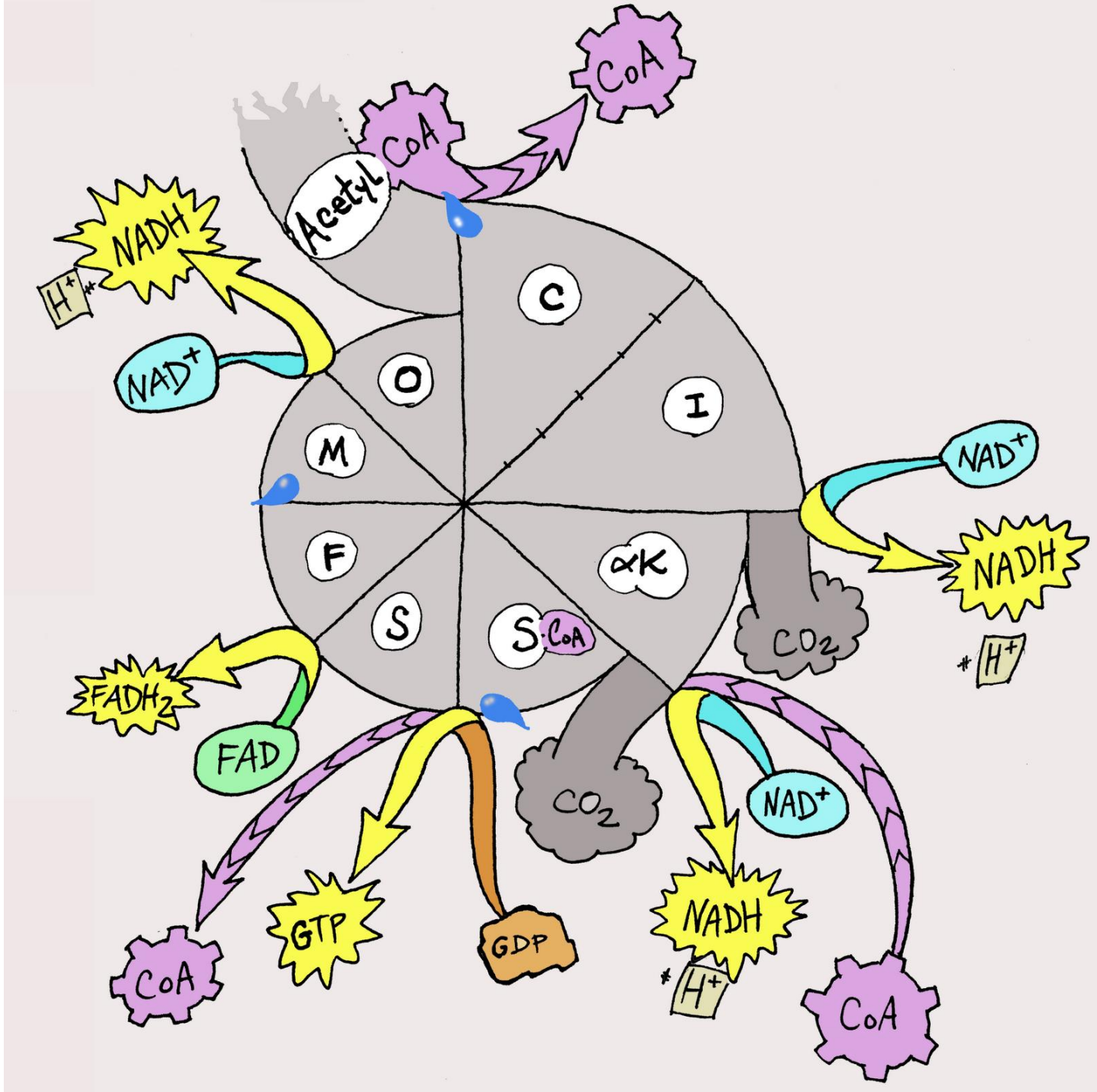
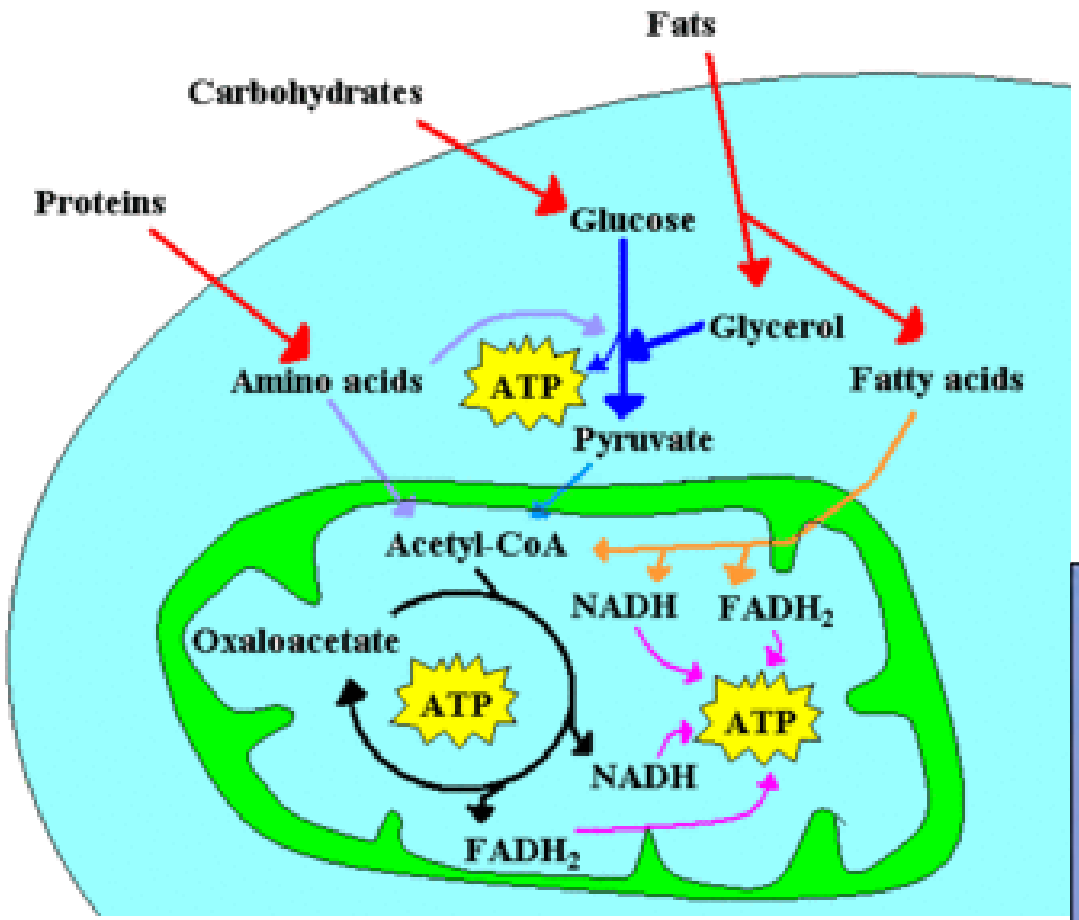
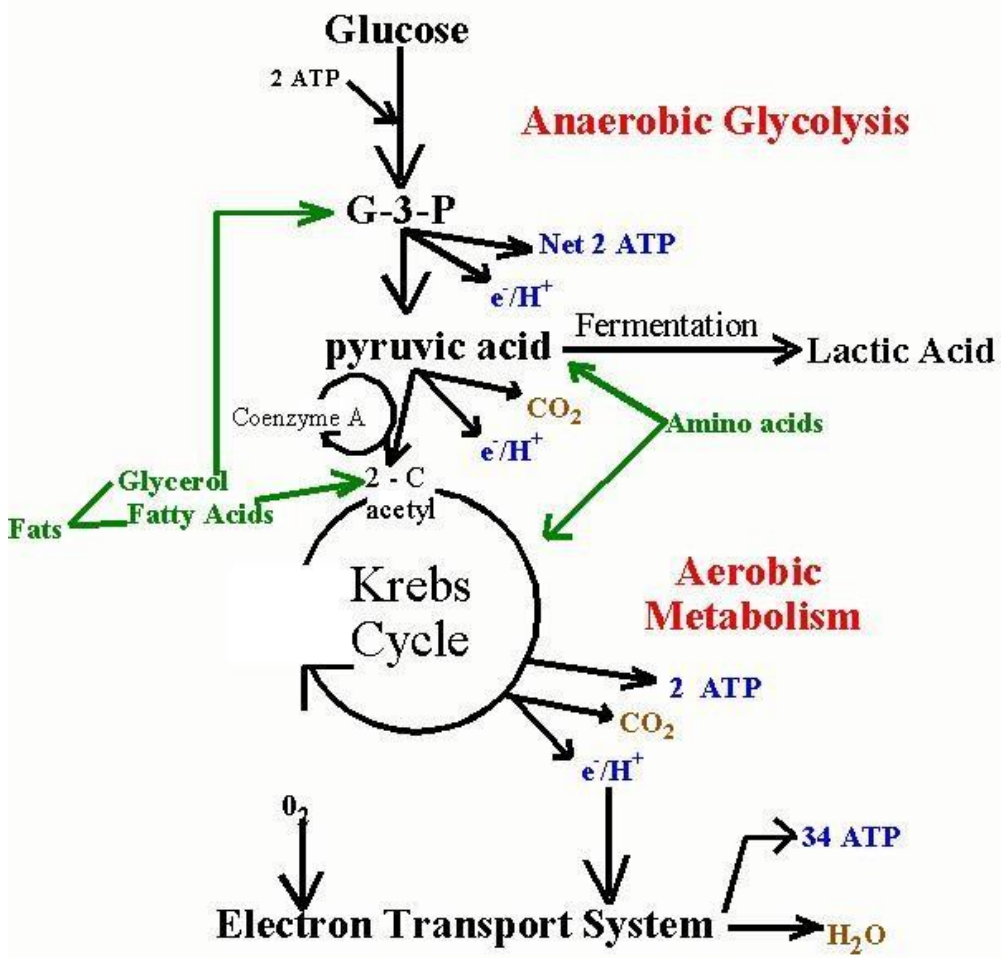
(Kreb's, Citric Acid, Tricarboxylic Acid) Cycle

