METABOLISM 1 :

Catabolic Reactions of the Cell

Life requires energy. As our discussion of biomolecules pointed out, the major functional components of the cell are mostly polymers - long chains of smaller individual molecular units. Each addition of a small link to the chain costs energy. Chemical reactions that build up complex molecules from simple ones are known as *anabolic* reactions. Conversely, heterotrophic organisms such as animals ingest food made up of these large polymers, which, when broken down in the digestive process, release energy for maintaining and building that organism. Such chemical reactions, in which complex molecules are broken down to simpler components, are classified as *catabolic* reactions. Taken as a group of reactions within a cell or even an organism, they can be referred to as the cell's or organism's anabolism or catabolism. The sum total of both types of reactions is the cell's *metabolism*. Nearly all metabolic reactions are catalyzed by enzymes in order to keep up with the energy and material demands of the cell. In fact, the discussion of some of the metabolic processes in this chapter will almost seem to be laundry lists of enzymes. We will begin with one such list in describing the catabolism of the simple sugar, glucose, through the process of glycolysis.
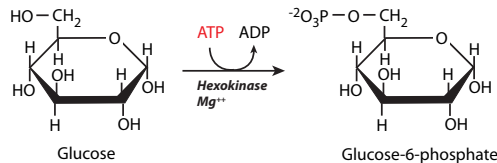
Glycolysis

Whether the cell is prokaryotic or eukaryotic, one of its basic methods for generating usable energy is glycolysis. This process uses glucose, which is the most common energy source for most cells. However, glucose cannot be directly broken down to provide energy for the cell: glycolysis is a process that breaks it down in a series of reactions to create adenosine triphosphate (ATP), which is the most common energy "currency" of the cell. That is, ATP *can* release usable energy in a single reaction.

Glucose, being a 6-carbon sugar, has a large amount of potential energy stored in its bonds. However, since it is thermodynamically stable, it would take the investment of a lot of external energy to release the energy of glucose in one step (e.g. lighting it on fire to break it down into CO_2 and H_2O), and not only is it impossible for cells to
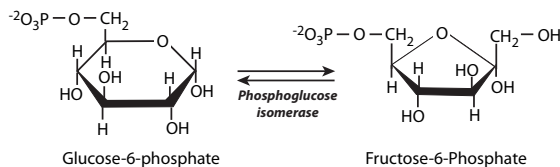
generate that kind of energy at once, the cell has no mechanism to use all the energy released at one instant in time. Most of it would be wasted as excess heat. Instead, the cell uses enzymes to destabilize and break down the sugar through a series of conversions into intermediate compounds. The basic process and enzymes involved are as follows.

1. Glucose is phosphorylated by hexokinase to make Glucose-6-Phosphate. The enzyme is so named because it is a kinase (puts a phosphate group on) that acts on a hexose (six-carbon sugar). In this case, it places the phosphate on the 6-carbon of glucose. However, hexokinase can also phosphorylate other hexoses such as fructose and mannose (all in the D- conformation). There are two major reasons this is good for the cell. Since glucose concentration is higher inside the cell than outside, there is pressure for it to move back out of the cell. By converting it to G6P, it is no longer part of the glucose concentration gradient, and it has a charged phosphate group making it nearly impossible to leak out of the membrane. The addition of the phosphate also increases the energy in the molecule, making it less thermodynamically stable, so that it can be broken down. This reaction requires the use of ATP as a phosphate donor and the energy needed to attach it. That is, energy is *used* in this step, not produced. Consider it an investment of energy though, since by the end of glycolysis, more ATP is produced than used.

Hexokinase requires ATP in the form of a complex (to the 2nd and 3rd phosphate groups) with a divalent cation, typically Mg^{++} in vivo. ATP alone is actually a competitive inhibitor of hexokinase. The product, G6P, also functions as an inhibitor, thus providing some measure of feedback regulation. In fact, muscle cells using glycogen stores convert the glycogen directly to G6P, so hexokinase activity is very low in those cells.


Glucose   Glucose-6-phosphate

2. Glucose-6-Phosphate is converted to Fructose-6-Phosphate by phosphoglucose isomerase. As the name implies, the isomerase simply rearranges the existing atoms within the G6P to make the F6P without removal or addition of any atoms.


Glucose-6-phosphate   Fructose-6-Phosphate

3. Fructose-6-Phosphate is phosphorylated by phosphofructokinase (PFK) to Fructose-1,6-bisphosphate. There is again an investment of an ATP to provide the phosphate group and the energy to attach it.

Fructose-6-Phosphate → Fructose-1,6-bisphosphate (Phosphofructokinase, Mg^{++}; ATP → ADP)
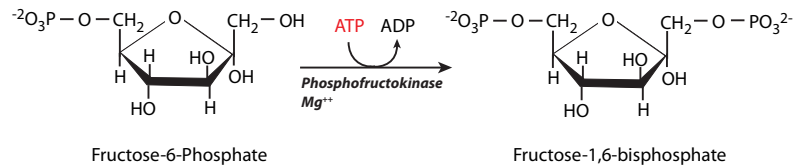
4. The Fructose-1,6-bisphosphate is cut in half by aldolase, yielding a molecule of dihydroxyacetone phosphate and a molecule of glyceraldehyde-3-phosphate.

Fructose-1,6-bisphosphate → (Aldolase) Glyceraldehyde-3-phosphate + Dihydroxyacetone phosphate
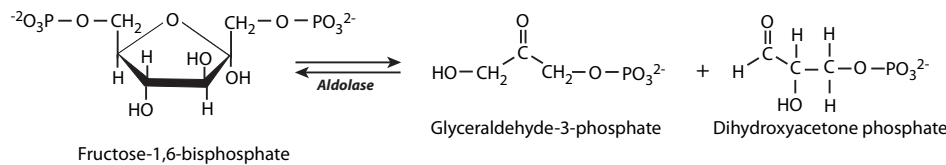
5. The G3P can participate in the next reaction, but the dihydroxyacetone phosphate, despite its similarity, cannot. So, it needs to be rearranged by triose phosphate isomerase, which converts it to another molecule of glyceraldehyde-3-phosphate.

Glyceraldehyde-3-phosphate ⇌ (Triose phosphate isomerase) Dihydroxyacetone phosphate
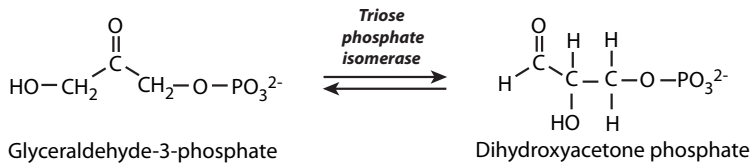
6. Each of the two molecules of G3P generated from the glucose molecule now undergo oxidation catalyzed by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the presence of NAD+ and inorganic phosphate (P_i). Each of these reactions produces 1,3-bisphosphoglycerate, which has a high-energy phosphate group, and NADH. NADH is a high energy electron carrier (electron comes from G3P). In eukaryotes with an aerobic environment, this NADH will likely be used to help generate ATP through the tricarboxylic acid cycle (aka Krebs cycle or citric acid cycle). In anaerobic situations, the NADH will participate in fermentation for reasons discussed in the next section.

Glyceraldehyde-3-phosphate + P_i ⇌ (Glyceraldehyde-3-phosphate dehydrogenase; NAD^+ → NADH) 1,2-Bisphosphoglycerate + H^+
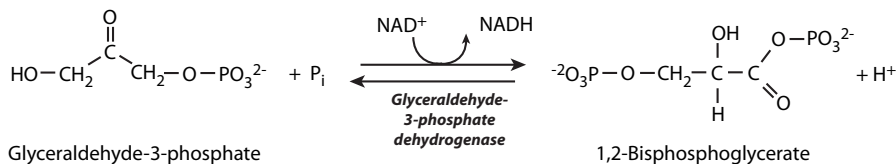
3. PFK is an important regulator of glycolysis. It is a tetrameric protein, and each subunit has two binding sites for ATP: one is the normal substrate site, the other is an inhibitory site such that binding of ATP lowers the enzyme's affinity for F6P. ATP is not the only regulator of PFK activity: AMP is also a positive regulator of PFK, and can increase it up to 5-fold.

4. There are two classes of aldolases: class I are found in animals and plants, while class II are found in fungi and bacteria. Class I require no cofactors, but class II require a divalent cation (physiologically usually Fe^{++} or Zn^{++}).

5. Triose phosphate isomerase is a "perfect enzyme" that catalyzes the formation of product as fast as the enzyme and substrate can make contact in solution (i.e. rate is purely diffusion-limited).

1.

1. Sulfhydryl of GAPDH initiates nucleophilic attack on G3P aldehyde.

2. Forms thiohemiacetal that is oxidized by NAD^+ to thioester. Resulting NADH is displaced by NAD^+.

2.

3.

3. Soluble inorganic phosphate attacks thioester to release 1,3-bisphosphoglycerate.
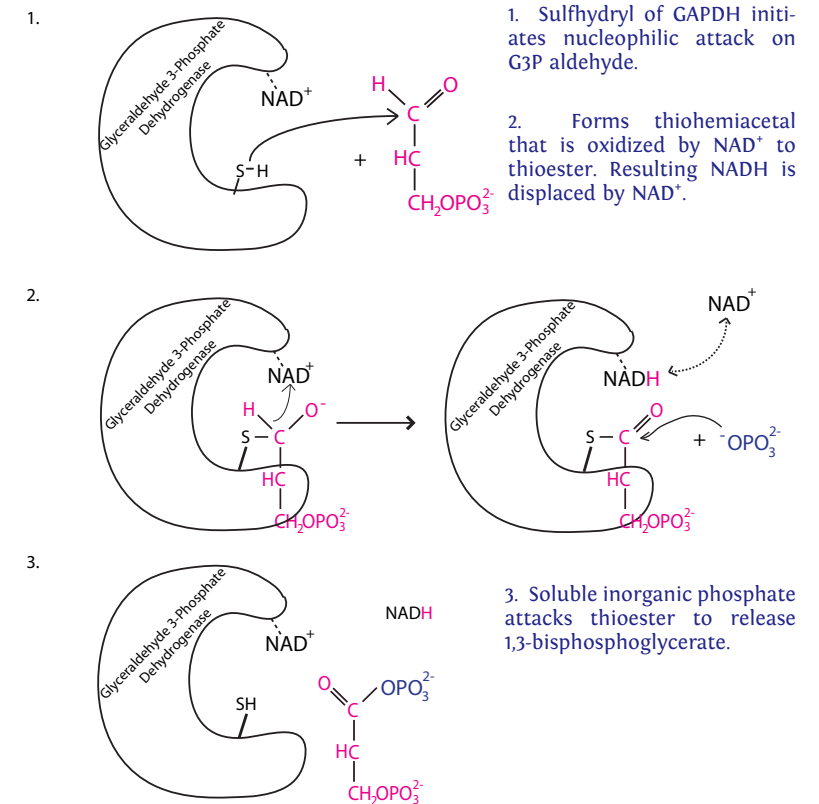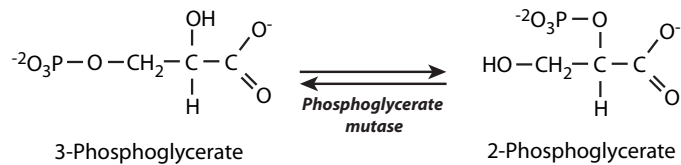
Figure 1. NAD^+ is reduced by glyceraldehyde-3-phosphate dehtdrogenase to form NADH. The NADH is released by substitution with another NAD^+.

