BB 422/622

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
- Introduction & overview; 2 phases
- Phase I
- Phase II
- Summary: logic, energetics, labeling studies

Other sugars
Pasteur: Anaerobic vs Aerobic
Fermentations: anaerobic fates of pyruvate
- Lactate-lactate dehydrogenase
- Exam-1 material Acetoacetate decarboxylase

- Exam-2 material Ethanol-pyruvate decarboxylase & alcohol dehydrogenase

Pyruvate oxidation:
aerobic fates of pyruvate
pyruvate dehydrogenase complex

Krebs' Cycle
How did he figure it out?
Overview
8 Steps
- Citrate Synthase
- Aconitase
- Isocitrate dehydrogenase
- Ketoglutarate dehydrogenase
- Succinyl-CoA synthetase
- Succinate dehydrogenase
- Fumarase
- Malate dehydrogenase

Energetics; Regulation
Summary

Oxidative Phosphorylation
Electron Transport
Chemiosmotic theory
ATP synthesis

Clinical Correlations

CLINICAL INSIGHT

Variations in K_M Can Have Physiological Consequences

Two enzymes play a key role in the metabolism of alcohol.

$$\underset{\text{Ethanol}}{CH_3CH_2OH} + NAD^+ \underset{}{\xrightleftharpoons{\text{Alcohol dehydrogenase}}} \underset{\text{Acetaldehyde}}{CH_3CHO} + NADH + H^+$$

$$CH_3CHO + NAD^+ + H_2O \xrightleftharpoons{\text{Aldehyde dehydrogenase}} CH_3COO^- + NADH + 2H^+$$

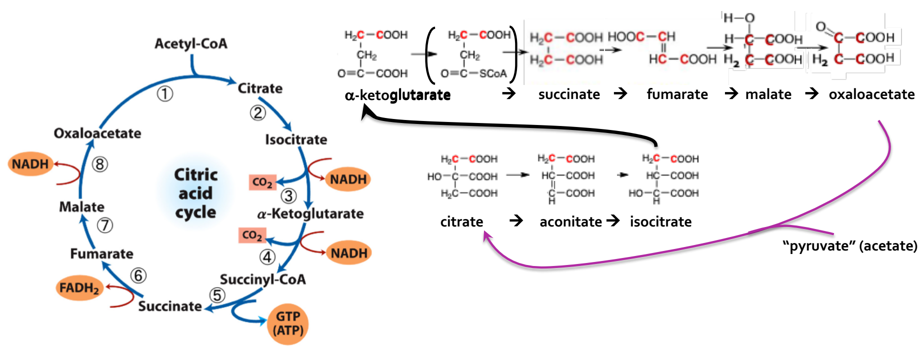
Some people respond to alcohol consumption with facial flushing and rapid heart beat, symptoms caused by excessive amounts of acetaldehyde in the blood. There are two different **acetaldehyde dehydrogenases** in most people, one with a low K_M and one with a high K_M.

The low K_M enzyme is genetically inactivated in some individuals. The enzyme with the high K_M cannot process all of the acetaldehyde, and so some acetaldehyde appears in the blood.

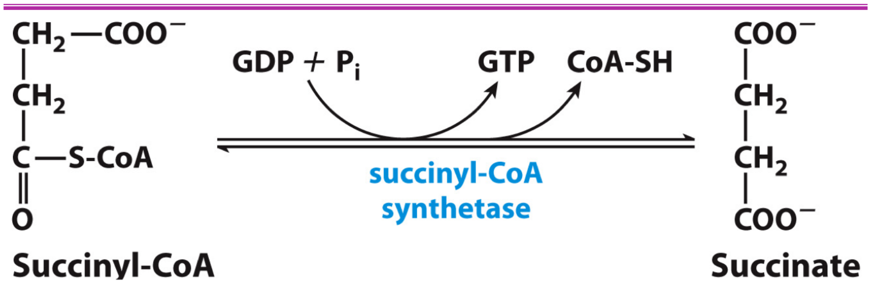
Knowing the values of the constants, K_m and V_{max}, for enzymes is important

