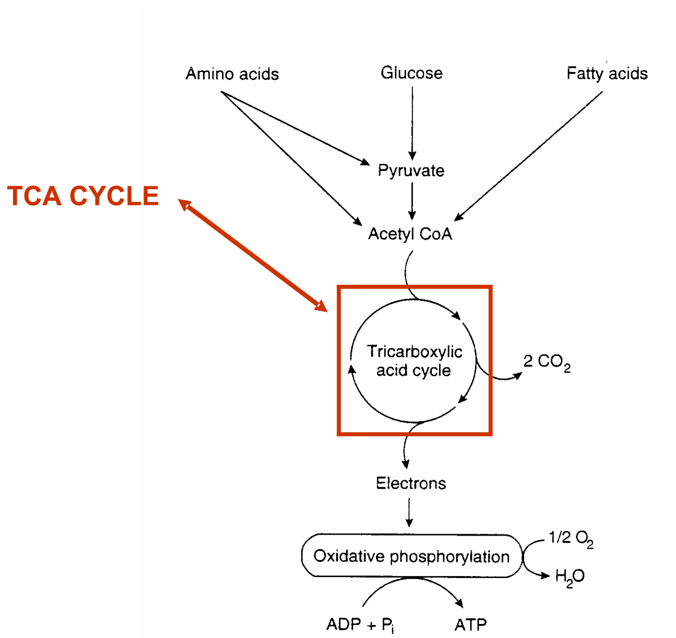
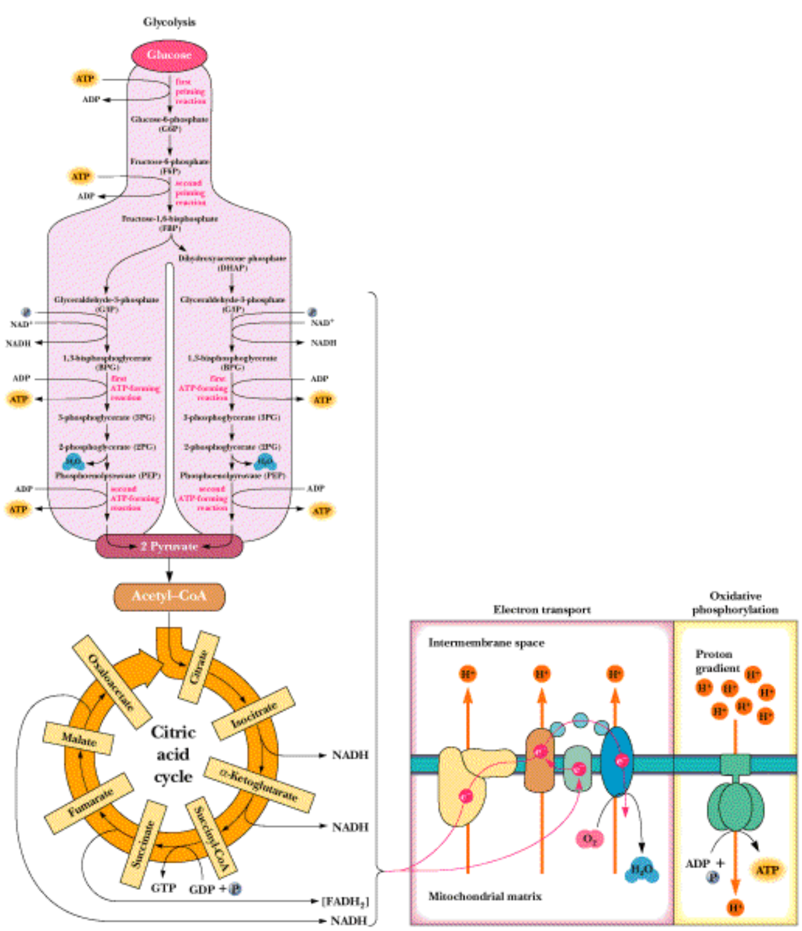
TCA CYCLE (Citric Acid Cycle)





- The **Citric Acid Cycle** is also known as:
  - **Kreb's cycle**
    - Sir Hans Krebs
    - Nobel prize, 1953
  - **TCA (tricarboxylic acid) cycle**

- The citric acid cycle requires **aerobic** conditions!!!!
  - Cells have evolved to use oxygen
  - **Oxygen** serves as the **final electron acceptor** as **pyruvate** (from glycolysis) is converted (oxidized) completely to $\mathbf{CO_2}$ and $\mathbf{H_2O}$
    - If cell is under anaerobic conditions energy production is not too efficient - ~10% of energy possible is generated
    - **Pyruvate** converted to **Acetyl-CoA by PDH** and then **Acetyl-CoA** enters the TCA cycle

Energy in the citric acid cycle

- Energy of the oxidation reactions is largely conserved as <u>reducing power</u>
- Coenzymes reduced:
  - **NAD^+/NADH**
  - **FAD/$FADH_2$**
- Reduced coenzymes used by electron transport chain and oxidative phosphorylation to make ATP

The Tricarboxylic acid (TCA) cycle (citric acid cycle) is **amphibolic** (both *catabolic* and *anabolic*)

The TCA Cycle Serves Two Purposes:

