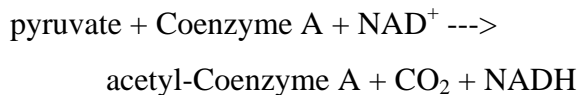


CHAPTER 14 - TRICARBOXYLIC ACID CYCLE AND PENTOSE PHOSPHATE PATHWAY

We have now gotten to the point in glucose metabolism where one glucose molecule has been cleaved into two molecules of pyruvate, with some released energy which has been stored in the form of NADH and ATP. We would now like to discuss the completion of glucose oxidation to the final end products carbon dioxide and water.

Under normal conditions when the cell is in need of a little energy but has enough oxygen, the pyruvate will be further metabolized to carbon dioxide. The first step in this conversion is transport of the pyruvate into the mitochondria. Remember that all of the steps in glycolysis are carried out in the cytoplasm. After getting into the mitochondria, the pyruvate is converted into acetyl-CoA by the action of several enzymes called the pyruvate dehydrogenase complex. The pyruvate dehydrogenase complex consists of three different enzyme systems which carry out three separate reactions. The net reaction is:



This reaction can be broken down into three separate components. The first reaction is the decarboxylation of pyruvate to hydroxyethyl-thiamine pyrophosphate complex + carbon dioxide. This reaction has a similar mechanism to that of pyruvate decarboxylase that we looked at the information of ethanol in yeast.

This reaction is carried out by the pyruvate dehydrogenase component of the pyruvate dehydrogenase complex. The product of the reaction is hydroxyethyl-thiamine pyrophosphate. Hydroxyethyl-TPP is oxidized to a acetyl group and concurrently transferred to a different cofactor, lipoic acid. This reaction is carried out by the enzyme dihydrolipoyl transacetylase, and the reaction involves both the transfer of the two carbon unit and an oxidation/reduction reaction: the oxidation of the hydroxyethyl group to an acetate group and the reduction of the lipoic acid cofactor.

The lipoic acid subunit contains a reactive disulfide unit. This reacts with the hydroxyethyl-TPP to allow transfer of the acetyl group and formation of a thioester with lipoic acid. This thioester bond, as you remember, is an energy rich bond, and the energy in this bond will need to be conserved. In carrying out this transfer, the sulfur on the lipoic acid is reduced

from the disulfide form to the sulfhydryl form; at the same time, the carbon atom is effectively oxidized from the aldehyde oxidation state to the carboxylic acid oxidation state.

The next step in the reaction involves the transfer of the acetyl group from lipoic acid to coenzyme A, via the action of the enzyme dihydrolipoyl transacetylase. The result is the final desired compound plus one molecule of reduced dihydrolipoamide. The lipoic acid is regenerated by dihydrolipoyl dehydrogenase. The electrons are transferred to NAD⁺ to form one mole of NADH.

Now, all of these enzymes are found in the pyruvate dehydrogenase complex. This complex, as isolated from *E. coli* cells has a molecular weight of about 4600 kd. It consists of about 60 polypeptide chains. In mammals, the molecular weight of the complex is about 9000 kd, and contains about 60 copies of the transacetylase and 20-30 copies of the other two enzymes. The trick in the mechanism is that two lipoic acid groups are involved in the transfer of one pyruvate to acetyl-CoA. The difficult part of the mechanism is that the acetyl-lipoic acid must interact with the active sites of two different enzymes. By physical measurements, these are estimated to be somewhere between 4.5-6.0 nm apart. The lipoic acid coenzyme subunit is covalently bound to a terminal amine group on lysine, but taking all of the distances into account a single lipoic acid complex can only move about 2.8 nm in space.

By introducing a second lipoic acid, now enough freedom of movement is allowed for the lipoyl intermediate to react with two different enzymes immobilized in the complex and to allow the generation of the final product.