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
ICDH & αKGDH ✓	• Steps 3–4: Oxidative decarboxylations to give 2 NADH
Suc-CoA Synthetase	• Step 5: Substrate-level phosphorylation to give GTP
	• Step 6: Dehydrogenation to give $FADH_2$
	• Step 7: Hydration
	• Step 8: Dehydrogenation to give NADH



The Citric Acid Cycle: Succinyl-CoA Synthetase



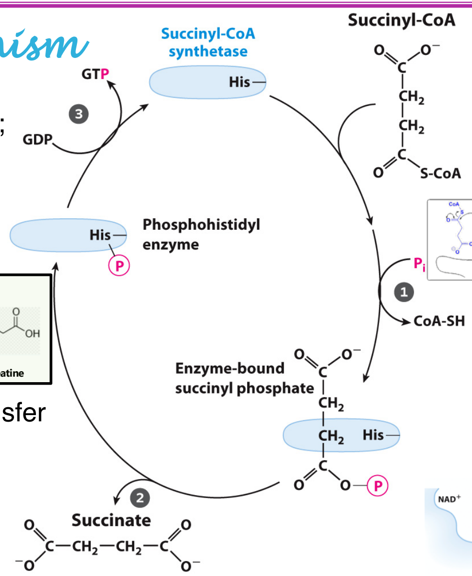
- This step was not appreciated in original cycle until discover of CoA and its role (Fritz Lipmann). Named for the reverse reaction.
- Substrate-level phosphorylation (like GAPDH + 1,3-BPG kinase)
- The energy of thioester allows for incorporation of inorganic phosphate.
- Goes through a phospho-enzyme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

(OMSGAP)

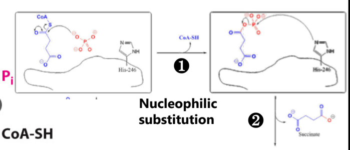
The Citric Acid Cycle: Succinyl-CoA Synthetase

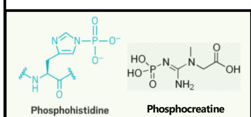
Mechanism

Phospho-His transfer to NDP; slightly downhill energetically

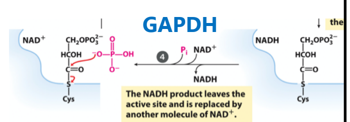


Phosphorolysis: Thioester to mixed anhydride; uphill energetically

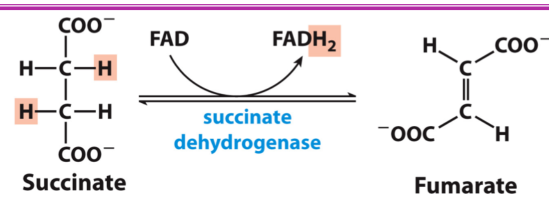




Phosphoryl transfer to His; downhill energetically



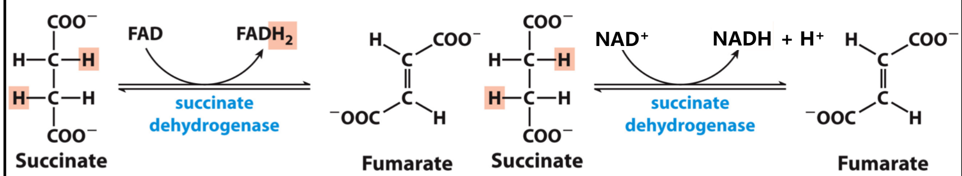
The Citric Acid Cycle: Succinate Dehydrogenase



