PROFESSOR: Let's look at storyboard two. We're going to look in more detail at carbohydrate catabolism at this point. We're eventually going to be doing the pathway of glycolysis, but we have to get there first. And it depends on what precursors are available to enter the pathway of glycolysis. One option that we'll look at a little bit later is to take in glucose from the blood by way of a glucose carrier, and then phosphorylate the glucose using either hexokinase or glucokinase.

The second option is to take glycogen, the polymeric storage form of glucose, and degrade it to glucose 1-phosphate, which will be then converted to glucose 6-phosphate which will enter the pathway of glycolysis. We're going to start with this pathway of glycogen breakdown, or glycogenolysis.

Panel A shows the structure of glycogen, which consists of glucose monomeric units connected by a bond between the 1 and 4 carbons in the alpha configuration. Chemistry is going to be happening at the non-reducing end, which is to the left. The reducing end, which is to the right, is connected to a scaffolding protein called glycogenin. We'll come back to glycogenin later in the course.

Let's look next at panel B. The reaction begins with the proteination of the red oxygen between the terminal two glucose moieties by a proton on the glycogen phosphorylase enzyme. The intermediate product is a resonant stabilized pair of positively charged species, or cations, an oxonium ion at the 1 prime oxygen, and a carbocation at the 1-carbon. The electrophilic carbocation is attacked by the negatively charged phosphate residue, which is non-covalently associated with glycogen phosphorylase to form glucose 1-phosphate.

Note that the glucose 1-phosphate that forms has the covalently attached phosphate on the alpha, or bottom face, as it's drawn in the figure. The structure of glycogen phosphorylase allows attack of it's phosphate from the bottom, giving rise to alpha isomer only of glucose 1-phosphate. The structure of the phosphorylase precludes access of its phosphate to the top face of the sugar molecule, so you get only one stereoisomer of glucose 1-phosphate out of this reaction. The other product shown at the bottom of panel C is the glycogen chain, which is

truncated or shortened by one glucose unit.

Now looking at the big picture, the epinephrine molecule produced as part of the stress response interacted with the cell membrane of the muscle cell. And that interaction ultimately activated glycogen phosphorylase to enable it to degrade glycogen, the storage form of glucose, and liberate glucose 1-phosphate, which is going to then find its way into glycolysis to generate fast energy to enable the student to be able to stand up in class and avoid the stressful situation. I'm not going to go through all of the details of the chemical reactions of glycolysis. You can find those in the book which does a good job of presenting those details.

In the first step of glycolysis, we see the phosphorylated hexose, glucose 6-phosphate, be converted by phosphoglucoisomerase into the furanose fructose 6-phosphate. The next step involves the phosphorylation of the fructose 6-phosphate by phosphofructokinase 1. ATP is the phosphate donor, and you form fructose 1,6-bisphosphate as the product. You'll notice that phosphofructokinase catalyzes one way, a thermodynamically irreversible reaction. This step, like hexokinase we talked about earlier, are sites of regulation of the pathway.

The word glycolysis comes from the Greek word sugar splitting, and we're now at the step where the splitting of the sugar from one 6-carbon compound into two 3-carbon compounds occurs. The enzyme that splits the sugar in half is called aldolase. And, again, I advise you to take a look at the book to see the detail of the chemical reaction.

Briefly, the enzyme is going to form a proteinated Schiff base at the number two carbon of the fructose 1,6-bisphosphate. That proteinated Schiff base is going to draw electrons all the way over from the hydroxyl group on carbon 4 of fructose 1,6-bisphosphate. That movement of electrons is going to result in cleavage of the molecule into two parts at the broken line shown in the figure.

Carbons 1, 2, and 3 are going to form DHAP, or dihydroxyacetone phosphate, and carbons 4, 5, and 6 are going to form GAP, or glyceraldehyde 3-phosphate. GAP and DHAP are the products of the aldolase reaction.

As an aside, this is also a branch point in the pathway. Dihydroxyacetone phosphate is an opportunity for the cell to make the glycerol backbones of lipids that we'll come to later. So we see here that glycolysis is indeed a resource that can be used for things other than energy generation. Now let's look at panel B.

Imagine that a cell is committed to make as much energy as it can. For example, the stand up, sit down scenario we talked about earlier. In that case, the enzyme TIM, or triosephosphate isomerase, is going to interconvert very quickly dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, and glyceraldehyde 2-phosphate is going to be the molecule that progresses along the glycolysis pathway.