7. The phosphate group on the 1-carbon of 1,3-bisphosphoglycerate is transferred to ADP by phosphoglycerate kinase to make 3-phosphoglycerate and ATP (finally!). From the two molecules of G3P entering step 6, we get two molecules of ATP to provide energy for the cell in this step. Recalling the earlier investment of ATP (in steps 1 and 3), the reaction has only "broken even" at this point. 2 in, 2 out.

1,2-Bisphosphoglycerate   3-Phosphoglycerate
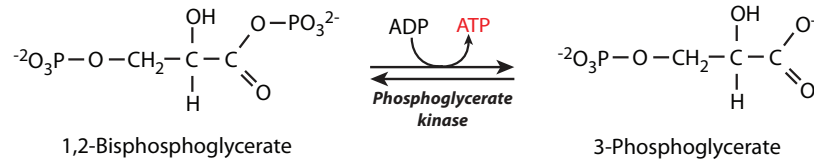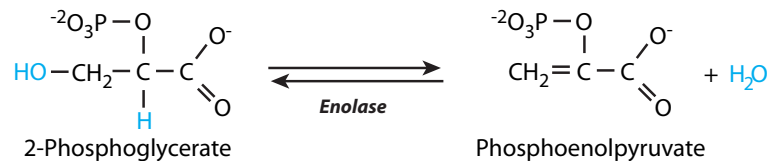
The name of the enzyme suggests that a phosphate is added to phosphoglycerate. This is not a mistake: remember that enzymes can catalyze reactions in either direction, depending on reaction conditions. Under conditions of high phosphoglycerate and ATP, phosphorylation of phosphoglycerate would occur. However, the physiological conditions are a relatively high concentration of the 1,3-bisphosphoglycerate in comparison to relatively low levels of phosphoglycerate thus driving the reaction "backwards" with respect to the naming of the enzyme.

8. The 3-phosphoglycerate is then rearranged by phosphoglycerate mutase to make 2-phosphoglycerate. This molecule has a higher free energy of hydrolysis than when the phosphate group is on the 3-carbon.

The action of phosphoglycerate mutase is not just the intramolecular phosphate group transfer that it seems to be at first glance. The enzyme must first be activated by phosphorylation, and it is the enzyme's phosphate that is added to the 2-carbon of 3PG. The doubly-phosphorylated intermediate then transfers its 3-phosphate to the enzyme, and 2PG is released.

3-Phosphoglycerate   2-Phosphoglycerate

9. That energy is used to create ATP, as the 2-phosphoglycerate undergoes dehydration by enolase to make phosphoenolpyruvate (PEP).

PEP is made because hydrolysis of the phosphate from 2PG does not release enough energy to drive phosphorylation of ADP to ATP. PEP hydrolysis, on the other hand, releases significantly more than needed.

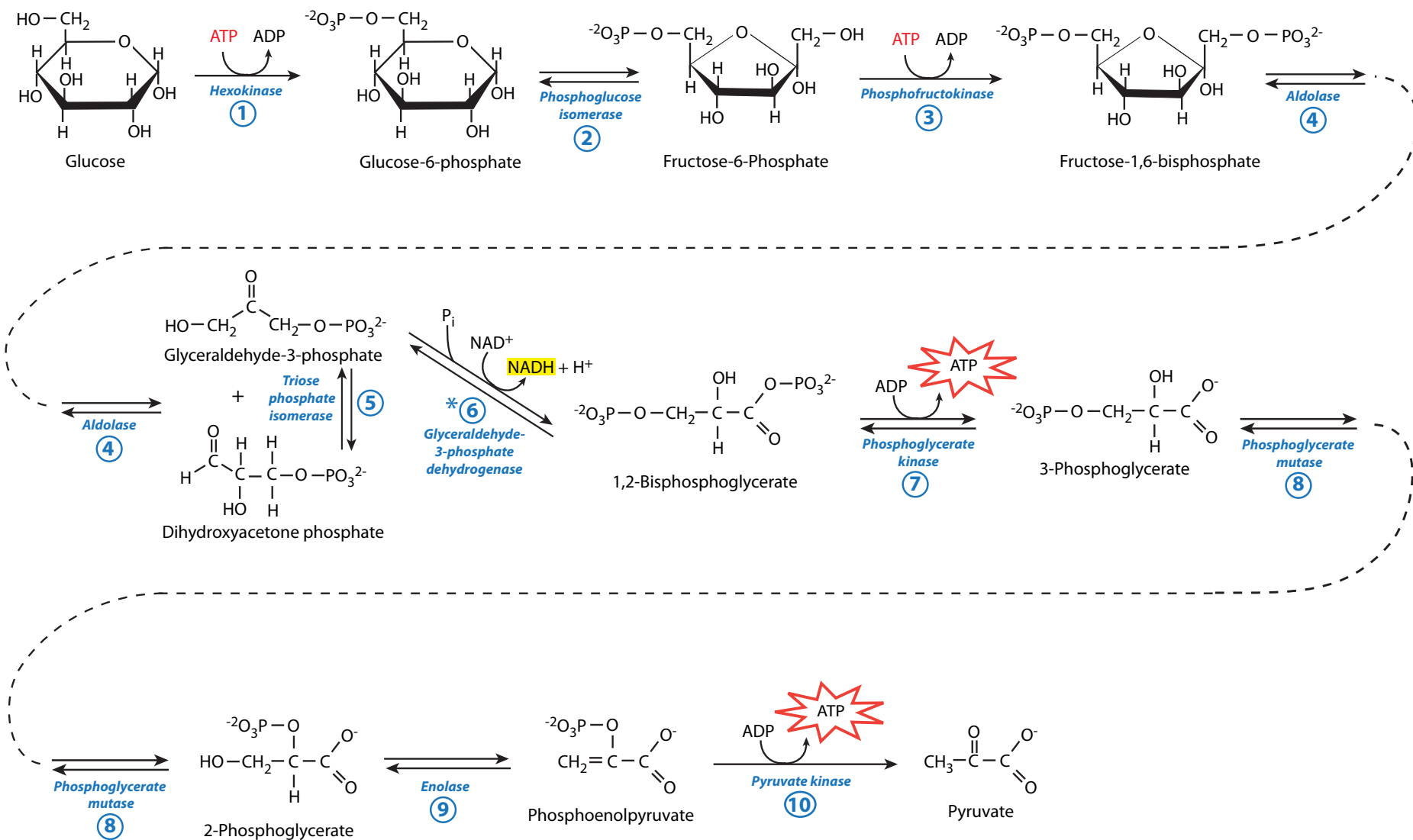2-Phosphoglycerate   Phosphoenolpyruvate

Figure 2. Overview of Glycolysis.

Bidirectional arrows indicate enzymes used for both glycolysis and gluconeogenesis. Unidirectional arrows indicate enzymes that only function in glycolysis. *Note that reactions 6-10 are occurring in duplicate (two G3P from one glucose).

10. Pyruvate kinase then transfers a high energy phosphate group from PEP to ADP, producing an ATP for use by the cell, and pyruvate.

Phosphoenolpyruvate   Pyruvate
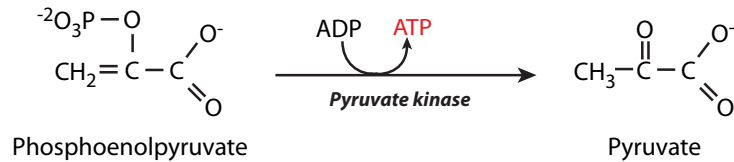
Keeping in mind the doubling of reactions from steps 6-10 (splitting of fructose-1,6-bisphosphate generates two G3P), the total usable energy production from glycolysis of a single molecule of glucose is 4 ATP and 2 NADH. However, the net ATP production is only 2 ATP if we remember the initial investment of two ATP in the early steps. *Not really anything to write home about.* Furthermore, although the NADH and pyruvate can participate in the tricarboxylic acid cycle in aerobic eukaryotic situations to generate a significant amount of ATP, in anaerobic situations, they do not produce usable energy.

Thus anaerobic ATP production, i.e. glycolysis, is far less efficient at extracting energy from a glucose molecule than aerobic ATP production, which can generate approximately 38 ATP per glucose. On the other hand, when a lot of ATP must be generated quickly, glycolysis is the mechanism of choice, in cells such as the fast-twitch fibers of skeletal muscle. These cells actually have very few mitochondria because glycolysis can produce ATP at a much higher (up to 100 times) rate than oxidative phosphorylation. What happens to the pyruvate and NADH? In aerobically metabolizing cells, they go to the mitochondria for the TCA cycle and oxidative phosphorylation. In anaerobes, they undergo fermentation.

Fermentation

When the average person hears the word "fermentation" he probably thinks about alcohol. As you no doubt recall, glycolysis gave us some usable energy in the form of ATP, and then there are the other products, NADH and pyruvate. As we shall see in the next section, if the cell is eukaryotic and oxygen is available, then those molecules can help make more ATP. If no oxygen is available or the cell is just a lowly prokaryote, it undergoes fermentation to produce either lactate or ethyl alcohol. Why does the cell need lactate or ethanol? It doesn't, although the lactate can contribute to overall metabolism.   What the cells do need is NAD^+, so that glycolysis can continue beyond step 6. Without fermentation, continued glycolysis would convert all of the NAD^+ to NADH, and then be stuck, unable to continue. So the primary reason for fermentation, whichever path it takes, is to regenerate NAD^+ from the NADH.

Pyruvate kinase requires not only divalent Mg^{++} as with most other kinases, but also K^+. The enzyme works in two steps: the ADP attacks the PEP phosphorus to make ATP and enolpyruvate. Enolpyruvate is then converted to its keto- tautomer.

Note that the NADH produced by glycolysis in the cytoplasm does not *directly* participate in oxidative phosphorylation in the mitochondria since the inner mitochondrial membrane is impermeable to it, but it sends a "virtual equivalent" into the mitochondria via one of two pathways: the aspartate-malate shuttle combines malate-α-ketoglutarate antiports, aspartate-glutamate antiports, and metabolite interconversion by transaminase with malate dehydrogenase to oxidize NADH cytoplasmically and use the energy generated to reduce NAD+ in the mitochondrial matrix; the other pathway is a DHAP shuttle system, in which NADH is used to reduce dihydroxyacetone phosphate to glycerol-3-P using a cytoplasmic glycerol-3-phosphate dehydrogenase, and the cycling the DHAP to glycerol-3-P via a flavoprotein dehydrogenase embedded in the inner mitochondrial membrane. This flavoprotein dehydrogenase takes the electrons from glycerol-3-P to make $FADH_2$, which can participate in the electron transport chain.

The DHAP or glycerophosphate shuttle is less efficient than the malate-aspartate shuttle, generating approximately 2 ATP vs 2.7 ATP per NADH. However, it can operate even when the concentration of cytoplasmic NADH is low, as happens in tissues/cells with a very high metabolic rate (including skeletal muscle and brain), while the malate-aspartate shuttle (prevalent in liver and heart) is sensitive to the relative concentration of NADH and NAD^+.

In lactate fermentation, the pyruvate is converted to lactate by lactate dehydrogenase. This reaction requires the oxidation of NADH, which thus provides NAD$^+$ to the cell for continued glycolysis.

