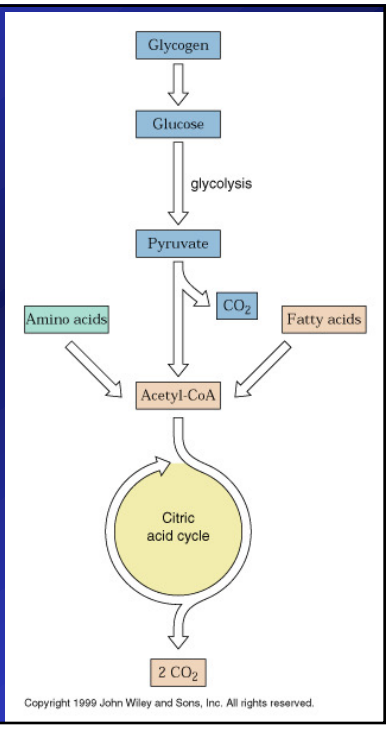
Chapter 16 - Citric Acid Cycle

TCA (tricarboxylic acid cycle)
Citric acid cycle and Krebs cycle
Named after Sir Hans Krebs, Nobel Laureate. He worked as an assistant professor for Otto Warburg (Nobel Prize 1931) and his position terminated 1933 and at, Sir Fredrick Gowland Hopkin's (Nobel prize 1929) request he left Germany to hold a Rockefeller Studentship at the School of Biochemistry, Cambridge. In 1953 he earned the Nobel Laureate in Medicine for his discovery of the citric acid cycle

1

The Krebs cycle is a central pathway for recovering energy from three major metabolites: carbohydrates, fatty acids, and amino acids.

Most enter the cycle through Acetyl~CoA. The two carbons entered at this step are lost as CO_2 (the reason you breath out CO_2). The carbon atoms that enter by A-CoA leave after the second turn of the cycle.



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Synthesis of the TCA

- Reactions take place in mitochondria - thus transport of reactants and products are important
- Overall reaction involves the entry of a 2 carbon compound (acetyl CoA) into the cycle with the loss of 2 CO_2 and formation of 3 NADH, $FADH_2$ and GTP or ATP.

Acetyl CoA + 2 O_2 +12 ADP + 12 Pi -> 2 CO_2 +CoASH +12 ATP + H_2O

- No net change in the concentration of the 4 carbon compound oxaloacetate.
- The carbons lost as CO2 are from previous A-CoAs not from the reactant A-Co

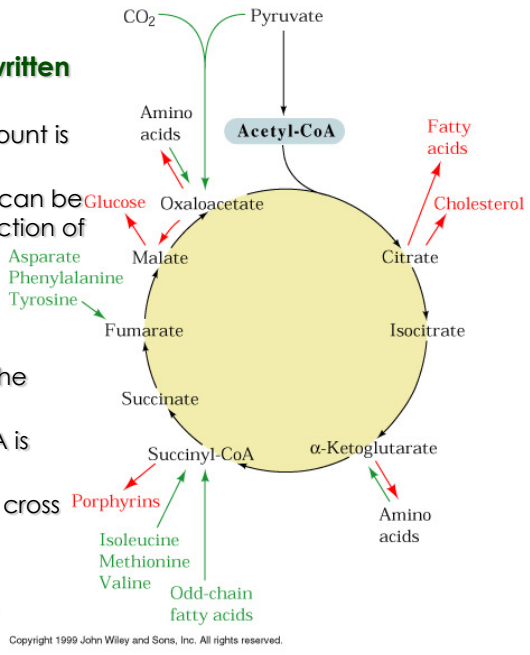
3

Think of why this is a cycle vs. pathway - not because it is written that way.

- Oxaloacetate - only a small amount is needed - catalytic role
- Anapleurotic - "filling up" cycle can be used as entry and exit for production of other essential metabolites

The TCA

- Takes place in mitochondria in the matrix
- Like glycolysis pathway, the TCA is highly regulated
- Sources of Acetyl CoA (another cross road metabolite)
  - glycolysis - via PDH
    - ß oxidation of fatty acids
    - selected amino acids



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Free energy considerations of the Citric Acid Cycle

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

Reaction	Enzyme	$\Delta G^{\circ\prime}$ (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

Table 17-2
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The rxns with –ΔG directionally drive the pathway. As these rxns are thermodynamically favorable, they MUST be regulated.

