Chapter 12 - The Citric Acid Cycle

- The citric acid cycle (tricarboxylic acid cycle) is **amphibolic** (both catabolic and anabolic)
- The cycle is involved in the aerobic catabolism of carbohydrates, lipids and amino acids
- Intermediates of the cycle are starting points for many biosynthetic reactions
- Enzymes of the cycle are in the mitochondria (eukaryotes) or the cytosol of bacteria

Energy in the citric acid cycle

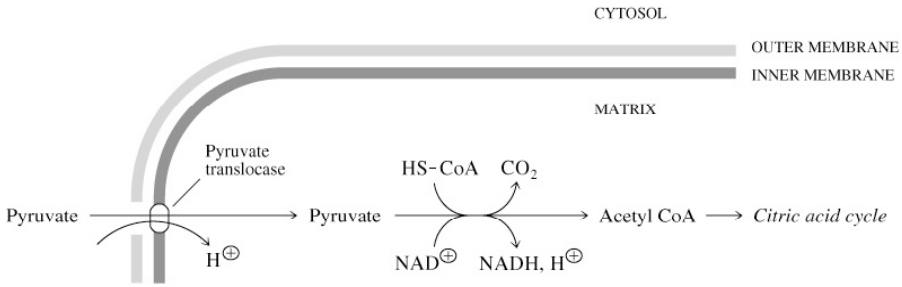
- Energy of the oxidation reactions is largely conserved as reducing power
- Coenzymes reduced:

$NAD^+ \longrightarrow NADH$

Ubiquinone (Q) $\longrightarrow$ Ubiquinol (QH_2)

12.1 Entry of Pyruvate into the Mitochondrion

- **Fig 12.1** Pyruvate translocase transports pyruvate into the mitochondria in symport with H^+



12.2 Conversion of Pyruvate to Acetyl CoA

- Pyruvate dehydrogenase complex (PDH complex) is a multienzyme complex containing:

  3 enzymes + 5 coenzymes + other proteins

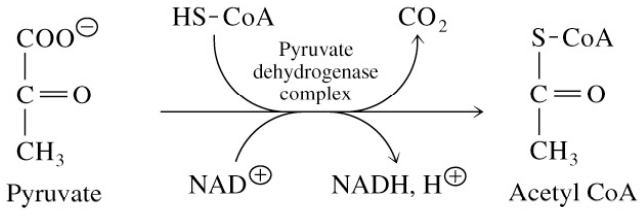
  (+ ATP coenzyme as a regulator)

  E_1 = pyruvate dehydrogenase

  E_2 = dihydrolipoamide acetyltransferase

  E_3 = dihydrolipoamide dehydrogenase

Overall reaction of pyruvate dehydrogenase complex



(12.1)

Table 12.1 Components of the PDH Complex

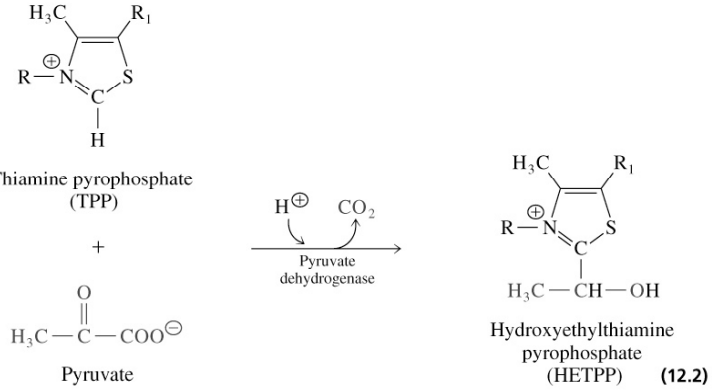
TABLE 12.1 Components of the pyruvate dehydrogenase complex in mammals and in *E. coli*

Enzyme	Coenzyme	Oligomeric form		Number of oligomers per complex	
		Mammals	*E. coli*	Mammals	*E. coli*
Pyruvate dehydrogenase(E_1)	TPP	$\alpha_2\beta_2$	α_2	20–30	12
Dihydrolipoamide acetyltransferase(E_2)	Lipoic acid, HS-CoA	α_{60}	α_{24}	1	1
Dihydrolipoamide dehydrogenase(E_3)	FAD, NAD⊕	α_2	α_2	6	6
E_3-binding protein	Lipoic acid	Monomer	—	12	—
Kinase	ATP	$\alpha\beta$	—	2–3	—
Phosphatase	None		—	>3	—

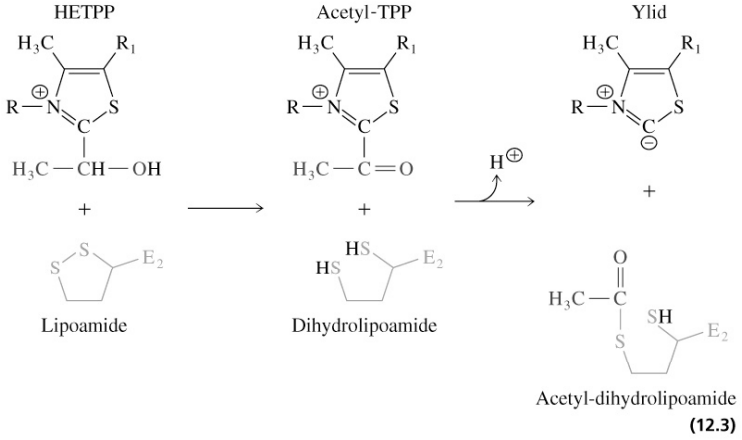
[Adapted from Patel, M. S., and Roche,T. E. (1990). Molecular biology and biochemistry of pyruvate dehydrogenase complexes.*FASEB J.* 4:3224–3233.]

The five steps of the PDH complex

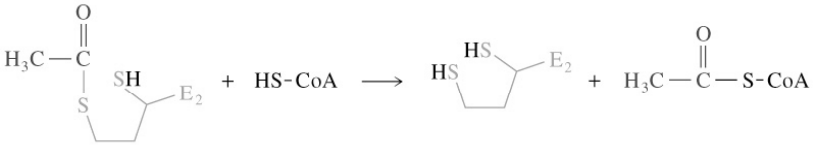
Step 1: Catalyzed by E_1



Step 2: The second step is also catalyzed by E_1



Step 3: E_2 transfers the lipoamide-bound acetyl group to HS-CoA forming acetyl CoA



Acetyl-dihydrolipoamide Dihydrolipoamide Acetyl CoA

(12.4)