- We've seen this chemistry by enolase/aconitase, but those did a dehydration; not seen an alkane → 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 dbl bond, hydrate, oxidize to ketone)
- Famous competitive inhibitor: malonate (O**M**SGAP)
- 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
  - A.K.A. Complex II *(more later)*
- Near equilibrium/reversible ($\Delta G^{\circ\prime} = -0.5$ kcal/mol); [fumarate] kept low

The Citric Acid Cycle: Succinate Dehydrogenase

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



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

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

$= +0.031\ V^* - (+0.031\ V)$

$= 0.0\ V$

These are REDUCTION values for each half-reaction

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

$= -0.320\ V - (+0.031\ V)$

$= -0.351\ V$

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

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

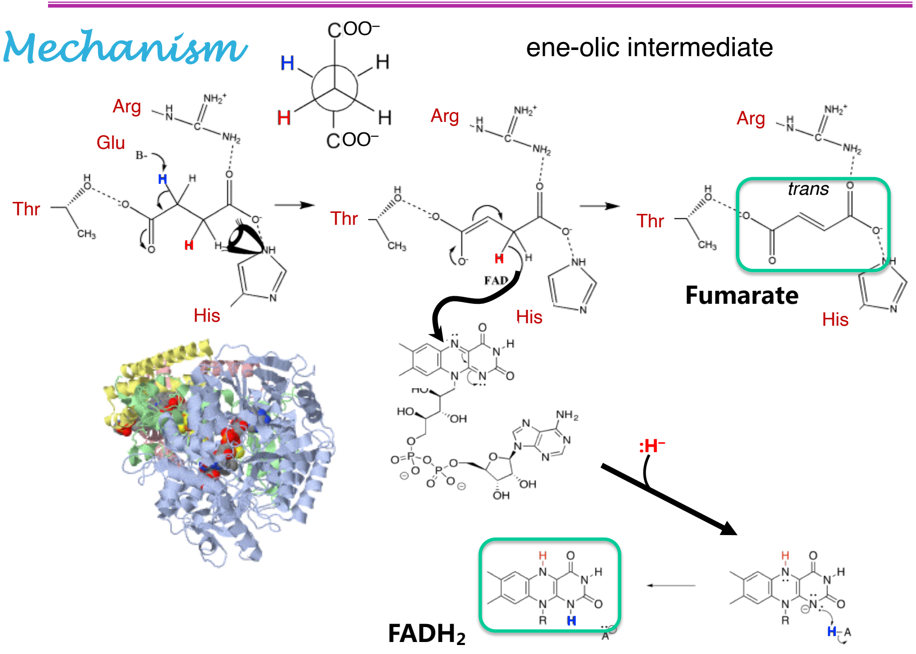
$= +16\ kcalmol^{-1}$

Standard Redox Potentials E'_0

Half reaction		Half reaction	$E'_0(V)$
Succinate + CO_2 + $2H^+$ +$2e^-$	↔	α-ketoglutarate + H_2O	-0.670
Acetate + $2H^+$ +$2e^-$	↔	acetaldehyde	-0.581
$2H^+$ +$2e^-$	↔	H_2	-0.421
α-ketoglutarate + CO_2 + $2H^+$ +$2e^-$	↔	citrate	-0.380
Cystine + $2H^+$ +$2e^-$	↔	2 cysteine	-0.340
NAD^+ + $2H^+$ +$2e^-$	↔	NADH + H^+	-0.320
$NADP^+$ + $2H^+$ +$2e^-$	↔	NADPH + H^+	-0.324
Acetaldehyde + $2H^+$ +$2e^-$	↔	ethanol	-0.197
Pyruvate + $2H^+$ +$2e^-$	↔	lactate	-0.185
Oxaloacetate + $2H^+$ +$2e^-$	↔	malate	-0.166
FAD + $2H^+$ +$2e^-$	↔	$FADH_2$	0.031
Fumarate + $2H^+$ +$2e^-$	↔	succinate	0.031
Ubiquinone + $2H^+$ +$2e^-$	↔	ubiquinol	0.045
2 cytochrome $b_{(ox)}$ +$2e^-$	↔	2 cytochrome $b_{(red)}$	0.070
2 cytochrome $c_{(ox)}$ +$2e^-$	↔	2 cytochrome $c_{(red)}$	0.254
2 cytochrome $a_{3(ox)}$ +$2e^-$	↔	2 cytochrome $a_{3(red)}$	0.385
1/2 O_2 + $2H^+$ +$2e^-$	↔	H_2O	0.816

