

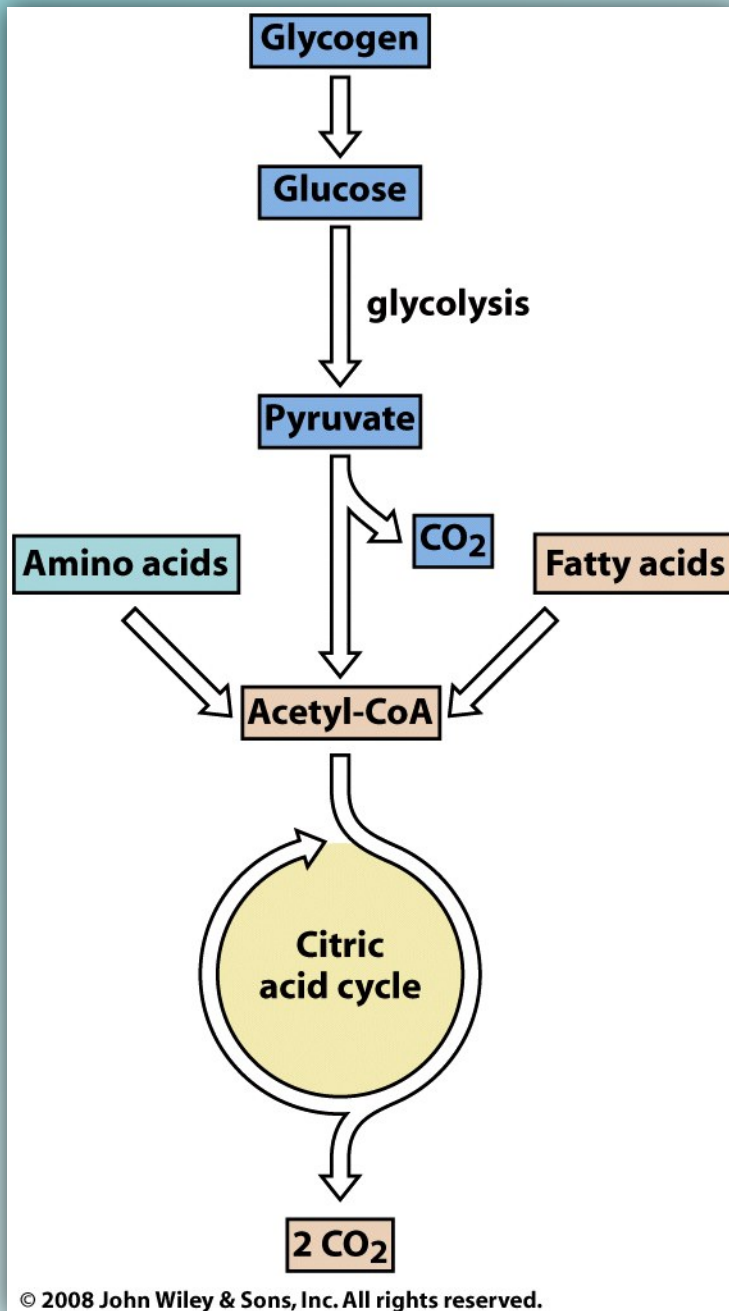
The Tricarboxylic Acid Cycle

(Krebs cycle; citric acid cycle)

CHEM 420 – Principles of Biochemistry
Instructor – Anthony S. Serianni

Chapter 21: Voet/Voet, *Biochemistry*, 2011
Fall 2015

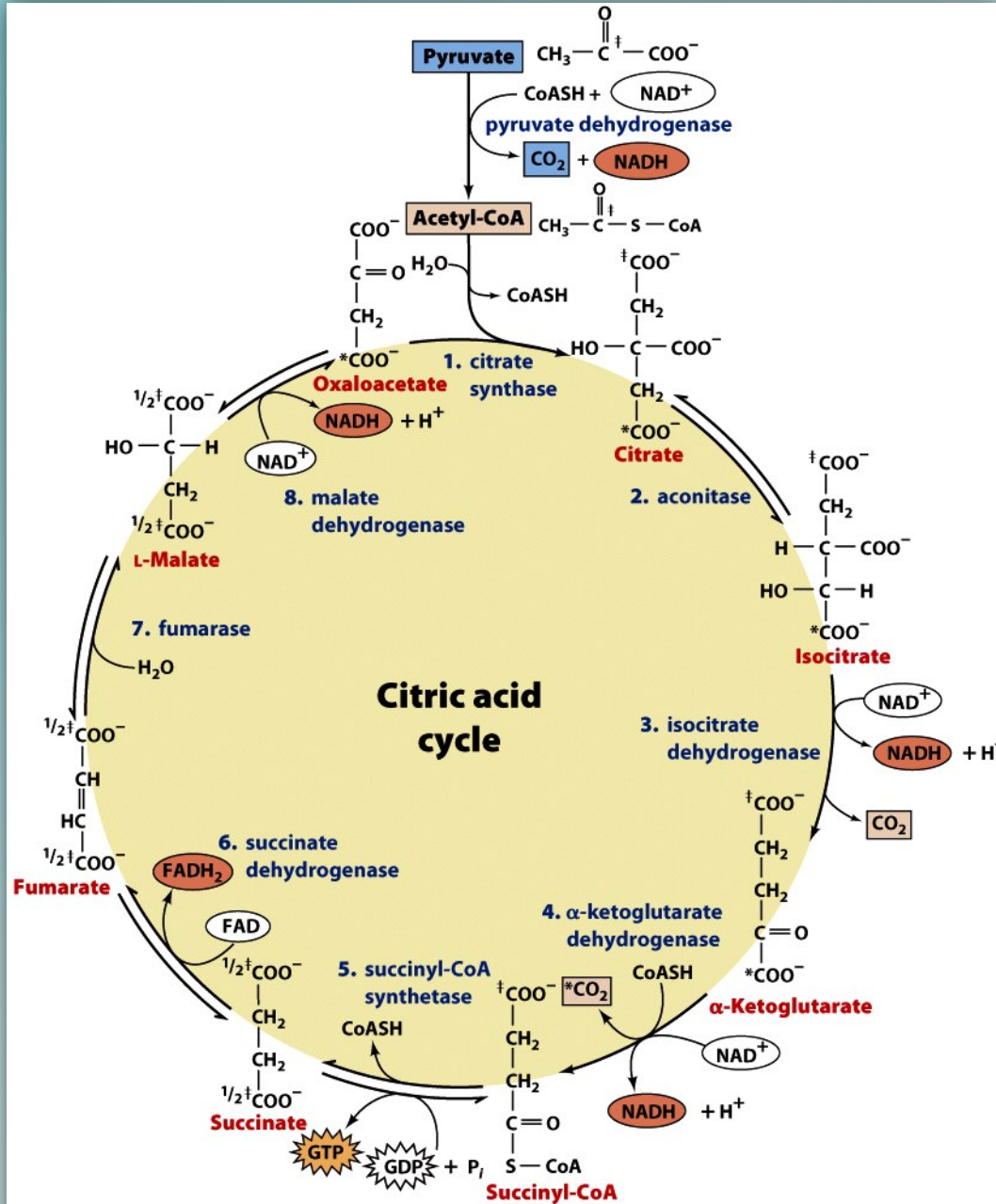
November 20 & 23



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 (NADH and FADH₂); there is one substrate-level phosphorylation reaction. The C₂ acetyl fragment of acetyl CoA is converted to 2 molecules of CO₂.

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

Reaction	Enzyme	$\Delta G^{\circ'}$ (kJ · mol⁻¹)	ΔG (kJ · mol⁻¹)
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

