# AEROBIC RESPIRATION[1]

***Review*;** In the last set of notes we learned some of the basic types of reactions involved in cellular work and energy conservation. Recall that we focused on:

- Substrate-level phosphorylations and
- Oxidation of long-term energy storage compounds by the coenzyme $NAD^+$

In regard to the latter, we learned that these electrons cannot remain indefinitely on the oxidized form of $NAD^+$ (i.e., on NADH) because otherwise the cell would quickly run out of $NAD^+$. Reactions that require it would be unable to proceed. For instance, this lack of $NAD^+$ would stop glycolysis and rob the cell of a source of ATP. To get around this problem, other compounds are used by cells to oxidize the NADH back to $NAD^+$. The examples we saw were pyruvate (leading to lactate) or acetaldehyde (leading to ethanol).

These reactions can be viewed as energetically wasteful. The electrons carry considerable potential energy but it is not used to produce ATP. Instead, the energy is incorporated in compounds where the energy normally cannot be extracted – at least not under the conditions that resulted in its production.

One of the great biochemical inventions are pathways that allow the energy to be removed from these electrons and used to synthesize ATP. The particular pathway we will consider uses **$O_2$ as the final electron acceptor** and we have come to call it **aerobic respiration**. In these notes we will consider the specific processes that use $O_2$ plus high-energy electrons from coenzymes to produce ATP. This process is called **oxidative phosphorylation**. We will see that it is restricted to the inner membrane and immediately adjacent areas of the inner-membrane space and matrix of the mitochondria (review mitochondria notes if needed). We will also consider, in very general terms, the reactions that feed oxidative phosphorylation. These include the famous Krebs cycle of the mitochondrial matrix, the $\beta$-oxidation pathway for "burning" fatty acids, and some other reactions, including those we have already considered in glycolysis.

## ***Oxidative Phosphorylation – the Electron Transport System and Chemiosmosis***

- Oxidative phosphorylation is a complex process that is centered on the inner membrane of the mitochondria. The "players" are:
  **Reduced coenzymes** (NADH and $FADH_2$) that are produced mainly in mitochondria matrix
- **The electron transport system or respiratory chain** – a series of compounds able to undergo oxidation and reduction. Some of them are proteins that contain metal ions; others lack metals. Some of them change color according to oxidative state

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- **A proton ($H^+$) or pH gradient** across the inner membrane. A high concentration of $H^+$ in the inner membrane space with a relatively low [$H^+$] in the matrix.
- **ATP Synthase**: Transmembrane proteins that can allow $H^+$ to move from the inner membrane space to the matrix and in the process synthesize ATP.

**The Electron Transport System (ETS)**: The electron transport system (ETS) is essentially made of compounds collectively called cytochromes. Cytochromes (literally "cell colors") were so named because microscopists noticed that mitochondria change color depending on whether or not $O_2$ was present. These are the compounds responsible for the colorr change.

The members of the ETS are organized functionally into chains. The first member of the chain oxidizes a coenzyme, either NADH or $FADH_2$ (see below) thereby regenerating the oxidized forms of these compounds.

