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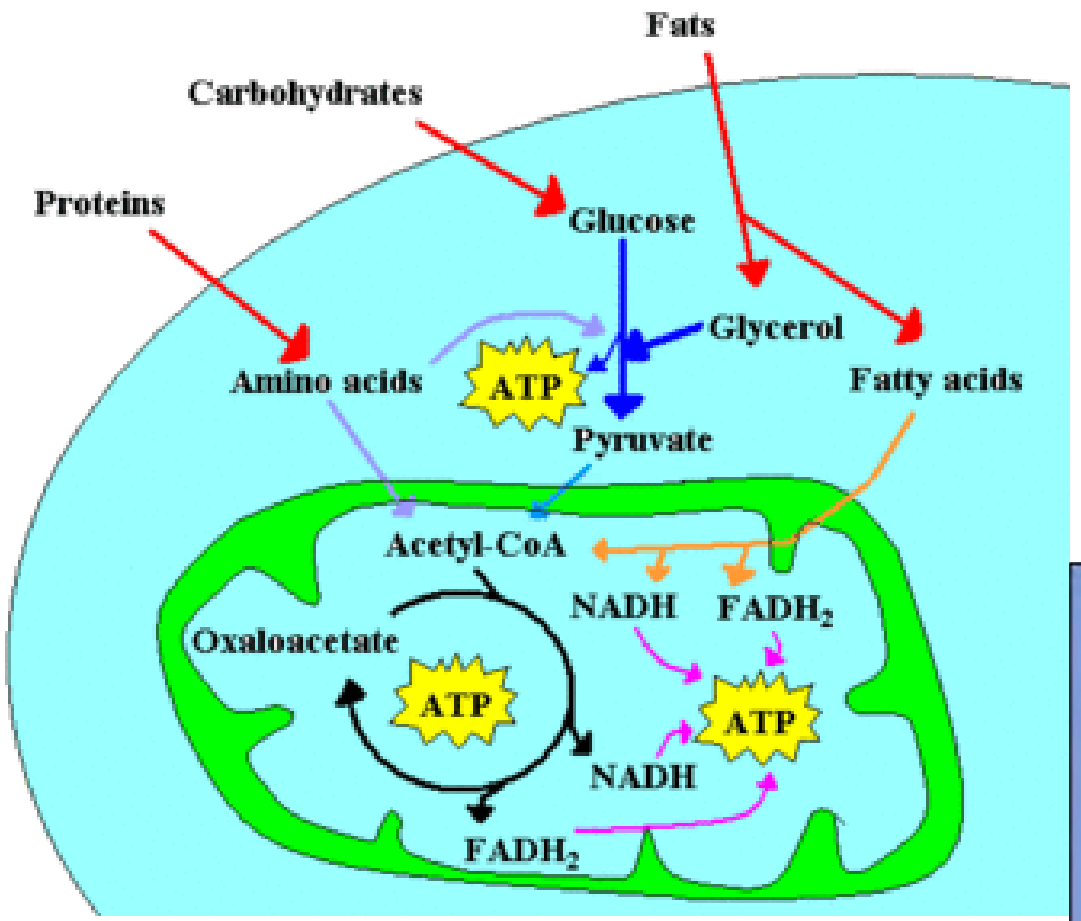
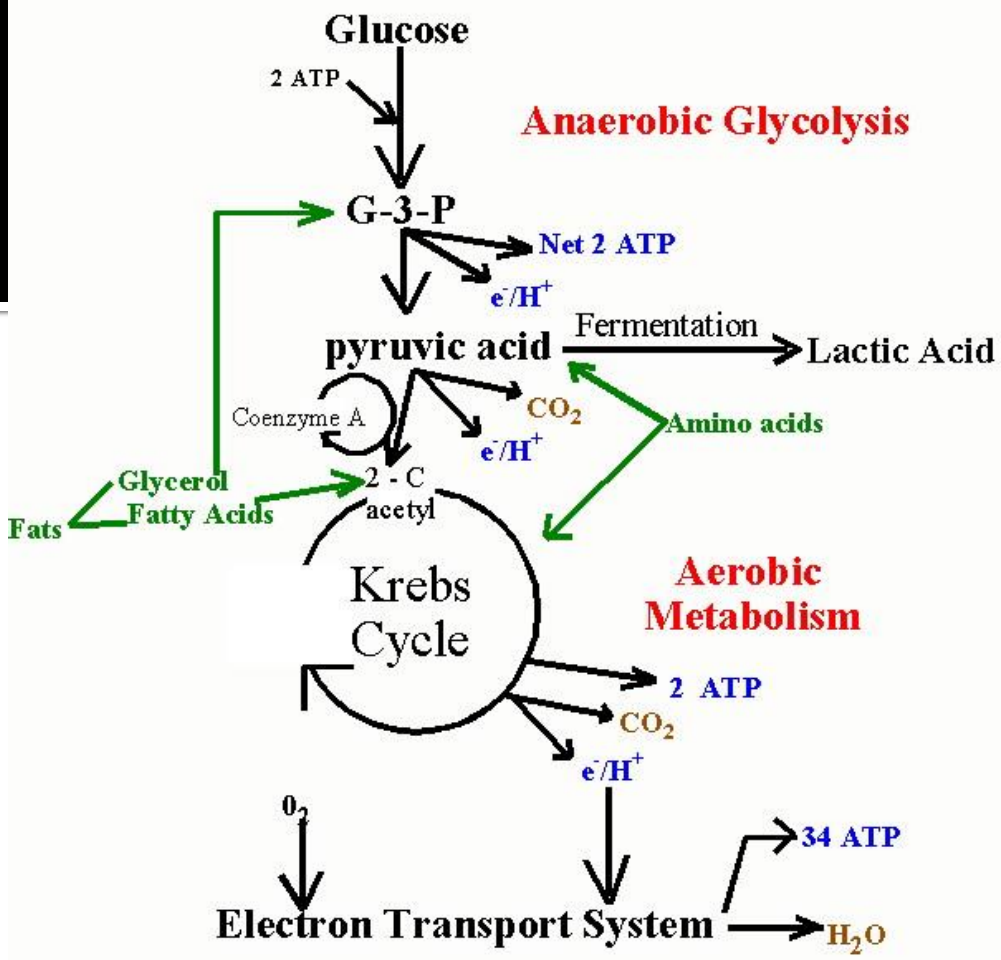
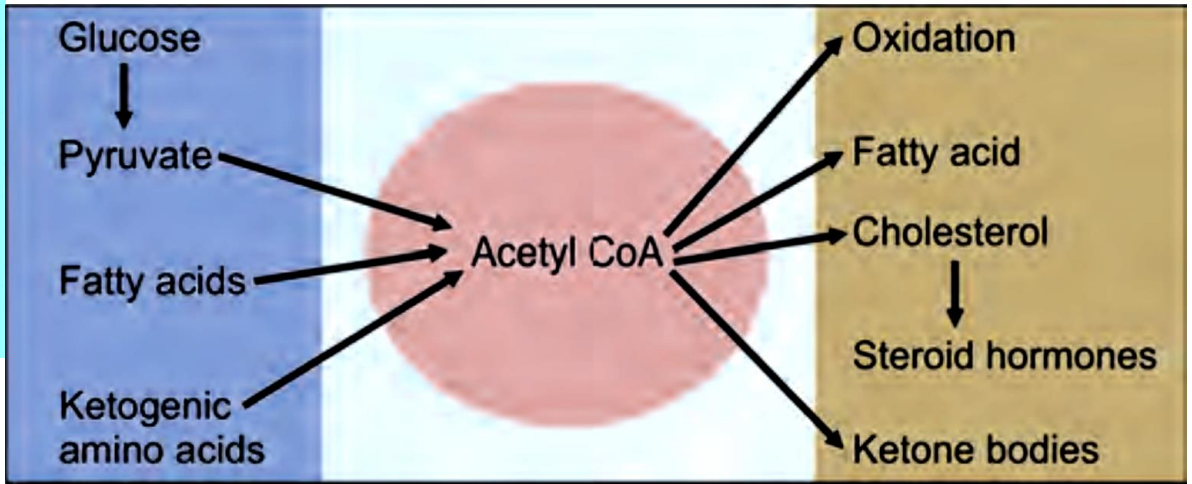
(Kreb's, Citric Acid, TCA) Cycle

How does it fit?



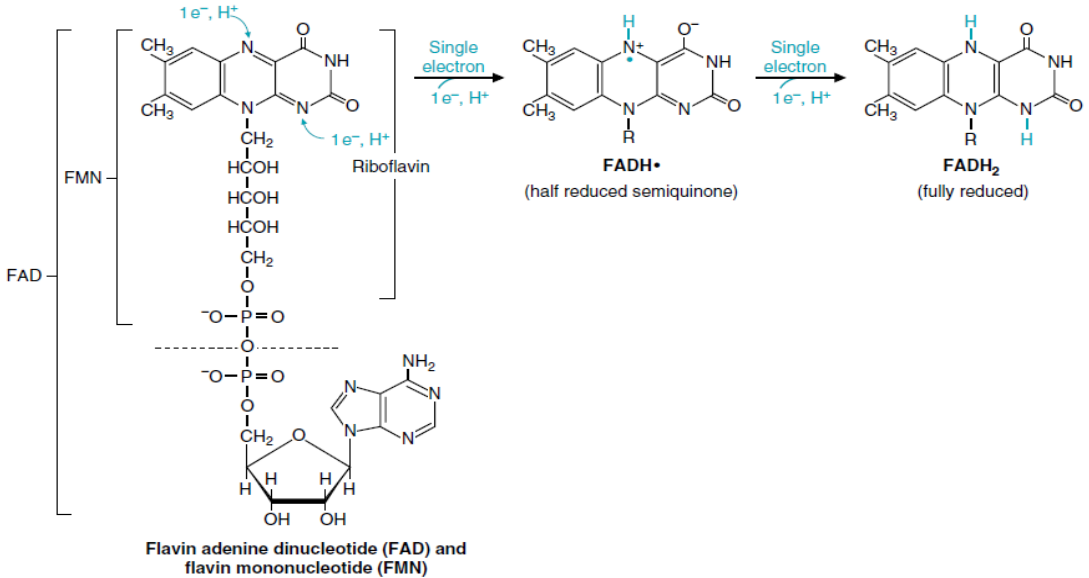




Electron (energy) Carrying Molecules (NAD+, FAD)

FAD

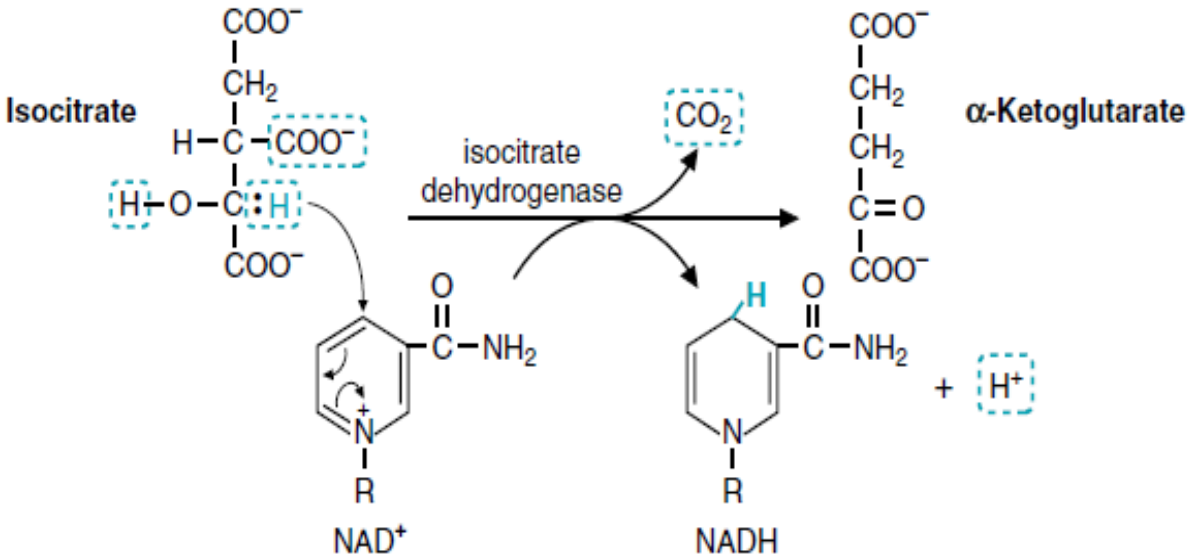
- Single electrons (H•), different sources
- Succinate to fumarate, lipoate to lipoate disulfide in α-KG
- FAD must remain tightly, sometimes covalently, attached to its enzyme
- E^{o} for enzyme-bound FAD varies



