Biochemistry -I
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Lecture # 25
Metabolism - I

The final chapter of this course deals with metabolism. In metabolism we need certain nutrients. We are going to learn about especially in the metabolism of carbohydrates how the food that we are taking in is broken down.

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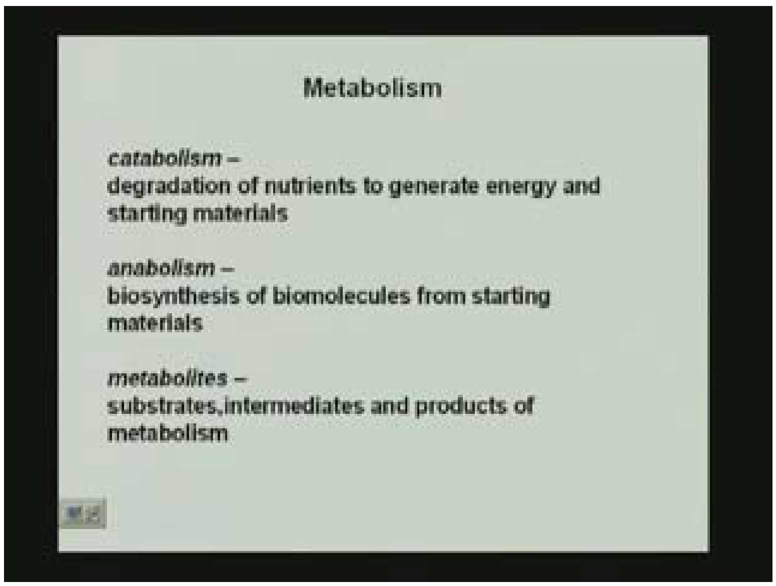

Metabolism actually as you have learnt from your school days is comprised of catabolism which is the degradation of nutrients to generate energy and starting materials which is what we are going to do basically and anabolism is the biosynthesis of biomolecules from starting materials which also actually happens in our body because we create proteins and proteins are synthesized in the body which is a process that would require anabolism. Catabolism is the breaking down of the nutrients i.e. 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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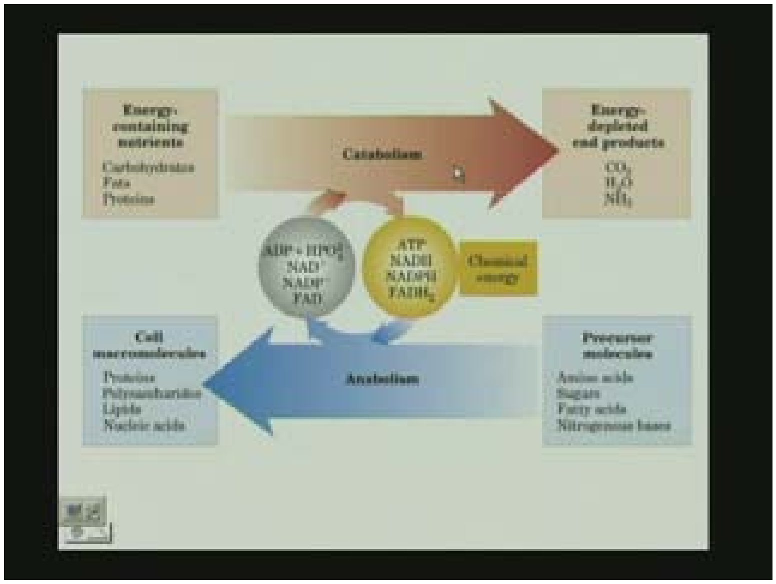

If we just look at this whole picture, I will show you another one also where we will see how the whole process actually takes place. We have the energy containing nutrients that we taken in our diet; carbohydrates, fats and proteins. In the catabolism that is in the breakdown of these processes or the breakdown of these units we actually have energy depleted end products that are finally carbon dioxide, water and ammonia. And we have in this process then the use or the utilization of certain aspects or certain cofactors that we have already looked.

We have ADP and ATP in a large extent. We have NAD^+, FAD and $NADP^+$ and so on and so forth. All of these actually will then either breakdown 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 acids, the sugars, the fatty acids and the nitrogenous bases that in a process of anabolism that is in the formation of the macro molecules will ultimately lead to the cell macro molecules such as proteins, polysacoharides, lipids and nucleic acids. 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 bits and pieces some of them get lost in energy depleted products, some of them form the precursor molecules further formation of the generation of other proteins, polysacoharides and lipids and nucleic acids.

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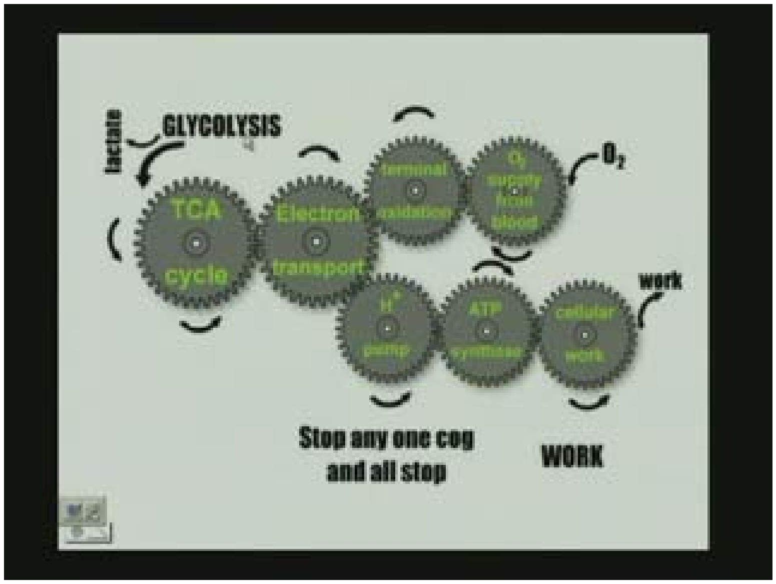

Now if we look at actually the whole process it is extremely interesting. This is like a gear system. We have the process of Glycolysis which we are going to study in our breakdown of glucose in the metabolism that we study. The Glycolysis leads to something called the TCA cycle which is the Tri Carboxylic Acid cycle that has electron transport. As we just looked at the electron transport has a proton pump to it. The proton pump then uses ATP synthase to produce cellular work where we get work. This oxygen again comes in from the blood utilized in the ATP synthase in the electron transport system where we have the reduction of the oxygen to water. This is the way all of these are interconnected actually. And if you just look at a whole picture of all the metabolic pathways there is actually a beautiful picture on the net that has all the metabolic pathways that actually take place in our body.

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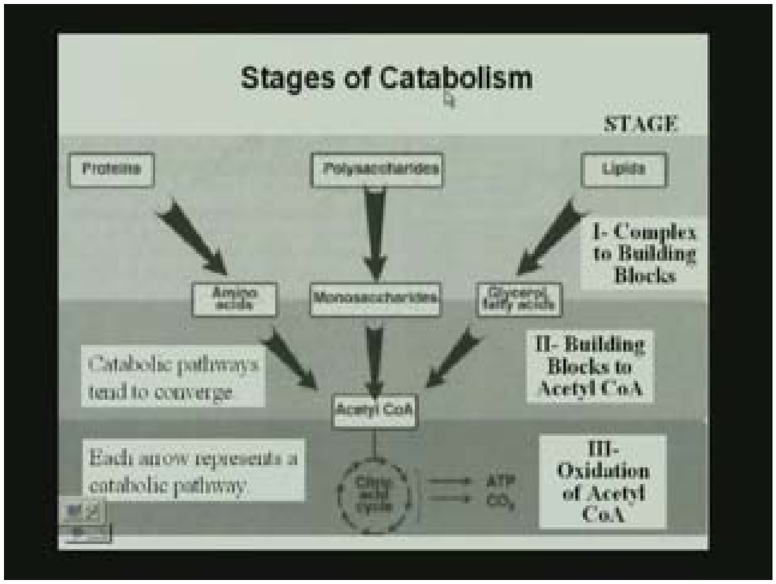

What I am going to give you is just probably a small like a drop in the huge ocean of all those metabolic pathways that actually take place. What we actually intake? We are looking at stages of catabolism. Our intake is proteins, polysacoharides, lipids that is fats, proteins and carbohydrates basically that is what we intake. The smaller blocks of proteins, what is the breakdown of proteins going to give you? It is going to give you amino acids. The breakdown of polysacoharides is going to give you monosacoharides, the breakdown of lipids is going to give you fatty acids or glycerol.

