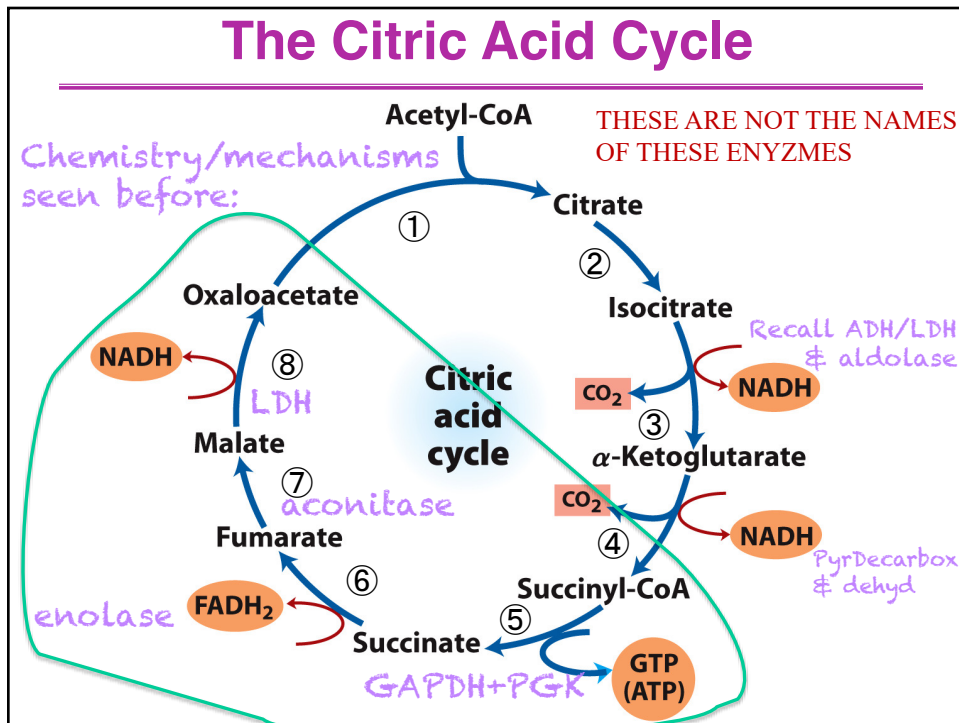
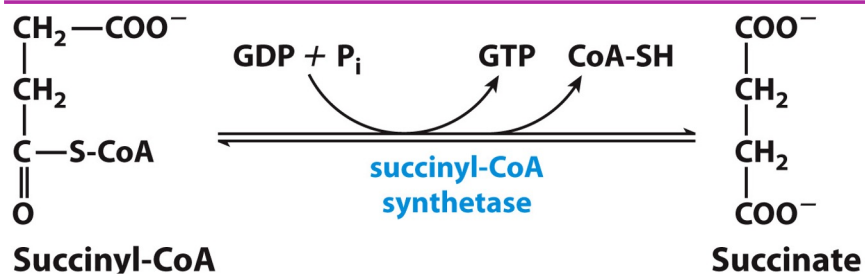


The Citric Acid Cycle



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The Citric Acid Cycle: Succinyl-CoA Synthetase

