

# ANAEROBIC METABOLISM<sup>1</sup>

## Introduction

*About the Next Three Sets of Class Notes:* Previously, we learned something of the characteristics of biological catalysts. We have seen that enzymes are optimized to perform a single task. However, isolated processes (such as a single type of catalyzed reaction) do not by themselves do much. Moreover, even if they produce an obviously useful result, they require the input of very specific molecules that may not be continuously available. Typically then, enzymes work together in biochemical pathways. In pathways, the products of one enzyme's reaction serve as the substrates for the next. All must work together and so their function coevolves. The catabolic processes of metabolism are excellent examples of biochemical pathways. As we study them, you will be exposed to some of the nitty-gritty detail of the processes. However, the main purpose of this will be to illustrate a series of "big ideas" dealing with metabolism. What are these big ideas?

- The importance of oxidation and reduction in biological systems. We will see that the main way that energy is removed from highly reduced storage compounds, such as carbohydrates and lipids, is by oxidation of C-H bonds.
- Chemical work as a transfer or reorganization of chemical bonds. For us, these bonds will be covalent and we will see that chemical work often involves cleaving off parts of a molecule or transferring groups that are especially easily moved about. The so-called "high-energy" phosphate bond is the prime example of the latter.
- Integrated function and regulation. We will see that as demand for energy changes, the processes adjust to meet demand.

As we study these pathways, look at how individual reactions are integrated and regulated as a functioning whole. Think about how the players (the enzymes) must be adapted to work with each other. And finally, when we consider the outputs and evolutionary ages of the processes think about what the terms "primitive" and "advanced" mean in modern biology.

*Overview of Anaerobic Metabolism:* So-called anaerobic metabolic processes<sup>2</sup> are the most ancient catabolic processes. They can be defined as pathways

- whose purpose is to conserve energy in the form of ~P (WHY?) and

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<sup>2</sup> It is probably not a good idea to use the terms anaerobic and respiration together since respiration historically implies breathing. Breathing, of course, is closely tied to aerobic metabolism. This said, it is not uncommon to hear microbiologists talk about anaerobic respiration and if respiration is thought of as the catabolic pathways that yield ATP for other reactions, then this is certainly a valid way to speak.

- which use a substance besides oxygen as the final acceptor of electrons produced as a by-product of dismantling the fuel molecules (redox creeps in once again!).

**An important note:** Anaerobic metabolism **often** occurs in situations where there is no oxygen. In fact many organisms are **obligate anaerobes** that usually cannot even tolerate O<sub>2</sub> let alone use it for respiration. **However, in many animals anaerobic metabolism may occur even when there are large amounts of O<sub>2</sub> present.** This is especially the case when the organism needs to generate ~P at a higher rate than it can do by aerobic processes alone. This is what happens in our bodies when we exercise heavily.

### Glycolysis

Organisms use many versions of anaerobic metabolism. We will study only two of these anaerobic pathways. These are mostly the same and only differ in their final few steps. Both of these involve a process called **glycolysis** (the "breaking of glucose"). Glycolysis is the most universal of energy-generating pathways. It is found in most species, regardless of the taxon. If we look at the cells that make up these individuals, we see that glycolysis is found in nearly all cell types. This includes cells that have essentially no mitochondria and therefore are incapable of aerobic respiration (see Cell Tour notes).

You have such cells in your own body. They are called type II muscle fibers and they are very important in certain types of activity (those that require large amounts of force).

Glycolysis occurs in the cytosol. The steps of the process are controlled by a few less than 10 types of enzyme molecules that are either floating around free or are bound to cellular structures such as contractile fibrils. Thus, the pathway is not localized in a specialized organelle, as is the case with the aerobic pathways.

Let's look at the process. Again, the details are less important to us than the "big ideas". When details matter, you will be warned!

**1. Starting Points:** Glycolysis starts with either glucose or glycogen (but see below). More about this shortly. For the moment, let's start with glucose. Many of your cells do just that (for example, your brain) while others definitely prefer glycogen (muscle).

