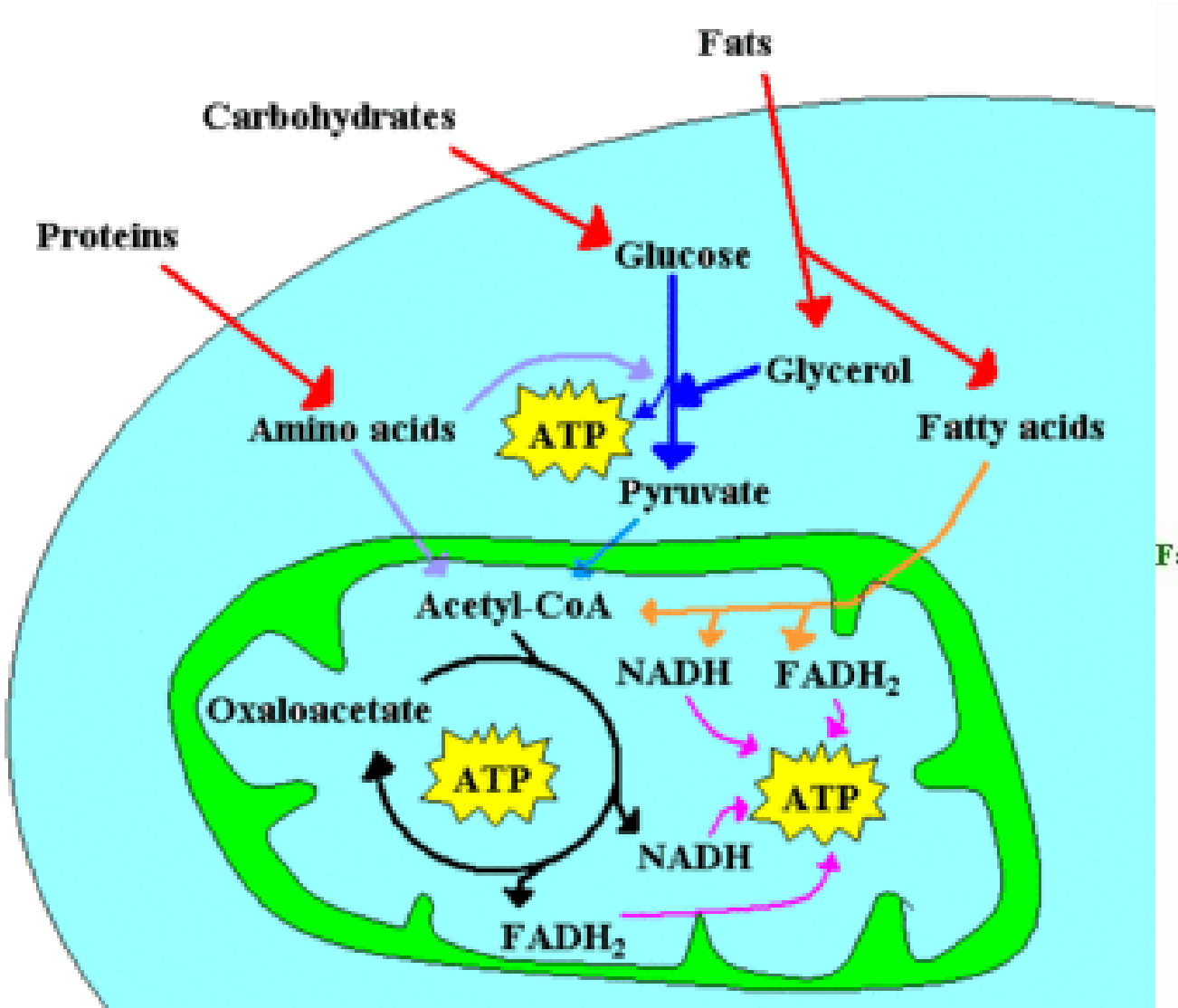
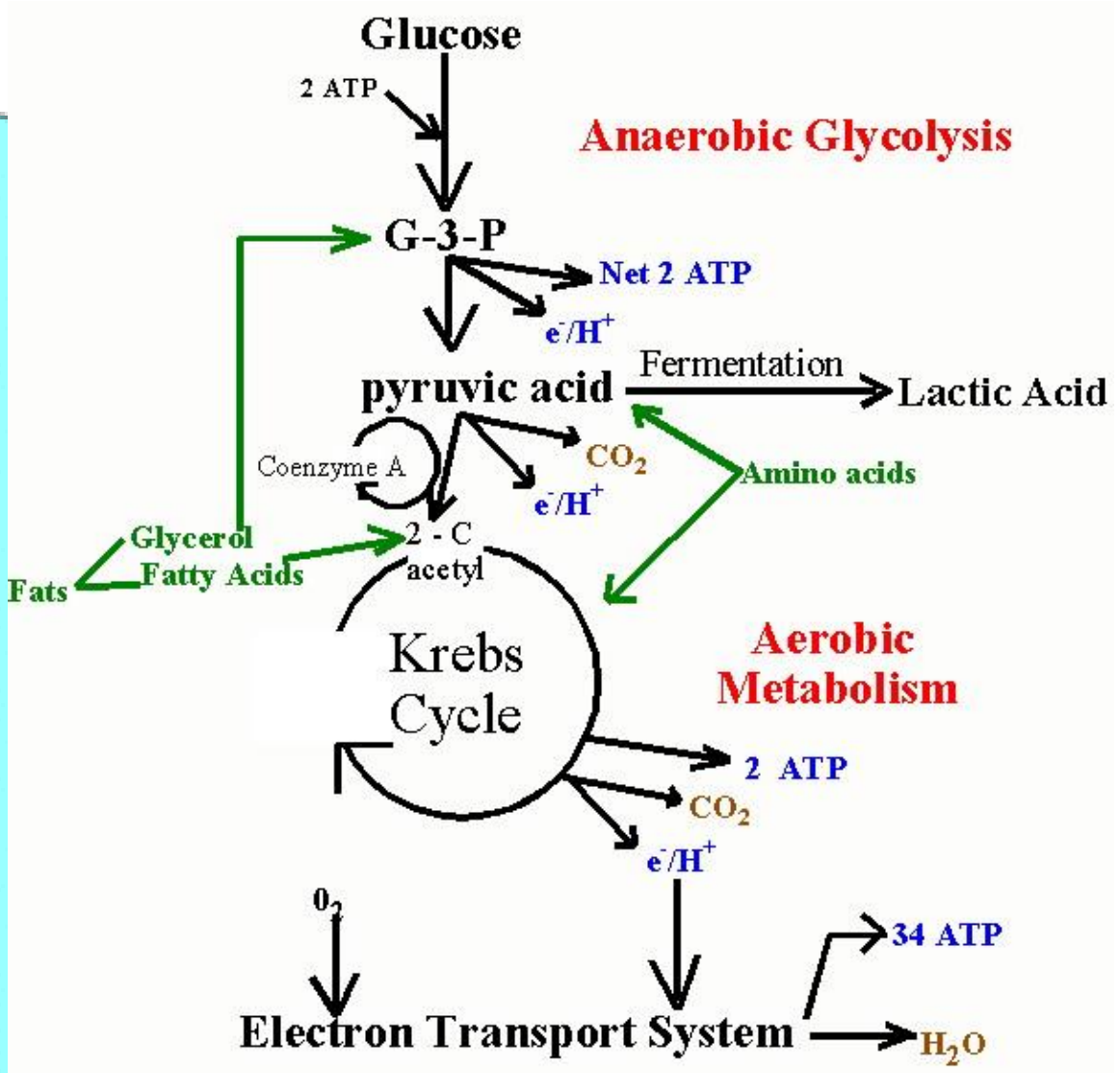
Nafith Abu Tarboush
DDS, MSc, PhD
natarboush@ju.edu.jo
www.facebook.com/natarboush

(Kreb's, Citric Acid, TCA) Cycle

How does it fit?

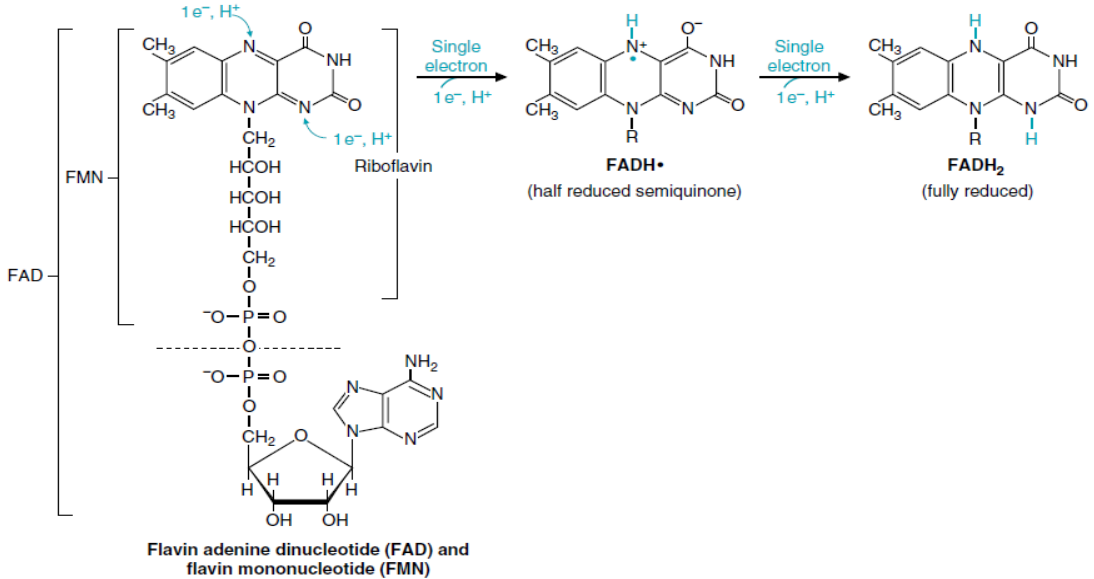




Electron (energy) Carrying Molecules (NAD+, FAD)

