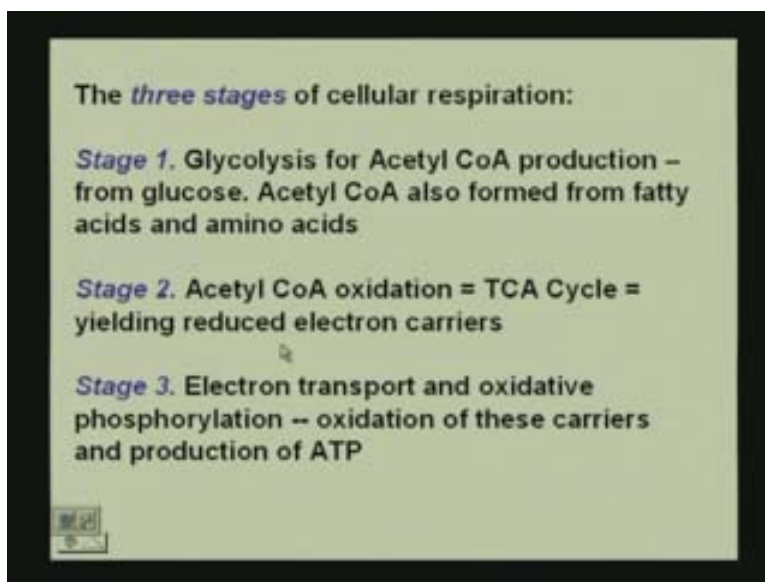


Biochemistry - I
Prof. S. Dasgupta
Department of Chemistry
Indian Institute of Technology, Kharagpur
Lecture # 27
Metabolism - III

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

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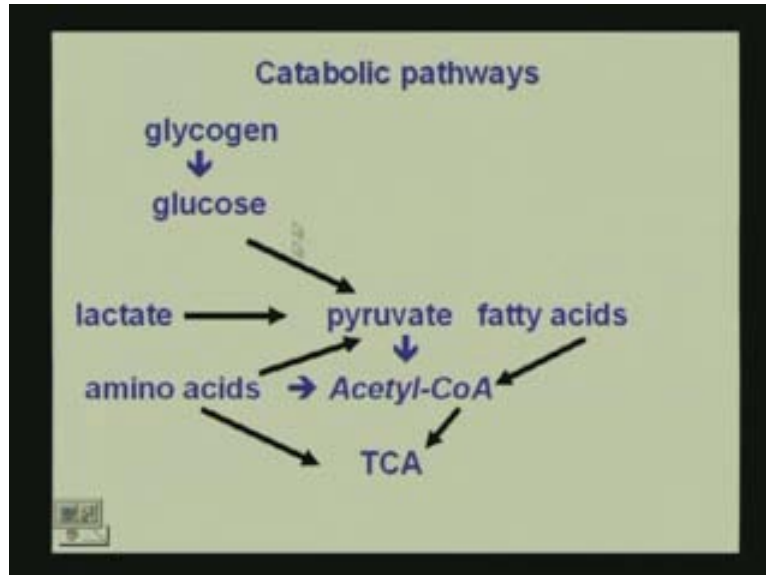


Now if we look at the three stages of cellular respiration, there are actually the first stage that we have gone through that is glycolysis for acetyl coenzyme A production. We have already seen the breakdown of glucose to pyruvate and we will see now how pyruvate actually gets to acetyl coenzyme A that 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 and if we consider the catabolic pathways of all the break down 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 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 step but

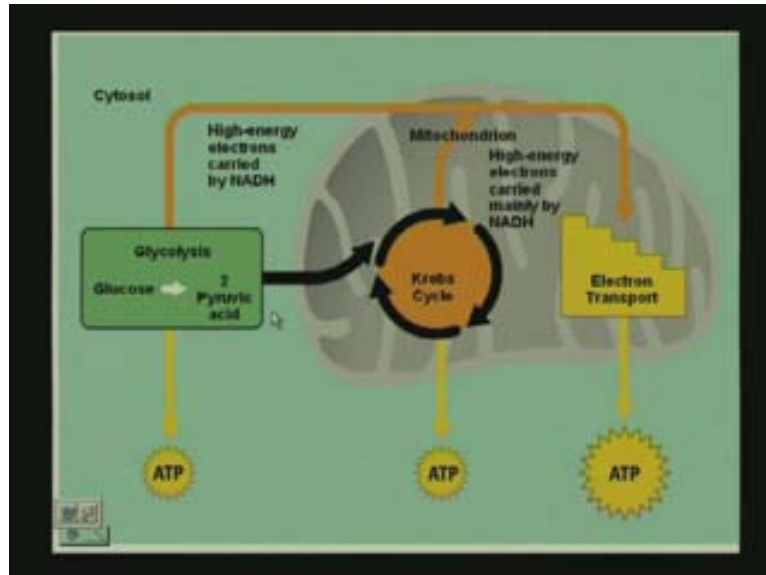
relatively since these involved some of the similar enzymes, we do have the pyruvate getting back to the glucose.

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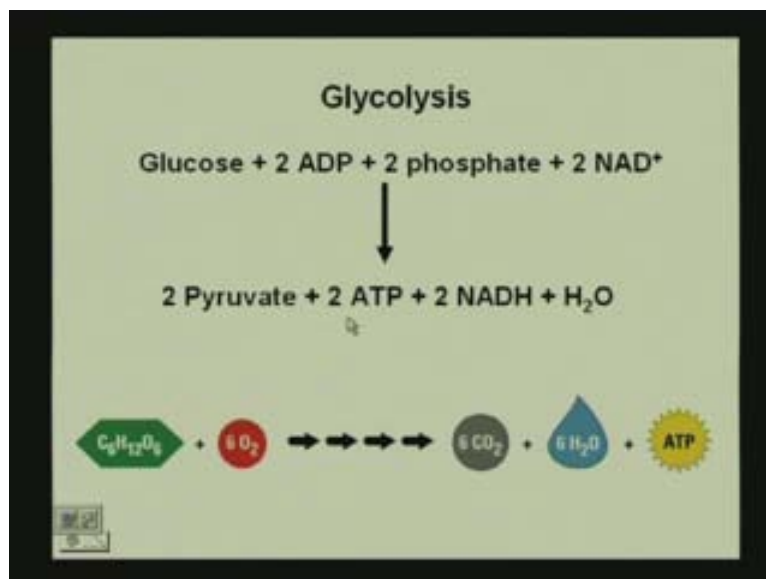
So we also saw how the lactate is formed from the pyruvate. So we have lactate also getting to pyruvate and amino acids have actually entry points at three points where it can get to pyruvate, you can get to acetyl CoA or you could get directly to the tricarboxylic acid cycle. Fatty acids get into acetyl CoA, so we have considered so far the break down of glucose to pyruvate. Now in general we have these three overall steps, all of them produce ATP. That is our major concern in the production of energy. Production of energy in the form of ATP which is ultimately going to be used for the break down of these high energy phosphate bonds that is going to release a large amount of energy for our bodily functions.

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So we have the first step glycolysis where from glucose we get 2 pyruvic acid molecules. Here we have the kerbs cycle which we are going to study right now and the electron transport that also resulted in the production of ATP. So these are the three stages that we are going to have. Now if you look at the two stages that have been mentioned, the stage two and stage three the Krebs cycle and the electron transport they occur in the mitochondria. This is the picture of the mitochondria where you can recognize the inner folds called the crystate of the mitochondria. And this glycolysis step occurs in the cytosol of the cell. So this mitochondria is in the cell, the cytosol is where the glycolysis steps occur and here we have in a mitochondria Krebs cycle and electron transport.

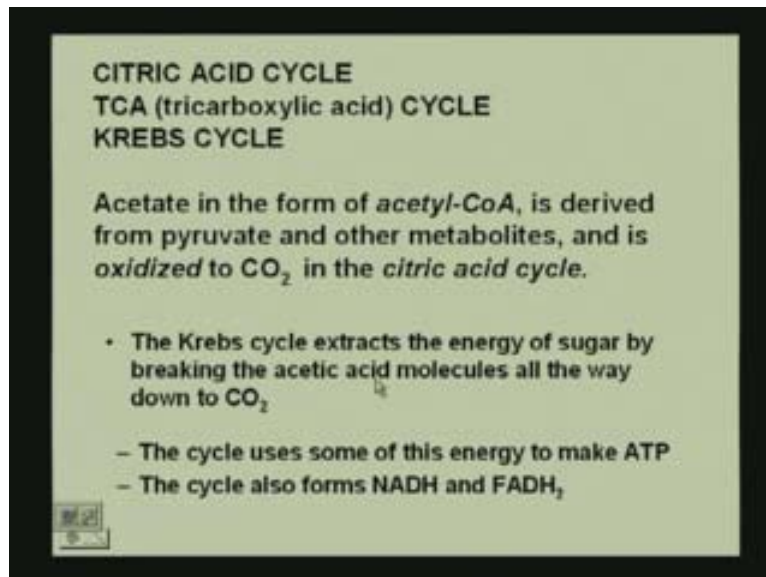
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The electron transport we know occurs in the inner mitochondrion membrane where we have complexes 1 through 4 and the $F_0 F_1$ ATPase that creates the ATP from the proton motive force. So this is what we have done previously where we consider glycolysis, the glucose get into 2 pyruvate. We have gone through all these steps but ultimately glucose is going to be broken down into carbondioxide and water with ATP.

