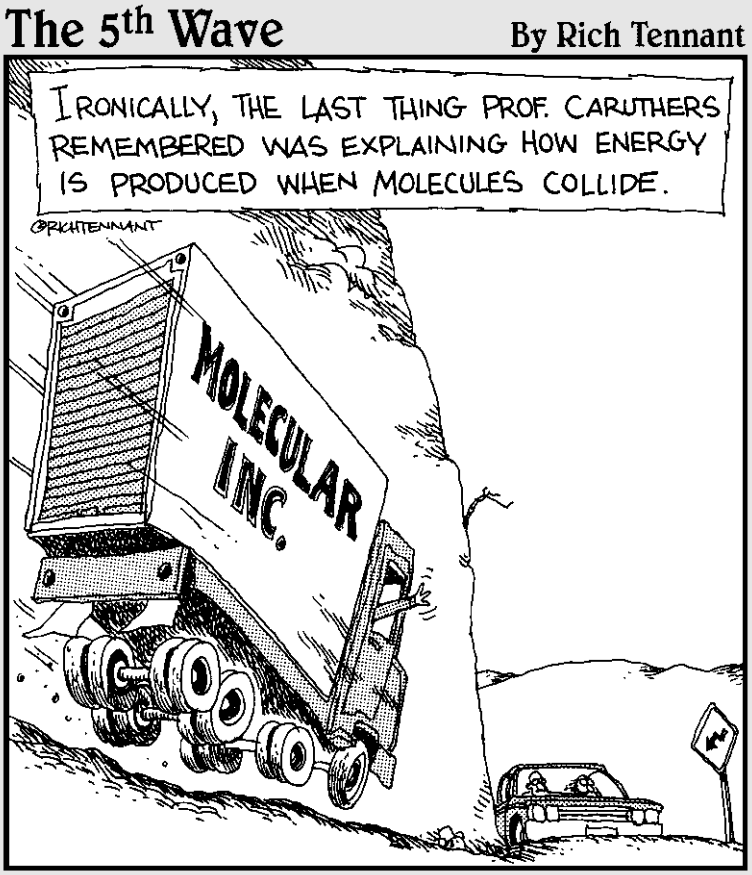
Part IV
Bioenergetics and Pathways



In this part . . .

For anyone to do anything requires energy, and this is where we focus on the way life obtains and uses it. Here we take a gander at energy needs and follow the trail of where that energy goes and why. The main character in this part is your good buddy ATP, and running through this episode is where you'll find the citric acid cycle. Finally, we tackle nitrogen chemistry.

Chapter 12

Life and Energy

In This Chapter

- Learning about ATP and energy
- Visiting the nucleoside triphosphate family
- Considering AMP, ADP, and ATP
- Going without food

The chapters in this part examine metabolism — all the processes involved in maintaining a cell. Metabolism has two components: catabolism and anabolism. *Catabolism* deals with the breaking down of molecules, whereas *anabolism* deals with the building up of cells. Both processes take place in the mitochondria. All metabolic processes involve energy: They either absorb energy (*endergonic*) or produce it (*exergonic*).

The key energy molecule is *adenosine triphosphate,* abbreviated *ATP,* which forms as a product of the common catabolic pathway.

ATP: The Energy Pony Express

Determining the basic reaction processes involved in the production and use of energy is called *bioenergetics*. This study has developed bioenergetic principles that allow us to examine energy at the microscopic level.

Fortunately, ATP is recycled within the body. The typical daily requirement for an adult is over 140 pounds of ATP per day. However, the amount of ATP present in your body at any one time is only about one-tenth of a pound. That means each ATP molecule in your body is recycled about 1,400 times each day. Now that is effective recycling — and you don't even have to put anything into a blue container.

ATP and free energy

The *free energy content* (G) is the intrinsic energy present in a molecule. In a reaction, the change in this energy is written as ΔG. The change in energy is equal to the energy of the products minus the energy of the reactants. The value of ΔG is the key: If a reaction produces energy, ΔG represents the maximum possible amount of energy that the reaction may produce. If a reaction requires energy, ΔG represents the minimum possible amount of energy that a reaction will require. Reactions producing energy have a negative value of ΔG and are *spontaneous.* Reactions requiring energy have a positive value of ΔG and are *nonspontaneous.*

Spontaneity bears no relation to speed. Spontaneous reactions may be very rapid or very slow.

The conditions under which a reaction occurs may alter the value of ΔG. (The "ideal" or standard value of ΔG is ΔG°.) The formula for modifying the free energy for the equilibrium reaction A ⇌ B is:

$$\Delta G = \Delta G^\circ - RT \ln [B] / [A] = \Delta G^\circ - RT \ln K$$

According to this relationship, the free energy change, ΔG, comes from a modification of the standard free energy value. R is the universal gas constant ($8.314\ J \times mol^{-1}K^{-1}$ or $1.987\ cal \times mol^{-1}K^{-1}$). T is the absolute temperature. K is the equilibrium constant found by dividing the concentration of the product, [B], by the concentration of the reactant, [A].

In many bioenergetic studies, *calories* are the unit instead of joules (J). The relationship is 1 calorie = 4.184 J (exactly) or 1 kilocalorie = 4.184 kJ.

In research, it is often better to use ΔG°'. This modification of ΔG stems from the use of the biologically more realistic value of pH = 7 ($[H^+] = 10^{-7}$ M) instead of the standard pH = 0 ($[H^+] = 1$ M). Some relationships between K and ΔG°' are shown in Table 12-1.

Table 12-1 Relationships Between ΔG°' and K

$\Delta G^{\circ\prime}\ kJ \times mol^{-1}$	K
–17.1	1,000
–11.4	100
–5.7	10
0	1

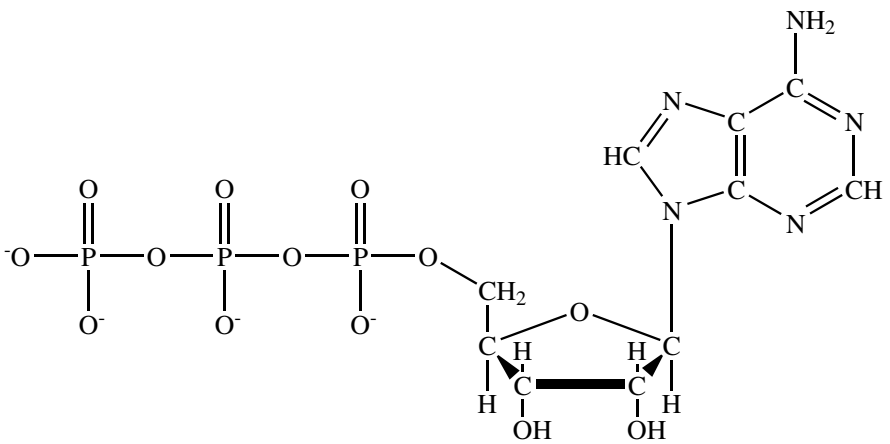
$\Delta G^{\circ\prime}$ kJ × mol^{-1}	K
5.7	0.1
11.4	0.01
17.1	0.001

Table 12-1 shows that the larger K is, the more *exergonic* (spontaneous) the reaction. For example, if K = 1000, the concentration of the product, [B], is 1,000 times that of the reactant, [A], and 17 kJ per mole will be released. It is important to remember that, in biological systems, variations in [A] and [B] must be taken into account in addition to $\Delta G^{\circ\prime}$. For example, increasing the reactant concentration promotes the reaction, whereas increasing the product concentration inhibits the reaction.

ATP as an energy transporter

Cells utilize exergonic processes to provide the energy necessary for life processes, and the key supplier of this energy is ATP (Figure 12-1). ATP supplies the energy required to force endergonic reactions to take place, to provide mechanical energy (muscle movement), light energy (in fireflies), and heat energy (to maintain body temperature).

Hydrolysis of the terminal phosphate of ATP yields ADP and inorganic phosphate, indicated as P_i. The structure of ADP is shown in Figure 12-2. This hydrolysis releases 30.5 kJ × mol^{-1}.



Adenosine triphosphate (ATP)

Figure 12-1: Structure of ATP.

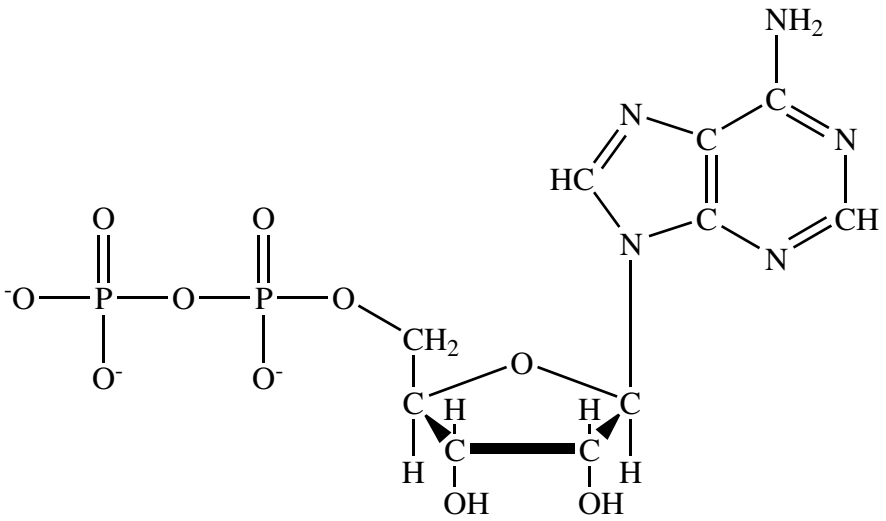


Figure 12-2: Structure of ADP.

Adenosine diphosphate (ADP)

Concentration variations lead to changes, usually minor, in energy.

Hydrolysis of the terminal phosphate of ADP yields AMP and inorganic phosphate, indicated as P_i. The structure of AMP is in Figure 12-3. This hydrolysis also releases 30.5 kJ × mol^{-1}. (This reaction is of less biological importance than the ATP to ADP hydrolysis.)

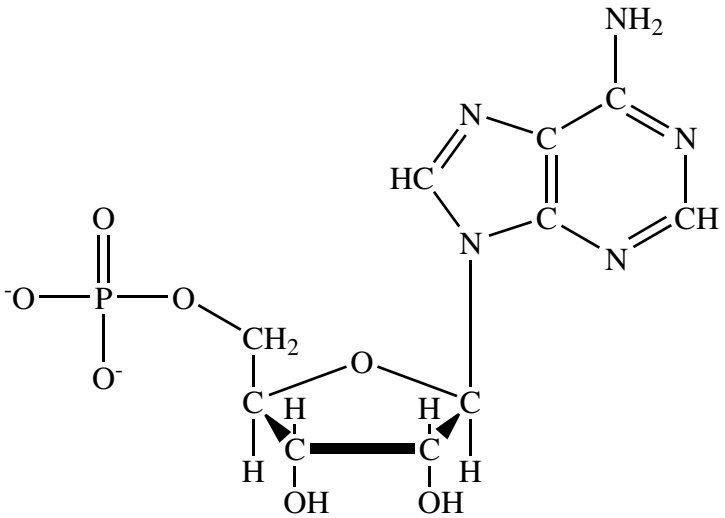
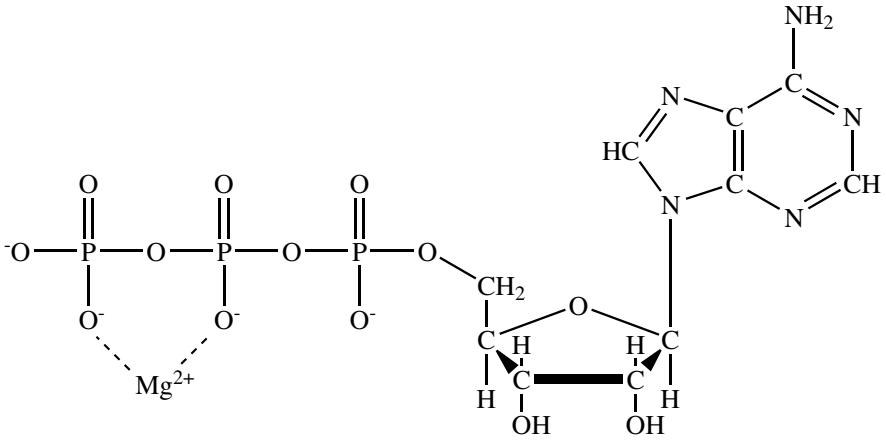


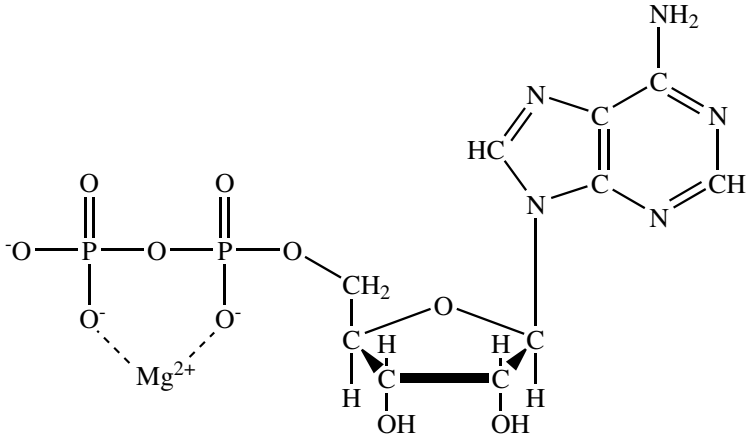
Figure 12-3: Structure of AMP.

Adenosine monophosphate (AMP)

It is also possible to go directly from ATP to AMP, cleaving a pyrophosphate, $P_2O_7^{4-}$, from the phosphate chain. Biochemists use PP_i to indicate pyrophosphate. This furnishes slightly more energy then a simple hydrolysis to release P_i (about 33.5 kJ × mol^{-1}). Under physiological conditions, the phosphate portions of ATP and ADP form a complex with magnesium ions. In certain circumstances, manganese (II) ions, Mn^{2+}, may take the place of Mg^{2+} ions. Figure 12-4 depicts the magnesium complexes with ATP and ADP.



Adenosine triphosphate (ATP)-Mg^{2+}



Adenosine diphosphate (ADP)-Mg^{2+}

Figure 12-4: Magnesium complexes with ATP and ADP.

The removal of the last phosphate involves the loss of the least amount of energy (14.2 kJ•mol^{-1}). This hydrolysis involves the cleavage of an ester bond instead of an anhydride bond. In general, the hydrolysis of an ester bond involves less than half the energy of the hydrolysis of an anhydride bond.

It's Relative: Molecules Related to ATP

A few other biomolecules can provide energy equivalent to that which comes from the hydrolysis of ATP. GTP is an example of such a molecule. There are also a few molecules that supply *more* energy. Table 12-2 compares some of the high-energy molecules to ATP, and Figure 12-5 shows their structures.

Table 12-2 Energy Released ($\Delta G°'$) by Some High-Energy Molecules Related to ATP

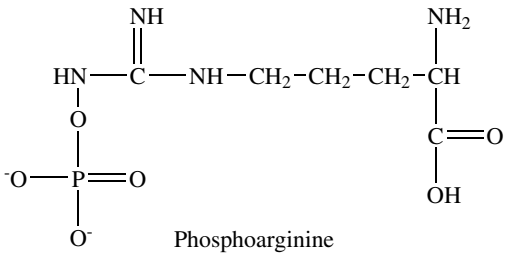
Biomolecule	*Energy released (kJ × mol^{-1})*
ATP	30.5
Phosphoarginine	32.2
Acetyl phosphate	43.3
Phosphocreatine	43.3
1,3-Bisphosphoglycerate	49.6
Phosphoenolpyruvate	62.2

Phosphopyruvate, 1,3-bisphosphogylcerate, and acetyl phosphate are important for the transfer and conservation of chemical energy. Phosphoarginine and phosphocreatine are important molecules for storing metabolic energy. Phosphocreatine is stored in muscles and can be quickly converted to ATP to give energy for muscle contraction. Production of phosphocreatine occurs when ATP concentration is high — high ATP concentration is needed to overcome the energy deficit of 12.8 kJ × mol^{-1}. The reverse, phosphate transfer to form ATP from ADP, occurs at low ATP concentrations. Phosphoarginine behaves similarly in certain invertebrates.

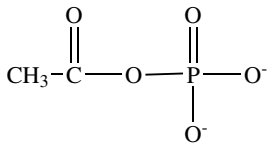
The nucleoside triphosphate family

The predominant energy transfer molecule, as we have been saying, is ATP. But other nucleoside triphosphates (such as CTP, GTP, TTP, and UTP) may also serve this energy transfer function. These five molecules also supply part of the energy necessary for DNA and RNA synthesis. All the nucleoside

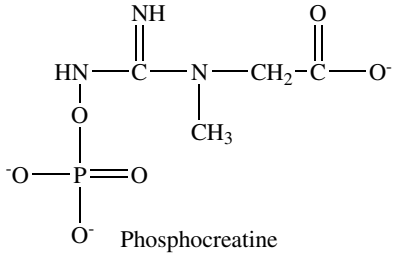
triphosphates have about the same energy yield. (Note that ATP is necessary for the synthesis of the remaining nucleoside triphosphates.)



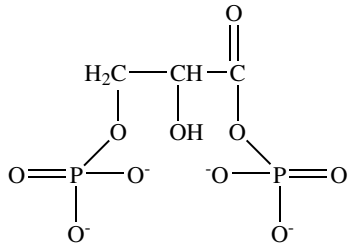
Phosphoarginine



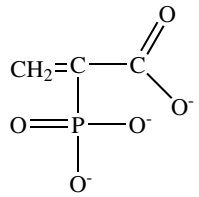
Acetyl phosphate



Phosphocreatine



1,3-Bisphosphoglycerate



Phosphoenolpyruvate

Figure 12-5: Structures of some high-energy molecules.

The biosynthesis of the ribonucleoside triphosphates, in general NTP, begins with the production of the appropriate monophosphate, NMP. The stepwise addition of the next two phosphate groups requires two enzymes of low specificity. These enzymes are nucleoside monophosphate kinase and nucleoside diphosphate kinase. (The term *kinase* refers to a transferase enzyme that transfers a phosphate group of a nucleoside triphosphate.) The general reactions are shown in Figure 12-6.

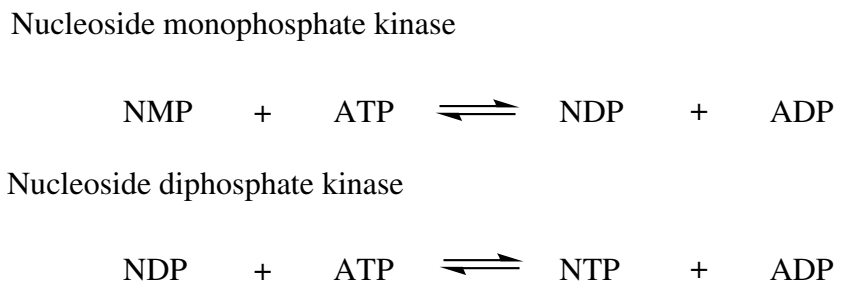


Figure 12-6: Two of the reactions catalyzed by the kinase enzymes.

The formation of the deoxyribonucleoside triphosphates, dNTP, follows two different paths. In one path, a multienzyme system converts the appropriate nucleoside diphosphate to the corresponding deoxyribonucleoside diphosphate. Then nucleoside diphosphate kinase catalyzes the formation of the deoxyribonucleoside triphosphate. The other path occurs in certain microorganisms where there is a direct conversion of NTP to dNTP.

As easy as 1, 2, 3: AMP, ADP, and ATP

It is possible to hydrolyze ATP either to ADP plus phosphate, P_i, or to AMP plus pyrophosphate, PP_i. (The pyrophosphate will undergo further hydrolysis to two phosphates, 2 P_i.) ADP and P_i are the immediate precursors for the reformation of ATP. To produce ATP starting with AMP utilizes the enzyme adenylate kinase. This enzyme catalyzes the transfer of a phosphate group from an ATP to an ADP. This reaction results in the formation of two ADP molecules. (Adenylate kinase also catalyzes the reverse reaction.)

The easy transfer of phosphate groups between nucleotides creates a metabolic network for the transfer of energy. The key to this network is the intercellular production of ATP.

Where It All Comes From

One of the purposes of the food we eat, of course, is to supply energy, with carbohydrates and fats being the major sources of energy. Digestion breaks polysaccharides into glucose and other monosaccharides, whereas fats are broken into glycerol and fatty acids. Catabolism converts these energy sources primarily to ATP. Proteins are broken into amino acids, which usually do not serve as energy sources. (We explain the details of these reactions later in this book.) Glucose produces 36 ATP molecules. This is an average of 6 ATPs per carbon. The step-by-step energy change for glucose is in Table 12-3. Other carbohydrates give a similar yield.

Table 12-3 ATP Yield for Each Step in the Metabolism of Glucose

Chemical Steps	*Number of ATP Molecules Produced*
Activation (conversion of glucose to 1,6-fructose diphosphate)	-2
Oxidative phosphorylation 2(glyceraldehyde 3-phosphate $\rightarrow$ 1,3-diphosphoglycerate), producing 2 NADH + H^+ in cytosol	4
Dephosphorylation 2(1,3-diphosphoglycerate $\rightarrow$ pyruvate)	4
Oxidatie decarboxylation 2(pyruvate $\rightarrow$ acetyl CoA), producing 2 NADH + H^+ in mitochondrian	6
Oxidation of two C_2 fragments in citric acid and oxidative phosphorylation common pathway, producing 12 ATP for each C_2 fragment	24
Total	36

Each fat molecule hydrolyzes to a glycerol and three fatty acid molecules. Glycerol produces 20 ATPs per molecule. The energy production from a fatty acid will vary with the identity of the particular acid. Stearic acid, $C_{18}H_{36}O_2$, produces a total of 146 ATPs per molecule. This amounts to an average of 8.1 ATPs per carbon. The step-by-step energy change for stearic acid is shown in Table 12-4. Other fatty acids give a similar yield.

