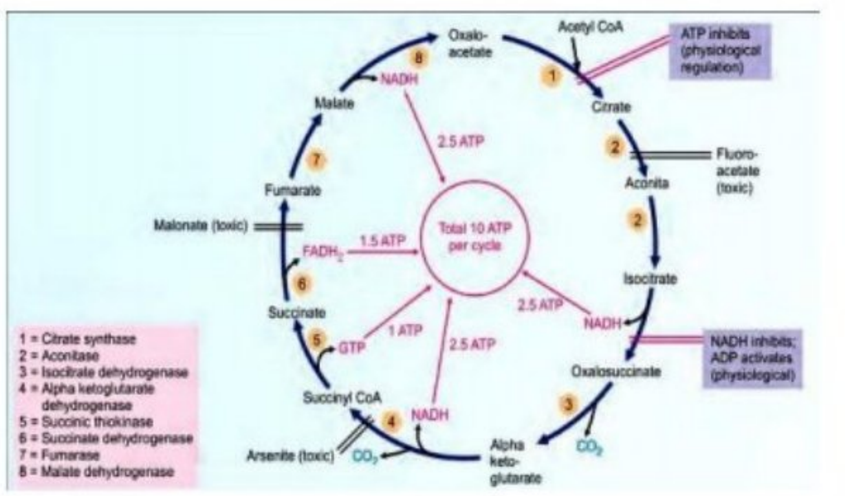
- Like all pathways, overall net -ΔG (-228 kcal/mole), not 100%.
- **3** NADH, FAD(H2), and GTP (10ATP), 207 Kcal, 90%.
- Three reactions have large (-ve) values. (**Which restricts the movement in only one direction).**
- Physiologically irreversible, low products.

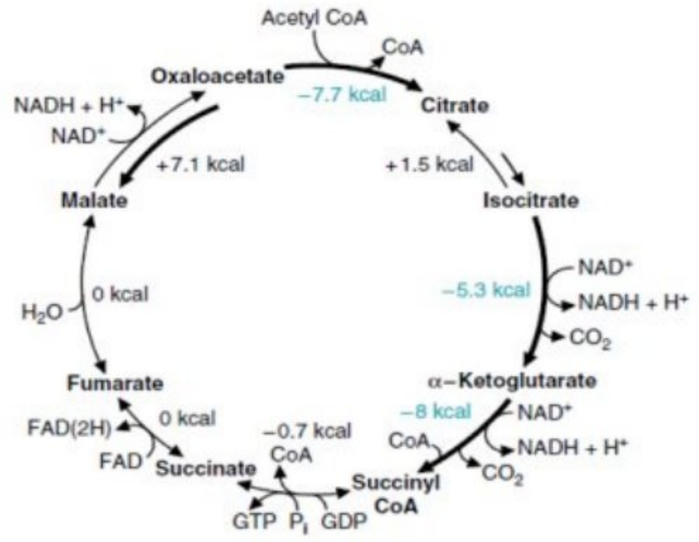
TABLE 19.1: ATP generation steps

Step No	*Reactions*	*Co-enzyme*	*ATPs (old-calculation)*	*ATPs (new calculation)*
3	Isocitrate → alpha keto glutarate	NADH	3	2.5
4	Alpha keto glutarate → succinyl CoA	NADH	3	2.5
5	Succinyl CoA→Succinate	GTP	1	1
6	Succinate → Fumarate	$FADH_2$	2	1.5
8	Malate → Oxalo acetate	NADH	3	2.5
		Total	12	10

Ponder the figure, for god's sake.

there are old calculations and new ones, memorize both just in case.





The concept of 2.5 ATP molecules is cursed, to be clearer just say that 2 NADH molecules give 5 ATP molecules.

Important: to calculate the efficiency of the cycle, we must divide the actual result on the expected one, 207 kcal (actual)/ 228 kcal (expected)= 90%, and this is the most efficient machine in the world.

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

Side note: O2 needs 4 e- to be fully reduced to water (a note to make the halves concept clearer, there is no ½ O2 that accepts 2 e- as we have been taught).