**2. Glucose needs to be "activated" before it can be dismantled.** This is done by attaching two phosphates to either end of the glucose. These phosphates come from ATP<sup>3</sup>. So, put another way, it takes two high-energy

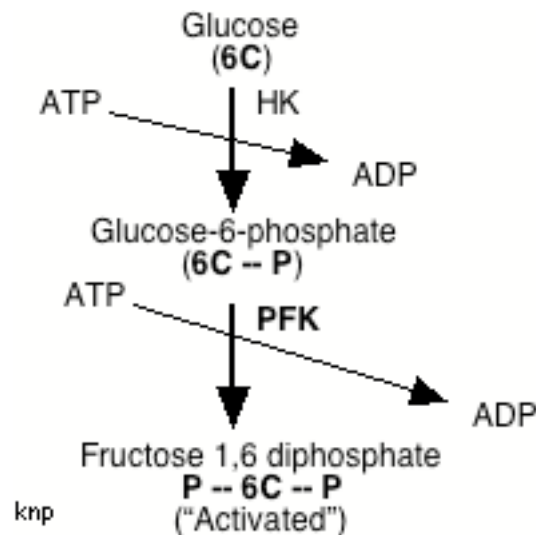
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<sup>3</sup> Here is our first illustration of the fact that "high-energy bonds" are easily transferred to other compounds – in this case the terminal phosphate group from ATP to glucose or glucose derived compounds.

phosphates (two ATPs) to activate the glucose for glycolysis. Note that this **activation happens before any energy is given up by the glucose.**

Don't think of adding these phosphates as providing activation energy in the way you might heat up a mixture, but it does have some features in common with that process.

Here is a **summary of the activation steps:** (don't memorize this unless you want to!)



*What you should remember:*

1. Two ~P bonds have been used in activation and we have yet to get anything back. What makes them high energy is that the ~Ps can easily be moved onto a variety of other compounds.
2. However, the phosphates that have been added to make P -- 6C -- P are no longer "high energy" phosphates. Eventually they will become so again, but that will take some major re-arrangements of the glucose molecule. Energy will need to be transferred back into the bond to make it "high-energy" (easily transferred). So, although like "high energy phosphates" in that they are also covalent bonds, they are not easily transferred to most other compounds. **They are thus "low energy" and not especially useful for doing chemical work.**
3. Notice that **each step is catalyzed by its own enzyme**. The enzyme **PFK** (phosphofructokinase) in the second reaction is especially important and we will discuss it in the next class.

**Remember PFK; however, you need not learn the names of the other compounds!!**

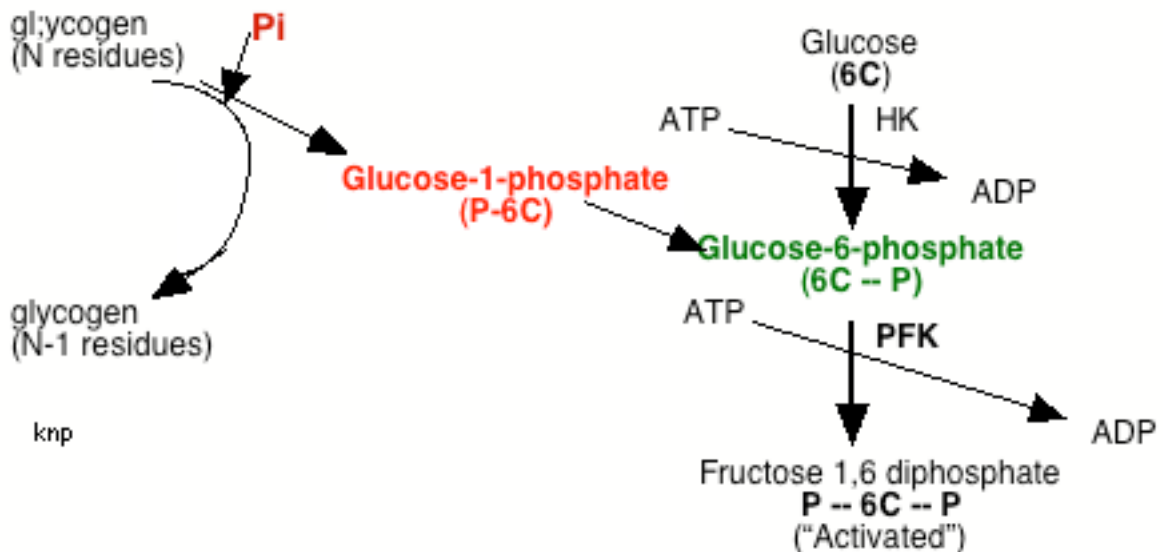
Now is a good time to show how glycogen is used. In the process we can also show a short pathway that feeds into glycolysis but is not considered part of glycolysis.

Pathways, like many things humans and observe and think about, are partially **artificial "constructs"** of the human mind. You will note below that there is no obvious separation between glycogen breakdown and glycolysis (except that they can be regulated separately).

