Biochemistry
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Lecture - 25
Metabolism – I

We come to the final chapter of this course that deals with metabolism. Okay. Now in metabolism, we need certain nutrients we are going to learn how the food that we have taken especially in the metabolism of carbohydrates the food that we are taking how that is broken down. Okay.

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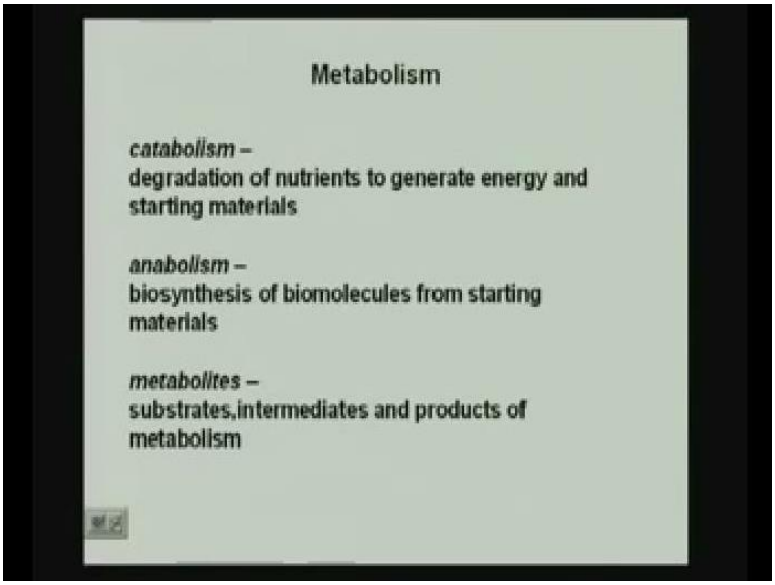

Now metabolism actually is you have learnt from your school days comprised of catabolism which is the degradation of nutrients to generate energy and starting materials which is what we are going to do basically. And anabolism is the biosynthesis of biomolecules from starting materials which also actually happens in our body because we create proteins, protein are synthesized in the body which is a process that would require anabolism.

Catabolism is the breaking down of the nutrients that are ultimately going to provide the energy for the actions or whatever work that we do. The metabolites that take part in these processes actually are substrates and intermediates and of course there will be a large number of enzymes that are going to be actually part of the whole system.

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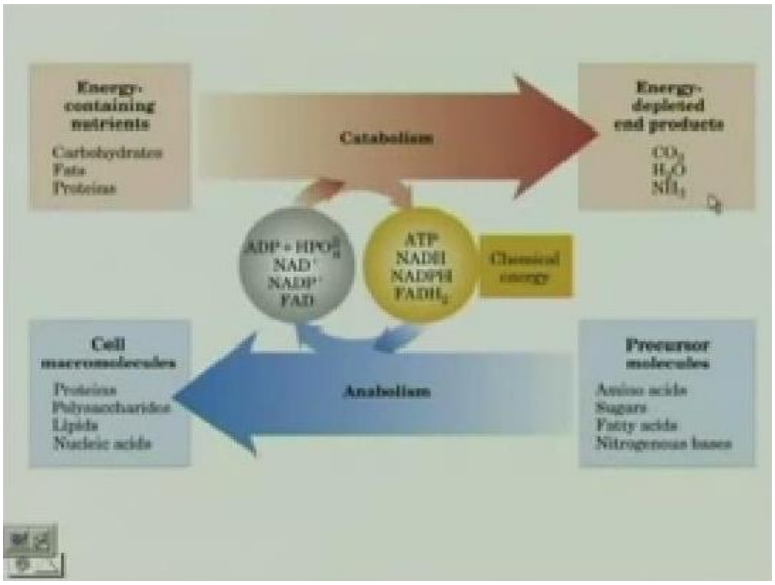

Okay. Now, if we just look at this whole picture and I will show you another one also where we will how the whole process actually takes place we have energy containing nutrients that we take in our diet carbohydrates, fats and proteins. In the catabolism, that is in the breakdown of these processes or the breakdown of these units we actually have energy depleted end products that are finally carbon dioxide water and ammonia. Okay.

And we have in this process then the use or the utilization of certain aspects or certain co-factors that we have already looked at we have ADP and ATP in a large extent we have NAD plus we have FAD we have NADP plus and so on and so forth. Now all of these actually, will then either break down or form in different ways giving us finally the chemical energy.

Then we have certain precursor molecules that are also found in our body where we have the amino acid, the sugars, the fatty acid and the nitrogen as bases that in a process of anabolism that is in the formation of the micro molecules will ultimately lead to the cell micromolecules such as proteins polysaccharide, lipids and nucleic acids. Okay. So eventually, it is just like a whole cyclic process that it is the food that we take that is ultimately broken down.

And then after the breaking down the bit and pieces some of them get lost in energy depleted products, some of them formed a precursor molecules for the formation of the generation of other protein polysaccharides and lipids and nucleic acids.

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Now if we look at actually the whole process it is extremely interesting. Okay. This is like a system of a gear system. Okay. We have the process of glycolysis which we are going to study in our break down of breakdown of glucose. Okay. In the metabolism, that we are studying, the glycolysis leads to something called the TCA cycle which is the tricarboxylic acid cycle that has electron transport the electron transport we just looked at has a proton pump to it.

The proton pump then uses ATP synthesis to produce ATP that is cellular work where we get work. This oxygen again comes in from the blood utilized in the ATP synthesis in the electron transport system where we have the reduction of the oxygen to water. Now this is the way all of these are interconnected actually and if you just look at a whole picture of all the metabolic pathways.

That is actually beautiful picture on the net that has all the metabolic pathway it actually takes place in our body what I am going to give you is just probably a small like drop in the huge ocean of all those metabolic pathways it actually takes place.

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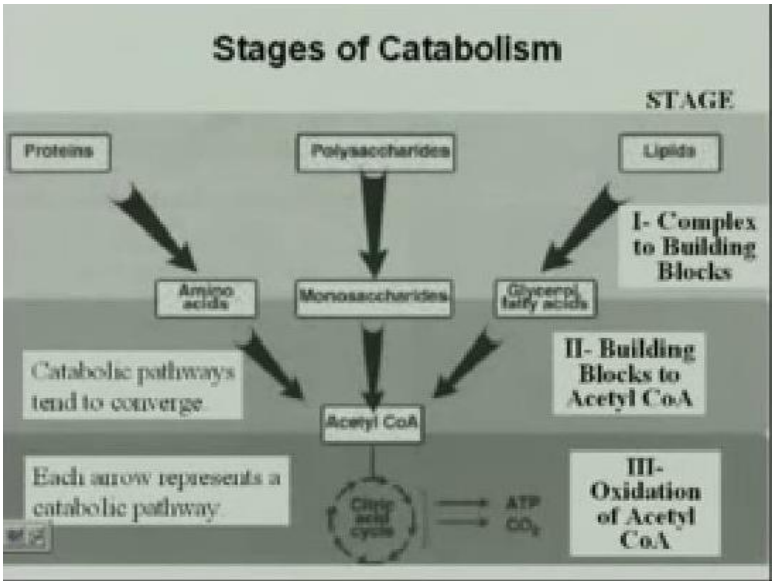

Okay. Now, what we actually intake, we looking at stages of catabolism, our intake is proteins, polysaccharides, lipids that is fats, proteins and carbohydrates basically that is what we intake. The smaller blocks of proteins break down. What is the breakdown of proteins giving to give you? Amino acids, the breakdown of polysaccharides is going to give you monosaccharides. The breakdown of lipids is going to give you fatty acid or glycerol.

Fine, now each of these again are further going to be broken down and converge to Acetyl coenzyme A, which is an extremely important part in the catabolic pathway that is going to take you again to the citric acid cycle that requires the oxidation of Acetyl coenzyme A. So basically what we are getting at is we are going to look at just this part here monosaccharide the breakdown of glucose that is what we are going to look at.

But if you consider the emptying polysaccharides that are present in the body that are possible for breaking down you realize that each of them is an enzymatic reaction that is required for the breaking down from its polymeric form to the monomeric form which is finally going to be broken down into other forms.

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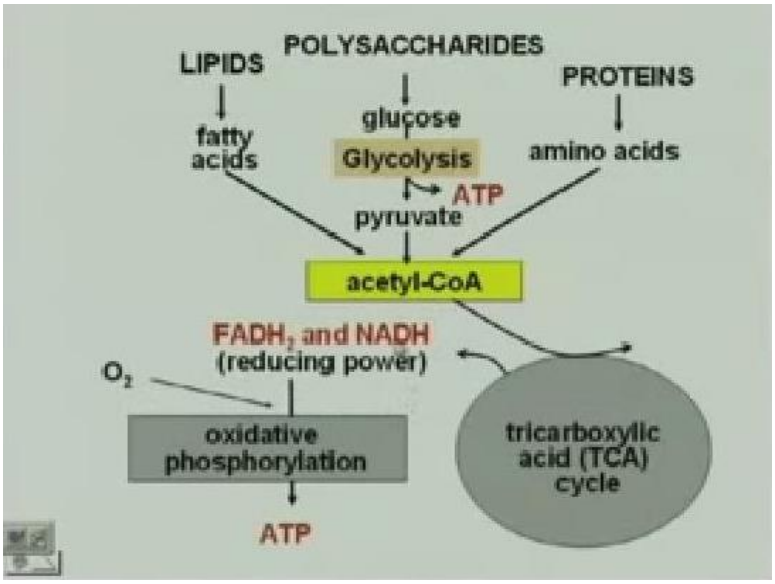

So, what we have is we have our lipids, polysaccharides, and proteins. Lipids breaking down into fatty acids that form the Acetyl-CoA, polysaccharides breaking down into glucose the monomer unit in the process of glycolysis forming pyruvate which is what we will see in the process of glycolysis that we will study.

Proteins again breaking down into amino acids that again are utilized in this Acetyl-CoA which finally is used in the tricarboxylic acid cycle which is going to be the broken down of the tricarboxylic acids, the oxaloacetic and so on and so forth. Then we have this process where have FADH2 plus NADH giving you with oxygen oxidative phosphorylation in the production of ATP. Okay.

This a part that we have studied in a bit detail and now what we are going to look at, is we are going to look at glucose going to pyruvate in the process called glycolysis and in the event producing ATP also. But requiring ATP also in a number of steps and finally Acetyl-CoA is that is going to be utilized in the tricarboxylic acid cycle that is also known as the Krebs cycle.

