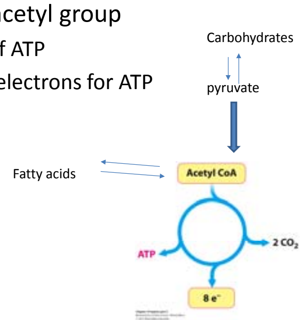
Citric Acid Cycle

Chapter 19
Stryer Short Course

Overview of Cycle

- Oxidation of acetyl group
  - Production of ATP
  - High energy electrons for ATP

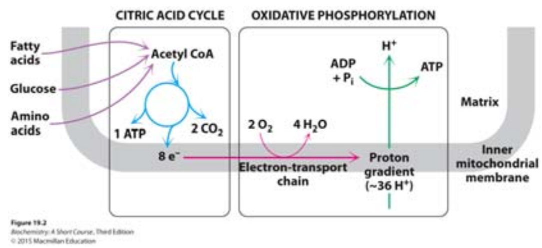


Fate of Acetyl CoA

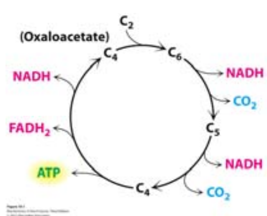
- Storage of energy as fatty acid
- ATP production (harvest of high potential electrons)
- Formal reaction:

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

Where we are going…



Citric Acid Cycle



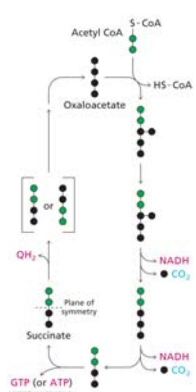
Stage 2: regeneration

Stage 1: Oxidation

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

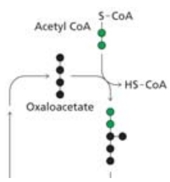
Carbon Flow

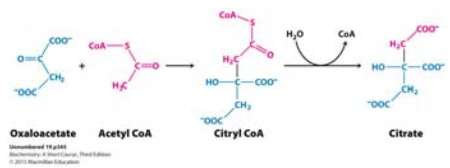
- Each cycle is **net** oxidation of acetyl CoA
  - Not actual loss of carbon from acetyl CoA
- C-13 incorporation experiments
- 4-carbon compounds act "catalytically" in oxygen consumption
  - Cyclic pathway!



1. Citrate Synthase

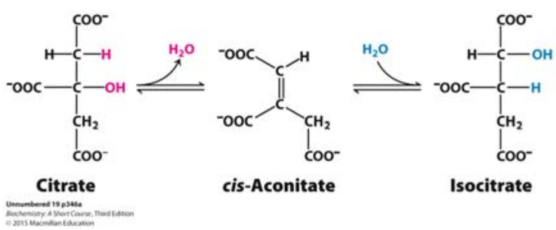
- Highly exothermic—lysis of high energy bond
- Used to drive reaction in presence of small [oxaloacetate]





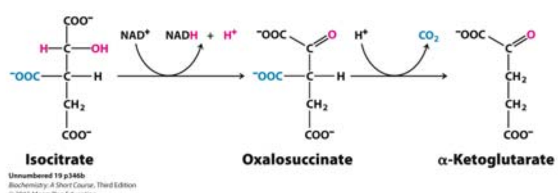
2. Aconitase

- Overall purpose: oxidative decarboxylation
- This step: put hydroxyl in correct position



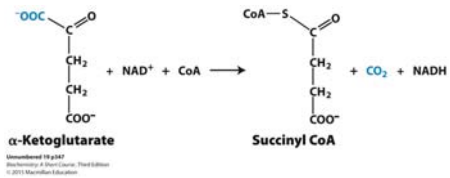
3. Isocitrate Dehydrogenase

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

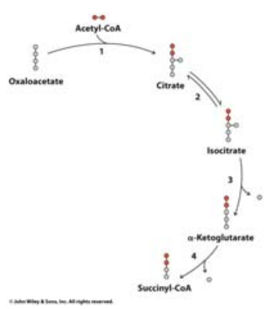


4. α-Ketogluterate Dehydrogenase Complex

- Analogous to pyruvate dehydrogenase complex
- Second decarboxylation, but this is α-decarboxylation
- Forms NADH and high energy bond