Chemical structure of acetyl-CoA

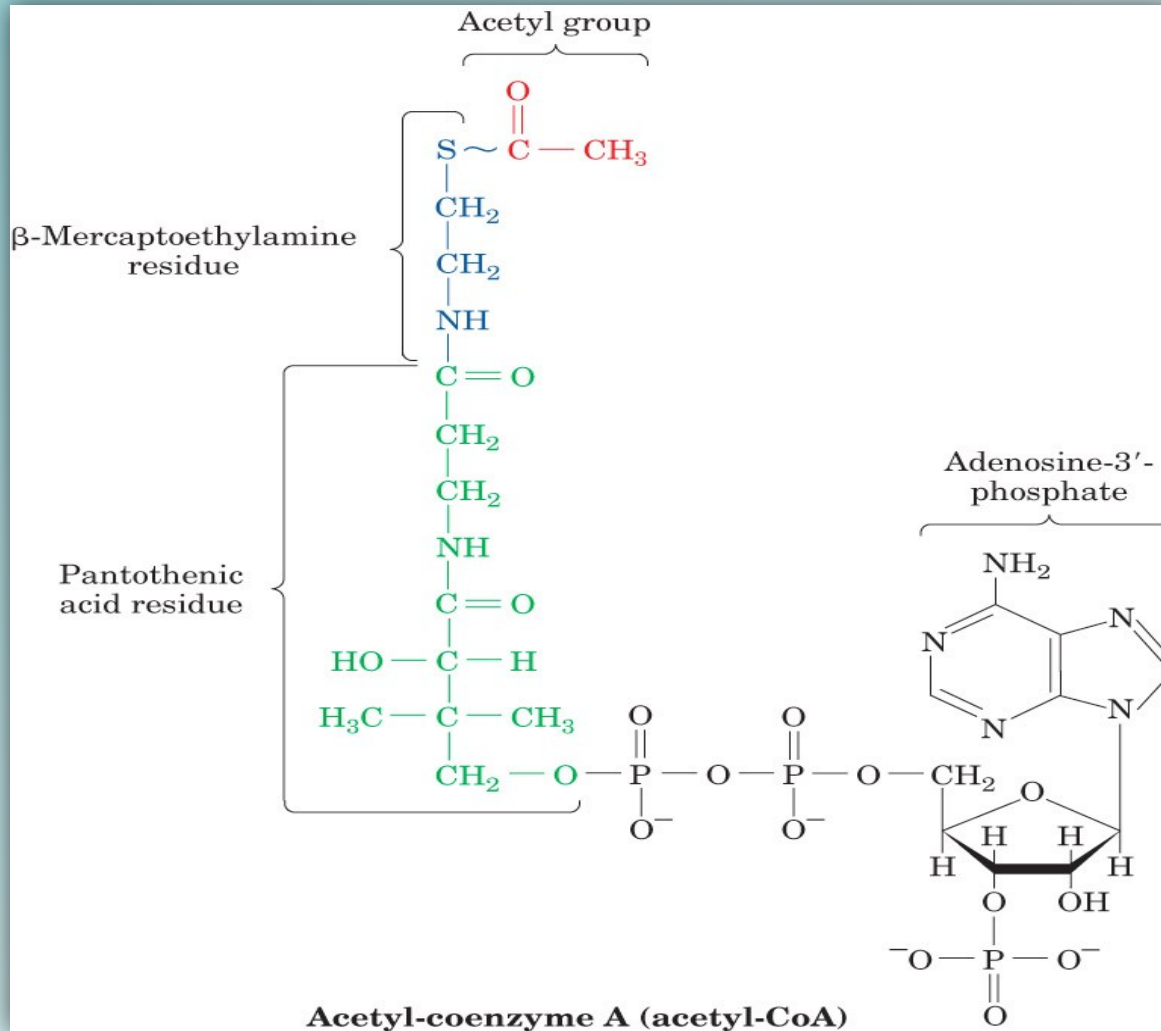
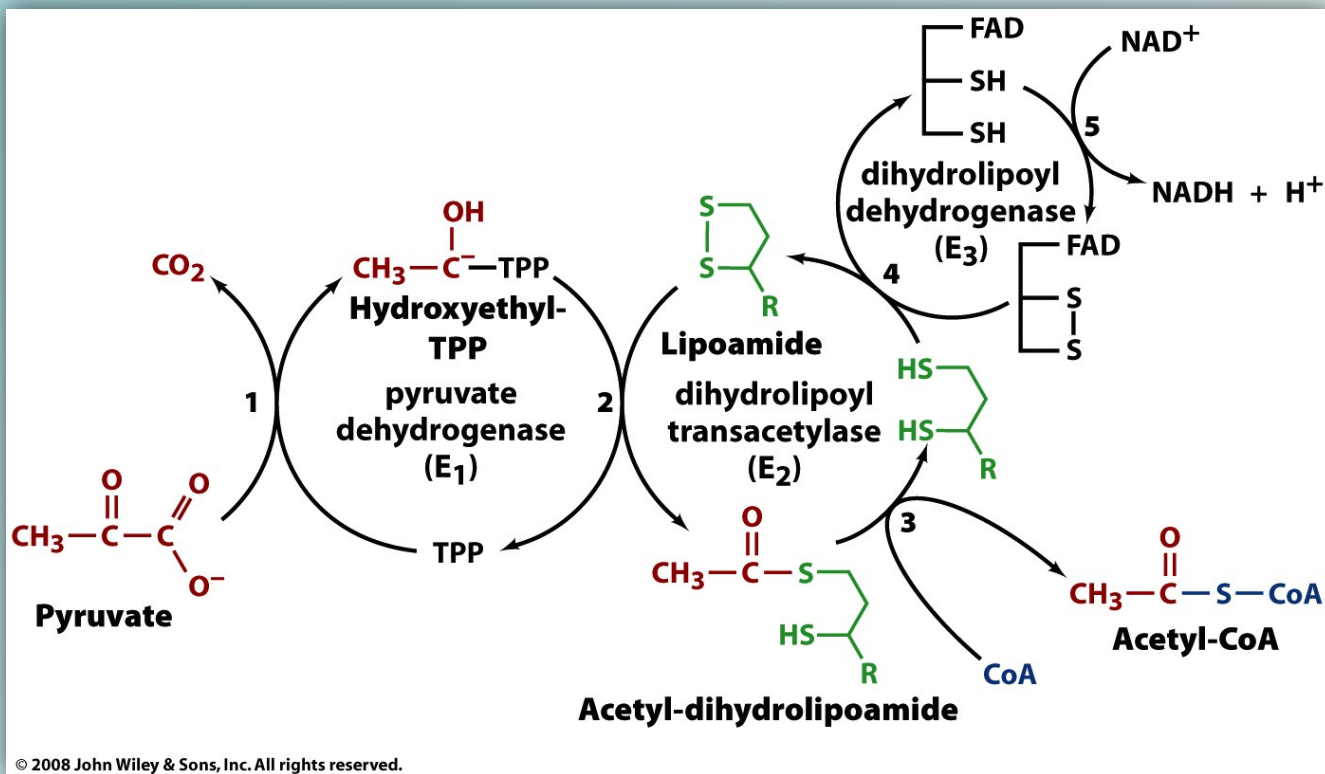
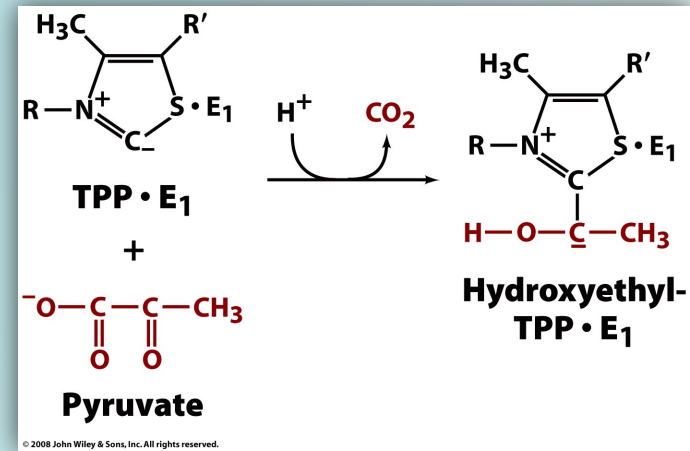
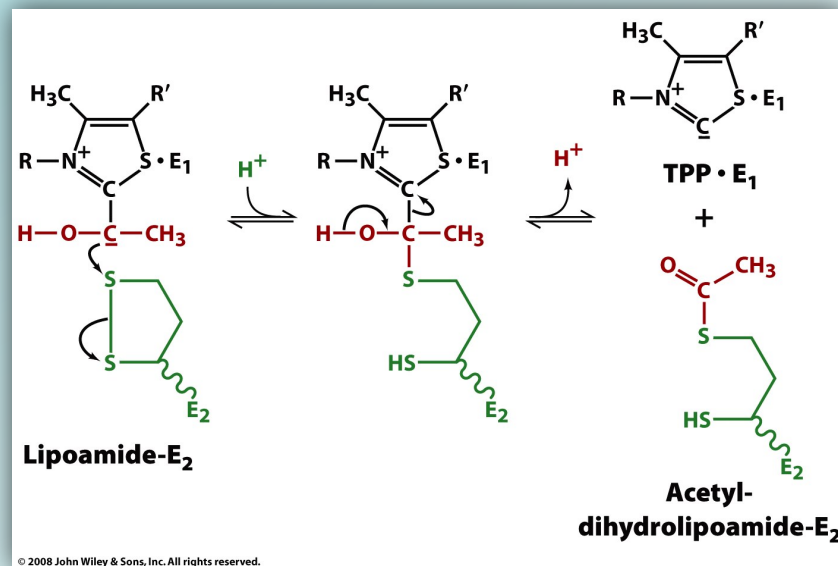
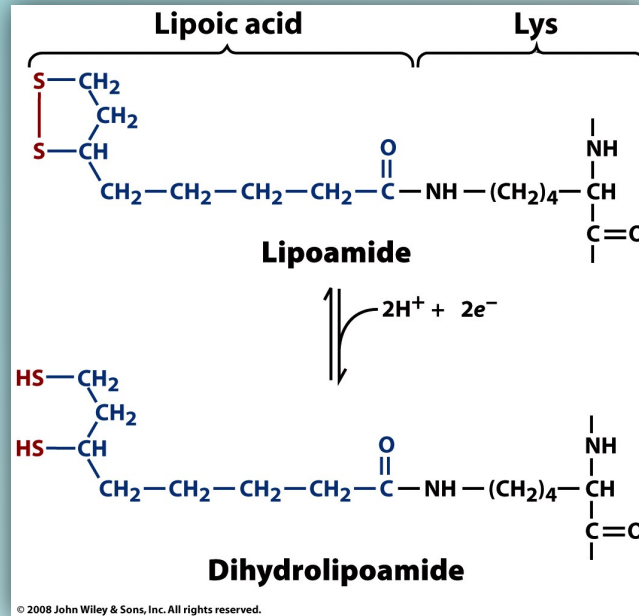


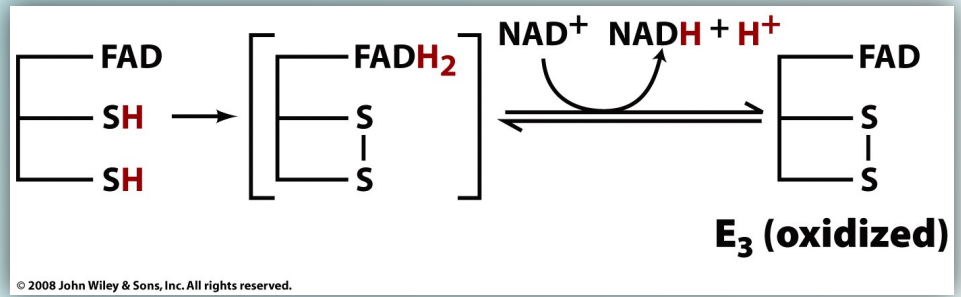
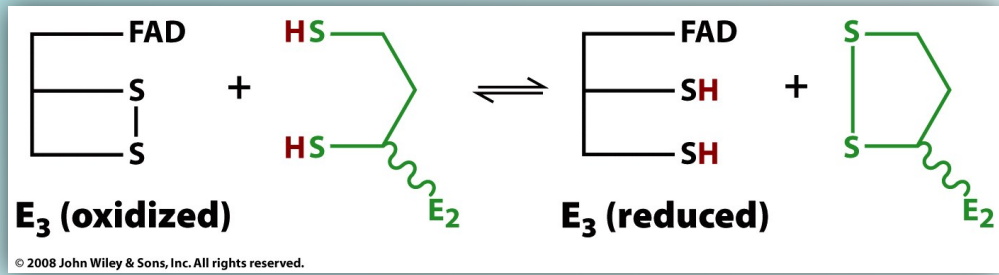
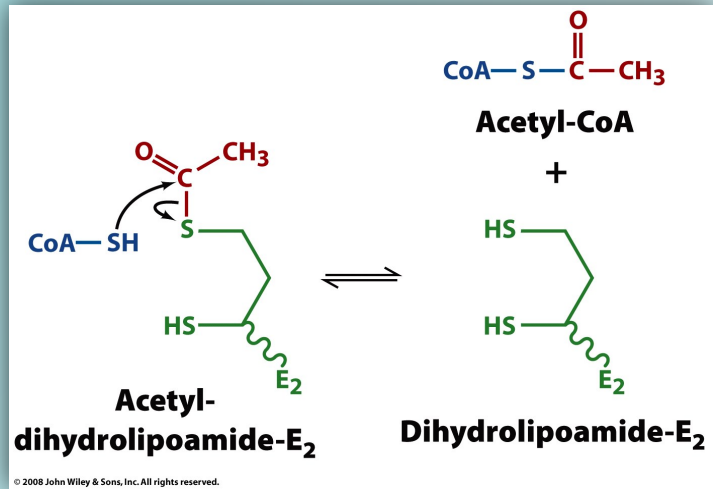
Table 17-1**The Coenzymes and Prosthetic Groups of Pyruvate Dehydrogenase**

