

Biological Regulation: The Control of Metabolism¹

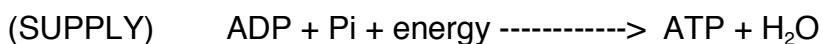
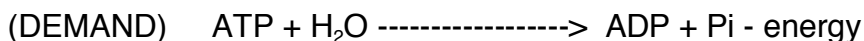
The Regulation of Metabolism

In this class we will tie together much of what we have learned in the past and add one new wrinkle. If you have learned the previous material well, these notes should enable you to glimpse how organisms pull off a feat that is not found in the inorganic world -- they regulate their internal conditions over a vast range of conditions.

You have certainly encountered the term **homeostasis** in your previous studies of biology. Recall that the term itself means constant conditions. The physiologist Walter Cannon coined the term in about 1920 and what he had in mind was the ability of animals to, in some cases, precisely control their internal environments in the face of factors that should cause large changes. In fact, no organism is absolutely homeostatic in regards to any important variable but in some cases organisms are quite exacting in their ability to regulate important factors. Although we will not cover it, in other cases organisms simply do not possess the ability to regulate variables, either because they don't need to, because it is expensive metabolically, or because it might actually be better for them not to regulate. So when we consider different species and different physiological factors, we find a continuum from **conformers** who don't regulate at all through all degrees of precision of **regulation** to something that approaches homeostasis. There are advantages to each level of regulation or lack thereof and it is a mistake to always regard homeostasis as the best possible state.

We will see that regulation is achieved using a variety of mechanisms. Chief among these are (a) buffers (this may or may not have to do with pH -- there are all kinds of buffers); (b) chemical and physical equilibria; and (c) metabolic pathway regulation where the ability of certain proteins to catalyze crucial reactions is increased or decreased. We will especially focus on this one.

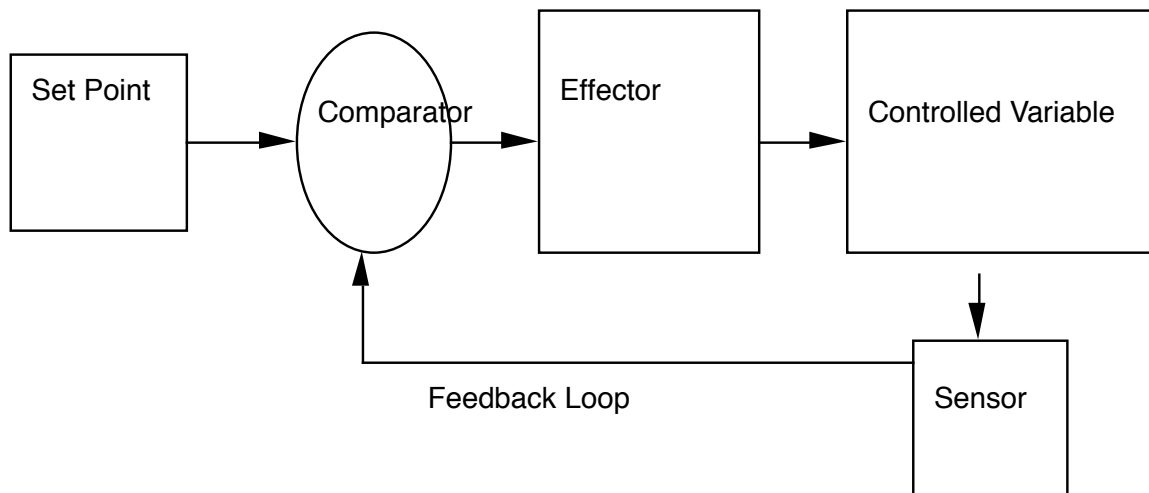
Recall from two sets of notes ago that the purpose of the "Supply" reactions was to keep adequate supplies of ATP present regardless of demand. Recall that we said that there was very little ATP actually present in cells and that in some cells, especially muscles, the demand process could easily use up all of the ATP in less than 2 s. (in humans for instance). Recall that order to achieve a nearly constant level of ATP required that as demand increased, the supply reactions would also need to increase. As long as the rate of these two reactions were equal, the [ATP] would remain constant:



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Finally, recall that there is means that directly turns up the rate of ATP production when demand increases. So, we will need a number of systems to stabilize ATP.