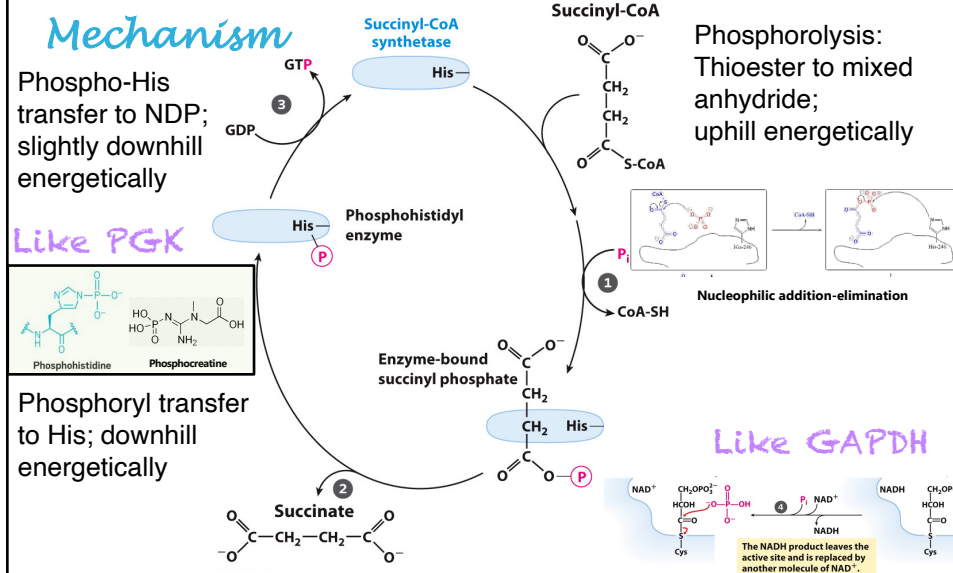


- This step was not appreciated in original cycle until discover of CoA and its role (Fritz Lipmann). Named for the reverse reaction.
- Substrate-level phosphorylation (like GAPDH + 1,3-BPG kinase)
- The energy of thioester allows for incorporation of inorganic phosphate.
- Goes through a phospho-enzyme intermediate
- Produces GTP, which can be converted to ATP
- Slightly thermodynamically favorable/reversible ($\Delta G^\circ = -0.7 \text{ kcal/mol}$).
 – product concentration kept low to pull forward

(OMSGAP)

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The Citric Acid Cycle: Succinyl-CoA Synthetase



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The Citric Acid Cycle

Citrate Synthase

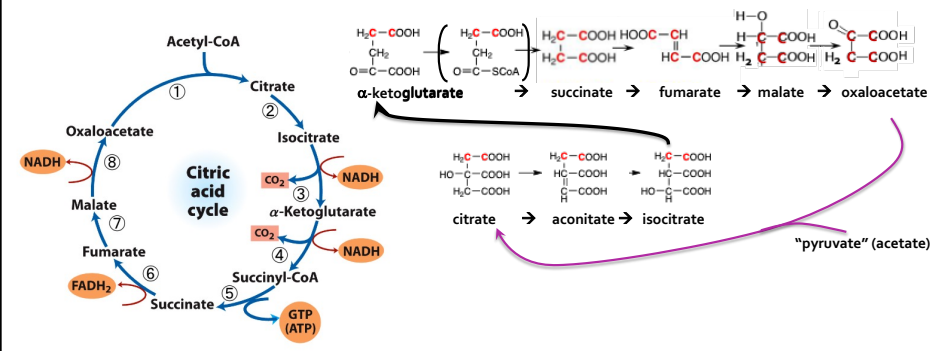
- ✓ Step 1: C-C bond formation between acetate (2C) and oxaloacetate (4C) to make citrate (6C)

Aconitase

Isocitrate Dehydrogenase & α-ketoglutarate DHC

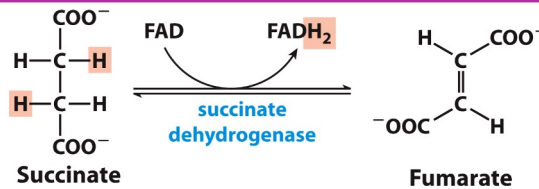
Succinyl-CoA Synthetase

- ✓ Step 2: Isomerization via dehydration/rehydration
- ✓ Step 3–4: Oxidative decarboxylations to give 2 NADH
- ✓ Step 5: Substrate-level phosphorylation to give GTP
- Step 6: Dehydrogenation to give FADH₂
- Step 7: Hydration
- Step 8: Dehydrogenation to give NADH



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The Citric Acid Cycle: Succinate Dehydrogenase

