Medical Committee
The University of Jordan

SLIDE SHEET

SLIDE : 4-TCA Cycle

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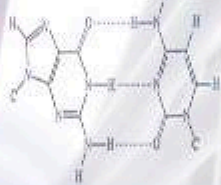
Biochemistry



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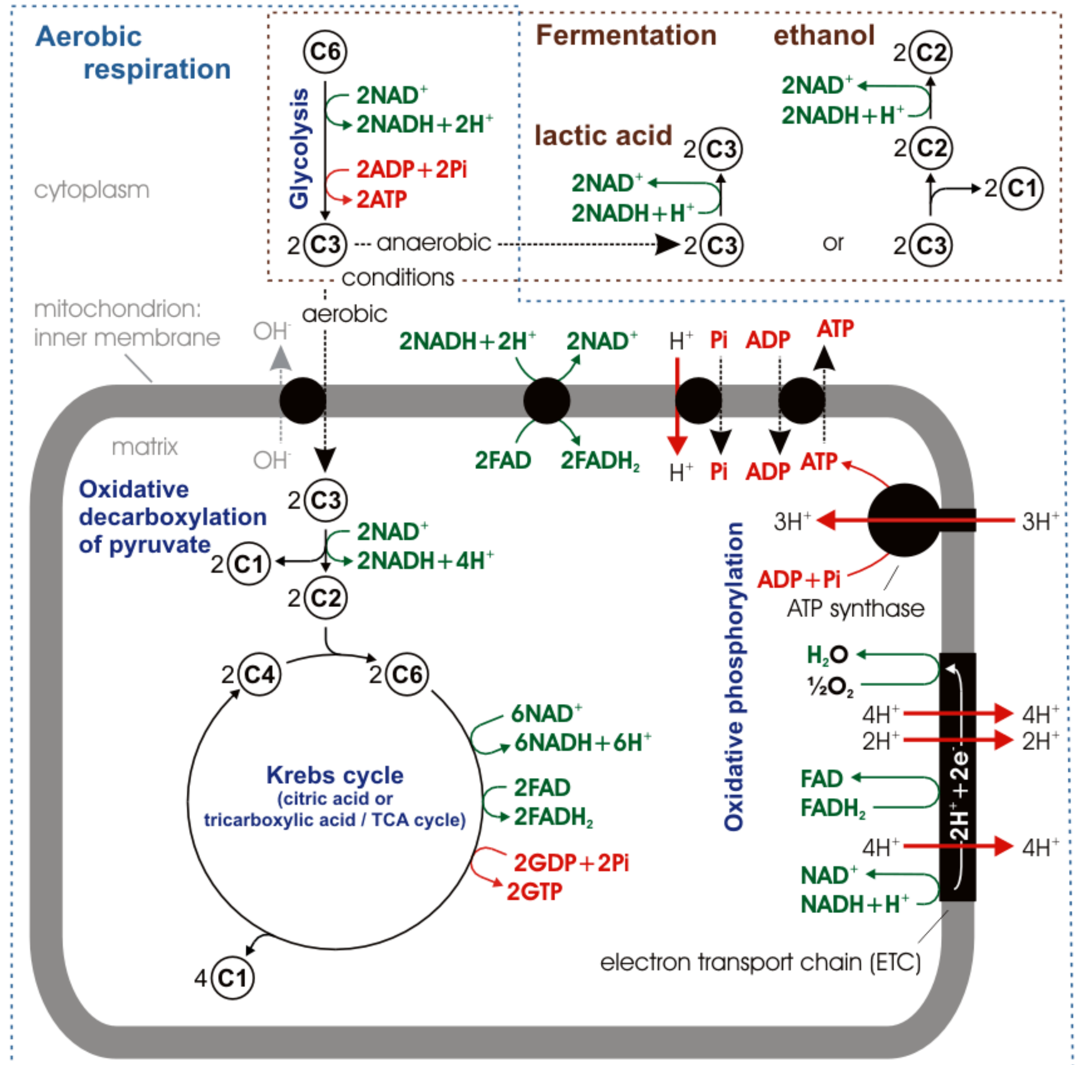
(Kreb's, Citric Acid, TCA) Cycle

Objectives

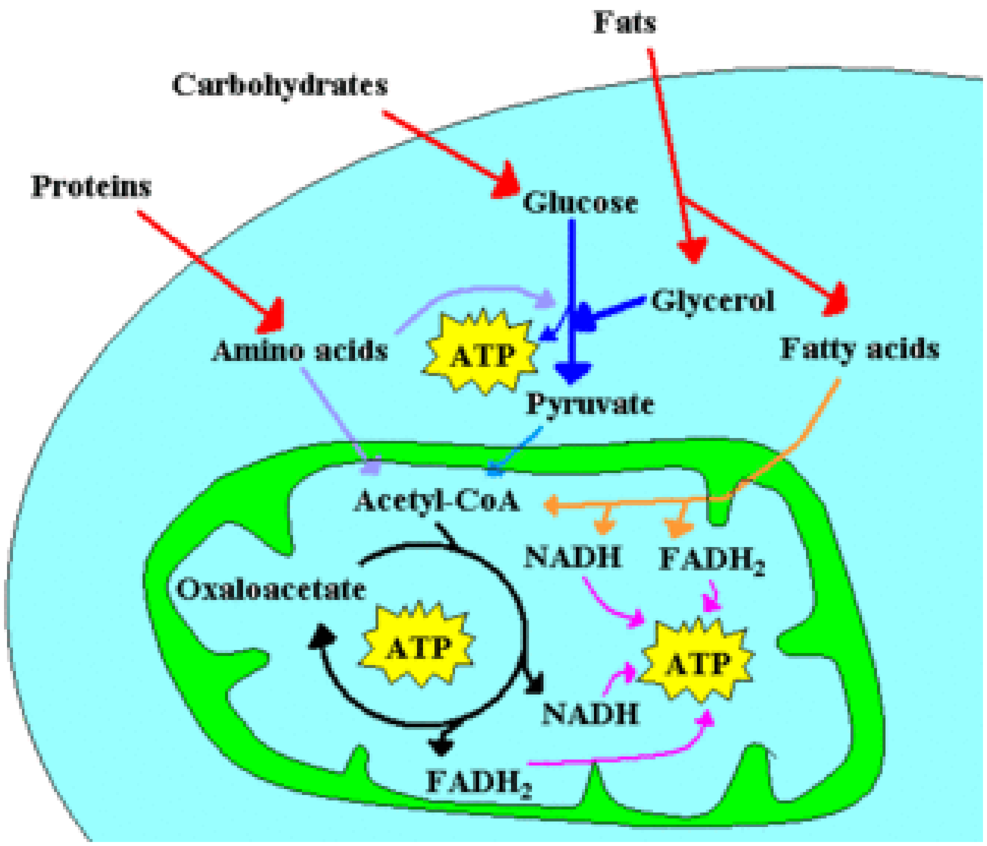
- Why do we need it?
- Location & where is it in the picture?
- Electron (energy) carrying molecules
- Components
- Enzymes & cofactors
- Regulation
- Intermediates & anaplerotic reactions

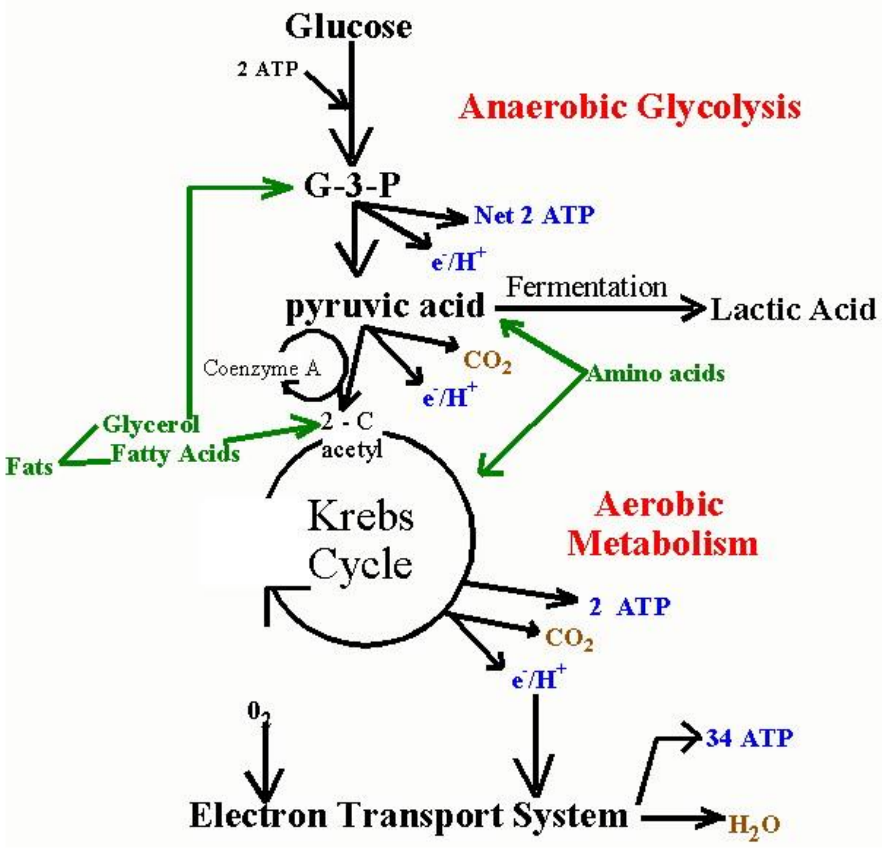
Why do we need it?