Table 12-4 ATP Yield for Each Step in the Metabolism of Stearic Acid

Chemical Steps	*Happens*	*ATP Molecules Produced*
Activation (stearic acid → stearyl CoA)	Once	-2
Dehydrogenation (acetyl CoA → transenoyl CoA), producing $FADH_2$	8 times	16
Dehydrogenation (hydroxyacyl CoA → keto acyl CoA), producing $NADH + H^+$	8 times	24
C_2 fragment (acetyl CoA → common catabolic pathway), producing 12 ATP per C_2 fragment	9 times	108
Total	146	

What happens if you stop eating?

Starvation is the total deprivation of food. Here is what happens during starvation: Initially, the body utilizes its glycogen reserves. Then it moves on to its fat reserves — the first ones are those around the heart and kidneys. Finally, the body relies on the reserves found in the bone marrow. Early in a total fast, the body metabolizes protein at a rapid rate. The amino acids are converted to glucose, because the brain prefers glucose. These proteins come from the skeletal muscles, blood plasma, and other sources in a process that produces a quantity of nitrogen-containing products, which need to be excreted. Excretion requires large quantities of water, and the resulting loss of water may lead to death by dehydration. If the starvation continues, the brain chemistry adjusts to accept fatty acid metabolites, which uses the last of the fat reserves. Finally, the body resorts to structural proteins, systems begin to fail rapidly, and death follows quickly.

Chapter 13

ATP: The Body's Monetary System

In This Chapter

- Checking out carbohydrate metabolism and examining the citric acid cycle
- Finding out about electron transport and oxidative phosphorylation
- Seeing how biosynthesis takes place

Here we examine a number of general processes that either produce or consume energy. Breaking down molecules often produces energy. The breakdown of one molecule is often coupled with the synthesis of another, and this other synthesized molecule is often adenosine triphosphate, or ATP. *Catabolism* is the breaking down of molecules to provide energy. *Anabolism* is the building of molecules. These two processes combine to give metabolism. *Metabolism* comprises all reactions in biological systems.

As you can see in Chapter 12, the "currency" in biological systems is ATP. There are other energy-containing molecules, but the rate of exchange to ATP is the reference. The breakdown of certain molecules produces the currency of ATP, and there is a cost involved in the synthesis of other molecules. Polysaccharides and fats are like "banks" that store energy for later use.

Metabolism 1: Glycolysis

The *Embden-Meyerhof pathway,* or *glycolysis,* is a primitive means of extracting energy from organic molecules. The process converts glucose to two lactic acid molecules in an anaerobic (without oxygen) process. Nearly all forms of life, whether a person or a jellyfish, utilize glycolysis. All carbohydrates follow this pathway. Aerobic (utilizing oxygen) processing of carbohydrates uses pyruvate derived from glycolysis. (Alcoholic fermentation also produces pyruvate from glucose. The glucose is converted to two ethanol molecules and two CO_2 molecules.) Glycolysis is a two-part process, which we label Phase I and Phase II. Figures 13-1 and 13-2 help illustrate the upcoming, ahem, rather *involved* discussion. You may want to refer back to these figures as you read.

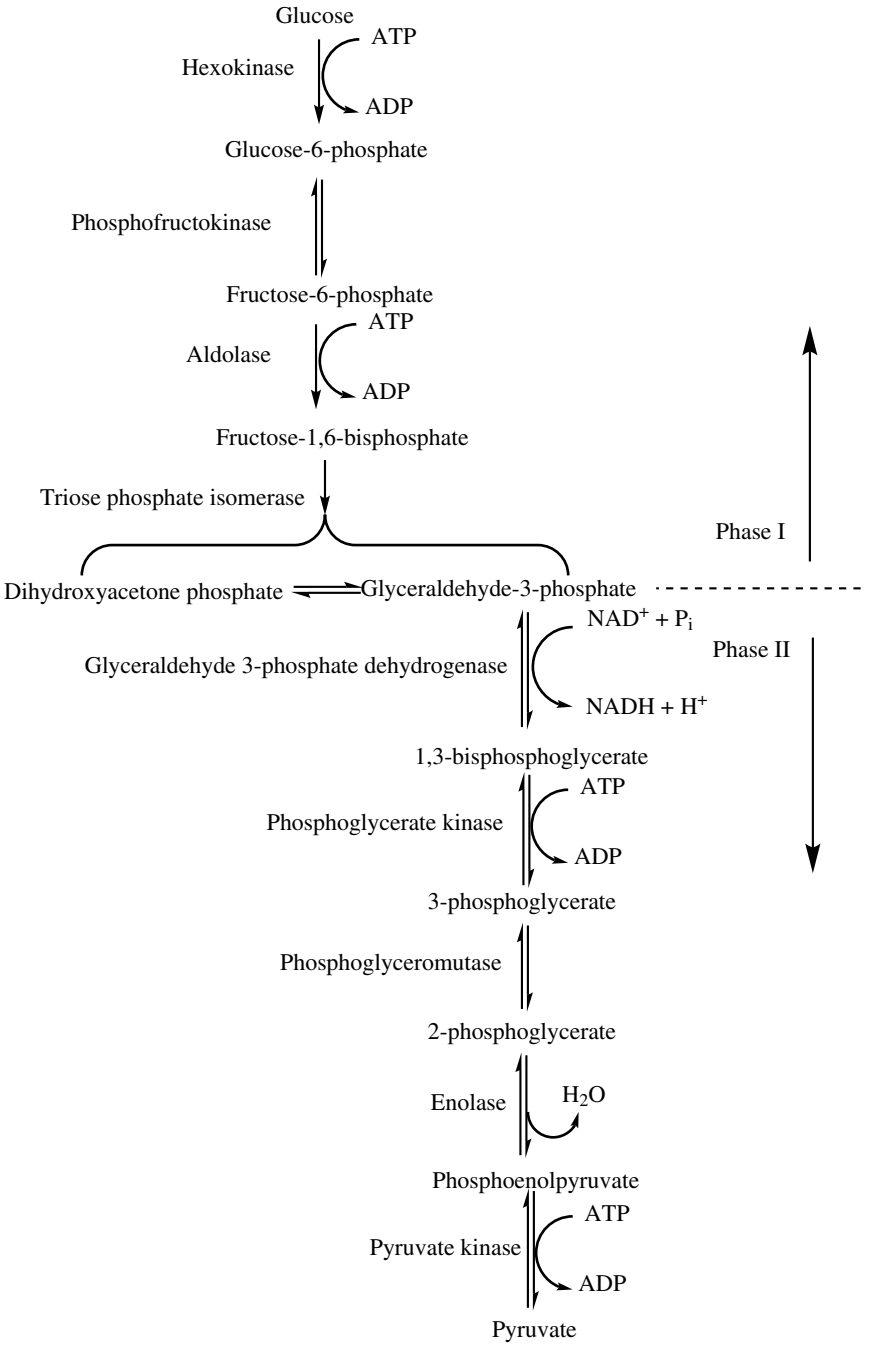


Figure 13-1: Steps in glycolysis.

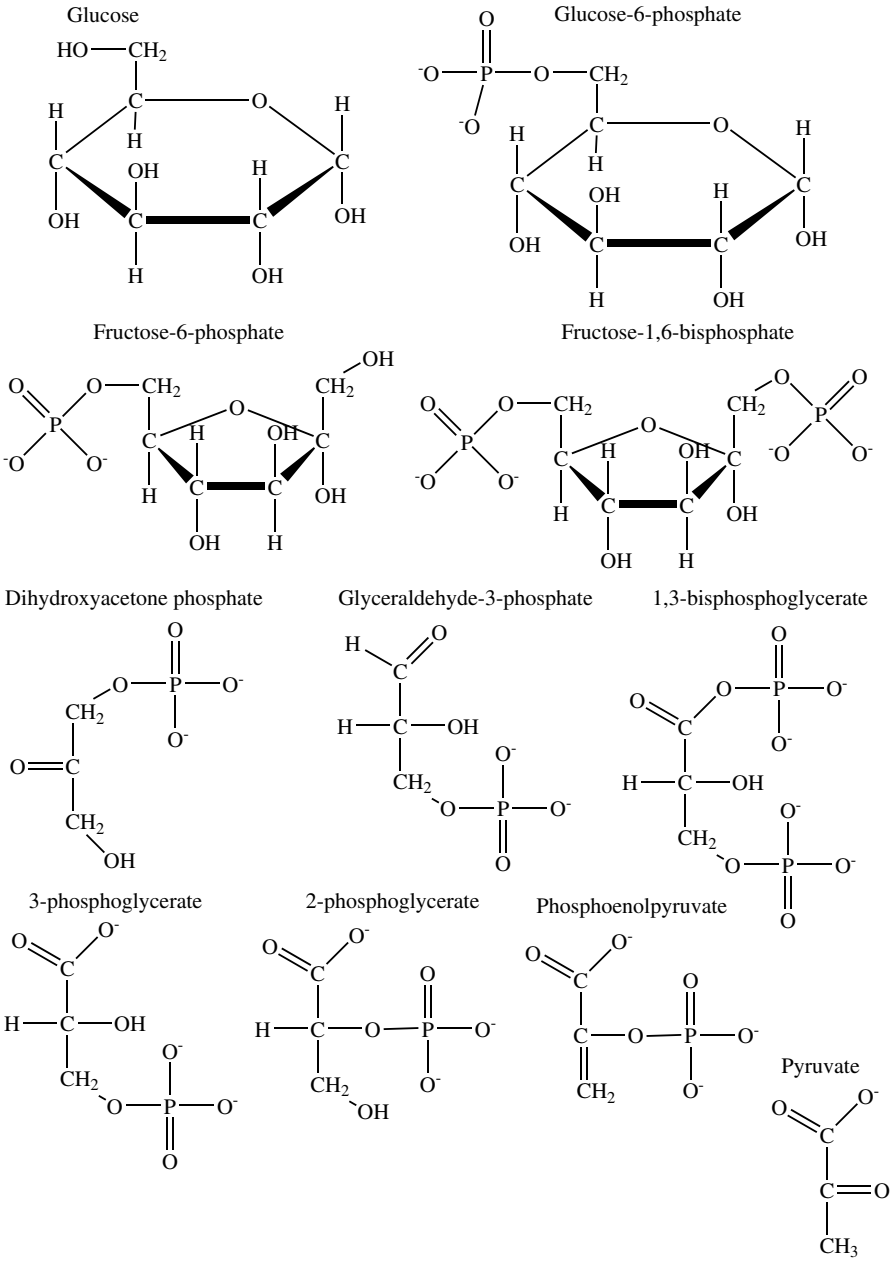


Figure 13-2: Molecules involved in glycolysis.

Glucose: Where it all starts

As we mentioned, glycolysis occurs in two phases: Phase I and Phase II.

Phase I

As glucose enters the cell, it undergoes immediate phosphorylation to glucose-6-phosphate — the first step in Phase I. The phosphate comes from ATP, and the enzyme hexokinase, with the aid of Mg^{2+}, catalyzes the transfer. Thus, the first step in the production of energy requires an investment of energy, which is necessary to activate the glucose in a reaction that is not easy to reverse. In addition, the presence of the charged phosphate group makes it difficult for this and other intermediates to diffuse out of the cell.

The enzyme phosphoglucose isomerase then catalyzes the isomerization of glucose-6-phosphate to fructose-6-phosphate. This results in a compound with a primary alcohol group, which is easier to phosphorylate than the hemiacetal originally present. Fructose-6-phosphate then reacts with another molecule of ATP to form fructose-1,6-bisphosphate. The enzyme for this step is phosphofructokinase — (try saying that ten times fast!) — and this enzyme requires Mg^{2+} to be active. This is the major regulatory step in glycolysis. ATP inhibits this enzyme, whereas AMP activates it.

Aldolase enzymatically cleaves the fructose-1,6-bisphosphate into two triose phosphates. These triose phosphates are dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. The dihydroxyacetone phosphate isomerizes to glyceraldehyde-3-phosphate to complete Phase I. Triose phosphate isomerase catalyzes this isomerization. (You see why we suggested following along with Figures 13-1 and 13-2?)

The net result of Phase I is the formation of two molecules of glyceraldehyde-3-phosphate, which costs two ATP molecules, and produces no energy.

Phase II

Phase II begins with the simultaneous phosphorylation and oxidation of glyceraldehyde-3-phosphate to form 1,3-bisphosphoglycerate. Glyceraldehyde-3-phosphate dehydrogenase catalyzes this conversion. Inorganic phosphate is the source of the phosphate. NAD^+ is the coenzyme and oxidizing agent. NAD^+ reduces to NADH.

There is a high-energy acyl phosphate bond present in 1,3-bisphosphoglycerate. Phosphoglycerate kinase, in the presence of Mg^{2+}, catalyzes the direct transfer of phosphate from 1,3-bisphosphoglycerate to ADP. This results in the formation of ATP and 3-phosphoglycerate. Because the formation of ATP involves direct phosphate transfer, this process is called *substrate-level phosphorylation* to avoid confusion with *oxidative phosphorylation* (discussed later). Phosphoglyceromutase then catalyzes the transfer of a phosphate

group from C-2 to C-3, thus converting 3-phosphoglycerate to 2-phosphoglycerate. After that, dehydration occurs to form phosphoenolpyruvate (PEP), which contains a nigh-energy phosphate bond. The enzyme catalyzing the reaction is enolase.

The final, irreversible step is a second substrate-level phosphorylation. Here, an ADP molecule receives a phosphate group from the PEP. The enzyme pyruvate kinase is necessary for this step. This enzyme requires not Mg^{2+}, but also K^{+}. Pyruvate is the other product. Whew!

During Phase II, two molecules of glyceraldehyde-3-phosphate (from Phase I) form two molecules of pyruvate with the formation of four molecules of ATP and two molecules of NADH.

The pyruvate produced by glycolysis has several fates. When there is plenty of oxygen, the pyruvate enters the Krebs cycle, the electron transport chain, and oxidative phosphorylation pathways as Acetyl-CoA. This results in the production of more ATP and the total conversion to CO_2. If oxygen is lacking, vertebrates (you included) convert pyruvate to a related substance, lactate. Other organisms, such as yeast, convert pyruvate to ethanol and CO_2 — and that is why we have beer. These latter two possible fates yield less energy than the oxygen-rich fate.

Miles per gallon? Energy efficiency

Glycolysis is the initial conversion of carbohydrate to energy. After that there is the production of two ATP molecules, two NADH molecules, and two pyruvate molecules. The energy content of the ATP molecules is only 2 percent of the total energy present in each glucose molecule. This shows the relative inefficiency of anaerobic energy production. Fortunately, the pyruvate molecules will undergo further aerobic oxidation to increase this energy yield. The total energy output of anaerobic and aerobic oxidation of glucose is 30–32 ATP molecules, which accounts for about 30 percent of the total energy present in glucose. Much of the remaining energy is available as heat for warm-blooded animals.

Going in reverse: Gluconeogenesis

Gluconeogenesis is a series of reactions that generate glucose from non-carbohydrate sources. This pathway is necessary when the supply of carbohydrates is inadequate (something that is rare in our lives). The non-carbohydrate sources include lactate, pyruvate, some amino acids, and glycerol. In many ways, gluconeogenesis is the reverse of glycolysis. Figure 13-3 summarizes the steps of gluconeogenesis. (The formation of glucose in plants utilizes the process of photosynthesis.)

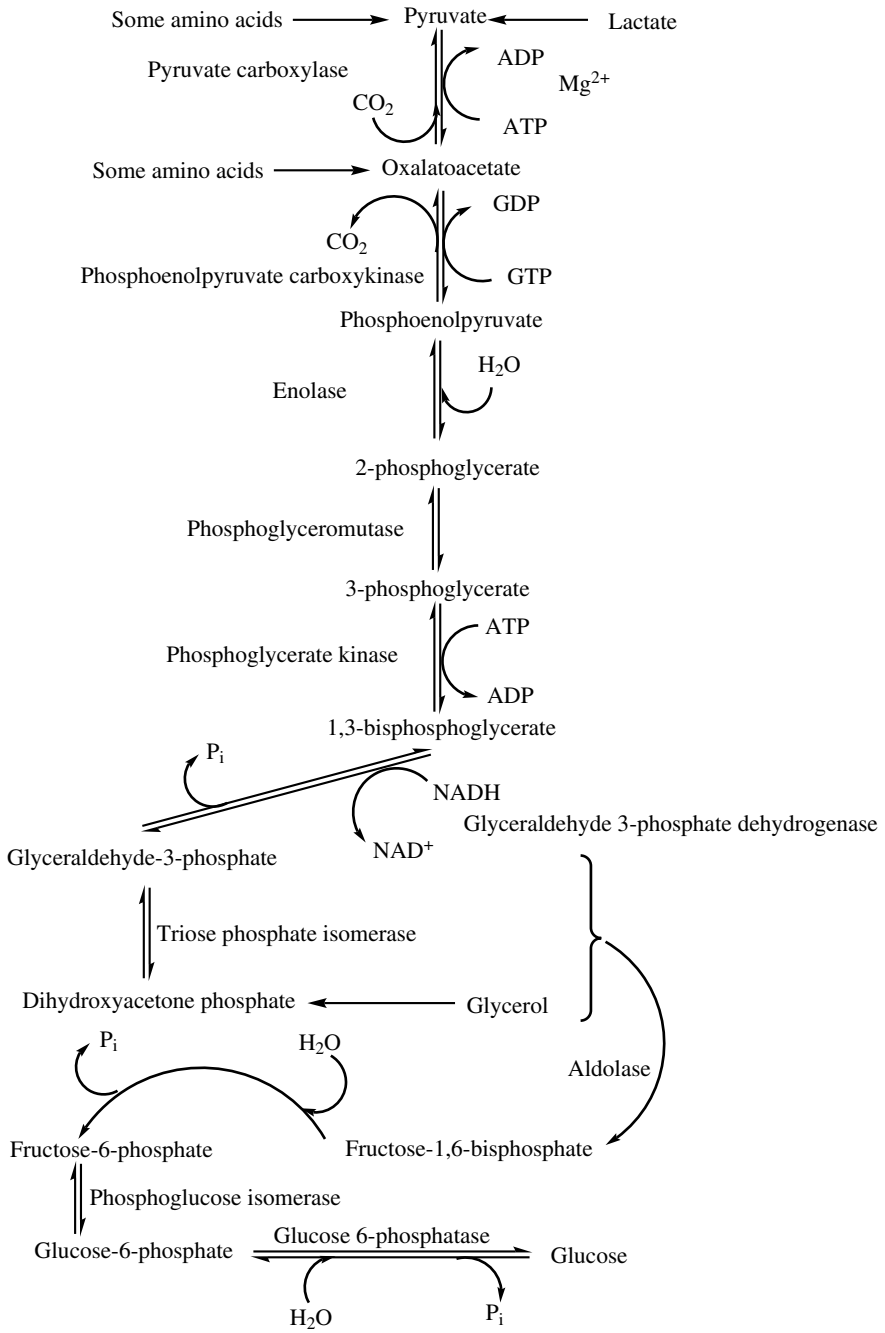


Figure 13-3: Steps in gluconeogenesis.

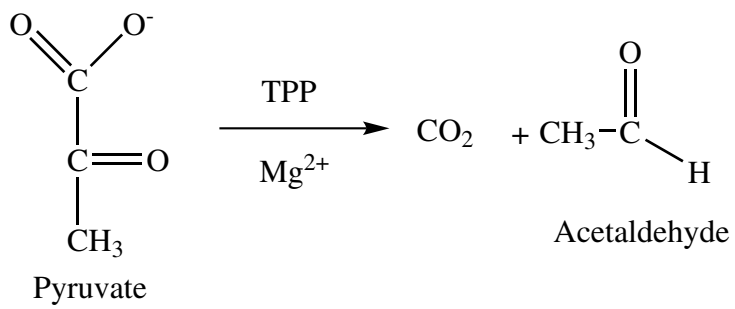
The presence of many of the same intermediates enables the use of many of the same enzymes in both glycolysis and gluconeogenesis. The differences (four enzymes) between the two systems allow regulation, so that the processes don't cancel each other. Regulation is also possible by isolating the two pathways in different organs. Other carbohydrates may also form.

Alcoholic fermentation: We'll drink to that

Under anaerobic conditions, yeast and other organisms convert pyruvate to ethanol and carbon dioxide. This process is accompanied by the oxidation of NADH to NAD^+. The NAD^+ is used in glycolysis. During this process, there is a net generation of two ATP molecules.

The first step in alcoholic fermentation is the decarboxylation of pyruvate to carbon dioxide and acetaldehyde. The enzyme pyruvate decarboxylase, along with the cofactors Mg^{2+} and TPP (thiamin pyrophosphate), catalyze this step. The enzyme alcohol dehydrogenase, along with the coenzyme NADH, catalyzes the conversion of acetaldehyde to ethanol. Makes you really appreciate that shot of tequila, doesn't it? Figure 13-4 summarizes these steps.

1. Pyruvate decarboxylase reaction



2. Alcohol dehydrogenase reaction

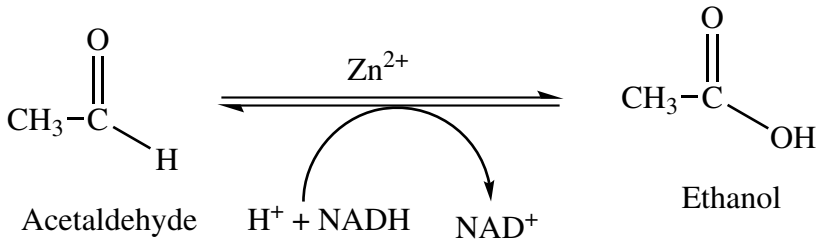


Figure 13-4: Steps in alcoholic fermentation.

Metabolism II: Citric Acid (Krebs) Cycle

The *citric acid cycle* and *oxidative phosphorylation* are the aerobic processes of catabolism that produce energy (ATP). The citric acid cycle is also known as the *Krebs cycle* and also as the *tricarboxylic acid cycle* (TCA). The primary entry molecule for this series of reactions is acetyl-CoA (short for acetyl-coenzyme A). The sources of acetyl-CoA are pyruvate from glycolysis, certain amino acids, or the fatty acids present in fats. The structure of acetyl-CoA is shown in Figure 13-5. Note: these processes take place in the *mitochondria,* the energy factories of the cell.

