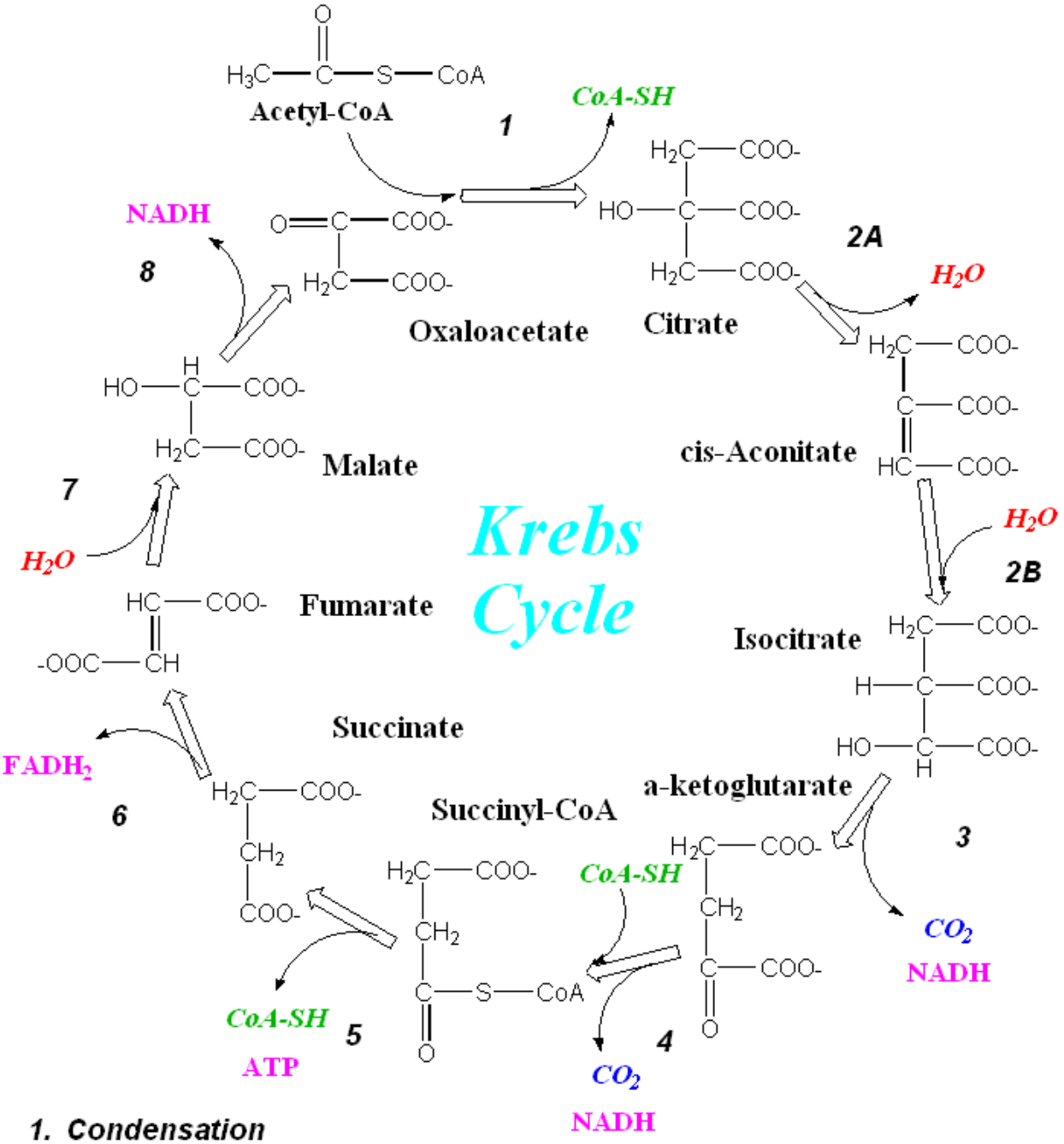
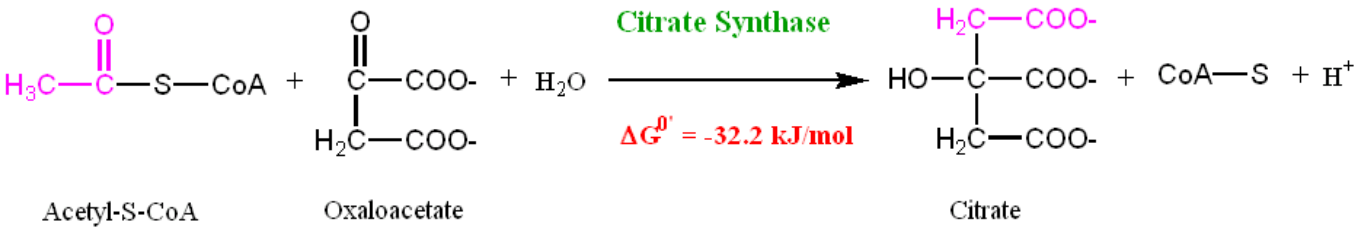
The Citric Acid (Krebs, TCA) Cycle