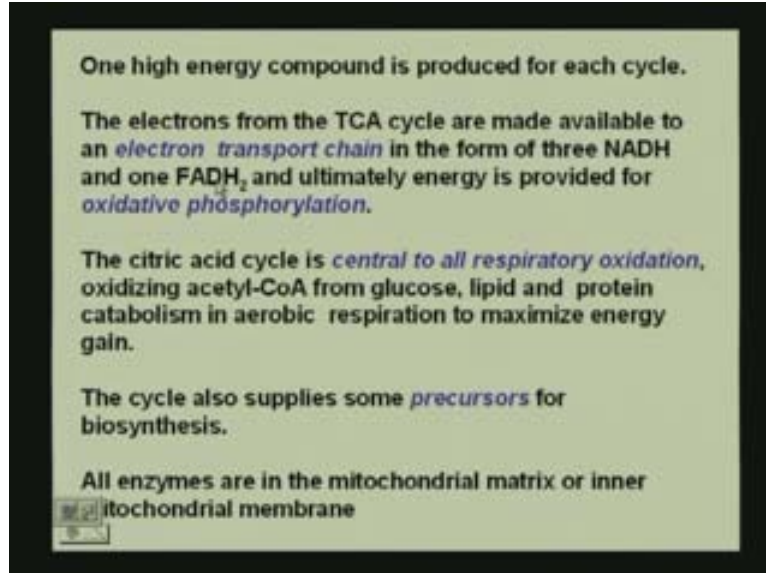
So our next step now is to see how pyruvate is going to get to carbondioxide. This is formed by the kerbs cycle, the 3 different names to the cycle it is called the CAC or the citric acid cycle, the TCA or the tricarboxylic acid cycle or the kerbs cycle, since it was most of these reactions was determined by hand scripts. Now acetate in the form of acetyl CoA is derived from pyruvate. So the key step after pyruvate is obtained in the break down of glucose is the formation of the acetate. This acetate is taken up by coenzyme A to form acetyl CoA and then later on it is oxidized to CO_2 in this citric acid cycle.

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So what happens is the Krebs cycle actually extracts the energy of the sugar by breaking the acetic acid molecules all the way down to carbon dioxide enzymatically. And the cycle uses some of this energy to make ATP and it also forms NADH and $FADH_2$ and later what we are going to see is, we are going to see an energy balance and see exactly the amount of energy we are going to get from a single molecule of glucose. Now in the Krebs cycle, these are the key features of the kerbs cycle. We have one high energy compound produced for each cycle. What we mean by a higher energy compound is actually gtp. Gtp is formed in this case but a triphosphate bond being formed.

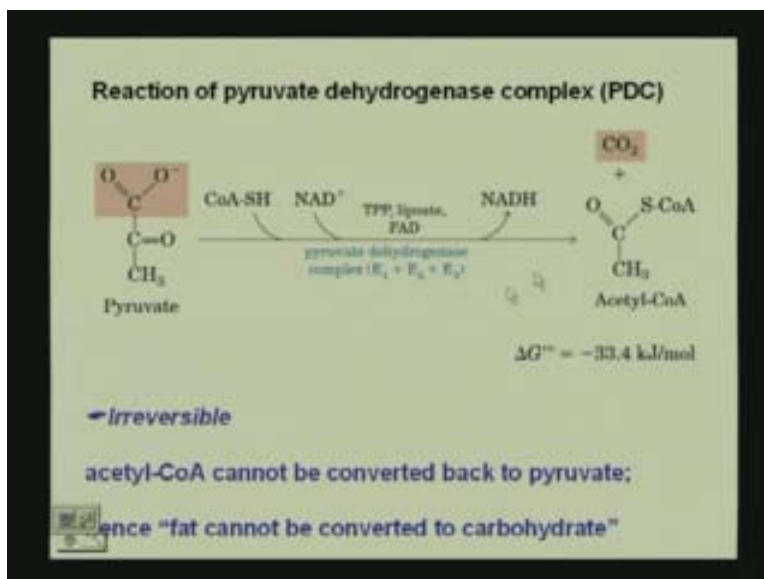
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Like in glucose we had ATP being formed that is the formation of a high energy compound. The electrons from the tricarboxylic acid cycle are made available to an electron transport chain in the form of 3 NADH and 1 FADH₂. So these are also formed in the reactions in the Krebs cycle and this NADH and FADH₂ is utilized in oxidative phosphorylation for the production of ATP where we require these cofactors in the complexes 1, 2, 3 and 4 also. The citric acid cycle you have to remember is central to all respiratory oxidation and it oxidizes acetyl CoA that is obtained from glucose lipid and protein catabolism.

So you understand that this acetyl CoA is an extremely important component because it is formed from the break down, not only of carbohydrates but also of lipids and of proteins. And the cycle also supplies some precursors for other biosynthesis, for the biosynthetic methods in the formation of proteins and other biological macromolecules and all these enzymes as I showed in one of the previous slides are in the mitochondrial matrix or in the inner mitochondrial membrane. These are for the Krebs cycle, glycolysis occurs in the cytosol of a cell.

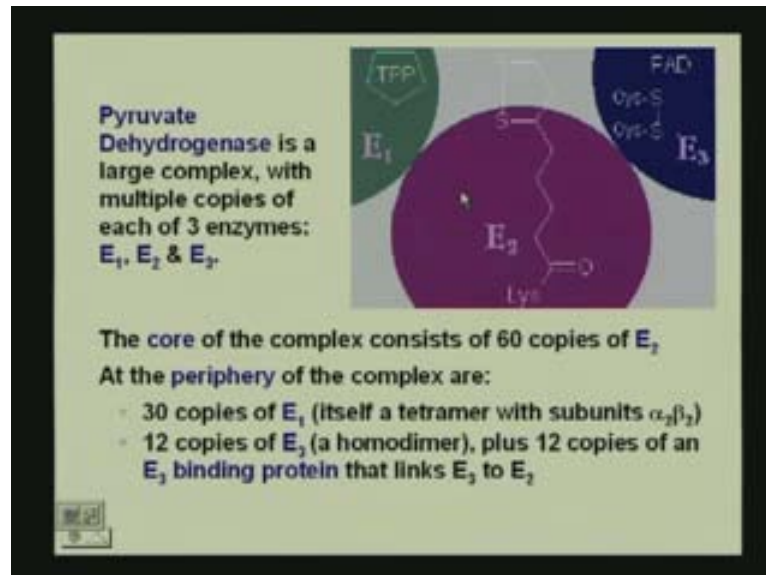
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Now this is the major reaction, what we have here is we have pyruvate. Pyruvate is $\text{CH}_3\text{COCOO}^-$ minus that is pyruvate. We have here coenzymeA that is we will look at the structure in a moment, NAD plus this goes to NADH and we form acetyl CoA with the release of carbondioxide. The enzyme for this is pyruvate dehydrogenase complex, so it is a complex of enzymes actually 3 enzymes E₁, E₂ and E₃. **We will see what those components are in a moment.**

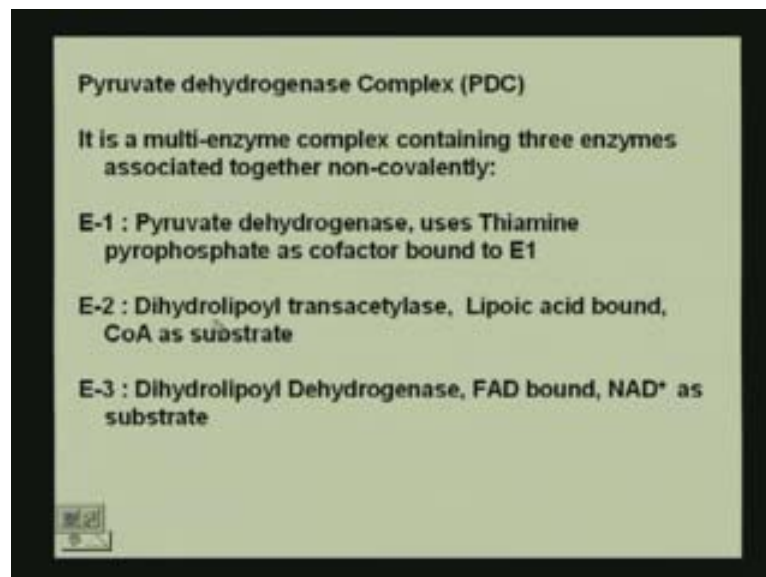
This is called the PDC the pyruvate dehydrogenase complex that comprises of 3 enzymes. Now once acetyl CoA is formed it does not get back to pyruvate which makes this reaction irreversible and which means basically that fat actually cannot be converted to carbohydrate because even though you get to the same acetyl CoA because fat break down would also get you to acetyl CoA. But since acetyl CoA cannot get back to pyruvate you cannot get back to glucose because pyruvate can get back to glucose in which process the process is called gluconeogenesis. So we can get from pyruvate to carbohydrate and had this step been reversible, since acetyl CoA is formed from the break down of fatty acids, we should had been able to obtain carbohydrate from fats. But that is not possible due to the irreversibility of this step.

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Now this has 3 enzymes E_1 , E_2 and E_3 . Now we won't go into the details of the mechanism or how the procedure actually takes place but nevertheless we need to know some thing about the complex.

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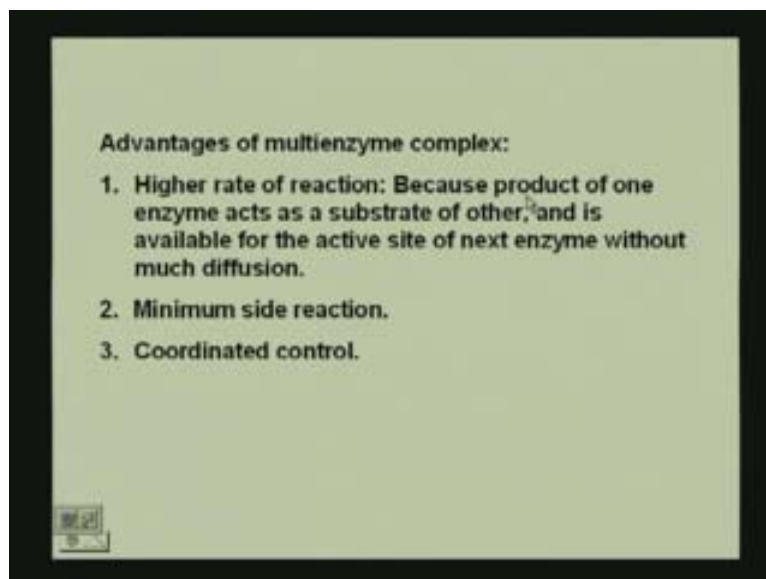


It has, as I have been mentioning E_1 , E_2 and E_3 , there are 60 copies of E_2 in the core of the complex, 30 copies of E_1 and 12 copies of E_3 . So you can imagine that this is a huge complex in the way it actually acts. Each of these have their own cofactors associated with it. So E_1 is actually pyruvate dehydrogenase that uses tpp as the cofactor. E_2 is

dihydrolipoyl transacetylase that is lipoic acid bound and uses coenzyme A as the substrate. So as soon as we have coenzyme A we know that we are going to create now Acetyl coenzyme A, the acetate coming from the pyruvate and in the next one we have E_3 where FAD is the cofactor and NAD plus is the substrate.