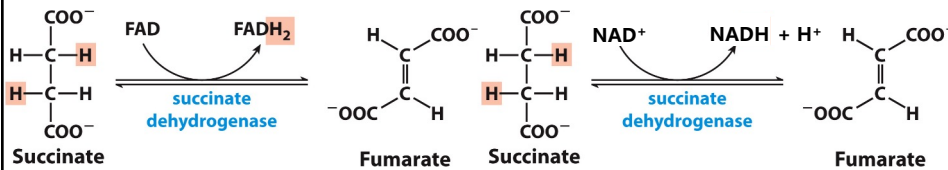


- We've seen this chemistry by enolase/aconitase, but those did a dehydration; not seen an alkane \rightarrow alkene oxidation. Most often it uses FAD as a cofactor.
- But we have seen these next 3 steps. If it worked once it will work again: Aconitase and ICDH (put in double-bond, hydrate, oxidize alcohol to ketone)
- Famous competitive inhibitor: malonate (OMSGAP)
- Reduction requires FADH_2 (generally true for alkane to alkene oxidation)
 - Reduction potential of carbon-hydrogen bond is too low for production of NADH.
 - The 2 hydrogens are removed stereo-specifically.
 - FAD is covalently bound at His, unusual
 - Has a series of 3 iron-sulfur clusters (*for re-oxidation of FADH_2 by transfer of electrons to electron-transport chain;more later*)
- Bound to mitochondrial inner membrane
 - Also known as (AKA) Complex II (*.....more later*)
- Near equilibrium/reversible ($\Delta G^\circ = -0.5 \text{ kcal/mol}$); [fumarate] kept low

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The Citric Acid Cycle: Succinate Dehydrogenase

Why use FAD, and not NAD^+ , for this reaction?