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

NAD

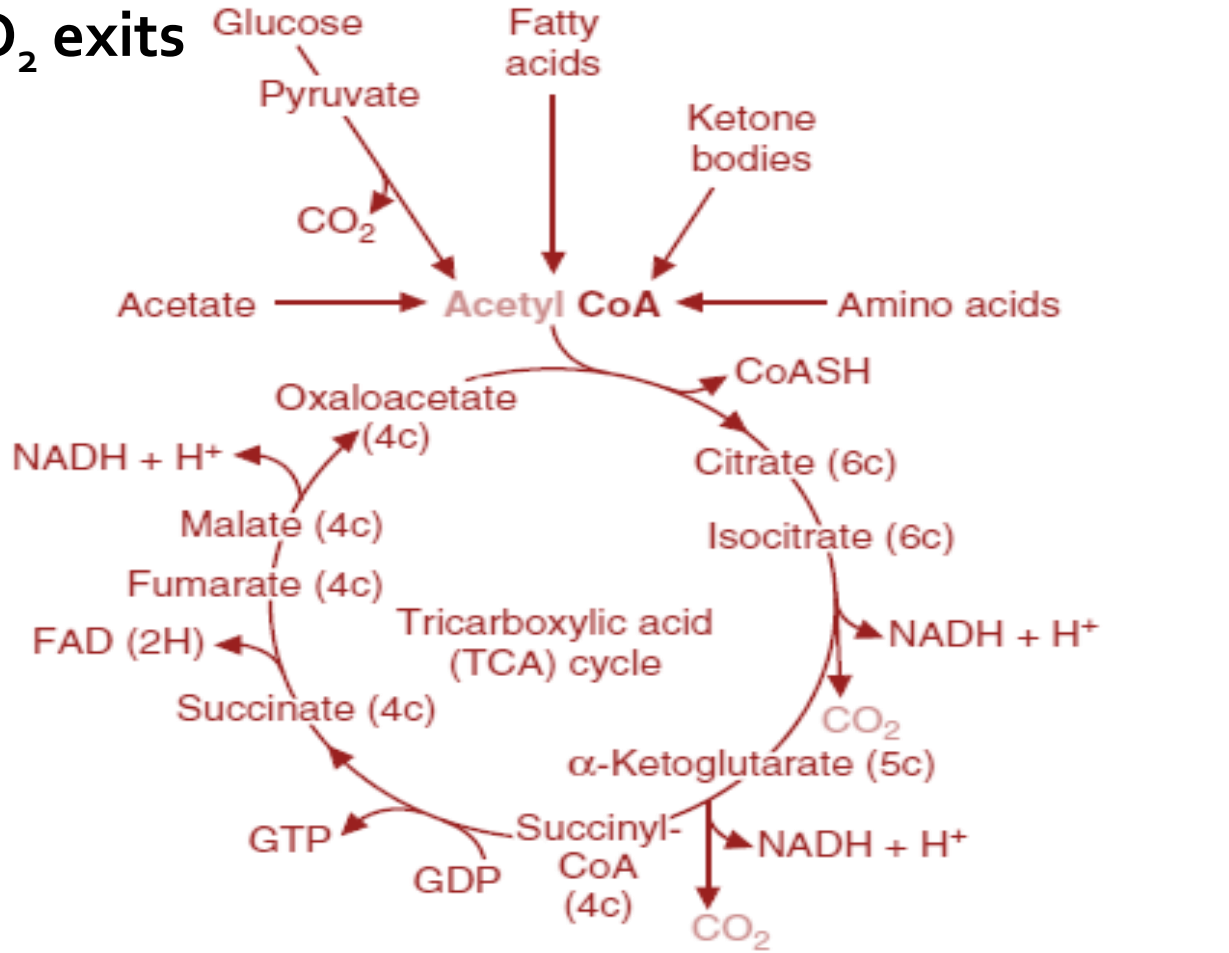
- Pair of electrons (H-), same source
- Alcohols to ketones by malate dehydrogenase & isocitrate dehydrogenase
- NADH plays a regulatory role in balancing energy metabolism



Components & stepwise reactions

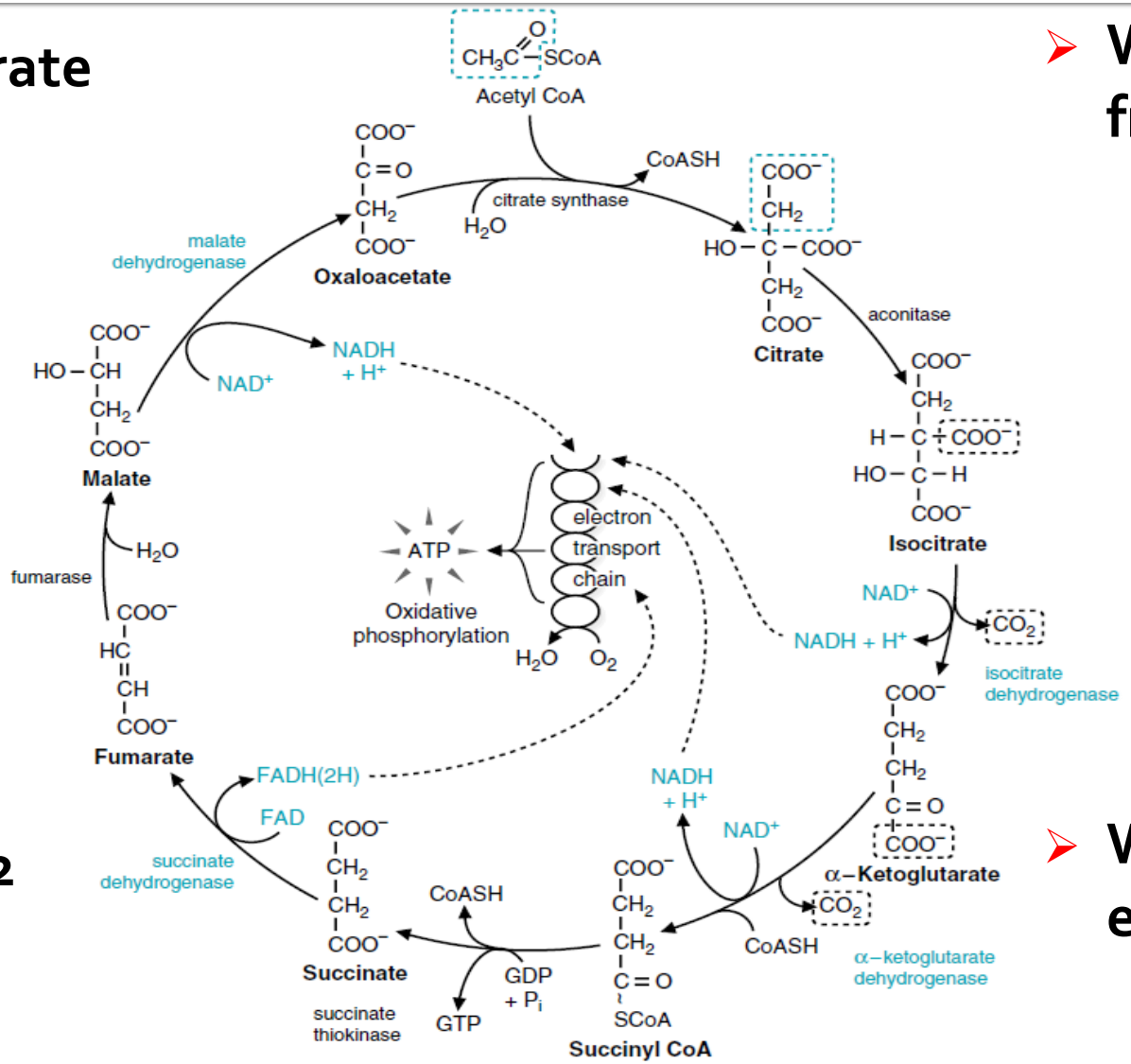
- No O_2 introduced, two CO_2 exits

CIA Sent Soldiers For My Office



Does Acetyl-CoA exit as 2 CO_2?

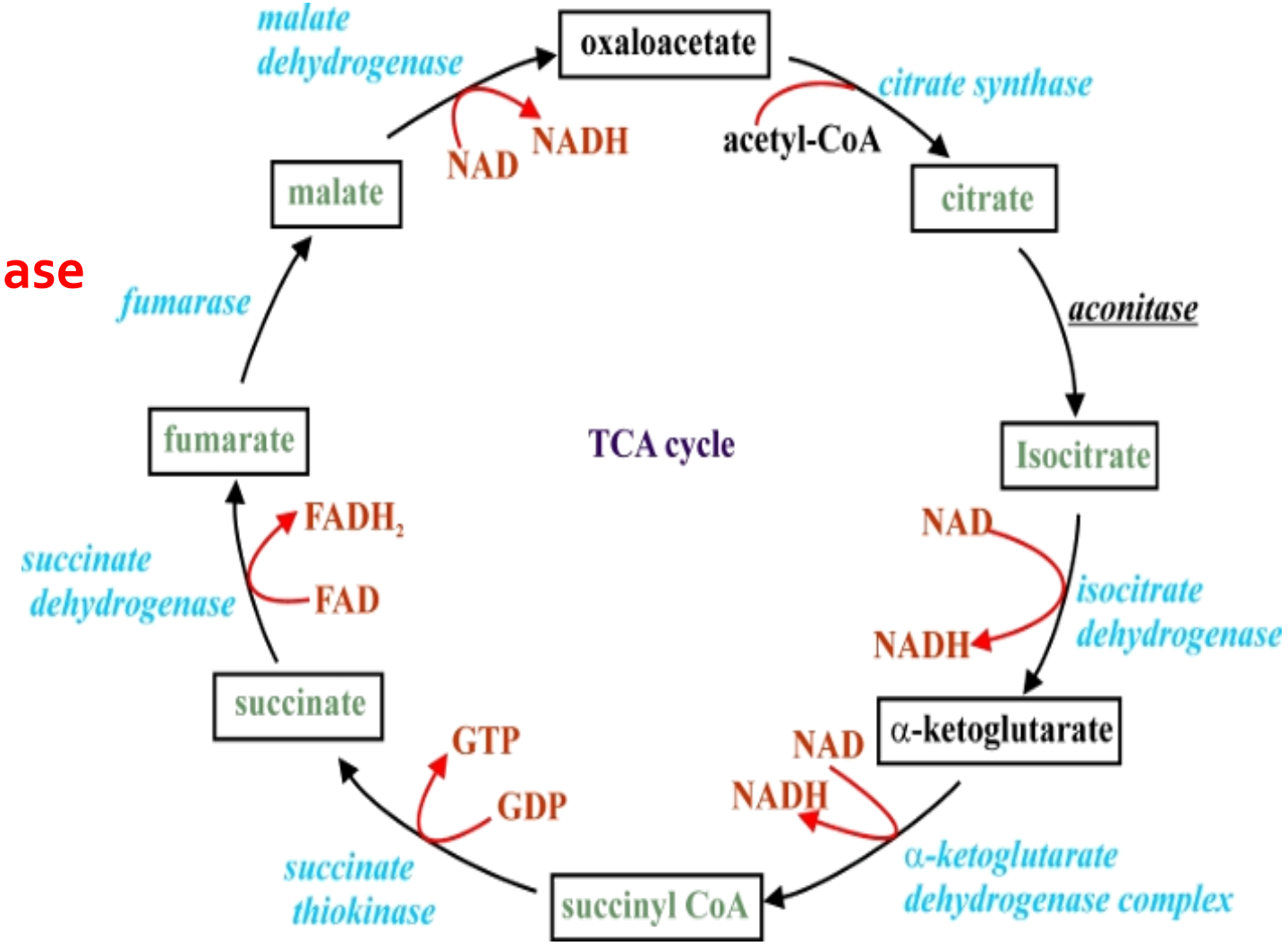
- Why to make Isocitrate from citrate?