- A route to make ATP eventually (most ATP is generated through oxidative phosphorylation)
- There is no life without TCA cycle!



How does it fit?

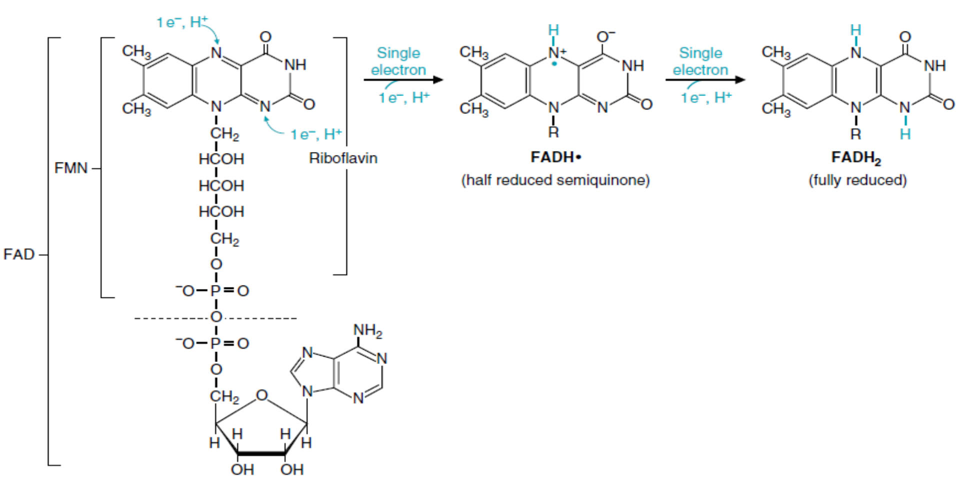




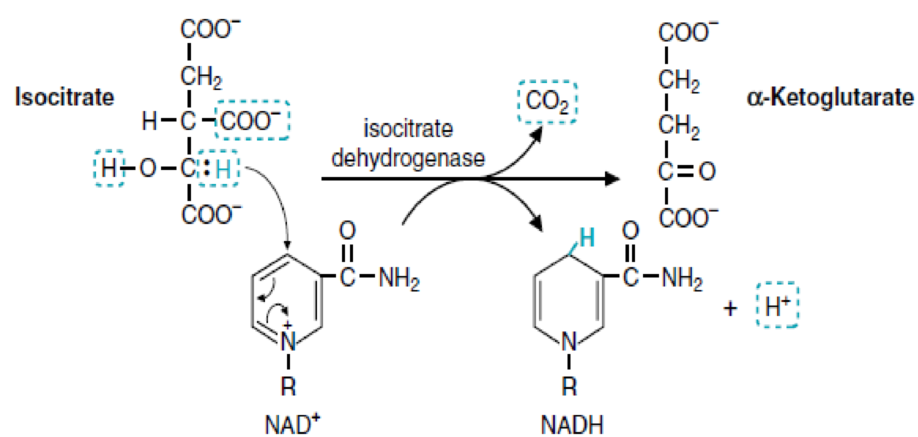
Electron (energy) Carrying Molecules (NAD+, FAD)

- 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*° for enzyme-bound FAD varies

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

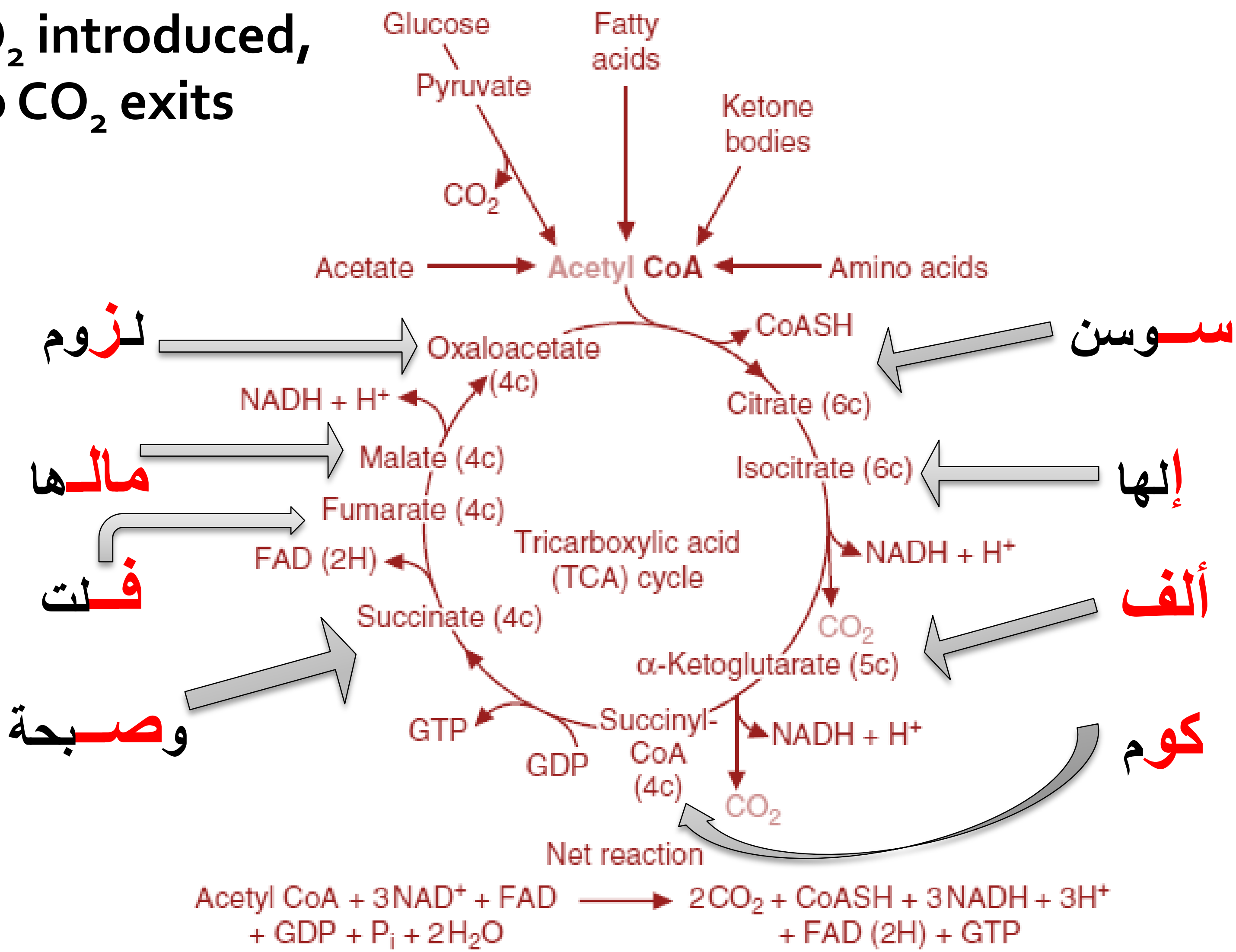


Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)