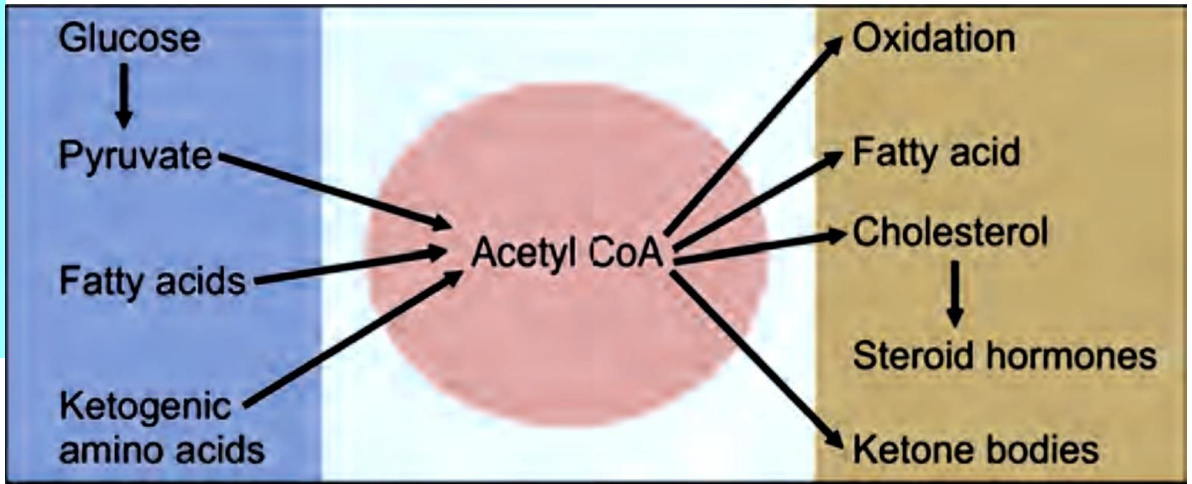
Dr. Diala Abu-Hassan, DDS, PhD



Sources and Uses of Acetyl-CoA



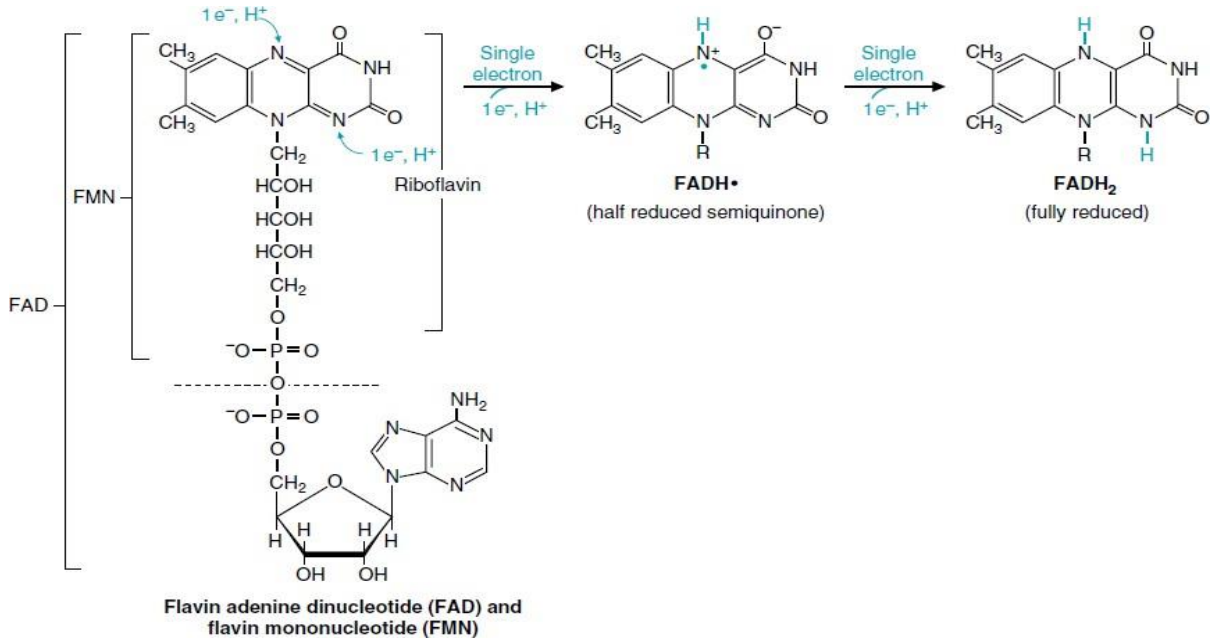




Electron (energy) carrying Molecules (NAD+, FAD)

FAD

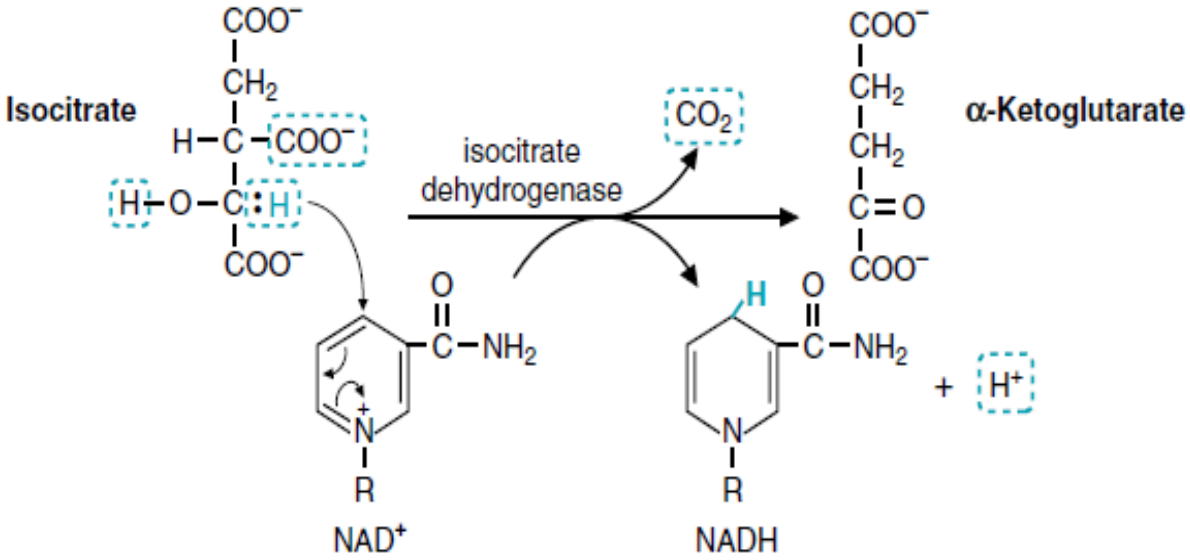
- Single electrons (H•), different sources
- Succinate to fumarate, lipoate to lipoate disulfide in α-KG
- FAD must remain tightly, sometimes covalently, attached to its enzyme
- E^{o} for enzyme-bound FAD varies



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

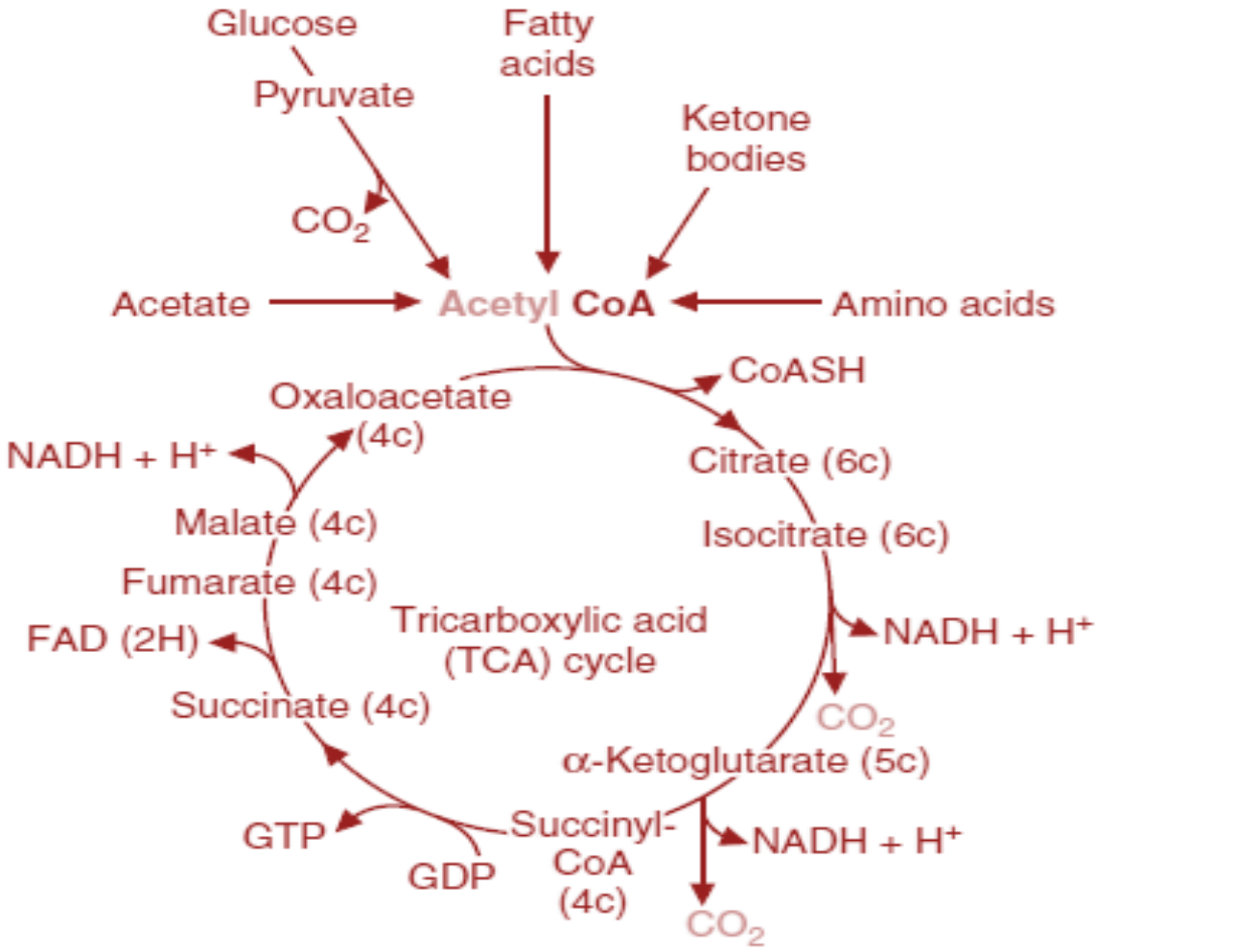
NAD

- Pair of electrons (H-), same source
- Alcohols to ketones by malate dehydrogenase & isocitrate dehydrogenase
- NADH plays a regulatory role in balancing energy metabolism