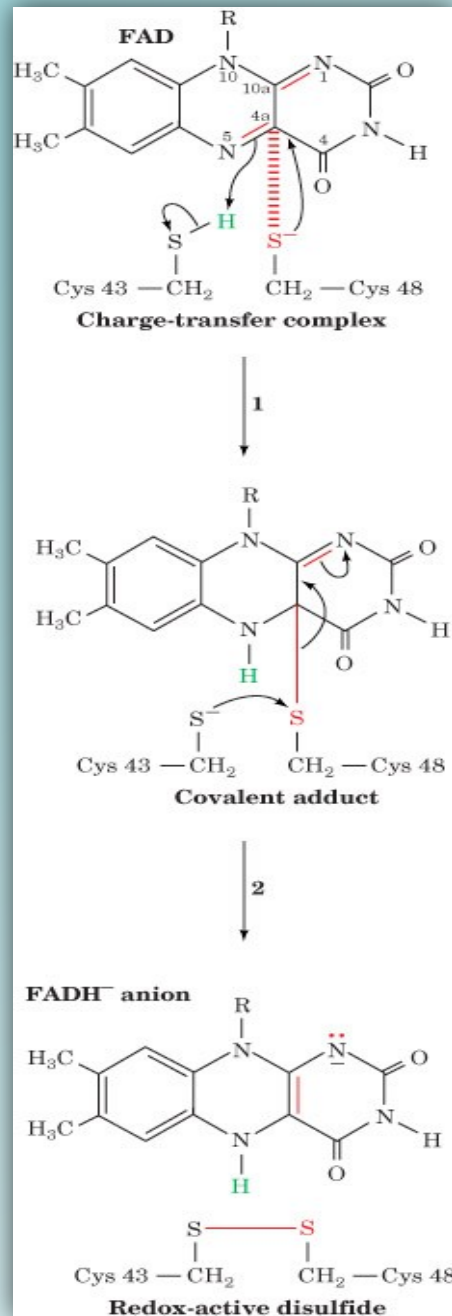
Cofactor	Location	Function
Thiamine pyrophosphate (TPP)	Bound to E₁	Decarboxylates pyruvate yielding a hydroxyethyl-TPP carbanion
Lipoic acid	Covalently linked to a Lys on E₂ (lipoamide)	Accepts the hydroxyethyl carbanion from TPP as an acetyl group
Coenzyme A (CoA)	Substrate for E₂	Accepts the acetyl group from lipoamide
Flavin adenine dinucleotide (FAD)	Bound to E₃	Reduced by lipoamide
Nicotinamide adenine dinucleotide (NAD⁺)	Substrate for E₃	Reduced by FADH₂

The pyruvate dehydrogenase complex: oxidative decarboxylation of an α -ketoacid (pyruvate)

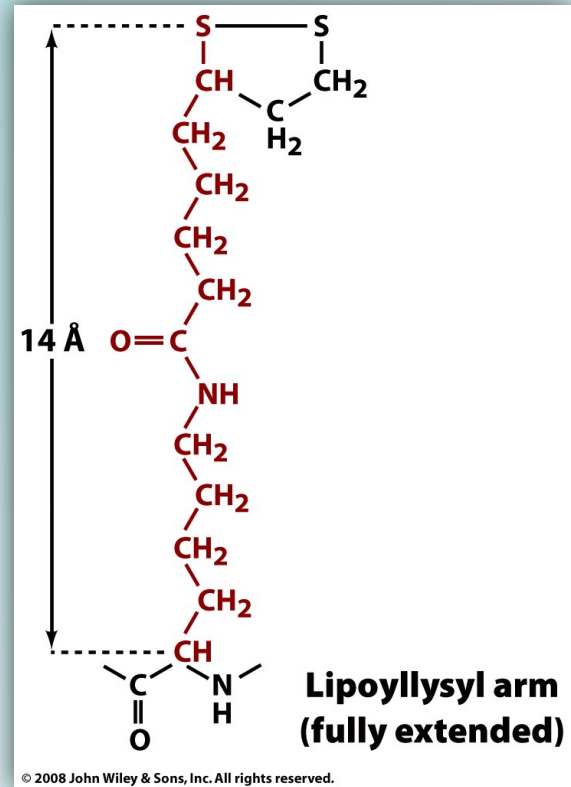
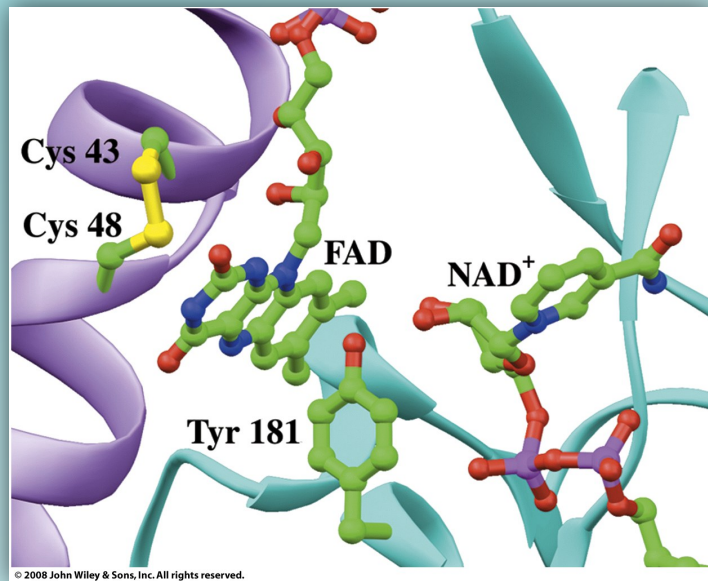