$$\Delta E^\circ = E^\circ(\text{reduction}) - E^\circ(\text{oxidation})$$

$$\Delta E^\circ = E^\circ(\text{FAD}) - E^\circ(\text{Fumarate})$$

$$= +0.031 \text{ V}^* - (+0.031 \text{ V})$$

$$= 0.0 \text{ V}$$

$$\Delta E^\circ = E^\circ(\text{NAD}) - E^\circ(\text{Fumarate})$$

$$= -0.320 \text{ V} - (+0.031 \text{ V})$$

$$= -0.351 \text{ V}$$

$$\Delta G^\circ = -n \mathcal{F} \Delta E^\circ$$

$$= -(2)(23.06 \text{ kcalV}^{-1} \text{ mol}^{-1})(-0.35 \text{ V})$$

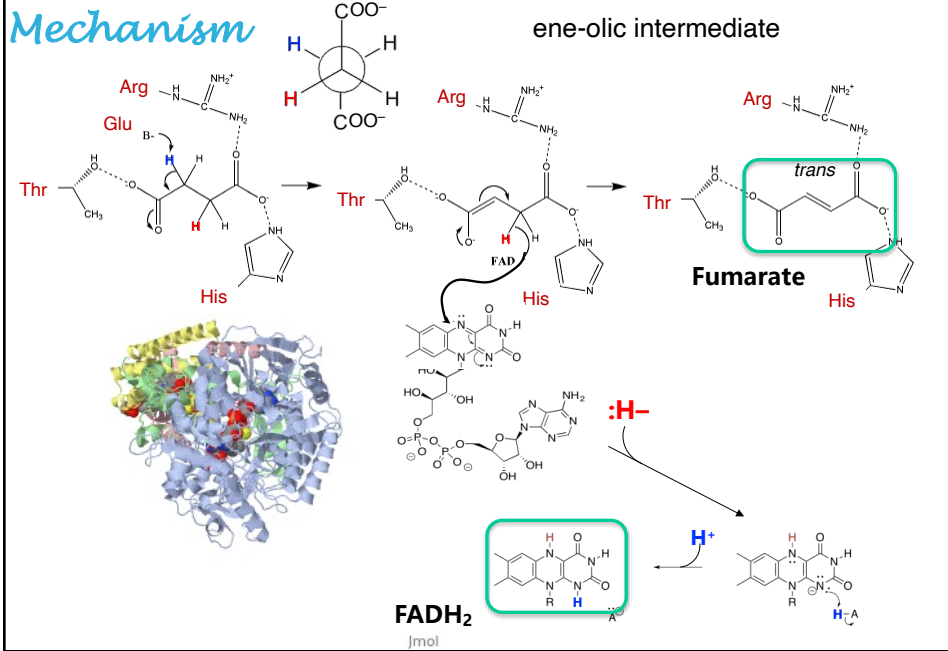
$$= +16 \text{ kcalmol}^{-1}$$

These are REDUCTION values for each half-reaction

Half reaction	Standard Redox Potentials E°	Half reaction	$E^\circ(\text{V})$
Succinate + $\text{CO}_2 + 2\text{H}^+ + 2e^-$	\leftrightarrow	Isotiglate + H_2O	-0.070
Acetate + $2\text{H}^+ + 2e^-$	\leftrightarrow	Acetaldehyde	-0.581
$2\text{H}^+ + 2e^-$	\leftrightarrow	H_2	-0.413
Isobutyrate + $\text{CO}_2 + 2\text{H}^+ + 2e^-$	\leftrightarrow	Isobutane	-0.380
Cysteine + $2\text{H}^+ + 2e^-$	\leftrightarrow	2 cysteine	-0.340
$\text{NAD}^+ + 2\text{H}^+ + 2e^-$	\leftrightarrow	$\text{NADH} + \text{H}^+$	-0.330
$\text{NADP}^+ + 2\text{H}^+ + 2e^-$	\leftrightarrow	$\text{NADPH} + \text{H}^+$	-0.324
Acetylaldehyde + $2\text{H}^+ + 2e^-$	\leftrightarrow	ethanol	-0.197
Pyruvate + $2\text{H}^+ + 2e^-$	\leftrightarrow	lactate	-0.185
Oxalacetate + $2\text{H}^+ + 2e^-$	\leftrightarrow	malate	-0.166
$\text{FAD} + 2\text{H}^+ + 2e^-$	\leftrightarrow	FADH_2	0.003
Fumarate + $2\text{H}^+ + 2e^-$	\leftrightarrow	succinate	0.031
Ubiquinone + $2\text{H}^+ + 2e^-$	\leftrightarrow	ubiquinol	0.045
2 cytochrome $b_{558} + 2e^-$	\leftrightarrow	2 cytochrome b_{558}	0.070
2 cytochrome $b_{560} + 2e^-$	\leftrightarrow	2 cytochrome b_{560}	0.254
2 cytochrome $b_{566} + 2e^-$	\leftrightarrow	2 cytochrome b_{566}	0.385
$\text{O}_2 + 2\text{H}^+ + 2e^-$	\leftrightarrow	H_2O	0.816

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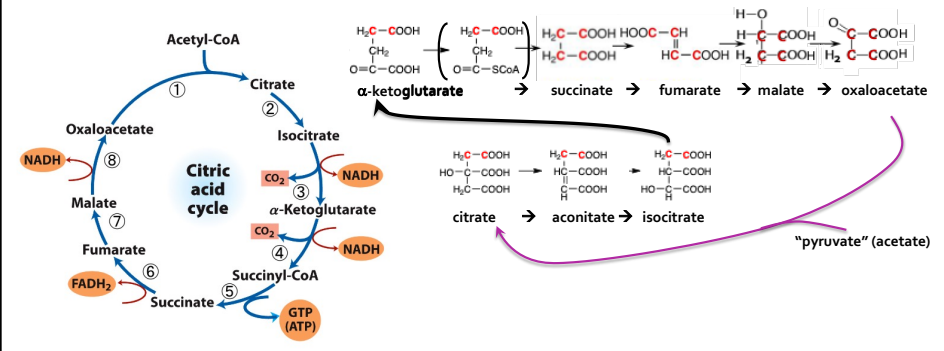
The Citric Acid Cycle: Succinate Dehydrogenase



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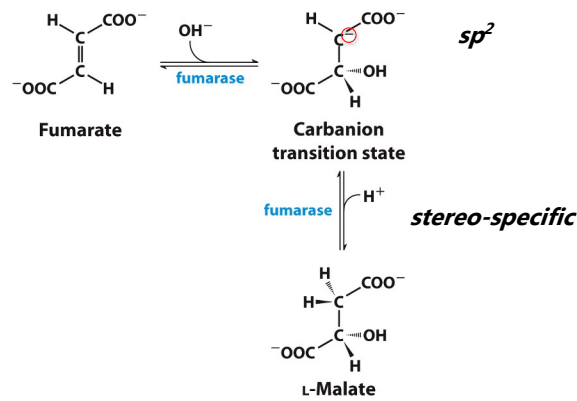
The Citric Acid Cycle

- Citrate Synthase** ✓ • Step 1: C-C bond formation between acetate (2C) and oxaloacetate (4C) to make citrate (6C)
- Aconitase** ✓ • Step 2: Isomerization via dehydration/rehydration
- Isocitrate Dehydrogenase & α-ketoglutarate DHC** ✓ • Steps 3–4: Oxidative decarboxylations to give 2 NADH
- Succinyl-CoA Synthetase** ✓ • Step 5: Substrate-level phosphorylation to give GTP
- Succinate dehydrogenase** ✓ • Step 6: Dehydrogenation to give FADH₂
- Step 7: Hydration
- Step 8: Dehydrogenation to give NADH



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The Citric Acid Cycle: Fumarase



• Stereospecific

- Addition of water is always trans and forms L-malate.
- OH⁻ adds to fumarate... then H⁺ adds to the carbanion.
- Cannot distinguish between "acetates," no 3-point attachment, so either can gain -OH

• Slightly thermodynamically favorable/reversible ($\Delta G^\circ = -0.9 \text{ kcal/mol}$).