NOW - we're in the mitochondria as acetyl-CoA. What happens next? Let me now give you an overview of the reactions in the tricarboxylic acid cycle, also called the citric acid cycle, also called the Krebs cycle, to allow you to see where we are going. I'll then return to describe the enzymes which perform the steps in the reaction.

I've given you a photocopy tonight which describes the reactions of the citric acid cycle, which you may use for reference.

The first reaction in the citric acid pathway that I would like to discuss is the isomerization of citrate to isocitrate. The enzyme which catalyzes this reaction is aconitase. The enzyme is called aconitase because the intermediate in the isomerization reaction is cis-aconitate.

As you can see, citrate undergoes a dehydration reaction to form cis-aconitate, followed

by a hydration reaction to form isocitrate. The standard free energy change for this reaction is +6.3 kJ/mol; At equilibrium, the ratio of citrate:aconitate:isocitrate is 90:6:4.

Fluoroacetate is a poison found in the leaves of some plants in Australia, Africa, and South America. Animals who graze mistakenly on these plants die of severe metabolic poisoning. Fluoroacetate will be converted into fluoroacetyl-CoA by the enzyme acetate thiokinase (an enzyme usually used to metabolically process acetate floating around in the cell), which is then utilized by citrate synthase to form citrate. The fluorocitrate is not a substrate for the aconitase, which blocks up metabolism and is ultimately harmful to the cell. By the way, difluoroacetate is also a poison, but not so potent as fluoroacetate, while trifluoroacetate is relatively benign. Why is the toxicity of the three compounds different?

The next step in glycolysis is the oxidative decarboxylation of isocitrate to α -keto-glutarate. The enzyme catalyzing this step is isocitrate dehydrogenase. The reaction is a two step process, first involving oxidation of the secondary alcohol to form a ketone (with the energy of the oxidation in electrons passed from isocitrate to NAD^+ to form reduced NADH), forming the intermediate oxalosuccinate. Oxalosuccinate is a β -ketoacid, which is susceptible to decarboxylation; the carbon dioxide is lost to form the final product α -ketoglutarate. The standard free energy change associated with this reaction is about -20.9 kJ/mol.

The next step in the citric acid cycle is a second oxidative decarboxylation step to convert α -ketoglutarate to succinyl-CoA. The series of enzymes which perform this reaction is the α -ketoglutarate dehydrogenase complex. This enzyme complex is similar to the pyruvate dehydrogenase complex, and acts upon α -ketoglutarate in the same way that pyruvate is acted upon by pyruvate dehydrogenase. The first intermediate is the hydroxyethyl-derivatized thiamine, formed after loss of carbon dioxide from α -ketoglutarate, followed by transfer of this group to lipoic acid, followed by transfer of the succinate group to Coenzyme A, followed by the oxidation of reduced lipoic acid to oxidized lipoic acid with concurrent reduction of NAD^+ .

The next step in the citric acid cycle is the release of succinate from succinyl-CoA with corresponding phosphorylation of a nucleotide diphosphate. This time, GDP is phosphorylated to form GTP. The enzyme catalyzing this reaction is succinate thiokinase. The reaction mechanism is not entirely worked out for this enzyme, but likely involves the initial displacement of succinate from succinyl-CoA by inorganic phosphate, forming free coenzyme-A

and succinyl-phosphate. The phosphate group is apparently removed from succinate by a specific histidyl nitrogen, with release of succinate. The enzyme bound phosphate then adds to GDP to form GTP. The standard free energy change associated with this reaction is -2.9 kJ/mol .

At this point, we have oxidized the two carbons from acetate to carbon dioxide. To complete the cycle, we need to oxidize the succinate back to oxaloacetate to allow it to condense with another acetyl-CoA and perform another round of the cycle. The first step to regenerate oxaloacetate is to oxidize succinate to fumerate. The reaction is catalyzed by the enzyme succinate dehydrogenase. The oxidation of succinate is coupled to the reduction of the electron carrier flavin adenine dinucleotide, whose structure is:

Succinate dehydrogenase is a flavoprotein; that is, FAD is a tightly bound cofactor. As we will discuss when we talk about oxidative phosphorylation, NADH is a stronger reducing agent than FADH_2 . In simple terms, this means that more energy is stored in NADH than in FADH_2 . The use of the FAD cofactor in this reaction is required because there is not enough energy released in the oxidation of succinate to allow for the reduction of NAD^+ . We'll discuss these redox reactions and relationships soon. This reaction has a standard free energy change of approximately 0.