1. **Oxidize Acetyl-CoA to CO_2 to produce energy**

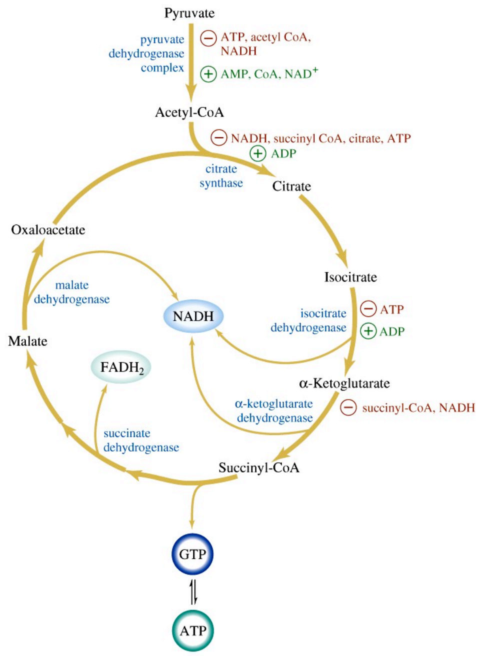


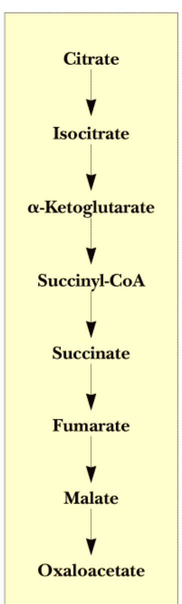
Figure 16-10 Concepts in Biochemistry, 3/e

- **ATP (GTP)**
- **Reducing power of NADH and $FADH_2$**

- The cycle is involved in the aerobic catabolism of carbohydrates, lipids and amino acids

2. **Supply precursors for biosynthesis of carbohydrates, lipids, amino acids, nucleotides and porphyrins**

- Intermediates of the cycle are starting points for many biosynthetic reactions
- The cycle itself is not a pathway for a net degradation of any cycle intermediates
- Cycle intermediates can be shared with other pathways, which may lead to a re-supply or net decrease in cycle intermediates
- Reactions feeding into the cycle replenish the pool of cycle intermediates



Fundamental Differences between Glycolysis and TCA Cycle:

1. Glycolysis is a **linear** pathway; TCA cycle is **cyclic**
2. Glycolysis occurs in the **cytosol** and TCA is in the **mitochondrial** matrix
3. Glycolysis does **not require** oxygen; TCA **requires** oxygen (aerobic)

Summary of the citric acid cycle

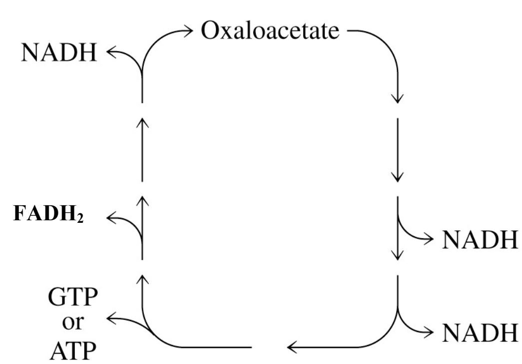
For **each acetyl-CoA** that enters the cycle:

(1) **Two** molecules of **CO_2** are released

(2) Coenzymes **NAD^+ and FAD** are reduced

(3) One **GDP (or ADP) is phosphorylated**

(4) The initial acceptor molecule oxaloacetate is **reformed**



Energy conservation by the cycle

- Energy is conserved in the reduced coenzymes NADH, $FADH_2$ and one GTP

- NADH, $FADH_2$ can be oxidized to produce ATP by oxidative phosphorylation
- Energy is also conserved in either ATP or GTP- produced by **substrate-level phosphorylation** (from the thioester bond in succinyl-CoA.)
- The use of many steps in the oxidation of acetyl CoA to CO_2 enables conservation of most of the energy as work with little lost as heat

Table 16.2
The reactions of the citric acid cycle

Reaction Number[a]	Reaction	Enzyme	Prosthetic Group	Reaction Type[b]
1	Acetyl-CoA + oxaloacetate + H_2O ⇌ citrate + CoA	Citrate synthase		3, 4
2	Citrate ⇌ *cis*-aconitate ⇌ isocitrate	Aconitase	Fe–S	4
3	Isocitrate + NAD^+ ⇌ α-ketoglutarate + CO_2 + NADH + H^+	Isocitrate dehydrogenase		1, 4
4	α-Ketoglutarate + NAD^+ + CoA ⇌ succinyl-CoA + CO_2 + NADH + H^+	α-Ketoglutarate dehydrogenase complex	Lipoamide, FAD, TPP	1, 4
5	Succinyl-CoA + P_i + ADP or GDP ⇌ succinate + ATP or GTP + CoA	Succinyl-CoA synthetase		2
6	Succinate + FAD (enzyme bound) ⇌ fumarate + $FADH_2$ (enzyme bound)	Succinate dehydrogenase	FAD, Fe–S	1
7	Fumarate + H_2O ⇌ L-malate	Fumarase		4
8	L-Malate + NAD^+ ⇌ oxaloacetate + NADH + H^+	Malate dehydrogenase		1

[a] The reaction numbers correspond to the steps in Figure 16.8.
[b] Reaction type: 1, oxidation–reduction; 2, phosphoryl group transfer; 3, hydrolysis; 4, nonhydrolytic cleavage (addition or elimination); 5, isomerization–rearrangement; 6, bond formation coupled to ATP cleavage.