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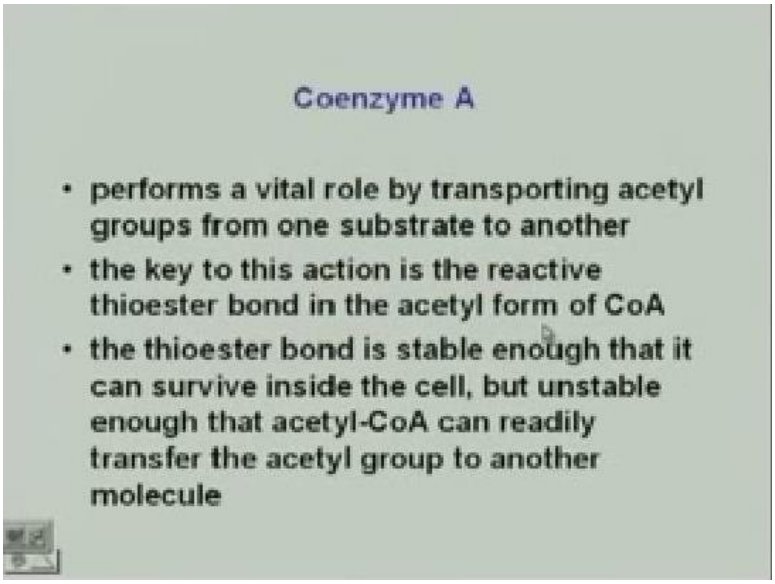

Okay. Now before I get into the process of glycolysis this is something that we looked at before when we consider the vitamins. I mentioned that each of these vitamins is now realized is the precursor for a large number of cofactors and pro sending group. Okay. Coenzyme A is another such compound that is extremely important in the formation or transfer rather of the acetyl group that is a transfer of two carbon system for any of the process that occur.

What we have in coenzyme A is actually an ADP part an adenine dinucleotide part, the phosphorylated one because that is a phosphate at this position if you can see and we have pantothenic acid. Okay. That is one vitamine, and to it we have mercaptoethylamine attached.

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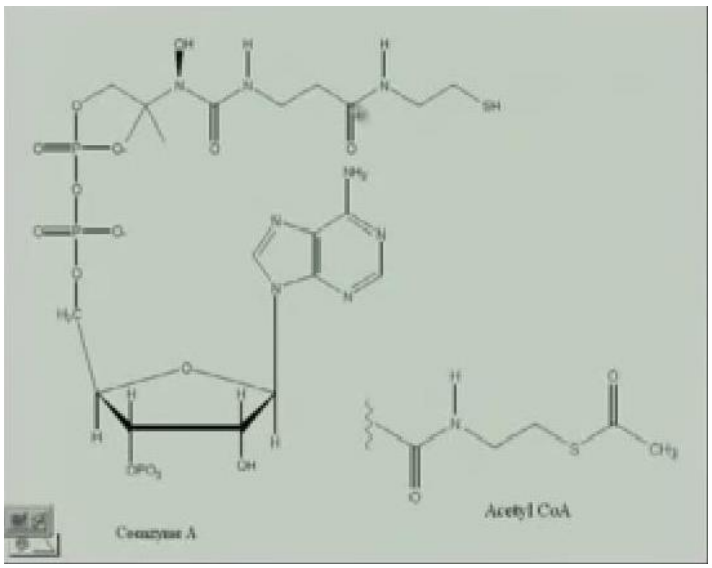

Now, this SH if acetated is called acetyl-CoA. Okay. So whenever we look at any metabolic

pathway you will see acetyl-CoA come into the picture a large number of times. Okay. But you have remembered it is derived from that vitamin and it is nothing but a phosphorylated ADP linked with and oxygen to the pantothenic acid that is linked to mercaptoethylamine.

This unit is coenzyme A if this thiol group is acetylated it becomes Acetyl-CoA and that's exactly as to how it is referred to in all of the metabolic pathways, Acetyl-CoA. Okay. So it is this acetyl part that is important and the rest you realize is derived from phosphorylated ADP vitamin pantothenic acid and mercaptoethylamine.

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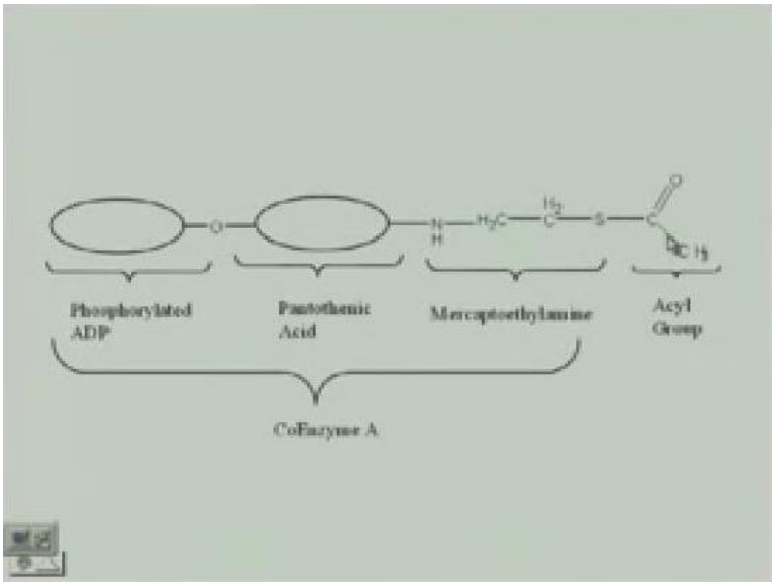

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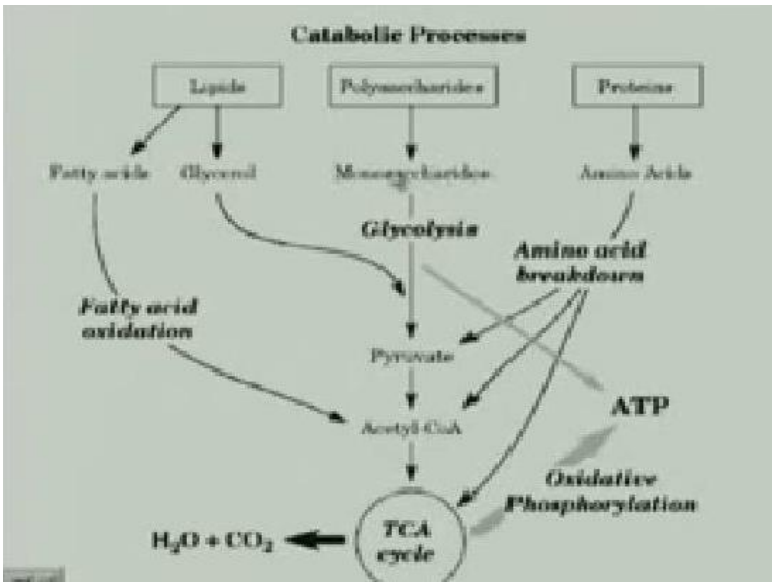

Now if you look at all the catabolic processes that occur, the glycolysis pathway will lead us to pyruvate. Pyruvate all of the breakdown getting to Acetyl-CoA. Acetyl-CoA being the

main part of the TCA cycle that ultimately breaks it's down to carbon dioxide and water. Okay. Now, this is obviously going to involve a large number of enzymes. Okay. And we will look at each of those enzymes and how they act.

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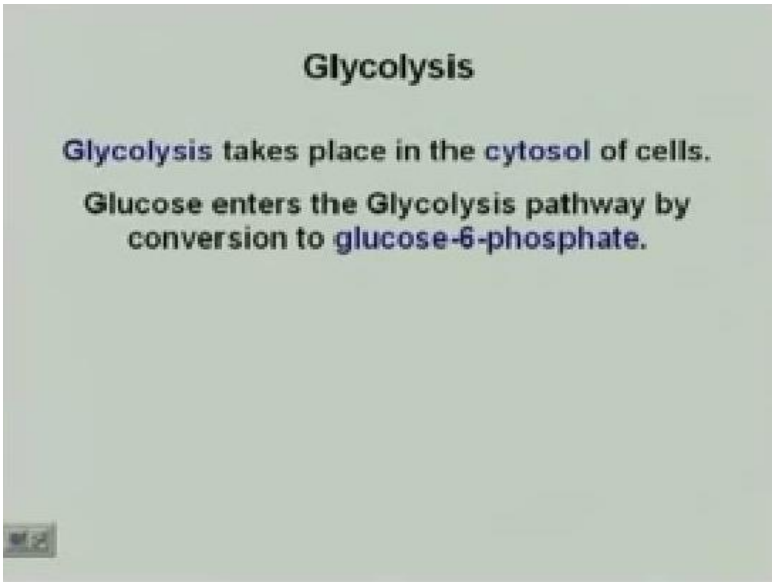

Okay. Now glycolysis, the process of glycolysis take place in the cytosol of cells and glucose enters the glycolysis pathway by the formation of glucose-6-phosphate. Now glucose-6-phosphate means that we are going to have a large number of steps that are actually going to get you to pyruvate. There are 10 steps involved, which means they are 10 enzymes involved and we have this form pyruvate.

And we have 3 steps that are regulated in the formation of pyruvate from glucose in the 10 steps that form the process of glycolysis. Okay. So we have glucose go to pyruvate in 10 steps and we have 3 of those steps that are regulated and it is occurring in the cytosol of the cells. If we do not have oxygen then this pyruvate forms lactate. Okay.

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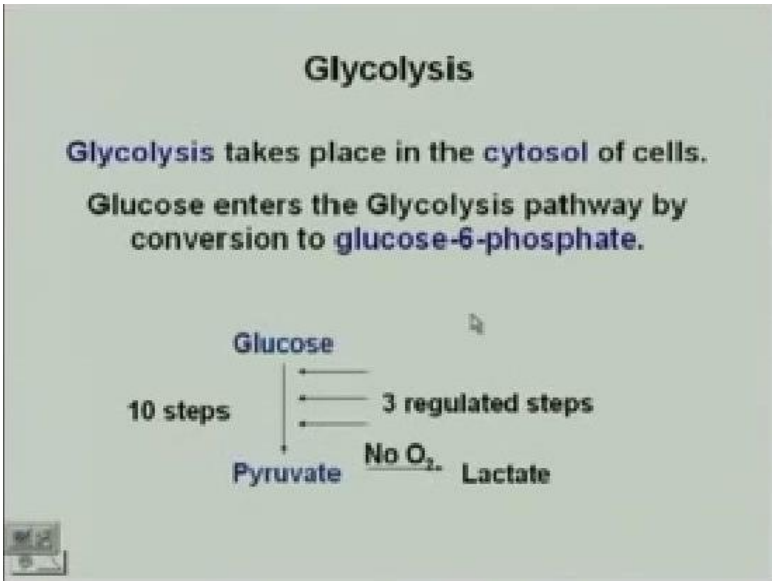

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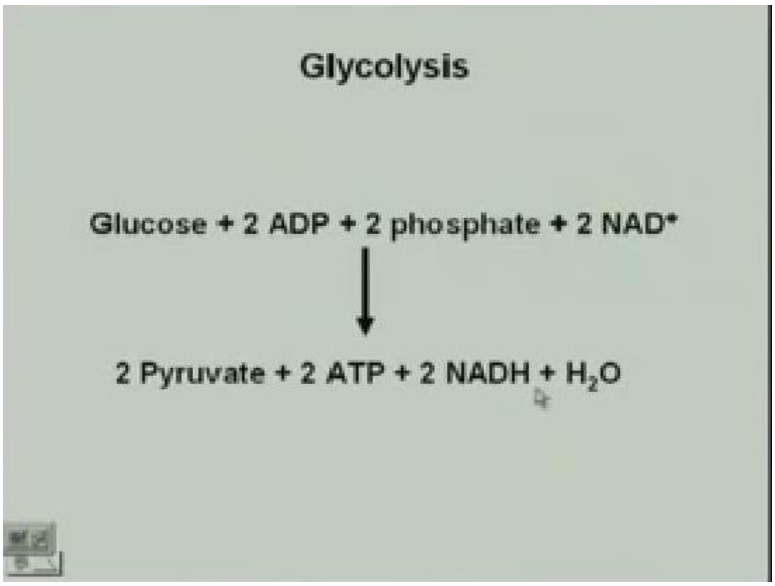

This is the overall reaction, we have glucose plus two ADP plus two pi that is phosphate, plus two NAD+ go to two pyruvates plus two ATP so you are generating ATP here also. Plus 2NADH + H2O. Okay. So we are going to look at each of these steps and see how you have ATP consumption, ATP production and finally we are going to take an account of where we are using it ATP where we are producing ATP. Okay.