FAD

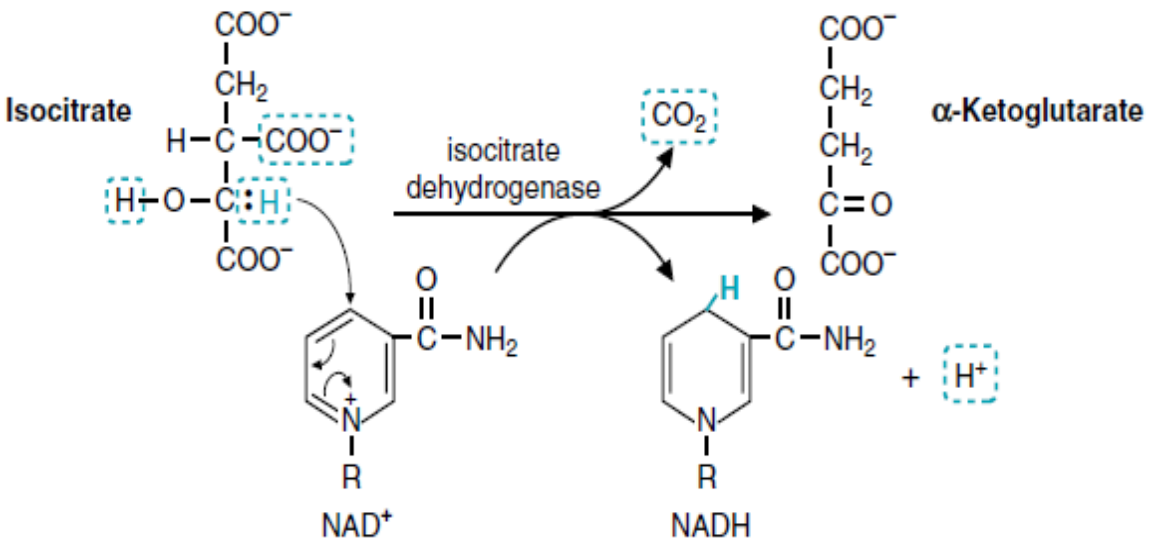
- Single electrons (H•), different sources
- Succinate to fumarate, lipoate to lipoate disulfide in α-KG
- FAD must remain tightly, sometimes covalently, attached to its enzyme
- E^{o} for enzyme-bound FAD varies



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

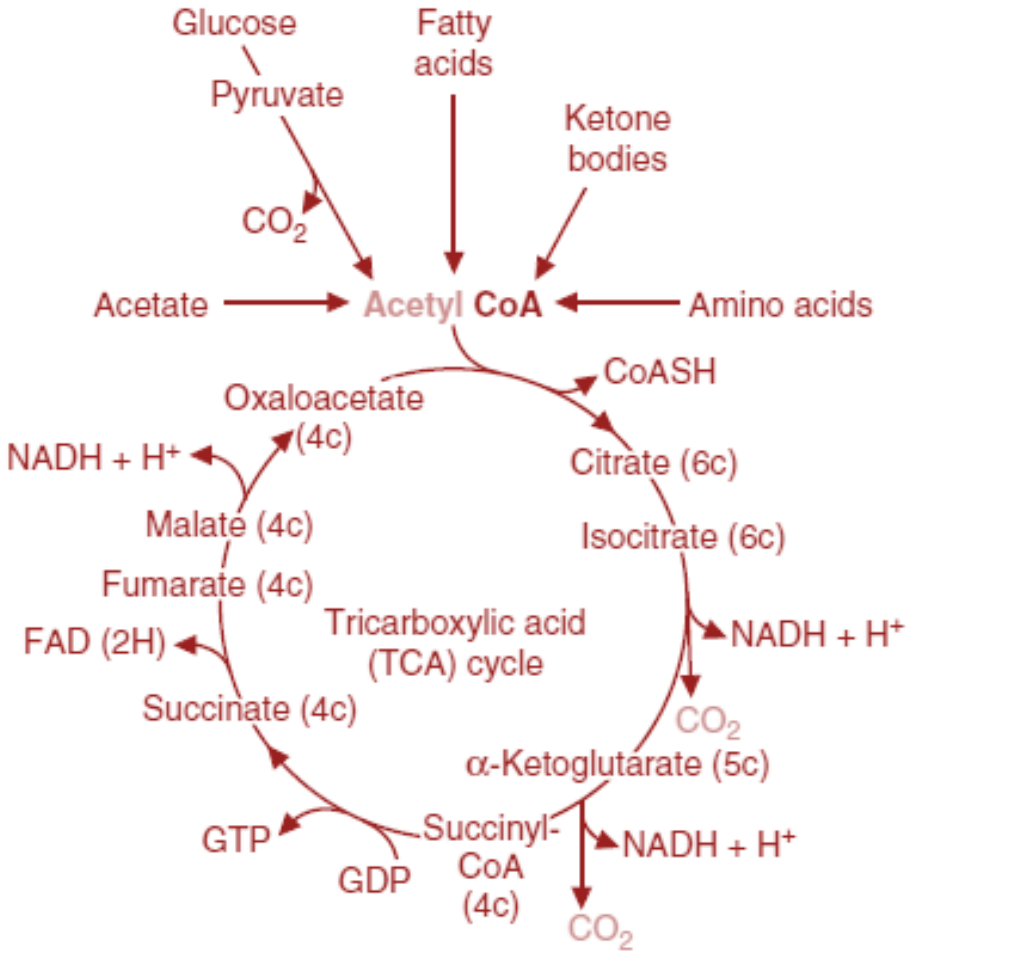
NAD

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



Components & stepwise reactions

- No O_2 introduced, two CO_2 exits

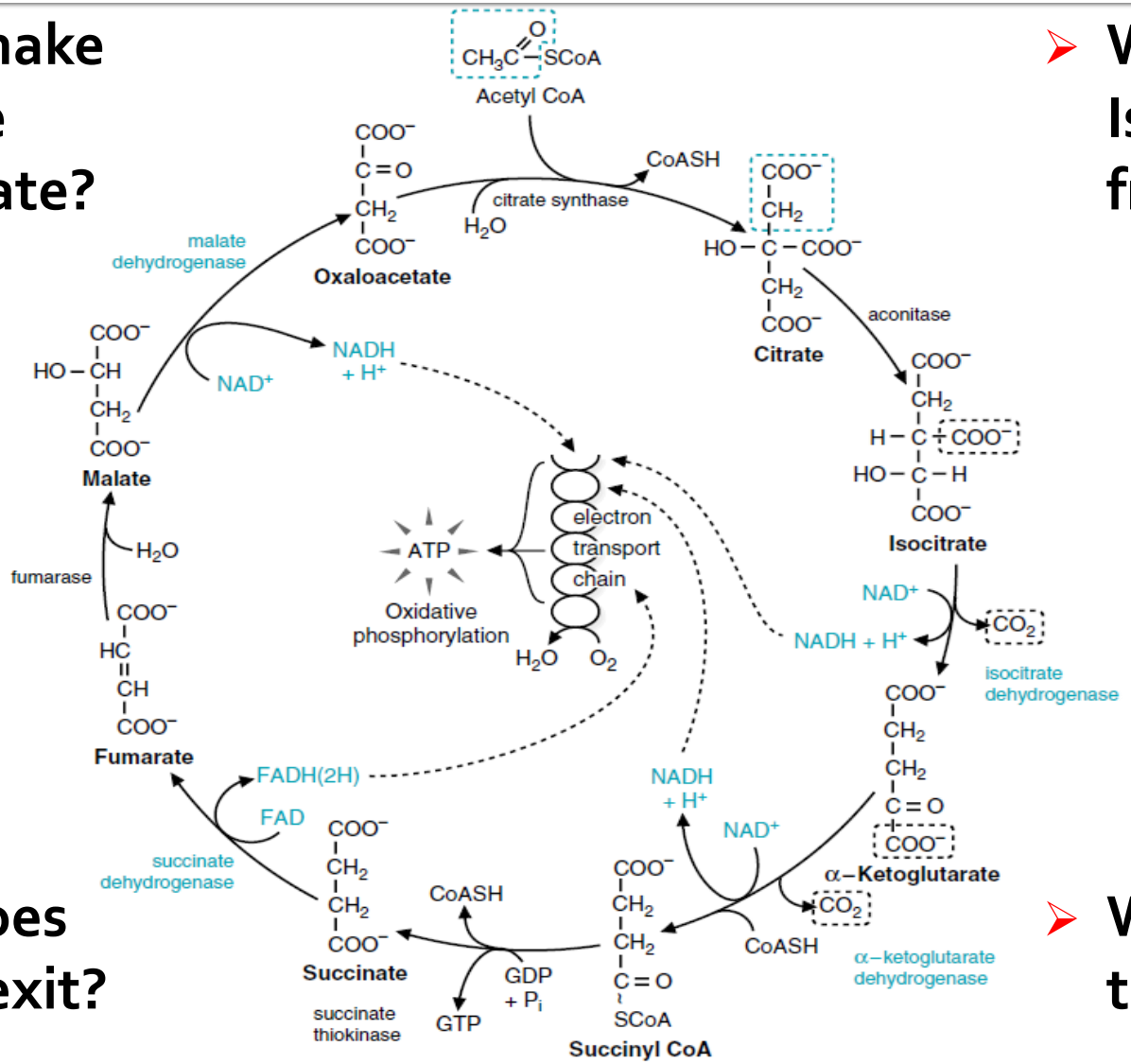


Net reaction

$$\text{Acetyl CoA} + 3\text{NAD}^+ + \text{FAD} + \text{GDP} + P_i + 2H_2O \longrightarrow 2CO_2 + \text{CoASH} + 3\text{NADH} + 3H^+ + \text{FAD (2H)} + \text{GTP}$$

Does Acetyl-CoA exit as 2 CO_2?

- Why to make Isocitrate from citrate?