Step 4: E_3 FAD group oxidizes reduced lipoamide of E_2 forming $FADH_2$

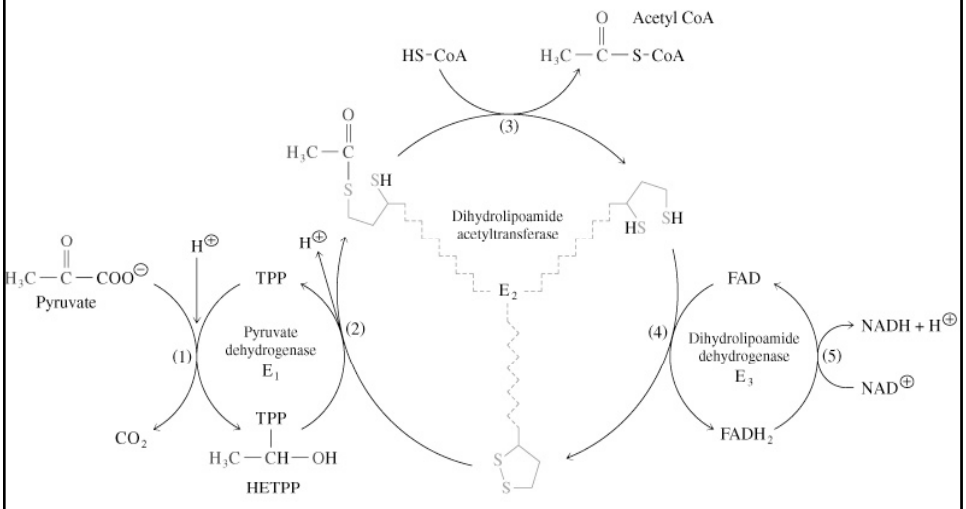
Dihydrolipoamide Lipoamide **(12.5)**

Step 5: E_3-$FADH_2$ reduces NAD^+ to regenerate E_3-FAD and NADH

- The oxidation of E_3-$FADH_2$ regenerates the original holoenzyme completing the catalytic cycle
- NADH dissociates from the complex

$$E_3\text{-}FADH_2 + NAD^+ \longrightarrow E_3\text{-}FAD + NADH + H^+$$

Fig 12.2 Reactions of the PDH complex

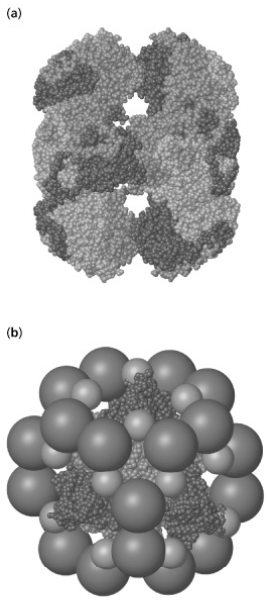


Roles of the coenzymes of the PDH complex

- NAD^+ and HS-CoA are cosubstrates
- TPP, lipoamide and FAD are prosthetic groups
- ATP is a regulator of the PDH complex
- Lipoamide (on E_2) acts as a "swinging arm" to transfer the two carbon unit from the active site of E_1 to the active site of E_3 (substrate channeling)

Fig 12.3

- Structure of the PDH complex
- (a) Core of the complex (24 E_2 chains)
- (b) Model of the entire complex: 12 E_1 dimers (blue), 6 E_3 dimers (green) surround the core



12.3 The Citric Acid Cycle Oxidizes AcetylCoA

- **Table 12.2**

TABLE 12.2 The enzymatic reactions of the citric acid cycle

Reaction	Enzyme
1. Acetyl CoA + Oxaloacetate + H_2O ⟶ Citrate + HS-CoA + H^+	Citrate synthase
2. Citrate ⇌ Isocitrate	Aconitase (Aconitate hydratase)
3. Isocitrate + NAD^+ ⟶ α-Ketoglutarate + NADH + CO_2	Isocitrate dehydrogenase
4. α-Ketoglutarate + HS-CoA + NAD^+ ⟶ Succinyl CoA + N + ADH CO_2	α-Ketoglutarate dehydrogenase complex
5. Succinyl CoA + GDP(or ADP) + P_i ⇌ Succinate + GTP(or ATP) + HS-CoA	Succinyl-CoAsynthetase
6. Succinate + Q ⇌ Fumarate + QH_2	Succinate dehydrogenase complex
7. Fumarate + $_2HO$ ⇌ L-Malate	Fumarase (Fumarate hydratase)
8. L-Malate + NAD^+ ⇌ Oxaloacetate + NADH + H^+	Malate dehydrogenase
Net equation:	
Acetyl CoA + 3 NAD^+ + Q + GDP(or ADP) + P_i + 2 H_2O ⟶ HS-CoA + 3 NADH + QH_2 + GTP(or ATP) + 2 CO_2 + $2H^+$	

Summary of the citric acid cycle

- For each acetyl CoA which enters the cycle:
  - (1) Two molecules of CO_2 are released
  - (2) Coenzymes NAD^+ and Q are reduced
  - (3) One GDP (or ADP) is phosphorylated
  - (4) The initial acceptor molecule (oxaloacetate) is reformed

Fig 12.4

- Citric acid cycle (Four slides)

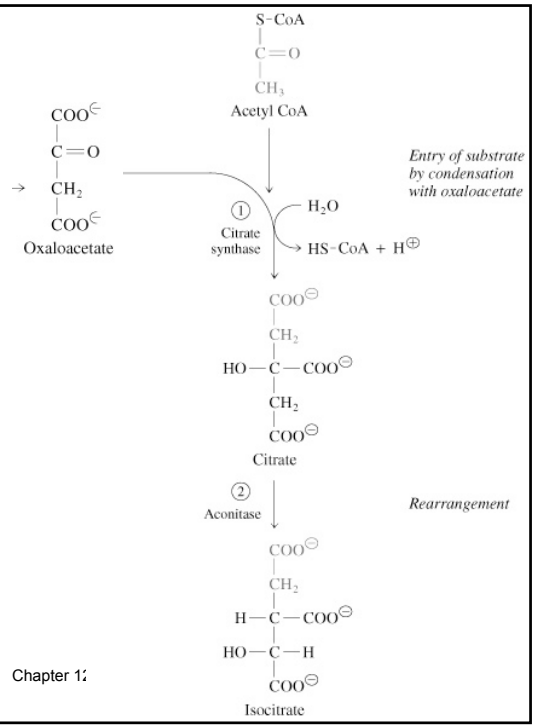


Fig 12.4 (continued)