1. *Condensation*
2. *Dehydration*
2. *Hydration*
3. *Oxidative decarboxylation*
4. *Oxidative decarboxylation*
5. *Substrate level phosphorylation*
6. *Dehydrogenation*
7. *Hydration*
8. *Dehydrogenation*

Step 1: Condensation

In step 1 of the Krebs cycle, the two-carbon compound, ***acetyl-S-CoA***, participates in a condensation reaction with the four-carbon compound, ***oxaloacetate***, to produce ***citrate***:



- This reacion is moderately *exergonic*. Thermodynamically, the equilibrium is in favor of the products. Thus, this is considered to be the first *committed* step of the Krebs cycle
- Being the first committed step, this is a likely step to have some kind of regulatory control mechanism (which will effectively regulate the entire cycle)
- The Krebs cycle is also known as the *citric acid cycle*. Citrate is a tricarboxylic acid, and the Krebs cycle is also known as the *tricarboxylic acid* (or *TCA*) cycle

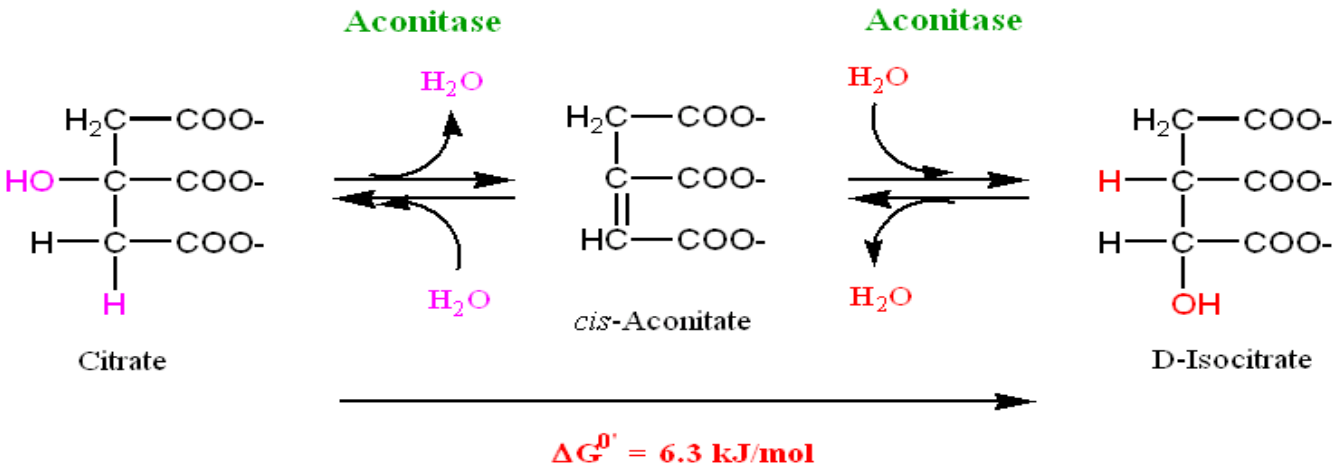
Step 2. Isomerization of Citrate

As we will see later on in the Krebs cycle, there will be a *decarboxylation* reaction.

