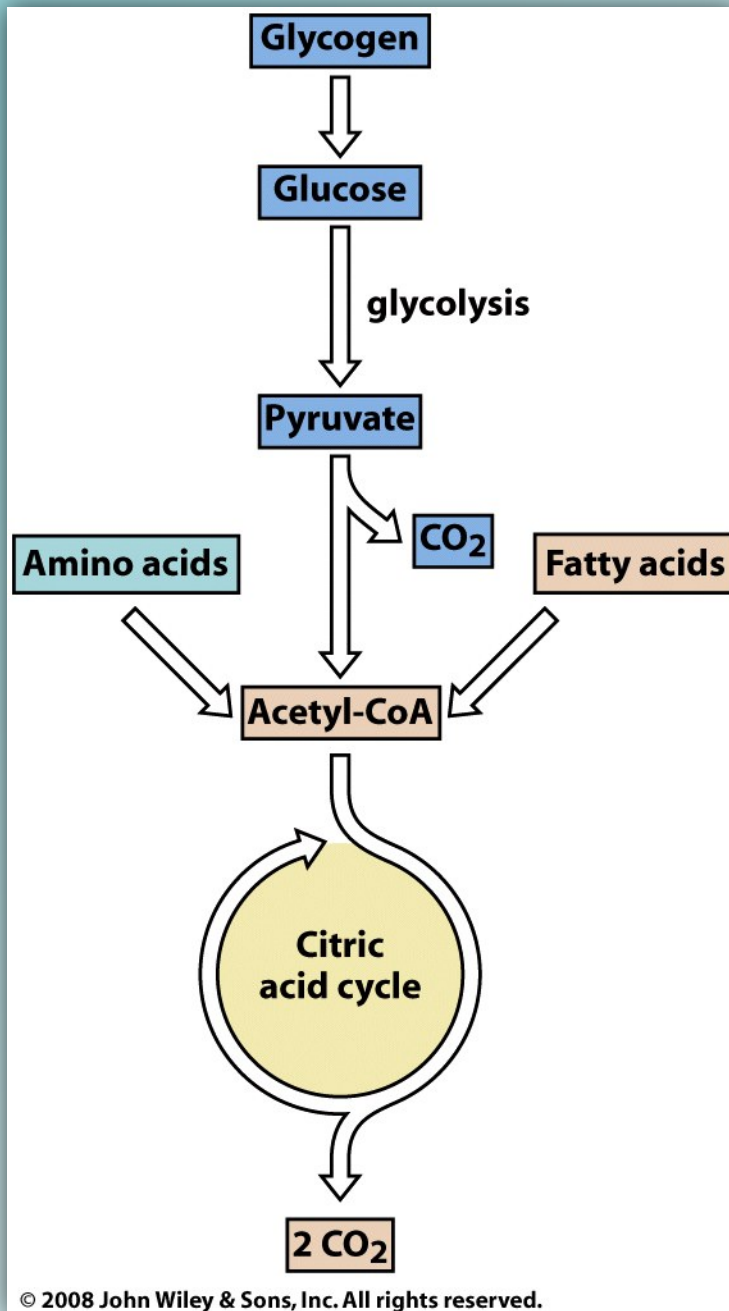


# CHEM 539

Molecular Metabolism: Pathways and Regulation

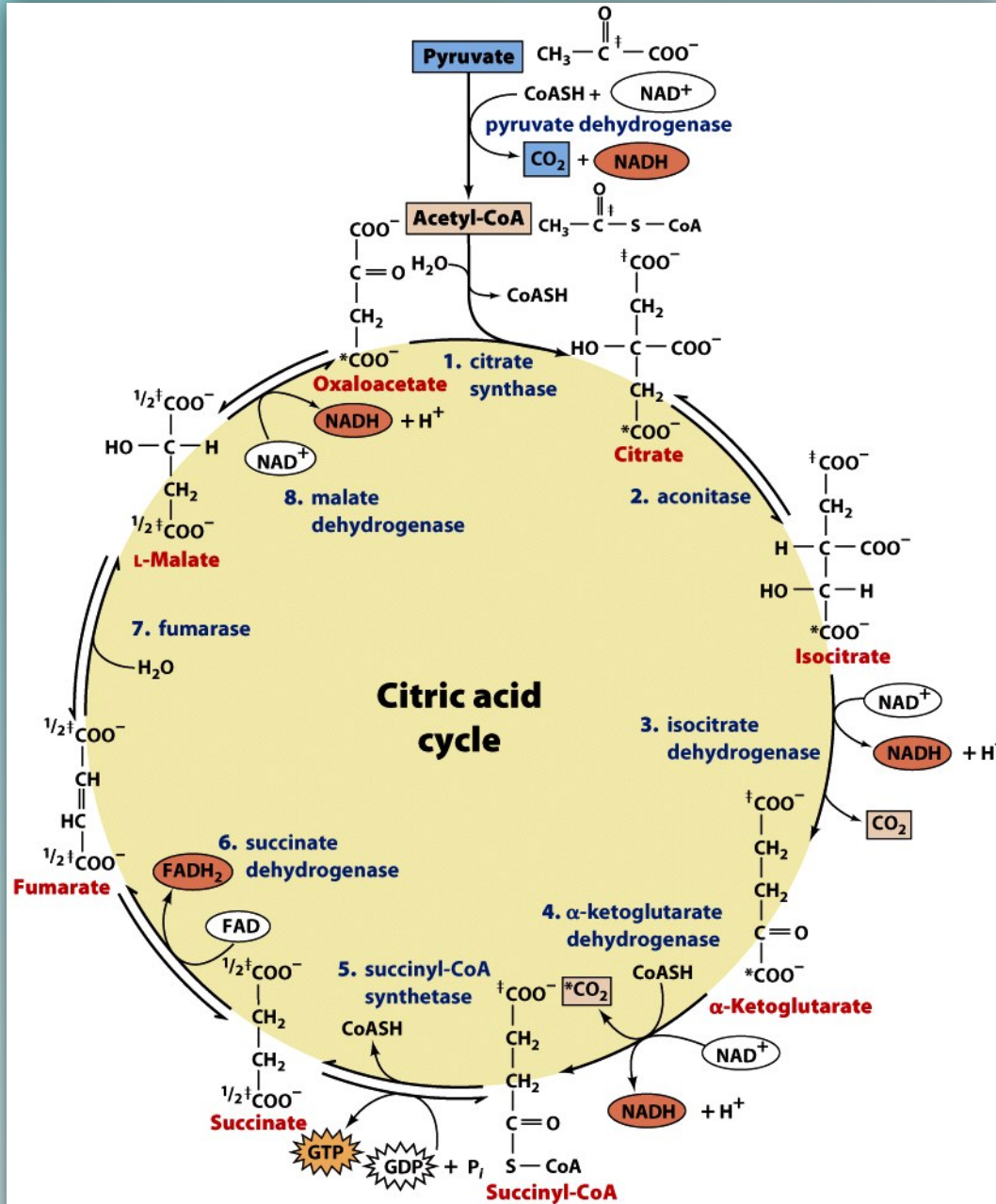
**PPT Set 4: The tricarboxylic acid cycle**  
(Krebs cycle; citric acid cycle)



## Integration of glycolysis with TCA

All TCA reactions are mitochondrial (matrix); the PD reaction is also mitochondrial.

# Summary of the nine TCA cycle reactions

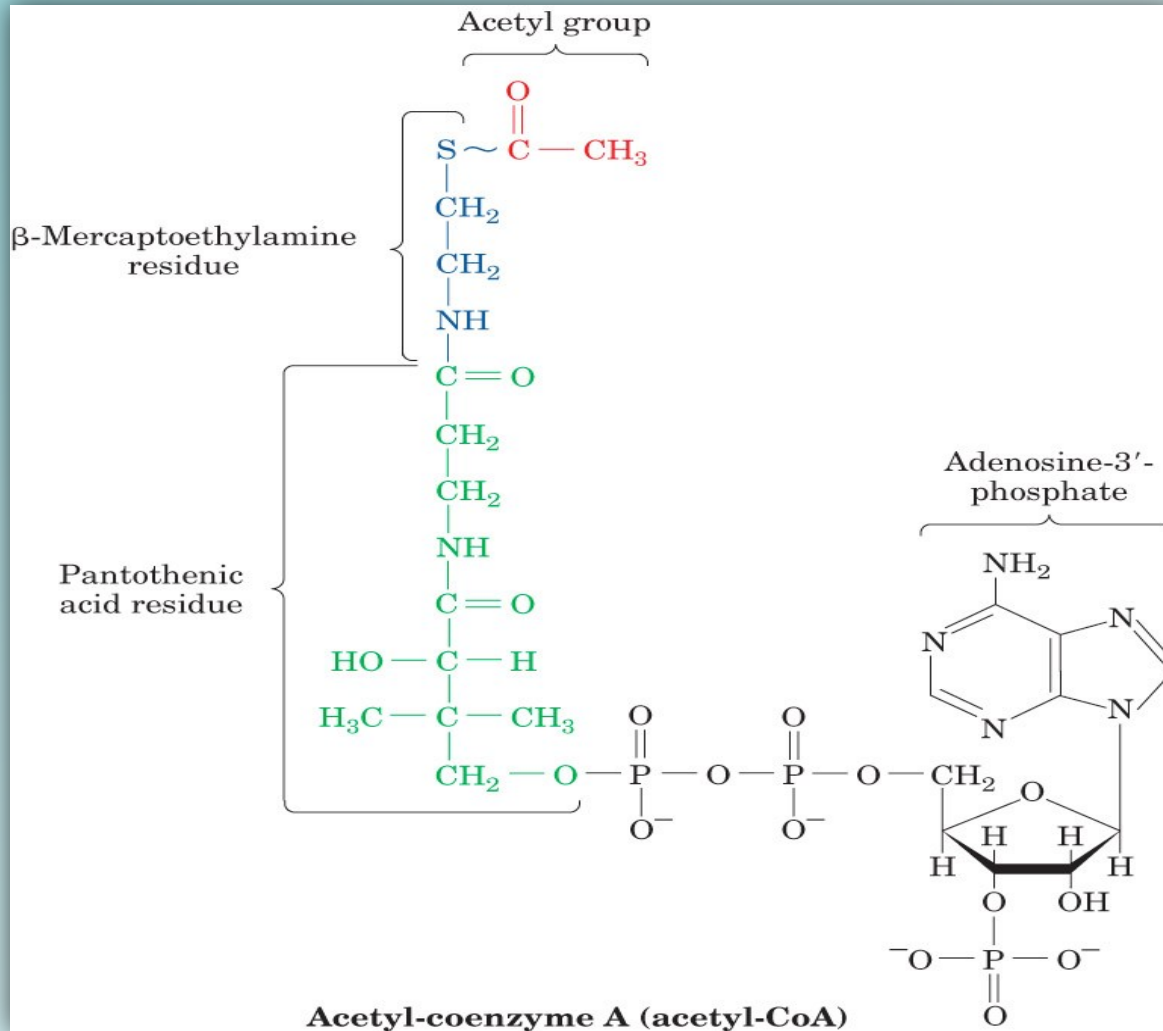


Five reactions produce reducing equivalents ( $\text{NADH}$  and  $\text{FADH}_2$ ); there is one substrate-level phosphorylation reaction. The  $\text{C}_2$  acetyl fragment of acetyl CoA is converted to 2 molecules of  $\text{CO}_2$ .