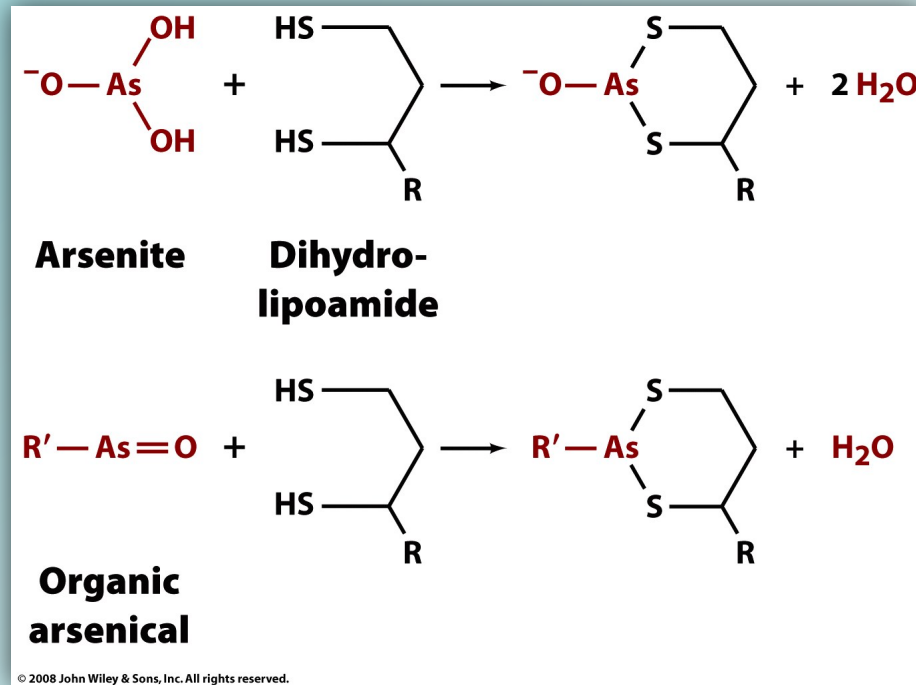




Dihydrolipoyl dehydrogenase mechanism

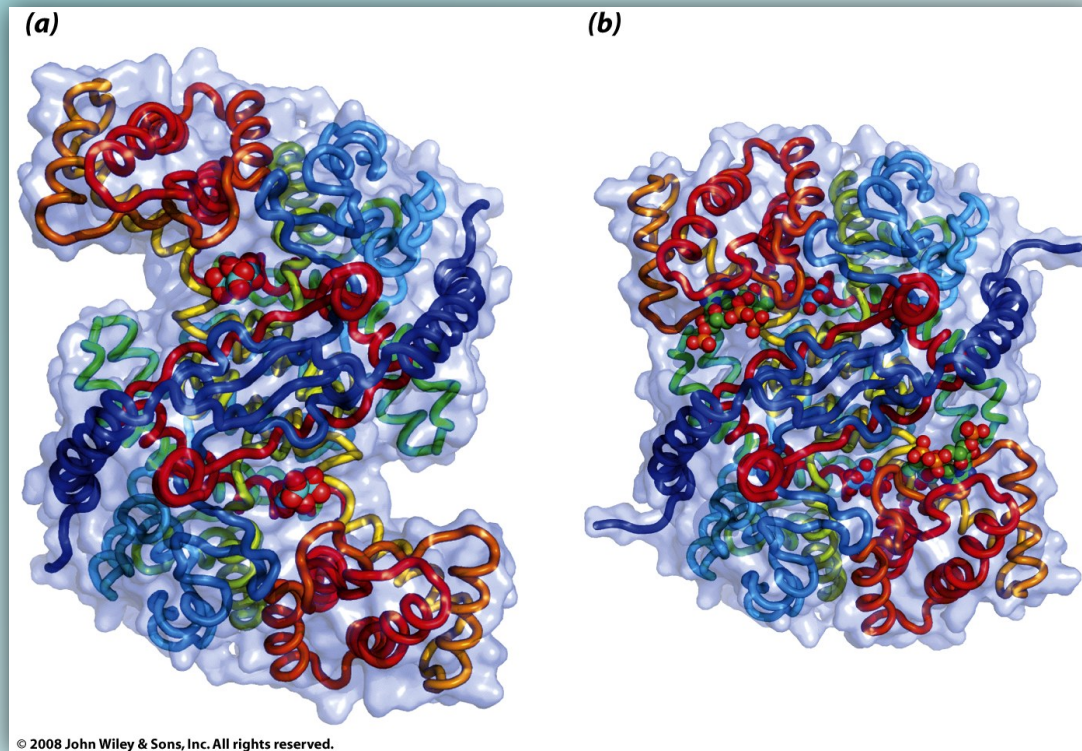


Molecular basis of arsenic poisoning

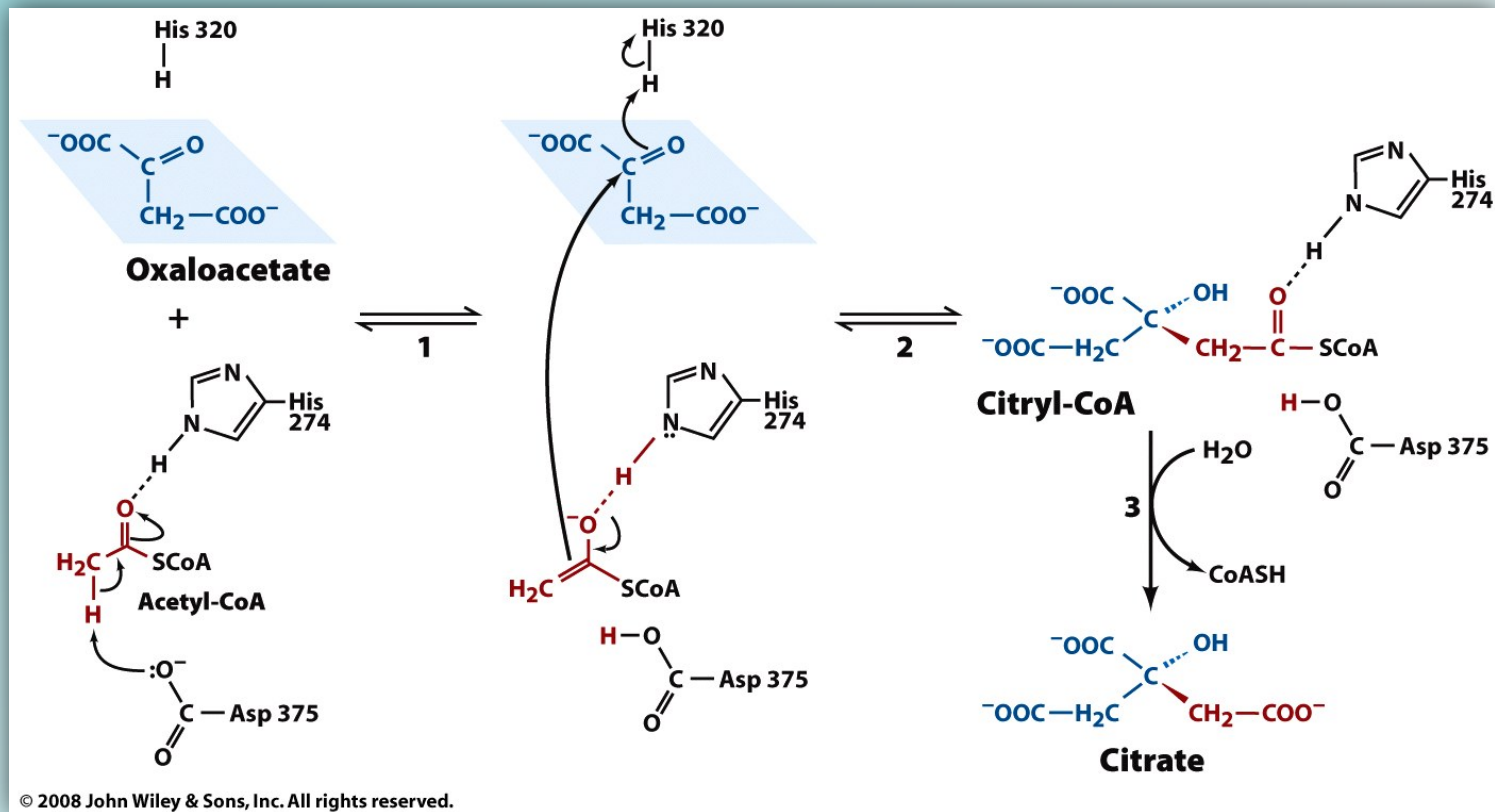


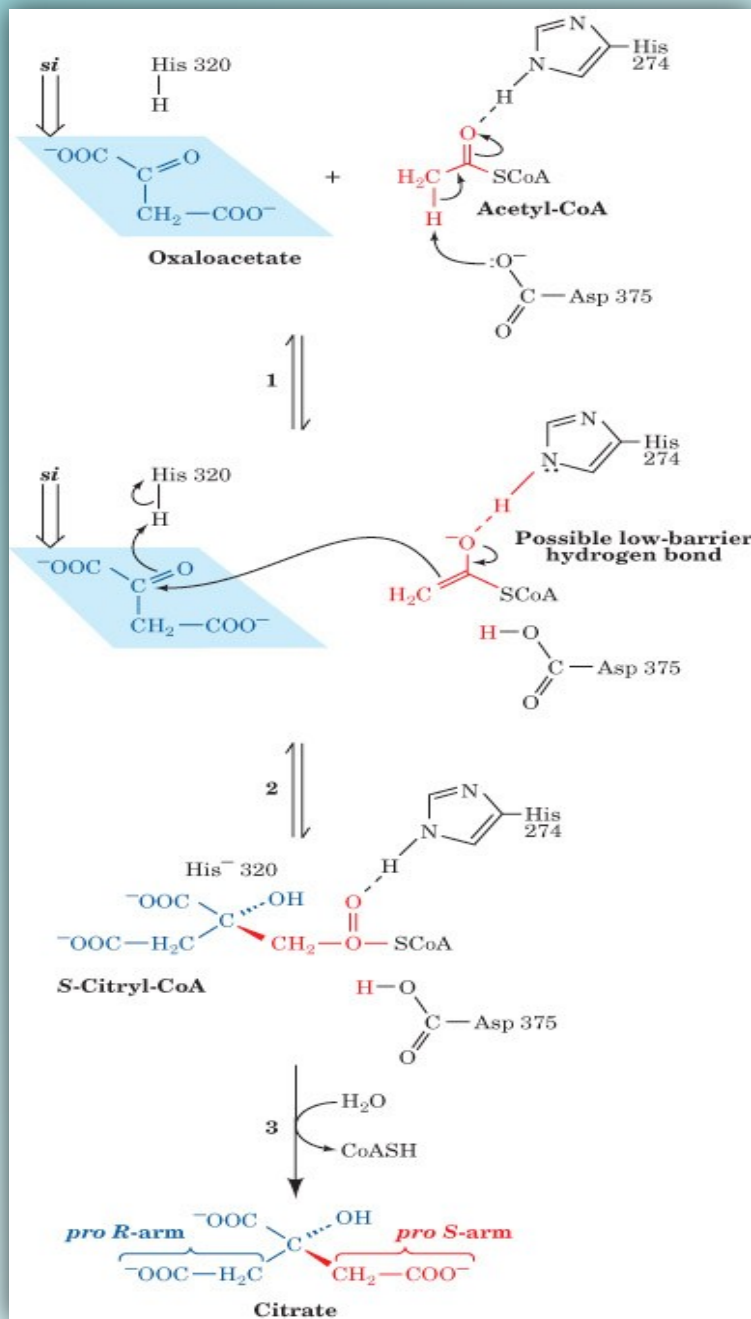
The pyruvate and α -ketoglutarate dehydrogenase complexes are susceptible to inactivation by arsenic.

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



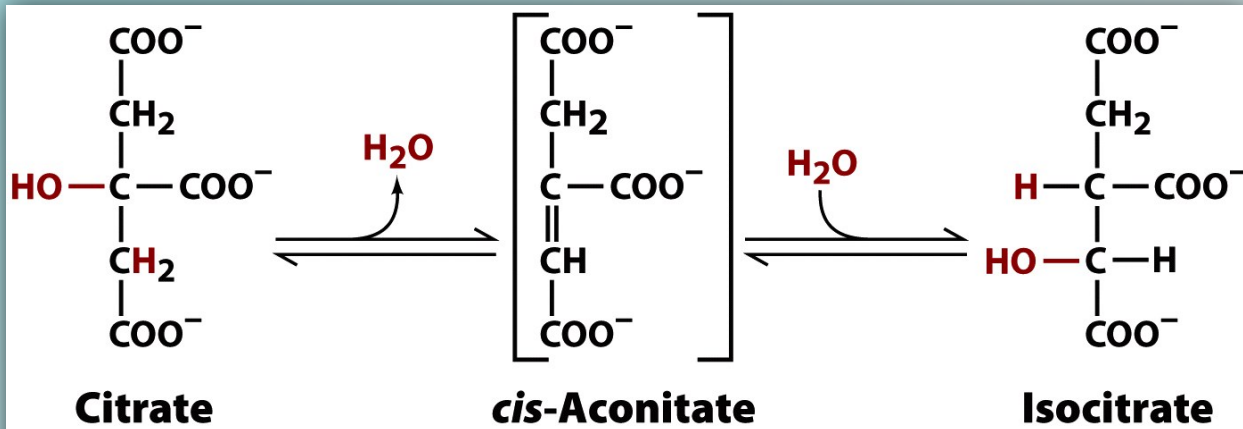
Mechanism of the citrate synthase reaction