Going from succinate to fumerate added a double bond to our four-carbon molecule. The next step is the hydration of the double bond to form L-malate. The reaction is carried out by the enzyme fumerase:

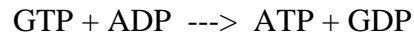
After we get through the reactions, we'll go back and discuss the fate of the carbons in acetyl-CoA and the stereospecificity of some of the reactions here. Fumerase catalyzes a trans addition of water to the double bond to form L-malate. If the addition of water was nonspecific, the result would be equal concentrations of the L and D isomers. The standard free energy change for this reaction is -3.8 kJ/mol .

The final step in the citric acid cycle is the oxidation of malate to form oxaloacetate. The enzyme which carries out this oxidation, with concurrent reduction of NAD^+ , is malate dehydrogenase. The reaction is:

The standard free energy change for this reaction is about $+7.1 \text{ kcal/mol}$, meaning that the reaction is thermodynamically unfavorable. Under equilibrium conditions in the

mitochondria, the concentration of oxaloacetate is about 100,000 times less than the concentration of malate. This keeps the concentration of oxaloacetate low, which we mentioned was the need for the very favorable free energy change associated with the citrate synthase reaction.

Let us now look at the final tally for the conversion of pyruvate through the citric acid cycle. Pyruvate has been converted to 3 molecules of carbon dioxide. We made one molecule of NADH/pyruvate in making acetyl-CoA, another at the oxidation of isocitrate, a third at the oxidation of alpha-ketoglutarate, a fourth at the oxidation of malate. One molecule of FADH₂ was formed at the oxidation of succinate, while one molecule of GTP was formed by the enzyme succinate thiokinase. (Just as point of fact, GTP can form ATP by the action of the enzyme nucleoside diphosphokinase, by the reaction:



Extra hydrogens and oxygens are provided by water - the final stoichiometry balance includes the use of 2 molecules of water and the release of two protons.

So, final tally from pyruvate is 4 NADH, 1 FADH₂, 1 GTP. To get the total energy tally from glucose, double this (since two pyruvate are formed from glucose) and add to the energy gain in going from glucose to pyruvate (which was 2 ATP, 2 NADH). So the final energy stored is: 3 NTP, 6 NADH, 1 FADH₂. When we study ox phos, we'll convert these reduced cofactors to ATP.

STEREOCHEMICAL ASPECTS OF THE CITRIC ACID CYCLE

What are the fates of the carbons in pyruvate as they enter the citric acid cycle and carbon dioxide is released? Let's start with pyruvate, and just for the sake of argument, label the carbons in pyruvate in red. One CO_2 is immediately released by the action of pyruvate dehydrogenase; Acetyl-CoA is formed. This acetyl-CoA enters the citric acid cycle by condensation with oxaloacetate (which I will draw in white), forming this 6-carbon compound.

Let us look at a model of citrate. Let's look at this central carbon. Is it chiral? No - it is not chiral because two of the substituents, these two CH_2COO^- arms, are chemically the same. However, the two arms are philosophically different – what do I mean by this? Suppose that we take one of the CH_2 groups of citrate and replace it with a bromine. If we now examine the stereochemistry of citrate, we see that it is now a chiral molecule and the configuration at the center carbon is now ? rather than #. When citrate is synthesized from acetyl-CoA and oxaloacetate, the acetate group derived from acetyl-CoA corresponds to the *pro-S* arm of citrate, while the *pro-R* arm is derived from oxaloacetic acid.

