Citric Acid Cycle

Pratt & Cornely, Ch 14

Overview

- Compartmentalization
  - Glycolysis: Cytosol
    - Pyruvate carried into mitochondria via transporter
  - GNG: Starts in mitochondria, but mainly cytosol
  - Citric Acid Cycle: mitochondria

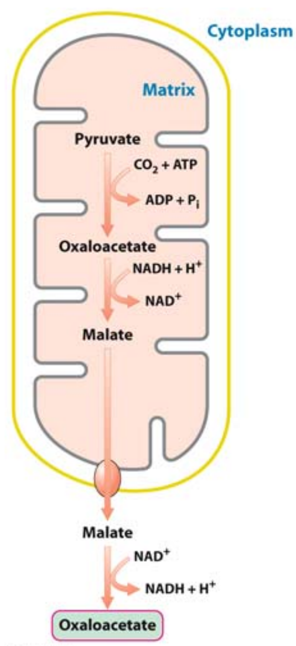
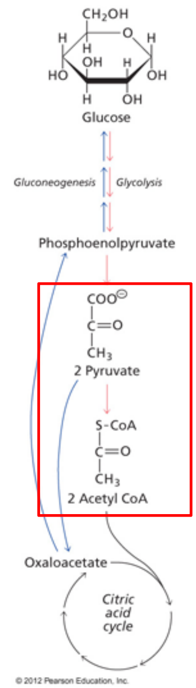


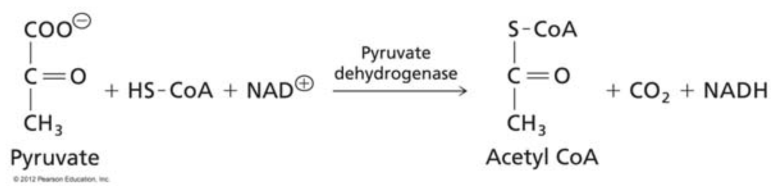
Figure 17.4
Biochemistry: A Short Course, Third Edition

Overview

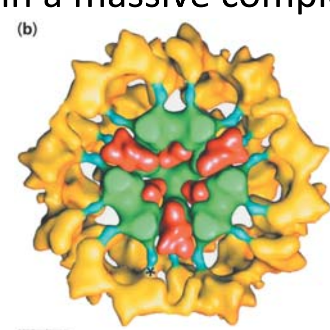


- Glycolysis
- Pyruvate dehydrogenase complex
  - Commitment of carbon away from carbohydrates
- Into the citric acid cycle

Pyruvate Dehydrogenase Complex

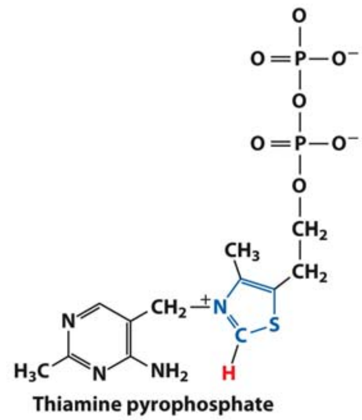


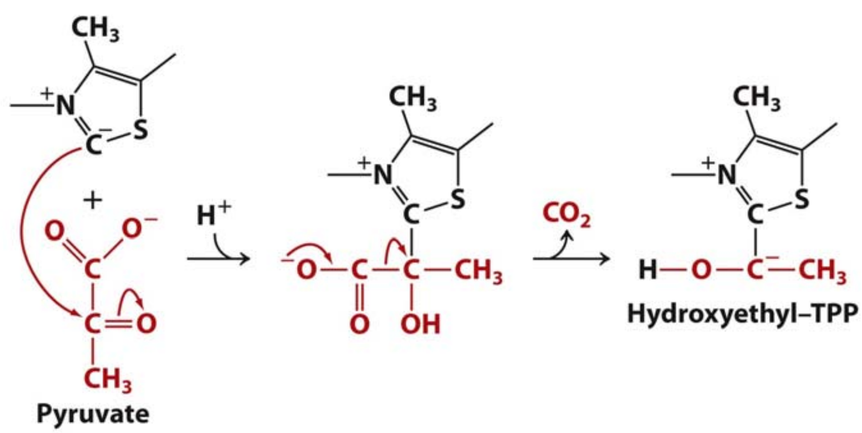
- Three distinct enzymes—in a massive complex
- Five chemical steps
- What cofactors needed?