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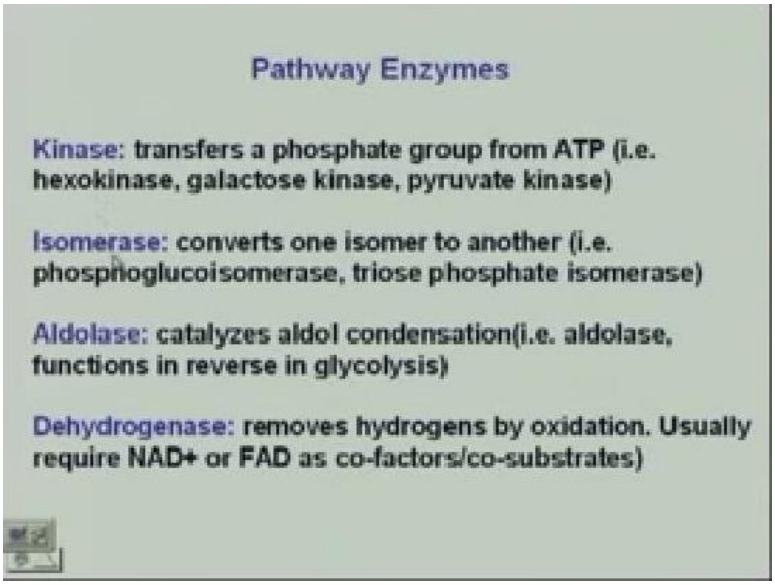

Now, before we get into that we have to see what enzymes are involved in the pathway, in the metabolic pathway. We have kinases, isomerase, aldolase, dehydrogenases. Okay. Kinases are a specific class of transferases and kinases are those that transfer of phosphate group. This we have looked at before or I have mentioned before where we can have we will see hexokinase will be a part of our glycolysis mechanism.

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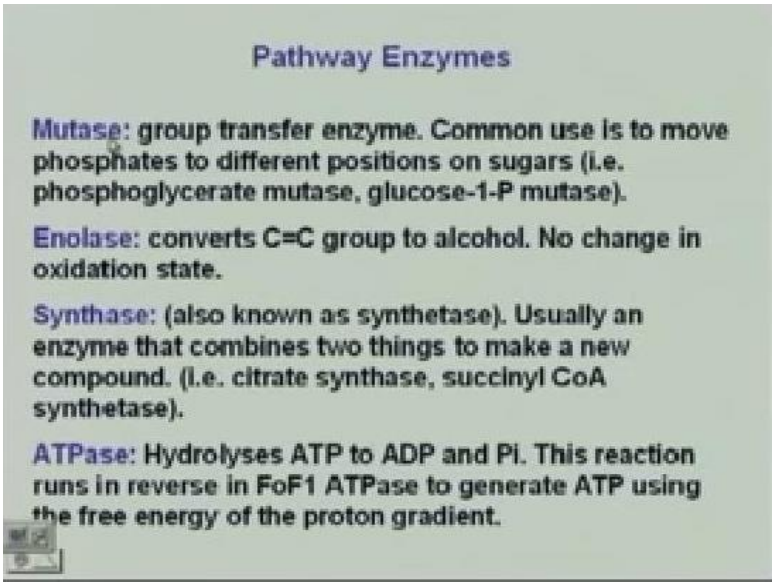

So we have kinases that transfer of phosphate group from ATP to a specific substrate we have isomerases, what isomerases going to do? They are going to convert, one isomer to another that is what the function of isomerases is. If we look at aldolases, they are going to catalyze aldol condensations. All of you know what aldol condensations are? So we have kinases, isomerases, aldolases, we have dehydrogenases.

Dehydrogenases, we have looked at also where we have the removal of hydrogen by oxidation. Okay. Then what are the other enzymes we have a few more. We have mutases that are actually group transfer enzymes. They transfer phosphate from one position of the substrate to another position. Okay. So say you have you have a glucose-1-phosphate to form a glucose-6 phosphate you will need a mutase. Okay.

So, the mutase is going to group transfer enzyme that is going to transfer say, the common news would be the transfer of phosphate to a different position and example being glucose-1-P mutase which will actually transfer the phosphate from the carbon 1 to 6. Okay. Forming from glucose-1 phosphate it will form glucose-6 phosphate in that case you would need mutase.

If you want an enolase you would covert a C double C group to an alcohol. Okay. Because you have to remember that when we are looking at each of these steps, we are finally going to breakdown glucose into carbon dioxide and water that is our final aim. Okay. So, we have to look at how this can be accomplished with this in the biological way with the use of these certain enzymes. We have synthase that is also known as synthetase, what does that do?

Just combine two molecules together, synthesis. We have ATPase, that are going to hydrolyses ATP to ADP and Pi and this is in reverse to ATP synthetase, what was ATP synthase doing? It was taking ADP and Pi and producing ATP, this hydrolyses ATP to ADP and Pi. Okay. So these are the pathway enzymes that are going to be used in every step of the way as we go on now.

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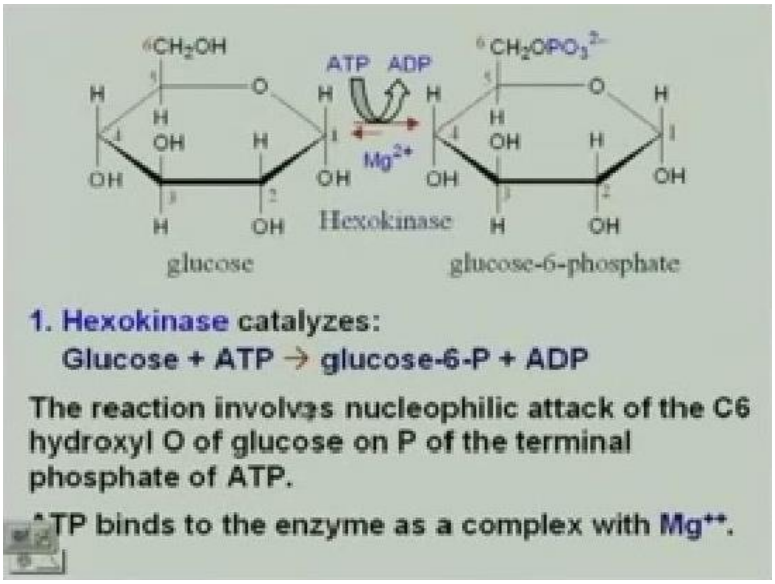

The first step, the step number one is the use of hexokinase. So, the first step in the glycolysis after you had broken down the polysaccharide to glucose. Okay. We now have glucose we are not going into how the polysaccharide is broken down or how even the other dietary nutrients have broken down like fats or amino proteins or whatever we are just going to look at the metabolism of carbohydrates. Just looking at the breakdown of glucose, the glucose breakdown will ultimately lead us to pyruvate and it will involve 10 steps.

This is step number 1 where from glucose we form glucose -6-phosphate that means that the sixth carbon atom is no longer OH it is now phosphorylated. It is phosphorylated by taking a phosphate from ATP in the event ATP becomes ADP. Okay. This is a coupled reaction we will look at the energetics of these steps. The glucose going to glucose-6-phosphate it has a delta g that is positive the ATP to ATP has a delta g that is negative.

A coupled reaction will give a favorable forward reaction to this and the reaction actually involves a nucleophilic attack of hydroxyl OH that is attached to carbone -6 to the gamma phosphate of ATP resulting in the formation of ADP and in this case the enzymes that is involved is hexokinase. Hexo means, it is going to act on a six-member sugar ring.

It is a kinase because it is helps in the transfer of the phosphate and the ATP binds to the enzyme as a complex with magnesium. Okay. Now, why would we have magnesium there? What is ATP? ATP has a large number of negative groups, right. There are three phosphates one after the another what magnesium does,

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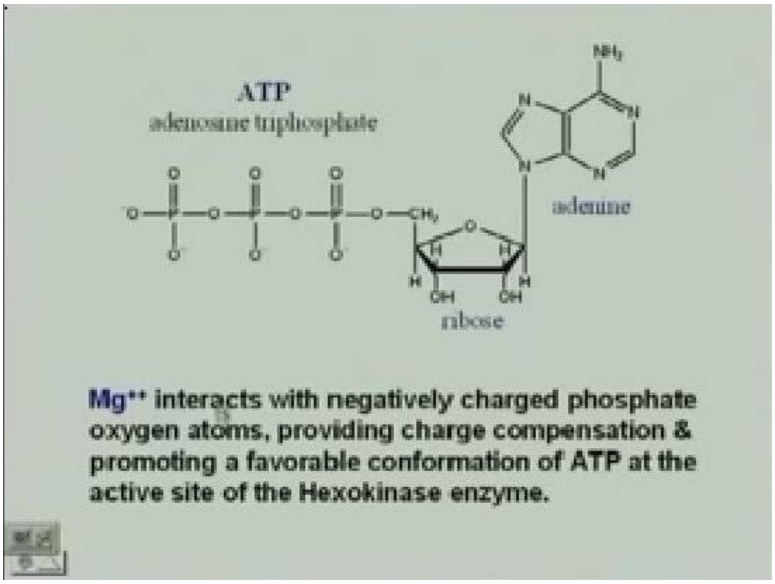

it interacts with the negatively charged phosphate oxygen atoms and provides charged compensation and also promotes a favorable confirmation of ATP at the active site of the hexokinase enzyme because you have to realize that when we looking we are not going to look at the details of how the enzymes is working but what we have here is we have magnesium in this set.

So that the ATP can be favorably interacted with the magnesium because we are finally going to break the final phosphate bond here. And what is going to happen? Where is the phosphate going to go? It is going to be attached to the sixth carbon atom of glucose in the formation of glucose-6 phosphate from glucose. Okay. So that is our step number 1. So step number 1, is glucose to glucose-6 phosphate the enzyme is hexokinase and you have ATP going to ADP and Pi and not Pi, Pi is attached to the 6-carbon so ADP.