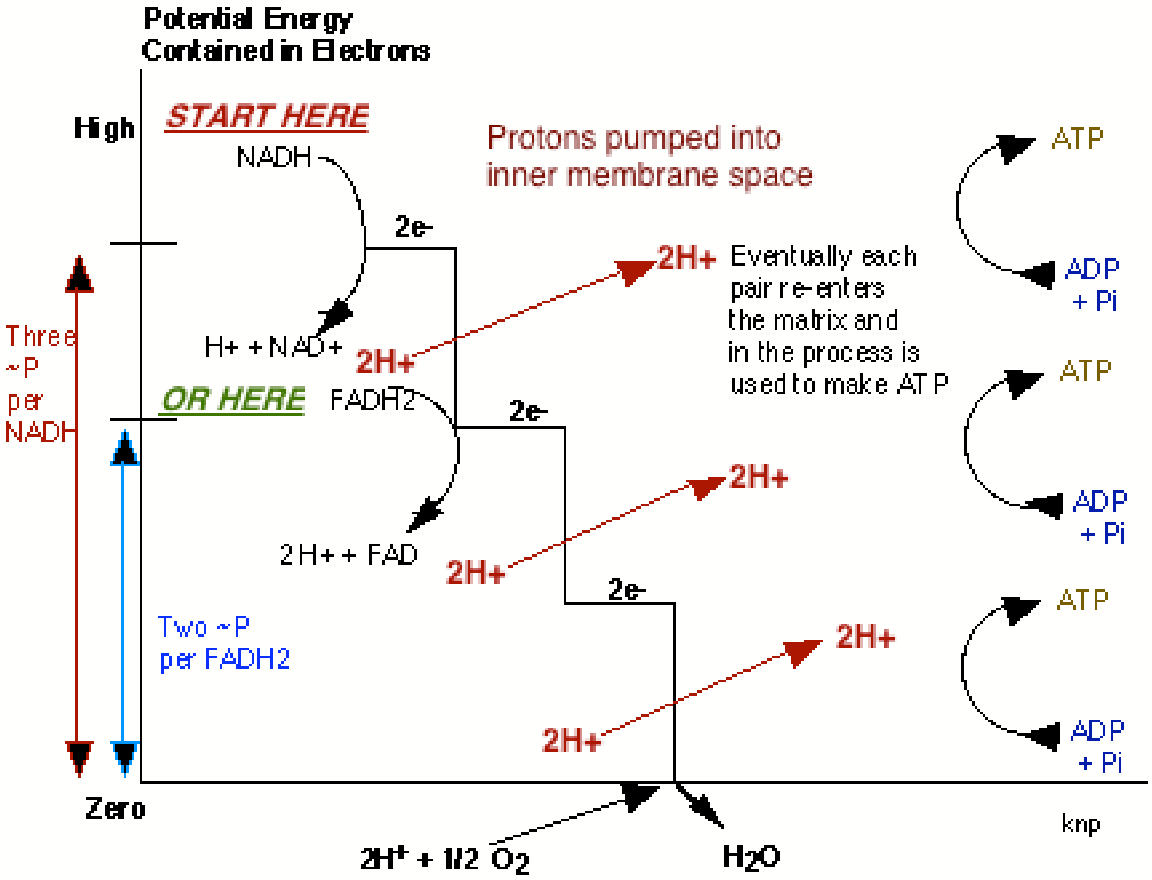
**What is $FADH_2$?** This is the reduced form of a coenzyme that is used to **oxidize compounds and remove electrons that are less energetic than those picked up by $NAD^+$.** The general reaction is like that of $NAD^+$ to NADH (see last set of notes):

Electron donor + FAD --------------> oxidized (former) donor + $FADH_2$

In going from FAD to $FADH_2$ two electrons and two protons were taken from the donor by the FAD. Again, these electrons contain a lot of potential energy but not as much as the ones carried by NADH.

These electrons are then handed along to the next member of the chain and in turn to the next member etc. until finally $O_2$ is reached. Each passage goes to the progressively stronger oxidizing agent. Many members of the chain are are physically inner-connected within the inner membrane. When $O_2$ is reached, water is formed. Thus water constitutes the waste product of the ETS.

In the process, energy is removed from the electrons at a number of points. The energy is used to transport a pair of protons from the matrix to the inner membrane space. One way to look at the energetics of the ETS is to envision a stairway:



Imagine a ball moving down the stairs. It is analogous to the electrons. Each time it drops one level of the stairs, energy is released. Notice that:

- When **NADH** is the source of the electrons, a total of **three energy drops occur**.
- On the other hand, since the electrons taken from **FADH$_2$** are less energetic and require a stronger oxidizing agent, they enter a bit later in the scheme and only drop down **two** stairs.

Now, what causes each energy drop? As mentioned above and shown in the diagram, **energy is taken from the electrons and is used to transport a pair of H$^+$ out of the matrix to the inner membrane space**.