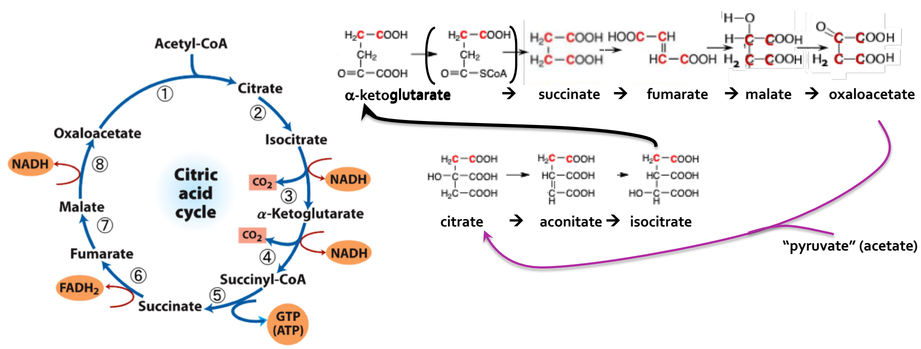
The Citric Acid Cycle: Succinate Dehydrogenase

Mechanism

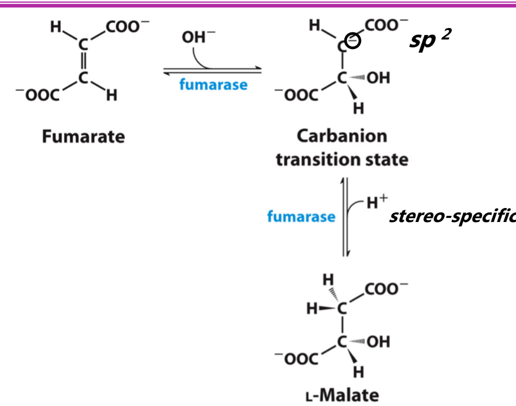


The Citric Acid Cycle

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
ICDH & αKGDH	✓	Steps 3–4: Oxidative decarboxylations to give 2 NADH
Suc-CoA Synthetase	✓	Step 5: Substrate-level phosphorylation to give GTP
Succinate dehydrogenase	✓	Step 6: Dehydrogenation to give $FADH_2$
		Step 7: Hydration
		Step 8: Dehydrogenation to give NADH



