Biochemistry
Prof. S. Dasgupta
Department of Chemistry
Indian Institute of Technology – Kharagpur

Lecture - 26
Metabolism - II

We 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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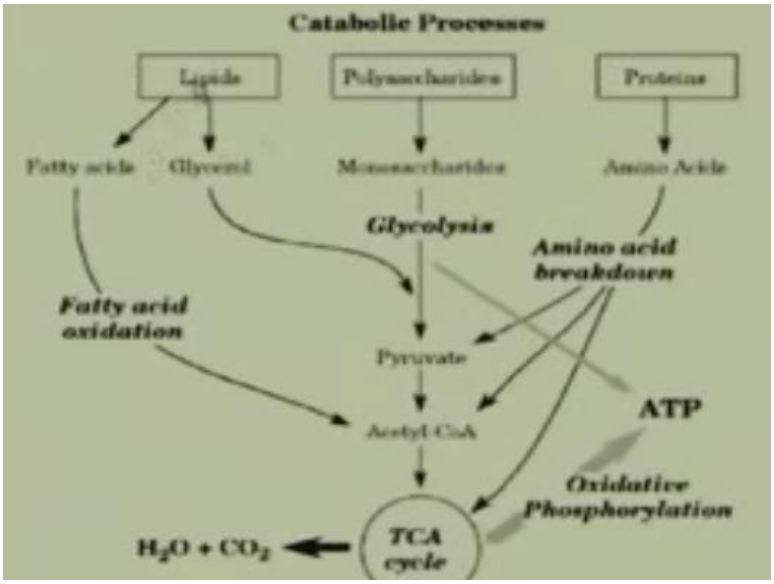

The overall catabolic processes, look at the breakdown of lipids, polysaccharides and proteins. Now, the breakdown of lipids gets into components of fatty acids and glycerol, the polysaccharides break down to monosaccharides and the proteins break down to amino acids. Now, in the anabolic processes we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

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 offshoot of that is the production of ATP where we studied oxidative phosphorylation in the different complex processes that require a number of electron

transfer cofactors as well as certain enzymes.

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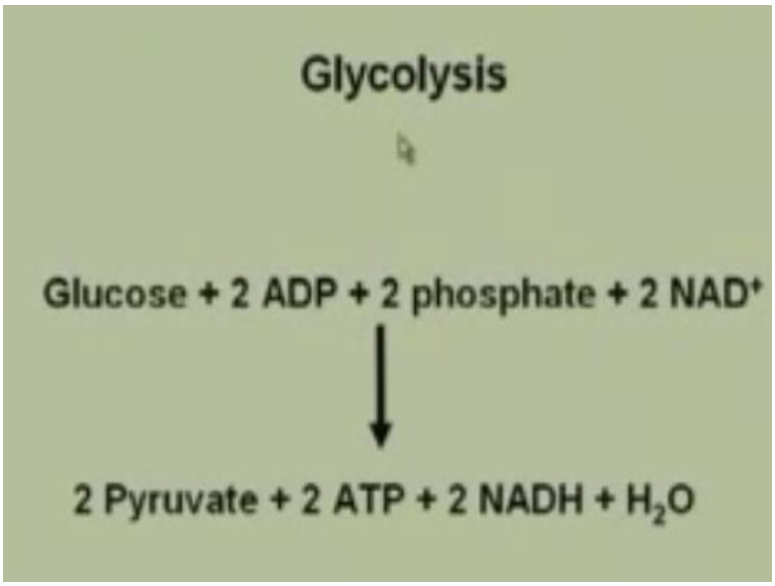

The overall equation for glycolysis is the breakdown of glucose into two pyruvates. Now what we have here is we see how ATP is produced as we continue with all the steps. We will see how ATP is produced in some of the steps, but in the first set that we did, we found ATP consumption.

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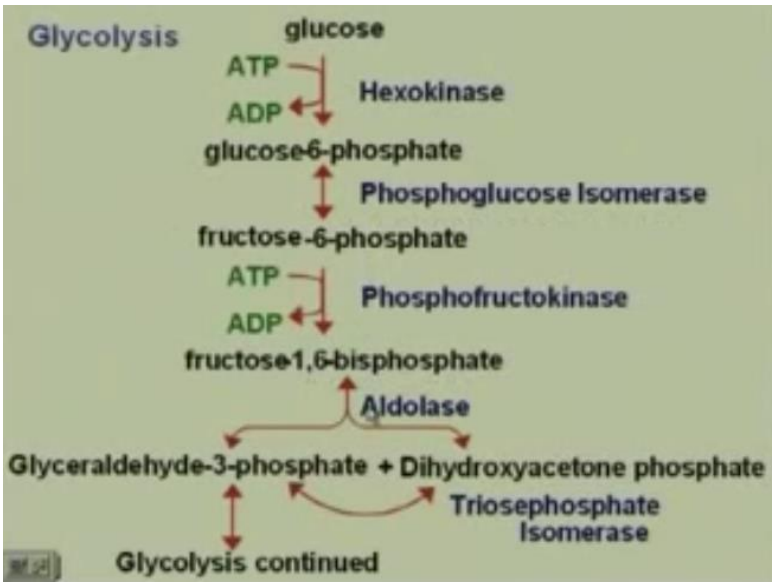

This is the number of steps that we considered in our last class. We had glucose going to glucose-6-phosphate, the enzyme being hexokinase. We have to remember that a kinase is a transferase that transfers a phosphate group. In this process, we have the breakdown of ATP to

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 require another ATP to be broken down and the enzyme used there was another kinase, but in this time it was phosphofructokinase. After this particular step, we have aldolase coming to the picture.

Aldolase is what actually breaks up the 6-carbon member ring into two 3-carbon member rings where we have glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. Now these two are inter transferable by the enzyme triosephosphate isomerase and we also learnt that the equilibrium of this enzyme is such that dihydroxyacetone phosphate is preferred to be formed in larger quantities.

But to shift the equilibrium to the right where glyceraldehyde-3-phosphate will be utilized because that is what continues the glycolysis process, so we have to have glyceraldehyde-3-phosphate that is going to continue the glycolysis or continue with the breakdown and we have to remember here that we now have two 3-carbon units instead of the 6-carbon unit that we started off with.

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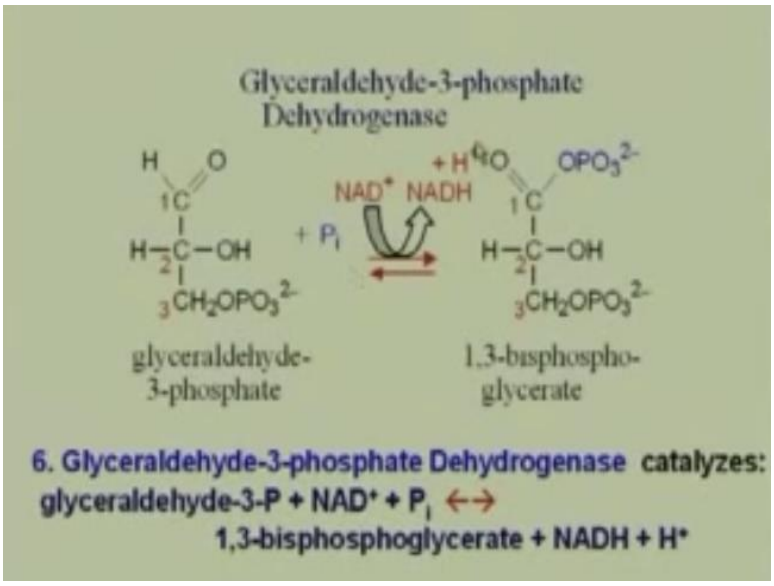

So, this is our step number 6. If you remember, we reached glyceraldehyde-3-phosphate after the action of aldolase on fructose-1,6-bisphosphate. So, our next step is the formation of 1,3-bisphospho-glycerate from glyceraldehyde-3-phosphate. So we are now adding a phosphate, but we have not used ATP in this step. We are using Pi, so the reaction here is glyceraldehyde-3-phosphate plus NAD-plus plus Pi in a reversible step giving you 1,3-bisphosphoglycerate plus NADH plus H-plus, so this is our sixth step where what has been done is we have added a phosphate using NAD-plus forming NADH in a reversible step.

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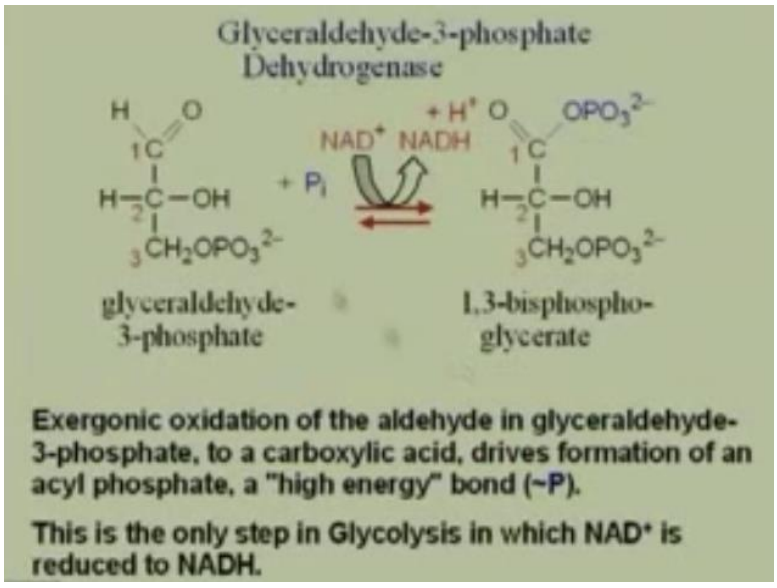

Now in this case what happens, this is the only step in glycolysis where NAD-plus is actually reduced to NADH. In this step that uses glyceraldehyde-3-phosphate dehydrogenase because you

have this hydrogen being removed from the aldehyde group and you have a phosphate introduced by the Pi that comes in as a factor to form 1,3-bisphospho-glycerate and what we have here now is if you notice, we are going to actually create an acid from this, okay, because this now has form where we have seen double bond OPO3 2 minus. okay., this was initially an aldehyde, okay and we are gradually getting to an acid.

