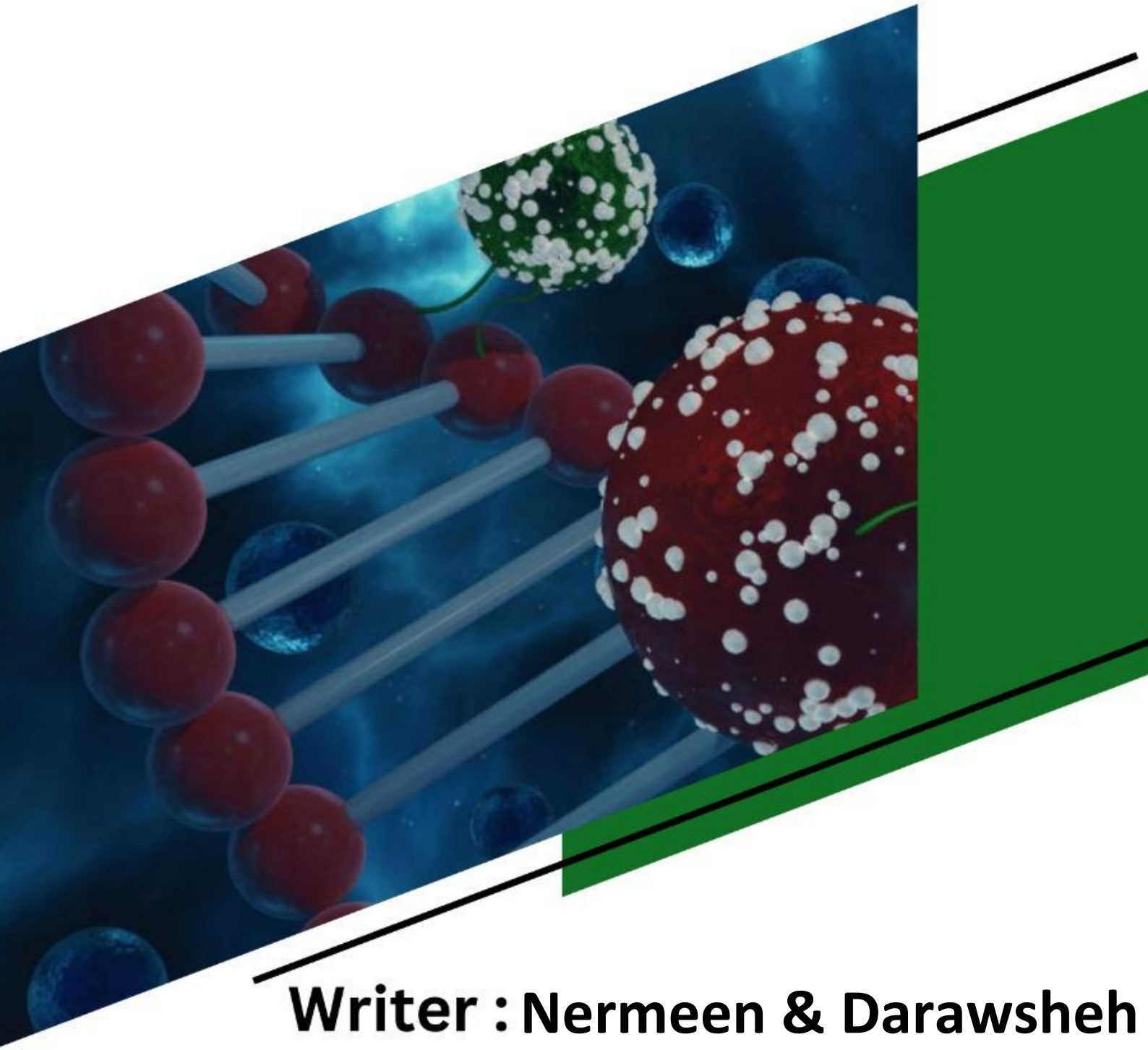
Doctor 021

METABOLISM

Sheet no. 5



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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.