The next enzyme in the citric acid cycle, which is aconitase, can tell the difference between the two arms. Aconitase carries out an isomerization reaction by sequential dehydration and hydration reactions. The dehydration reaction produces a double bond between the central carbon and one of the methylene groups, specifically the *pro-R* hydrogen, the one derived initially from the oxaloacetate. Dehydration and hydration results in isocitrate, where the acetate group derived from acetyl-CoA is left unscathed.

Now, the reactions proceed by two oxidative carboxylations to form α -ketoglutarate, then succinate. In the first reaction, the released carbon dioxide is derived from oxaloacetate - it is from the branched carbonyl which is not part of either acetate substituent.

In the second reaction, the carbon dioxide released is adjacent to the ketone group. Another way to describe this is to say that it is derived from the oxaloacetate initially. Therefore, in going from citrate to succinyl-CoA, two molecules of carbon dioxide are removed, but both originate from the oxaloacetate already in the citric acid cycle.

Let us look at how aconitase can and does distinguish between the CH_2COO group on citrate which came from oxaloacetate and the same group which came from acetyl-CoA. Let me

describe how the enzyme does this. This enzyme, like most enzymes, requires one particular orientation of the substrate in the binding site. If the substrate bound to its binding site solely at the CH_2COO part of citrate, then the two pathways should occur equally. A more likely explanation from experimental evidence is that citrate interacts with aconitase at three sites. Let me place one CH_2COO group on that central carbon directly into the blackboard and then place the other three groups around this carbon in a tetrahedral fashion, so that each group is sticking out from the blackboard a bit. Let us assume for the time being that this is the correct orientation necessary for dehydration of the CH_2COO group in this position. Can we put this other CH_2COO group in the proper position on the enzyme? NOT WITHOUT REQUIRING A DIFFERENT POSITIONING OF THE CARBOXYL AND HYDROXYL GROUPS! Since they have defined positions on the enzyme as well, these are required.

So, aconitase recognizes a particular orientation of citrate in the active site which results in the formation of a double bond on the citrate between bonds from the original oxaloacetate. The position of the two CH_2COO groups is defined by citrate synthase - the oxaloacetate is essentially locked into place by the enzyme so that there is a single orientation of the two CH_2COO groups in the final citrate.

Let's follow our label just a little further. Succinate is made - This compound is neither chiral, nor prochiral. A prochiral carbon is one like the one found in citrate - Prochiral groups are sterically inequivalent and can be converted to chiral compounds in a single step. When succinate is oxidized to fumarate, a trans double bond is formed. Water can add across that double bond either by addition to a red or white carbon - this is both the theorized and experimental result. Final oxidation to oxaloacetate places 1/2 of the red carbons in the carboxyl group alpha to the carbonyl group in one compound, and in the other case the red carbons are found in the CH_2COO group.

Let's continue to follow this molecule through to oxaloacetate. In doing so, we find that half of our acetate originally from acetyl-CoA ends up as this half of the oxaloacetate, while half ends up as the other half of the oxaloacetate.

QUESTION: What will happen to these red carbons during the next cycle through?
What percentage of the label will remain after the second pass?

TCA CYCLE AND GLYCOLYSIS AS THE SOURCE OF BIOSYNTHETIC PRECURSORS

Let's look again at the sum of glycolysis and the TCA cycle and observe how synthetic precursors are generated. The first point of generation of biosynthetic precursors is pyruvate. At the point of pyruvate, a number of events can happen. Pyruvate can be used to make back glucose by gluconeogenesis. It can also make lactate or ethanol, depending upon the organism. It can also be used to biosynthesize amino acids, as we will see in a few weeks. If the pyruvate is converted to acetyl-CoA, its fate is pretty well decided. If the acetyl-CoA is in the cytosol, it will go on to make lipids. If the acetyl-CoA is in the mitochondria, the acetyl group will be donated to the citric acid cycle.