Table 16-2 Concepts in Biochemistry, 3/e

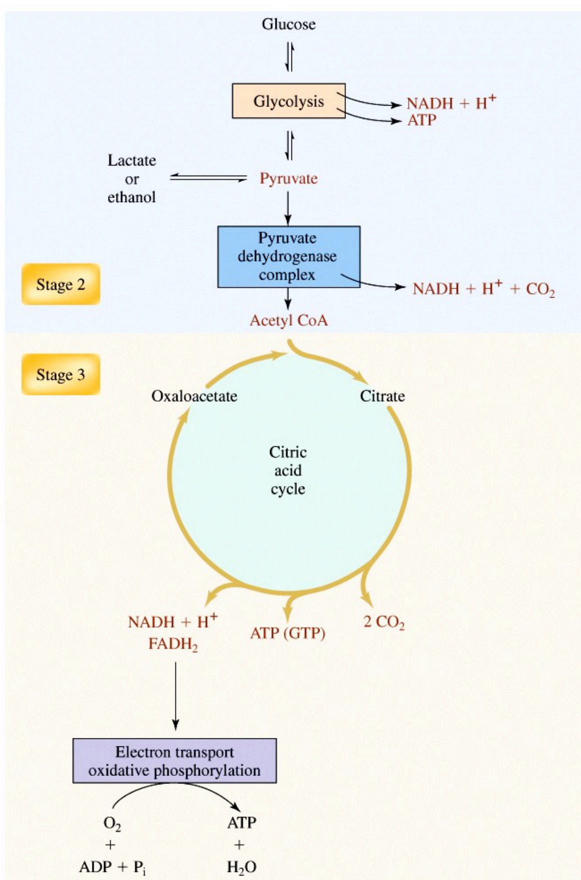


Figure 16-1 Concepts in Biochemistry, 3/e

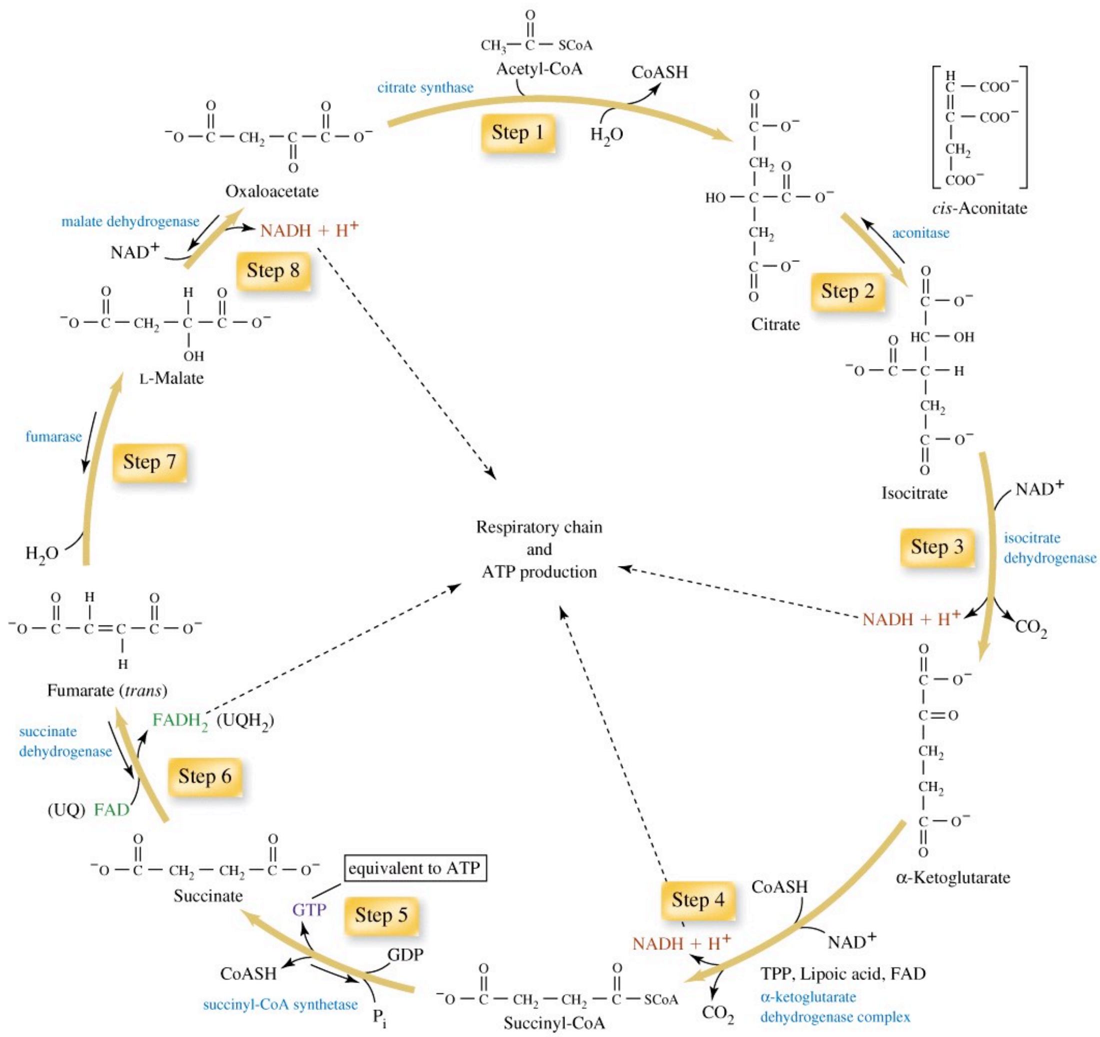


Figure 16-8 Concepts in Biochemistry, 3/e
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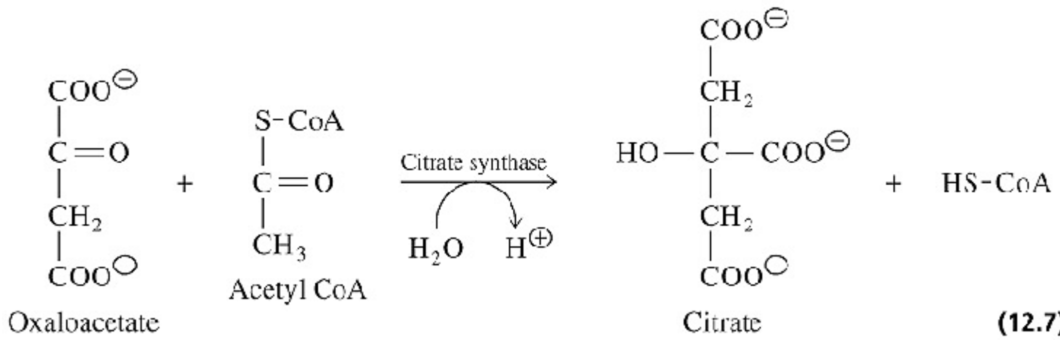
8 REACTIONS OF THE TCA CYCLE:

1. Formation of Citrate

- Citrate formed from condensation of acetyl CoA and oxaloacetate
- Addition of acetyl to the keto double bond of OAA = aldol condensation
- Only cycle reaction with C-C bond formation
- No energy of ATP hydrolysis needed
- **Synthase** is an enzyme that catalyzes addition to a double bond or elimination to form a double bond without needing ATP hydrolysis