Pyruvate Dehydrogenase (E_1)

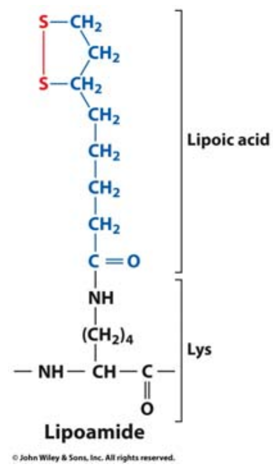
- TPP cofactor: Decarboxylation of α carboxyketones
- Stabilization of "acyl anion"
- Draw mechanism of decarboxylation

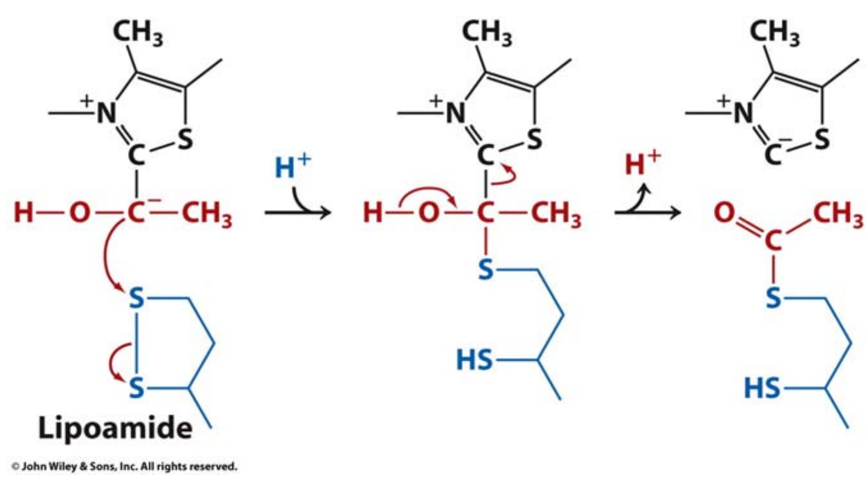




Dihydrolipoamide Acyltransferase (E_2)

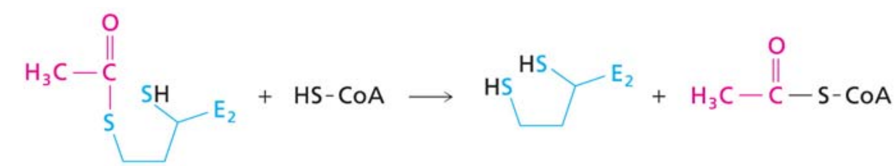
- Transfer catalyzed by E1
- Serves as an linker to “swing” substrate through subunits
- Mechanism of redox





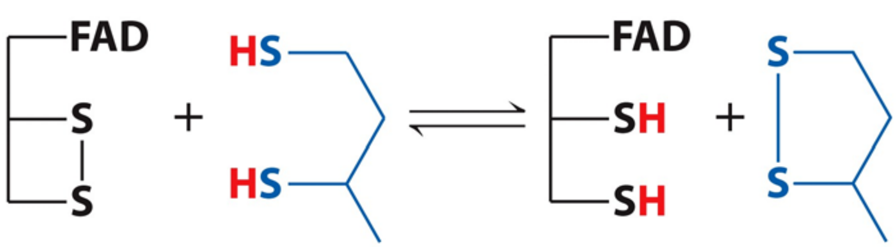
Step 3: transfer

- Maintenance of high energy bond
- Acetyl CoA product is made
- Lipoamide still reduced—not catalytically viable at this point

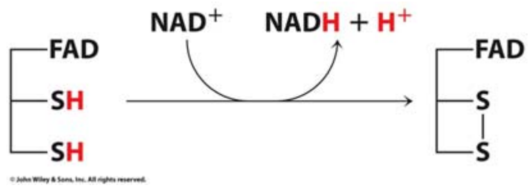


Dihydrolipoamide dehydrogenase (E_3)

- Redox of prosthetic FAD/$FADH_2$ through a disulfide bond in the protein
- Still not a regenerated catalyst!