In the next step of glycolysis, glyceraldehyde 3-phosphate will be oxidized by glyceraldehyde 3-phosphate dehydrogenase, or GAPDH. Again, take a look at the mechanism in the book. In brief, it involves attack by a thiol residue of a GAPDH cysteine on the aldehydic carbon of glyceraldehyde 3-phosphate. The product is a thiohemiacetal which is then oxidized. Its electrons are transferred as hydride to NAD+ on the enzyme to form NADH. This is the only oxidation step in the pathway of glycolysis.

The oxidation of the hemiacetal produces the thioester, and that thioester is a very high energy compound. The thioester is attacked by inorganic phosphate to form an acetylphosphate, another high energy compound, which is called 1,3-bisphosphoglycerate. The acetylphosphate of 1,3-bisphosphoglycerate then phosphorylates ADP to form ATP in the first ATP forming step of glycolysis.

Keep in mind that two molecules of GAP have been formed in the upstream part of the pathway. So for each molecule of glucose, you're getting two molecules of ATP at this step. The enzyme that does this phosphorylation is phosphoglycerate kinase. After 1,3-bisphosphoglycerate has lost its terminal phosphate from the 1-carbon, it forms the acid 3-phosphoglycerate. As a reminder, mutases are enzymes that move a functional group from one atom to another on the same molecule.

The next enzyme in the pathway is phosphoglycerate mutase, which in effect moves the phosphate from the 3 to the 2-carbon, forming 2-phosphoglycerate, which is the next intermediate in the glycolysis pathway. Although it's easy to say that the phosphate is quote unquote "moved," that is somewhat inaccurate. If you look at the details of the step in the book, you'll see that the reaction starts with the transfer of a phosphate from the mutase protein to the substrate 3-phosphoglycerate, forming an intermediate bis-phosphorylated product 2,3-bisphosphoglycerate.

As an aside, this is the same powerful allosteric effector that JoAnne described when she taught us about how small molecules can dramatically reduce the affinity of hemoglobin for

oxygen. In the case of the mutase, however, the enzyme will take the phosphate off of the 3-hydroxyl of 2,3-bisphosphoglycerate and the enzyme will re-phosphorylate itself. So the final product of the phosphoglycerate mutase reaction is 2-phosphoglycerate.

Now let's turn to storyboard four. In panel A, we see the enzyme enolase, which removes the hydrogen from the 2-carbon of 2-phosphoglycerate. This is not an easy task. The PK of that hydrogen is about 30. Nevertheless, the reaction does occur and liberates water. The product is phosphoenolpyruvate, usually abbreviated PEP, or PEP, a very high energy compound.

We're now almost at the end of the pathway of glycolysis. And as I mentioned earlier, one usually looks for highly exergonic steps near the beginnings or ends of pathways to see where the pathway is regulated. Pyruvate kinase, or PK, the last step in the pathway is such a regulation point.

The pyruvate kinase reaction occurs in two steps. In the first step, phosphoenolpyruvate phosphorylates ADP to form ATP. The enol product then undergoes enol-keto tautomerization, yielding the ketone pyruvate. And pyruvate is the end of the pathway.

Let's turn now to story board five, panel A. Let's take a look at this pathway from a higher altitude. First, as I just mentioned, the pathway is regulated at the top and bottom specifically at the hexokinase step, the glycogen phosphorylase step, and the pyruvate kinase step.

It's also regulated in the middle, specifically at the phosphofructokinase one step. Regulation can be allosteric, which we shall see as the case with PFK. It also can be covalent. We saw that this is the case with glycogen phosphorylase, or GP. The last lecture, we'll take a look at regulation of these enzymes in great detail.

As a second issue, let's now look at the pathway as drawn in summary form in panel B of this storyboard. We start with a single molecule of the 6-carbon sugar glucose. Hexokinase or glucokinase will utilize one ATP to form a phosphorylated intermediate.

Phosphofructokinase-1 will use a second ATP to form a doubly phosphorylated hexose, fructose 1,6-bisphosphate. Bis-phosphorylated hexose will split into two trioses, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. These are inter-convertible.

The chemical species glyceraldehyde 3-phosphate is subjected to oxidation. Because we get two molecules of GAP per molecule of glucose, GAP oxidation will produce two molecules of NADH. In the next step, we're going to make two ATP's using the enzyme phosphoglycerate

kinase.

At this point, we're ATP neutral. We've consumed two ATP's and we've generated two ATP's. And lastly, the enzyme pyruvate kinase is going to generate an additional two ATP's. I put those in a box, because these are the two net ATP's for the whole pathway. So that's the pathway of glycolysis.