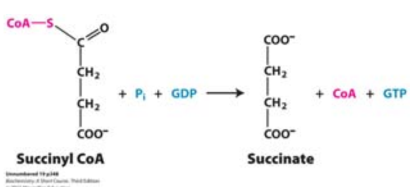


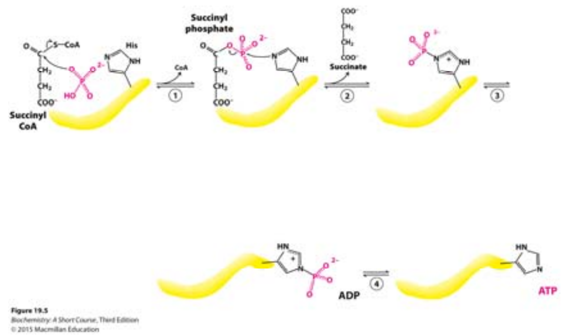
Carbon Review



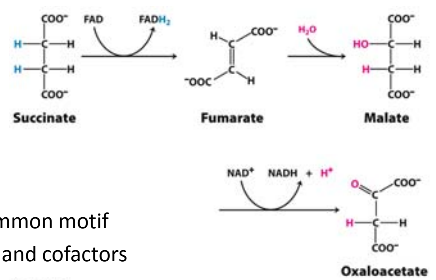
5. Succinyl CoA Synthetase

- Synthetase means ATP (GTP) involved
- High energy bond used to do substrate-level phosphorylation





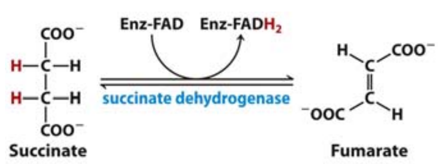
Reactions 6-8



- Common motif
- DH and cofactors

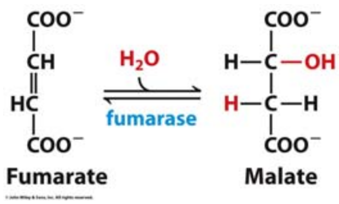
6. Succinate Dehydrogenase

- Oxidation to form C=C releases less energy
- FAD is bound redox reagent



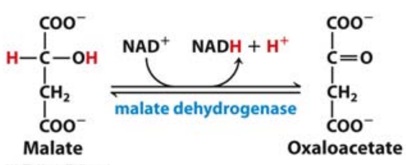
7. Fumarase

- Hydration reaction sets up another oxidation



8. Malate Dehydrogenase

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



Recognize Reaction Type

Table 19.1 Citric acid cycle

Step	Reaction	Enzyme	Prosthetic group	Type*	$\Delta G^{\circ\prime}$ kJ mol^{-1}	$\Delta G^{\circ\prime}$ kcal mol^{-1}
1	Acetyl CoA + oxaloacetate + H_2O → citrate + CoA + H^+	Citrate synthase		a	−31.4	−7.5
2a	Citrate ⇌ *cis*-aconitate + H_2O	Aconitase	Fe-S	b	+8.4	+2.0
2b	*cis*-Aconitate + H_2O N isocitrate	Aconitase	Fe-S	c	−2.1	−0.5
3	Isocitrate + NAD^+ ⇌ α-ketoglutarate + CO_2 + NADH	Isocitrate dehydrogenase		d + e	−8.4	−2.0
4	α-ketoglutarate + NAD^+ + CoA ⇌ succinyl CoA + CO_2 + NADH	α-ketoglutarate dehydrogenase complex	Lipoic acid, FAD, TPP	d + e	−30.1	−7.2
5	Succinyl CoA + P_i + ADP ⇌ succinate + ATP + CoA	Succinyl CoA synthetase		f	−3.3	−0.8
6	Succinate + FAD (enzyme-bound) ⇌ fumarate + $FADH_2$ (enzyme-bound)	Succinate dehydrogenase	FAD, Fe-S	e	0	0
7	Fumarate + H_2O ⇌ L-malate	Fumarase		e	−3.8	−0.9
8	L-Malate + NAD^+ ⇌ oxaloacetate + NADH + H^+	Malate dehydrogenase		e	+29.7	+7.1

*Reaction type: a, condensation; b, dehydration; c, hydration; d, decarboxylation; e, oxidation; f, substrate-level phosphorylation.