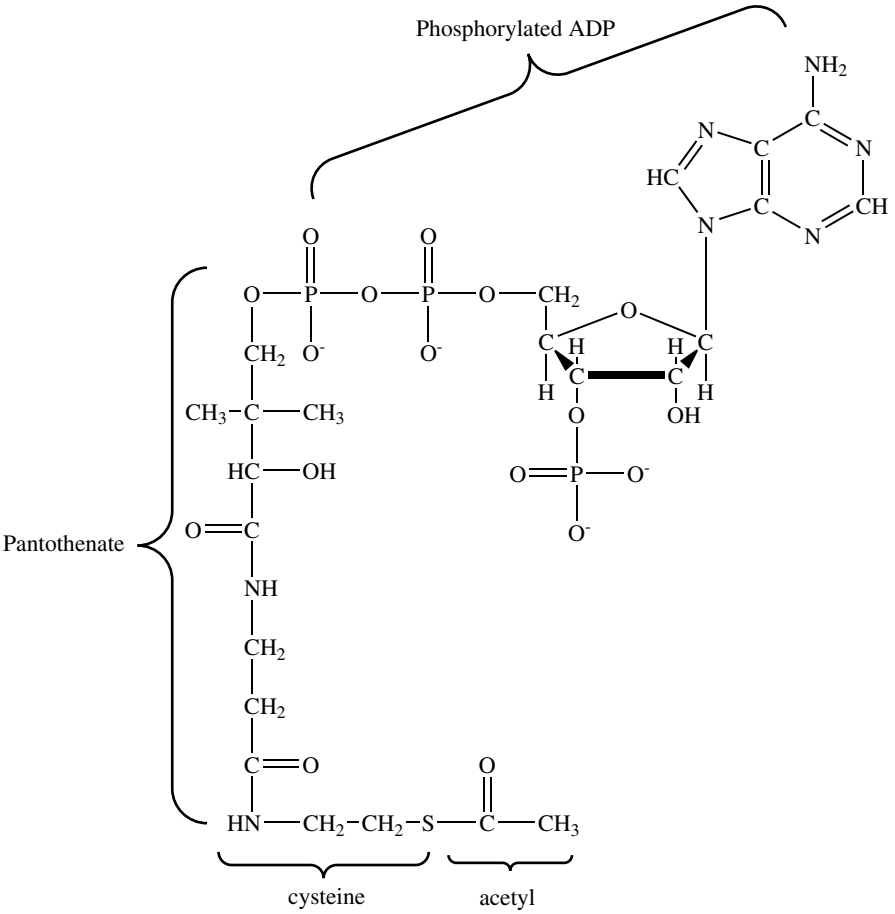


Figure 13-5: Structure of acetyl-CoA.

In addition to being an energy source, acetyl-CoA is the starting material for the synthesis of a number of biomolecules. In the next few sections, we discuss the citric acid cycle. The general cycle is shown in Figure 13-6, and the structures are shown in Figure 13-7.

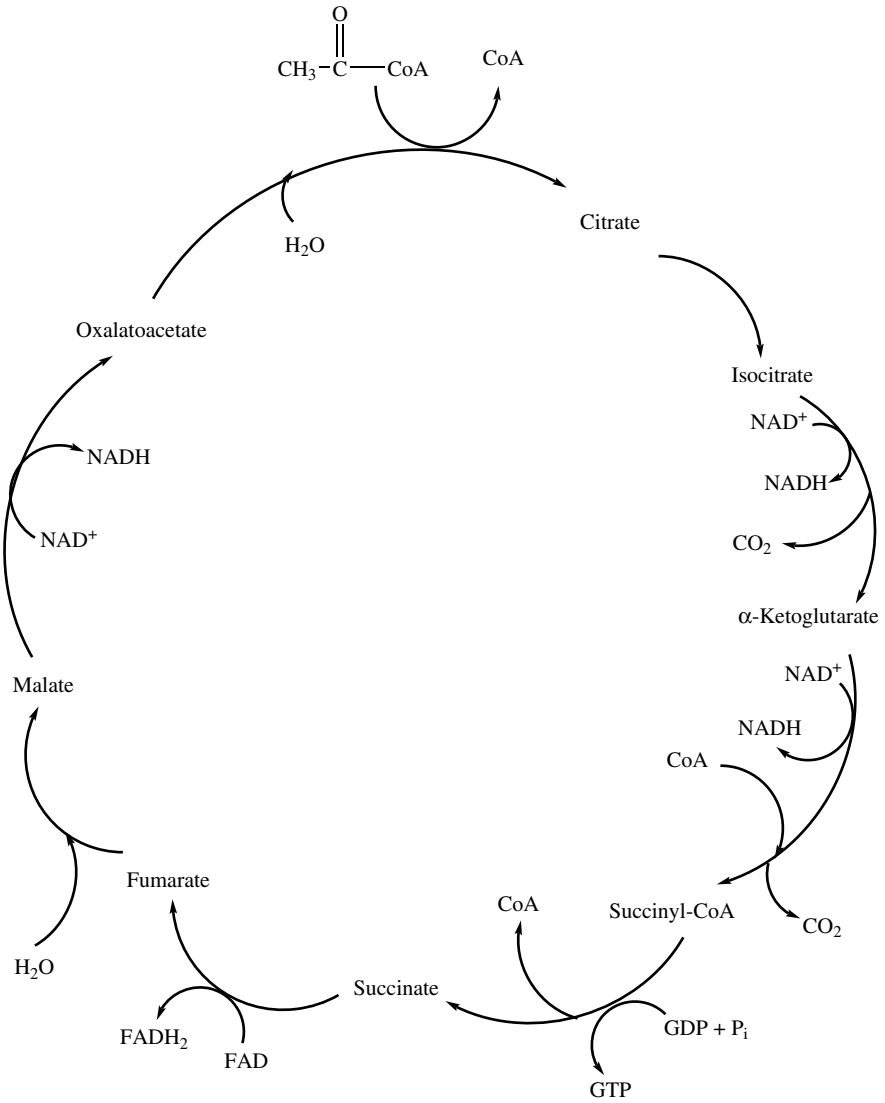
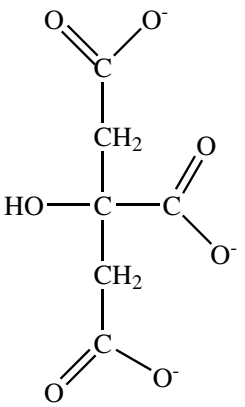
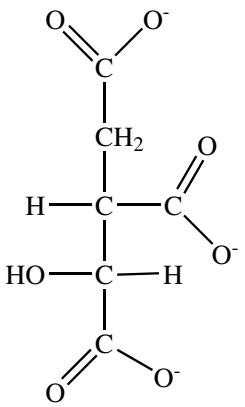


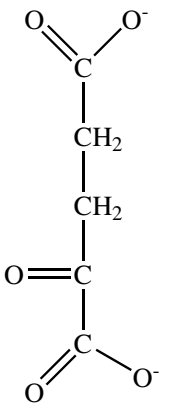
Figure 13-6: Citric acid (Krebs) cycle.



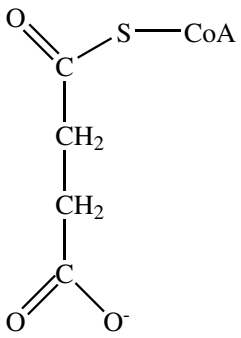
Citrate



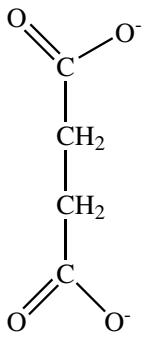
Isocitrate



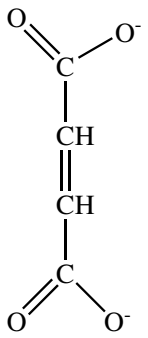
α-Ketoglutarate



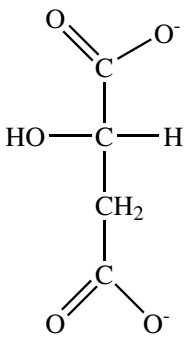
Succinyl-CoA



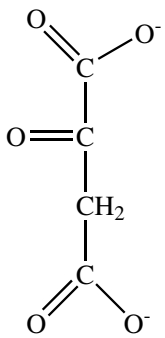
Succinate



Fumarate



Malate



Oxalatoacetate

Figure 13-7: Structures of molecules involved in the citric acid (Krebs) cycle.

Let's get started: Synthesis of acetyl-CoA

The synthesis of acetyl-CoA is a multi-step process. Figure 13-8 shows a simplified version of this process.

These steps are coupled to preserve the free energy produced by the decarboxylation. In the first step, pyruvate combines with TPP (thiamin pyrophosphate) and undergoes decarboxylation. The pyruvate dehydrogenase component of the multi-enzyme complex catalyzes this step. During the second step, the TPP undergoes oxidation, which yields an acetyl group (refer back to Figure 13-8). This acetyl group transfers to lipomide. In this reaction, the oxidant is the disulfide group of lipomide, and acetyllipoamide forms in this step. The pyruvate dehydrogenase component also catalyzes this reaction. In the final step, the acetyl group of acetyllipoamide transfer to CoA to form acetyl CoA. The catalyst for this reaction is dihydrolipoyl transacetylase.

However, the process does not end with the formation of acetyl CoA. It is necessary to regenerate the oxidized form of lopoamide. The enzyme dihydrolipoyl dehydrogenase catalyzes this step. The two electrons from the oxidation transfer to FAD and then to NAD^+. Some of the important intermediates in these steps are shown in Figure 13-9.

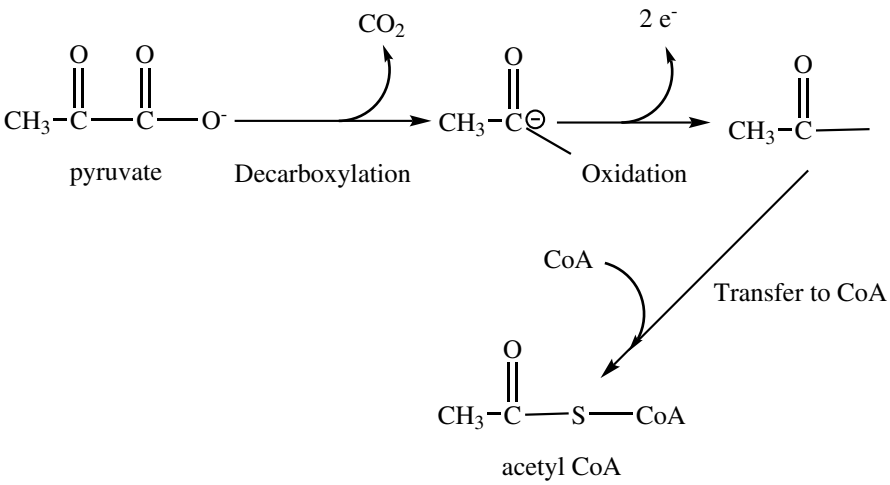
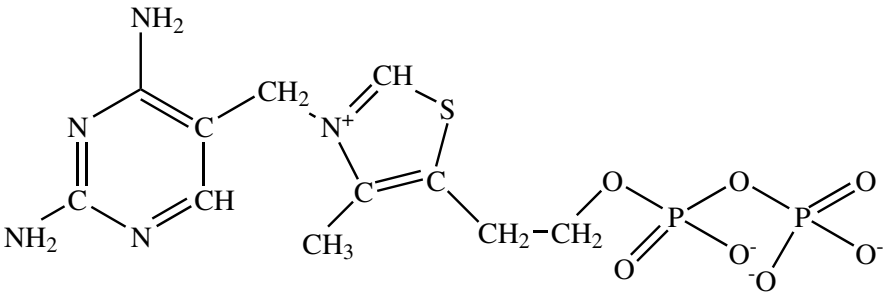
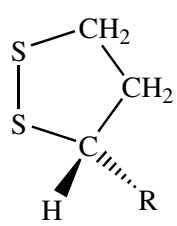


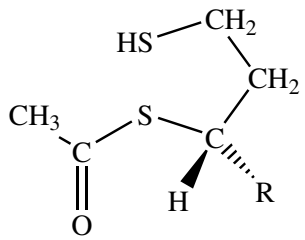
Figure 13-8: Simplified scheme for the formation of acetyl CoA.



Thiamine pyrophosphate (TPP)



Lipoamide



Acetyllipoamide

Figure 13-9: Structures of TPP, lipomide, and acetyllipo-amide.

Three's a crowd: Tricarboxylic acids

When acetyl-CoA enters the citric acid cycle, it interacts, in the presence of citrate synthase, with oxaloacetate. This interaction results in the transfer of the acetyl group to the oxaloacetate to form citrate. The hydrolysis of the thioester linkage of the acetyl-CoA releases a large amount of energy.

The enzyme aconitase, with Fe^{2+} as a cofactor, catalyzes the isomerization of citrate to isocitrate. For a time, cis-aconitate, derived aconitase, was thought to be a part of the citric acid cycle. However, even though the structure of cis-aconitate is related to the other tricarboxylic acids, it is *not* part of the citric acid cycle. The structure of cis-aconitate is in Figure 13-10.

Just a little gas: Oxidative decarboxylation

The next step is the conversion of isocitrate to α-ketoglutarate. The molecule passes through the intermediate oxalosuccinate. The isocitrate binds to the enzyme isocitrate dehydrogenase. During this process, the coenzyme NAD^+ undergoes reduction. Both ATP and NADH are negative factors in the allosteric regulation of isocitrate dehydrogenase, whereas ADP is a positive factor. This is an important mechanism to control the production of ATP.

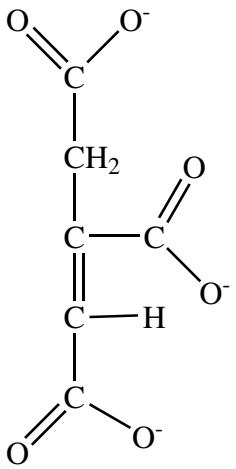


Figure 13-10: Structure of cis-aconitate.

Production of succinate and GTP

The conversion of α-ketoglutarate to succinate requires two steps. The α-ketoglutarate must bind to the enzyme to form an α-ketoglutarate dehydrogenase complex. This reaction requires the same cofactors as needed for the formation of acetyl-CoA. The result of this reaction is the elimination of carbon dioxide and the formation of succinyl-CoA. This process is irreversible under physiological conditions.

In the second step, succinyl-CoA separates to form succinate and release energy, which is harnessed by the conversion of GDP to GTP. This substrate-level phosphorylation is catalyzed by succinyl-CoA synthetase. (GTP contains about the same energy as ATP and can substitute for ATP.)

Oxaloacetate regeneration

The regeneration of oxaloacetate completes the cycle, requiring three reactions which, together, convert a methylene to a carbonyl group. First, a hydrogen atom is removed from each of two adjacent carbon atoms, resulting in the formation of a double bond. Next, a water molecule adds to the double bond. Finally, the removal of two hydrogen atoms yields the appropriate α-keto group. Succinate dehydrogenase catalyzes the first of these reactions. The prosthetic group, FAD, accepts the two hydrogen atoms by covalently binding to the enzyme. Fumarase catalyzes the next step. The final oxidation utilizes the enzyme malate dehydrogenase with the coenzyme NAD^+. The oxaloacetate is now ready to begin the cycle again.

Amino acids as energy sources

Although carbohydrates are the most readily available energy source, there are situations where amino acids can serve as energy sources. This is important for carnivores (like ourselves), who live on a high protein diet. The utilization of amino acids as energy sources is also important during hypoglycemia, fasting, and starvation.

The process begins with the removal of the amino group. This usually occurs through *transamination,* which is the transfer of an amino group from one molecule to another. Any amino acid other than threonine, proline, and lysine will undergo this process. Usually, the amino group transfers to the keto carbon of α-ketoglutarate, oxalatoacetate, or pyruvate to form glutamate, aspartate, or alanine, respectively. Specific transaminases are necessary and the coenzyme pyridoxal phosphate catalyzes this process. A second transamination is involved in the process of transforming aspartate and alanine to glutamate.

Oxidative deamination converts glutamate to α-ketoglutarate. This process, which occurs primarily in the liver, releases an ammonium ion. The reverse reaction, glutamate synthesis, is one of the few reactions that occurs in animals in which inorganic nitrogen is converted into organic nitrogen. The ammonium ion resulting from oxidative deamination may enter one or more biosynthetic pathways or the urea cycle. Most vertebrates convert the ammonium ion to urea, which is excreted in the urine. Most marine organisms, including fish, eliminate ammonia directly, whereas birds, insects, and reptiles convert the ammonium ion to uric acid.

The products of transamination, oxidative deamination, and further modification of the remaining portion of the amino acid produce one of the intermediates in glycolysis or the citric acid cycle. This is the fate of all the amino acids — some of the amino acids go through one intermediate, whereas others require more intermediates. Figure 13-11 shows where each of the amino acids enters glycolysis or the citric acid (Krebs) cycle. Some of the amino acids have more than one entry point.

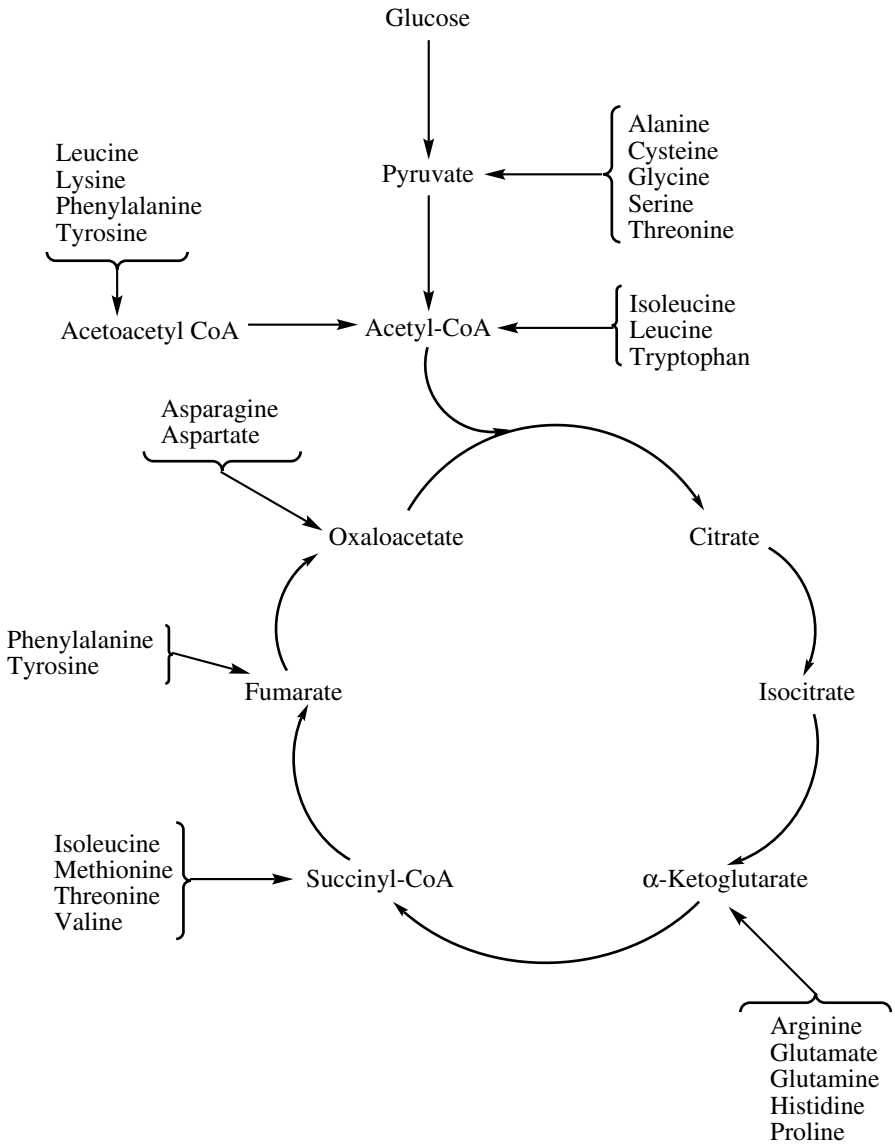


Figure 13-11: Fate of the amino acids.

Electron Transport and Oxidative Phosphorylation

The production of NADH and $FADH_2$ by the citric acid cycle supplies the materials for the next phase: oxidative phosphorylation. These reduced coenzymes transport the electrons derived from the oxidation of pyruvate. The final fate of these electrons is the reduction of oxygen to water.

The details of oxidation phosphorylation are not as easy to study as glycolysis and the citric acid cycle because the processes take place within the mitochondria, where many of the proteins involved are integrated into the walls. In addition, many of the processes are coupled. The separate components of a *coupled* process must not only be in close proximity, but often need to be in a specific arrangement.

The electron transport system

A number of species in the mitochondria must undergo oxidation-reduction reactions. Oxidation involves a loss of electrons, whereas reduction involves a gain of electrons. These processes are coupled in that the electrons lost must equal the electrons gained. The reduction potential indicates how easily a molecule undergoes oxidation or reduction. The molecular players that are important to the electron transport system are the pyridine-linked dehydrogenases, flavin-linked dehydrogenases, iron-sulfur proteins, ubiquinones, and cytochromes.

Off on a tangent: Dealing with reduction potentials

The standard for reduction potentials is the reaction:

$$2\ H^+(aq) + 2\ e^- \quad H_2(g)$$

Under standard conditions (25°C, P_{H2} = 1 atm, and $[H^+]$ = 1.0 M), the standard reduction potential is $E^\circ = 0.00$ V. Under physiological conditions in humans the value is –0.42 V (designated as E'°), because the conditions are not standard.

Table 13-1 lists a number of physiological reduction potentials. We show you how to use these entries later. The values in the table are arranged in order of increasing potential. The higher the value, the better the reaction is at oxidation, and the lower the value, the better the reaction is at reduction.