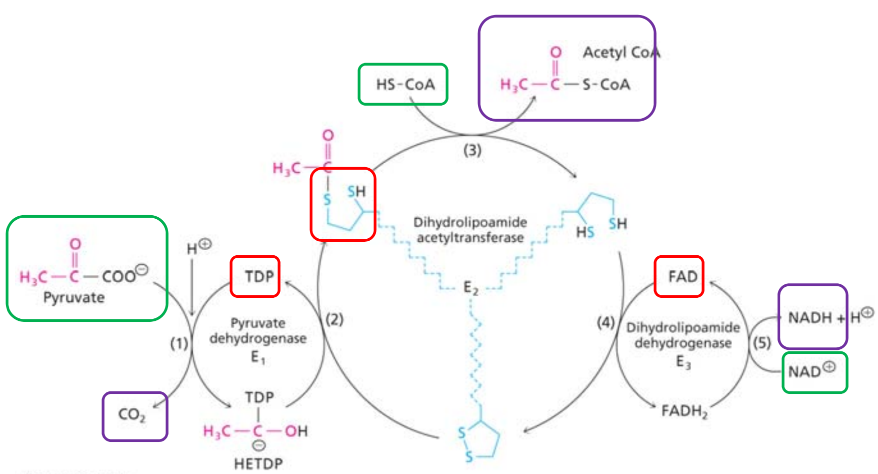


Step 5: NADH produced



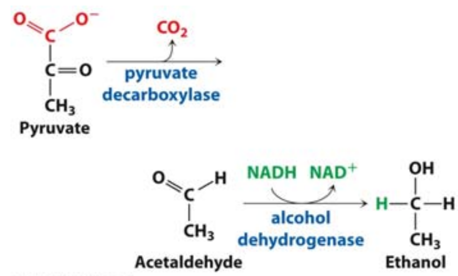
- Prosthetic group is restored by stoichiometric NAD^+
- Step 1 uses proton, step 5 regenerates
- Oxidation energy of one carbon atom used to
  - Produce high energy thioester
  - Produce NADH

Overall Reaction



Problem 6

- Using pyruvate DH as an example, propose a mechanism for the TPP dependent yeast pyruvate decarboxylase reaction in alcohol fermentation.



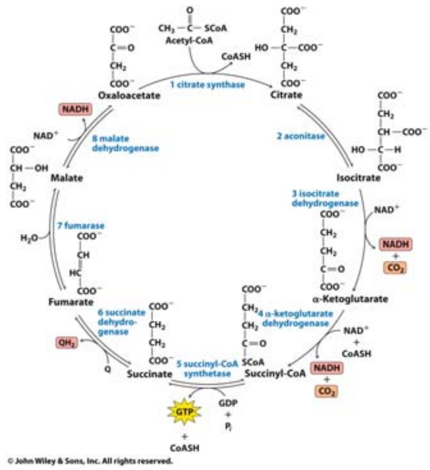
Fate of Acetyl CoA

- Storage of energy as fatty acid, OR
- ATP production (harvest of high potential electrons)

Formal reaction:

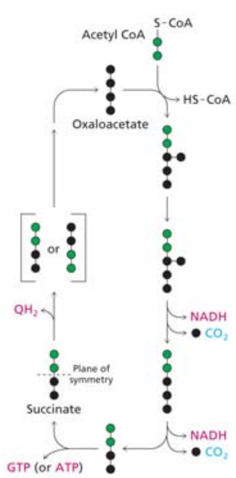
$$CH_3\text{—}C(=O)\text{—S-CoA} + 2\,H_2O + OH^{\ominus} \longrightarrow 2\,CO_2 + \text{HS-CoA} + 7\,H^{\oplus} + 8e^{\ominus}$$

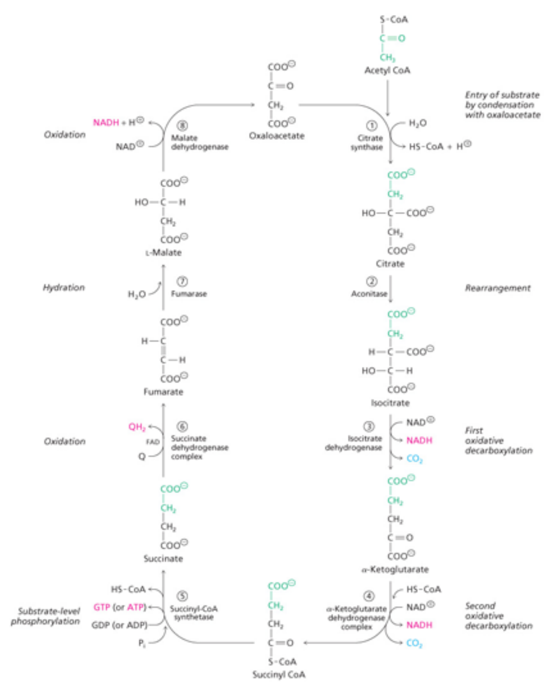
Citric Acid Cycle



- Cyclic pathway
  - CO_2 is the product
- Substrate level NTP
- NADH stores high energy electrons
  - Oxidation of alcohol or oxidative decarboxylation
- QH_2 stores high energy electrons
  - Alkane reduction to pi bond