Pyruvate → Lactate (via Lactate Dehydrogenase, NADH → NAD$^+$)

Pyruvate   Lactate
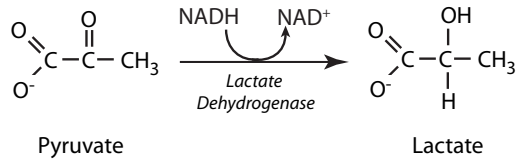
For many cells, the lactate is a waste product and excreted. In fact, this is the case with most muscles: the lactate is carried by the blood from the muscle cells to the liver, where it can be converted to glucose. Thus, although lactate is formed at high rates when muscles are overworked and become fatigued, it is not directly the cause of muscle fatigue. As oxygen availability cannot keep up with aerobic ATP production, and larger and larger proportions of the ATP generated come from glycolysis with fermentation. The current model of muscle fatigue posits that it is due to acidification of the muscle cell as it undergoes rapid glycolysis.

However, in some tissues and cell types, particularly in the heart and brain of higher animals, cell membranes are highly permeable to lactate, the cells can readily convert the lactate to pyruvate, and since these are highly oxygenated tissues, the pyruvate is then used for the TCA cycle and oxidative phosphorylation to generate ATP. In fact, some non-neuronal support cells in the brain (astrocytes) generate and excrete copious lactate which is taken up by the neighboring neurons to fuel ATP production.

In alcohol fermentation, pyruvate is first acted upon by pyruvate decarboxylase, which liberates a CO_2 molecule and produces acetaldehyde. Acetaldehyde is then acted upon by alcohol dehydrogenase, using NADH, generating NAD$^+$ and ethanol. Here, like with lactate fermentation, the desired product is the regenerated NAD+. Ethanol is excreted, and in most animals, is converted to acetaldehyde and then acetic acid, before finally ending up as acetyl-CoA.

Pyruvate → Acetaldehyde + CO_2 (via Pyruvate Decarboxylase)

Pyruvate   Acetaldehyde

Acetaldehyde → Ethanol (via Alcohol Dehydrogenase, NADH → NAD$^+$)

Ethanol
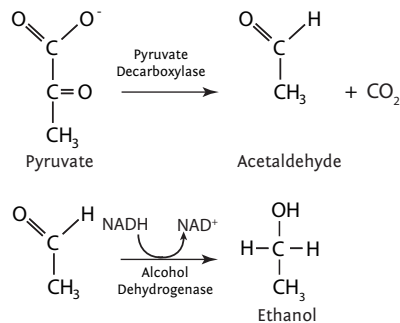
As with glycolysis, fermentation can and does take place in cells that are able to make ATP by oxidative phosphorylation. The relative contribution of glycolysis and oxidative phosphorylation to the cellular ATP pool is determined dynamically by physiological conditions.

The TCA cycle

So you're a hot young eukaryote sporting all kinds of fancy internal membranous organelles, with a need to prove yourself better than the old guard prokaryotes — what do you do? Well, make scads of ATP, of course! And seemingly effortlessly at that, using only the dregs left over after glycolysis has taken its pass at a glucose molecule: NADH and pyruvate. Glycolysis in eukaryotes, as befits its prokaryotic origins, happens in the cytoplasm. The TCA cycle happens inside the matrix of the *mitochondria*, a double-membraned organelle.

The pyruvate needs to make its way from the cytoplasm, through both outer and inner mitochondrial membranes, and into the mitochondrial matrix. How does this work? The outer membrane is porous, being riddled with large relatively nonspecific anion channels known as voltage-dependent anion channels (VDACs), and will readily admit pyruvate. In contrast, the inner mitochondrial membrane is highly impermeable, and entry of pyruvate is specifically regulated by a pyruvate transporter protein.
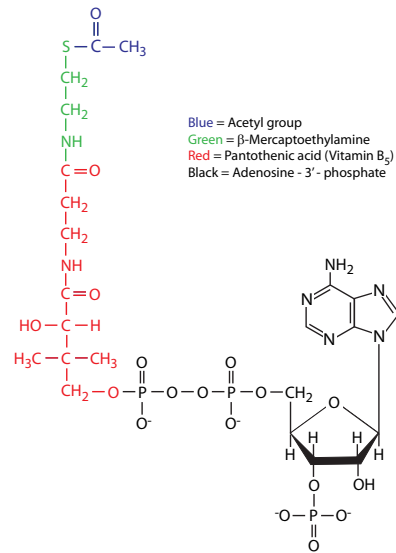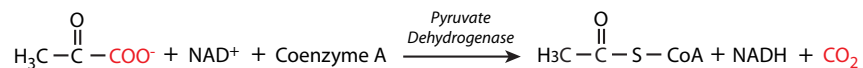
Figure 3. Acetyl-Coenzyme A is composed of four distinct molecular parts.

1. Once the pyruvate enters the mitochondrial matrix, the pyruvate dehydrogenase complex (consisting of three enzyme subunits E1, E2, and E3) converts it to acetyl-CoA (fig. 3) for entry into the tricarboxylic acid cycle (TCA). This reaction generates NADH and liberates CO_2.

$$H_3C-\overset{\overset{O}{\|}}{C}-COO^- + NAD^+ + \text{Coenzyme A} \xrightarrow{\substack{\textit{Pyruvate}\\\textit{Dehydrogenase}}} H_3C-\overset{\overset{O}{\|}}{C}-S-CoA + NADH + CO_2$$

Now consider the breakdown of glucose. Recall that the complete breakdown of that six-carbon sugar should yield six single-carbon molecules of carbon dioxide. In glycolysis, the glucose is broken down into two molecules of three-carbon pyruvate. As the pyruvate is converted to acetyl-CoA, one CO_2 is generated per molecule of pyruvate. That leaves just four carbons (in two 2-carbon molecules of acetyl-CoA) out of the original glucose 6. The TCA cycle will liberate each of those carbons as CO_2 as well. Knowing the reactions in which the remaining carbons are released is a good way to study the first half of the TCA cycle.

As an integral part of coenzyme A, vitamin B_5, or pantothenic acid, is needed for the TCA cycle, and therefore, for normal efficient generation of ATP. However, unlike some other vitamins, B_5 deficiency is rare, and usually associated with deficiency in other vitamins or general malnourishment. On the other hand, deficiency in another B vitamin involved in pyruvate dehydrogenase activity (fig. 4), thiamine (B_1), can lead to disease symptoms known as beriberi.

Arsenic, or more specifically arsenic-containing compounds such as arsenite and arsenate, are poisonous to cells by interfering with this reaction. The arsenic compound can interact with dihydrolipoamide, resulting in cyclization by bonding of both sulfhydryl sulfurs to the arsenic atom. This prevents E2 from working, and acetyl-CoA cannot be generated for ATP production via TCA cycle and oxidative phosphorylation. It should be noted that these arsenic compounds also affect other sulfhydryl-containing compounds, and within the context of the TCA cycle, it can also inactivate α-ketoglutarate dehydrogenase, which is similar to the pyruvate dehydrogenase.

Beriberi symptoms are classified in two groups: wet beriberi affects the cardiovascular system with symptoms such as enlarged heart, lung congestion, shortness of breath, swelling of lower legs, congestive heart failure; dry beriberi (also known as Wernicke-Korsakoff syndrome) affects the nervous system. Symptoms include polyneuritis in both central and peripheral nervous system, leading to pain, tingling, loss of sensation in extremities, loss of muscle function or paralysis of the lower legs, vomiting, nystagmus, and eventually mental confusion, speech difficulties, coma and death.

1. Pyruvate dehydrogenase complex (fig. 4) is actually an amalgamation of three enzymes. That is, there are three subunits to the complex: pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3). These three subunits are associated by noncovalent bonds. The pyruvate dehydrogenase subunit E1 acts first, using the cofactor thiamine pyrophosphate (TPP) to help remove a CO_2 from the pyruvate to generate hydroxyethyl-TPP.

This is immediately used as a substrate by E2, resulting in regeneration of TPP and reactivation of pyruvate dehydrogenase, and also making the intermediate acetyl-dihydrolipoamide. Coenzyme A, which is also a substrate for E2, has a sulfhydryl group that attacks the acetyl group of acetyl-dihydrolipoamide. The acetyl group is immediately transferred to Coenzyme A to form the Acetyl-CoA that enters the TCA cycle.

The final step is for the dihydrolipoamide to be oxidized back to lipoamide by E3. It is this oxidation step that generates the NADH from NAD+.
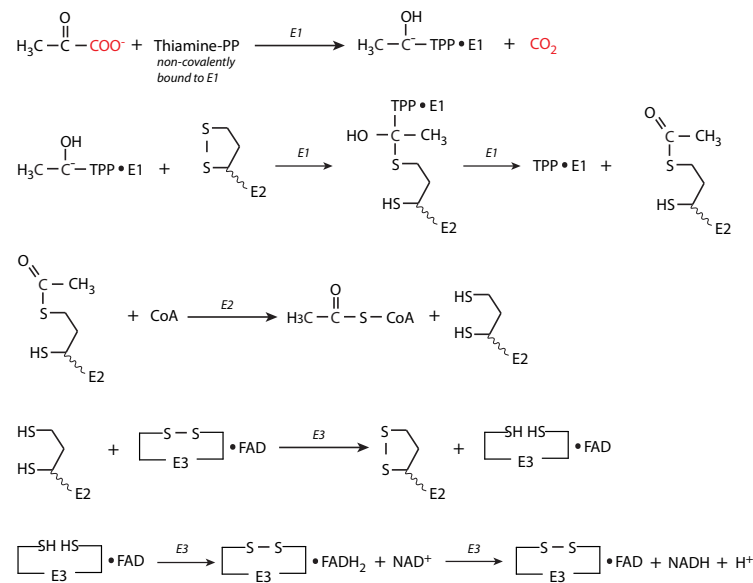

Figure 4. Pyruvate dehydrogenase is a complex of three enzymatic subunits, E1, E2, and E3, which work in sequence as depicted here.

2. Acetyl-CoA enters the tricarboxylic acid cycle as a substrate of citrate synthase, which adds it to oxaloacetate to make citrate. This is the reason that this cycle is also called the citric acid cycle. Citrate, having three carboxyl groups, is a tricarboxylic acid, leading to the name that this text will use. The other common name for this is the Krebs cycle, as it was first proposed by Hans Krebs in 1937.
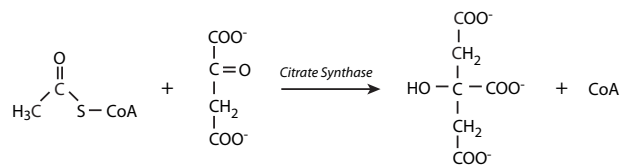
3. In the next step, aconitase rearranges citrate to make isocitrate.
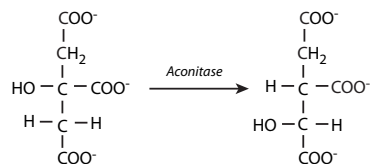
2. Citrate synthase is a dimeric enzyme that in its native form has a binding cleft for oxaloacetate. Binding of oxaloacetate causes a conformational shift closing the oxaloacetate binding site, locks it in and simultaneously reveals the acetyl-CoA binding site. The current model for this reaction involves three steps: Acetyl-CoA is converted to an enol intermediate, which attacks the oxaloacetate to form citronyl-CoA (S-citryl-CoA), which is then hydrolyzed to citrate and Coenzyme A.
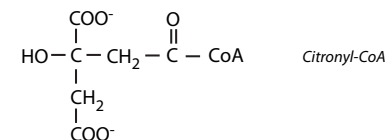
3. Aconitase pushes citrate into a cis-aconitate intermediate, which is then converted to isocitrate. Interestingly, while aconitase contains an Fe-S cluster, it does not appear to participate in redox reactions as is usually the case for such groups. Instead, its purpose is to hold the cis-aconitate in its place within the enzyme as it [the cis-aconitate] undergoes a bizarre molecular flip on its way to isocitrate.