The result is that the concentration of these ions increases in the inner membrane space.

**This creates an electrical gradient (review the Donnan Equilbrium in the cell membrane materials).**

**It also creates an osmotic gradient (since the pumping tends to cause the inner membrane space to swell --- WHY?), and it creates a *p*H gradient with a low *p*H in the inner membrane space.**

For our purposes, these are all the same. I would argue that it is best to term the result an electrochemical (related to a Donnan) gradient since that emphasizes energetics.

Now, notice that the result of this pumping of $H^+$ must be to make the matrix negatively charged relative to the inner membrane space. This tends to favor the movement of $H^+$ back into the matrix, if there is a path. Likewise, the $H^+$ concentration gradient also favors the same movement. Thus, there is a significant amount of energy stored in this gradient and carried by these protons.

However, **$H^+$ can only re-enter the matrix at the sites of proteins called <u>ATP SYNTHETASE</u> (a.k.a. the "$F_0F_1$ ATPase").** If :

- ATP synthetase binds ADP and Pi, it will undergo an **allosteric shape change**
- This alllows the binding of a pair of $H^+$
- This in term induces an allosteric change that transports the $H^+$ through the inner membrane.
- This movement is associated with changes that bring the ADP and Pi together to form ATP.
- In the end, the protons are released back into the matrix, as is the ATP.

Thus, the entry of two $H^+$ has driven the synthesis of ATP.

There are transporters in the inner membrane that allow the ATP to leave (and others that import ADP and Pi from the cytosol).

If the cell is resting and there is relatively little ADP and $P_i$ around, will $H^+$ find it easy to re-enter the matrix? Explain.
Under these conditions will it be easy for the ETS to accept electrons at a high rate? Explain.

The end result of all this is that **per NADH:**

- **One pair of electrons and protons is removed.**
- **These electrons move through the ETS; the protons go into solution.**
- **The electrons have enough energy to pump 3 pairs of protons into the inner membrane space.**
- **These can re-enter and form 3 high-energy phosphate bonds (3 ~P).**
- **The spent electrons finally combine with oxygen to give water, the waste product of the ETS.**

By <u>contrast, each **$FADH_2$** also gives up two electrons and two protons</u>. However,

- **The two protons go into solution.**
- **These electrons add later in the ETS scheme**
- **The electrons only have enough energy to cause two pairs of protons to be pumped.**
- **As a result only two ATP can be synthesized from ADP and Pi.**
- Once again, **one water molecule is synthesized as a waste**.

So, **NADH generally contains enough energy for 3 ~P** and **$FADH_2$ is its poor relative that only has the means to fuel the synthesis of 2 ~P**. One is not better than the other or fundementally different beyond the fact that FAD is specialized to oxidize bonds that contain less potential energy then those oxidized by $NAD^+$.

If NADH is the electron source, how many mols of ATP will be synthesized by the ETS-ATP synthase system per mol of $O_2$ used? What if $FADH_2$ is the fuel?

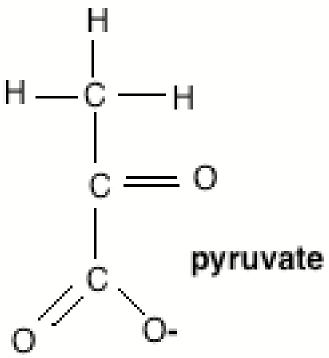
Answers: NADH – 6; $FADH_2$ – 4.

**Feeding the ETS: Sources of Electrons**

Electrons, carried by either NADH or $FADH_2$, can come from the cytosol or the mitochondria. We know the cytosol source. It is glycolysis and we considered the reaction that produced it in the last set of notes. Thus, to the extent that a cell can act aerobically, NADH produced by glycolysis is oxidized by the ETS.