Now, each of these again are further going to be broken down and converge to acetyl coenzyme A which is a 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; monosachorides the breakdown of glucose that is what we are going to look at. But if you consider the ampting polysacoharides 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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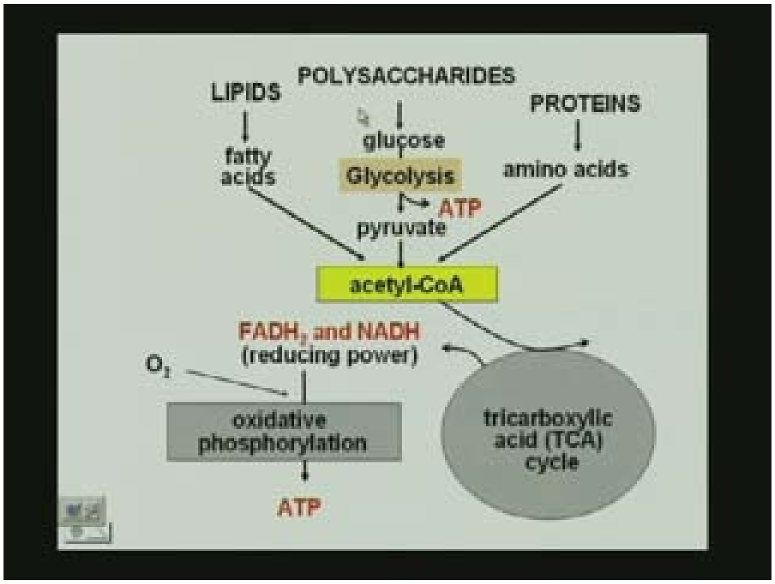

So what we have is we have our lipids, polysacoharides and proteins, lipids breaking down into fatty acids that form the acetyl CoA, polysacoharides breaking down into glucose the monomaric unit in the process of Glycolysis forming pyruvate which is what we will see in the process of Glycolysis that we will study, proteins again breaking down into amino acids and again are utilized in this acetyl CoA which finally is used in the Tri Carboxylic Acid cycle which is going to be the breakdown of the Tri Carboxylic Acids the oxaloacetate and so on and so forth. Then we have this process where we have $FADH_2$+ NADH giving you with oxygen oxidative phosphorylation in the production of ATP. This is a part that we have studied in a bit detail.

Now what we are look at is we are going to look at the 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 that is going to be utilized in the Tri Carboxylic Acid cycle that is also known as the Krebs cycle.

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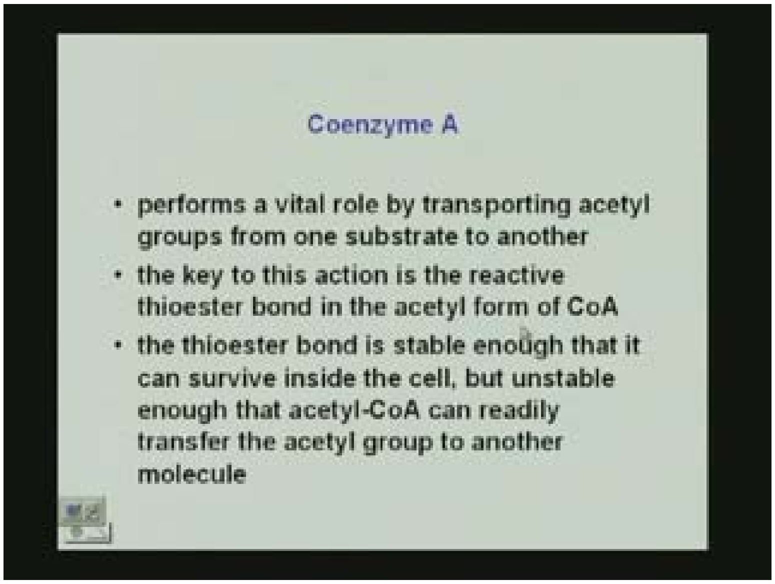

Now, before I get into the process of Glycolysis this is something that we looked at before when we considered the vitamins. I mentioned that each of these vitamins you now realize is the precursor for a large number of cofactors and prosthetic groups. Coenzyme A is another such compound that is extremely important in the formation or the transfer rather of the acetyl group that is the transfer of the two carbon system for any of the processes that occur.

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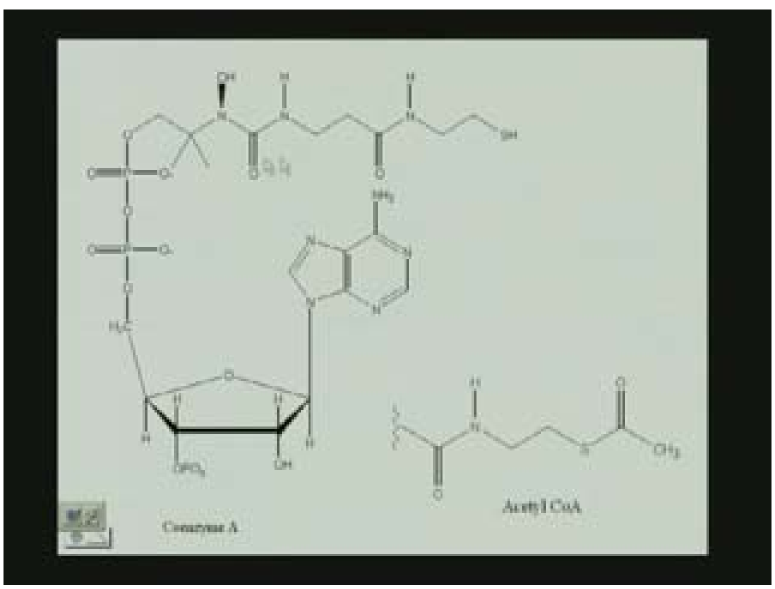

What we have in coenzyme A is actually an ADP part an Adenine Dinucleotide part, a phosphorylate 1 because there is a phosphor at this position if you can see and we have pantothenic acid that is one vitamin and to it we have mercapto ethanylamine attached. Now this SH if acetylated is called acetyl CoA. So whenever we look at any metabolic pathway you will see acetyl CoA come into the picture a large number of times. But you have to remember it is derived from that vitamin and it is nothing but a phosphorylated ADP linked with the oxygen to pantothenic acid that is linked with marcaptoethylamine and this unit is coenzyme A. If this thiol group is acetylated it becomes acetyl CoA and that is exactly as how it is referred to in all of the metabolic pathways acetyl CoA. So it is this acetyl part that is important and the rest is you realize is derived from phosphorylated ADP, vitamin pantothenic acid and marcaptoethylamine.

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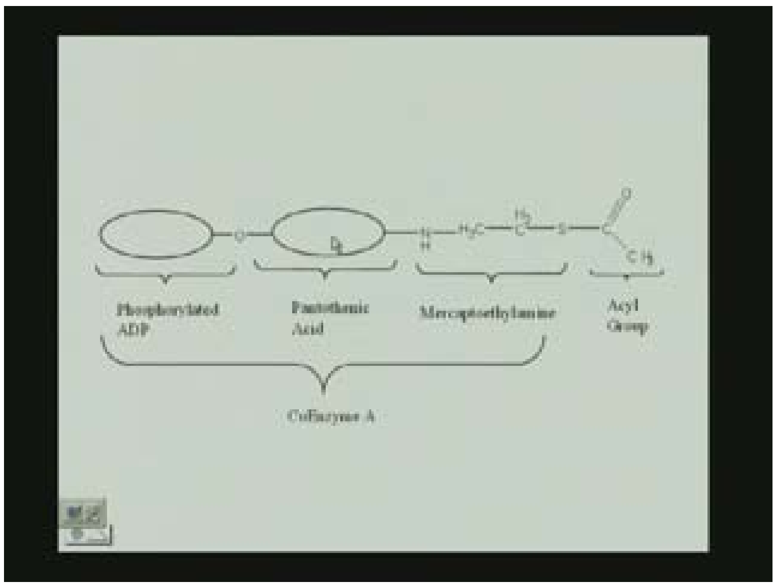

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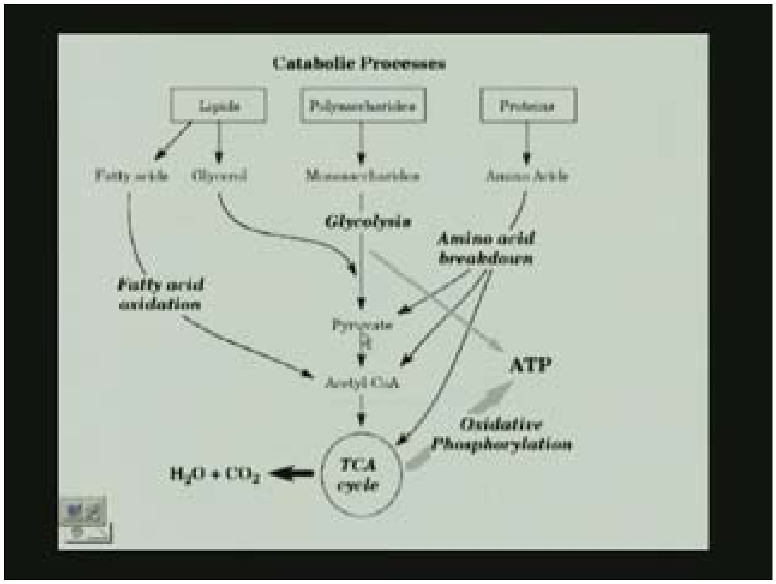

Now if you look at all the catabolic processes that occur the Glycolysis path way will lead us to pyruvate, pyruvate all of the breakdown getting to acetyl CoA, acetyl CoA being the main part of the TCA cycle that ultimately breaks it down to carbon dioxide and water. Now this is obviously going to involve a large number of enzymes and we will look at each of these enzymes and how they act.