**Recall that glycogen is a 1→4 polymer of glucose with frequent 1→6 branch points.** It is the **main storage form of glucose in the body.** It is particularly important in metabolism in muscles and is the generally preferred fuel for these cells. (It is also found in the liver in large amounts but it is not much used for the liver's metabolism. Instead it acts as a general storage of glucose for release to the rest of the body, in particular the brain.)

So, when muscles need hexose (glucose) for their metabolism they can either use their small store of free glucose, get some from the blood (where it may be coming from liver glycogen), or, more commonly, go to the muscle's own store of glycogen.

When cells start glycolysis with glycogen, they split a hexose off using Pi. This gives a 6C--P compound very much like the one shown above. After another step, it is converted to the same 6C-P compound shown in the first reaction given above:



Notice that in the short term, if the muscle starts with glycogen, it takes one less high-energy phosphate to activate the hexose! (compare this with the last diagram).

Is this “lower activation cost” the free lunch we've all been looking for? Absolutely not. Although it does only cost one ~P to activate fully the hexose taken from glycogen, **this cost was more than paid for when the glycogen was synthesized**. Look at the reactions below that show how glucose is added to glycogen:

Don't learn this

glucose + ATP -----> glucose-6-phosphate + ADP --> glucose -1- phosphate  
glucose -1 - phosphate +UTP --> UDP-glucose + 2 Pi + glycogen(n)

-----> glycogen(n+1) + UDP

You need not learn these reactions but **note that 2 ~P were used to put the glucose on the glycogen in the first place!** There is no free lunch! -- but sometimes, its nice to have a loan that you can pay back (albeit at a high interest rate) later when you have less need for money (or ATP)!

Now let's summarize the rest of glycolysis and then look at it in a bit more detail afterwards:

**3. The fully activated P--6C--P gets broken down into TWO 3C--P fragments**<sup>4</sup>.

This is the "lysis" part of the term glycolysis. Each of these fragments will undergo the same reactions. For our purposes, the important remaining events are:

**4. The next step involves the oxidization of the 3C-P by the coenzyme NAD<sup>+</sup>**.

**NAD<sup>+</sup>** is the **oxidized form** of this coenzyme. The reduced form is called **NADH**.

So, each 3C-P is oxidized by one NAD<sup>+</sup> to give one NADH. This NAD<sup>+</sup>/NADH has a very important role that we will consider shortly.

**5. Next come two substrate-level phosphorylations** (with a series of isomerizations in between). Following the oxidation-reduction, the phosphate on each 3C becomes “high energy”. Thus, its transfer ability has increased and it is transferred easily to ADP to form ATP; this is called **substrate-level phosphorylation**. (these reactions are, of course, catalyzed).

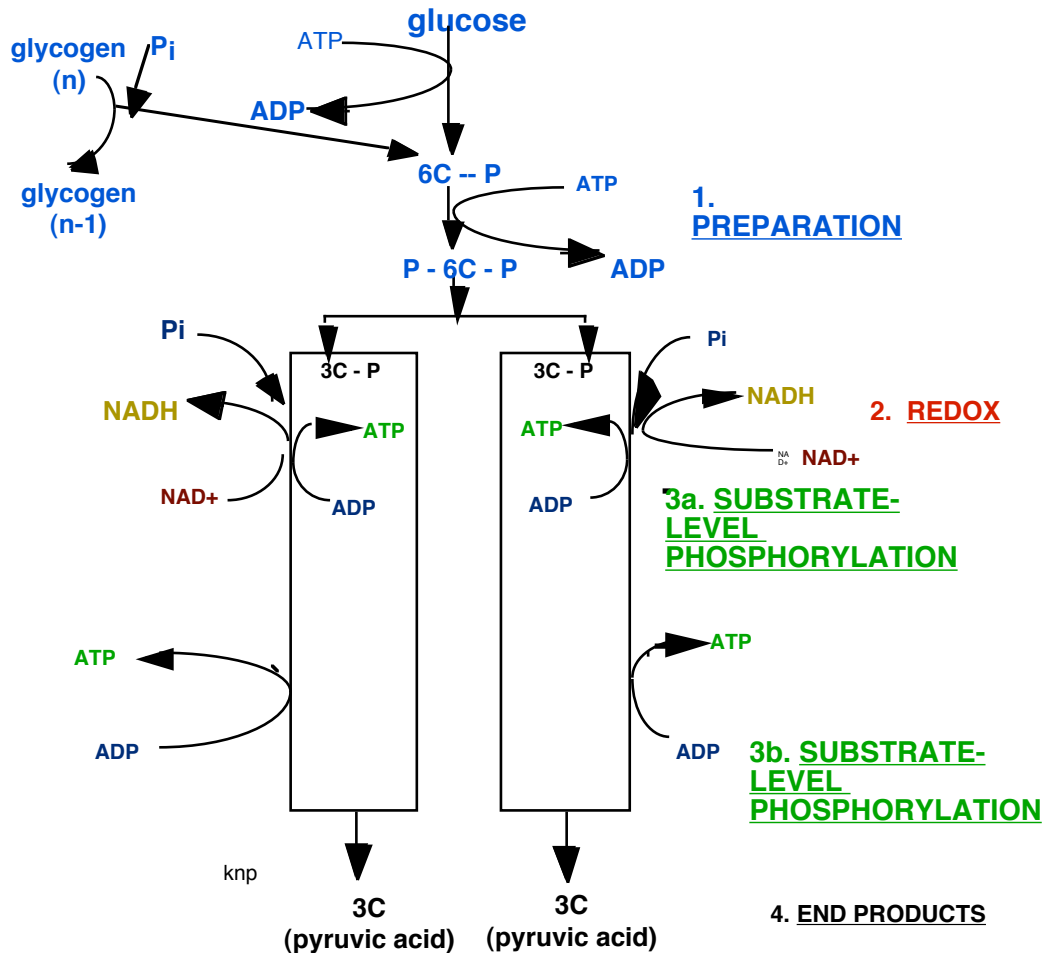
**6. After the second substrate-level phosphorylation we are left with two 3C molecules of pyruvate**.