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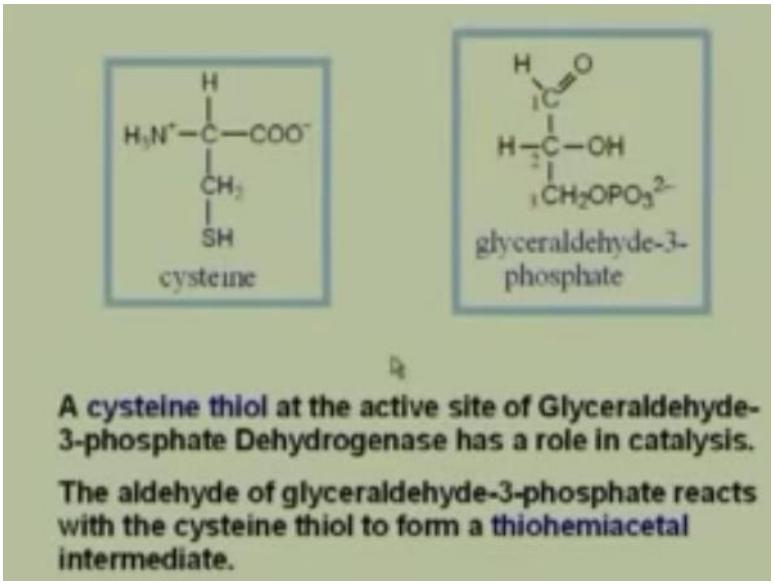

Now, what is going to happen is in this case, there is a cysteine thiol. All of us know that in the cysteine residues, we have the SH thiol group. okay., now what happens is this cysteine thiol is present at the active site of the glyceraldehyde-3-phosphate dehydrogenase and that actually forms an intermediate with the acetaldehyde of the glyceraldehyde-3-phosphate dealdehyde that reacts with the cysteine thiol to form what is called a thiohemiacetal, okay.

So what happens here like we learnt yesterday on another reactions, we had lysine coming to the picture. Here we have cysteine. We will also see one of the enzymes has histidine, so these are the particular side chains of the enzymes that are going to take part in the overall reactions for the transformations to occur.

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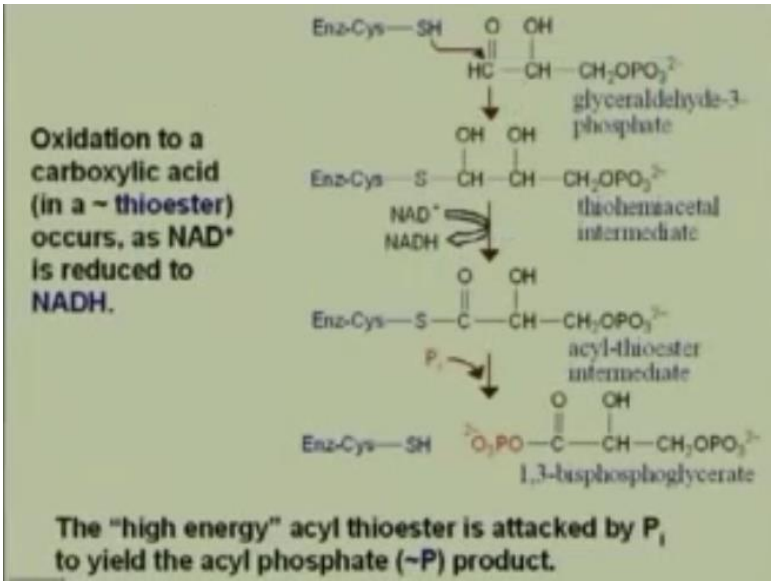

Now, in this case what we have here is our enzyme with the cysteine group attached to it. What we have here, you can see the enzyme has the SH group attached to this. Now what is happening here, this SH creates a thiohemiacetal intermediate by reacting with the aldehyde of the glyceraldehyde-3-phosphate. okay. So this is our glyceraldehyde, so this is carbon number 1, carbon number 2, carbon number 3. Okay.

Because, now remember, we have broken down our glucose into a three-carbon system, so the enzyme, the cysteine group that is present in the active site of glyceraldehyde-3-phosphate dehydrogenase is forming a thiohemiacetal intermediate with the aldehyde of glyceraldehyde-3-phosphate, okay so now we have this linked to the enzyme, okay. Now this part is linked to the enzyme.

Then, NAD plus is reduced to NADH, in the process we get an acyl-thioester intermediate. Where is this thioester, you see this CHOH is now C double bond O and the S is part of the cysteine, so it becomes a thioester and acyl-thioester intermediate. Now what happens is then the Pi, that is the phosphate comes and cleaves the S-C bond and your enzyme is regenerated and in the event what happens is the phosphate adds on to the first carbon atom.

So now you have 1, 3-bisphosphoglycerate. Is that clear. So what happens is we have this acyl-thioester that is attacked by the Pi, which actually cleaves the substrate, rather in this case it has

now become the product and regenerates the enzyme, so we have again enzyme cysteine with SH that now is ready to act upon another glyceraldehyde-3-phosphate. okay., basically what we have here is from glyceraldehyde-3-phosphate with the help of this cysteine of the enzyme.

We have the formation of the thiohemiacetal intermediate that then forms an acyl-thioester intermediate, which then is attacked by Pi that yields 1,3-bisphosphoglycerate and regenerates the enzyme, okay, so that is our step.

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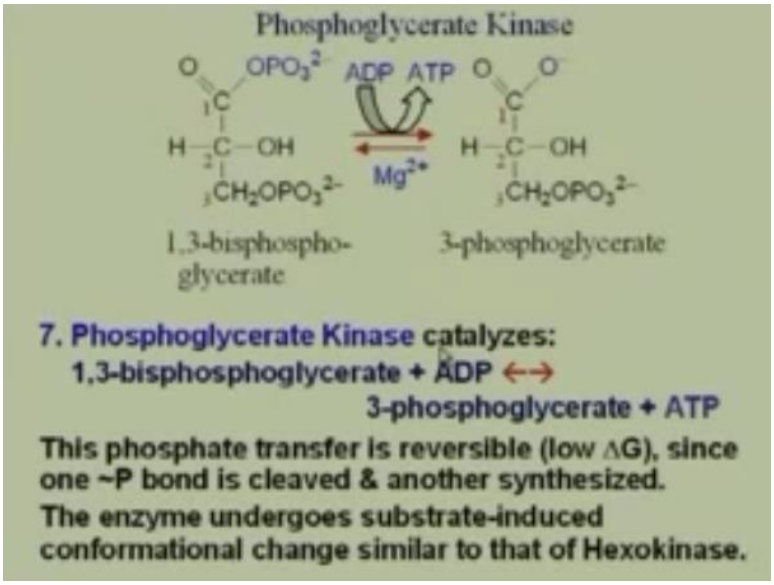

So, now we have landed up with 1,3-bisphosphoglycerate, now what happens is, this phosphate is taken up by ADP to produce ATP, okay, so we have now the first reaction where we have produced ATP in our glycolysis step, okay, so ADP one of the phosphates from 1,3-bisphosphoglycerate is the first one is lost to ADP, which produces ATP in the event, it forms 3-phosphoglycerate.

So we now have an acid here, not an aldehyde and carbon number 1. okay., and all of this remember is enzyme acting, okay. So initially we had, what was the first thing that we had here, we had glyceraldehyde-3-phosphate, so this was our aldehyde. I have to create pyruvic acid, okay, so I have to create an acetic group at position number 1. The first step in doing that is the formation of 1,3-bisphosphoglycerate.

In the formation of 1,3-bisphosphoglycerate, the enzyme glyceraldehyde-3-phosphate dehydrogenase utilizes a cysteine thiol to bring about the reaction. And in the next step we lose the phosphate to ADP, which produces ATP and we have 1,3-bisphosphoglycerate plus ADP go to 3-phosphoglycerate plus ATP.

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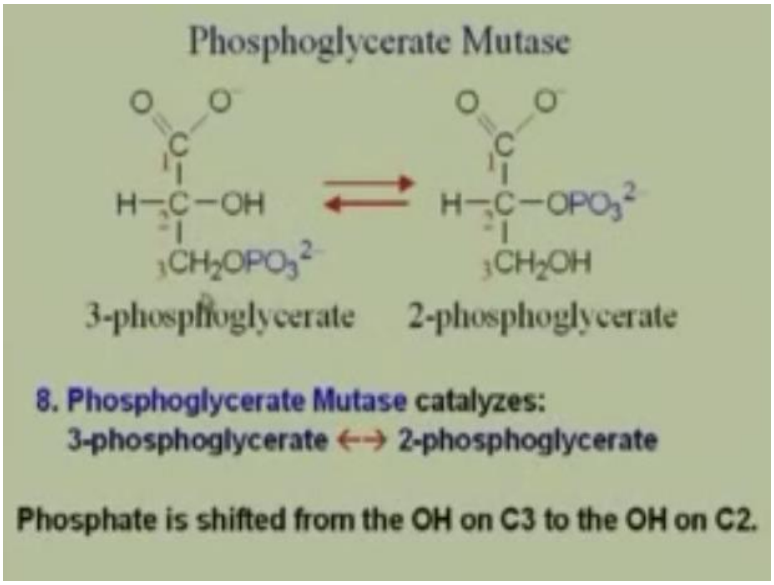

The next step is a mutase. If you remember, we considered in our last class all the enzymes that are actually going to take part in the glycolysis steps. The mutase shifts the phosphate from one position to the other, so the mutase enzyme shifts a moiety from one carbon atom to another. In this case, the 3-phospho is shifted to the second carbon, so the product is 2-phosphoglycerate. Okay.

So instead of 3-phosphoglycerate after the action of this enzyme phosphoglycerate mutase, which shifts the phosphate moiety from the third carbon atom to the second carbon atom, so the phosphate is shifted from the OH on C3 to the OH on C2, so we have a shift here.

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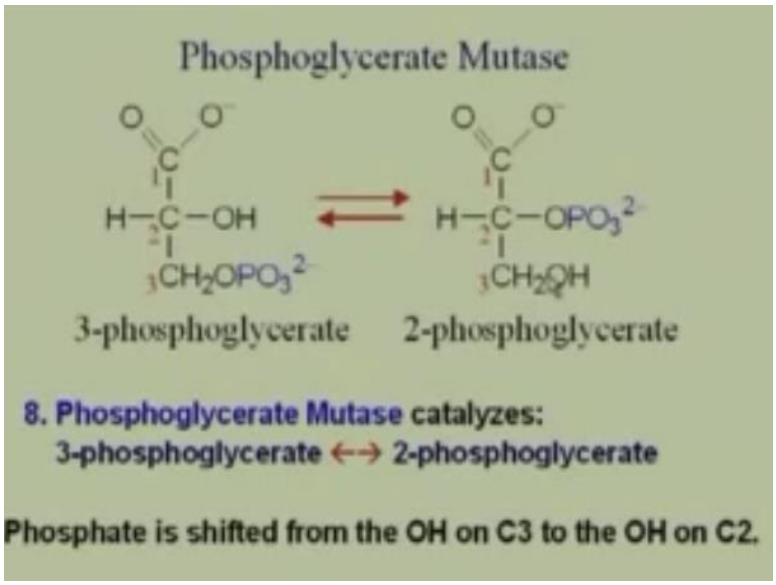

Now in this case, from 3-phosphoglycerate to 2-phosphoglycerate, the enzyme, which is phosphoglycerate mutase has a histidine, okay, and this histidine is present again where, in the active site of the enzyme and what it does is it helps in the phosphate transfer by accepting the phosphate and then donating it again. okay.

So the mechanism is such that the histidine accepts the phosphate from 3-phosphoglycerate and then donates it back, but to the second carbon atom, so this would obviously depend upon the position of where the carbon atoms are actually located in the active site of phosphoglycerate mutase, okay and in the intermediate that we have here, we have an additional, actually, 2,3-bisphosphoglycerate and this finally loses the phosphate, that is the third carbon atom into becoming 2-phosphoglycerate.