Left column:
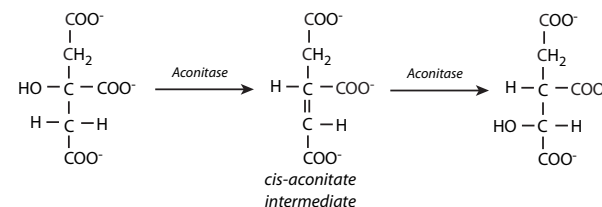
4. Isocitrate is a substrate for isocitrate dehydrogenase, which transfers a high energy electron from the isocitrate onto NAD+ to make NADH and α-ketoglutarate. This reaction also liberates one CO_2. For those keeping track at home, that leaves two more carbons from the six in glucose.

$$
\begin{array}{c}
COO^- \\
| \\
CH_2 \\
| \\
H-C-COO^- \\
| \\
HO-C-H \\
| \\
COO^-
\end{array}
\quad
\xrightarrow[\text{dehydrogenase}]{\text{Isocitrate}}
\text{(NAD}^+ \to \text{NADH} + CO_2\text{)}
\quad
\begin{array}{c}
COO^- \\
| \\
CH_2 \\
| \\
CH_2 \\
| \\
C=O \\
| \\
COO^-
\end{array}
$$
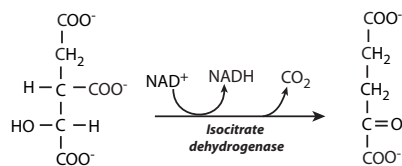
5. Alpha-ketoglutarate is also oxidized (by α-ketoglutarate dehydrogenase) generating NADH and succinyl-CoA. Like acetyl-CoA, this CoA-associated compound contains a high energy thioester bond. This reaction liberates the final CO_2 from the glucose.

$$
\begin{array}{c}
COO^- \\
| \\
CH_2 \\
| \\
CH_2 \\
| \\
C=O \\
| \\
COO^-
\end{array}
+ CoA + NAD^+
\xrightarrow[\text{dehydrogenase}]{\alpha\text{-ketoglutarate}}
\begin{array}{c}
COO^- \\
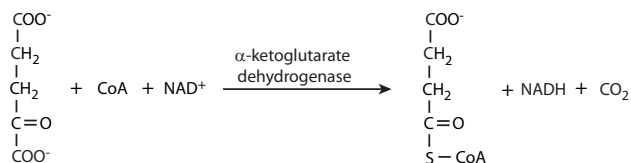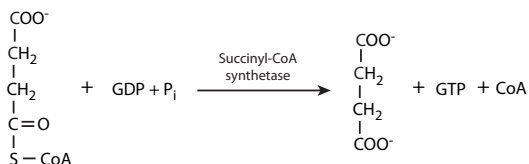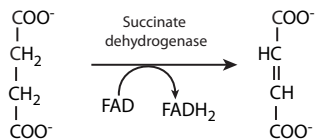| \\
CH_2 \\
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CH_2 \\
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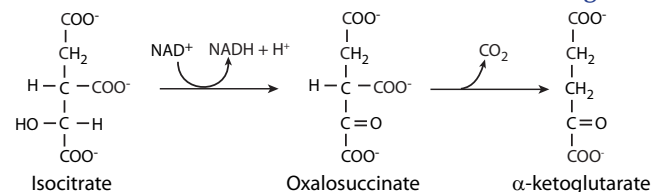C=O \\
| \\
S-CoA
\end{array}
+ NADH + CO_2
$$

6. The CoA is regenerated by succinyl-CoA synthetase, which also forms succinate and GTP or ATP. This GTP is energetically an ATP equivalent, and made in animal cells. Bacterial and plant homologues of this enzyme use ADP and make ATP. Formation of this ATP/GTP is possible because the high-energy thioester bond of succinyl-CoA is broken.

$$
\begin{array}{c}
COO^- \\
| \\
CH_2 \\
| \\
CH_2 \\
| \\
C=O \\
| \\
S-CoA
\end{array}
+ GDP + P_i
\xrightarrow[\text{synthetase}]{\text{Succinyl-CoA}}
\begin{array}{c}
COO^- \\
| \\
CH_2 \\
| \\
CH_2 \\
| \\
COO^-
\end{array}
+ GTP + CoA
$$

7. Next, the succinate is oxidized. The enzyme that does this, succinate dehydrogenase, is a little different from the other dehydrogenases because this one happens to be embedded in the inner mitochondrial membrane, and instead of transferring the electron to NAD^+, the electron is transferred to FAD, making $FADH_2$, and fumarate. The energy in $FADH_2$ can also be used to power ATP production similar to the energy in NADH.

$$
\begin{array}{c}
COO^- \\
| \\
CH_2 \\
| \\
CH_2 \\
| \\
COO^-
\end{array}
\xrightarrow[\text{FAD} \to \text{FADH}_2]{\text{Succinate dehydrogenase}}
\begin{array}{c}
COO^- \\
| \\
HC \\
\| \\
CH \\
| \\
COO^-
\end{array}
$$

Right column:

4. The NAD^+-dependent isocitrate dehydrogenase is actually found in two isoforms in mammals: an NAD+-utilizing isoform in the mitochondrial matrix, and an isoform that uses $NADP^+$ that is found in the cytosol as well as the mitochondria. The reaction starts with NADH-generating oxidation of isocitrate to oxaolosuccinate, which is then decarboxylated with the help of a Mn^{++} or Mg^{++} co-factor to release the carbon dioxide and form α-ketoglutarate.

$$
\underset{\text{Isocitrate}}{
\begin{array}{c}
COO^- \\
| \\
CH_2 \\
| \\
H-C-COO^- \\
| \\
HO-C-H \\
| \\
COO^-
\end{array}}
\xrightarrow{\text{NAD}^+ \to \text{NADH} + H^+}
\underset{\text{Oxalosuccinate}}{
\begin{array}{c}
COO^- \\
| \\
CH_2 \\
| \\
H-C-COO^- \\
| \\
C=O \\
| \\
COO^-
\end{array}}
\xrightarrow{CO_2}
\underset{\alpha\text{-ketoglutarate}}{
\begin{array}{c}
COO^- \\
| \\
CH_2 \\
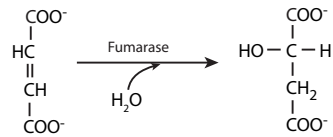| \\
CH_2 \\
| \\
C=O \\
| \\
COO^-
\end{array}}
$$

5. α-ketoglutarate dehydrogenase is very similar to pyruvate dehydrogenase complex structurally and mechanistically. There are three enzymes: the α-ketoglutarate dehydrogenase, a dihydrolipoyl transsuccinylase, and dihydrolipoyl dehydrogenase. Also similar to pyruvate dehydrogenase complex, the end product is a molecule containing a high energy thioester bond.

6. Succinyl-CoA synthetase first brings together the succinyl-CoA and inorganic phosphate (in solution within the mitochondrial matrix as well as the cytosol) to produce succinyl phosphate and liberate the CoA. Then the phosphate is transferred from the succinyl phosphate, to the enzyme itself temporarily, which then drops the succinate. And finally, the phosphate is transferred to GDP/ADP.

7. Even though the usual intro-class simplification is that $FADH_2$ is roughly an equivalent to NADH, the situation is actually more complicated. Unlike NAD^+, and for that matter, unlike most occurences of FAD, the FAD is covalently bound to the succinate dehydrogenase. Therefore, it is not a soluble metabolite, nor is it available to be reoxidized quite like NADH. It is, of course, reoxidized. But, this occurs within the context of the electron transport chain (where it is known as Complex II), with the help of Coenzyme Q.

8. Fumarase catalyzes the addition of water to the fumarate to generate malate.

$$COO^- \quad\quad\quad\quad COO^-$$
$$| \quad\quad\quad\quad\quad\quad\quad |$$
$$HC \quad\xrightarrow{\text{Fumarase}}\quad HO-C-H$$
$$|| \quad\quad\quad\quad\quad\quad\quad |$$
$$CH \quad\quad H_2O \quad\quad CH_2$$
$$| \quad\quad\quad\quad\quad\quad\quad |$$
$$COO^- \quad\quad\quad\quad COO^-$$

8. The carbon double bond of fumarate is attacked by a hydroxyl (OH-) to form a carbanion transition, which then takes on a proton (H+) from the enzyme to form the malate. The fumarase enzyme is protonated at the same time that it binds fumarate, and is deprotonated at the end to form the malate.

9. Malate is oxidized by malate dehydrogenase in the final reaction of the TCA cycle to generate more NADH, and oxaloacetate, the latter of which can then be added to

$$COO^- \quad NAD^+ \quad NADH \quad COO^-$$
$$| \quad\quad\quad\quad\quad\quad\quad\quad\quad |$$
$$HO-C-H \quad\xrightarrow{\hspace{2cm}}\quad C=O$$
$$| \quad\quad\quad\quad\quad\quad\quad\quad\quad |$$
$$CH_2 \quad\quad \text{\textit{Malate}} \quad\quad CH_2$$
$$| \quad\quad \text{\textit{Dehydrogenase}} \quad |$$
$$COO^- \quad\quad\quad\quad\quad\quad COO^-$$

9. Malate dehydrogenase is similar in structure to the lactate dehydrogenase and the alcohol dehydrogenase mentioned in the fermentation section. Energetically, the standard free energy change of this reaction is very highly positive (29.7 kJ/mol) but the oxaloacetate is quickly converted to citrate, so that more formation of oxaloactetate is favored over malate formation.

acetyl-CoA to start the cycle all over again. Now that the complete cycle has been described, it should be noted that the regulation of this cycle is primarily through acetyl-CoA and oxaloacetate availability, as well as NADH concentration. As respiration rate increases, NADH levels drop as they are oxidized to make ATP (see next section). This drop in [NADH] then causes an increase in oxaloactetate, which is then used by citrate synthase. [Acetyl-CoA] is regulated by its synthesis by pyruvate dehydrogenase. On the reverse side of regulation, both NADH and succinyl-CoA are strong inhibitors of a-ketoglutarate dehydrogenase. Thus as NADH is used up, the enzyme is disinhibited and increases its production of more NADH.