T<u>he exact way the electrons get into the mitochondria is a bit circuitous</u>. Moreover, this path has some <u>consequences on where the electrons enter the ETS</u> and therefore on the <u>amount of ATP produced</u>. These details are for your cell biology or biochemistry courses of the future. All you need to know for our course is that:
To a varying extent NADH produced by glycolysis can be oxidized (**indirectly**) by the ETS.
T**o the extent this happens, there will be an energy bonus** and
To the extent this happens, **no anaerobic products (such as lactate) accumulate**.

Notice that with the ETS operating, pyruvate is not needed as an electron acceptor. Look at the structure of pyruvate. It carries five highly reduced bonds (when compared to oxygen); energy can be harvested from these:



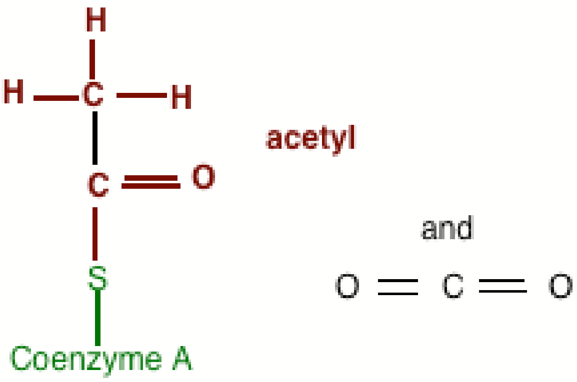
Pyruvate enters mitochondria. In the inner membrane space it is oxidized by NAD$^+$, decarboxylated, and combined with a different type of coenzyme called **Coenzyme-S-H**. This reaction is catalyzed by the enzyme **pyruvate dehydrogenase** (PDH)[2].

$$\text{pyruvate} + \text{CoenzymeA} + \text{NAD}^+ \xrightarrow{\text{PDH}} \text{acetylCoenzymeA} + \text{CO}_2 + \text{NADH} + \text{H}^+$$

Let's see what happened here:

- The COO- group (bottom of the molecule in diagram above) is broken off and replaced by the Coenzyme A molecule.
- This releases $CO_2$. In animals, the mitochondrial reactions are the source of most of the $CO_2$ molecules the animal eliminates in respiration.

So, we are now left with:



Incidentally, the Coenzyme A is a fairly large molecule compared with pyruvate. This coenzyme is not an electron carrier. Nevertheless, it is used in a great number of biosynthetic reactions

- At about the same time (we'll avoid the details, they are definitely for biochem class) the NADH is produced using electrons carrying energy that can be seen as being formerly associated with the reduced C-C bond that was broken and replaced by C-S. Don't sweat this; I just include it because someone always asks!

What is the fate of this NADH? How much ATP comes from this reaction? Will this reaction "go" if there is insufficient $O_2$ in the cell? Explain.

**Into the Matrix!**

No, we are not watching Neo and his compadres – sorry it's just acetylCoA going into the inner mitochondrion. Here, it enters one of the most central biochemical pathways in cells – the so-called **Krebs cycle**. We only need to know the generalities.

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[2] Notice once again the dehydrogenase performs an oxidation-reduction.

The acetyl CoA reacts with a 4-carbon molecule already present in the matrix called oxaloacetate. In the process the CoA is released and the result is a six-carbon compound (2 from the acetylCoA (see above) and 4 from the oxaloacetate) called citric acid. Citric acid has three COOH groups and therefore is a tricarboxylic acid. Thus, the other two names for the Krebs cycle – the Citric acid cycle and the Tricarboxylic acid cycle (TCA).