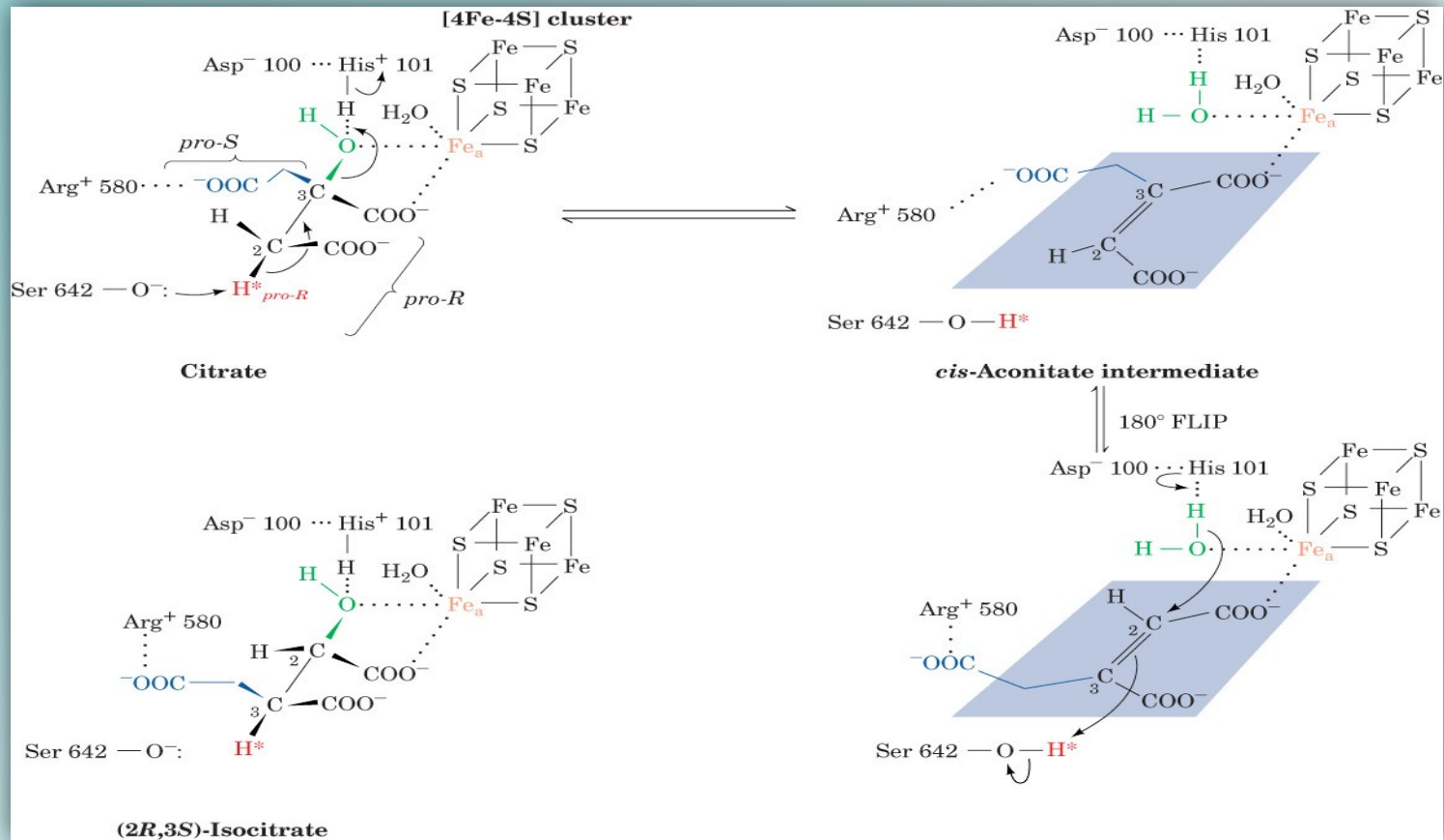


Citrate synthase: reaction stereochemistry

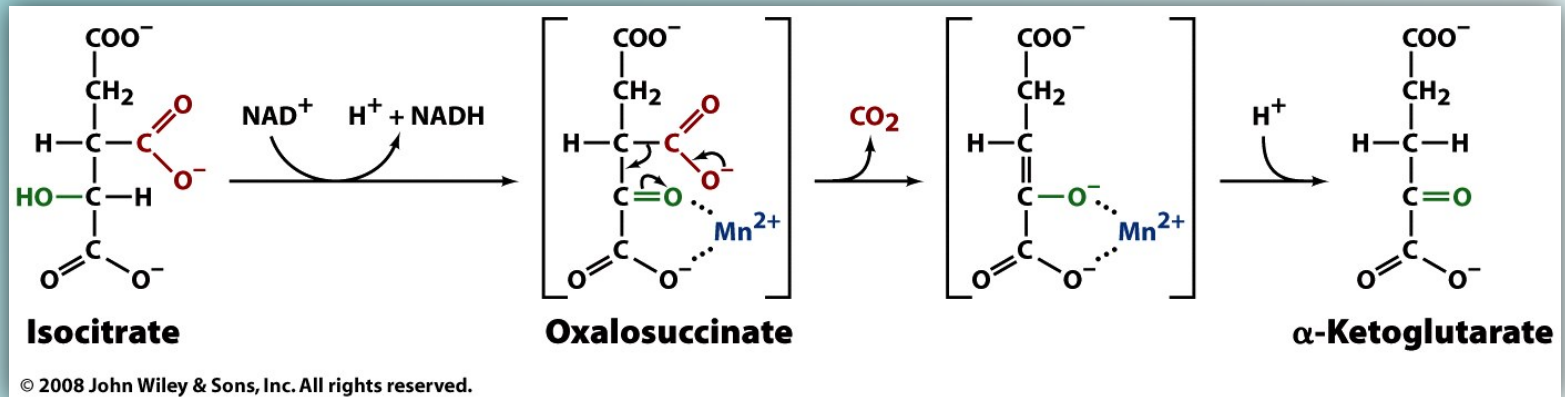
Aconitase



Aconitase reaction stereochemistry

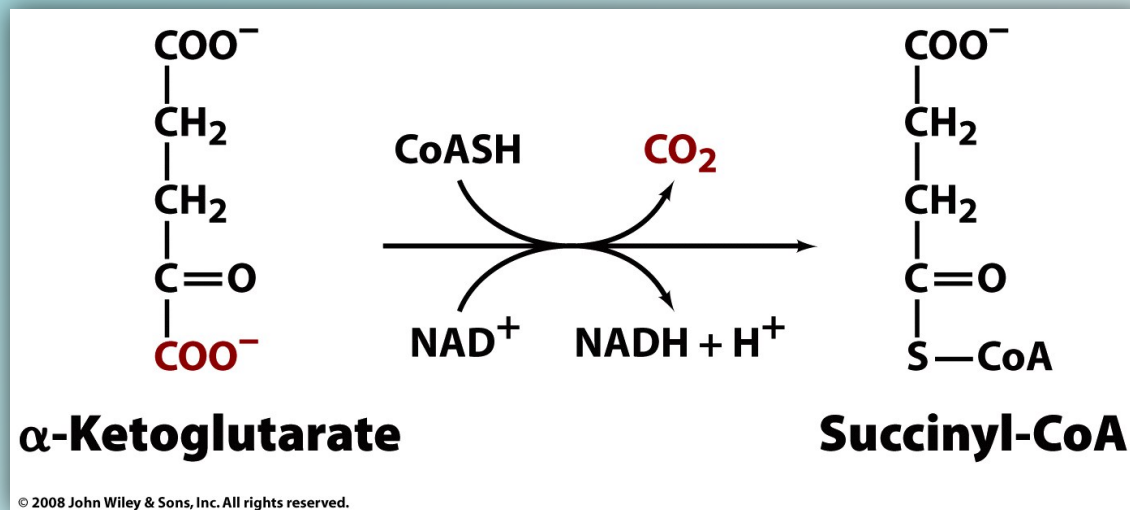


Isocitrate dehydrogenase (NAD⁺-linked dehydrogenase):



Involves oxidative decarboxylation of a β -ketoacid intermediate

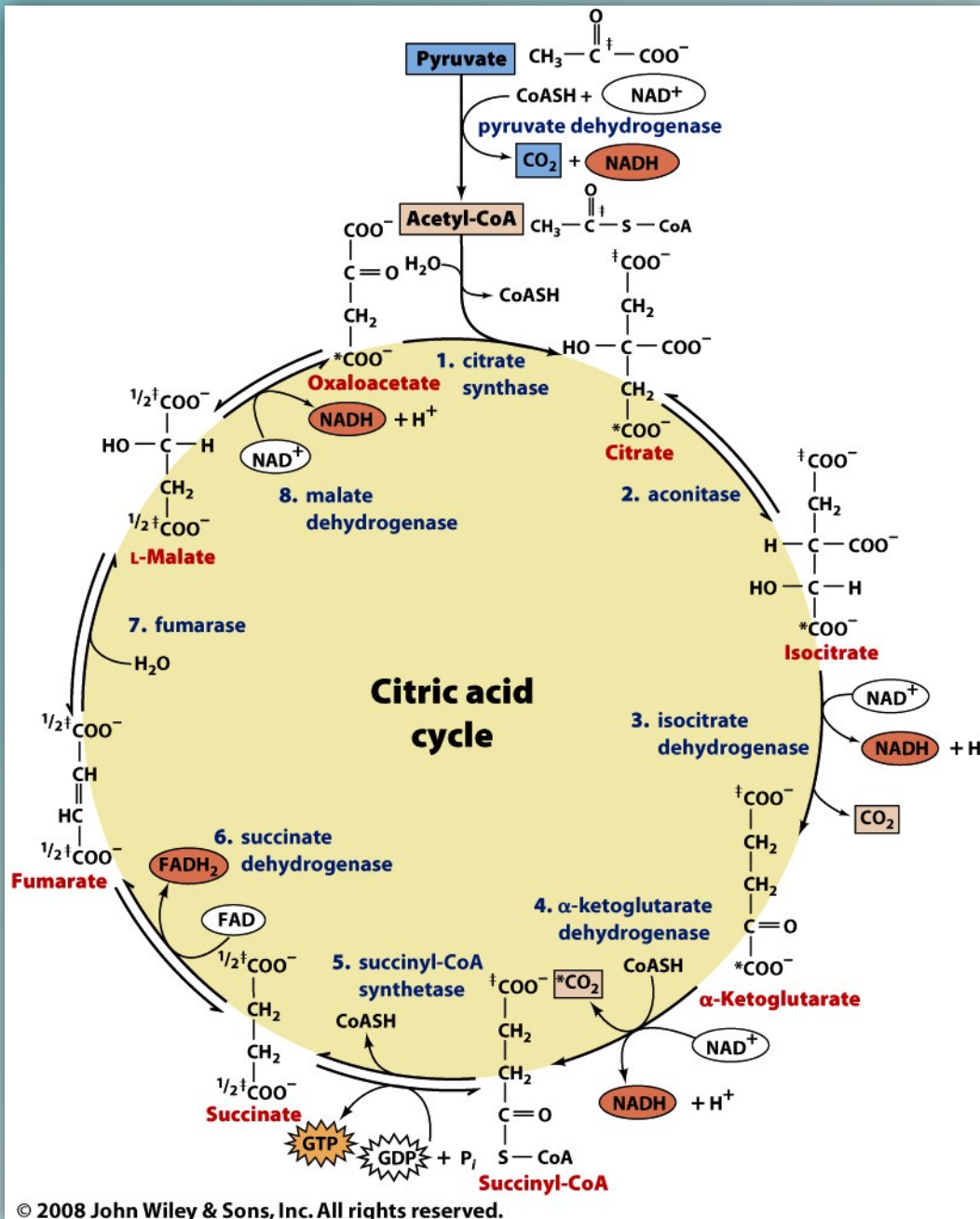
α -Ketoglutarate dehydrogenase (NAD⁺-linked dehydrogenase):



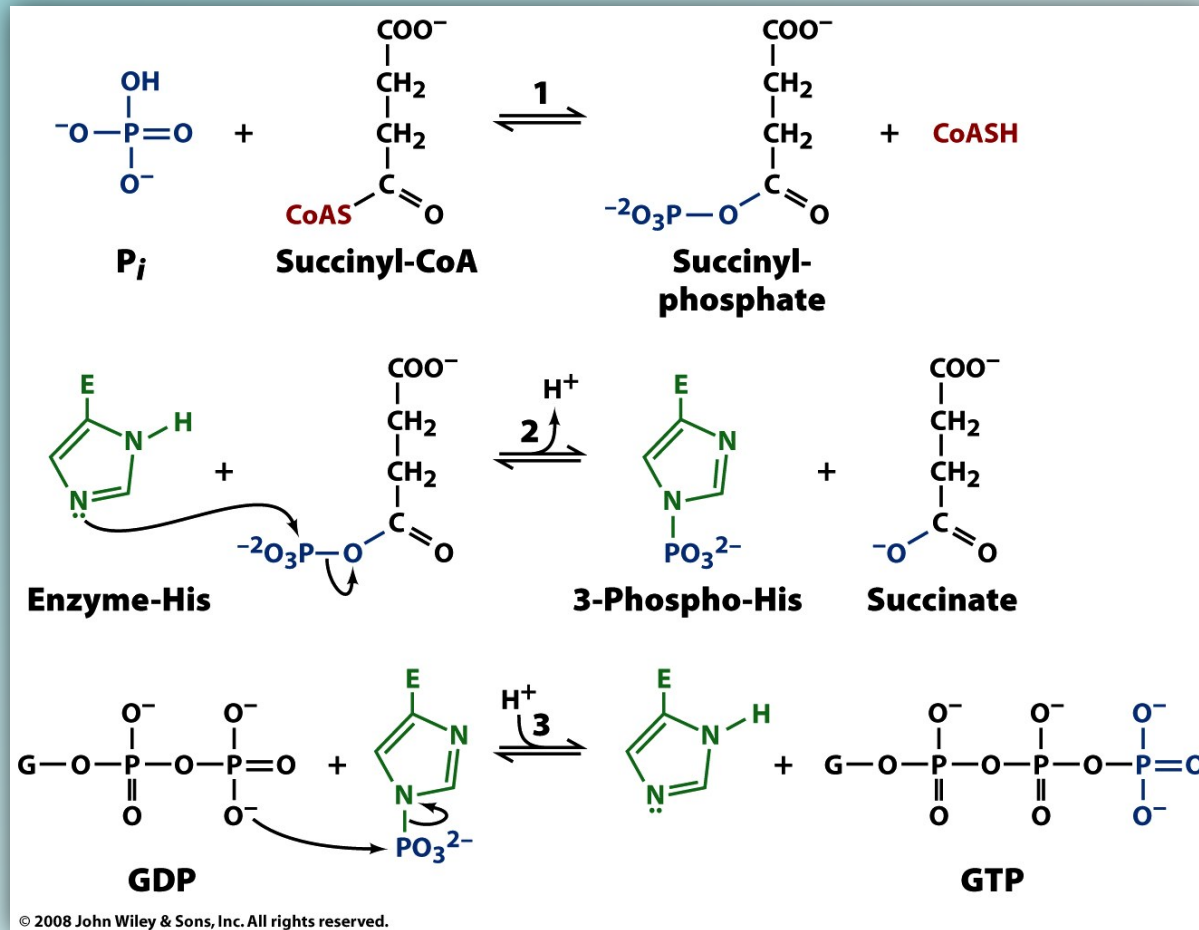
(mechanism is similar to that of pyruvate dehydrogenase;
oxidative decarboxylation of an α -ketoacid)