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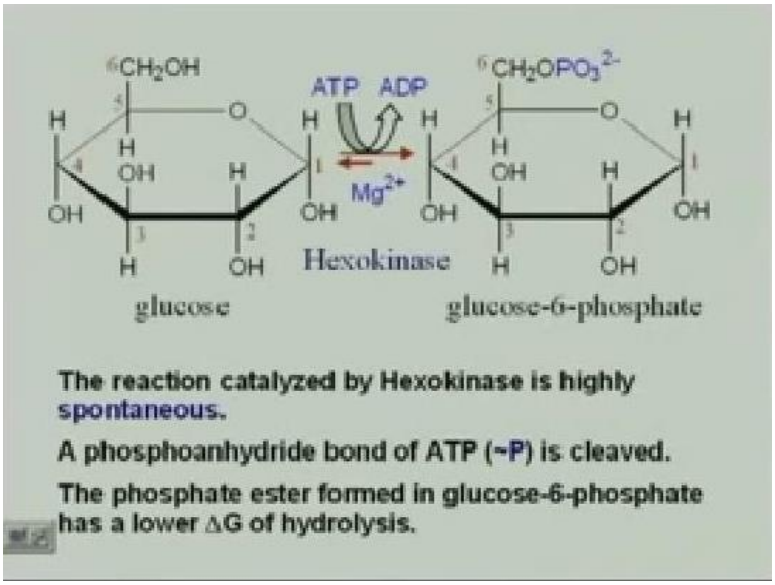

Okay. Now his reaction is highly spontaneous because the phosphoanhydride bond of ATP is cleaved. Okay. That is a high energy bond as we call it and the phosphate ester has a lower delta G of hydrolysis.

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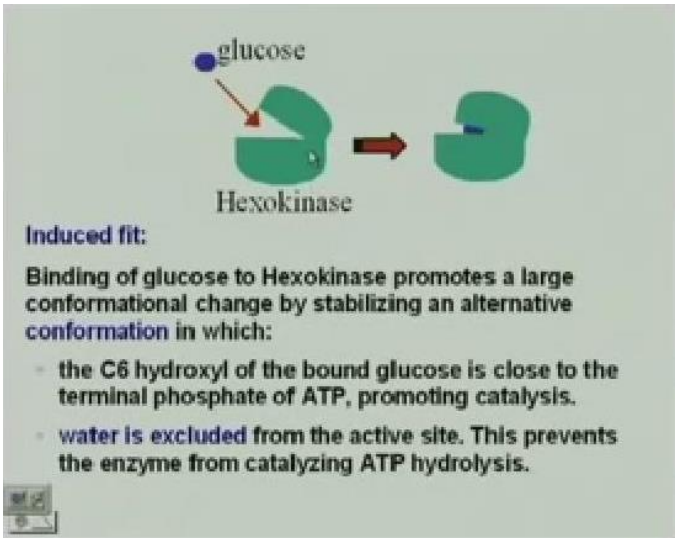

Now, what's happen in this case is you have what is called an induced fit. Remembered, we studied induced fit for the enzyme mechanism, the way they work. There is a lock and key mechanism and an induced fit mechanism. Usually, the enzymes of the glycolytic pathway have induced fit they are not ones that would just sit with the lock and key where at exact substrate would come and sit there.

It happens such that the binding of the glucose to this hexokinase promotes a conformational change in the protein in the enzyme rather and it stabilizes a different confirmation where by the ATP is positioned in such a way that the terminal phosphate can be transferred to the

substrate that is glucose. Okay. So, what happens is you normally would have hexokinase as soon as the sub substrate glucose comes into the picture there is the ATP that is positioned favorably so that the phosphate can be transferred from where from ATP to glucose.

Okay. And we also have this important point where water is excluded from the active site. Because what would happen if water would be there? ATP would be hydrolyzed. Okay. We do not want the hydrolysis of ATP; we want the phosphate to be transferred only to glucose. Okay. So once, the substrate comes to hexokinase it changes its confirmation so that it can accommodate glucose at the same time positioning ATP in such a manner.

So that it is favorably interacting and remember what happen? How did the reaction take place? It was the 6-OH the OH attached to the 6th carbon atom that actually went and attacked the phosphate.

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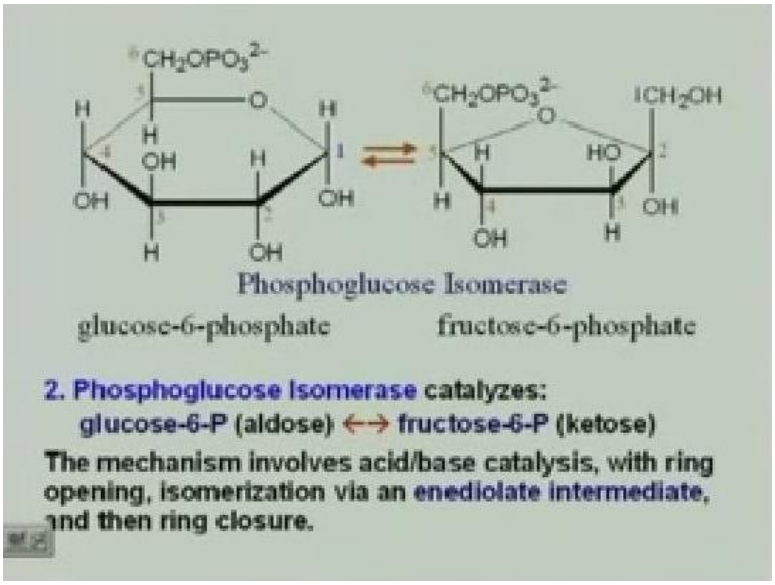

This is step number 2. In step number 2, you have glucose-6-phosphate go to fructose-6-phosphate. So what happens is, you have the formation of an isomer. So, the enzyme involved is an isomerase. Okay. What is this isomerase? It is phosphoglucose isomerase and what is happening here is the glucose-6 phosphate forms a fructose-6 phosphate where instead of the aldehydes now the aldose you have a ketose. Right?

You have the CH2OH up here now. Okay. So, we have glucose-6-phosphate go to fructose-6-phosphate, the enzyme involved is phosphoglucose isomerase and the mechanism actually involves acid base catalysis with ring opening via an enediolate intermediate and finally we

have ring closer. So we have an isomerase. So what was the first step? First step was glucose to glucose-6-phosphate. The next step is glucose-6-phosphate to fructose-6-phosphate.

What are the enzymes required? In the first step, we need a kinase in the second step we need an isomerase. Okay.

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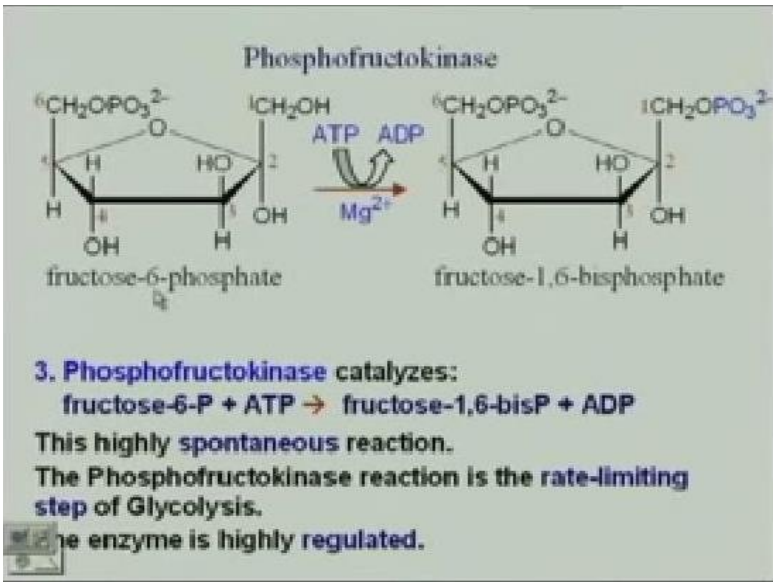

Third step, look at what is happening? We had fructose-6-phosphate go to fructose-1 6-bisphosphate. So what is happening? I have the addition of another phosphate at the one position so what is the enzyme that I need, a kinase. That is what you have to identify. You have a process you know the substrate, you know the product what is the enzyme involved? You know in this case that you have prepared fructose-6-phosphate from where? From glucose-6-phosphate.

And it was just an isomerization so you needed an isomerize, you are going now from fructose-6-phosphate to fructose-1,6-bisphosphate. Which means you have added another phosphate, which means the breakdown of another ATP, which means that you need a kinase and what kind of an kinase do you need? A fructose kinase, why a fructose kinase? Because you have a fructose and you are adding a phosphate to a fructose, that's it.

So you have fructose-6 phosphate that is going to from fructose-1,6 bisphosphate and this process is actually a highly spontaneous and this phosphofructokinase reaction is the rate limiting step of glycolysis we will see how that is later on when we studied the whole process. And this enzyme is extremely tightly regulated. There are some processes when we

go through the whole system you will see.

There are some processes in the glycolysis steps of reactions that are reversible there are some that are irreversible. Okay, means you cannot once glucose has got into glucose-6 phosphate there is no way it is going to get back to glucose. Okay.

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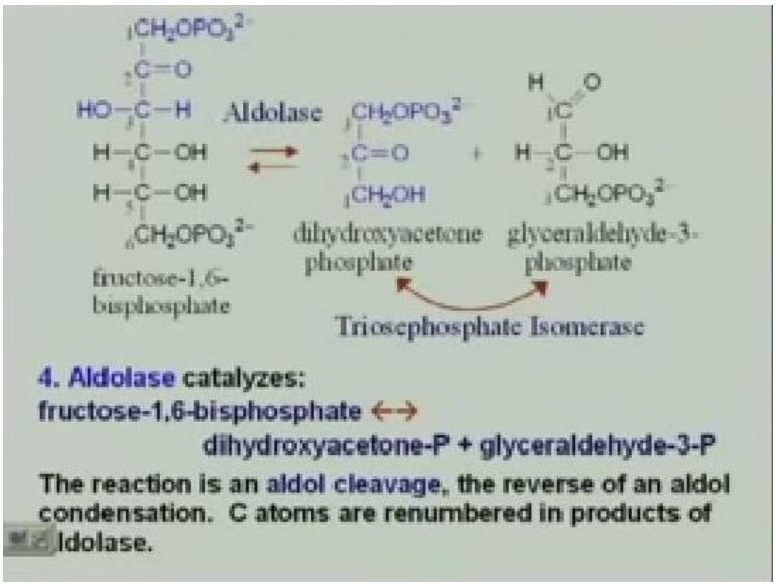

The next step, what we have here now is we have fructose-1,6-bisphosphate. Now what happens to fructose-1,6-bisphosphate if you look at the previous we have it in the ring formation here. Okay. In the next step, there is going to be the breakdown of this how many carbons do we have here 6. Glucose has 6 carbons, we have 6 carbons here. We have 1, 2, 3, 4, 5, 6 what is going to happen now is in the next step first there is ring opening.

Then the six membered ring is going to breakdown into two three member rings. Okay. Because you have remembered that finally we have to get to carbon dioxide and water. Okay. Unless, we start chopping up it is not going to be possible. So we have our glucose. So now we are going to go to a step where we are going to break the fructose-1, 6-bisphosphate into 2,3 carbon units.