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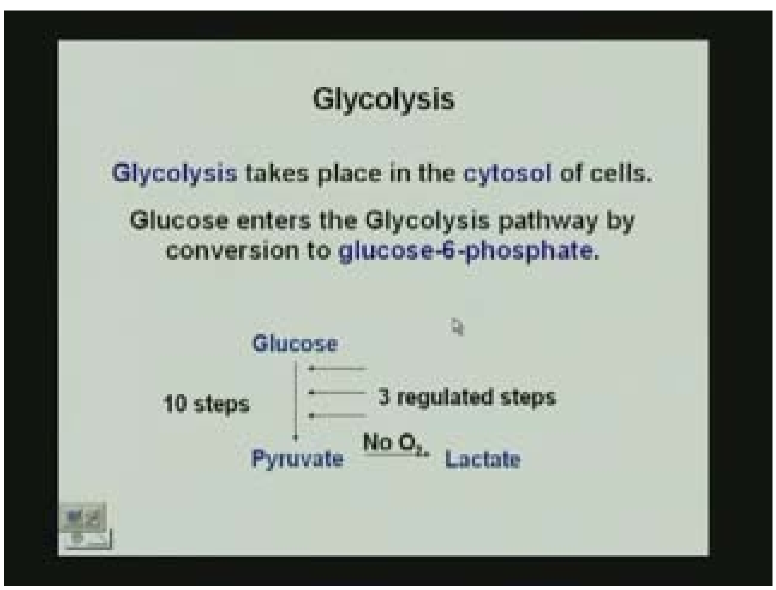

Glycolysis: the process of Glycolysis takes place in the cytosol of cells and glucose enters the Glycolysis pathway by the formation of glucose-6-phosphate. Glucose-6-phosphate means that we are going to have a large number of steps that are actually going to get you to pyruvate. There are ten steps involved which mean there are ten enzymes involved and we have this form pyruvate and we have three steps that are regulated in the formation of pyruvate from glucose in the ten steps that perform the process of Glycolysis. So we have glucose go to pyruvate in ten steps and we have three 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.

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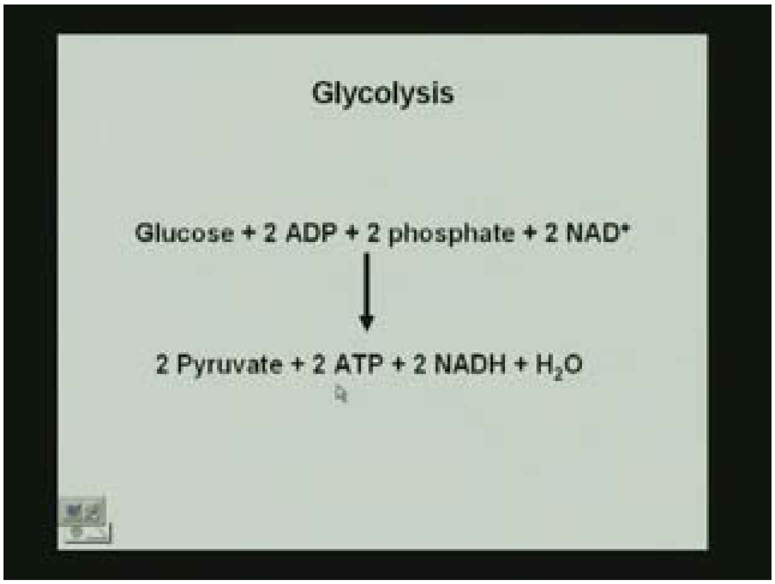

This is the overall reaction. We have glucose + 2 ADP + 2 Pi that is phosphate + 2 NAD + go to 2 pyruvate + 2 ATP so you are generating ATP here also + 2 NADH + H_2O. So we are going to look at each of these steps and see how you have ATP consumption, ATP production and finally we are going to take an account of where we are using ATP where we are producing ATP.

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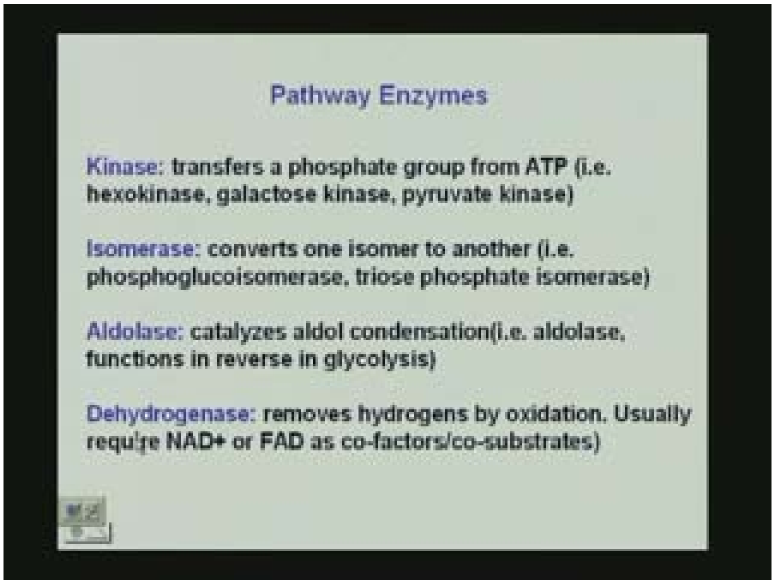

Now before we get into that we have to see why enzymes are involved in the metabolic pathway. We have kinases, isomerases, aldolases, dehydrogenases. Kinases are a specific class of transferases and kinases are those that transfer a phosphate group, this we have looked at before or I have mentioned before. We will also see how Hexokinase will be a part of our Glycolysis mechanism. So we have kinases that transfer a phosphate group from ATP to a specific substrate. We have isomerases, what are isomerases going to do? They are going to convert one isomer to another and that is what the function of isomerase 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 hydrogens by oxidation.

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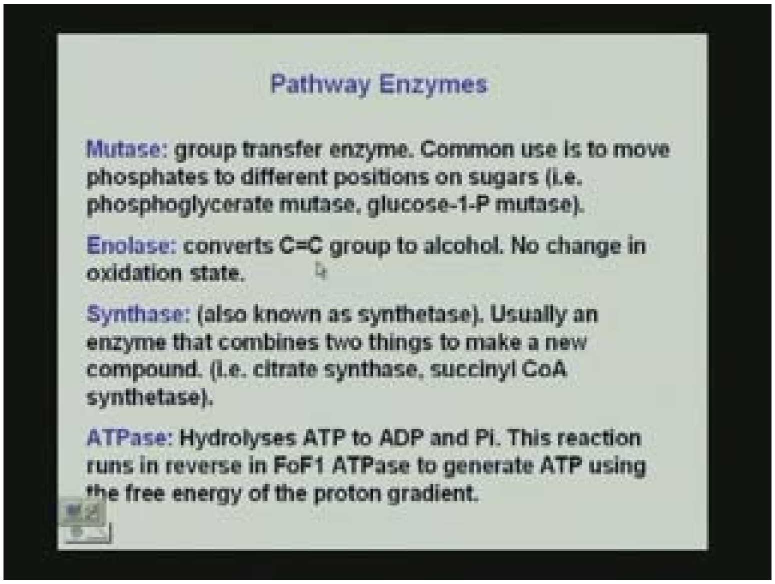

Then we have the other enzymes, we have a few more we have mutases that are actually group transfer enzymes. They transfer phosphate from one position of the substrate to another position. Say you have a glucose-1-P to form a glucose-6-phosphate you will need a mutase. So the mutase is going to be a group transfer enzyme that is going to transfer, the common use would be the transfer of phosphate to a different position an example being glucose-1-P mutase which would actually transfer the phosphate from carbon 1 to carbon 6. Forming from glucose-1-P it would form glucose-6-phosphate in that case you would need mutase.

If you want an enolase you would convert a C double bond C group to an alcohol 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. So we have to look at how this can be accomplished in the biological way with the use of these certain enzymes. We have synthase that is also known as synthetase, what does that do? It just combines two molecules together, synthesis. We have ATPases that are going to hydrolyses ATP to ADP and P_i and this is in reverse to ATPsynthase which was, what was ATP synthase doing? It was taking ADP and Pi and producing ATP, this hydrolyses ATP to ADP and P_i. So these are the pathway enzymes that are going to be used in every step of the way as we go along.