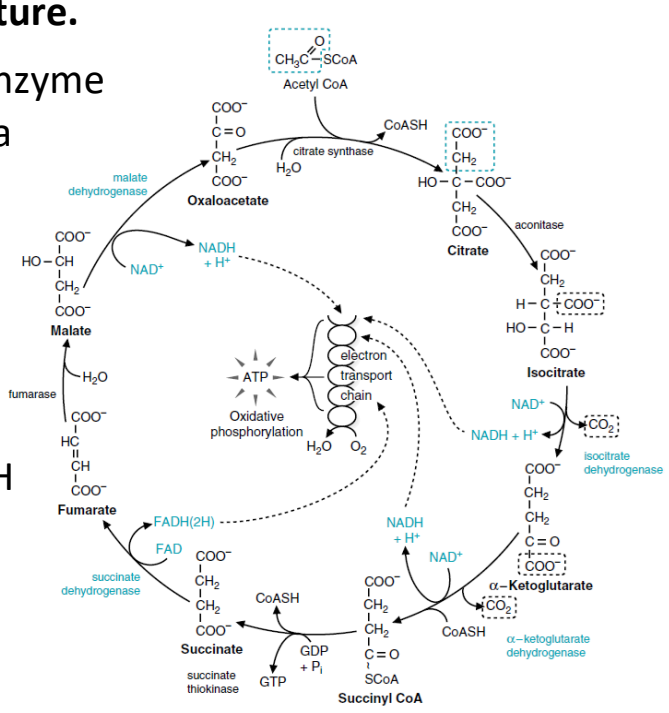
7- Fumarate → malate (hydration):

Fumarate has a double bond in its structure.

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

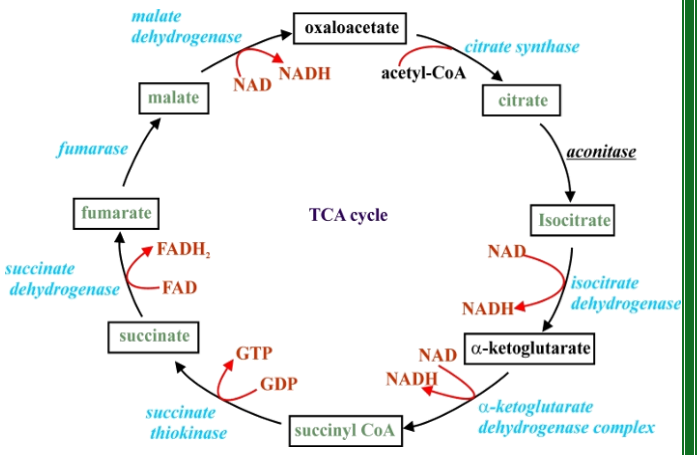
8- Malate → oxaloacetate (oxidized):

