Metabolism

Part II. The Tricarboxylic Acid (TCA), Citric Acid, or Krebs Cycle

George M. Bodner
Purdue University, West Lafayette, IN 47907

In 1935, Albert Szent-Györgyi found that four-carbon dicarboxylate ions such as the succinate, fumarate, or malate ions catalyzed the uptake of O_2 when added to suspensions of minced pigeon-breast muscle tissue and postulated that these intermediates were linked by a sequence of enzyme-catalyzed reactions,

$$\text{succinate} \rightarrow \text{fumarate} \rightarrow \text{malate} \rightarrow \text{oxaloacetate}$$

Shortly thereafter, Carl Martius and Franz Knoop extended this pathway by outlining the steps by which these suspensions oxidize the citrate ion to succinate,

$$\text{citrate} \rightarrow \text{isocitrate} \rightarrow \alpha\text{-ketoglutarate} \rightarrow \text{succinate}$$

In 1937, Hans Krebs found that citrate is readily formed from oxaloacetate and suggested that this pathway was in fact a cycle of reactions that was responsible for the aerobic oxidation of fuel molecules. Concern over whether citric acid (or more accurately the citrate ion) was the first product of this cycle led Krebs to propose calling this sequence of enzyme-catalyzed reactions the *tricarboxylic acid cycle*.

The tricarboxylic acid (TCA), citric acid, or Krebs cycle differs from glycolysis in several ways. First, and foremost, it is a cyclic rather than linear pathway that catalyzes the oxidation of fuel molecules without a net production or consumption of the intermediates along the pathway. Second, glycolysis occurs in the soluble portion of the cell cytoplasm, whereas the enzymes that catalyze the TCA cycle are bound to the inner walls of the mitochondria. Third, anaerobic glycolysis can recycle its NAD^+ coenzymes and therefore stand on its own. The TCA cycle cannot stand alone; it is tightly coupled to electron transport and oxidative phosphorylation whose enzymes are also found on the inner walls of the mitochondria.

The Bridge between Glycolysis and the TCA Cycle

The end product of glycolysis under aerobic conditions is the pyruvate ion. The starting material for the TCA cycle, however, is a substance known as acetyl coenzyme A. The bridge between these pathways therefore involves converting pyruvate into acetyl CoA,

$$CH_3\overset{O}{\overset{\|}{C}}CO_2^- \rightarrow CH_3\overset{O}{\overset{\|}{C}}—S—CoA$$

Formally, at least three things occur in this reaction: decarboxylation of an α-ketocarboxylic acid to an aldehyde,

$$CH_3\overset{O}{\overset{\|}{C}}CO_2H \rightarrow CH_3\overset{O}{\overset{\|}{C}}H + CO_2$$

oxidation of the aldehyde to a carboxylic acid,

$$CH_3\overset{O}{\overset{\|}{C}}H \rightarrow CH_3CO_2H$$

and reaction between the carboxylic acid and a thiol to form a thioester,

$$CH_3CO_2H + HS—CoA \rightarrow CH_3CO—S—CoA$$

This reaction is too complex for a single enzyme and is catalyzed by a pyruvate dehydrogenase complex which contains three enzymes and five coenzymes. Two of the enzymes catalyze oxidation-reduction reactions (pyruvate dehydrogenase and dihydrolipoyl dehydrogenase), and the third catalyzes the transfer of an acetyl group (dihydrolipoyl transacetylase). The five coenzymes are built from essential vitamins or "vital amines". Three of the coenzymes act as oxidizing/reducing agents (lipoic acid, flavin adenine dinucleotide or FAD, and nicotinamide adenine dinucleotide or NAD^+). A fourth coenzyme activates pyruvate for the loss of CO_2 (thiamine pyrophosphate or TPP), and the fifth acts as an acetyl group carrier (coenzyme A).

The reactions carried out by the pyruvate dehydrogenase complex are shown in Figure 1. The first step involves attack on the pyruvate ion by a thiamine pyrophosphate (TPP) coenzyme bound to the pyruvate dehydrogenase enzyme. The C—H group in the thiazole ring at the heart of the TPP coenzyme is acidic enough to add across the carbonyl group of the pyruvate ion. This intermediate then loses CO_2 to form a carbanion that is stabilized by the positively charged nitrogen on the thiazole ring.