Regulation and Negative Feedback: A negative feedback control process is one that *tends to maintain some regulated variable at a more or less preset constant value*. The preset value or range is set by natural selection. Anytime a disturbance occurs to the value, a negative feedback system takes action to return the value to the set point. Here is a schematic of how an engineer would envision negative feedback:



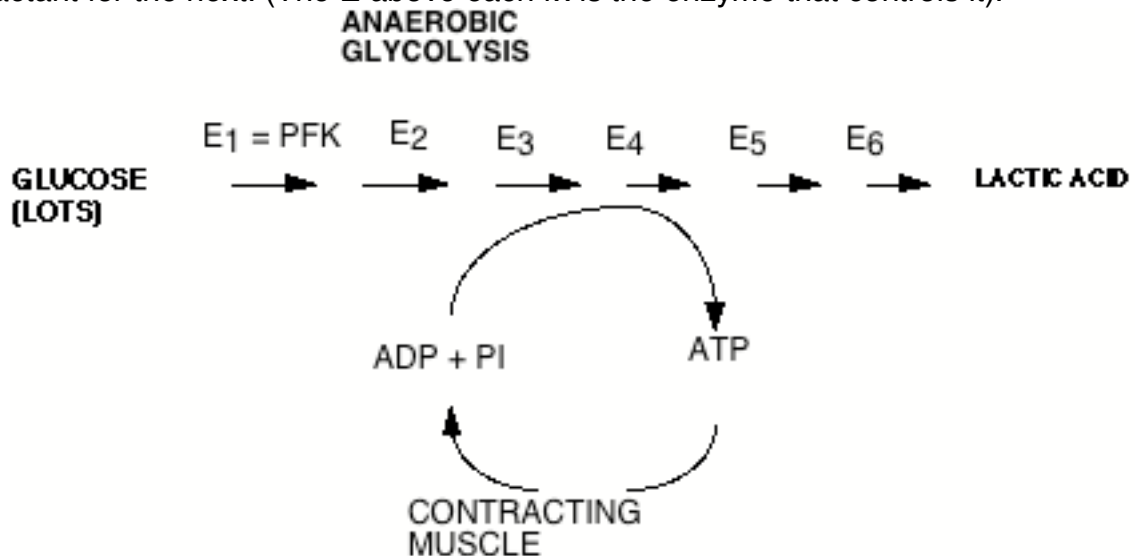
This diagram is quite useful. Let's understand what each of the boxes does:

- **Set Point:** this simply the ideal value for the controlled variable. Its actual value depends on conditions and so it is possible for it change. For example, during exercise we regulate our body temperature at a higher point then when we rest and so the set point changes. But for the purposes of discussion, let's assume that set points are constant. This is pretty much the case for [ATP].
- **Controlled variable:** This is whatever it is that we are regulating. So, in these notes it will be the [ATP] -- but it could be something like body temperature or blood glucose concentration.
- **Sensor:** something that senses the present value of the controlled variable. For [ATP] it is a protein, for core body temperature, there are a series of cells in a part of the brain known as the hypothalamus. Within these cells are proteins whose shape is very sensitive to temperature (one way to think about them is to assume that they are weakly held together by hydrophilic interactions – as the temperature changes, they change shape quite a bit since they are not very rigid).
- **Feedback loop:** a pathway to transmit the output of the sensor to the comparator (see next). In our example with ATP, this will all be inside of a single protein; with temperature, it is a series of short nerves that run within the hypothalamus.