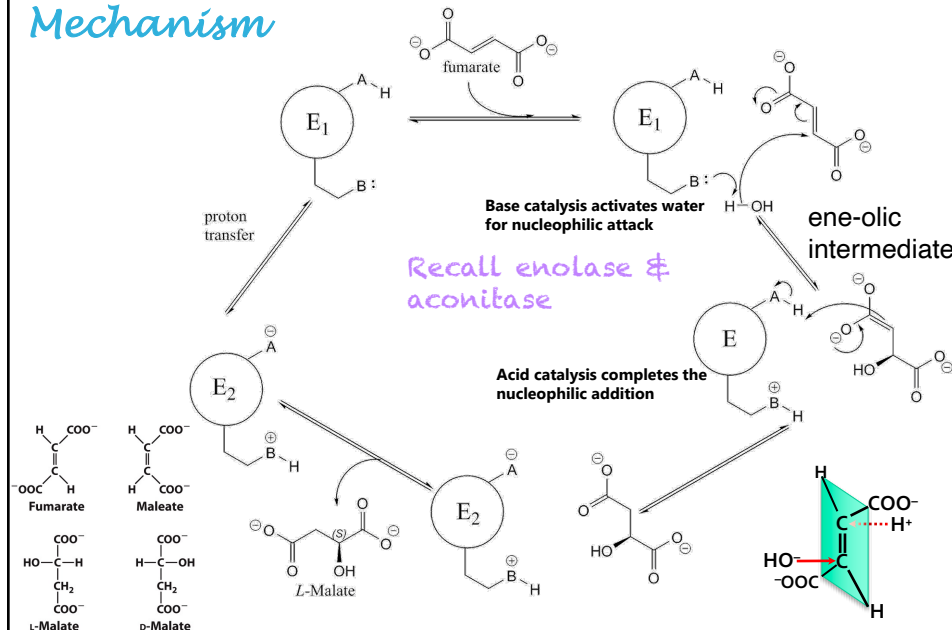
- product concentration kept low to pull reaction forward

$\Delta G'^\circ = -3.8 \text{ kJ/mol}$

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The Citric Acid Cycle: Fumarase

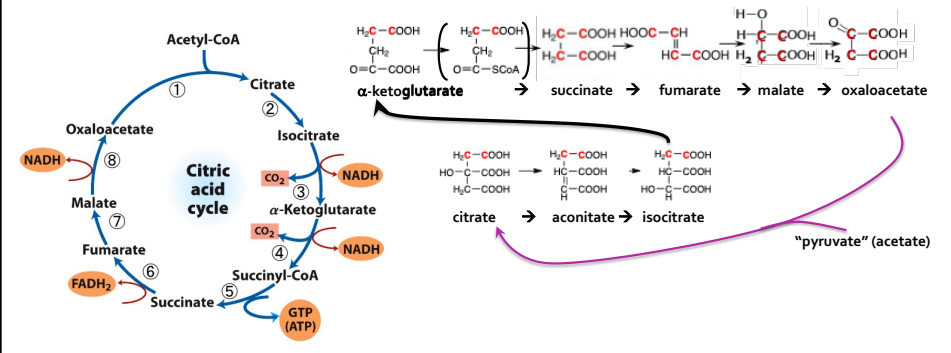
Mechanism



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The Citric Acid Cycle

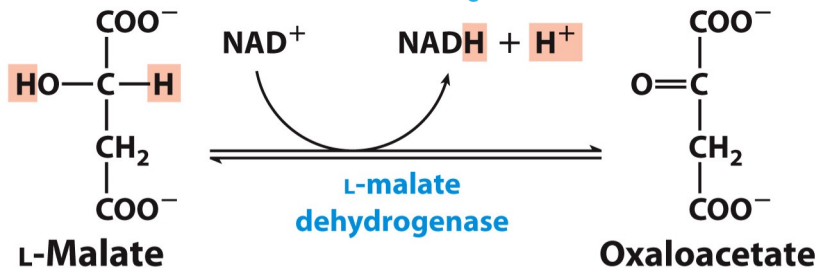
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- Step 8: Dehydrogenation to give NADH