**Table 17-2****Standard Free Energy Changes ( $\Delta G^{\circ'}$ ) and Physiological Free Energy Changes ( $\Delta G$ ) of Citric Acid Cycle Reactions**

<b>Reaction</b>	<b>Enzyme</b>	<b><math>\Delta G^{\circ'}</math> (kJ · mol<sup>-1</sup>)</b>	<b><math>\Delta G</math> (kJ · mol<sup>-1</sup>)</b>
1	Citrate synthase	-31.5	Negative
2	Aconitase	~5	~0
3	Isocitrate dehydrogenase	-21	Negative
4	$\alpha$ -Ketoglutarate dehydrogenase	-33	Negative
5	Succinyl-CoA synthetase	-2.1	~0
6	Succinate dehydrogenase	+6	~0
7	Fumarase	-3.4	~0
8	Malate dehydrogenase	+29.7	~0

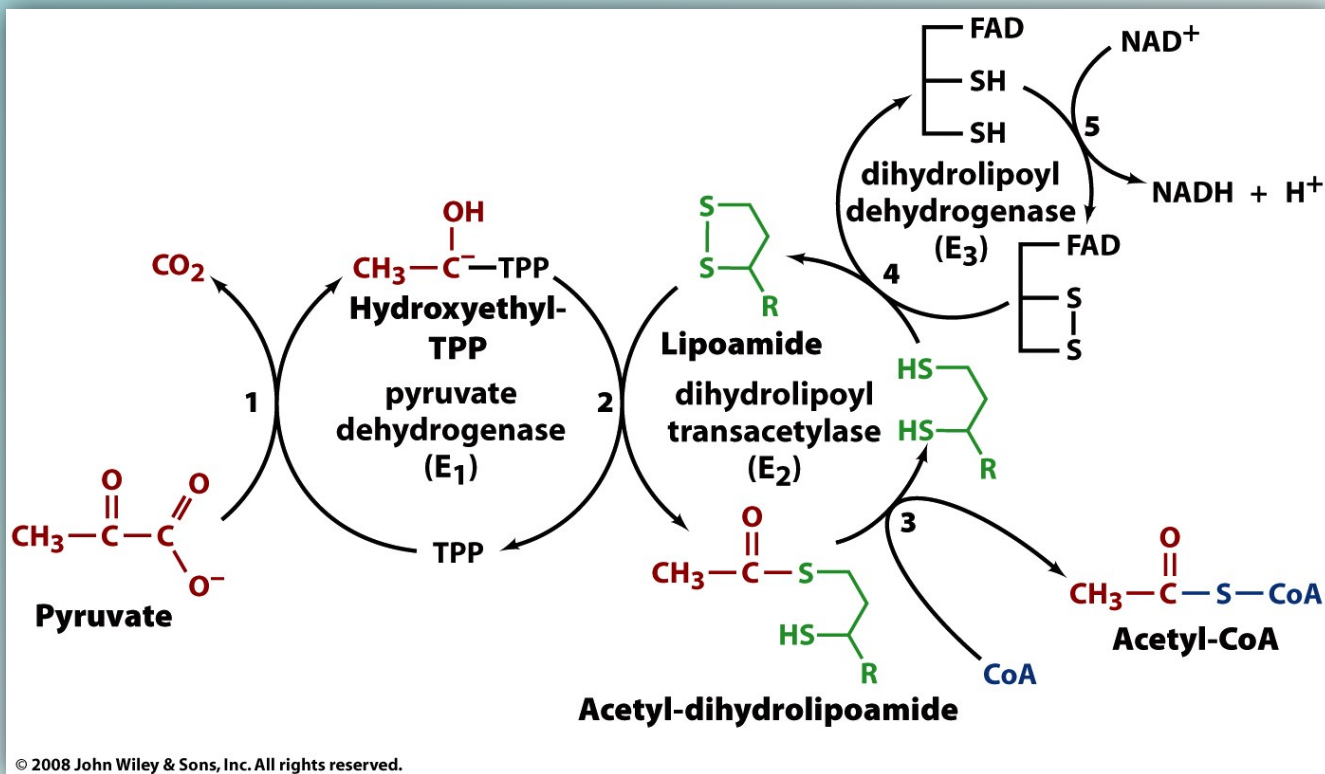
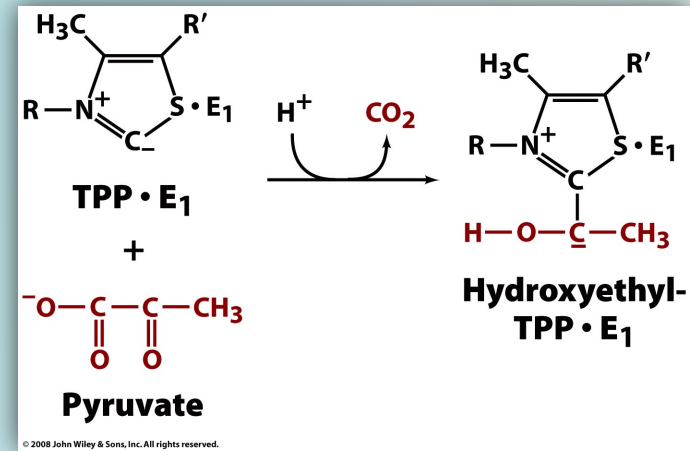
# Chemical structure of acetyl-CoA

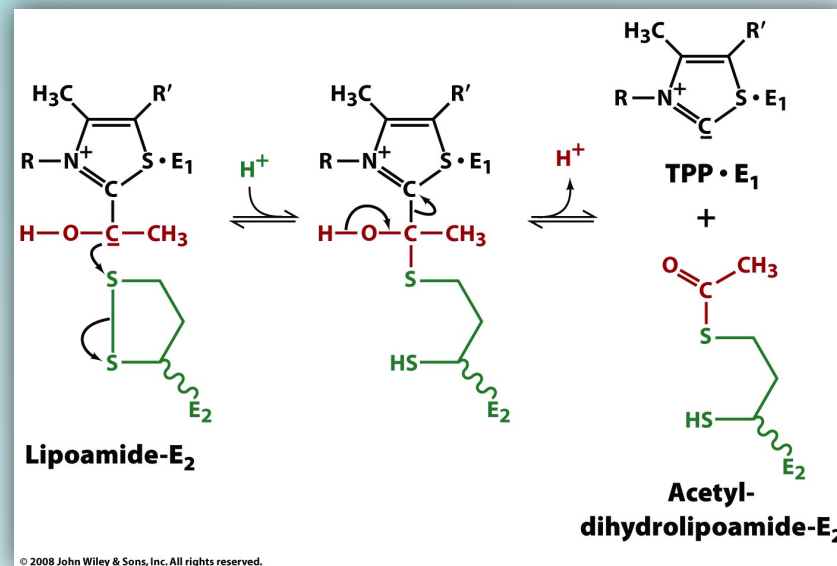
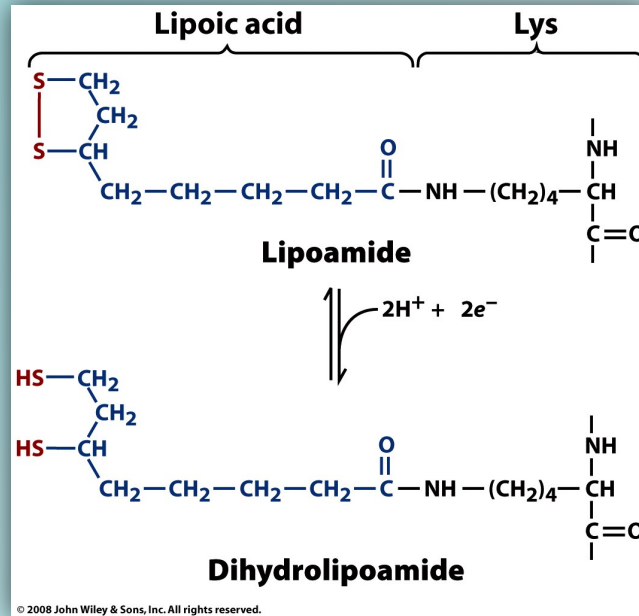


**Table 17-1****The Coenzymes and Prosthetic Groups of Pyruvate Dehydrogenase**

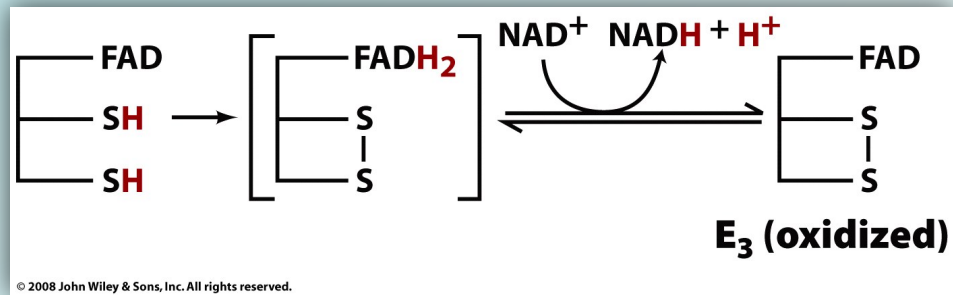
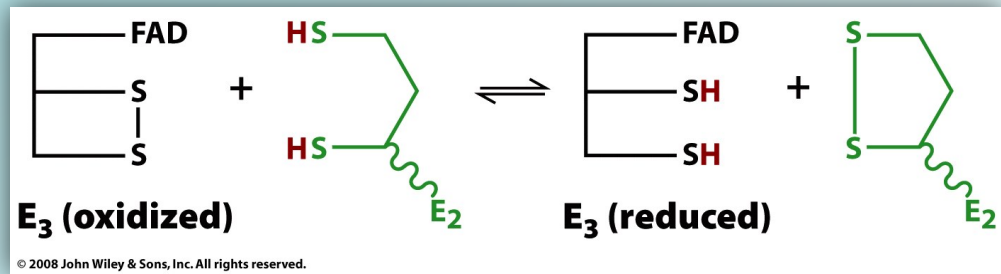
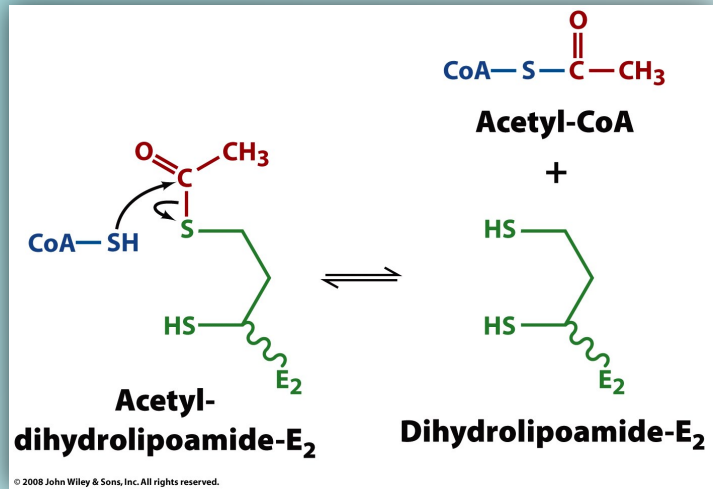
<b>Cofactor</b>	<b>Location</b>	<b>Function</b>
<b>Thiamine pyrophosphate (TPP)</b>	<b>Bound to E<sub>1</sub></b>	<b>Decarboxylates pyruvate yielding a hydroxyethyl-TPP carbanion</b>
<b>Lipoic acid</b>	<b>Covalently linked to a Lys on E<sub>2</sub> (lipoamide)</b>	<b>Accepts the hydroxyethyl carbanion from TPP as an acetyl group</b>
<b>Coenzyme A (CoA)</b>	<b>Substrate for E<sub>2</sub></b>	<b>Accepts the acetyl group from lipoamide</b>
<b>Flavin adenine dinucleotide (FAD)</b>	<b>Bound to E<sub>3</sub></b>	<b>Reduced by lipoamide</b>
<b>Nicotinamide adenine dinucleotide (NAD<sup>+</sup>)</b>	<b>Substrate for E<sub>3</sub></b>	<b>Reduced by FADH<sub>2</sub></b>