- **The 6 C compound is gradually degraded back to oxalacetic acid (4C)**. In the process, two molecules of $CO_2$ are released as waste products. Note that the $CO_2$ is nothing more than a carboxyl<u>ate</u> (COO-) group that gets knocked off the molecule. At the end of the cycle, the new molecule of oxaloacet<u>ate</u> can then react with another acetyl Coenezyme A to produce more citric acid and go through the cycle again.
- **This molecule (citric acid) will be slowly disassembled** so that **by the end we will end up with a molecule of oxaloacetate again.** Thus, the idea of a cycle.
- In the process, **two molecules of $CO_2$ will be released** as wastes. These can be seen as the carbons from the acetylCoA. By the time the cycle has completed itsefl, the equivalent of all of the carbon that entered in the form of pyruvate will have been released as $CO_2$ (one C in the PDH reaction and the other two from the Krebs cycle).
- The main way that e**nergy is removed is <u>in the form of high-energy electrons</u>**. Per turn of the cycle (*i.e.*, per 2C that enters) there are 4 pairs of electrons removed:

  **3 to NADH**

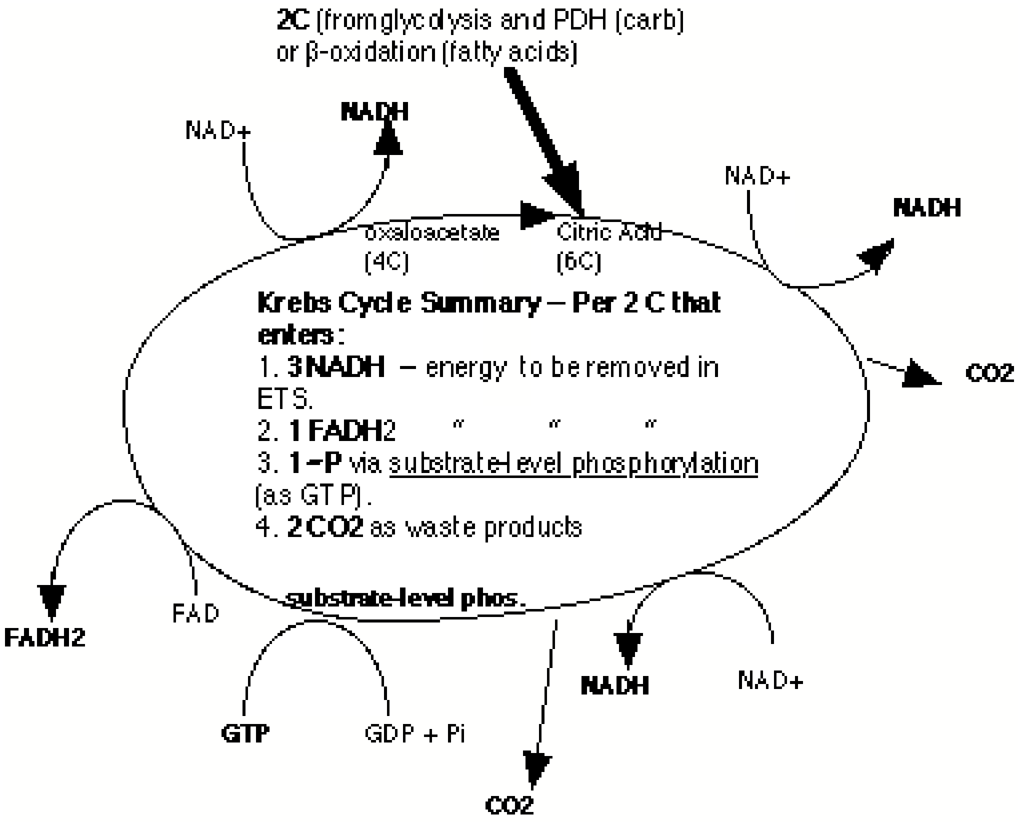
  - There is also **one** "**SUBSTRATE LEVEL PHOSPHORYLATION**" where an enzyme catalyzes the direct formation of a high-energy phosphate, just as in glycolysis. Note that in this case the reaction involves a different nucleotide phosphate:

    GDP + Pi ------------> GTP + $H_2O$

    As we learned earlier, **<u>GTP is the functional equivalent of ATP</u>** -- it is used as an energy source in certain types of reactions analogously to ATP; it also can transfer its terminal phosphate to ADP to ATP:

    GTP + ADP <-----------> GDP + ATP

The next figure summarizes the Krebs cycle:



Please don't' get the idea that there is one Krebs cycle or one ETS in each mitochondrion. There are millions of each. A good measure of the total number of capacity of aerobic reactions (Krebs and ETS) is:

1. The number of mitochondria,
2. Their collective volumes and
3. The degree of folding of the inner membrane

(Note that the folding relates more to the ETS but recall that the Krebs cannot work without the ETS).