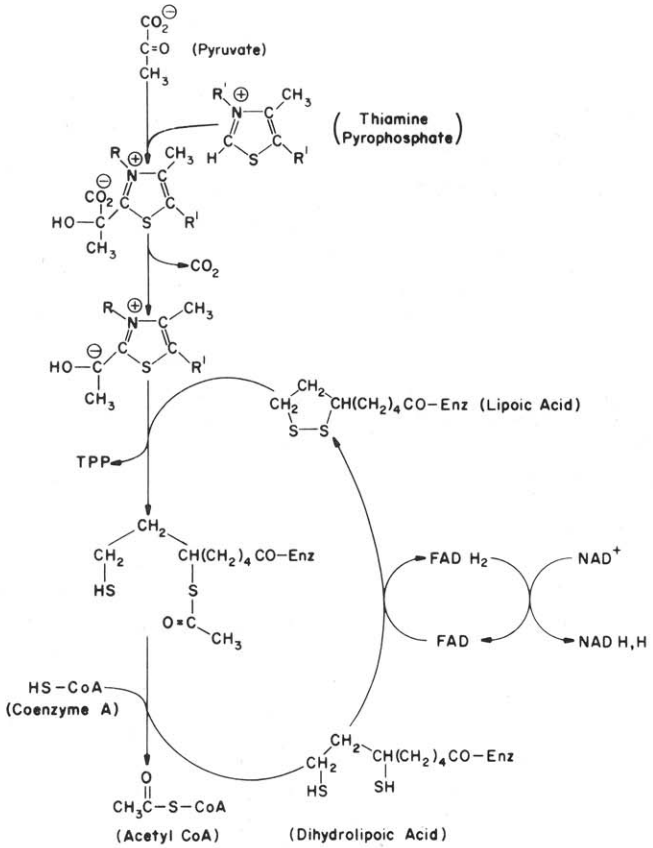


Figure 1. The reactions catalyzed by the pyruvate dehydrogenase complex that bridge the gap between glycolysis and the tricarboxylic acid cycle.

The carbanion is now oxidized by a lipoic acid coenzyme bound through the carbonyl group to the dihydrolipoyl transacetylase enzyme. The net effect of this reaction is simultaneously to regenerate the TPP coenzyme, reduce lipoic acid to dihydrolipoic acid, oxidize the carbanion to an acetyl group, and bind the acetyl group to the reduced form of lipoic acid as a thioester. The transacetylase enzyme then catalyzes the transfer of the acetyl group from dihydrolipoic acid to coenzyme A. Once again, the acetyl group is carried as a thioester, and acetyl CoA is therefore written as $CH_3CO—S—CoA$.

Dihydrolipoic acid is oxidized back to lipoic acid by an FAD coenzyme bound to the dihydrolipoyl dehydrogenase enzyme, and the reduced $FADH_2$ coenzyme is then oxidized back to FAD by NAD^+. This sequence of five reactions therefore transforms pyruvate to acetyl CoA with the net loss of a molecule of CO_2 and the net reduction of one NAD^+ coenzyme to NADH.

Why Synthesize Acetyl Coenzyme A?

Why does the cell convert pyruvate into acetyl CoA? It would be much easier to build an enzyme that simply catalyzed the oxidative decarboxylation of pyruvate to the acetate ion,

$$CH_3COCO_2^- \rightarrow CH_3CO_2^-$$

The advantage of making acetyl CoA revolves around the fact that thioesters have large enough standard free energies of hydrolysis to be included in the class of high-energy compounds. By coupling the synthesis of a thioester with the oxidative decarboxylation reaction, some of the energy released in this reaction can be captured.

Other Sources of Acetyl Coenzyme A

The degradation of carbohydrates such as glucose is not the only source of acetyl CoA. Catabolism of the amino acids lysine, leucine, isoleucine, tryptophan, phenylalanine, and tryosine also forms acetyl CoA. Furthermore, the β-oxidation spiral used to break down fatty acids such as steric acid, $CH_3(CH_2)_{16}CO_2H$, involves oxidation of these fatty acids to acetyl CoA. Because the pathways for the oxidation of carbohydrates, proteins, and lipids all converge at the first step of the TCA cycle where acetyl CoA is consumed, the TCA cycle is often called the central pathway of aerobic metabolism.