- What happens next depends on how able the cell is metabolizing the pyruvate aerobically.
- To the extent it can't, metabolism will be anaerobic. We will eventually consider two general ways by which anaerobic metabolism proceeds (so-called lactic acid fermentation and ethanol fermentation).

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<sup>4</sup> Details details! The breakdown of P-6C-P results in two different 3C-P compounds – one molecule of glyceraldehyde 3-phosphate (Ga3P) and one of dihydroxyacetone phosphate (DHAP). However, DHAP is easily isomerized to Ga3P and so we can think of the reaction as going from P-6C-P to two Ga3P molecules, both of which will go through the rest of glycolysis. (NO NEED TO LEARN THIS)

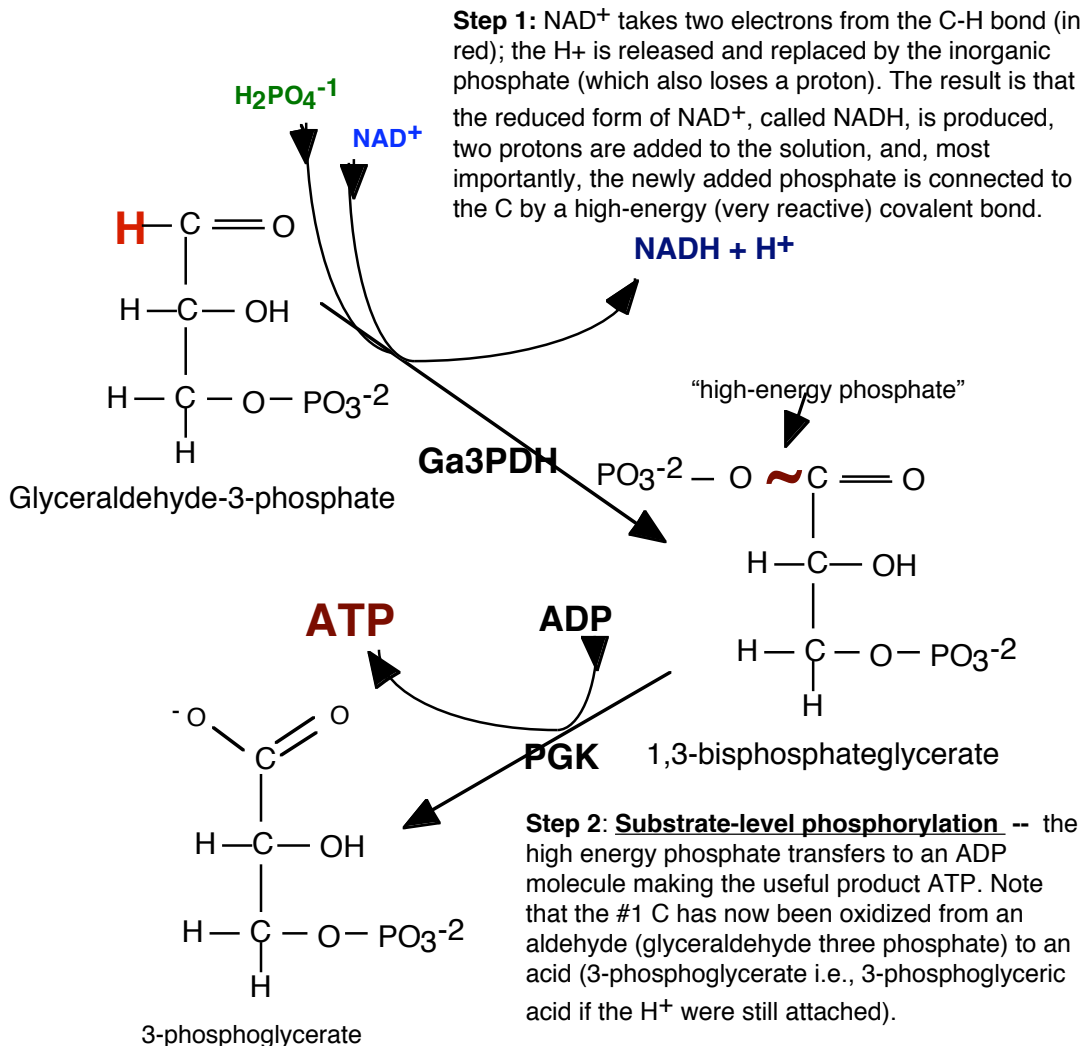
The figure below summarizes the process.