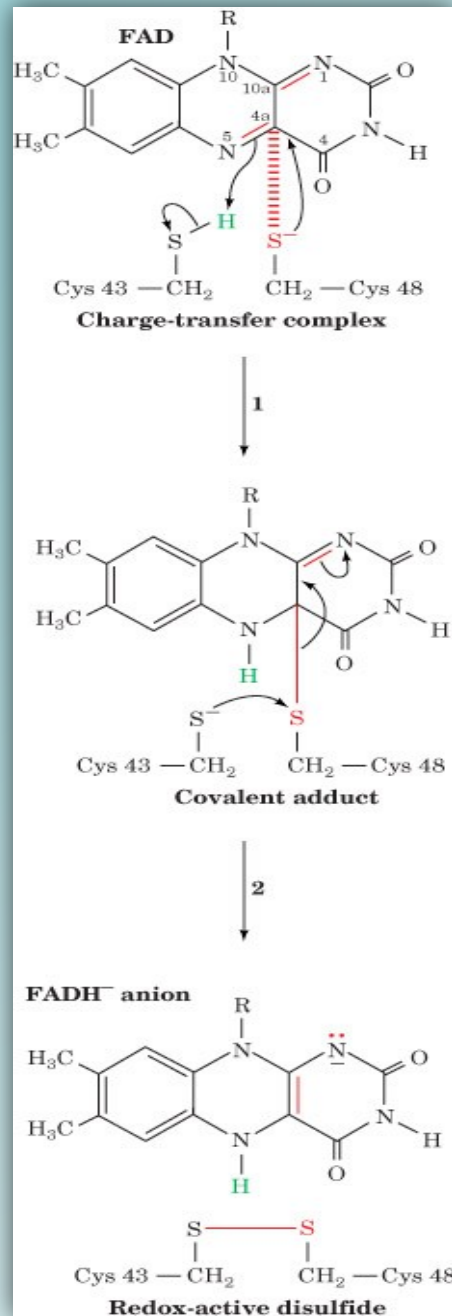
The pyruvate dehydrogenase complex: oxidative decarboxylation of an  $\alpha$ -ketoacid (pyruvate)



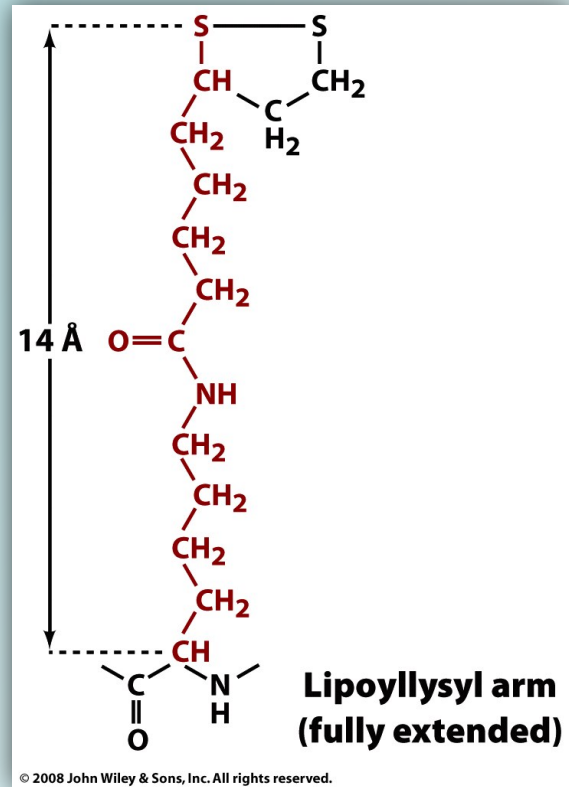
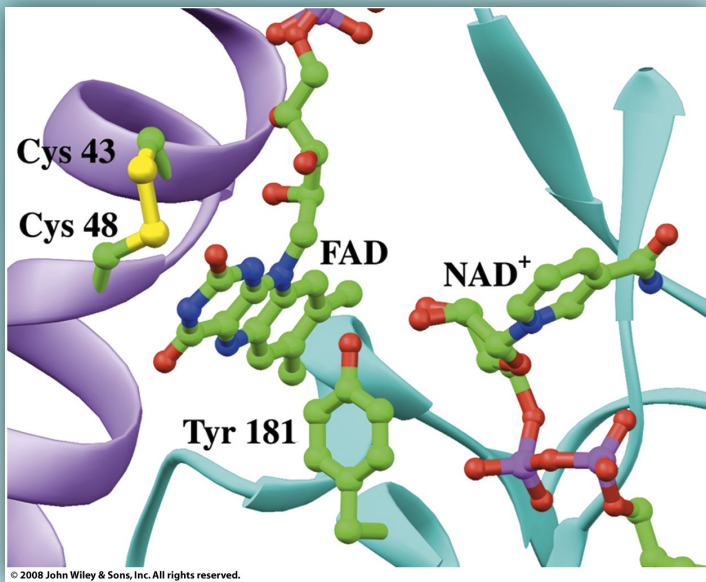