Table 13-1 Some Physiological Reduction Potentials (E'°)

	E'°(volts)
Ferredoxin-Fe^{3+} + e^- ⇌ Ferredoxin-Fe^{2+}	–0.43
2 H^+(aq) + 2 e^- ⇌ H_2(g)	–0.42
α-Ketoglutarate + CO_2 + 2 H^+ + 2 e^- ⇌ Isocitrate	–0.38
NAD^+ + H^+ + 2 e^- ⇌ NADH	–0.32
FAD + 2 H^+ + 2 e^- ⇌ $FADH_2$	–0.22
Riboflavin + 2 H^+ + 2 e^- ⇌ Riboflavin-H_2	–0.20
Dihydroxyacetone phosphate + 2 H^+ + 2 e^- ⇌ Glycerol 3-phosphate	–0.19
Pyruvate + 2 H^+ + 2 e^- ⇌ Lactate	–0.19
Oxaloacetate + 2 H^+ + 2 e^- ⇌ L-Malate	–0.17
Fumarate + 2 H^+ + 2 e^- ⇌ Succinate	+0.03
Cytochrome b-Fe^{3+} + e^- ⇌ Cytochrome b-Fe^{2+}	+0.08
Cytochrome c-Fe^{3+} + e^- ⇌ Cytochrome c-Fe^{2+}	+0.22
Cytochrome c_1-Fe^{3+} + e^- ⇌ Cytochrome c_1-Fe^{2+}	+0.23
Cytochrome a-Fe^{3+} + e^- ⇌ Cytochrome a-Fe^{2+}	+0.29
Cytochrome a_3-Fe^{3+} + e^- ⇌ Cytochrome a_3-Fe^{2+}	+0.38
1/2 O_2 + 2 H^+ + 2 e^- ⇌ H_2O	+0.82

Each reaction in Table 13-1 is known as a *half-reaction.* It takes two half-reactions — one oxidation and one reduction — to produce a complete (oxidation-reduction) reaction. The electrons lost (oxidation) must equal the electrons gained (reduction). For this reason, electrons only appear in the half-reaction, but never in the overall reaction.

By convention, the reactions in Table 13-1 all appear as *reduction* half-reactions. To convert any of these to an *oxidation* half-reaction, you must do two things. First, reverse the reaction, and then reverse the sign of E'°. In an oxidation-reduction reaction, the overall reaction is created by combining (adding) an oxidation reaction with a reduction reaction. Before adding the two reactions, though, make sure that the electrons in each reaction are equal. This may require multiplying one or both of the reactions by a value to make sure the

electrons are equal. (Multiply the reactions only — do not change the value of E'° [other than a sign change].) For example, look at the following reactions from the table:

$NAD^+ + 2\ e^-$ NADH –0.32

Cytochrome b-Fe^{3+} + e^- Cytochrome b-Fe^{2+} +0.075

Let's now change the first reaction to an oxidation:

NADH $NAD^+ + H^+ + 2\ e^-$ +0.32

If we now want to combine these reactions, we need to multiply the cytochrome reaction by two (so both reactions now involve two electrons):

2 Cytochrome b-Fe^{3+} + 2 e^- 2 Cytochrome b-Fe^{2+} +0.08

The number of electrons lost must equal to electrons gained. Also, notice that only the reaction is doubled, not the voltage. We can now combine these two reactions, canceling the electrons from both sides:

NADH $NAD^+ + H^+ + 2\ e^-$ +0.32 V

2 Cytochrome b-Fe^{3+} + 2 e^- 2 Cytochrome b-Fe^{2+} +0.08 V

NADH + H^+ + 2 Cytochrome b-Fe^{3+} 2Cytochrome b-Fe^{2+} + NAD^+ + $2H^+$ +0.40 V

The final reaction will have no electrons. Other species may cancel, if they appear on both sides of the reaction arrow. Any time the sum of the two potentials is positive, the reaction produces energy. Conversely, a negative value means the reaction requires energy. The greater the value of the sum, the greater the amount of energy produced.

Pyridine-linked dehydrogenases

In order for these enzymes to function, the coenzymes NAD^+ or $NADP^+$ are necessary. The coenzymes may be in either the oxidized or the reduced forms. If the general form of the substrate in the reduced form is $Z\text{-}H_2$, and in the oxidized form, it is Z, then the reaction will be:

$Z\text{-}H_2$ + NAD^+ (or $NADP^+$) Z + NADH (or NADPH) + H^+

There are more than 200 pyridine-linked dehydrogenases. The majority of NAD^+-linked dehydrogenases are involved in aerobic respiration. Most of the $NADP^+$-linked dehydrogenases are involved in biosynthesis.

Flavin-linked dehydrogenases

Enzymes (E) of this type require FAD or FMN as tightly bound prosthetic groups or coenzymes. Again, the species may be in either the oxidized or the reduced forms. The general reactions of this type are:

$$Z\text{-}H_2 + E\text{-}FAD \quad Z + E\text{-}FADH_2$$

$$Z\text{-}H_2 + E\text{-}FMN \quad Z + E\text{-}FMNH_2$$

NADH dehydrogenase, which contains the prosthetic group FMN, is the enzyme responsible for transporting electrons from NADH to the next acceptor in the electrons transport chain. There are other flavin-linked dehydrogenases — for example, succinate dehydrogenase.

Iron-sulfur proteins

The chief characteristics of iron-sulphur proteins are the presence of iron and sulfur, as S^{2-}. The electron transporting ability of these proteins is the Fe^{2+}/Fe^{3+} couple. Several of these proteins are associated with the electron transport chain, where they are complexed to other respiratory species. Examples include succinate dehydrogenase, with two iron-sulfur centers, and NADH dehydrogenase, with four iron-sulfur centers.

Ubiquinones

The *ubiquinones* are a group of coenzymes that are fat-soluble. Coenzyme Q (CoQ) is an example of an ubiquinone. The oxidation-reduction center is a derivation of quinine, and the fat-solubility is enhanced by the presence of a long hydrocarbon chain, containing a series of isoprene units. Many of the different ubiquinones differ only in the number of isoprene units present. The oxidized form of coenzyme Q is simply CoQ whereas the reduced form is $CoQH_2$. The general structures of both the oxidized and reduced forms of a ubiquinone appear in Figure 13-12.

Cytochromes

The *cytochromes* are a group of proteins containing a heme group. Like the iron-sulfur proteins, the oxidation-reduction couple is Fe^{2+}/Fe^{3+}. The three general classes of cytochromes are a, b, and c. The derivation of the class names relates to spectral studies done during the first isolation of these molecules. Cytochromes occur in both the mitochondria and the endoplasmic reticulum. The heme group, present in all cytochromes, is like the heme groups present in myoglobin and hemoglobin. In all cases, the central portion of the group is identical; differences derive from the attachment of side-chains to the heme core. Figure 13-13 shows the heme core and where the side-chains normally attach.

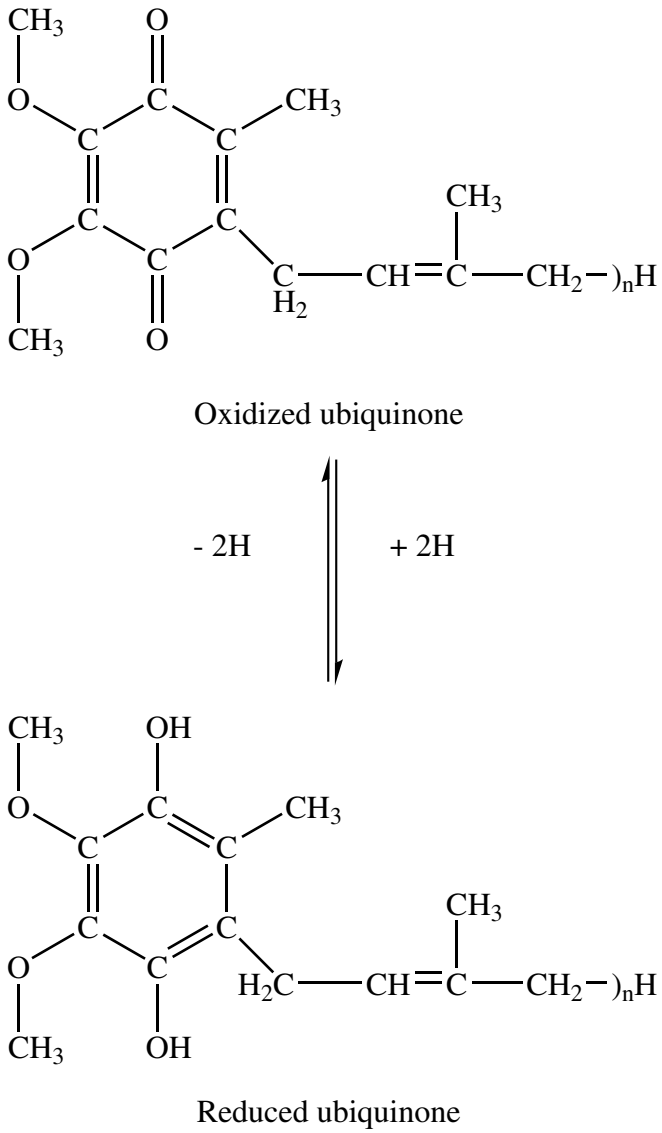


Figure 13-12: General structures of the oxidized and reduced forms of a ubiquinone.

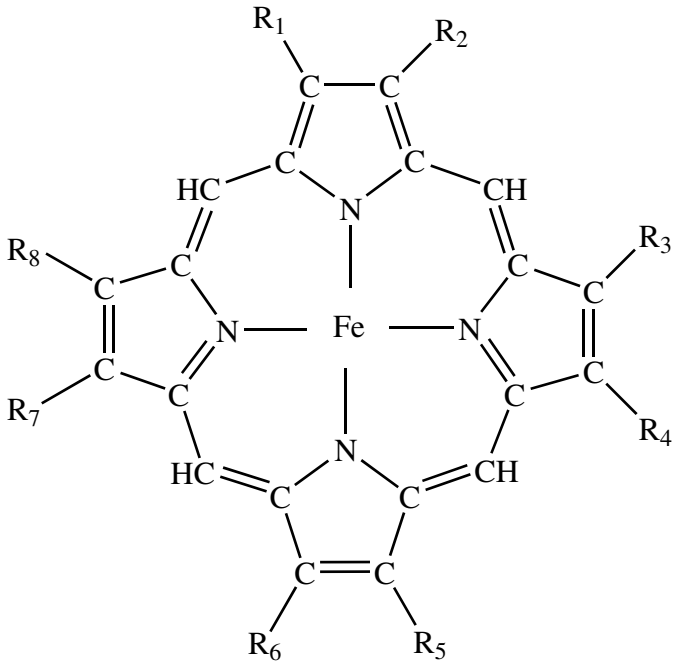


Figure 13-13: The heme core and attachment sites (R).

Five cytochromes (a, a_3, b, c, and c_1) have been identified as part of the electron transport chain of mammals. Cytochrome c, or *cyt c,* is easy to extract from cells, and therefore it is the most studied of the cytochromes. The structure of cytochrome c from different species is important to the study of biochemical evolution. Cytochromes a and a_3, cyt aa_3, occur together as a complex containing not only the expected two heme groups, but also two copper ions. The copper ions are part of another oxidation-reduction couple (Cu^{+}/Cu^{2+}). This complex, known as *cytochrome oxidase,* is the terminal cytochrome, which transfers electrons to O_2.

Interpersonal relationships

The members of the electron transport chain are grouped into four complexes with coenzyme Q (CoQ) and cytochrome c (cyt c) serving as links. One way of indicating the sequence of events in the electron transport chain appears in Figure 13-14. Figure 13-15 illustrates the same sequence emphasizing the cyclic nature of the steps. The processes take place in four complexes with linking CoQ and cytochrome c. These complexes are part of the inner mitochondrial membrane.

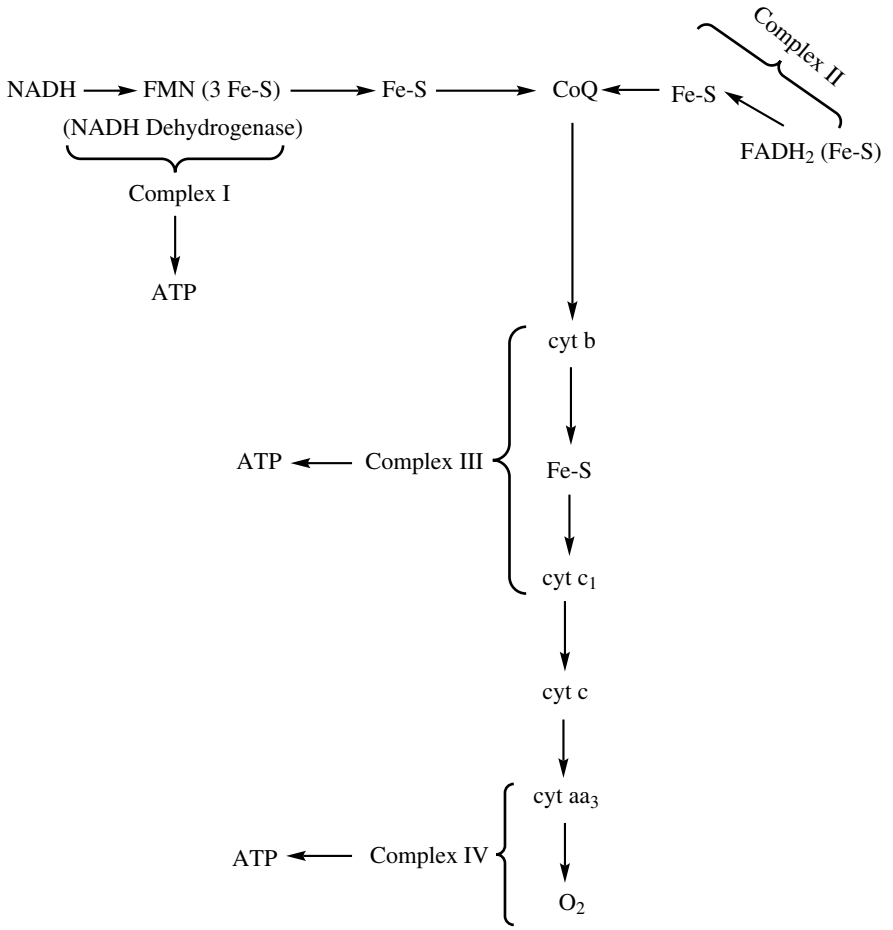


Figure 13-14: Steps in the electron transport chain.

Figure 13-15: Electron transport chain, emphasizing the cyclic nature of each of the processes.

The script: Oxidative phosphorylation

The processes of oxidative phosphorylation and the electron transport chain are closely coupled. It is only possible to oxidize the reduced forms of the coenzymes $FADH_2$ and NADH in the presence of ADP. The oxidations couple with the ADP transforming to ATP (phosphorylation).

If we calculate the oxidation-reduction potentials for NADH and $FADH_2$ reducing oxygen, we find:

$NADH \quad NAD^+ + H^+ + 2\ e^-$	+0.32 V
$\underline{1/2\ O_2 + 2\ H^+ + 2\ e^- \quad H_2O}$	$\underline{+0.82\ V}$
$1/2\ O_2 + H^+ + NADH \quad H_2O + NAD^+$	+1.14 V

And:

$FADH_2 \quad FAD + 2\ H^+ + 2\ e^-$	+0.22 V
$\underline{1/2\ O_2 + 2\ H^+ + 2\ e^- \quad H_2O}$	$\underline{+0.82\ V}$
$1/2\ O_2 + FADH_2 \quad H_2O + FAD$	+1.04 V

In both cases, the combination of the potentials is positive. Positive potentials refer to spontaneous processes, and spontaneous processes produce energy. Each NADH is capable of supplying sufficient energy to produce 2.5 ATP, and each $FADH_2$ can produce 1.5 ATP.

The play: Proposed mechanisms

The current proposed mechanism for oxidative phosphorylation is the *chemiosmotic hypothesis.* This hypothesis assumes that the hydrogen ion gradient is a significant factor promoting the conversion of ADP to ATP. The processes occurring in the four complexes present in the inner mitochondrial membrane result in a net transfer of hydrogen ions across the membrane.

The hydrogen ion transfer results in an increase in the hydrogen ion concentration in the space between the inner and outer mitochondrial membranes. It is necessary to move hydrogen ions back across the membrane. This transfer of hydrogen ions is necessary in the synthesis of ATP.

The box office: ATP production

The reactions from the anaerobic oxidation of glucose (glycolysis) and the aerobic oxidation of glucose result in the production of 32 molecules of ATP from every molecule of glucose. These reactions are:

Anaerobic:

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

Aerobic:

$$2\ \text{Pyruvate} + 5\ O_2 + 30\ ADP + 30\ P_i \rightarrow 6\ CO_2 + 34\ H_2O + 30\ ATP$$

Sum:

$$\text{Glucose} + 2\ NAD^+ + 5\ O_2 + 32\ ADP + 32\ P_i \rightarrow 6\ CO_2 + 36\ H_2O + 2\ NADH + 2\ H^+ + 32\ ATP$$

Involving the fats: β-oxidation cycle

Fatty acids may also serve as a source of ATP. Accomplishing this requires a series of reactions, known as β-*oxidation,* or the *fatty acid spiral,* to break down the fatty acid molecule. This series of reactions is a cyclic process. Some of the processes are oxidations, which require the coenzymes NAD^+ and FAD. This process also occurs in the mitochondria. The initiation of fatty acid oxidation requires activation of the relatively unreactive fatty acid molecule. The activated form is analogous to acetyl-CoA. In this case, the coenzyme A binds to the fatty acid to form a fatty acyl-CoA. Activation requires acyl-CoA synthetase and one molecule of ATP. The ATP uses two phosphates and becomes AMP.

At the inner mitochondrial membrane, the fatty acyl-CoA combines with the carrier molecule carnitine. Carnitine acyltransferase catalyzes this transfer. The fatty acyl-carnitine transports into the mitochondrial matrix, where it converts back to fatty acyl-CoA. With the mitochondrial matrix, a series of four reactions constitute the cycle known as β-oxidation. The name of this process refers to the oxidation of the second (β) followed by the loss of two carbons from the carboxyl end of the fatty acyl-CoA. Each trip around the cycle results in the removal of two carbon atoms, and the cycle continues until all the carbon atoms are removed. Figure 13-16 illustrates the general aspects of the cycle.

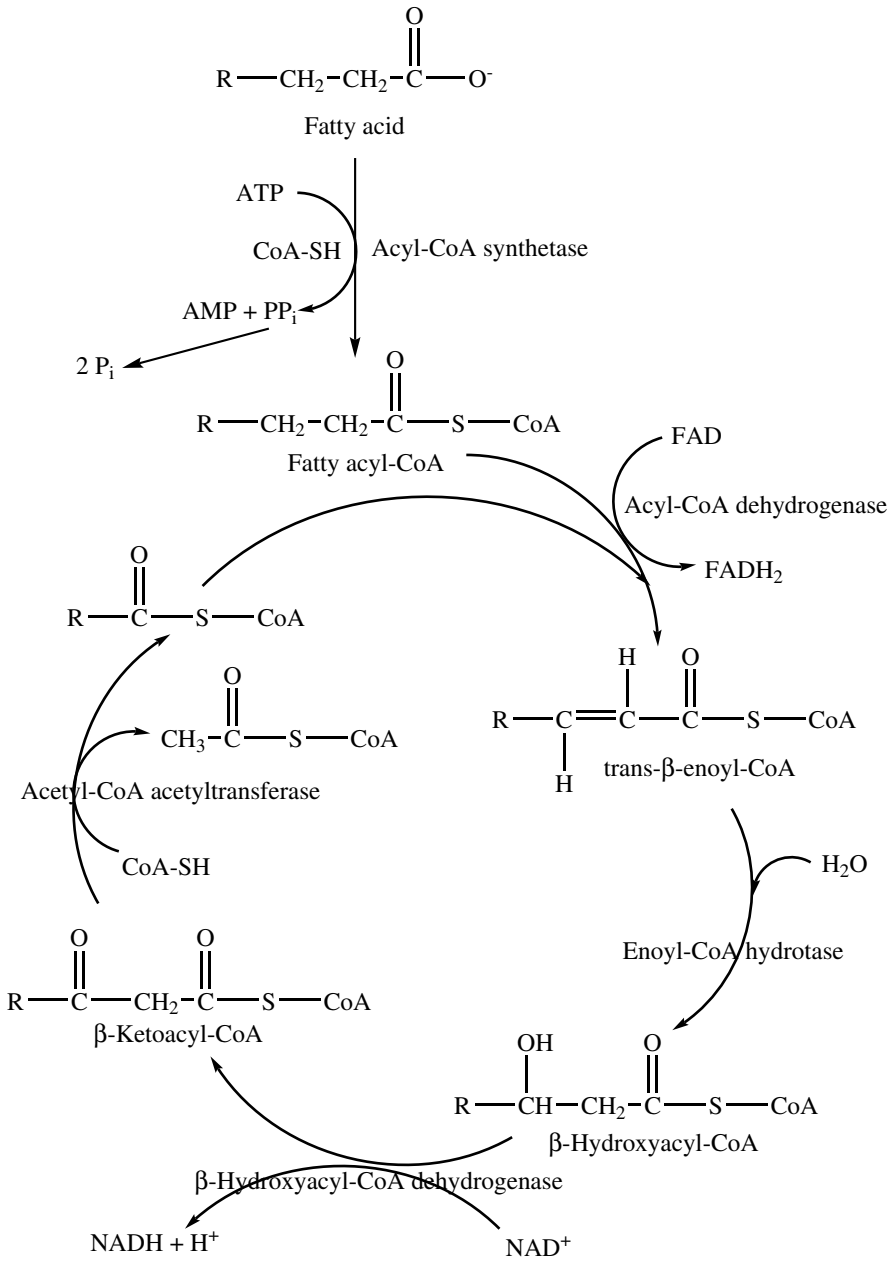


Figure 13-16: General steps in the β-oxidation cycle.

The first step in the cycle is an oxidation, with the catalyst being acyl-CoA dehydrogenase. During this step, coenzyme FAD accepts two hydrogen atoms. One of the hydrogen atoms is from the α carbon, and the other is from the β carbon atom. The process is stereospecific, producing the trans form. Elsewhere, the $FADH_2$ undergoes re-oxidation to FAD with the production of 1.5 molecules of ATP.