So now we have already formed the acid, we are gradually getting to the step where we are going to form an enol and then a pyruvate.

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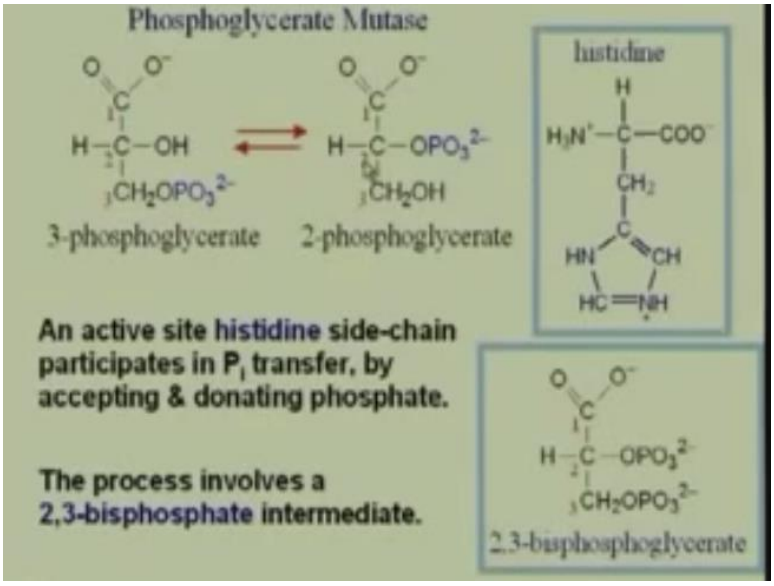

In the ninth step, we have the enolase. Enolase actually, we have now reached two phosphoglycerate. We had 3-phosphoglycerate, we have now formed 2-phosphoglycerate by what enzyme, a mutase. After 2-phosphoglycerate, we have now an enolate intermediate. What is that enolate intermediate. It has formed with the loss of H plus here. It has formed a double bond at this position between carbon atoms 1 and 2, we have a double bond formation.

Then, with the loss of OH we have a double bond between carbons 2 and 3. okay. So this negative charge of the oxygen will come back here, this forms a double bond here and the OH is lost. Is that clear, the mechanism, so we the O come back here to form the double bond, then this double bond shifts here, the OH is lost, so what we get is, we get phosphoenolpyruvate, so we are gradually getting to our final step, which is going to be formation of pyruvate, from where, from glucose. okay.

So we have in our ninth step, enolase that catalyzes the formation of phosphoenolpyruvate from 2-phosphoglycerate, which is actually nothing but a dehydration reaction. So, essentially you are losing this hydrogen and this OH, okay into forming a double bond between carbon atoms 2 and 3, that is essentially what is happening, so you have CH2 double bond C with the phosphate attached and C double bond O and O minus a carboxylate group, okay.

So essentially you are having a dehydration where you are losing this hydrogen and this OH and

you are forming a double bond. Now, what else do you have to lose to form the pyruvate, the phosphate, so that will be our next step, but there are some other notes here where we have the dehydration reaction is magnesium dependent, the two magnesium ions interact with oxygen atoms of the substrate carboxyl group at the active site.

So what happens is we have a magnesium interacting with the oxygen atoms of the carboxylate group of 2-phosphoglycerate that helps stabilize, so we have two O minuses here, right, so what happens is the magnesium ion stabilize this enolate ion and then we have an extraction of the hydrogen from carbon number 2, when a lysine actually helps in doing that. Okay. So the active site in this case would be the presence of the lysine and the presence of two magnesium ions that would help stabilize the enolate intermediate.

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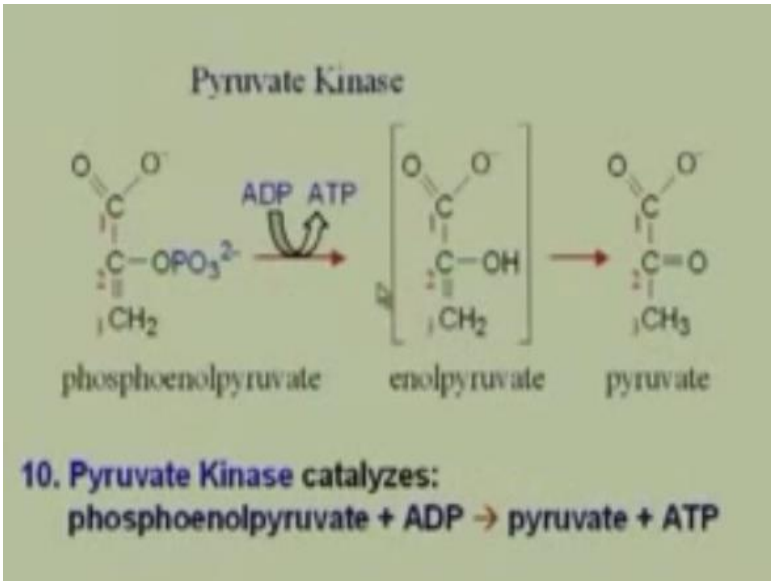

So our next step now is the formation of pyruvate where we have to lose the phosphate. okay, So, what we have is this is the last step where we have formed pyruvate. Pyruvate is CH3-C double bond O COH, that is pyruvic acid. So we have phosphoenolpyruvate that was formed from what?

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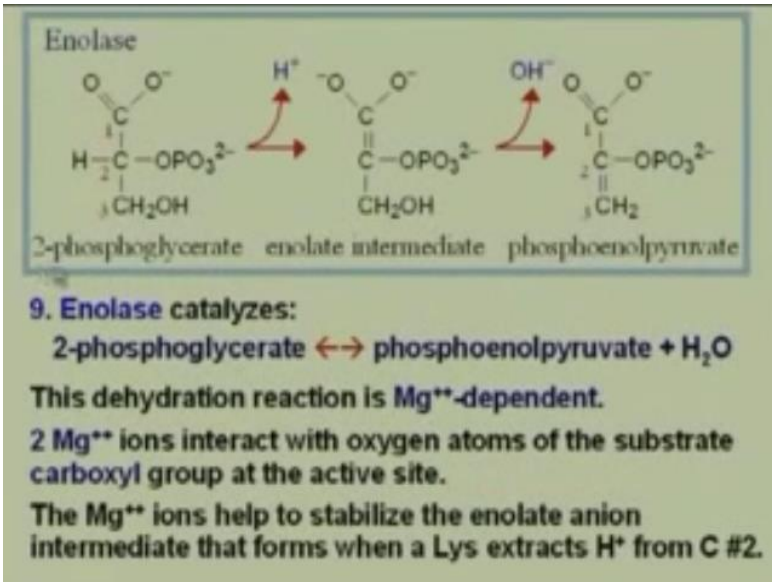

It was formed from two phosphoglycerates. From two phosphoglycerates, we formed phosphoenolpyruvate and we now know that to form pyruvic acid, we have to have CH3 C double bond O COOH or other pyruvate would be CH3-C double bond O COO minus, okay. So we have to lose this phosphate.

And who takes up the phosphate ADP, okay. So now we form another ATP here in this step of glycolysis, so and the enzyme here is pyruvate kinase that is shifting the phosphate, remember the kinase shifts the phosphate. It is taking the phosphate from phosphoenolpyruvate and giving it to ADP and forming ATP. The intermediate is enolpyruvate that actually then forms the pyruvate, so our last step is phosphoenolpyruvate plus ADP going to pyruvate plus ATP, okay.

So eventually we have broken down the glucose and we have finally formed pyruvate. This pyruvate is then later on going into through Acetyl-CoA, it is going to go into the tricarboxylic acid cycle and from there we will have the production of carbon dioxide and water, which we will see in a later class.

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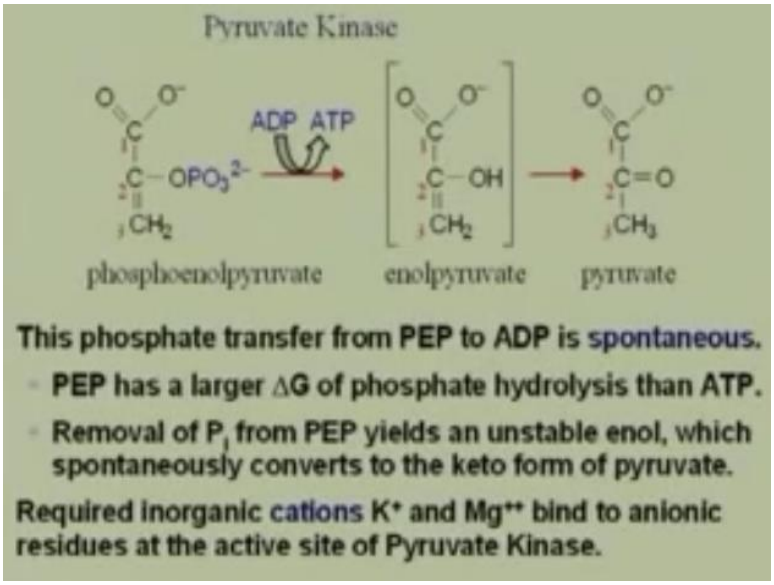

Okay, now the formation of the phosphate transfer from PEP, what is PEP, phosphoenolpyruvate. okay, two ADP is a spontaneous reaction. okay, PEP has a larger delta G of phosphate hydrolysis than ATP, what does it mean, it is going to lose a phosphate very easily and ADP will take up this phosphate to form ATP and because you have these anionic residues.

There are certain cations that bind to the anionic residues of the active site of pyruvate kinase to bring about this particular reaction, okay, so remember that apart from the electron transfer cofactors, apart from the site changes that are present, since we have carboxylate anions or enolate ions, we have some cations, specifically that they use to stabilize the intermediates, okay. So if we look at the overall glycolysis steps now, we have 10 steps, okay.

So if we just reiterate we have glucose in the first step going to glucose-6-phosphate. You have to remember that you have to form the pyruvate from the glucose, so the first step is glucose with the help of hexokinase and ATP forming glucose-6-phosphate. The next step is an isomerase where we formed fructose-6-phosphate. We then need to form glucose-1,6-bisphosphate, which means that we need another kinase and another ATP, okay.

This you should remember in this form, so we have glucose, glucose-6-phosphate and since you have to form fructose-1,6-bisphosphate, you have to form fructose-6-phosphate, so you need an isomerase that is going to form fructose-6-phosphate from glucose-6-phosphate with the kinase

you have fructose-1,6-bisphosphate and aldolase acts upon the open ketone form of fructose-1,6-bisphosphate.

That is what I showed you yesterday with the lysine acting there and we have now a breakup into two 3-carbon moieties. They are dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. The glyceraldehyde-3-phosphate is going to continue the glycolysis.