- Why to make Isocitrate from citrate?
- Where does the CO_2 exit?
- Where does the CO_2 exit?

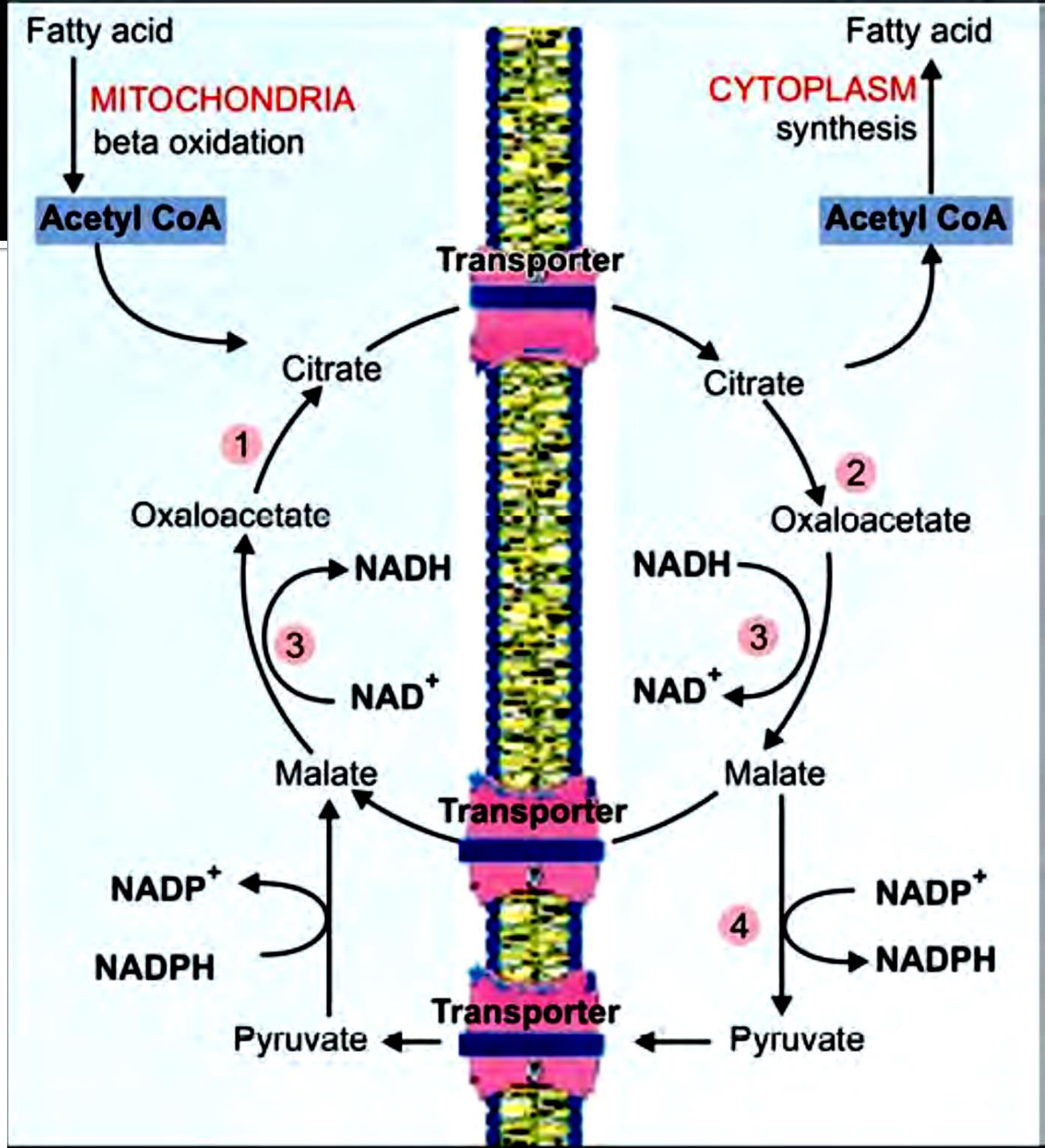
Enzymes of the TCA Cycle

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



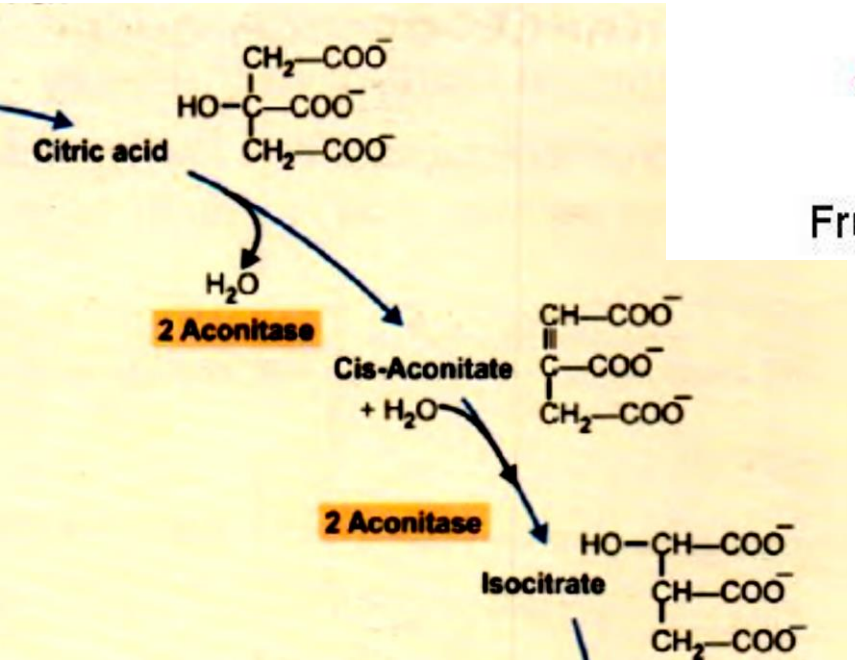
Formation of citrate

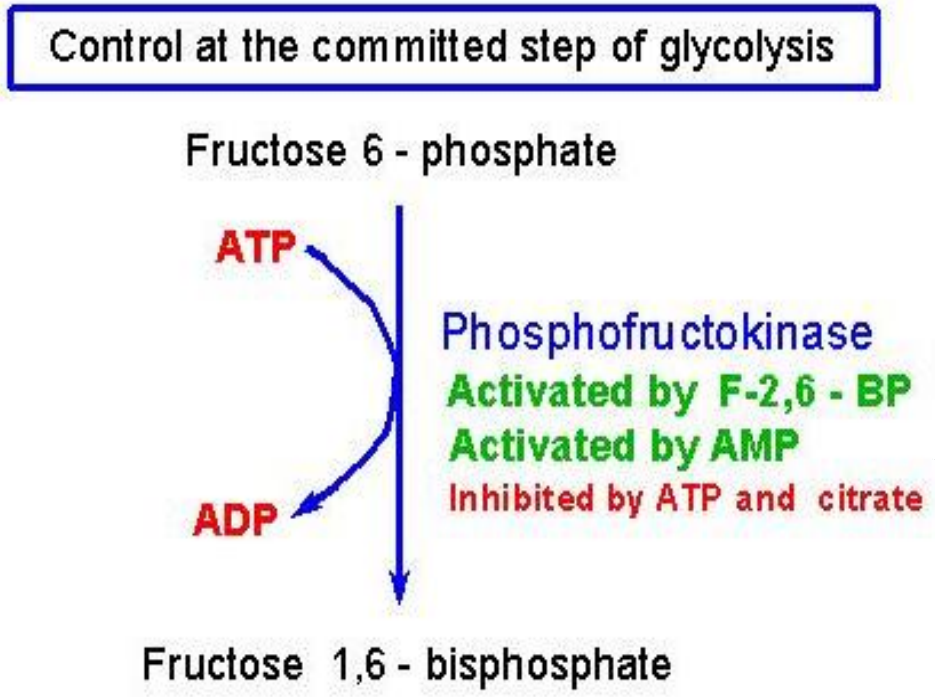
- What drives the reaction forward?
- Is it reversible or irreversible?
- Can it be reversed?
- ATP-Citrate lyase or ATP-Citratase!

