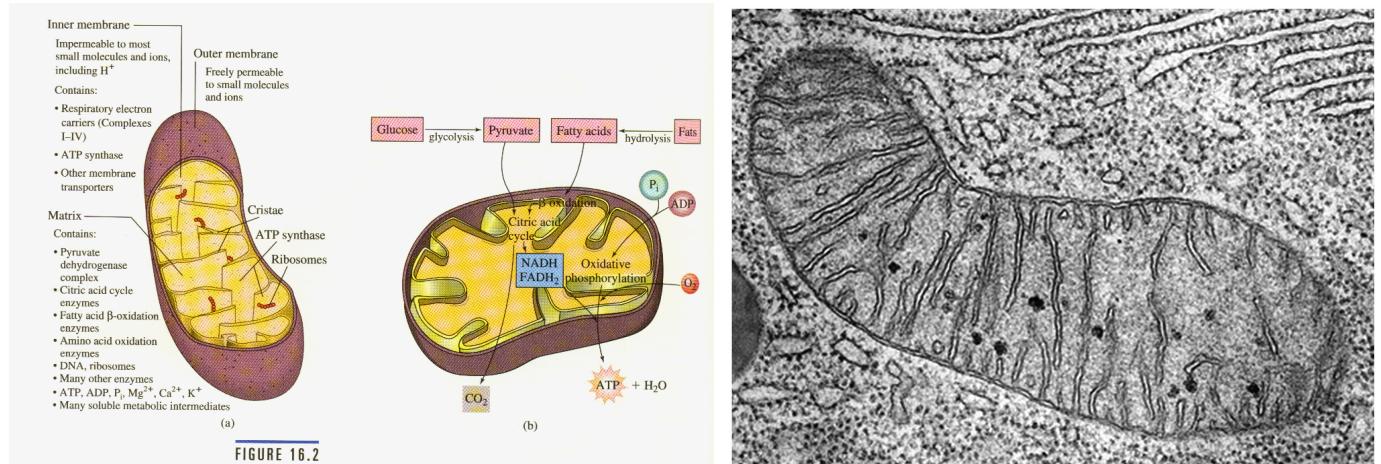


# PYRUVATE DEHYDROGENASE COMPLEX

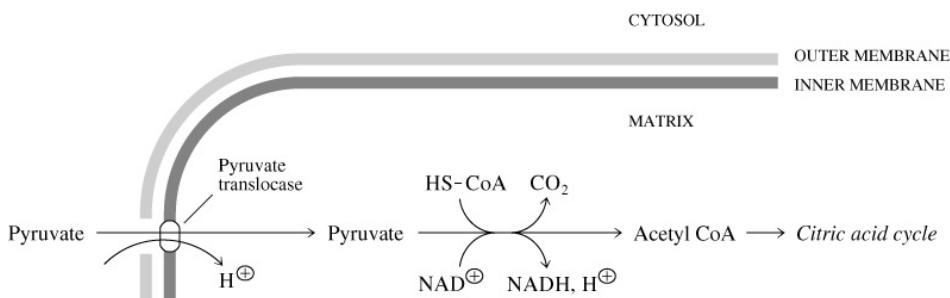
- The pyruvate dehydrogenase complex and the citric acid cycle enzymes exist in the matrix of the **mitochondrion** in eukaryotes
  - Pyruvate is generated by glycolysis in the cytosol and needs to be moved into the mitochondria

## MITOCHONDRIAL STRUCTURE



- Mitochondria have a **TWO** membrane system
    - **Outer Membrane:** Permeable to small molecules
    - **Inner Membrane:** NOT permeable – Has specific integral membrane protein transporters
    - Region between the two membranes = intermembrane space
    - Inner membrane is highly folded and forms boundary to fluid filled interior = **MATRIX**
      - Matrix has a “gel-like” consistency
      - Contains the proteins of the TCA cycle
    - Other proteins responsible for further aerobic metabolism are either in the matrix or are bound to the inner membrane

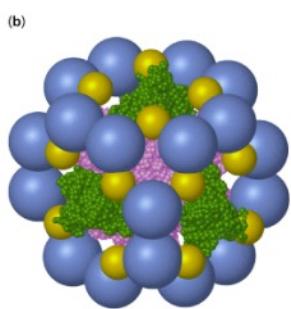
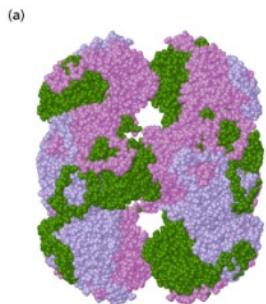
### **Pyruvate generated in Cytosol enters the Mitochondrion (Aerobic fate of Pyruvate)**



- Diffuses through the outer membrane
  - **Pyruvate translocase** transports pyruvate **into** the mitochondria in **symport** with **H<sup>+</sup>**
    - Integral membrane protein in inner membrane

## CONVERSION OF PYRUVATE TO ACETYL CoA

The pyruvate dehydrogenase complex LINKS GLYCOLYSIS TO THE TCA CYCLE! - also occurs in mitochondria



- Pyruvate dehydrogenase complex (PDH complex) is a multienzyme complex containing:
  - 3 enzymes + 5 coenzymes + other proteins (+ ATP coenzyme as a regulator)
  - E1 = pyruvate dehydrogenase
  - E2 = dihydrolipoamide acetyltransferase
  - E3 = dihydrolipoamide dehydrogenase

**Table 16.1**  
Enzymes and coenzymes of the pyruvate dehydrogenase complex

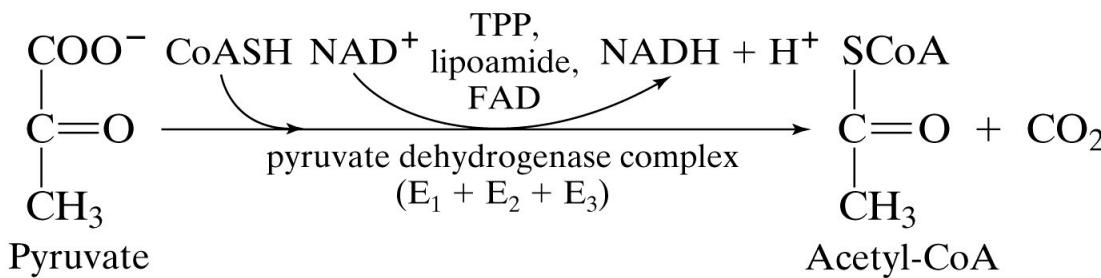
Enzyme	Abbreviation	Coenzyme
Pyruvate dehydrogenase	E <sub>1</sub>	Thiamine pyrophosphate (TPP)
Dihydrolipoyl transacetylase	E <sub>2</sub>	Lipoamide, coenzyme A (CoASH)
Dihydrolipoyl dehydrogenase	E <sub>3</sub>	Flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD <sup>+</sup> )