The Citric Acid Cycle: Fumarase

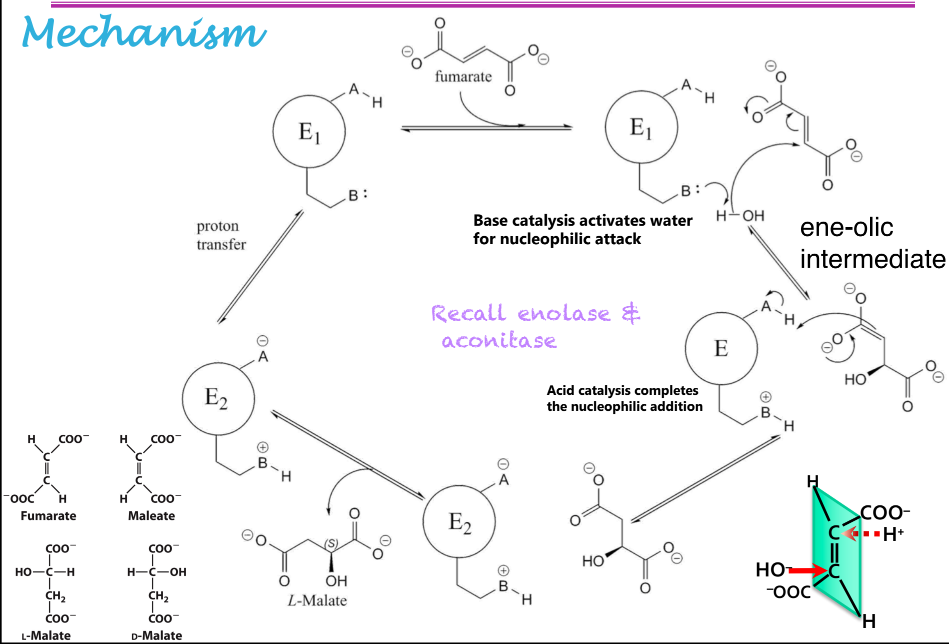


- Stereospecific

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

  - 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 ($\Delta G^{o'}$ = –0.9 kcal/mol).
  - product concentration kept low to pull reaction forward

The Citric Acid Cycle: Fumarase



The Citric Acid Cycle

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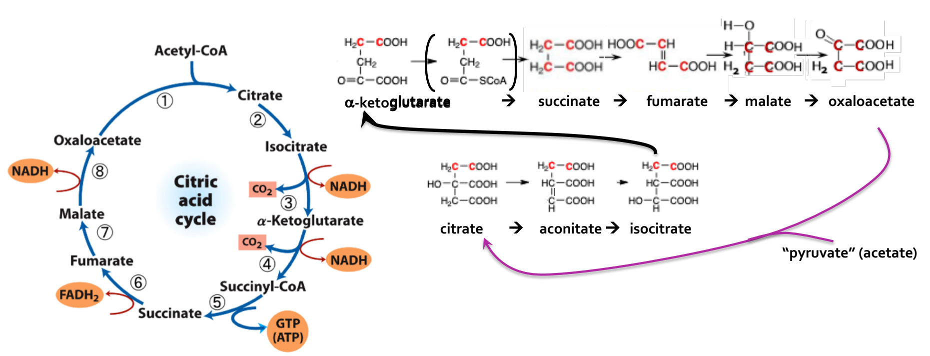
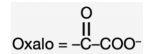
Aconitase	✓	• Step 2: Isomerization via dehydration/rehydration
ICDH & αKGDH	✓	• Steps 3–4: Oxidative decarboxylations to give 2 NADH
Suc-CoA Synthetase	✓	• Step 5: Substrate-level phosphorylation to give GTP
SucDH	✓	• Step 6: Dehydrogenation to give $FADH_2$
Fumarase	✓	• Step 7: Hydration
		• Step 8: Dehydrogenation to give NADH



The Citric Acid Cycle: Malate Dehydrogenase



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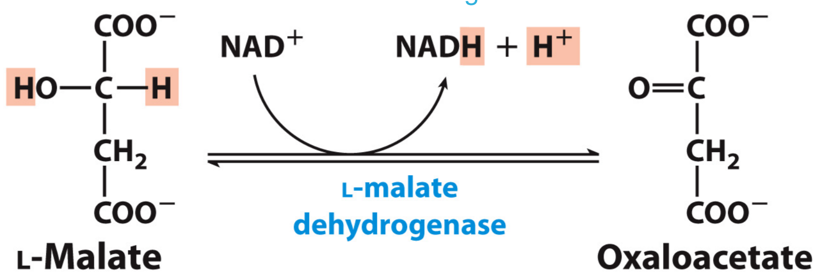
Oxidation of Alcohol to a Ketone and Regeneration of Oxaloacetate



- Final step of the cycle
- Regenerates oxaloacetate (α-ketosuccinate) for citrate synthase
- Highly thermodynamically **UNfavorable** ($\Delta G^{o\prime}$ = +7.1 kcal/mol).
  - Similar to LDH & ADH
  - Reversible
  - oxaloacetate concentration kept VERY low by citrate synthase
    - pulls the reaction forward (–7.7 + 7.1 = –0.6)