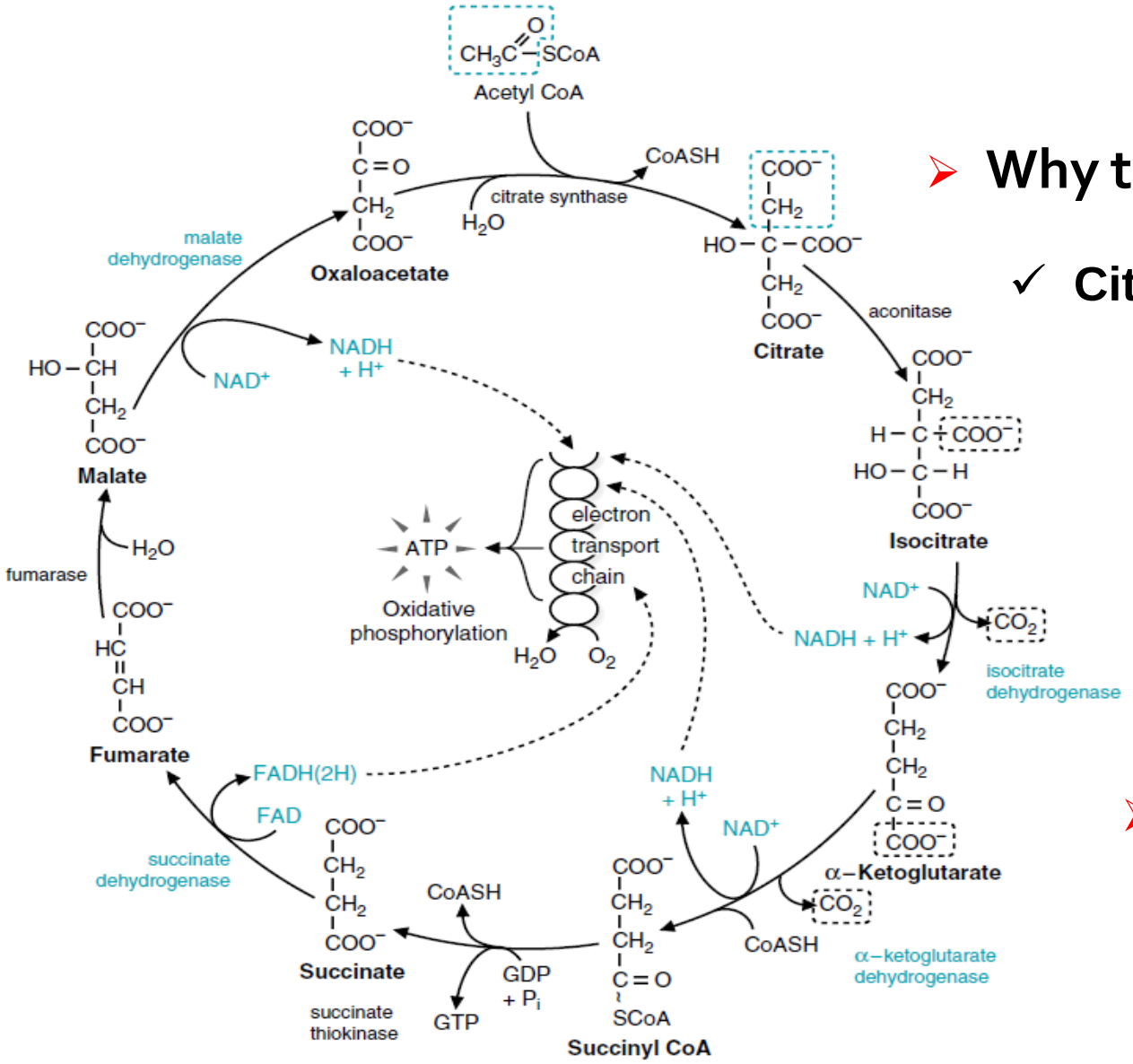


Stepwise Reactions

- No O_2 introduced
- Two CO_2 exits



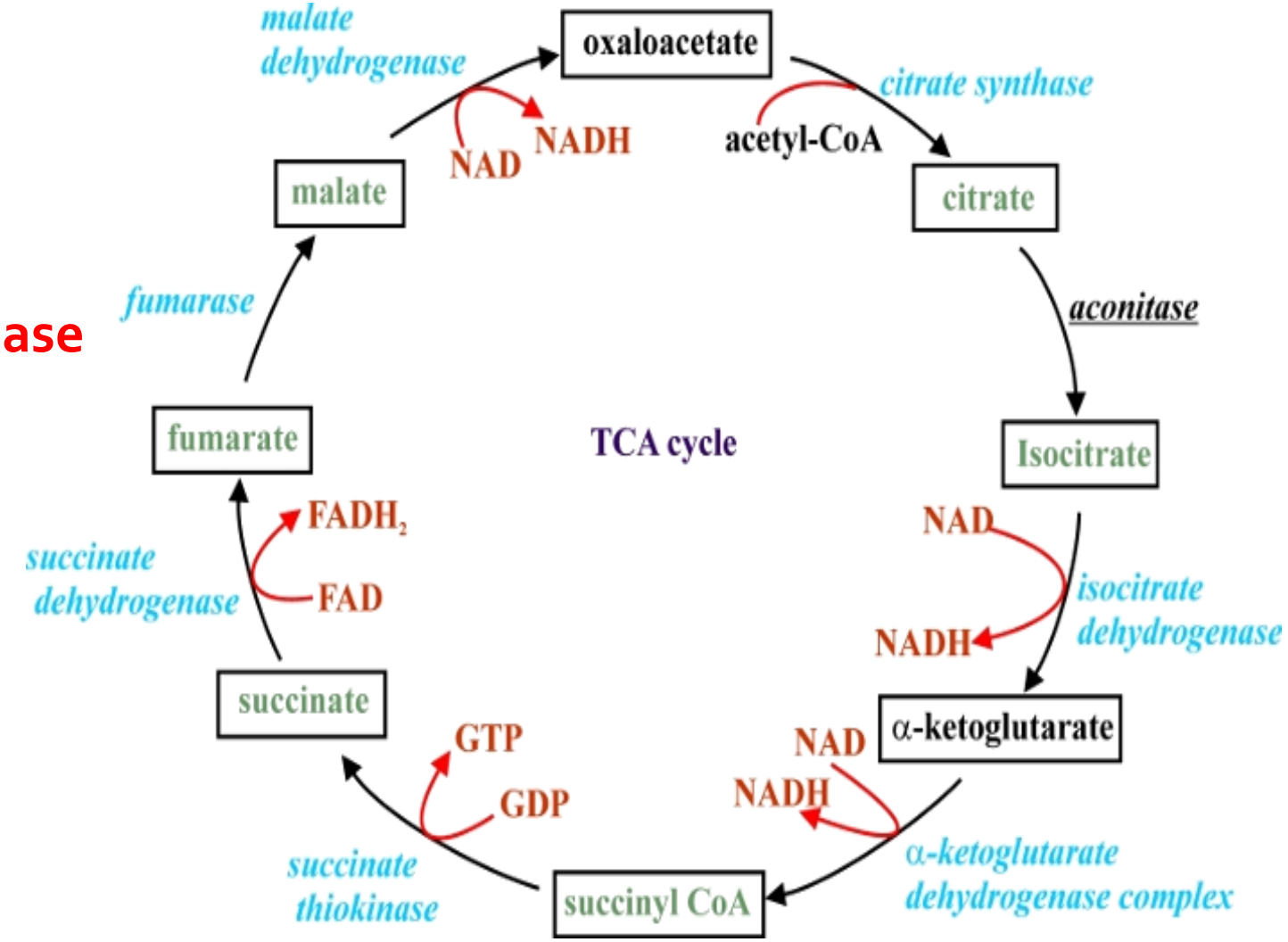
Does Acetyl-CoA exit as CO2?



- **Why to make Isocitrate from citrate?**
  - ✓ **Citrate is a tricarboxylic acid**
- **Where does the CO_2 exit?**

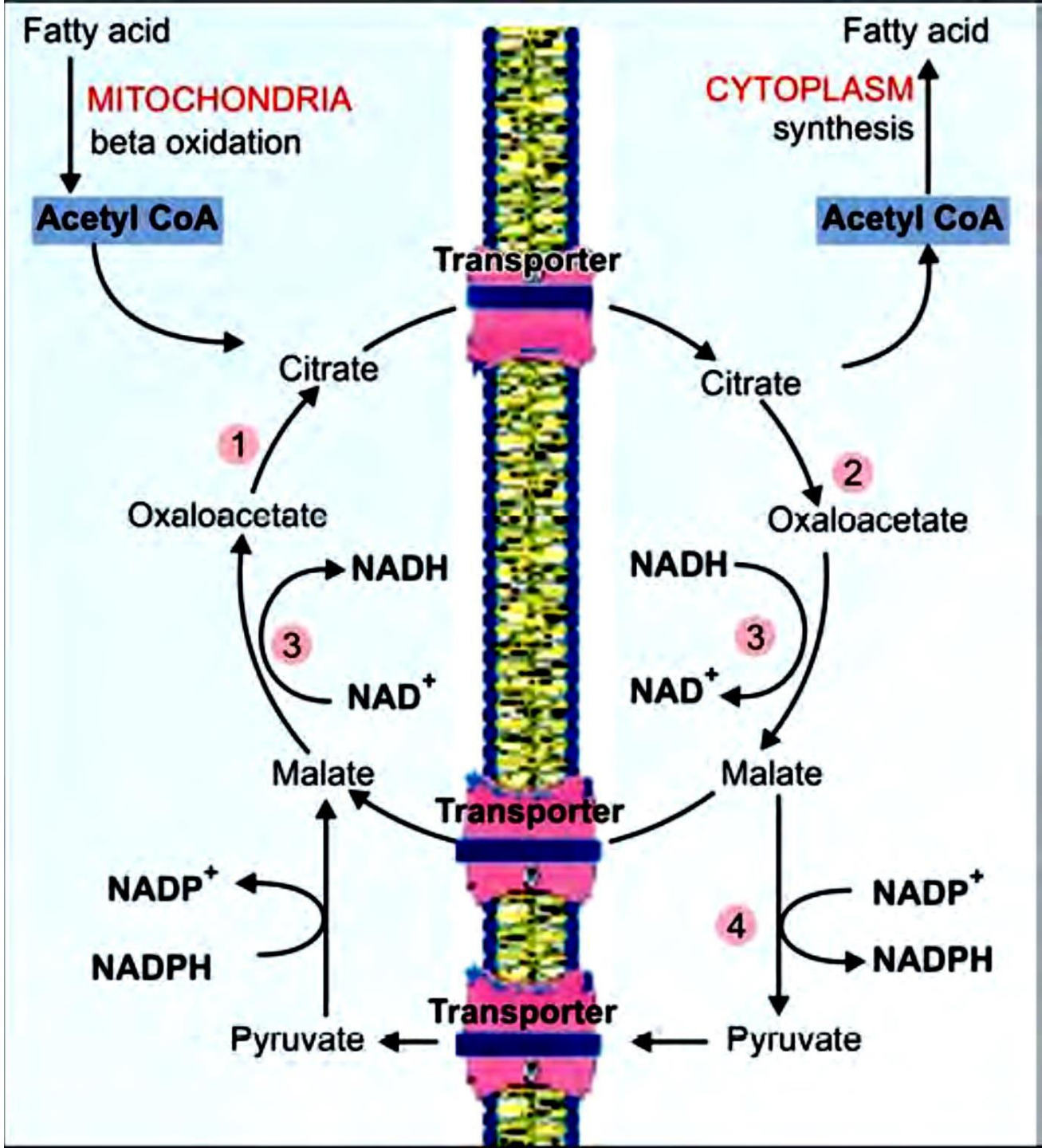
Enzymes of the TCA Cycle

- Citrate synthase (not an allosteric enzyme)
- Aconitase
- Isocitrate dehydrogenase
- α-ketoglutarate dehydrogenase
- Succinate thiokinase
- Succinate dehydrogenase
- Fumarase
- Malate dehydrogenase



Step 1: Formation of Citrate

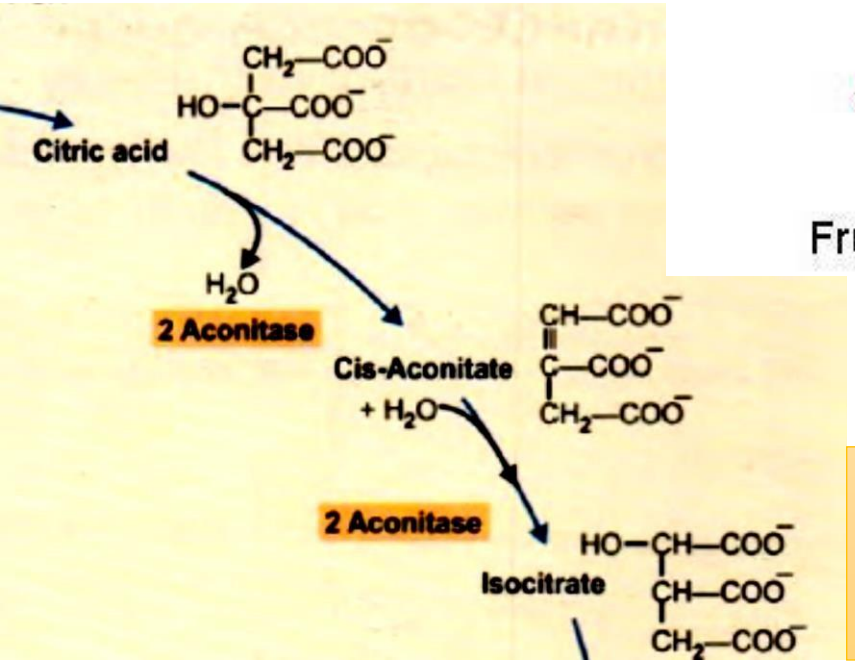
- ✓ Citrate synthase is inhibited by its product, citrate.
- ✓ Substrate availability is another way of regulation for citrate synthase.
- ✓ The binding of oxaloacetate causes a conformational change in the enzyme that generates a binding site for acetyl CoA.
- ✓ Citrate provides a source of acetyl CoA for synthesis of fatty acids and activates their synthesis
- ✓ Citrate inhibits phosphofructokinase (glycolysis)