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

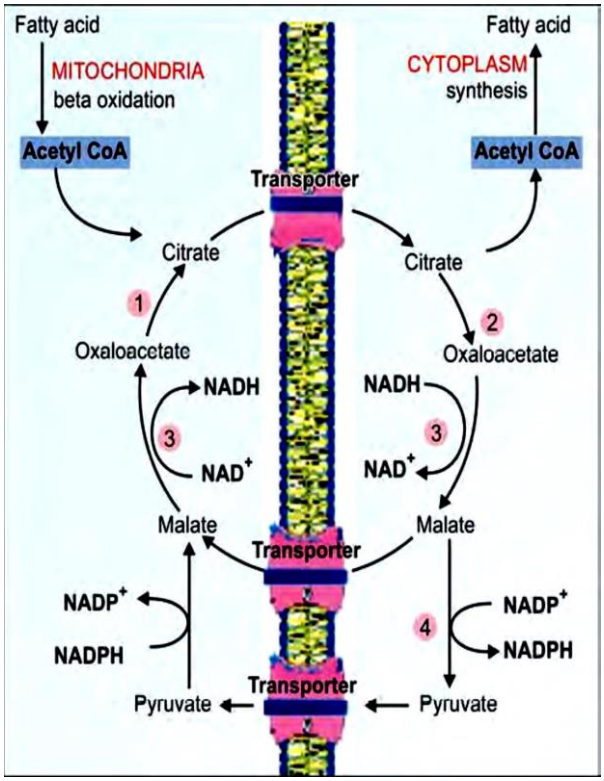


❖ **What drives the reaction forward?**

FORMATION OF CITRATE

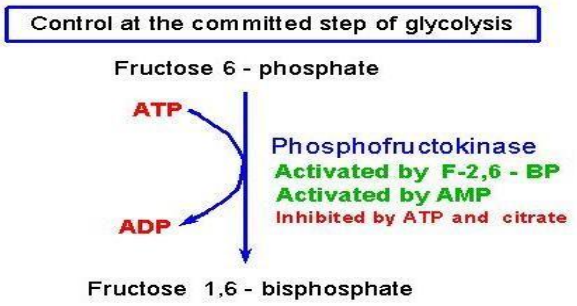
- **What drives the reaction forward?**
  Hydrolysis of acetyl CoA.
- **Is it reversible or irreversible?**

  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

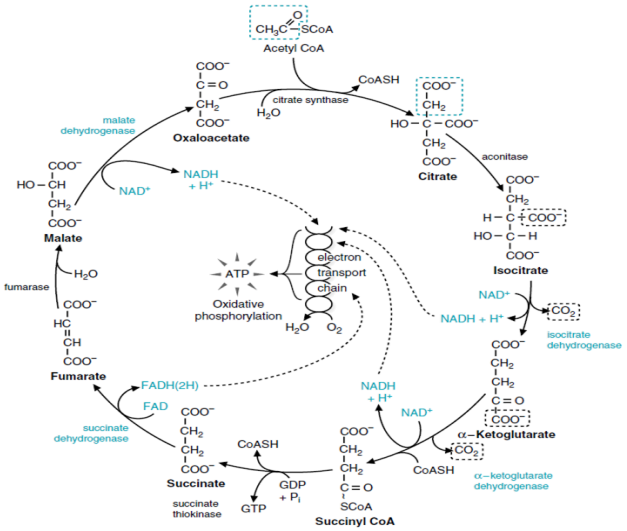
- **Oxidative decarboxylation.**
- **3^0 alcohol to 2^0 alcohol.**
- Citrate can exit the mitochondrial matrix to the cytosol and control the rate-limiting step of glycolysis.



- 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

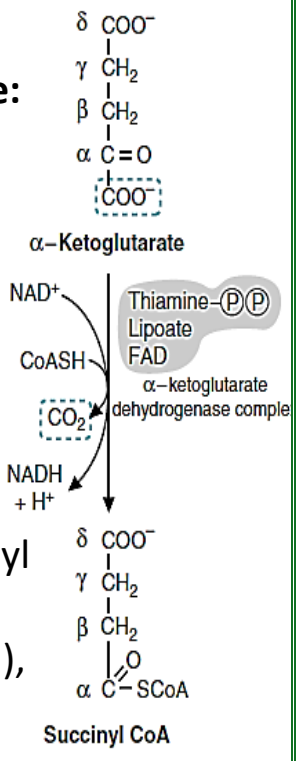
- **Oxidative decarboxylation.**
- **Thiamine pyrophosphate, lipoic acid, and FAD.**
- **Keto group oxidized to acid, CoASH, succinyl CoA.**
- **Energy conserved as NADH, thioester bond.**
- **The only irreversible step in the whole reaction cycle (there is none enzymes that can reverse this reaction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