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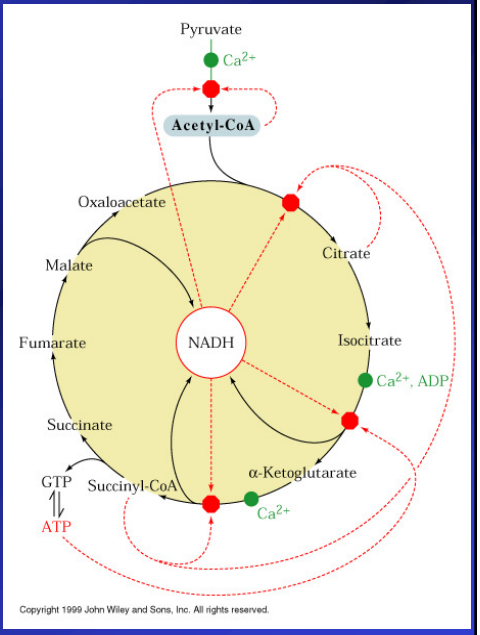
Regulation of the Citric Acid Cycle

Cycle enzymes

Citrate synthase
inhibited by S-CoA, citrate, NADH, and ATP

Isocitrate dehydrogenase
inhibited by NADH & ATP
stimulated by NAD^+ & ADP

α-ketoglutarate dehydrogenase
Inhibited by S-CoA, NADH and high ATP/AMP ratio



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Amphibolic functions of the Citric Acid Cycle

Anaplerotic flux must balance cataplerotic flux in order to retain homeostasis of cellular metabolism.

① **Amphibolic:** Biochemical pathway that involves both catabolism (break down of molecules) and anabolism (construction of molecules).

② **Red arrows:** Intermediates drawn off by cataplerotic reactions (extract intermediates for biosynthesis) for use in anabolic pathways.

③ **Green arrows:** Anaplerotic reactions replenish cycle intermediates.

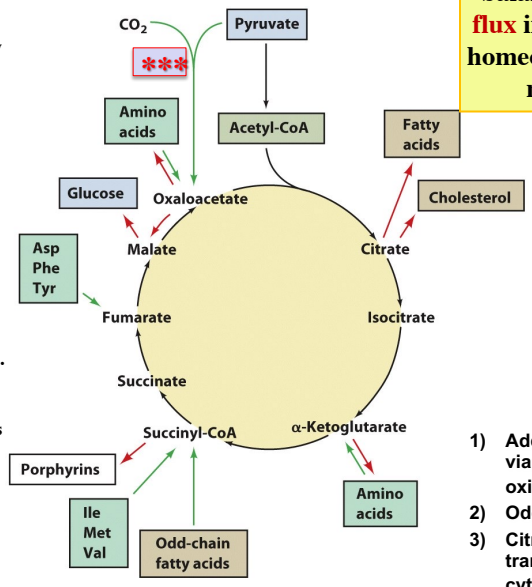

Figure 17-17
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1) Addition of amino acids via transamination and oxidative deamination
2) Odd chain fatty acids add
3) Citrate can be transported into the cytosol to be converted to FA and cholesterol

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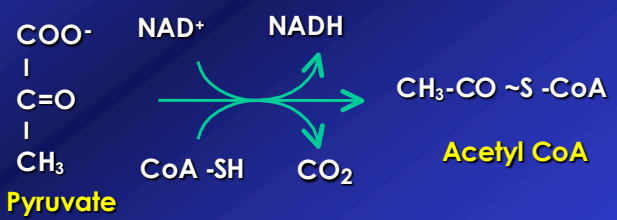
Getting there - don't forget PC!

Pyruvate Dehydrogenase (**PDH**) - Entry of glucose metabolites into cycle is through formation of acetyl-CoA by oxidative decarboxylation of pyruvate

- In eukaryotes, all of the TCA enzymes and the PDH are found in the mitochondria. Either in the inner compartment or the matrix of the mitochondrion.
- Pyruvate is made in the cytosol and transported by a H+ / pyruvate symporter.