In the meanwhile, let's briefly examine two reactions in some detail. Our purpose is to use these reactions as examples of important energy transfer processes.

- The first involves oxidation of a **H-C** bond by the coenzyme NAD<sup>+</sup>. This compound is very important player in the cells and in catabolism. More about it shortly.
- The second reaction illustrates the **substrate-level phosphorylations** alluded to previously. A high-energy phosphate ( $\sim P$ ) was created as a result of the oxidation-reduction (step 1). Thus, this  $\sim P$  presently resides on the substrate molecule (see the structure in the middle of the page). The next reaction transfers the  $\sim P$  from the substrate to the ADP. Put another way, the substrate molecule phosphorylated the ADP to make ATP – thus the term "substrate-level phosphorylation".

## An Illustration of Oxidation-Reduction and Substrate-Level Phosphorylation



(note: **Ga3PDH** and **PGK** refer to the enzymes that catalyze each reaction).

### Useful Facts and Questions:

1. PGK stands for phosphoglycerokinase. A **kinase** is an enzyme that transfers high-energy phosphate bonds. They may or may not remain high energy after the transfer – in this case they do but in many others they do not. The enzyme PFK mentioned earlier as being important is also a kinase – it moves phosphate from ATP to F6P to give fructose 1,6-diphosphate (FDP). As mentioned previously, these phosphates are NOT high energy.
2. Ga3PDH stands for glyceraldehyde 3-phosphate **dehydrogenase**. Dehydrogenases are enzymes that are involved in oxidation-reduction reactions

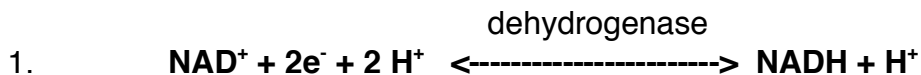
(obviously!). Typically, they require a **coenzyme** such as  $\text{NAD}^+$ ,  $\text{NADP}^+$  or  $\text{FAD}$ . These compounds, which are not proteins, **work with the enzymes by picking up or transferring the high-energy electrons associated with the redox reaction.**

**Questions:**

1. Where did the energy contained in the C-H bond of the #1 C go? You should be able to list three places.
2. What does this tell you about the relative amounts of energy in that bond as compared to say, the energy carried in the terminal phosphate of ATP or in the electrons of NADH?
3. Explain how this illustrates that oxidized states contain less energy.
4. Energy-wise, in the previous example, what is pushing the process whereby some ATP is being created?

**About  $\text{NAD}^+$  and NADH**

Notice that **glycolysis REQUIRES THAT THERE ALWAYS BE  $\text{NAD}^+$  PRESENT**. You should think of  $\text{NAD}^+$  and NADH as comprising a small, very limited pool of these vital substances.  $\text{NAD}^+$  and NADH are interconverted via the following general oxidation/reduction reaction:

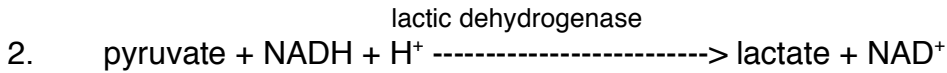


where the two (high energy) electrons and two protons were removed from some substrate molecule (such as 3C-P in the example on the last page).