- **Comparator:** something that detects any difference between the set point and the sensed value of the controlled variable. **If there is a difference, an error signal is produced.** This error signal operates the next step in the chain:
- **Effector:** The processes or whatever that change the present value of the controlled variable back towards the set point value. In our example this will be an enzyme that speeds up or slows down all of glycolysis or the Krebs cycle. In the case of core body temperature, the effectors include skeletal muscles for shivering, smooth muscles to regulate blood flow, sweat glands, etc.

A familiar example: the thermostat. Here the regulated variable is the room temperature, the set point is the desired value, the sensor is a thermometer in the thermostat, the comparator is a simple device that compares the set point and present temperature and produces an error signal if necessary. The effector would be the furnace or air conditioner.

Negative Feedback and the Regulation of Metabolism: We'll use anaerobic glycolysis as an example, but everything I will say also applies to aerobic metabolism. Recall that anaerobic glycolysis is an example of a biochemical pathway. A series of enzymes (about 10 types) perform a step-by-step disassembly of the glucose molecule. Remember that each enzyme performs a specific reaction. Think of this pathway as an orderly disassembly line where each enzyme is a worker who performs a specific task and then passes the results on to the next worker. Thus the product of one enzymes reaction is the reactant for the next. (The E above each rx is the enzyme that controls it).



Now, each enzyme is present in sufficient concentration so that its reaction and the speed of the entire process (pathway) can be very high. There is also normally a large amount of glucose present in the cell and we know that our body temperature is reasonably high (even at rest). So, **given that there are lots of**

enzyme molecules and plenty of substrate (glucose) one would expect the reaction rate to always be very high. *But it isn't.*

The body is economical and reaction rates are only high when needed. Anaerobic glycolysis only runs when more ATP is needed – we don't waste glucose and energy by running the process at full speed when not needed anymore than we normally floor the accelerator on car when it is out of gear waiting at a traffic light. Such a procedure is highly wasteful. How do we turn the glycolysis pathway up and down? We use a negative feedback process that senses the level of ATP and its products ADP and Pi.

An enzyme early in the process regulates the entire glycolytic process. It should make good "economic" sense to you to regulate a step early in the pathway and not later on. In glycolysis the enzyme is near the beginning and is called **phosphofructokinase** or **PFK** for short. Most of the other enzymes are not regulated in any significant manner and therefore they don't need regulatory sites in addition to their active sites.

Let's consider PFK in some detail. **PFK is a quaternary protein and has two allosteric regulatory sites and a catalytic site.**

- One of the regulatory sites is for an **activator site**. In this case the **activator is AMP**. Why should AMP be an activator? Well, for reasons we will not go into, the [AMP] increases as the [ADP] goes up. Thus, it is an excellent activation signal. If the muscle starts using ATP and therefore needs more, the ADP will increase. So, if AMP concentration goes up enough (which will only happen when [ATP] goes down), it binds to PFK, induces an allosteric change, and makes the active site into a better fit for PFK's substrates. Let's reiterate -- AMP and ATP can never both be at high or low concentration at the same time.
- The **inhibitory site** is on a different polypeptide chain. The **inhibitor for PFK is a high concentration of ATP**. If too much ATP is made, it is best to slow or stop the glycolysis. High concentrations of ATP cause it to bind to PFK's inhibitor site and this in turn causes the active site to change to a shape that makes it a poorer catalyst.