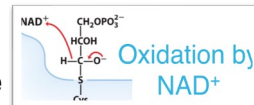
86

The Citric Acid Cycle: Malate Dehydrogenase

Oxidation of Alcohol to a Ketone and Regeneration of Oxaloacetate



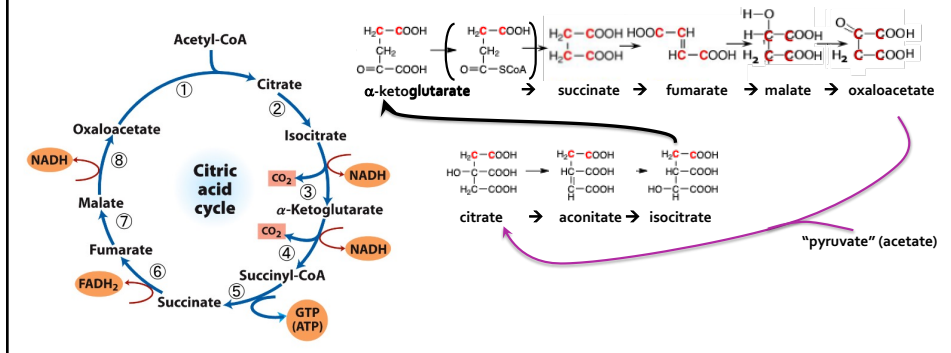
- Final step of the cycle
- Regenerates oxaloacetate for citrate synthase
- Highly thermodynamically **UNfavorable** ($\Delta G^\circ = +7.1 \text{ kcal/mol}$).
 - Like GAPDH/LDH
 - Reversible
 - oxaloacetate concentration kept VERY low by citrate synthase
 - pulls the reaction forward ($-7.7 + 7.1 = -0.6$)



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The Citric Acid Cycle

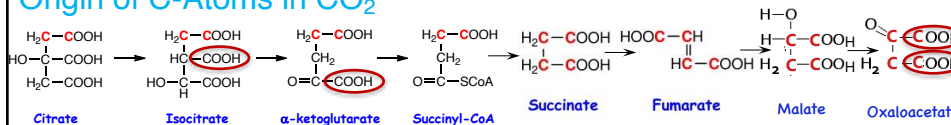
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- Fumarase** ✓ ✓ • Step 7: Hydration
- Malate Dehydrogenase** ✓ ✓ • Step 8: Dehydrogenation to give NADH



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The Citric Acid Cycle

Origin of C-Atoms in CO₂



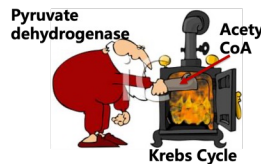
Energetics: why the cycle only turns in one direction

$\Delta G^{\circ} =$

-7.7	+3.2	-2.0	-7.2	-0.7	0	-0.9	+7.1	each step
-7.7	-4.5	-6.5	-13.7	-14.4	-14.4	-15.3	-8.2	cumulative

- **Overall energetics makes cycle irreversible:**
–Citrate synthase, IDH, and KDH
- Carbons from acetyl-CoA are red.
- All CO₂ generated during the citric acid cycle is produced before succinyl-CoA is made.
- In one turn of the citric acid cycle, neither of the acetyl-CoA carbons is lost.
- Both of the CO₂ molecules lost were present on the oxaloacetate used to begin the cycle.

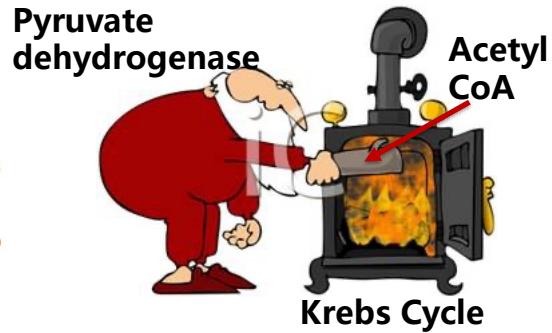
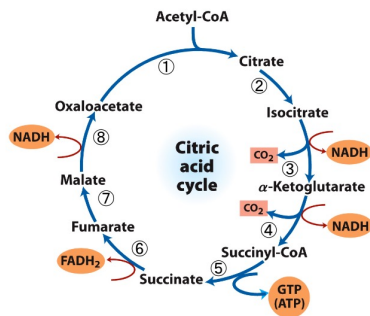
- What about kinetics?
- Nature of a cycle; acts as a unit and goes as fast as the available carbon