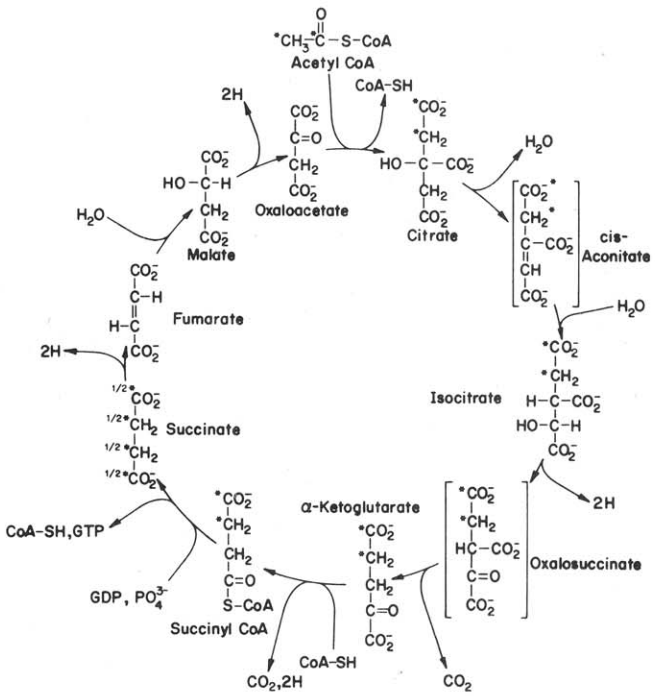
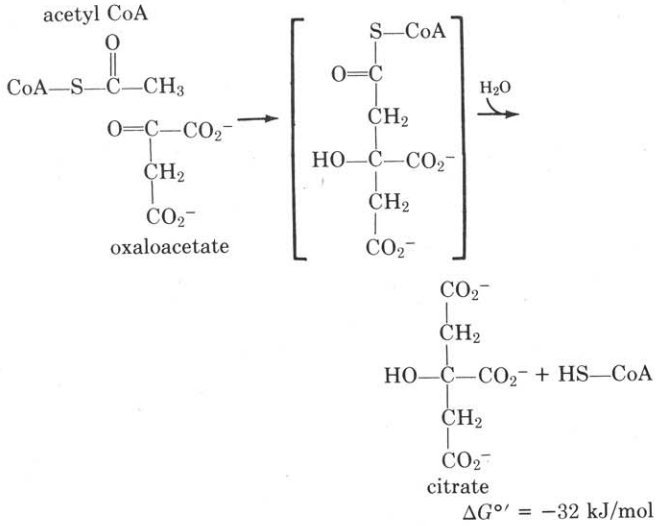


Figure 2. The tricarboxylic acid cycle. The asterisks mark the fate of ^{14}C-labeled acetyl CoA injected into this cycle.

The Tricarboxylic Acid Cycle

Step 1: Aldol Condensation

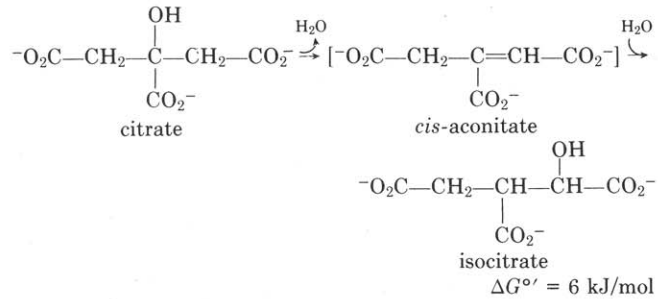
The first step in the TCA cycle shown in Figure 2 is an aldol condensation of acetyl CoA and oxaloacetate to give an enzyme-bound thioester intermediate. Hydrolysis of this thioester gives the citrate ion and coenzyme A.



This reaction is catalyzed by an enzyme known as condensing enzyme or citrate synthetase, and the equilibrium lies heavily on the side of the products ($K_c = 4.4 \times 10^5$) because the free energy of hydrolysis of the thioester drives the reaction to completion.