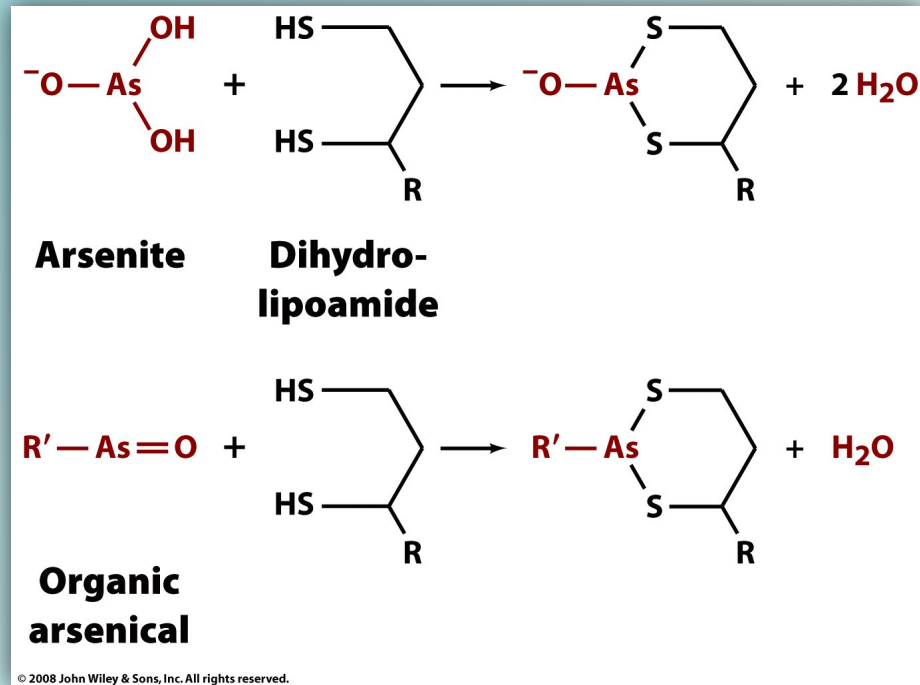




## Dihydrolipoyl dehydrogenase mechanism

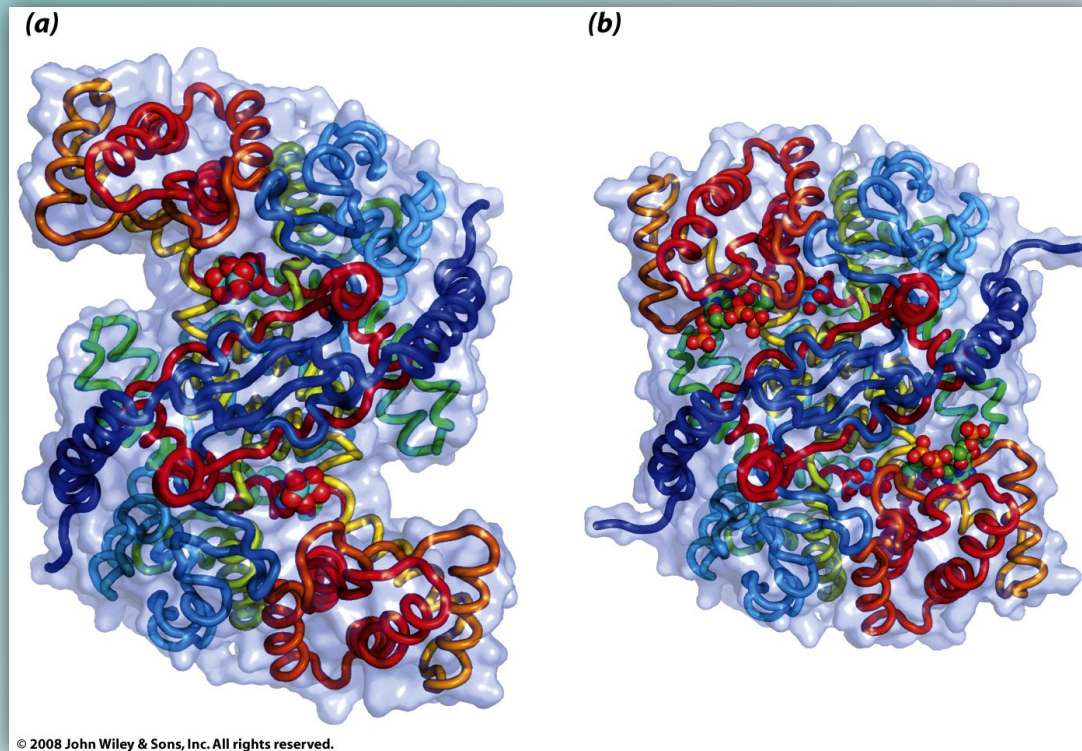


# Molecular basis of arsenic poisoning

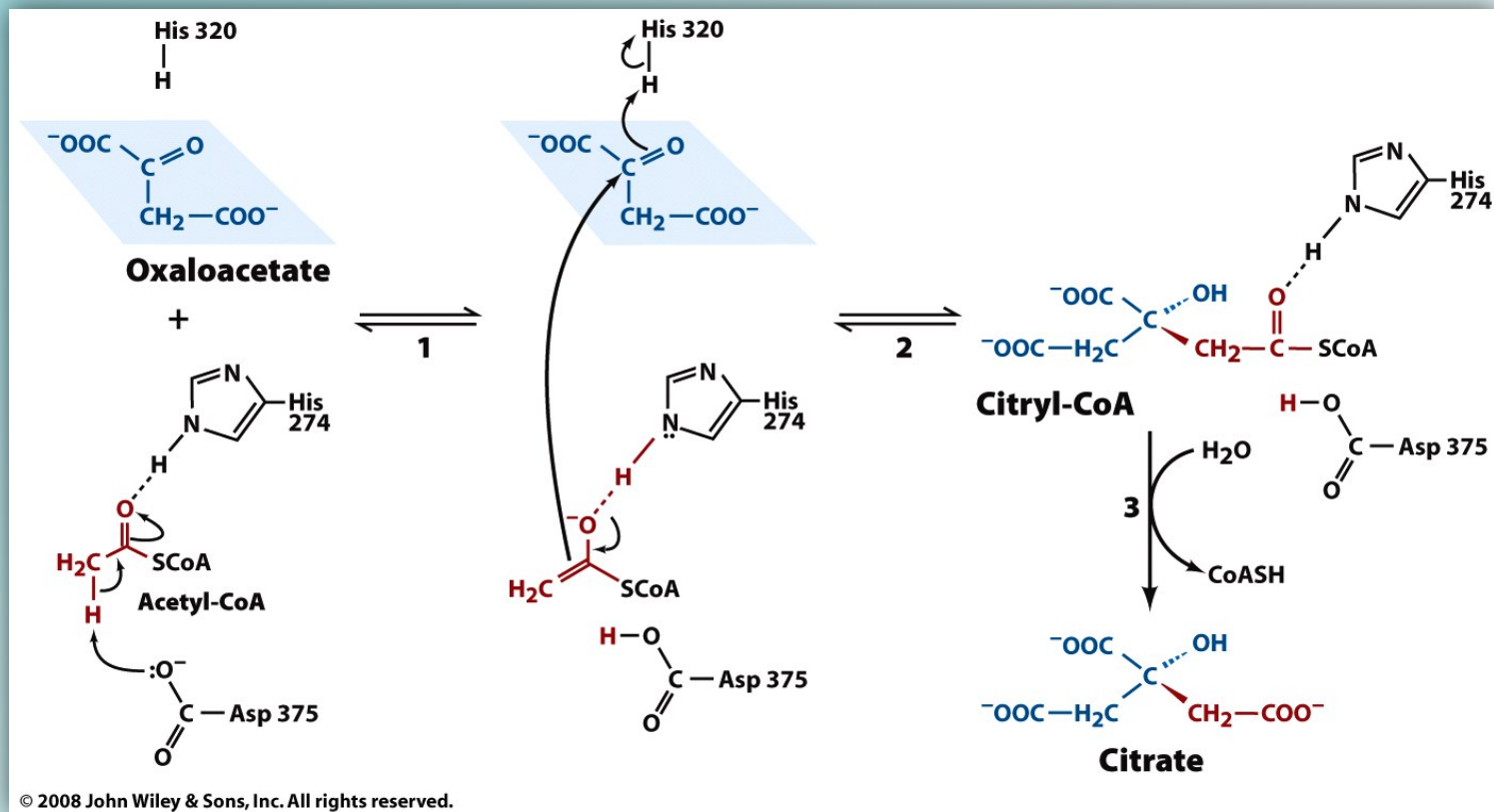


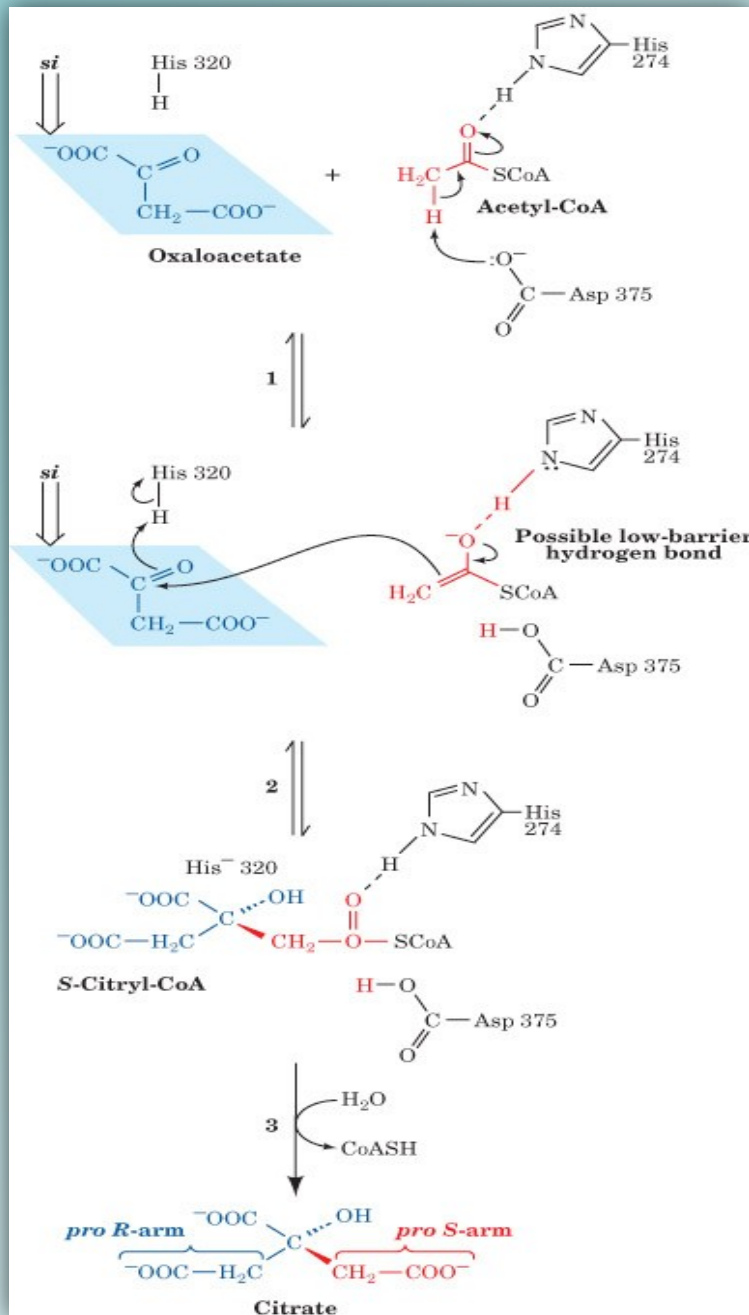
The pyruvate and  $\alpha$ -ketoglutarate dehydrogenase complexes are susceptible to inactivation by arsenic.

Major conformational change occurs when citrate synthase binds its substrate (CS is a homodimer)



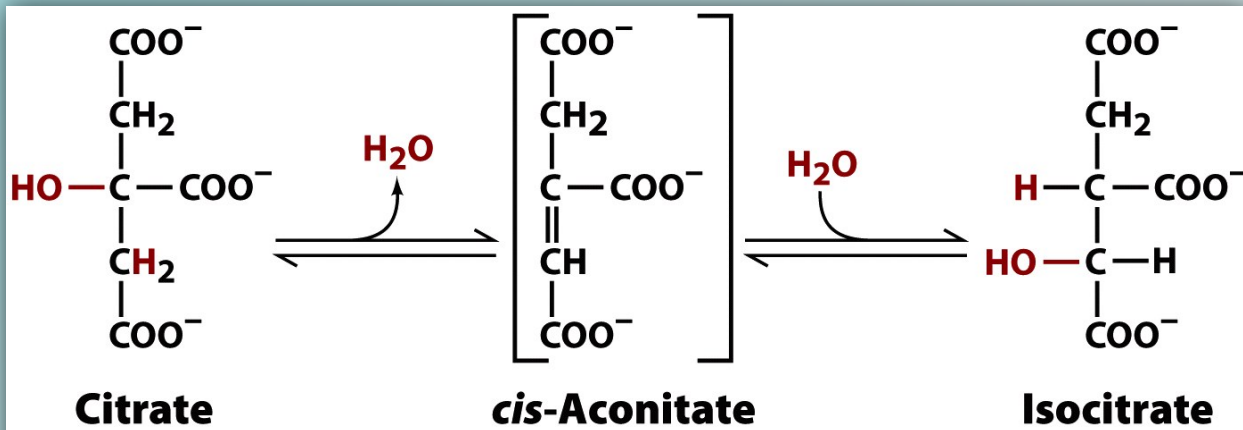
# Mechanism of the citrate synthase reaction





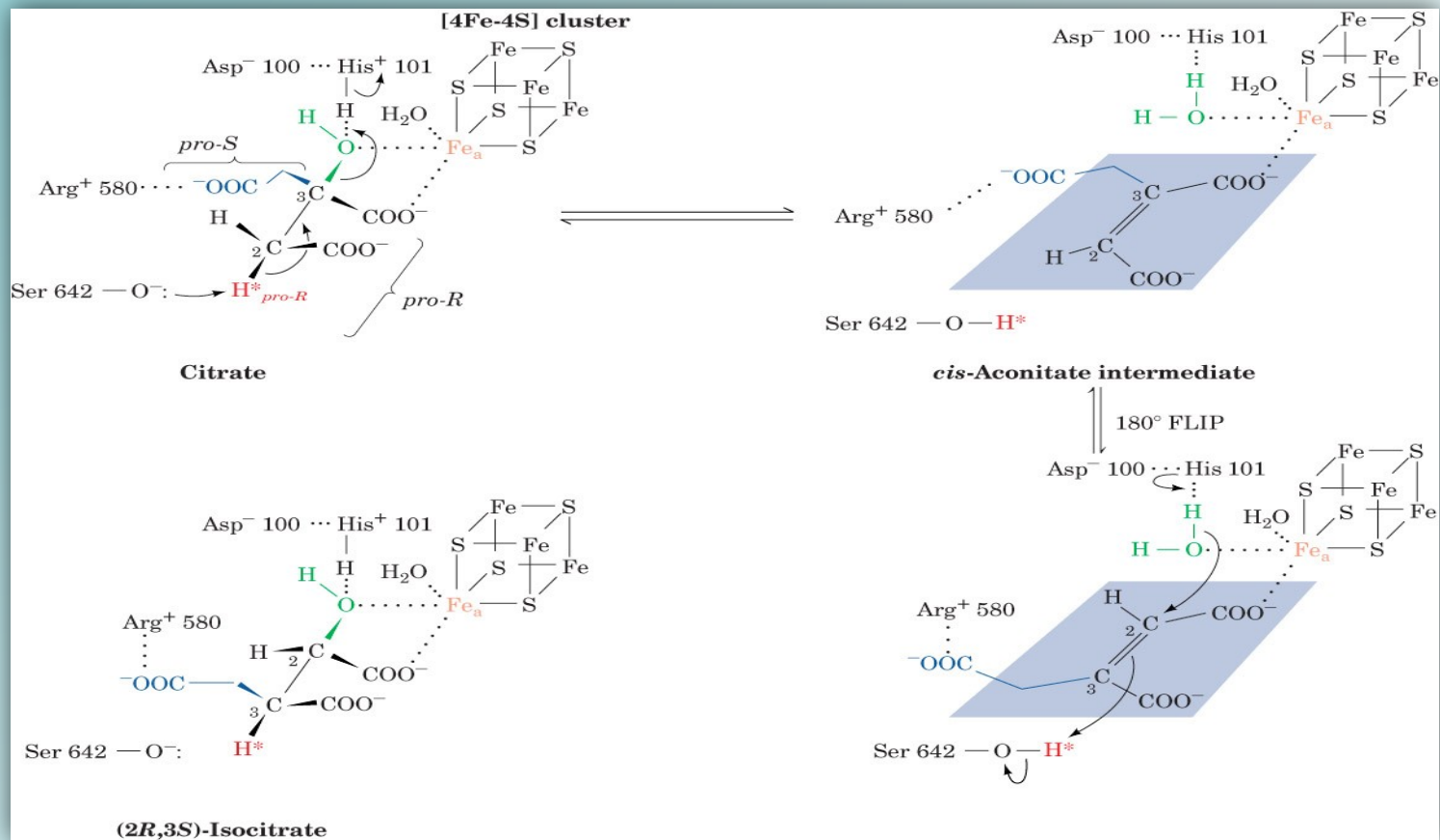
## Citrate synthase: reaction stereochemistry