The trans-alkene undergoes hydration to form a secondary alcohol in the second step. The catalyst is the enzyme enoyl-CoA hydratase — a stereospecific enzyme yielding only the L isomer. Next, the secondary alcohol undergoes oxidation to form a ketone. The oxidizing agent is NAD^+. The enzyme catalyzing this oxidation is β-hydroxy-acyl-CoA dehydrogenase. The re-oxidation of NADH to NAD^+ via the electron transport chain produces two molecules of ATP.

The final step involves the cleavage of the β-ketoacyl-CoA with a molecule of CoA. This produces acetyl-CoA and a fatty acyl-CoA two carbon atoms shorter than the original. The enzyme from this step is β-ketothiolase (or simply thiolase). The new fatty acyl-CoA goes around the cycle to be shortened by two carbon atoms. An unsaturated fatty acid also goes through similar steps, but needs one or two additional enzymes.

The energy yield from a fatty acid is larger than from glucose. The process begins with the activation of the fatty acid, which costs the equivalent of two ATP molecules. Each trip around the cycle yields ten molecules of ATP, a molecule of $FADH_2$, and a molecule of NADH. The NADH and $FADH_2$ ultimately yield four additional molecules of ATP. Thus, each trip around the cycle produces 14 molecules of ATP. In addition, the final trip around the cycle produces not one but two molecules of acetyl-CoA.

Not so heavenly bodies: Ketone bodies

Some of the excess acetyl-CoA will form a group of relatively small molecules called *ketone bodies.* This is especially important when there is a build up of acetyl-CoA. A build up may occur when the rate of production is too high or if it is not used efficiently. Two acetyl-CoA molecules combine in the reverse of the last step in β-oxidation to produce acetoacetyl-CoA. Acetoacetyl-CoA reacts with water and another acetyl-CoA to form β-hydroxy-β-methylglutaryl-CoA, which in turn cleaves to acetoacetate and acetyl-CoA. Most of the acetoacetate undergoes reduction to β-hydroxybutyrate (a small amount decarboxylates to acetone and carbon dioxide). These steps appear in Figure 13-17.

The other guy

When a fat molecule breaks down, the results are a glycerol and three fatty acid molecules. The fatty acid molecules enter the β-oxidation cycle and produce energy. Catabolism of the glycerol also serves as a source of energy. First, the glycerol is phosphorylated to glycerol 1-phosphate (= glycerol 3-phosphate). This uses one molecule of ATP. Oxidation of glycerol 1-phosphate generates dihydroxyacetone phosphate, which can enter the glycolysis pathway. The net energy production is from 16.5 to 18.5 molecules of ATP.

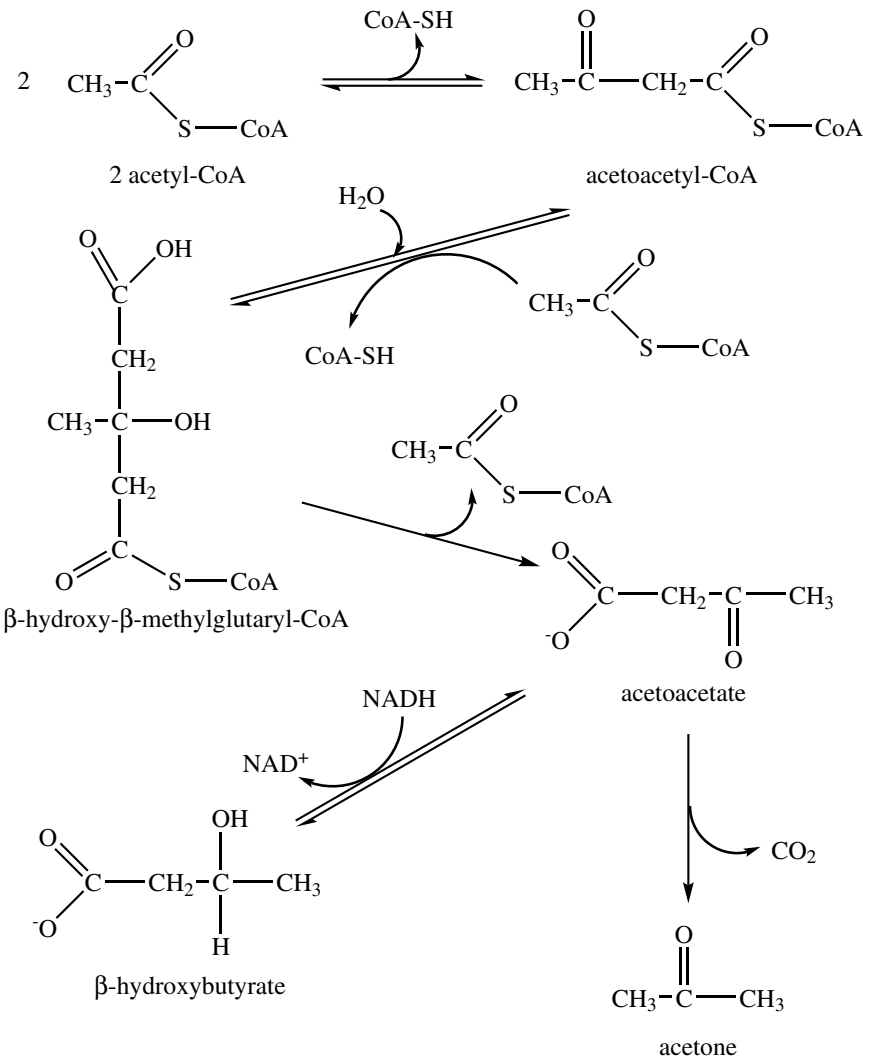


Figure 13-17: Formation of the ketone bodies.

As a group, acetone, β-hydroxybutyrate, and acetoacetate are the ketone bodies.

This process occurs primarily in the liver, and the β-hydroxybutyrate and acetoacetate then enter the bloodstream for use by other tissues. During prolonged starvation, ketone bodies may serve as the major energy source for some tissues. The kidneys excrete excess ketone bodies. Normal blood levels are about 1 mg of ketone bodies per 100 mL of blood.

In starvation or diabetes mellitus, a form of diabetes, cells may not receive sufficient carbohydrate for energy, leading to an increase in the rate of fatty acid oxidation to compensate for the energy deficit. As the amount of acetyl-CoA increases, there is insufficient oxaloacetate in the citric acid cycle available for oxidation of this acetyl-CoA. (The oxaloacetate concentration is lower because of the necessity of using it for glucose synthesis.) This leads to an increase in the production of ketone bodies and an increase of ketone bodies in the bloodstream. At 3 mg of ketone bodies per 100 mL, a condition known as *ketonemia* arises — a high concentration of ketone bodies in the urine. Ketonemia and ketonuria are two aspects of ketosis.

Two of the ketone bodies are in the form of acids. The build up of ketone bodies leads to an overwhelming of the blood buffers. The decrease in blood pH may reach 0.5 units lower than the normal pH (7.4), leading to acidosis, a serious condition, which, among other things, leads to difficulty in oxygen transport by hemoglobin. Dehydration results as the kidneys eliminate large quantities of liquid trying to remove the excess acid. Severe acidosis may result in a coma that may result in death.

Mammals cannot convert acetyl-CoA to carbohydrates. It is possible to convert carbohydrates to fats, but not to do the reverse.

Investing in the Future: Biosynthesis

One aspect of metabolism, catabolism, is to produce the energy required for life. Another aspect, anabolism, is to supply the materials for growth and replacement. Food supplies the raw fuel for metabolism. A number of pathways are available to allow for flexibility. It is necessary to block some pathways to overcome Le Châtelier's Principle, partly because an enzyme will catalyze both the forward and the reverse reaction.

Nearly all intermediates in catabolic processes are also intermediates in anabolic processes. Molecules may easily change from one pathway to another. In general, anabolic processes require the energy produced by catabolic processes. We've already seen one aspect of anabolism — gluconeogenesis. Earlier, we saw how this process, related to glycolysis, could generate glucose and other carbohydrates. We examine other biosynthesis processes in this section.

Fatty acids

Production of the fatty acids is necessary to form the membrane lipids. But the main reason for fatty acid synthesis is to convert excess dietary carbohydrate to fats for storage. The key molecule for this is acetyl-CoA.

The liver is the primary fatty acid synthesis site in humans, and humans can synthesize all the fatty acids but two: linoleic acid and linolenic acid. Linoleic acid and linolenic acid are also essential fatty acids, required components of the diet. Acetyl-CoA from glycolysis or β-oxidation reacts with bicarbonate ion in a reaction (Figure 13-18) powered by ATP and catalyzed by acetyl-CoA carboxylase, forming the three-carbon molecule malonyl-CoA.

The release of insulin triggers a series of steps that result in the activation of acetyl-CoA carboxylase. Release of insulin indicates high food levels. Both glucagon and epinephrine inhibit the enzyme, through a series of steps. In mammals, the enzymes necessary to synthesize palmitic acid from acetyl-CoA and malonyl-CoA are present in a complex known as *fatty acid synthase.* In plants and bacteria, the enzymes are present as separate molecules. Synthesis proceeds two carbon atoms at a time, which is why all the natural fatty acids contain an even number of carbon atoms.

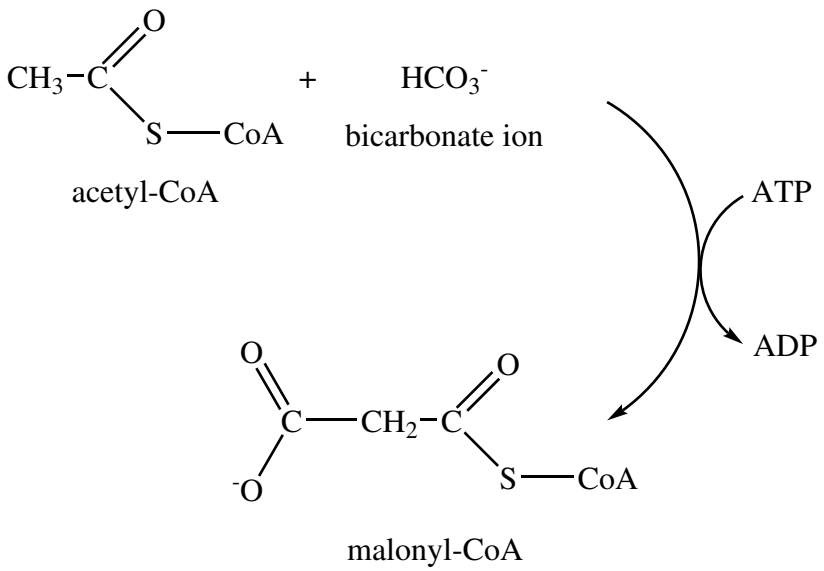


Figure 13-18: Synthesis of malonyl-CoA.

Synthesis begins when a molecule of acetyl-CoA links to an acyl carrier protein, ACP, and a malonyl-CoA does the same with another ACP. The two ACP-linked molecules then join and release a carbon dioxide molecule, an ACP, and an acetoacetyl-ACP. Next are three steps that are the reverse of the first three steps of β-oxidation. First, NADPH reduces the ketone group to an

alcohol. Then dehydration of the alcohol leaves a double bond between the second and the third carbon atoms. The coenzyme NADPH again serves as a reducing agent to produce butyryl-ACP. The sequence repeats with butyryl-ACP replacing the acetyl-ACP. These steps are in Figure 13-19.

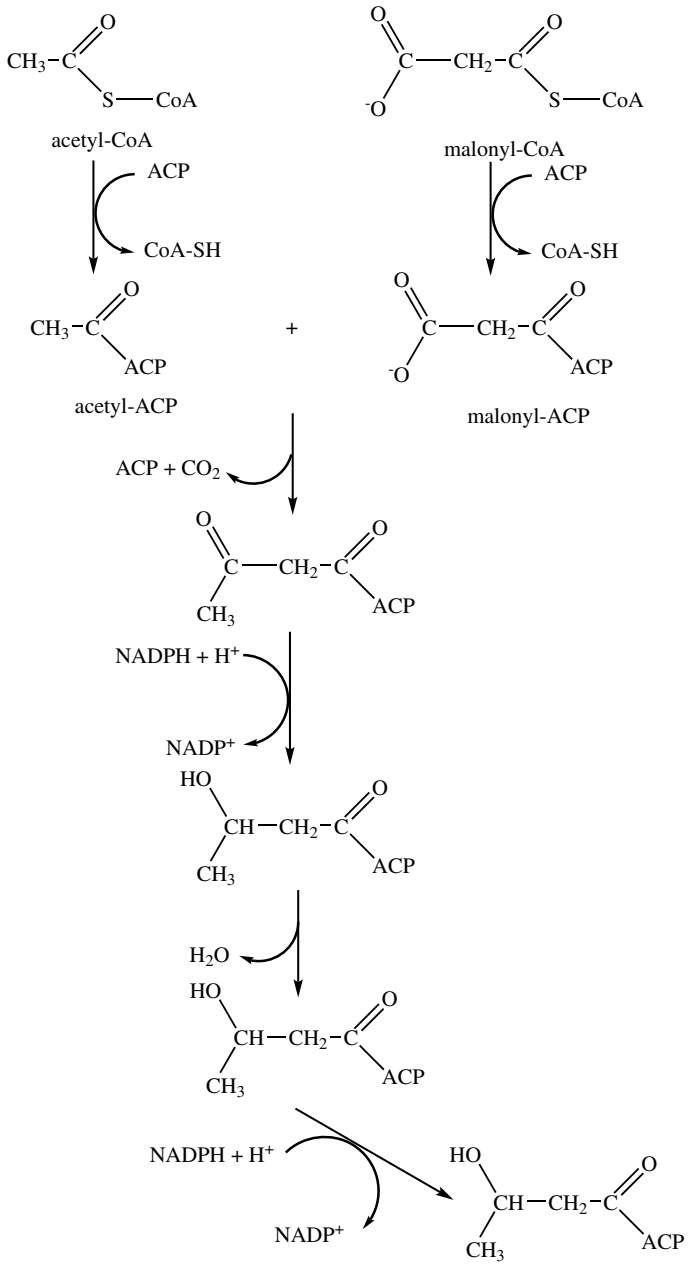


Figure 13-19: Fatty acid synthesis.

The series of synthesis steps continues up to palmitic acid (16 carbon atoms). The overall reaction is:

$$8 \text{ acetyl-CoA} + 7 H^+ + 14 \text{ NADPH} + 7 \text{ ATP} \rightarrow \text{palmitic acid} + 8 \text{ CoA} + 14 \text{ NADP}^+ + 7 \text{ ATP} + 7 P_i$$

Once the palmitic acid forms, additional reactions, where necessary, can lengthen or shorten the chain. These require different enzyme systems. Partial oxidation of a saturated fatty acid yields an unsaturated fatty acid.

Membrane lipids

Like other molecules, it is necessary to synthesize the membrane lipids from their constituents. In the previous section, we explained how to synthesize the fatty acids. These fatty acids need to be activated with acetyl-CoA in order to produce the appropriate acyl-CoA. The reduction of dihydroxyacetone, from glycolysis, yields glycerol 3-phosphate. The glycerol 3-phosphate combines with the appropriate acyl-CoA molecules to yield a phosphatidate (Figure 13-20). The phosphitidate then reacts with an activated serine or an activated choline to form the appropriate phosphoglyceride.

The formation of the spingolipids follows a similar path. In this case, sphingosine replaces glycerol. The synthesis of sphingosine begins with the reaction of palmitoyl-CoA, with serine in the presence of acid. This reaction yields Coenzyme A, carbon dioxide, and the precursor of sphingosine. Oxidation of the precursor yields sphingosine (Figure 13-21).

An acyl-CoA can then add a fatty acid to the amine group to produce N-acylsphingosine (ceramide). The reaction of the alcohol on the third carbon of the ceramide with activated phosphocholine yields sphingomyelin.

The reaction of ceramide with an activated monosaccharide begins the synthesis of the glycolipids. To complete the synthesis, it is necessary to add additional activated monocaccharides (UDP-glucose being one example).

Cholesterol is another membrane lipid. It helps to control the fluidity of cell membranes and is also the precursor of the steroid hormones. The entire synthesis takes place in the liver, where acetyl-CoA molecules are joined. Thus, the cholesterol molecule is built up two carbon atoms at a time.

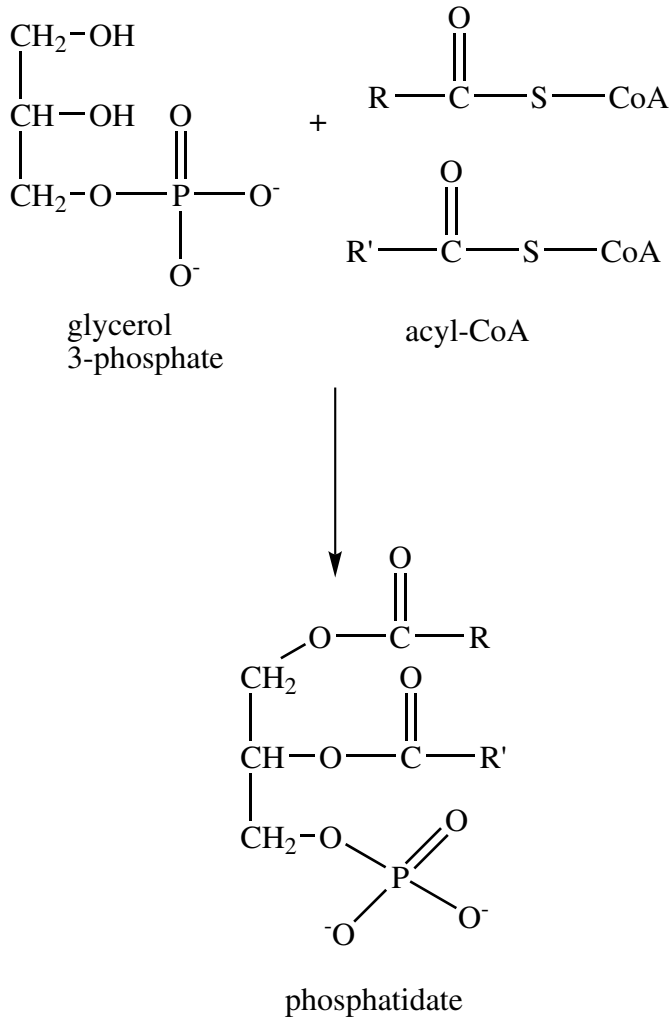


Figure 13-20: Formation of phosphatidate.

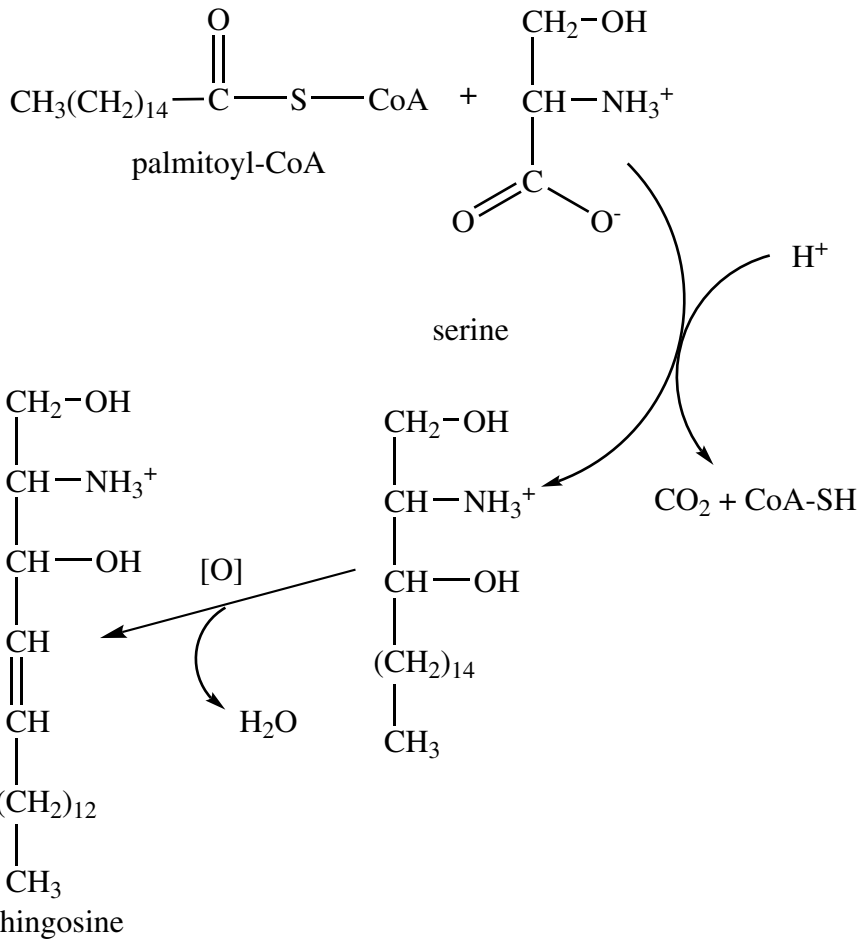


Figure 13-21: Formation of sphingosine.

Amino acids

Synthesis of amino acids becomes necessary when insufficient quantities are present in the diet. Adult humans can only synthesize 11 of the 20 amino acids. The amino acids that humans cannot synthesize are known as the *essential amino acids,* and these are a necessary requirement in the diet. Table 13-2 list the essential and non-essential amino acids.

Table 13-2 Essential and Non-essential Amino Acids

Essential	*Non-essential*
Histidine	Alanine
Isoleucine	Asparagine
Leucine	Aspartate
Lysine	Cysteine
Methionine	Glutamine
Phenylalanine	Glutamate
Threonine	Glycine
Tryptophan	Proline
Valine	Serine

Arginine is essential for children, but not for adults. Tyrosine is non-essential in the presence of adequate quantities of phenylalanine. Glutamate is important to the synthesis of five amino acids. Glutamate may form by the reduction of α-ketoglutaric acid, an intermediate from the Krebs cycle. The process is shown in Figure 13-22.

In the forward direction, this is a synthesis reaction, whereas the reverse reaction is an important oxidative deamination from the catabolism of amino acids. Glutamate, when necessary, serves as an intermediate in the biosynthesis of alanine, aspartate, asparagine, glutamine, proline, and serine. The transamination in Figure 13-23 illustrates the formation of alanine.