The citric acid cycle provides several sources of biosynthetic precursors. Citrate can be transported from the mitochondria to the cytosol and be converted by ATP-citrate lyase to form Acetyl-CoA and oxaloacetate, which can be used for fatty acid synthesis and gluconeogenesis, respectively. Acetyl-CoA in the cytoplasm is also the starting point for the synthesis of ketone bodies, which are released into the blood stream as an alternate energy source in times of fasting or starvation. α -ketoglutarate and oxaloacetate are both precursors for amino acid biosynthesis, and succinate is the starting point for heme and porphyrin biosynthesis.

Now, if we remove biosynthetic precursors from the TCA cycle, how does the cycle get replenished? If we remove intermediates, this means that less oxaloacetate is available for condensation with acetyl-CoA, and there is less capacity in the mitochondria to produce energy.

When the cell is metabolizing carbohydrates (which is the time you'd like maximum flux through the citric acid cycle), the cycle is replenished in mammals by the action of the enzyme pyruvate carboxylase. This reaction is termed an anaplerotic reaction; this is from the Greek word meaning "filling up". Pyruvate carboxylase catalyzes the addition of carbon dioxide to pyruvate at the expense of ATP:

The final product is oxaloacetate, which is one of the citric acid cycle intermediates. Remember that in a cycle the addition of 1 component results in reequilibration of concentrations and effective replenishing of all components. Pyruvate carboxylase is a mitochondrial enzyme, and we will discuss its mechanism when we talk about gluconeogenesis.

Amino acids can also be used to replenish citric acid cycle intermediates. There is a reaction called transamination which converts α -keto acids into amino acids, and back again, in one step. The amino acids aspartic acid and glutamic acid can be transaminated (with a different α -keto acid accepting the amino group) to the α -keto acids α -ketoglutarate and oxaloacetate, both citric acid cycle intermediates.

REGULATION OF THE CITRIC ACID CYCLE

The sites of regulation in the citric acid cycle represent important branch points in the cycle. The first and most important point of regulation is the enzyme directly before the cycle, that is, the pyruvate dehydrogenase complex.

There are two advantages in having a multienzyme complex. The first is that the substrate is quickly passed from enzyme to enzyme in the pathway. This both increases the local substrate concentration (helping to drive the formation of product) and prohibits the substrate and intermediates from diffusing away from the next enzyme in the pathway. The second advantage, the one relevant to this discussion, is that multienzyme complexes increase the opportunity for enzyme regulation. The formation of acetyl-CoA in the mitochondria forces the pyruvate to enter the citric acid cycle. All of these factors make pyruvate dehydrogenase a prime candidate for regulation.

How is the complex regulated? The affinity of the first enzyme in the pyruvate dehydrogenase complex, pyruvate dehydrogenase, for pyruvate is reduced by an increase in the energy charge (high ratio of ATP/ADP) and by a high concentration of acetyl-CoA. The overall rate of the reaction is also decreased by an increase in the ratio of NADH/NAD⁺. These effects slow the conversion of pyruvate when the energy load in the cell is high.

The pyruvate dehydrogenase complex is also regulated by specific phosphorylation /dephosphorylation reactions. Each complex contains a few molecules of a protein kinase and protein phosphatase. The kinase catalyzes the phosphorylation of 3 specific serine hydroxyl groups on the pyruvate decarboxylase enzyme, rendering it relatively inactive. The activity of the kinase is increased by the same factors which directly affect the decarboxylase; high ATP,

NADH, and acetyl-CoA. The activity of the phosphatase, which removes the inactivating phosphate groups from the decarboxylase, is enhanced by high levels of calcium and also by the hormone insulin. High levels of calcium are often generated in response to hormonal stimulation of the cell. Protein phosphorylation/dephosphorylation is a commonly used regulatory mechanism in mammalian cells.