Having taken the 2 pyruvates created during glycolysis through the TCA cycle to complete oxidation into CO_2, what is our intrepid eukaryotic hero left with? Two ATP-equivalents (GTPs) six NADH, and two $FADH_2$. This hardly seems to be a treasure trove of usable energy worth boasting about. Fortunately, the mitochondrion is not finished. Next, the high energy electrons will take a ride on the electron transport chain, and via the magic of oxidative phosphorylation, produce ATP by the bucket.
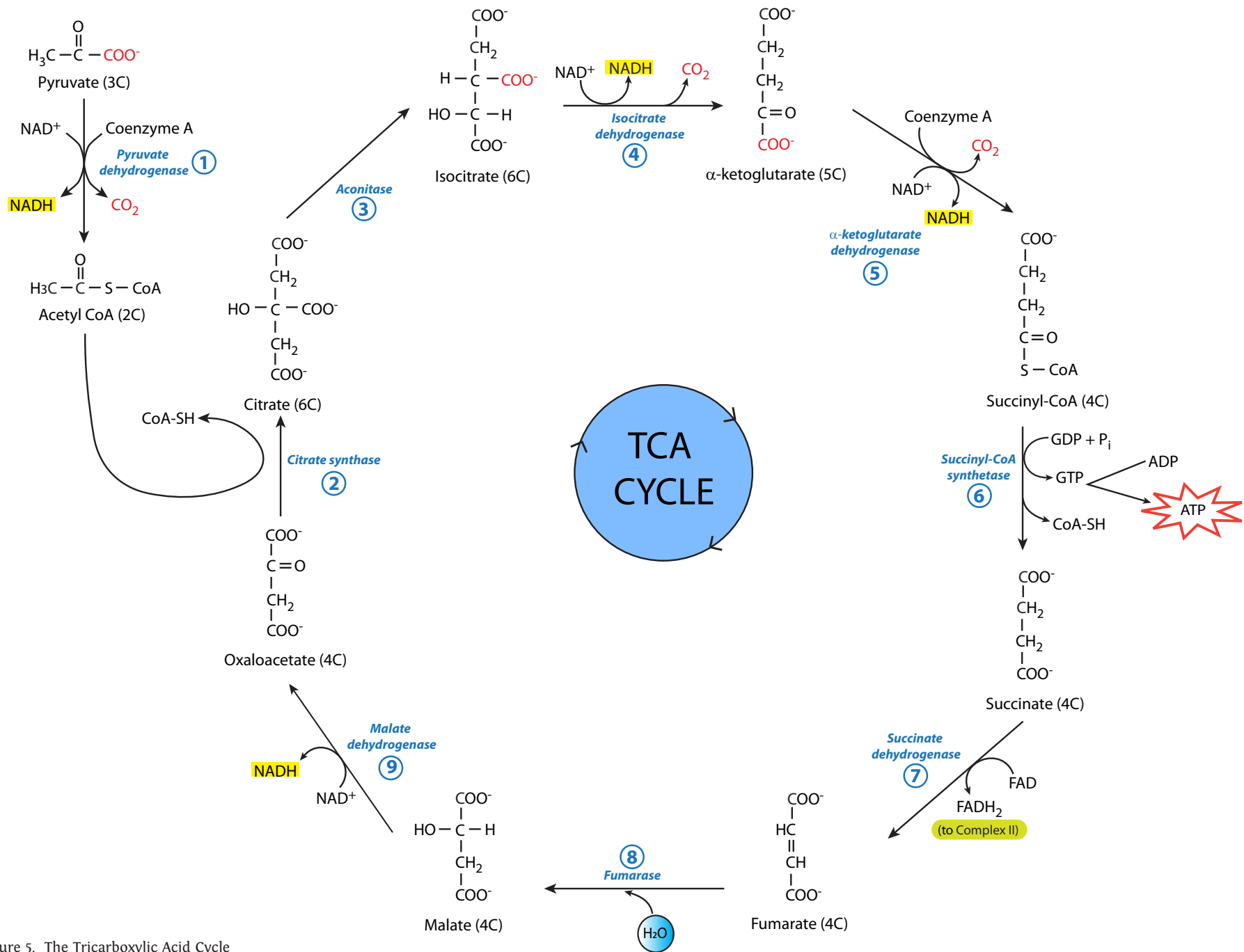
Figure 5. The Tricarboxylic Acid Cycle

Oxidative Phosphorylation

Oxidative phosphorylation denotes the phosphorylation of ADP into ATP, utilizing the energy from successive electron transports (hence the "oxidative"). The basic concept is that oxidation of NADH, being highly exergonic, can generate the energy needed to phosphorylate ADP. Since oxidation of NADH by oxygen can potentially release 52 kCal/mol (218 kJ/mol), and the energy needed to phosphorylate ATP is approximately 7.5 kCal/mol (30.5 kJ/mol), we should be able to expect the formation of several ATP per oxidized NADH. Indeed, this is what happens, although not directly. As noted with the breakdown of glucose, a one-step oxidation would generate too much energy for cellular processes to handle, and most would be wasted. So instead of oxidizing NADH directly with O_2, the electrons are transferred to a series of gradually lower-energy carriers until finally reaching oxygen. This sequence is the *electron transport chain*.

The electron transport chain is based on the activity of four major enzyme complexes (conveniently called complexes I-IV) embedded in the inner mitochondrial membrane, along with some small easily diffusible electron carriers to move the electrons from one complex to the next. These complexes are present in extremely high numbers as befits their necessity in generating energy, comprising nearly 75% of the inner membrane mass (in comparison, the plasma membrane of an average eukaryotic cell has a protein concentration closer to 50%). An overview of the process is shown in figure 6: as previously noted, electrons are stripped from NADH, and eventually end up on oxygen. As the electrons are moved to lower-energy carriers, energy is released and used to pump protons from the mitochondrial matrix into the intermembrane space.

Complex I is an NADH dehydrogenase. Shown in yellow in figure 6, its purpose is to remove a pair of electrons from NADH and transfer them onto ubiquinone (Coenzyme Q or CoQ), a small hydrophobic electron carrier that can then carry the electrons to complex III. This is a multistep process that involves first transferring the electrons onto an associated flavin mononucleotide (FMN) molecule, which then transfers the electrons to a set of iron-sulfur moieties connected to the enzyme complex itself (structure in fig. 7). Finally, the electrons are moved onto ubiquinone. As these transfers occur, the energy that is released during these transfers powers the pumping of 4 H^+ ions across the inner mitochondrial membrane. Complex I is inhibited by rotenone, a pesticide used primarily against insects and fishes.

We'll take a mental pass on complex II for now, and hit it at the end of this roll call. The reasons will be apparent then.
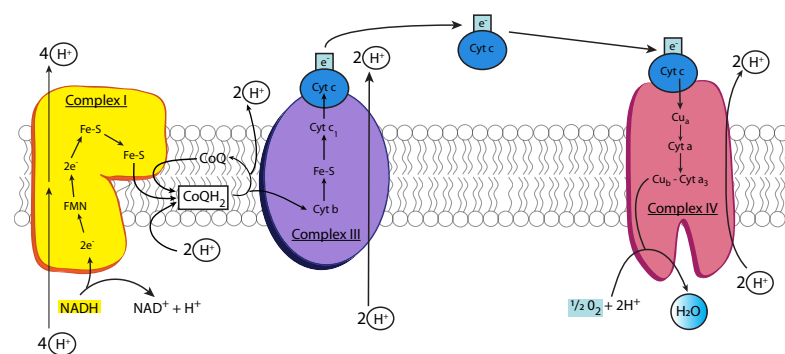

Figure 6. The primary electron transport pathway in mitochondria. Complexes I, III, and IV are shown. Complex II is pictured in fig. 10. The complexes are all buried in the inner mitochondrial membrane. Protons are being pumped from the matrix to the intermembrane space utilizing energy sapped from the high energy electrons as they move from a higher-energy carrier to a lower-energy carrier.
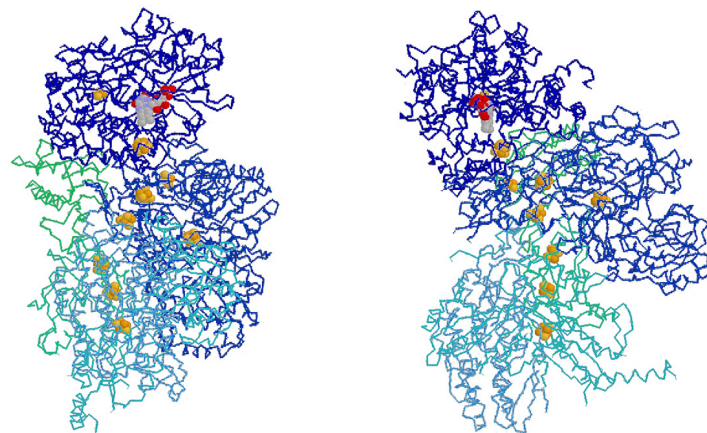
Figure 7. Although the size of complex I varies somewhat across species, the rough L-shaped three-dimensional conformation is constant. The FMN is located in the larger portion of the complex, while the ubiquinone docking site is located in the short branch. In the figure above, which depicts two aspects (rotated 90°) of NADH dehydrogenase complex, the FMN is shown in grey and red, while Fe-S centers are shown in orange and yellow. The figure was generated from data in the RCSB Protein Data Bank.

Complex III is also known as the cytochrome bc_1 complex (fig. 6, purple). The purpose of this complex is to pass the electrons from ubiquinone onto cytochrome c. The use of ubiquinone is important here, because it is stable with either two, or just one, extra electron. Cytochrome c, on the other hand, can only carry one electron. So, this complex docks ubiquinone, and holds it until it has passed its first electron onto cytochome c, which then moves onto complex IV, and then its second electron onto another cytochrome c. With each transfer, two protons are pumped across the membrane.

Finally, cytochrome c drops the electron off to complex IV, cytochrome c oxidase (fig. 6, red). Cytochrome c oxidase accomplishes the final step: transferring electrons onto oxygen atoms to make water. The really interesting thing about this process is that the enzyme must hold onto the electrons as they are transferred one at a time from cytochrome c, until it holds four electrons. Then, it can transfer one pair to each of the oxygen atoms in molecular oxygen (O_2). It is very important to do this because transferring any less than all four electrons would lead to the creation of reactive oxygen species (ROS) that could cause damage to the enzymes and membranes of the mitochondria.

In fact, some well known poisons act at exactly this point. Both cyanide and carbon monoxide can bind with higher affinity than oxygen at the heme in complex IV. Since neither can accept electrons, the effect is just as though no oxygen was available.

Although cytochrome c oxidase is sometimes abbreviated COX, it is *not* the target of the COX-2 inhibitors that are used pharmaceutically in pain management, e.g. Bextra, Celebrex, or Vioxx. That refers to a family of enzymes known as the cyclooxygenases.

Oxygen is absolutely required. If oxygen is not available, there is no place to transfer the electrons, and very quickly, the electron transport chain is halted and carriers such as cytochrome c and CoQ cannot release their electrons and eventually there are no more available carriers. Similarly, when that happens, NAD^+ is not regenerated, so the TCA cycle is also stuck. This leaves only the anaerobic non-oxygen-requiring glycolysis-fermentation cycle for generating ATP.

We now return to complex II (see fig. 10). We mentioned complex II as succinate dehydrogenase when discussing the TCA cycle. It also participates in the electron transport chain by passing electrons to ubiquinone. However, rather than transferring electrons that originated from NADH like the other three complexes of the electron transport chain, the electrons originate from the covalently bound electron carrier $FADH_2$ (flavin adenine dinucleotide), which received the electrons from succinate, as described in the TCA cycle section. Once the electrons have been passed to ubiquinone, it then moves on to complex III to drop off those electrons to cytochrome c, and the rest of the electron transport chain continues. FAD, the oxidized form of $FADH_2$, is then ready to participate in the next redox cycle.