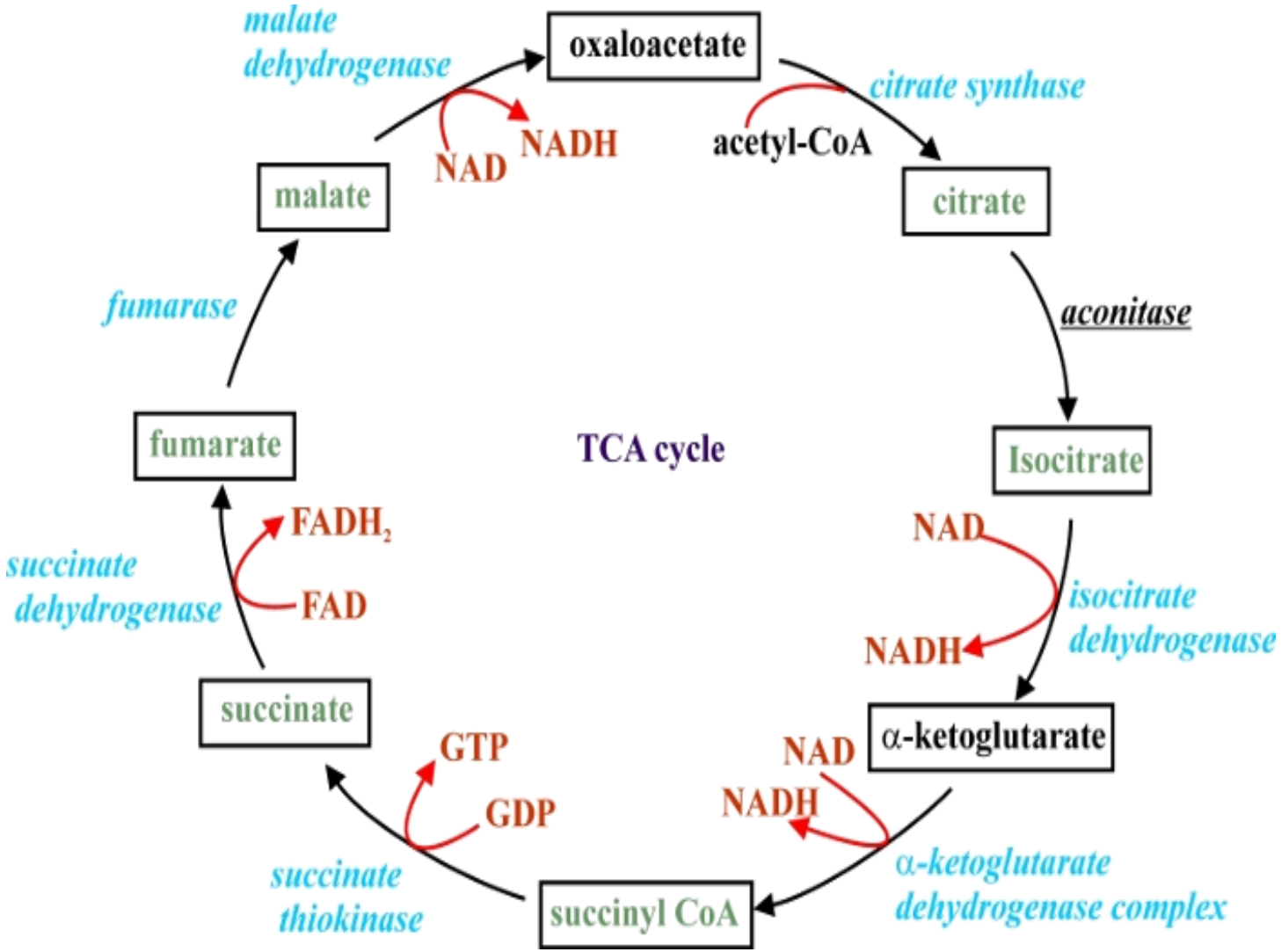
- Where does the CO_2 exit?

- Why to make Isocitrate from citrate?

- Where does the CO_2 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° to 2° alcohol