Table 16-1 Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons

### Structure of the pyruvate dehydrogenase (PDH) complex

### Overall reaction of pyruvate dehydrogenase complex

- Multienzyme Complex (36 subunits!)
- pyruvate + CoASH + NAD<sup>+</sup> → acetyl-CoA + CO<sub>2</sub> + NADH + H<sup>+</sup>



Unnumbered figure pg 487 Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons

## Roles of the coenzymes of the PDH complex

- TPP (thiamine pyrophosphate)
  - Active form of thiamine
    - Vitamin B1
    - Beans, green vegetables, sweet corn, egg yolk, liver, corn meal, brown rice
    - Deficiency = beriberi
  - TPP often used for **decarboxylation** reactions

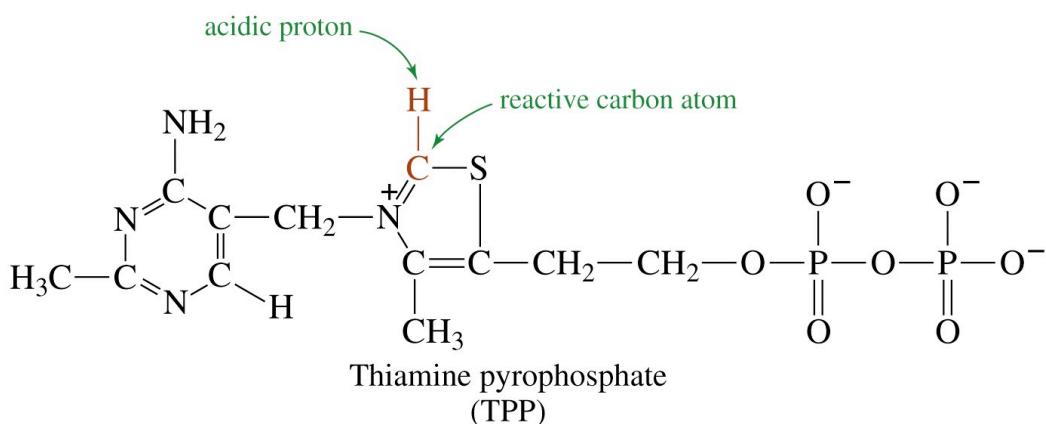
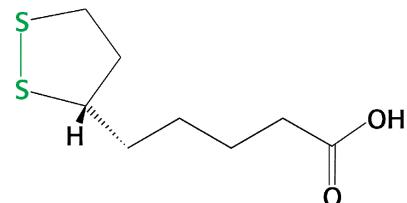


Figure 16-4 Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons

## - Lipoic Acid

- Acetyl transfer and oxidation reactions



## - FAD and NAD<sup>+</sup>

- Oxidizing agent/electron acceptors → Get reduced (will be later reoxidized)

## - Coenzyme A (CoA-SH)

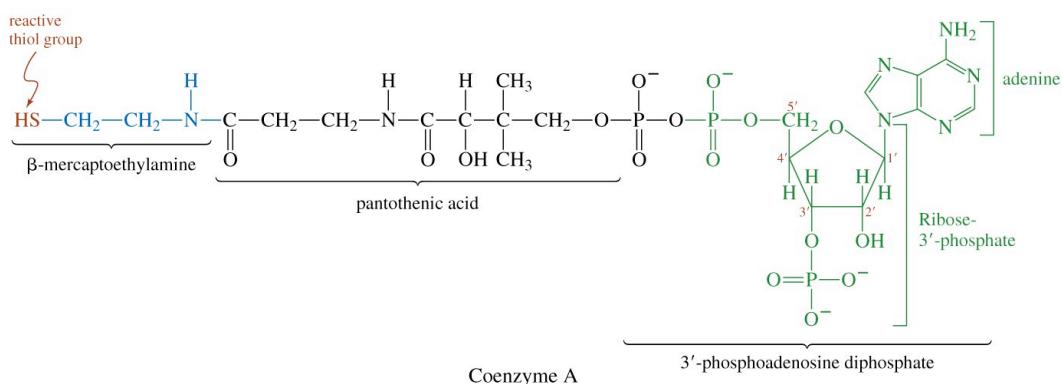
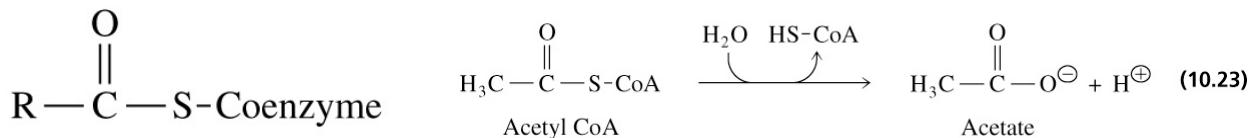


Figure 16-6 Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons

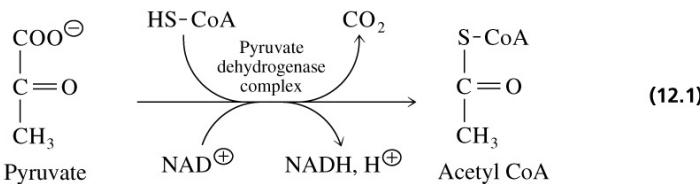
- Synthesized from the vitamin pantothenic acid
- Has a free thiol (-SH) group

- Coenzyme A has a free thiol group (CoASH) that can form **thioesters** which are **energy-rich compounds** (high free energies of hydrolysis -  $\Delta G^\circ = -31 \text{ kJ/mol}$ )
  - Energizes molecules
  - Makes more unstable and more prone to react and release energy