NET RESULT OF THE CYCLE AND IT'S SIGNIFICANCE

Fats are burned in the fire of carbohydrates. **Acetyl CoA (lipid-based ones) can never be turned into CO2 without the presence of oxaloacetate (carb based).**

Fat cannot be converted to glucose because pyruvate dehydrogenase reaction is an absolutely irreversible step.

But excess carbs turn into acetyl CoA, molecules of acetyl CoA join forming fatty acids.

Box 19.1: Significance of citric acid cycle

1. Complete oxidation of acetyl CoA
2. ATP generation
3. Final common oxidative pathway
4. Integration of major metabolic pathways
5. Fat is burned on the wick of carbohydrates
6. Excess carbohydrates are converted as neutral fat
7. No net synthesis of carbohydrates from fat
8. Carbon skeletons of amino acids finally enter the citric acid cycle
9. Amphibolic pathway
10. Anaplerotic role.

TABLE 19.2: Stoichiometry of the TCA cycle

Acetyl CoA	→	$2\ CO_2$ +CoA-SH
Oxaloacetate		Oxaloacetate
FAD		$FADH_2$
$3NAD^+$		3 NADH
GDP + PI GTP		

*Amphibolic: not anabolic, not catabolic but both.

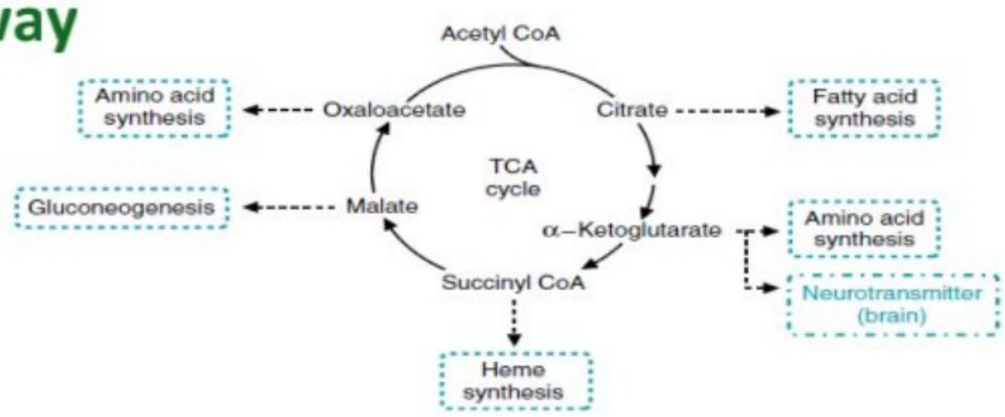
Don't ignore those 10 points, please.