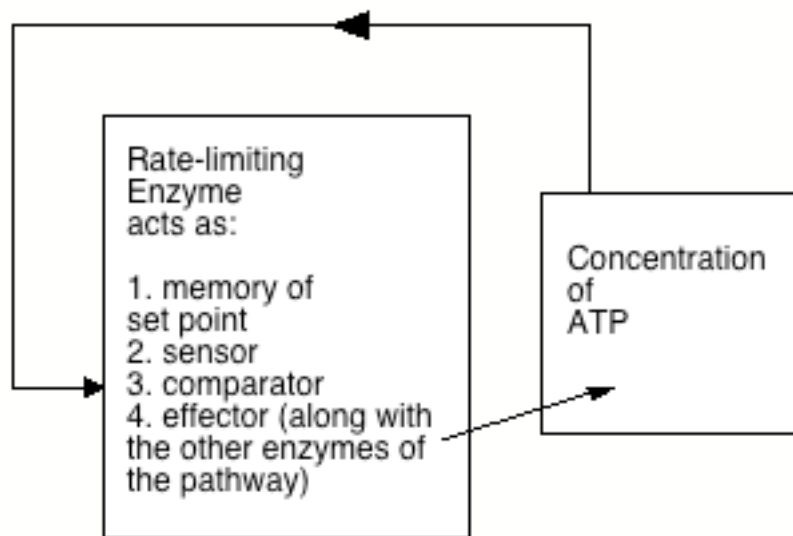
Notice that there are two binding sites for ATP on PFK -- one in the catalytic area where ATP acts as a substrate and the other on the inhibitory site. Do you expect the affinity of PFK for ATP to be the same at each site? Explain.

Make a schematic drawing of PFK showing its quaternary structure and the three binding sites just mentioned.

The entire glycolysis pathway is turned up or down in response to the cells need for ATP. When **lots of ATP is used up and more is needed, [AMP] increases**. The **PFK senses this when AMP binds to its activator site** and at the same time there is no ATP on the inhibitor site. The result is that the PFK molecules undergo an allosteric change that enhances their catalytic ability. As a

result, the overall rate of the PFK reaction increases. On the other hand, if the demand for ATP decreases but glycolysis is still moving along (such as at the end of exercise), the levels of ATP start to increase. As ATP continues to increase, there is less and less AMP. ATP begins to bind to the inhibitor site and meanwhile, there are very few PFK molecules with AMP on the activator site. The PFK undergoes an allosteric change to decrease its catalytic ability and the velocity of the reaction decreases. As a result, the rate of the entire glycolytic pathway decreases and less ATP is made, in line with the lower demand.

Thus, this pathway helps regulate the concentration of ATP and is an example (actually two examples) of negative feedback (one example is the activation, the other the inhibition). Here is a diagram that looks at the regulation scheme for ATP using anaerobic glycolysis but that uses the terminology of feedback control:



A very similar process occurs with the Krebs cycle. Once again, it is the first couple of enzymes that are most crucial for control.

From what you have read above -- does AMP and ATP bind loosely or strongly to the activator and inhibitor site? Explain.

The Roles of Substrate, Fuels, Oxygen, and ADP in Controlling Metabolism

Aerobic metabolism and the balance of aerobic and anaerobic metabolism is partially determined by the availability of O_2 and ADP. Note that *the availability of fuel molecules is usually not a problem unless the organism is starving or has not engaged in vigorous exercise for an extended period of time (ask about marathons, for instance)*.

About ADP: this one is easy. We have already seen that the catalytic ability of some allosteric, rate-limiting enzymes such as PFK is regulated by ADP and (closely related) AMP. Moreover, *substrate-level phosphorylations all require either ADP or GDP*. If there isn't much of either, the reactions that use them will

not proceed. If these reactions do not proceed, then the entire pathway moves along slowly, if at all. The same is also true of the rate of electron acceptance and transfer by the ETS.

Explain why the entire pathway slows if one reaction slows. Make an analogy to an assembly line.

With regard to the ETS:

(a) the synthesis of ATP from ADP by ATP synthetase is not directly part of the ETS. How then could the availability of ADP affect the rate of the ETS?

(b) Will changes in the availability of ADP to oxidative phosphorylation also affect the rate of the Krebs cycle? Explain.

Would you ever expect that [ADP] would be low and yet glycolysis would be activated at the same time?