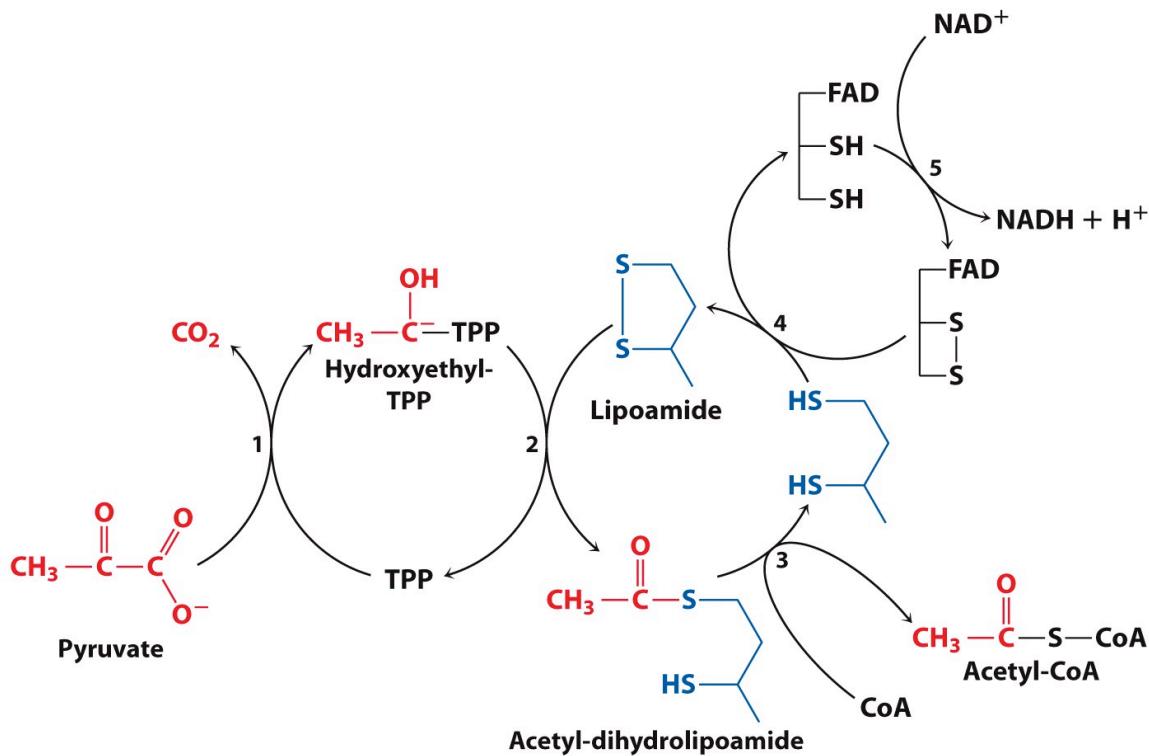
**Thioester linkage**  
(joins thiol with carboxylic acid)

**SUMMARY:**

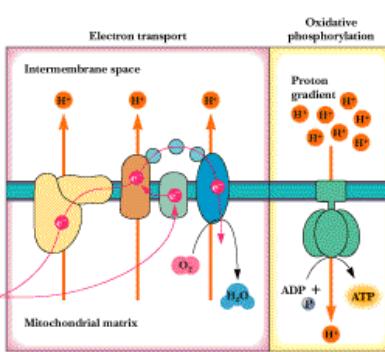
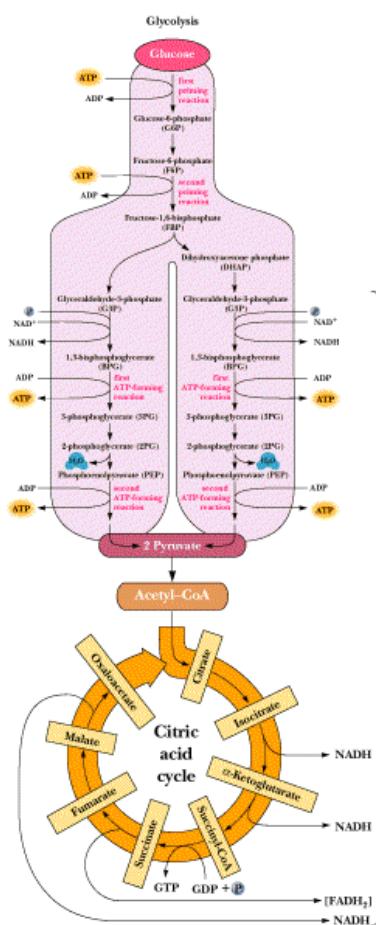
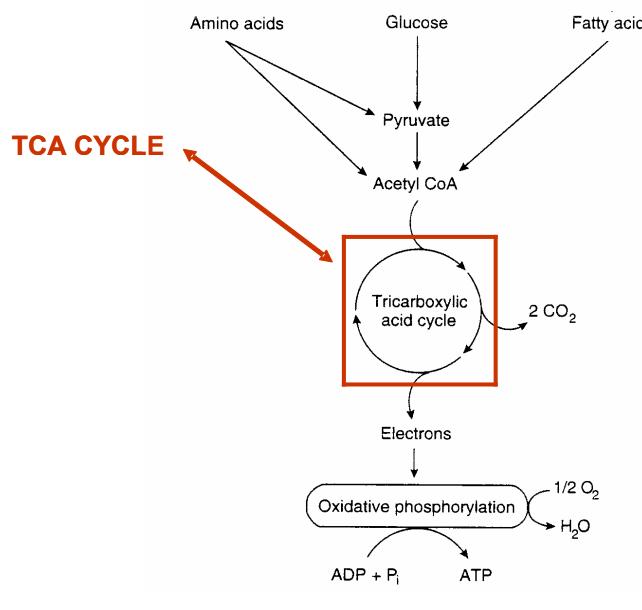


- Net reaction is **SIMPLE** – Process in **COMPLEX**!
- Pyruvate is now activated ready to enter the TCA cycle as Acetyl-CoA!