Pyruvate Dehydrogenase

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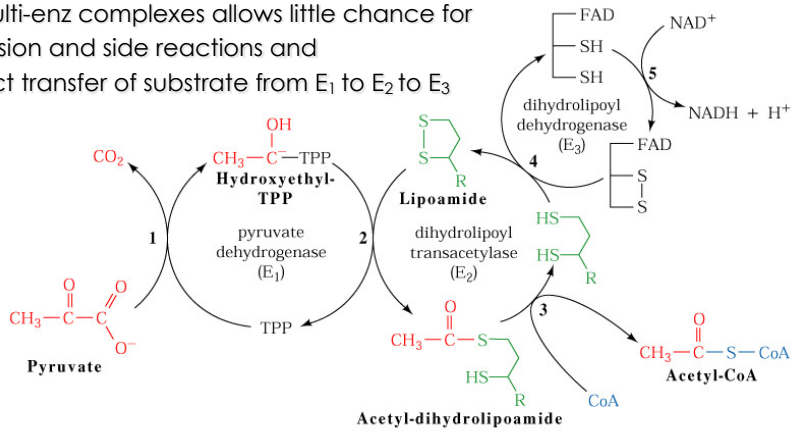
PDH Exists as large multiunit complex

- Coenzymes - Vitamin B1- thiamine pyrophosphate (TPP), panthanoic acid (CoA), riboflavin (FAD), Niacin (NAD^+) and lipoamide
- (3 different subunits)

E_1 Pyruvate Dehydrogenase - 24 copies / E_2 Dihydrolipoyl Translacetylase - 24 copies / E_3 Dihydrolipoyl Dehydrogenase - 12 copies

- increases local concentration of substrate for each subunit
- multi-enz complexes allows little chance for diffusion and side reactions and direct transfer of substrate from E_1 to E_2 to E_3



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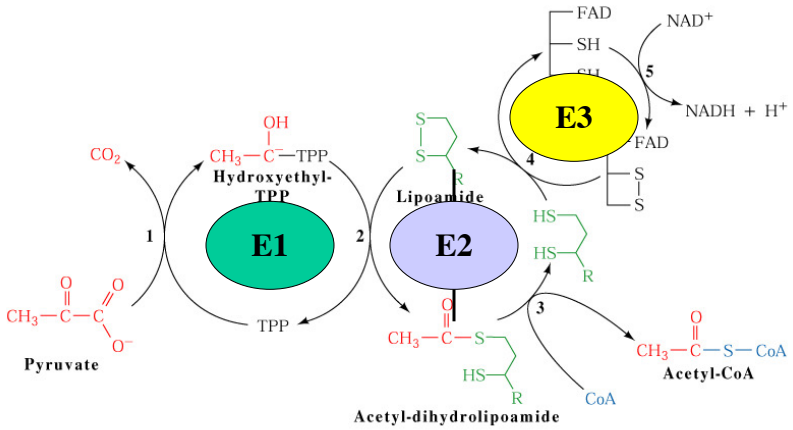
The three enzymes of the PDH

E1 - Pyruvate dehydrogenase

E2 Dihydrolipoyl Transacylase

E3 - Dihydrolipoyl Dehydrogenase



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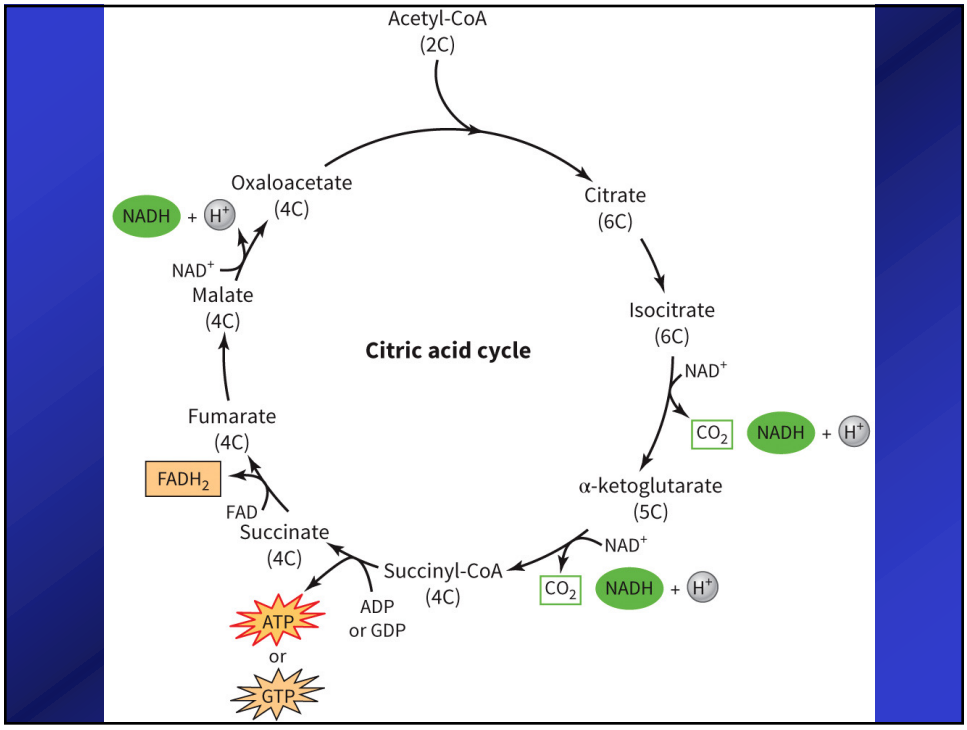