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The Citric Acid Cycle

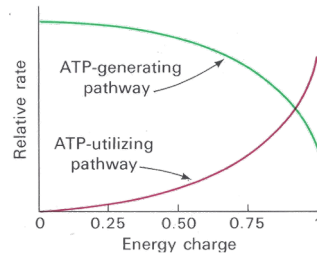
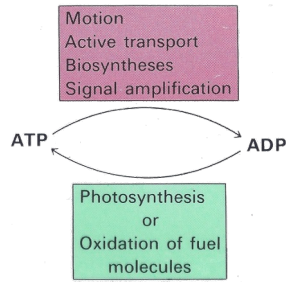
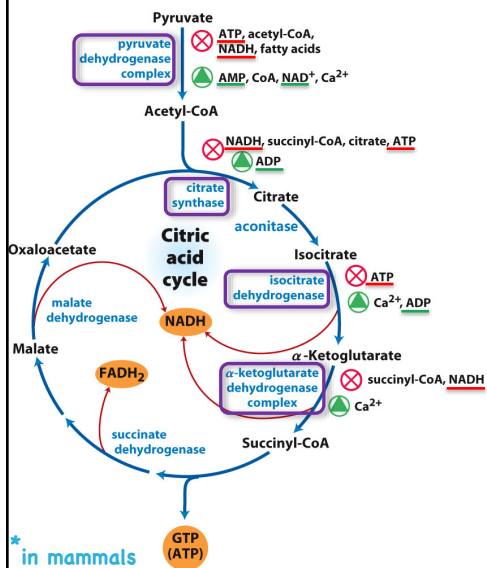
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- Fumarase** ✓ • Step 7: Hydration
- Malate Dehydrogenase** ✓ • Step 8: Dehydrogenation to give NADH



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The Citric Acid Cycle

Regulation*

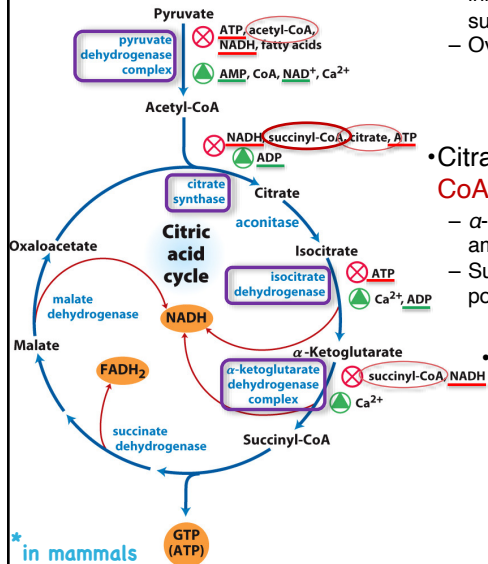


* in mammals

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The Citric Acid Cycle

Regulation*



- General regulatory mechanism
 - activated by substrate availability
 - inhibited by **product accumulation** (acetyl-CoA, succinyl-CoA, citrate)
 - Overall products of the pathway are **NADH** and **ATP**
 - affects all regulated enzymes in the cycle
 - **inhibitors**: NADH and ATP
 - **activators**: NAD⁺, ADP, and AMP
- Citrate synthase is also inhibited by **succinyl-CoA**. Classic feedback inhibition
 - α-Ketoglutarate is an important branch point for amino acid metabolism.
 - Succinyl-CoA communicates flow at this branch point to the start of the cycle.
- Regulation of isocitrate dehydrogenase controls citrate levels; important for fatty acid metabolism. Energy Charge.
 - Aconitase is reversible.
 - Inhibition of IDH leads to accumulation of isocitrate and reverses aconitase.
 - Accumulated citrate leaves mitochondria and inhibits phosphofructokinase-1 in glycolysis.