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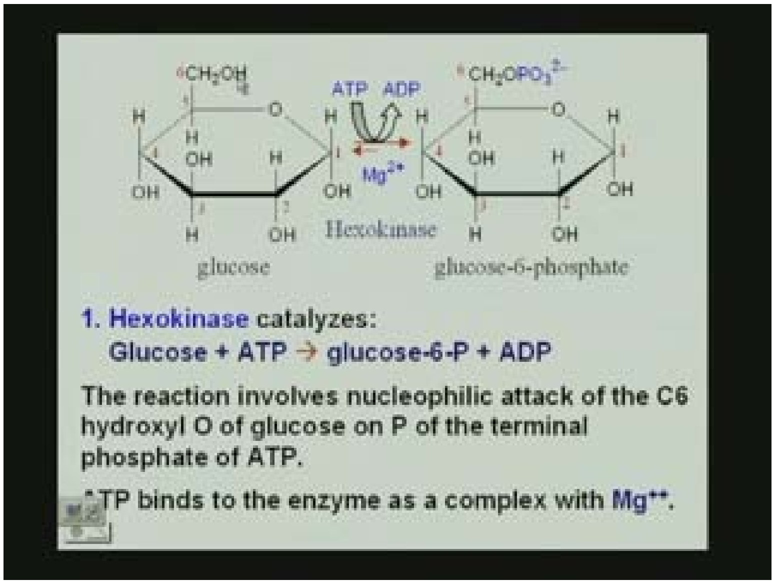

We have step number 1 which is the use of Hexokinase. Hexokinase is the first step in Glycolysis after you have broken down polysacoharide to glucose. We now have glucose, we are not going in to how the polysacoharide is broken down or how even the other dietary nutrients are broken down like fats or amino acids, 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 ten steps, this is step number 1 where from glucose we form glucose-6-phosphate. That means the 6 carbon atoms is no longer OH it is now phosphorylated. It is phosphorylated by taking a phosphate from ATP, in the event ATP becomes ADP. This is a coupled reaction. We will look at the energetics of these steps. The glucose going to glucose-6-phosphate has a delta G that is positive.

The ATP to ADP has a delta G that is negative, and the coupled reaction will give a favorable forward reaction to this and the reaction actually involves a nucleophilic attack of the hydroxyl OH that is attached to carbon 6 to the gamma phosphate of ATP resulting in the formation of ADP and in this case the enzyme that is involved is Hexokinase and Hexo means it is going to act on a 6 membered sugar ring. It is a Kinase and it helps in the transfer of the phosphate and the ATP binds to the enzyme as a complex with magnesium.

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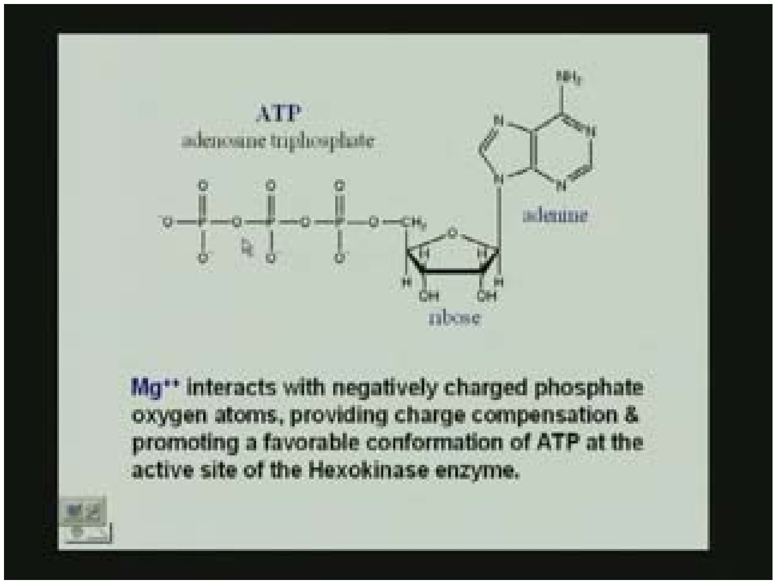

Now, why would we have magnesium there, what is ATP? ATP has a large number of negative groups. There are three phosphates one after the other, what magnesium does is it interacts with the negatively charged phosphate oxygen atoms and provides charge compensation and also promotes a favorable conformation of ATP at the active site of the Hexokinase enzyme because you have to realize that when we are looking we are not going to look at the details of how the enzyme is working. But what we have here is we have magnesium in the 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-phosphatefrom glucose, 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 P_i is attached to sixth carbon so ADP.

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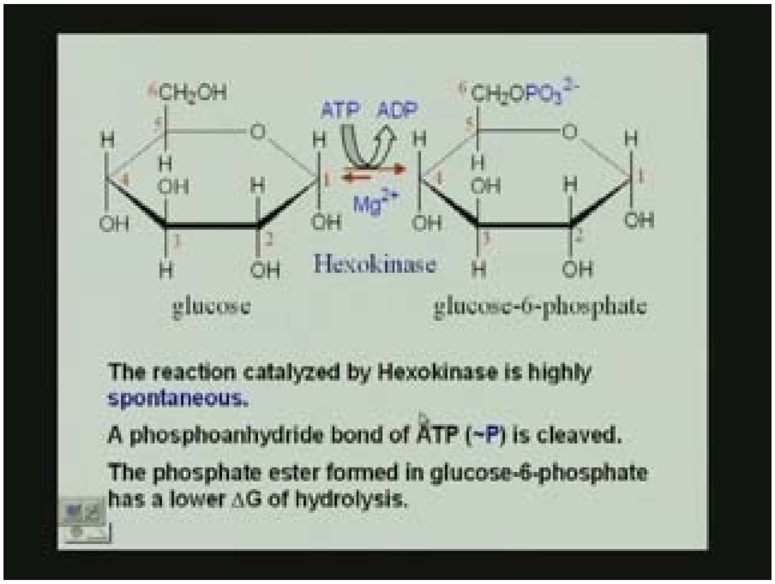

Now this reaction is highly spontaneous because the phosphoanhydride bond of ATP is cleaved. That is a high energy bond as we call it and the phosphate ester has a lower delta G of hydrolysis.

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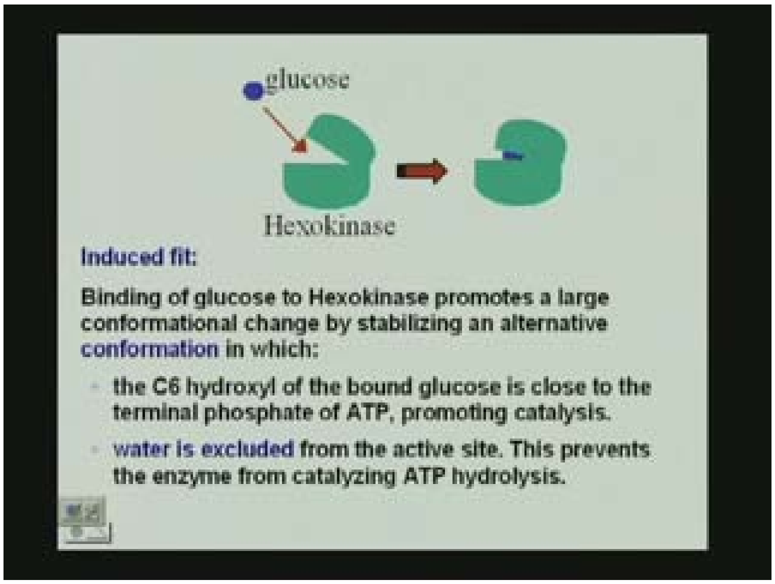

Now what happens in this case is you have what is called an induced fit. Remember, we studied induced fit for the enzyme mechanisms the way they work. There is a lock and key mechanism and an induced fit mechanism. Usually the enzymes of the glycolytic pathway have induced fit. They are not ones that would just sit with a lock and key where an 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 conformation where by the ATP is positioned in such a way that the terminal phosphate can be transferred to the substrate that is glucose. So what happens is you normally would have Hexokinase and as soon as the substrate glucose comes into the picture there is the ATP that is positioned favorably so that the phosphate can be transferred from ATP to glucose.

And we also have this important point where water is excluded from the active site. What would happen if water would be there? The ATP would be hydrolyzed. We don't want the hydrolysis of ATP but we want the phosphate to be transferred only to glucose. So once the substrate comes to Hexokinase it changes its conformation 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 happened, how did the reaction take place? It was the sixth OH the OH attached to the sixth carbon atom that actually went and attacked the phosphate along here.