# Aconitase

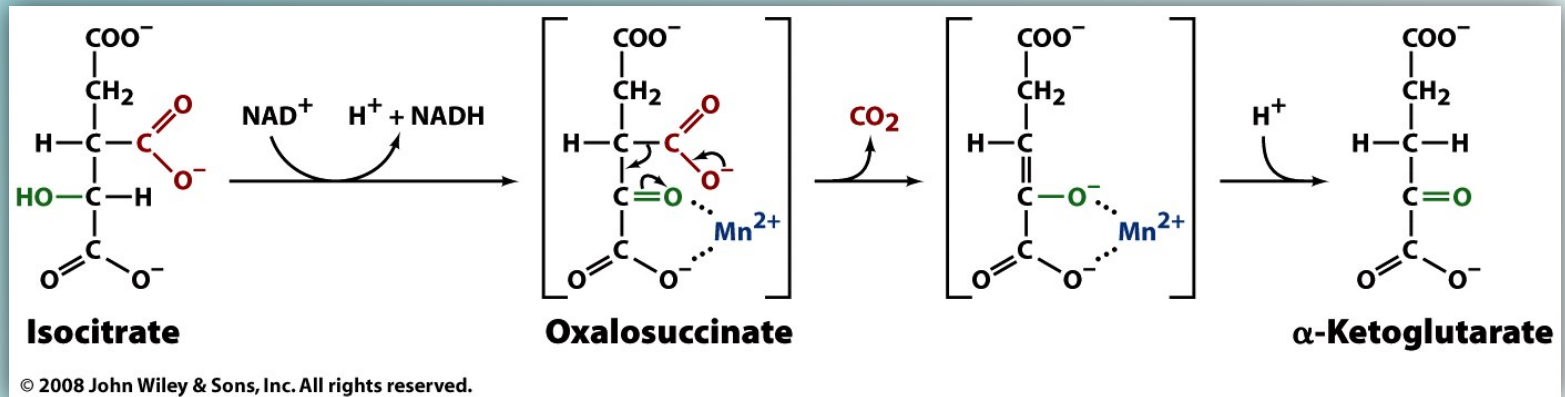




# Aconitase reaction stereochemistry

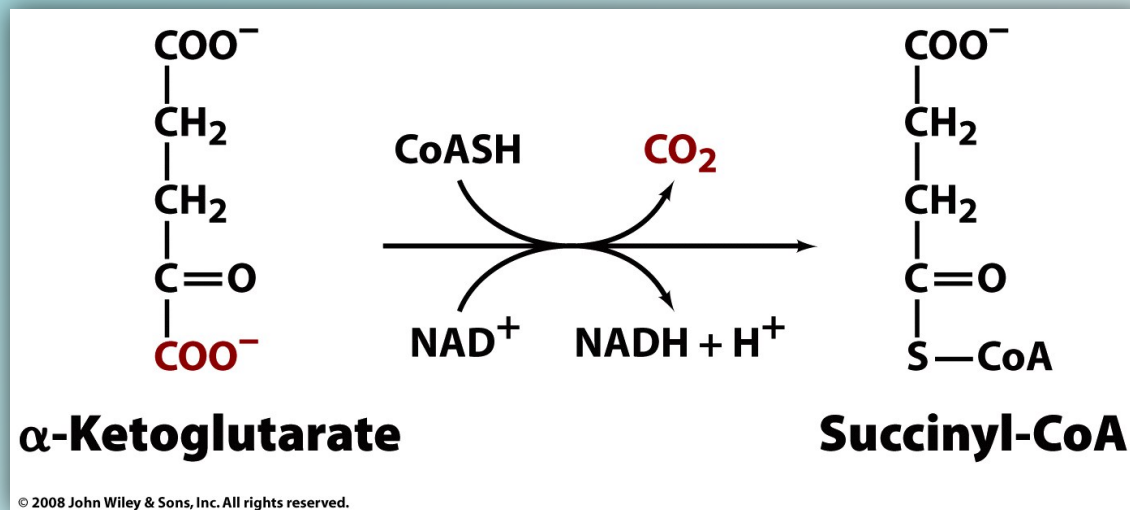


## Isocitrate dehydrogenase (NAD<sup>+</sup>-linked dehydrogenase):



Involves oxidative decarboxylation of a  $\beta$ -ketoacid intermediate

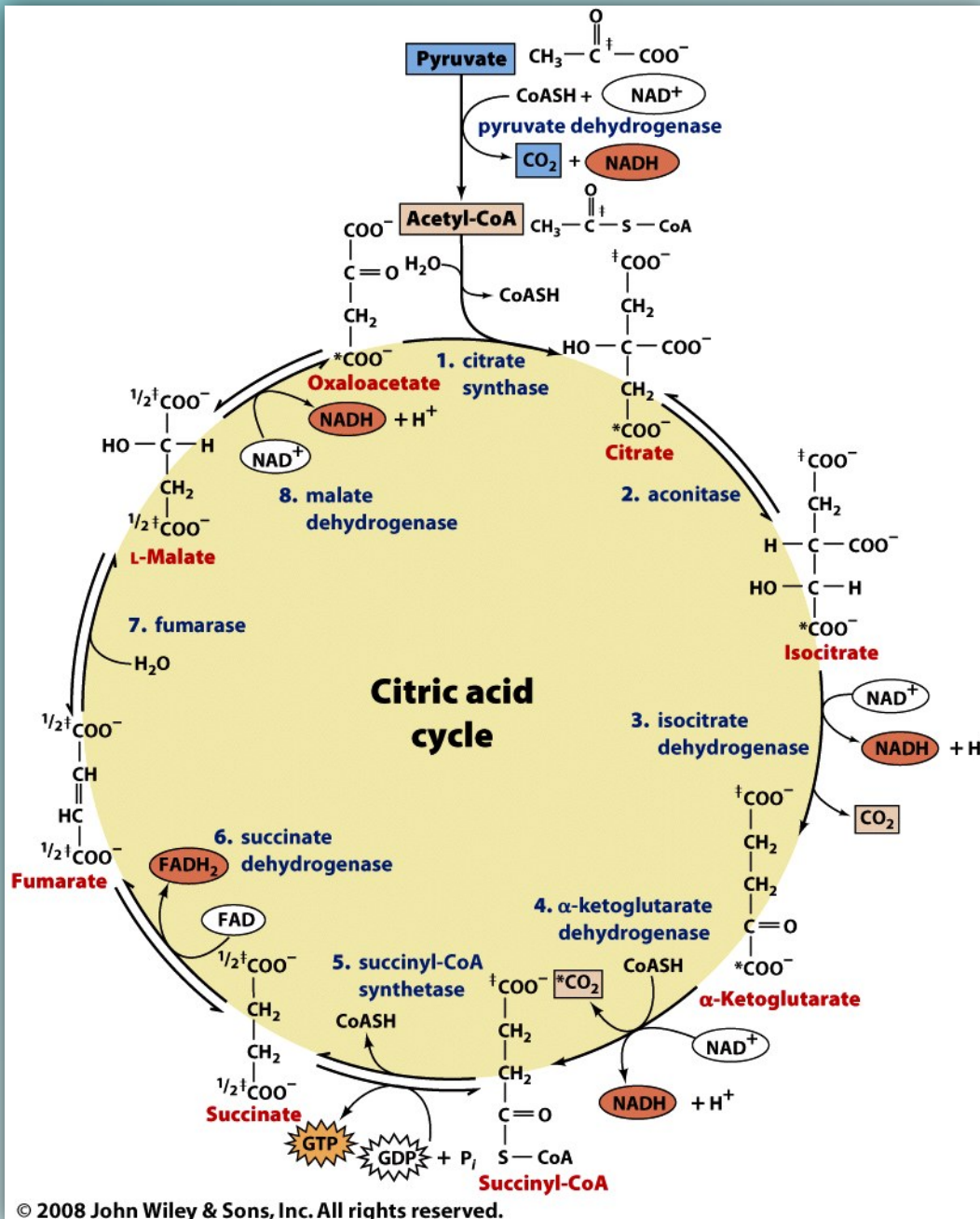
## $\alpha$ -Ketoglutarate dehydrogenase (NAD<sup>+</sup>-linked dehydrogenase):



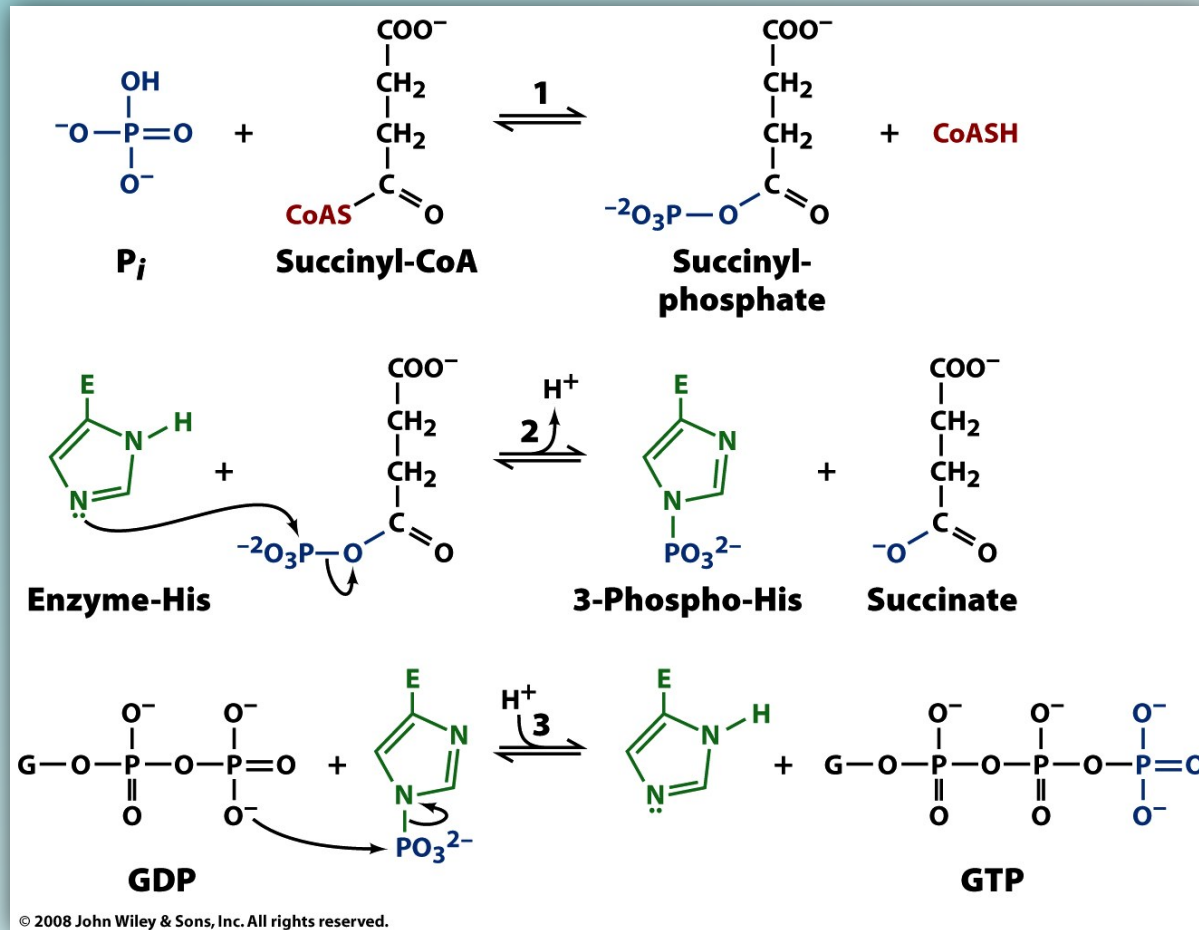
(mechanism is similar to that of pyruvate dehydrogenase;  
oxidative decarboxylation of an  $\alpha$ -ketoacid)

# Summary of the nine TCA cycle reactions

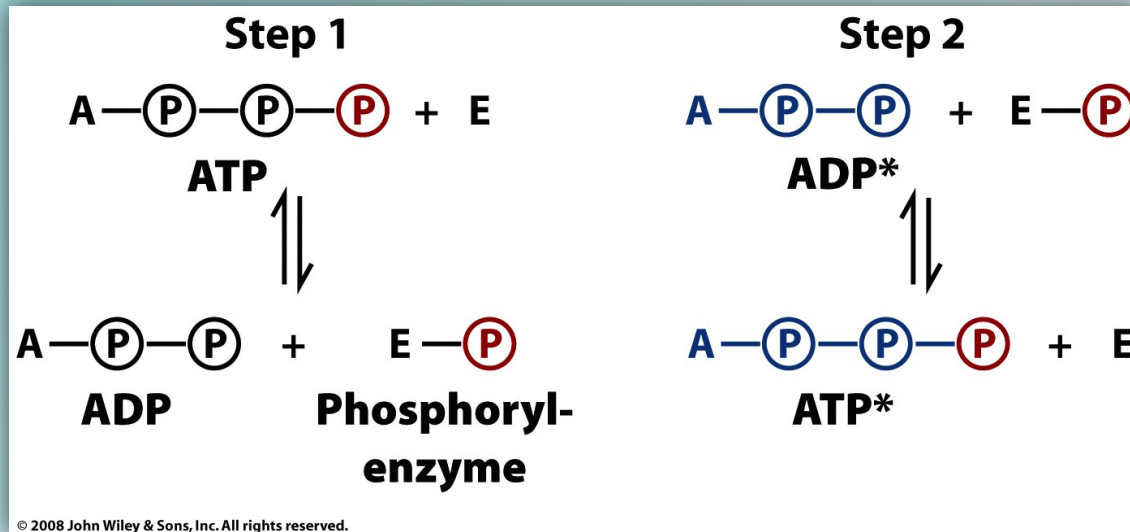
Five reactions produce reducing equivalents (NADH and FADH<sub>2</sub>); there is one substrate-level phosphorylation reaction. The C<sub>2</sub> acetyl fragment of acetyl CoA is converted to 2 molecules of CO<sub>2</sub>.