### 5 Reactions of the Pyruvate Dehydrogenase Complex



## 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  $\text{CO}_2$  and  $\text{H}_2\text{O}$
- 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 reducing power
- Coenzymes reduced:
  - $\text{NAD}^+/\text{NADH}$
  - $\text{FAD/FADH}_2$
- 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<sub>2</sub> to produce energy

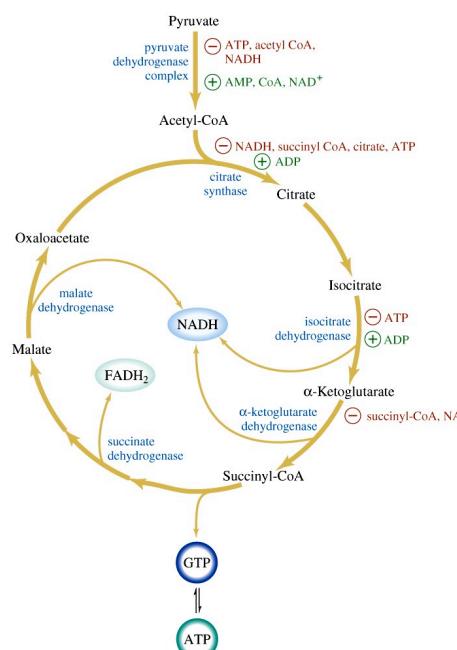


Figure 16-10 Concepts in Biochemistry, 3/e  
© 2006 John Wiley & Sons

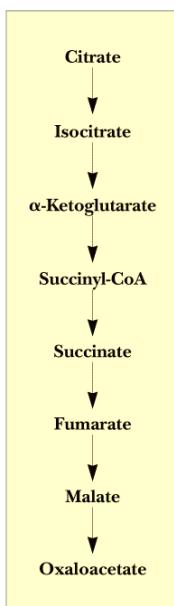
- ATP (GTP)

- Reducing power of NADH and FADH<sub>2</sub>

- 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:

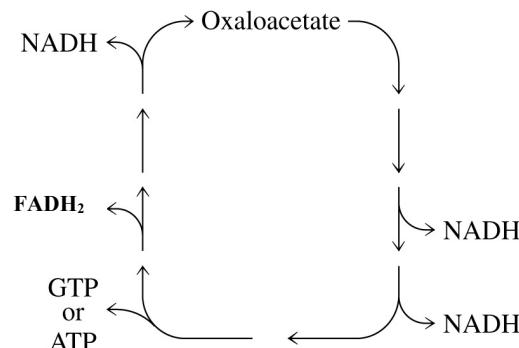
1. Glycolysis is a **linear** pathway; TCA cycle is **cyclic**
2. Glycolysis occurs in the **cytosol** and TCA is in the **mitochondrial matrix**
3. Glycolysis does **not require** oxygen; TCA **requires** oxygen (aerobic)

## Summary of the citric acid cycle

For each acetyl-CoA that enters the cycle:

- (1) Two molecules of CO<sub>2</sub> are released
- (2) Coenzymes NAD<sup>+</sup> 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<sub>2</sub> and one GTP

- NADH, FADH<sub>2</sub> 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<sub>2</sub> enables conservation of most of the energy as work with little lost as heat

**Table 16.2**  
The reactions of the citric acid cycle

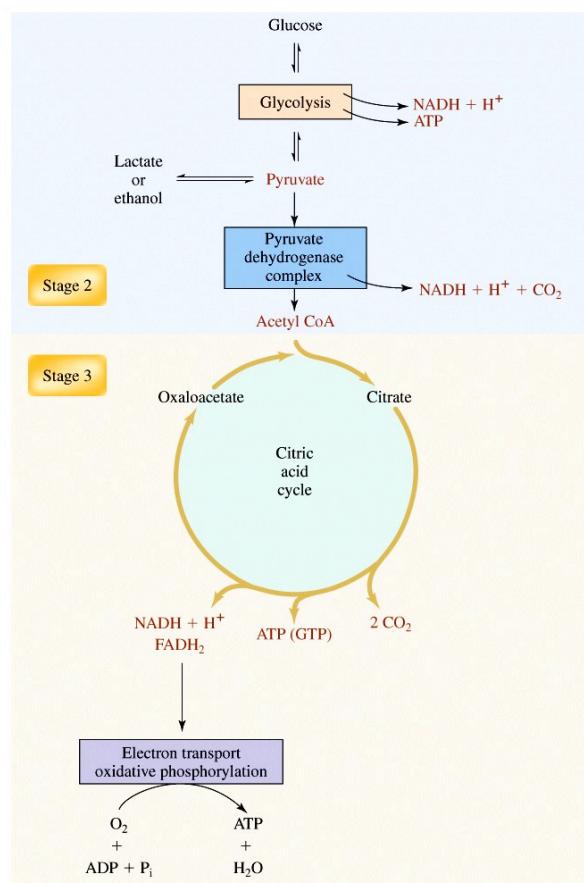
Reaction Number <sup>a</sup>	Reaction	Enzyme	Prosthetic Group	Reaction Type <sup>b</sup>
1	Acetyl-CoA + oxaloacetate + H <sub>2</sub> O ⇌ citrate + CoA	Citrate synthase		3, 4
2	Citrate ⇌ cis-aconitate ⇌ isocitrate	Aconitase	Fe-S	4
3	Isocitrate + NAD <sup>+</sup> ⇌ α-ketoglutarate + CO <sub>2</sub> + H <sup>+</sup>	Isocitrate dehydrogenase		1, 4
4	α-Ketoglutarate + NAD <sup>+</sup> + CoA ⇌ succinyl-CoA + CO <sub>2</sub> + NADH + H <sup>+</sup>	α-Ketoglutarate dehydrogenase complex	Lipoamide, FAD, TPP	1, 4
5	Succinyl-CoA + P <sub>i</sub> + ADP or GDP ⇌ succinate + ATP or GTP + CoA	Succinyl-CoA synthetase		2
6	Succinate + FAD (enzyme bound) ⇌ fumarate + FADH <sub>2</sub> (enzyme bound)	Succinate dehydrogenase	FAD, Fe-S	1
7	Fumarate + H <sub>2</sub> O ⇌ L-malate	Fumarase		4
8	L-Malate + NAD <sup>+</sup> ⇌ oxaloacetate + NADH + H <sup>+</sup>	Malate dehydrogenase		1

<sup>a</sup>The reaction numbers correspond to the steps in Figure 16.8.