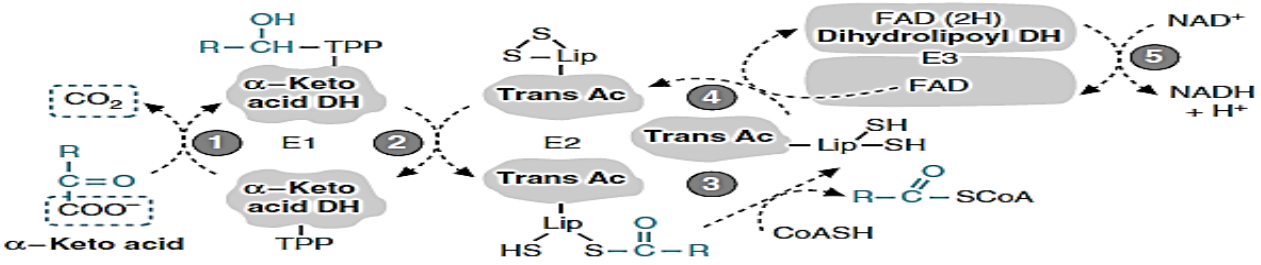
  Remember: one way of enzyme regulation is to complex one enzyme whose product is the substrate of the second one.



- **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).

- **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).

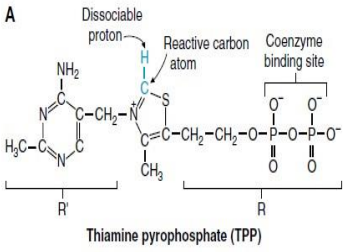
E3:

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.

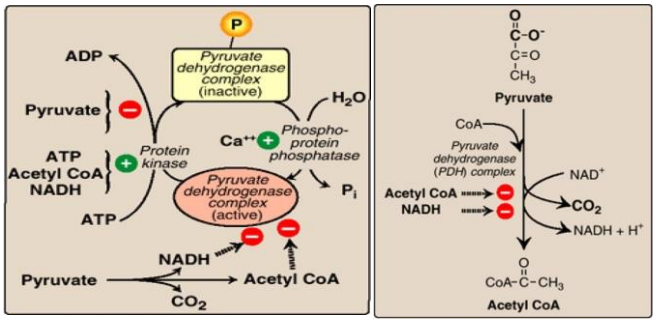
THIAMINE PYROPHOSPHATE

- 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.**



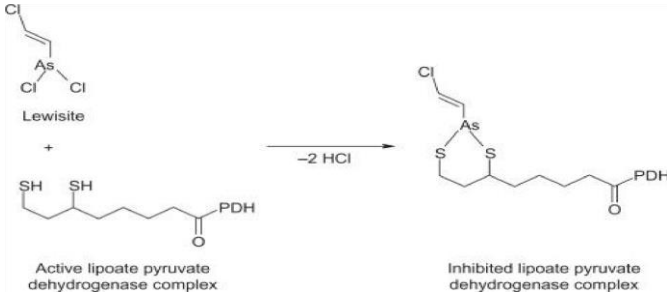
OXIDATIVE DECARBOXYLATION OF PYRUVATE

- 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.

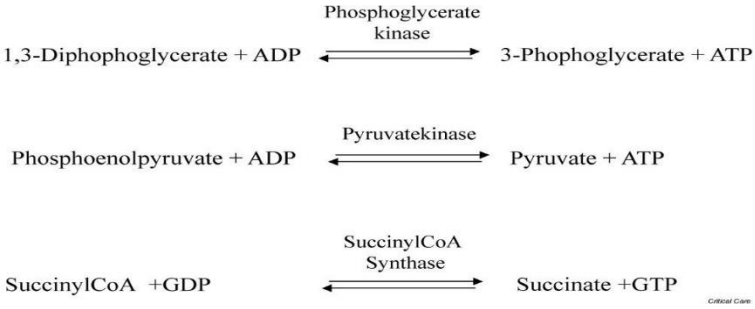
  Arsenic binds the 2 sulfurs of lipoic acid, breaks the disulfide bond and inhibits its action.