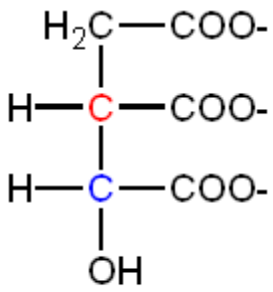
- Such decarboxylation reactions usually involve α- (or β-) keto acids
- The hydroxyl group of citrate can be oxidized to yield a keto group, but to form an α-keto acid it needs to be adjacent to one of the terminal carboxyl groups

Thus, step 2 involves moving the hydroxyl group in the citrate molecule so that we can later form an α-keto acid

- This process involves a sequential dehydration and hydration reaction, to form the *D-Isocitrate* isomer (with the hydroxyl group now in the desired α- location), with *cis-Aconitase* as the intermediate
- A single enzyme, *Aconitase*, performs this two-step process:



- This reaction is endergonic, so the equilibrium is in favor of the *reactants* and not the desired product. However, the exergonic character of the *next* reaction in the cycle helps shift the equilibrium of *this* reaction towards the right.
- There are two asymmetric centers in the D-Isocitrate molecule. Eeach can adopt either the L- or D- rotamer, thus there are 4 possible isomers of this molecule



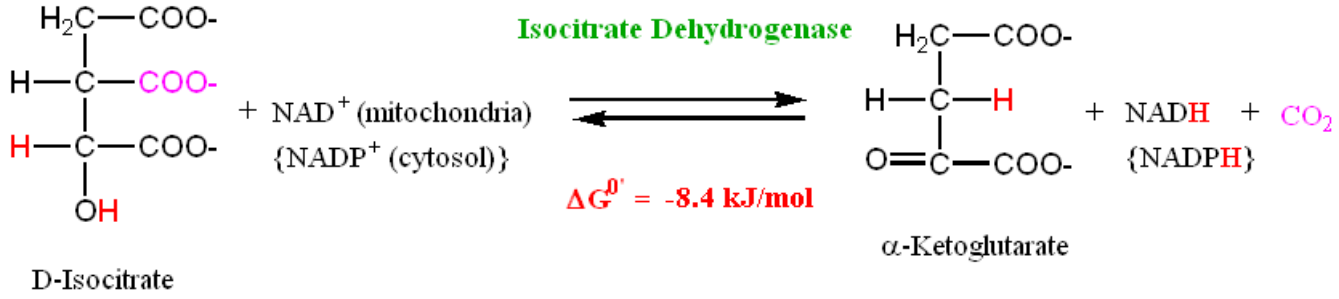
D-Isocitrate

- *Aconitase* only produces the single form of Isocitrate (D-Isocitrate). Thus, *Aconitase* is a *stereospecific* enzyme

Note: the stereospecificity of Aconitase was established by introducing carboxyl-labeled Acetate into the Krebs cycle. The conversion of Acetate into Acetyl-CoA can subsequently result in the labeling of Citrate. Although Citrate is a symmetric molecule, the labeled carboxyl-group always ends up on the γ- carbon group in D-Isocitrate

Step 3: Generation of CO_2 by an NAD^+ linked enzyme

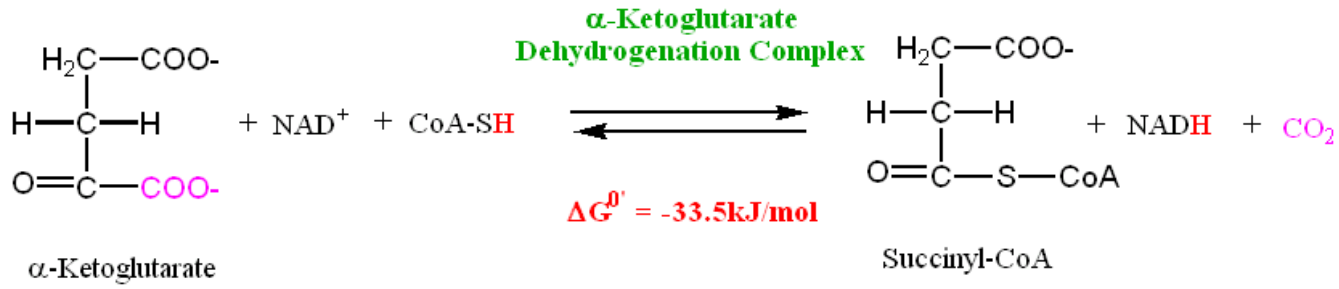
- The Krebs cycle contains two oxidative decarboxylation steps; this is the first one
- The reaction is catalyzed by the enzyme *Isocitrate dehydrogenase*