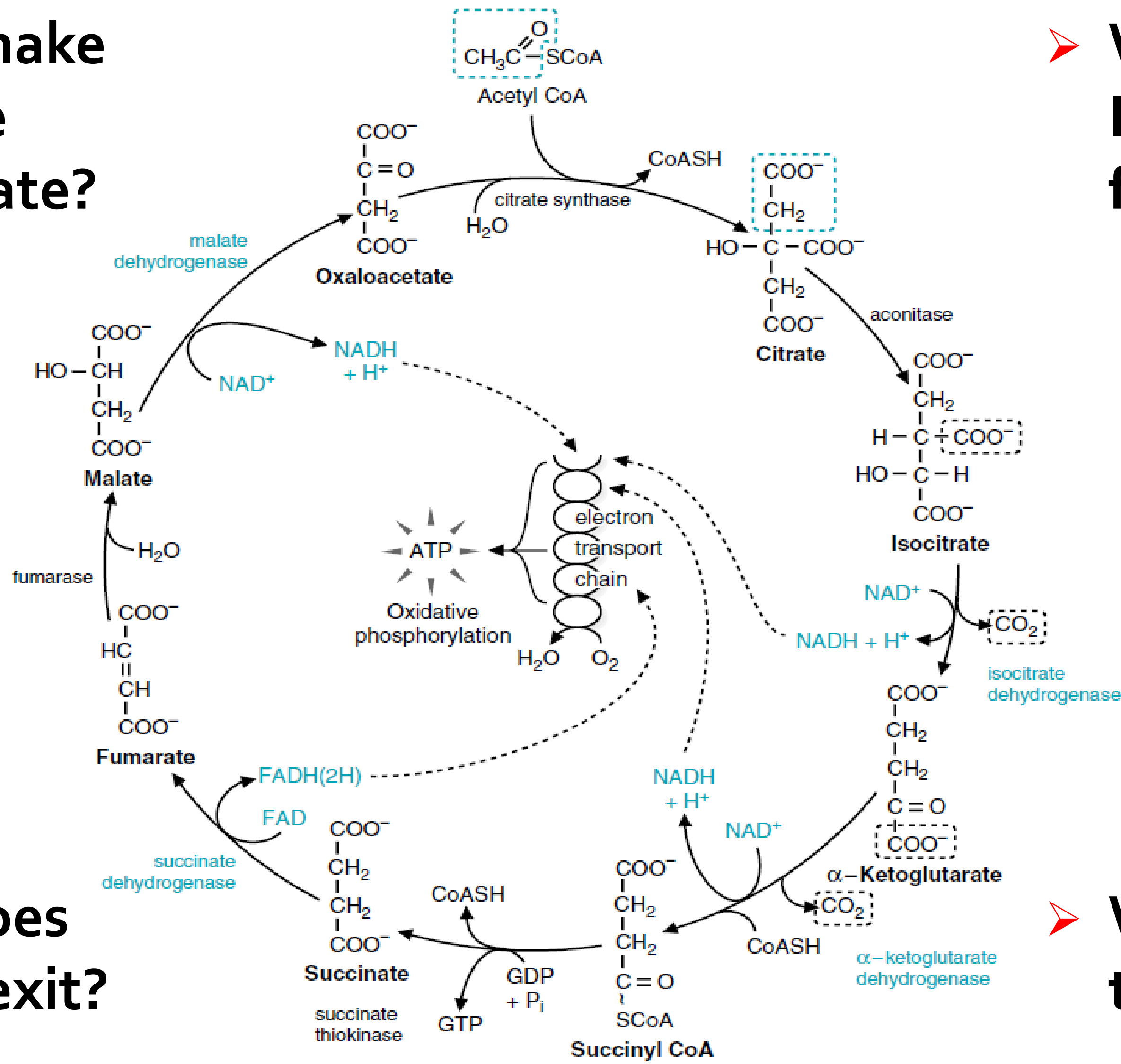
Components & stepwise reactions

- No O_2 introduced, two CO_2 exits



Does Acetyl-CoA exit as 2 CO2?

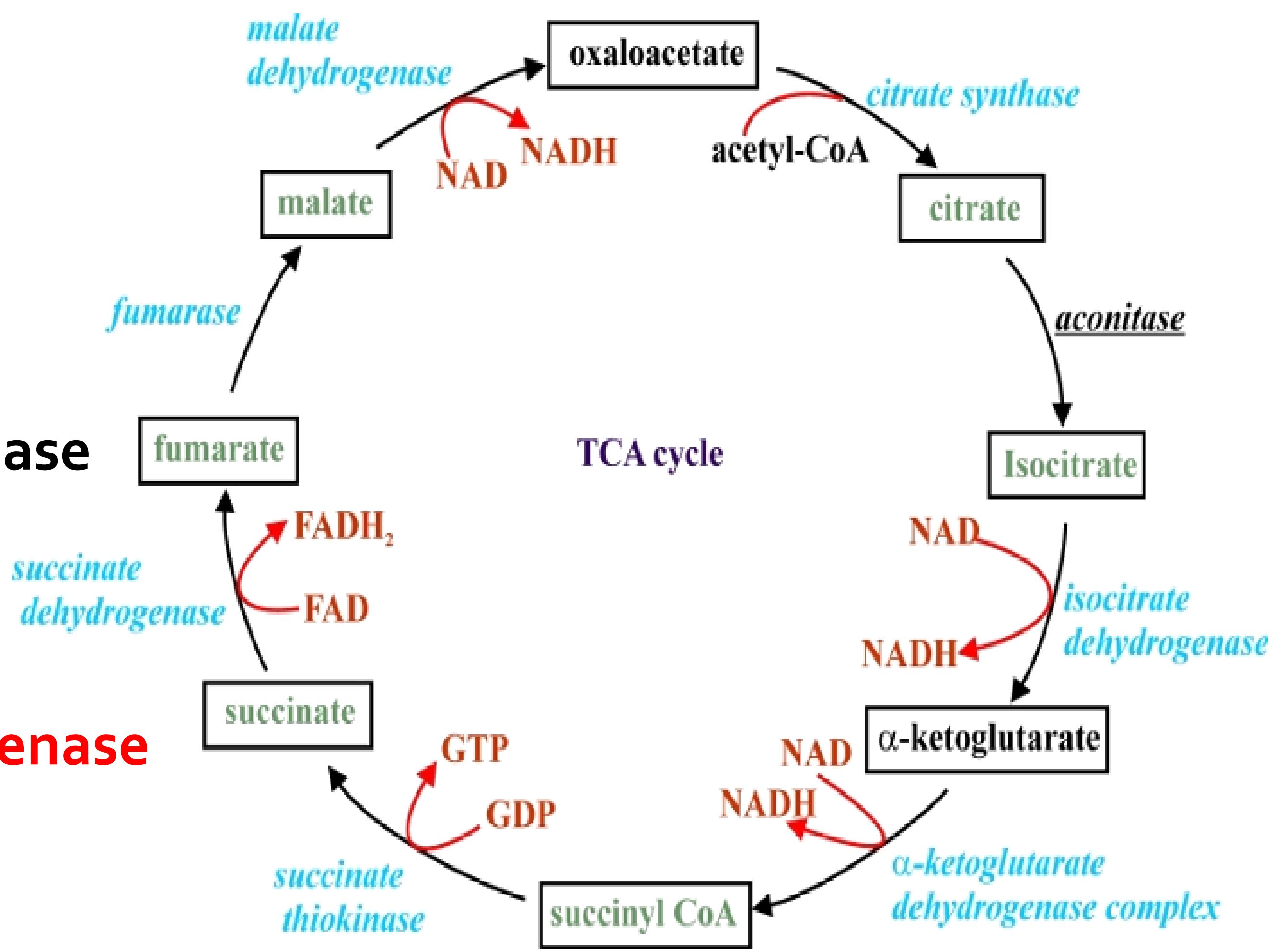
- Why to make Isocitrate from citrate?



- Why to make Isocitrate from citrate?
- Where does the CO2 exit?
- Where does the CO2 exit?