The Citric Acid Cycle

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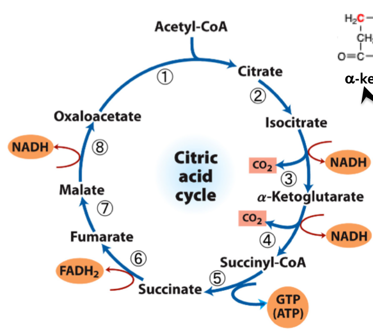
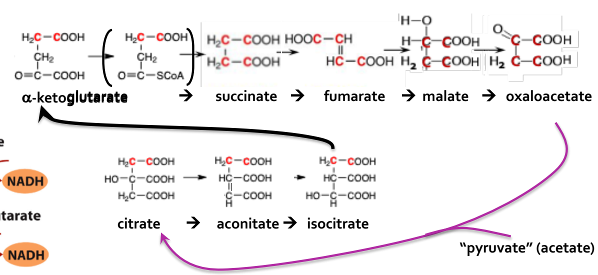
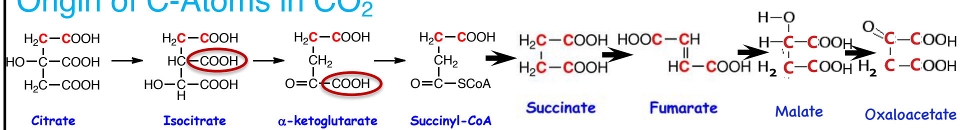
Suc-CoA Synthetase	✓	Step 5: Substrate-level phosphorylation to give GTP
SucDH	✓	Step 6: Dehydrogenation to give $FADH_2$
Fumarase	✓	Step 7: Hydration
Malate DH	✓	Step 8: Dehydrogenation to give NADH





The Citric Acid Cycle

Origin of C-Atoms in CO_2

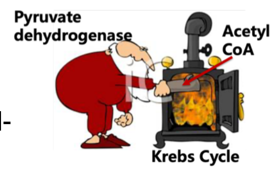


Energetics: why the cycle only turns in one direction

$\Delta G^{o\prime} =$

-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 acetate are red.
- All CO_2 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 CO_2 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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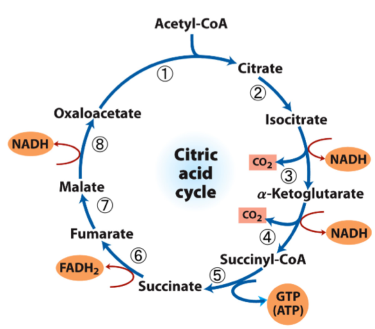
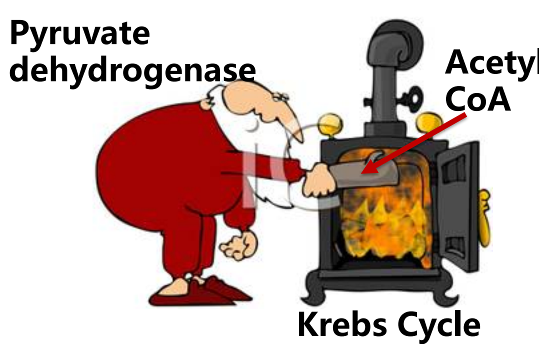
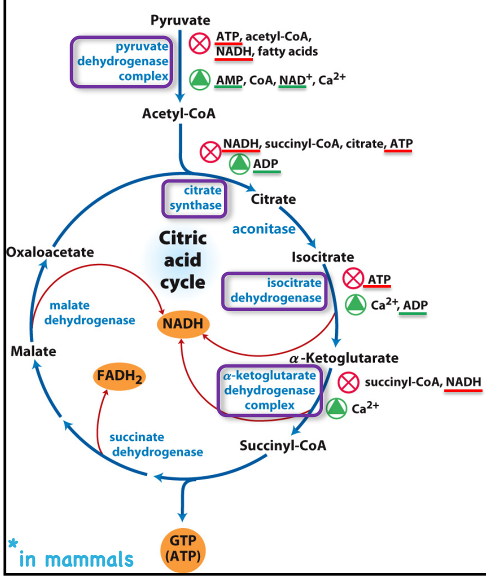
Malate DH	✓	• Step 8: Dehydrogenation to give NADH