Replacing pyruvate in the preceding reaction with oxaloacetate yields aspartate.

It is possible to convert excess phenylalanine to tyrosine by a simple oxidation in the presence of phenylalanine hydroxylase (Figure 13-24).

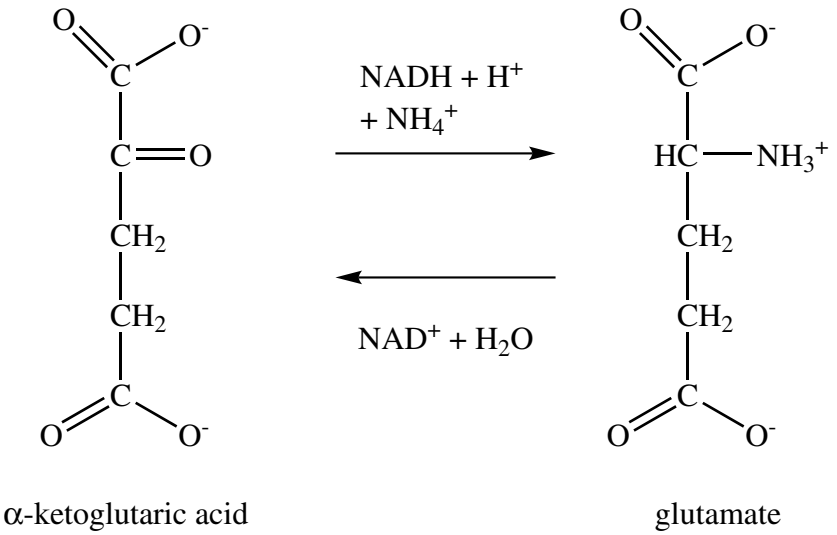


Figure 13-22: Equilibrium between glutamate and α-ketoglutaric acid.

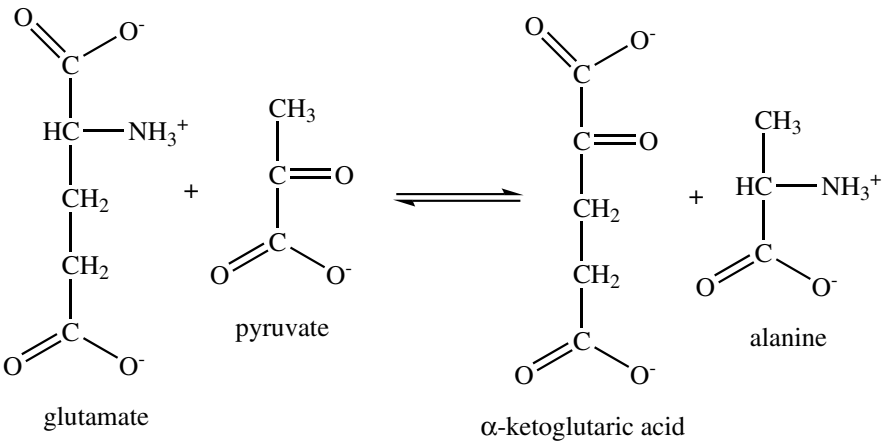


Figure 13-23: Formation of alamine.

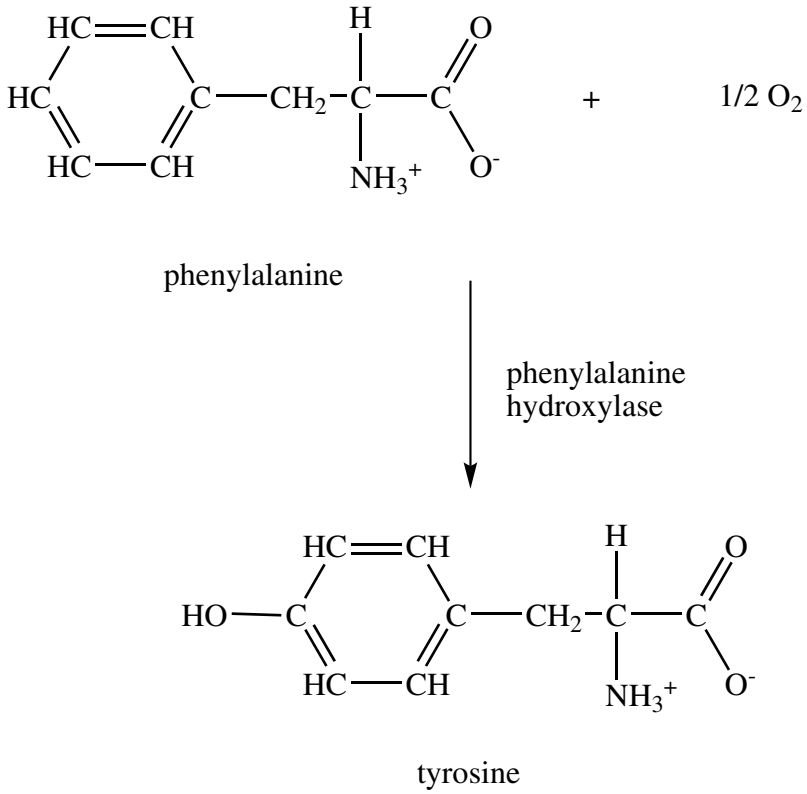


Figure 13-24: Synthesis of tyrosine.

Methionine serves as the source of sulfur for the synthesis of cysteine. Serine serves as the base of the rest of the molecule. *Serine* is the product of a three-step process beginning with 3-phosphoglycerate. The process starts with the oxidation by NAD^+ of the secondary alcohol group. The ketone thus formed undergoes transamination with glutamate to form 3-phosphoserine. Finally, hydrolysis of the phosphate ester yields serine (Figure 13-25).

The formation of proline is a four-step process beginning with glutamate. The process is shown in Figure 13-26.

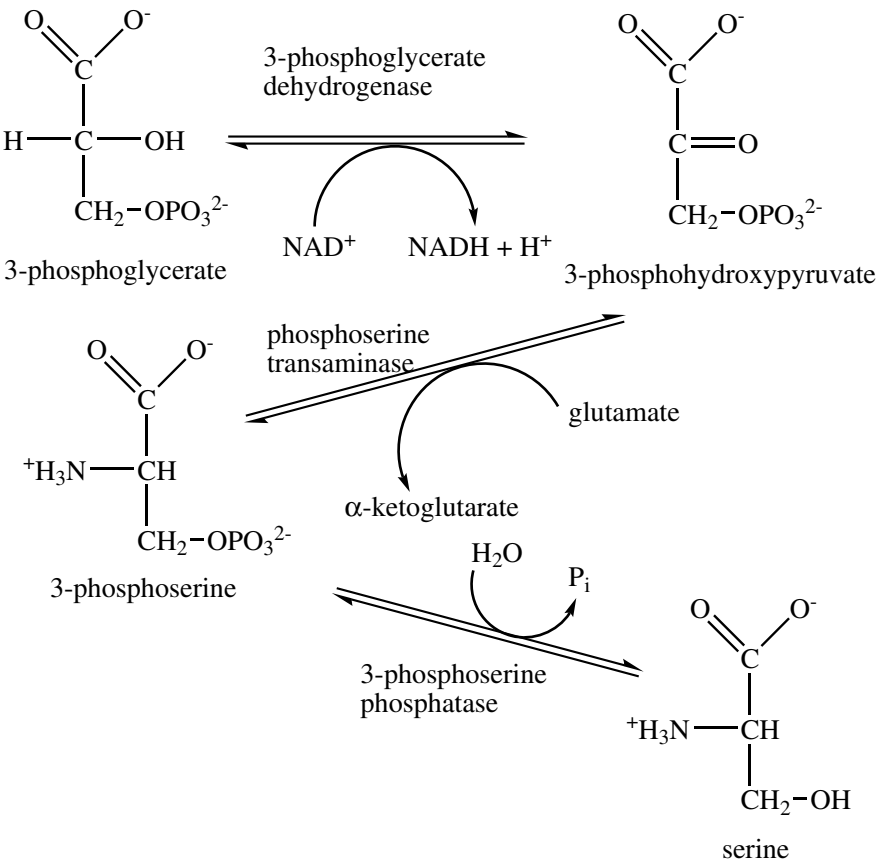


Figure 13-25: Synthesis of serine.

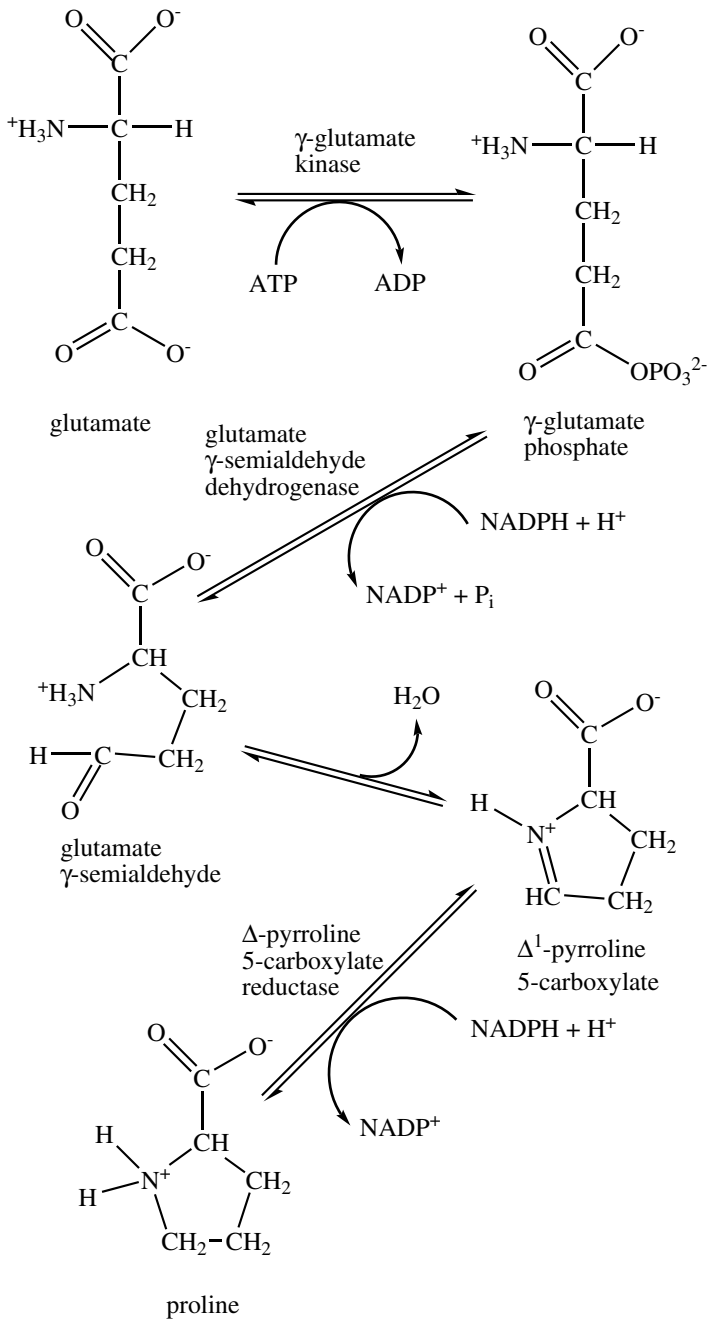


Figure 13-26: Synthesis of proline.

Chapter 14

Smelly Biochemistry: Nitrogen in Biological Systems

In This Chapter

- Talking about purine and pyrimidine
- Examining catabolism and discussing the urea cycle
- Considering amino acids
- Finding out about metabolic disorders

In this chapter, we investigate the role of nitrogen in biomolecules. Nitrogen occurs primarily in the amino acids (proteins) and in nucleic acids (purines and pyrimidines), many of which have a distinctive and generally unpleasant aroma, hence our chapter title. A few other molecules, such as hemoglobin, also contain nitrogen. Humans eliminate nitrogen primarily in the urea.

Ring in the Nitrogen: Purine

Adenine and guanine are nitrogen bases that employ the purine ring system (Figure 14-1). The formation of these molecules is essential to the synthesis of both DNA and RNA. The biosynthesis of the purines generates the molecules in their nucleotide forms instead of the free base form.

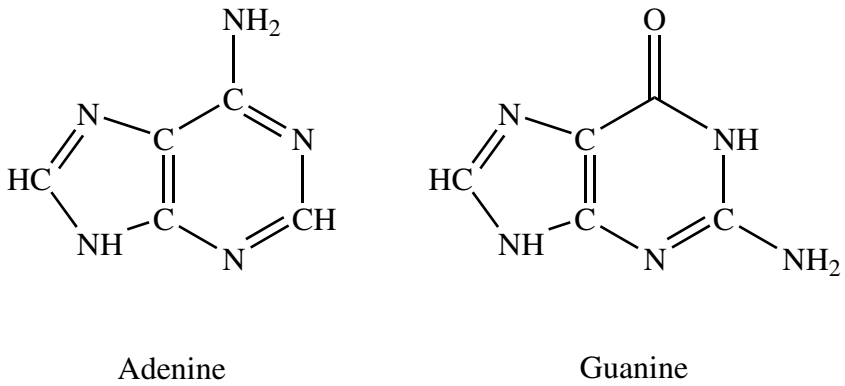


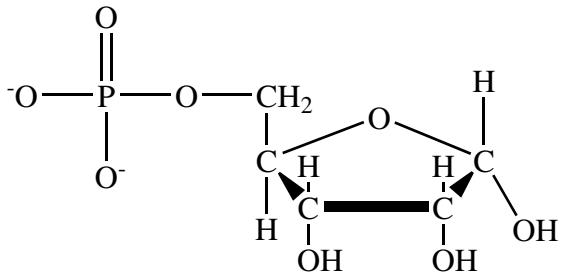
Figure 14-1: Purine nitrogen bases.

Biosynthesis of purine

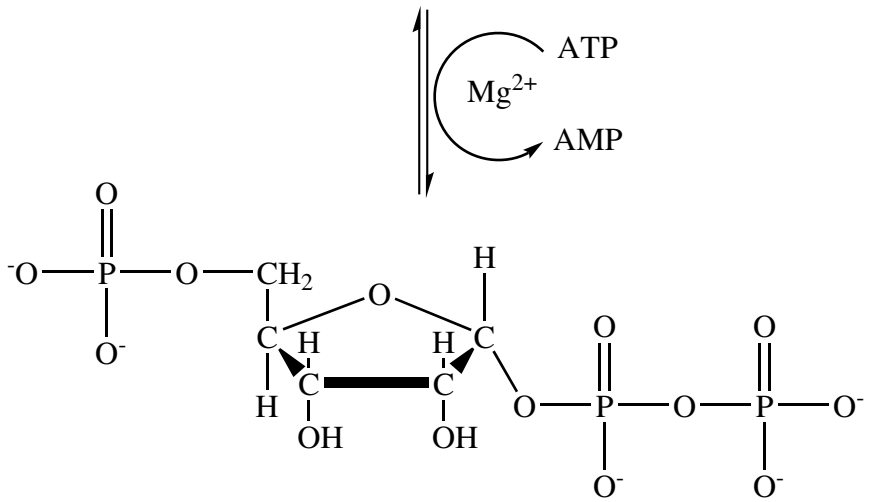
The synthesis of purine begins with the activation of D-ribose-5'- phosphate through pyrophosphorylation. In this reaction a pyrophosphate group from ATP is transferred to C-1 of an α-D-ribose-5'-phosphate. This gives a 5-phospho-α-D-ribose 1-pyrophosphate (PRPP) and AMP. The reaction is unusual because it involves the transfer of an intact pyrophosphate group (Figure 14-2). PRPP is also necessary for the synthesis of pyrimidines.

Inosine synthesis

PRPP goes through a series of ten steps (Figure 14-3) to become inosine 5'-phosphate or inosinic acid (IMP). Notice that throughout these ten steps the D-ribose-5'-phosphate portion of PRPP does not change. The ten enzymes necessary for these steps are in Table 14-1. Two additional, though different, steps are necessary to convert IMP to either AMP or GMP.



α-D-ribose 5-phosphate



5-phospho-α-D-ribose 1-pyrophosphate (PRPP)

Figure 14-2: Activation of D-ribose-5'-phosphate.

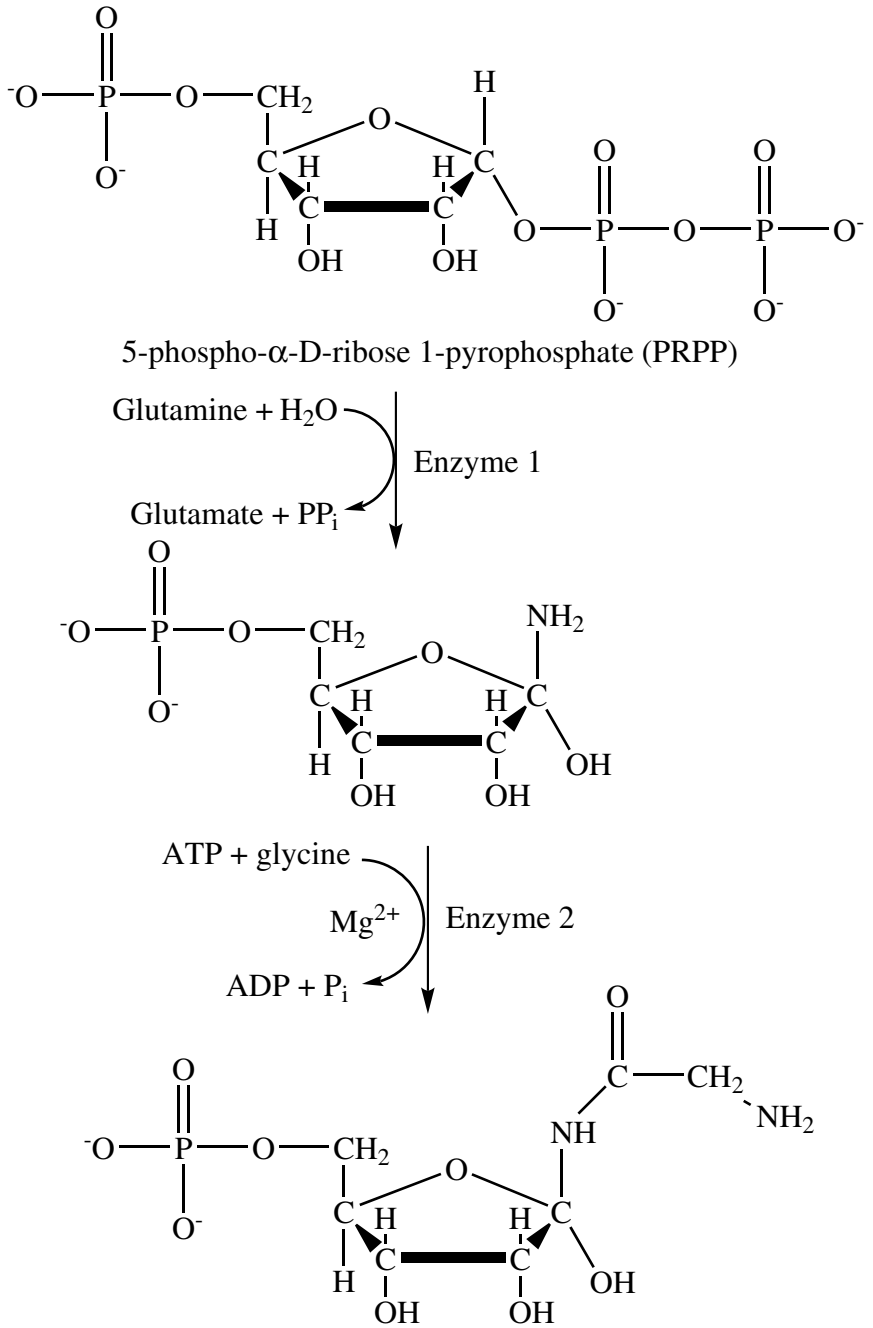
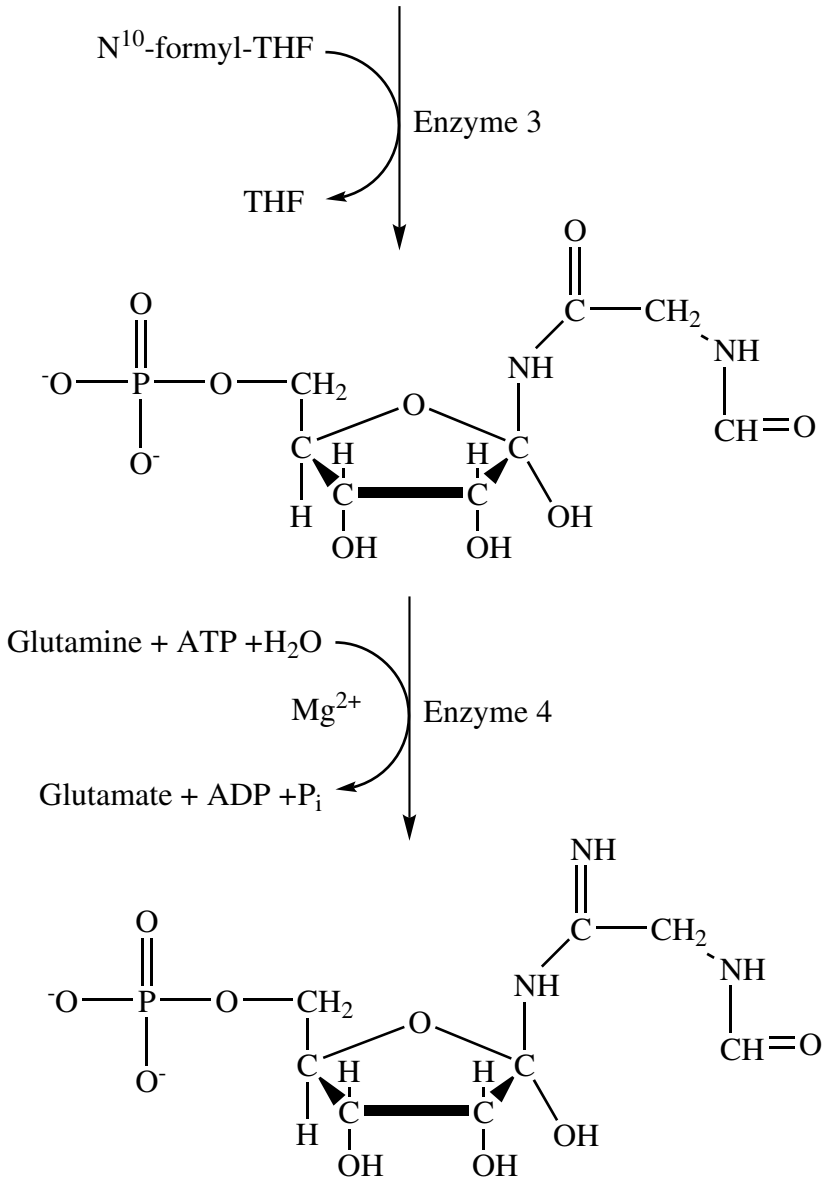
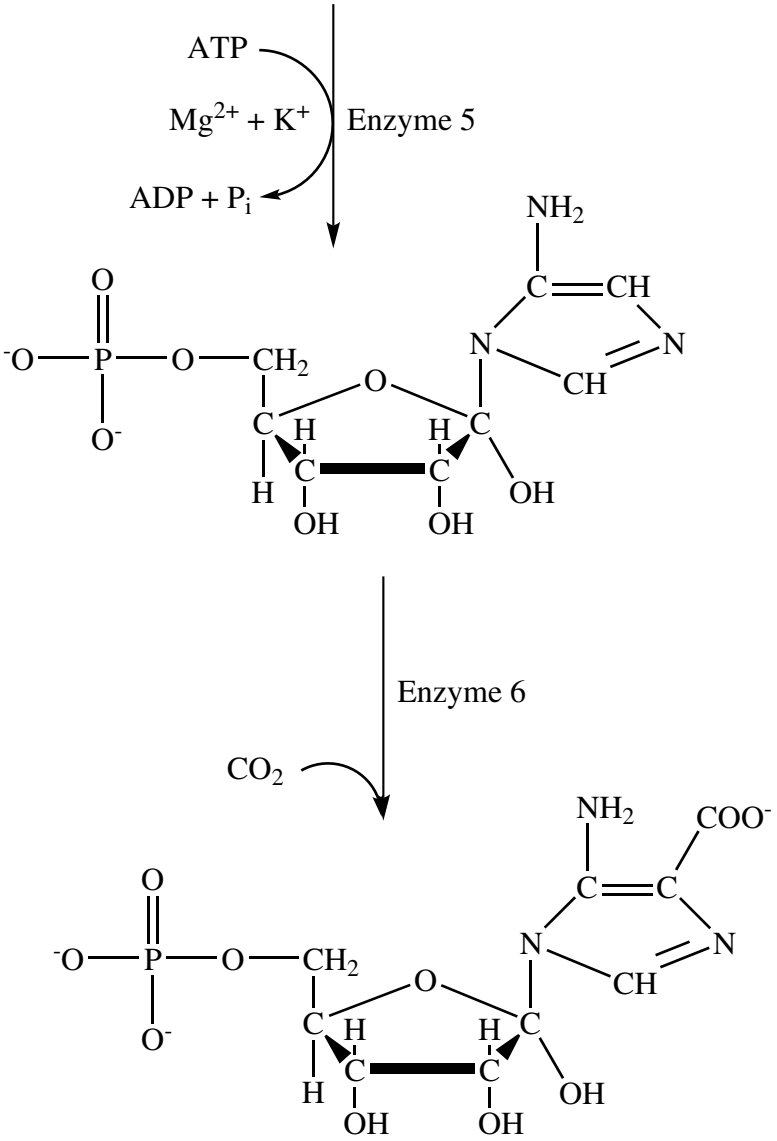