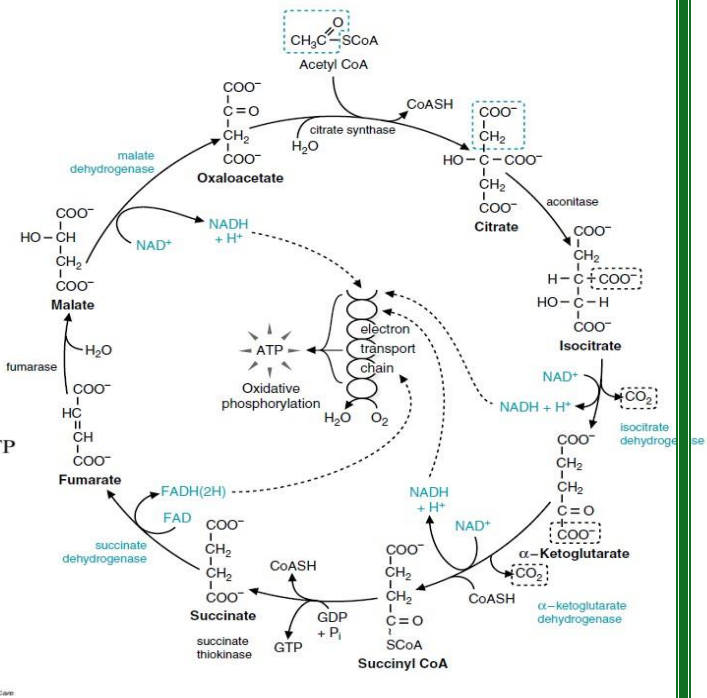


GENERATION OF GTP

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

Side note: what applies to ATP applies to GTP.

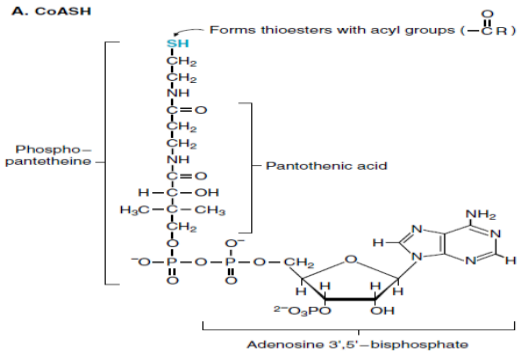


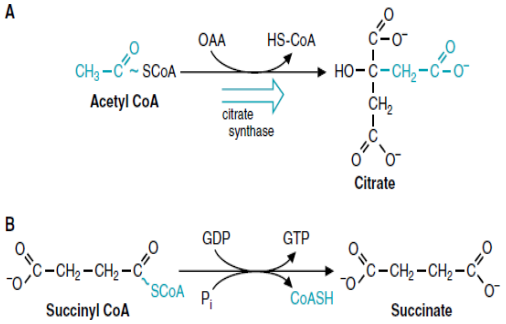


COA

- 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).

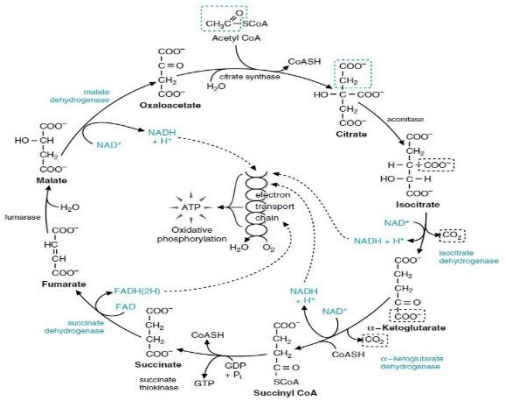
It is known for having a high energy value.