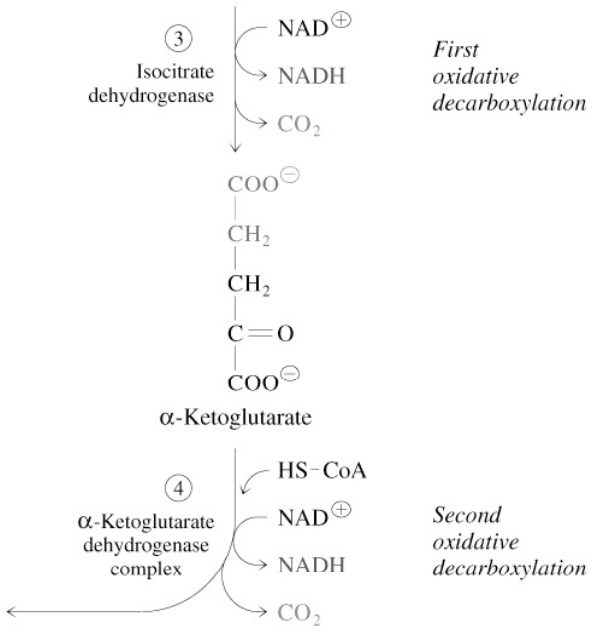


Fig 12.4 (continued)

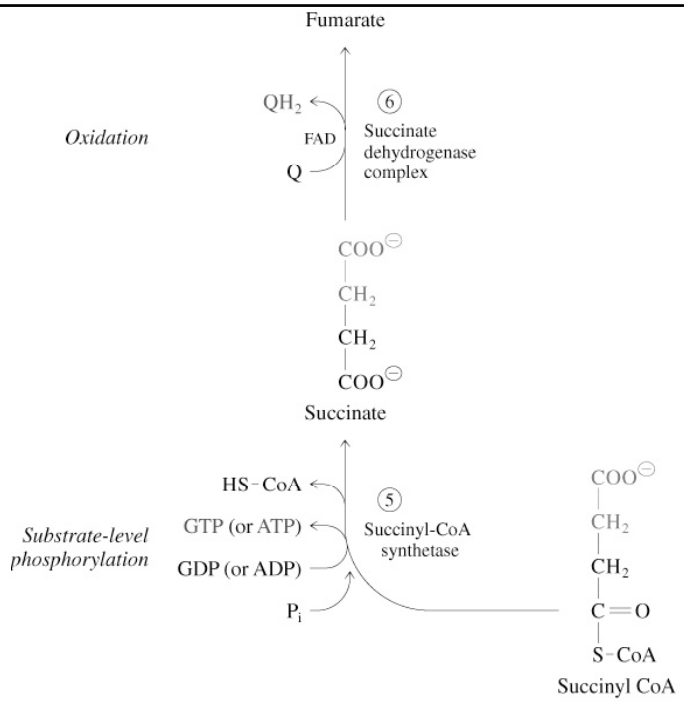


Fig 12.4 (continued)

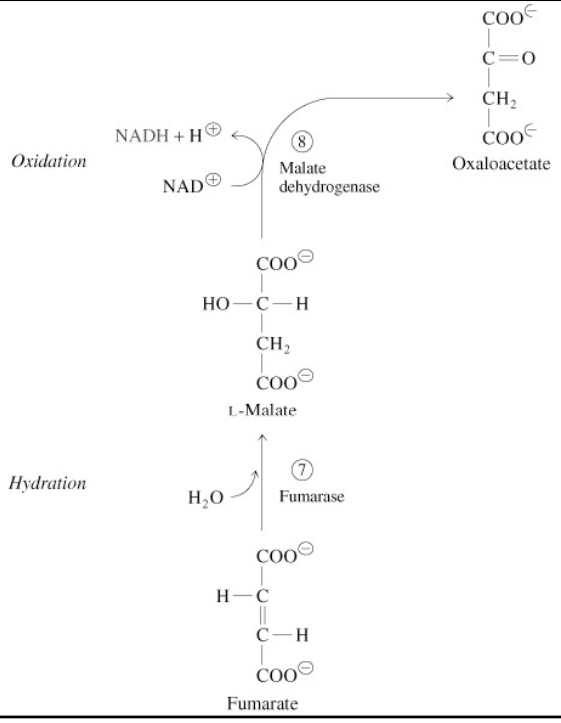


Fig 12.5

- Fates of carbon atoms in the cycle
- Carbon atoms from acetyl CoA (red) are not lost in the first turn of the cycle

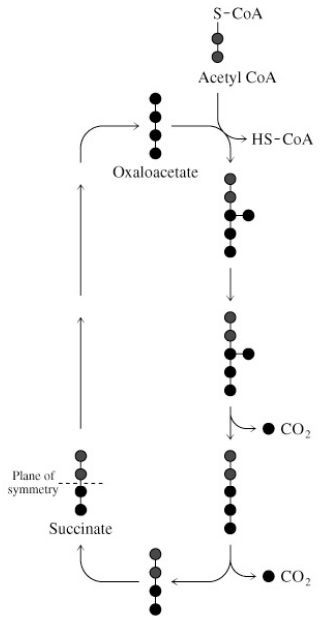
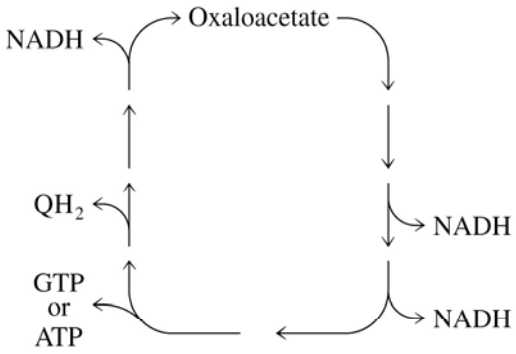


Fig 12.6 Energy conservation by the cycle

- Energy is conserved in the reduced coenzymes NADH, QH_2 and one GTP
- NADH, QH_2 can be oxidized to produce ATP by oxidative phosphorylation



12.4 The Citric Acid Cycle Can Be a Multistep Catalyst

- Oxaloacetate is regenerated
- The cycle is a mechanism for oxidizing acetyl CoA to CO_2 by NAD^+ and Q
- 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 resupply or net decrease in cycle intermediates

1. Citrate Synthase

- Citrate formed from acetyl CoA and oxaloacetate
- Only cycle reaction with C-C bond formation

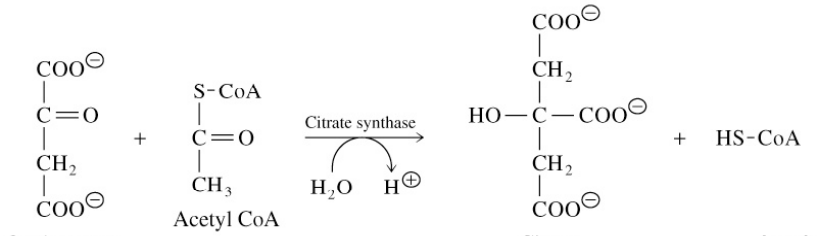


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Fig 12.7 Proposed mechanism of citrate synthase