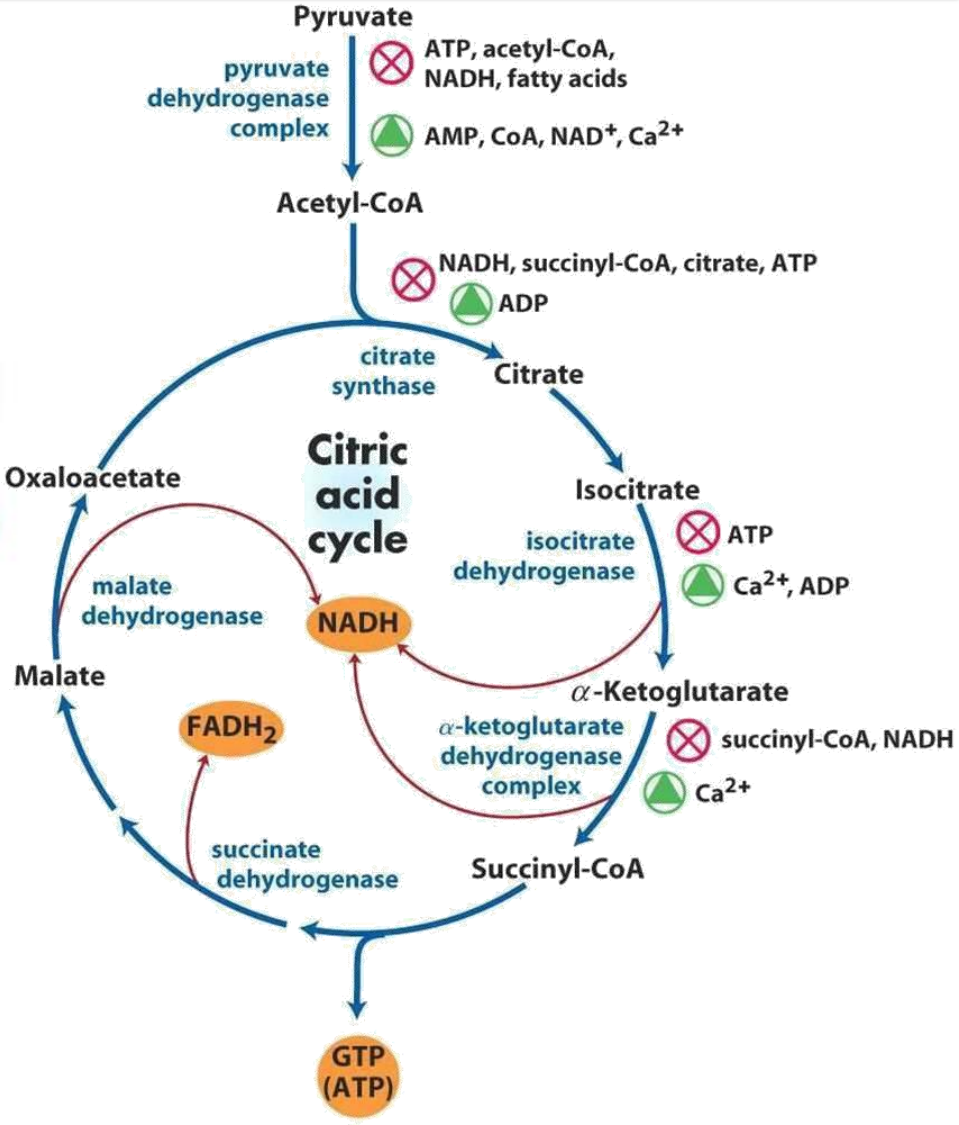


Formation and Oxidation of Isocitrate

- Oxidative decarboxylation
- 3° to 2° alcohol

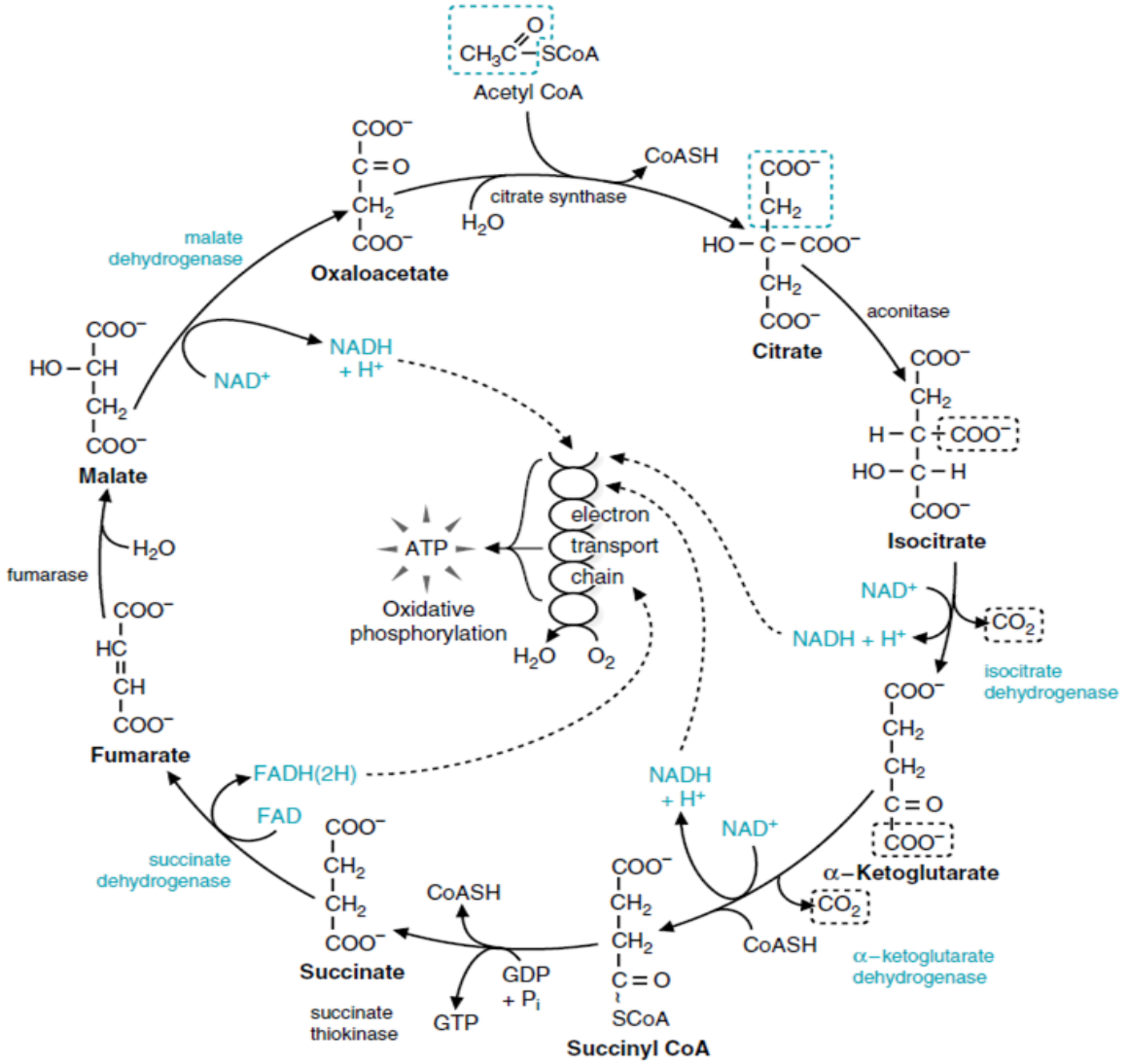






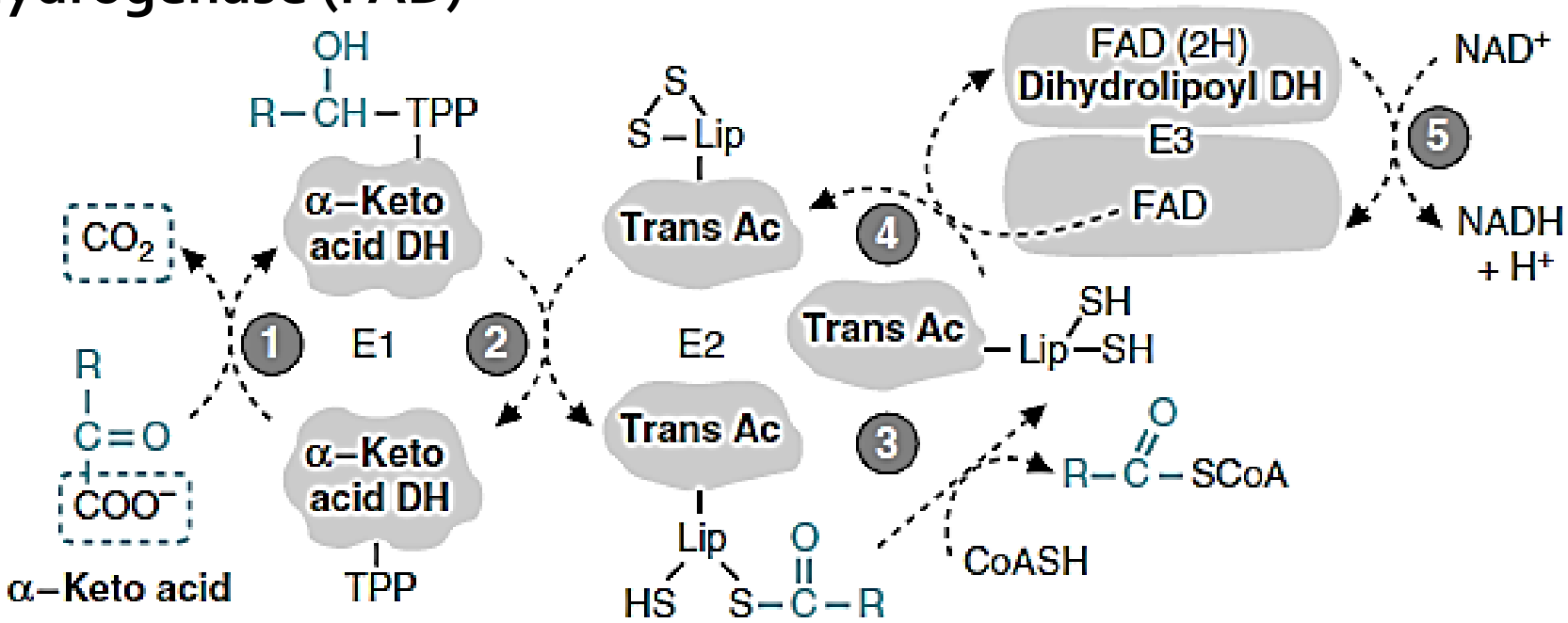
α-Ketoglutarate to Succinyl CoA

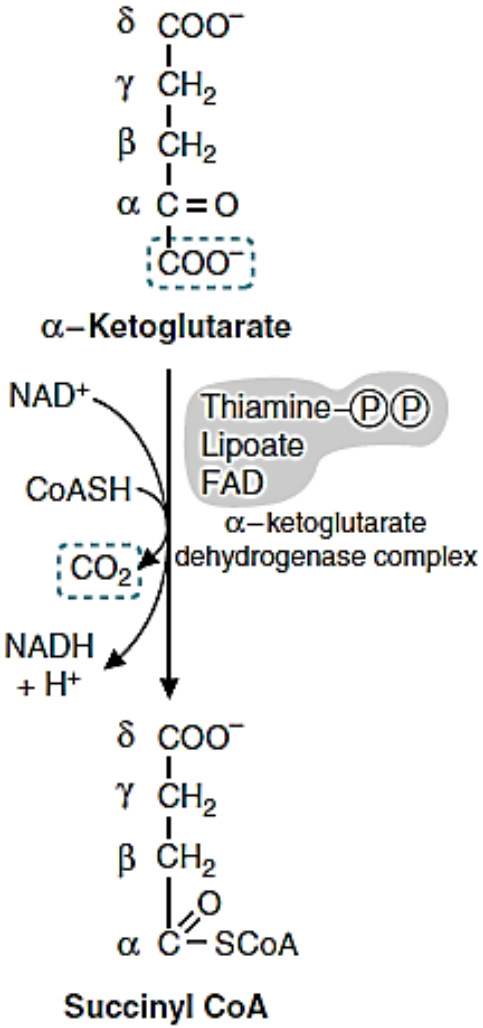
- Oxidative decarboxylation
- Thiamine pyrophosphate, lipoic acid, and FAD
- Keto group oxidized to acid, CoA-SH, succinyl CoA
- Energy conserved as NADH, thioester bond
- The only irreversible step in the whole reaction cycle



α-Ketoacid Dehydrogenase Complexes (TLCFN)

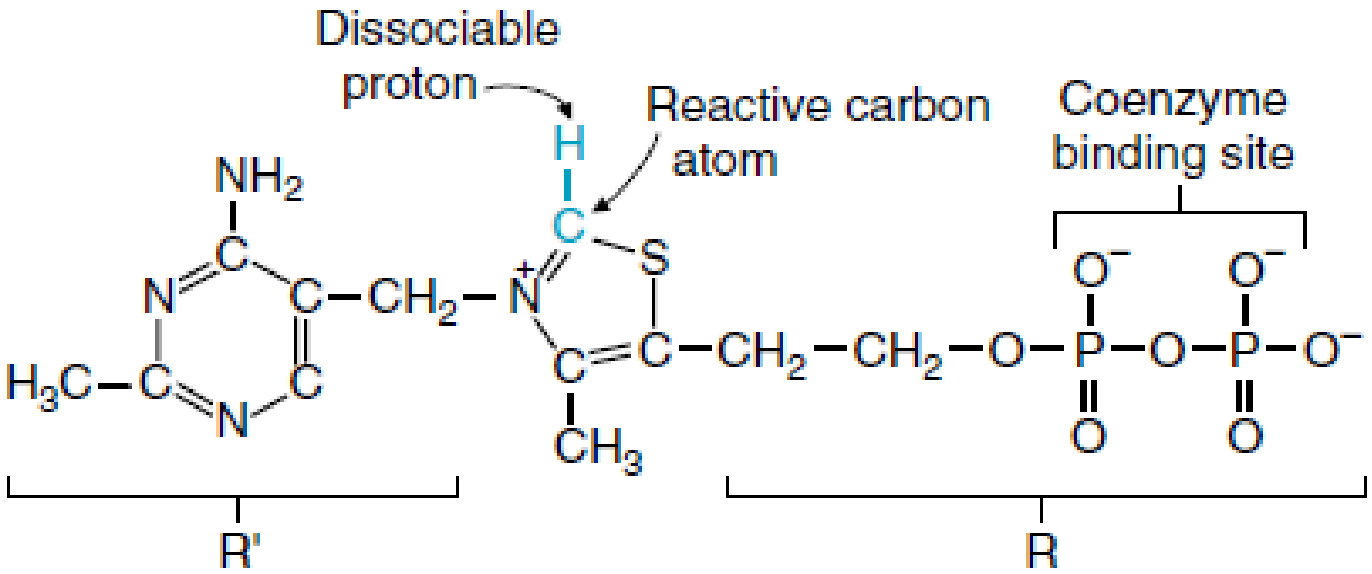
- (α-ketoglutarate, pyruvate, and branched chain α-keto acid) dehydrogenase complexes
- Huge enzyme complexes, multiple subunits of 3 different enzymes (no loss of energy, substrates for E2 and E3 remain bound → higher rate)
- E1, E2, & E3 are a decarboxylase (TPP), a transacylase (lipoate), & a dehydrogenase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