- **Both Hydrolysis Reaction and Non-hydrolytic cleavage (addition or elimination)**



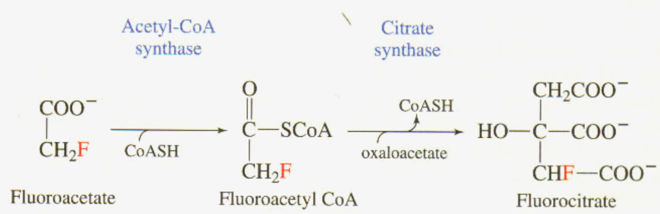
(12.7)

- **Locoweed is toxic because it accumulates fluoroacetate**

Locoweed contains fluoroacetate, which is transformed to toxic fluorocitrate in animals.

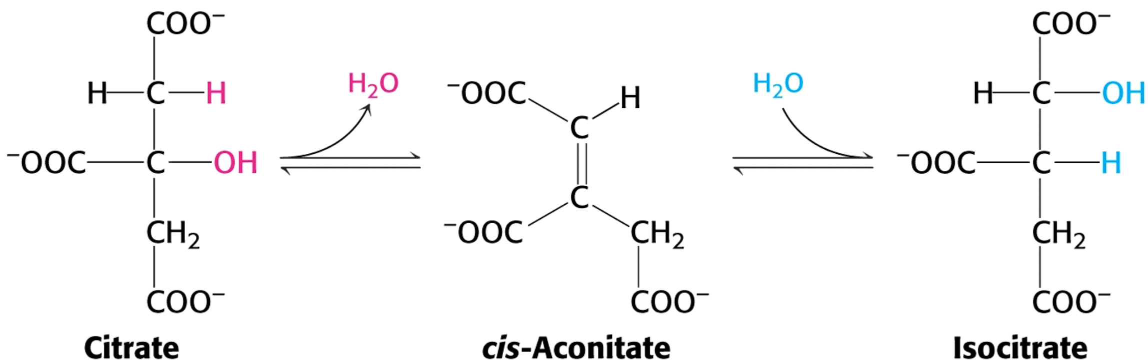
FIGURE 16.9

Metabolism of fluoroacetate to the toxic substance fluorocitrate. Fluoroacetate is activated by attachment to CoASH. Fluoroacetyl CoA can substitute for acetyl CoA as a substrate for citrate synthase. Instead of citrate, the toxic substance fluorocitrate is formed.