Table 19.1
Biochemistry: A Short Course, Third Edition

Overview

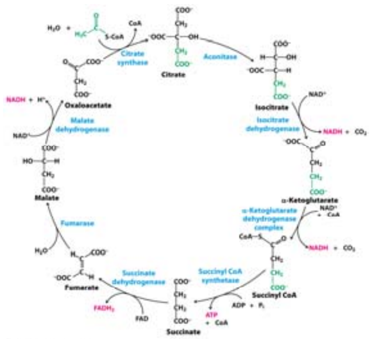
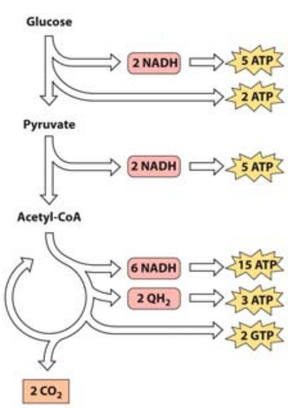


Figure 19.6
Biochemistry: A Short Course, Third Edition

ATP Harvest: By Enzyme

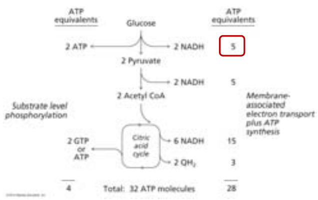
Table 13.2 Energy production in the citric acid cycle

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

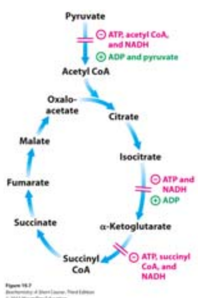


Net ATP Harvest from Glucose

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

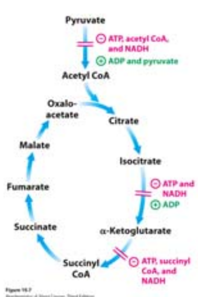


Regulation



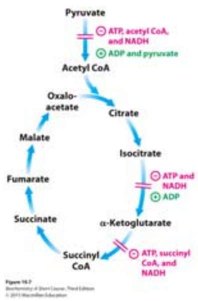
- Flux is generated through three irreversible steps
- NADH inhibits
- Product inhibition
- Energy charge

Regulation: Between Pathways



- Shutdown of CAC leads to citrate buildup
- Citrate goes into cytoplasm
  - Begins fatty acid synthesis
  - Inactivates glycolysis

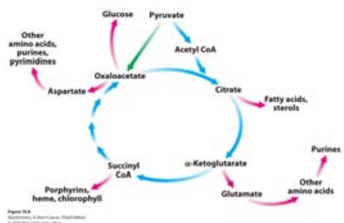
Regulation: High NADH



- Buildup of acetyl CoA
- Shuts down PDH and CAC
- Pyruvate builds up in mitochondria
- Acetyl CoA activates PEPCK: pyruvate to oxaloacetate
- High [NADH] helps shuttle oxaloacetate into cytoplasm for gluconeogenesis

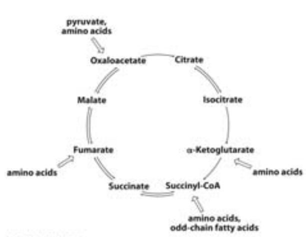
Anabolic Roles for CAC

- Not just for degradation
- Intermediates can be used for building
  - Amino acids
  - Gluconeogenesis
  - Fatty acids



Anaplerotic Reactions

- Problem with dual role: if intermediates used in biosynthesis, how can we oxidize acetyl CoA?
- "Filling up" reactions
  - Enhanced aerobic respiration (increase flux)
  - Gluconeogenesis pathway
- Key Reaction: Formation of oxaloacetate by pyruvate carboxylase
- Some amino acids can also serve if in high concentration



Key Anaplerotic Reaction

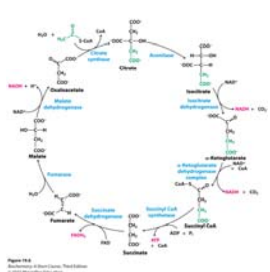
- If energy charge is high, citrate is backed up—oxaloacetate sent out for gluconeogenesis
- If energy charge is low, citrate is used up, and oxaloacetate builds up flux of cycle

Problem

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

No Net Carb Production

- In animals, acetyl CoA cannot produce **NET glucose**
- To make an oxaloacetate in the CAC requires the use of an oxaloacetate!



Glyoxylate Pathway

- Makes acetyl-CoA into oxaloacetate in non-cyclic path
- Allows plants (seeds) to use stored fat to make net glucose
- At expense of bypassing oxidation reactions (NADH production)