# Succinyl CoA synthetase mechanism

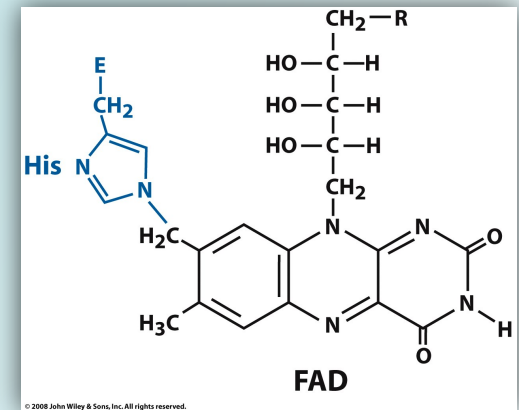
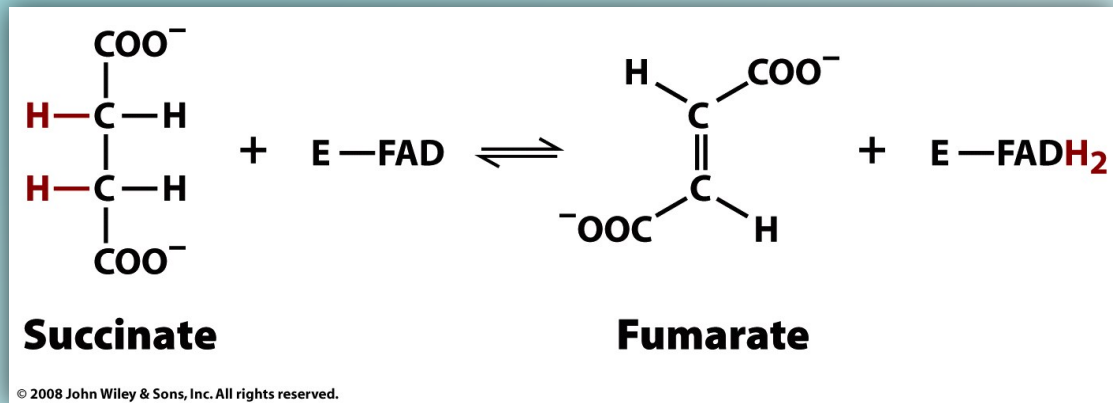


## Succinyl CoA synthetase:

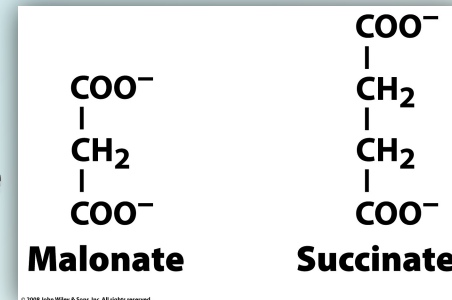


**Evidence for the involvement of a phosphoryl-enzyme intermediate; *the only substrate-level phosphorylation reaction of the TCA cycle***

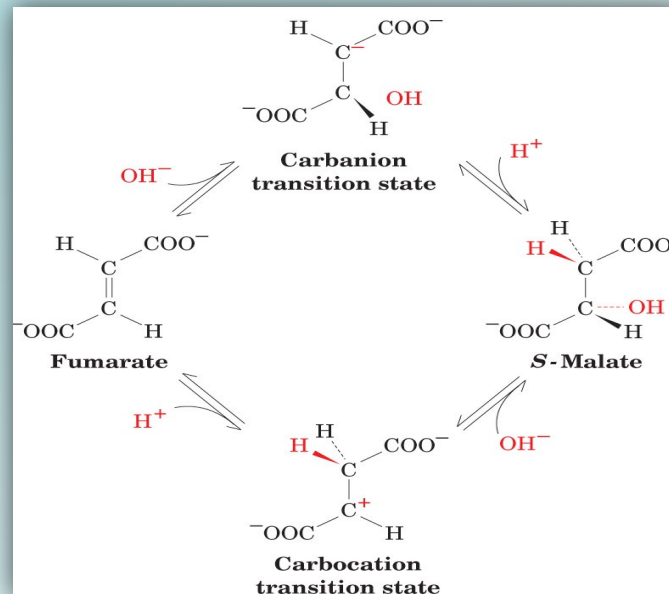
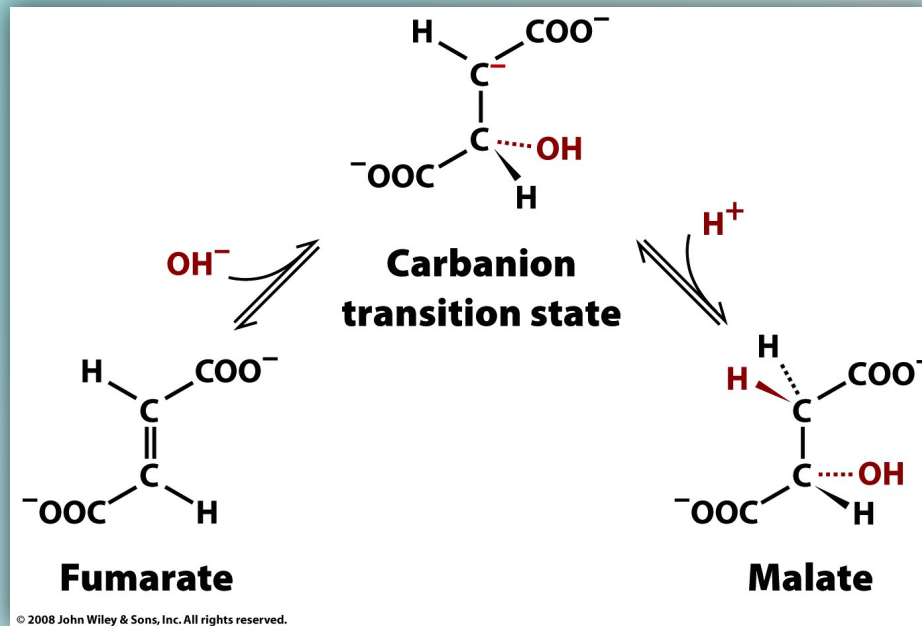
## Succinate dehydrogenase (a flavin-linked dehydrogenase):



Inhibited by malonate and succinate

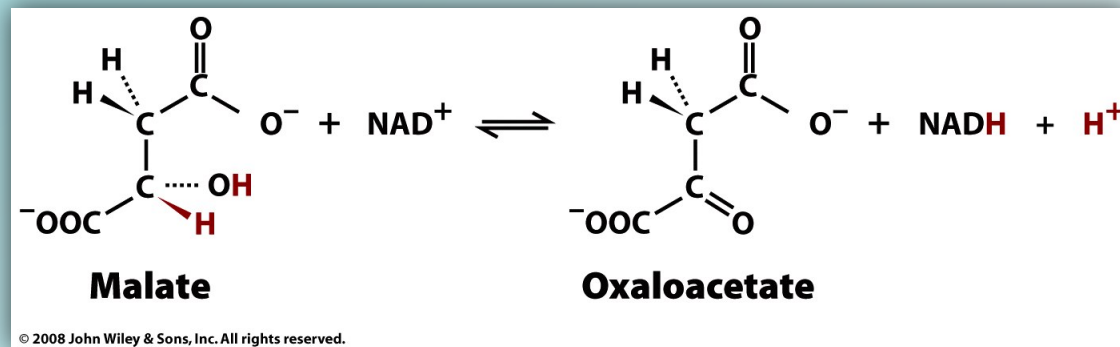


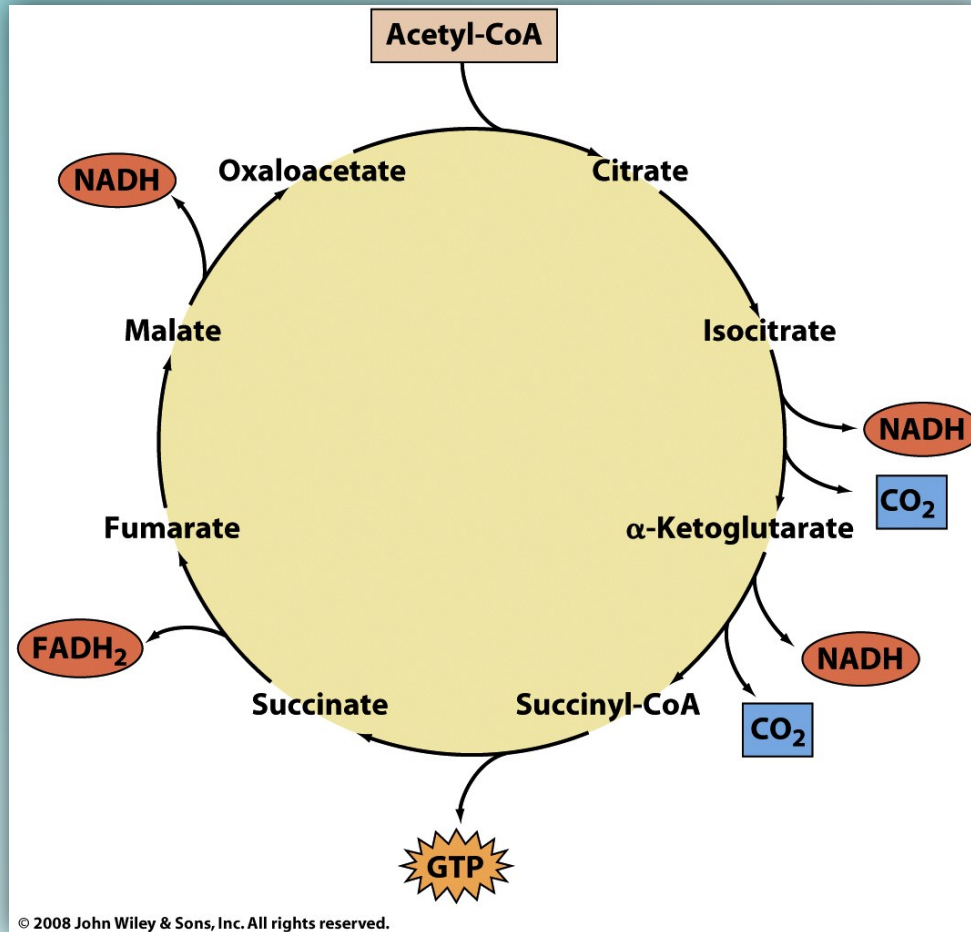
# Fumarase:





## Malate dehydrogenase (NAD<sup>+</sup>-linked dehydrogenase):

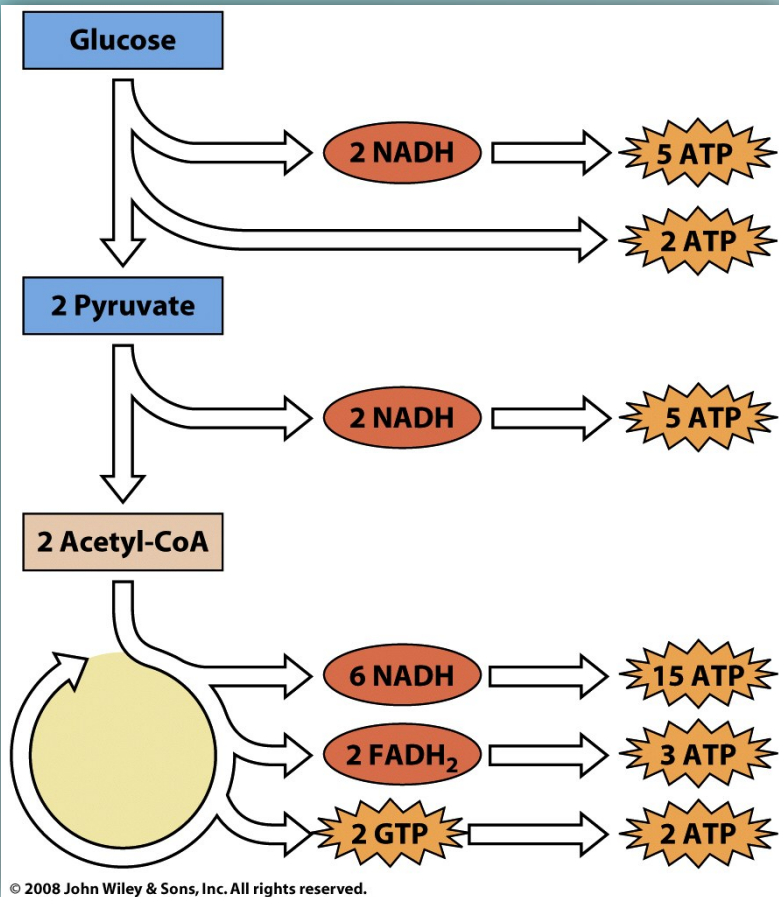




## Products generated by the TCA cycle

For each pyruvate entering the cycle:  
4 NADH  
1 FADH<sub>2</sub>  
1 GTP

All three carbons of pyruvate are lost as CO<sub>2</sub>.



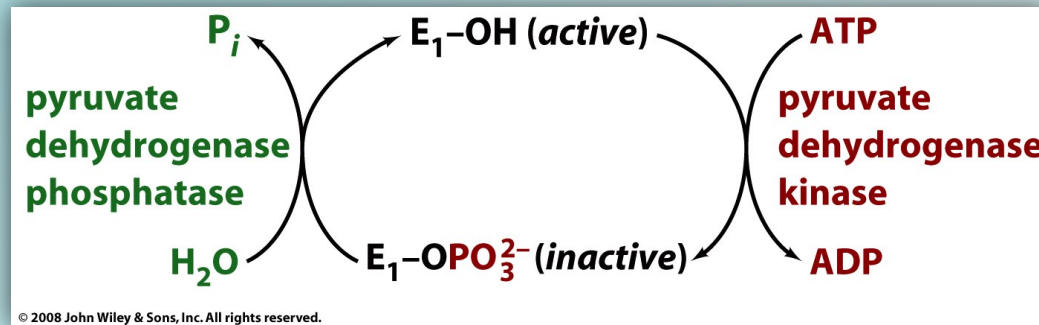
## Summary of ATP production from glycolysis, TCA and electron transport/oxidative phosphorylation

2.5 ATP per NADH and 1.5 ATP per FADH<sub>2</sub> entering ET/OP

32 molecules of ATP are produced per glucose under aerobic conditions.

# Regulation of the TCA cycle

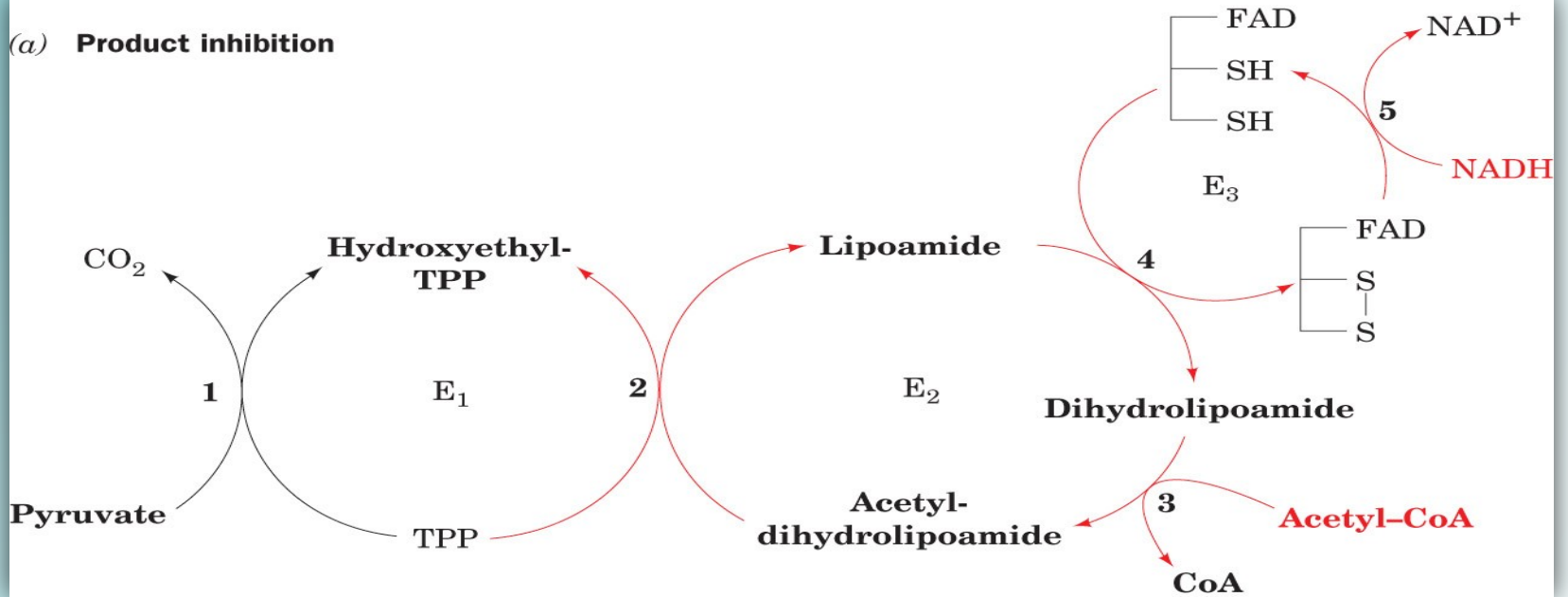
Pyruvate dehydrogenase is regulated by product inhibition (NADH and acetyl CoA) and by covalent modification (phosphorylation of Ser).



PDK is activated by NADH and acetyl CoA and inhibited by pyruvate,  $Ca^{2+}$  and ADP; insulin and  $Ca^{2+}$  activate PDP.

PDK and PDP are part of the PD complex.

(a) **Product inhibition**



(b) **Covalent modification**

*Activators*

$Mg^{2+}$   
 $Ca^{2+}$

pyruvate  
dehydrogenase  
phosphatase

$H_2O$

$P_i$

$E_1-OH$  (*active*)

$E_1-OPO_3^{2-}$  (*inactive*)