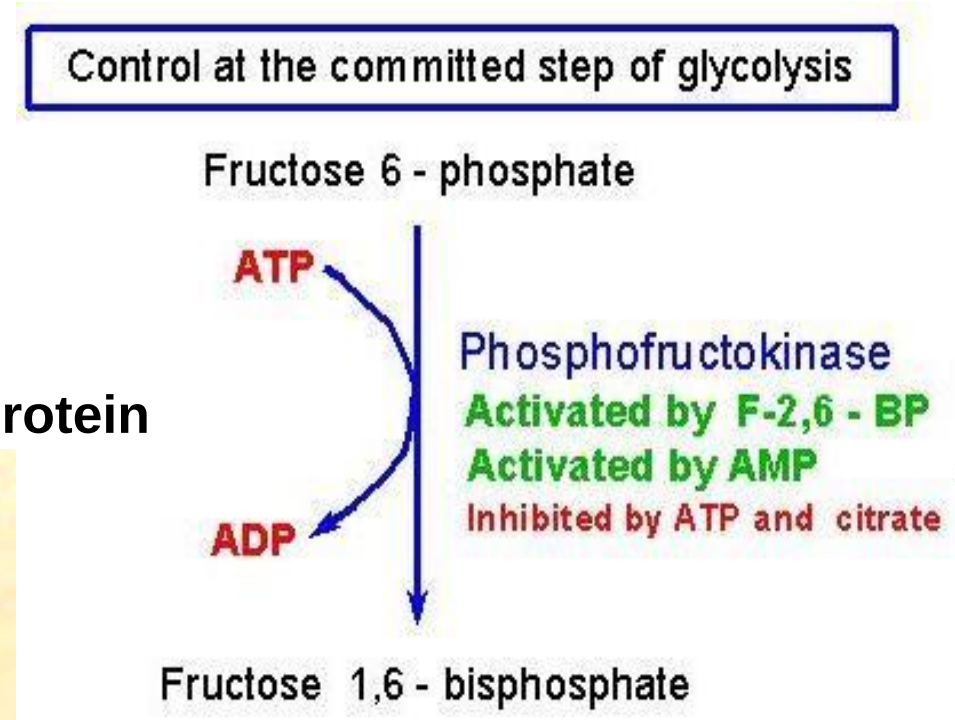


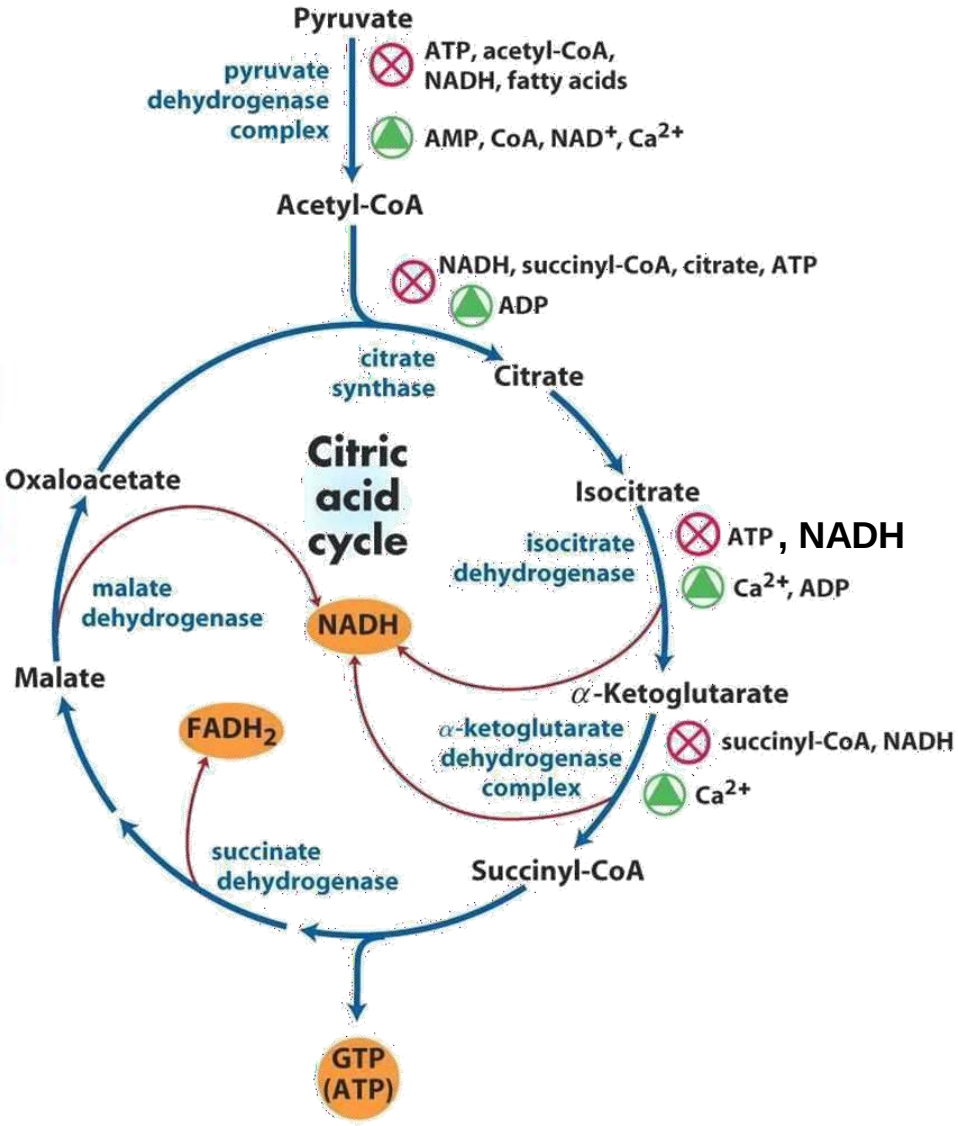
Step 2+3: Formation and Oxidation of Isocitrate

- **Oxidative decarboxylation (irreversible)**
- **3° to 2° alcohol**

✓ **Aconitase is an Fe-S protein**



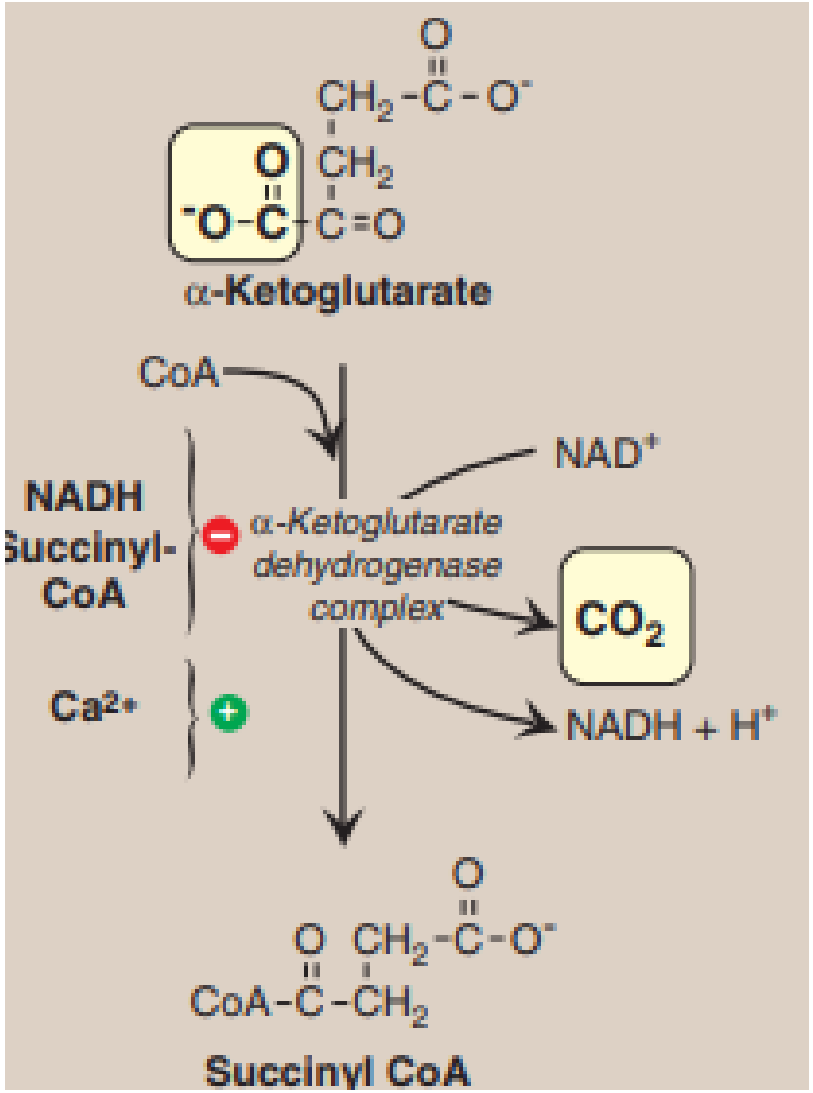




Application: Aconitase is inhibited by fluoroacetate that is used as a rat poison

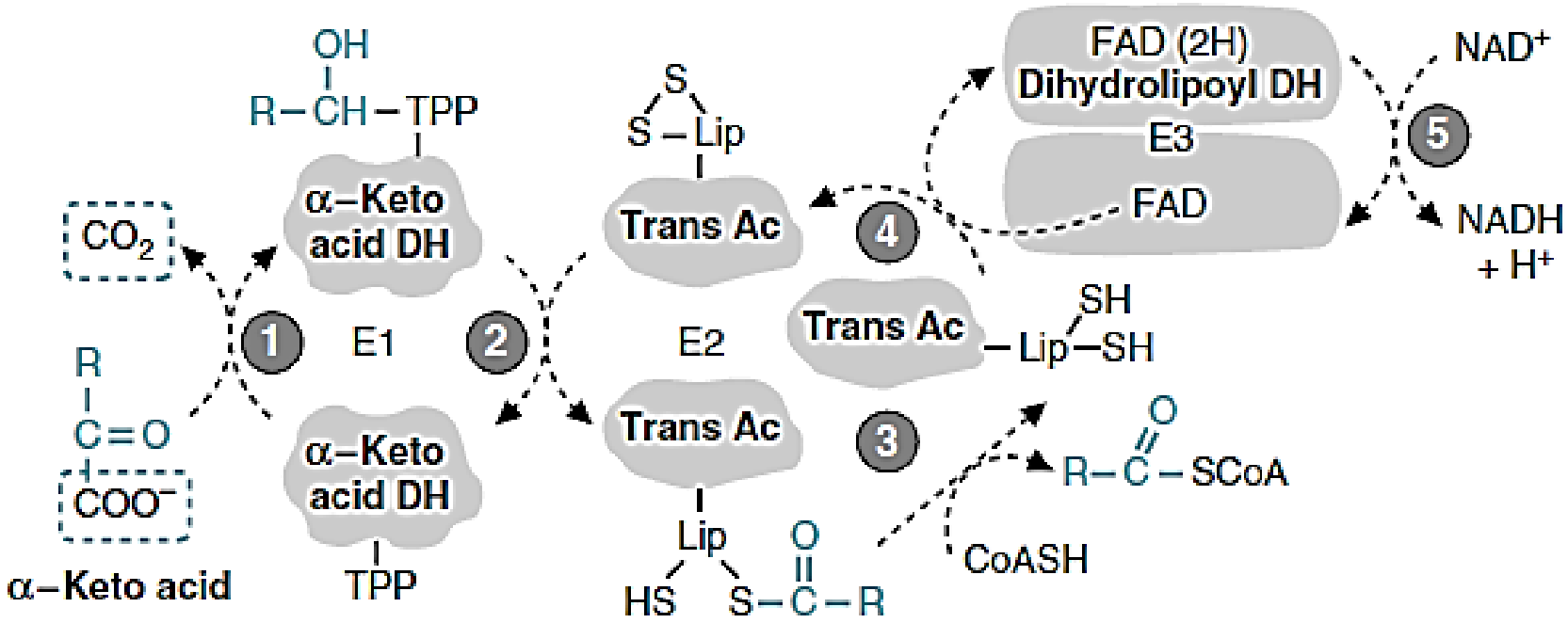
Step 4: α-Ketoglutarate to Succinyl-CoA

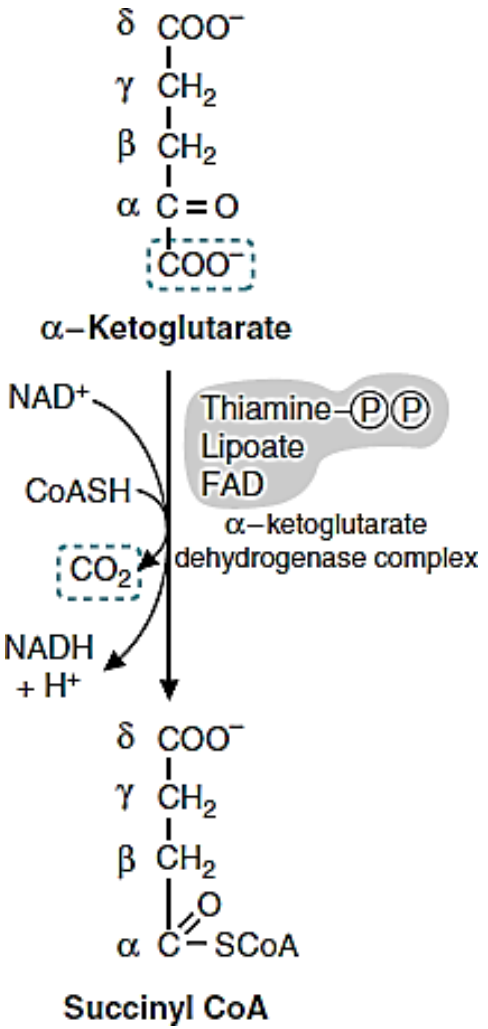
- **Oxidative decarboxylation**
- **α-ketoglutarate dehydrogenase complex, a multimolecular aggregate of three enzymes**
- **Thiamine pyrophosphate, lipoic acid, FAD, NAD+, and CoA**
- **Energy conserved as NADH, thioester bond**



α-Ketoacid Dehydrogenase Complexes (TLCFN)