Summary of the nine TCA cycle reactions

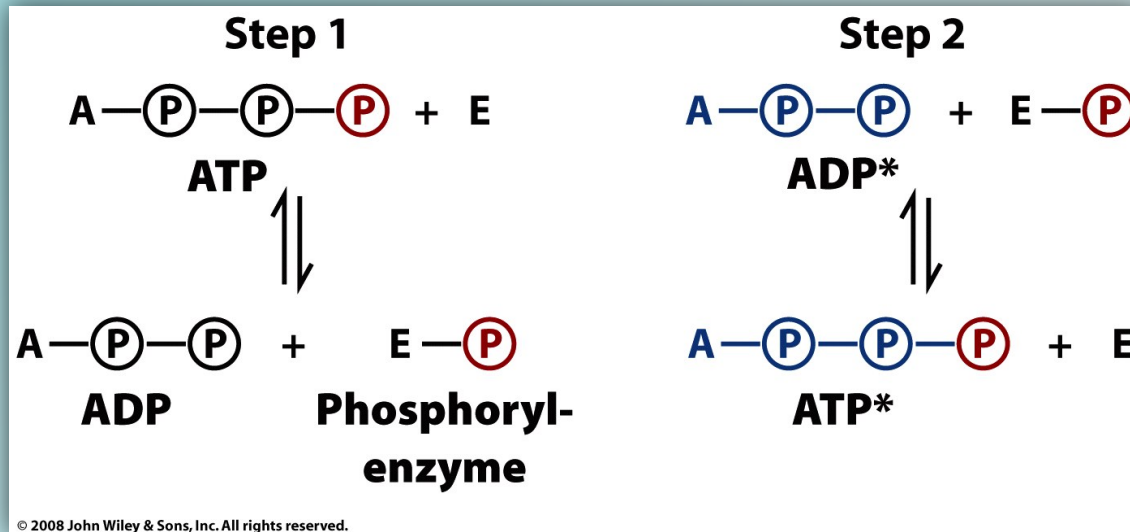
Five reactions produce reducing equivalents (NADH and FADH₂); there is one substrate-level phosphorylation reaction. The C₂ acetyl fragment of acetyl CoA is converted to 2 molecules of CO₂.



Succinyl CoA synthetase mechanism

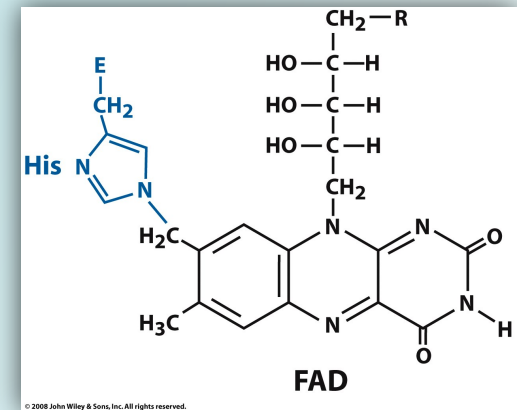
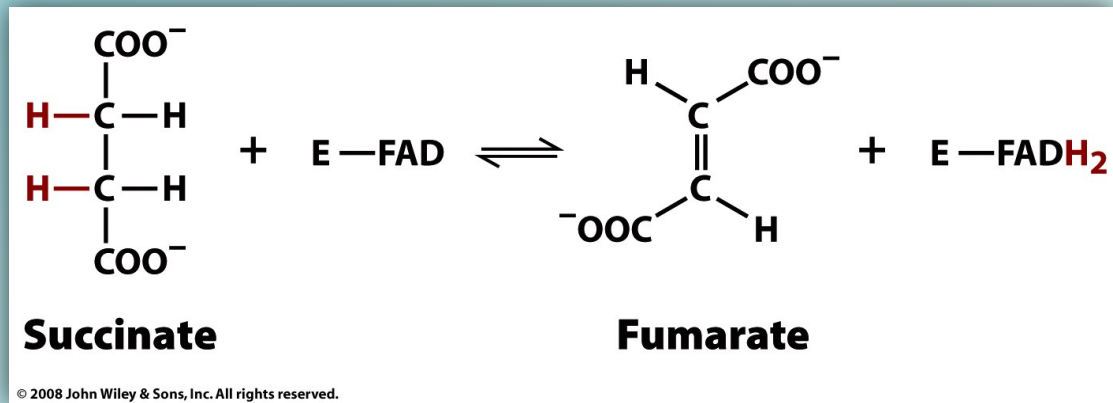


Succinyl CoA synthetase:

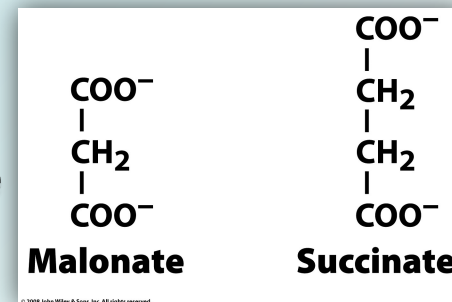


Evidence for the involvement of a phosphoryl-enzyme intermediate; *the only substrate-level phosphorylation reaction of the TCA cycle*

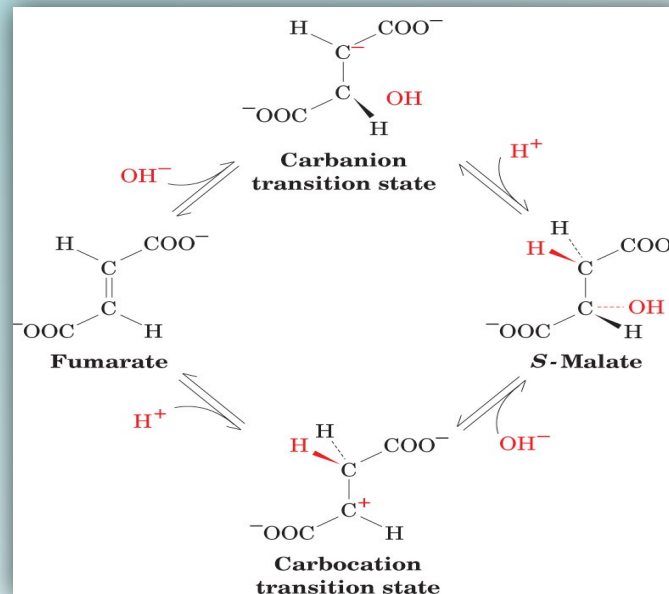
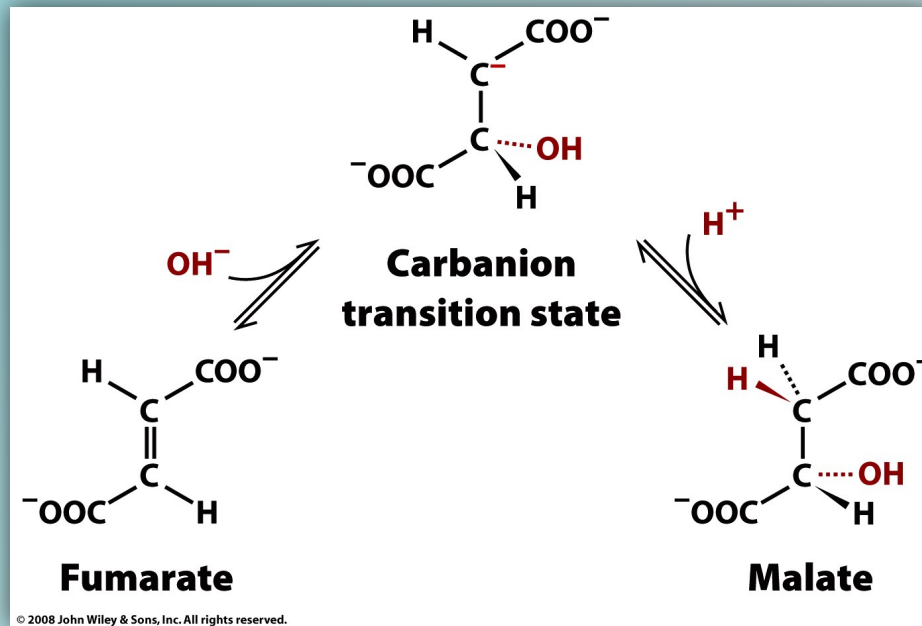
Succinate dehydrogenase (a flavin-linked dehydrogenase):



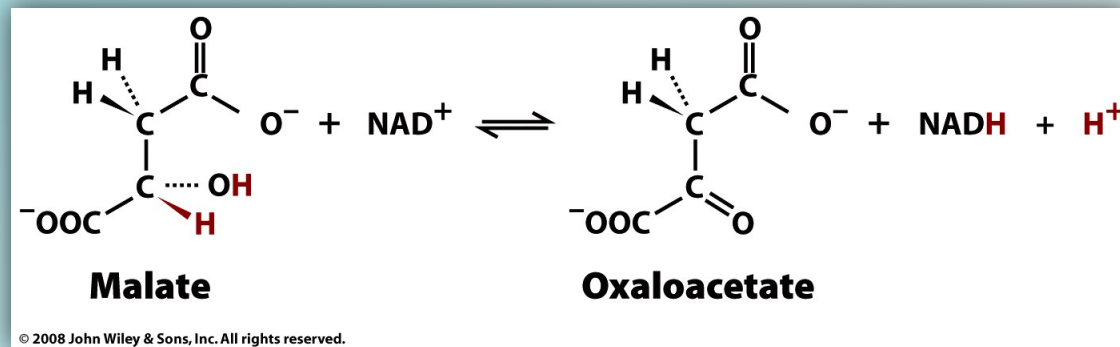
Inhibited by malonate and succinate

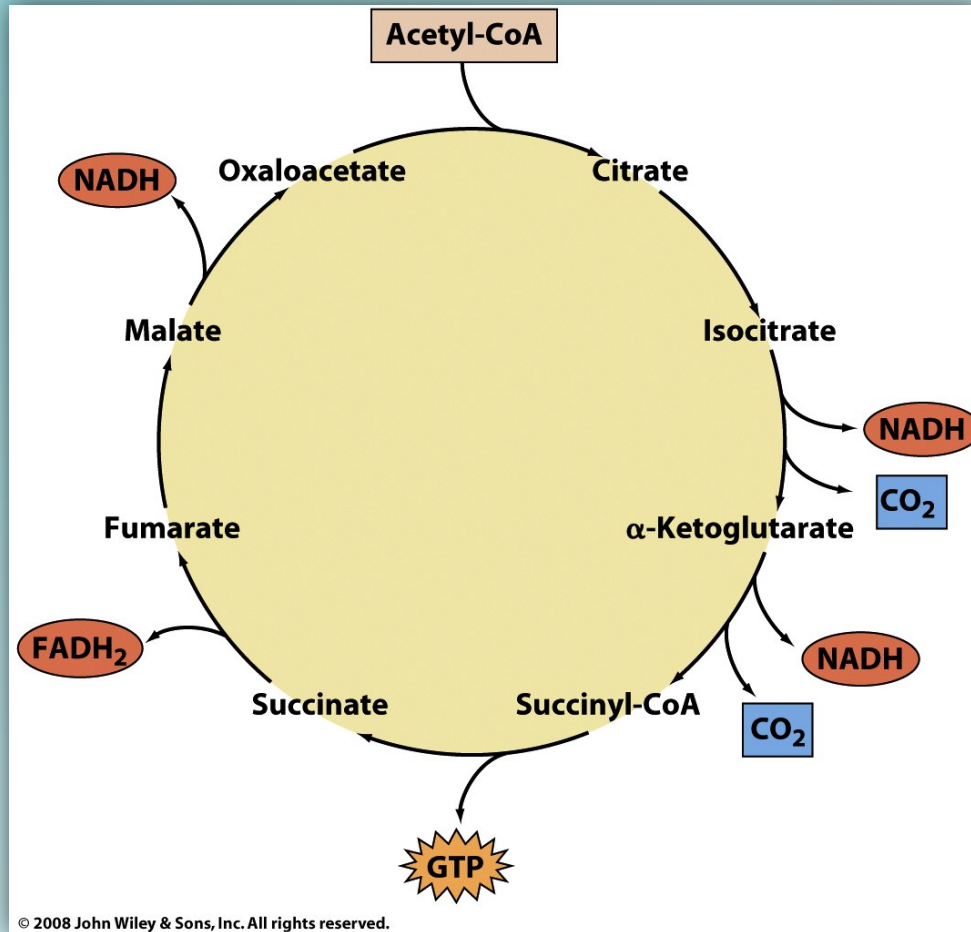


Fumarase:



Malate dehydrogenase (NAD⁺-linked dehydrogenase):

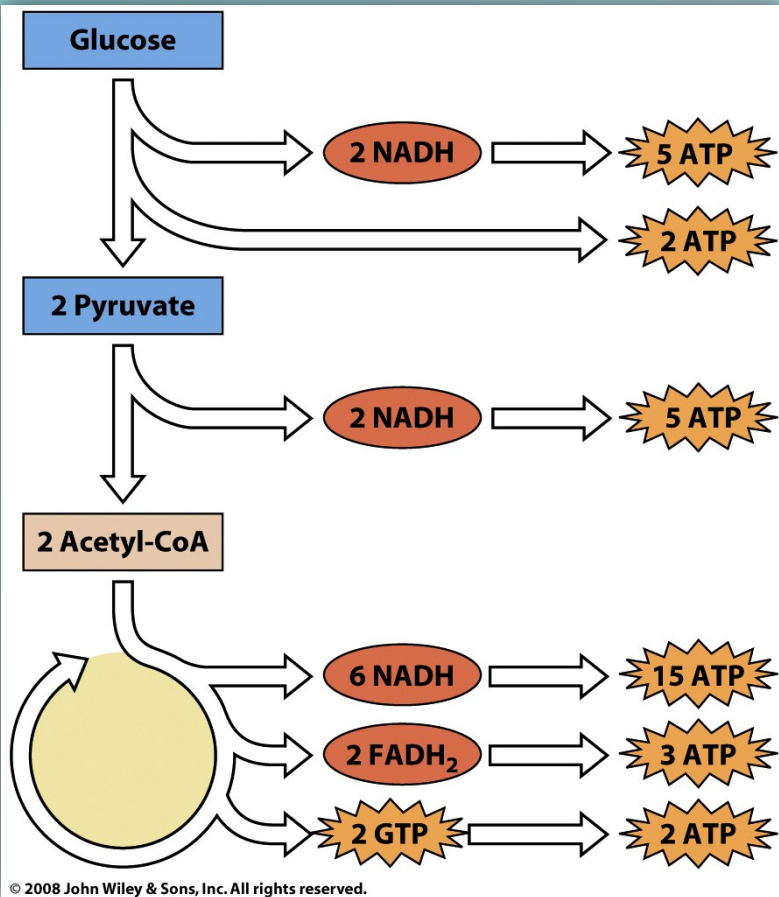




Products generated by the TCA cycle

For each pyruvate entering the cycle:
4 NADH
1 FADH₂
1 GTP

All three carbons of pyruvate are lost as CO₂.



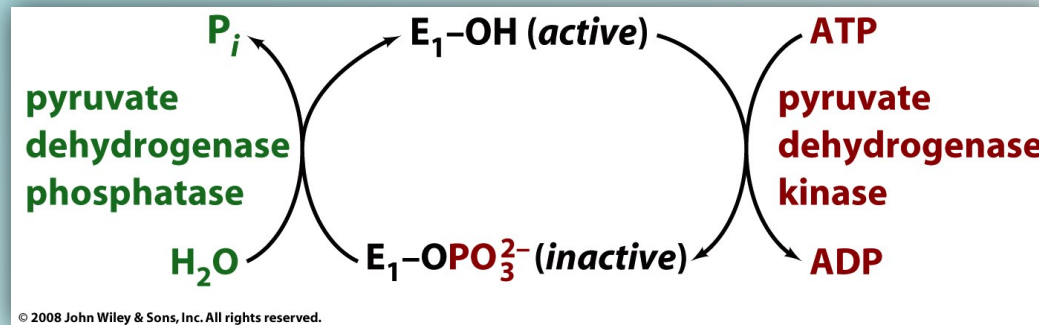
Summary of ATP production from glycolysis, TCA and electron transport/oxidative phosphorylation

2.5 ATP per NADH and 1.5 ATP per FADH₂ entering ET/OP

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

Regulation of the TCA cycle

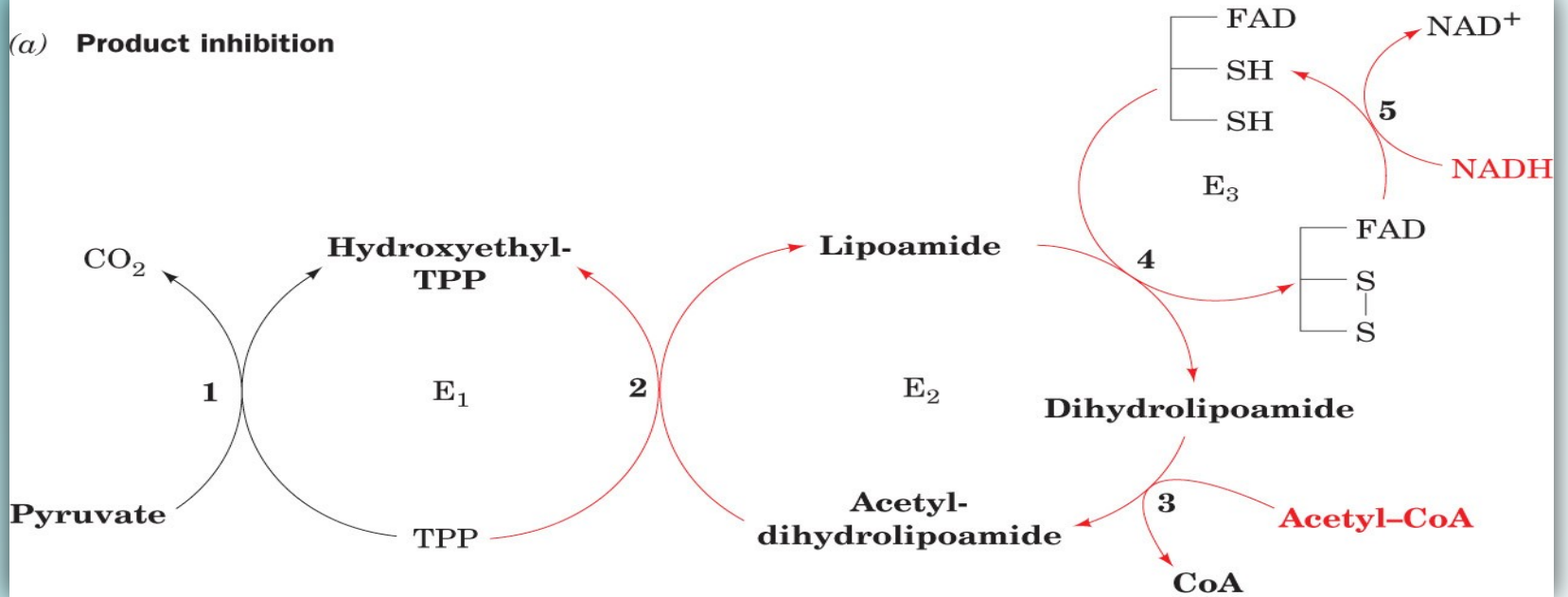
Pyruvate dehydrogenase is regulated by product inhibition (NADH and acetyl CoA) and by covalent modification (phosphorylation of Ser).