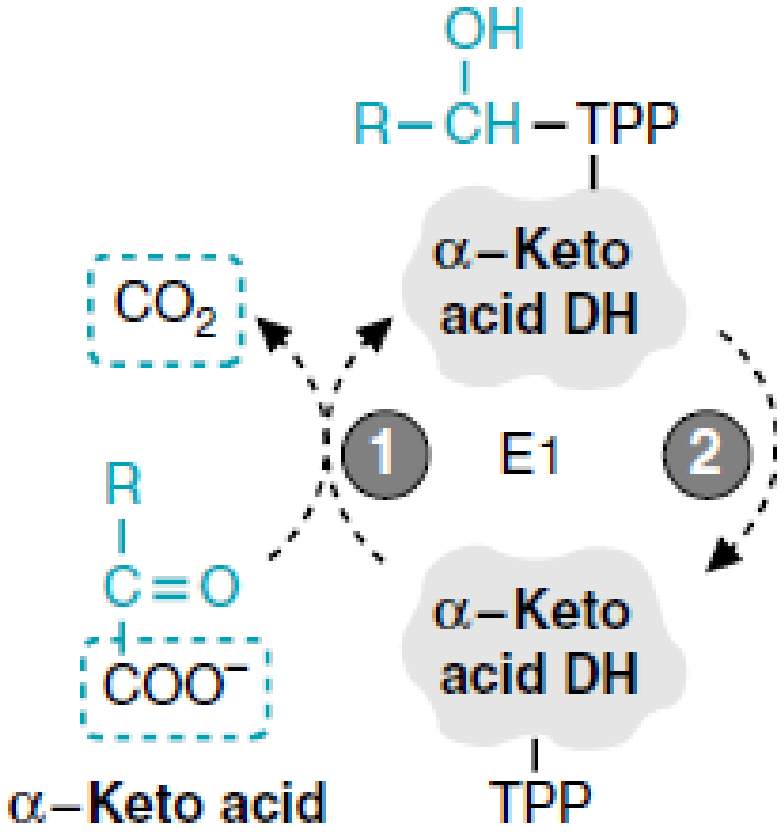


Thiamine Pyrophosphate

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

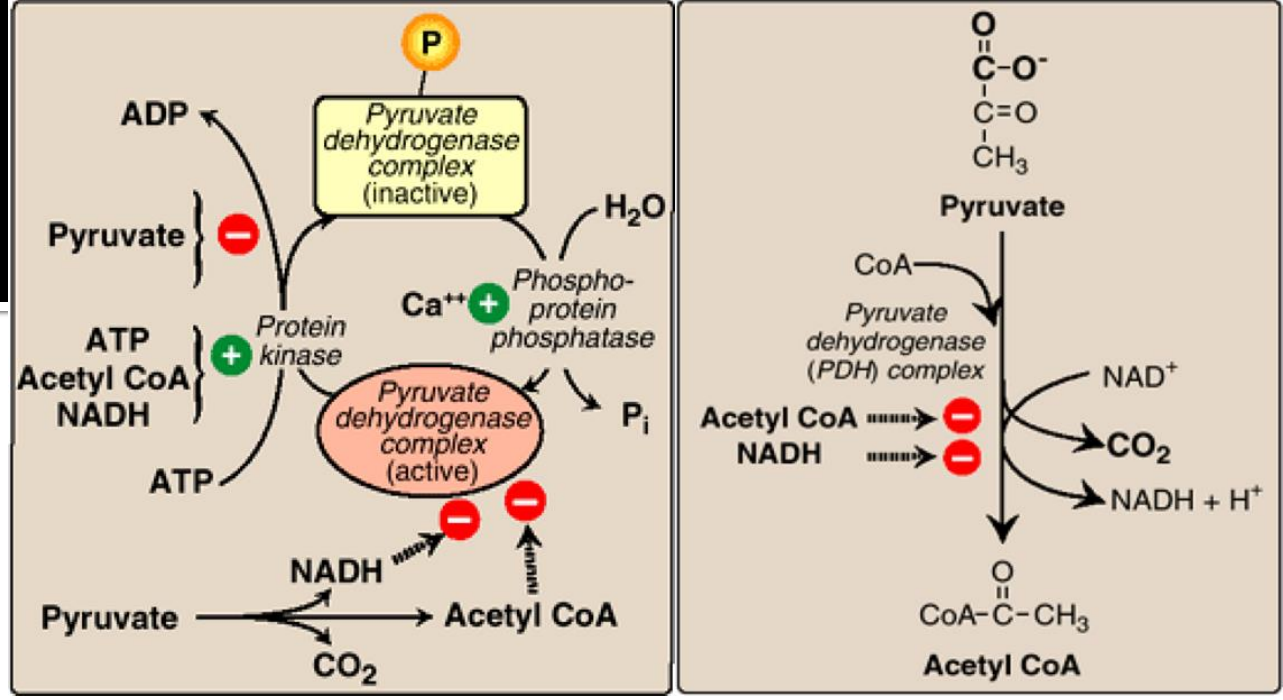


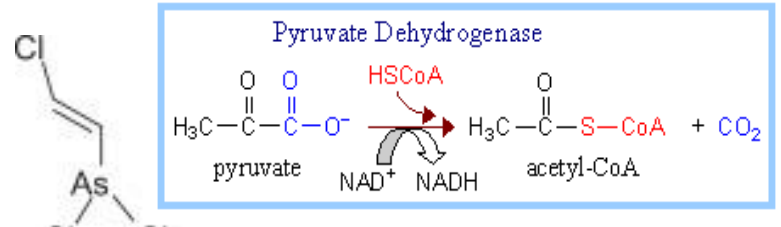
Thiamine pyrophosphate (TPP)



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**





Generation of GTP

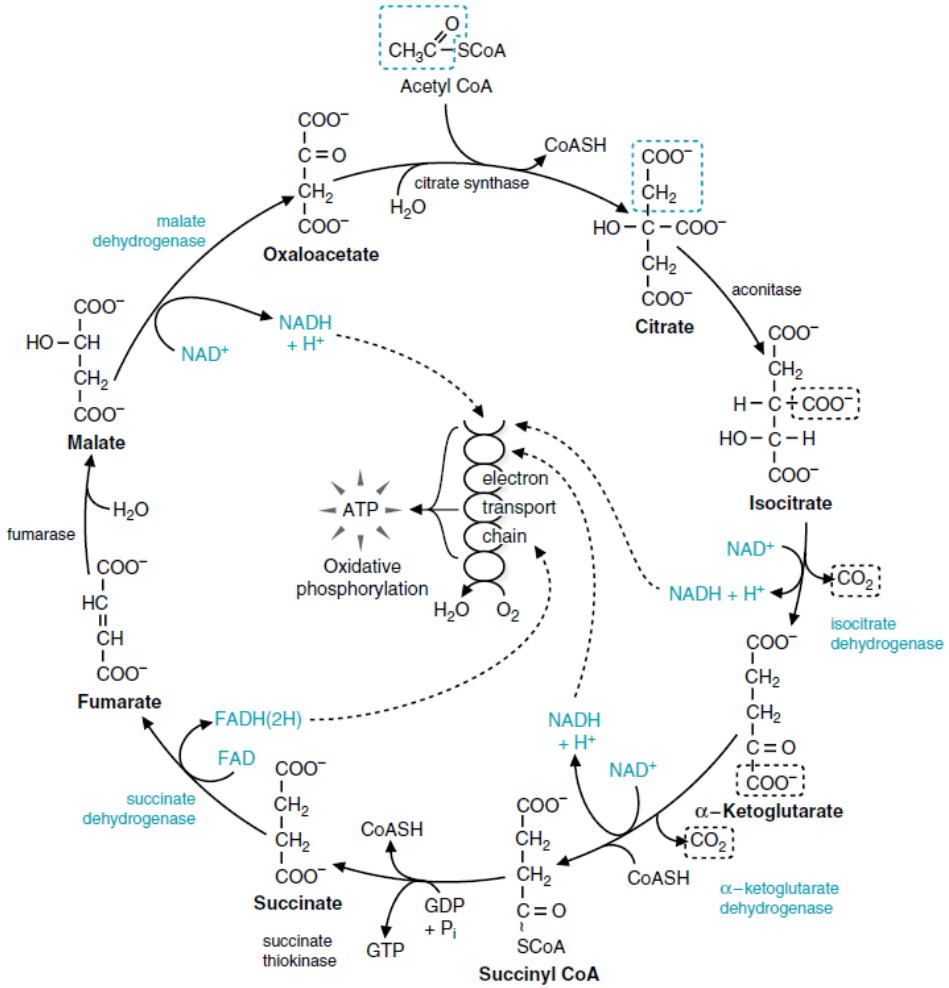
- **Succinyl CoA thioester bond, succinate thiokinase, substrate level phosphorylation**

GTP +ADP ↔ GDP + ATP

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

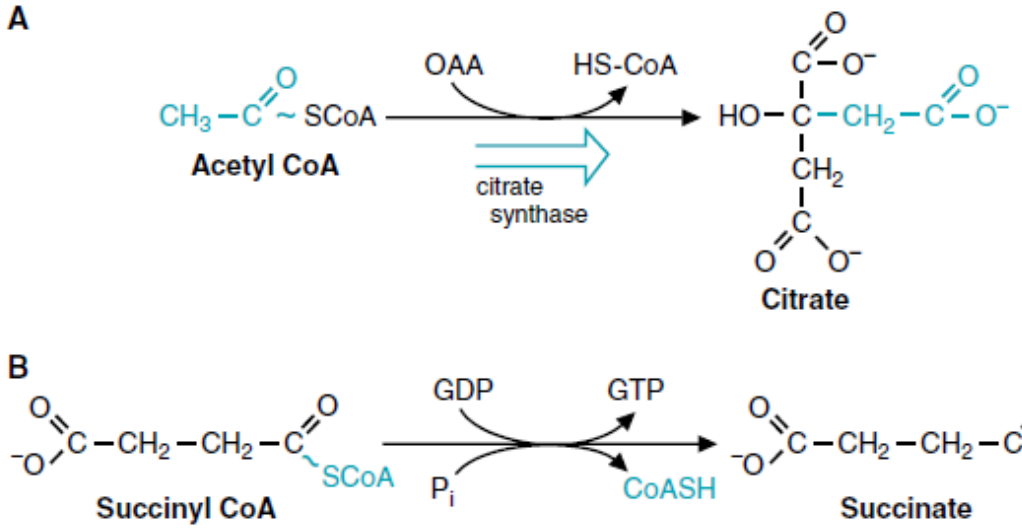
Phosphoenolpyruvate + ADP ⟶ (Pyruvatekinase) ⟵ Pyruvate + ATP

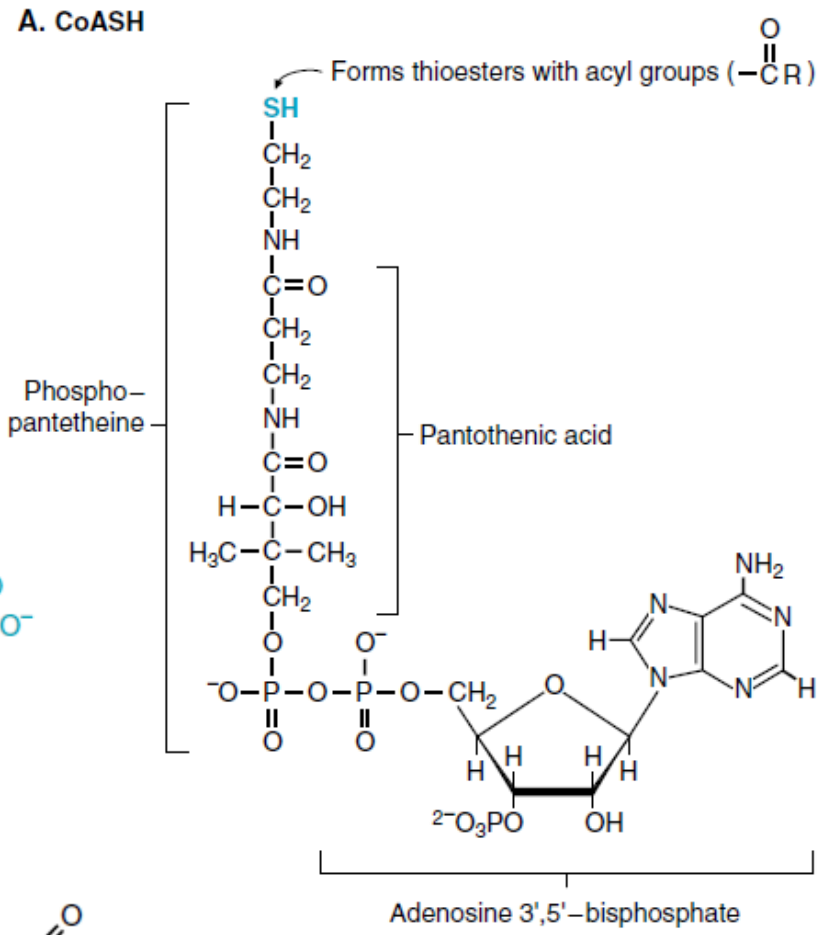
SuccinylCoA +GDP ⟶ (SuccinylCoA Synthase) ⟵ Succinate +GTP