Step 2: Citrate Isomerization

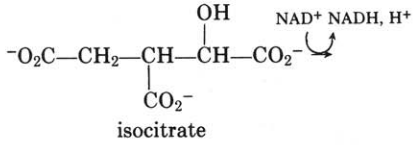
The symmetric citrate ion is now converted into an optically active isomer. This reaction is catalyzed by an enzyme known as aconitase because it is presumed to pass through an enzyme-bound intermediate known as the *cis*-aconitate ion. According to this hypothesis, aconitase catalyzes a reversible dehydration/hydration reaction which moves the —OH group from the third to the fourth carbon atom of the citrate ion.



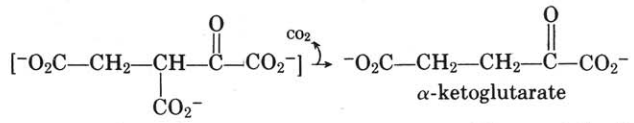
The net effect of this reaction is to convert a 3° alcohol, which cannot undergo oxidation, into a 2° alcohol that can be oxidized.

Step 3: Oxidation of Isocitrate

The isocitrate ion is now oxidized by NAD^+ in a reaction catalyzed by isocitrate dehydrogenase to form an enzyme-bound intermediate which loses CO_2 to give the α-ketoglutarate ion.



isocitrate



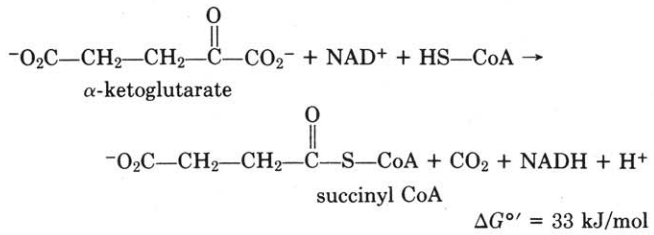
oxalosuccinate — α-ketoglutarate

$\Delta G^{\circ\prime} = 21$ kJ/mol

The loss of CO_2 can be understood by noting that the presence of a carboxylate ion on C_3 and a carbonyl on C_2 makes the intermediate a β-ketoacid, and β-ketoacids readily undergo decarboxylation reactions.

Step 4: Oxidation of α-Ketoglutarate

The α-ketoglutarate ion is now transformed to succinyl CoA by a series of reactions which proceed by the same five-step mechanism as the degradation of pyruvate to acetyl CoA.

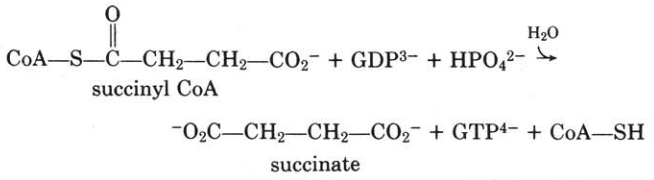


$\Delta G^{\circ\prime} = 33$ kJ/mol

Once again, the cell captures some of the energy given off in an oxidative decarboxylation reaction to synthesize a high-energy thioester bond.

Step 5: Hydrolysis of Succinyl CoA

The standard free energy of hydrolysis of thioesters is large enough to drive substrate-level phosphorylation in which ATP is created from ADP and the phosphate ion. The first step in this process involves capturing the energy given off during hydrolysis of the thioester to form GTP (guanosine triphosphate),



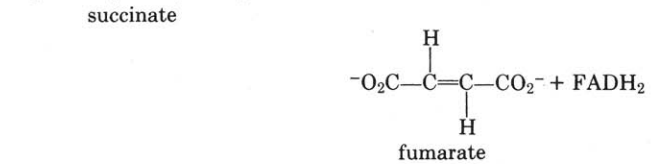
$\Delta G^{\circ\prime} = -3$ kJ/mol

which can then be used to convert ADP to ATP.

$$GTP^{4-} + ADP^{3-} \rightarrow GDP^{3-} + ATP^{4-}$$

Step 6: Oxidation of Succinate

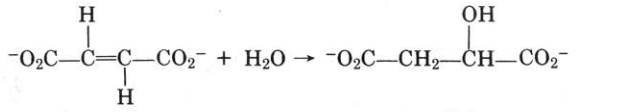
The last three steps are designed to recover oxaloacetate. Succinate is first oxidized to fumarate by an FAD coenzyme covalently bound to the succinate dehydrogenase enzyme.