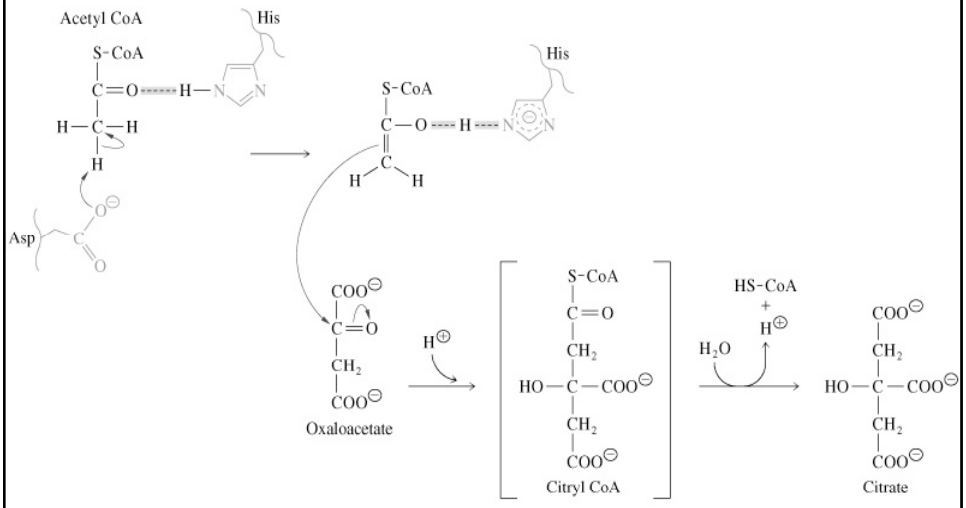
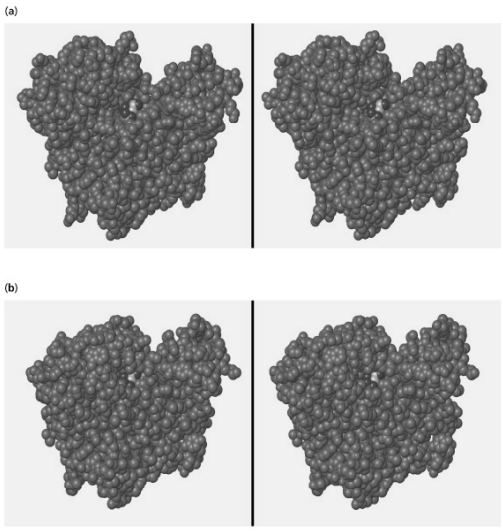


Fig 12.8 Stereo views of citrate synthase

(a) Open conformation

(b) Closed conformation

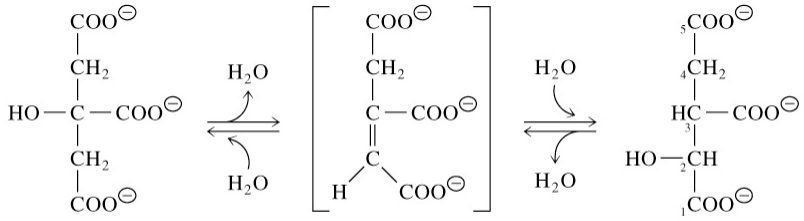
Product citrate (red)



2. Aconitase

- Elimination of H_2O from citrate to form C=C bond of *cis*-aconitate
- Stereospecific addition of H_2O to *cis*-aconitate to form 2*R*,3*S*-Isocitrate

Reaction of Aconitase



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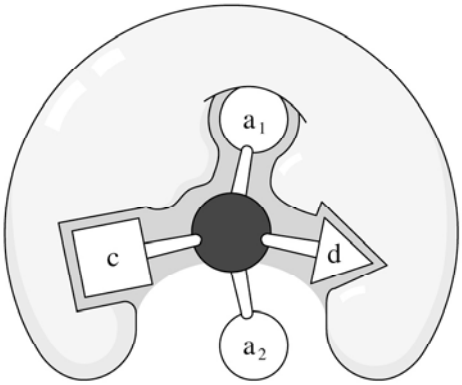
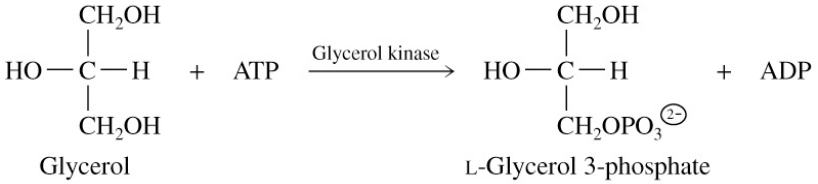
Box 12.1 Three point attachment of prochiral substrates to enzymes

- Chemically identical groups a_1 and a_2 of a prochiral molecule can be distinguished by the enzyme



Glycerol kinase converts prochiral substrate (glycerol) into a chiral product (L-glycerol 3-phosphate)

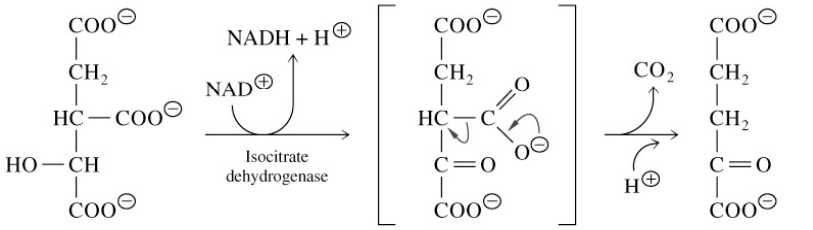


Glycerol L-Glycerol 3-phosphate

3. Isocitrate Dehydrogenase

- Oxidative decarboxylation of isocitrate to α-ketoglutarate (α-kg) (a metabolically irreversible reaction)
- One of four oxidation-reduction reactions of the cycle
- Hydride ion from the C-2 of isocitrate is transferred to NAD^+ to form NADH
- Oxalosuccinate is decarboxylated to α-kg

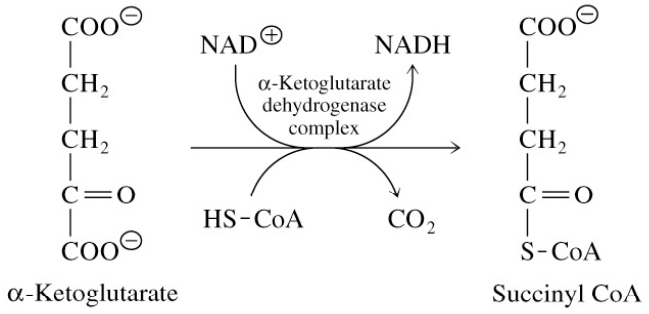
Isocitrate dehydrogenase reaction



Isocitrate — Oxalosuccinate — α-Ketoglutarate

(12.9)