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The Citric Acid Cycle

Yield (TCA): $\text{Acetyl-CoA} + 3\text{NAD}^+ + \text{FAD} + \text{GDP} + \text{P}_i + 2 \text{H}_2\text{O} \rightarrow$

$2\text{CO}_2 + 3\text{NADH} + \text{FADH}_2 + \text{GTP} + \text{CoA} + 3\text{H}^+$

Yield (from pyruvate):

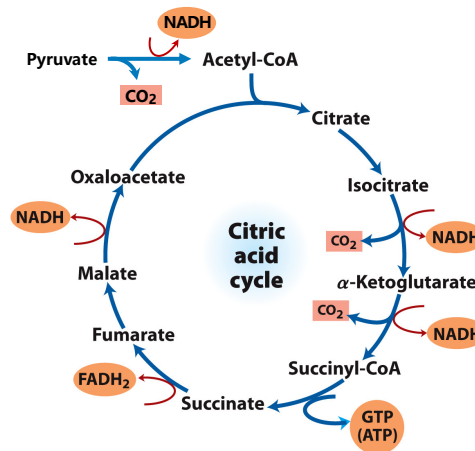
$\text{Pyruvate} + 4\text{NAD}^+ + \text{FAD} + \text{GDP} + \text{P}_i + 2 \text{H}_2\text{O} \rightarrow 3\text{CO}_2 + 4\text{NADH} + \text{FADH}_2 + \text{GTP} + 3\text{H}^+$

From acetyl-CoA:

- Net oxidation of two carbons to CO_2
 - equivalent to two carbons of acetyl-CoA
 - but NOT the exact same carbons
- Energy captured by electron transfer to NADH and FADH_2
- Generates 1 GTP, which can be converted to ATP
- Cycle acts as a unit; it's basically a furnace for burning carbon



.....except we don't have the water yet!



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Pyruvate Oxidation & Citric Acid Cycle

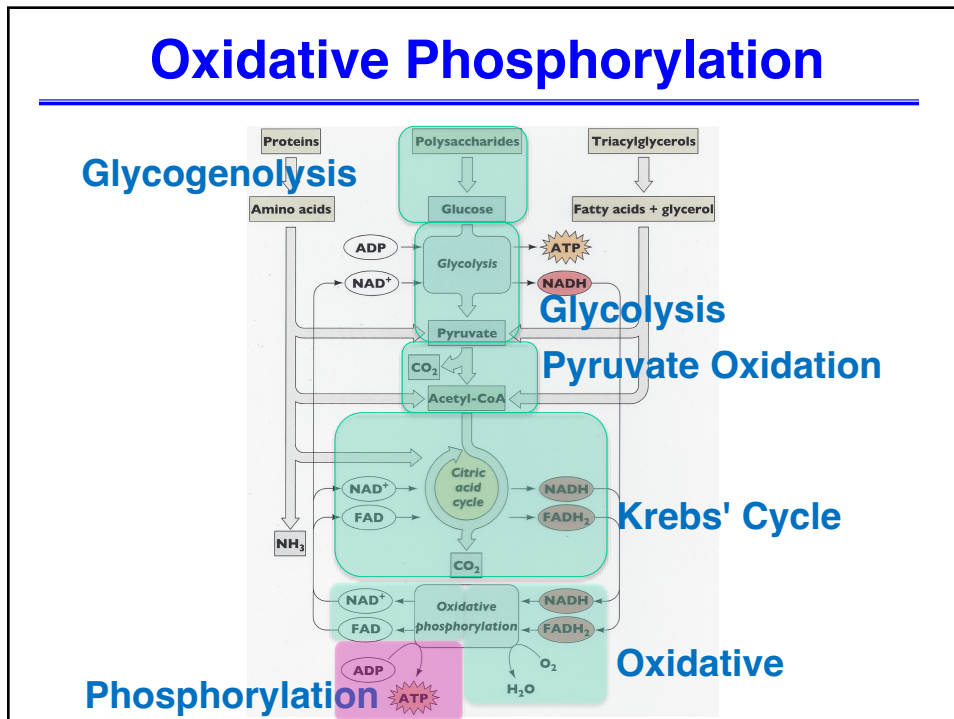
Summary

We learned that:

- a large multi-subunit enzyme, **pyruvate dehydrogenase** complex, converts pyruvate into acetyl-CoA
- **several cofactors** are involved in reactions that harness the energy from pyruvate
- the citric acid cycle is an **important catabolic process**: it makes reduced cofactors (**NADH & FADH₂**), plus **GTP**, that could yield **ATP**
- the rules of organic chemistry help to **rationalize reactions** in the citric acid cycle
- the citric acid cycle is largely **regulated by** availability of substrates and product inhibition (especially **NADH and ATP**)

Oxidative Phosphorylation

2



3

Oxidative Phosphorylation

Learning goals:

- Function of electron-transport chain in mitochondria.....
make water (finish the reaction: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$)
- Building up the proton-motive force
- Synthesis of ATP in mitochondria, chloroplasts, & bacteria