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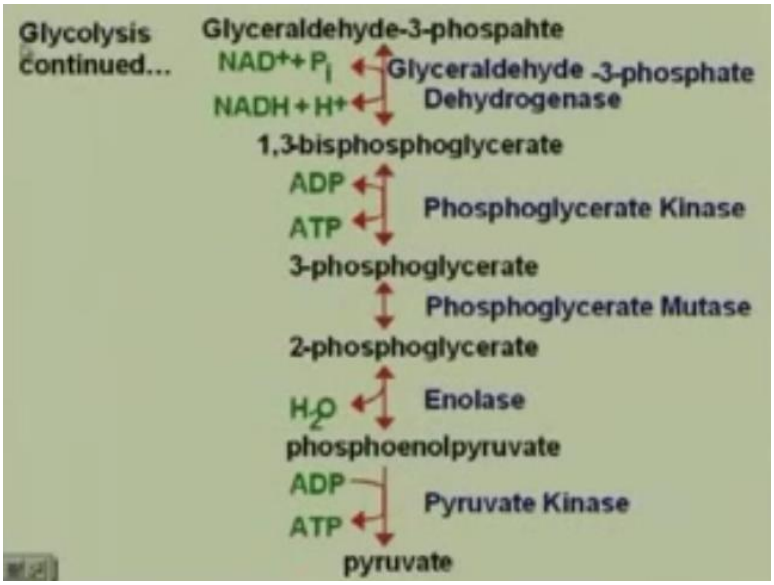

So what is our glycolysis continued, we have glyceraldehyde-3-phosphate with glyceraldehyde-3-phosphate dehydrogenase with the help of NAD plus and Pi form 1,3-bisphosphoglycerate, okay. So you remember in this step when we have the dehydrogenase you are forming from the glyceraldehyde, you are forming glycerate. That is important. Because you have to form finally pyruvate, which means you have to have what moiety, a carboxylic acid moiety, right, so this is the step where you have that oxidation actually take place from the aldehyde to the acid.

So if you have a redox reaction what is the enzyme that you need, a dehydrogenase or an oxidase or some such enzyme that is going to bring about the redox reaction and for that you need an electron transfer cofactor, which in this case is NAD plus, okay. So this is what you have to remember where you have glyceraldehyde-3-phosphate that takes up NAD plus and a Pi to form 1,3-bisphosphoglycerate, okay, then it has to lose the phosphate, as simple as that.

Now we needed the bisphosphoglycerate to oxidize the aldehyde, okay. So for the oxidation of the aldehyde, which in turn reduced NAD plus to NADH okay because this is a redox reaction, right. What is getting oxidized in this case, glyceraldehyde is getting oxidized to form glycerate. What is getting reduced. NAD plus is getting reduced to NADH and the enzyme is the dehydrogenase, okay.

So we have glyceraldehyde-3-phosphate going to glyceraldehyde-3-phosphate dehydrogenase and in the event it forms 1,3-bisphosphoglycerate. Now that you have formed a glycerate, you have to lose the phosphate to form the pyruvate, okay. So the first step in the loss of one of the phosphates is the phosphoglycerate kinase that is going to lose the phosphate that is attached to the carboxylic first carbon atom of the glycerate and you have 3-phosphoglycerate formed, fine.

After you form 3-phosphoglycerate, there is a mutase reaction, which shifts the phosphate moiety 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 of pyruvic acid, which then forms pyruvate after the loss of the phosphate and who takes up this phosphate, ADP, okay.

So that comprises the whole series of steps where glucose is broken down into pyruvate. Now what we have to see is we have to do a balance of energy, okay. We have to see how many ATPs are taken up, how many ATPs are produced and to see whether the actual breakdown of glucose is giving us any energy at all. There is one thing that we have to remember that per glucose, there are two 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, okay.

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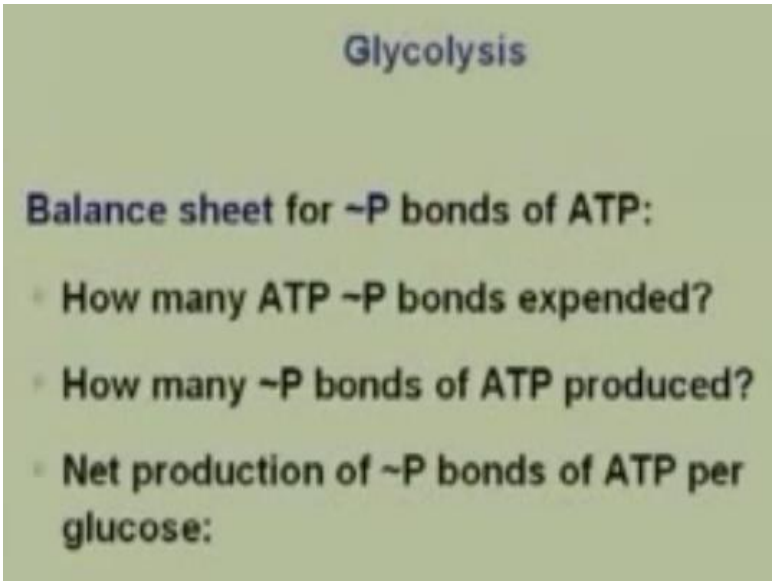

So our balance sheet for the phosphate bonds of ATP. How many ATP bonds are broken. How many are broken.

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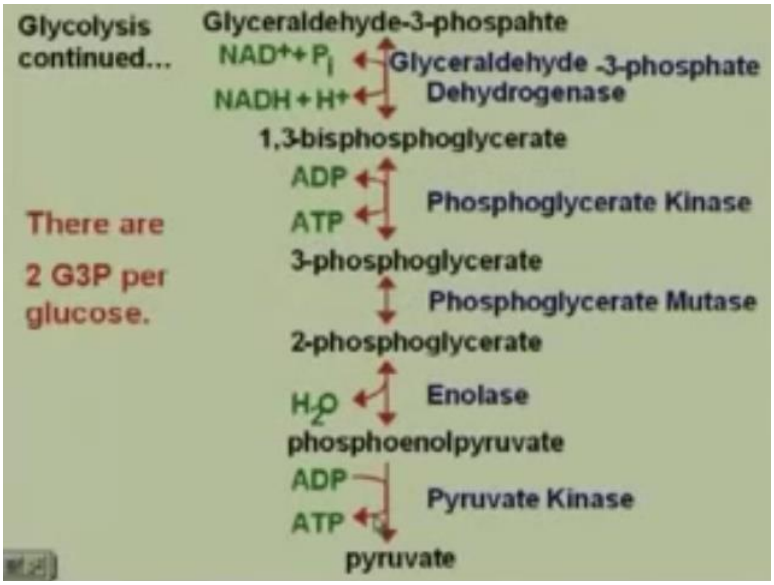

This is our whole series. We have one broken when we form glucose to glucose-6-phosphate, right, another broken when we formed fructose-1,6-bisphosphate from fructose-6-phosphate. Fine, so our answer for the first question is 2. How many phosphate bonds of ATP are produced. How many are produced, is it 2 from every molecule of glucose, 4. Why, because we have two of these, glyceraldehyde-1, glyceraldehyde-3-phosphate is going to create two ATPs, right.

But in our previous step, what do we have, we have two of these moieties, because we have

broken down, what the 6-member ring in 6 carbons into two 3-carbon moieties. Because of the two, that is why I specifically mention that there are two G3Ps that is glyceraldehyde-3-phosphates per glucose, so we have four. So how many bonds of ATP are produced, four and the net production therefore per glucose is two, because we are utilizing two and recreating four, so eventually we get some energy after we break down a molecule of glucose.

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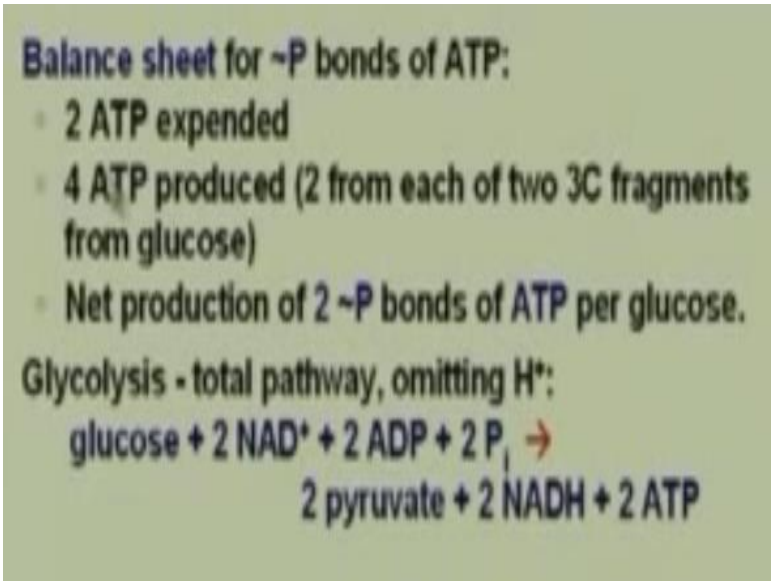

Okay, so this is what we have. We have two ATP expended, four ATP produced that is two from each of the two 3-carbon fragments from glucose and the net production there is two phosphate bonds of ATP or two ATP rather per glucose. okay, so our overall step is going to be glucose plus 2NAD plus, where did we use this NAD plus. We used one here. Is it just one here, two here because we have again two of G3P okay, so this is where we are using two of them.

Then two ADP and 2Pi. Where was Pi used again, in the same step where you had NAD plus we used a Pi to form the phospho and what does this finally form, two pyruvates from one glucose, two 3-carbon moieties, two NADH plus two ATP, so that is our total pathway.

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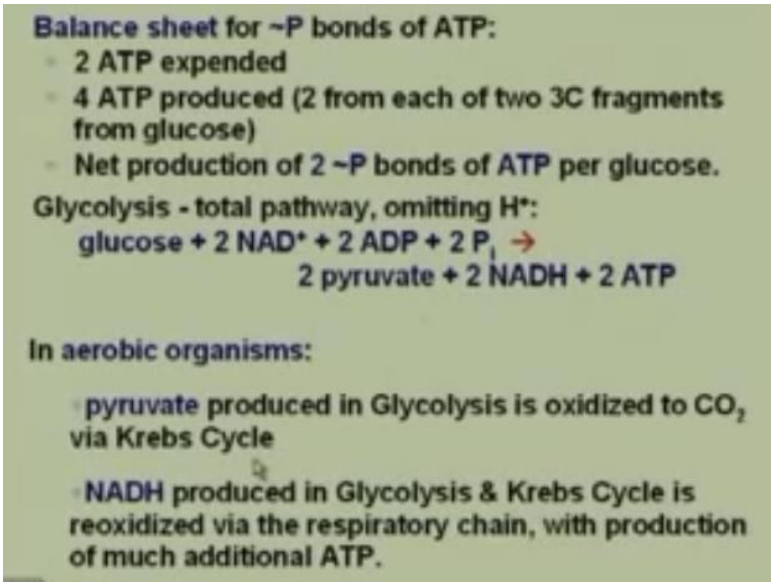

Now, in aerobic organisms, you have pyruvate that is produced in glycolysis, oxidized to carbon dioxide. That is what we are going to see when we do the Krebs cycle. This is also known as the TCA cycle and the NADH. You have NADH produced here this NADH that is produced in glycolysis and also in the Krebs cycle is reoxidized. What is it mean to be reoxidizing NADH, means you have to get back NAD plus, because you need NAD plus for the glycolysis and now it is already NADH.