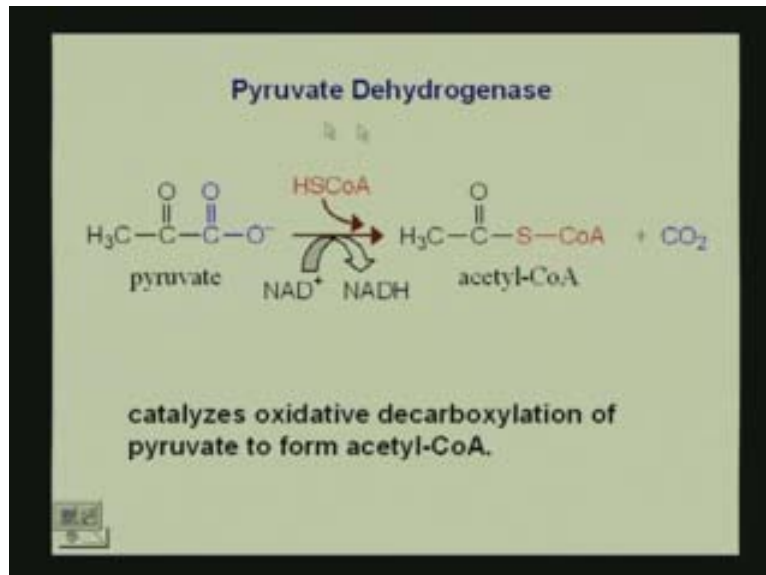
Now the advantages of having this multienzyme complex is that usually for other reactions that we have been seeing for glycolysis or that we will see for the krebs cycle have a single enzyme that acts on it. This complex is a multienzyme complex and the utility of the multienzyme complex actually shows how important the formation of acetyl CoA actually is and how tightly it has to be regulated as well. So what happens when we have this multienzyme complex is we have a higher rate of reaction because the product of one enzyme actually acts as a substrate for the next enzyme. So as soon as E_1 acts, E_2 will come into the picture then E_2 acts, E_3 comes into the picture.

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So we have a series an assembly line actually going on where we have the product of one being the substrate of the next enzyme making the reaction go faster in a sense. Because the enzyme itself does not have to get back to its original form right at that point. We also have minimum side reactions and which is most important coordinated control of what is going on in the reaction.

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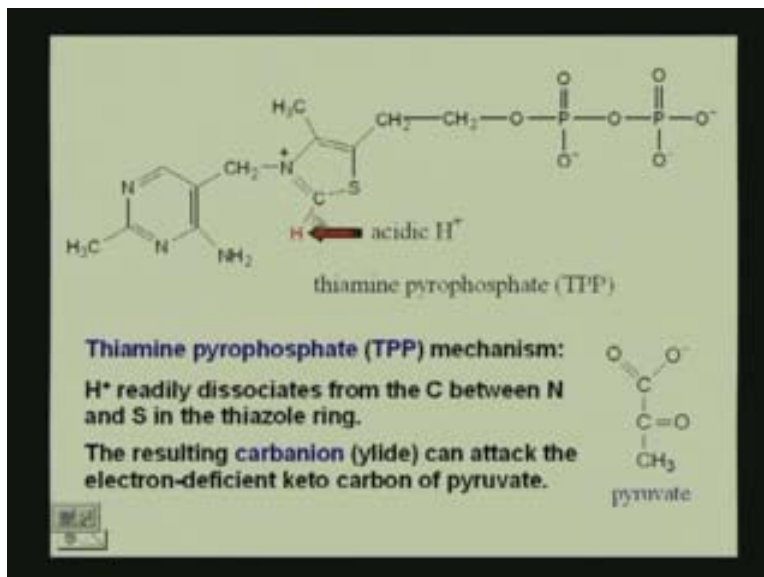
The overall reaction is pyruvate getting in to acetyl coenzyme A with the release of carbondioxide with this enzyme. So these are the 3 complex, the 3 enzymes E₁, E₂, E₃ and these are the 3 prosthetic groups that are attached to the specific enzymes. TPP, lipoamide and FAD to E₁, E₂, E₃ that is this 3 enzymes that comprise the PDC, the pyruvate dehydroganase complex.

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Pyruvate Dehydrogenase Subunits

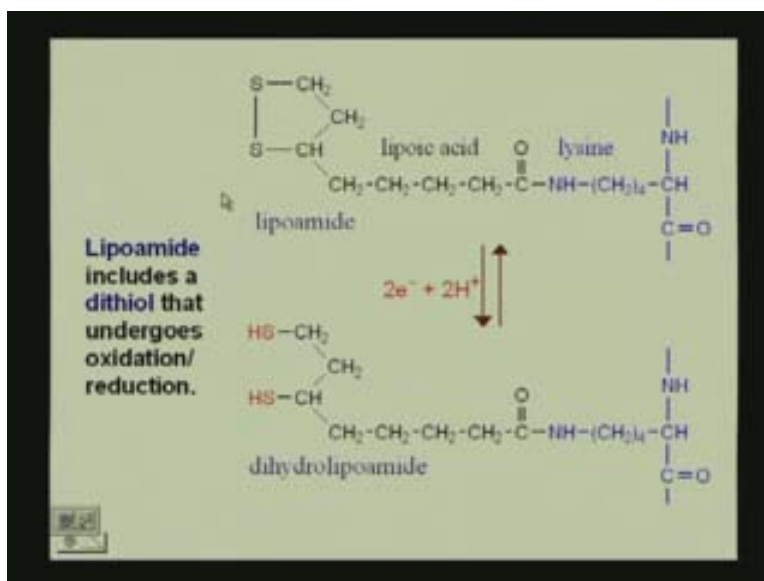
Enzyme	Abbreviated	Prosthetic Group
Pyruvate Dehydrogenase	E ₁	Thiamine pyro-phosphate (TPP)
Dihydrolipoyl Transacetylase	E ₂	Lipoamide
Dihydrolipoyl Dehydrogenase	E ₃	FAD

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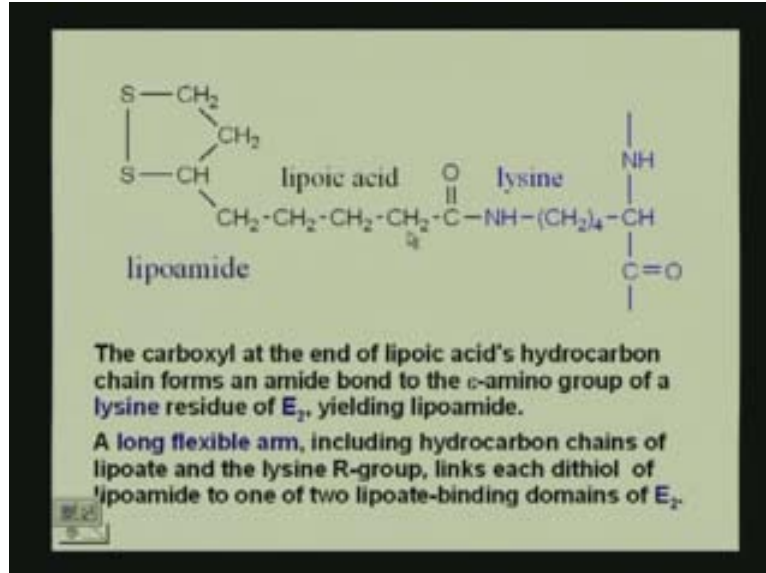


Now in E₁ you all remember that when we studied vitamins we had vitamin B₁ thiamine that actually formed TPP. In TPP we have an acidic H plus and if you remember we spoke about this acidic H plus and how it is important in certain reactions and we will see how it is important in this one. So we have this acidic hydrogen that actually disassociates from the ring and creates a carbanion, this negative then attacks the... What happens here, this attacks then the carbonyl of the ketone of pyruvate resulting in the release of carbon dioxide. So what do you have here? You have CH₃ CO that is the acetyl.

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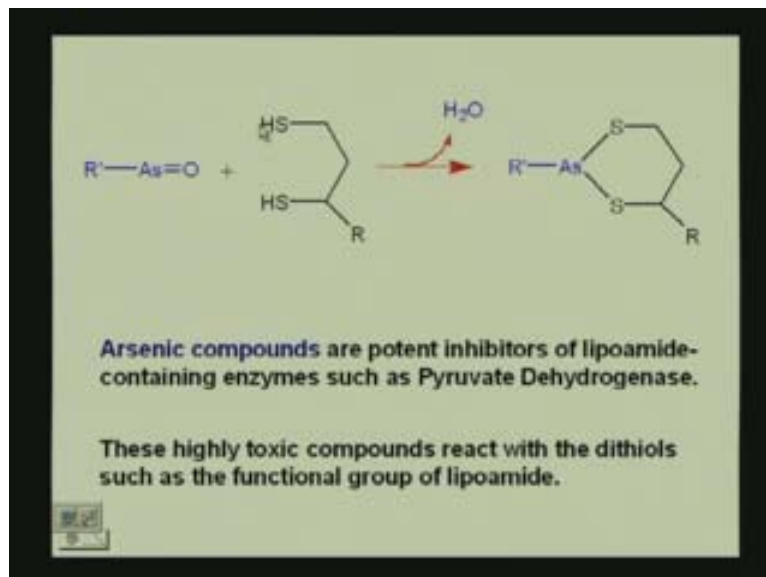


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So the acetyl is formed with the help of TPP that is part of E_1 of the PDC that is the pyruvate dehydrogenase complex. In the second part we actually have a lipoic acid that is linked to lysine in the enzyme E_2 and here we have a certain reaction which is going to get into the reduction of this disulphide, the vicinal disulphide here where we have SH SH that is going to be dihydrolipoamide. Now the reason why we are going through this is this because we have to understand how acetyl CoA is actually being formed. We have acetate now, we have got rather the acetyl. Where did the acetyl come from? It came from E_1 where what happen to E_1 in the previous step? We released CO_2 and we had acetyl that is now linked to this.