About O_2 : The amount of O_2 that is in an aerobic cell is always measured relative to the cells need to generate $\sim P$. As long as sufficient O_2 is present so that all $\sim P$ is generated aerobically there is plenty of O_2 . But obviously the actual amount will be quite different when cells are metabolically active as compared to when they are quiescent. This is most obvious in muscle cells which, almost instantaneously can under go extreme changes in demand for $\sim P$. The quantity of O_2 present under resting conditions will not be able to accept all the electrons made by glycolysis and the Krebs cycle during periods of high demand for $\sim P$. So, aerobic metabolism will be limited by the amount of O_2 being delivered to the muscles. As with the demand and supply sides of metabolism; the supply of O_2 follows the demand. Systems above those of the cell (circulatory and respiratory) are required to deliver additional O_2 and make it possible for the ETS to speed up. In the meanwhile, the cell must find alternative ways to obtain the $\sim P$ needed by contraction.

If there is too little O_2 , what happens to the relative amounts of NADH compared to NAD^+ ? Explain how any change occurs. What happens to the reaction rates of the Krebs cycle if there is low O_2 (relative to need).

Notice that in cells with the ability to engage in anaerobic metabolism (*i.e.*, cells with enzymes such as lactic dehydrogenase) that if NADH starts to increase, the anaerobic pathway will begin to accept electrons from the NADH and regenerate NAD^+ . Notice that this could happen even though the cell was also using O_2 to accept electrons from NADH at the same time). Thus, in animal cells it is quite common for anaerobic and aerobic pathways to be operating at the same time and for the relative amount of energy generated from each to vary.

The way this usually works is that some of the high-energy electrons produced by glycolysis are used to reduce some of the pyruvate to lactate. The balance of

these electrons and pyruvate go to mitochondria and are handled oxidatively by the ETS and Krebs cycle.

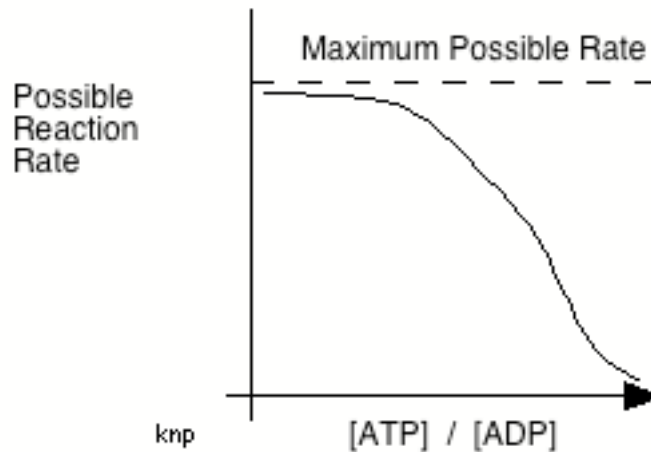
Be aware that in mammals this sort of dual metabolism is most likely to happen when an individual pushes themselves near to or beyond their maximum aerobic ability to generate $\sim P$. They will generate as much $\sim P$ as they can aerobically but will try to get additional $\sim P$ by also running anaerobic metabolism at the same time.

Review : In the last section we learned that one of the most potent ways to control the rate of the reactions in a metabolic pathway was to control how well one or more enzymes functioned. Another way to think about this is to view the process of activating or inhibiting these critical "rate-controlling enzymes" as the equivalent of **increasing or decreasing the amount of fully functional enzyme molecule**. For example, in the case of PFK, an increase in ADP and AMP above their normal resting concentrations (always coupled with a decrease in ATP) leads to more ADP or AMP attaching to the regulatory site on the PFK molecules. Each time particular PFK molecule binds an ADP or AMP, it becomes more activated. The more PFK molecules that are affected, the more activated in general the PFK reaction becomes. The reason for this is simply that more and more PFK molecules have undergone an allosteric change that makes them excellent (instead of mediocre) catalysts.