The only way you can get it back is via the respiratory chain. That is the oxidative phosphorylation, which we studied with production of a lot of additional ATP, which we started with the proton pump. There you saw there was mainly FADH and NADH that was used there, right.

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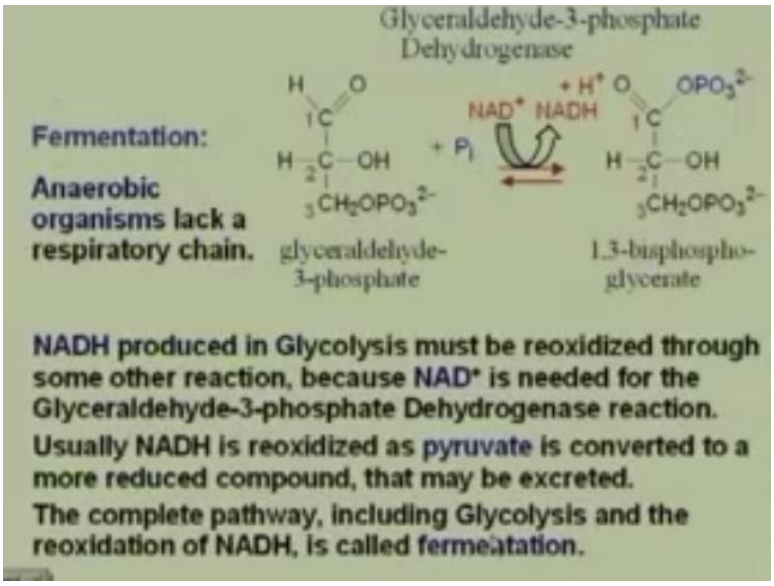

Now, another thing that we have to look at is anaerobic organisms. When we have aerobic organisms, we have the formation of pyruvate, okay, but in this case what happens there is a formation of lactate instead of pyruvate. Where for example even in our muscles where there is a lack of oxygen at times, pyruvate is formed into lactate, okay. Now in anaerobic organism, what is anaerobic organism means they function without the presence of oxygen, okay.

They lack a respiratory chain in that sense, but in the formation of the glyceraldehyde-3-phosphate, two 1,3-bisphosphoglycerate where we have the NAD plus and the Pi, this is one of the reactions in glycolysis, okay. Now the NADH produced in glycolysis must be reoxidized through some other reaction because it is needed for this reaction and the NADH is reoxidized as pyruvate is converted to more reduced compound later on that may be excreted.

This is one of the cases and the complete pathway including glycolysis and the reoxidation is actually called fermentation. We will see that.

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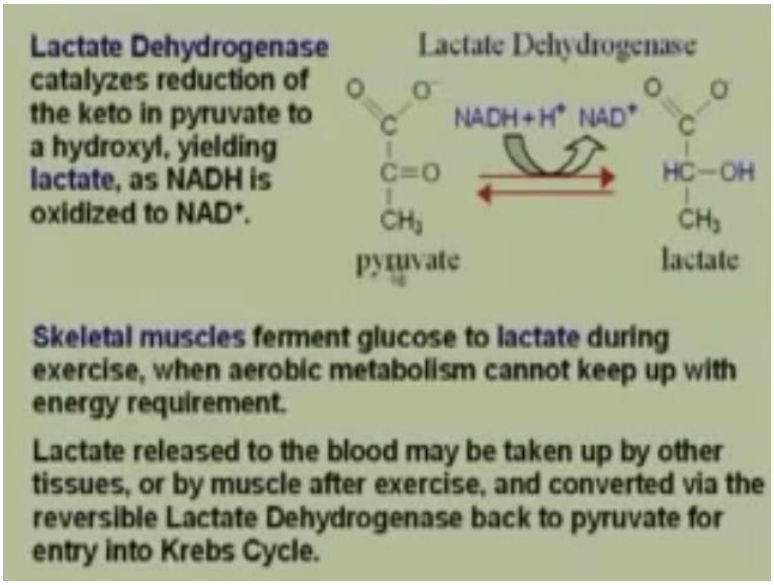

What we have here is now we created pyruvate from glucose, that was our final step in a glycolytic cycle, right, now we can reform NAD plus. Remember what did we take in the bisphosphoglycerate formation. We took NAD plus and made NADH out of it, right. Now, lactate dehydrogenase it actually reduces the keto in the pyruvate and in the event, it oxidizes NADH to NAD plus. Again, it is a redox reaction, so the enzyme is going to be a dehydrogenase, right.

Now, for example, this is what happens in skeletal muscles that ferment glucose to lactate during exercise when aerobic metabolism cannot keep up with the energy requirement, so what happens is this pyruvate is further, what happens, there is a reduction of the keto in the pyruvate and this reduction will eventually do what, oxidize NADH to NAD plus, okay. So, the lactate released to the blood may be taken up by other tissues or by the muscle after exercise and convert it via the reversible lactate dehydrogenase back to pyruvate to get in to the Krebs cycle.

So this is sort of a stop-cap situation where we would have in the lack of aerobic metabolism that would lead to the lactate formation where there is not enough oxygen present.

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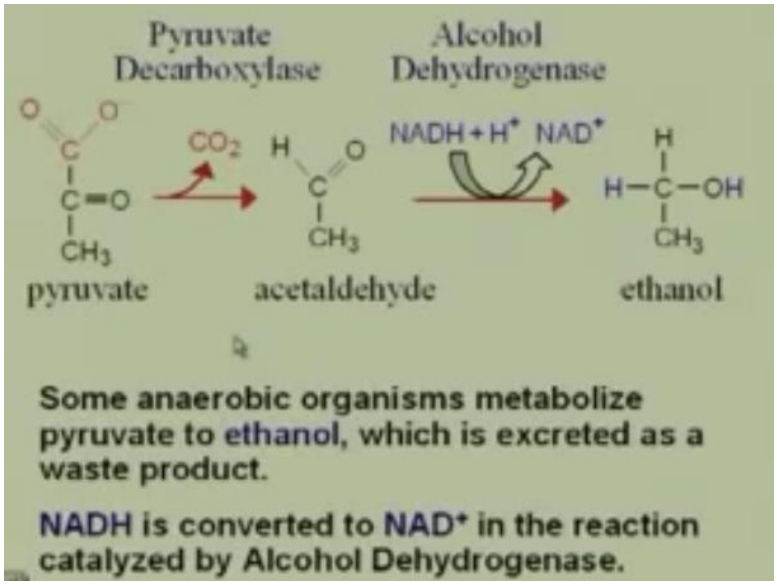

Now some anaerobic organisms metabolize pyruvate to ethanol. This process you have learnt fermentation, like yeast. This is a reaction that yeast often do where you have industrial chemistry, where you learn about a lot of this, where we have NADH go to NAD plus by alcohol dehydrogenase. In the first step, the pyruvate loses carbon dioxide to form acetaldehyde, okay. That is one step of the reaction.

This acetaldehyde then in the presence of alcohol dehydrogenase forms ethanol, okay and in the event NADH is oxidized to NAD plus. So, they metabolize pyruvate to ethanol, which is actually a waste product, okay.

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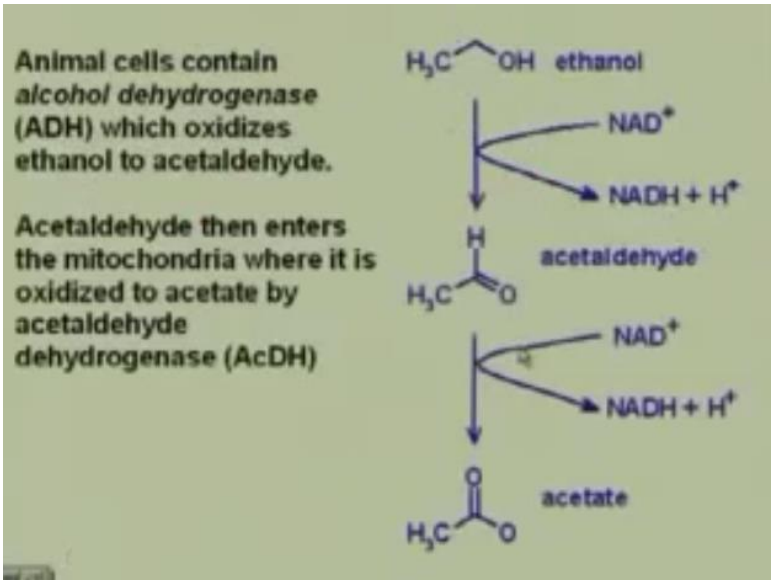

This is a process in fermentation. So animal cells also contain alcohol dehydrogenase, which oxidizes ethanol to acetaldehyde, okay. So we have ethanol, then we have NAD plus that is getting reduced to NADH and what is it doing, it is oxidizing ethanol to acetaldehyde, okay and then further oxidation is also possible to acetate where you have NAD plus going to NADH plus H plus, so what are you doing in this case.

You are reducing NAD plus in the event oxidizing acetaldehyde to acetate. In fact, the animal cells that contain alcohol dehydrogenase in the forming of acetaldehyde, this is what results in the hangover that you get from alcohol consumption. You hear of hangovers from alcohol consumption. What happens is this ethanol gets oxidized to acetaldehyde in the body by this particular enzyme alcohol dehydrogenase and this acetaldehyde actually reacts with certain proteins to give you that numb feel, okay. So it is all biochemistry that goes on.

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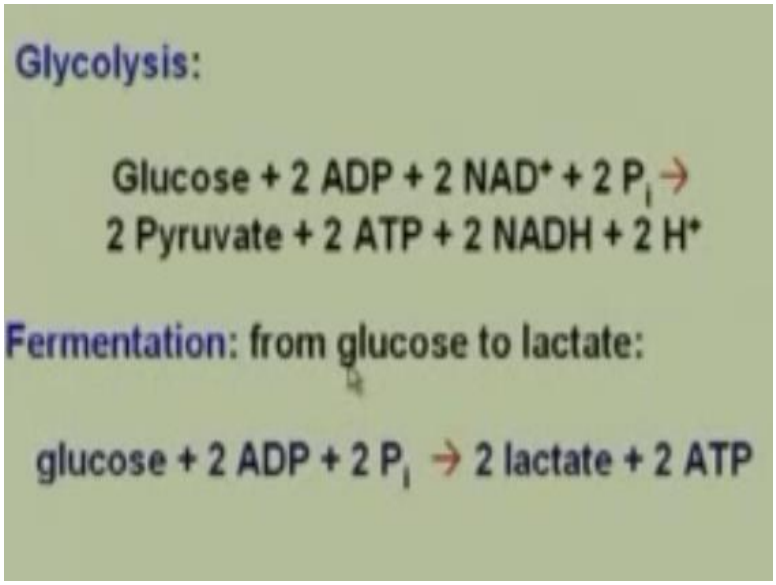

So this is our final step of glycolysis where we have our glucose, 2ADP, 2NAD plus 2phosphate that forms 2 pyruvate, 2 ATP, 2NADH plus 2H plus. In the fermentation instead of pyruvate, we are going to get lactate, okay, we have the glucose with 2ADP plus 2Pi will give us 2 lactate plus 2ATP because remember when we are forming the pyruvate to the lactate, we just go back a step. This NADH, NAD plus does not come into the picture, right.