$\Delta G^{\circ\prime} \cong 0$ kJ/mol

Step 7: Hydration of Fumarate

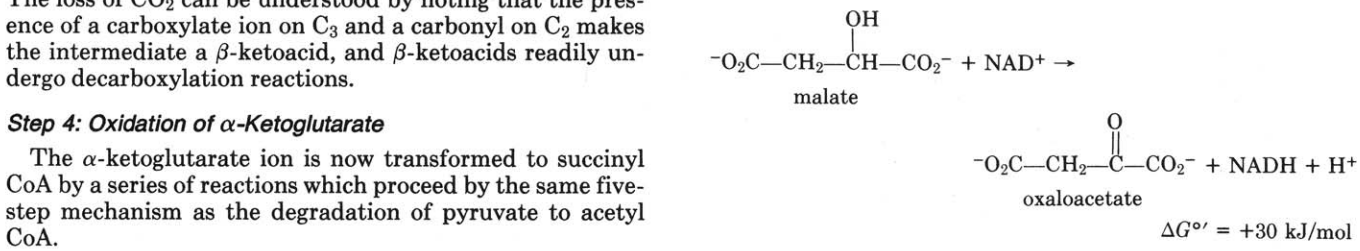
Fumarate hydratase or fumarase then catalyzes the stereospecific addition of water to form the L-malate ion.



$\Delta G^{\circ\prime} = 4$ kJ/mol

Step 8: Oxidation of Malate

Malate dehydrogenase then catalyzes the NAD^+ oxidation of L-malate to give a dicarboxylate ion which can be thought of as the product of a fusion of the oxalate and acetate ions, thus the name oxaloacetate.



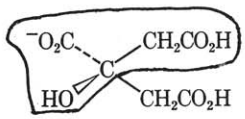
$\Delta G^{\circ\prime} = +30$ kJ/mol

This energetically unfavorable reaction is pulled to completion by the rapid rate at which oxaloactate condenses with acetyl CoA to form citrate, thereby reactivating the cycle.

The Results of Isotopic Labeling Experiments

Asterisks are used in Figure 2 to follow the fate of isotopically labeled acetyl CoA through the TCA cycle. If both carbon atoms of the acetyl group in acetyl CoA were initially labeled with ^{14}C, the citrate ion formed in the first reaction would be labeled at C_1 and C_2. Because the citrate ion is symmetric, it seems just as likely that aconitase would move the —OH group on C_3 to the isotopically labeled C_2 and unlabeled C_4 positions. Experimentally, we find that aconitase invariably transfers the —OH group to the unlabeled C_4 position. This raises an interesting question: How does aconitase catalyze an asymmetric reaction on the symmetric citrate ion?

The citrate ion is symmetric or *achiral* because the two ends of the molecule are mirror images of each other. The top and bottom halves of the molecule are different, however, and the citrate ion is therefore *prochiral*; it has the potential for acting as if it were chiral.



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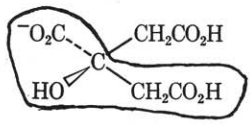


bottom

Asymmetric enzymes such as aconitase can recognize and preferentially bind to one end of this molecule and thereby catalyze asymmetric reactions on what appears to be a symmetric substrate.

The Net Effect of the TCA Cycle

A single turn around the TCA cycle produces one molecule of ATP, two molecules of CO_2, and four reduced NADH or $FADH_2$ coenzymes.

$$CH_3CO—S—CoA + 3\ NAD^+ + FAD + ADP^{3-} + HPO_4^{2-} + 2\ H_2O \rightarrow 2\ CO_2 + HS—CoA + 3\ NADH + FADH_2 + ATP^{4-} + 2\ H^+$$

The NADH and $FADH_2$ coenzymes are oxidized back to NAD^+ and FAD in a complex series of reactions known as electron transport that eventually reduce O_2 to water.

$$NADH + H^+ + \tfrac{1}{2}O_2 \rightarrow NAD^+ + H_2O$$
$$FADH_2 + \tfrac{1}{2}O_2 \rightarrow FAD + H_2O$$

Electron transport is tightly coupled to a process known as oxidative phosphorylation which captures, in the form of

ATP, some of the energy given off during this oxidation. Three ATP are produced each time an NADH coenzyme is recycled, and two ATP result from the oxidation of an $FADH_2$ coenzyme.