4. The α-Ketoglutarate Dehydrogenase Complex



(12.10)

Structure of α-Ketoglutarate dehydrogenase complex

- Similar to pyruvate dehydrogenase complex
- Same coenzymes, identical mechanisms

E_1 - α-ketoglutarate dehydrogenase (with TPP)

E_2 - succinyltransferase (with flexible lipoamide prosthetic group)

E_3 - dihydrolipoamide dehydrogenase (with FAD)

5. Succinyl-CoA Synthetase

- Free energy in thioester bond of succinyl CoA is conserved as GTP (or ATP in plants, some bacteria)

Fig 12.9

- Mechanism of succinyl-CoA synthetase (continued on next slide)

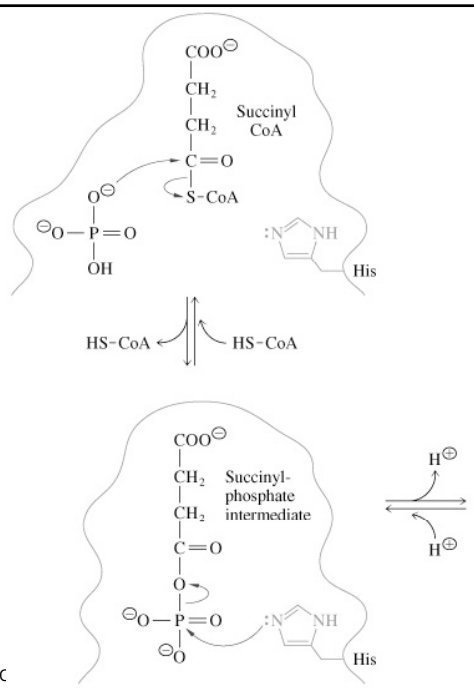
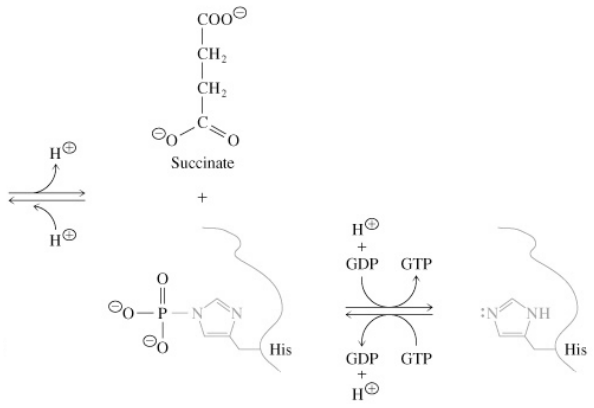


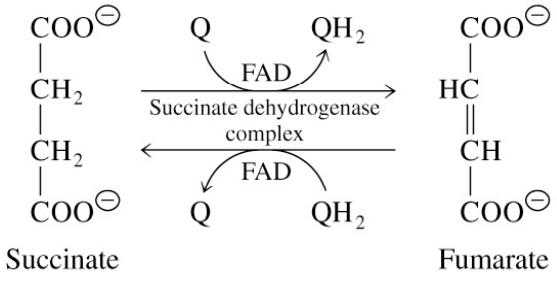
Fig 12.9 (continued)



6. The Succinate Dehydrogenase (SDH) Complex

- Located on the inner mitochondrial membrane (other components are dissolved in the matrix)
- Dehydrogenation is stereospecific; only the *trans* isomer is formed
- Substrate analog malonate is a competitive inhibitor of the SDH complex

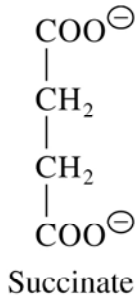
Reaction of the succinate dehydrogenase complex



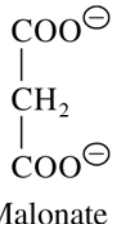
(12.12)

Fig 12.10 Succinate and malonate

- Malonate is a structural analog of succinate
- Malonate binds to the enzyme active site, and is a competitive inhibitor



Succinate



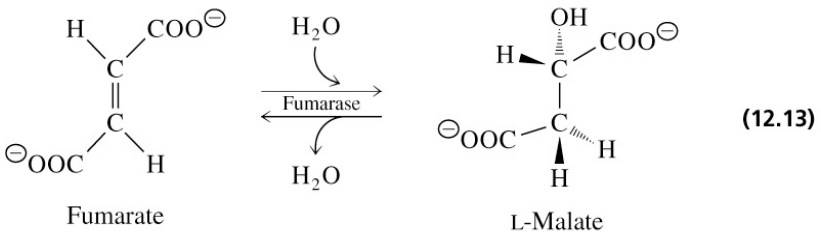
Malonate

Structure of the SDH complex

- Complex of several polypeptides, an FAD prosthetic group and iron-sulfur clusters
- Electrons are transferred from succinate to ubiquinone (Q), a lipid-soluble mobile carrier of reducing power
- $FADH_2$ generated is reoxidized by Q
- QH_2 is released as a mobile product

7. Fumarase

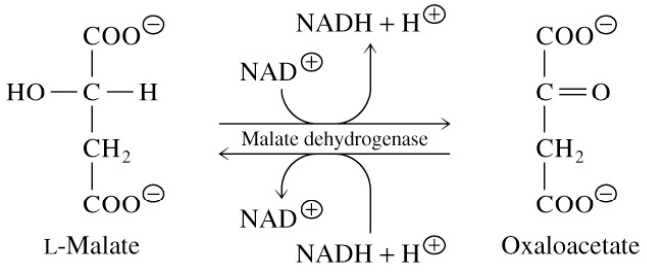
- Stereospecific *trans* addition of water to the double bond of fumarate to form *L*-malate



Fumarate L-Malate

(12.13)

8. Malate Dehydrogenase

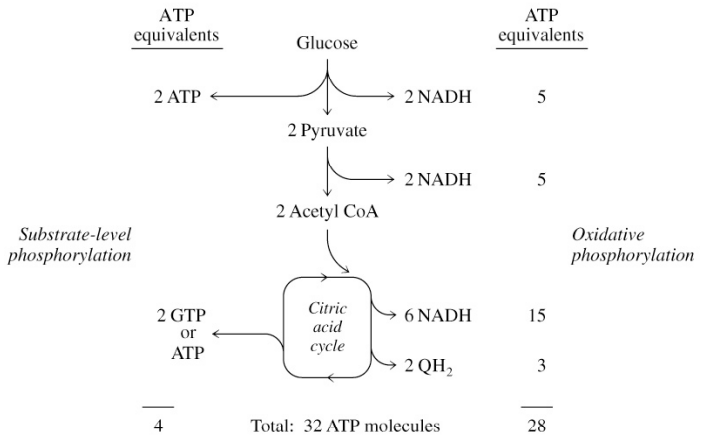


(12.14)