- The reaction involves dehydrogenation to *Oxalosuccinate*, an unstable intermediate which spontaneously decarboxylates to give *α-Ketoglutarate*
- The reaction is *exergonic*, with a $\Delta G^{0'}$ = -20.9 kJ/mol. This helps drive the preceding (endergonic) reaction in the cycle
- In addition to decarboxylation, this step produces a reduced nicotinamide adenine dinucleotide (NADH) cofactor, or a reduced nicotinamide adenine dinucleotide phosphate (NADPH) cofactor
- If the NAD^+ cofactor is *reduced*, then the *D-Isocitrate* must be *oxidized* when forming *α-Ketoglutarate*. Thus, this step is referred to as an ***oxidative decarboxylation*** step

Step 4: A Second Oxidative Decarboxylation Step

- This step is performed by a multi-enzyme complex, the *α-Ketoglutarate Dehydrogenation Complex*



- The multi-step reaction performed by the *α-Ketoglutarate Dehydration Complex* is analogous to the *Pyruvate Dehydrogenase Complex*, i.e. an α-keto acid undergoes oxidative decarboxylation with formation of an acyl-CoA
- Overall, this oxidative decarboxylation step is more exergonic than the first oxidative decarboxylation step

Summary of Kreb cycle reactions up to this point

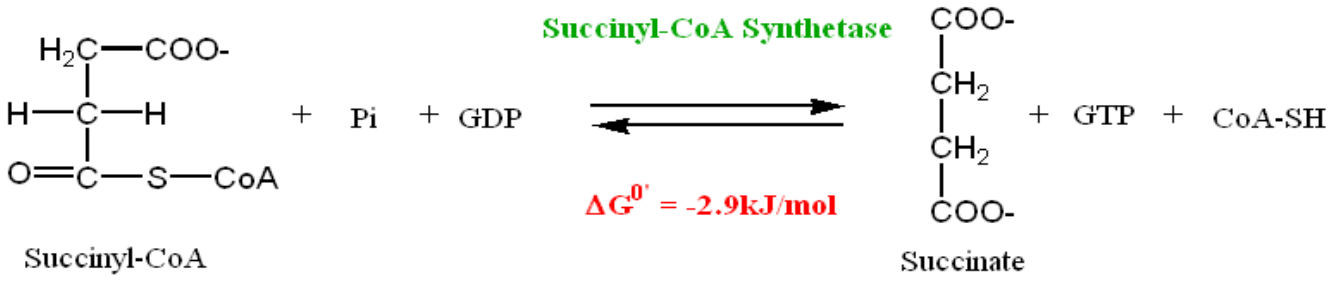
Two carbons have been added to *Oxaloacetate* by the action of *Citrate Synthase* (and *Acetyl-CoA*)

- Two carbons have been lost as CO_2 by oxidative decarboxylation steps
- Two oxidized NAD^+ cofactors have been reduced to NADH
- Due to the stereospecific action of *Aconitase*, the two carbons added are *not* the same two carbons lost in the oxidative decarboxylation steps

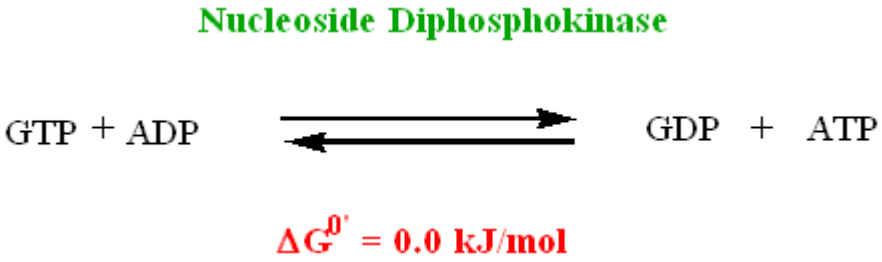
In the remaining steps of the Krebs cycle, the Succinyl-CoA is converted back into the original substrate for the cycle: Oxaloacetate