Because if we look at this reaction, we had the NAD plus and the Pi here forming NADH, but

that is not, the NADH is utilized in that pyruvate reaction to lactate, okay. So, we do not have the NAD plus NADH feature in the fermentation where we are creating lactate from glucose, okay. Because that pyruvate to lactate conversion requires NAD plus NADH, okay.

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Glycolysis Enzyme/Reaction	$\Delta G^{o'}$ kJ/mol	ΔG kJ/mol
Hexokinase	-20.9	-27.2
Phosphoglucose Isomerase	+2.2	-1.4
Phosphofructokinase	-17.2	-25.9
Aldolase	+22.8	-5.9
Triosephosphate Isomerase	+7.9	-ve
Glyceraldehyde-3-P Dehydrogenase & Phosphoglycerate Kinase	-16.7	-1.1
Phosphoglycerate Mutase	+4.7	-0.6
Enolase	-3.2	-2.4
Pyruvate Kinase	-23.0	-13.9

So, if we now look at all the enzymes that are involved, there are 10 enzymes here for the 10 steps in glycolysis. Hexokinase, isomerase, so now you should be able to tell me exactly what each of these steps are doing. Hexokinase creates glucose-6-phosphate from glucose. Isomerase creates fructose-6-phosphate from glucose-6-phosphate. Phosphofructokinase creates the fructose-1,6-bisphosphate from the fructose-6-phosphate.

Aldolase breaks it up into dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. The isomerase interconverts dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. Then we have glyceraldehyde-3-phosphate dehydrogenase, then we have phosphoglycerate kinase that is going to create the bisphosphoglycerate. The bisphosphoglycerate has a mutase acting on it where creates from 3-phosphoglycerate, it goes to 2-phosphoglycerate.

Then we have the enolase that creates the phosphoenol pyruvate that then loses. Because when you have the enolase, you have to have an enol present there, so you have a phosphoenol pyruvate that phosphoenol pyruvate loses the phosphorus by pyruvate kinase to form pyruvate, so these are all the steps of glycolysis okay and if we now look at the specific free energy

changes involved in the steps, we have a delta G zero prime.

The prime means a biological standard where the temperature is taken as 37 degree centigrade instead of 25 degree centigrade, because you are looking at the free energy changes that are happening in the body when the reactions are taking place. In each of the kinase reaction, this is just the delta G with the concentrations that we have, this is delta G zero.

So how we do we calculate delta G using delta G zero and you need the concentrations of the reactants and the products, that is all you need. How do you do, you calculate an equilibrium constant for a delta G zero process, right and then you have the reaction quotient that is going to depend upon the concentration of the reactants and products that you have and you can calculate a delta G associated with the process provided you know the delta G zero, fine.

So considering that the concentrations of the products and the reactants can be calculated or can be obtained. We get a delta G associated with these enzymes, 10 of these enzymes and if you notice the kinase enzymes are the ones that are most spontaneous, okay. They have high kilojoule per mole value and the reason why they have this is because they have, this is a highly spontaneous reaction where it is going to lose a phosphate. These are the ones that use couple reactions with the ATP that provides the energy actually to give you the product, okay.

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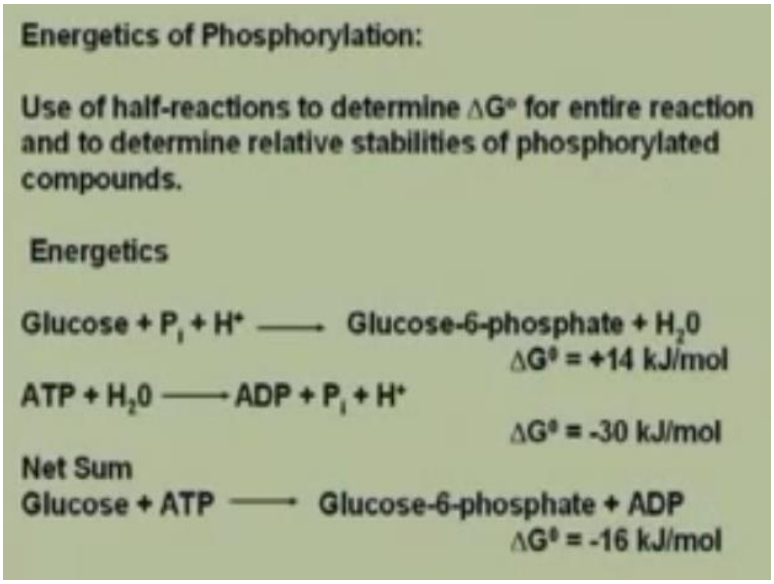

So what we have is if we look now at the energetics of phosphorylation we are looking at the first step. Hexokinase, okay the glucose actually needs the phosphate to become glucose-6-phosphate. The delta G zero associated with that is actually plus 14 kilojoules per mole. So there is no way it is going to happen by itself, okay. But you have a couple reaction that is ATP, the hydrolysis of ATP the high energy phosphate bond that is actually going to break ATP into ADP plus Pi.

This Pi is going to be supplied to glucose. This free energy change is minus 30 kilojoules per mole, okay. so we have this couple reaction, so we use the half reactions to determine the delta G zeros for the whole reactions, okay and we have therefore an overall reaction that is going to be glucose plus ATP giving us glucose-6-phosphate plus ADP with a favourable standard free energy change that we have here, okay.

Now depending on the concentrations of glucose-6-phosphate that you have and the concentration of glucose and the relative amounts of ATP and ADP that you have, the free energy change of the reaction is going to change, right.

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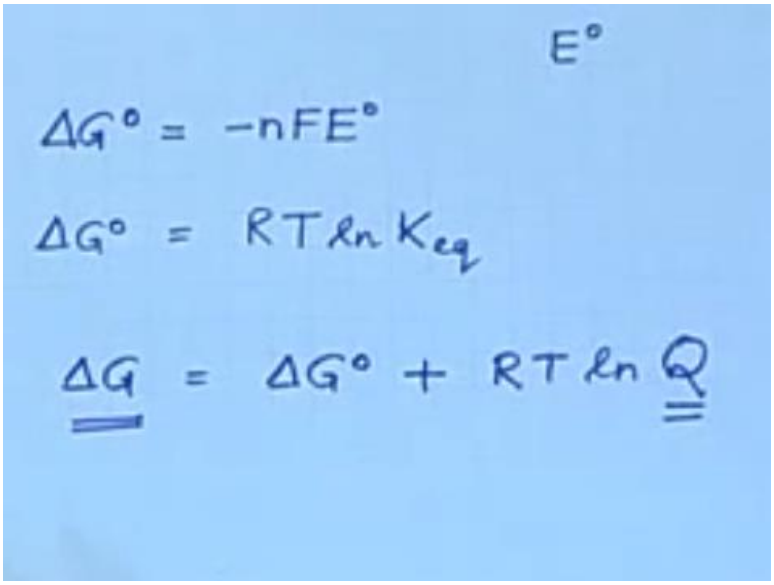

Because what is that reaction that we have? If we look at a delta G zero value, now what you can have is for each of these you can have an E zero also associated with it because remember you have a redox reaction taking place, okay. In some cases, you would have a redox reaction taking

place where you would have this -nFE, right. From this you can determine the equilibrium constant. What is the equation? Equilibrium right. Then you can have a delta G, what is that? That is delta G zero plus RT Ln Q. What is this Q?

This Q is your reaction quotient. What is the reaction Quotient? It is the ratio of the concentrations of the products to the reactants, okay. So based on that you can find a delta G associated with this. Now if we look at this first reaction that we have here, the fact that glucose-6-phosphate concentration is low, okay. What is that Q in this case what is the Q going to be? It is going to be glucose-6-phosphate ADP divided by glucose ATP concentrations that is what Q is going to be.

Now if you have a high concentration of products that could more than compensate the delta G zero value negative that you have here and make an overall delta G of the reaction positive, okay.

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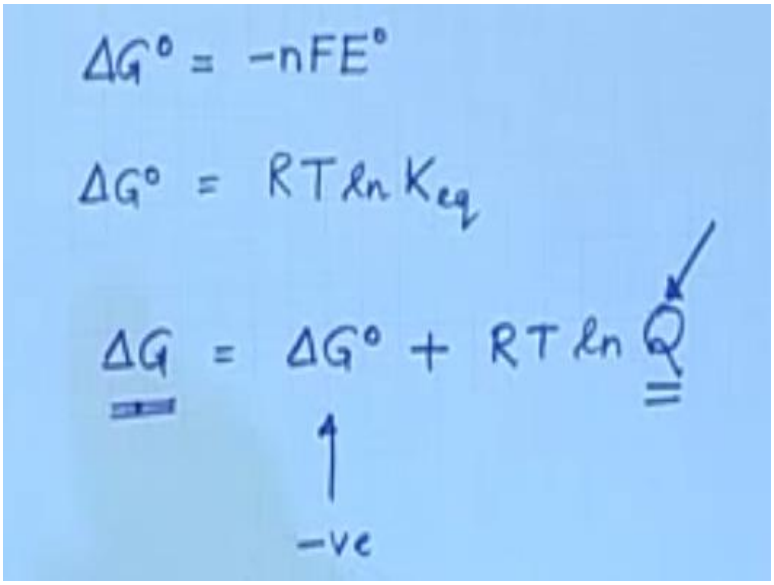

What am I taking about, I am talking about this factor. This is negative but the ratio of the products to the reactants is important here, okay. You cannot have too higher concentration of the products because that will overcompensate for the negative delta G zero you have here making this non-spontaneous, okay. This is what happens in most biological reactions. The product concentrations are pretty low, making the reaction spontaneous because your delta G

zero of the couple reaction is negative, okay.

So the concentration of glucose-6-phosphate in this particular reaction is actually low and the overall Gibbs free energy that you get is minus 27 kilojoules per mole. So what you can do with this value is actually determine what the ratio of products to the reactants are. You know what delta G zero is, you know what delta G is, right? You can find the ratio of the products to the reactants and when you are considering, if it is not mentioned a delta G zero prime value means you use a temperature of 37 degrees centigrade, okay.

So that would be how you would actually calculate the energetics of every step of glycolysis, okay. We can calculate the energetics associated with every step of the glycolysis and find the overall energetics associated with this.