***in table 19.2, GTP must be on the products side, be careful.**

TCA CYCLE INTERMEDIATES

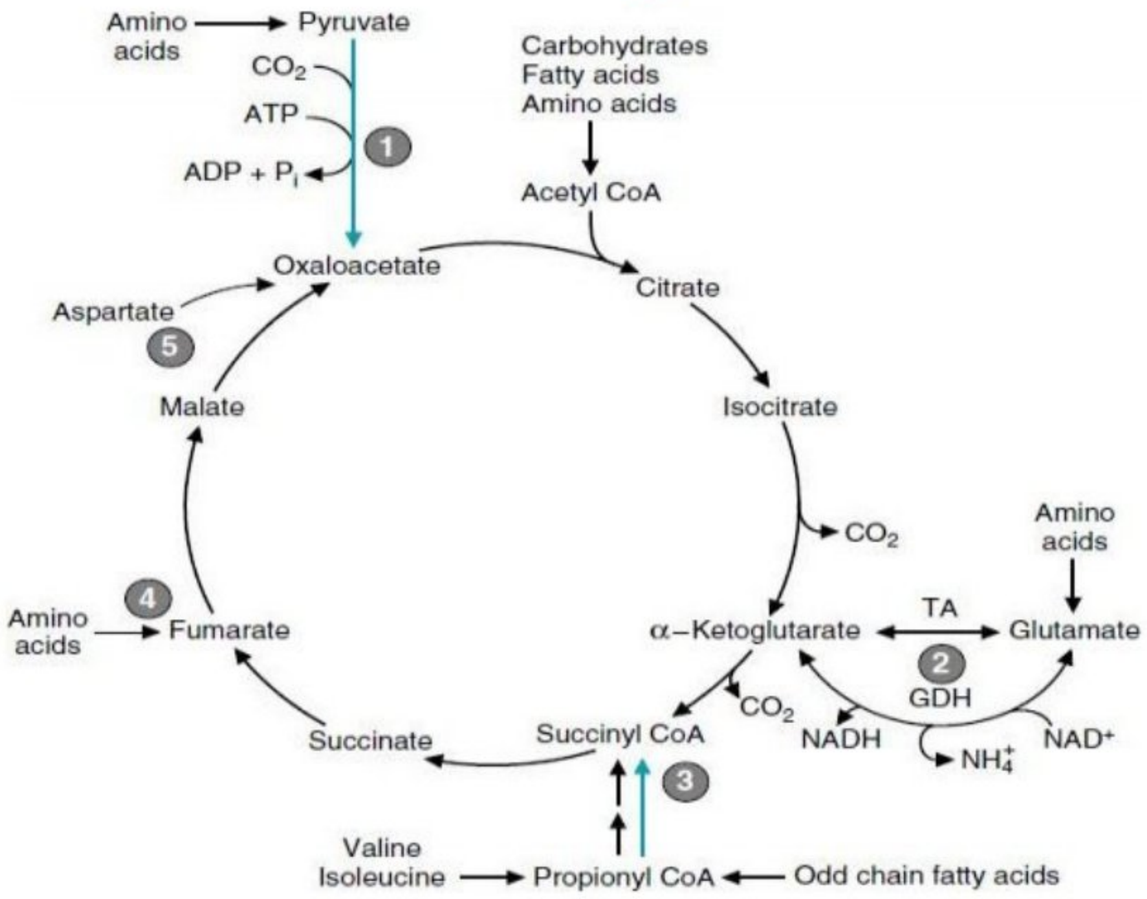
- **Intermediates are Precursors for Biosynthetic Pathways (citrate, acetyl CoA, fatty acid synthesis, liver) (fasting, malate, gluconeogenesis, liver) (Succinyl CoA, heme biosynthesis, bone marrow) (α-ketoglutarate, glutamate, GABA, a neurotransmitter, brain) (α-ketoglutarate, glutamine, skeletal muscle to other tissues for protein synthesis) and the pathway oxaloacetate → aspartate applies to the last ().**