When the ATP produced by recycling the NADH and $FADH_2$ coenzymes is added to the ATP synthesized during the hydrolysis of succinyl CoA, the net effect of a single turn around the TCA cycle is 12 molecules of ATP.

3(NADH → NAD^+)	3 × 3 = 9 ATP
1($FADH_2$ → FAD)	1 × 2 = 2 ATP
1 (succinyl CoA → succinate)	1 × 1 = 1 ATP
	total = 12 ATP/CH_3CO—S—CoA

A fourth NAD^+ coenzyme was reduced to NADH when pyruvate was oxidized to acetyl CoA in the step bridging glycolysis and the TCA cycle. Three more ATP are produced when this coenzyme is recycled, and a total of 15 ATP are therefore synthesized for each pyruvate ion oxidized in the mitochondria of the cell.

Another ATP was produced when phosphoenolpyruvate was hydrolyzed to the pyruvate ion during glycosis, and an NADH coenzyme was produced when glyceraldehyde 3-phosphate was oxidized to 1,3-diphosphoglyceric acid. Because glycolysis occurs in the cytosol but oxidation of the NADH coenzyme by electron transport occurs within the mitochondria, recycling of the NADH coenzyme from glycolysis is slightly less efficient. Only two ATP are produced when this coenzyme is oxidized to NAD^+.

A total of 18 ATP therefore result from the degradation of a single glyceraldehyde 3-phosphate ion. Because each glucose molecule gives two of these ions, oxidation of glucose by glycolysis coupled with the TCA cycle generates a total of 36 molecules of ATP. Complete oxidation of glucose to CO_2 and H_2O therefore has the potential for liberating 18 times as much energy as the anaerobic degradation of glucose to lactate or ethanol described in the first paper in this series.

Efficiency of Aerobic Oxidation of Glucose

The standard free energy for the oxidation of glucose to CO_2 and H_2O,

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O \qquad \Delta G^0 = -2{,}870\ \text{kJ/mol}$$

is often compared to the standard free energy of hydrolysis of ATP

$$ATP^{4-} + H_2O \rightarrow ADP^{3-} + HPO_4^{2-} + H^+ \qquad \Delta G^0 = -31\ \text{kJ/mol}$$

to estimate the efficiency with which electron transport and oxidative phosphorylation capture energy. Assuming that 36 ATP are produced per molecule of glucose consumed, these standard free energies suggest that slightly less than 40% of the energy given off is captured as ATP.

This calculation is misleading, however, because the concentrations of metabolic intermediates in biological systems are far from the standard state concentration of 1.00 . . . *M*. When one adjusts the free energy of hydrolysis of ATP to reflect more accurately concentrations of ATP, ADP, and phosphate in a typical cell[1], 36 ATP correspond to the capture of as much as 65% of the energy released during the oxidation of glucose.[2]

The Balanced Chemical Equation for the Oxidation of Glucose

The balanced chemical equation for the oxidation of glucose,

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$

is also somewhat misleading because it leads one to believe that some of the O_2 consumed in this reaction is incorporated into CO_2, whereas in practice all of the O_2 is reduced to water.

A total of 12 molecules of H_2O are actually produced for each molecule of glucose consumed in this reaction. Each glucose molecule is split into two glyceraldehyde 3-phosphate ions; there are six places where either NAD^+ or FAD coenzymes are reduced each time a glyceraldehyde 3-phosphate ion is oxidized; and a molecule of water is generated each time one of these coenzymes is recycled.

$$NADH + H^+ + \tfrac{1}{2}O_2 \rightarrow NAD^+ + H_2O$$
$$FADH_2 + \tfrac{1}{2}O_2 \rightarrow FAD + H_2O$$

The overall stoichiometry of the reaction can be understood by noting that water is also consumed in the TCA cycle. One molecule of water is consumed during the hydrolysis of the thioester intermediate produced by the aldol condensation in Step 1. A second water molecule is consumed during the hydrolysis of succinyl CoA in Step 5, and a third molecule of water is consumed during the hydration of fumarate in Step 7. A total of six water molecules are therefore consumed for each molecule of glucose that enters the TCA cycle.