Of course, the number and development of mitochondria only reflects **capacity for aerobic metabolism**; the degree of aerobic metabolism will depend on:

1. The amount of $O_2$ present (to allow the ETS to run) and on
2. The demand for ATP.

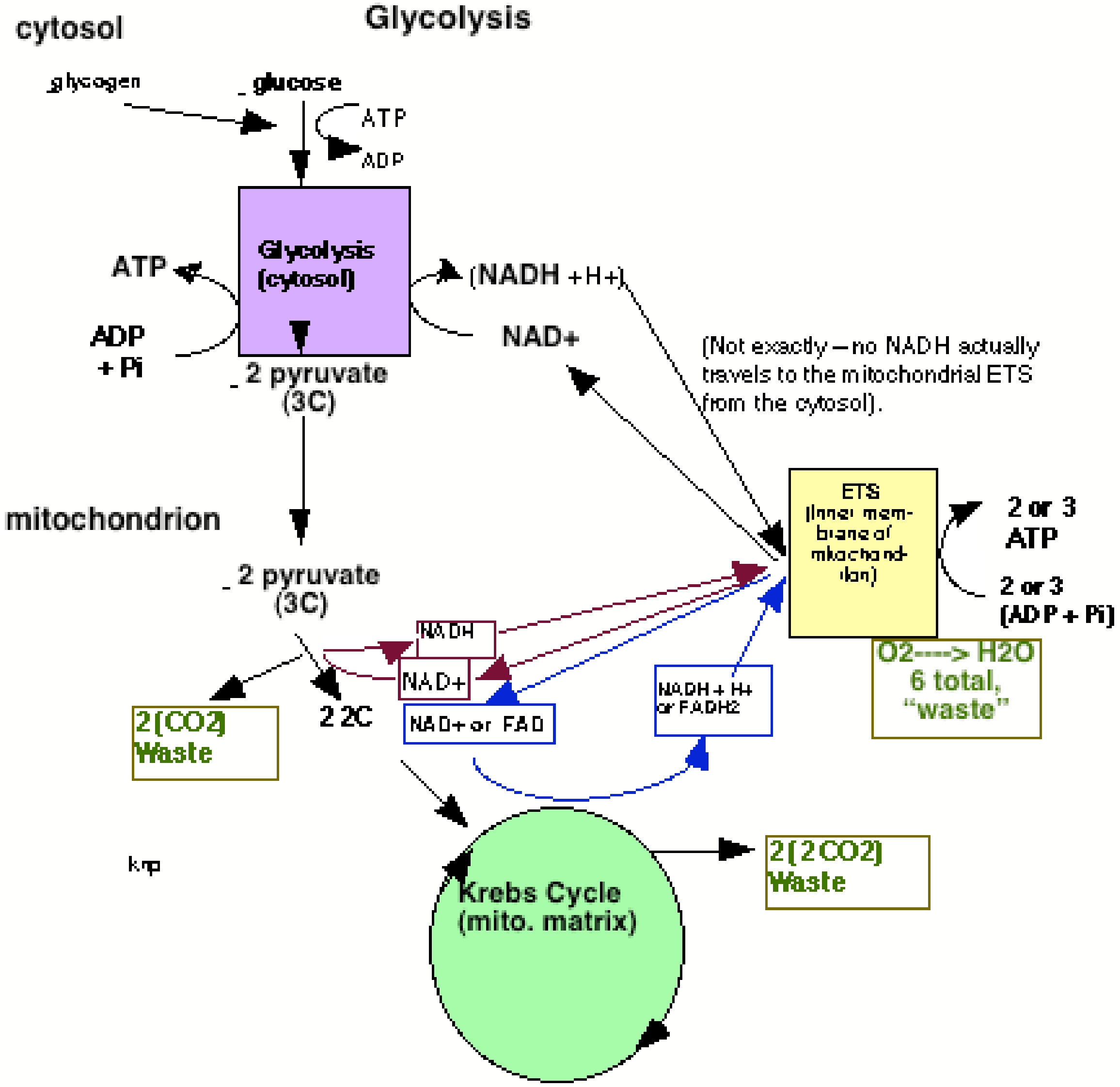
But that is the next class.