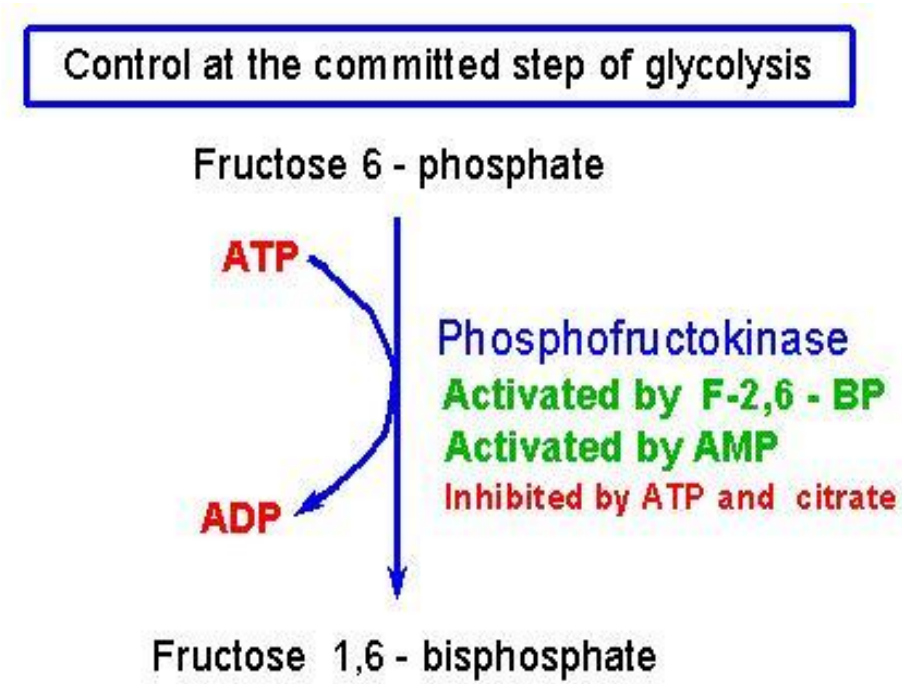
Enzymes of the TCA Cycle

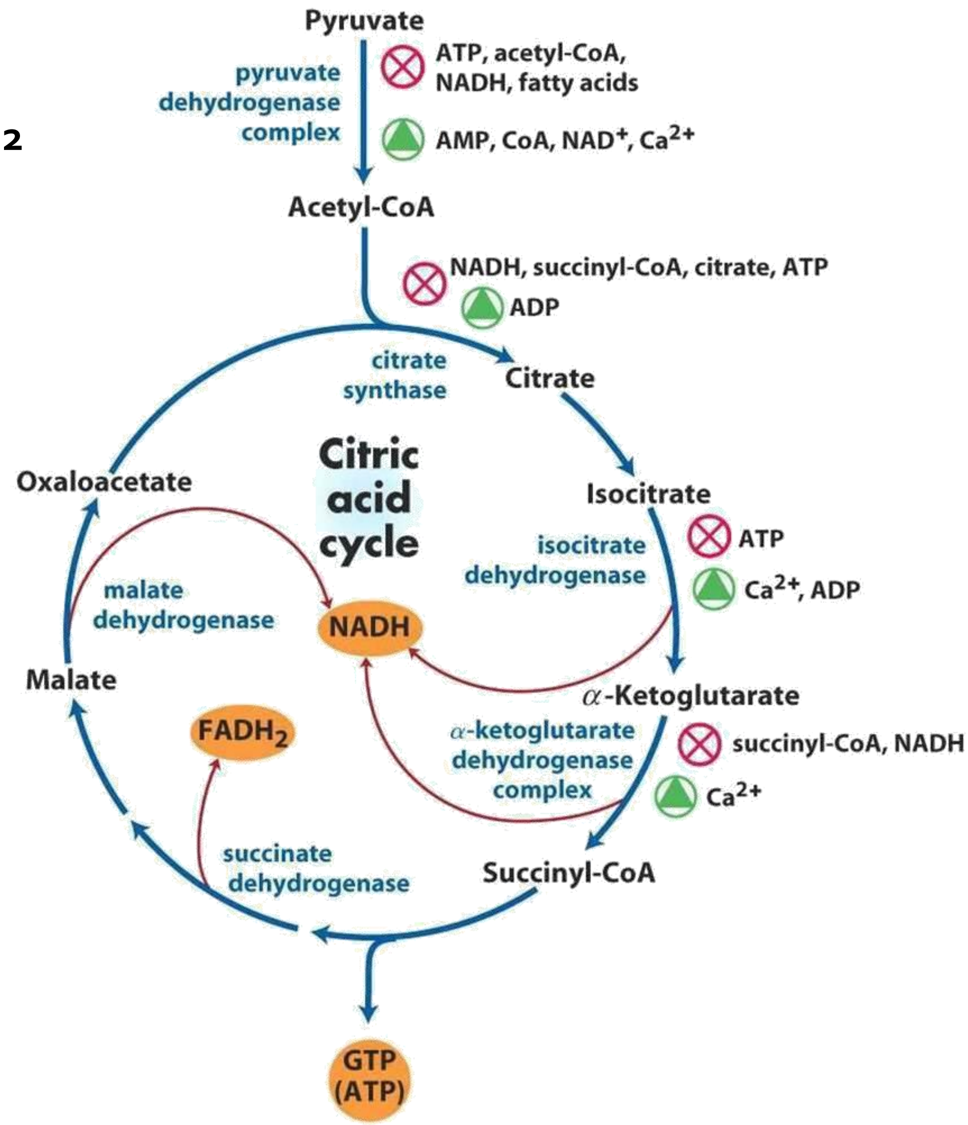
- Citrate cynthase
- Aconitase
- Isocitrate dehydrogenase
- α-ketoglutarate dehydrogenase
- Succinate thiokinase
- Succinate dehydrogenase
- Fumarase
- Malate dehydrogenase



Formation and Oxidation of Isocitrate

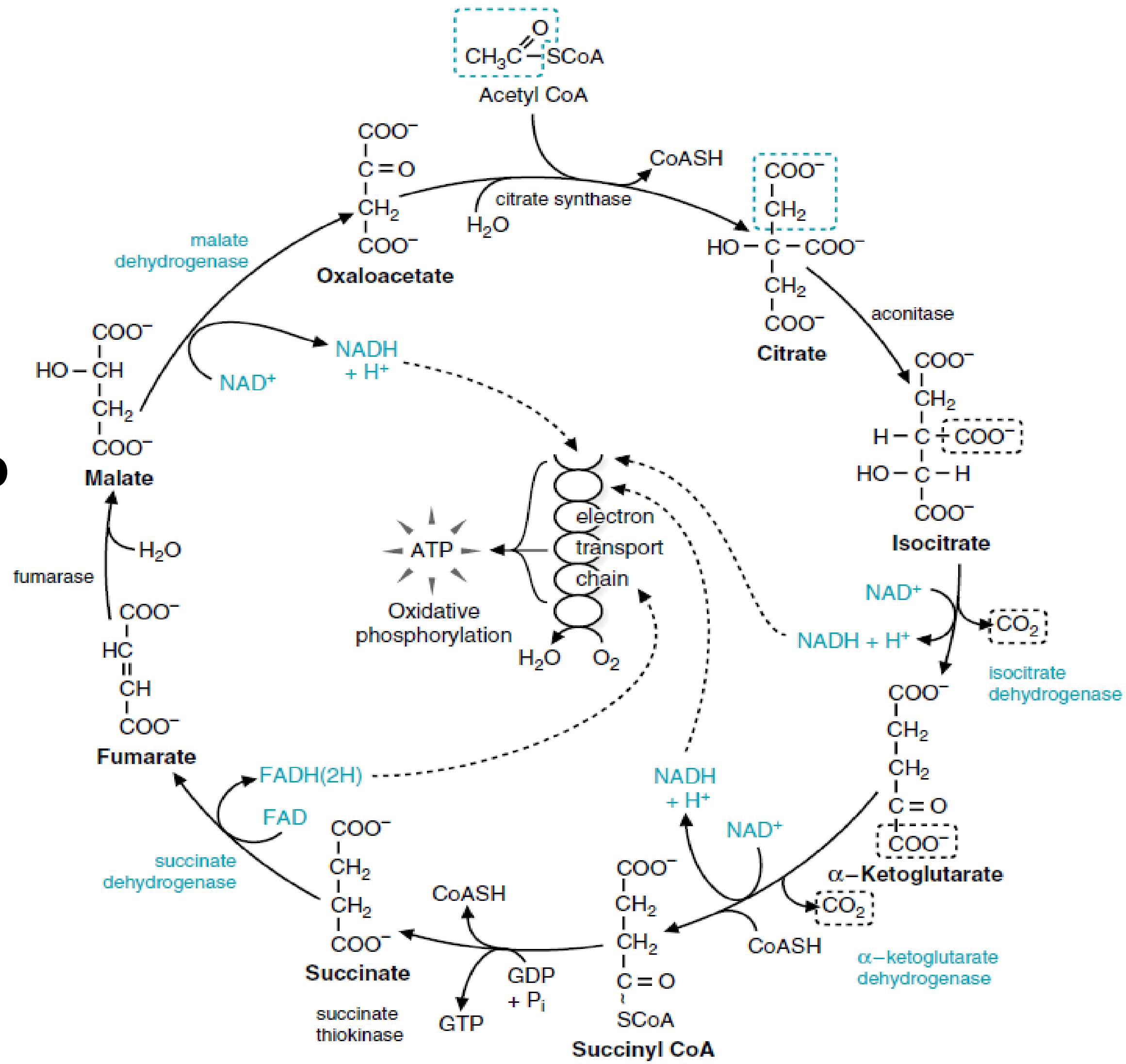
- Oxidative decarboxylation, CO_2
- 3^o to 2^o alcohol