$ATP$

pyruvate  
dehydrogenase  
kinase

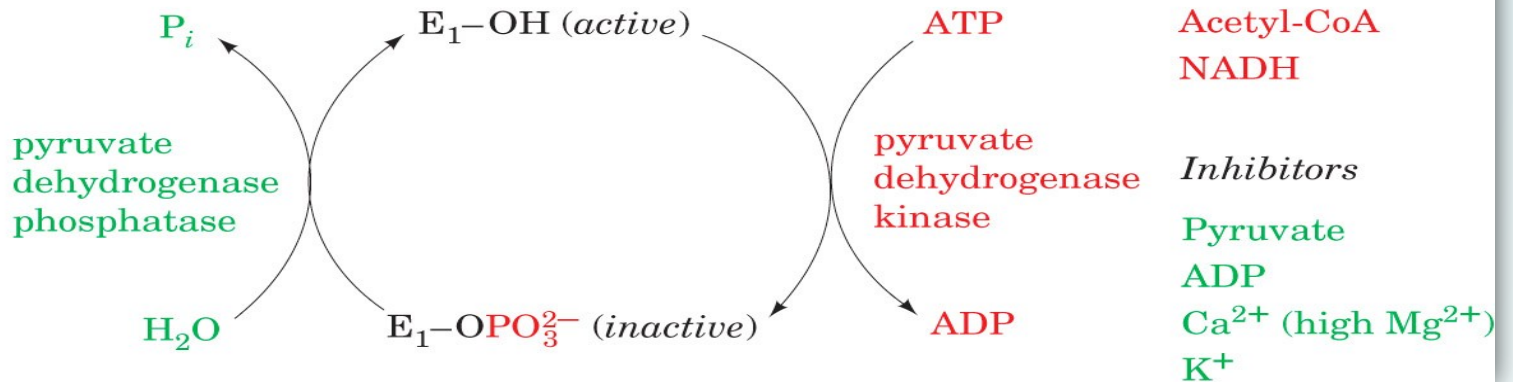
$ADP$

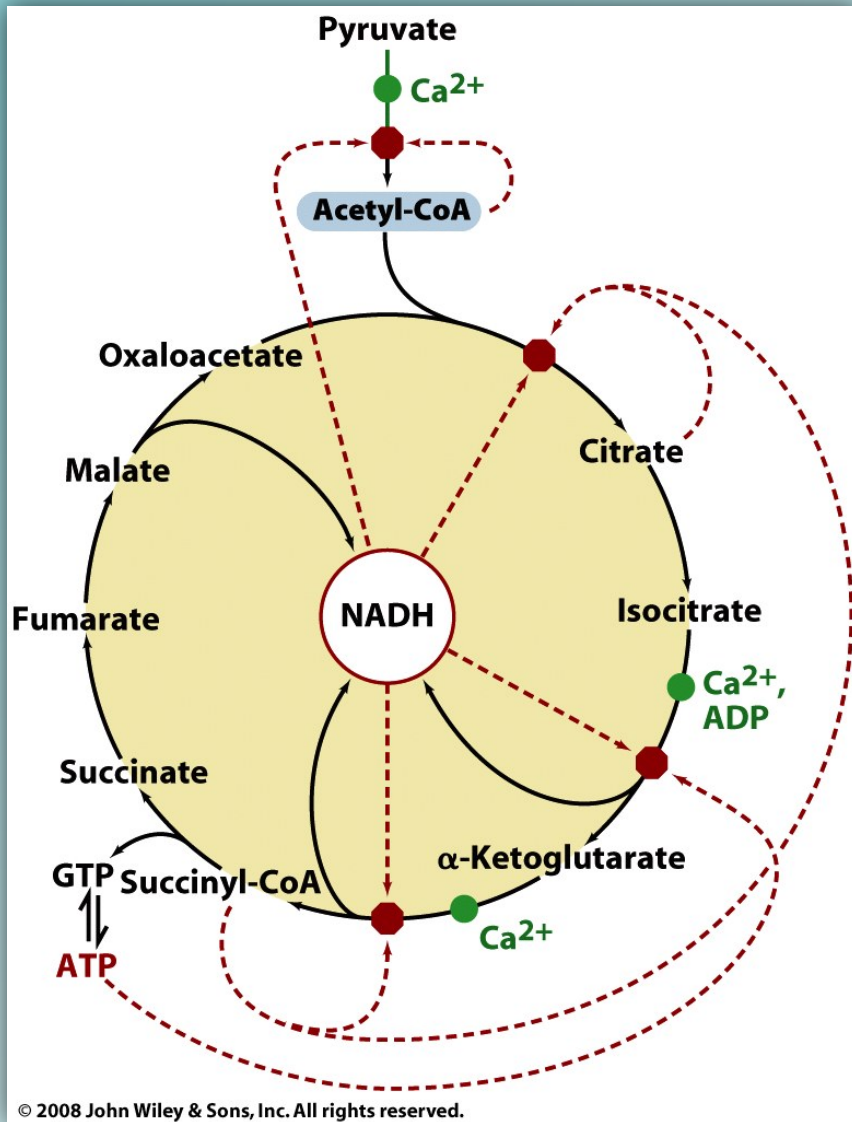
*Activators*

Acetyl-CoA  
NADH

*Inhibitors*

Pyruvate  
ADP  
 $Ca^{2+}$  (high  $Mg^{2+}$ )  
 $K^+$





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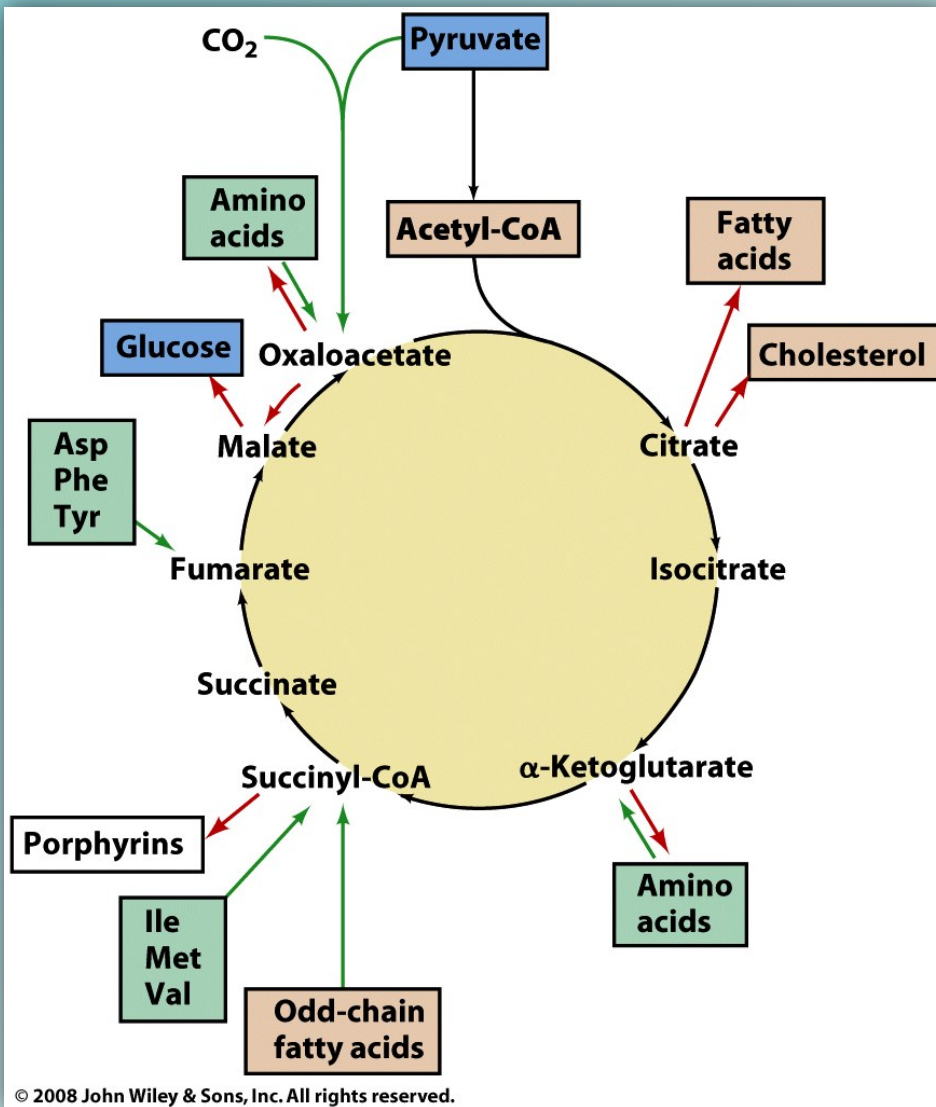
## Regulation of the TCA cycle

Red symbols indicate points of inhibition; green symbols are points of activation.

**Table 17-2** Standard Free Energy Changes ( $\Delta G^\circ$ ) and Physiological Free Energy Changes ( $\Delta G$ ) of Citric Acid Cycle Reactions

Reaction	Enzyme	$\Delta G^\circ$ (kJ · mol <sup>-1</sup> )	$\Delta G$ (kJ · mol <sup>-1</sup> )
1	Citrate synthase	-31.5	Negative
2	Aconitase	~5	~0
3	Isocitrate dehydrogenase	-21	Negative
4	α-Ketoglutarate dehydrogenase	-33	Negative
5	Succinyl-CoA synthetase	-2.1	~0
6	Succinate dehydrogenase	+6	~0
7	Fumarase	-3.4	~0
8	Malate dehydrogenase	+29.7	~0

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A summary of the amphibolic functions of the TCA cycle, showing *catabolic* (red) and *anabolic* (green) reaction paths

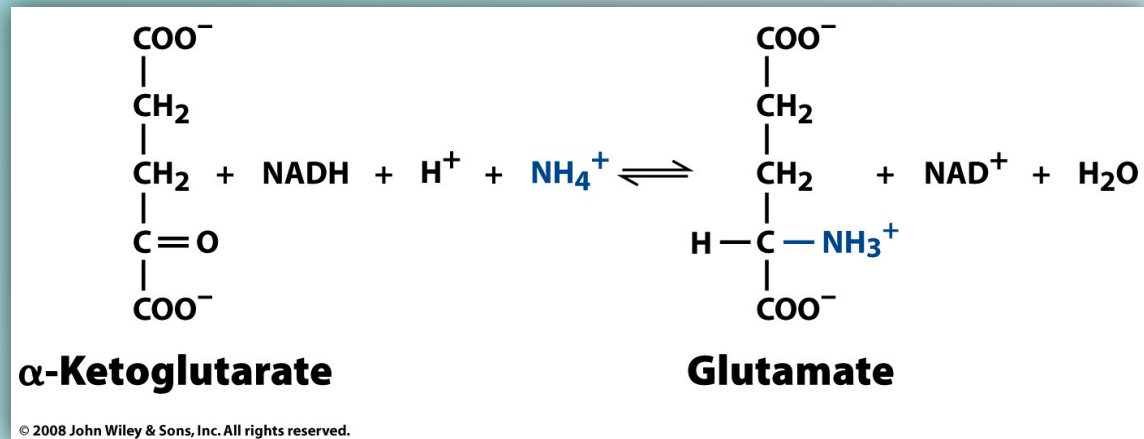
Malate to glucose: gluconeogenesis (*transport problem*)

Citrate to fatty acids: fatty acid biosynthesis (*transport problem*)

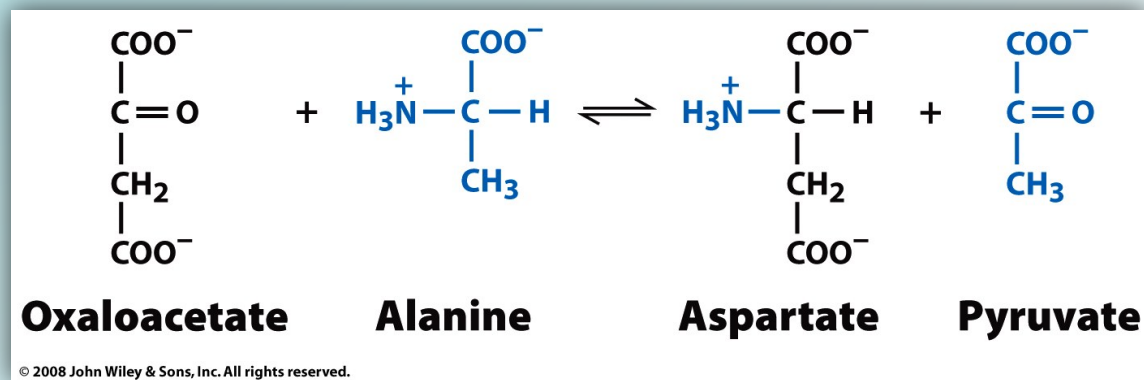
OAA and  $\alpha$ KG to amino acids: amino acid biosynthesis

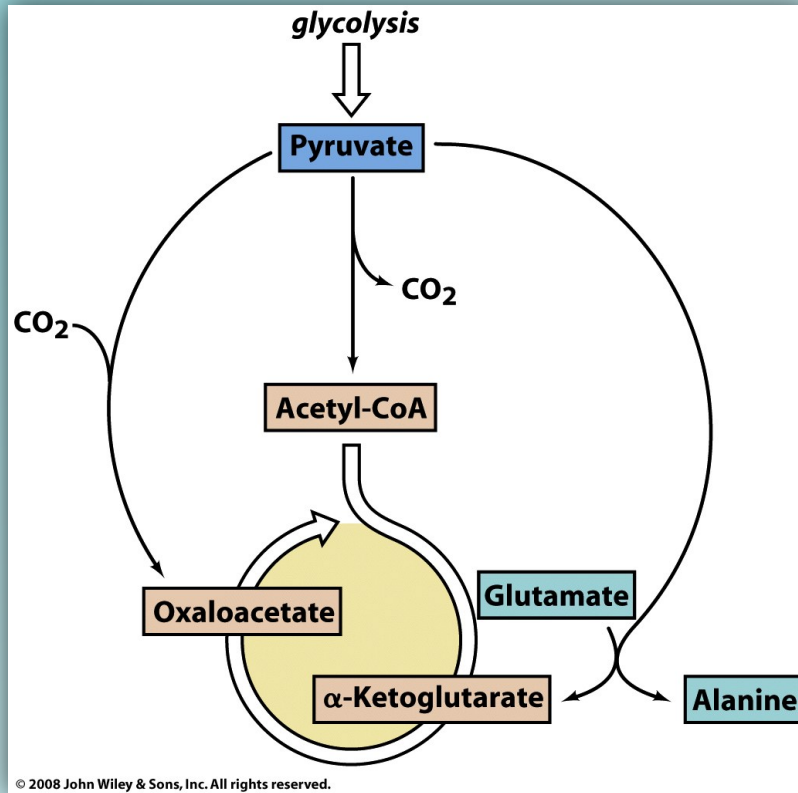


## Conversion of $\alpha$ KG to the amino acid, L-glutamate, by glutamate dehydrogenase (reductive amination)



## Transamination of OAA with alanine (requires PLP)





## Ways to replenish TCA intermediates

Pyruvate carboxylase connects glycolysis with TCA directly; PC requires biotin for activity.

## Glyoxalate pathway: microorganisms and plants

Allows net conversion  
of 2 molecules of acetyl CoA  
to OAA instead of 4  
molecules of CO<sub>2</sub>: valuable  
for gluconeogenesis

Organisms that lack the  
glyoxalate cycle cannot  
undertake the net  
synthesis of glucose  
from acetyl CoA; *i.e.*,  
they cannot convert fats  
to carbohydrates.