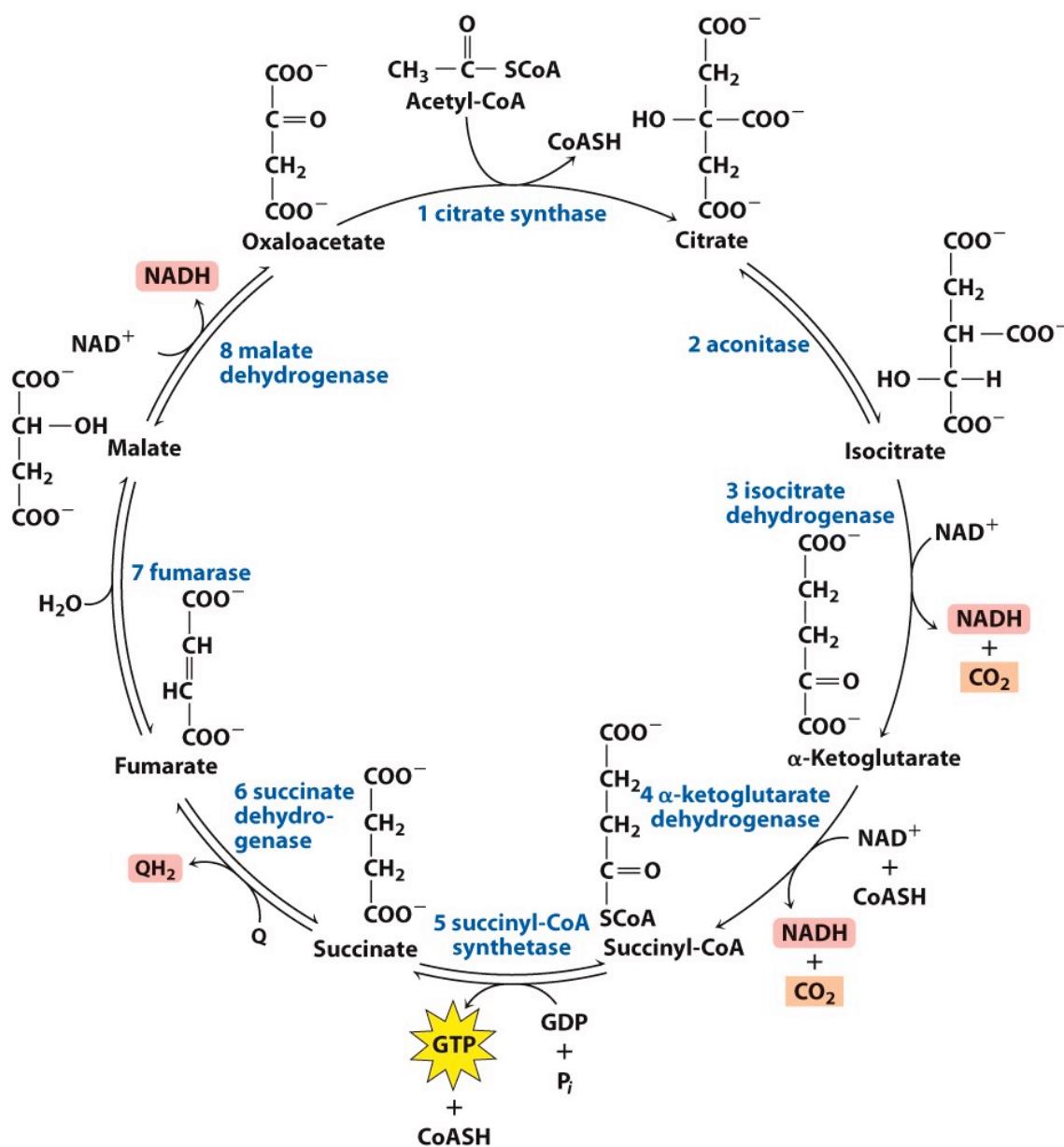
<sup>b</sup>Reaction type: 1, oxidation-reduction; 2, phosphoryl group transfer; 3, hydrolysis; 4, nonhydrolytic cleavage (addition or elimination); 5, isomerization-rearrangement; 6, bond formation coupled to ATP cleavage.

**Table 16-2 Concepts in Biochemistry, 3/e**

© 2006 John Wiley & Sons



**Figure 16-1 Concepts in Biochemistry, 3/e**  
© 2006 John Wiley & Sons



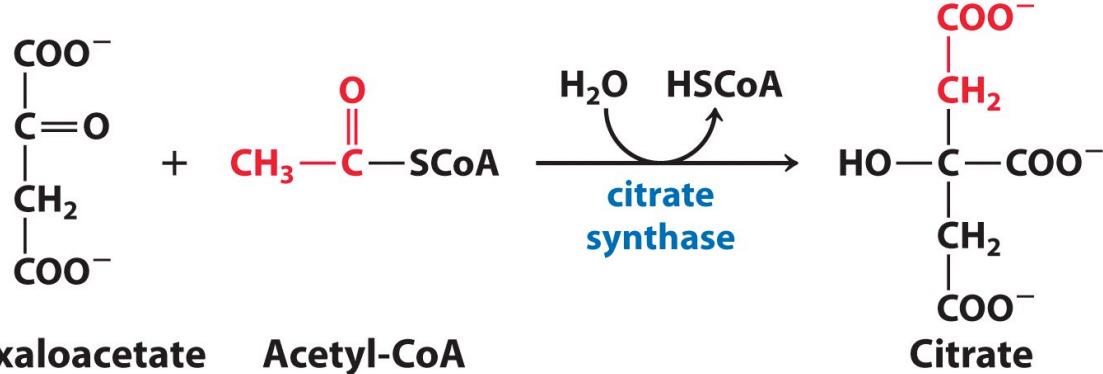
8

© John Wiley &amp; Sons, Inc. All rights reserved.

## 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)**



© John Wiley & Sons, Inc. All rights reserved.

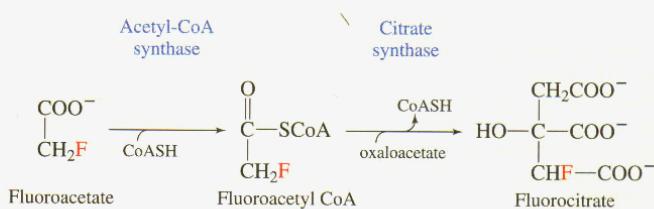
- Locoweed is toxic because it accumulates fluoroacetate



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

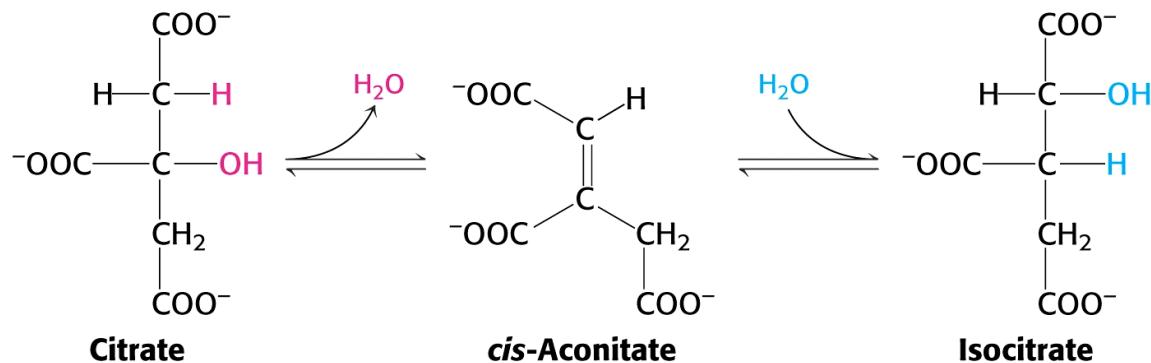
FIGURE 16.9

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.