I want to give you a little bit of a preview of coming attractions at this point. What you'll notice is that the pathway involves an oxidation step in which we consumed two NAD+ molecules and generated two NADH's. NAD+ is derived from a vitamin. We'll only have limited amounts of it. We need to find a way to regenerate the NAD+ in order to process the next molecule of glucose, and we'll see that nature has several ways to solve that problem.

Nature actually has three ways to regenerate NAD+. The first we'll call alcoholic fermentation. The second is homolactic fermentation, and the third is respiration. Alcoholic fermentation and homolactic fermentation occur in the absence of oxygen. That is, anaerobically. Respiration by definition is an aerobic process.

Now we'll look at each of these mechanisms of regeneration of NAD+ in some detail, but also, in addition to NAD+, you're going to have to generate a number of other products that can be useful to the cell. We'll see those later.

Let's look at panel C. Under anaerobic conditions, yeast will take pyruvate and convert it initially to acetaldehyde, and then reduce the acetaldehyde to ethanol. These are the reactions of alcoholic fermentation.

Yeast uses an enzyme called pyruvate decarboxylase to process the pyruvate. Pyruvate decarboxylase, or PDC, has on it a covalently attached thiamin pyrophosphate. Thiamin is derived from vitamin B1.

As we go through the pyruvate decarboxylase reactions, at the outset I want you to keep in mind that PDC, pyruvate decarboxylase, is very similar to the front end of the chemical reaction series that's conducted by an enzyme present in mammals like us. That enzyme complex has pyruvate dehydrogenase, which we'll come to a little later when we talk about respiration.

The thiazole ring in TPP forms an ylide. That means that despite the fact that the PKA of the

thiamin pyrophosphate is about 19, you are able to form a carbanion at the carbon of the thiazolium ring system. That carbanion attacks the middle carbon of pyruvate, converting it from a ketone to an alcohol.

Now you have the thiazolium ring system with the positive charge beta to the carboxylate of pyruvate. That system readily decarboxylates as shown, liberating thiamin pyrophosphate and the product, acetaldehyde. The next enzyme in this small pathway is alcohol dehydrogenase which utilizes NADH, which came in, in principle, from the GAPDH step of glycolysis.

Alcohol dehydrogenase uses the glycolysis-derived NADH to reduce the aldehyde functionality of acetaldehyde to form the product of this pathway, ethanol. Ethanol is an alcohol, hence the name alcoholic fermentation.

So looking at this small pathway in total, what you see is that you form CO2 as a first product, which could be the bubbles in a carbonated beverage or what makes bread rise, and form ethanol as the other major product. And, of course, you get your NAD+ back, which you can then return to glycolysis, specifically the GAPDH step of glycolysis, to enable metabolic processing of the next molecule of glucose. So this is the pathway that yeast and other alcohol-forming organisms use to maintain redox neutrality within the cell.

I'm on storyboard six, and we're going to start with panel D. The second general mechanism that we're going to look at that concerns the regeneration of NAD+ for glycolysis is called homolactic fermentation. This occurs in mammals and in lactic acid bacteria. And like alcoholic fermentation, it is also a process that occurs anaerobically. That is, in the absence of oxygen.

As you can see, pyruvate is a keto acid, and the ketone at the number 2 carbon can be easily reduced. In this case, NADH will transfer hydride to the ketone in order to reduce it to the alcohol lactate. The net reaction here involves consumption of one NADH and the production of one NAD+. And this NAD+ of course, can go back and be utilized to enable oxidation of the next molecule of glucose passing through the glycolytic pathway.

When a mammal is running hard, this is the pathway by which we achieve redox neutrality and glycolysis. When we exercise intensely, lactate is produced in excess to keep the glycolytic pathway active. The lactate causes the blood pH to go down. That is, the blood becomes more acidic because lactic acid has a low PKA. I also want to point out that this anaerobic pathway is also the basis for production of lactate by lactic acid bacteria, which is critical to the manufacturing of yogurt.

Let's look now at panel E. The third pathway to regenerate NAD+ for glycolysis is respiration. We're going to be going through respiration in some detail later, but right now I'm going to give you a very high level view of it.

In the way of an introduction, the mitochondrial intermembrane is very well equipped to be able to transport electrons. Those electrons will travel along in an electron transport chain to oxygen, reducing the oxygen we breathe into water. This is a highly energy generating process, and the energy that's generated is part of the driving force for the synthesis of ATP.

The details of how a respiring organism generates ATP is covered later. For right now, however, let's just say that the mitochondrial membrane oxidizes NADH to regenerate the NAD+ needed to sustain glycolysis. And, again, we'll see the details of how this happens later.