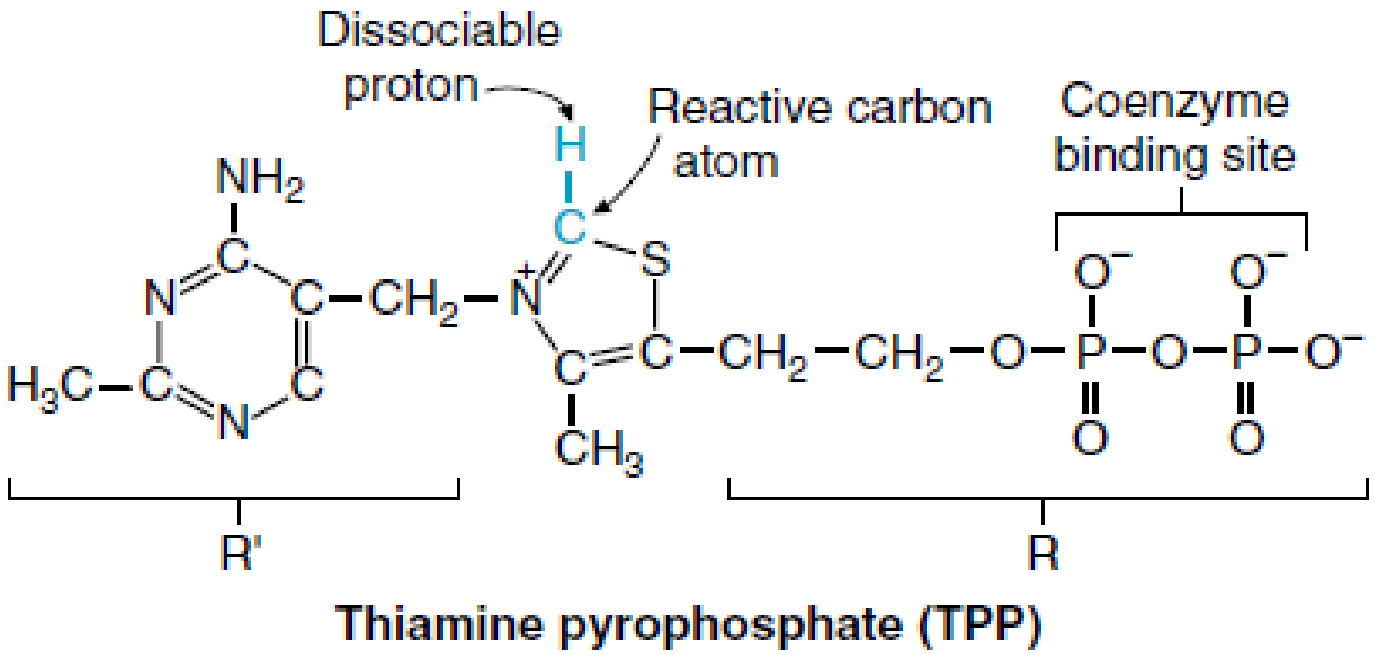
- (α-ketoglutarate, pyruvate, and branched chain α-keto acid) dehydrogenase complexes
- Huge enzyme complexes, multiple subunits of 3 different enzymes (no loss of energy, substrates for E2 and E3 remain bound → higher rate)
- E1, E2, & E3 are a decarboxylase (TPP), a transacylase (lipoate), & a dehydrogenase (FAD)

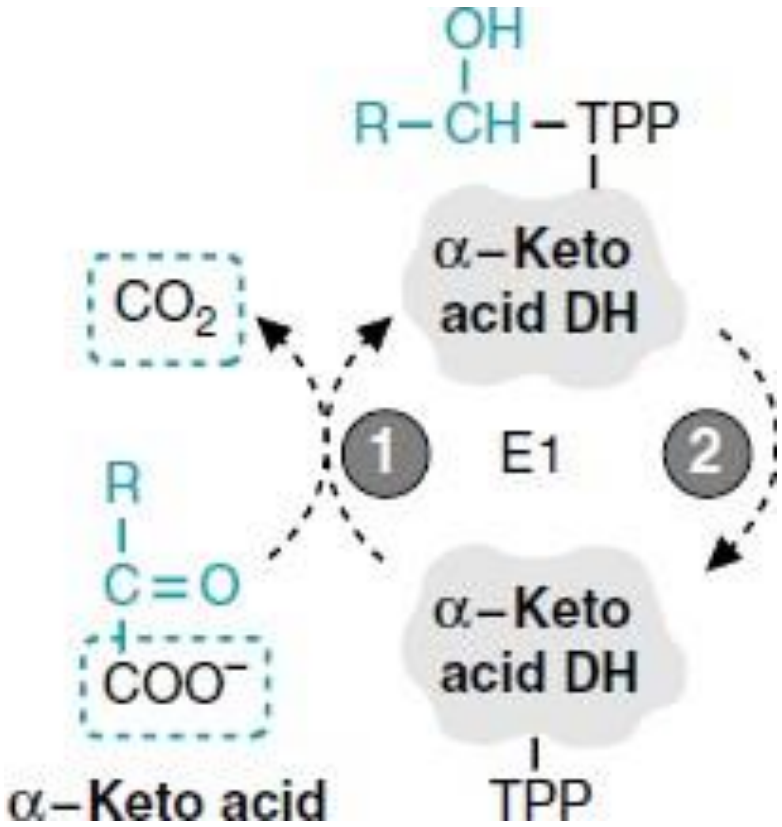




Thiamine pyrophosphate

- **Thiamine (vitamin B1) deficiency, α-ketoglutarate, pyruvate, & branched chain α-keto acids accumulate in the blood**





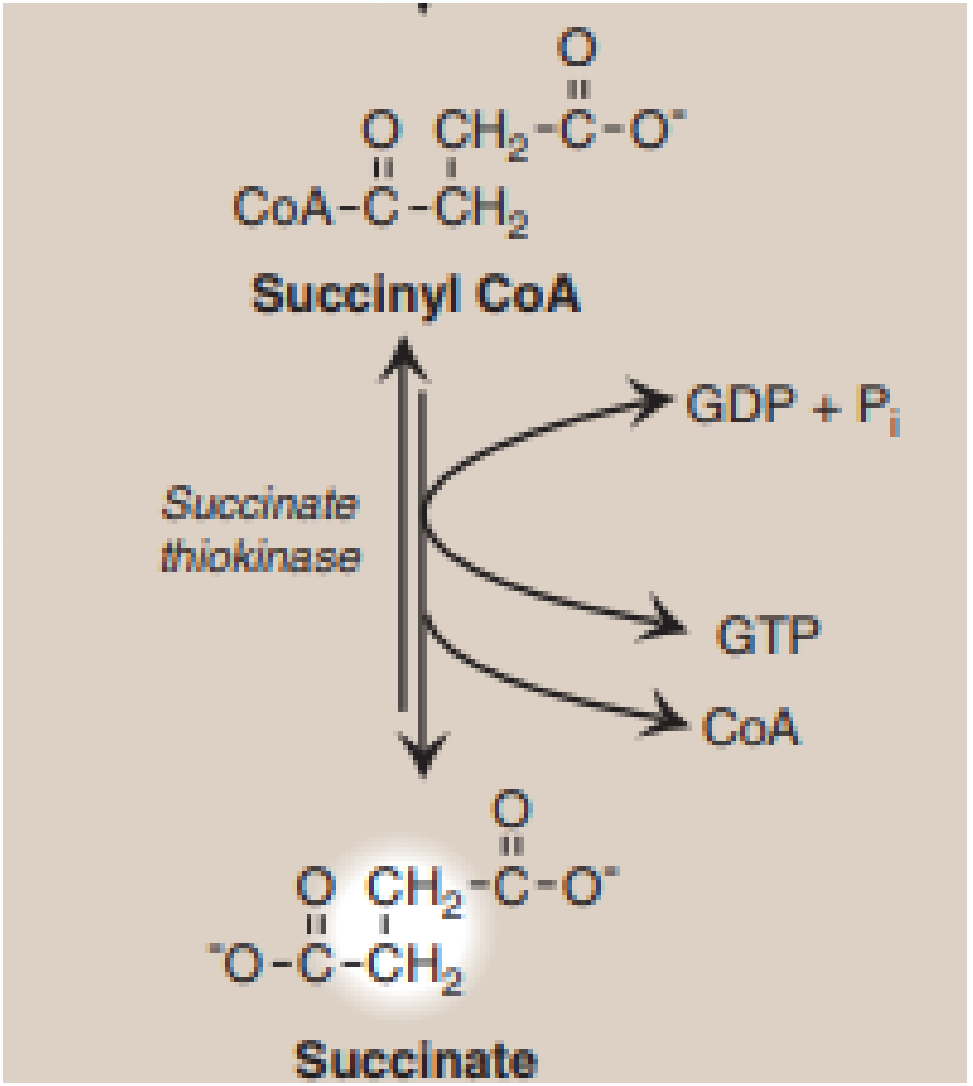
Step 5: Cleavage of succinyl CoA and Generation of ATP

- Succinate thiokinase (succinyl CoA synthetase—named for the reverse reaction) cleaves the high-energy thioester bond of succinyl CoA

- Succinyl CoA has a thioester bond (CoASH & an acyl group)
- GTP is produced by <u>substrate level phosphorylation</u>

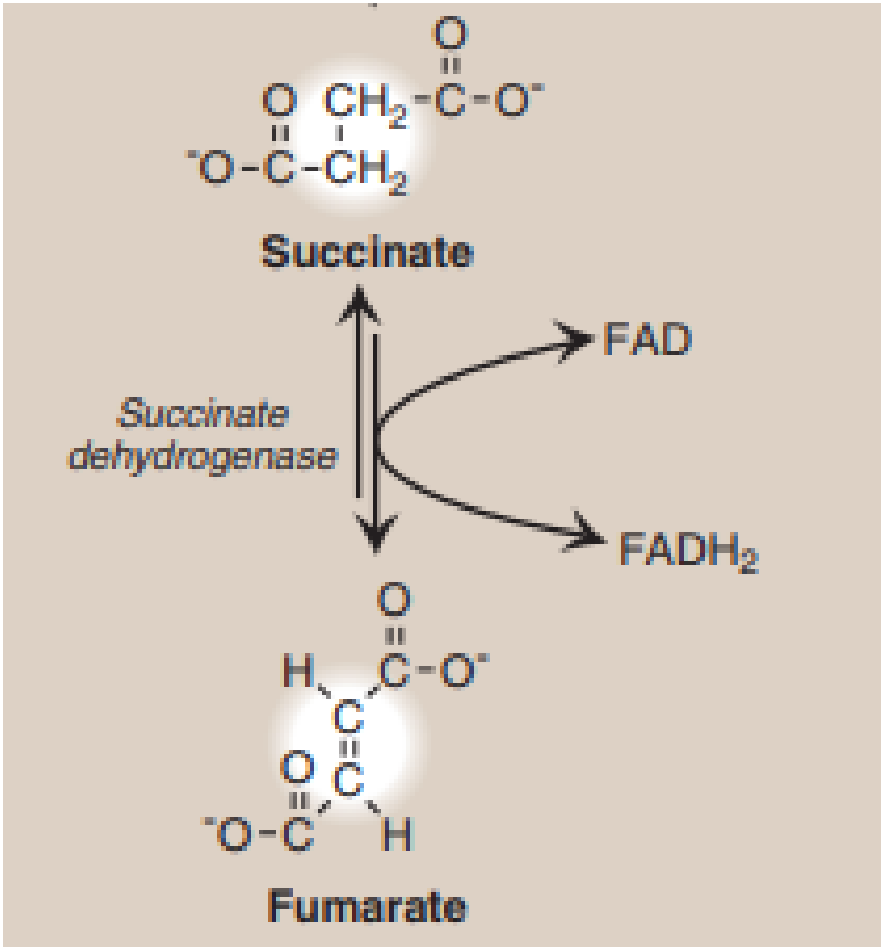
  ✓ GTP and ATP are energetically interconvertible by the nucleoside diphosphate kinase reaction

  GTP + ADP ↔ GDP + ATP



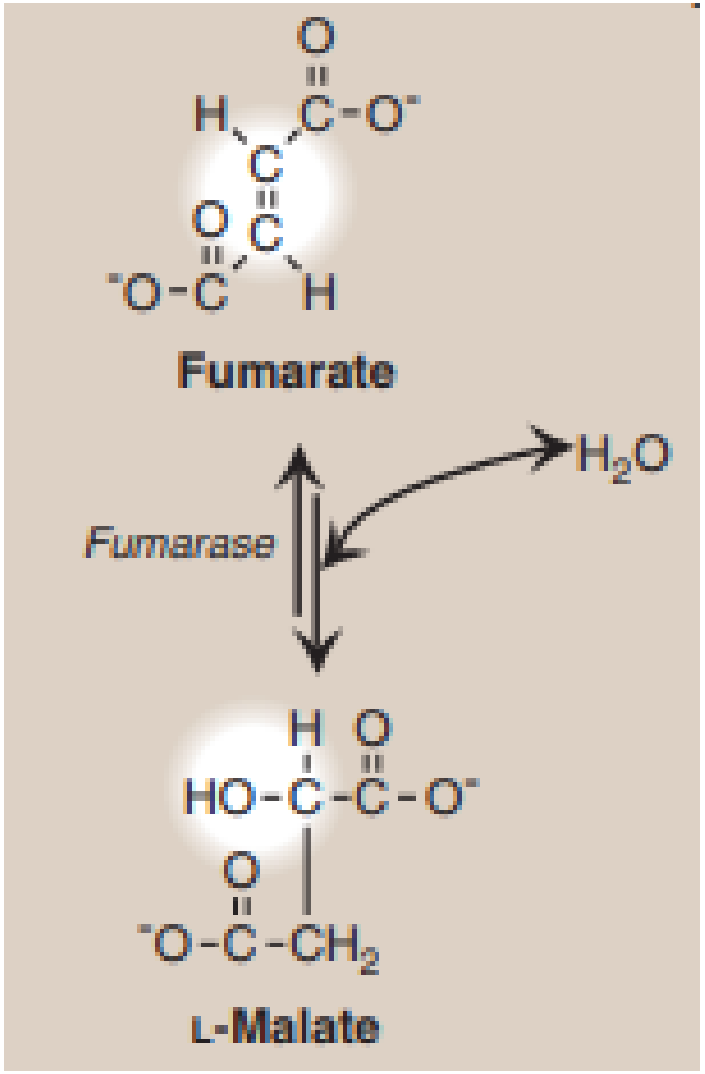
Step 6: Oxidation of succinate

- Succinate is oxidized to fumarate by succinate dehydrogenase
- FAD (its coenzyme) is reduced to FADH2
- FAD, rather than NAD+, is the electron acceptor because the reducing power of succinate is not sufficient to reduce NAD+
- Succinate dehydrogenase is the only enzyme of the TCA cycle that is embedded in the inner mitochondrial membrane.
- Succinate dehydrogenase functions as Complex II of the electron transport chain