Figure 14-3: The ten steps necessary to convert PRPP (5-phospho-α-D-ribose 1-pyrophosphate) into inosine 5'-phosphate.


N10-formyl-THF
Enzyme 3
THF
Glutamine + ATP +H2O
Mg2+
Enzyme 4
Glutamate + ADP +Pi


ATP
$Mg^{2+} + K^{+}$
Enzyme 5
$ADP + P_i$
Enzyme 6
CO_2

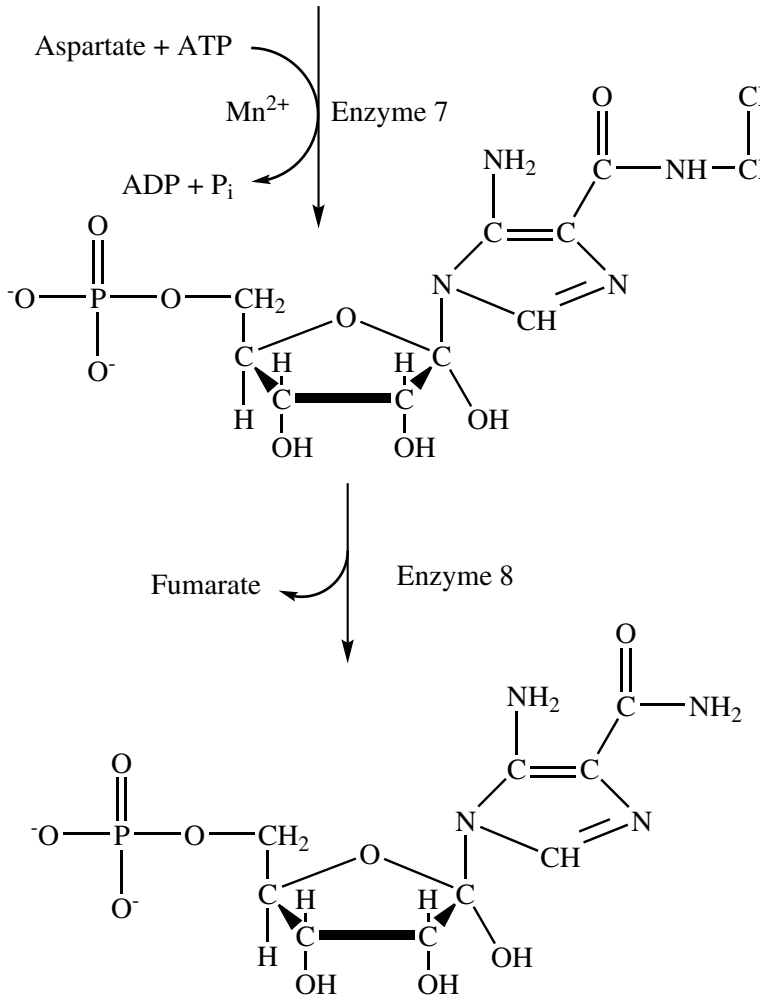

Aspartate + ATP
Mn^{2+}
Enzyme 7
ADP + P_i
Fumarate
Enzyme 8

Table 14-1 Ten Enzymes Necessary for Inosine Synthesis

Enzyme	*Name*
1	Amidophosphoribosyl transferase
2	Phosphoribosylglycinamide synthetase
3	Phosphoribosylglycinamide formyltransferase
4	Phosphoribosylformylglycimamide synthetase
5	Phosphoribosylaminoimidazole synthetase
6	Phosphoribosylaminoimidazole carboxylase
7	Phosphoribosylaminoimidazole-succinocarboxamide synthesase
8	Adenylosuccinate lyase
9	Phosphoribosylaminoimadazolecarboxamide formyltransferase
10	IMP cyclohydrolase

AMP synthesis

To convert IMP into AMP, it is necessary to transfer an amino group from an aspartate. This transfer requires two steps, and the energy to add aspartate to IMP comes from the hydrolysis of a GTP. The process is then completed by the loss of fumarate. The enzyme adenylosuccinate synthetase catalyzes the first step, and the enzyme adenylosuccinate lyase catalyzes the second. Figure 14-4 illustrates the process.

GMP synthesis

The conversion of IMP to GMP begins with the IMP dehydrogenase catalyzed oxidation to xanthosine 5'-phosphate. The coenzyme for this step is NAD^+. GMP synthetase catalyzes the next step — the amine transfer from glutamate. The energy for this step is supplied by the hydrolysis of ATP (Figure 14-5).

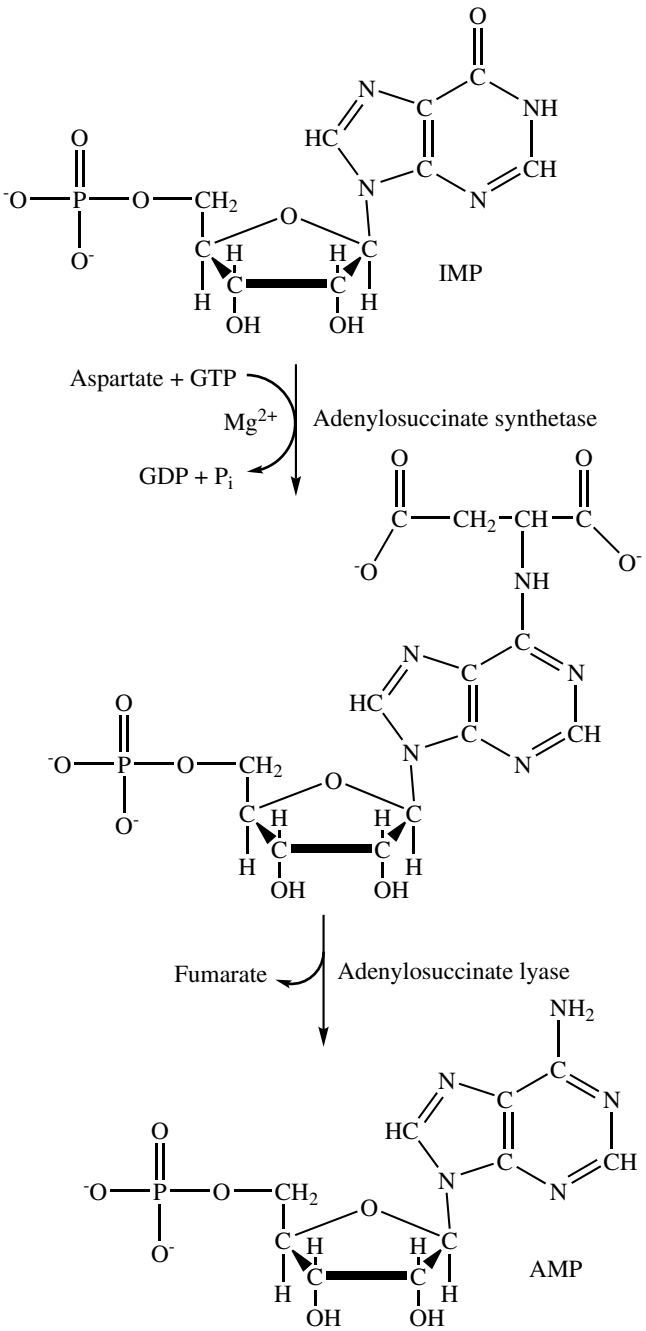


Figure 14-4: Conversion of IMP to AMP.

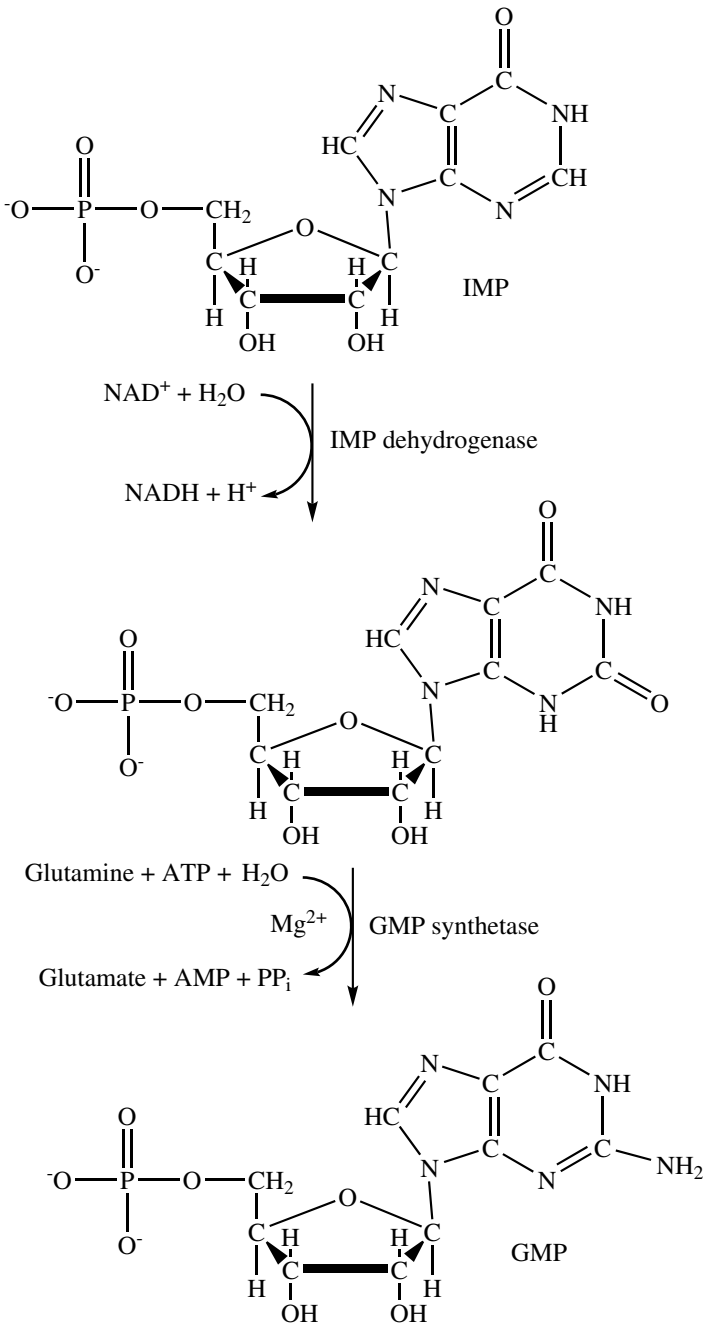


Figure 14-5: Conversion of IMP to GMP.

How much will it cost?

The biosynthesis of both AMP and GMP requires the hydrolysis of several high-energy bonds. To produce IMP from D-ribose 5-phosphate requires the hydrolysis of five high-energy bonds (one PP_i and five ATP). To convert IMP to AMP requires the hydrolysis of one more high-energy bond (from GTP). And to convert IMP to GMP requires the hydrolysis of two high-energy bonds — one ATP and one PP_i.

Anaerobic organisms, such as the bacteria responsible for tetanus or botulism, must oxidize four glucose molecules at two ATP per glucose to meet the energy requirement. An aerobic organism, like you, for example, needs to oxidize only one glucose molecule at 36 or 38 ATP per glucose. The preceding processes require a substantial amount of energy. Sometimes this energy requirement may be lessened by metabolic processes known as the *salvage pathways.* In the salvage pathways, nitrogen bases are recycled instead of synthesized. The nitrogen bases are then converted to nucleotides.

Pyrimidine Synthesis

The biosynthesis of pyrimidines follows a different path from purine synthesis. In this case, synthesis of the base takes place before attachment to the ribose. Ring synthesis requires bicarbonate ion, aspartic acid, and ammonia. Although it is possible to use ammonia directly, it usually comes from the hydrolysis of the side chain of glutamine.

First step: Carbamoyl phosphate

The initial step is to transfer a phosphate from an ATP to a bicarbonate ion to form carboxyphosphate, which in turn undergoes an exchange where ammonia replaces the phosphate to form carbamic acid. Whew! — got that? A second ATP transfers a phosphate to carbamic acid to form carbamoyl phosphate. Figure 14-6 summarizes these steps.

The primary enzyme for the process in Figure 14-6 is carbamoyl synthetase. One region of the enzyme is responsible for the synthesis of carbamic acid, whereas a second region hydrolyzes ammonia from glutamine. A third region completes the process, and a channel connects the three regions.

Next step: Orotate

The next step in pyrimidine synthesis is the formation of orotate, which will be joined to a ribose. It begins with the enzyme aspartate transcarbamoylate,

which joins aspartate to carbamoyl phosphate with the loss of phosphate. This forms carbamoylaspartate. Carbamoylaspartate cyclizes to dihydroorotate, which is oxidized by NAD^+ to orotate (Figure 14-2).

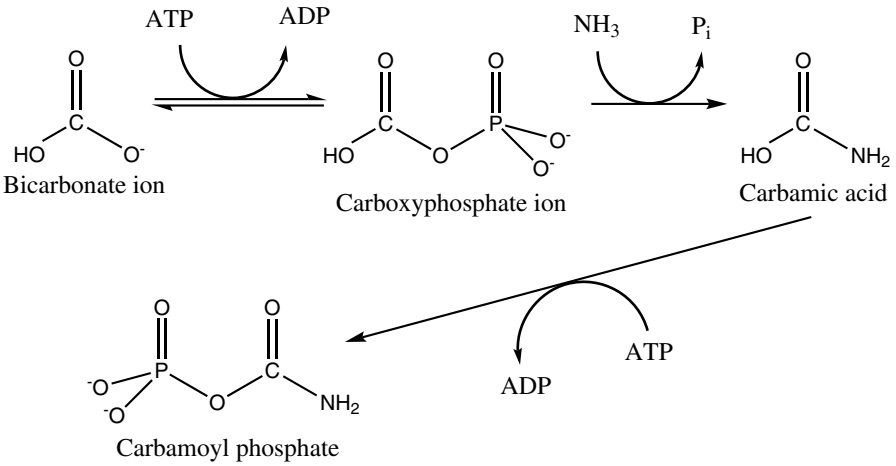


Figure 14-6: Synthesis of carbamoyl phosphate.

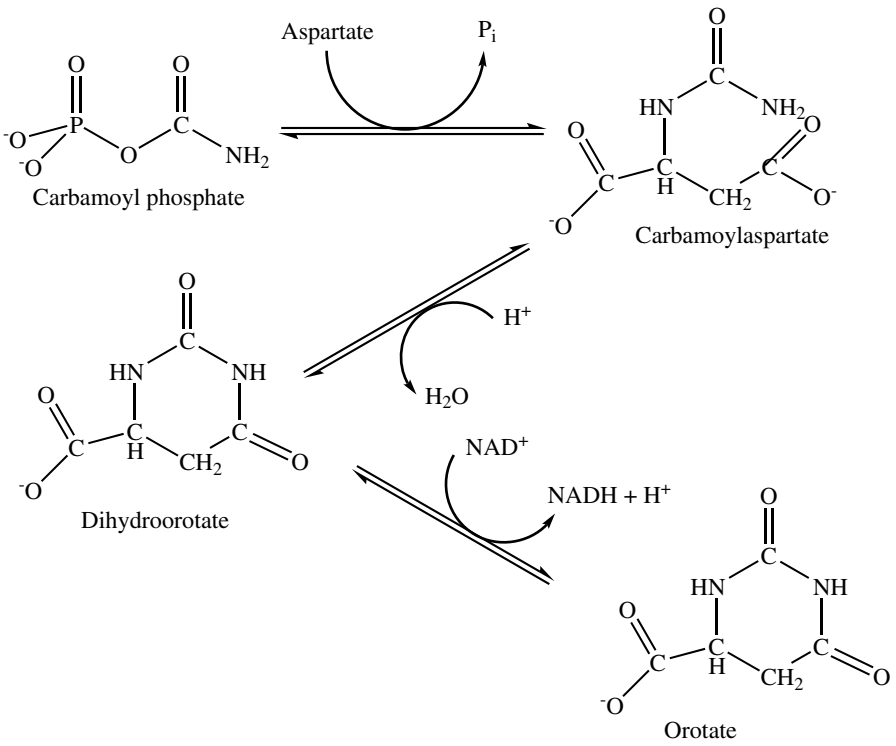


Figure 14-7: Formation of orotate from carbamoyl phosphate.

Orotate joins with 5-phosphoribosyl-1-pyrophosphate (PRPP) to form orotidylate, with pyrophosphate hydrolysis providing the energy necessary. The enzyme pyrimidine phosphoribosyltransferase is responsible for this reaction. The enzyme orotidylate decarboxylase catalyzes the decarboxylation of orotidylate to uridylate (UMP). Figure 14-8 illustrates these steps.

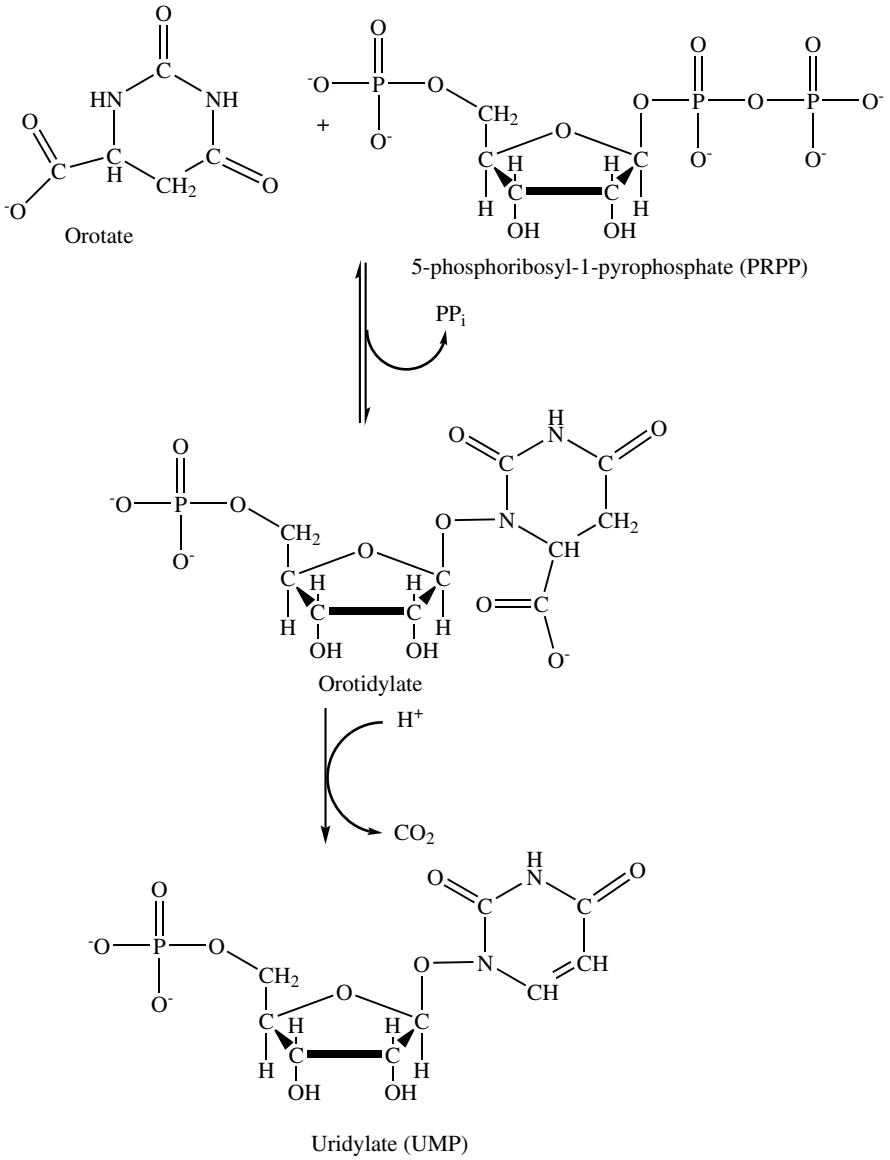


Figure 14-8: Conversion of orotate to uridylate (UMP).