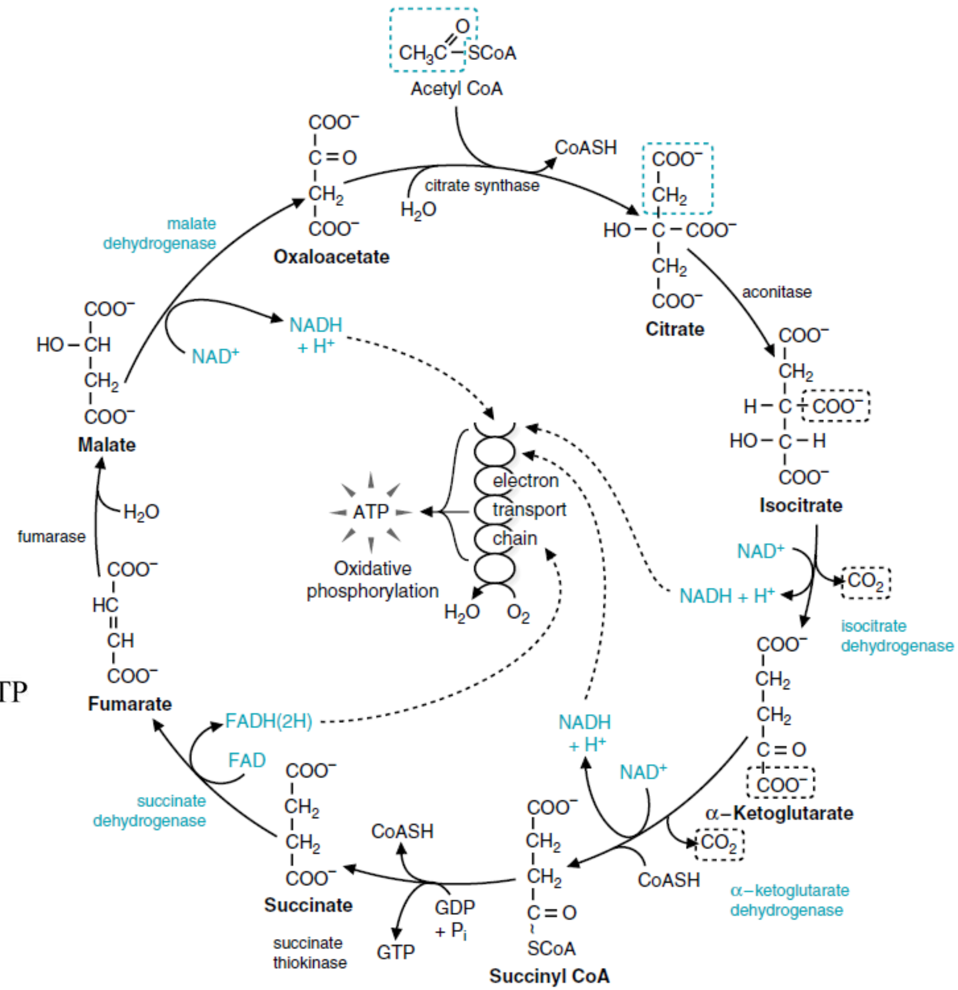
α-Ketoglutarate to Succinyl CoA

- Oxidative decarboxylation
- Thiamine pyrophosphate, lipoic acid, and FAD
- Keto group oxidized to acid, CoA-SH, succinyl CoA
- Energy conserved as NADH, thioester bond



Generation of GTP

- Succinyl CoA thioester bond, succinate thiokinase, substrate level phosphorylation



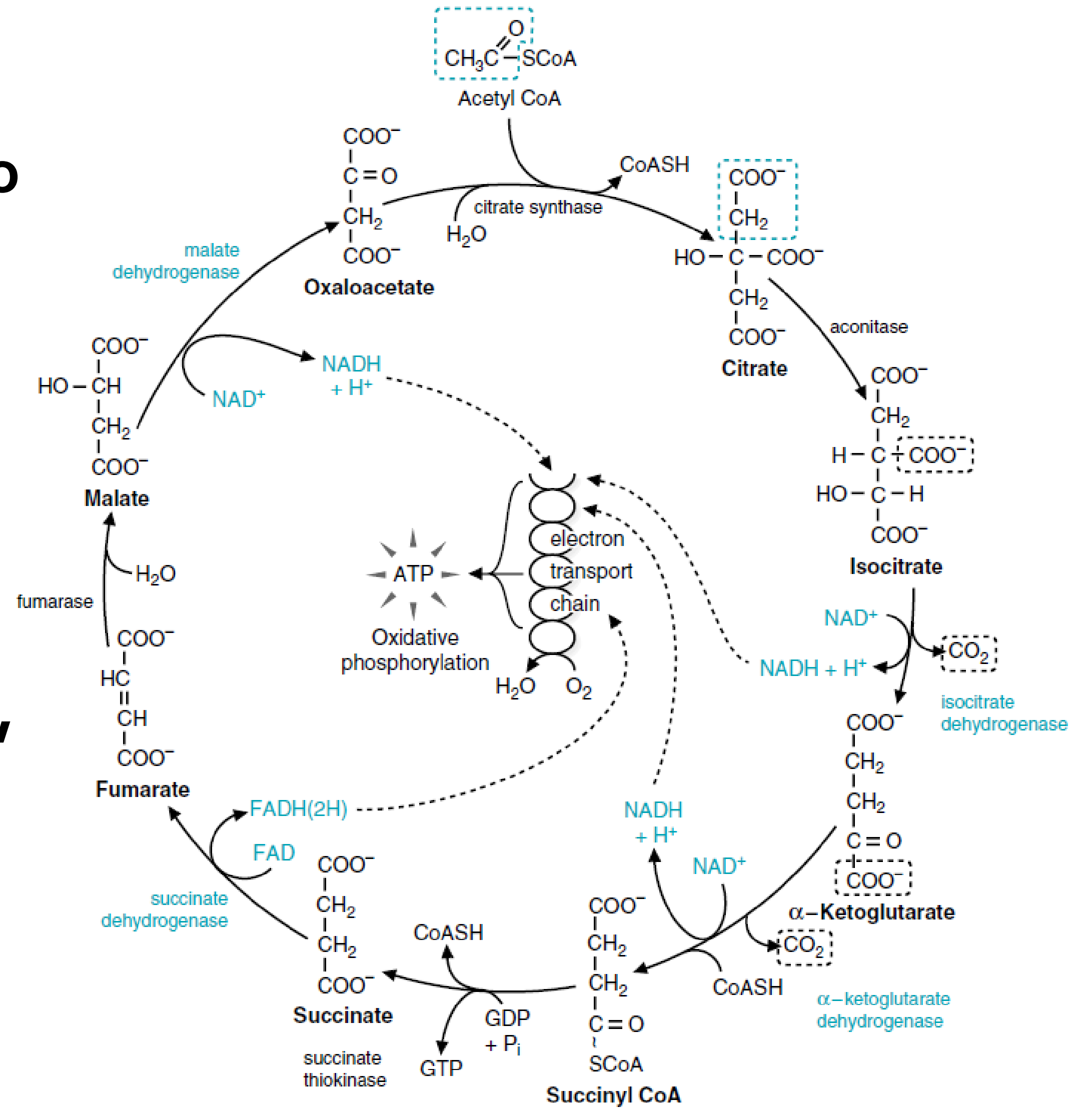
1,3-Diphophoglycerate + ADP ⇌ (Phosphoglycerate kinase) 3-Phophoglycerate + ATP