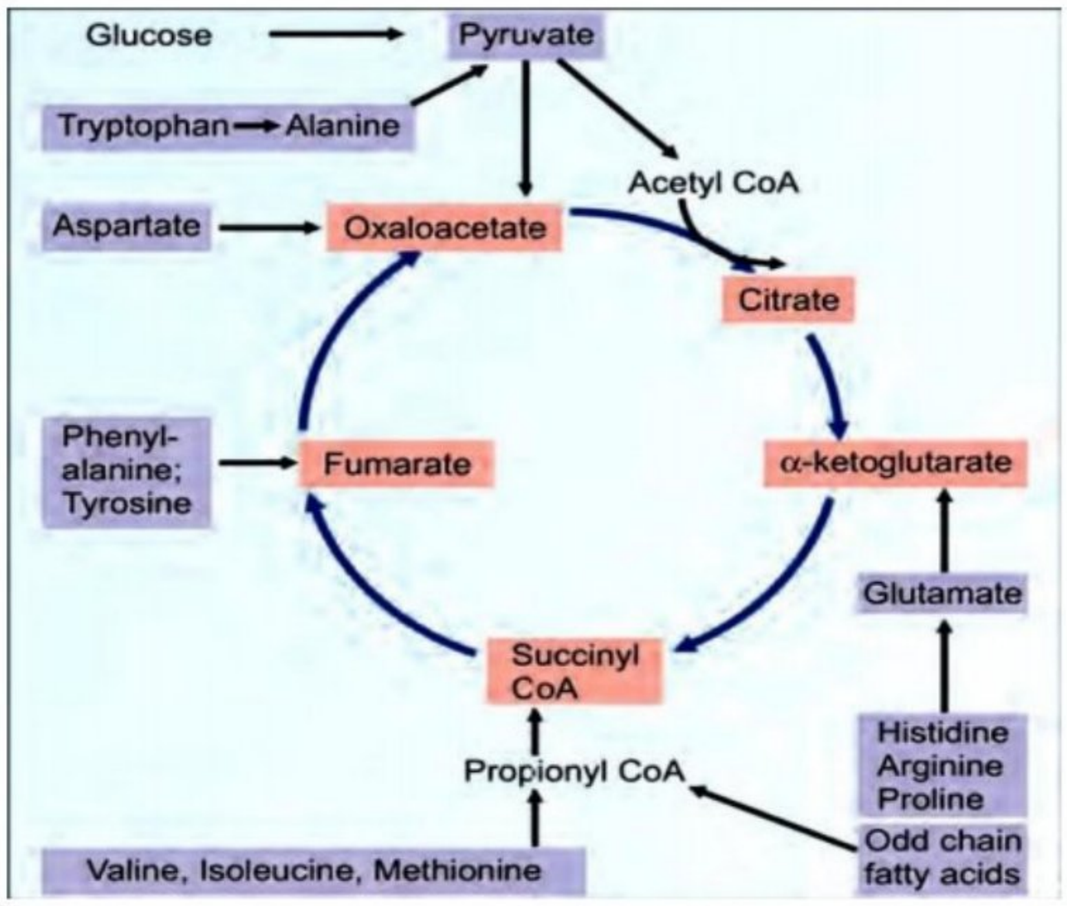
each () in the paragraph above is a pathway the TCA cycle intermediates can be used in.



ANOTHER ANAPLEROTIC ROUTES

Amino acid degradation



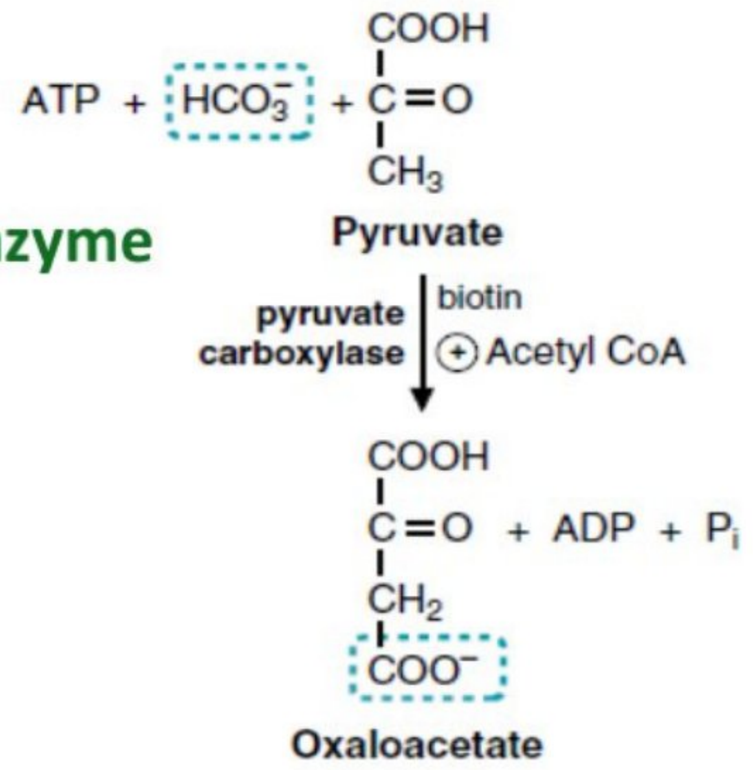


On the other hand, when I have a deficiency in in one of the cycle intermediates, there are some pathways that can synthesize those intermediates, for example, reverse the pathway α-ketoglutarate →glutamine, and you will get α-ketoglutarate out of glutamine by transamination, and the same for other intermediates, most of them can be produced by amino acid degradation.