Step 5: Substrate-Level Phosphorylation

Succinyl-CoA is a high potential energy molecule. The energy stored in this molecule is used to form a high energy phosphate bond in a Guanine nucleotide diphosphate (GDP) molecule:



- Most of the GTP formed is used in the formation of ATP, by the action of *Nucleoside Diphosphokinase*

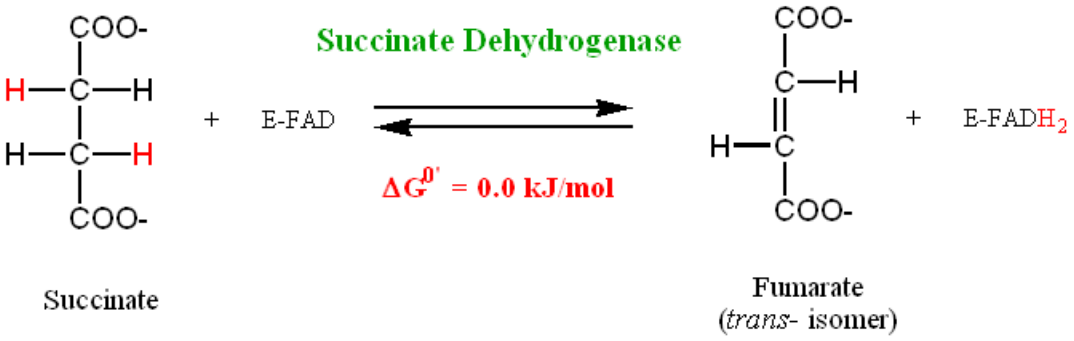


- In plants and bacteria ATP is formed directly in the *Succinyl-CoA Synthase* catalyzed reaction by phosphorylation of ADP directly. In animals, GDP is the substrate in the reaction with formation of GTP (which is then used to form ATP by *Nucleoside Diphosphokinase*)

Step 6: Flavin-Dependent Dehydrogenation

The Succinate produced by *Succinyl CoA-Synthetase* in the prior reaction needs to be converted to Oxaloacetate to complete the Krebs cycle.

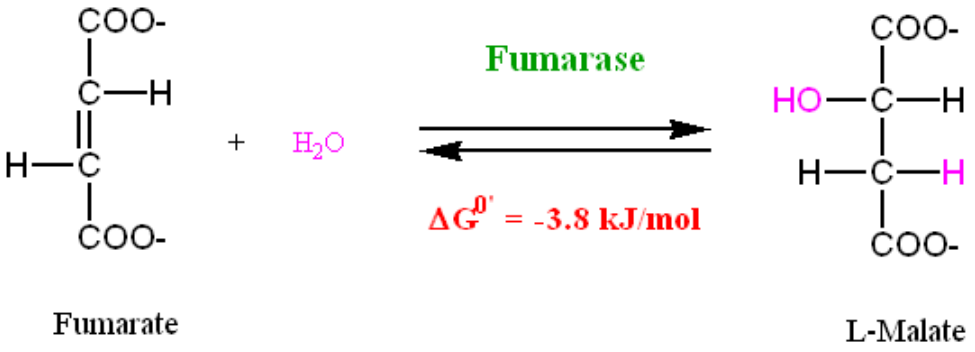
- Both Succinate and Oxaloacetate are 4-carbon compounds
- The first step in the conversion is the dehydrogenation of Succinate to yield Fumarate



- In this reaction a C-C bond is being oxidized to produce a C=C bond. This oxidation is energetically more costly than oxidizing a C-O bond.
- The *redox coenzyme* for this reaction is therefore FAD, rather than NAD^+ (FAD is a more powerful oxidizing agent compared to NAD^+)
- FAD is covalently bound to the *Succinate Dehydrogenase* molecule (via a histidine residue)
- The $FADH_2$ has to be oxidized for the enzyme activity to be restored. This oxidation occurs via interaction with the mitochondrial electron transport system (bound to mitochondrial inner membrane).
- *Succinate Dehydrogenase* is tightly bound to the <u>mitochondrial inner membrane</u>
- *Succinate Dehydrogenase* is stereo-specific: the *trans*- isomer (Fumarate) is produced and not the *cis*- isomer (Maleate)