12.5 Reduced Coenzymes Fuel the Production of ATP

- Each acetyl CoA entering the cycle nets:
  - (1) **3 NADH**
  - (2) **1 QH_2**
  - (3) **1 GTP** (or **1 ATP**)
- Oxidation of each NADH yields **2.5 ATP**
- Oxidation of each QH_2 yields **1.5 ATP**
- Complete oxidation of **1 acetyl CoA = 10 ATP**

Fig 12.11 Glucose degradation via glycolysis, citric acid cycle, and oxidative phosphorylation



NADH fate in <u>anaerobic</u> <u>glycolysis</u>

<u>Anaerobic</u> <u>glycolysis</u>

- NADH produced by G3PDH reaction is <u>reoxidized</u> to NAD^+ in the pyruvate to lactate reaction
- NAD^+ recycling allows G3PDH reaction (and glycolysis) to continue anaerobically

NADH fate in aerobic glycolysis

- Glycolytic NADH is not reoxidized via pyruvate reduction but is available to fuel ATP formation
- Glycolytic NADH (cytosol) must be transferred to mitochondria (electron transport chain location)
- Two NADH shuttles are available (next slide)

NADH shuttles

- Malate-aspartate shuttle (most common)

  One cytosolic NADH yields **~ 2.5 ATP**
  (total **32 ATP/glucose**)

- Glycerol phosphate shuttle

  One cytosolic NADH yields **~1.5ATP**
  (total **30 ATP/glucose**)

12.6 Regulation of the Citric Acid Cycle

- Pathway controlled by:
  - (1) Allosteric modulators
  - (2) Covalent modification of cycle enzymes
  - (3) Supply of acetyl CoA
  - (4) Regulation of pyruvate dehydrogenase complex controls acetyl CoA supply

Fig 12.12 Regulation of the PDH complex

- Increased levels of acetyl CoA and NADH inhibit E_2, E_3 in mammals and *E. coli*

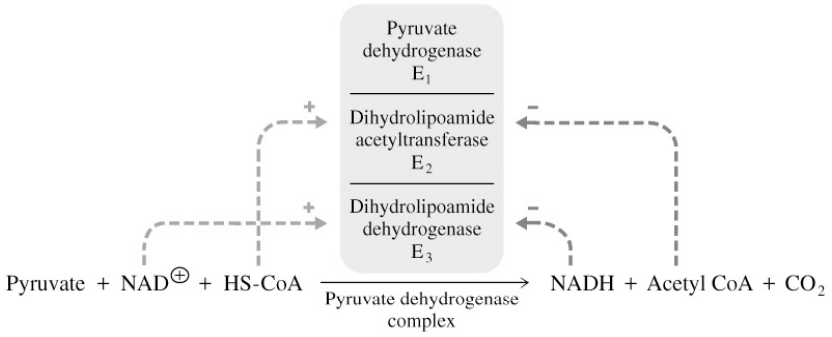
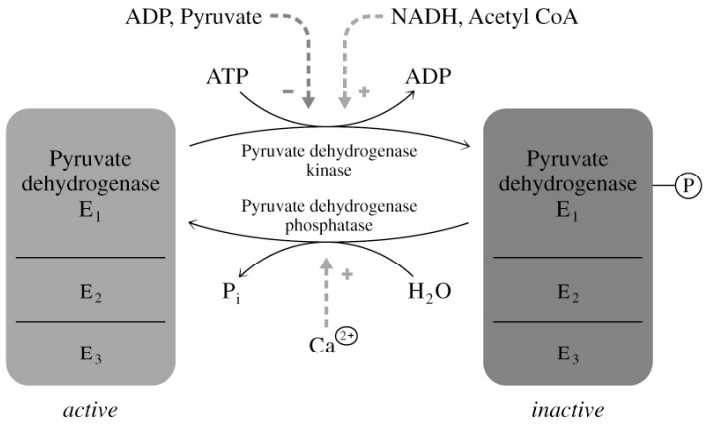


Fig 12.13 Regulation of mammalian PDH complex by covalent modification

- Phosphorylation/dephosphorylation of E_1



Further regulation of the PDH complex

Pyruvate dehydrogenase kinase (PDK)

- PDK is activated by NADH and acetyl CoA (leads to inactivation of the PDH complex)
- PDK is inhibited by pyruvate and ADP (leads to activation of the PDH complex)

Pyruvate dehydrogenase phosphatase (PDP)

- PDP activity is stimulated by Ca^{2+} (leads to an activation of the PDH complex)

Regulation of isocitrate dehydrogenase (ICDH)

Mammalian ICDH

- Allosteric effectors: (+) Ca^{2+}, ADP, (-) NADH (not subject to covalent modification)

E. coli ICDH

- Bifunctional kinase/phosphatase enzyme phosphorylates/dephosphorylates ICDH
- Bifunctional enzyme is reciprocally regulated by intermediates of glycolytic and citric acid cycles