**?** What would happen to the rate of the Krebs cycle if:
there is little oxygen present (explain)
there is plenty of oxygen AND plenty of ATP -- *i.e.*, very little ADP and Pi (explain)

**?** Calculate the total number of ~P that are synthesized during one turn of the Krebs cycle – (i.e., starting with acetylCoA reacting with oxaloacetate). Include ~P obtained by both oxidative phosphorylation and by substrate-level phosphorylation.

### A Review of Aerobic Respiration of Carbohydrates

Let's put all of this together in pictorial form. The figure on the next page shows what happens -- notice the linkage of glycolysis to the mitochondrial reactions so that $NAD^+$ is regenerated and all pyruvate is removed and converted to $CO_2$.



### The Question of Efficiency

Recall that in thermodynamic terms, the **degree to which a process conserves energy (if that is its purpose such as in respiration) or the degree to which it produces useful work is its efficiency**. Efficiency is usually expressed as a percentage. Therefore:

Efficiency = (Energy Conserved or Useful Work / Total Energy Released) *100

(We firsts saw this equation in the last set of notes). Let's use it to find the efficiency of aerobic metabolism of carbohydrates. We have seen earlier that if glucose is totally oxidized that 696 Kcal of energy are released:

$C_6H_{12}O_6 + 6\ O_2$ ------------> $6\ CO_2 + 6\ H_2O$ - 686 Kcal (= 2872 kJ)

In oxidative respiration, glucose is totally oxidized as shown in eq. 4. Now, the whole point of respiration was to conserve as much of this energy as possible. Let's see how well organisms do under standard conditions (recall from thermodynamics notes and/or ask me about this in class). Let's assume that starting with one glucose we get a net gain of 36 ~P. Under so-called standard conditions, allowing one mol of ~P bonds to move to equilibrium will result in the release of 7.3 Kcal. Thus, the total energy released is 7.3 Kcal/ mol * 36 mols = 283 Kcal. So, the efficiency is:

Efficiency (standard conditions) = 283 / 686 *100 = **38%** (we'll call this 40%)

If we make the calculation for true cellular conditions where each ~P is worth about 10.5 Kcal[3], we get:

Efficiency (actual conditions) = 385 / 686 Kcal * 100 = **55%**

What happens to the remaining energy? That is, if only 56% is conserved, what happens to the remaining 44% of the 686 Kcal?

**<u>β-Oxidation of Fatty Acids</u>**:

In most animals there are two principal energy storage compounds – carbohydrate and fat. Fat is largely broken down by a process called β-oxidation. This occurs exclusively within the mitochondria. In β-oxidation, fatty acids are oxidized by $NAD^+$ and eventually broken down in a lot of 2C chunks of acetylCoA. You should remember this stuff from the PDH reaction with carbohydrates. What happens to acetylCoA from β-oxidation? The answer is simple and what you would expect – it enters the Krebs cycle and is broken down.