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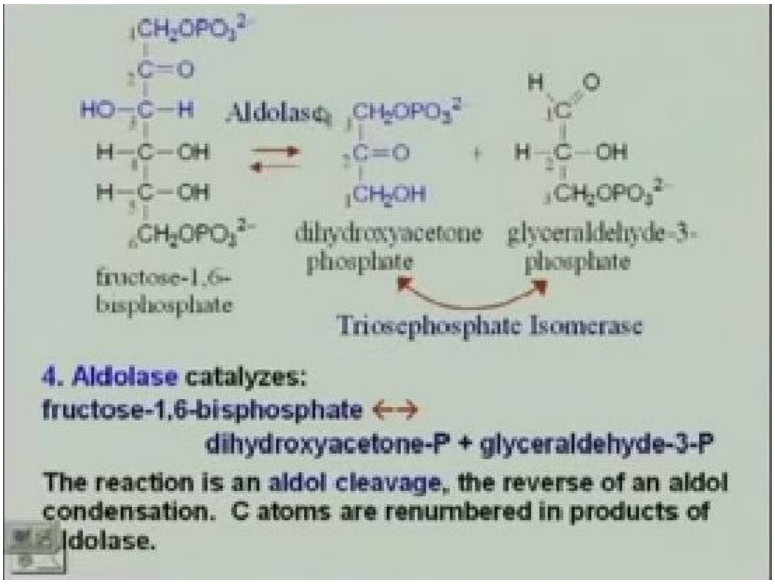

The process here is a reverse of aldol condensation. It is aldol cleavage we have here the fructose-1, 6-bisphosphate. Okay. Which is now, not in its ring form but it in its open form where you can see the ketone, it is the ketose, right. So it has to have this C double bound O there are two phosphate attached to it now.

One, at the one position and one at the 6 position, fine. We have now an aldolase which is a reaction that is going to involve aldol cleavage and there is going to be a reverse of aldol condensation which means that you are going to have a break at this position here where we are going to have the formation of 2, 3 carbon units one of them is dihydroxyacetone phosphate. If you look at this it's this is acetone CH3, CO, CH3 as acetone, right. This is acetone CH3.

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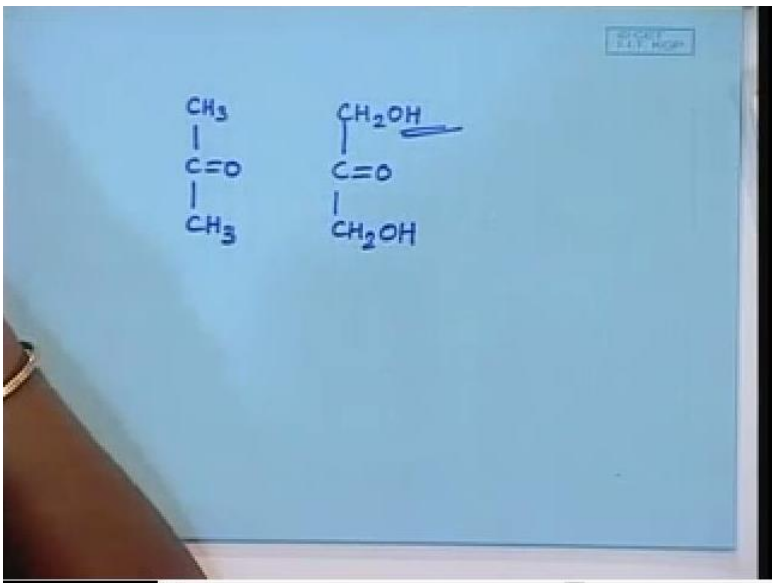

This is acetone. Dihydroxyacetone, is that right now if we have dihydroxyacetone phosphate we have phosphorylated one of these. Okay. So, that is exactly what we have.

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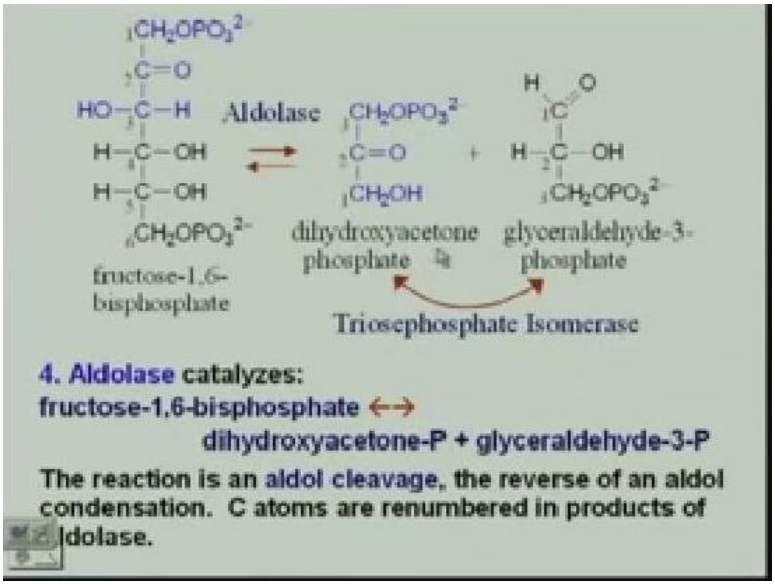

Here we have dihydroxyacetone phosphate and we have glyceraldehyde-3-phosphate. This is glyceraldehyde you recognize this CHO of aldehyde. The CH OH and CH2OH which would have been here but we now have it phosphorylated. So, the phosphor now is distributed in the 2, 3 carbon units, right. So, this is the ketone form. This is the aldehyde form. What are these? Isomers. So you can go from one to the other by what enzyme? An isomerase.

What is this isomerase going to be named? It works on a three carbon unit so what triosephosphate isomerase. Okay. So, you see if you see at the nomenclature it's actually very simple all you have to know is what the substrate are and what the products are. In this case, we have an aldolase with the reaction being an aldol cleavage. We have the formation of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate so this step is where you have the breakdown of the 6 membered or the 6 carbon unit. Okay.

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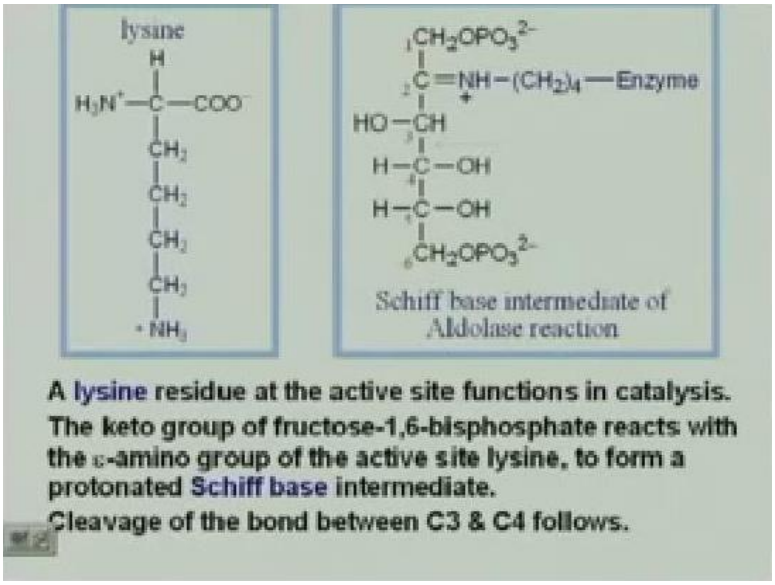

Now, if you look at how the 6 carbon unit or the aldolase actually works there is a lysine residue, Where? Where is this lysine residue? In the enzyme aldolase. Obviously, it has to be there because the aldolase is what is acting on the substrate what is a substrate? The substrate is fructose-1, 6-bisphosphate that is your substrate what are you products? Your products are dihydroxyacetone phosphate and glyceraldehyde-3-phosphate.

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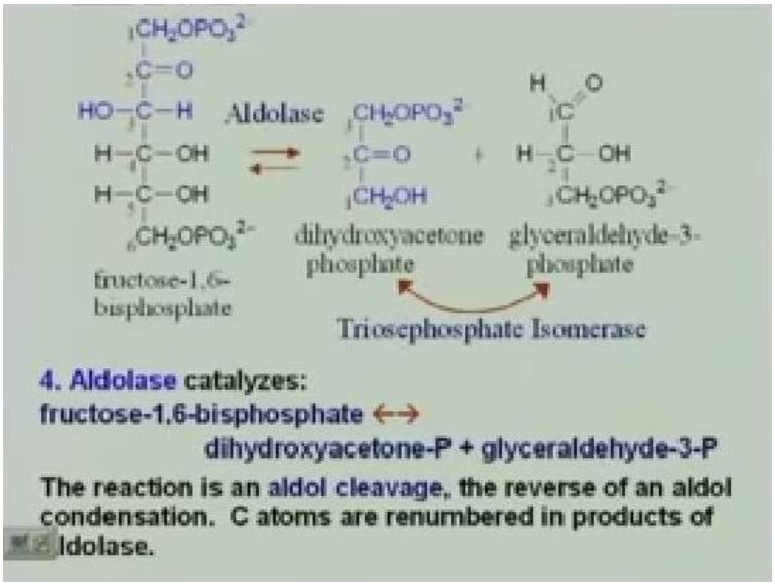

Okay. So, you have glyceraldehyde-3-phosphate and dihydroxyacetone phosphate which later on you will see it's written as DHAP and G3P.

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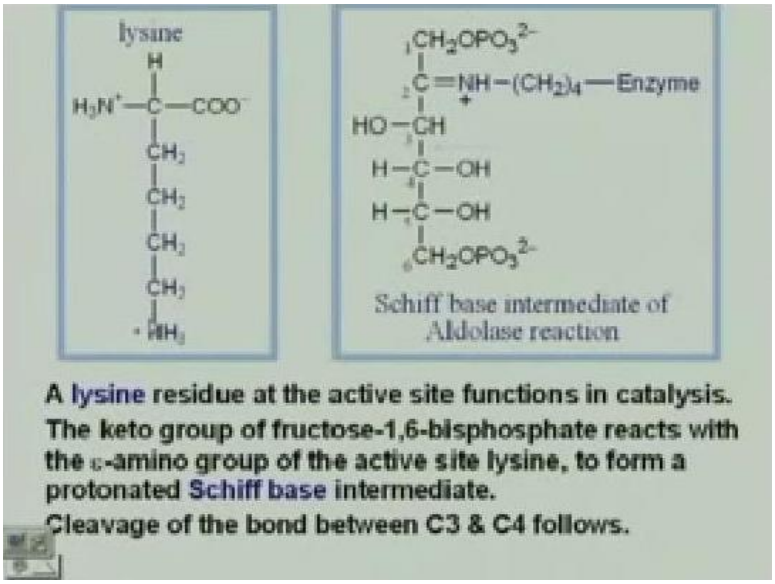

Okay. So we have a lysine residue in aldolase that is present at the active site. What this lysine does is the keto group of fructose-1,6-bisphosphate reacts with the amino group of the lysine. Okay, and it forms the protonated Schiff base then there is the cleavage between carbon atoms 3 and 4. We are not going into details of all this but what you have to know is you have the breakdown.