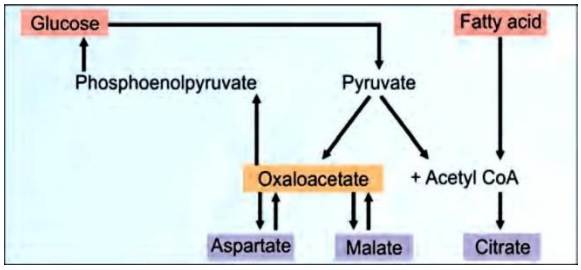
OXIDATION OF SUCCINATE TO OXALOACETATE

- **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

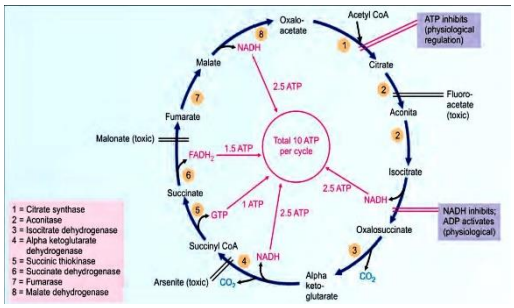
- **Like all pathways, overall net -ΔG (-228 kcal/mole), not 100%.**
- **3 NADH, FAD(H2), and GTP (10ATP), 207 Kcal, 90%.**
- **Three reactions have large (-ve) values. (Which restricts the movement in only one direction).**
- **Physiologically irreversible, low products.**

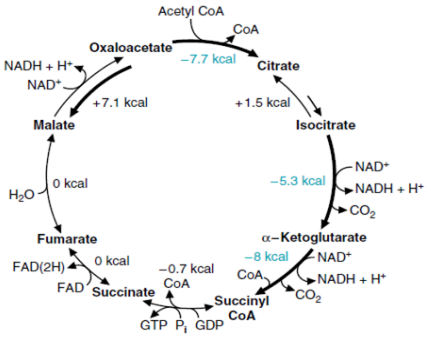
TABLE 19.1: ATP generation steps

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

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.

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.

TABLE 19.2: Stoichiometry of the TCA cycle

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

*Amphibolic: not anabolic, not catabolic but both.

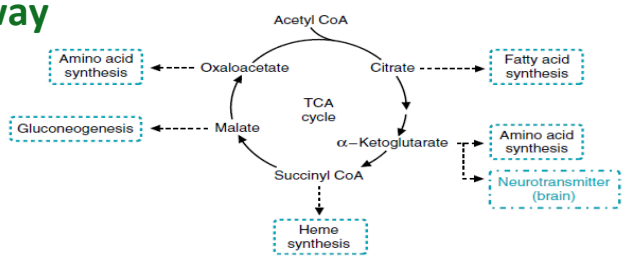
Don't ignore those 10 points, please.

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

TCA CYCLE INTERMEDIATES

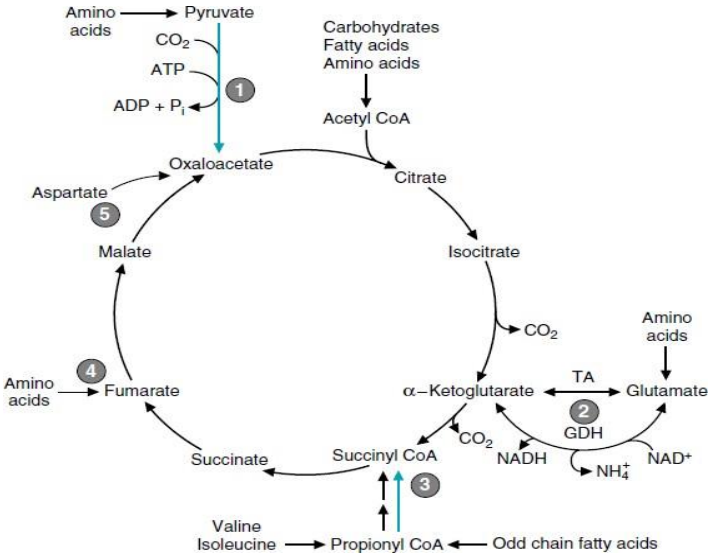
- **Intermediates are Precursors for Biosynthetic Pathways (citrate, acetyl CoA, fatty acid synthesis, liver) (fasting, malate, gluconeogenesis, liver) (Succinyl CoA, heme biosynthesis, bone marrow) (α-ketoglutarate, glutamate, GABA, a neurotransmitter, brain) (α-ketoglutarate, glutamine, skeletal muscle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