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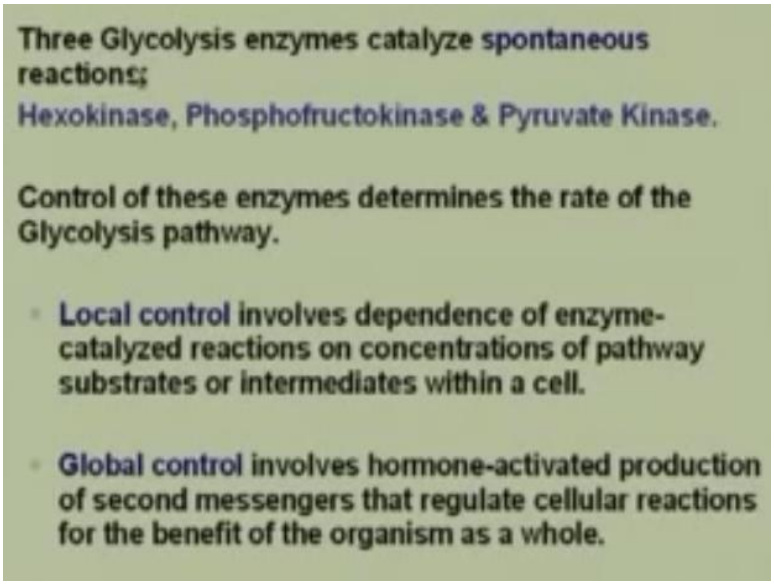

Now the spontaneous reactions that we did see that was with this particular case this enzyme is hexokinase, right.

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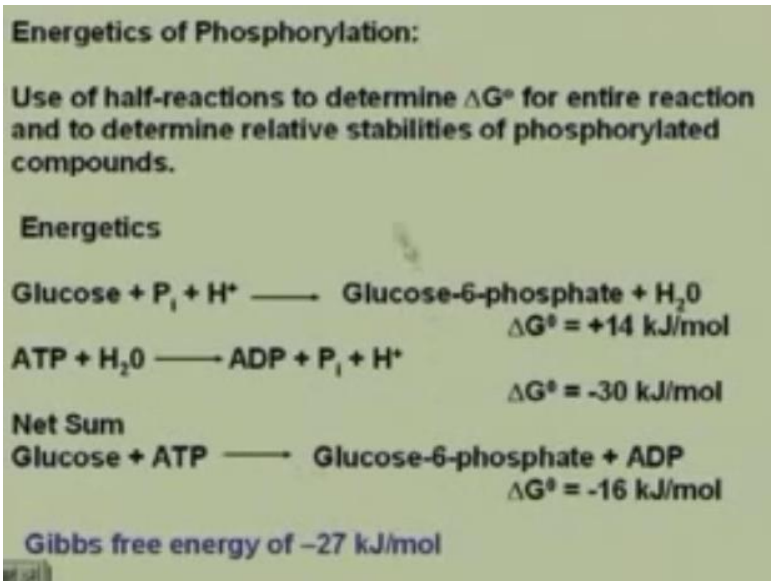

And the other ones that I showed you what? Phosphofructokinase and pyruvate kinase, okay. These are three enzymes that actually catalyze spontaneous reactions and the control of these enzymes actually determines the rate of glycolysis, okay. Because each of these particular reactions if you go back and look at the glycolysis pathway, its these three reactions that are irreversible. So once glucose forms glucose-6-phospate it is tracked in the cell, okay.

So the control of these enzymes actually determines the rate of the glycolysis pathway. So there are two types of control. We have local control and global control. Local control means that involves the dependence of the enzyme-catalyzed reactions on concentrations of pathway substrates or intermediates within cell. So what is this local control this is exactly what I was speaking in the last line where we have the delta G values associated with the amount of product and reactant you have, okay.

So if my product concentration gets too high what is going to happen? My reaction will be non-spontaneous, okay. So that is where you would have local control. If glucose-6-phosphate has too higher concentration the delta G associated with the expression that we have written here will make this non-spontaneous, okay.

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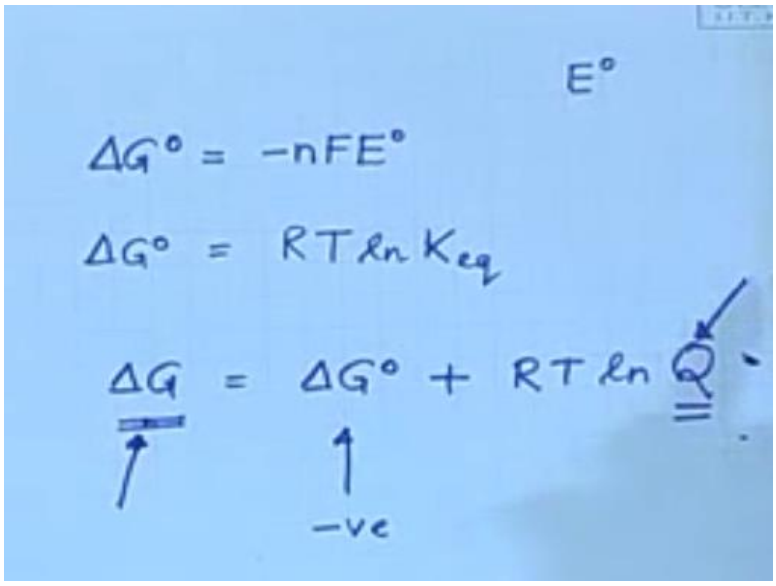

So this is regulated by the amount of glucose-6-phosphate that is found because remember I mentioned in the last class that glucose-6-phosphate actually inhibits hexokinase. So what is going to happen if it inhabits hexokinase, then this enzyme cannot act on another glucose molecule, which means that the concentration of glucose-6-phosphate is getting too high making this Q too large which is offsetting delta G zero preventing a reaction from taking place, okay.

You do not want that to happen, okay. If you want the glycolysis reaction do go forward, then you have to have this regulation. You do not want too much of glucose be broken down, okay. That is where the regulation takes place, okay. So we have local control which involves the dependents of enzyme-catalyzed reactions on concentrations of pathway substrates or intermediates within a cell.

You now realize what this means that the concentration of the intermediates the concentrations of the products and the substrates are extremely important in determining the dependence of these catalyzed reactions. Then you have global control that actually involves hormone-activated production of second messengers, these are certain messengers that are there in the body that actually regulate the cellular reactions for the benefit of the organism as a whole.

So this is like more global approach where it would work on the cell itself preventing the cell from acting or preventing certain reactions taking place altogether, okay. But at the local level

the concentration dependences are due to the variations in the product and the reactant. okay. Now let us look at one such example.

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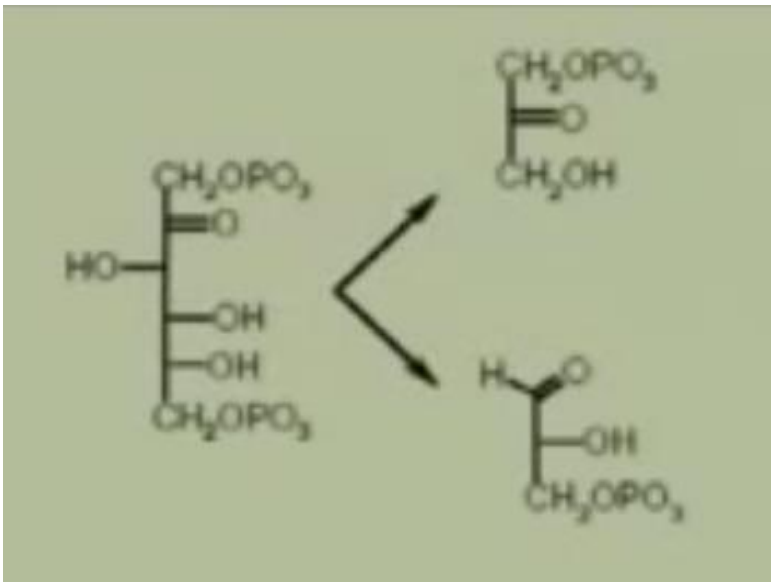

This is one step of the glycolytic pathway. okay. If you look at this, you see it has two phosphates attached carbon number 1 and carbon number 6 you have a ketose. So this is fructose-1,6-bisphosphate. It has broken down into dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. So the enzyme that has acted here is aldolase, okay, in a reverse aldol reaction. So this is what you should be able to recognize.

This is the key step in the glycolysis why, because it is actually breaking down the glucose. Previously all the steps that led to fructose-1,6-bisphosphate still have the 6-carbon atoms in them, okay. This step the aldolase step is the one that you are breaking the 6-carbon unit into 3-carbon units, okay.

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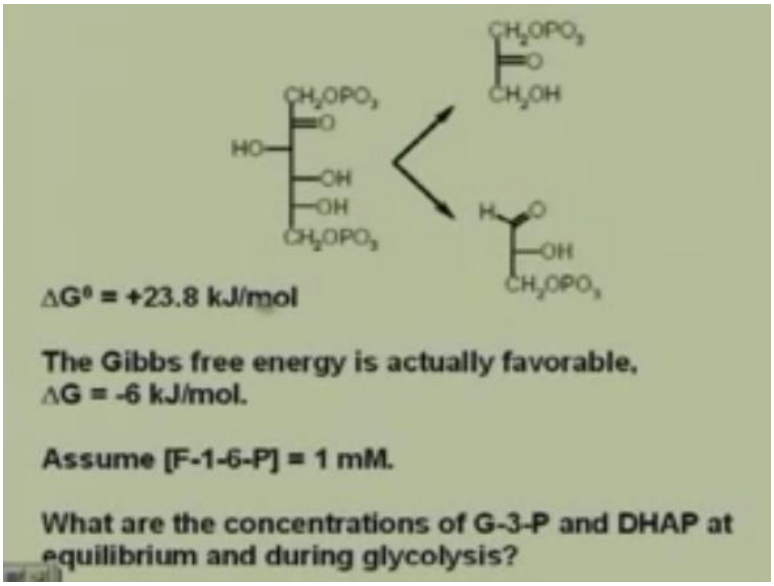

Now we have a delta G zero of this particular reaction having a value of plus 23.8 kilojoules per mole, okay. The Gibbs free energy is actually favorable. What does that mean I have a delta G.

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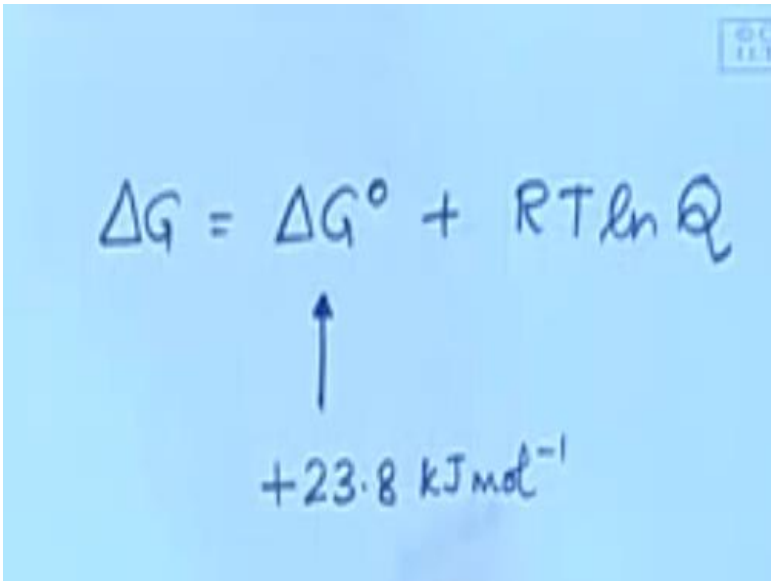

I have a delta G and I have a delta G zero plus RTlnQ. This value is plus 23.8 kilojoules per mole. This value is minus 6 kilojoules per mole. So where is the regulation coming from? It has to come from Q, right.