Phosphoenolpyruvate + ADP ⇌ (Pyruvatekinase) Pyruvate + ATP

SuccinylCoA +GDP ⇌ (SuccinylCoA Synthase) Succinate +GTP

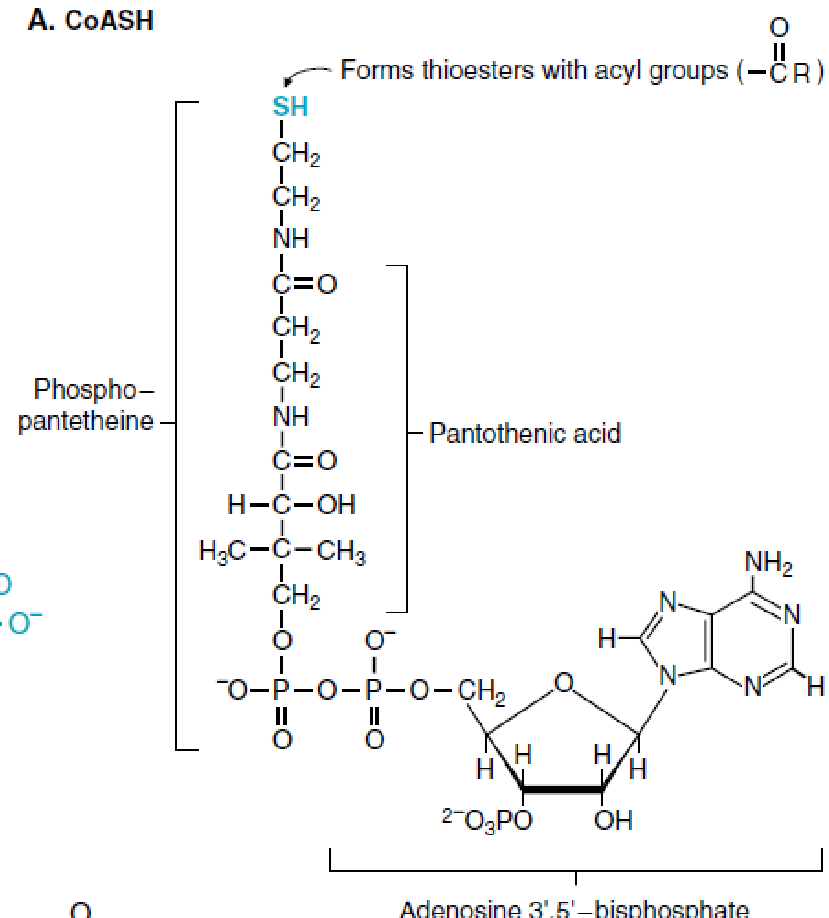
Oxidation of Succinate to Oxaloacetate

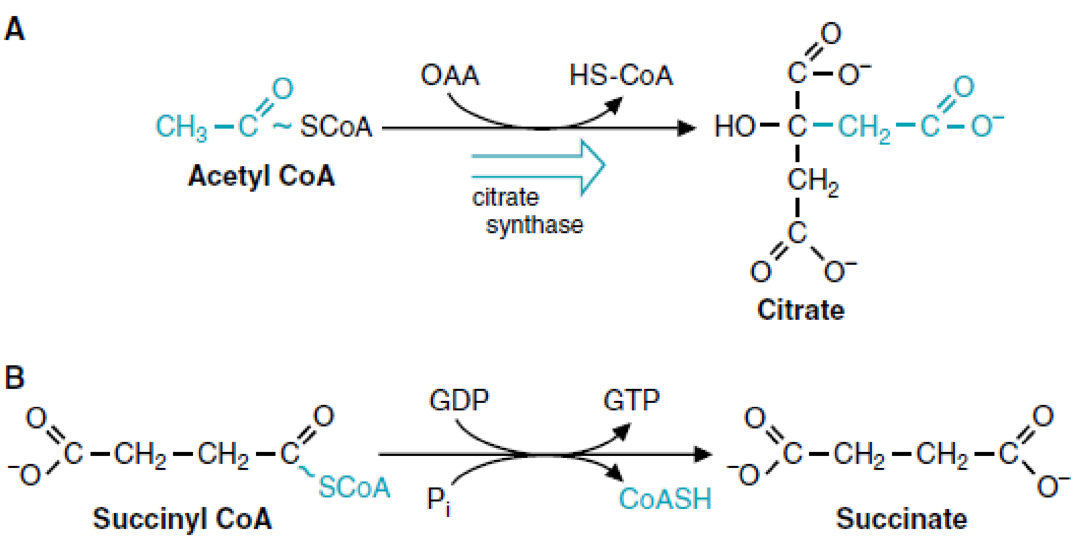
- Oxidation of succinate to fumarate, succinate dehydrogenase, FAD
- Fumarase, OH + H^+ from water, fumarate to malate
- Alcohol group of malate oxidized to a keto group, NADH



CoA

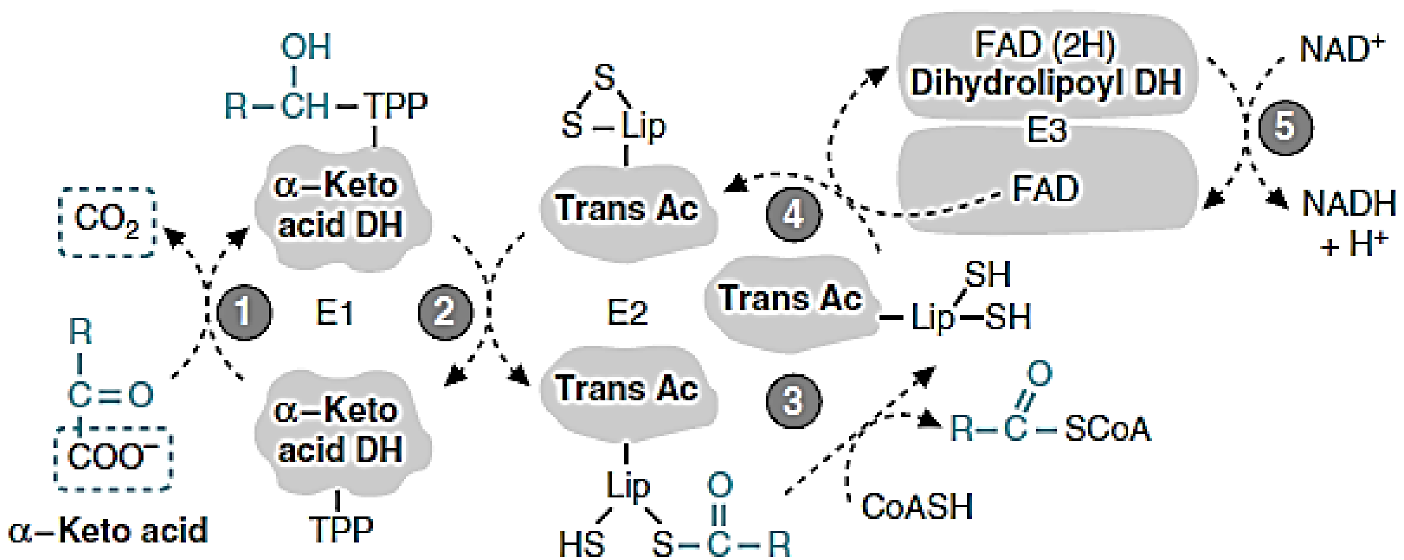
- Forms a thioester bond, CoASH & an acyl group (e.g., acetyl CoA, succinyl CoA)
- Sulfur vs. oxygen (carbon can be activated, -13kcal, GTP, keeps the reaction going)

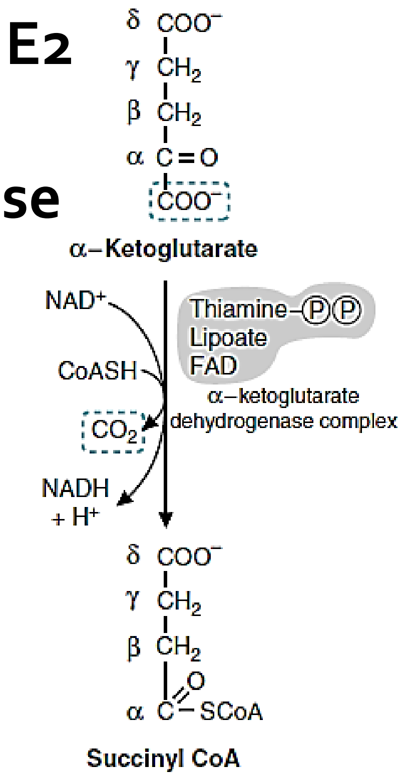




α-Ketoacid Dehydrogenase Complexes (TLCFN)