Control at the committed step of glycolysis

Fructose 6 - phosphate

ATP

ADP

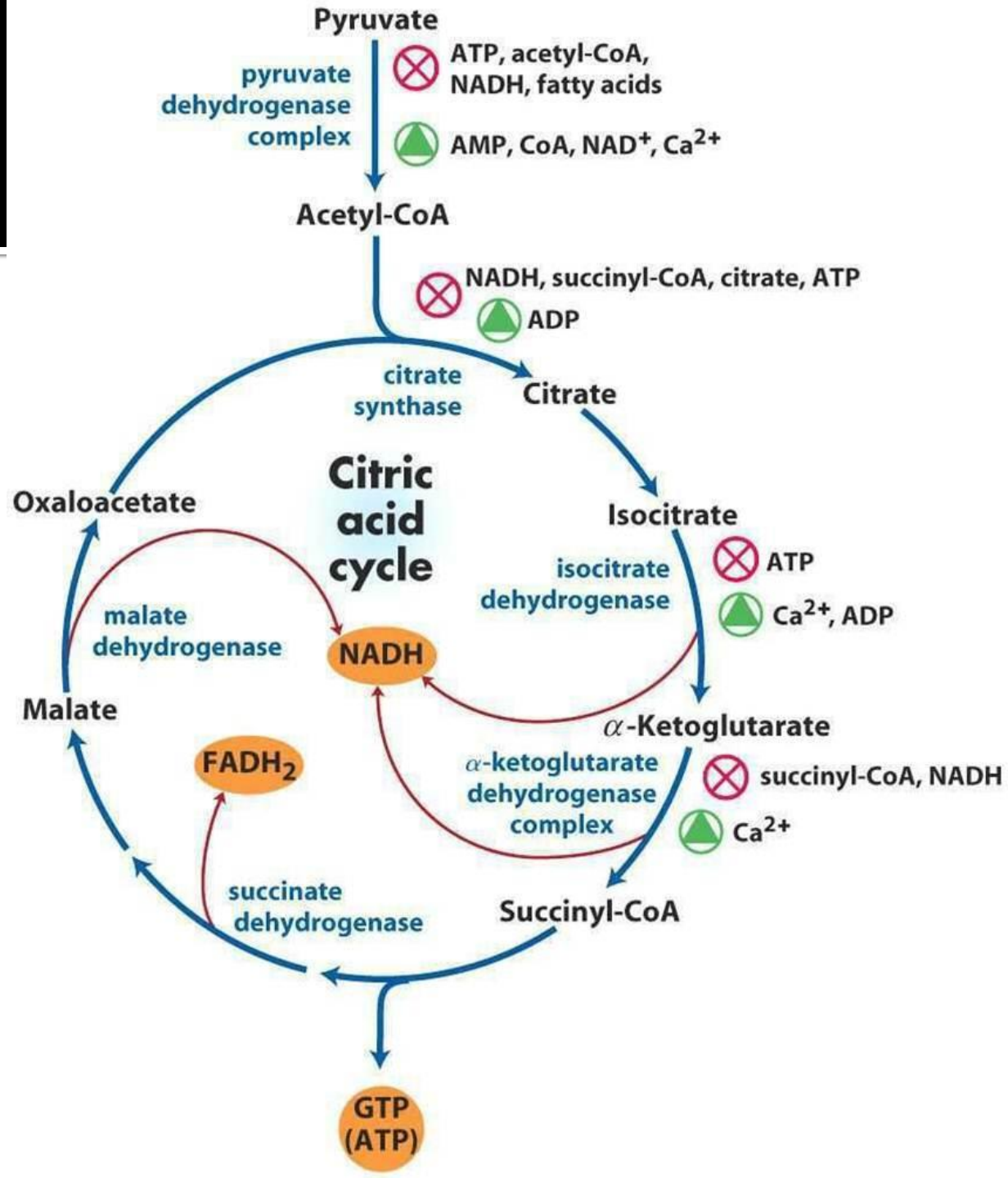
Phosphofructokinase

Activated by F-2,6 - BP

Activated by AMP

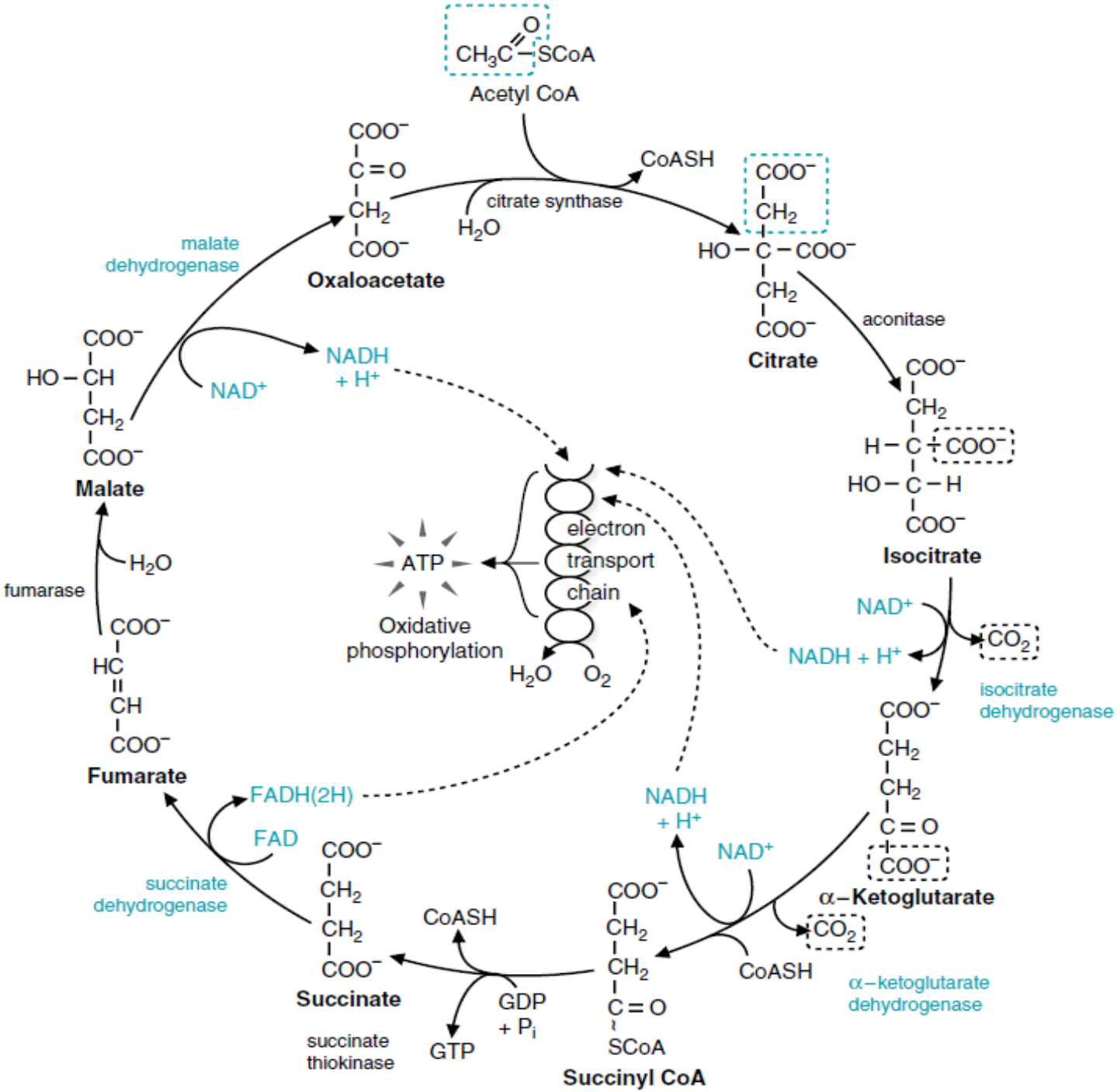
Inhibited by ATP and citrate

Fructose 1,6 - bisphosphate



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



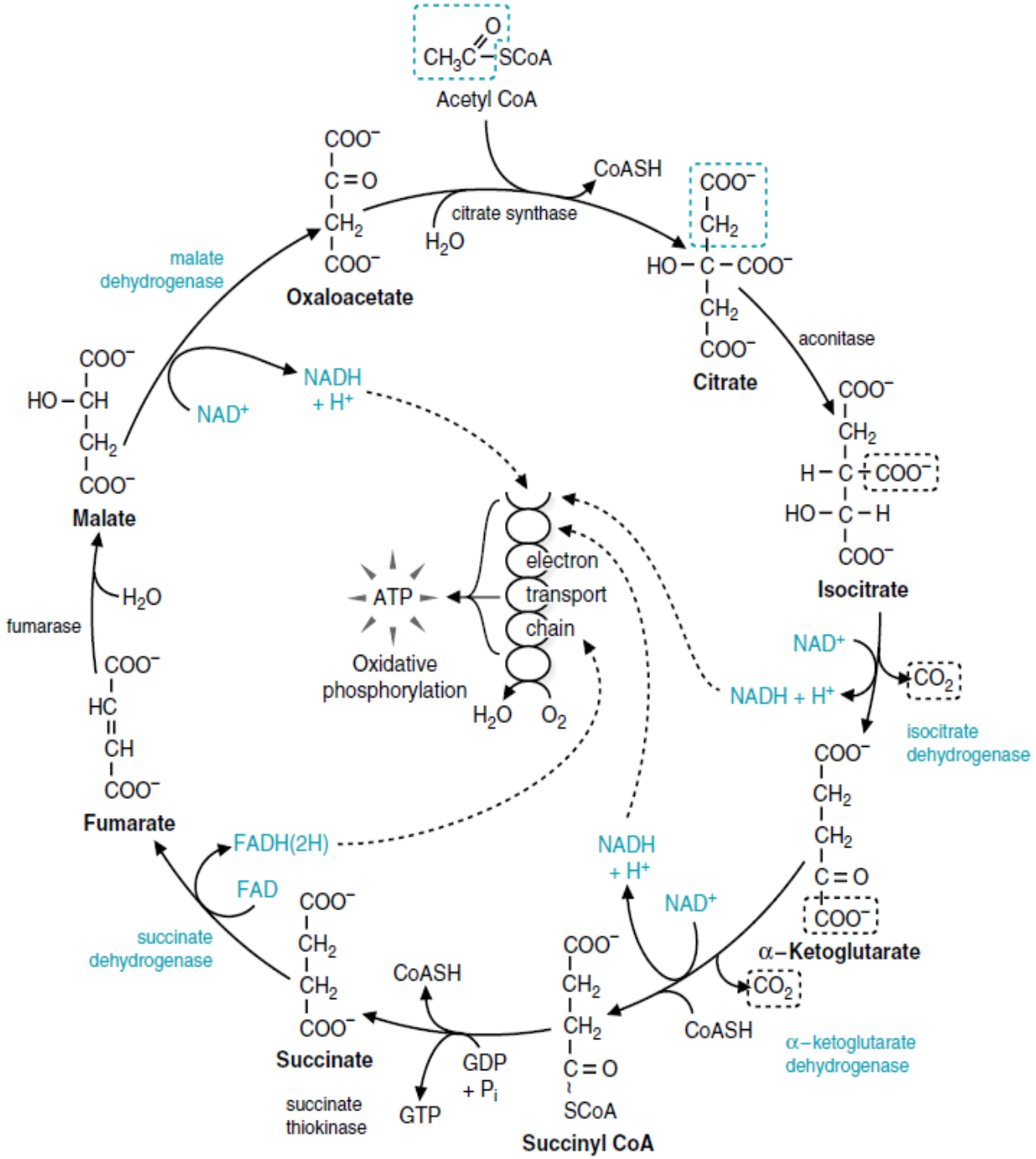
Generation of GTP

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

1,3-Diphophoglycerate + ADP $\xrightleftharpoons{\text{Phosphoglycerate kinase}}$ 3-Phophoglycerate + ATP

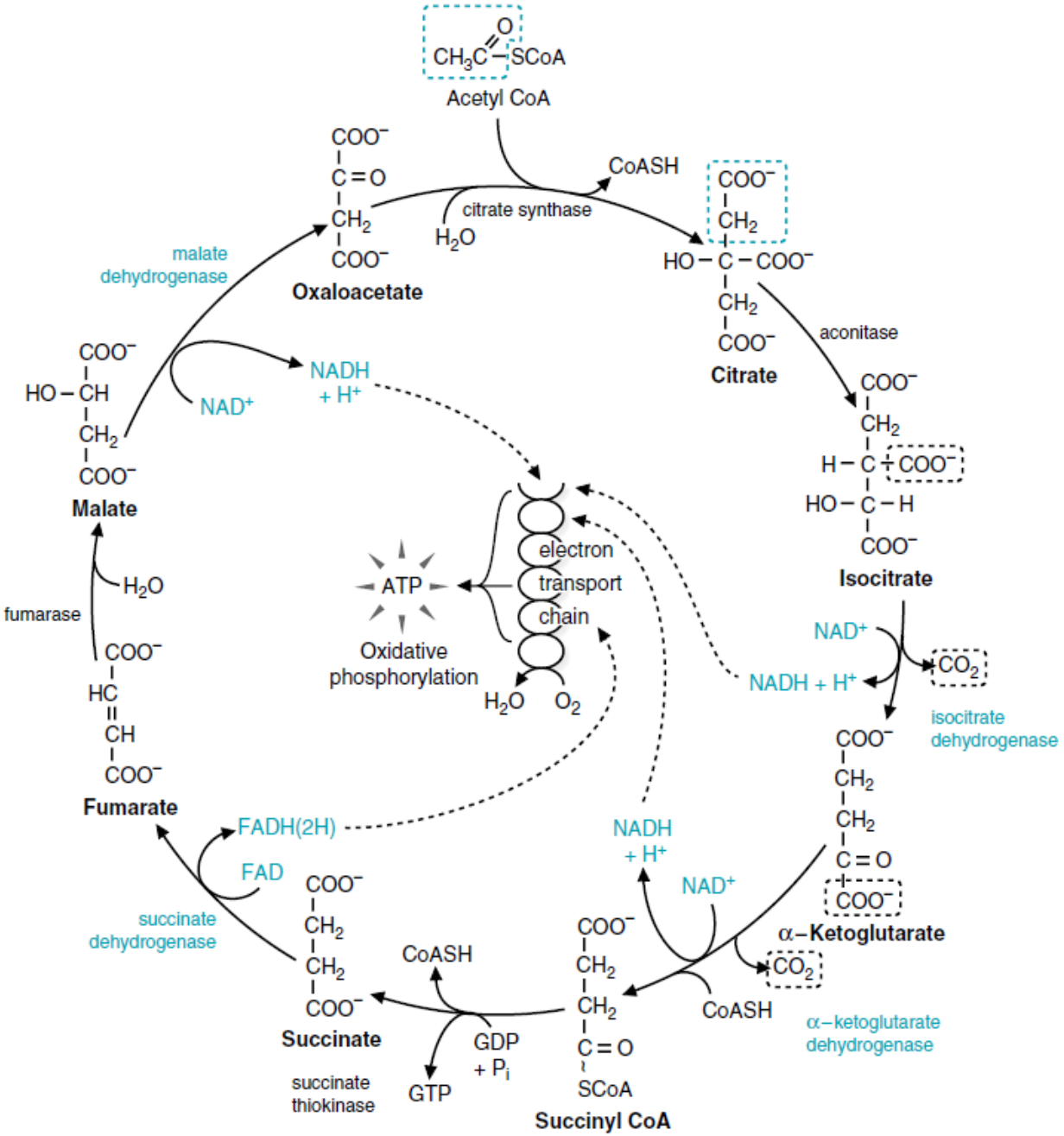
Phosphoenolpyruvate + ADP $\xrightleftharpoons{\text{Pyruvatekinase}}$ Pyruvate + ATP

SuccinylCoA +GDP $\xrightleftharpoons{\text{SuccinylCoA Synthase}}$ Succinate +GTP