The purpose of this electron transport chain, with respect to ATP generation, is the pumping of H^+ from the mitochondrial matrix into the intermembranous space. Since the concentration of protons is higher in the intermembrane space, it will take energy to move them against the concentration gradient, which is where our high-energy
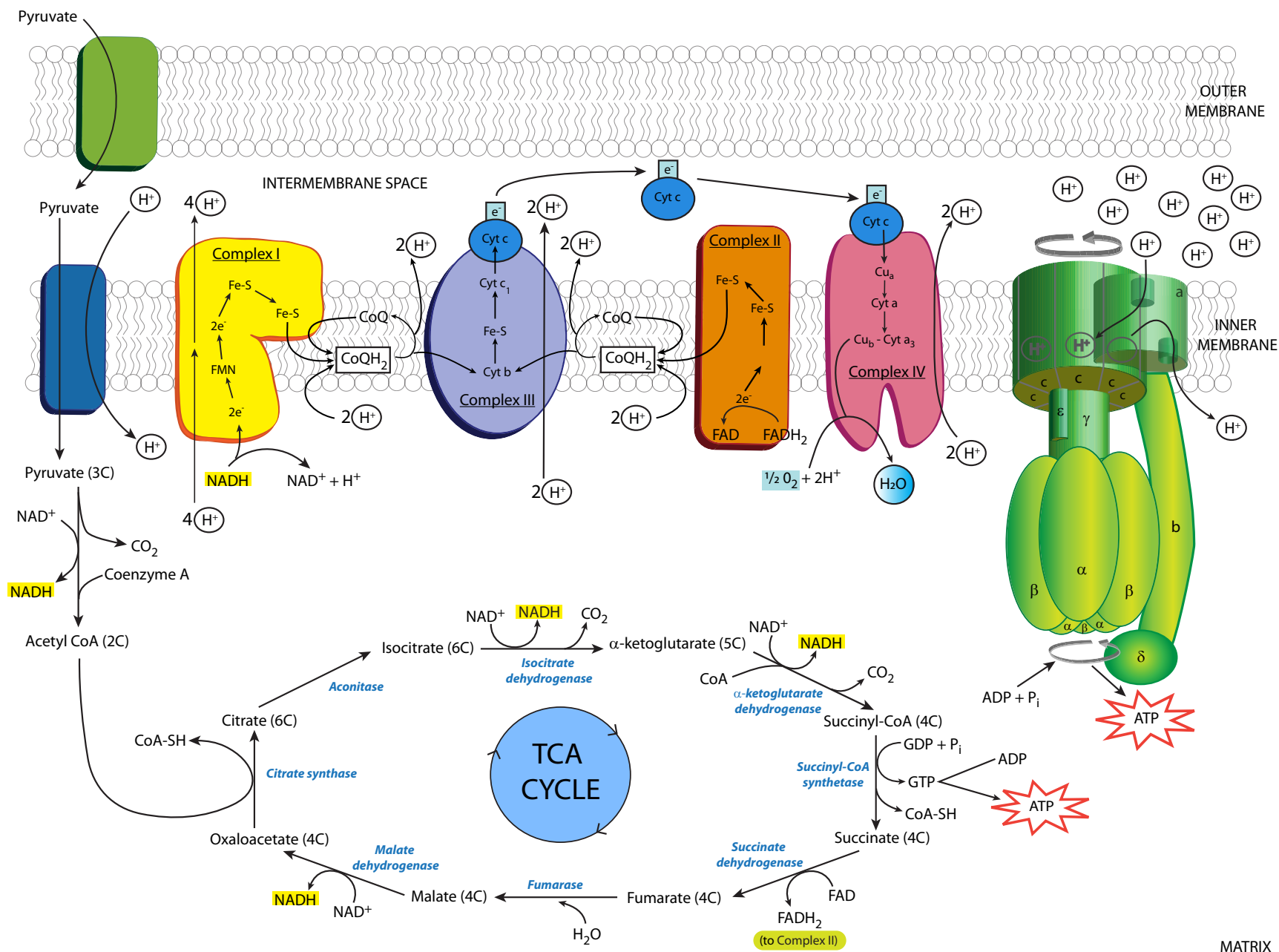
Figure 10. Catabolic reactions of the mitochondria.

electrons come into the picture. As they move from one carrier to the next, they are moving from a higher to a lower energy state. This implies that some energy is lost from the electron, and some of that energy is tapped by the enzymes of the electron transport chain to move protons from the matrix to the intermembrane space.

There are two methods by which the protons are moved: the redox loop, and the proton pump. The proton pump, which is the method by which complex IV moves protons, is the easier to understand: H^+ is bound on the matrix side of the enzyme in its reduced state (after it has received an electron), and a conformational shift occurs upon reoxidation to open the enzyme up to the intermembrane side, and the H^+ is released. The redox loop, which occurs in complex I, and in complex III in a variation called the Q cycle, essentially posits that an initial redox center requires the binding of both the high energy electron and a proton from the matrix side of the membrane. When the electron is transferred to the next redox center in the chain, a proton is released to the intermembrane space.

Whatever the mechanism, what is the point of all this proton pumping? As you might suspect, using up energy to pump an ion against its concentration gradient isn't done for the fun of it. Rather, this generates significant potential energy across the inner mitochondrial membrane. And, it so happens that there is an enzyme that can convert that energy into the physiologically useful chemical form of ATP. This enzyme is, not surprisingly, named ATP synthase (fig. 8). It is also referred to in some texts as the F_1F_0-ATPase, based on its reverse activity (at the expense of ATP, it can pump protons), and the fact that it can be broken down into two major functional units: F1 which can hydrolyze but not synthesize ATP and is a soluble protein, and F0 which is an insoluble transmembrane protein.

The ATP synthase is an extraordinary example of an enzyme that transforms the energy inherent in a concentration gradient across a membrane into mechanical energy, and finally into chemical bond energy. It is descriptively called a "rotary engine" because the very generalized sequence of events is as follows: protons flow down their gradient through a proton channel subunit of the ATP synthase, in flowing down the gradient, energy is released, this energy causes rotation of a multisubunit "wheel"-like subunit attached to a spindle/axle (γ subunit) which also spins. The spinning of this asymmetrically shaped spindle unit causes conformational changes in the catalytic subunit (made of the α and β subunits) it is attached to, changing an ADP+Pi binding site to a catalytic site that can "squeeze" the molecules together into an ATP, and then finally open up to release the ATP (fig. 9).
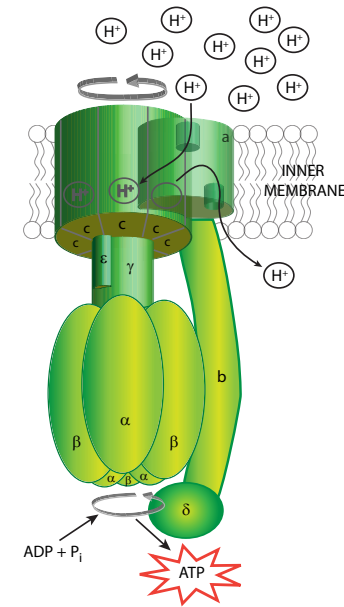

Figure 8. ATP synthase. As protons pass through the ATP synthase, they release energy by going from high concentration to low. This energy drives the rotational movement of the shaft and the generation of ATP.
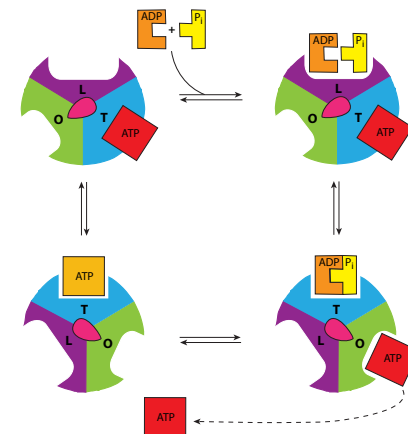

Figure 9. ATP synthase head rotation. The rotating spindle causes asymmetric changes to the shape of the three potential binding sites, cycling them through the loose (L) conformation that binds ADP and Pi, the tight (T) conformation that literally squeezes the two sustrates together into ATP, and the open (O) conformation that allows ATP.

Of course, it isn't quite that simple (fig. 8). Starting with the initial movement of protons, as they move from the intermembrane space into the ATP synthase, they enter a small hydrophilic channel (a) and then bind onto one of the c-subunits of the "water wheel" c-ring. Binding of the H$^+$ to the c-subunit causes it to lose affinity for the a-subunit, allowing it to spin, and simultaneously causes a conformational change that essentially pushes off against the a-subunit, initiating the movement. Once it has spun around almost a complete turn, the H$^+$ is positioned by another channel (b), which funnels it from the c-subunit into the matrix. The c-subunit structure is connected to an asymmetric spindle that is itself connected to the catalytic subunits.

Uncoupling Electron Transport from ATP Synthesis

So, that is oxidative phosphorylation. It productively utilizes the energy of the proton gradient across the inner mitochondrial membrane (created by oxidation-powered pumps) to drive ATP formation at an approximate rate of 3 protons to 1 ATP. The system is normally highly self-regulated due to impermeability of the inner mitochondrial membrane to H+. If the ATP is not used up quickly, then its concentration slows the action of ATP synthases, which slow the movement of protons out of the intermembrane space. This buildup of protons will eventually be enough that the free energy needed to transfer a proton into the intermembrane space (from the electron transport chain) will not be sufficient to overcome the concentration gradient. Electron transport is slowed, and working backwards, the chain reaction slows respiration rates in general. As the cell/organism requires more energy and uses up the ATP more quickly, protons flow more quickly and the electron transport chain is disinhibited. Thus there is a direct association between respiration rate and physiological energy need.

Interestingly, there is an exception to this tight coupling of the electron transport chain and formation of ATP. The purpose of brown fat (aka brown adipose tissue), which is most often found in newborn and hibernating mammals, is to generate non-shivering (non-movement-based) heat to keep the animal warm. This is accomplished by uncoupling the electron transport chain from the ATP synthesis. This uncoupling is a hormonally controlled process based on the presence of a mitochondrial proton channel called thermogenin. The hormone norepinephrine increases production of free fatty acids, which open the thermogenin channel. This allows protons to flow from the intermembrane space back into the matrix without having to go through ATP synthase. Because of this, the electron transport chain can keep chugging away, ATP levels do not build up, there is no reduction in respiration rate, and the excess energy not being used in ATP production is released as heat.

In fact, 2,4-dinitrophenol, which is used in a variety of research and industrial applications today, was at one time used as dieting drug (in the 1930's) because through a different mechanism, it too uncoupled electron transport from ATP synthesis. Its mechanism of action derived from its ability to carry and release protons as it freely diffused through the mitochondrial membrane (since it is a small hydrophobic molecule). As this continues, cells catabolize more and more stores of carbohydrates and fats, which is the reason for the interest by dieters. Unfortunately for some of those dieters, this pharmacological means of uncoupling the electron transport chain from the ATP synthesis had no regulation other than the amount of DNP taken. In cases of overdose, respiration rates could rise dramatically while producing little ATP and a great deal of heat. In fact, overdose illness and death are generally due to the spike in body temperature rather than lowered ATP availability. Unfortunately, there are still some dieters and bodybuilders who self-medicate with DNP despite the dangers.

Structure of Electron Carriers

Though they have been mentioned frequently in the earlier parts of this chapter, the structures of the electron transport chain participants, and particularly of the moieties that temporarily hold extra electrons, have not been addressed. So, now is the time to do so. The major players are the flavin mononucleotide (FMN) that plays a role in complex I, ubiquinone (Coenzyme Q), the lipid-soluble electron carrier, the heme groups of the cytochromes, and iron-sulfur clusters, found in complexes I, II, and III.

Flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD), are pictured in figure 11. Note the triple-ring structure and the three possible oxidation states. All three states are stable - the semiquinone state is not merely a transient form. This stability allows the conversion from carriers that can only handle one electron to carriers that can handle two electrons, and vice versa. The same holds true for ubiquinone - stable as ubiquinone (fully oxidized), semiubiquinone (radical state), and ubiquinol (fully reduced). Alternative nomenclature for these molecules is Coenzyme Q, CoQH+, and CoQH$_2$. Note the aromaticity gained by ubiquinone when it is reduced. This enhances its stability and its suitability as a receiver of electrons from NADH.