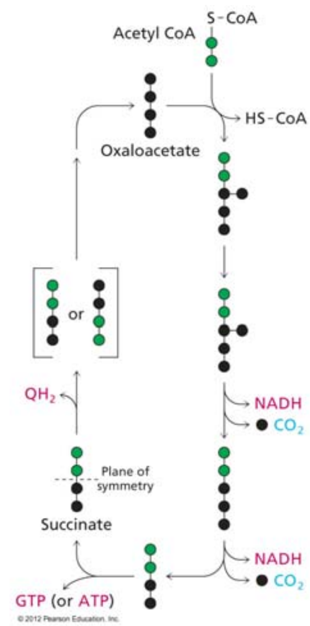
Overview





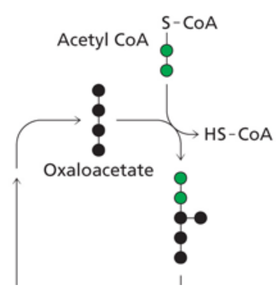
Carbon Flow

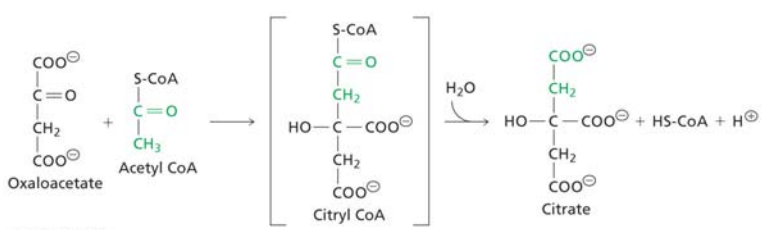
- Each cycle is **net** oxidation of acetyl CoA
  - Not actual loss of carbon from acetyl CoA
- C-14 incorporation experiments
- 4-carbon compounds act “catalytically” in oxygen consumption
  - Cyclic pathway!

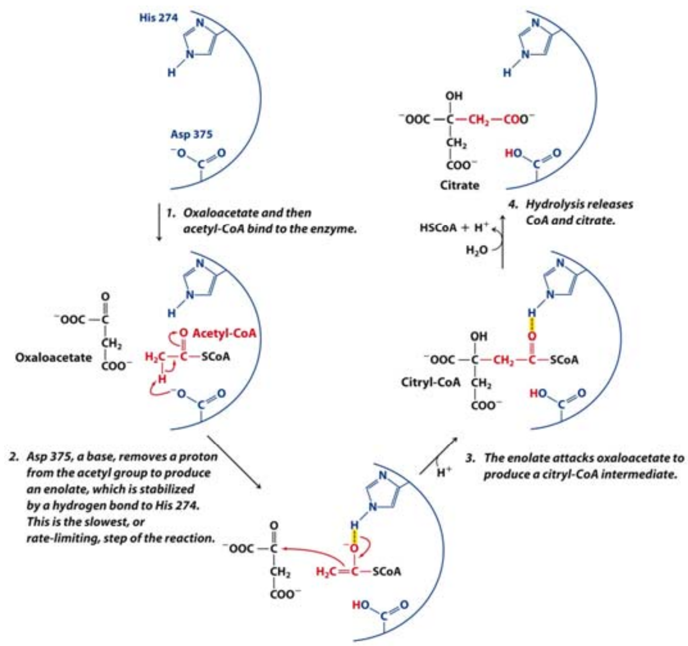


1. Citrate Synthase

- Highly exothermic—lysis of high energy bond
- Used to drive reaction in presence of small [oxaloacetate]

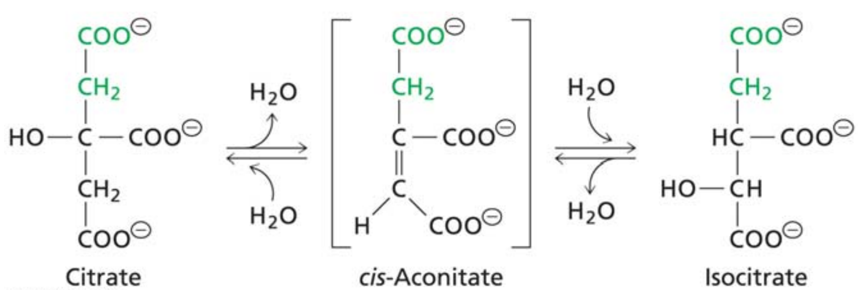






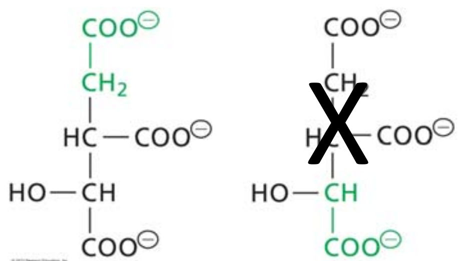
2. Aconitase

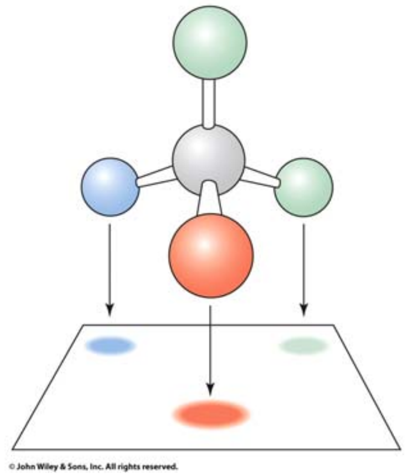
- Citrate is prochiral
- Green represents carbon from acetyl CoA
  - How can enzyme distinguish prochirality?