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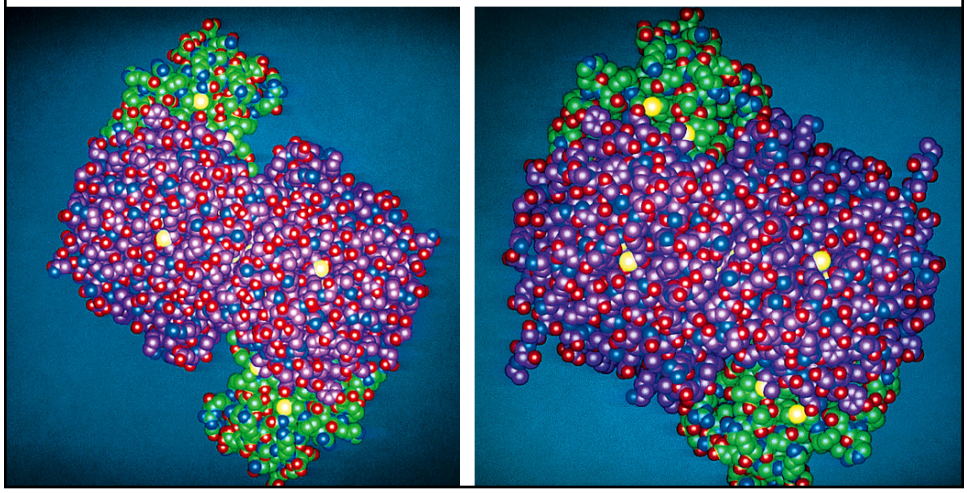
Reaction of the cycle

Citrate Synthase (CS) - **catalyzes the condensation of acetyl-CoA and OAA in a highly exergonic fashion.** There is a substantial conformational change in the enzyme when substrate binds. "hides" water from the active site and then forms A-CoA binding site. - ordered sequential reaction



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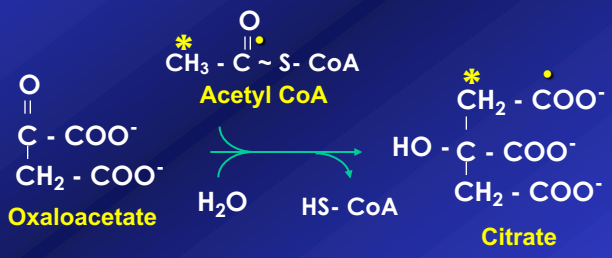
Citrate Synthase (CS)

-aldol condensation of Acetyl CoA and oxaloacetate
-involves two His and one Asp
- ordered reaction leading to tertiary changes
- induced fit caused by OAA binding forms reactive site



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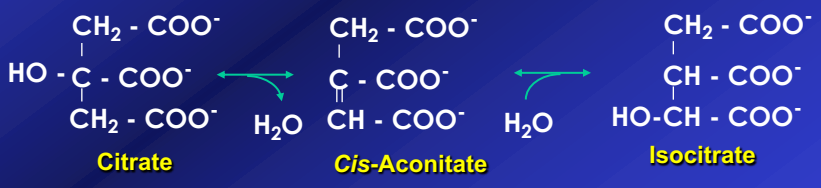
Aconitase - catalyzes the isomeration of citrate to isocitrate via steriospecific dehydration and rehydration. (a two step reaction).