**<u>Breakdown of Amino Acids</u>**:

Amino acids are less commonly used for energy. Usually when a protein is broken down to amino acids by a **<u>protease</u>**, the amino acids are reused (one of our next topics). However, sometimes the amino acids are used as fuel. Here are the bare outlines of this process:

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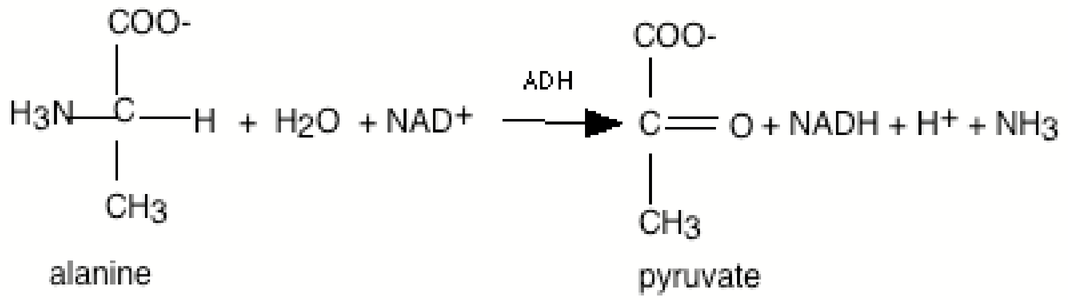
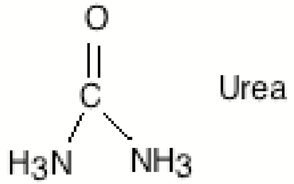
[3] Under cellular conditions, the reactions are further displaced from equilibrium than under standard conditions. The result is more reactions occur and more energy is liberated per mol.

- **The amino acid is deaminated**: this means the amine group is removed and it enters the cytosol as ammonia. Depending on the original amino acid, the remaining carbon skeleton (deaminated amino acid) will have a structure like one of any number of glycolytic or Krebs cycle intermediates (or it can easily be converted to one). Here is an example with the amino acid alanine and pyruvate:



- The pyruvate (or whatever carbon skeleton) is then oxidized by the pathways we have already considered.
- Ammonia can be toxic if it builds up and so either the cell eliminates it quickly or turns it into something that is non-toxic like uric acid or urea:



Study Questions (including Metabolism Overview and Anaerobic Glycolysis)

1. What are the demand and supply reactions? What is the role of the supply reactions with respect to the concentration of ATP?

2. What actions go along with oxidation and reduction in biological systems? Write a generalized equation for the oxidation of some compound X to its oxidized form Y using $NAD^+$ as an oxidizing agent and "X dehydrogenase" as the enzyme. What are dehydrogenases in general? What are the effects of low amounts of $O_2$ on the ability of the ETS to accept electrons? Why?

3. What is the role of $O_2$ in the ETS?

4. Why is the ETS generally able to produce 3 ~P from electrons derived from NADH while two come from electrons from $FADH_2$?

5. Why is it important that $NAD^+$ and FAD concentrations not get too low? What would happen to the Krebs cycle and PDH reactions?

6. What is the purpose of using two ~P bonds early in glycolysis?

7. When in glycolysis does the redox step occur relative to the occurrence of the substrate level phosphorylations?

8. What is the difference in reaction path followed by a catalyzed vs. uncatalyzed reaction (such as the oxidation of glucose)? Is there an overall energy difference in these two processes?

9. Speculate on the advantage of using glycogen as fuel during exercise as compared to glucose. Is there a cost to this advantage?

10. Explain how energy is transferred from the ETS to a proton gradient and then used to synthesize ATP.

11. Distinguish between each of the following:
    a. catabolism and anabolism
    b. cellular and cell respiration
    c. ATP yield from anaerobic & aerobic respiration
    d. substrate-level phosphorylation and chemiosmosis
    e. glycolysis, pyruvate oxidation, Krebs (citric acid) cycle and oxidative phosphorylation
    f. adenosine diphosphate (ADP) and adenosine triphosphate (ATP)
    g. pyruvate and pyruvic acid; citrate and citric acid

12. Identify each of the following:
    a. fermentation
    b. electron transport system (ETS)
    c. acetyl-CoA
    d. $FADH_2$
    e. cristae
    f. proton pump

13. Compare and contrast aerobic and anaerobic metabolism of carbohydrates with regards to efficiency, energy conserved, amount of substrate used to produce a given amount of ATP and waste products.

14. What is deamination?

15. Explain why $\beta$-oxidation absolutely requires mitochondria and $O_2$.