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

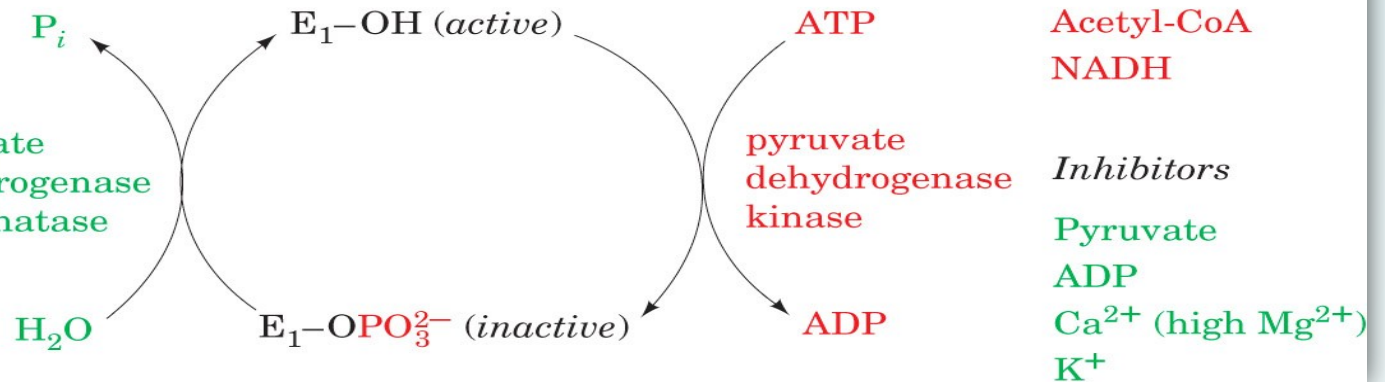
ADP

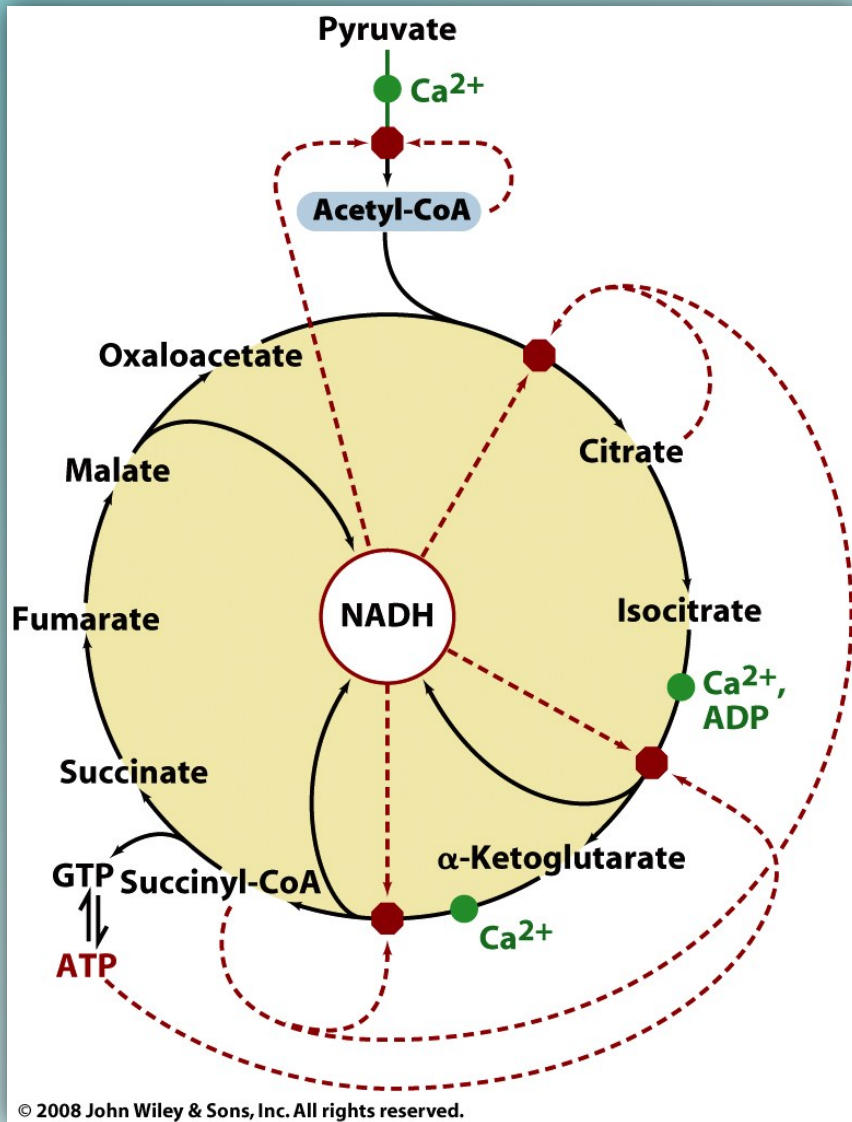
Activators

Acetyl-CoA
NADH

Inhibitors

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





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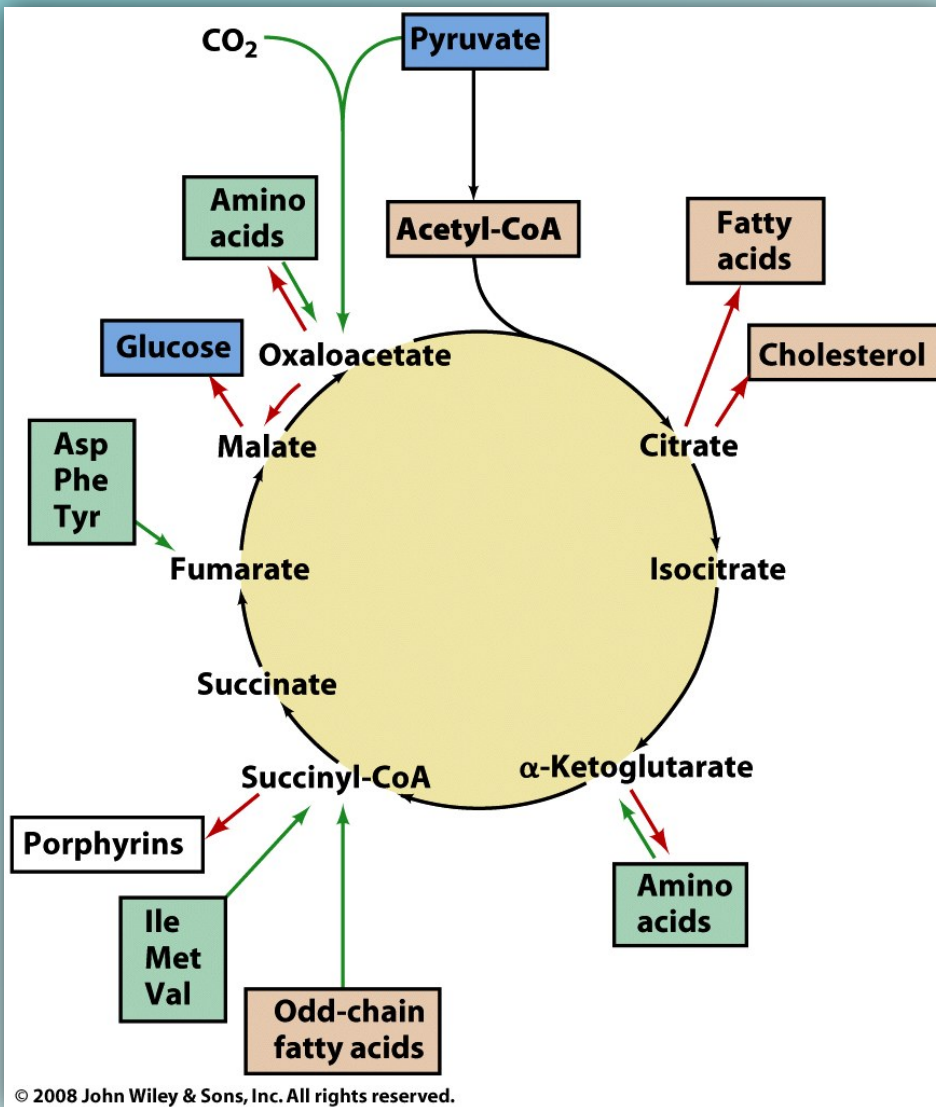
Regulation of the TCA cycle

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

Table 17-2 Standard Free Energy Changes (ΔG°) and Physiological Free Energy Changes (ΔG) of Citric Acid Cycle Reactions

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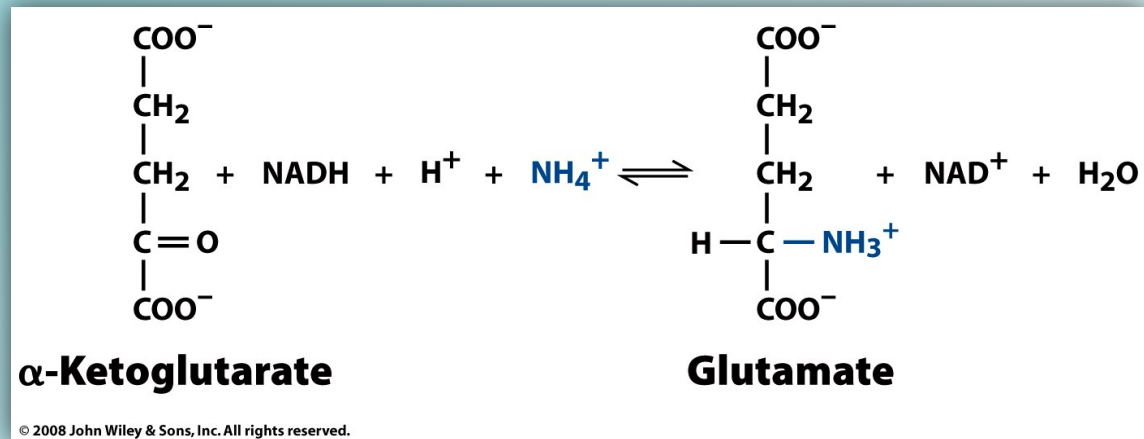
A summary of the amphibolic functions of the TCA cycle, showing *catabolic* (red) and *anabolic* (green) reaction paths

Malate to glucose: gluconeogenesis (*transport problem*)

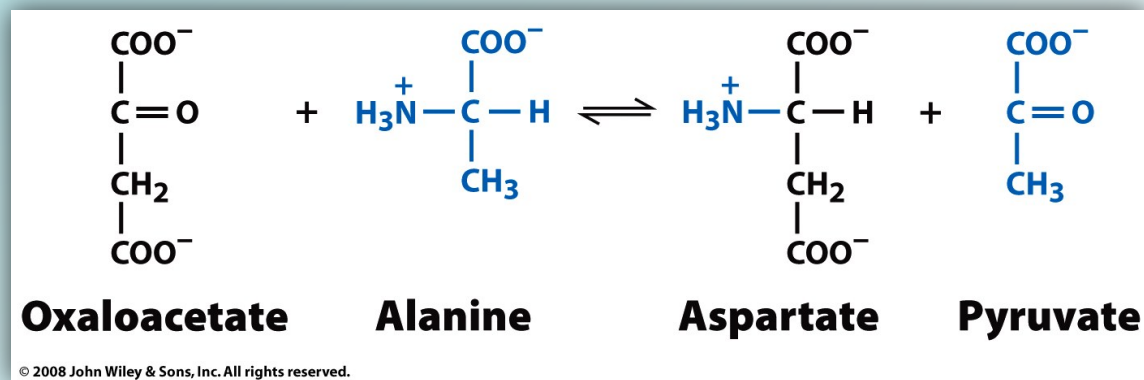
Citrate to fatty acids: fatty acid biosynthesis (*transport problem*)

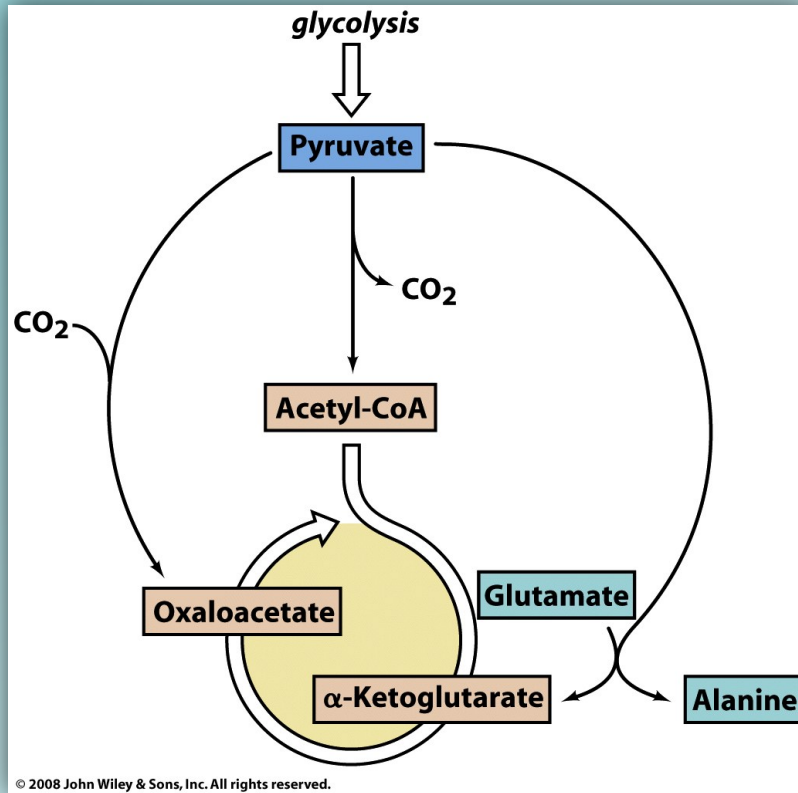
OAA and α KG to amino acids: amino acid biosynthesis

Conversion of α 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 CO₂: 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.