Notice what is being said here. You already know that there are thousands and thousands of PFK enzymes in each cell. At any moment in time, the overall rate of the reaction these molecules catalyze ($6C-P + ATP \rightarrow P-6C-P$) is dependent on the proportion of them that are in the allosteric form that makes them excellent catalysts. This in turn depends on the relative concentrations of ADP and ATP as compared to their "set point" values. So, if ATP is slightly lower than normal and therefore ADP is slightly higher, then more PFK molecules have:

- no ATP at their inhibitory regulatory site
- ADP at their activation regulatory site

This puts these PFK molecules into a form that makes them better catalysts. The more ADP increases, the more PFK molecules undergo this change. By contrast, notice that the opposite happens when ATP increases. But most importantly notice that there is a smooth response by the population of PFK molecules to changes in $[ATP]/[ADP]$. As the ratio increases (in favor of ATP) fewer and fewer PFK molecules are in a good catalytic form; as the ratio decreases, more and more function well and the overall reaction rate has the potential to be higher:



Notice also that there is a maximum possible reaction rate. ***This would be achieved only when all PFK molecules are fully activated very (low [ATP] & very high [ADP]) and when there is plenty of substrate and little product.*** This maximum rate is a measure of the number of enzyme molecules present in the cell and is called the **ENZYME ACTIVITY**.

Questions:

1. Draw a qualitative graph that shows the relationship between the enzyme activity (maximum possible rate under ideal conditions) and the number of enzyme molecules present in a cell.
2. Would the maximum rate be realized if an enzyme was fully activated but there were low concentrations of substrate and/or high concentrations of product? Why?

Immediate Sources of Stability: ~P Buffering

When demand for ~P suddenly increases, it takes a moment for glycolytic and mitochondrial metabolism to be turned up. ADP/AMP levels must increase and activate rate-limiting enzymes such as PFK. Meanwhile, pyruvate or fatty acids must become available in greater amounts for the mitochondria. And finally, more oxygen must be delivered. The problem is that this all takes a bit of time and the body needs the ATP NOW! ATP-requiring processes simply do not work without the ATP. So what happens that allows the body to function during energy demand transitions? The answer is that there are ~P buffering systems in all organisms.

You probably know a bit about **pH buffers**. These are mixtures of acid and base. If additional acid or base is added, the pH does not change as much as if the same amount of acid or base were added to water. The buffer reacts in part with what was added and tends to minimize the disturbance.

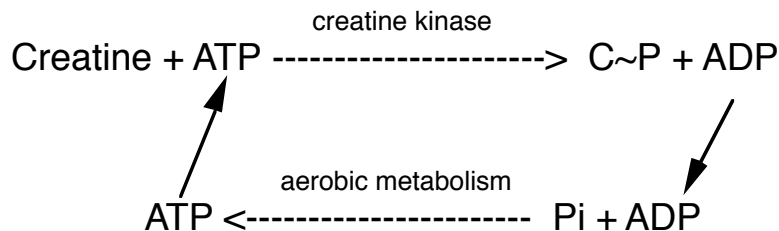
Notice that contrary to what many think, buffers do not prevent pH changes, they simply make them smaller than they would have been in the absence of a buffer.

There are other buffers besides pH buffers. The example we are concerned with is such a buffer. It helps keep the [ATP] from dropping too far. Like any buffer it has a certain capacity to perform this function but if the demand for ATP is too great over too long of a time period, the buffer can be overwhelmed.

Substances that "buffer" the [ATP] are called **phosphagens**. The phosphagen found in most vertebrates is called **creatine phosphate (CP or C~P)**.

CP is often drawn with the ~P to emphasize that the phosphate in this compound is "high energy" -- meaning that it is easily transferred to other compounds such as ADP. It is the equivalent in terms of energy of the two terminal phosphates in nucleotide triphosphates. Note that as with ATP, the "high energy" covalent bond is the result of the fact that both the phosphate and creatine groups have a large number of negative charges. It takes a great deal of energy to form this bond.