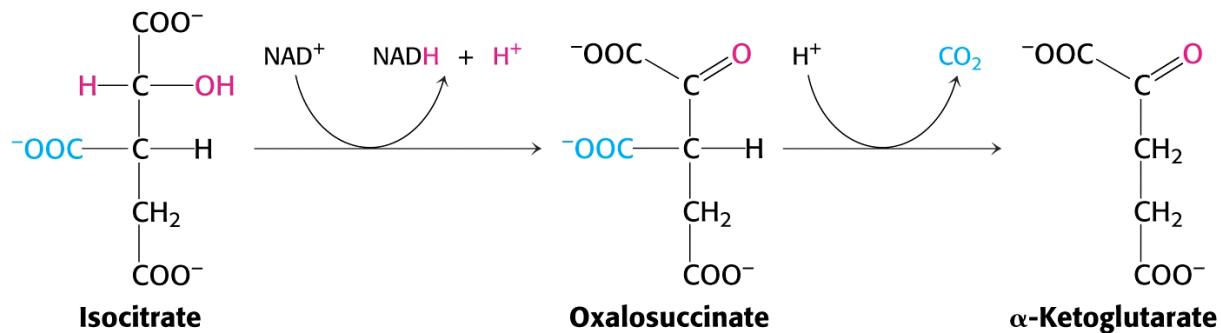
## 2. Aconitase

- 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<sub>2</sub>O from citrate to form C=C bond of *cis*-aconitate
  - Rearrangement allows the further oxidation of the molecule



### 3. Isocitrate Dehydrogenase

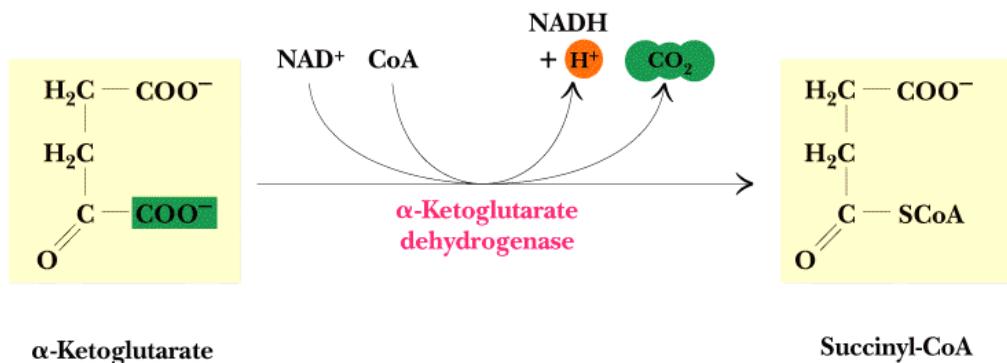
- First oxidative decarboxylation of isocitrate to  $\alpha$ -ketoglutarate ( $\alpha$ -kg)
- Metabolically irreversible reaction
- One of four oxidation-reduction reactions of the cycle
- Also a Non-hydrolytic cleavage reaction (addition or elimination)
- Hydride ion from the C-2 of isocitrate is transferred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



### 4. $\alpha$ -Ketoglutarate Dehydrogenase Complex

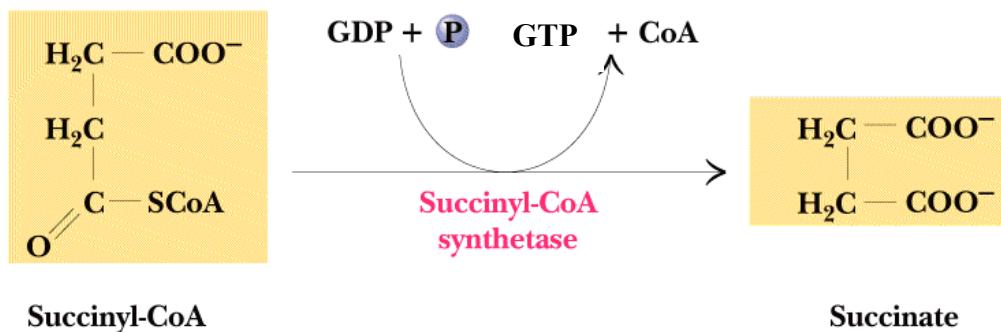
- Second oxidative decarboxylation reaction
- Also a Non-hydrolytic cleavage reaction (addition or elimination)
- $\alpha$ -Ketoglutarate converted to Succinyl-CoA
- Similar to pyruvate dehydrogenase complex except a succinyl group is activated, not acetyl
  - Same coenzymes, identical mechanisms
- Succinyl-CoA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

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



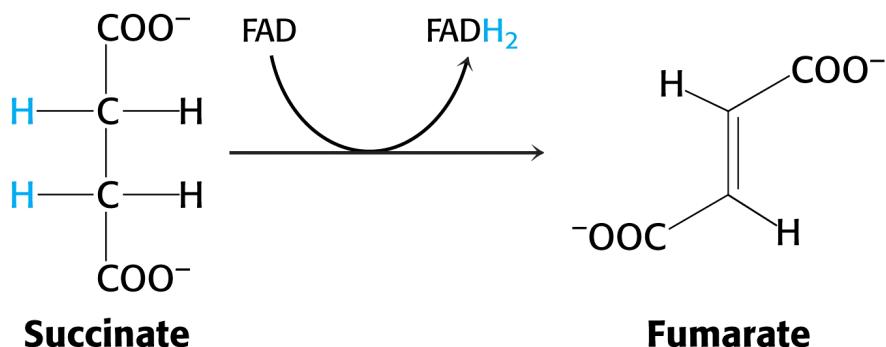
## 5. Succinyl-CoA Synthetase (Formation of succinate)

- 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  $\text{FADH}_2$  to  $\text{O}_2$  – release of energy drives ATP formation from  $\text{ADP} + \text{Pi}$