Heme groups (figure 12) are considerably larger, encompassing a porphyrin ring with an iron ion held in its center. This iron ion alternates between ferric (Fe^{3+}) and ferrous (Fe^{2+}) states as the heme group is oxidized and reduced, respectively. In the case of complex IV, the iron ion can form a complex with O$_2$, which can then receive the electrons being held by the ring structure. This large structure is particularly important because it needs to be able to transfer a total of 4 electrons to reduce O$_2$ to 2 H$_2$O.

Finally, Fe-S clusters (figure 12) can also act as electron carrying moieties. Like in the heme group, the iron atom can readily switch between the ferric and ferrous states.
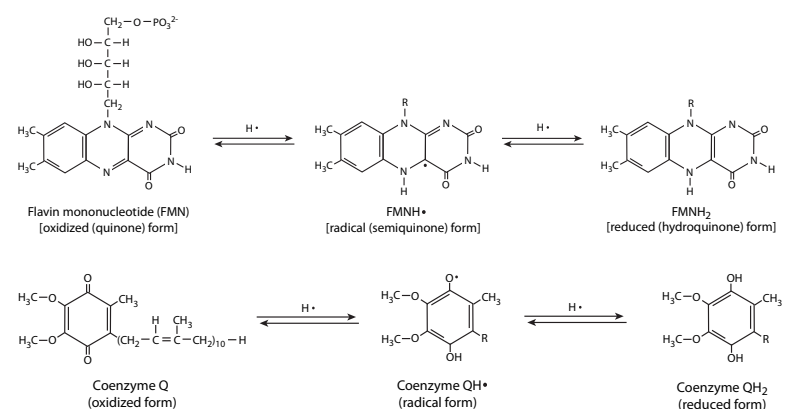

Figure 11. Flavin mononucleotide and Ubiquinone are electron carriers.
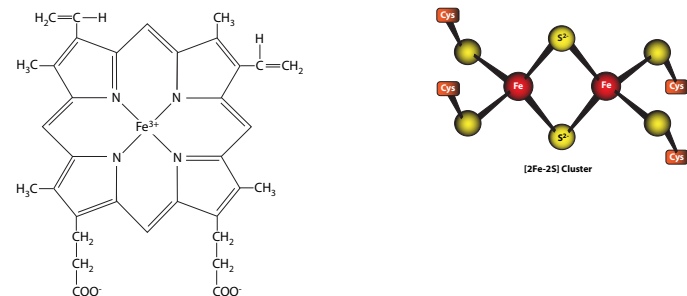
Figure 12. Left: The heme group shows one of the possible combinations of the groups attached to the outside of the porphyrin ring. Right: The Fe-S centers also have variations, such as 4 Fe and 4 S, or even just 1 Fe surrounded by the S atoms of the four cysteines.

Other Catabolic Reactions

Of course, for many organisms, the food used by cells is not in the form of simple glucose solutions, but made up of various polymeric biomolecules. The breakdown of those molecules is described in the next sections.

Starch and Glycogen Depolymerization

Glycogen and starch are long branched polymers of glucose that provide a rapidly available source of glucose molecules for glycolysis. In omnivores and herbivores, the primary source of carbohydrates (and thus glucose) is dietary starch. The catabolism of the amylose and amylopectin in humans begins in the mouth with salivary α-*amylase*. This enzyme breaks α(1-4) bonds of both starch molecules except at the ends and near branch points (in the case of amylopectin). Though the salivary enzyme is inactivated by the acidity of the stomach, a pancreatic α-amylase goes to work on starch that has reached the small intestine. The product of these digestions includes maltose, maltotriose, and dextrins. These are acted upon by other intestinal enzymes: α-*glucosidase* removes individual glucoses from oligosaccharides, and α-*dextrinase*, also known as debranching enzyme, can break α(1-6) bonds as well as the α(1-4) bonds.

Glycogen breakdown is different since most glycogen breakdown is occuring internal to the cells of an organism rather than in the digestive tract. The primary enzyme is *phosphorylase* (also known as glycogen phosphorylase), which breaks the bond of a terminal glucose to its neighbor by substituting a phosphate group. This generates glucose-1-phosphate, which can be converted to glucose-6-phosphate by *phosphoglucomutase*. The G6P, of course, can enter the glycolytic pathway. A *glycogen debranching enzyme* is also important, as the phosphorylase is unable to work closer than five glucose residues to a branch site.

The use of glycogen presents an interesting question: why use it as an energy storage molecule when fats are more abundant in most animals, and more efficient at packaging potential energy? As described in the next section, fatty acids can only be metabolized aerobically, so they cannot serve as a backup fuel source in anaerobic conditions. Furthermore, even in aerobic conditions, fatty acid catabolism cannot generate glucose, which is not only needed for cellular fuel, but in the bloodstream for feedback control mechanisms regulating organismic metabolism.

Fatty Acid Breakdown

Hormone-sensitive lipase in adipose tissue hydrolyzes the stored fat in those cells into glycerol and fatty acids. Glycerol can enter the glycolytic cycle via conversion to dihydroxyacetone phosphate (a two-step conversion using glycerol kinase and glycerol-3-phosphate dehydrogenase). The fatty acids are secreted from the adipose cells into the bloodstream where they bind to a carrier protein, albumin. This complex can then be brought inside of other cells by endocytosis, where they can be broken down as an energy source.
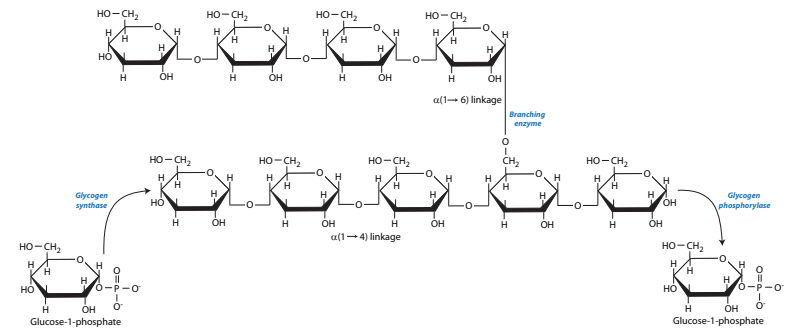

Figure 13. Glycogen is a storage form of glucose that is broken down by glycogen phosphorylase (linear α(1-4) bonds) and debranching enzyme (branchpoint α(1-6) bonds).

Phosphorylase is a homodimer that is allosterically controlled by glucose, G6P, and ATP negatively, and by AMP positively. In addition to allosteric binding sites for these molecules and a substrate binding site, phosphorylase also binds pyridoxal-5-phosphate as an essential cofactor. P5P is derived from pyridoxine, or vitamin B$_6$.

Much like the phosphoglycerate mutase in step 8 of glycolysis, phosphoglucomutase is a phosphorylated enzyme that temporarily transfers is phosphate group to the substrate to form a glucose-1,6-bisphosphate intermediate.

Debranching enzyme actually has two functions: it transfers a trisaccharide from a 4-sugar branch on the "1" side of an α(1-6) branching linkage to the end of a branch connected to the "6" side of the branchpoint. It then hydrolyzes the α(1-6) connecting the final glucose of the branch, leaving an unbranched chain of glucose for phosphorylase to attack.

The breakdown of fatty acids occurs by β oxidation inside the mitochondrial matrix (fig. 14). Since the inner mitochondrial membrane is impermeable to long-chain free fatty acids, they must first be activated to fatty acyl-CoA and linked to carnitine, an amino acid derivative synthesized from methionine and lysine (see Fig. 15). The first step is
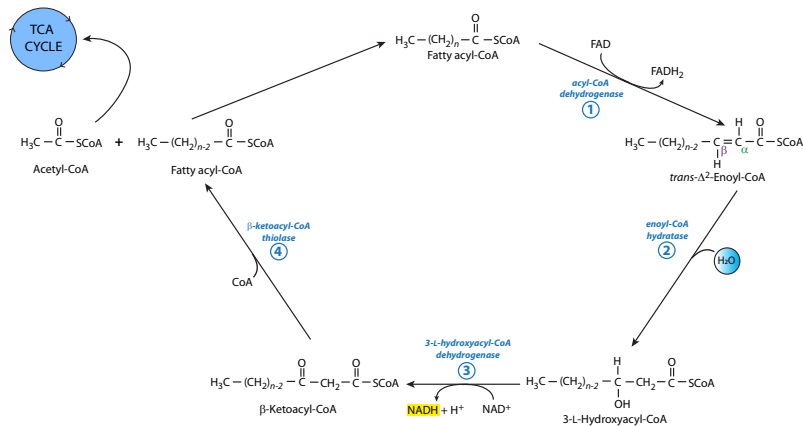

Figure 14. β oxidation of fatty acids happens in the mitochondrial matrix.

Figure 15. Fatty acid breakdown by Carnitine Palmitoyl Transferases.

performed by one of a family of enzymes known as acyl-CoA synthetases or thiokinases, and requires Coenzyme A and ATP hydrolysis. These reactions occur either on the cytoplasmic surface of the mitochondrial outer membrane or the endoplasmic reticulum, where acyl-CoA synthetases are embedded. In the second reaction, carnitine palmitoyltransferase I on the outside of the inner mitochondrial membrane links the acyl chain to carnitine, releasing CoA. The acyl-carnitine is transported into the mitochondrial matrix where carnitine palmitoyltransferase II releases the fatty acyl chain from the carnitine and reattaches it to an molecule of CoA.

In the mitochondrial matrix, β oxidation occurs in four steps to yield an acyl-CoA chain that is shortened by two carbons, and an acetyl-CoA that can then enter the TCA. .
The β refers to the second closest carbon to the one attached to CoA. The bond that will be broken is the bond between the α and β carbons. All even-numbered, fully saturated, fatty acids can thus be completely oxidized. The presence of double bonds in unsaturated fatty acids introduces complications to this process that must be addressed using additional enzymes that either move the double bond or remove it.

Most animals and plants generate even-numbered fatty acids; however, some marine animals (e.g. smelt, mullet) and some plants and bacteria synthesize odd-chain fatty acids as well. The same enzymes responsible for β oxidation of even-numbered fatty acids can handle odd-numbered fatty acids as well, except that the final degradation yields propionyl-CoA instead of acetyl-CoA.
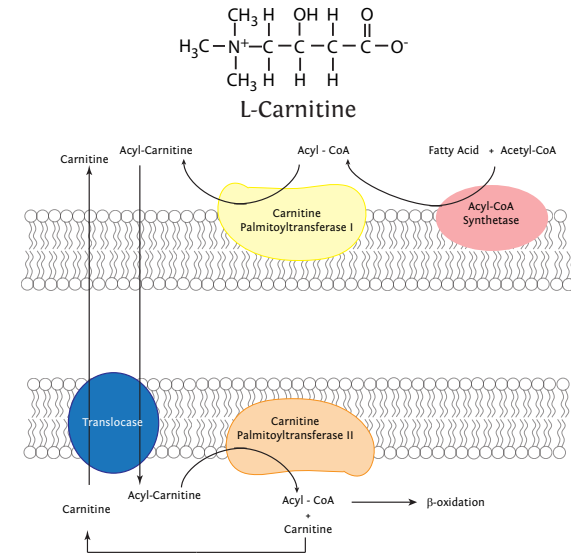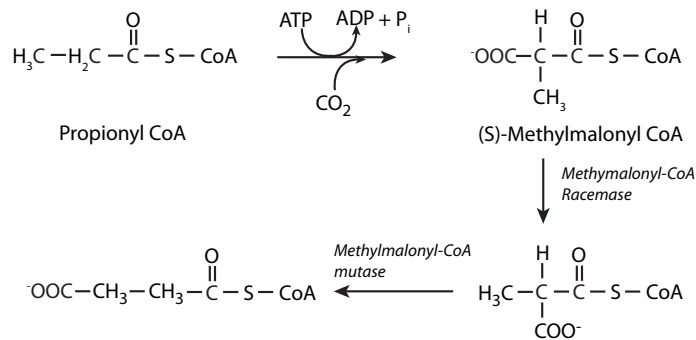
Carnitine deficiency syndromes can occur when there is either a dysfunctional mutation of carnitine palmitoyltransferase or a severe deficiency of intracellular carnitine. Since most of the carnitine in the body is found in cardiac and voluntary muscle, the usual symptoms are muscle weakness and cardiac arrhythmias, as well as hypoketosis. In neonates, the arrythmias can lead to death. Carnitine supplementation is a successful treatment in systemic carnitine deficiency due to either low carnitine intake or defects in the carnitine transporter embedded in the cell membranes. However, if the defect is in the palmitoyltransferase, supplementation will be unsuccessful.