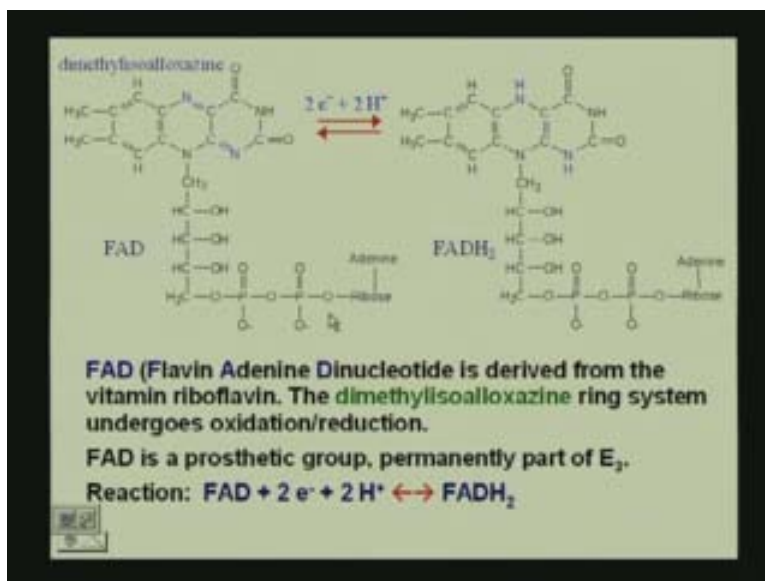
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So now we have to go on in our further steps. This is the part (Refer Slide Time: 15:00) where we have the dithiol that undergoes oxidation and reduction. One important thing of this is this dithiol reacts with the lipoic acid with the lysine of the...Where is this lysine? It is present in E₂ and this is the prosthetic group that is present with E₂ and this actually is involved in the acetyl part, the CH₃ CO part actually gets linked to this S in the reaction but we are not going into the mechanistic details of this. One important thing that you might want to know is this SH SH that is formed, the dihydrolipoamide is actually, sometimes you hear about arsenic poisoning. What happens is, this is what gets into inhibiting this lipoamide containing enzymes.

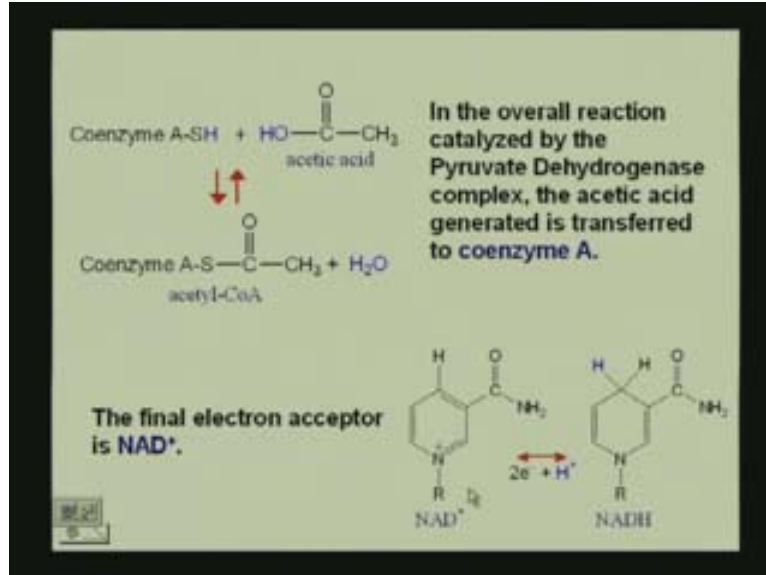
So what happens is the acetyl CoA is not produced because it acts on the enzyme E₂. This is what happens with arsenic poisoning and if you read a bit of the history of this, there was a certain tonic that was supposed to be made that actually what a tonic will sort of give you more energy in a sense and that act actually led to arsenic poisoning. Charles Darwin infact died of arsenic poisoning and this is the mechanistic thing that happened where this compound was actually formed that prevented the dehydrogenase complex from acting, preventing the formation of acetyl CoA, preventing the complete degradation of the glucose.

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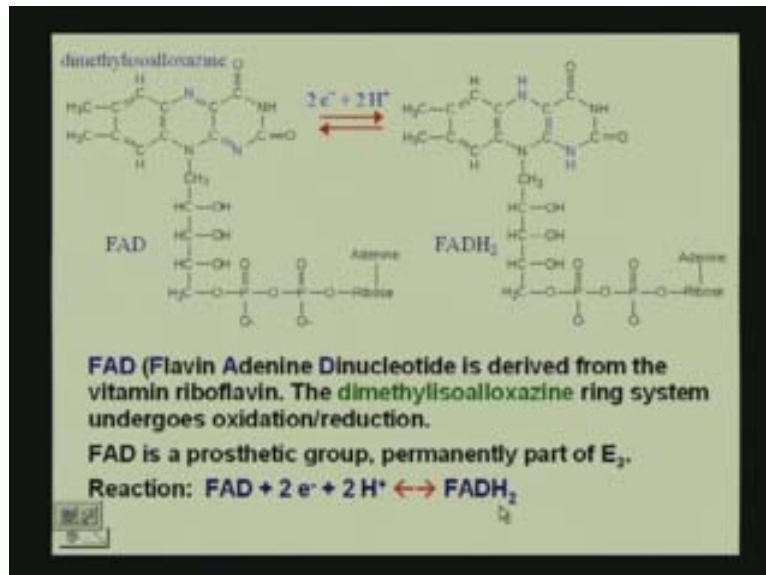
Then we also have FAD, FAD is a part of enzyme part E₃. We have E₁ that is TPP, E₂ that is lipoamide and E₃ that is our FAD. So basically what is happening we have coenzyme A that is the thiol with the acetic acid forming acetyl CoA and the final electron acceptor because FAD is going to get to FADH₂.

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But it has to get back to FAD so that it can react again. So the final electron acceptor is NAD plus that is the substrate for enzyme E₃ that will take up the H₂ that has been given to FAD and get it back to FAD. So FAD forms FAD plus, FAD H₂ here and to get this back to FAD this has to be taken up by some thing. Who is going to take it up? NAD plus is going to take it up in the third reaction. So ultimately the electron acceptor is the NAD plus which is why it is mentioned that NAD plus is the substrate for that particular mechanism.

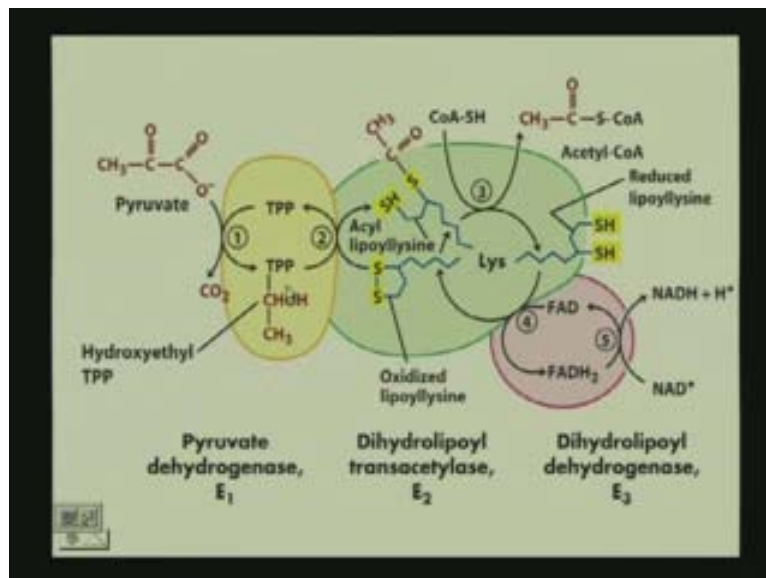
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This is essentially what is happening, the yellow one here is E₁, this green one is E₂ and the pink one here is E₃. The first step as we saw is we have the TPP. So here we have the loss of this carbondioxide, this is carbondioxide which is coming off here (Refer Slide Time: 18:32). We have now an acetyl part so this acetyl part is linked to the TPP because you had that elite formation there. So we have the acetyl part linked to the TPP which then transfers it to the lipoic acid part that is connected to the lysine in the enzyme E₂. So this is the lysine so you 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 lipoic acid chains that have the dithiol and the dithiol, one of them picks up the acetyl and has it linked to the sulfur of the lipoamide the lipoic acid part here.

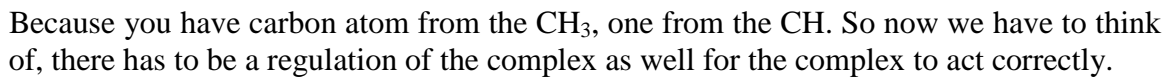
Now what happens is coenzyme A now comes into to the picture. Coenzyme A now has to form acetyl CoA, so it picks up this acetyl forming acetyl CoA and then you have the reduced SH SH. Now what must happen for this to act again? This has to get back to the dithiol then. So two of these SH have to be removed. How can they be removed? They are removed by FAD.

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FAD then comes into the picture, fix up the 2 hydrogen's and this gets back to your oxidized lipoyllysine. So this is just a 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 started from, so that they can go and act on a pyruvate. Eventually what happens is this gets to the oxidized part so this can now take up acetyl rather. Another acetyl can now get attached to this, FAD now been reduced to FADH₂. What must happen in this case is FADH₂ has to get back to FAD. So what comes into the picture then? NAD plus. NAD plus then acts as this and picks up the hydrogen. So that is basically the whole procedure that gets from your pyruvate to your acetyl CoA. This acetyl CoA is now going to enter the tricarboxylic acid cycle.