The roles of oxygen and water in the aerobic degradation of glucose can best be appreciated by following the fate of isotopically labeled O_2.

$$C_6H_{12}O_6 + 6\ O_2{}^* + 6\ H_2O \rightarrow 6\ CO_2 + 12\ H_2O^*$$

Water provides the oxygen atoms necessary to oxidize glucose to CO_2, and all of the molecular oxygen is reduced to water.

Anaplerotic Reactions

There are several points on the TCA cycle where key intermediates are removed to provide the building blocks for the biosynthesis of carbohydrates, proteins, lipids, nucleic acids, and specialized molecules such as chlorophyll. Oxaloacetate and α-ketoglutarate, for example, are starting materials for the synthesis of amino acids such as arginine, asparagine, aspartic acid, glutamic acid, glutamine, isoleucine, lysine, methionine, proline, and threonine. Citrate is used for the biosynthesis of both fatty acids and heme proteins, and succinyl CoA is the starting material for the synthesis of the pyrrole rings found in hemoglobin, chlorophyll, and the cytochromes.

Not surprisingly, there are also points where key intermediates are injected into the TCA cycle when biomolecules are degraded. Oxaloacetate, α-ketoglutarate, succinyl CoA, and fumarate, for example, are all products of the break down of amino acids.

Because it effectively acts as a catalyst to oxidize acetyl CoA to CO_2 and H_2O, the TCA cycle cannot, by itself, produce or consume any of these intermediates. The only way to either replenish pools of depleted TCA intermediates or metabolize intermediates present in excess is through *anaplerotic* (literally: filling up) reactions which provide shortcuts across the cycle.

The most important example of anaplerosis is a reversible reaction in which pyruvate is converted to oxaloacetate by the enzyme pyruvate carboxylase.

$$\underset{\text{pyruvate}}{CH_3{-}\overset{\overset{O}{\|}}{C}{-}CO_2^-} + CO_2 + ATP^{4-} + H_2O \rightarrow$$

$$^-O_2C{-}CH_2{-}\overset{\overset{O}{\|}}{C}{-}CO_2^- + ADP^{3-} + HPO_4^{2-} + 2\ H^+$$
oxaloacetate

$$\Delta G^{\circ\prime} = 2\ \text{kJ/mol}$$

This reaction compensates for the drain on the TCA cycle

[1] Minakami, S.; Yoshikawa, H. *Biochem. Biophys. Res. Comm.* **1965**, *18*, 345, as cited in Stryer, L. "Biochemistry", 2nd ed.; Freeman: San Francisco, 1981; p 276.

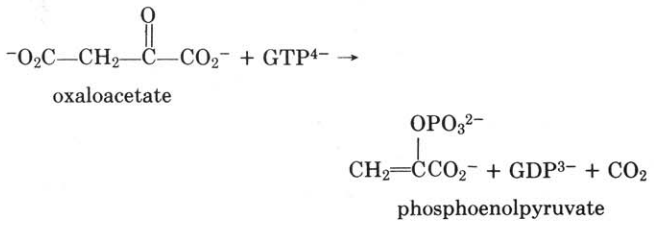
[2] Lehinger, A. L. "Principles of Biochemistry"; Worth: New York, 1982.

that occurs when intermediates that lead to oxaloacetate are removed from the cycle for biosynthesis. Alternatively, when the degradation of proteins produces an excess of the intermediates leading to oxaloacetate, this reaction can be reversed to convert some of this excess into pyruvate which can be oxidized to acetyl CoA and then burned in the TCA cycle to provide energy.

Gluconeogenesis

Anaplerotic reactions provide us with a basis for understanding how people can survive on high-protein/low-carbohydrate diets. The catabolism of amino acids gives rise to TCA intermediates that are eventually converted to oxaloacetate. Some of the oxaloacetate is decarboxylated to pyruvate which is then converted to acetyl CoA and oxidized by the TCA cycle.

Oxaloacetate can also be decarboxylated by an enzyme known as pyruvate carboxykinase to form phosphoenolpyruvate (PEP).



Consideration of the free energy of reaction for the steps in glycolysis suggests that PEP is close enough in energy to glucose to allow *gluconeogenesis* (literally: synthesis of new glucose) to occur by essentially reversing the steps by which PEP was formed from glucose.