CoA

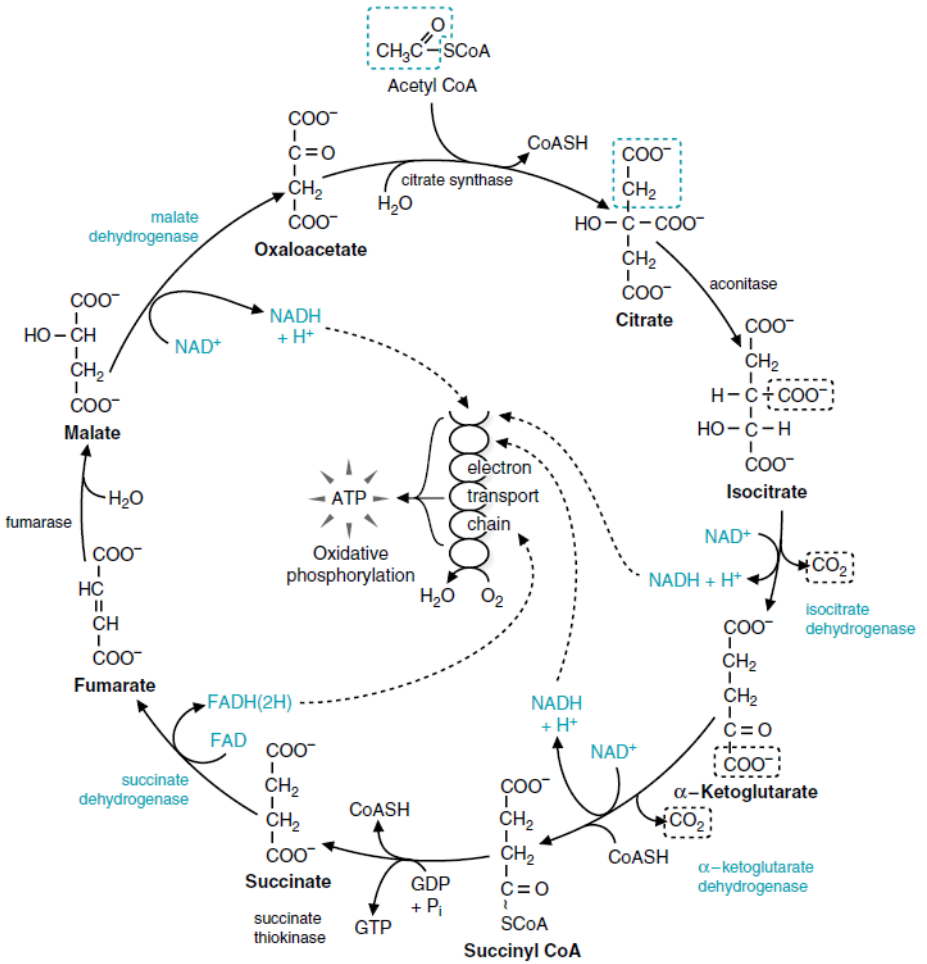
- Forms a thioester bond, CoASH & an acyl group (e.g., acetyl CoA, succinyl CoA)
- Sulfur vs. oxygen (carbon can be activated, -13kcal, GTP, keeps the reaction going)





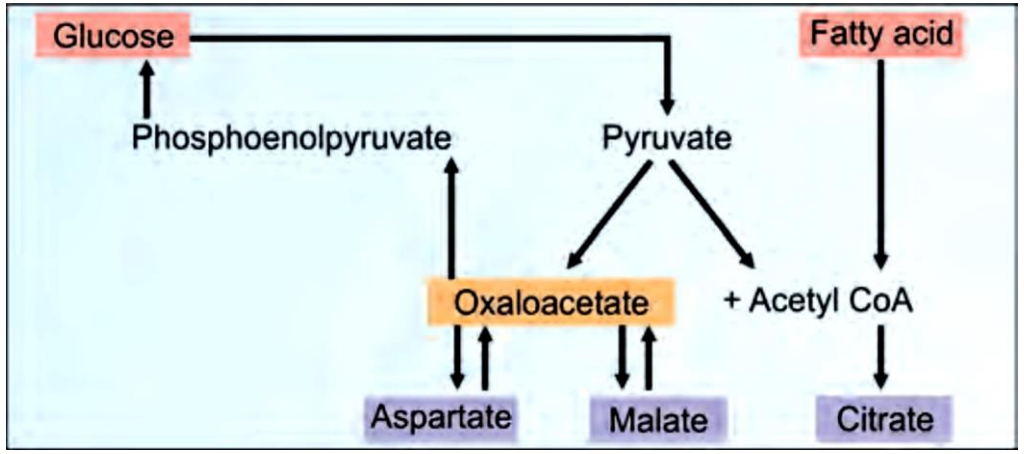
Oxidation of Succinate to Oxaloacetate

- 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 catalyst
- An important junction point in metabolism



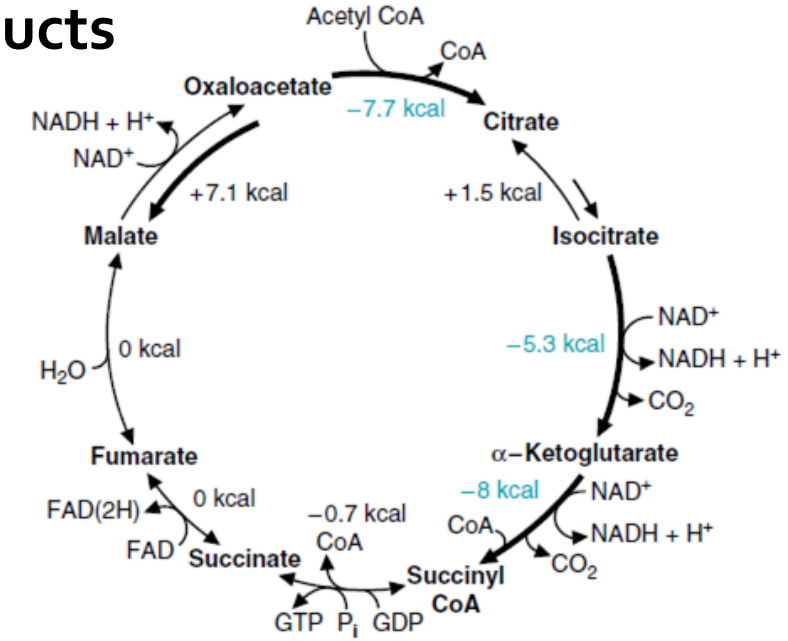
Bioenergetics of TCA Cycle

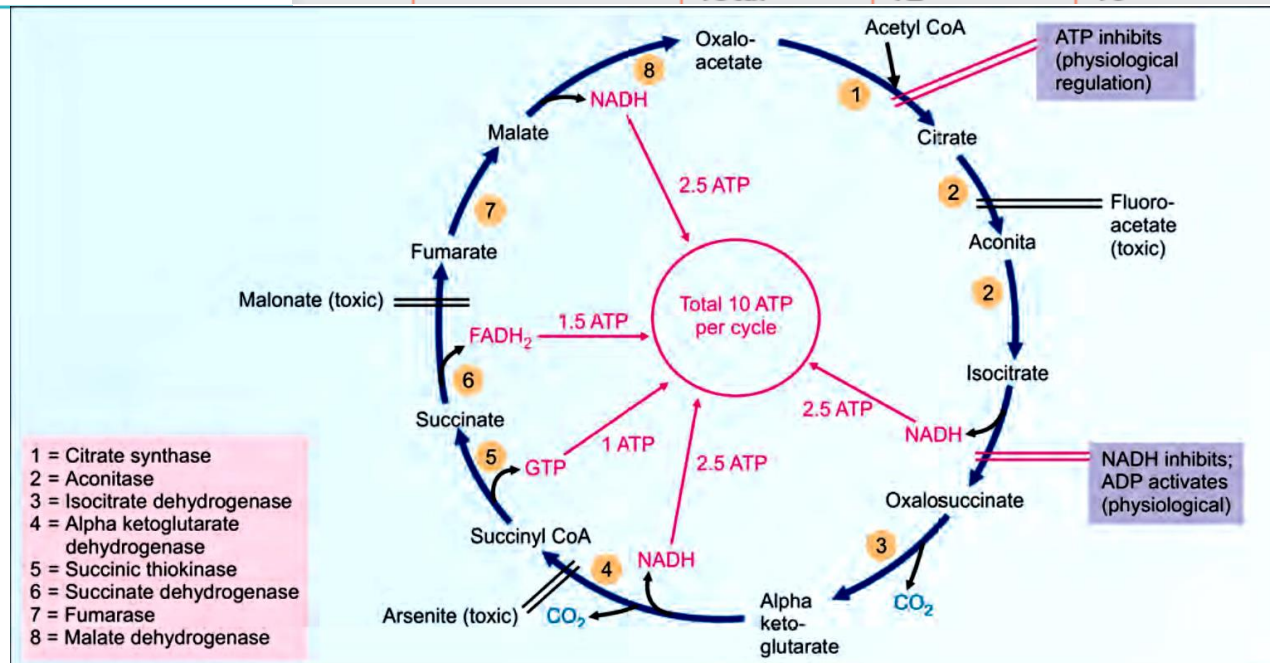
- Like all pathways, overall net -ΔG (-228 kcal/mole), not 100%
- NADH, FAD(H2), and GTP (10ATP), 207 Kcal, 90%
- Three reactions have large (-ve) values
- Physiologically irreversible, low products

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

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 the cycle & 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

Why?

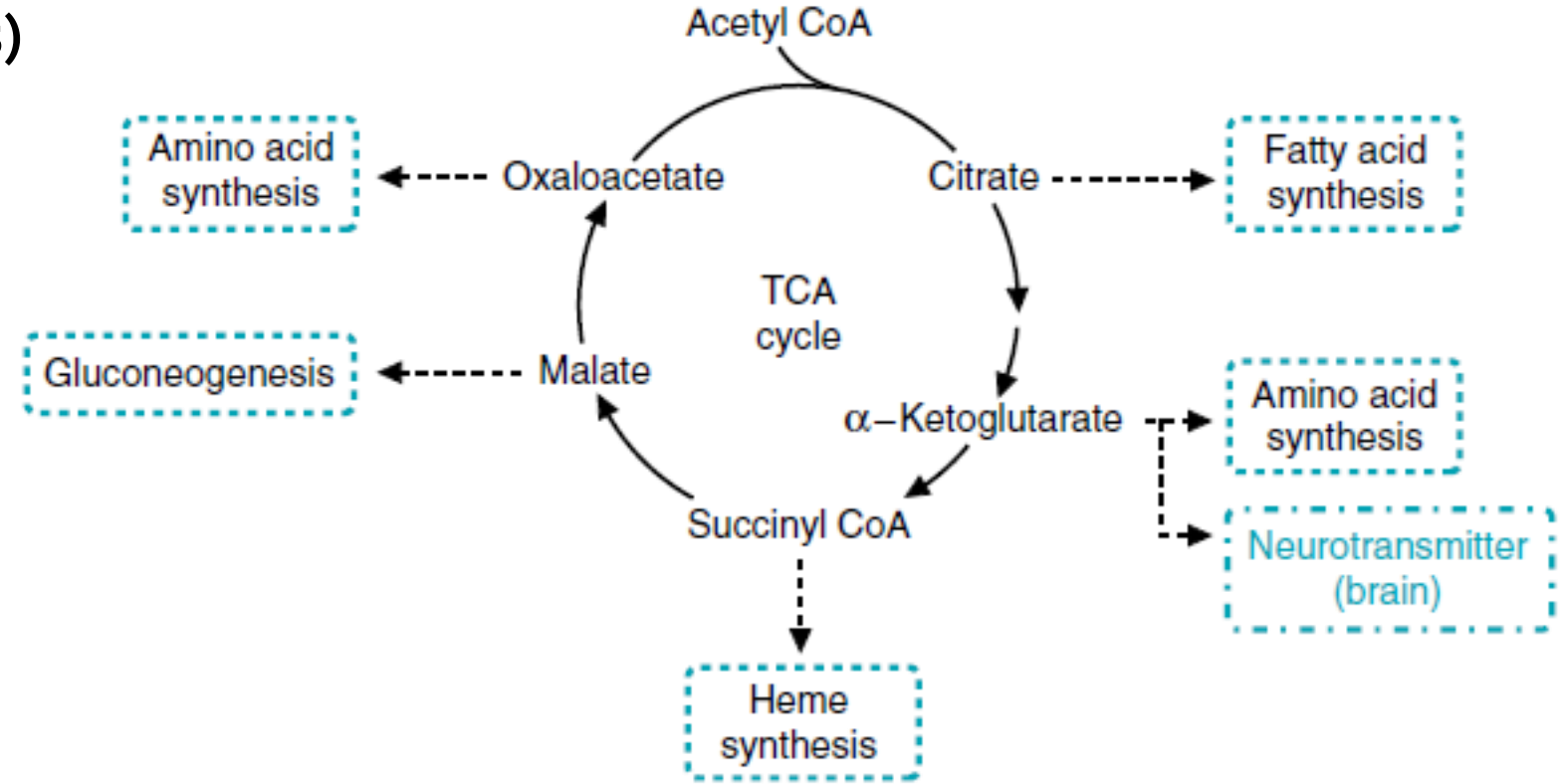
Box 19.1: Significance of citric acid cycle

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.