ANAPLEROTIC REACTIONS

- **Pathways or reactions that replenish the intermediates of the TCA cycle**
- **Pyruvate Carboxylase is a major anaplerotic enzyme (requires <u>biotin</u>) (the most important)**
- **Found in many tissues, liver, kidneys, brain, adipocytes, and fibroblasts.**
- **Very high conc. In liver and kidney**

(Gluconeogenic pathway).



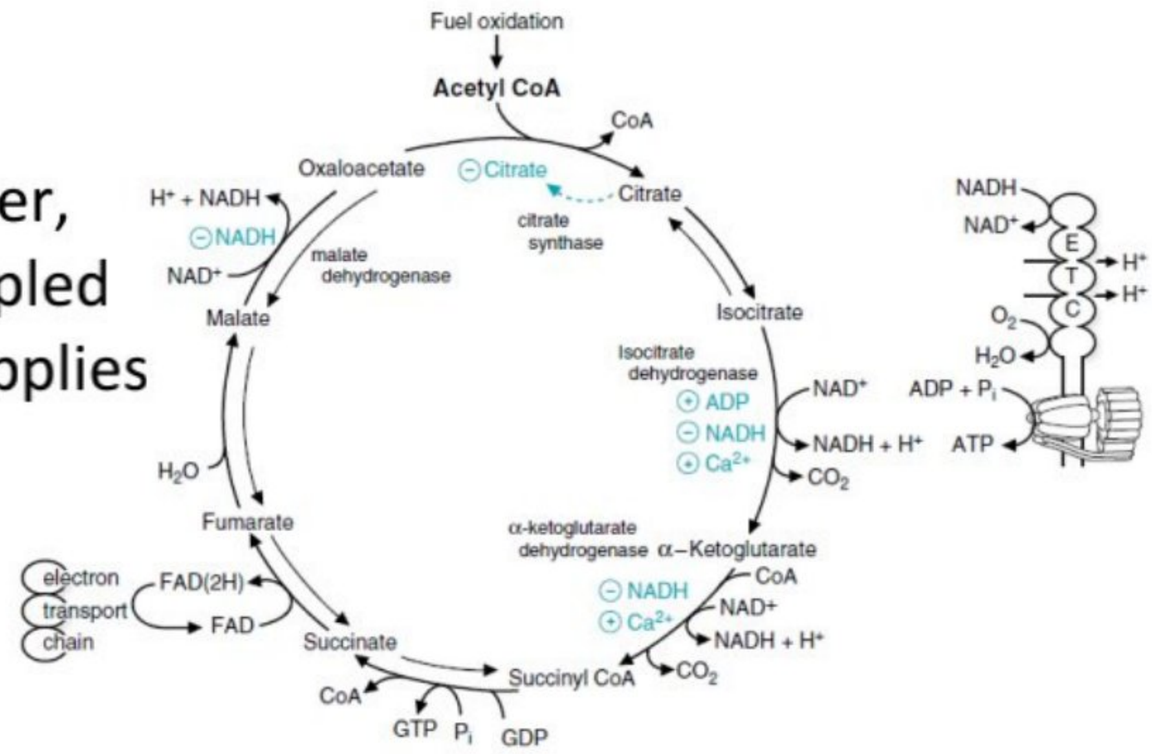
- **Activated (acetyl CoA). (Acetyl CoA presence activates the reaction, because if you have a lot of acetyl CoA you will need a lot of oxaloacetate to bind with it)**

Side note: pyruvate is a ketoacid that can be transferred into alanine by transamination

REGULATION OF TCA CYCLE

➢ Correspond to ETC (ATP/ADP)

ATP and ADP are coupled to each other, any increase in either one will be coupled to a decrease in the other, this also applies to (NADH/NAD+) Ratio.



And by those ratios, the cycle can sense the amount of energy that needs to be synthesized.

- **Two major messengers (feedback):(a) phosphorylation state of adenines, (b) the reduction state of NAD.**
- **Adenine nucleotides pool and NAD pool are relatively constant.**

Citrate synthase works as a regulator, remember (if we want to regulate a pathway, we have to regulate the first step), citrate and ATP work as feedback inhibitors, if you have lots of them, the cycle will slow down.