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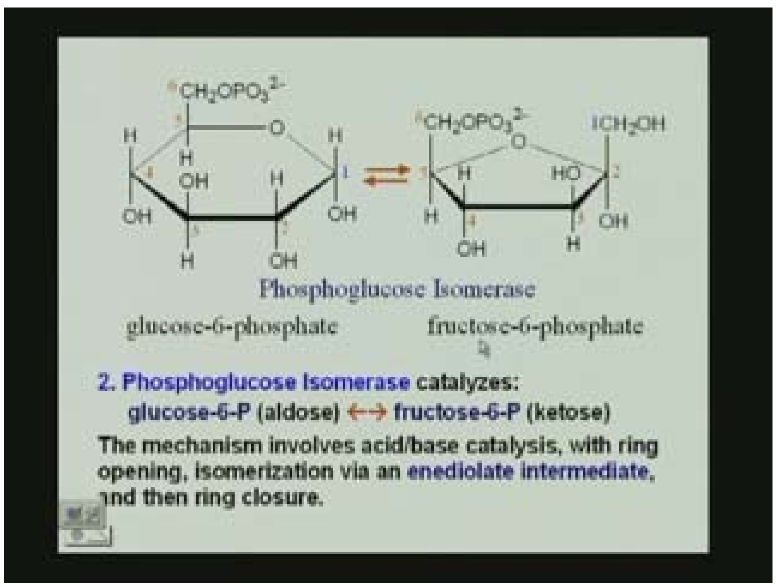

This is step number 2. In step number 2 you have glucose-6-phosphate go to fructose-6-phsphate. So what happens is you have the formation of an isomer so the enzyme involved is an isomerase. What is this isomerase? It is a Phosphoglucose Isomerase and what is happening here is the glucose-6-phosphate forms a fructose-6-phosphate where instead of the aldehyde now the aldose you have a ketose. You have the CH2 OH up right here now so you have glucose-6-phosphate go to fructose-6-phosphate. The enzyme involved is Phosphoglucose Isomerase and the mechanism actually involves acid base catalysis with the ring opening via an ene diolate intermediate and finally we have ring closure. So we have an isomerase so what was the first step? The first step was glucose to glucose-6-phosphate and the next step is glucose-6-phosphate to fructose-6-phosphate. What do the enzymes require? In the first step we need the kinase and in the second step you need an isomerase.

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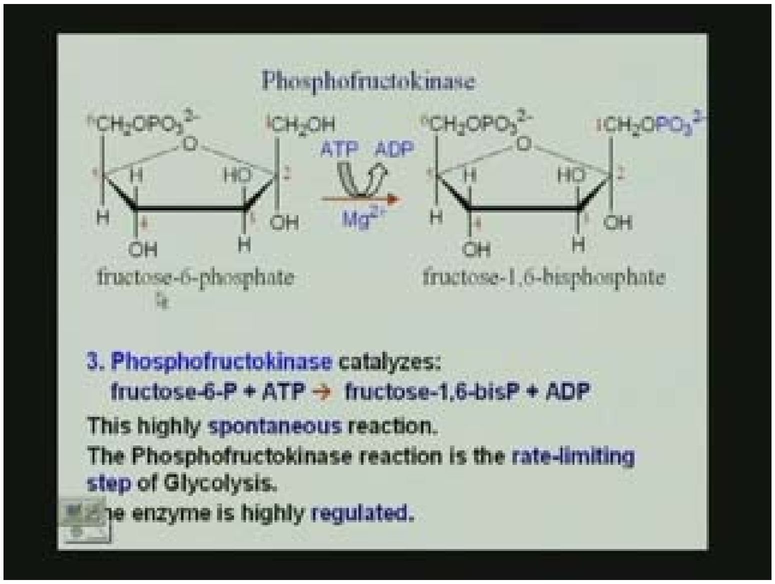

Third step; look at what is happening. We had fructose-6-phosphate go to fructose-1.6-bis phosphate. So what is happening? I have the addition of another phosphate at the one position so what is the enzyme that I need? I need a kinase and that is what you have to identify. You have a process you know the substrate, you know the product what is the enzyme involved. You know in this case that you have prepared fructose-6-phosphate from glucose-6-phosphate and it was just an isomerazation so you needed an isomerase.

You are going now from fructose-6-phosphate to fructose-1.6-bis phosphate which means you have added another phosphate which again means the breakdown of another ATP and that you need a kinase. And what kind of a kinase do you need? A fructokinase. Why a fructokinase is because you have a fructose and you are adding a phosphate to a fructose that is it. So you have fructose-6-phosphate that is going to form fructose-1.6-bis phosphate and this process is actually highly spontaneous and this phosphofructokinase reaction is the rate limiting step of Glycolysis.

We will see how that is later on when we study the whole process and this enzyme is extremely tightly regulated. And when we go through the whole system you will see that there are some processes in the Glycolysis steps of reactions that are reversible and there some that are irreversible. Once glucose has got in to glucose-6-phosphate there is no way it is going to get back to glucose.

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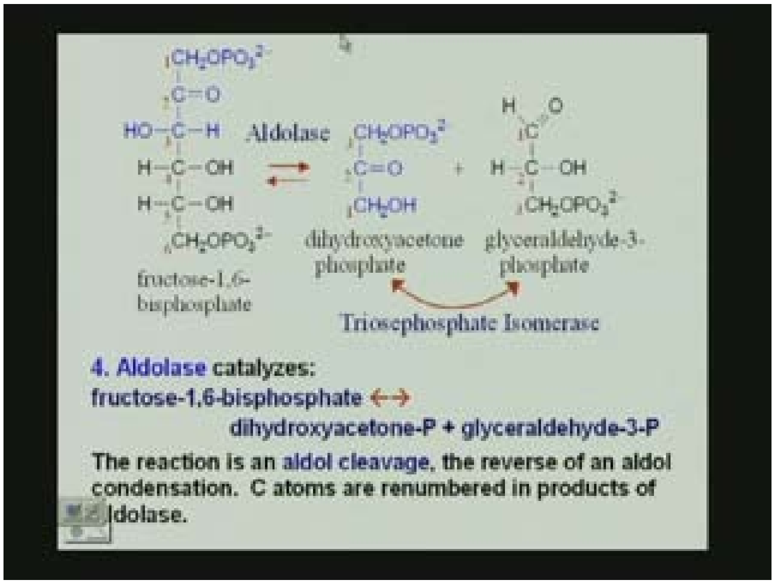

The next step what we have here now is we have fructose-1.6-bis-phosphate. Now what happens to fructose-1.6-bis-phosphate is we have it in the ring formation here. In the next step there is going to be the breakdown of this, how carbons do we have here? We have 6, glucose has 6 carbons, and we have 6 carbons here. We have 1, 2, 3, 4, 5, 6 and what is going to happen now is in the next step first there is ring opening then the six membered ring is going to break down into two three member rings. Because you have to remember that finally we have to get to carbon dioxide and water. So unless we start chopping up it is not going to be possible. Hence we have our glucose and now we are going to go to a step where we are going to break the fructose-1.6-bis-phosphate into two three carbon units.

The process here is a reverse of aldol condensation. It is aldol cleavage. We have here the fructose-1.6-bis-phosphate which is now not in its ring form but in its open form where you can see the ketone. It is a ketose so it has to have this C double bond O. there are two phosphates attached to it now, one at the one position and one at the six position. We have now an aldolase which a reaction that is going to involve aldol cleavage and there is going to be a reverse of aldol condensation which means you are going to have a break at this position here where we are going to have the formation of two three carbon units one of them is Dihydroxyacetone phosphate. If you look at this, this is acetone CH3 CO CH3 is acetone, this is acetone CH3 this is acetone Dihydroxyacetone is that.

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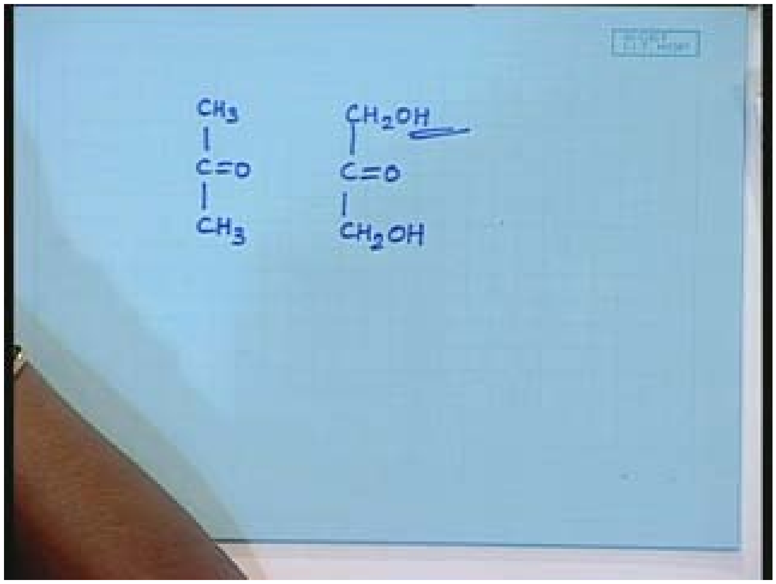

Now if we have Dihydroxyacetone phosphate we have phosphorylated one of these. So that is exactly what we have. Here we have (Refer Slide Time: 29:12) Dihydroxyacetone phosphate and we have Glyceraldehyde-3-phosphate.