The breakdown from the 6 carbon to the 3 carbon by the enzyme aldolase, the enzyme has a lysine residue that interacts the alpha the epsilon the amino group of the lysine interacts, where is this lysine? It is in the active site of aldolase. It interact with the keto group of fructose-1, 6-bisphosphate and it breaks it up. Okay. There is cleavage between carbon atom 3 and 4.

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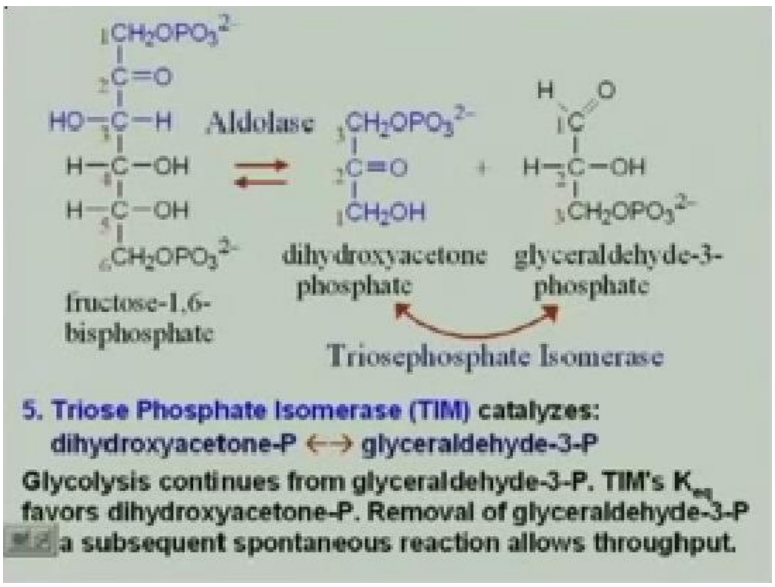

Okay. So, this is just the triose phosphate isomerase that is going to be interconverting dihydroxyacetone phosphate and glyceraldehyde-3 phosphate. So in this protein, actually is pretty interesting in its structure also but we are not going to go into the details of that it's called TIM where it has TIM barrel actually associated with it. Okay.

We have the glycolysis that is going to continue from glyceraldehyde-3 phosphate. Now, the equilibrium constant now so what is the triosephosphate isomerizing, what is it doing? It is converting dihydroxyacetone phosphate to glyceraldehyde-3-phosphate. So, this is an isomerization reaction. The equilibrium constant is such that it favors dihydroxyacetone phosphate. Okay.

So, this is favored but the glycolysis steps the further steps actually continue from glyceraldehyde-3-phosphate. So, if the equilibrium shifts to dihydroxyacetone phosphate what does it mean? It means, I may not have sufficient glyceraldehyde-3-phosphate to continue with the glycolysis.

I will repeat that once more. We have the enzyme triose phosphate isomerase. The equilibrium constant of triosephosphate isomerase is such that it favors dihydroxyacetone phosphate. Okay. When you have equilibrium, your equilibrium either shifts to the left or the right to the reaction or the products. In this case dihydroxyacetone phosphate is preferred based on its equilibrium constant.

But the process of glycolysis will only continue with glyceraldehyde-3-phosphate. So what has to be done? Your equilibrium has to be shifted to the right. So what is usually done is this glyceraldehyde-3-phosphate as soon as it if formed it is then utilized in the next step. So, if it is utilized in the next step what happens? Your equilibrium is shifted to the right which means some more of the dihydroxyacetone phosphate has to be isomerized to glyceraldehyde-3-phosphate. Its regulated, you see how it is regulated. Okay.

The equilibrium is such that it shifted to the left. But if you require the breakdown of the glyceraldehyde-3-phosphate you break it down. What happens then? Your equilibrium is moved in such a way that the dihydroxyacetone phosphate forms glyceraldehyde-3-phosphate. Okay. Zakir

So then what happens then there is removal of the glyceraldehyde-3-phosphate and the subsequent spontaneous reaction. Okay. But it doesn't happen as it is, the equilibrium shifted to the left but with the removal of the product that is glyceraldehyde-3-phosphate it obviously moves to isomerize more of the DHAP.

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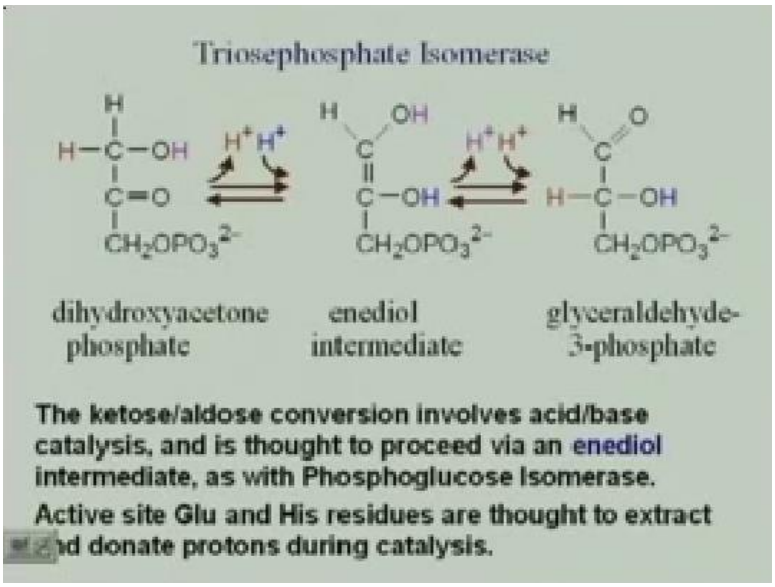

Okay. Now, this is how it actually this is the intermediate that you have which is the enediol intermediate where you have the ketose aldose conversion which actually involves an acid base catalysis and it the isomerase that actually takes place is phosphoglucose isomerase or rather what happens here is? When we have dihydroxyacetone phosphate what are we talking of? We are talking of the ketose, right.

When we have the glyceraldehyde-3-phosphate we are talking of an aldehyde, right. So, there has to be an enediol intermediate that is going to take you from the ketose the aldehyde it's similar to fructose and glucose. What is glucose? Glucose is an aldose and fructose is a ketose you have an isomerization there also, right. That takes you from glucose to fructose it's exactly the same thing but here you are working on a 3 carbon atom instead of a 6 carbon that's the difference.

Okay. So, we have the dihydroxyacetone phosphate we have the glyceraldehyde-3-phosphate and these are formed from a enediol intermediate so when we are talking of the triosephosphate isomerase. We are talking of this equilibrium, right. It is this equilibrium that we are talking about. So, the equilibrium actually will shift to left side but with the removal of the glyceraldehyde-3-phosphate. What will happen?

There will be more formation of the glyceraldehyde-3-phosphate that will eventually be used in the other steps of glycolysis. Okay.

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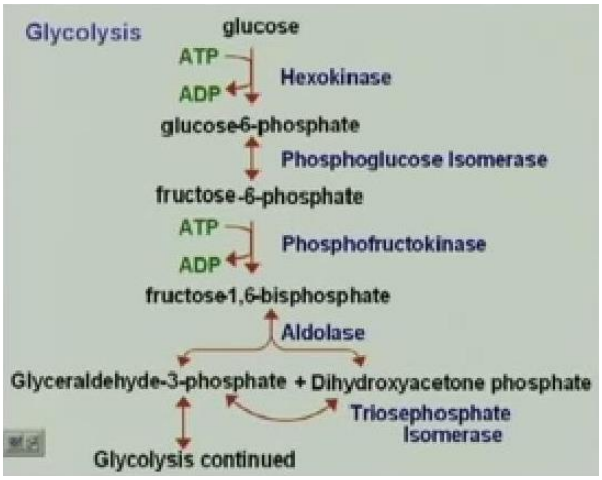

So, this is what we are going to do for glycolysis for today because there are some other discussions that I want to make regarding hexokinase. Okay. Now we have glucose so these are the steps. We have done up till we just broken down the 6 member ring let's put it that way. We have broken down the 6-member ring, we will see now what is going to happen to the 3 carbon ring. Okay.

The 3 carbon not the ring, the 3 carbon system now the 3 carbon system will eventually get into the tricarboxylic acid cycle and that tricarboxylic acid cycle will ultimately produce carbon dioxide and water. So you will have it broken down. But what we started off with was we started off with glucose in the first step we had glucose go to glucose-6-phosphate, right. Now, since it took up a phosphate it required the breakdown of an ATP it's just summarizing the steps that we have done so far.

So we have glucose to glucose-6-phosphate that required the breakdown of an ATP and the enzyme was hexokinase step number 1 in our glycolysis. Step number 2, was the formation of fructose-6-phosphate from glucose-6-phosphate which is nothing but an isomerization where we have an aldose go to a ketose. The enzyme is a phosphoglucose isomerase. Okay. Step number 3, where we have our fructose-6-phosphate form fructose 1,6-bisphosphate what is happening there?

I am adding another phosphate in the addition of a phosphate I have to break down another

ATP and I need another kinase, right. So I have fructose-6-phosphate that has taken up, will take up phosphate from the ATP into forming fructose-1, 6-bisphosphate with the help of phosphofructokinase.

The next step now is the breakdown of the 6 carbon to 2, 3 carbons and we have aldolase. Aldolase is going to break down fructose-1, 6-bisphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate and if you noticed here it is glyceraldehyde-3-phosphate that will allow the glycolysis to continue. So we need an isomerase that is going to transform our dihydroxyacetone phosphate to glyceraldehyde-3-phosphate. Okay.

Now in the steps that we wrote here if you noticed the steps that involve the addition of the phosphate are irreversible. Okay. So glucose forms glucose-6-phosphate with the formation of AT by the breakdown of ATP to ADP that step is one way. Okay. That means once glucose enters the cell and forms glucose-6-phosphate it is trapped into being broken down. Okay. If it is not required to be broken down then glucose will actually go for storage as glycogen.

Okay. But once it forms glucose-6-phosphate it has to be broken down. Okay. So if we look at the features actually of hexokinase, this enzyme is inhabited by its product. Okay. Now biochemically that makes absolute sense because if hexokinase is broken down by glucose-6-phosphate, is inhibited by glucose-6-phosphate then what is you are going to do? It is going to prevent the formation of glucose 6 phosphate from glucose.

So it is going to be regulated in nature so as soon as there is sufficient glucose-6-phosphate or sufficient glucose to be broken down it will stop itself from acting because you do not want all the glucose to form glucose-6-phosphate because once the glucose-6-phosphate is formed it has to be broken down it's end of the glycolytic cycle there is no way it can go back. Okay.

But if hexokinase is inhibited then what happens glucose can still go someplace else and be stored as glycogen in the liver say but once hexokinase has acted on glucose there is no way it can go back, right. So glucose-6-phosphate acts as an inhibitor to hexokinase so that further glucose breakdown is not possible.