Fig 12.14 *E. coli* ICDH models

(a) Phosphorylated form inactive, (b) Active form

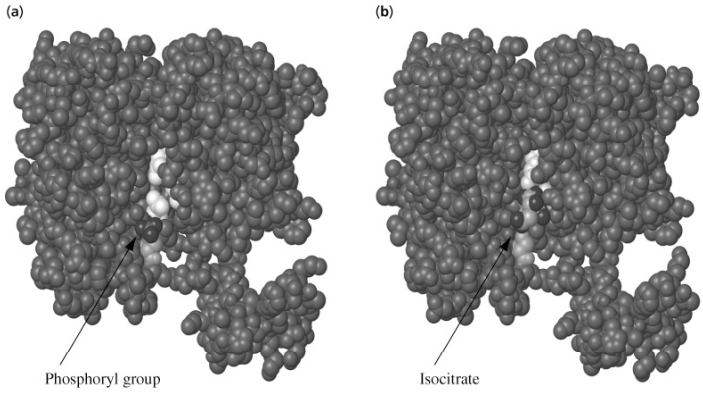


Fig 12.15 Covalent regulation of *E. coli* ICDH

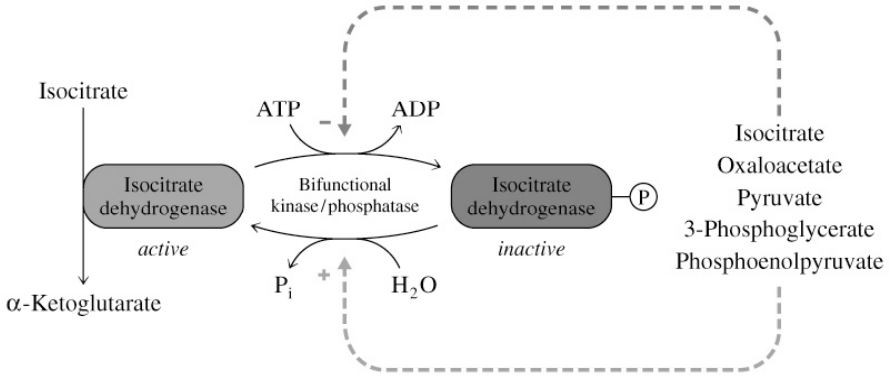


Fig 12.16 Regulation of the PDH complex and the citric acid cycle

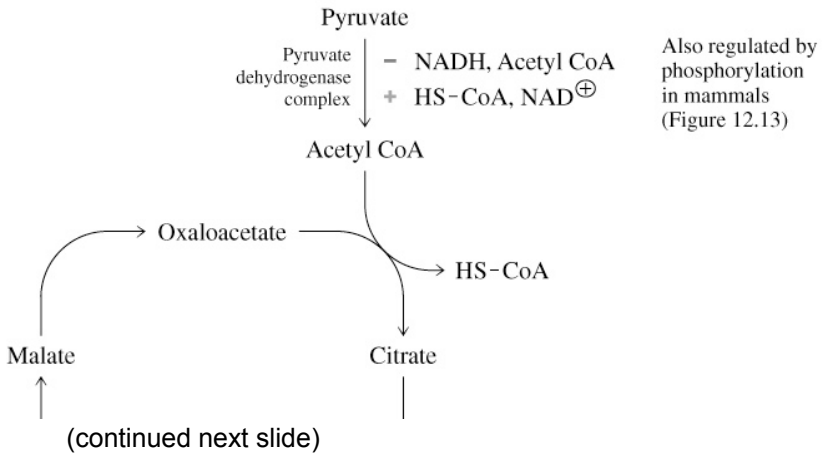


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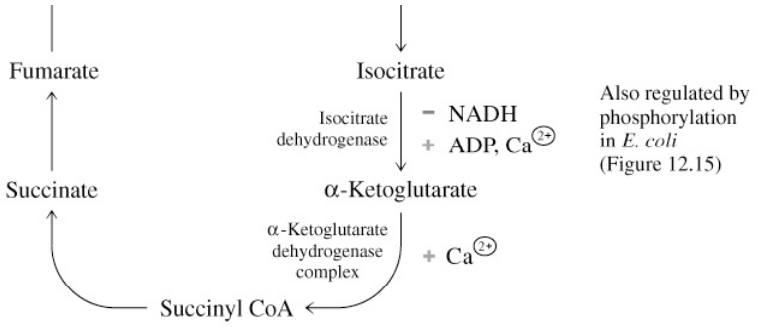
Fig 12.16 (continued)



12.7 Entry and Exit of Metabolites

- Intermediates of the citric acid cycle are precursors for carbohydrates, lipids, amino acids, nucleotides and porphyrins
- Reactions feeding into the cycle replenish the pool of cycle intermediates

Fig 12.17 Routes leading to and from the citric acid cycle

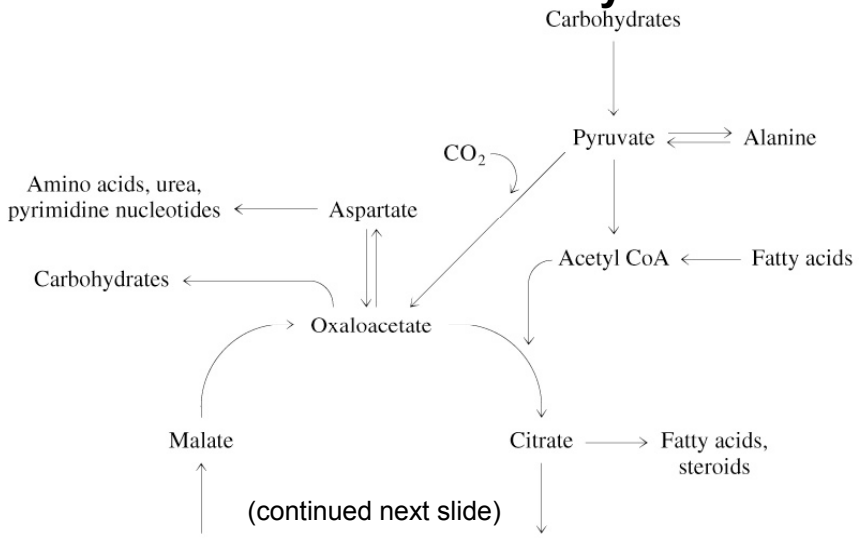
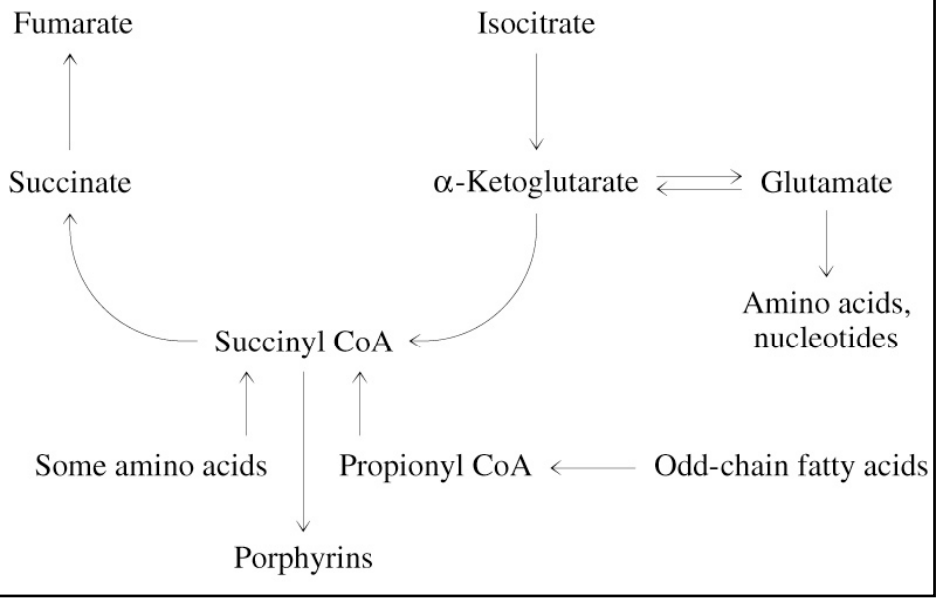


Fig. 12.17 (continued)



Anaplerotic reactions

- **Anaplerotic** (filling up) reactions replenish citric acid cycle intermediates
- **Pyruvate carboxylase** is a major anaplerotic reaction in mammalian tissues