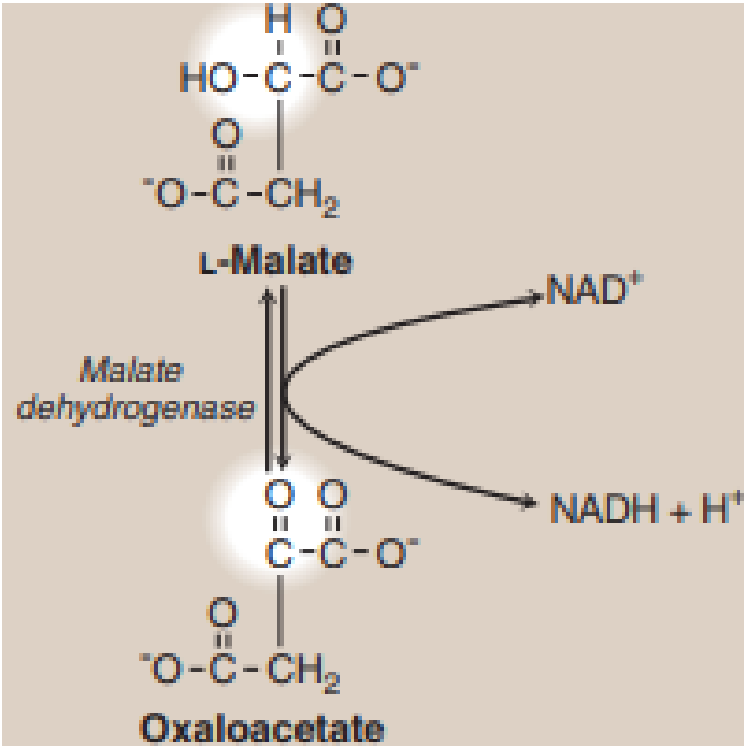
Step 7: Hydration of fumarate

- Fumarate is hydrated to malate by fumarase (fumarate hydratase)
- A reversible reaction

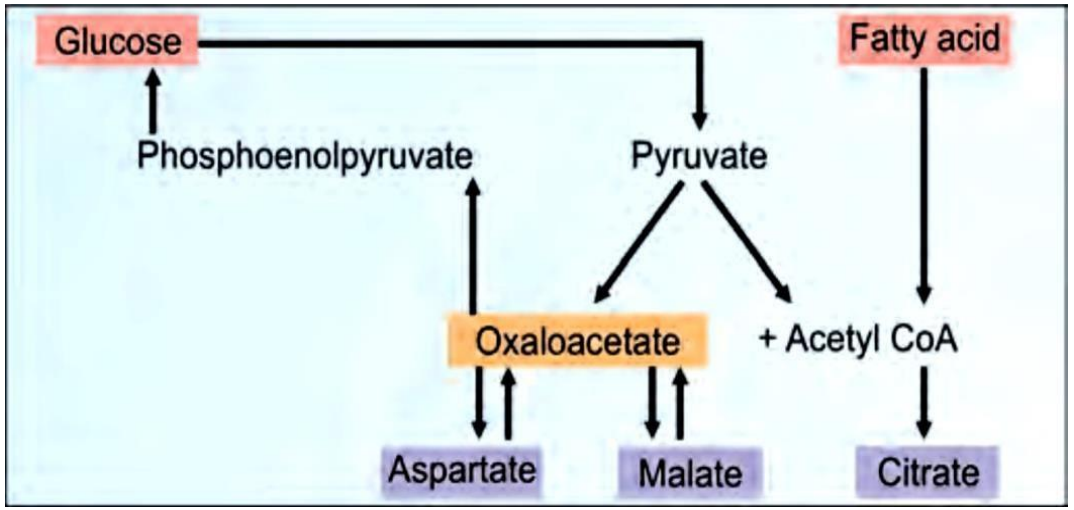


Step 8: Oxidation of malate

- Malate is oxidized to oxaloacetate by malate dehydrogenase
- The Alcohol group of malate oxidized to a keto group
- This reaction produces the third and final NADH of the cycle.
- The ΔG^0 of the reaction is positive, but the reaction is driven in the direction of oxaloacetate by the highly exergonic citrate synthase reaction.



Oxaloacetate as a junction point



- **An important junction point in metabolism**

Bioenergetics of TCA Cycle

- Like all pathways, overall net -ΔG (-228 kcal/mole)
- 3NADH, FAD(H2), and GTP= (10ATP)
- Three reactions have large (-ve) values
- Physiologically irreversible, low products

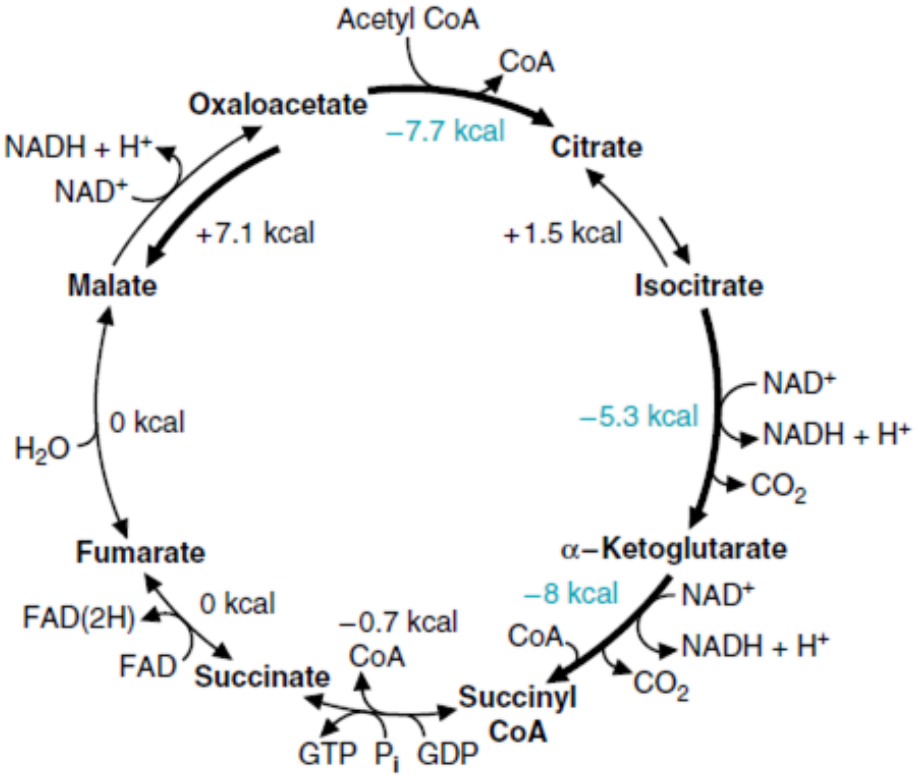
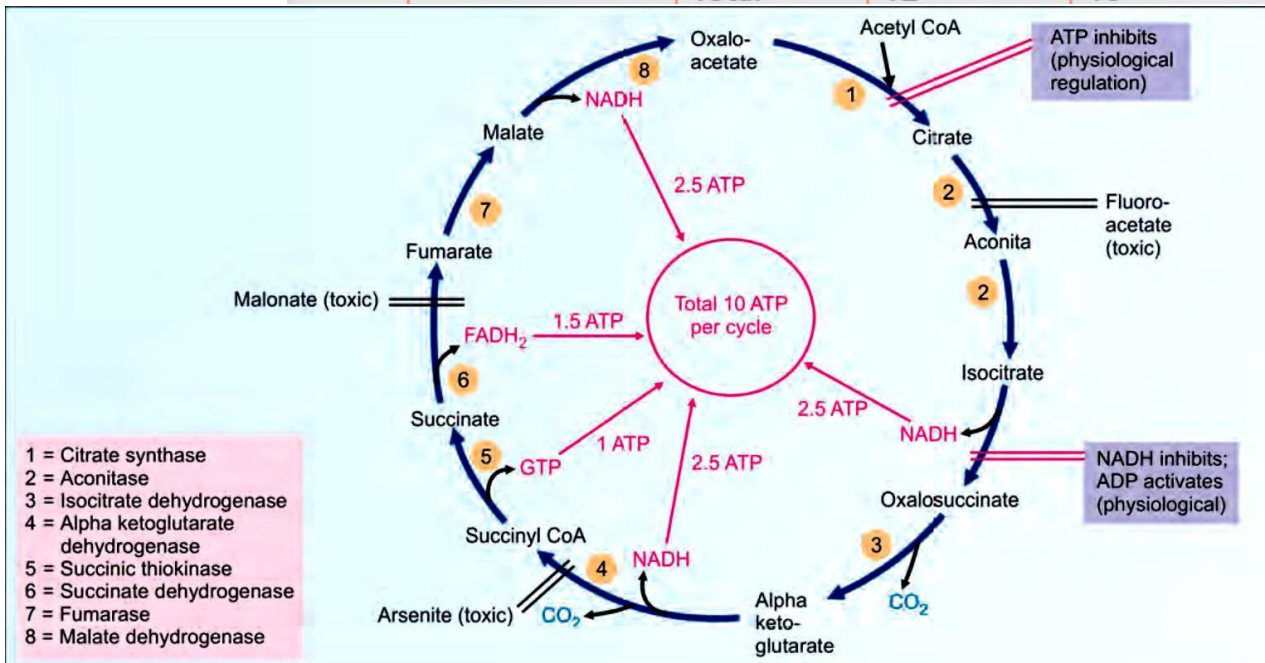


TABLE 19.1: ATP generation steps

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



Net Result of TCA Cycle and its significance

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

Box 19.1: Significance of citric acid cycle

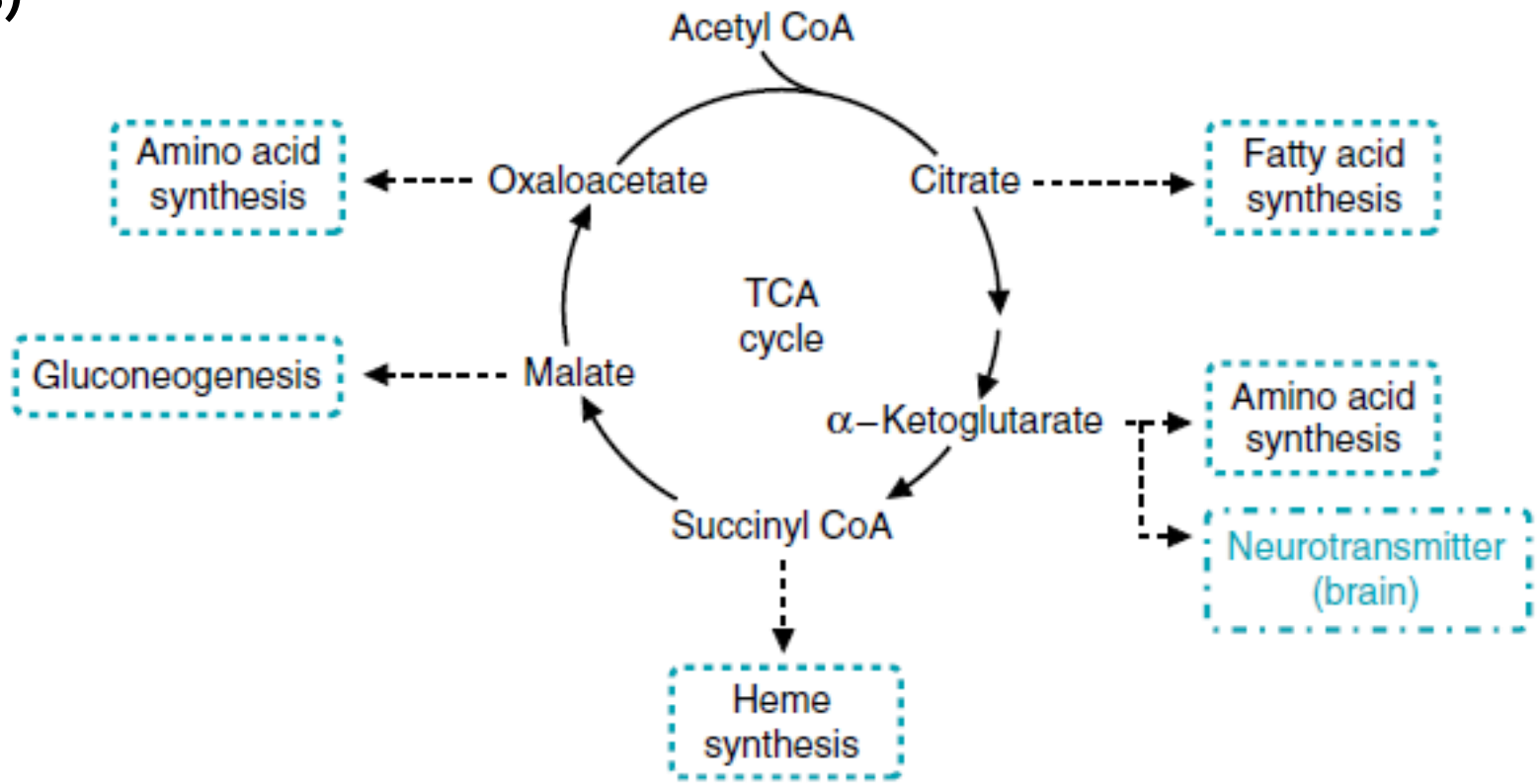
1. Complete oxidation of acetyl CoA
2. ATP generation
3. Final common oxidative pathway
4. Integration of major metabolic pathways
5. Fat is burned on the wick of carbohydrates
6. Excess carbohydrates are converted as neutral fat
7. No net synthesis of carbohydrates from fat
8. Carbon skeletons of amino acids finally enter the citric acid cycle
9. Amphibolic pathway → A biochemical pathway, which involves both catabolism and anabolism
10. Anaplerotic role. ← Chemical reactions that form intermediates of a metabolic pathway

Why?