In cells such as muscles that can use a lot of ATP in a very short time there are typically much more C~P than ATP. The two are in equilibrium with each other. Creatine phosphate is formed in resting muscles when ATP transfers its outermost high-energy phosphate to the compound creatine (see the appendix to these notes that deals with creatine supplements to see creatine's structure). The enzyme required to do this is **creatine kinase**:



Notice that this reaction only occurs when the muscle is resting -- that is, when its demand for ATP is low. In such as resting cell, as soon as this reaction is complete, the ADP will be phosphorylated by the ETS glycolysis or Krebs cycles. If there is still extra creatine present, it will tend to react with ATP until most creatine is converted to CP. Eventually, a typical resting muscle cell in a human will end up approximately the following ratios of compounds:

1 ADP: 10 ATP: 50 C~P

Don't learn the values above; they are just to give you an idea what cellular conditions are like.

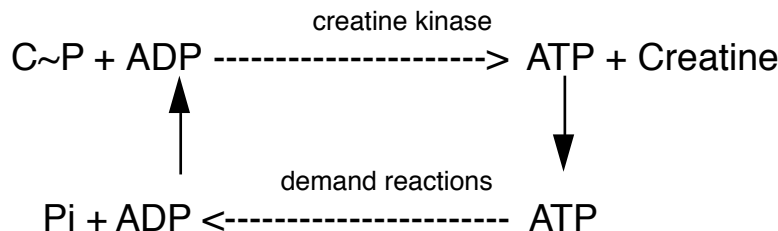
About creatine "supplements": Creatine supplements are of some use for people trying to develop strength since the fastest way to get stronger is to lift the largest amount of weight you can safely handle. If sufficient C~P is present, the athlete

is less likely to experience fatigue due to accumulation of lactic acid. You should be able to easily grasp the idea for taking creatine supplements. If one increases the [creatine] in their cells, they should be able to store even greater amounts of ~P as C~P. Within limits this is true. Creatine supplements can result in significant and useful increases in C~P. However, don't get the idea that the more you take, the more of an increase in C~P you get. As levels get higher, the excess creatine is simply broken down. You might be able at best to double the [C~P].

Adverse effects of taking creatine supplements are not known for certain but it is believed that one risk of long-term heavy use is kidney damage. Short term, it can cause dehydration. But honestly, not much is known. If you feel you must use this substance, the best advice is to use the lowest recommended amount and to not use them for more than a few weeks at a time.

The process will continue until nearly all of the creatine in the cell is converted to C~P, at which point it stops. The C~P just sits around and waits for periods of heavy use. Unlike the cell's ATP pool which is always being used and resynthesizes, C~P is only used during periods of exceptionally high demand for ATP. Thus, many cells which never experience such high demands do not have high levels of C~P. Think of C~P as money in the bank. The reaction just shown (last page) is the process of filling the bank account with money.