Last step: Cytidine

The final nucleotide, cytidine, forms from uridinemonophosphate (UMP). The first step is to change UMP into UTP. UMP kinase transfers a pyrophosphate from ATP to UMP. Figure 14-9 shows this process.

Back to the Beginning: Catabolism

Catabolism, remember, is the breaking down of molecules to provide energy. In many cases, a complete breakdown is not necessary, because the products from a partial breakdown can be reused when necessary.

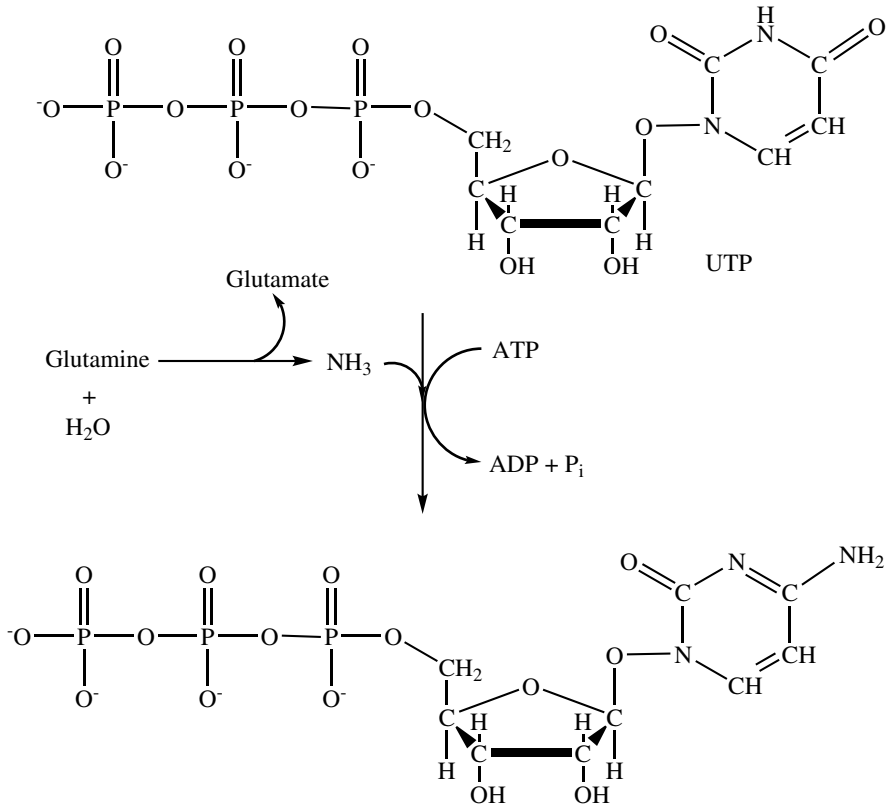


Figure 14-9: Conversion of UTP to CTP.

Nucleotide catabolism

The breakdown of the nucleotides begins with the removal of a phosphate group (from C-5). Next, a phosphate attaches to C-1 to give the sugar-1-phosphate, and the base leaves. In humans and many other species, uric acid (Figure 14-10) is the product of further degradation of purines. Other biochemical species further degrade uric acid into other products.

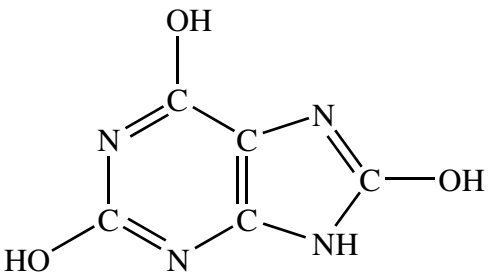


Figure 14-10: Structure of uric acid.

Amino acid catabolism

Hydrolysis of proteins yields the separate amino acids. It is possible to recycle these amino acids, use them in the synthesis of other amino acids, or produce energy from them. Through transamination it is possible to transfer an amino group from any amino acid (other than lysine, proline, or threonine), and an α-keto acid. The general category of enzymes that catalyzes this reaction is a transaminase, and the general reaction is shown in Figure 14-11. Nitrogen destined for elimination transfers to α-ketoglutarate to form glutamate. Transamination is important in the biosynthesis of alanine, aspartate, and glutamate.

Oxidative deamination of glutamate forms α-ketoglutarate (to be recycled), an ammonium ion (to enter the urea cycle) and, indirectly, 3 ATP. Glutamate dehydrogenase and either NAD^+ or $NADP^+$ are necessary for this.

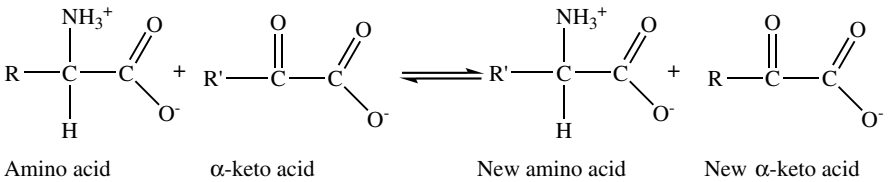


Figure 14-11: General transamination reaction.

The deaminated amino acid (α-keto acid) is further broken down. The α-keto acid may be broken down to pyruvate or some other material the body can

use to form glucose. These acids are called *glucogenic.* The alternative is to break down the α-keto acid to acetyl CoA and acetoacetic acid. These acids are called *ketogenic.* To further confuse you, some amino acids may be both glucogenic and ketogenic (see Table 14-2). These are the two possible fates of the carbon skeleton of the amino acids. The degradation of the amino acid transforms the carbon skeletons into intermediates in the citric acid cycle or into materials convertible to glucose.

Table 14-2 Glucogenic and Ketogenic Amino Acids

Glucogenic: Alanine, arginine, asparagine, aspartate, ccysteine, glutamate, glutamine, glycine, histidine, methionine, proline, serine, threonine, valine
Ketogenic: Leucine
Both: Isoleucine, lysine, phenylalanine, tyrosine, tryptophan

The general process is cyclic, with the various amino acids entering at different points. The basic scheme is shown in Figure 14-12.

Heme catabolism

The other important nitrogen compound in red-blooded organisms is *heme*. This species occurs in both hemoglobin and myoglobin. Hemoglobin is released as aged red blood cells are destroyed. The globin portion hydrolyzes to the appropriate amino acids. The iron separates from the heme and is stored in ferritin. Through a series of steps, bilirubin forms from the heme. The gall bladder temporarily stores bilirubin until the organism eliminates it.

Process of Elimination: The Urea Cycle

The catabolism of nitrogen-containing compounds yields recyclable nitrogen compounds and ammonia. Glutamine serves as temporary storage and transportation of the nitrogen — however, even small amounts of ammonia are toxic to humans. For this reason, ammonia must be converted to a less toxic form for elimination. The first step involves the conversion of ammonia, as the ammonium ion, to carbamoyl phosphate. The enzyme utilized for this conversion is carbamoyl phosphate synthetase. Figure 14-13 illustrates this reaction.

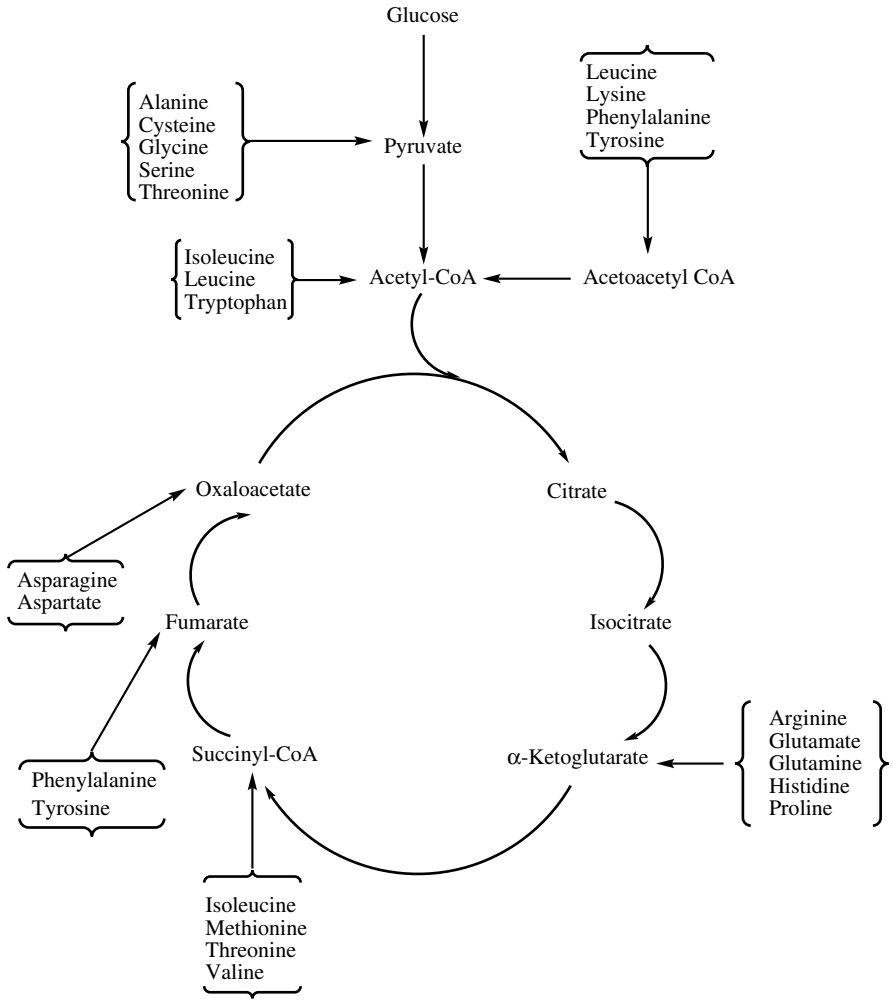


Figure 14-12: Fates of the amino acids.

Carbamoyl phosphate enters the urea cycle by joining to ornithine to produce citrulline, with the enzyme ornithine transcarbamoylase catalyzing this reaction. The enzyme arginosuccinate synthetase, with energy from the hydrolysis of ATP, joins aspartate to citrulline to form arginosuccinate. Arginosuccinase then catalyzes the splitting of arginosuccinate to fumarate

and arginine. The enzyme arginase completes the cycle by cleaving arginine into urea (for elimination) and ornithine (for recycling). The urea cycle and compounds involved in it are shown in Figures 14-14 and 14-15.

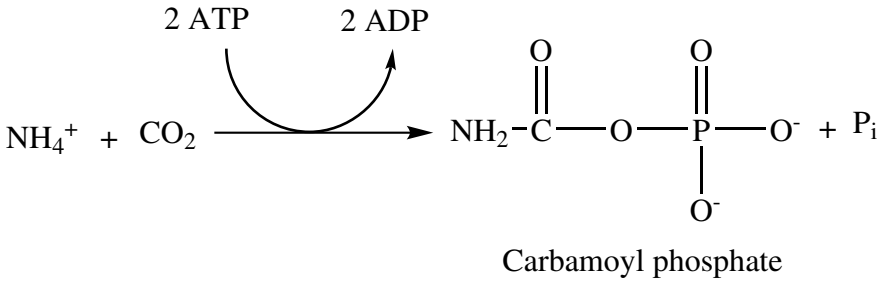


Figure 14-13: Formation of carbamoyl phosphate.

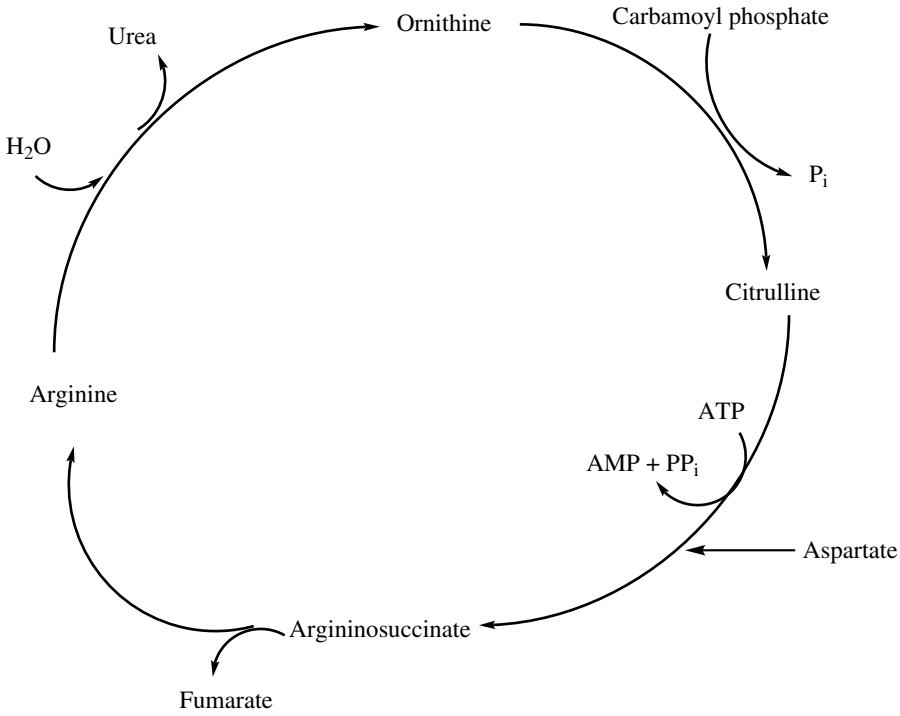


Figure 14-14: Overview of the urea cycle.

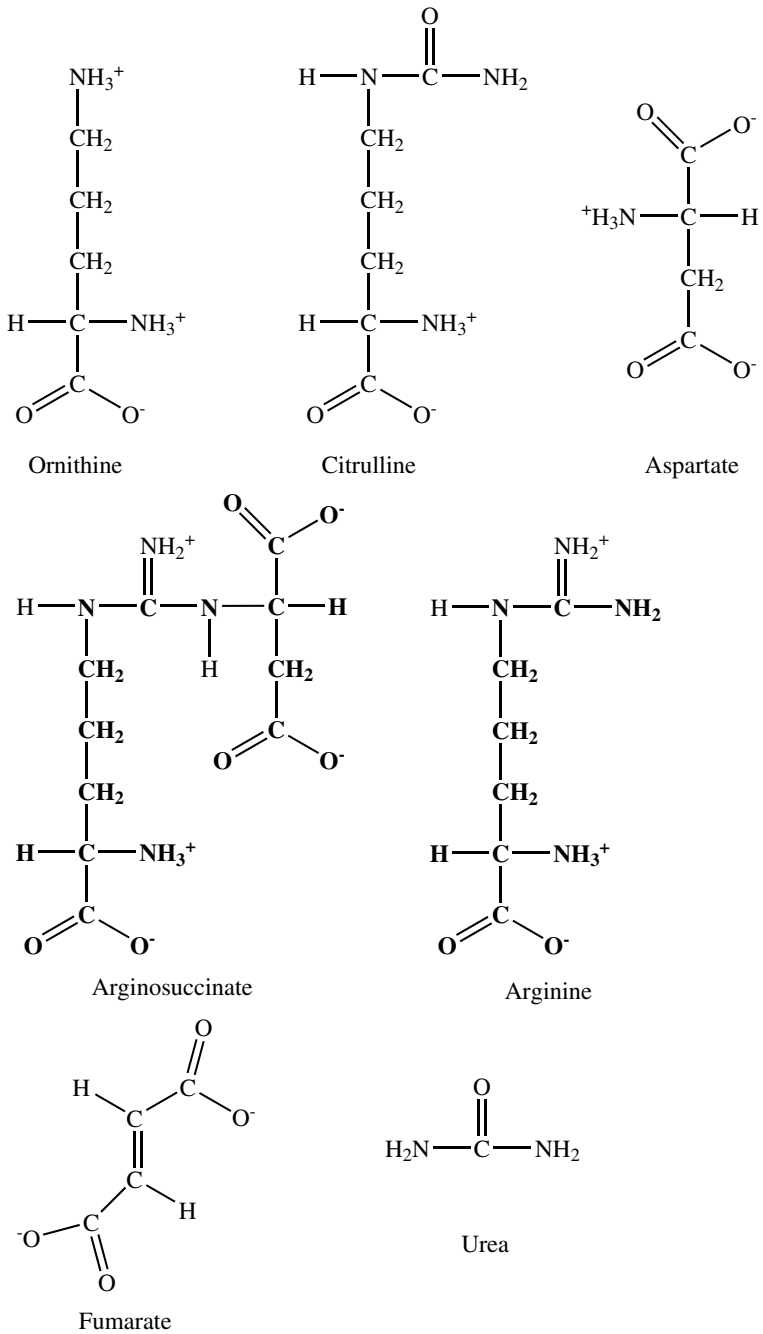


Figure 14-15: Compounds from the urea cycle.

Amino Acids Once Again

The synthesis of proteins requires 20 amino acids. If not readily available, humans can synthesize ten of these amino acids. These are the non-essential amino acids. The remaining ten amino acids, the essential amino acids, must come from the diet. Table 14-3 summarizes these amino acids.

Table 14-3 Essential and Non-Essential Amino Acids

Essential Amino Acids	*Non-Essential Amino Acids*
Arginine*	Alanine
Histidine	Asparagine
Isoleucine	Aspartate
Leucine	Cysteine
Lysine	Glutamate
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Proline
Tryptophan	Serine
Valine	Tyrosine

** Not essential in adults*

A *complete protein* supplies all essential amino acids. Not all proteins are complete — many are *incomplete proteins*. In order to avoid disorders due to amino acid deficiencies, the human diet must contain complete proteins.

Transamination is important in the biosynthesis of alanine, aspartate, and glutamate. It is easy to convert aspartate to asparagines and glutamate to glutamine. The synthesis of proline requires four steps beginning with glutamate. The synthesis of serine begins with the glycolysis intermediate 3-phosphoglycerate, and after three steps serine forms. It is easy to convert serine to glycine. If sufficient phenylalanine is available, the catalyzed oxidation converts it to tyrosine. If sufficient methionine is available, the body can convert some of the excess to cysteine. Arginine comes from the urea cycle, but infants do not get sufficient quantities from this source.

Metabolic Disorders

When something is out of whack with an organism's metabolism, problems arise that must be treated.

Gout

Gout is the result of overproduction of uric acid, which leads to the precipitation of sodium urate in regions of the body where the temperature is lower than normal (37°C). These low temperature regions are commonly found in the joints of the extremities. Sodium urate may also precipitate as kidney stones. Treatment is partially dietary and partly with drugs. Dietary restrictions include limiting the intake of foods high in nucleic acids (meats) and alcohol, which aggravates the conditions. Doctors often prescribe drugs that inhibit the enzyme that produces uric acid.

Gout may also be the result of faulty carbohydrate metabolism. A deficiency in glucose-6 phosphatase forces phosphorylated carbohydrates to form ribose 5-phosphate instead of glucose. Excess ribose 5-phosphate leads to excess PRPP, which, in turn, stimulates the synthesis of purines. The excess purines cause the production of more uric acid.

Lesch-Nyhan syndrome

Lesch-Nyhan syndrome is another example of defective purine catabolism leading to excess uric acid. Patients with this disorder normally excrete 4–5 times as much uric acid as gout patients do. This is a genetic disease that is a recessive X-linked trait, the trait is carried by the mother and is passed on to her son. There is no treatment for this disease at the present time.

Albinism

Albinism, a recessive trait, is an inborn error of tyrosine metabolism. Tyrosine is the precursor of melanin, the pigment responsible for hair and skin color. In at least one form of albinism, the problem appears to be due a deficiency of the enzyme tyrosinase. A variation of albinism involves a temperature-sensitive form of tyrosinase. The enzyme is only effective at lower than normal temperatures, as found in the extremities. This form of tyrosinase is responsible for the coloration of Siamese cats.

Alkaptonuria

Alkaptonuria is a benign condition that manifests itself as a darkening of the urine. The condition is the result of a problem in the catabolic breakdown of phenylalanine and tyrosine. A defective enzyme leads to an accumulation, and subsequent elimination, of one of the reaction intermediates.

Phenylketonuria

Phenylketonuria, or PKU, is the result of a deficiency in the enzyme phenylalanine 4-monooxygenase, which results in a problem in phenylalanine metabolism. The consequence is an accumulation of phenylalanine in the blood. High levels of phenylalanine enhance transamination to form abnormally high levels of phenylpyruvate. High levels of phenylpyruvate damage the brains of infants with the condition.

The high levels of phenylalanine lead to competitive inhibition of the enzymes responsible for melanin production from tyrosine. Because little tyrosine converts to melanin, afflicted infants have light blonde hair and fair skin (similar to albinism).

Early diagnosis in infants is important to prevent brain damage. One test for PKU is to add $FeCl_3$ to the patient's urine. Phenylpyruvate reacts with iron ions to produce a green color. Another test is to assay for phenylalanine 4-monooxygenase activity. Treatment consists of maintaining a diet low in phenylalanine until at least the age of three.