Pyruvate + CO_2 + ATP + H_2O ⟶

Oxaloacetate + ADP + P_i

Phosphoenolpyruvate carboxylase

- Anaplerotic reaction in plants and bacteria
- Supplies oxaloacetate to the citric acid cycle

Phosphoenolpyruvate + HCO_3^- ⟶

Oxaloacetate + P_i

12.8 The Glyoxylate Cycle

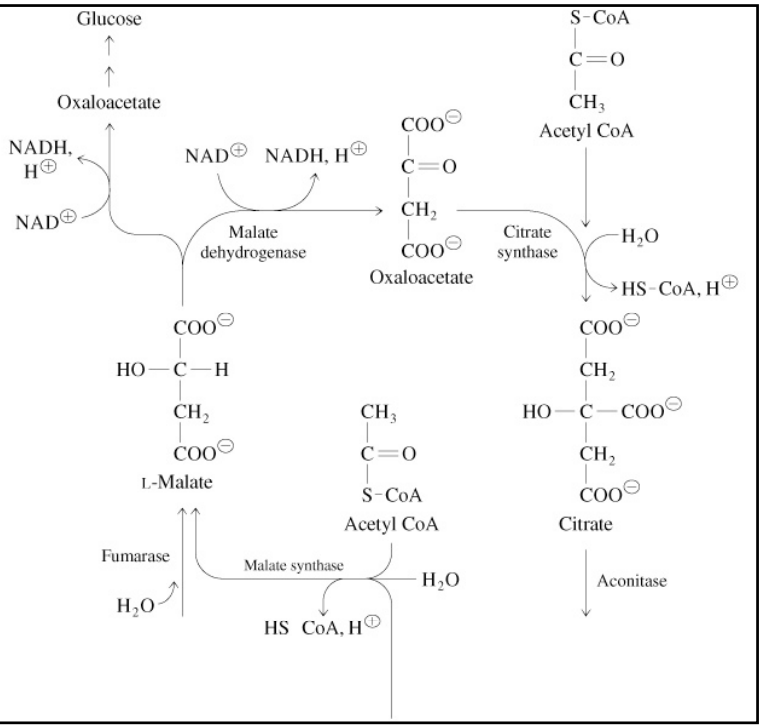
- Pathway for the formation of glucose from noncarbohydrate precursors in plants, bacteria and yeast (not animals)
- Glyoxylate cycle leads from 2-carbon compounds to glucose
- In animals, acetyl CoA is not a carbon source for the net formation of glucose (2 carbons of acetyl CoA enter cycle, 2 are released as 2 CO_2)

Glyoxylate cycle - formation of glucose

- Formation of glucose from acetyl CoA (or any substrate that is a precursor to acetyl CoA)
- Ethanol or acetate can be metabolized to acetyl CoA and then to glucose via the glyoxylate cycle
- Stored seed oils in plants are converted to carbohydrates during germination

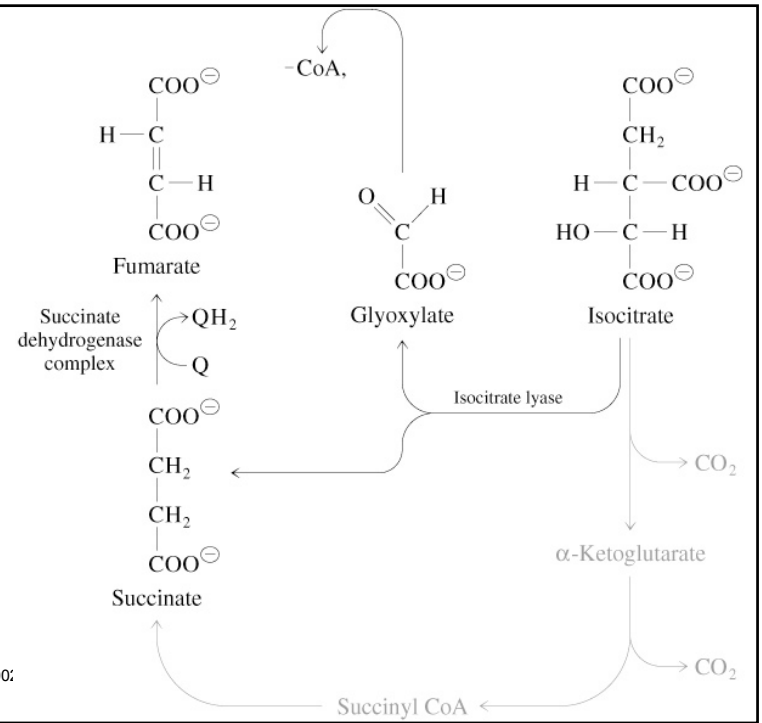
Fig 12.18

Glyoxylate Cycle (2 slides)



Prentice Hall c2(

Fig 12.18 (cont)



Prentice Hall c200

Fig 12.19 Isocitrate lyase: first bypass enzyme of glyoxylate

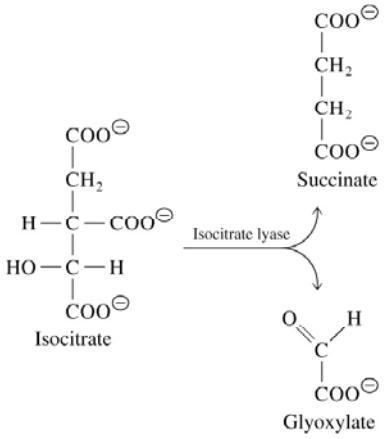
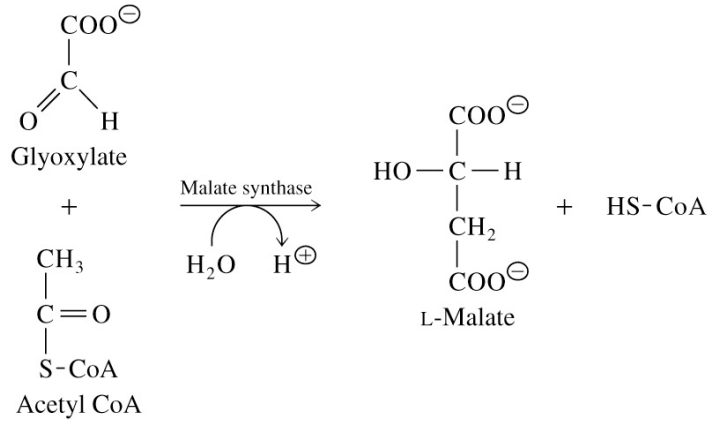


Fig 12.20 Malate synthase: second bypass enzyme of glyoxylate



Glyoxylate cycle in germinating castor beans

- Figure 12.21 (next slide)
- Conversion of acetyl CoA to glucose requires the transfer of metabolites among three metabolic compartments
  - (1) The glyoxysome
  - (2) The cytosol
  - (3) The mitochondrion