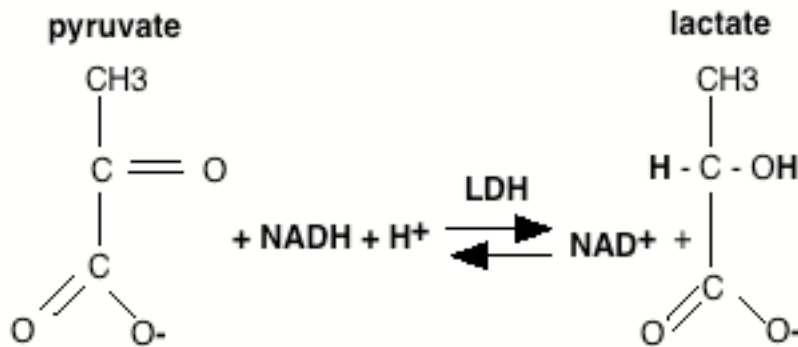
- If a dehydrogenase reaction proceeds to the right while meanwhile no other dehydrogenase reaction proceeds equally to the left, **there will be a decrease in  $\text{NAD}^+$  (or vice versa)**.
- Too little  $\text{NAD}^+$  means that a cell is unable to use glycolysis. No  $\sim\text{P}$  will be produced.
- **Cells do not (and can not) make large amounts of  $\text{NAD}^+$  *de novo* when it is needed** -- synthesis is complicated and cells simply do not make new  $\text{NAD}^+$  from precursors during periods of high metabolic demand.
- So, **in order to have sufficient  $\text{NAD}^+$  the cell must be able to re-oxidize NADH as fast as it is produced**. We will see when we look at aerobic metabolism that NADH is ultimately oxidized by oxygen.

With this understanding, we can finally define **ANAEROBIC METABOLISM**. It is ***a bit misleading to say that it is metabolism in the absence of  $\text{O}_2$***  and so I would like to discourage that. Instead, it is best to define anaerobic metabolism as a process for removing energy from long-term storage compounds (carbohydrates) and putting it into an immediately usable form (ATP) **WHERE SOMETHING BESIDES OXYGEN IS THE FINAL ACCEPTOR OF ANY HIGH**

**ENERGY ELECTRONS.** In the case of glycolysis in most animals, this other "final electron acceptor" is **pyruvic acid (pyruvate under cellular conditions)**. When pyruvate accepts electrons it is converted into lactate:



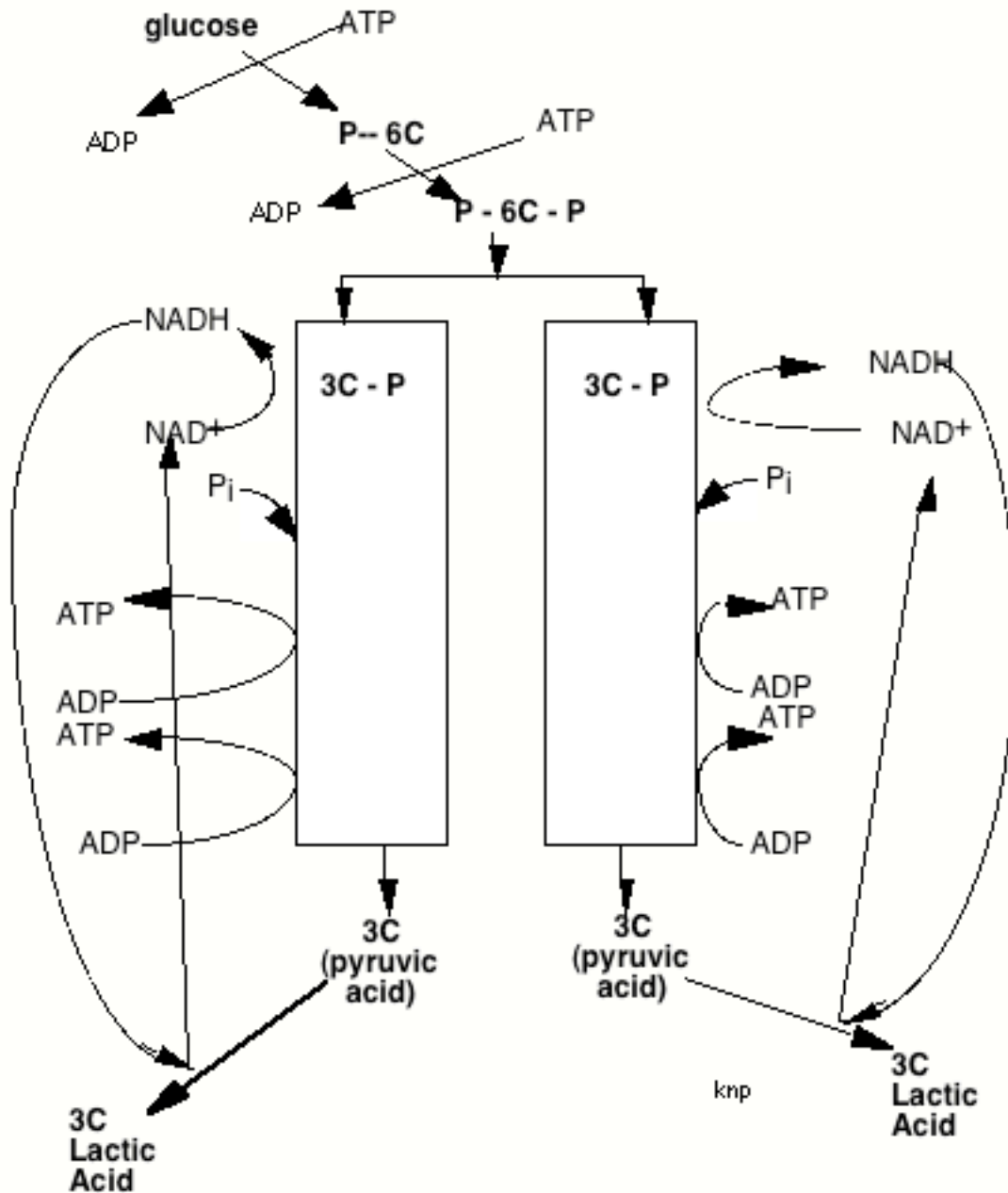
This is one of the few times we'll look at a structure, but this is a nice one because it is easy to see why pyruvate can be reduced and why **lactate is nothing more than "reduced pyruvate"**:



Notice that the central carbonyl in pyruvate is reduced with the addition of 2 electrons and one proton from the NADH and another from the solution.

Notice the "ate" ending again. It refers to the conjugate base of a carboxylic acid. Thus, lactate is the conjugate base of lactic acid. They are functionally the same except that one form is more likely to be found at low pH (lactic acid) and the other at pH values above 5 (lactate).

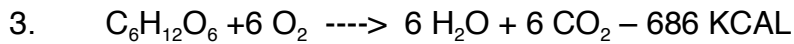
Notice that the result of reaction #2 is that  $\text{NAD}^+$  has been regenerated and it can now be used in earlier glycolytic reactions and thereby allow glycolysis to continue. Notice also that **no ~P is made in this step**. When glycolysis proceeds with pyruvate as the ultimate electron acceptor and with lactic acid as the end (note waste) product, we call it "LACTIC ACID (OR LACTIC ACID) FERMENTATION". There is a summary of lactic acid fermentation on the next page:



**ENERGETICS: Let's consider anaerobic GLYCOLYSIS IN TERMS OF ENERGY CONSERVATION.** Notice that the process only yields 4 new ~P. All come from substrate-level phosphorylations (see above). Moreover, recall that it took two to start the process. So the **net gain was only 2 ~P per glucose that entered the pathway**. If glycogen is the source of the 6 C) compound we get 3 ~P instead of two -- see earlier notes.

**HOW EFFICIENT IS ANAEROBIC METABOLISM?** It really depends on how you look at it. One method is to assume that the energy "available for harvest by glycolysis equals the energy that is released when glucose is fully

burned with  $O_2$ . This can be found using a device called a **bomb calorimeter**. For glucose, the amount of energy released when it is burned completely and the waste products of the burning are given as:



Efficiency is generally given as **useful** work (energy) or power divided by the total energy (or total power) used to produce the useful work. In our example the useful work are the net 2 or 3  $\sim P$  bonds. So, for standard conditions:

$$4. \quad E = 2 * 7.3 \text{ Kcal} / 686 \text{ Kcal} * 100 = 2\%$$

On the other hand, if you look at efficiency in terms of the **amount of energy that is released in going from glucose to lactate**, which is about **47 Kcal**, then the efficiency is quite high. Once again under standard conditions:

$$5. \quad E = 2 * 7.3 \text{ Kcal} / 47 \text{ Kcal} * 100 = 31 \%$$

**Lactate as a Waste:** It is often said that lactate is a waste product and in a sense it is. A cell that is producing it cannot hope to make any further use of it until the conditions that caused it to be produced are removed. On the other hand, it contains more energy than does pyruvate.

Why do you know that lactate contains more potential energy than pyruvate?