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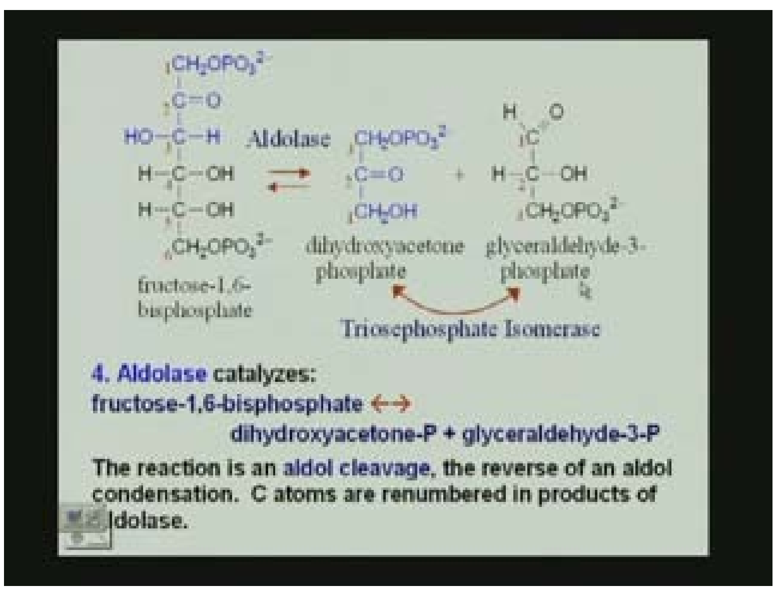

This is Glyceraldehyde you recognize the CHO of the aldehyde the CH OH and CH2 OH which would have been here but we now have it phosphorylated. So the phosphor now is distributed in the two three carbon units. So this is the ketone form, this is the aldehyde form. What are these? These are isomers. So you can go from one to the other by an isomerase enzyme. What is this isomerase going to be named? it works on a three carbon unit so a triosephosphate isomerase.

If you look at the nomenclature it is actually very simple. All you have to know is what the substrates 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 six membered or the six carbon unit.

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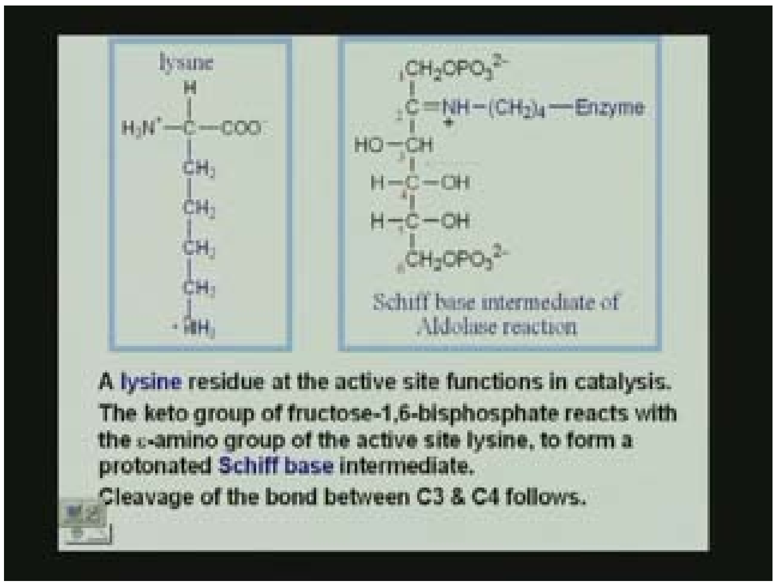

Now, if you look at how the six carbon unit or the aldolase actually works there is a lysine residue in the enzyme aldolase. Obviously it has to be there because the aldolase is what is acting on the substrate. What is the substrate? The substrate is fructose-1.6-bis-phosphate that is your substrate. What are your products? Your products are Dihydroxyacetone phosphate and Glyceraldehyde-3-phosphate. So you have Glyceraldehyde-3-phosphate and Dihydroxyacetone phosphate which later on you will see that it is written as dhap and g3p. So we have a lysine residue in aldolase that is present at the active site.

What this lysine does is a keto group of fructose-1.6-bis-phosphate reacts with the amino group of the lysine and it forms a protonated Schiff base then there is a cleavage between carbon atoms 3 and 4. We are not going to go into details of all this but what you have to know is you have the breakdown, the breakdown from the six carbon to the three carbon by the enzyme aldolase. The enzyme has a lysine residue that interacts with the amino group of the lysine and where is this lysine it is in the active site of aldolase. It interacts with the keto group of fructose-1.6-bis-phosphate and it breaks it up, that is cleavage between carbon atoms 3 and 4.

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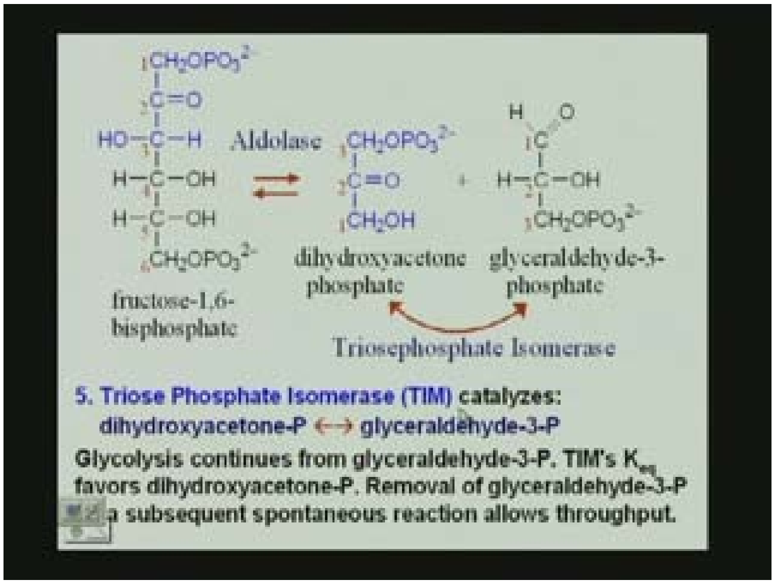

Therefore this is just the triose phosphate isomerase that is going to be inter converting dihydroxyacetone phosphate and Glyceraldehyde-3-phosphate. This protein actually is pretty interesting in its structure also but we not going into the details of that, it is called TIM and it has a TIM barrel actually associated with it. We have the Glycolysis that is going tocontinue from Glyceraldehyde-3-phosphate. Now what is that triose phosphate isomerase doing? It is converting Dihydroxyacetone phosphate to Glyceraldehyde-3-phosphate. This is an isomeration reaction. The equilibrium constant is such that it favors Dihydroxyacetone phosphate. So this is favored but the Glycolysis steps the further steps actually continue from Glyceraldehyde-3-phosphate. Therefore 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 triose phosphate isomerase is such that it favors Dihydroxyacetone phosphate. When you have equilibrium your equilibrium either shifts your left or right to the reactants or products. In this case Dihydroxyacetone phosphate is preferred based on its equilibrium constant. But the process of Glycolysis only will continue with Glyceraldehyde-3-phosphate. So what has to be done is your equilibrium has to shift to your right. So what is usually done is this Glyceraldehyde-3-phosphate as soon as it is formed it is then utilized in the next step. thus if it is utilized in the next step what happens is your equilibrium is shifted to right which means some more of the Dihydroxyacetone phosphate has to be isomerized to Glyceraldehyde-3-phosphate. It is regulated. You see how it is regulated. 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 is your equilibrium is moved in such a way that the Dihydroxyacetone phosphate forms in the Glyceraldehyde-3-phosphate.

Then what happens is there is removal of the Glyceraldehyde-3-phosphate and subsequent spontaneous reaction. 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 dha.

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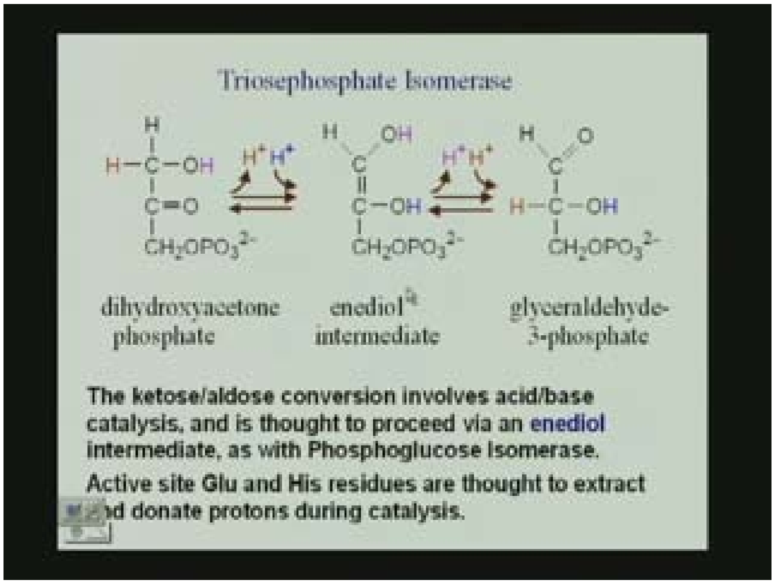

This is the intermediate you have which is the enediol intermediate where you have the ketose/aldose conversion which actually involves acid base catalysis and the isomerase that actually takes place is a Phosphoglucose Isomerase or rather what happens here is when we have Dihydroxyacetone phosphate what are we talking of is we are talking of ketose.