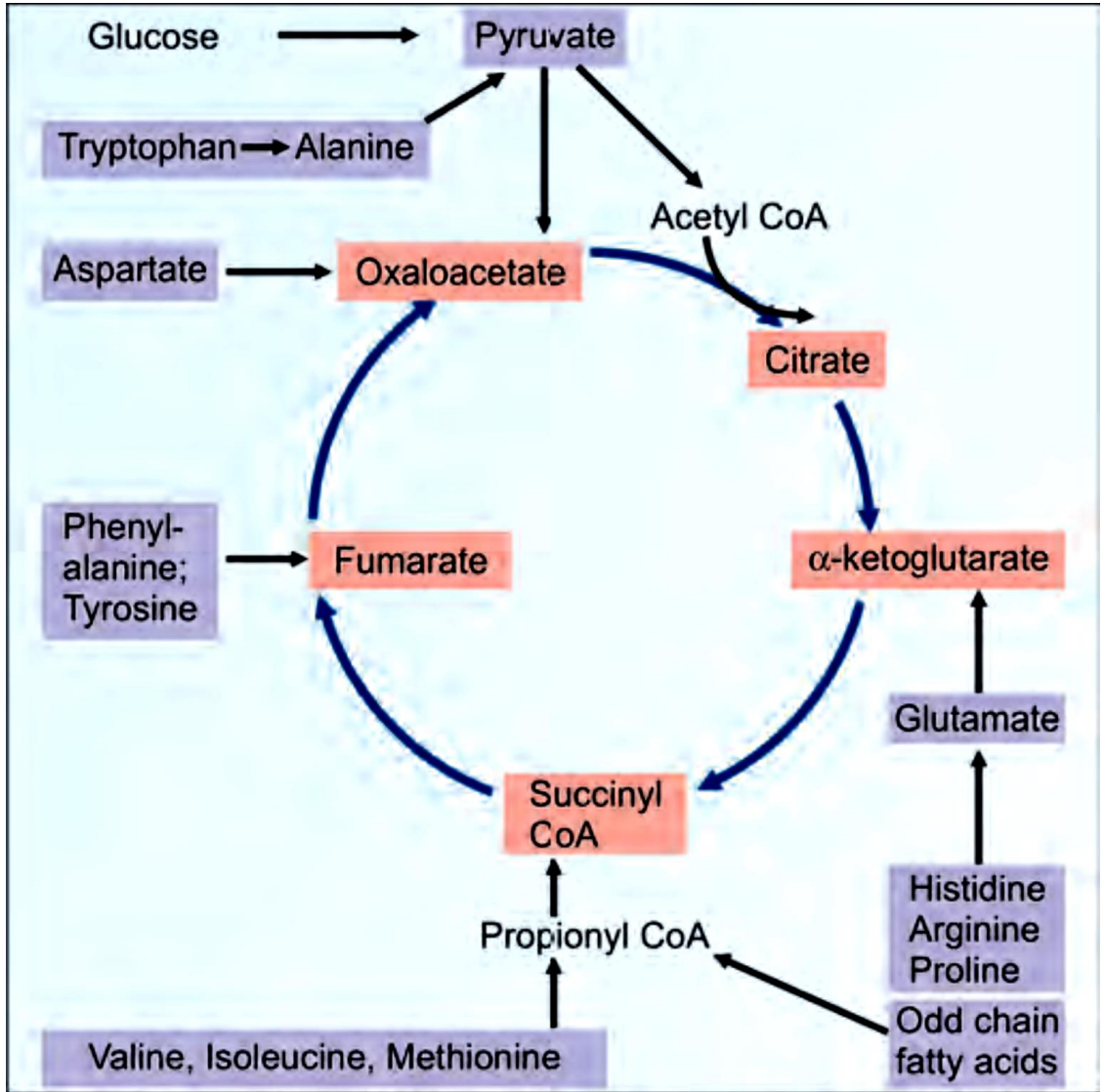
- ✓ Fats are burned in the fire of carbohydrates
- ✓ Fat cannot be converted to glucose because pyruvate dehydrogenase reaction is an absolutely irreversible step

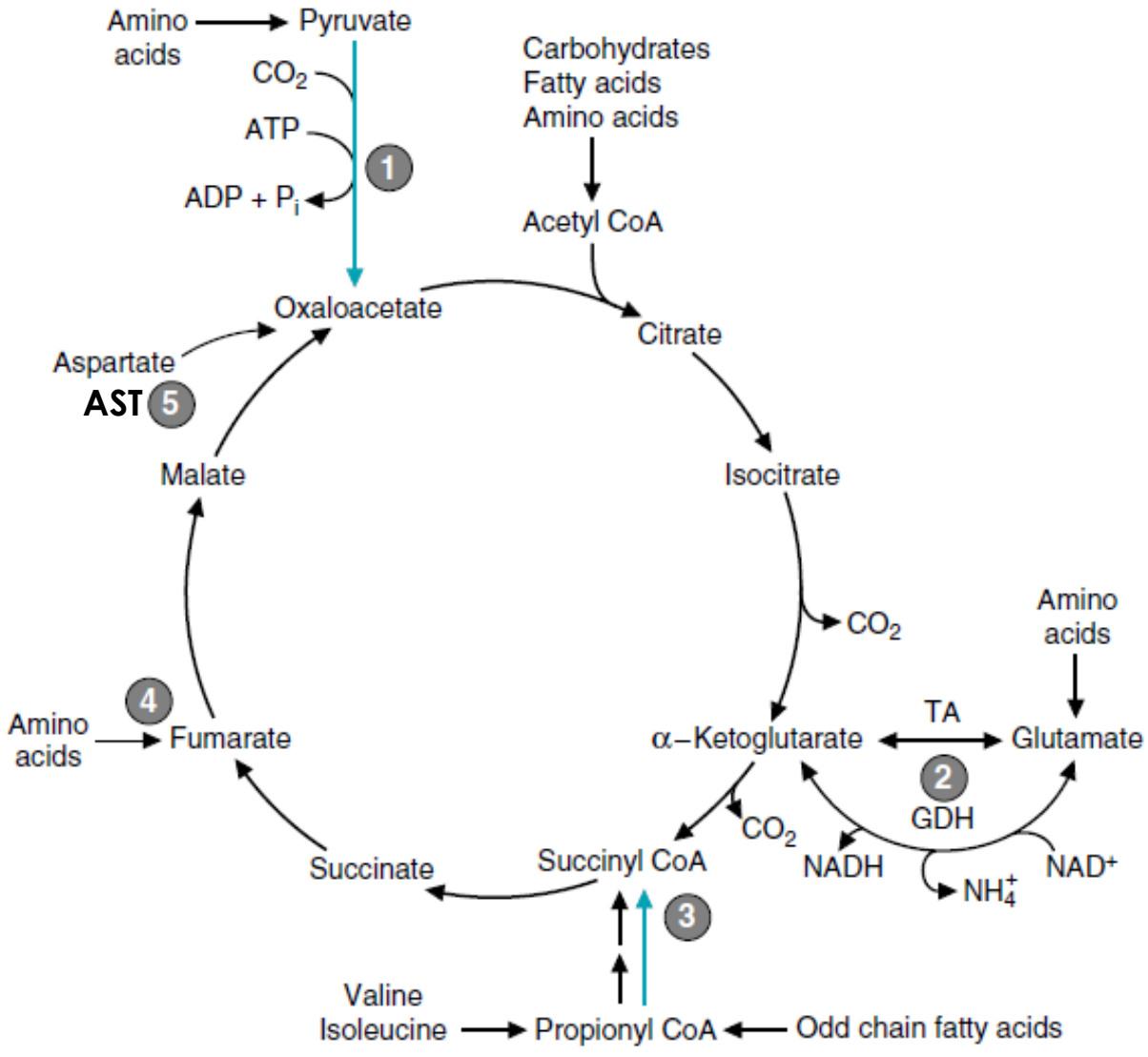
TCA Cycle Intermediates

- **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)**



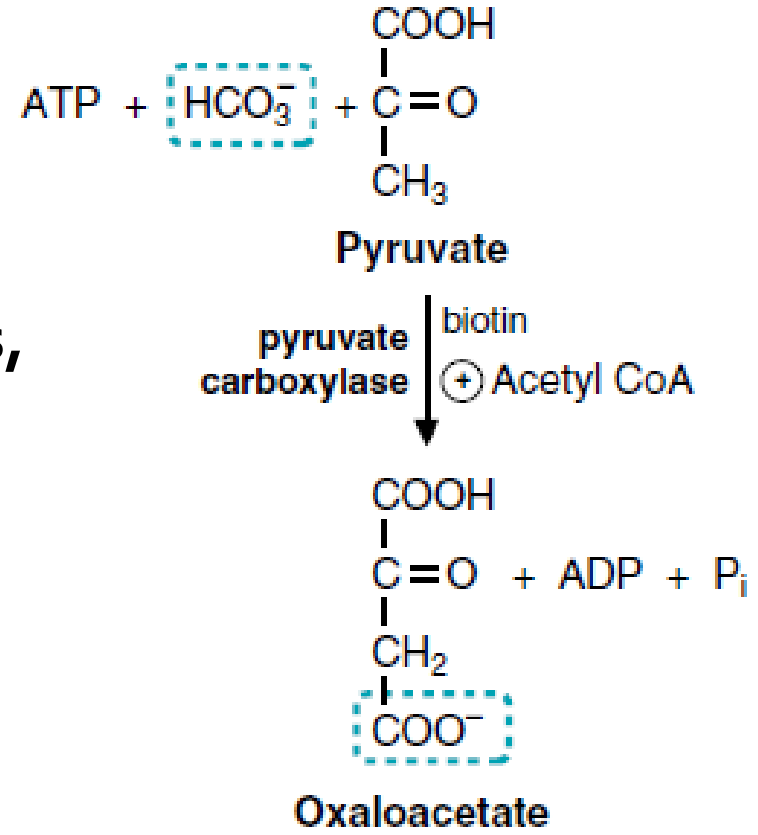
Other Anaplerotic Routes (Amino Acid Degradation)