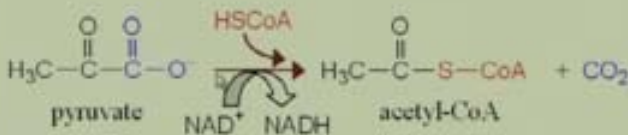
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Regulation of Pyruvate Dehydrogenase Complex

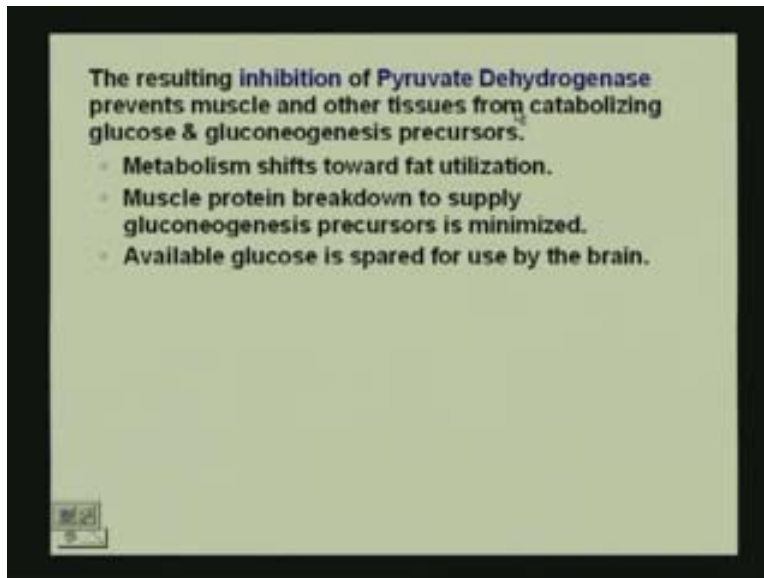
Product inhibition by NADH & acetyl CoA:

- NADH competes with NAD⁺ for binding to E₃.
- Acetyl CoA competes with CoA for binding to E₂.



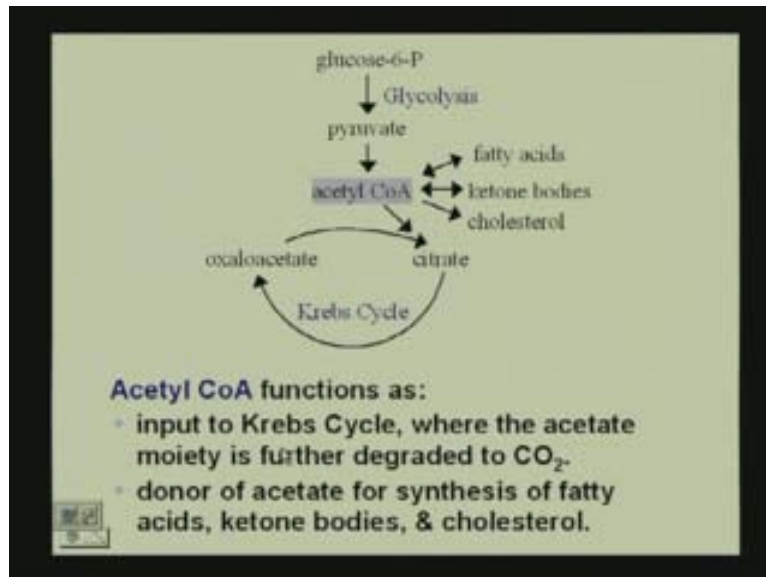
Now when we have E_3 , NADH competes with NAD plus for binding to E_3 . So it is inhibited by the product. Where did E_3 come into the picture? We had FAD going to $FADH_2$ and since NAD plus came into the picture there to form NADH. It competes with NAD plus for binding. So it is inhibited by the product and acetyl CoA competes with coenzymeA for binding to E_2 where this acetyl Co A was a product of the enzyme E_2 . So we now have product inhibition by NADH and acetyl CoA, this as I mentioned is the overall reaction.

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Now the resulting inhibition of pyruvate dehydrogenase prevents muscle and other tissues from catabolizing glucose and gluconeogenesis precursors. So basically it is just a regulation of the whole system.

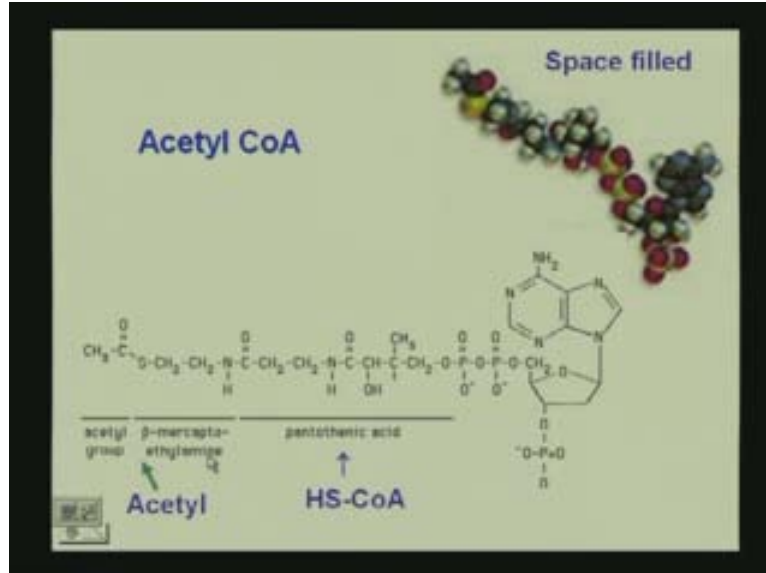
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This product inhibition you have to recognize is the regulation of the whole enzymatic procedure. So this is what we have. We have pyruvate, glucose getting to pyruvate and now we have acetyl CoA that is going to be the input to Krebs cycle where this acetate is going to be broken down in to carbondioxide. And we are going to have this acetyl CoA also involved in other components where further synthesis of fatty acids, ketone bodies and cholesterol but that is beyond what we are going to do. We need to know that acetyl CoA is going to the Krebs cycle and we need to know what is going on in the Krebs cycle.

This is acetyl CoA if you remember we have the acetyl group here, the beta mercaptoethylamine part, the pantothenic acid part that was derived from the vitamin and what is this part? Adenine, we have a phosphor part here also, we have to go and check it up. So this is acetyl coenzyme A.

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Now what do we have here? We have the basic features of the citric acid cycle where we have the PDC and its control, reactions of the TCA cycle and we have as I mentioned before the reactions of glycolysis that are localized in the cytosol and these take place in the mitochondria. The metrics and the respiration, the ultimate oxidation phosphorylation takes place in the inner mitochondria membrane.

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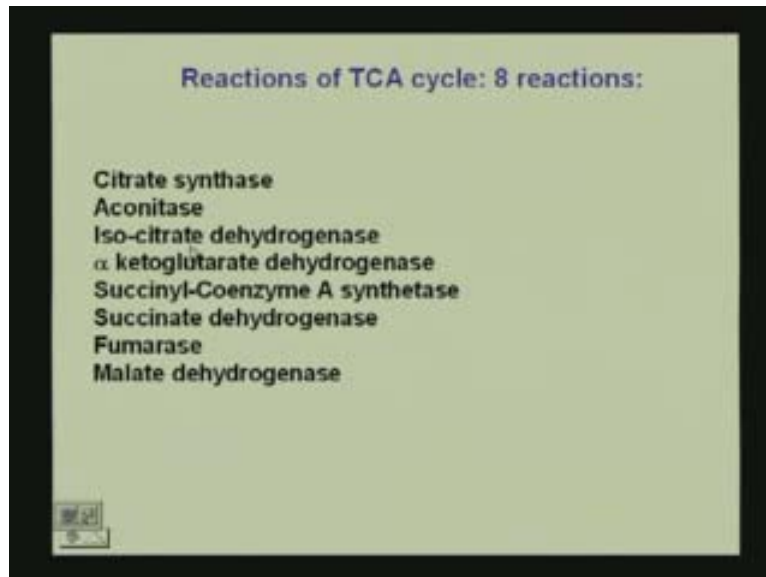
- Citric Acid cycle or Tricarboxylic Acid cycle or Krebs Cycle
- Pyruvate Dehydrogenase Complex (PDC) and its control
- Reactions of TCA cycle or CAC
- Regulation of TCA cycle
- Reactions of Glycolysis are localized in Cytosol, and do not require any oxygen.

Pyruvate dehydrogenase and TCA cycle reactions take place in mitochondria where oxygen is utilized to generate ATP by oxidative phosphorylation.

Consumption of oxygen (respiration) depends on the rate of glycolysis and TCA reactions.

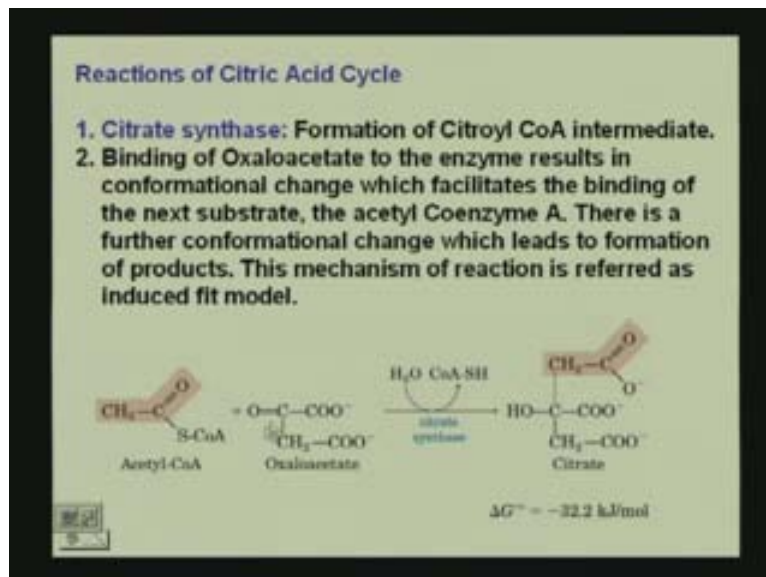
These are the enzymes, there are eight reactions going on here. We have citrate, synthase, aconitase we will go step by step in each of these like we went for glycolysis.