- citrate -> isocitrate
- isomerization reaction
- 2 steps removal and addition of water
- Iron required - not heme
- iron-sulfur protein - bound by cys residues



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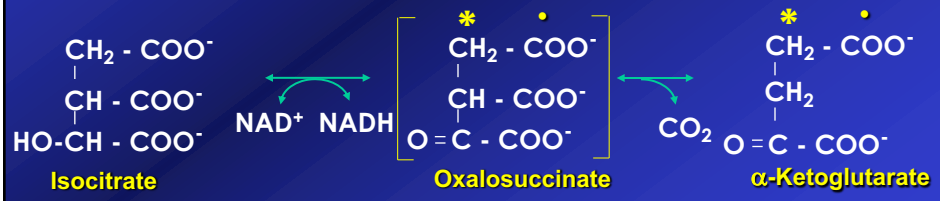
Isocitrate Dehydrogenase (IDH) - catalyzes the oxidative decarboxylation of isocitrate to produce an α-ketoglutarate and the first loss of CO_2 – remember that to get from citrate to OAA, you need to lose two carbons. This step also produces NADH.

- isocitrate -> [oxalosuccinate] -> α ketoglutarate
- first oxidative conversion
- 2 steps - all on same enzyme

  1 - oxidation of alcohol to ketone

  NAD^+ -> NADH (reduced) worth 3 ATPs

  2 - ß decarboxylation



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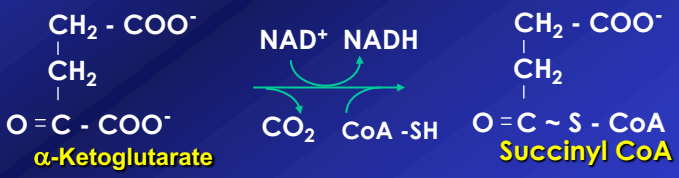
Alpha Ketoglutarate Dehydrogenase (α KGDH)

- another enzyme multienzyme complex similar to the PDH complex. αKGDH catalyzes the second oxidative decarboxylation. Note that the two carbons released as CO_2 in this round of the cycle are not the carbons that entered the cycle as acetyl-CoA

- generates CO_2, NADH and succinyl CoA
- analogous to PDH
- includes the same E_3 complex



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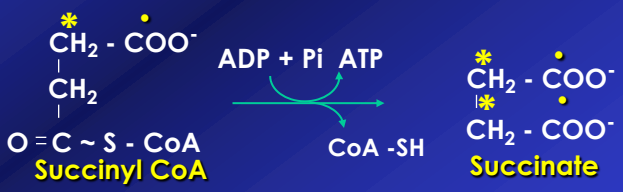
Succinate Thiokinase (STK) - AKA Succinyl-CoA synthetase –

The GTP is easily converted to ATP by NDPK. In some tissues and specific species this is an ATP specific enzyme

- succinyl CoA + GDP -> succinate + GTP + CoASH
- substrate level phosphorylation
- Hydrolysis of thioester bond of succinyl CoA -delta $G^{o'}$
- GTP used by G proteins or converted to ATP by NDPK
- two forms exist - in birds ATP is produced



Succinate is symmetric and SDH does not differentiate between The COOH groups, all four carbons are labeled after one turn

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Succinate Dehydrogenase (SDH)

- Succinate + FAD -> fumarate + $FADH_2$
- only non-matrix enzyme
- found bound to inner mitochondrial membrane
- facilitates transfer of FADH2 electrons to electron transport system
- iron sulfur center
- FAD generally acts to oxidize C-C to C=C
- While NAD^+ -> C-OH to C=O (aldehydes or ketones)
- FAD is covalently bound to protein - consequence?

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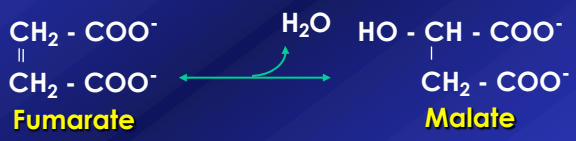
Fumarase

- fumarate -> malate
- specific addition of H_2O



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Malate Dehydrogenase (MDH)