Prochirality

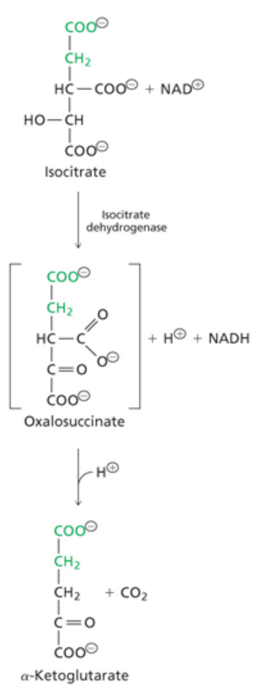
- Only one compound produced





3. Isocitrate Dehydrogenase

- Oxidative decarboxylation
- Spontaneous in β-ketoacids
- NADH
- α-ketoglutarate is a **key** intermediate



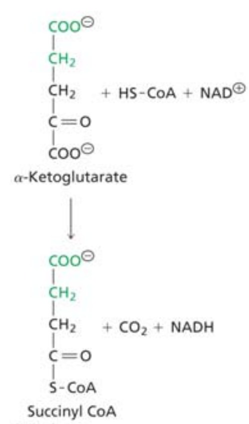
- Draw a full mechanism for conversion of isocitrate to α-ketoglutarate:

4. α-Ketoglutarate Dehydrogenase Complex

- Analogous to which enzyme?

  ____

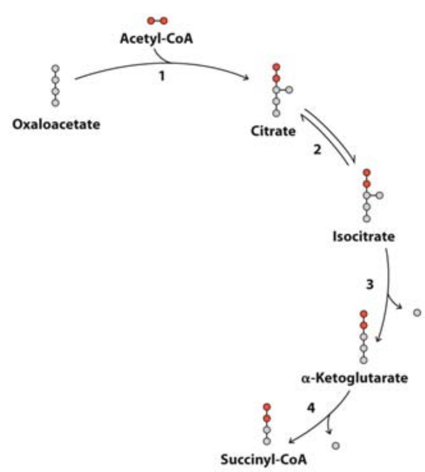
- Second decarboxylation, but this is an α-decarboxylation
  - Contrast mechanism
- High energy bond retained



Problem 28

- A patient with an αKG DH deficiency exhibits a small increase in [pyruvate] and a large increase in [lactate] so that the [lactate]/[pyruvate] ratio is many times larger than normal. Explain.

Carbon Review

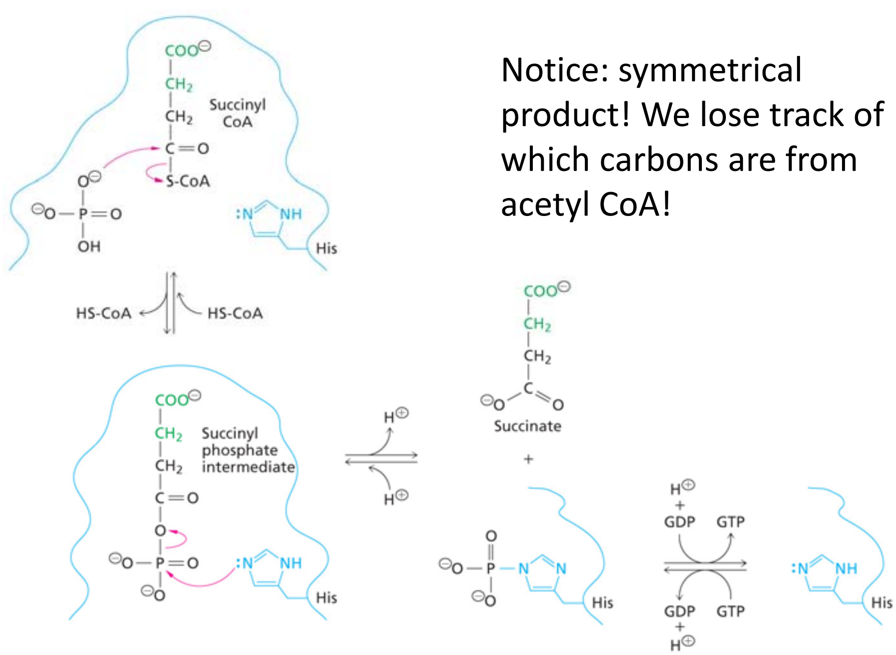


5. Succinyl CoA Synthetase

- Synthetase means ATP (or GTP) involved
- High energy bond used to do substrate-level phosphorylation
  - Good leaving group to activate P_i
  - Covalent catalysis
  - GDP → GTP