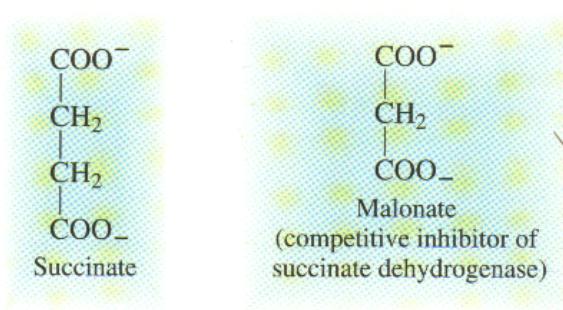


## 6. The Succinate Dehydrogenase (SDH) Complex

- 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<sub>2</sub>
  - NAD<sup>+</sup> functions in reactions that interconvert hydroxyl and carbonyl groups
- Dehydrogenation is **stereospecific**; only the *trans* isomer is formed
- Also known as Complex II of the electron transport chain – direct feed of electrons from FADH<sub>2</sub> into the electron transport chain.



- 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

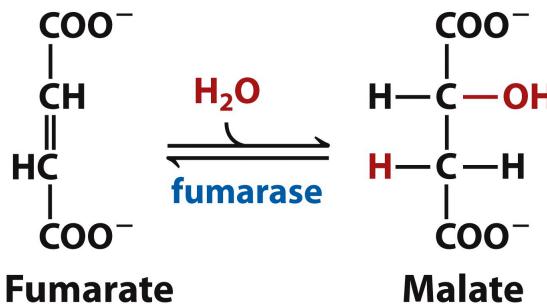


**FIGURE 16.10**

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

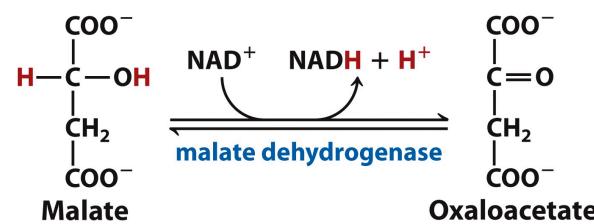
- Stereospecific *trans* addition of water to the double bond of fumarate to form **L-malate**



- 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<sub>2</sub>

- CO<sub>2</sub> leaves at steps 3 and 4

### 2. 3 NAD<sup>+</sup> 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 FADH<sub>2</sub>

- Step 6 – Succinate dehydrogenase

### 4. 1 phosphoanhydride bond formed in ATP or GTP

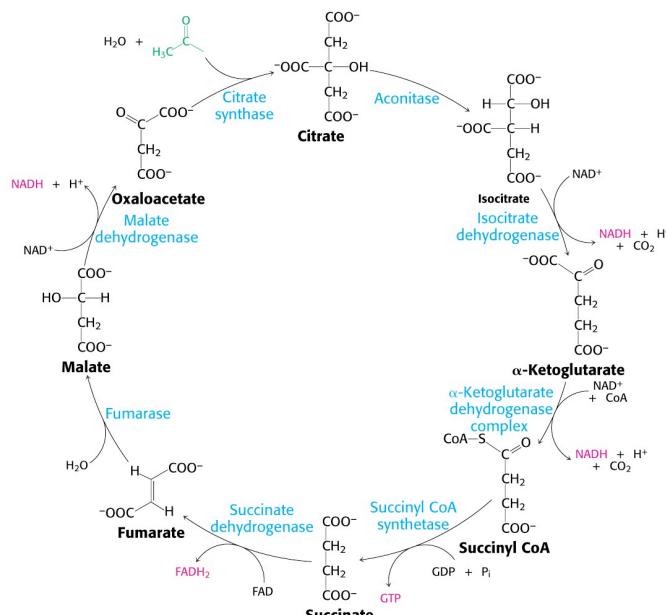
- Substrate level phosphorylation at step 5: Succinyl-CoA Synthetase
- Generated from energy stored in CoA thioester

So, per pyruvate:

4 NADH (one from pyruvate dehydrogenase complex + 3 TCA)

1 ATP or GTP

1 FADH<sub>2</sub>



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<sub>2</sub>
  - (3) 1 GTP (or 1 ATP)
- Oxidation of each NADH yields 2.5 ATP
- Oxidation of each FADH<sub>2</sub> yields 1.5 ATP
- Complete oxidation of 1 acetyl CoA = 10 ATP

Table 16.3

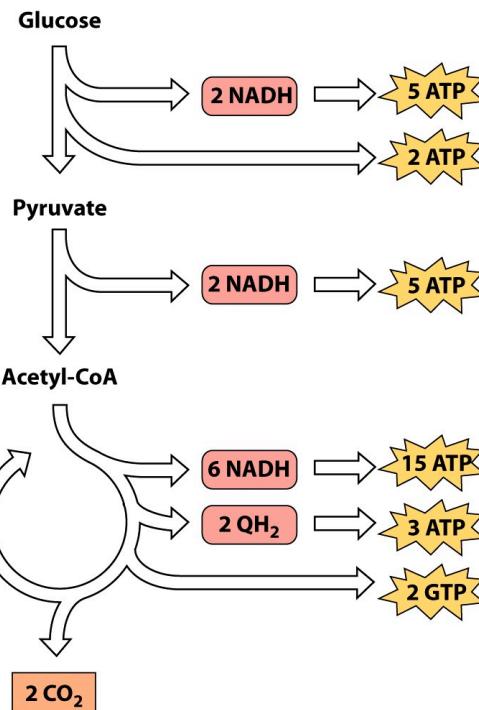
The ATP, NADH, and FADH<sub>2</sub> balance sheet for the pyruvate dehydrogenase complex and the citric acid cycle

Reaction Number <sup>a</sup>	Reaction	ATP (GTP) Change <sup>b</sup>	NADH Change <sup>b</sup>	FADH <sub>2</sub> Change <sup>b</sup>
		(per pyruvate)		
<i>Pyruvate oxidation</i>				
	Pyruvate dehydrogenase complex	0	+1	0
	Pyruvate oxidation total	0	+1	0
<i>Citric acid cycle</i>				
3	Isocitrate + NAD <sup>+</sup> $\rightleftharpoons$ $\alpha$ -ketoglutarate + CO <sub>2</sub> + NADH + H <sup>+</sup>	0	+1	0
4	$\alpha$ -Ketoglutarate + NAD <sup>+</sup> + CoA $\rightleftharpoons$ succinyl-CoA + CO <sub>2</sub> + NADH + H <sup>+</sup>	0	+1	0
5	Succinyl-CoA + GDP or ADP + P <sub>i</sub> $\rightleftharpoons$ succinate + GTP or ATP + CoASH	+1	0	0
6	Succinate + FAD $\rightleftharpoons$ fumarate + FADH <sub>2</sub>	0	0	+1
8	L-Malate + NAD <sup>+</sup> $\rightleftharpoons$ oxaloacetate + NADH + H <sup>+</sup>	0	+1	0
	Citric acid cycle total	+1	+3	+1
	Grand Total	+1	+4	+1