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In the first step we have acetyl CoA oxaloacetic acid in the presence of citrate synthase. What does citrate synthase mean? It is going to form citrate, so we have oxaloacetate here. Now one thing when you consider or study the citric acid cycle you have to keep count of the carbon atoms and you have keep count of what is going where. So if you label any of this, this is how these reactions would actually decipher.

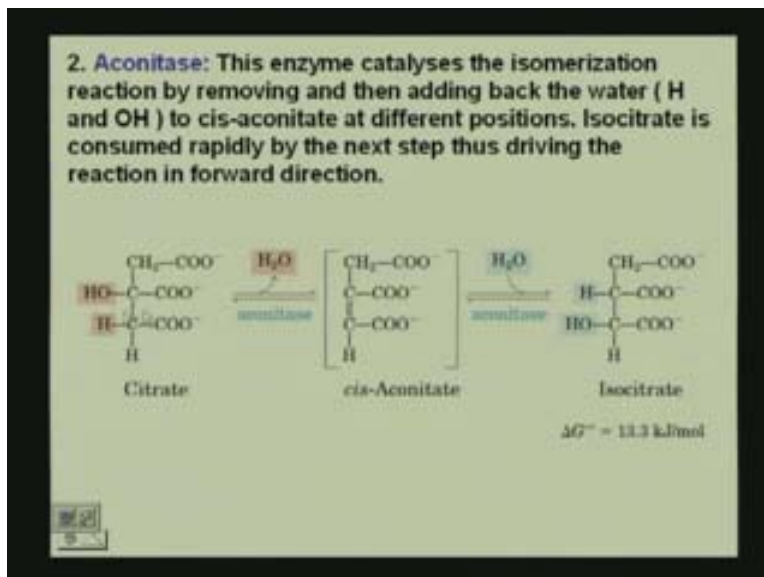
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If you labeled say the acetyl this carbon which carbondioxide it was forming can be determined. So when we have this acetyl CoA and we have oxaloacetate, oxaloacetate has 4 carbon atoms. We are adding acetyl to it, so now this has 6 carbon atoms. Four

from your oxaloacetate and two from the acetyl part of the acetyl CoA. So now we have synthesized citrate using citrate synthase.

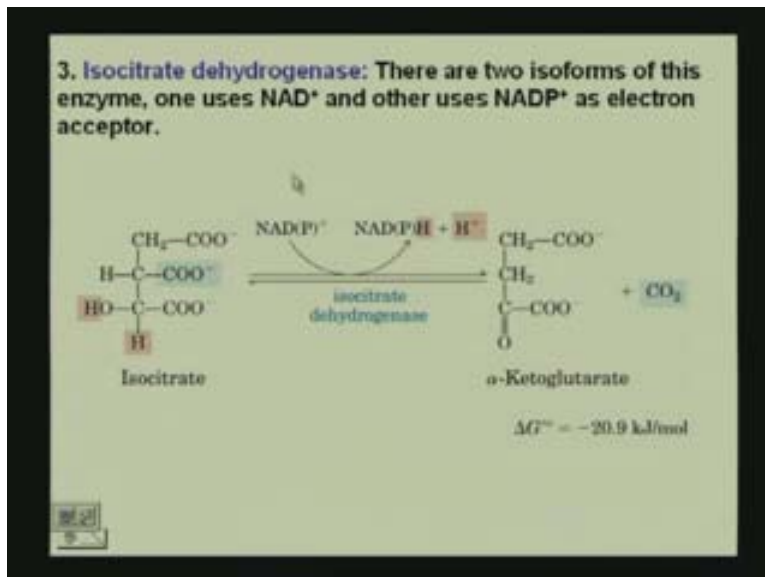
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So now we have citrate, we have aconitase that is nothing but an isomerase that forms isocitrate where you have just the interchanging of the H and the OH on carbon atoms 1 and 2. So you have citrate to isocitrate. Actually the ΔG° prime if you see here is a positive quantity but since there is some equilibrium any little bit of that is formed is pushed into going into the next step. So eventually what does happen is the reaction is going in the forward direction because the product is being removed. So that is our second step so our first step was the formation of citrate by citrate synthase. The next step is the formation of isocitrate.

The third step is isocitrate dehydrogenase. What is that mean? It means it is going to remove now water. We have H plus removed here in NAD and in some cases there are actually 2 forms of the enzymes that are called 2 isoforms of the enzyme. One of them prefers NAD plus as the cofactor, one of them prefers NAD plus as the cofactor but both of them would ultimately result in the removal of these 2 hydrogen's and the CO₂ here.

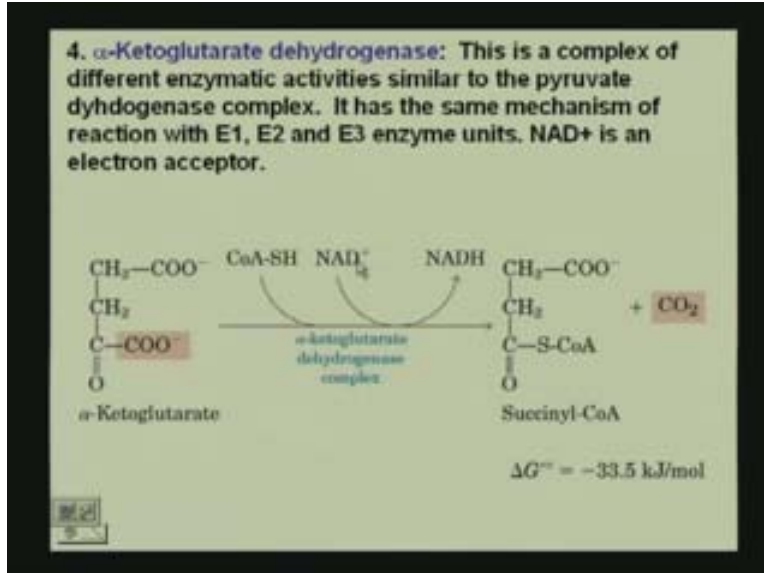
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So you have to remember that if you are acetyl carbon atoms were labeled, they would not be in the carbon dioxide released here because this still there. So we now have formed alpha ketoglutarate, this is glutric acid. We have the alpha, this is the keto part so alpha ketoglutarate. So that is our third step.

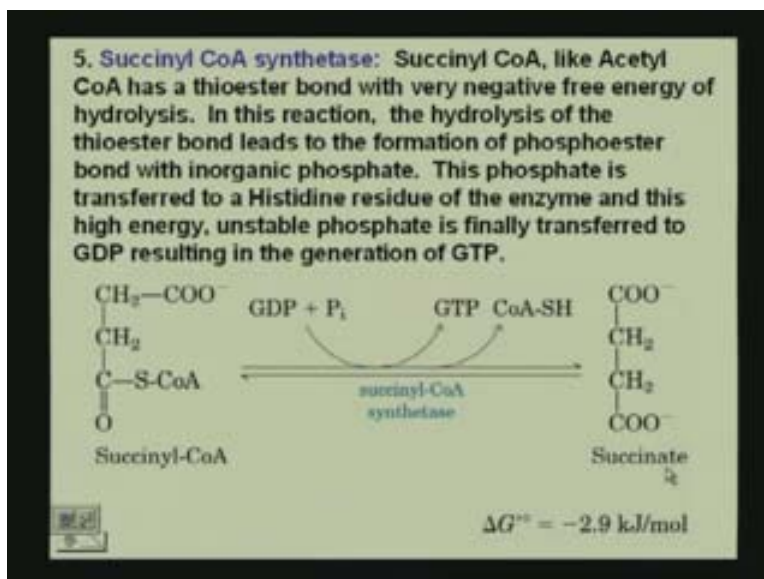
The fourth step is alpha ketoglutarate dehydrogenase. Again we have NAD plus and NADH. Now NADH is being formed here and later on when I show you the whole cycle we have to keep count of how many NADH are being formed, how many FADH_2 are being formed and how many ATP are being formed because that is going to tell us how much energy we are going to get. It is not as difficult or as complicated as it seems. It is pretty simple actually, if you just follow the steps round the cycle.

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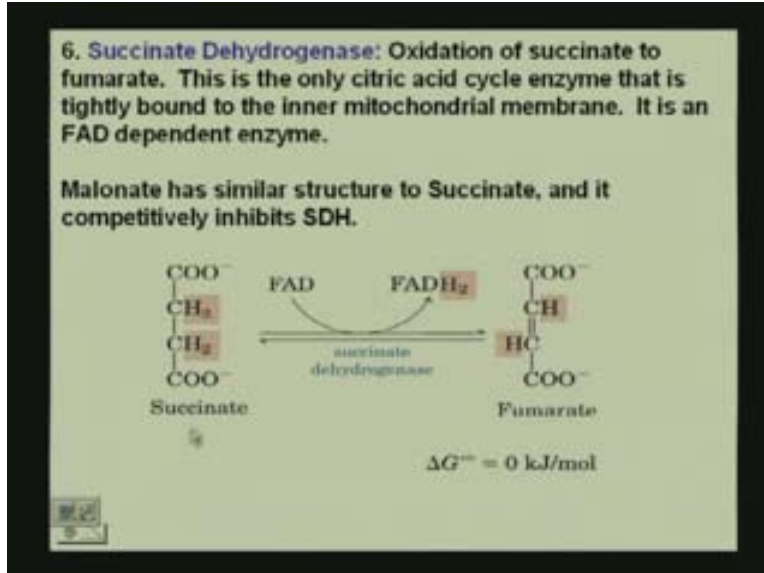
So what we have here is we have alpha ketoglutarate going to succinyl coenzymeA. If these are just cycles, this has fewer steps than the glycolysis cycle 8 compared to 10. Then we have succinyl coenzymeA go to succinate and this is the step that results in the production of GTP, the high energy bond. So it is the step succinyl CoA synthetase where it has a thioester bond, a high energy thiol ester bond that actually forms GTP from GDP plus P_i . So we have succinyl CoA going to succinate.

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Step number 6 is succinate dehydrogenase where you see how we are getting smaller and smaller.