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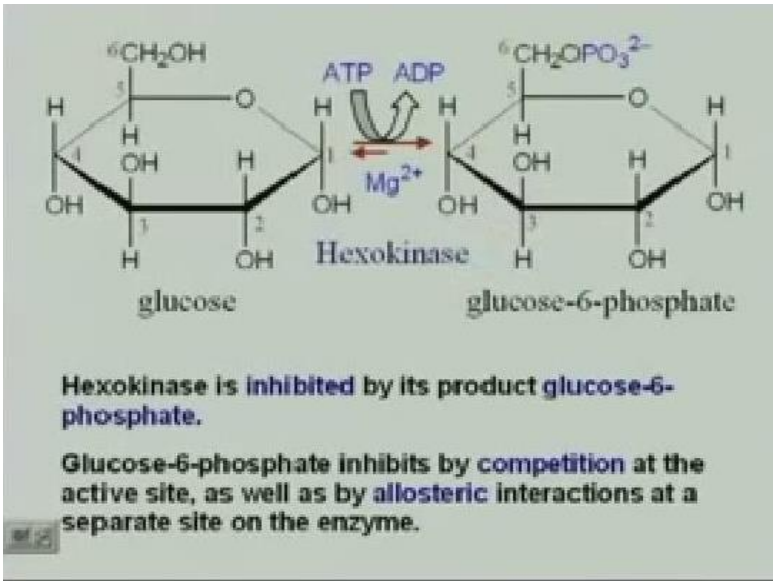

Here, that's exactly what I was saying. Hexokinase is inhibited by its product glucose-6-phosphate and the way it inhabits hexokinase is by competition at the active site as well as by allosteric interactions at a separate site on the enzyme. What does it means? It means that it will change the active side confirmation to such an extent that it will not be able to bind the substrate glucose.

And remember I showed you a rough cartoon of hexokinase where it acts it has an induced fit as soon as glucose comes into the picture right and then the ATP is positioned in such a manner that it forms glucose-6-phosphate, okay. But if glucose-6-phosphate actually inhibits hexokinase then it will either inhibit by sitting at the active site itself or it could inhibit at another positon where it could affect the active side so that the substrate cannot bind.

So once the substrate cannot bind, it means that the enzyme is inhibited. If the enzyme is inhibited what's happens? Then the product will not be formed. If the product is not formed in this case it means that glucose breakdown does not occur as simple as that. Okay. So it is extremely tightly regulated. So, the cells trap glucose by phosphorylating it.

So once glucose is phosphorylating, it's trapped. It has to be broken down, right. But this can be prevented if this glucose-6-phosphate inhibits the enzyme. If hexokinase is inhibited and glucose cannot form glucose-6-phospate fine so the product inhibition of hexokinase ensures that cells will be continue to accumulate glucose from the blood. Okay. If glucose-6-phosphate within the cell is in sufficient quantity. Okay.

Because once your glucose-6-phosphate is in sufficient quantity it means that this can continue in the glycolytic steps but if you need it then only glucose is broken down, unnecessary glucose is not broken down in the cell. Okay. It is only when the glucose carbohydrate metabolism is required is glucose broken down in the cell because once this first step of the glycolytic cycle takes it is irreversible. Okay.

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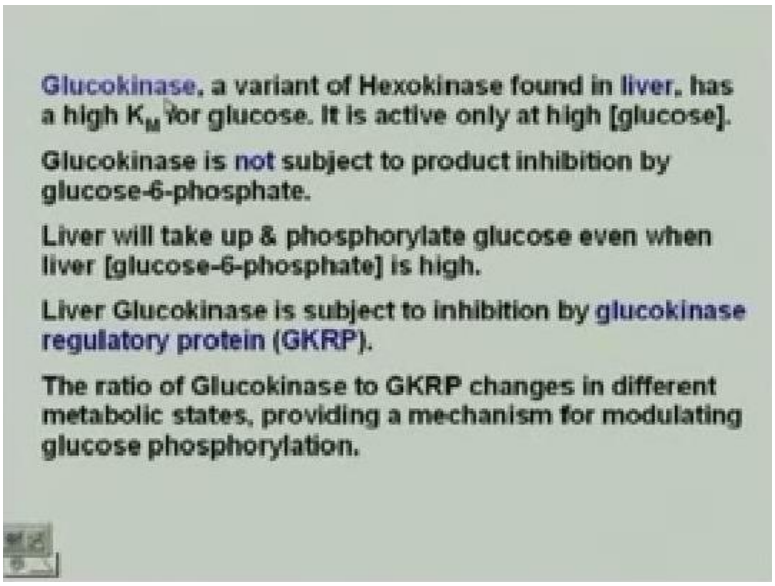

Now, glucokinase which is a variant of hexokinase is found in the liver. This has a high KM value all of you know what the KM value is now, Michaelis-Menten constant for glucose and is active only at high glucose concentrations. So the glucokinase acts at high glucose concentrations so when there is a high level of glucose concentration, the glucokinase enzyme comes into the picture the glucokinase enzyme is not subjected to product inhibition by glucose-6-phosphate.

What does that mean? It means that glucokinase will act on glucose. Why? It is not inhibited by glucose-6-phosphate. So any glucose that comes into contact with glucokinase will have a phosphate transfer to it, as simple as that. Why is that? Because the glucokinase is not subject to product inhibition by glucose-6-phosphate so glucokinase actually works at high glucose concentrations.

So when there is high glucose, glucokinase will transfer of phospate to the glucose at high glucose concentrations. Now, liver will, that means where is this glucokinase found? It is found in the liver what it does is it takes up and phosphorylate glucose even when the glucose-6-phosphate is high. Why is that? Because it is not inhibited by glucose-6-phosphate,

glucose-6-phosphate has nothing to do with glucokinase.

If it was hexokinase then glucose-6-phosphate would have inhibited the enzyme but glucose glucokinase is not inhibited by glucose-6-phosphate so it is immaterial whether glucose-6-phosphate is high or low. It doesn't matter.Liver glucokinase is actually inhibited by the glucokinase regulatory protein. What does that do? You have to remember that when we are considering an inhibition it is to prevent the enzyme from forming its product. Okay.

Now naturally, there are certain steps that you would not want the product to be formed for example in the hexokinase set. In the hexokinase step you realize that breaking down of all the glucose present is extremely an unnatural. Okay. And you would not obviously want that to happen because you want some stored glucose. Okay. So the product inhibition in the case of hexokinase is an extremely cleaver way of preventing the glucose from being broken down.

But in the case in the liver the glucokinase is present not hexokinase, it's a variant of hexokinase but it acts only when the glucose level is very high. Okay. The liver glucokinase is subject to inhibition by another protein and the protein will regulate when glucokinase is going to act. Okay. So it is not inhibited by a product it is inhibited by another protein that is going to regulate the action of glucokinase as to when it should be acting on a glucose and when it should not be acting on a glucose. Okay.

This glucokinase regulatory protein actually would work then when we have high glucose. Okay. Because that I mean when we have low glucose because high glucose would actually have glucokinase work on it.

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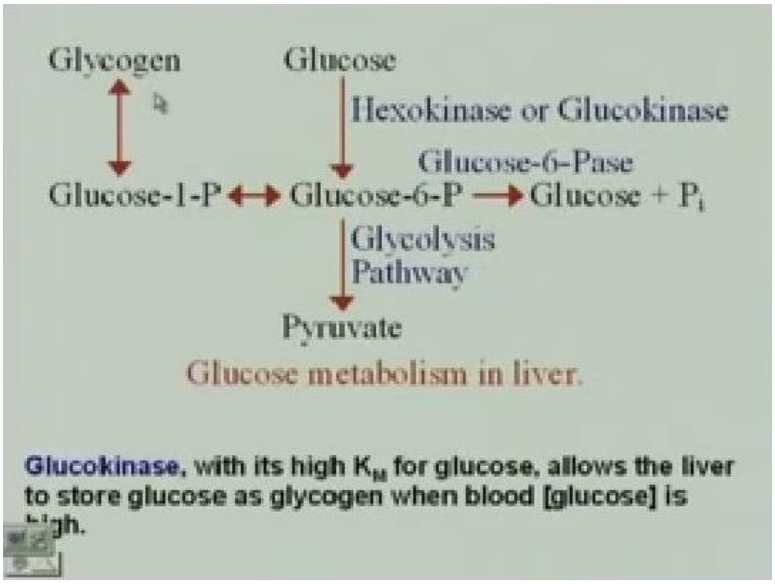

So what happens in this case glucokinase with its high KM value for glucose allows the liver to store glucose as glycogen when the blood glucose level is high. Okay. So when the blood glucose level concentration is high it will allow the storage of glucose as glycogen. Now what happens is we have therefor this is what we would have in the liver where we would have the glucose acted upon by the glucokinase to form glucose-6-phosphate.

It would not be broken down or rather hexokinase would be inhibited by glucose-6-phosphate but glucokinase. Okay. And then we would have from the glucose-6-phosphate once this is formed in the cell it is tracked.

Okay. So, we have a regulatory mechanism that actually works here. Then glucose-6-phosphatase catalyzes hydrolytic release of Pi from glucose. So what happens if you look at the steps here you had glucose-6-phosphate formed but there is another protein that is glucose-6-phosphatase that can release the phosphate but this happens only in the liver. Okay. Where the glucose can be stored right in the other cells.

What happens is see the enzymes glucokinase and glucose-6- phosphatase are both found in liver but not in most in other body cells. Why would it not be in most other body cells? Because then it would not get the energy. Glucose has to be broken down if all the steps prevented the breakdown of glucose then obviously you would not get energy but in the liver the excess glucose is stored as glycogen. Okay.

Because there are, these specific enzymes that are present in the liver that can form the

glycogen from glucose. Okay. So what we actually looked at, is we looked at the first few steps of glycolysis where what we have ultimately come to today is we have broken down the six membered ring of glucose to 2, 3 member rings. Now, we are going to see how those 3-members rings or rather glyceraldehyde-3-phosphate actually will fall pyruvate.

Okay. And finally how that in anaerobic condition goes to lactide or goes through acetyl-CoA, to the tricarboxylic acid cycle where it is finally broken down. We will see that in our next class. Thank you.

Continue our lecture on glycolysis we started off yesterday where we considered the different metabolic processes that actually go on in the body and if we look at the first slide here

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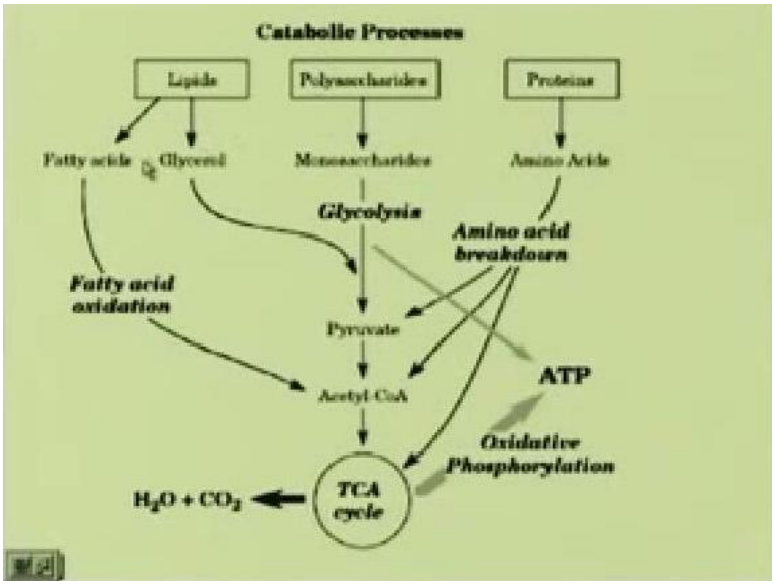

The overall catabolic processes, look at the breakdown of lipids polysaccharides and proteins. Now the breakdown of lipids gets into components of fatty acid and glycerol. The polysaccharides break down to monosaccharides and the proteins breakdown to amino acids. Now in the anabolic processes we have these broken down amino acids and other factors that actually got on into building up the other micromolecules that are required for our bodily function.