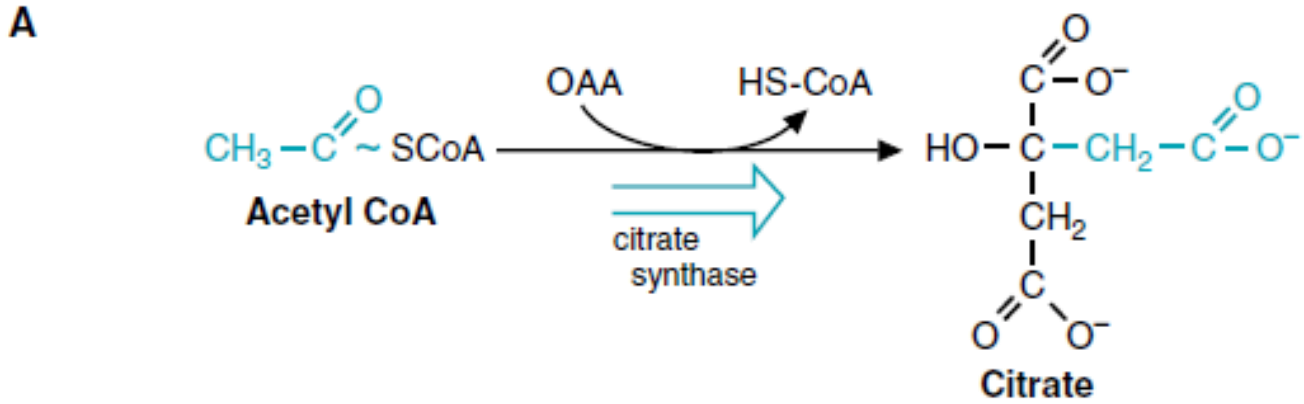
Oxidation of Succinate to Oxaloacetate

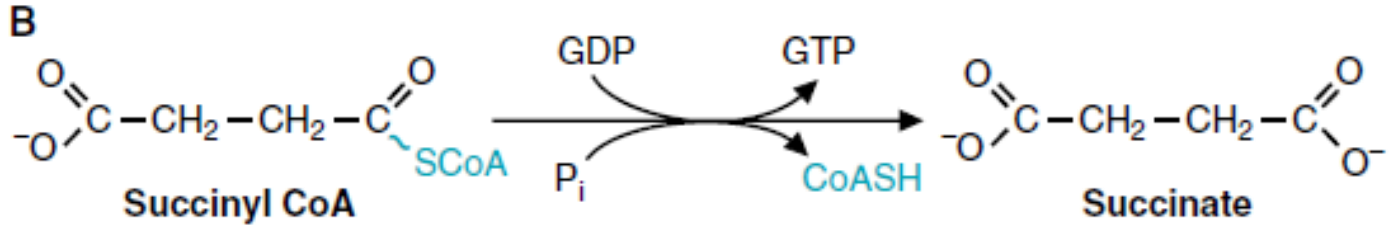
- Oxidation of succinate to fumarate, succinate dehydrogenase, FAD
- Fumarase, OH + H^+ from water, fumarate to malate
- Alcohol group of malate oxidized to a keto group, NADH

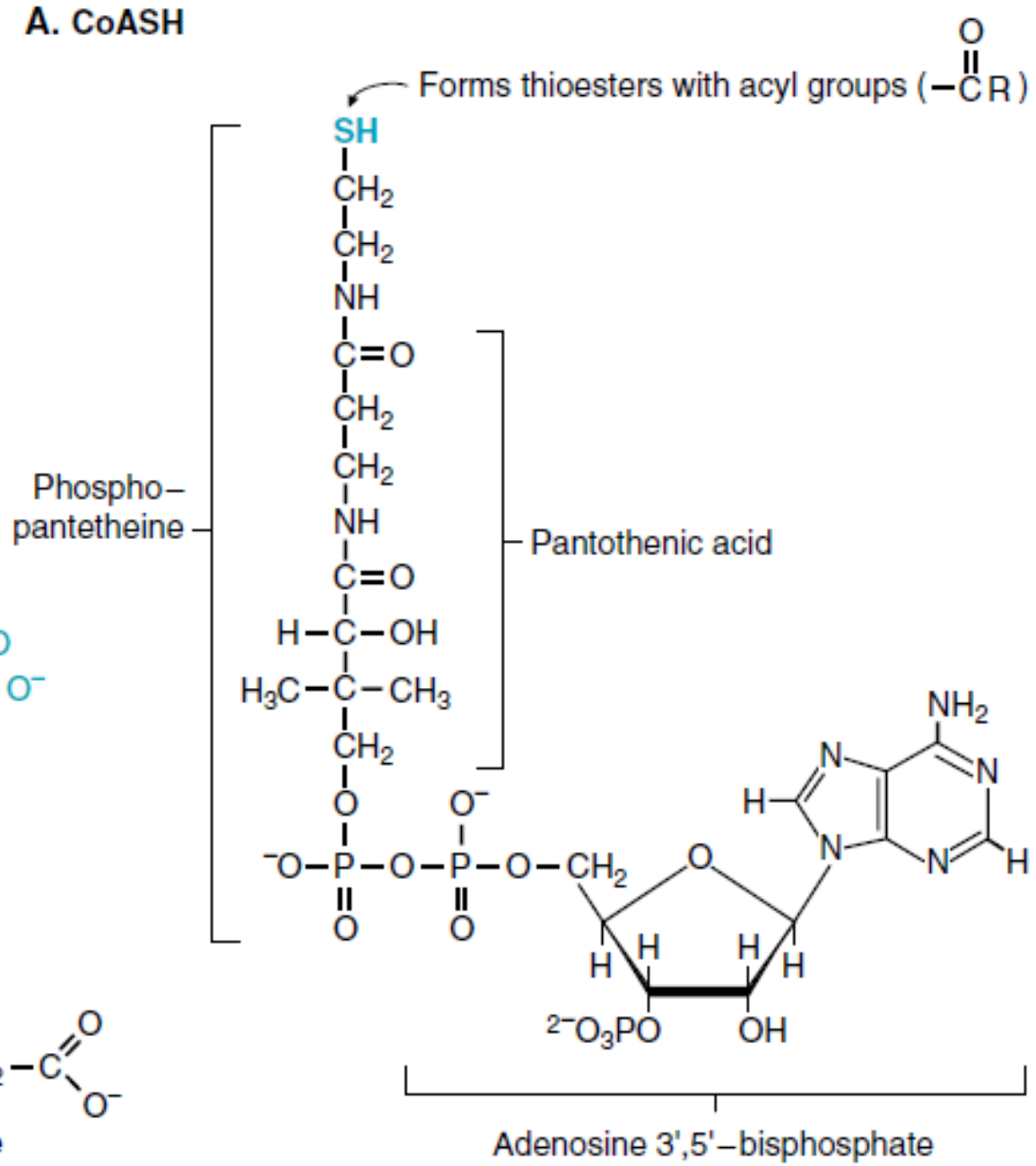


CoA

- 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)

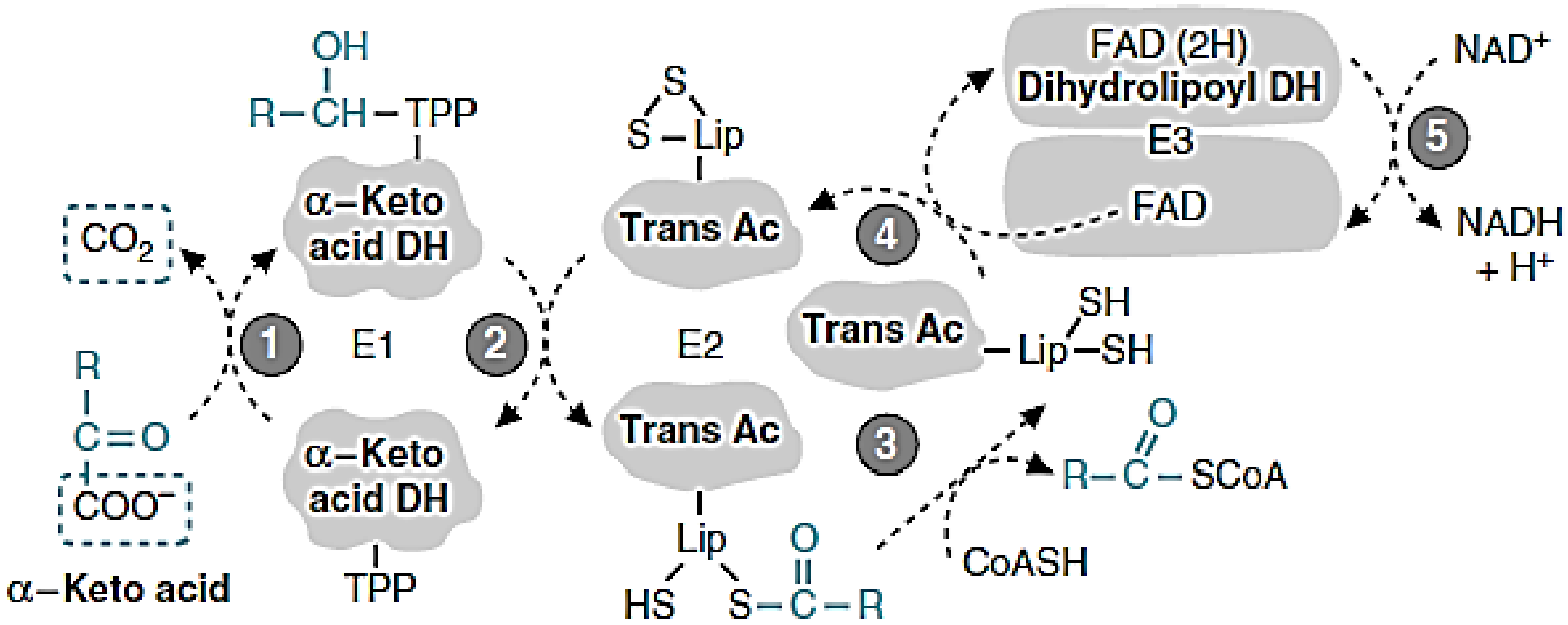


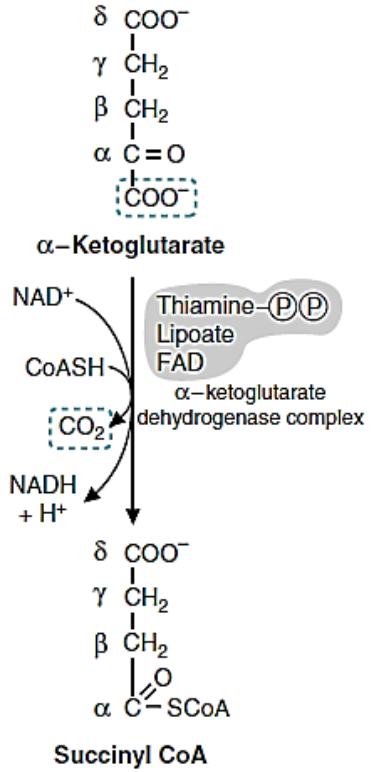




α-Ketoacid Dehydrogenase Complexes (TLCFN)