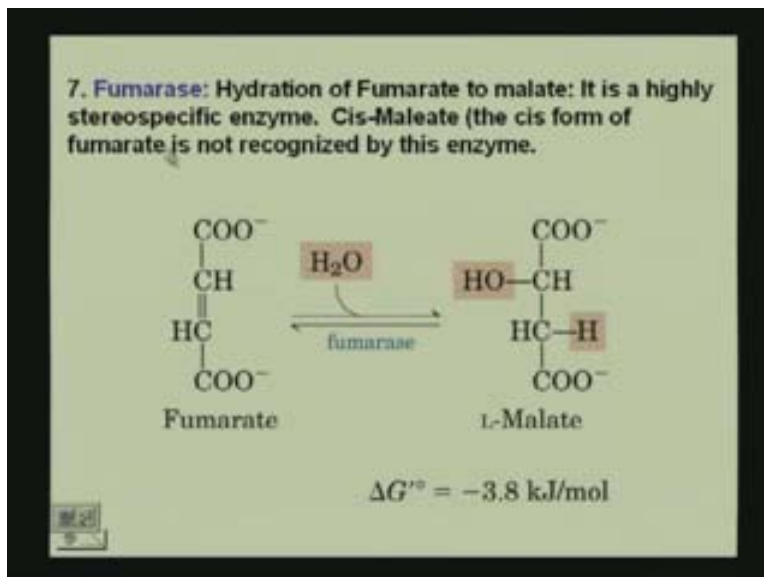
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The succinate is now; we have looked at the enzyme before when we looked at FAD going to FADH₂. If you remember when I mentioned how FAD goes to FADH₂. We consider succinate going to fumarate and in this case we have the removal of 2 hydrogen atoms and this is in the inner mitochondrion membrane and it happens spontaneously.

So we now have formed fumarate. How many carbons do we have now? 4. How many did citrate have? 6. The citrate that we considered any way will show you the here. The citrate had 1, 2, 3, 4, 5, and 6. Oxaloacetate that we started off with had 4, we added 2 carbons to make it 6.

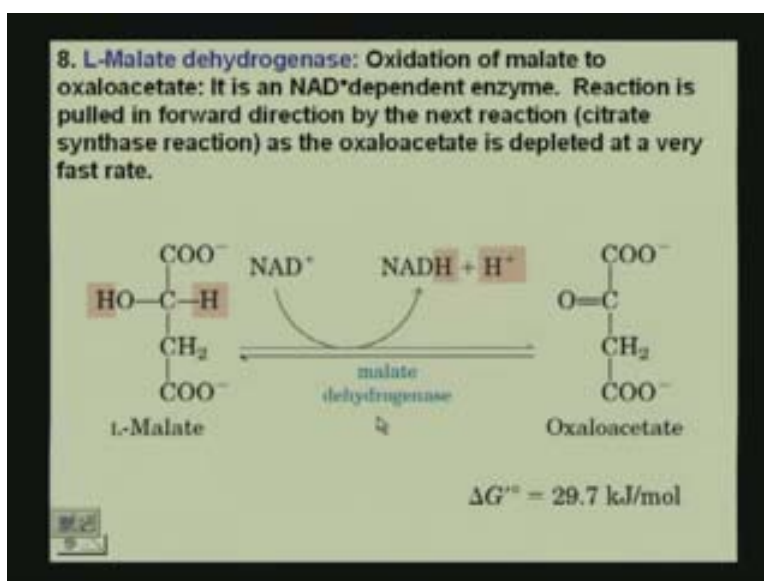
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Then we still had 6 because we have gone to just a isomeric form of it, then we lost a carbon here, so 1, 2, 3, 4, 5 now. Then we lost a carbon here in alpha keto, in forming succinyl CoA we lost another carbon. So we are now down to 4, so now we still have 4 from succinyl CoA to succinate. We still have 4 because we have just gone for a dehydrogenase.

Fumerate to malate, this is fumerase where we have H₂O forming malate. Then we have malate back to oxaloacetate, finished. So you have oxaloacetate that is ready to pick on another acetyl CoA to form citrate. So that is the whole cycle, so eventually what we have is if we look at the overall steps we have to now figure out where the NADH were formed.

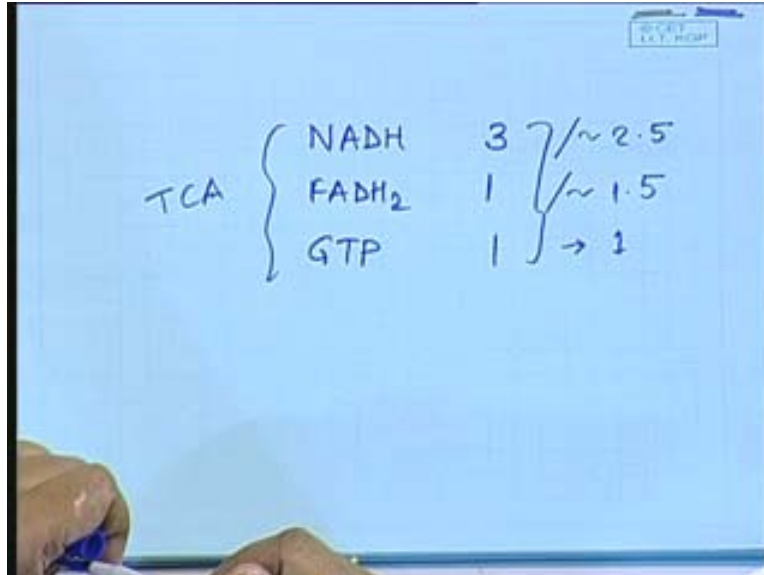
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So let us go back. Let us go back and in the first we do not have any production of NADH (Refer Slide Time: 32:25). So in step number one we have formed citrate. Step number 2 (Refer Slide Time: 32:33) we still do not have NADH formed. We have just an isomerase reaction going to isocitrate. Step three (Refer Slide Time: 32:40) we have lost CO₂ we have produced one NADH. So we keep track of that. So we have formed one NADH. In the fourth step (Refer Slide Time: 32:50) we have another NADH, so we have two. In this (Refer Slide Time: 32:54) step we have one GTP that has to be kept count at another counter. So we have another counter for GTP and ATP production because this GTP is ultimately forms ATP. We have (Refer Slide Time: 32:10) one FADH₂ so 2 NADH 1 FADH₂ one GTP, then another (Refer Slide Time: 33:20) NADH.

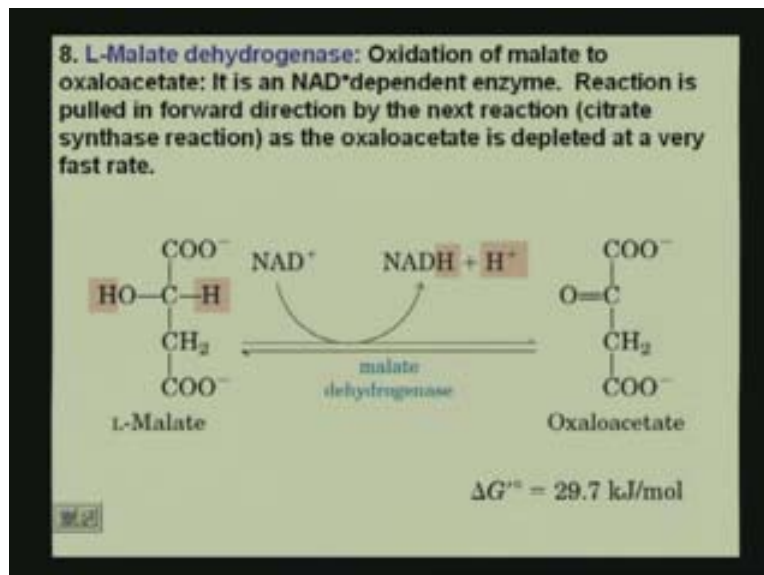
So how many do we have now? We have (Refer Slide Time: 34:26) 3 NADH. Let us just keep track of it. We have NADH three, FADH₂ we have one and GTP we have one. So these are all coming from a TCA cycle. Now this NADH and FADH₂ where is it going to go? It is going to go for oxidative phosphorylation in the production of ATP and when we looked at the overall reactions of oxidative phosphorylation.

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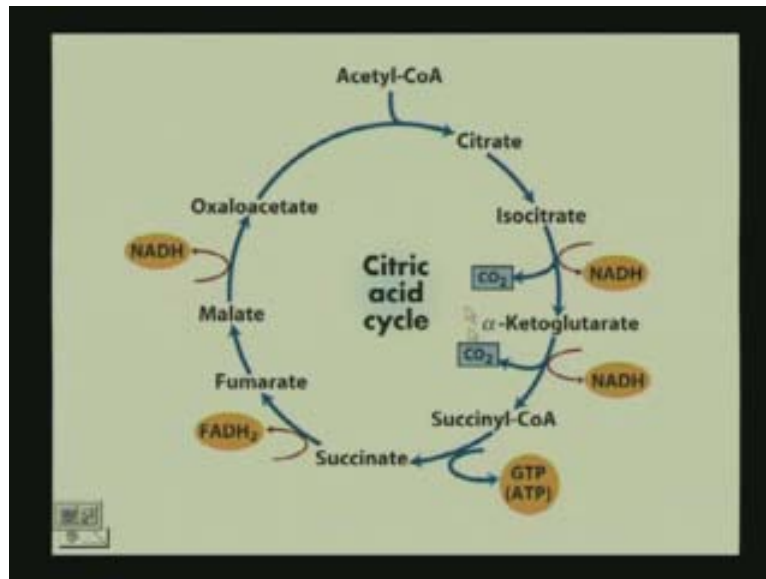
What happened to NADH and FADH₂? NADH had a reaction in which it produced approximately 2.5 ATP and this produced approximately 1.5 ATP and this is one ATP. So that is an energy, this is per NADH. So we will get back to the energy calculations once we complete this. So now we figured out that in the 8 because we are back to oxaloacetate now. So we have 3 NADH produced, 1 FADH₂ produced and 1 ATP set.

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So here is our whole cycle, acetyl CoA comes into the picture forms citrate, from oxaloacetate form citrate, isocitrate NADH produced. So here is one NADH so we have oxaloacetate. How many carbons here? 4 How many carbons here? 2, 6 we still have 6 we have lost one 5, lost another one 4, 4, 4. How many NAD do you have? One NADH two NADH, three NADH.