When we have the Glyceraldehyde-3-phosphate we are talking of an aldehyde so there has to be an enediol intermediate that is going to take you from the ketose to the aldehyde. It is similar from fructose to glucose. What is glucose? Glucose is an aldose and fructose is a ketose. You have an isomerization there are also that takes you from glucose to fructose. It is exactly the same thing but here you are working on a three carbon atom instead of a six carbon, so that is the difference.

We have the Dihydroxyacetone phosphate, we have the Glyceraldehyde-3-phosphate and these are formed from an enediol intermediate. So when we are talking of the triose phosphate isomerase we are talking of this equilibrium. It is this equilibrium that we are talking about. So the equilibrium will actually shift to the left side but with the removal of the Glyceraldehyde-3-phosphate what will happen is there will be more formation of the Glyceraldehyde-3-phosphate that will eventually be used in the other steps of Glycolysis.

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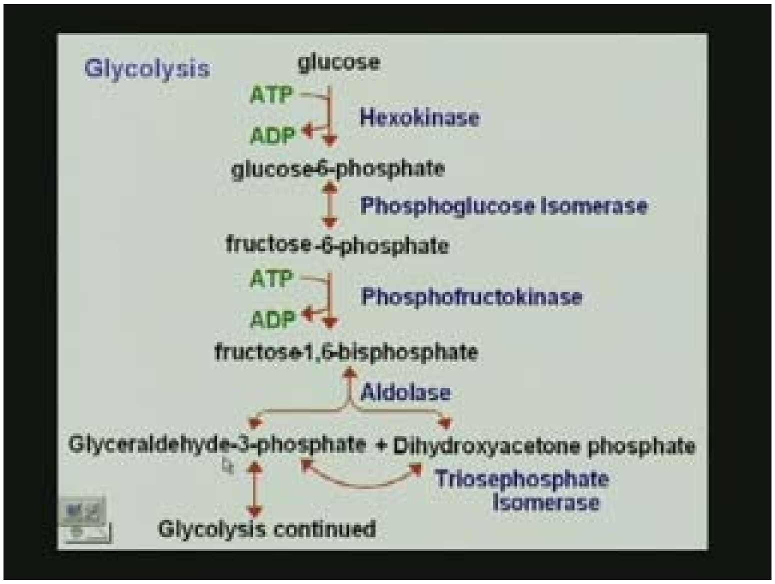

This is what we are going to do for Glycolysis today because there are some other discussions I want to make regarding Hexokinase. These are the steps. We have just broken down the six membered ring, let us put it that way. We will see now what is going to happen to the three carbon ring. Now the three carbon system will eventually get into the Tri Carboxylic Acid cycle and that Tri Carboxylic Acid cycle will ultimately produce carbon dioxide and water so you haven' broken down. But what we started off with is we started off with glucose.

In the first step we had glucose go to glucose-6-phosphate. Now since it took up a phosphate it required the breakdown of an ATP, it is 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, it is nothing but an isomeration where we have an aldose go to a ketose. The enzyme is a Phosphoglucose Isomerase.

Step number 3 is where we have our fructose-6-phosphate form fructose-1.6-bis-phosphate. What is happening there is I am adding another phosphate. In the addition of a phosphate I have to breakdown another ATP and I need another kinase. So I have fructose-6-phosphate that will take up phosphate from the ATP into forming fructose-1.6-bis-phosphate with the help of phosphofructokinase.

The next step now is the breakdown of the six carbon to 2 3 carbons and we have aldolase, aldolase is going to breakdown fructose-1.6-bis-phosphate into Glyceraldehyde-3-phosphate and Dihydroxyacetone phosphate. And if you notice here it is Glyceraldehyde-3-phosphate that will allow the Glycolysis to continue. So we need an isomerase that is going to transform a Dihydroxyacetone phosphate to Glyceraldehyde-3-phosphate.

Now in the steps that we wrote here if you notice the steps that involve the addition of the phosphate are irreversible. So, glucose forms glucose-6-phosphate with the breakdown of ATP to ADP that step is one way. That means once glucose enters the cell and forms glucose-6-phosphate it is struck into being broken down. If it is not required to be broken down then glucose will actually go for storage as glycogen. But once it forms glucose-6-phosphate it has to be broken down. So, if we look at the features actually of Hexokinase this enzyme is inhibited by it is product. Now biochemically that makes absolute sense because if Hexokinase is inhibited by glucose-6-phosphate then what is it 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.

Therefore as soon as there is sufficient glucose-6-phosphate are sufficient glucose to be broken down it will stop itself from acting because you don't want all the glucose to form the glucose-6-phosphate because once the glucose-6-phosphate is formed it has to be broken down it is entered the glycolitic cycle there is no way it can go back. But if Hexokinase is inhibited then what happens? Glucose can still go elsewhere and be stored as glycogen in the liver sink. But once Hexokinase has acted on glucose there is no way it can go back. So glucose-6-phosphate acts as a inhibited to the Hexokinase so that further glucose breakdown is not possible.

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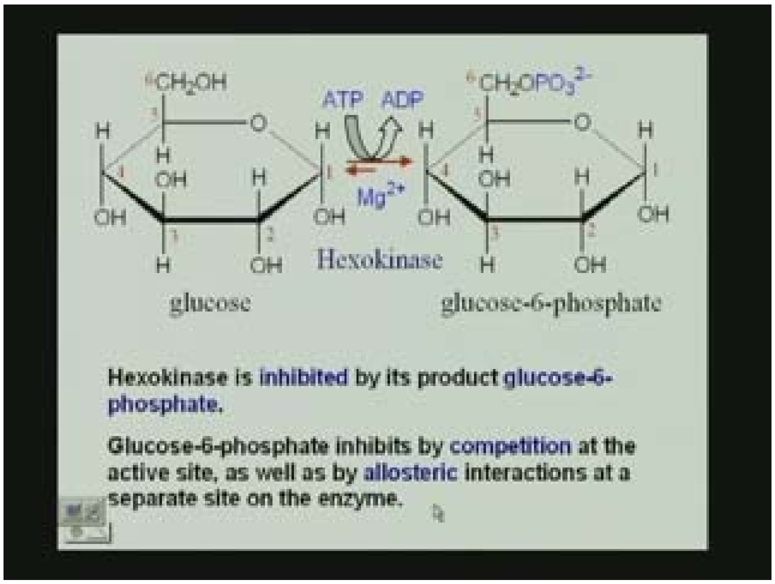

Hexokinase is inhibited by its product glucose-6-phosphate and the way it inhibits Hexokinase is by competition at the active site as well as by allosteric interactions at a separate site on the enzyme. What does that mean? It means that it will change the active site conformation to such an extent that it will not be able to bind the substrate glucose. And remember I showed you a rough cartoon of Hexokinase where it has an induced fit as soon as glucose comes into the picture then the ATP is positioned in such a manner that it forms glucose-6-phosphate.

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 position where it could affect the active site so that the substrate cannot bind. So once the substrate cannot bind it means that the enzyme is inhibited and if the enzyme is inhibited then the product will not be formed. If the product is not formed in this case it means that the glucose breakdown does not occur, as simple as that. So it is extremely tightly regulated. Thus the cells trap the glucose by phosphorylating it.