- malate + NAD^+ -> malate + NADH
- oxidation to reform oxaloacetate

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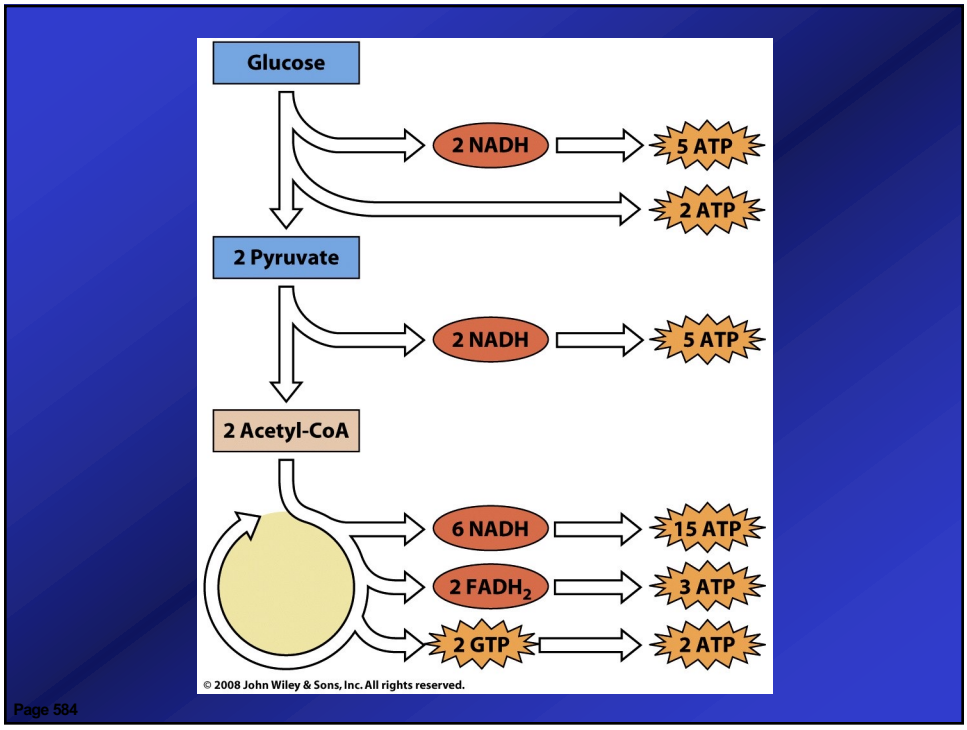

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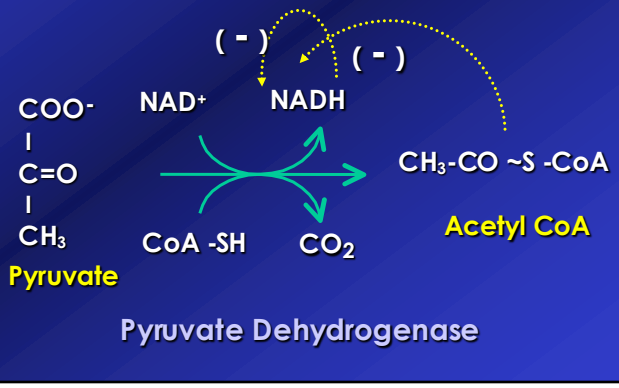
Regulation of PDH and the cycle

Regulated to meet needs of cell - don't waste energy

1 Feed back inhibition at PDH

- ACoA and NADH are allosteric inhibitors
  - Act by inhibiting E2 - shifting the equilibrium twords the acylated form. Leads to TTP build up and will decrease decarboxylation



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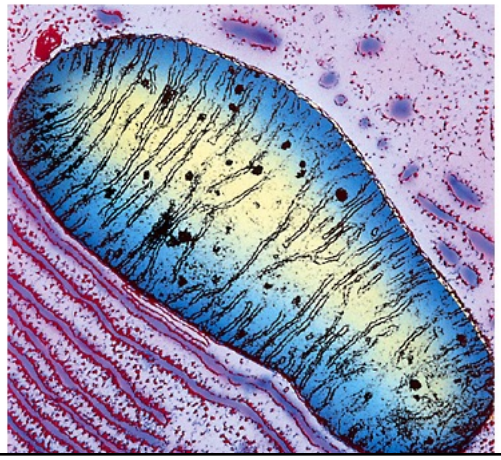
Mitochondria

- Outer membrane is very porous
- Inner membrane very tight. Transfer into and out of matrix is controlled - important in H^+ and shuttling reducing equivalents.
- membranes are topologically sided - different charge, lipids and proteins



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