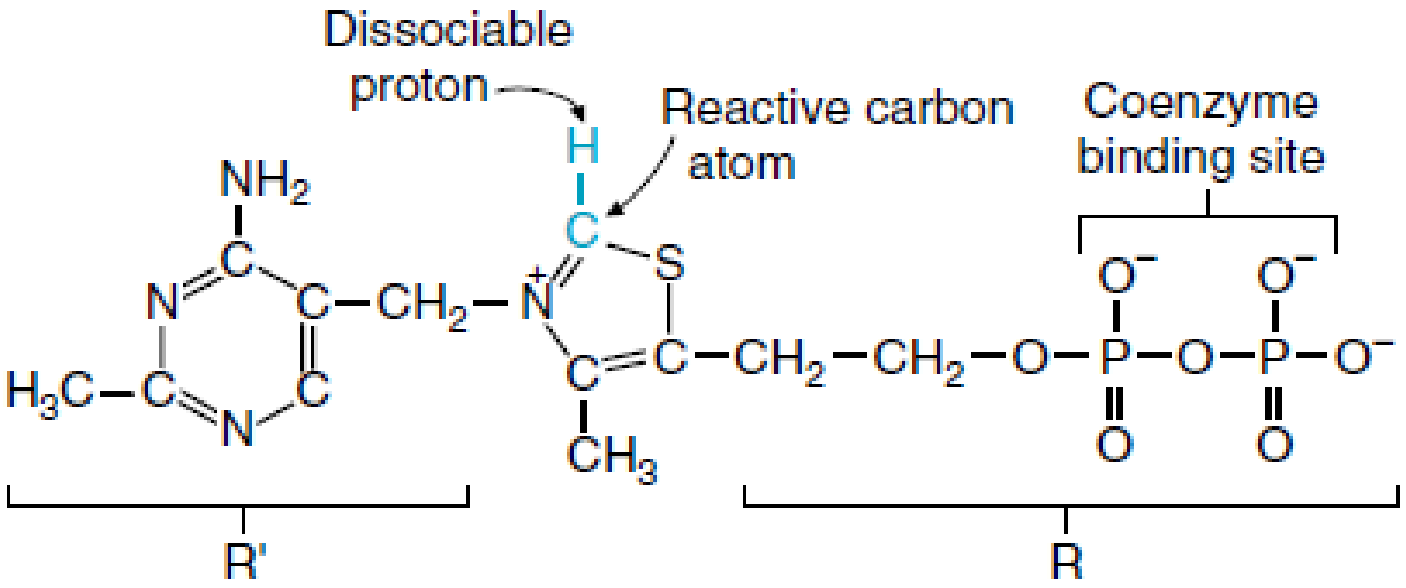
- (α-ketoglutarate, pyruvate, and branched chain α-keto acid) dehydrogenase complexes
- Huge enzyme complexes, multiple subunits of 3 different enzymes (no loss of energy, substrates for E2 and E3 remain bound → higher rate)
- E1, E2, & E3 are a decarboxylase (TPP), a transacylase (lipoate), & a dehydrogenase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



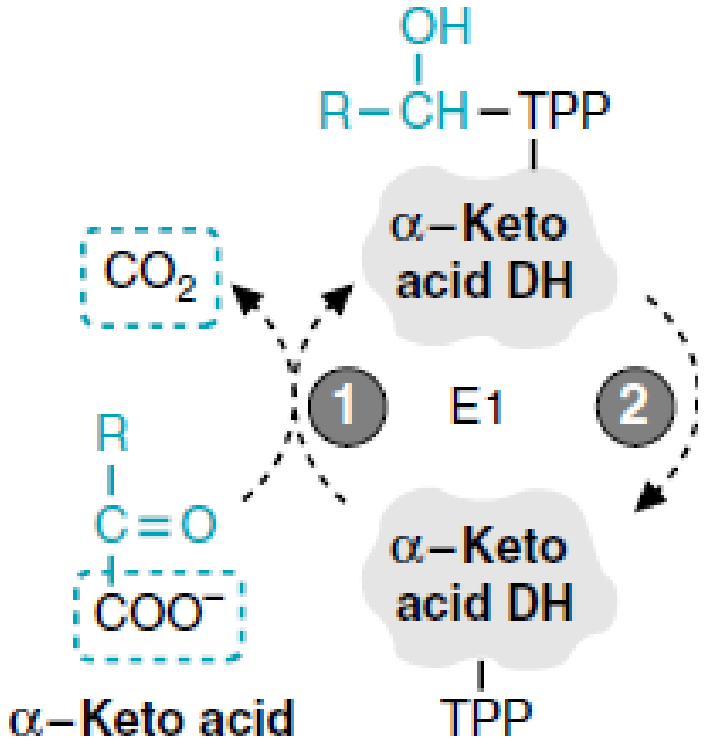


Thiamine Pyrophosphate

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

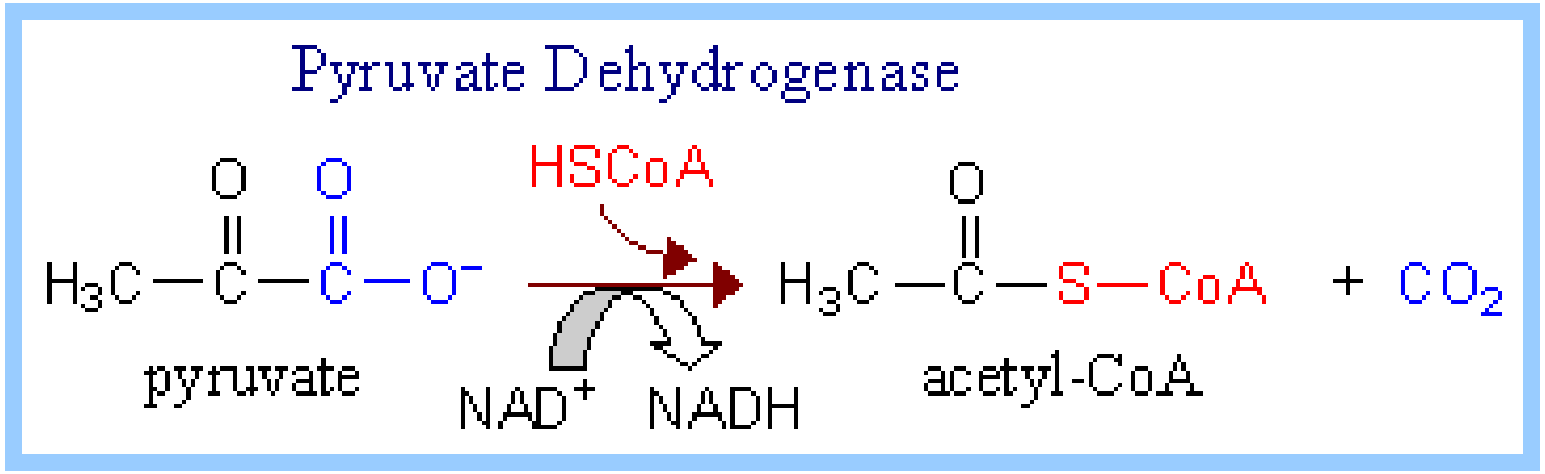


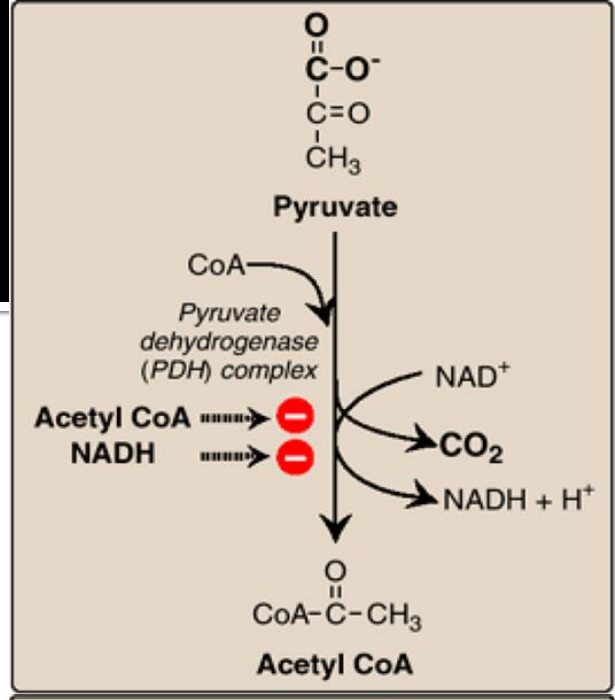
Thiamine pyrophosphate (TPP)