Although lactate contains lots of energy, cells still need to be rid of lactate. That is because **its presence in large quantities is associated with a lowering of pH**. The more lactate that is produced the more  $H^+$  are present and the lower the pH. So, cells really do need to get rid of this stuff.

Single cell organisms solve the problem by dumping lactate into the environment (kind of like we handle our wastes!). That's the secret of yogurt. The release of lactic acid by yogurt-making bacteria allows them to keep going metabolically. Meanwhile, the lactic acid curdles milk (milk contains large amounts of proteins, particularly those called caseins; their structure is changed as the pH decreases). The acid also adds a nice sour tangy taste.

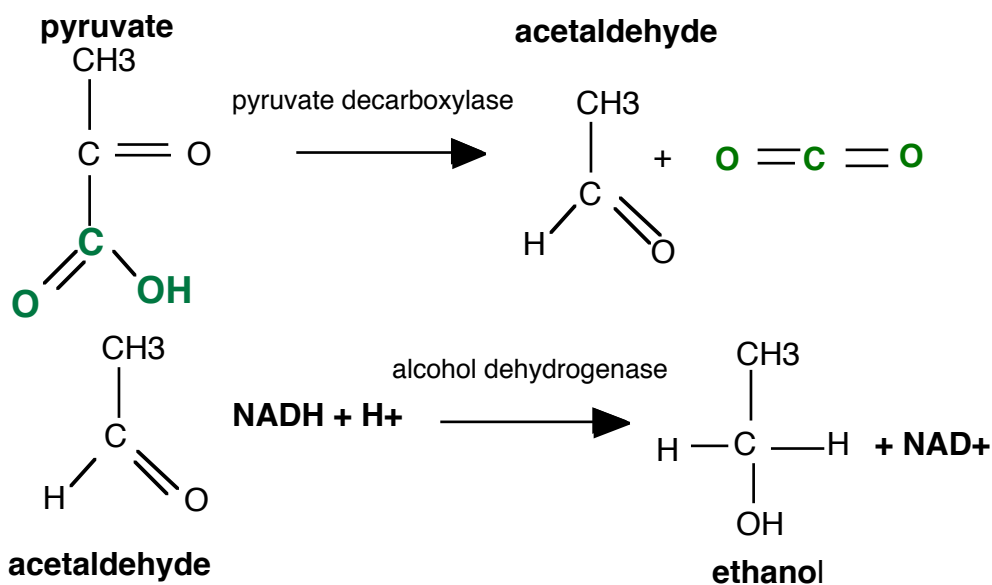
Explain why a change in pH should be especially effective in changing the 3<sup>o</sup> structure of proteins.

In animals, lactate can often be handled in other parts of the organism's body and therefore it is not truly a waste. Once again the cells producing it dump it into the environment but in this case the environment is the blood. Getting rid of the lactic acid helps to keep the cell that is producing its proteins from under

going changes in their 3-D structures. Nearly all of the lactic acid you produce in vigorous exercise is either **used as fuel** in your **heart** (during and after the exercise) or is **turned back into glucose**. Lactate can be burned aerobically in the heart since O<sub>2</sub> is never a problem. All that happens is that reaction #5 is reversed and the resulting NADH and pyruvate go to the heart's mitochondria and are oxidized (we'll learn more about this in the next class). **Alternately, your liver will convert it back to glucose and store it as glycogen**. This process (and others) whereby the liver produces "new" glucose from simpler molecules such as lactic acid (or amino acids or fats) is called gluconeogenesis.

In turning lactate back to glucose -- will exactly 2 ~P be required for the process (the number that were produced during glycolysis), or will greater or even less amounts of energy be required? Explain your answer. (Hint: think about the second law of thermodynamics).

There are **OTHER TYPES OF ANEROBIC PATHWAYS**, Some are extremely complex, some use carbohydrates, others use fats. We will only consider one additional version of anaerobic metabolism because it is important to us and is fairly widespread in microorganisms. This process is the **fermentation of glucose to ethyl alcohol (ethanol) – ETHANOL FERMENTATION**<sup>5</sup>. Nearly all of the process is identical to what we have learned above. The only difference is what happens after you make pyruvate. As with the production of lactate, we will need to regenerate NAD<sup>+</sup> for use in earlier reactions but without using the ETS and oxygen. In yeast and similar organisms, lactic acid is not made. Instead:



<sup>5</sup> Again, remember that the process that yields lactate is often called lactate fermentation.

Both CO<sub>2</sub> and ethanol are easily eliminated from the yeast. In traditional alcoholic beverage making, the CO<sub>2</sub> provides fiz and the alcohol provides the kick!