So what we can do is we can actually calculate assuming that fructose-1,6-bisphosphate is 1 millimolar. I have these in equal concentration, right, because its breaking down its forming from this. So I can actually find out the concentrations of glyceraldehyde-3-phosphate and

dihydroxyacetone phosphate at equilibrium. How can I do that at equilibrium? It will just be that equilibrium constant that I get from the delta G zero value. What do I get from the delta G zero value?

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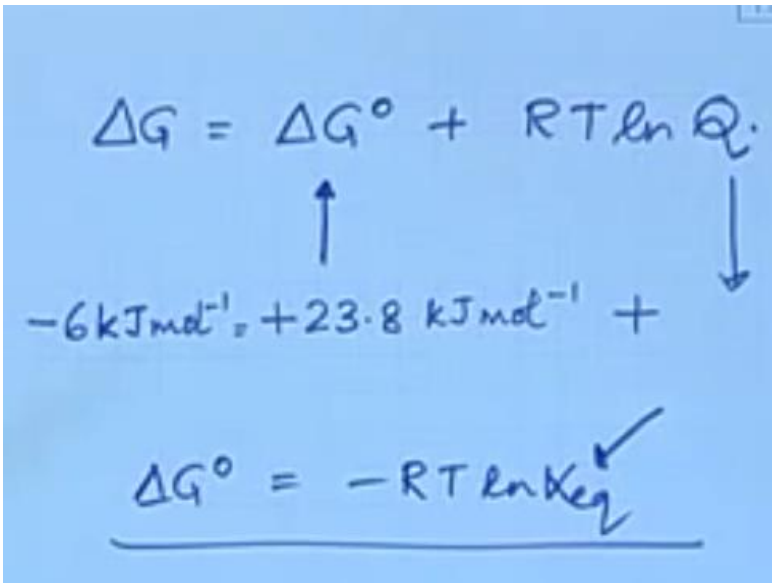

So I can get this because I know what my delta G zero is. I can also get so I can get the ratio of the products to the reactants. Based on considering the 1 millimolar concentration of fructose-1,6-bisphosphate and I can also find the same of ratio during glycolysis when my reaction is actually favorable. okay. so this whole idea gives you the energetics that are associated with each of these steps. Each of these steps has associated with it this energy, okay.

So each of these energy values can tell me what is actually happening. And what delta G zero the reaction actually have the equilibrium, okay. so this completes our discussion on glycolysis where we have actually broken down glucose to form pyruvate. Our next step will now be to see how there is further degradation of pyruvate in the tricarboxylic acid cycle where finally carbon di-oxide and water will be formed. We will do that in our next class. Thank you.

In the last step of our metabolism of carbohydrates, we are going to consider today the tricarboxylic acid cycle or the Krebs cycle.

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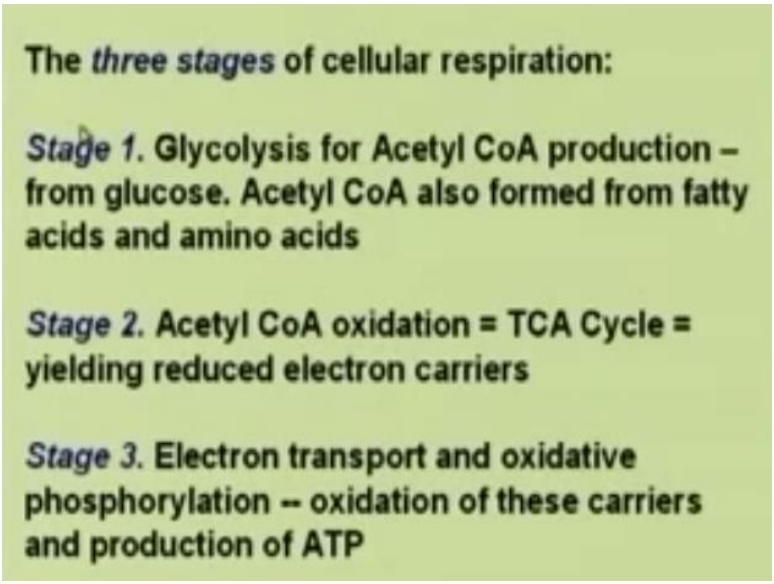

Now if we look at the three stages of cellular respiration there are actually, the first stage that we are gone through that is glycolysis for acetyl coenzyme A production. We have already seen the breakdown of glucose to pyruvate and we will see now how pyruvate actually gets to acetyl conenzyme A that then gets into the tricarboxylic acid cycle, which eventually leads to the production of carbon-dioxide.

And we have already considered the third step where we have electron transport and oxidative phosphorylation that leads to the production of ATP. So these are actually the three stages of cellular respiration.

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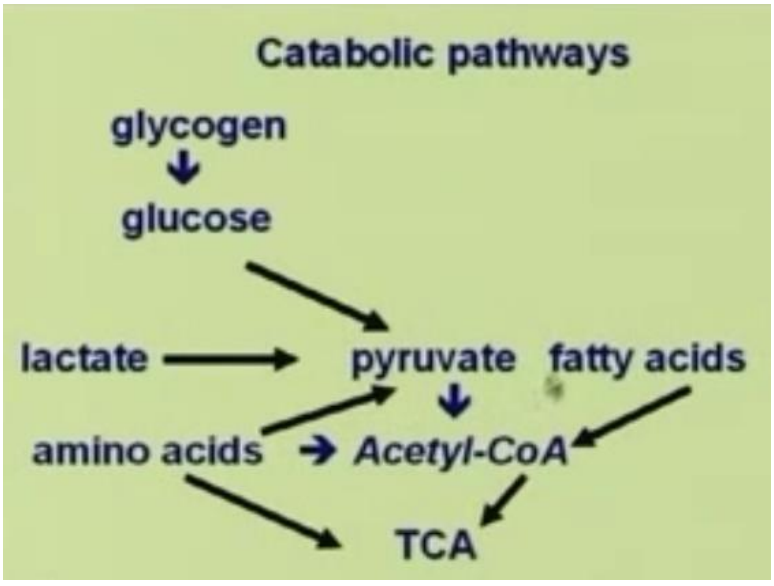

And if we consider the catabolic pathways of all the breakdown whether it is amino acids or fatty acids or glucose, they ultimately lead to acetyl CoA, which is the key component of the TCA cycle which is we will see. So what happens is, what we have studied in glycolysis is glucose getting to pyruvate. Now the reverse of this is also possible in a process that is called gluconeogenesis, where glucose is formed from pyruvate.

It is not the exact reverse of the glycolysis steps, but relatively since these involved some of the similar enzymes, we do have the pyruvate getting back to the glucose. So we also saw how the lactate is formed from the pyruvate. So we have lactate also getting to pyruvate and amino acids have actually entry points at three points where it can get to pyruvate, you can get to acetyl CoA or you could get directly to the tricarboxylic acid cycle. Fatty acid gets into acetyl CoA okay. So we have considered so far the breakdown of glucose to pyruvate.

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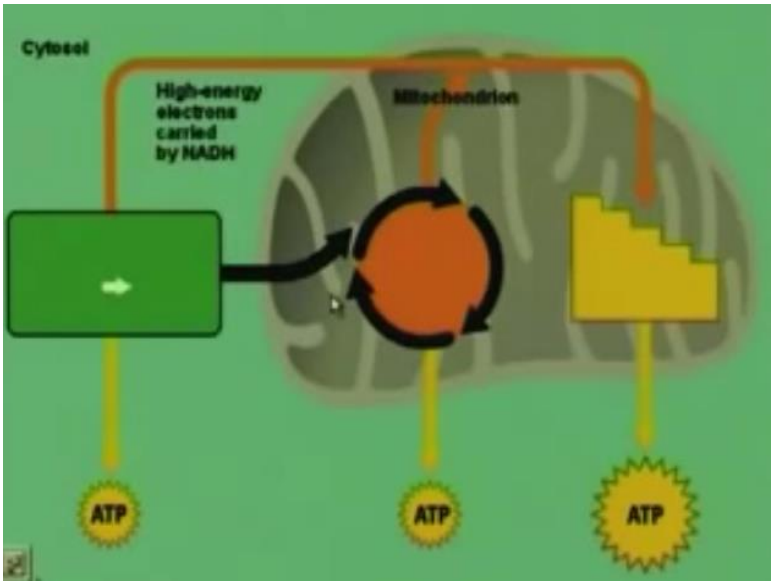

Now in general, we have these three overall steps all of them produce ATP okay. That is our major concern in the production of energy.

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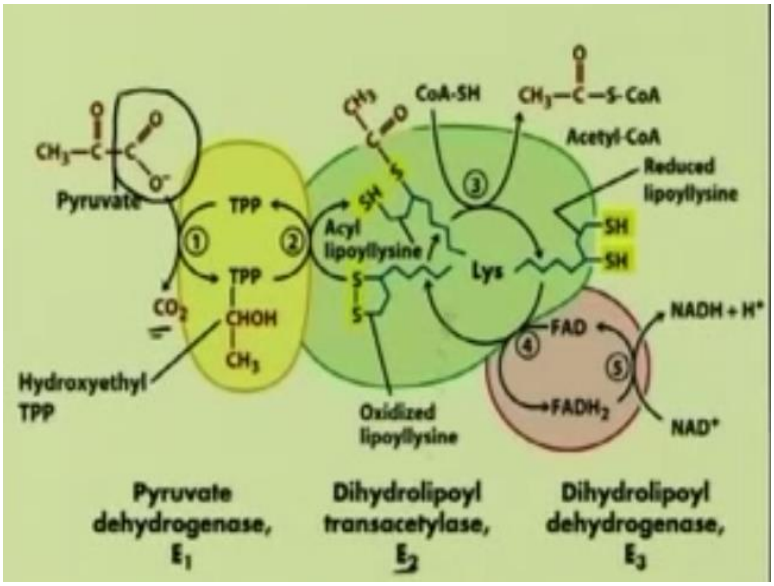

So this is the Lysine. So we see the lysine here and what are these chains. These are the lipoic acid chains. Can you see the lipoic acid chains? These are the lipoyllysine chains. That have the dithyol and the dithyol, one of them picks up the acetyl and has it linked to the sulphur of the lipoamide, the lipoic acid part here. Now what happens is coenzyme A now comes into the picture.

Coenzyme A now has to form the acetyl CoA, so it picks up this acetyl forming acetyl CoA and then you have the reduced SH, SH. Now what must happen for this to act again. This has to get back to the dithiol then. So two of these Hs have to be removed. How can they be removed? They removed by the FAD. FAD then comes into the picture, picks up the two hydrogens and this gets back to your oxidized lipoyllysine. Is that clear?

So this is just the general procedure where you would have these, because you realize that you have the acetate formed here directly already, but you have to have these enzymatic steps to get the enzymes back to where they are started from. So that they can go and act on another pyruvate. Eventually what happens is this gets to the oxidized part. So this can now take up another acetyl, another acyl rather, okay. Another acyl can now get attached to this. FAD has now been reduced to FADH2.