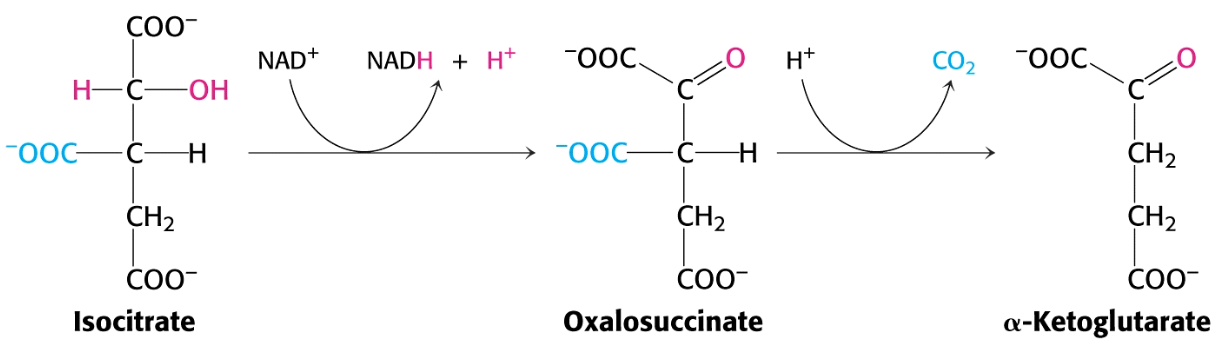
2. Aconitase

- Isomerization of **citrate (3° alcohol)** to **isocitrate (2° alcohol)**
- Aconitase contains an **iron-sulfur center** as a prosthetic group
- Catalyzes a **lyase** reaction that results in rearrangement of citrate with a tertiary alcohol to isocitrate with a secondary alcohol
  - **Non-hydrolytic cleavage (addition or elimination)**
  - Goes through an enzyme bound cis-aconitate intermediate
  - Elimination of H_2O from citrate to form C=C bond of *cis*-aconitate
  - Rearrangement allows the further oxidation of the molecule



3. **Isocitrate Dehydrogenase**

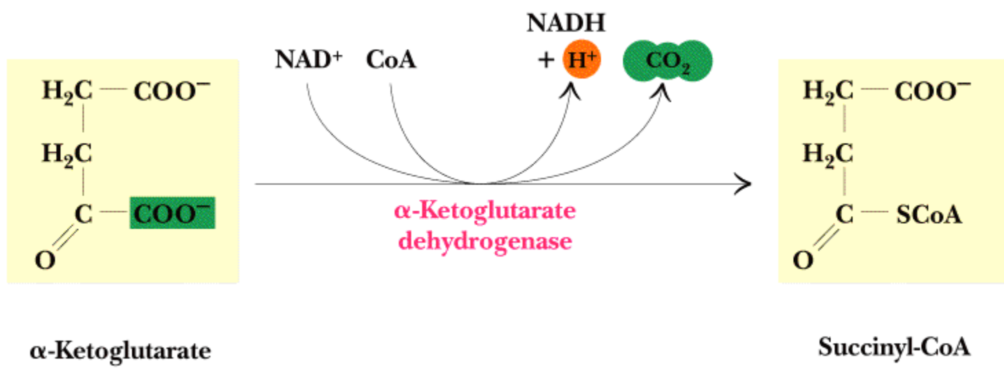
- **First oxidative decarboxylation** of **isocitrate** to **α-ketoglutarate (α-kg)**
- Metabolically irreversible reaction
- One of four **oxidation-reduction reactions** of the cycle
- Also a **Non-hydrolytic cleavage reaction (addition or elimination)**
- Hydride ion from the C-2 of isocitrate is transferred to NAD^+ to form NADH
- Oxalosuccinate is **decarboxylated** to α-ketoglutarate



4. **α-Ketoglutarate Dehydrogenase Complex**

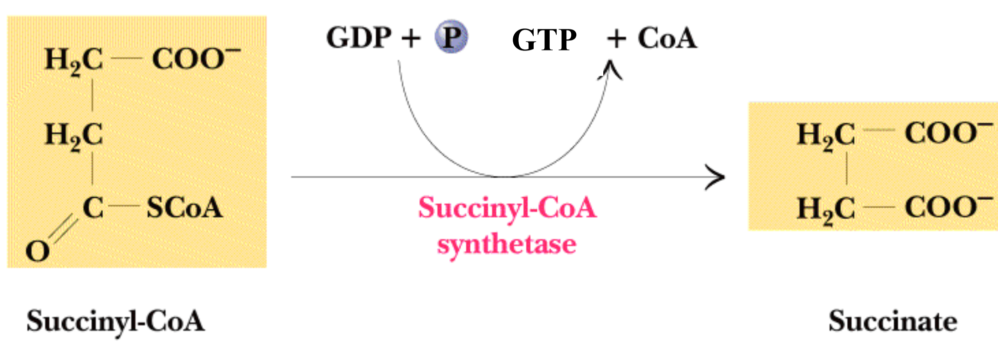
- **Second oxidative decarboxylation reaction**
- Also a **Non-hydrolytic cleavage reaction (addition or elimination)**
- α-Ketoglutarate converted to Succinyl-CoA
- Similar to **pyruvate dehydrogenase** complex except a succinyl group is activated, not acetyl
    - Same coenzymes, identical mechanisms
- **Succinyl-CoA thioester** is VERY high energy
- Generates **NADH**