Step 7: Hydration of a Carbon-Carbon Double Bond

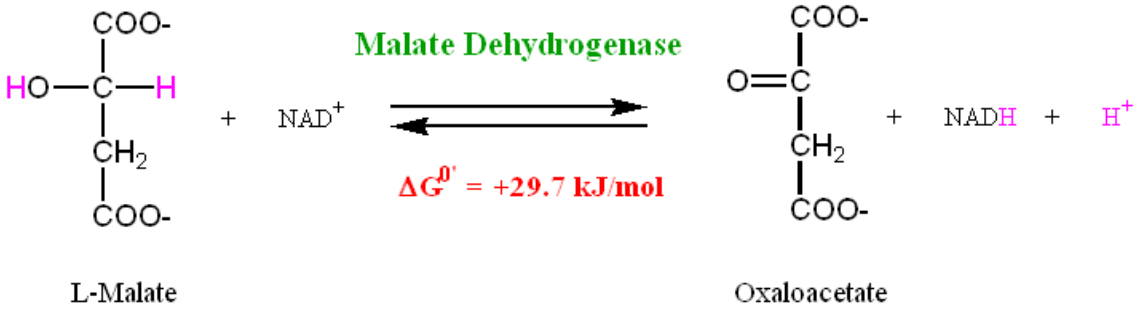
Fumarate undergoes a stereo-specific hydration of the C=C double bond, catalyzed by *Fumarate Hydratase* (also known as *Fumarase*), to produce L-Malate:



- *Fumarase* is a stereo-specific enzyme: it will only hydrate Fumarate, it will not hydrate Maleate. Furthermore, the enzyme can not use D-Malate as a substrate in the reverse reaction

Step 8: A Dehydrogenation Reaction that will Regenerate Oxaloacetate

L-Malate (Malate) is dehydrogenated to produce Oxaloacetate by the enzyme *Malate Dehydrogenase*



- This is a highly endergonic reaction ($\Delta G^{0'}$ = +29.7 kJ/mol) and so the equilibrium strongly favors the *reactants* over the products.
- However, the *next* step in the Krebs cycle (i.e. the *first* step in the process) is the highly exergonic reaction ($\Delta G^{0'}$ = -32.2 kJ/mol) catalyzed by *Citrate Synthase* and this keeps the levels of Oxaloacetate low ($<10^{-6}$ M), thus allowing the above reaction to proceed
- The formation of Oxaloacetate completes the Krebs cycle

Stoichiometry and Energetics of the Citric Acid Cycle

Reaction	Enzyme	$\Delta G^{0'}$ (kJ/mol)
Acetyl-CoA + Oxaloacetate + H_2O ⇨ Citrate + CoA-SH + H^+	*Citrate Synthase*	-32.2
Citrate⇔ *cis*-Aconitate + H_2O	*Aconitase*	+6.3
cis-Aconitase + H_2O ⇔ Isocitrate		
Isocitrate + NAD^+ ⇔ α-Ketoglutarate + CO_2 + NADH	*Isocitrate Dehydrogenase*	-8.4
α-Ketoglutarate + NAD^+ + CoA-SH ⇔ Succinyl-CoA + CO_2 + NADH	*α-Ketoglutarate Dehydrogenase*	-33.5
Succinyl-CoA + Pi + GDP ⇔ Succinate + GTP + CoA-SH	*Succinyl-CoA Synthetase*	-2.9
Succinate + E-FAD ⇔ Fumarate + E-$FADH_2$	*Succinate Dehydrogenase*	0
Fumarate + H_2O ⇔ L-Malate	*Fumarase*	-3.8
L-Malate + NAD^+ ⇔ Oxaloacetate + NADH + H^+	*Malate Dehydrogenase*	+29.7
NET:		-44.8

Overall Reaction:

Acetyl-CoA + ~~Oxaloacetate~~ + ~~H_2O~~ ⇨ ~~Citrate~~ + ~~CoA-SH~~ + H^+
~~Citrate~~⇔ ~~*cis-Aconitate*~~ + ~~H_2O~~
~~*cis-*Aconitase~~ + H_2O ⇔ ~~Isocitrate~~
~~Isocitrate~~ + NAD^+ ⇔ ~~α-Ketoglutarate +~~ CO_2 + NADH
~~α-Ketoglutarate +~~ NAD^+ + ~~CoA-SH~~ ⇔ ~~Succinyl-CoA~~ + CO_2 + NADH
~~Succinyl-CoA +~~ Pi + GDP ⇔ ~~Succinate~~ + GTP + CoA-SH
~~Succinate +~~ E-FAD ⇔ ~~Fumarate~~ + E-$FADH_2$
~~Fumarate~~ + H_2O ⇔ ~~L-Malate~~
~~L-Malate~~ + NAD^+ ⇔ ~~Oxaloacetate~~ + NADH + H^+

$$\text{Acetyl-CoA} + 2H_2O + 3NAD^+ + Pi + GDP + FAD \Rightarrow 2CO_2 + 3NADH + GTP + \text{CoA-SH} + FADH_2 + 2H^+$$

One turn of the citric acid cycle generates:

- One high-energy phosphate through substrate-level phosphorylation
- Three NADH
- One $FADH_2$

Catabolism of Glucose through Glycolysis and the Krebs Cycle

- Each molecule of Glucose produces two molecules of Pyruvate

$$\text{Glucose} + 2NAD^+ + 2ADP + 2Pi \Rightarrow 2\text{Pyruvate} + 2NADH + 2H^+ + 2H_2O + 2ATP$$

- Action of *Pyruvate Dehydrogenase* on Pyruvate:

$$\text{Pyruvate} + \text{CoA-SH} + NAD^+ \Rightarrow CO_2 + \text{Acetyl-CoA} + NADH$$

- The overall catabolism of Glucose to 2 Pyruvate molecules:

$$\text{Glucose} + 2NAD^+ + 2ADP + 2Pi \Rightarrow \cancel{2\text{Pyruvate}} + 2NADH + 2H^+ + 2H_2O + 2ATP$$
$$\cancel{2\text{Pyruvate}} + 2\text{CoA-SH} + 2NAD^+ \Rightarrow 2CO_2 + 2\text{Acetyl-CoA} + 2NADH$$

$$\text{Glucose} + 4NAD^+ + 2ADP + 2\text{CoA-SH} + 2Pi \Rightarrow 2CO_2 + 2\text{Acetyl-CoA} + 4NADH + 2H^+ + 2H_2O + 2ATP$$

- The GTP formed in the animal *Succinyl-CoA Synthetase* reaction in the Krebs cycle is readily converted to ATP (by *Nucleoside Diphosphokinase*)

$$\cancel{2\text{Acetyl-CoA}} + 4H_2O + 6NAD^+ + 2Pi + 2ADP + 2FAD \Rightarrow 4CO_2 + 6NADH + 2ATP + \cancel{2\text{CoA-SH}} + 2FADH_2 + 4H^+$$
$$\text{Glucose} + 4NAD^+ + 2ADP + \cancel{2\text{CoA-SH}} + 2Pi \Rightarrow 2CO_2 + \cancel{2\text{Acetyl-CoA}} + 4NADH + 2H^+ + 2H_2O + 2ATP$$

$$\text{Glucose} + 10NAD^+ + 4ADP + 2H_2O + 4Pi + 2FAD \Rightarrow 6CO_2 + 10NADH + 4ATP + 2FADH_2 + 6H^+$$

Yield of ATP

At this point the yield of ATP is 4 moles per mole of Glucose as it passes through the Krebs cycle

- This is not much more than the 2 moles which would have been produced from glycolysis
- However, NADH and $FADH_2$ are energy rich molecules
- Their oxidation is highly exergonic and is coupled with the production of ATP from ADP
- Oxidation of 1 mole NADH produces 3 moles ATP
- Oxidation of 1 mole $FADH_2$ produces 2 moles ATP
- Thus total ATP yield = (10 x 3) + (2 x 2) + 4 = 38 moles ATP per mole Glucose

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