The enzymes within the cycle which are regulated are citrate synthase, isocitrate dehydrogenase, and α -ketoglutarate dehydrogenase. Let's start with the last enzyme, which is a multienzyme complex similar in structure to the pyruvate dehydrogenase complex. Therefore you would expect that this enzyme complex is regulated in the same fashion as pyruvate dehydrogenase. Stryer states that this is so; however, other sources are less certain about the type and extent of regulation of this enzyme. This enzyme is likely a regulatory point, because α -ketoglutarate is a major synthetic precursor for the synthesis of amino acids and proteins.

Citrate synthase is another point of regulation. Citrate synthase is an allosteric enzyme, inhibited allosterically by ATP, thereby decreasing the enzyme's affinity for acetyl-CoA.

Isocitrate dehydrogenase is the final point of regulation in the cycle. The enzyme is allosterically activated by ADP, which enhances its affinity for substrates. The binding of isocitrate (the substrate), NAD⁺, Mg⁺², and ADP are mutually cooperative, that is, the binding of one will enhance the binding of the other. The enzyme is inhibited by both NADH (this is product inhibition; it displaces NAD⁺) and ATP.

INHIBITION OF CITRIC ACID CYCLE ENZYMES

I would like to make two additional points about the citric acid cycle. The first is the inhibition of the enzyme succinate dehydrogenase. It was known before the discovery of the citric acid cycle by Hans Krebs that this enzyme was inhibited by malonate. In comparing the structures of the substrate and inhibitor, the two are very similar, except that there is no site for dehydrogenation on malonate. This is the classic example of a competitive inhibitor - malonate binds to the active site without converting to product. The ability to selectively and completely inhibit one enzyme in the cycle allowed the isotopic labeling experiments which clarified the pathway to occur. Without this inhibition (and without purified enzymes), any labeled pyruvate would scramble through many cycles of the pathway before the reaction could be stopped and analyzed.

There are other simple compounds which act as very potent metabolic inhibitors by disrupting the activities of enzymes of the citric acid cycle. Arsenic compounds are toxic because they covalently bind to sulfhydryl compounds, keeping them from working as redox centers. For example, arsenic compounds will inhibit the lipoamide part of the pyruvate dehydrogenase complex, preventing the synthesis of acetyl-CoA and essentially starving cells to death.

Finally, I would like to emphasize the importance of pyruvate and its regulation to the citric acid cycle. Pyruvate is directly involved in the cycle in two ways:

1. In conversion to acetyl-CoA, two carbon units are provided for oxidation and energy production.
2. By the action of pyruvate carboxylase, more oxaloacetate is formed to replenish cycle intermediates.

Acetyl-CoA regulates both the pyruvate decarboxylase component of the pyruvate dehydrogenase complex and the pyruvate carboxylase enzyme. To illustrate this regulation, let us take an isolated mitochondrion in which the citric acid cycle is functioning only oxidatively; that is, no input of intermediates is required.

At this point, conditions change, and some α -ketoglutarate is removed from the cycle for biosynthetic purposes. This decreases the concentration of cycle intermediates, translating into a decrease in the concentration of oxaloacetate, resulting in decrease flux of acetyl-CoA into the cycle. This will increase the concentration of acetyl-CoA. This will inhibit the activity of pyruvate decarboxylase (both by reducing the affinity of the enzyme for pyruvate and activating protein kinase) and will activate pyruvate carboxylase. Activating pyruvate carboxylase will result in net synthesis of oxaloacetate, which will increase the flux of acetyl-CoA into the citric acid cycle, decreasing the concentration of acetyl-CoA, activating pyruvate decarboxylase and reducing the carboxylase activity.