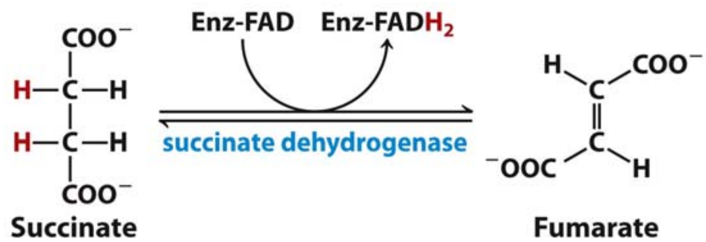
$$\text{Succinyl CoA} + P_i + \text{GDP} \longrightarrow \text{Succinate} + \text{HS-CoA} + \text{GTP}$$

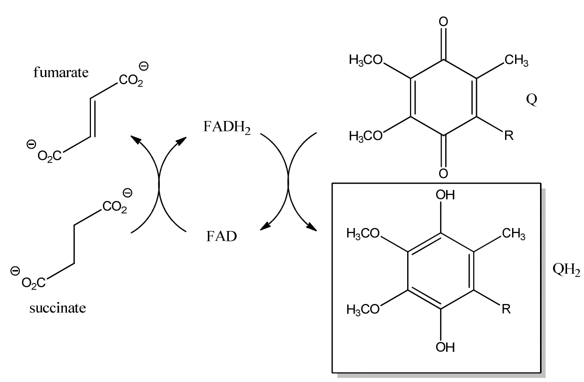
Notice: symmetrical product! We lose track of which carbons are from acetyl CoA!



6. Succinate Dehydrogenase

- Oxidation to form C=C releases less energy
- FAD is bound (prosthetic) redox reagent
- In turn, Q is reduced

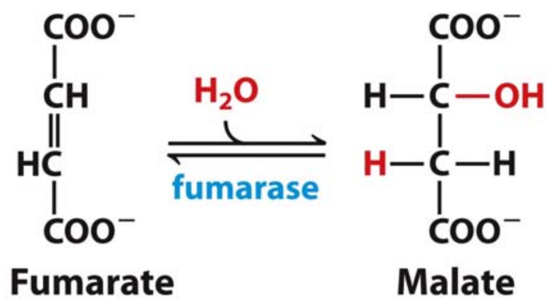




- Q is membrane bound cofactor—revisit its fate in chapter 15!

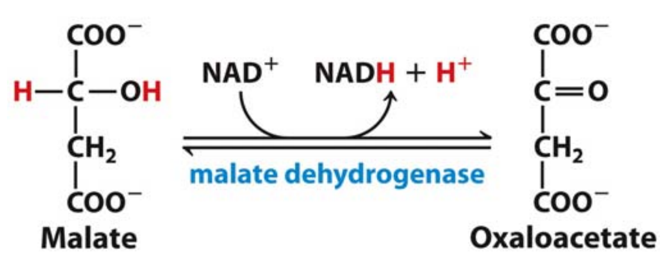
7. Fumarase

- Another prochiral molecule—enzyme makes L-malate exclusively
- Hydration reaction sets up another oxidation



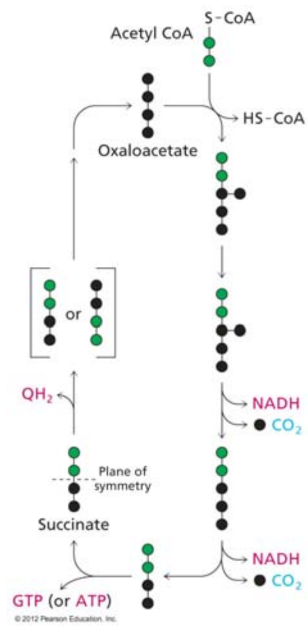
8. Malate Dehydrogenase

- Large positive standard free energy
- Driven by low [oxaloacetate]
  - Coupled back to reaction #1