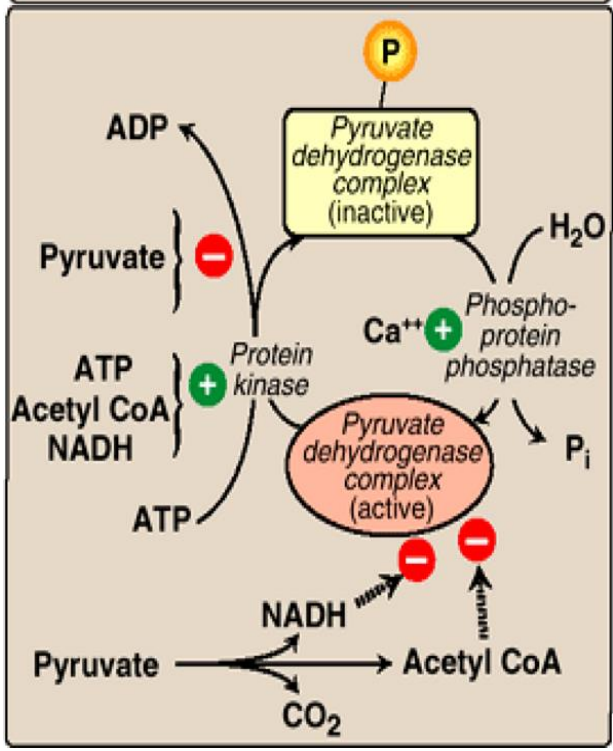


Oxidative decarboxylation of pyruvate

- **Component enzymes**
- **Coenzymes**
- **Regulation of the pyruvate dehydrogenase complex**
  - Pyruvate dehydrogenase deficiency: A deficiency in E_1 component is the most common biochemical cause of congenital lactic acidosis (X-linked, no treatment)
- **Mechanism of arsenic poisoning**



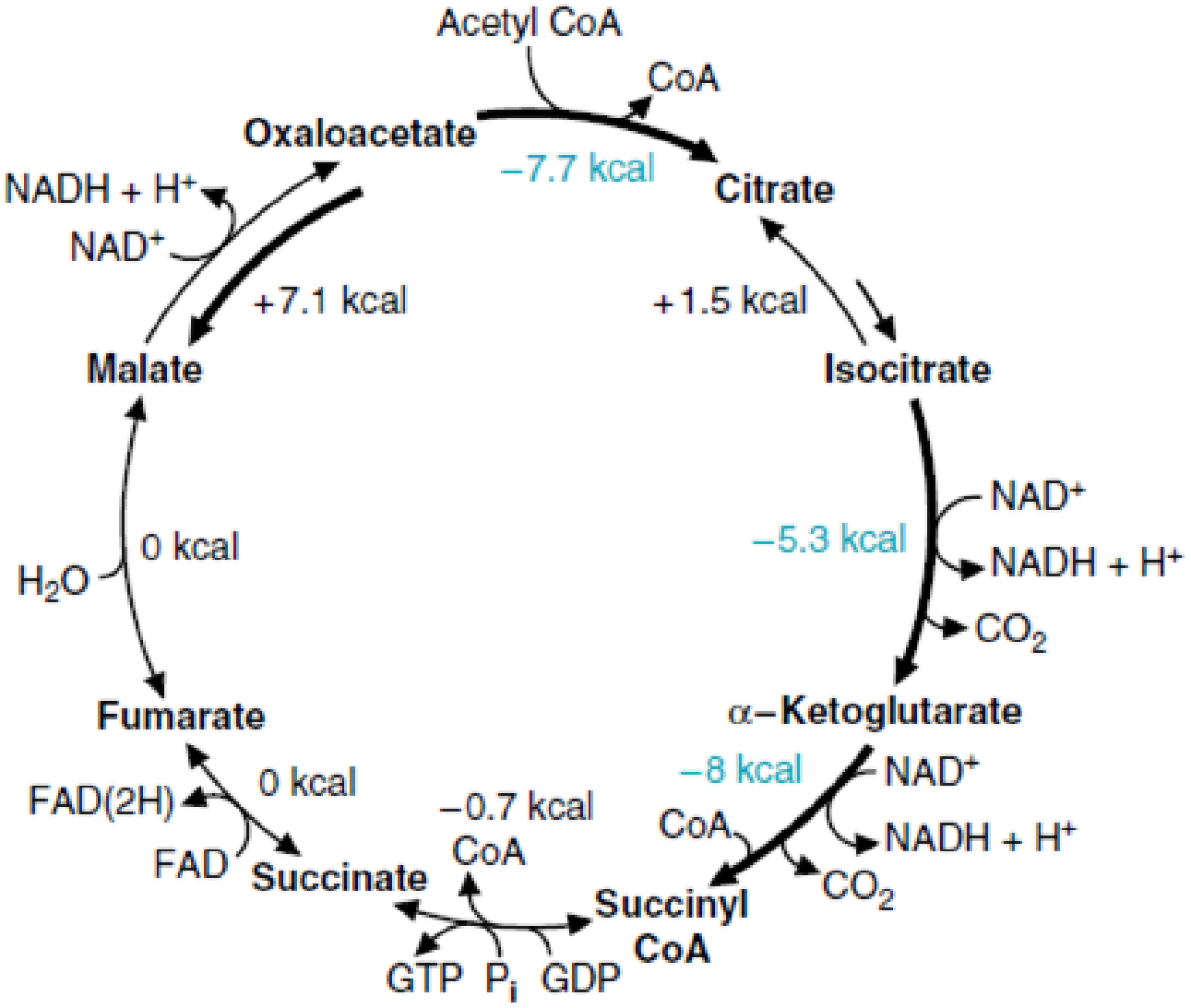




Bioenergetics of the TCA Cycle

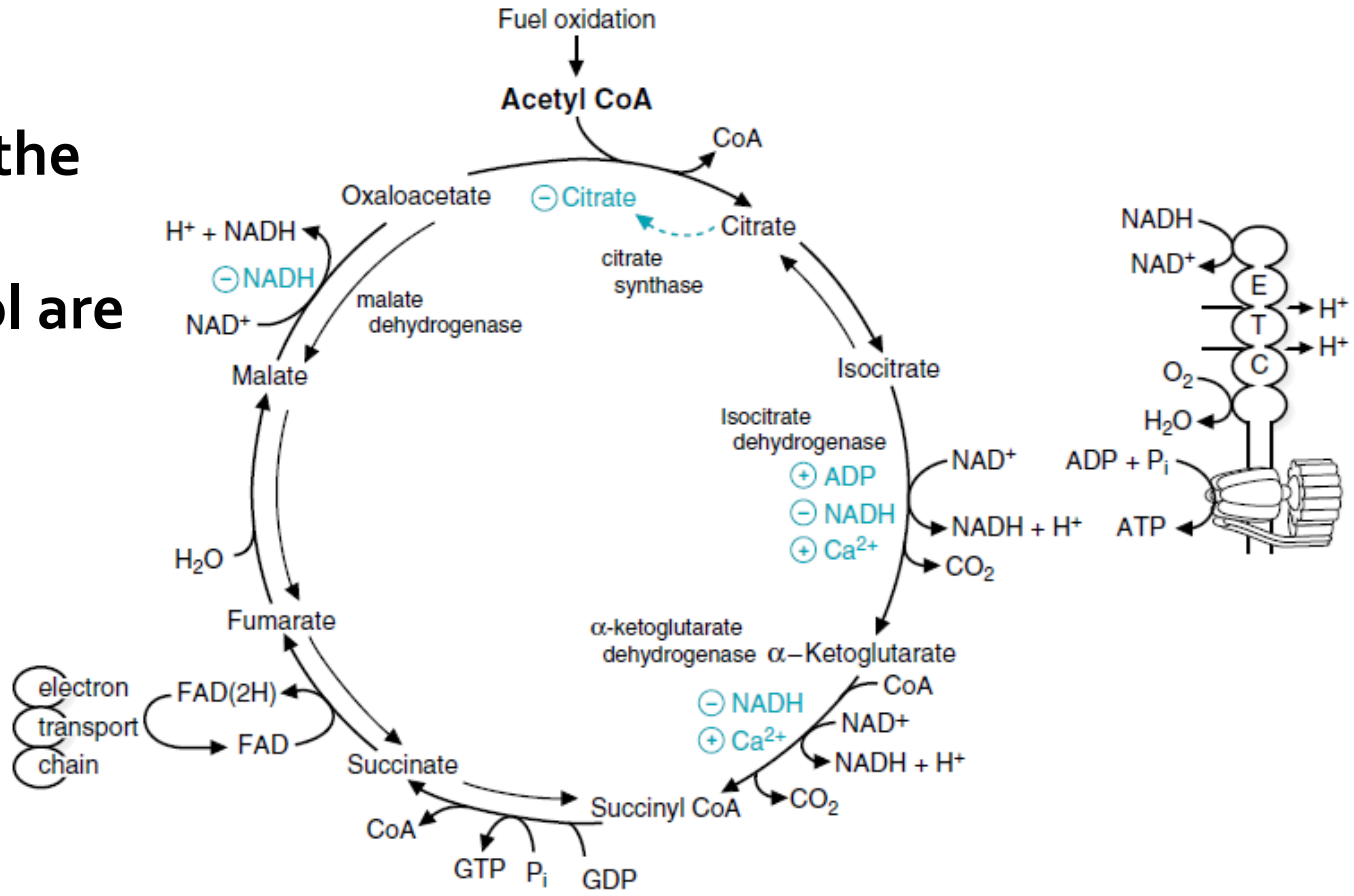
- Like all pathways, overall net -ΔG (-228 kcal/mole), not 100%
- NADH, FAD(H2), and GTP (10ATP), 207 Kcal, 90%
- Three reactions have large (-ve) values
- Physiologically irreversible, low products