Discussion of Dichloroacetate paper

Questions:

1. Why is there lactic acidosis? That is, if pyruvate is reduced to lactate, the carboxylate group in both cases is not protonated. How do excess protons get into the bloodstream?
2. Sodium bicarbonate is the standard treatment for lactic acidosis. Why might this be ineffective or deleterious in some patients?
3. Where is the site of action of dichloroacetate?
4. Insulin was not administered as part of the study because insulin is a “known activator of pyruvate dehydrogenase”. How does insulin activate pyruvate dehydrogenase?
5. Go to Table 2. What effect did dichloroacetate treatment have on lactate levels? What affect did it have on bicarbonate levels? What is the cause for the increase in bicarbonate?
6. What affect does dichloroacetate have on the levels of alanine? What is the origin of this effect?
7. What affect does dichloroacetate have on beta-hydroxybutyrate levels? What is the origin of this effect?
8. What is your assessment of the success of dichloroacetate in this study?
9. How many patients exhibited long term survival after this study?

PENTOSE PHOSPHATE PATHWAY

It is time for us to shift gears (just a little). Up until now we've discussed how sugars such as glucose are metabolized in order to generate energy in terms of ATP. This ATP can progress to carry out cellular energetic or biosynthetic functions. The other compound generated catabolically from glucose has been NADH. NADH is converted to ATP by oxidative phosphorylation, which we will talk about next week.

The point of this next topic is: if reducing equivalents are generated during catabolic processes, are they required for anabolic processes? The answer, boys and girls, is yes. How are these reducing equivalents supplied? If you said NADH, you would have blown double Jeopardy, Don Pardo, because the answer isn't NADH, it's NADPH! NADPH looks just like NADH, except that it has a P. This is a phosphate group attached to the 2' position of the adenosine part of the nicotinamide cofactor. As a rule, NADPH is used in anabolic processes; NADH is formed in catabolic processes.

As you might have noticed, we have not mentioned the formation or use of NADPH at all as of yet in our discussion of glycolysis and glycogen metabolism. NADPH is used up during biosynthetic processes. It is regenerated by a cycle branching from glycolysis called the pentose phosphate pathway (also called the hexose monophosphate shunt). In a general description of the pentose phosphate pathway, glucose-6-phosphate is converted to ribose-5-phosphate with generation of two molecules of NADPH from two molecules of NADP⁺. The total series of reactions is a bit more complicated, as we will discover.

The pathway can be divided into two parts – the oxidative part and the nonoxidative part. In the oxidative part, glucose-6-phosphate is converted into ribulose-5-phosphate, a pentose phosphate. In the nonoxidative part, the pentose phosphate (which has 5 carbons) has to be modified into something that can reenter the glycolytic pathway. We'll discuss the particulars of that in a moment.

Let's first describe the oxidative pathway and the enzymes which catalyze the steps. **SOMEONE DESCRIBE THE FIRST REACTION IN THE SEQUENCE.** The first step of the pathway is the oxidation of glucose-6-phosphate to 6-phosphoglucono- Δ -lactone with concurrent reduction of NADP⁺ to NADPH:

The reaction is catalyzed by the enzyme glucose-6-phosphate dehydrogenase. A relatively common genetic disorder occurs when individuals are deficient in glucose-6-phosphate dehydrogenase. A deficiency in this enzyme can lead to hemolytic anemia; this was mentioned in the hemolytic anemia paper. The deficiency is primarily evidenced in erythrocytes, and is only seriously harmful when the affected patient is given certain types of drugs or supplements. This is often observed with patients who are given antimalarial drugs; these are given to people before they travel to a part of the world where malaria is prevalent. The most commonly prescribed drugs are mefloquine, doxycycline, or Malarone.TM These drugs are absorbed by the malarial parasite and cause oxidative damage to them.

The same drugs are absorbed by our cells, and since the drugs are transported around our body in the blood, our blood cells are the first cells exposed to this material. In a normal patient, the drugs are taken up into red cells and reduced into harmless compounds, with glutathione as the source of electrons. The oxidized glutathione is then converted back into its reduced form by glutathione reductase:

Glutathione reductase required NADPH as a source of electrons. In a normal red blood cell, this is no problem; the pentose phosphate pathway can quickly regenerate the depleted NADPH and the cells stay happy. However, in cells without the glucose-6-phosphate dehydrogenase, the red blood NADPH begins low and is quickly used up before any more can be made. The red blood cells then experience oxidative damage, and many will die and lyse (hence the hemolytic anemia!)