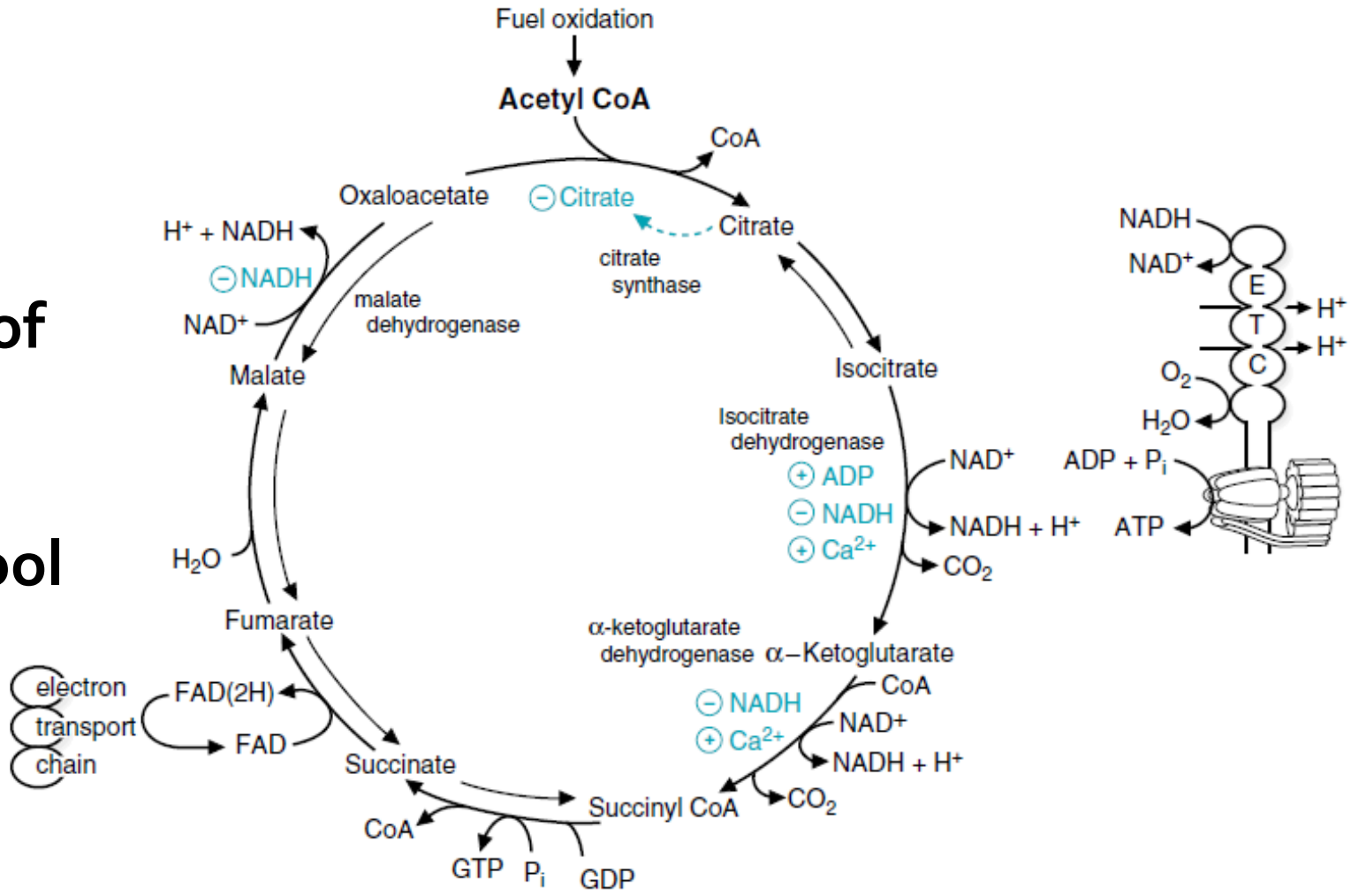
Anaplerotic Reactions

- 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)



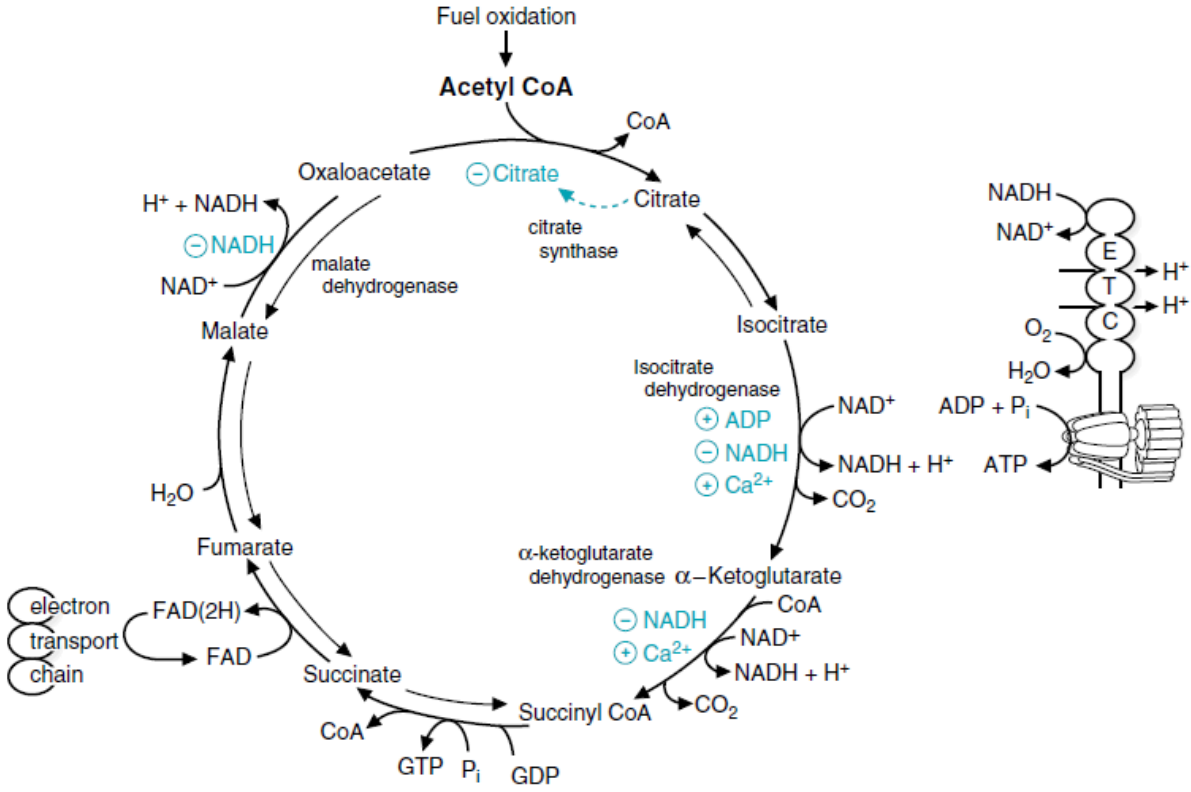
Regulation of the TCA Cycle

- 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



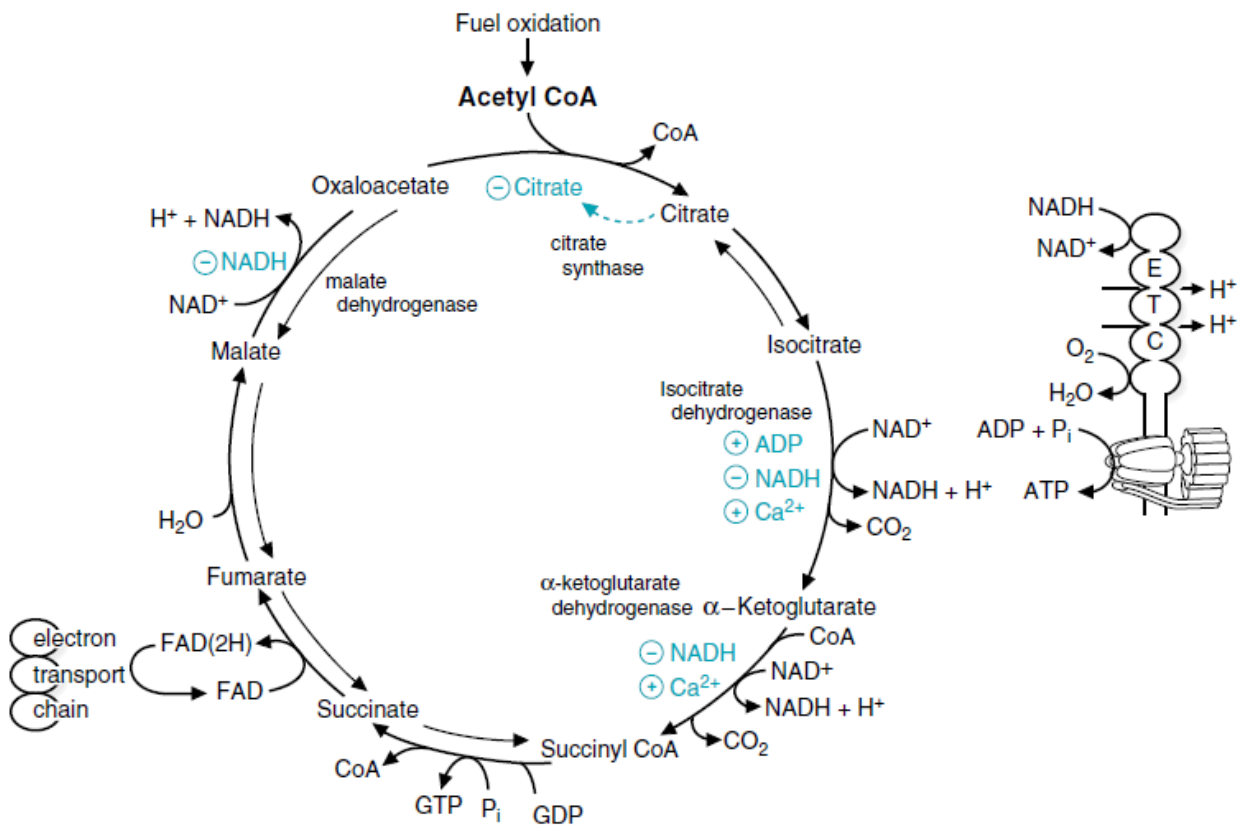
Regulation – Citrate & Citrate Synthase

- 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



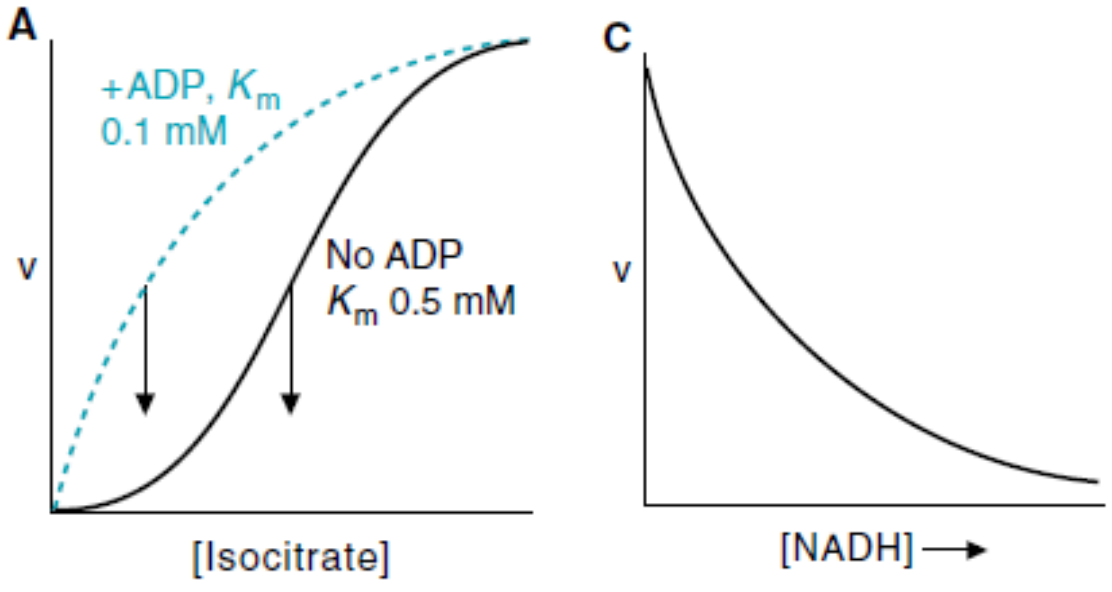
Isocitrate DH

- 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)