Carbon Flow

- Practice C-14 labeling problems using this basic chart

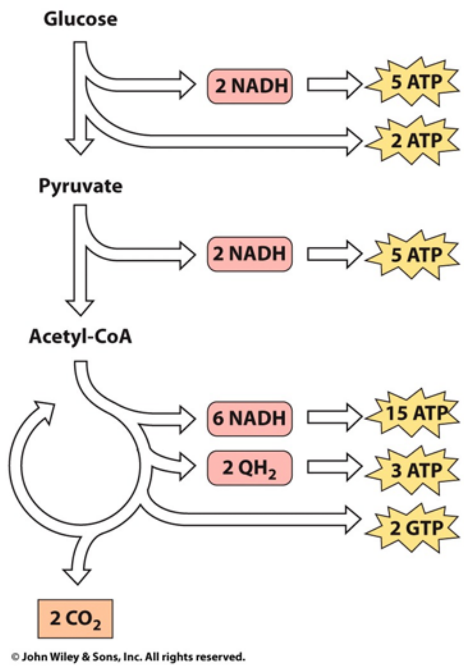


ATP Harvest: By Enzyme

Table 13.2 Energy production in the citric acid cycle

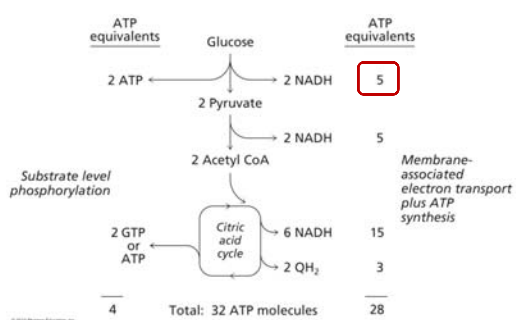
Reaction	Energy-yielding product	ATP equivalents
Isocitrate dehydrogenase	NADH	2.5
α-Ketoglutarate dehydrogenase complex	NADH	2.5
Succinyl-CoA synthetase	GTP or ATP	1.0
Succinate dehydrogenase complex	QH_2	1.5
Malate dehydrogenase	NADH	2.5
Total		10.0

Chapter 15 will explain how we get these ATP equivalent numbers

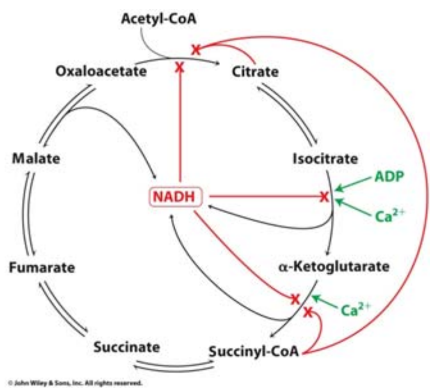


Net ATP Harvest from Glucose

- Glycolysis = 5-7 ATP
  - 3 or 5 ATP from cytosolic NADH
  - In some cases, NADH transport costs 2 ATP equivalents
- Pyruvate DH = 5 ATP
- Citric Acid Cycle = 20 ATP
- Total: 30-32 ATP/glucose in humans



Regulation

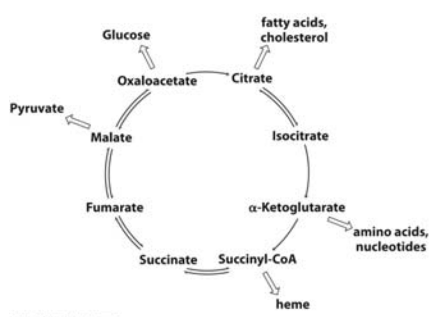


- Flux is generated through three irreversible steps
- NADH inhibits
- Citrate: product inhibition
- Succinyl CoA is last irreversible product: feedback inhibition
- Ca^{+2}: hormone mediated signal for need for energy
- ADP: need of energy

Anabolic Role for CAC

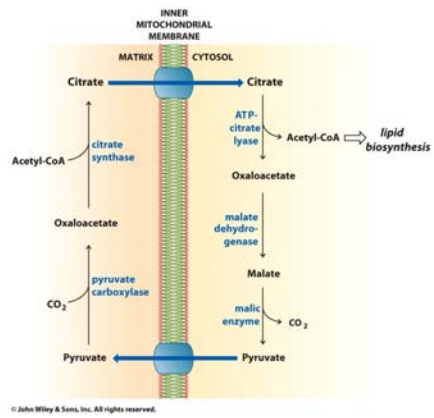
Remember that CAC is for more than ATP!