Carnitine is widely sold as a dietary supplement for increasing weight loss by enhancing fat catabolism. The basic idea is obvious: carnitine is needed for long-chain fatty acid breakdown, so more carnitine = more fat burned. However, that only holds true if carnitine levels are below saturation levels for the palmitoyltransferases. Because 75% of the carnitine in the body must be ingested (only 25% is synthesized), this is a mild possibility, depending on diet. Currently, the biomedical community has not reached a consensus on the efficacy of carnitine supplementation on fatty acid oxidation in carnitine-sufficient persons.

$$H_3C-H_2C-\overset{\overset{\displaystyle O}{\|}}{C}-S-CoA \xrightarrow[\text{CO}_2]{\text{ATP} \quad \text{ADP}+P_i} {}^-OOC-\overset{H}{\underset{CH_3}{\overset{|}{C}}}-\overset{\overset{\displaystyle O}{\|}}{C}-S-CoA$$

Propionyl CoA (S)-Methylmalonyl CoA

Methylmalonyl-CoA Racemase

$${}^-OOC-CH_3-CH_3-\overset{\overset{\displaystyle O}{\|}}{C}-S-CoA \xleftarrow[\text{mutase}]{\textit{Methylmalonyl-CoA}} H_3C-\overset{H}{\underset{COO^-}{\overset{|}{C}}}-\overset{\overset{\displaystyle O}{\|}}{C}-S-CoA$$

Propionyl-CoA is converted to succinyl-CoA through a series of three enzymes: propionyl-CoA carboxylase, methylmalonyl-CoA racemase, and methylmalonyl-CoA mutase. The succinyl-CoA could theoretically enter the TCA cycle, but recall that the succinyl-CoA is simply recycled and never actually consumed by the TCA cycle. Thus, in order for the succinyl-CoA to contribute to the energy needs of the cell, it must first be converted to malate (steps 6-8 of TCA cycle), which is then converted to pyruvate by malic enzyme, also known as decarboxylating malate dehydrogenase. Pyruvate can then enter and be consumed by the TCA cycle.

In addition to oxidation in the mitochondria, fatty acids also undergo β oxidation in peroxisomes. However, generally, the oxidation in peroxisomes is limited, and the purpose is to shorten long fatty acids in preparation for final degradation in the mitochondria.

In addition to the more common single-chain fatty acids, cells will also encounter branched fatty acids, which block β oxidation is alkyl group is on the β carbon. In these cases, phytanic acid for example, α oxidation is necessary to generate an intermediate with the alkyl group on the α carbon. This is then followed by β oxidation to completion.

Finally (with respect to fatty acid catabolism), it must be noted that in liver especially, a large part of the acetyl-CoA generated by oxidation of fatty acids does not enter the TCA cycle. Instead, it is converted into acetoacetate or D-β-hydroxybutyrate, which along with acetone, are known, somewhat bizarrely, as ketone bodies. These molecules are water soluble, and transported through the bloodstream as energy sources for a variety of tissues, even including brain, which typically only uses glucose as fuel since fatty acids cannot pass through the blood-brain barrier. However, ketone bodies can penetrate and are used by brain cells under starvation conditions.

Vitamin B_{12}, or 5'-deoxyadenosylcobalamin, is a coenzyme component of methylmalonyl-CoA mutase, but it is not made by either plants or animals. It is only made by certain bacteria, some of which live in the intestinal tracts of herbivores. Herbivores thus absorb the B_{12} for their use, and carnivores obtain their B12 from eating herbivores. Defects in methylmalonyl-CoA mutase or severe deficiency in vitamin B_{12} (most often in vegetarians) can lead to methymalonyl aciduria/acidemia, that can be fatal in untreated infants due to acidosis. However, depending on the cause, it can be treated with high doses of B12 and/or by avoiding dietary odd-chain fats and proteins rich in isoleucine, leucine, or methionine, which also catabolize to propionyl-CoA. Pernicious anemia, in which usually elderly patients have very low levels of red blood cells and hemoglobin, as well as neurodegeneration, is also related to B_{12}. However, it is usually not due to a vitamin deficiency, but rather to the insufficient secretion of *intrinsic factor*, which binds B_{12} in the stomach and then is taken into intestinal cells by receptor-mediated endocytosis.

Ketoacidosis is a condition in which ketone bodies are being produced much faster than they are used. This leads to a buildup of the molecules in the bloodstream, which lowers the pH, since the molecules are acidic. An easy diagnostic of ketoacidosis is a sweet somewhat fruity smell (of acetone) on the breath. This condition can be an indication of diabetes, but may also occur when a person is consuming a high-fat/low-carb diet. When the body's metabolism is not using glucose/carbohydrates as the primary food source for either reason, fat is used instead, increasing production of ketone bodies. Left untreated, severe ketoacidosis can lead to cell damage as the blood acidifies, and compensation by increased exhalation of carbon dioxide and lead to respiratory failure in susceptible individuals.

Amino Acid Degradation

Proteins are broken down by a variety of proteases that hydrolyze the peptide bonds to generate smaller peptides and amino acids. Those amino acids that are not used for building new proteins may be broken down further to enter the metabolic processes discussed in this chapter. In their conversion to metabolic intermediates, the amino acids first undergo deamination. The primary goal of deamination is to excrete excess nitrogen (as urea) and then use or convert (to glucose) the remaining carbon skeleton. This deamination is a two-part process: the first step to deamination is usually a transamination catalyzed by an aminotransferase, in which the amino group of the amino acid is transferred to α-ketoglutarate which then yields a new α-keto acid of the

α-Ketoglutarate   α-Amino acid   Glutamate   α-Keto acid
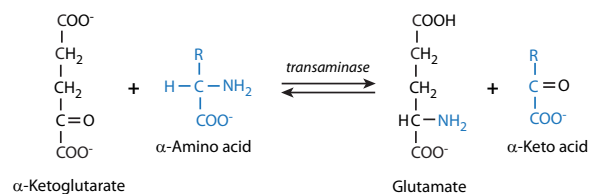
amino acid and glutamate.

The amino group of glutamate could then transferred to oxaloacetate to form α-ketoglutarate and aspartate. That series of transaminations transforms the original amino acid, but does not get rid of the amino group nitrogen. The alternative pathway is deamination of the glutamate by glutamate dehydrogenase, which generates α-ketogl-

Glutamate   α-Ketoglutarate
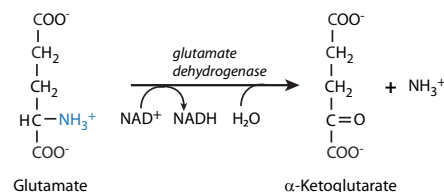
utarate and ammonia, using either NAD^+ or NADP as the oxidizing agent.

The amino acids break down into one of the following seven metabolic intermediates: pyruvate, acetyl-CoA, acetoacetate, α-ketoglutarate, succinyl-CoA, fumarate, and oxaloacetate as follows: 1) Ala, Cys, Gly, Ser, Thr, Trp break down to pyruvate; 2) Ile, Leu, Lys, Thr to acetyl-CoA; 3) Leu, Lys, Phe, Trp, Tyr to acetoacetate; 4) Arg, Glu, Gln, His, Pro to α-ketoglutarate; 5) Ile, Met, Val to succinyl-CoA; 6) Asp, Phe, Tyr to fumarate; 7) Asn, Asp to oxaloacetate.

There is a large variety of proteases, classified into one of six groups (as of 2008): serine proteases, metalloproteases, aspartic acid proteases, cysteine proteases, threonine proteases, and glutamic acid proteases. All of them work by forming a nucleophile at their active site to attack the peptide carbonyl group. They differ in the construction of their active sites, and the specificity of the target sequences to be cleaved. The MEROPS database (http://merops.sanger.ac.uk/) lists hundreds of enzymes and their specific recognition sites. As with other enzymes, recognition is based on formation of stabilizing hydrogen bonds between enzyme and target. In the case of proteases, many of the important stabilizing bonds must be formed right around the cleavage site, thus leading to specific recognition sequences.

Although cleavage is often thought of as a way of destroying the activity of a protein, in fact, specific cleavage of inhibitory parts of a protein can activate it. A prominent example of this (the caspase cascade) is discussed in the apoptosis section of the cell cycle chapter.

Some proteases are secreted and do their work extracellularly. These include digestive enzymes such as pepsin, trypsin, and chymotrypsin, as well as bloodstream proteases like thrombin and plasmin that help control clotting. The immune system also uses proteases to destroy invading cells and viruses.
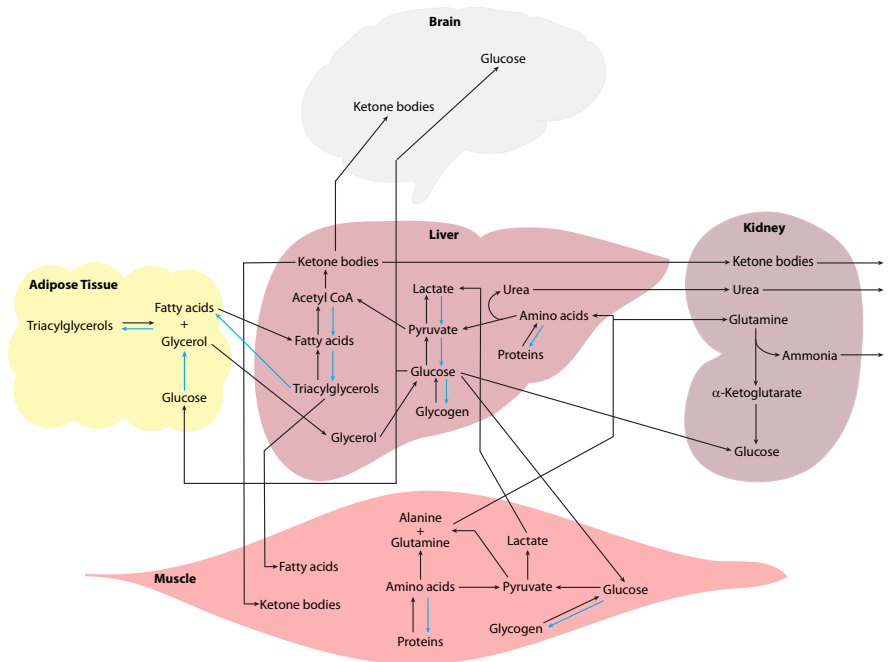
Figure 16. Overview of human major metabolites. Although most cells in the body carry out many of the metabolic activities described in this chapter and the next, the advantage of multicellular organisms is that certain cell types, tissues, or organs may become specialized to process particular metabolic reactions more efficiently than other cells, and thus take on a lot of that burden for the organism.