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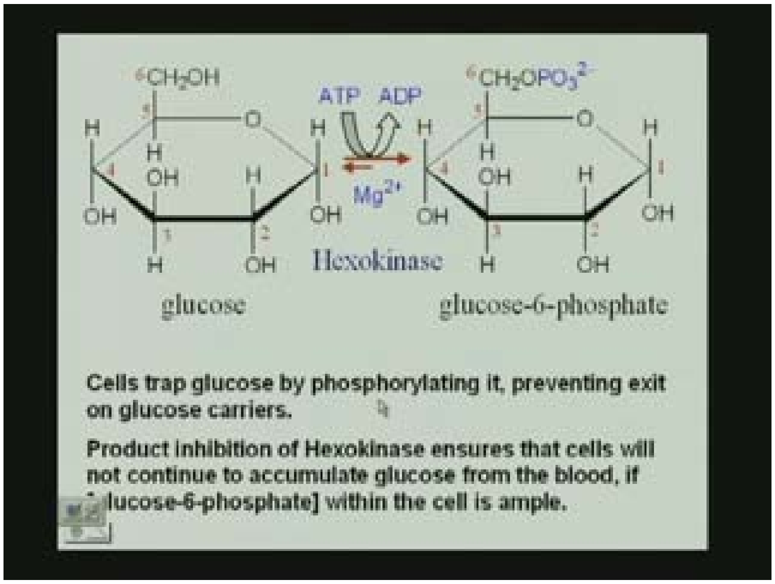

Once glucose is phosphorylated it is trapped, it has to be broken down. This can be prevented if this glucose-6-phosphate inhibits the enzyme. If Hexokinase is inhibited then glucose cannot form glucose-6-phosphate. So the product inhibition of Hexokinase ensures that cells will not continue to accumulate glucose from the blood if glucose-6-phosphate within the cell is insufficient quantity. Because once your glucose-6-phosphate is of insufficient quantity it means that this can continue in the glycolytic steps. But if you need it then only glucose is broken down. Unnecessarily glucose is not broken down in the cell. It is only when the glucocarbohydrate metabolism is required is glucose broken down in the cell because once this first step of the glycolytic cycle takes place it is irreversible.

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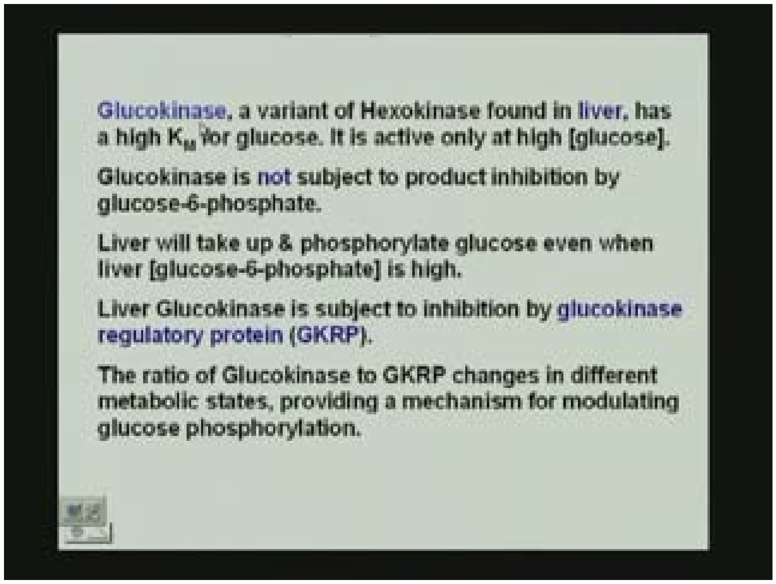

Now Glucokinase which is a variant of Hexokinase is found in the liver. This has a high Km value, all of you know what the Km value is now Michaelis Menten constant for glucose and it is acted only at high glucose concentration. So the Glucokinase acts at high glucose concentrations. Hence when there is a high level of glucose concentration the Glucokinase enzyme comes into the picture. The Glucokinase enzyme is not subject to product inhibition by glucose-6-phosphate. What does that mean? It means that Glucokinase will act on glucose because it is not inhibited by glucose-6-phosphate. So any glucose that comes into contact with the Glucokinase will have a phosphate transferred to it, as simple as that. Why is that? It is because the Glucokinase is not subject to product inhibition by glucose-6-phosphate. So Glucokinase actually works at high glucose concentration.

So, when there is high glucose Glucokinase will transfer a phosphate to the glucose at high glucose concentrations. That means where is this Glucokinase found? It is found in the liver. And what it does is it takes up and phosphorylates glucose even when the glucose-6-phosphate is high. Why is that? It is because it is not inhibited by glucose-6-phosphate. Glucose-6-phosphate has got nothing to do with Glucokinase. if it was Hexokinase then glucose-6-phosphate would have inhibited the enzyme. But Glucokinase is not inhibited by glucose-6-phosphate so it is immaterial whether the glucose-6-phosphate is high or low it does not matter. Liver Glucokinase is actually inhibited by a Glucokinase regulatory protein. What does that do? You have to remember that when you are considering an inhibition it is to prevent the enzyme from forming its product. Now naturally there are certain steps that you would not want the product to be formed for example in the Hexokinase step.

In the Hexokinase step you realize that breaking down of all the glucose present is extremely unnatural and you wouldn’t obviously want that to happen because you want some stored glucose. So the product inhibition in the case of Hexokinase is an extremely clever way of preventing the glucose from being broken down. But in the case in the liver the Glucokinase is present not Hexokinase. It is a variant of Hexokinase but it acts only when the glucose level is very high. Now the liver Glucokinase is subject to inhibition by another protein and the protein will regulate when Glucokinase is going to act. 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. This Glucokinase regulatory protein actually would work then when we have low glucose because high glucose we would actually have Glucokinase work on it.

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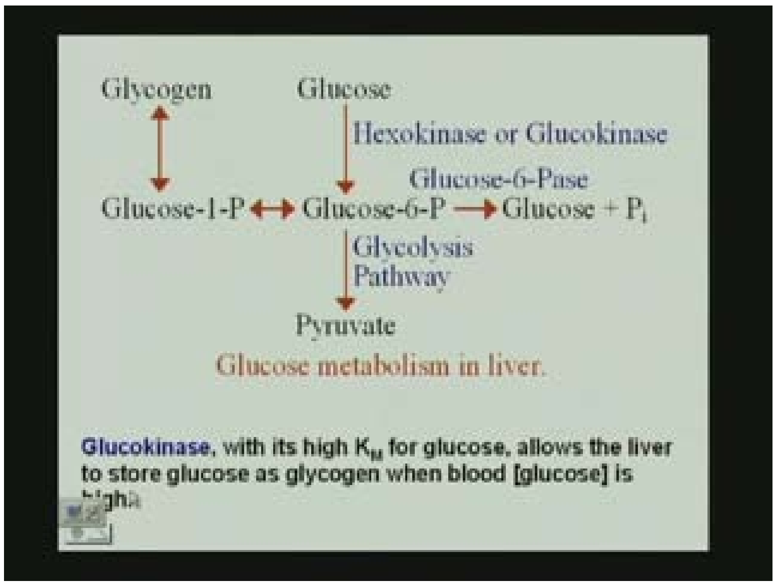

So what happens in this case Glucokinase with its high Km value for glucose allows the liver to store glucose as glycogen when the blood glucose level is high. So, when the blood glucose level concentration is high it will allow the storage of glucose as glycogen. Now what happens is therefore this is 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 not Glucokinase and then we would have from the glucose-6-phosphate once this is formed in the cell it is trapped. So we have a regulatory mechanism that actually works here and then glucose-6-phosphatase catalyses hydrolytic release of P_i from glucose.

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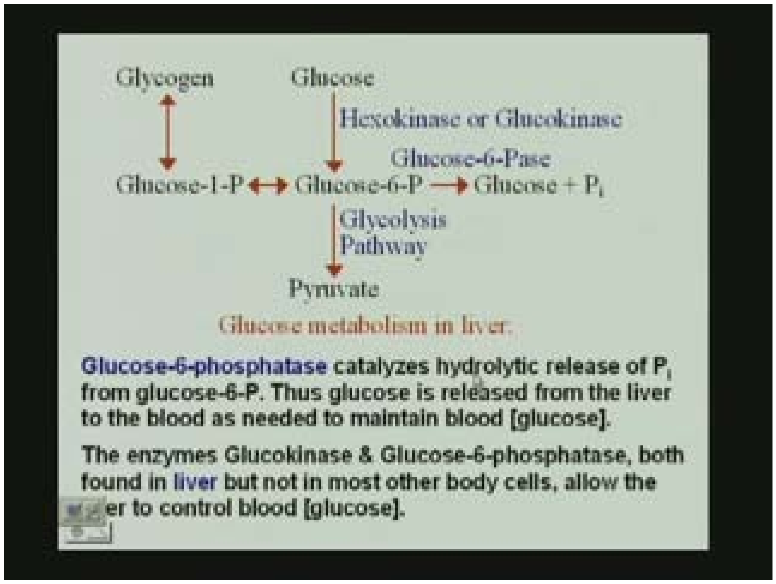

So what happens if you look at the step here you had glucose-6-phosphate formed but there is another protein that is glucose-6-phosphatase that can release the phosphate but this happens only in the liver where the glucose can be stored. In the other cells what happens is, the enzymes Glucokinase and glucose-6-phosphatase are both found in liver but not in most other body cells.

Why would it not be in most other body cells because there you wouldn't 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 because they are the specific enzymes that are present in the liver that can form glycogen from glucose. 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 membered rings. Now we are going to see how those three membered rings or rather Glyceraldehyde-3-phosphate actually will form pyruvate and finally how that will in anaerobic conditions goes to lactate or goes through acetyl CoA to the Tri Carboxylic Acid cycle where it is finally broken down. We will see that in our next class, thank you.