Now what we are interested in is the breakdown of the monosaccharides particularly the process of glycolysis that takes glucose and breaks it down into pyruvate and later on we will see how this pyruvate then gets on into the tricarboxylic acid cycle or the Krebs cycle to finally get to water and carbon dioxide. And an off shoot of that is the production of ATP

where we studied oxidative phosphorylation in the different complex process that require number of electron transfer co-factors as well as certain enzymes.

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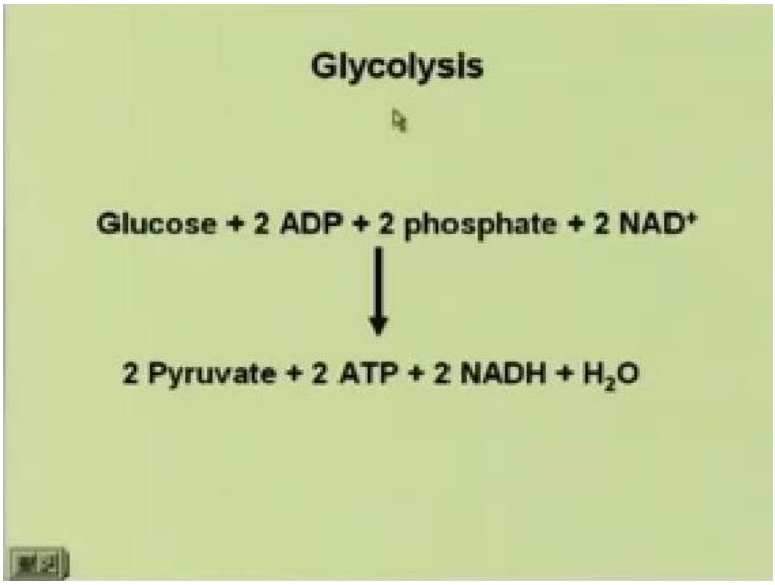

The overall equation for glycolysis is the breakdown of glucose into 2 pyruvates. Now, what we have here is we see how ATP is produced we will see as be continue with all the steps we will see how ATP is produced in some of the steps but in the first set that we did we found ATP consumption.

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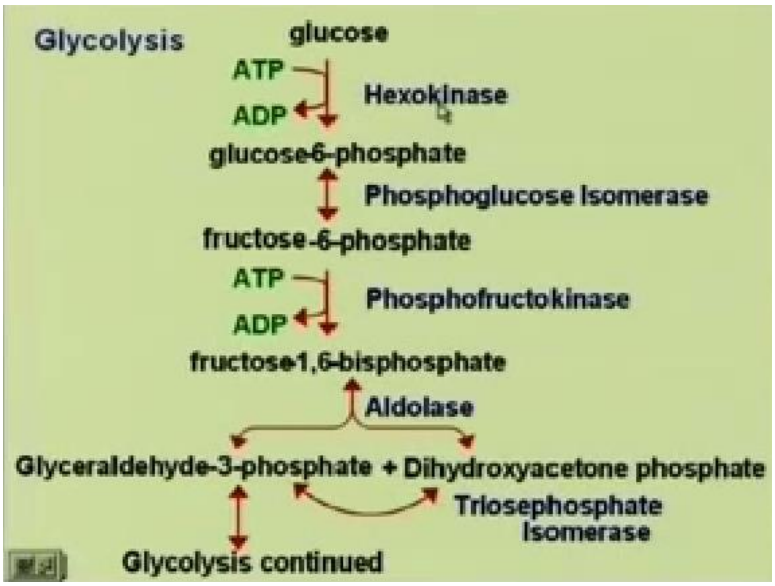

This is the number of steps that we considered in our last class we had glucose going to glucose-6-phosphate, the enzyme being hexokinase. We have to remember that a kinase is a transferase that transfers a phosphate group. So in this process we had the breakdown of ATP to ADP and the phosphate was transferred to the glucose this then went on to form the ketose

from aldose.

So we have fructose-6-phosphate that had the enzyme phosphoglucose isomerase acting on it because this is an isomer the aldose and the ketose that we have here. The fructose-6-phosphate then went on to form fructose 1, 6-bisphosphate where we required another ATP to be broken and the enzyme used there was another kinase but in this time it was phosphofructokinase.

After this particular step we have aldolase come into the picture. Aldolase is what actually breaks up the 6 carbon members ring into 2, 3 carbon members rings, getting oxidized in this case? Glyceraldehyde is getting oxidized to form glycerate. What is get introduced? NAD plus is get introduced to NADH and the enzyme is the dehydrogenase.

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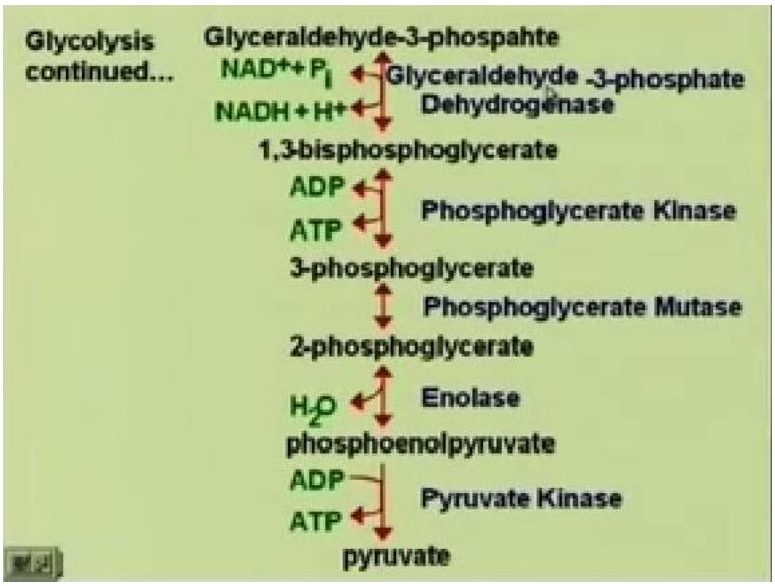

Okay. So we have glyceraldehyde-3-phosphate go into glyceraldehyde-3-phosphate dehydrogenase and in the event it forms 1.3-bisphosphoglycerate. Now, that you have formed the glycerate you have to lose the phosphate to form the pyruvate. Okay. So the first step in the loss of the one of the phosphates is a phosphoglycerate kinase that is going to lose the phosphate that is attached to the carboxylic first carbon atom of the glycerate. Okay.

And you have 3 phosphoglycerate formed. Fine, after you form 3 phosphoglycerate there is a mutase reaction which shifts the phosphate (()) (57:33) from the third carbon to the second carbon. So you have 3 phosphoglycerate form 2 phosphoglycerate then another enzyme that helps in the dehydration is enolase that results in phosphoenolpyruvate. So after

phosphoenolpyruvate, you have the enolic form pyruvic acid which then forms pyruvate after the loss of the phosphate and who takes up this phosphate ADP.

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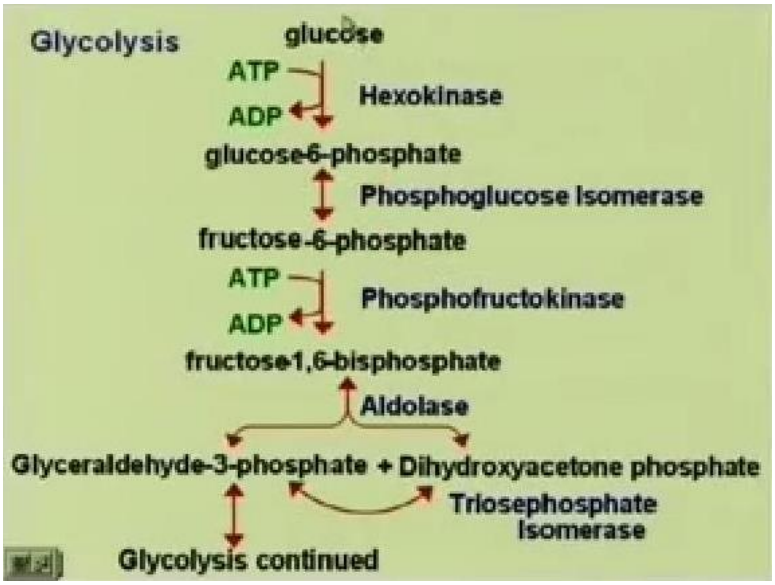

Okay. So that comprises the whole series of steps where glucose is broken down into pyruvate. Okay. Now, what we have to see is we have a do a balance of energy. Okay. We have to see how many ATPs are taken up? How many ATPs produced? And to see whether the actual breakdown of glucose is giving us any energy at all?

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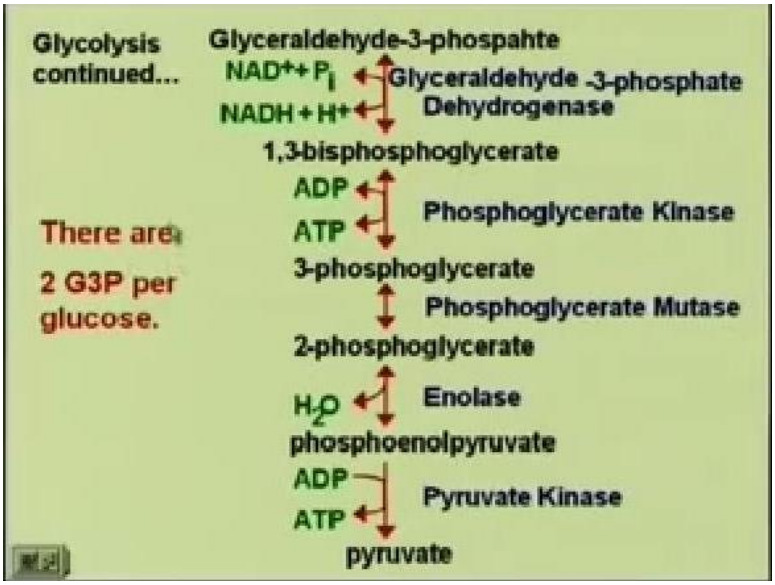

Okay. There is one thing that we have remember at per glucose there are 2 glyceraldehyde-3-phosphates. Okay. Because what is the previous step we have two of these. Okay. And in the triosephosphate isomerase we know that the equilibrium is shifted to this side because this is being consumed in the further steps.