- Purpose of step: Collect energy from α-ketoglutarate decarboxylation into the high energy succinyl-CoA molecule



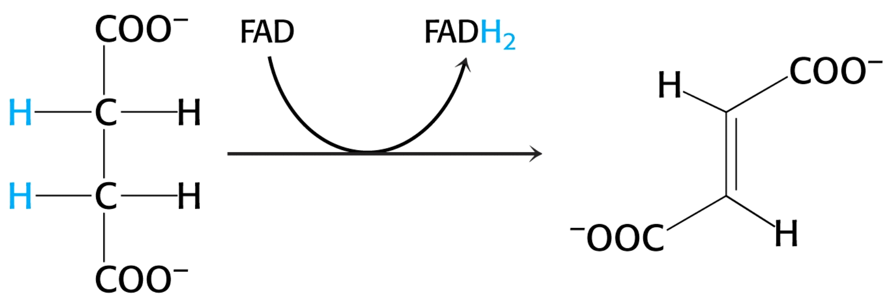
5. Succinyl-CoA Synthetase (Formation of succinate)

- Free **energy** in thioester bond of **succinyl CoA** is **conserved** as **GTP** (or ATP in plants, some bacteria)
- Enzyme: **Succinyl-CoA Synthetase**
    - Two forms in higher animals: One prefers ADP the other GDP)
    - **SUBSTRATE-LEVEL PHOSPHORYLATION** = Formation of ATP directly coupled to the reaction **(group transfer reaction)**
    - **Only** step where ATP (GTP) is formed directly in the TCA cycle
    - All other ATP is produced by *oxidative phosphorylation*
        - *Oxidative phosphorylation* is the oxidation of reduced co-factors NADH and $FADH_2$ to O_2 – release of energy drives ATP formation from ADP + Pi



6. The Succinate Dehydrogenase (SDH) Complex

- Located on the **inner mitochondrial membrane** (other components are in the matrix)
- **Oxidation-reduction** reaction that forms a carbon-carbon double bond
- Succinate is **oxidized** to fumarate, while FAD is **reduced** to $FADH_2$
    - NAD^+ functions in reactions that interconvert hydroxyl and carbonyl groups
- Dehydrogenation is <u>stereospecific</u>; only the *trans* isomer is formed
- Also known as Complex II of the electron transport chain – direct feed of electrons from $FADH_2$ into the electron transport chain.



Succinate **Fumarate**

- Substrate analog **malonate** is a **competitive inhibitor** of the SDH complex
- **Malonate** is a structural analog of **succinate**
- Malonate binds to the enzyme active site, and is a competitive inhibitor

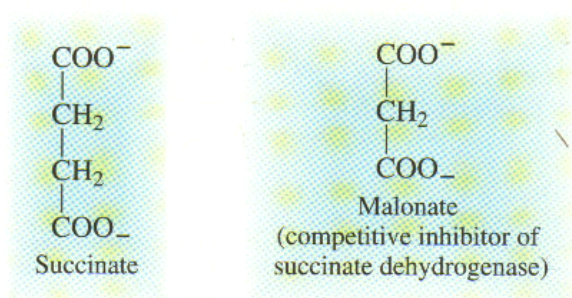
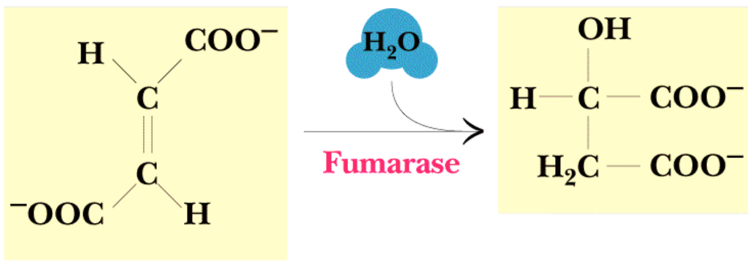


FIGURE 16.10

Structures of succinate, the normal substrate for succinate dehydrogenase, and a competitive inhibitor of succinate dehydrogenase, malonate. Malonate is toxic since it blocks a reaction in the citric acid cycle.

7. Fumarase

- Stereospecific *trans* addition of water to the double bond of **fumarate** to form ***L*-malate**