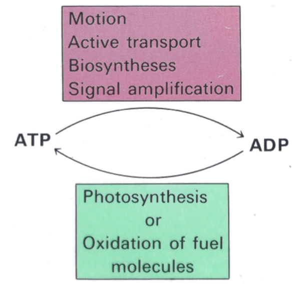


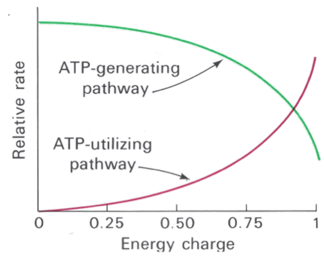


The Citric Acid Cycle

Regulation*



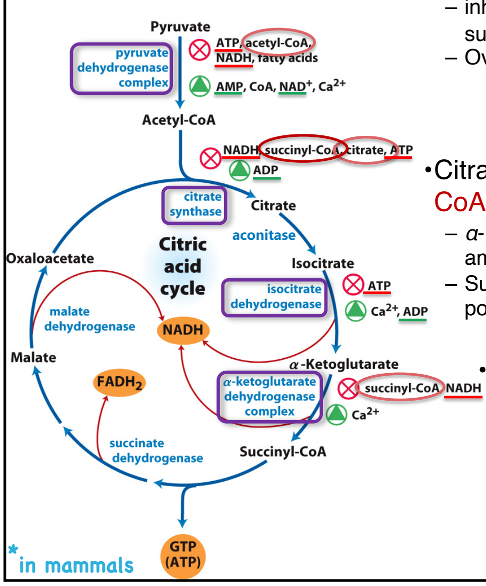




*in mammals

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 in glycolysis.

*in mammals

The Citric Acid Cycle

Yield (TCA): Acetyl-CoA + 3NAD$^+$ + FAD + GDP + P_i + 2 H_2O → $2CO_2$ + 3NADH + $FADH_2$ + GTP + CoA + 3H$^+$

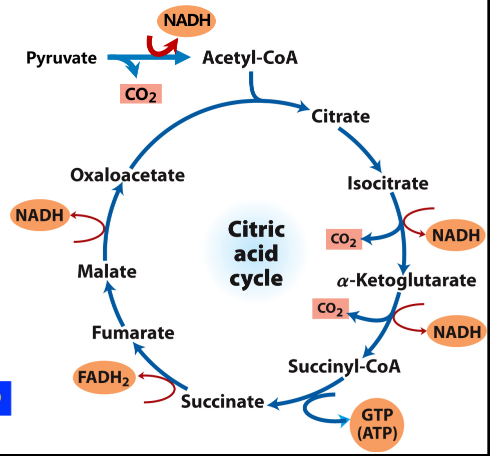
Yield (from pyruvate):
Pyruvate + 4NAD$^+$ + FAD + GDP + P_i + 2 H_2O → $3CO_2$ + 4NADH + $FADH_2$ + GTP + 3H$^+$

From acetyl-CoA:

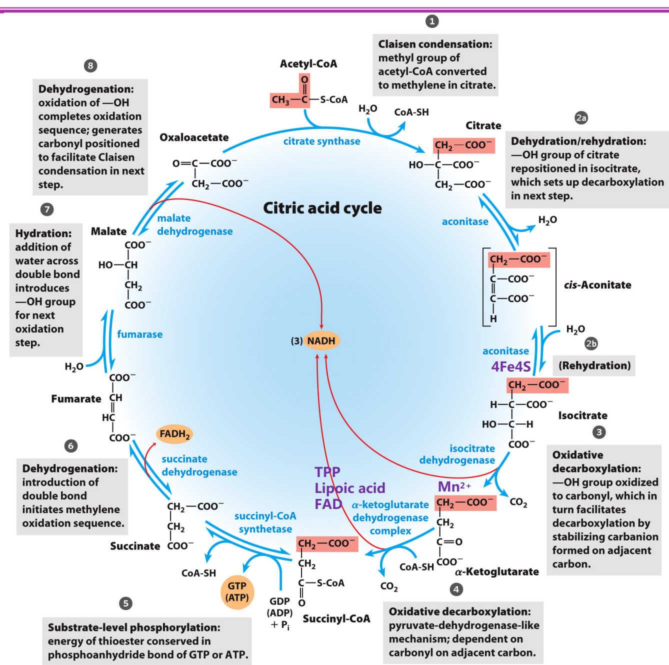
- Net oxidation of two carbons to CO_2
  - equivalent to two carbons of acetyl-CoA
  - but NOT the exact same carbons
- Energy captured by electron transfer to NADH and $FADH_2$
- Generates 1 GTP, which can be converted to ATP
- Cycle acts as a unit; its basically a furnace for burning carbon

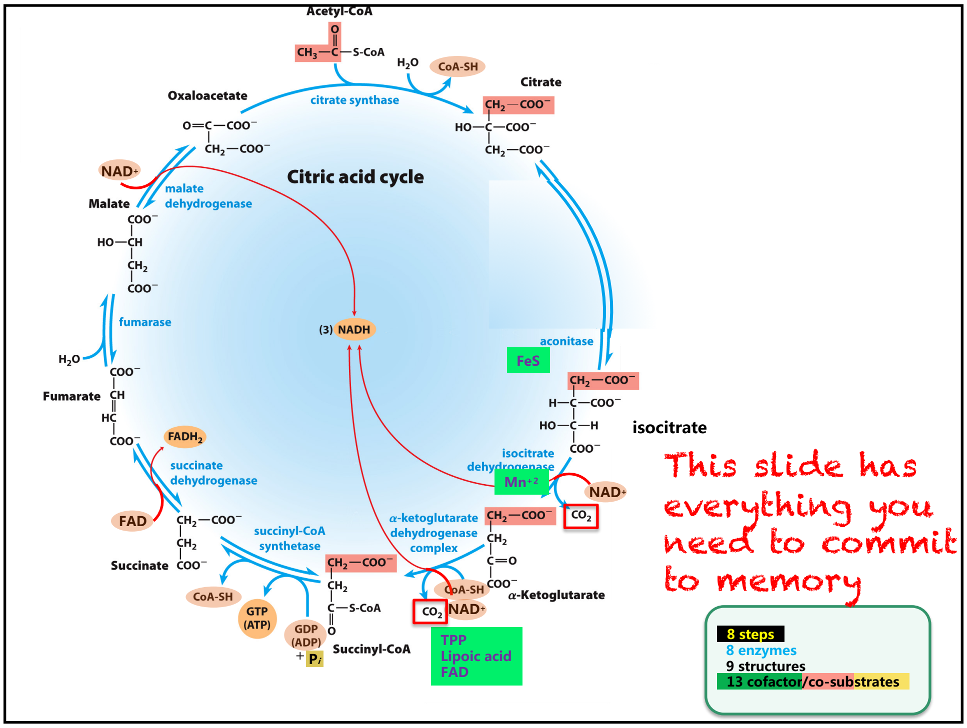
$C_3H_6O_3 + 3O_2 \longrightarrow 3CO_2 + 3H_2O$

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



The Citric Acid Cycle: Summary





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 & $FADH_2$), 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)