- ✓ **Fats are burned in the fire of carbohydrates,** the efficient burning of **fats** for energy depends on the presence of **carbohydrates** to provide the necessary oxaloacetate for the Krebs cycle
- ✓ **Fat cannot be converted to glucose because pyruvate dehydrogenase reaction is an absolutely irreversible step**

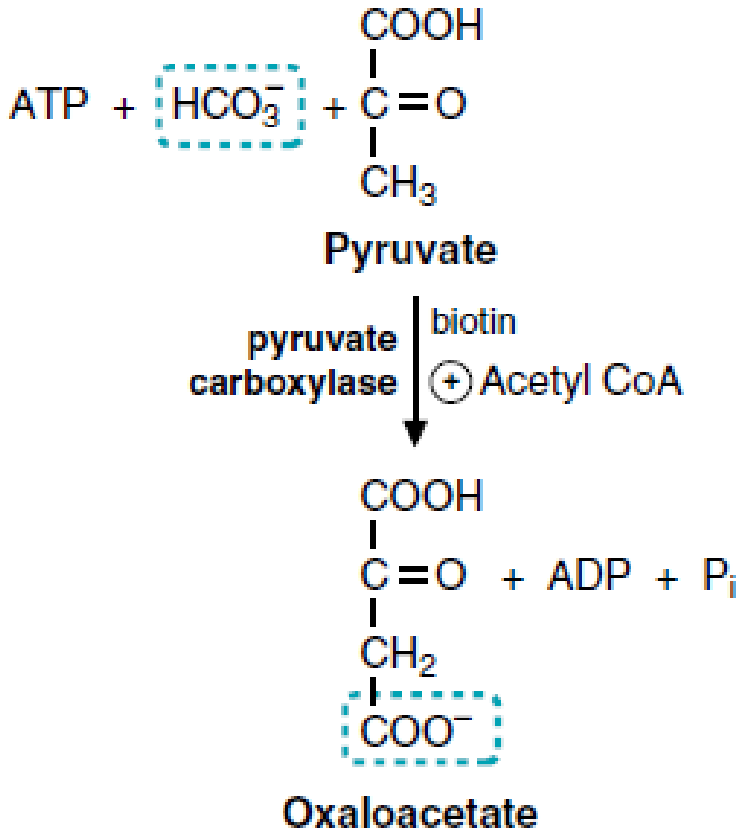
TCA Cycle Intermediates Interactions with Other Pathways

- **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)**

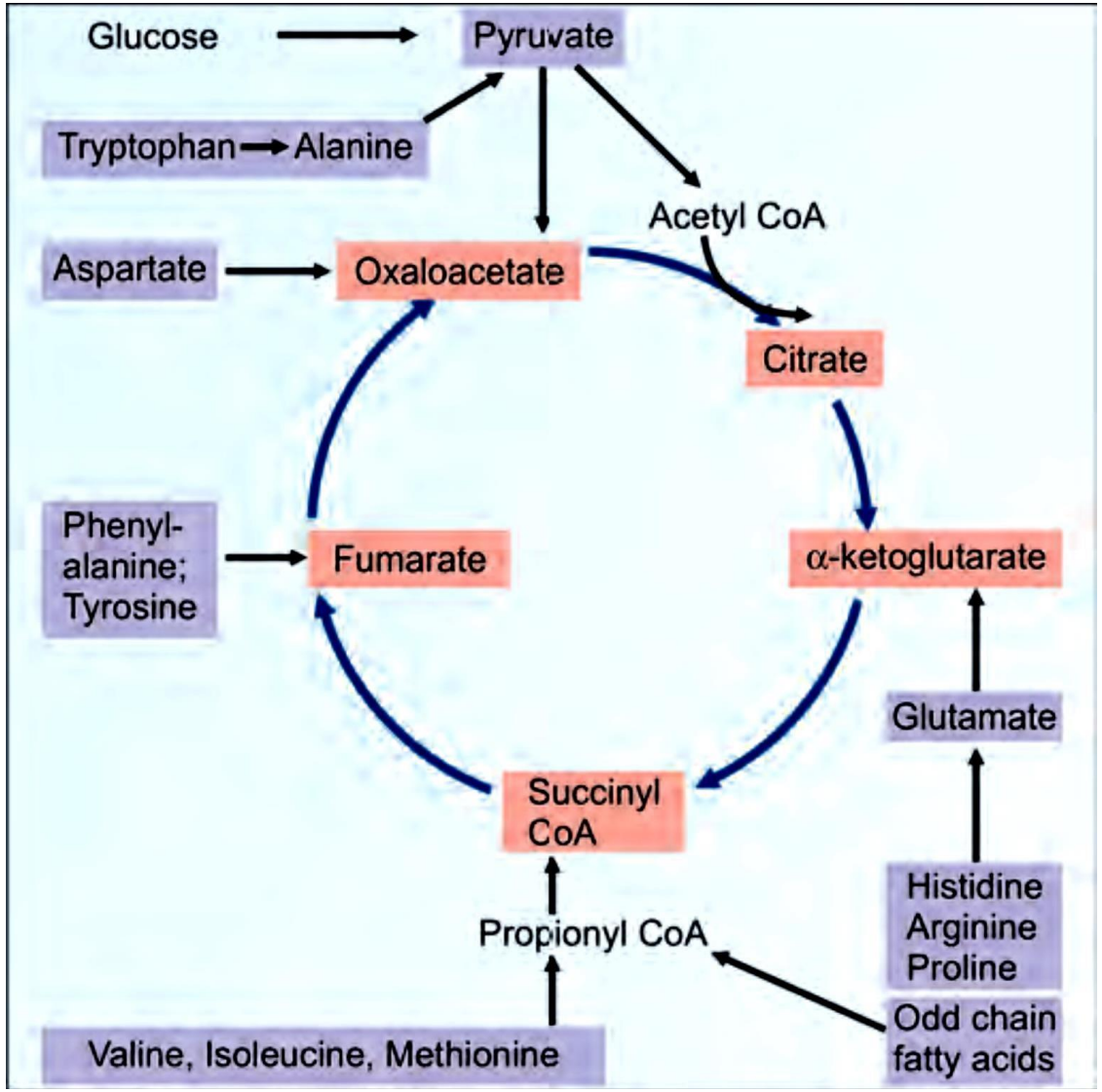


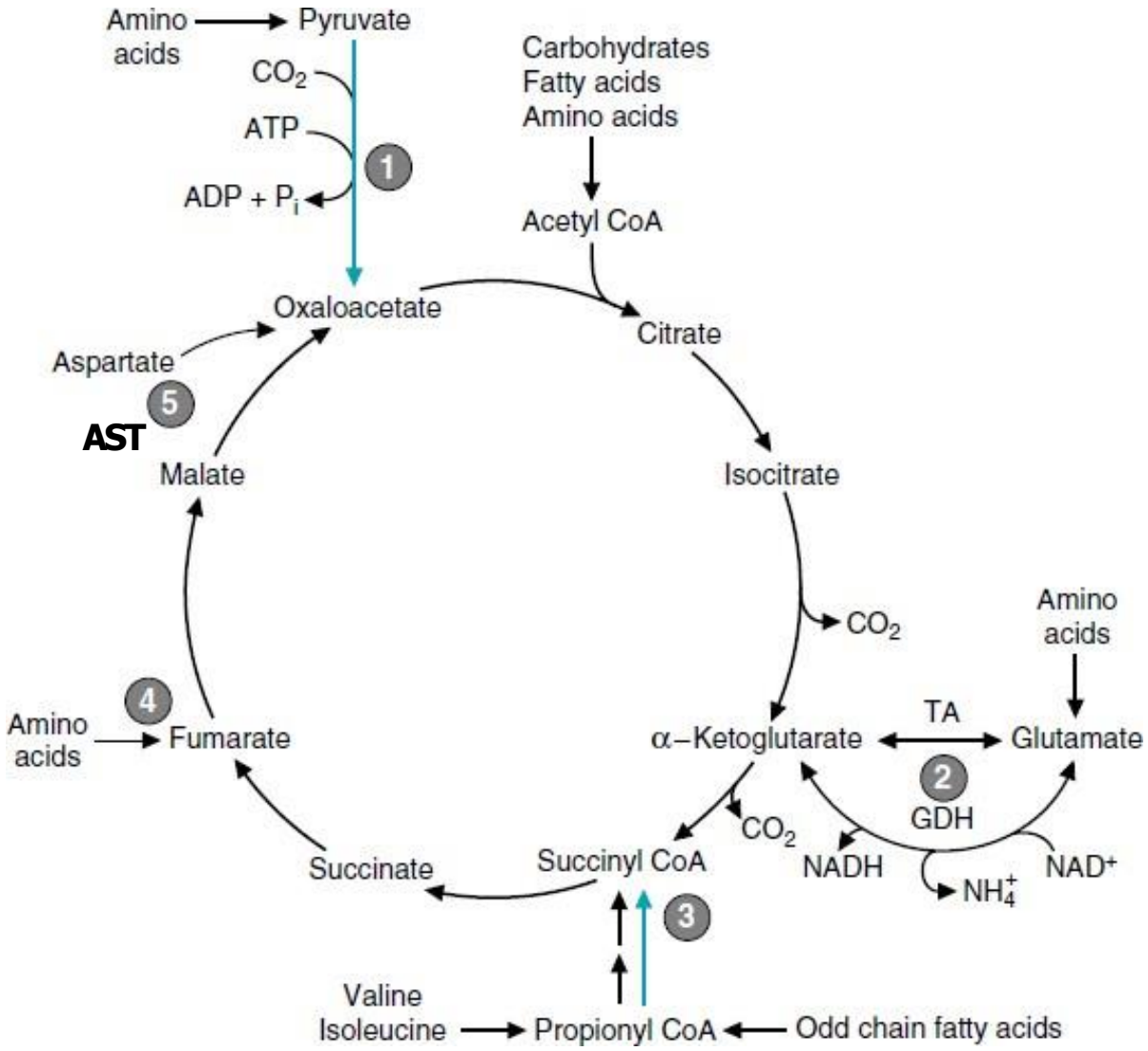
Anapleiotropic Routes

- Pathways or reactions that replenish the intermediates of the TCA cycle
- Pyruvate Carboxylase is a major anaplerotic enzyme (requires biotin)
- Found in many tissues, liver, kidneys, brain, adipocytes, and fibroblasts
- Very high conc. In liver and kidney (gluconeogenic pathway)
- Activated (acetyl CoA)