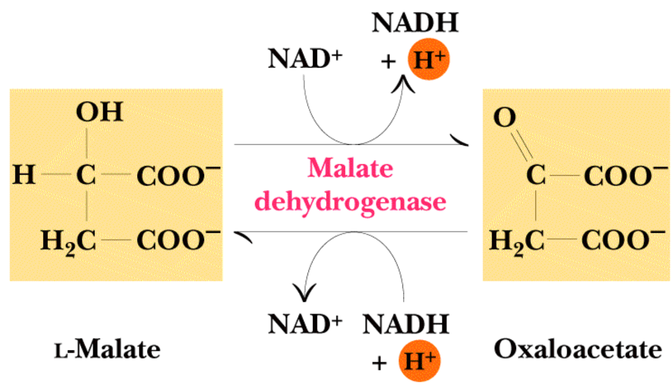


Fumarate **Malate**

- Only forms the L-isomer
- **Non-hydrolytic cleavage reaction**

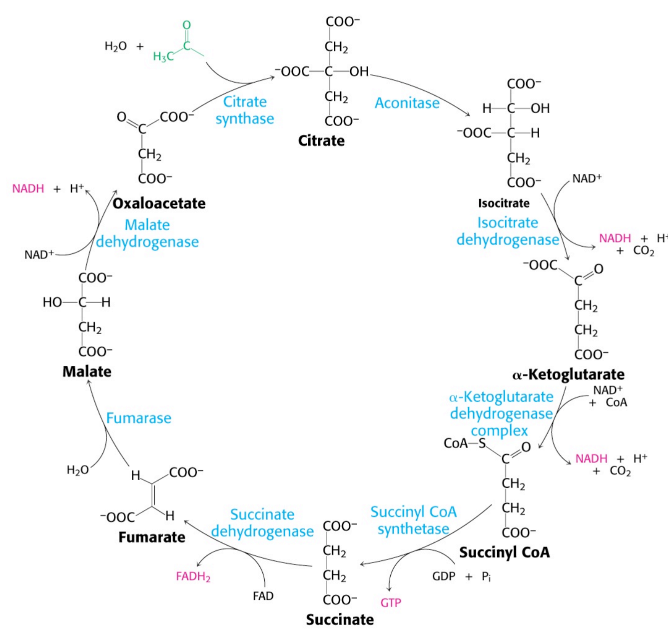
8. Malate Dehydrogenase

- Regeneration of **oxaloacetate** from **L-malate**
- Enzyme: **malate dehydrogenase (oxidation – reduction reaction)**
- Generates **NADH**



OVERALL SUMMARY OF TCA CYCLE:

1. **Oxidation of Acetyl-CoA to CO_2**
   - CO_2 leaves at steps 3 and 4
2. **3 NAD+ are reduced to NADH by dehydrogenase reactions**
   - Steps 3, 4, and 8
   - isocitrate dehydrogenase
   - α–ketoglutarate dehydrogenase
   - malate dehydrogenase
3. **1 molecule of FAD reduced to FADH2**
   - Step 6 – Succinate dehydrogenase
4. **1 phosphoanhydride bond formed in ATP or GTP**
   - Substrate level phosphorylation at step 5: Succinyl-CoA Synthetase
   - Generated from energy stored in CoA thioester



So, per pyruvate:

4 NADH (one from pyruvate dehydrogenase complex + 3 TCA)
1 ATP or GTP
1 $FADH_2$

ANIMATION:
http://www.wiley.com/college/fob/anim/ **Chapter 16**

- Fig. 16-2 -- The Reactions of the Citric Acid Cycle

ENERGY FROM THE TCA CYCLE:
Reduced Coenzymes Fuel the Production of ATP

- Each acetyl CoA entering the cycle nets:
  - (1) **3 NADH**
  - (2) **1 $FADH_2$**
  - (3) **1 GTP** (or **1 ATP**)
- Oxidation of each NADH yields **2.5 ATP**
- Oxidation of each $FADH_2$ yields **1.5 ATP**
- Complete oxidation of **1 acetyl CoA** = **10 ATP**

Table 16.3
The ATP, NADH, and $FADH_2$ balance sheet for the pyruvate dehydrogenase complex and the citric acid cycle

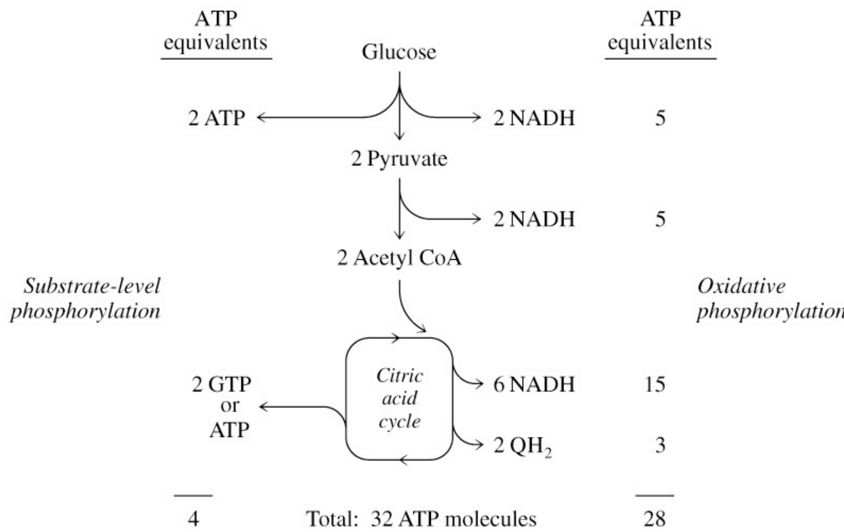
Reaction Number[a]	Reaction	ATP (GTP) Change[b] (per pyruvate)	NADH Change[b] (per pyruvate)	$FADH_2$ Change[b] (per pyruvate)
	Pyruvate oxidation			
	Pyruvate dehydrogenase complex	0	+1	0
	Pyruvate oxidation total	0	+1	0
	Citric acid cycle			
3	Isocitrate + NAD^+ $\rightleftharpoons$ α-ketoglutarate + CO_2 + NADH + H^+	0	+1	0
4	α-Ketoglutarate + NAD^+ + CoA $\rightleftharpoons$ succinyl-CoA + CO_2 + NADH + H^+	0	+1	0
5	Succinyl-CoA + GDP or ADP + P_i $\rightleftharpoons$ succinate + GTP or ATP + CoASH	+1	0	0
6	Succinate + FAD $\rightleftharpoons$ fumarate + $FADH_2$	0	0	+1
8	L-Malate + NAD^+ $\rightleftharpoons$ oxaloacetate + NADH + H^+	0	+1	0
	Citric acid cycle total	+1	+3	+1
	Grand Total	+1	+4	+1