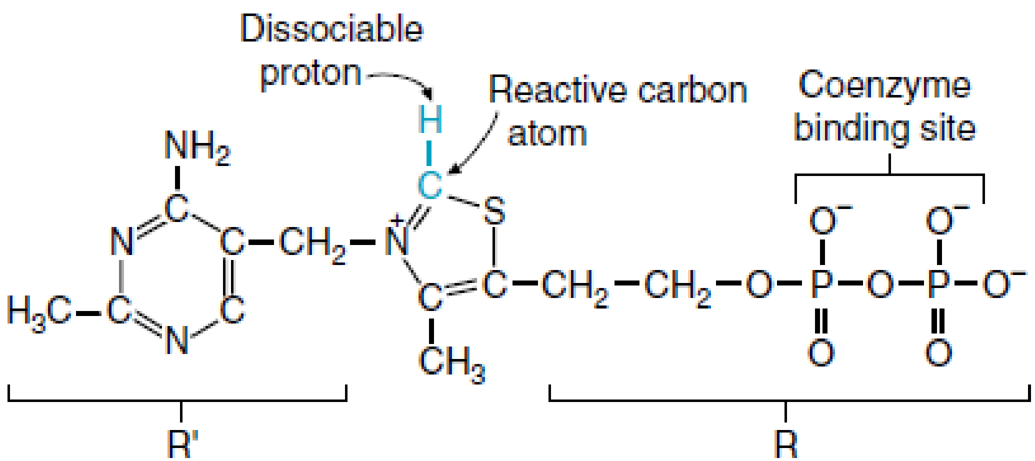
- (α-ketoglutarate, pyruvate, and branched chain α-keto acid) dehydrogenase complexes
- Huge enzyme complexes, multiple subunits of 3 different enzymes (no loss of energy, substrates for E2 and E3 remain bound → higher rate)
- E1, E2, & E3 are a decarboxylase (TPP), a transacylase (lipoate), & a dehydrogenase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



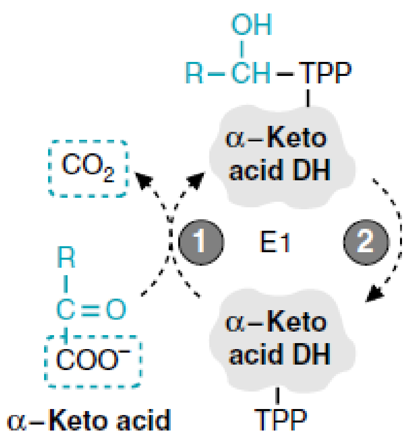


Thiamine Pyrophosphate

- Thiamine deficiency, α-ketoglutarate, pyruvate, & branched chain α-keto acids accumulate in the blood



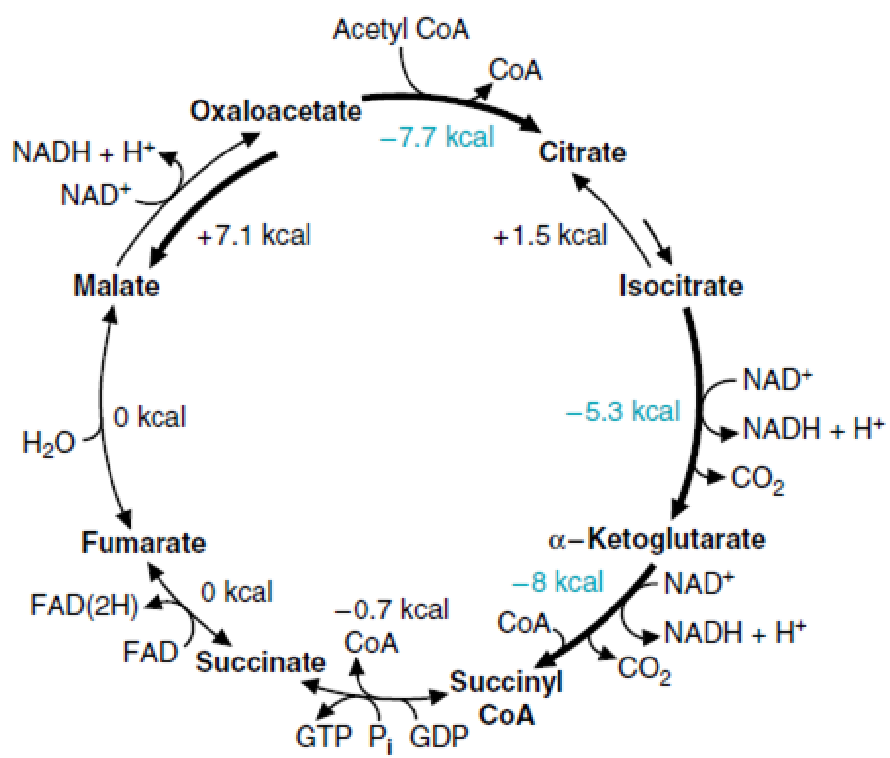
Thiamine pyrophosphate (TPP)



Bioenergitics of the TCA Cycle

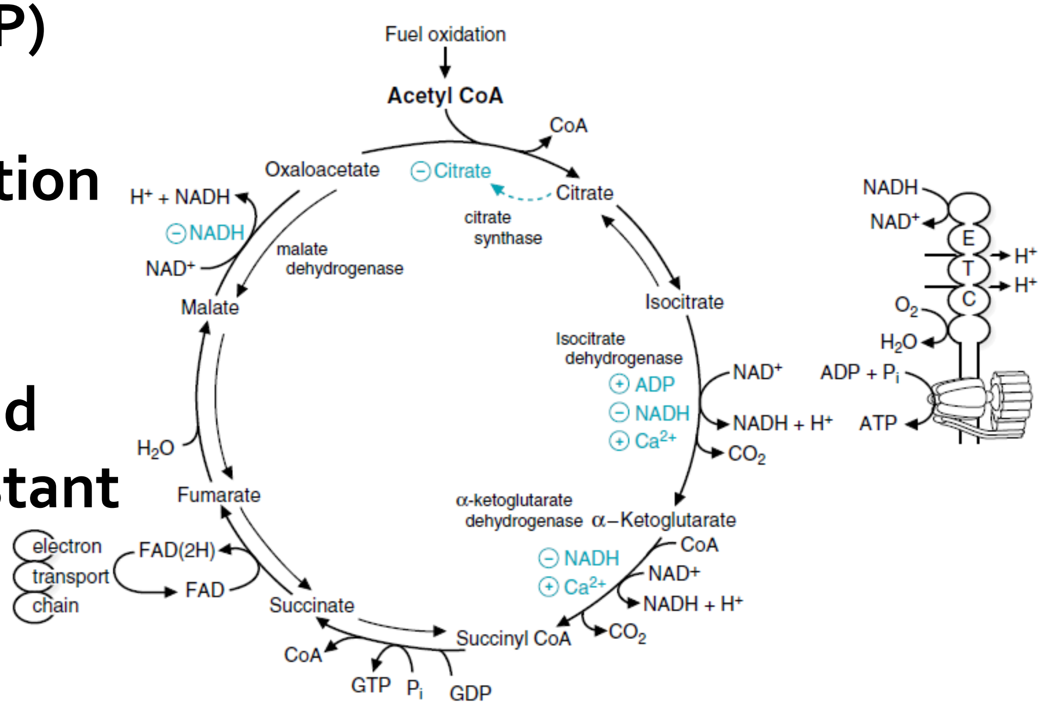
- Like all pathways, overall net -ΔG (-228 kcal/mole), not 100%
- NADH, FAD(H2), and GTP (10ATP), 207 Kcal, 90%
- Three reactions have large (-ve) values
- Physiologically irreversible, low products
- Aconitase (+ΔG), maintains 20 times of citrate/Isocitrate, Acetyl CoA in cytosol for fatty acid and cholesterol synthesis
- Malate DH (+ΔG), favors malate over oxaloacetate, fasting, liver, substrate for gluconeogenesis