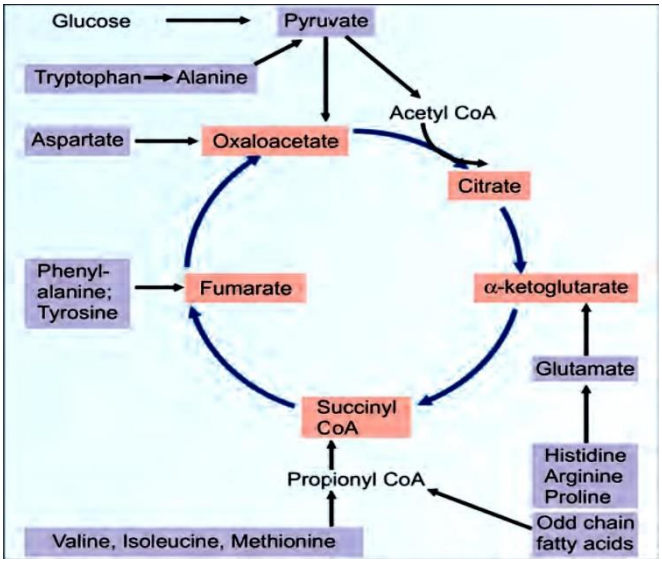
each () in the paragraph above is a pathway the TCA cycle intermediates can be used in.



ANOTHER ANAPLEROTIC ROUTES

Amino acid degradation



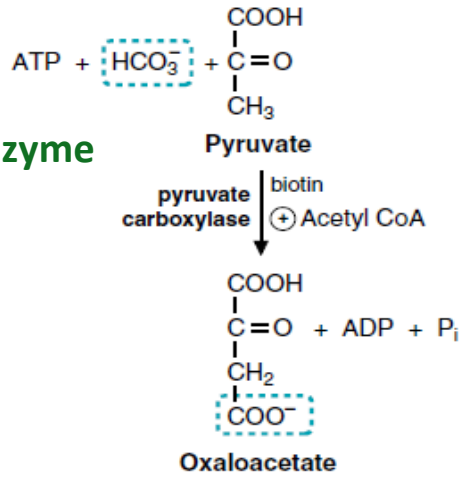


On the other hand, when I have a deficiency in in one of the cycle intermediates, there are some pathways that can synthesize those intermediates, for example, reverse the pathway α-ketoglutarate →glutamine, and you will get α-ketoglutarate out of glutamine by transamination, and the same for other intermediates,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



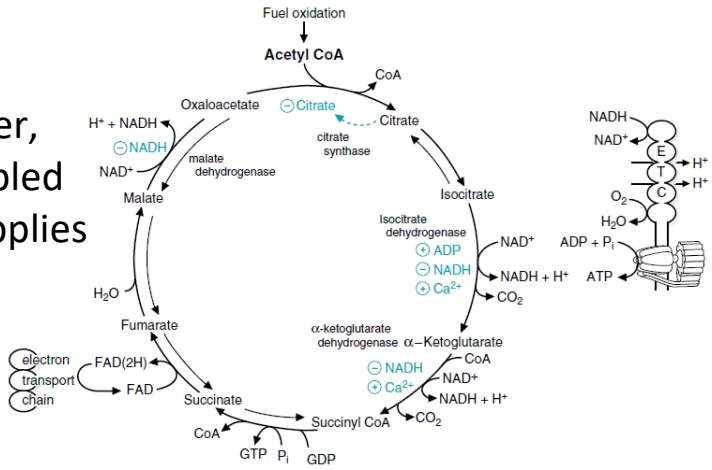
- **Activated (acetyl CoA). (Acetyl CoA presence activates the reaction, because if you have a lot of acetyl CoA you will need a lot of oxaloacetate to bind with it)**

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 <u>oxaloacetate</u> & <u>citrate</u> (inhibitor)**
- **<u>ATP</u> 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.

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