[a] The reaction numbers correspond to the steps in Figure 16.8.
[b] A + number indicates a production of ATP, NADH, or $FADH_2$. For example, for each pyruvate that is converted to acetyl-CoA by the pyruvate dehydrogenase complex, 1 NADH is formed.

Table 16-3 Concepts in Biochemistry, 3/e

Glucose degradation via glycolysis, citric acid cycle, and oxidative phosphorylation

AEROBIC
TOTAL/glucose = 32 ATP



If anerobic – Lactate is formed from pyruvate after glycolysis by *lactate dehydrogenase* and the NADH formed is USED. **Therefore, net gain of 2 ATP/glucose, not 32! (Hence 5-10% efficiency)**

- Occurs in muscles during exercise because they go into oxygen debt.
- Soreness due to H^+ from lactic acid
- Metabolism in muscles returns to normal when oxygen replenished

**Should be able to determine #ATP produced given a starting and stopping point in glycolysis or citric acid cycle. (i.e. know where NADH/$FADH_2$ or ATP are made and how many.

Regulation of the Citric Acid Cycle

- Regulation depends on the ENERGY LEVEL of cells – key to keep energy level constant
- When cells have lots of energy (ATP, NADH), the reactions involved in making more are slowed
- The reverse is also true.
- Pathway controlled by:
  - (1) Small molecule modulators (products of the cycle can inhibit)
  - (2) Covalent modification of cycle enzymes
  - (3) Supply of acetyl CoA

Regulation of the PDH complex

- Highly regulated

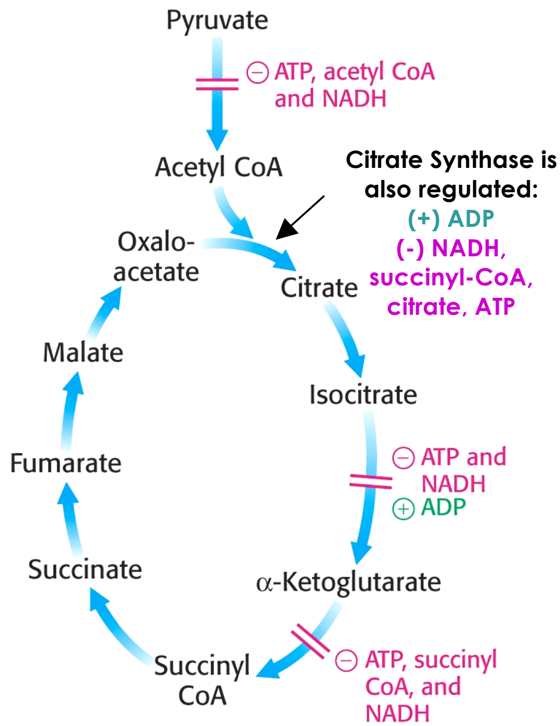
- Regulation of pyruvate dehydrogenase complex controls acetyl CoA supply
- Gatekeeper to aerobic metabolism
- Represents the committed step because pyruvate can still go back to glucose (gluconeogenesis) but acetyl-CoA cannot go back to glucose.
- Inhibitors: Indicators of high energy status
    - NADH, ATP, Acetyl-CoA, Fatty acids (degraded to form acetyl-CoA)
- Stimulators: Indicators of low energy status
    - AMP, NAD^+, Coenzyme A (CoA-SH)

Regulation of citrate synthase

- Inhibitors: NADH, ATP, succinyl-CoA, citrate
- Stimulators: ADP

Regulation of isocitrate dehydrogenase (ICDH)

- Inhibitors: NADH and ATP
- Stimulators: NAD^+, ADP and Ca^{+2}



POINTS OF REGULATION

Regulation of α-ketoglutarate dehydrogenase complex

- Inhibitors: NADH, ATP and succinyl-CoA
- Stimulators: NAD^+, ADP, AMP

ANIMATION:

http://www.wiley.com/college/fob/anim/ **Chapter 16**

- Fig. 16-14 -- Regulation of the Citric Acid Cycle

Table 16.4

The important regulatory enzymes of pyruvate and acetyl-CoA metabolism

Enzyme Name	⊕ Modulators	⊖ Modulators	Comments
Pyruvate dehydrogenase complex	AMP, NAD^+, CoA	ATP, acetyl-CoA, NADH	Also regulated by covalent modification
Citrate synthase	ADP	NADH, succinyl-CoA, citrate, ATP	Activity depends on metabolite concentrations
Isocitrate dehydrogenase	ADP	ATP	Activity depends on metabolite concentrations
α-Ketoglutarate dehydrogenase complex	—	Succinyl-CoA, NADH	Activity depends on metabolite concentrations