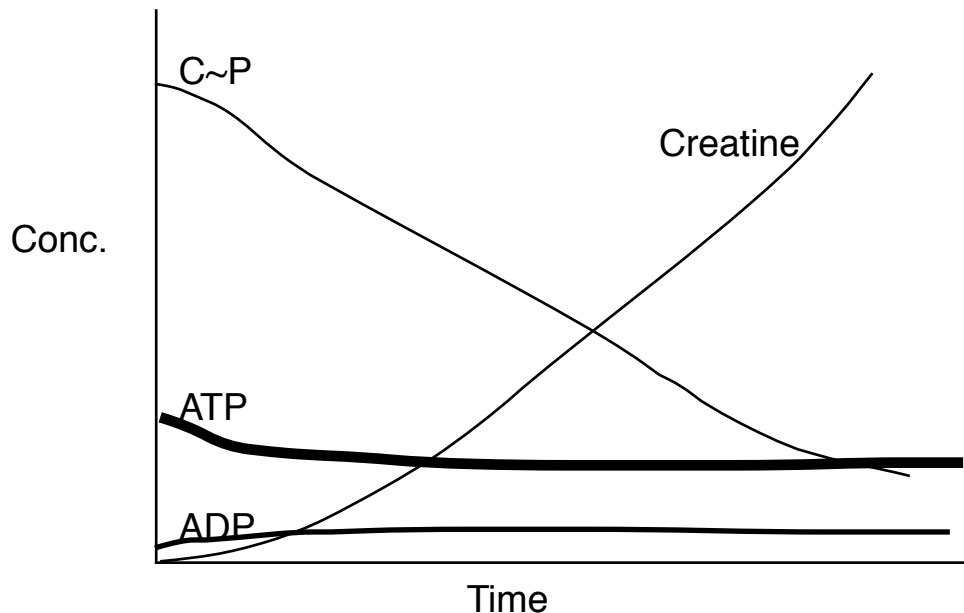
What happens when the demand for ATP is far greater than the rate at which it can be synthesized? This happens, for instance, when vigorous exercise starts. We will learn below that glycolysis, the Krebs cycle, and the ETS are all "turned down". Moreover, with respect to aerobic metabolism, there are probably not tremendously large amounts of O₂ present and so high rates of aerobic metabolism are not possible. But **the [ATP] must be kept above an acceptable minimum**. This is done by reversing the process shown on the last page (see top of next page)"



Notice that the C~P **buffers** the stores of ATP -- it prevents them from changing as rapidly as they would if the C~P stores had not been there. The result is that [ATP] does not change nearly as much as it would have during the time that glycolysis and aerobic metabolism are being activated. Notice also that the same enzyme (creatine kinase, CK) is used in both reactions (synthesis and breakdown of C~P). **Muscles tend to have very large amounts of CK**. This is important during exercise because it ensures that C~P will rapidly transfer ~P to

ADP -- no waiting time to find an enzyme molecule as would be the case if there was less CK.

Here is a graph that summarizes what happens to ATP, ADP, C~P and C during the first couple of seconds of an activity transition (this one is for going from rest to exercise):



Although the [ATP] does drop and the [ADP] does increase a comparable amount, the changes are very small. What really changes is the [C~P] and [C]. The decrease in C~P is what prevents large changes in [ATP] and [ADP].

Important Note: Some change in [ATP] and [ADP] is actually good. We have seen earlier that a small change is required to signal to the cell that something has happened and therefore that the reaction rate of glycolysis or the Krebs cycle needs to be increased or decreased.

Review Questions:

1. Be able to explain how a negative feedback system operates. Explain how the turning up of glycolysis by high [AMP] and down by high [ATP] can both be examples of negative feedback. What is the regulated variable in this system?
2. Explain what phosphagens are and what they do. Why is there very little phosphagen in glandular or neural tissue but great amounts in muscles.
3. Imagine a situation where a cell is producing ~P at a high rate, but it is doing it all aerobically. Assume that O₂ is being delivered at the greatest rate possible to the cells. Now suppose that the demand for ~P increases (in exercise this would

be the result of doing something like increasing speed). Explain what happens to:

- (a) ADP levels
- (b) the rate of glycolysis
- (c) the rate of the Krebs cycle
- (d) the rate of \sim P synthesis in the ETS
- (e) the total rate of \sim P synthesis in the cell
- (f) the amount of lactic acid present

4. Terms: phosphagen, comparator, negative feedback, error signal, controlled (regulated) variable, regulation, homeostasis, phosphagen, creatine phosphate, allosteric regulator (and site), PFK (phosphofructokinase).

Review With Earlier Material (and think!):

5. If a cell has few mitochondria but must generate large amounts of \sim P, how will it do it?

6. When mammals (any species) are forced into exercise beyond what they are used to performing, the amount of lactic acid they produce in doing this exercise gradually becomes less over time. What has happened? What changes would you expect to see on the microscopic level.