Later, I'll also cover the ways that redox neutrality is maintained in a mammalian cell. In brief, NAD+ is generated from NADH in aerobes by a series of reactions that I call quote unquote "the shuttles," which will be covered in section 12.

Before we go on, let me give you a little recap of where we are. We've seen that there are a couple of optional beginnings for glycolysis. It can begin with intake of glucose from the blood, or it can begin with the breakdown of glycogen by glycogenolysis.

The formal pathway takes glucose as glucose 6-phosphate down to pyruvate. We get a total of two ATP's in that process, and we produce two NADH's. Now, however, we've got to have a way to be able to regenerate our NAD+ from those NADH's in order to be able to make the pathway ready to process the next molecule of glucose. Accordingly, nature developed three endings to the pathway that result in the regeneration of NAD+. These endings are alcoholic fermentation, homolactic fermentation, and respiration.

Looking at that picture in panel E once again, in us, respiration happens in the mitochondria and primarily in the mitochondrial intermembrane and in the jelly-like mitochondrial matrix. Pyruvate generated in the cytoplasm-- that's the compartment where glycolysis occurs-- goes through the porous outer membrane of the mitochondria. Then, the pyruvate encounters the membrane-bound pyruvate dehydrogenase complex, which is our next topic. In bacteria, which are in many ways like mitochondria, respiration happens in the cellular membrane and in the cell's cytoplasm.

Let's take a look at panel A of storyboard seven. We're about to start our discussion of respiration, which is the oxidative metabolism of all metabolic fuels via the common intermediate acetyl CoA. In mammals, as I said earlier, these are mitochondrial reactions. At the outset, I also want to point out that we have seen that carbohydrates can be metabolized either anaerobically or aerobically. As we'll see when we use lipids as our metabolic fuels, they can only be metabolized aerobically. Lipids break down to acetyl CoA, which is then oxidized by the TCA cycle.

Let's take a look at panel B. When we talked about alcoholic fermentation earlier, I said that yeast have a pyruvate decarboxylase complex, and I said that the reactions of the pyruvate decarboxylase complex are very similar to the reactions in the early part of the pyruvate dehydrogenase reaction, which is a little bit more complex. Pyruvate dehydrogenase has three activities, E1, E2, and E3.

As with pyruvate decarboxylase, E1 has a thiamine pyrophosphate unit, TPP, and ylide on the TPP attacks the middle carbon of the pyruvate. Specifically, it's ketone carbon. At this point, you're going to want to take a look at detailed notes that I've provided as supplemental material. This supplemental material will be referred to as slide one, slide two, and so on.

Looking at slide one, you can see that decarboxylation happens exactly the same way that I described for the pyruvate decarboxylase system. Now take a look at slides two through six. In the case of PDH, pyruvate dehydrogenase, unlike the situation with PDC, pyruvate decarboxylase, restructuring of the carboxyethyl group is going to result in the formation of our carbanion that's going to attack the disulfide of lipoic acid.

Looking at slide seven, you'll see the conversion of the hydroxyethyl to a keto functionality jettisons the TPP, resulting in a thioester in which there is an acyl group connected to lipoic acid. At this point, the thiol of coenzyme A attacks the keto oxygen of the thioester, producing acetyl CoA, which is going to become a very important molecule as we move ahead. The second product is reduced lipoic acid.

Technically, the formation of reduced lipoic acid is the oxidation step of the pyruvate dehydrogenase reaction. The decarboxylation step that happened a few steps earlier is basically the production of CO2 that we eventually will breathe out when we exhale.

Now let's look at slide eight. The reducing equivalents on the E2 subunits present as reduced lipoic acid will move across the E2 subunit toward the E3 subunit. The E3 subunit has an

oxidized disulfide bond on it, which was created by the connection of two cysteines on the protein. That oxidized disulfide is then reduced by transfer of the reducing equivalents from the reduced lipoic acid to the disulfide.

And then finally, the reducing equivalents are passed from the reduced disulfide to FAD to form FADH2. That FADH2 passes along its reducing equivalents to NAD+ forming NADH. This NADH is soluble and will move to its next location. Specifically, this NADH will return to the mitochondrial membrane-- actually to another place in the mitochondrial membrane-- an enzyme called complex one, and be oxidized in the electron transport chain.

Overall, one pyruvate enters the PDH complex. We lose its carboxylate as CO2. We generate from pyruvate's residue an acetyl coenzyme A. And at the very end, we get an NADH, which will then go on to the electron transport complex to be oxidized. The formation of NAD+, as I mentioned above, is critical to allow further oxidation of reduce. That is, energy rich molecule such as glucose.