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



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

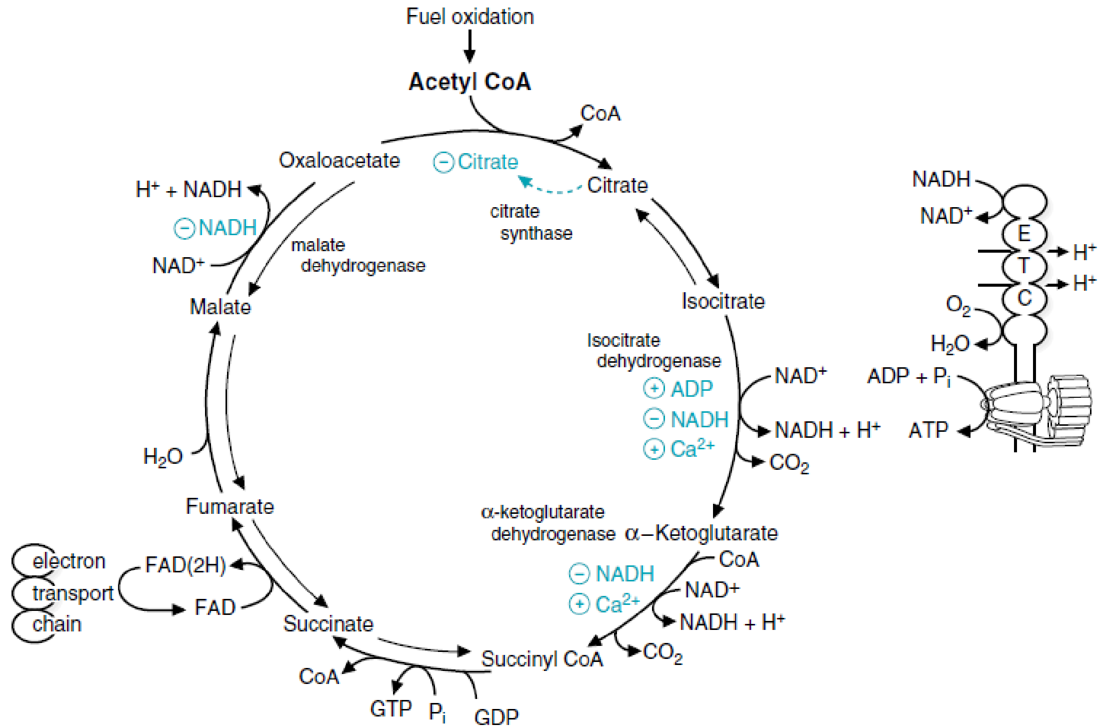


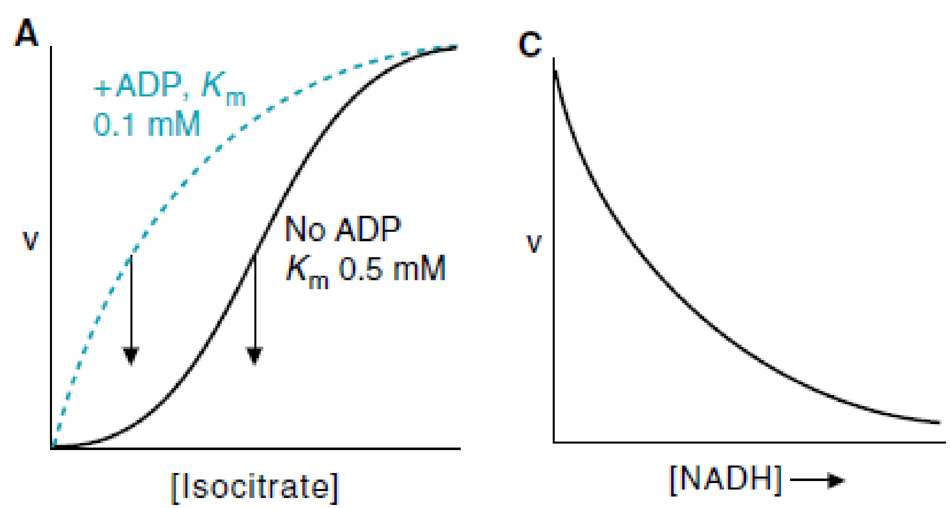
Citrate Synthase

- The first step, no allosteric regulation
- Rate regulated by oxaloacetate & citrate (inhibitor)
- Malate-oxaloacetate equilibrium has [ ] < K_m, NADH/NAD^+ (acetyl-CoA will be used for ketone bodies)

Isocitrate DH

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



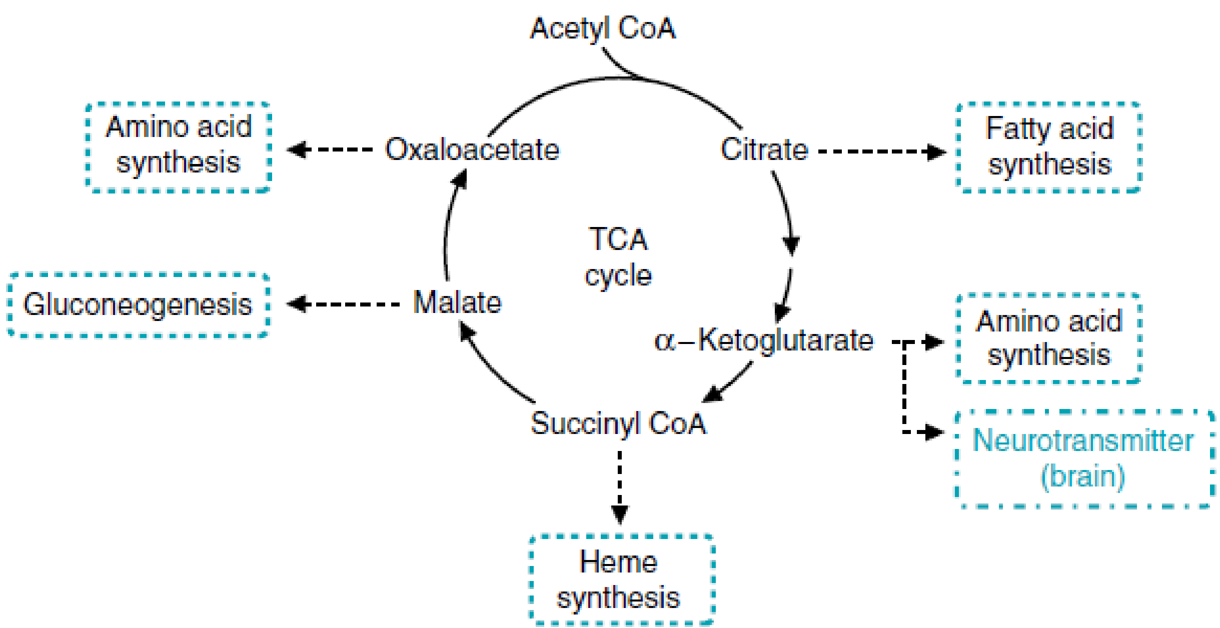


α-Ketoglutarate DH

- Inhibited by NADH and succinyl CoA, GTP
- Activated by Ca^{+2}, muscle contraction

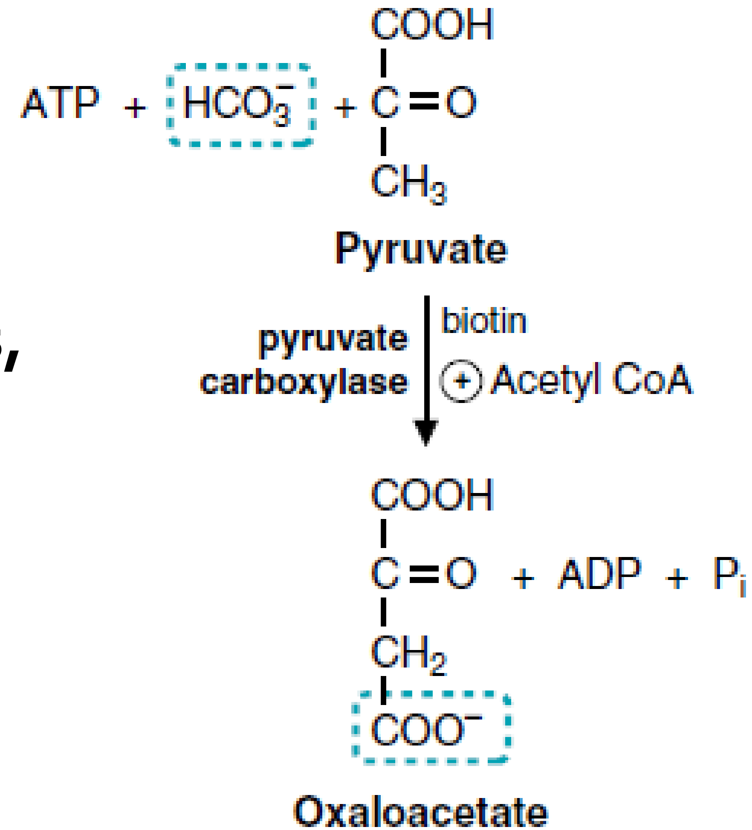
TCA Cycle Intermediates

- **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)**



Anaplerotic Reactions

- 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 Anaplerotic Routes (Amino Acid Degradation)