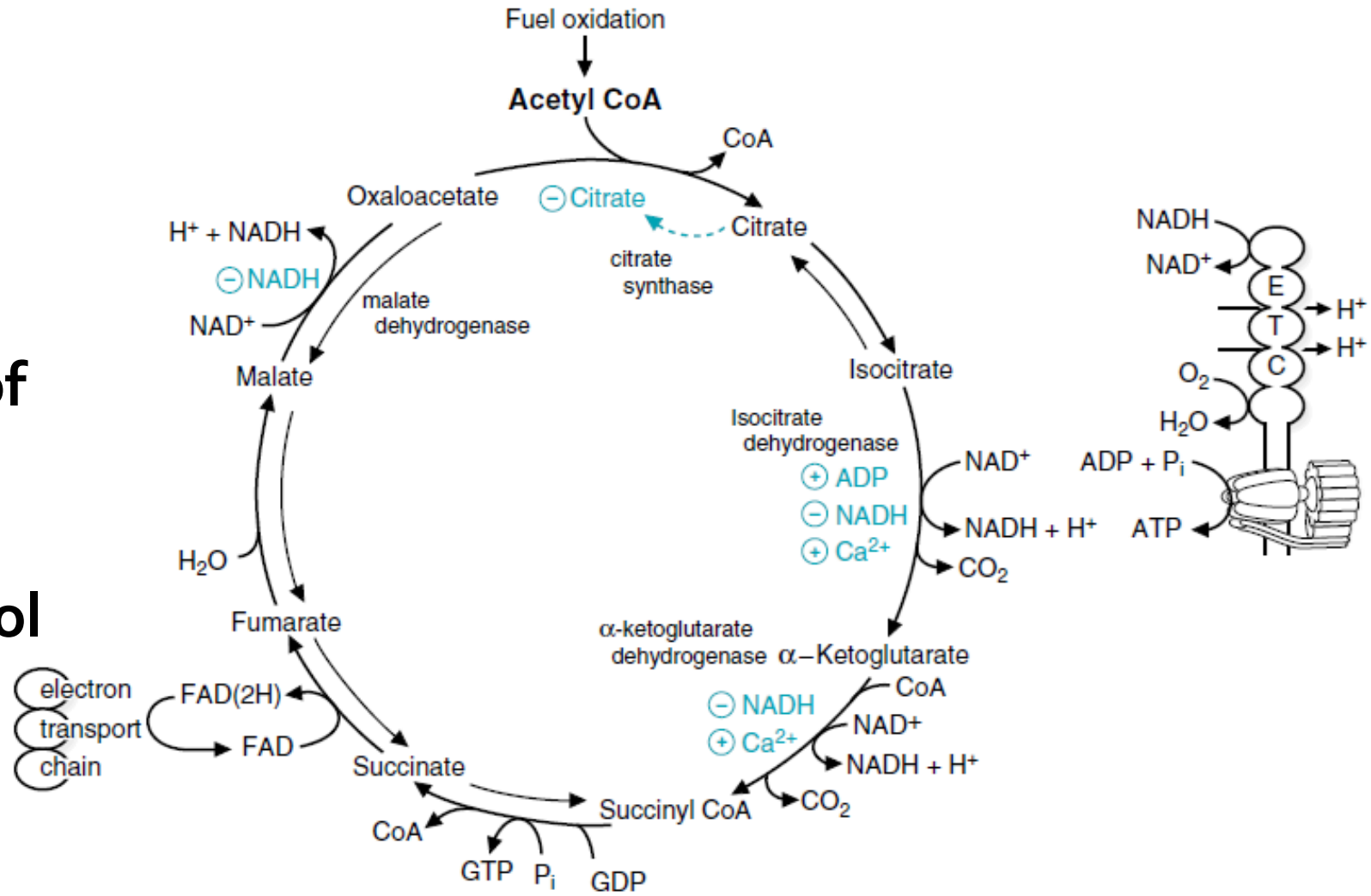
Other Anapleiotropic Routes (amino Acid Degradation)





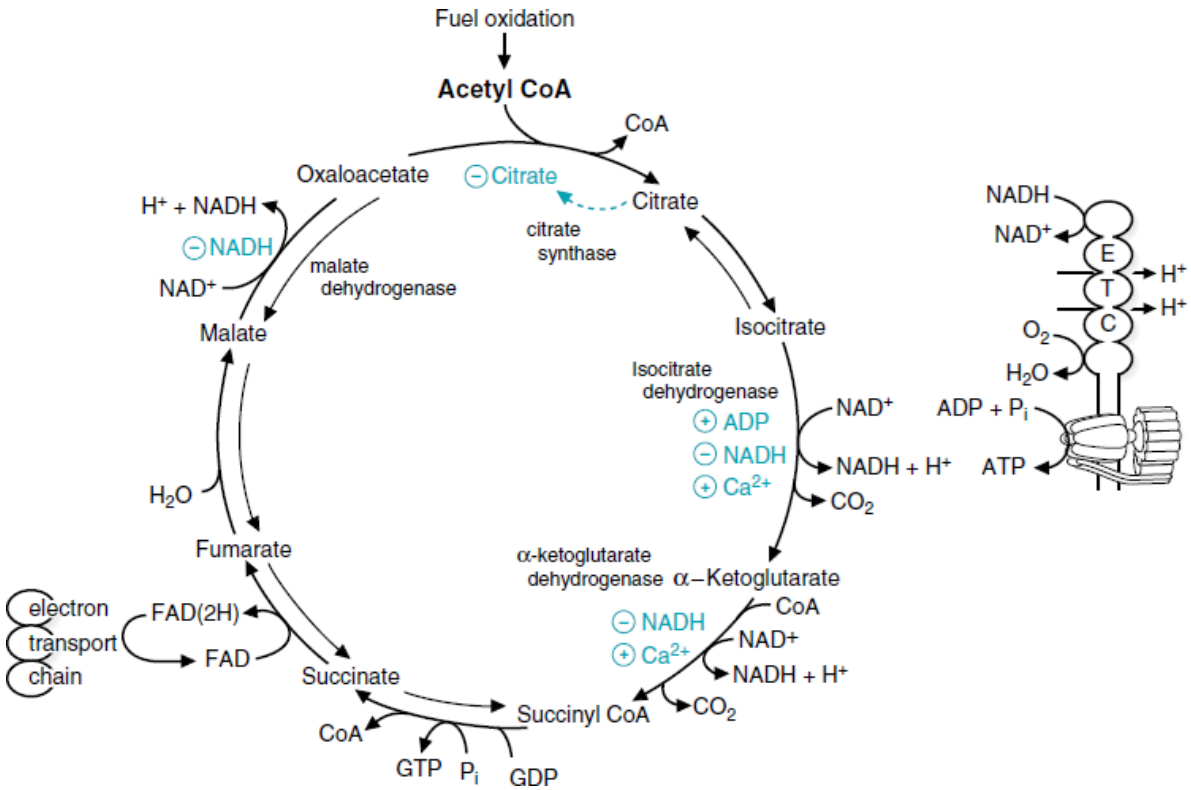
Regulation of the TCA Cycle

- Correspond to ETC (ATP/ADP)
- Two major messengers (feedback): (a) phosphorylation state of adenines, (b) the reduction state of NAD
- Adenine nucleotides pool and NAD pool are relatively constant



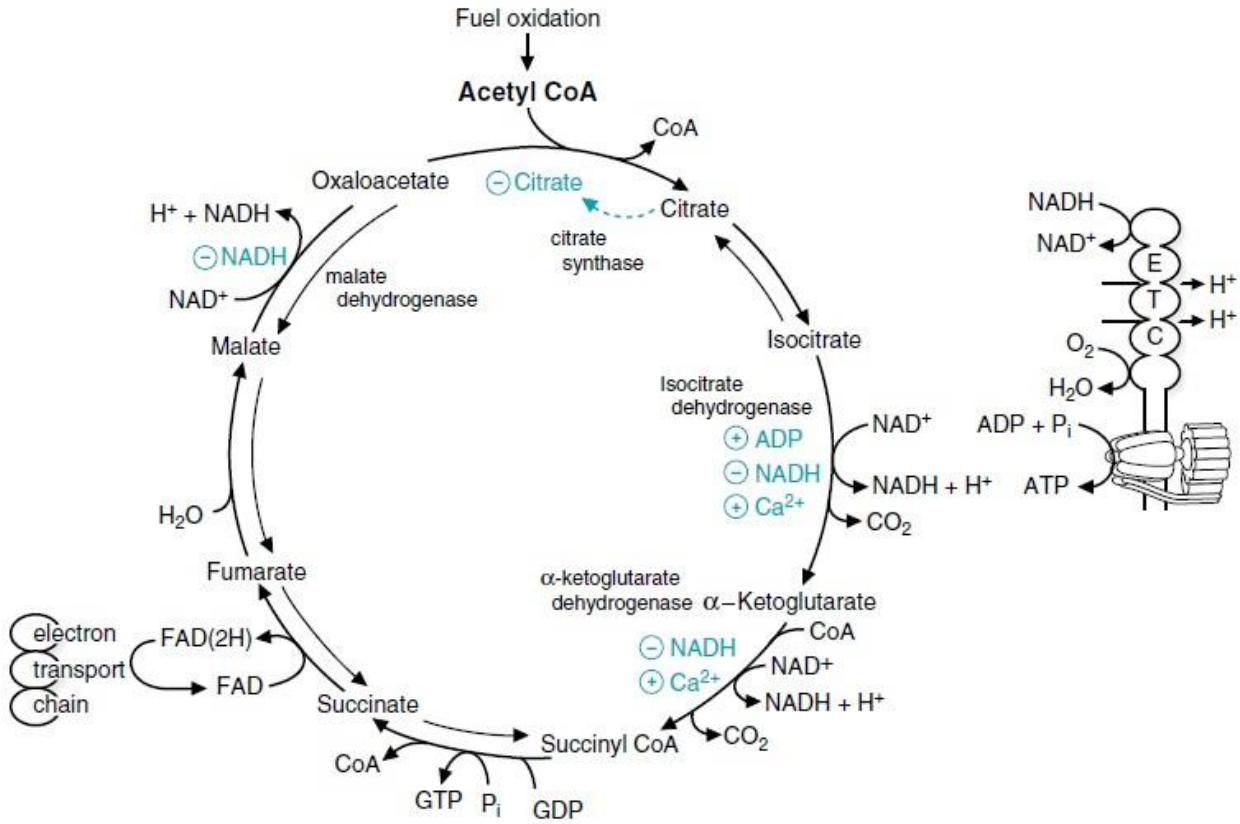
Regulation-Citrate and Citrate Synthase

- 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



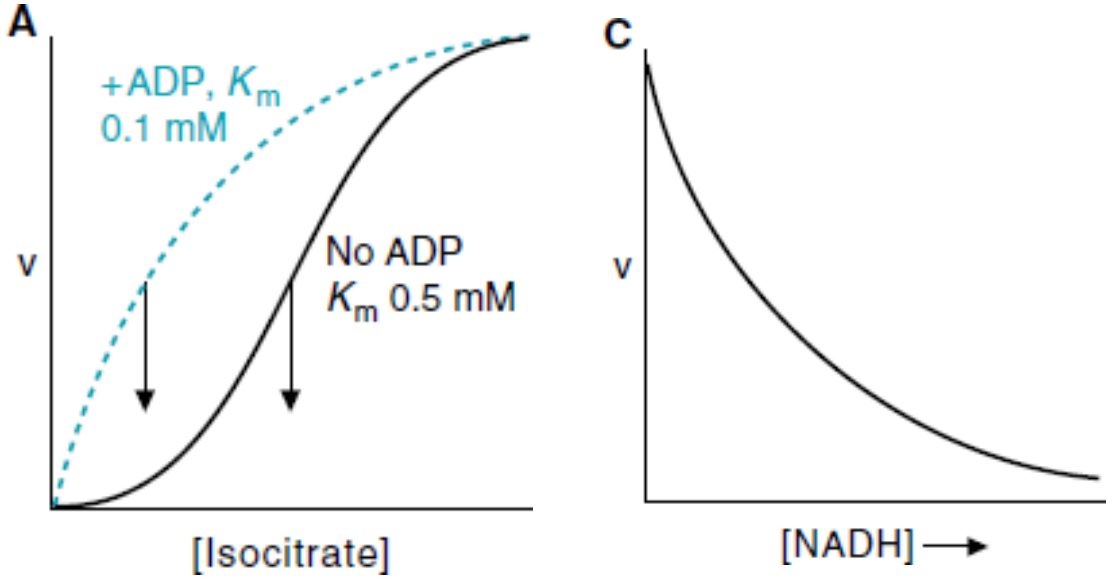
Isocitrate DH

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



α-Ketoglutarate DH

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



Inhibitors of TCA Cycle (Physiological?)

- ✓ A. **Aconitase** (citrate to aconitate) is inhibited by fluoroacetate (non-competitive inhibition)
- ✓ B. **Alpha ketoglutarate dehydrogenase** (alpha keto
- ✓ glutarate to succinyl CoA) is inhibited by Arsenite (non-competitive inhibition)
- ✓ C. **Succinate dehydrogenase** (succinate to fumarate) is inhibited by malonate (competitive inhibition)