REGULATION-CITRATE AND CITRATE SYNTHASE

- **Rate regulated by oxaloacetate & citrate (inhibitor)**
- **ATP acts as an allosteric inhibitor of citrate synthase**
- **Effect of citrate:**
  - **Allosterically inhibits PFK, the key enzyme of glycolysis**
  - **Stimulates fructose-1,6-bisphosphatase, a key enzyme of gluconeogenesis.**
  - **Activates acetyl CoA carboxylase, a key enzyme of fatty acid synthesis.**

ISOCITRATE DH

- **Best regulation (rate-limiting).**
- **Allosterically: activated by (ADP, Ca^{+2}).**
- **Inhibition by (NADH).**
- **No ADP vs. ADP (K_M), a small change in ADP, great effect.**

It’s the only enzyme in the cycle that gets affected by ADP levels, high ADP levels will activate this enzyme.

ALPHA-KETOGLUTARATE DH

- **Inhibited by: NADH, succinyl CoA, GTP.**
- **Activated by: Ca^{+2}.**

Side note: in both enzymes, calcium is an activator, NADH is an inhibitor.

إذا أردت أن تُطاع.. فاطلب المستطاع

REGULATION OF THE TCA CYCLE:

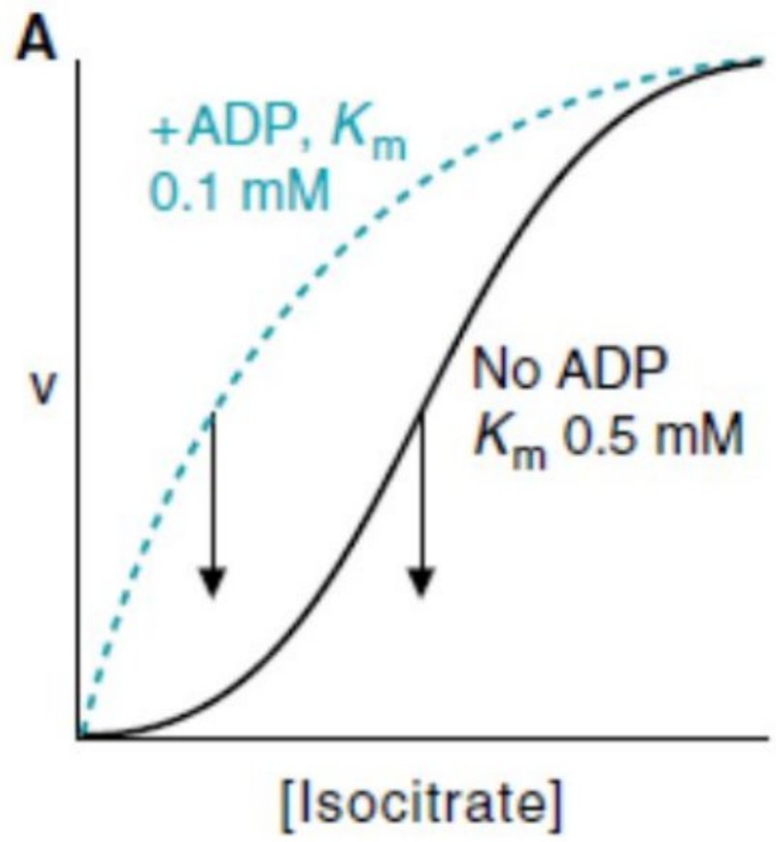
ISOCITRATE DH:

¬ **BEST REGULATION (RATE-LIMITING)**

¬ **ALLOSTERICALLY: ACTIVATED (ADP, CA+2)**

¬ **INHIBITION (NADH)**

¬ **NO ADP VS. ADP (KM), A SMALL CHANGE IN ADP, GREAT EFFECT**

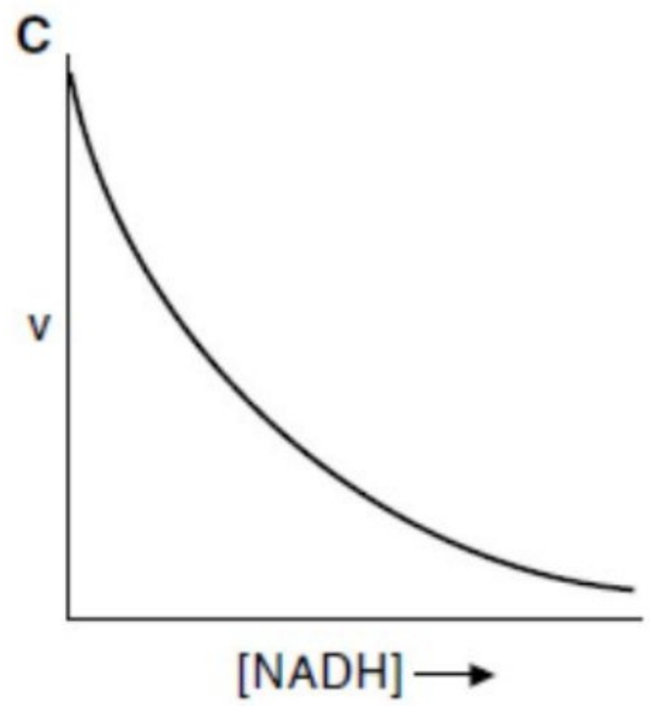


As we know isocitrate dehydrogenase is an allosteric enzyme so its curve will be sigmoidal in shape but when activating it by ADP the curve will be shifted to the left making it more hyperbolic, but it will never be hyperbolic (stay sigmoidal) as in the figure.

. ALPHA - KETOGLUTARATE DH :

¬ **Inhibited: NADH, succinyl CoA, GTP**

¬ **Activated: Ca+2**



As u notice darling both enzymes are activated by Ca+2

ISN'T THIS MYSTERIOUS!!!

As we know Ca+2 activates muscle contraction, but the new information is that it works as a second messenger…here is the tale:

Neurons and hormones control the body (homeostasis and metabolism).

Hormones bind to the cell surface and affect the enzyme phospholipase C which releases Ca+2, releasing Ca+2 stimulates kinase C that drives the phosphorylation of many molecules such as (enzymes and hormones) which control metabolism and energy in the body .

Inhibitors Of TCA Cycle:

all of the regulators that we've mentioned before were physiological regulators, now we will talk about non-physiological regulators.

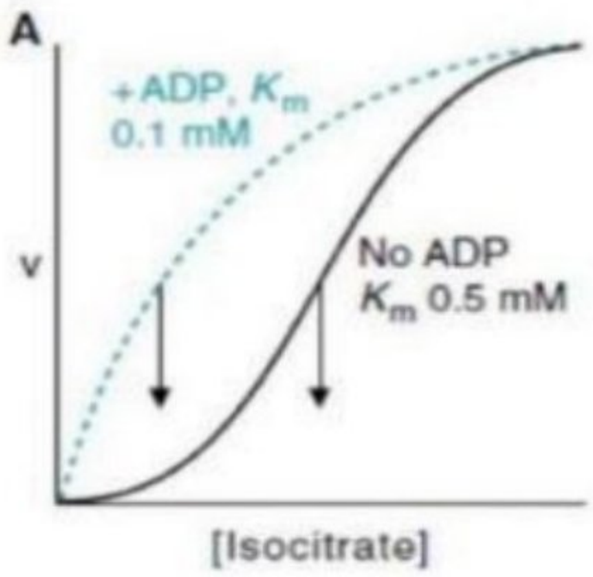
A. Aconitase (citrate to aconitate) is inhibited by fluoroacetate (noncompetitive inhibition).

B. Alpha ketoglutarate dehydrogenase (alpha keto glutarate to succinyl CoA) is inhibited by Arsenite (noncompetitive inhibition).

C. Succinate dehydrogenase (succinate to fumarate) is inhibited by malonate (competitive inhibition).

Malonate competes with Succinate on its receptors taking its place and inhibiting succinate function that's what we mean by saying competitive.

This schedule is just for recapping …..enjoy looking at it.

Citrate synthase	-It is the first enzyme in the cycle. It is a enzyme 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.