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

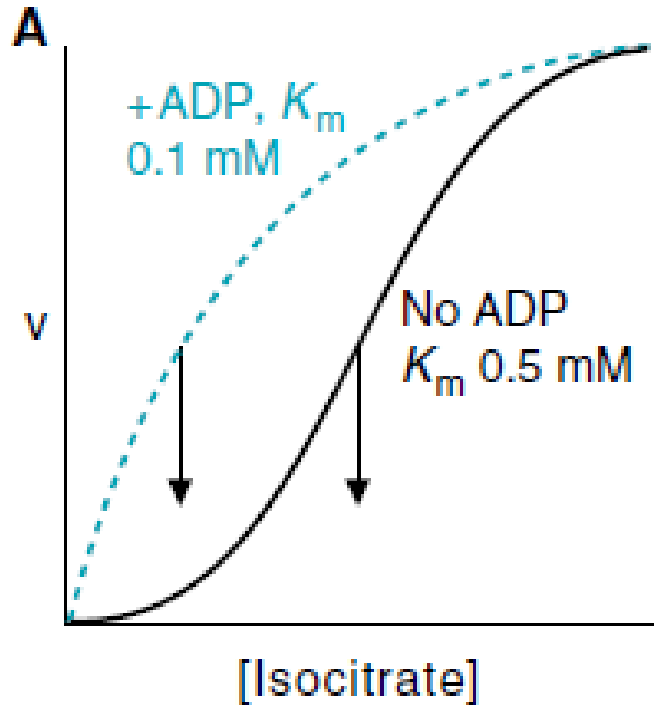


Citrate Synthase

- The first step, no allosteric regulation
- Rate regulated by oxaloacetate & citrate (inhibitor)

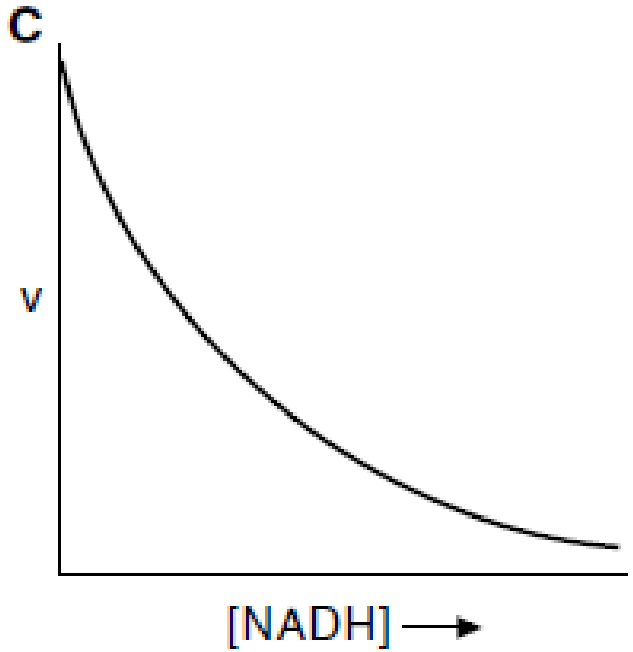
Isocitrate DH

- 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



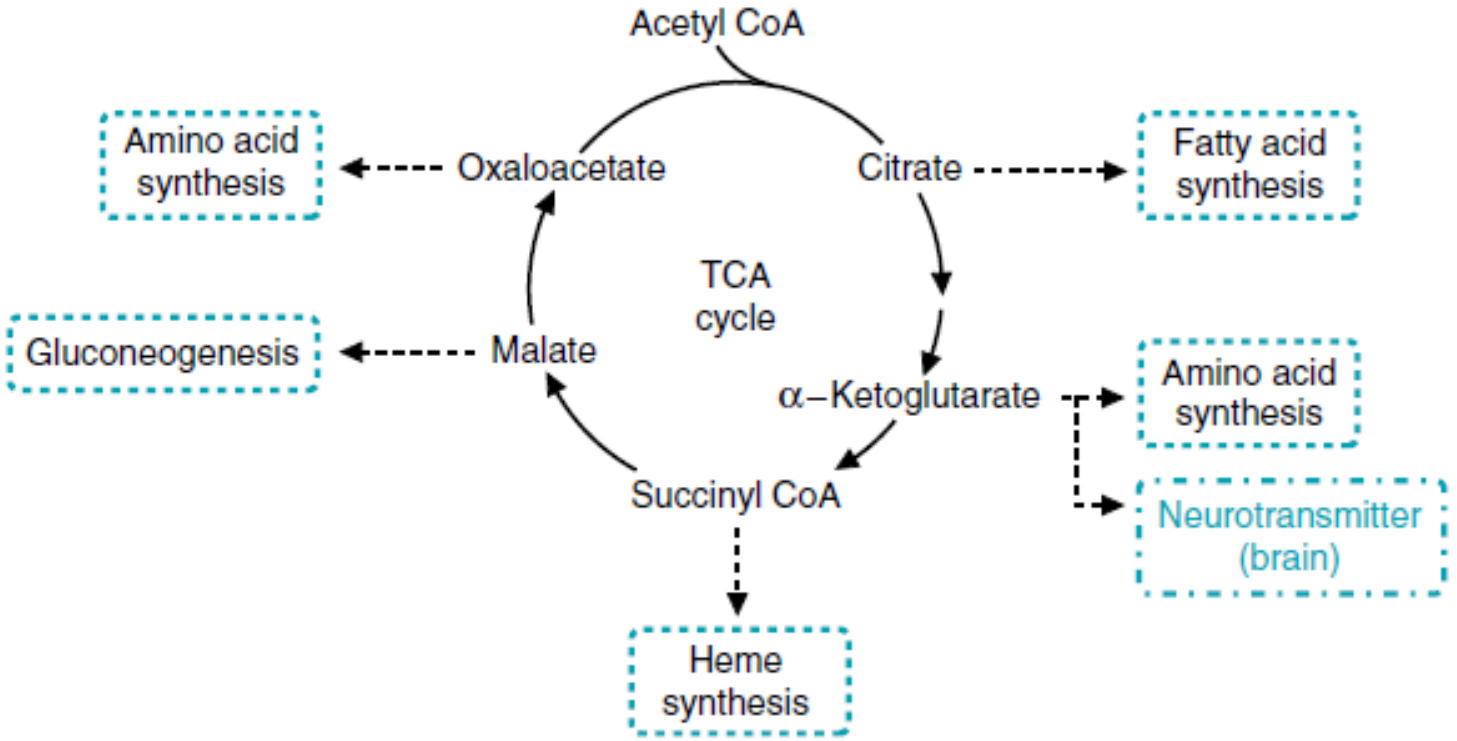
α-Ketoglutarate DH

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



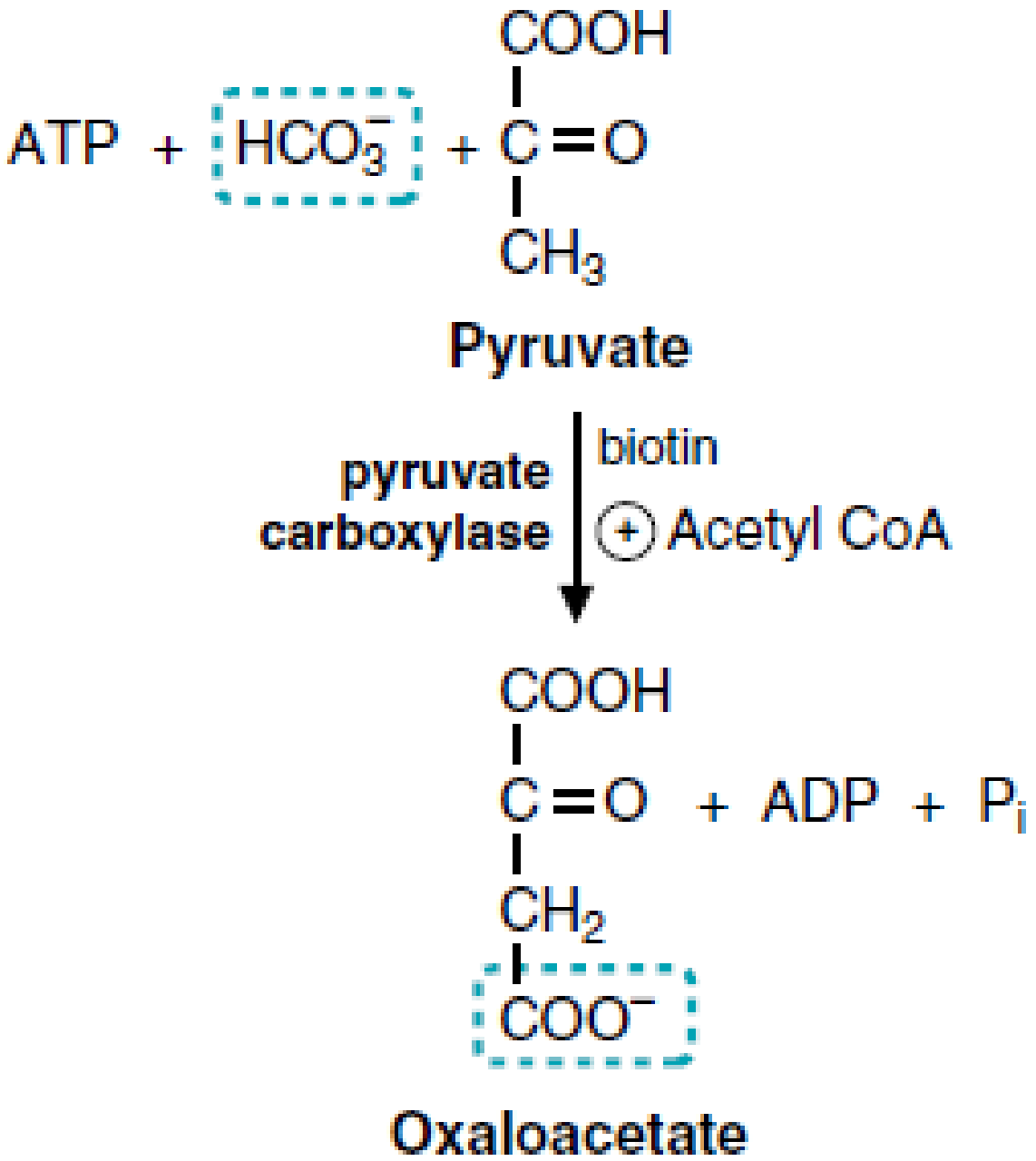
TCA Cycle Intermediates

- **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)