- Intermediates can be used for biosynthesis
  - Amino acids
    - aKG → glutamate
  - Gluconeogenesis
    - Through oxaloacetate
  - Fatty acids
    - Require transport of citrate



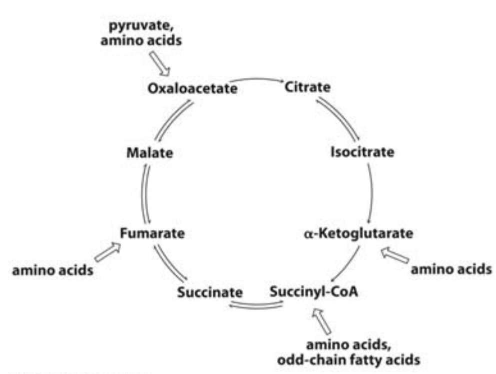
Citrate Transport System

- Fatty acid biosynthesis happens in the cytosol
- Acetyl-CoA cannot get across the mitochondrial membrane
- At cost of 2 ATP, acetyl-CoA gets across membrane in citrate form
- Oxaloacetate "taxi" gets back into matrix
- Full explanation in fatty acid metabolism chapter



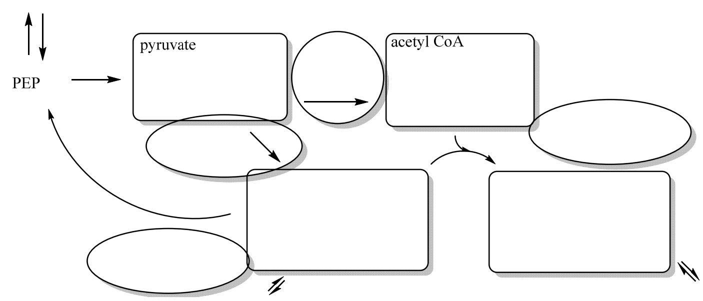
Anaplerotic Reactions

- Purposes of "filling up" reactions
  - Enhanced aerobic respiration (increase flux)
  - Gluconeogenesis pathway
- Replenish CAC intermediates through
  - Pyruvate carboxylase
  - Transamination
  - NOT through acetyl CoA



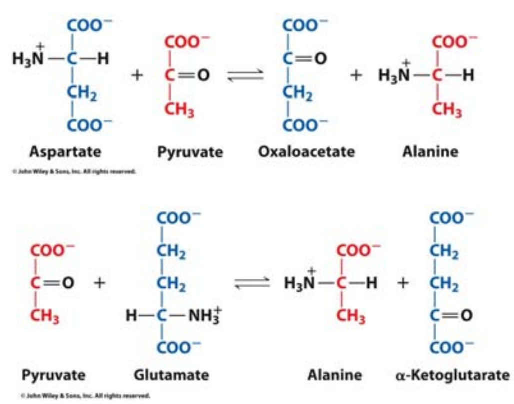
Problem 42

- Why is the activation of pyruvate carboxylase by acetyl-CoA a good regulatory strategy?



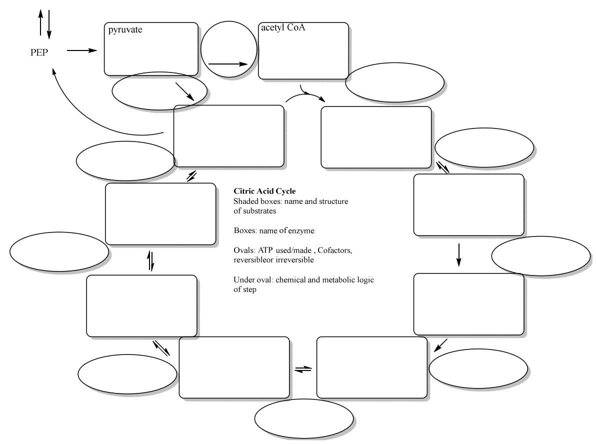
Transamination Stimulates CAC

- Quick rise of [pyruvate] in fast acting muscle boosts flux through CAC



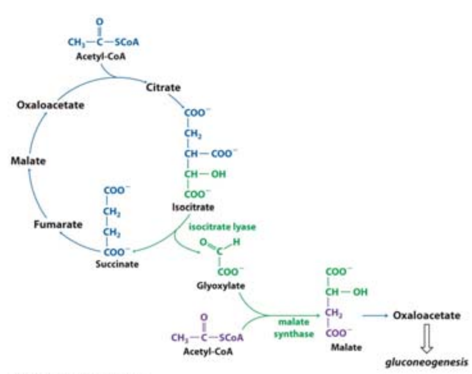
Acetyl CoA Cannot Fill Up CAC

- A key branch point of human metabolism
- Glucogenic vs. ketogenic
- No net glucose from acetyl CoA



Glyoxylate Pathway

- Plants (seeds) can use stored fat to make net glucose
- Makes acetyl-CoA into oxaloacetate in non-cyclic pathway
- At expense of bypassing two oxidation reactions (NADH production)



Problem 55

- Animals lack a glyoxylate pathway and cannot convert fats to carbohydrates. However, if an animal is fed a fatty acid with all its carbons labelled by C-14, some of the labeled carbons later appear in glucose. How is this possible?