<sup>a</sup>The reaction numbers correspond to the steps in Figure 16.8.<sup>b</sup>A + number indicates a production of ATP, NADH, or FADH<sub>2</sub>. 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  
© 2006 John Wiley & Sons

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

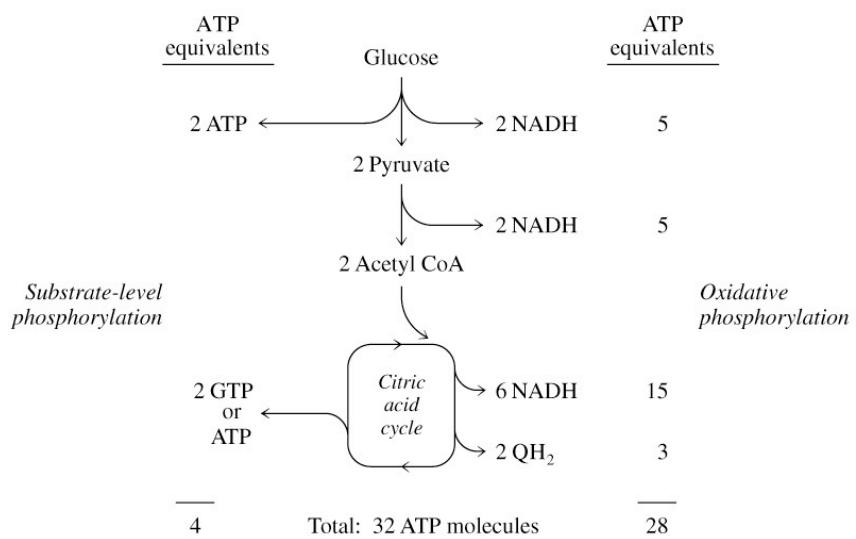
AEROBIC TOTAL/glucose = 32 ATP



If anaerobic – 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)

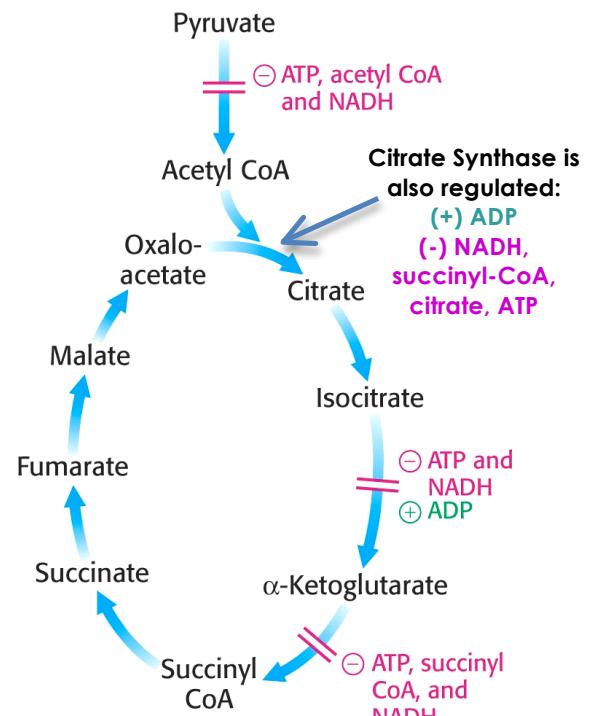
- Occurs in muscles during exercise because they go into oxygen debt.
- Soreness due to  $H^+$  from lactic acid
- Metabolism in muscles returns to normal when oxygen replenished

\*\*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<sub>2</sub> 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



## POINTS OF REGULATION

## 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<sup>+</sup>, 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:  $\text{NAD}^+$ , ADP and  $\text{Ca}^{+2}$

## Regulation of $\alpha$ -ketoglutarate dehydrogenase complex

- Inhibitors: NADH, ATP and succinyl-CoA
- Stimulators:  $\text{NAD}^+$ , 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, $\text{NAD}^+$ , 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
$\alpha$ -Ketoglutarate dehydrogenase complex	—	Succinyl-CoA, NADH	Activity depends on metabolite concentrations

**Table 16-4 Concepts in Biochemistry, 3/e**  
© 2006 John Wiley & Sons

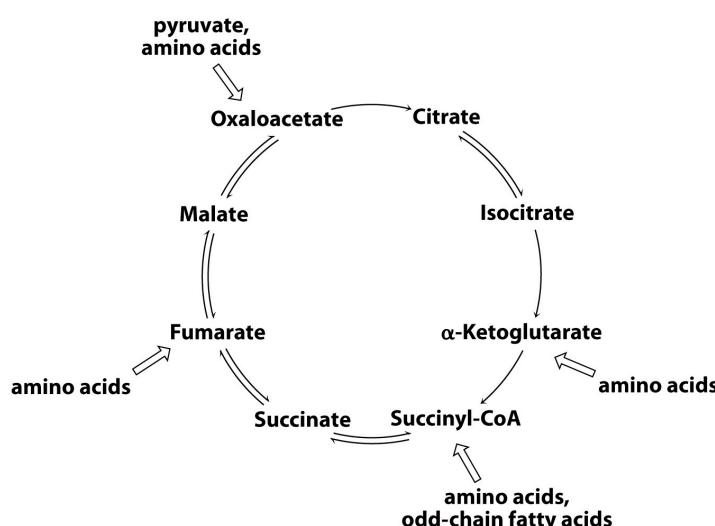
## Anaplerotic Reactions Replenish TCA Cycle Intermediates:

1. **Pyruvate Carboxylase** converts pyruvate to **oxaloacetate**.

Activated by Acetyl-CoA! Increases the flux through TCA cycle.

2. Degradation of odd numbered fatty acids yields **succinyl-CoA**

3. Degradation of **amino acids** produces other intermediates.



© John Wiley & Sons, Inc. All rights reserved.