Returning to the pathway, the lactone formed by the dehydrogenase is hydrolyzed by adding water to form 6-phosphogluconate:

The enzyme catalyzing this reaction is lactonase. The final step in the so-called oxidative segment of the pentose phosphate pathway is the oxidative decarboxylation of the gluconate to form ribulose-5-phosphate (note that the oxidation results in the formation of a beta ketoacid, which is unstable):

This step is catalyzed by 6-phosphogluconate dehydrogenase.

At this point, the metabolic function of the pathway has been fulfilled. A six-carbon sugar has been converted to a five-carbon sugar, and 2 NADPH have been generated. Unfortunately, the ribulose-5-phosphate isn't much good for anything – it must be converted into ribose-5-phosphate and xylulose-5-phosphate. The ribulose-5-phosphate is easily converted to

ribose-5-phosphate by the action of the enzyme phosphopentose isomerase:

The isomerase reaction converts the ribulose-5-phosphate (which is a ketopentose) to ribose-5-phosphate (an aldopentose). Ribose-5-phosphate is needed by many cells for what purpose? (NUCLEOTIDE SYNTHESIS). If the cells don't need ribose for nucleotide biosynthesis, then it can convert the five carbon pentose phosphate back to molecules that can reenter glycolysis. To do this, a second five carbon sugar, xylulose-5-phosphate, is needed; it is formed from ribulose-5-phosphate by the action of phosphopentose epimerase:

This sugar is formed by epimerizing C-3 of ribulose-5-phosphate, going from the D to L configuration at this position.

After the two 5-carbon sugars are in place, then the enzymes transketolase and transaldolase begin their function in regenerating 6 and 3 carbon sugars for insertion into glycolysis. To summarize the steps, which are described in detail in your textbook, you begin with two 5-carbon sugar phosphates.

$$5 + 5 = 3 + 7$$

$$7 + 3 = 4 + 6$$

$$4 + 5 = 6 + 3$$

To summarize, three 5-carbon sugars are converted into two 6-carbon sugars (fructose-6-phosphate). The first step in this complicated pathway involves the transfer of a 2-carbon unit from xylulose-5-phosphate to ribose-5-phosphate to yield glyceraldehyde-3-phosphate and sedoheptulose-7-phosphate, a 7 carbon sugar. The reaction is carried out by the transketolase; here is the mechanism for that enzyme:

The enzyme has a bound thiamine group that grabs onto the keto group of the xylulose-5-phosphate and rips off two carbons, leaving glyceraldehydes-3-phosphate. These are subsequently added to the aldehyde group on the ribose-5-phosphate.

Via the action of the transaldolase, the glyceraldehyde-3-phosphate can acquire a 3-carbon unit from sedoheptulose-7-phosphate to form fructose-6-phosphate, which can reenter glycolysis, and erythrose-4-phosphate. The mechanism for this enzyme is similar to that of aldolase, which has an active site lysine residue that forms a Schiff's base with the seven carbon

sugar at the site of the ketone. Four carbons fall off, and three carbons remain attached to the lysine. The glyceraldehydes-3-phosphate jumps in and grabs the three remaining carbons, resulting in fructose-6-phosphate.

The erythrose-4-phosphate can react with a molecule of xylulose-5-phosphate to form another fructose-6-phosphate plus a glyceraldehyde-3-phosphate, both of which can reenter glycolysis.

The regulation of this pathway occurs at the two oxidative steps, which are somewhat by the NADPH/NADP⁺ ratio in the cells. The glucose-6-phosphate dehydrogenase is the major site for regulation, since this is the point at which the glucose-6-phosphate decides whether or not it enters the pentose phosphate pathway. The enzyme is controlled by the amount of NADP⁺ in the cell. When NADP⁺ is low, then glycolysis is chosen. When NADP⁺ is high, then the pentose phosphate pathway is chosen.