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How many ATP? One, GTP it is converted to ATP because guanine and adenine both are purines and we have one FADH₂. So that is our count. What else do we have to remember? From glucose I have got two of these. So the whole thing is going to happen twice because glucose gave me 2 pyruvate and this is happening to each pyruvate. When I had glucose, well I have the steps for you just simple carbon atom steps.

So this is just the same thing, just showing you where the reaction are, where what is happening. Now we figure out what we have, so now we have to look at the conservation of energy of oxidation in the carbon citric acid cycle. We have 2 carbon acetyl group generated in the pyruvate dehydrogenase complex. There is complete oxidation of 2 carbons during one cycle, you saw that we had two CO₂ being released.

The two carbon atoms which enter the cycle become part of oxaloacetate for the next cycle. The two carbon atoms that have entered in the top, the CO₂'s are lost in from the middle. What was existing in the oxaloacetate? Remember I showed where the CO₂'s are coming off; those CO₂'s are not part of the acetyl CoA.

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The acetyl CoA shifts and in the formation the two carbon atoms which enter the cycle become part of the oxaloacetate and they are released in the third round. So you got to keep track because they come down one after the other so that is how it is being released and the energy released due to the oxidation is conserved in the reduction of three NAD plus one FAD and the synthesis of one GTP molecule which is converted to ATP.

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Conservation of energy of oxidation in the CAC:

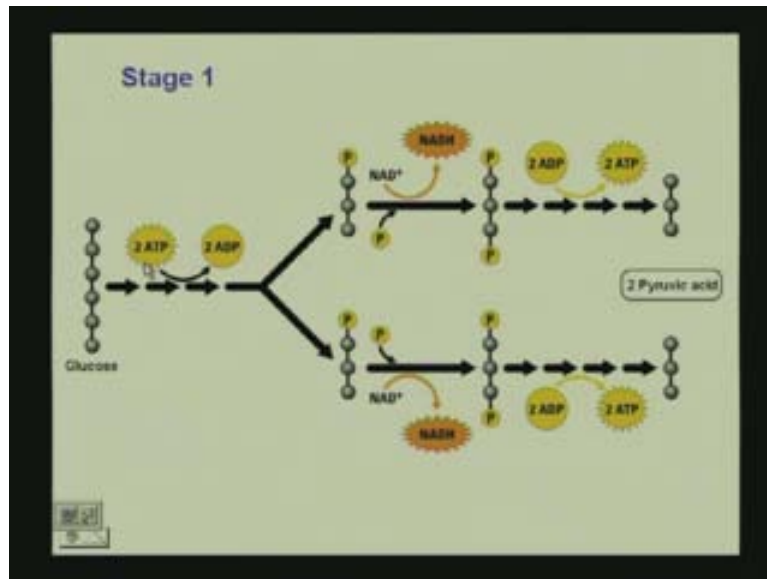
The two carbon acetyl group generated in PDC reaction enter the CAC, and two molecules of CO_2 are released in one cycle.

There is complete oxidation of two carbons during one cycle. The two carbons which enter the cycle become part of oxaloacetate, and are released as CO_2 only in the third round of the cycle.

The energy released due to this oxidation is conserved in the reduction of **3 NAD⁺**, **1 FAD** molecule and synthesis of one **GTP** molecule which is converted to ATP.

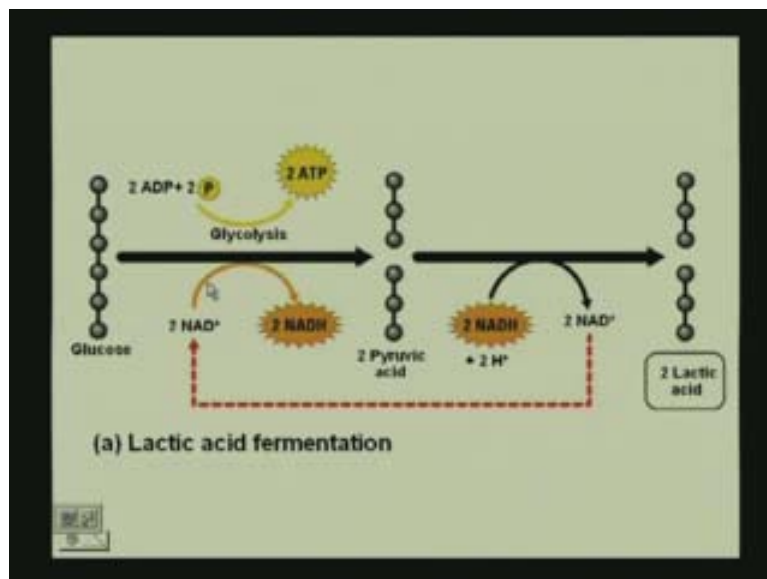
So this is our stage one, 6 carbon atoms we had 2 ATP remember that were taken up in the formation 2 steps there for the glucose 6 phosphate and for the 1, 6 bisphosphate. So we have 2 ATP broken down into 2 ADP. What were these two? Dihydroxyacetone phosphate and glyceraldehyde 3 phosphate, both of these still have phosphate. Then we had NADH being produced and we had 2 ATP being produced.

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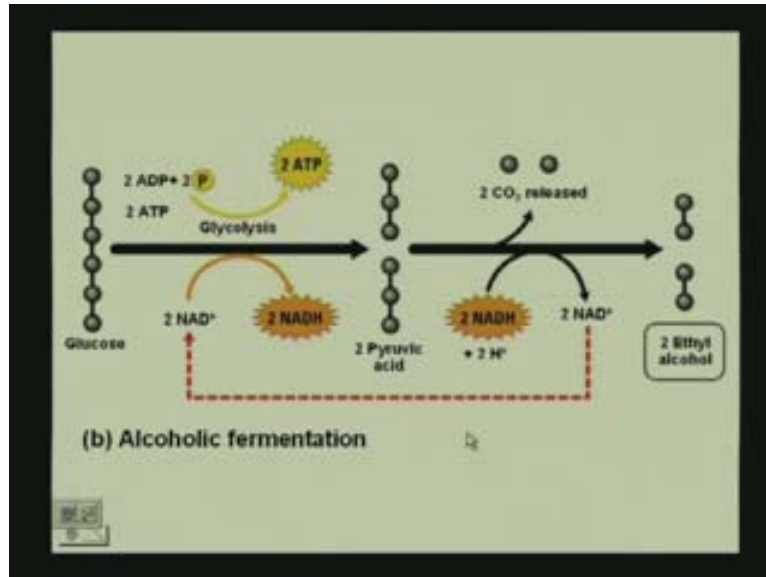
Remember then we said that since we had 4 ATP here and 2 ATP being used up, we eventually had 2 ATP to consider. So that is our stage one of our glycolysis.

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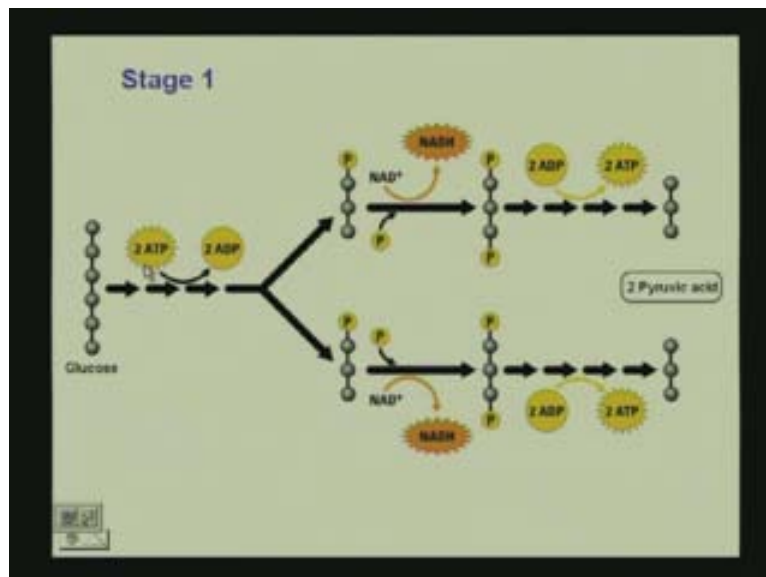
Now there are 2 other possibilities here where we can have lactic acid fermentation, in that case remember that we said we do not have that NAD plus because this NAD plus is being utilized again in the reaction. This is what we did in our last class. We also have alcohol formation that happens in yeast.

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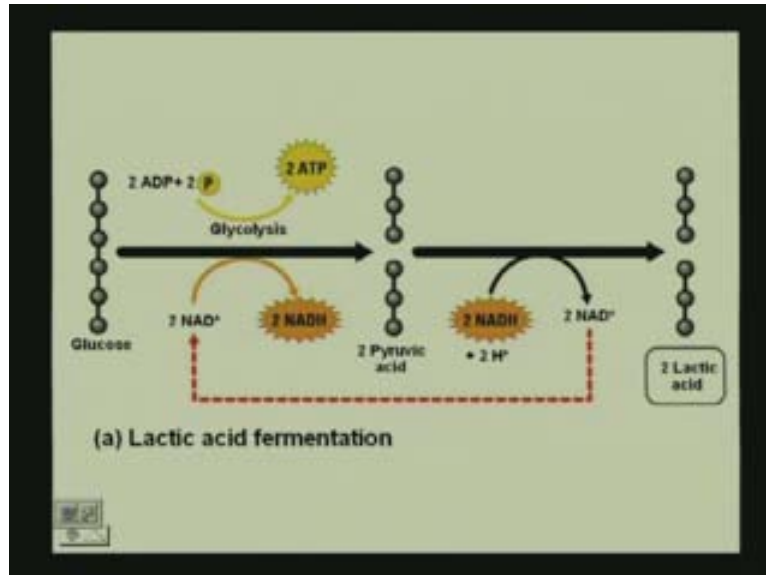
In the fermentation where we have alcoholic fermentation where the pyruvic acid, so we have to look at the fate of pyruvic acid. So what is happening say in this case glucose is forming pyruvic acid. Now the pyruvic acid can get into forming acetyl CoA that is going to be part of the tricarboxylic acid cycle.

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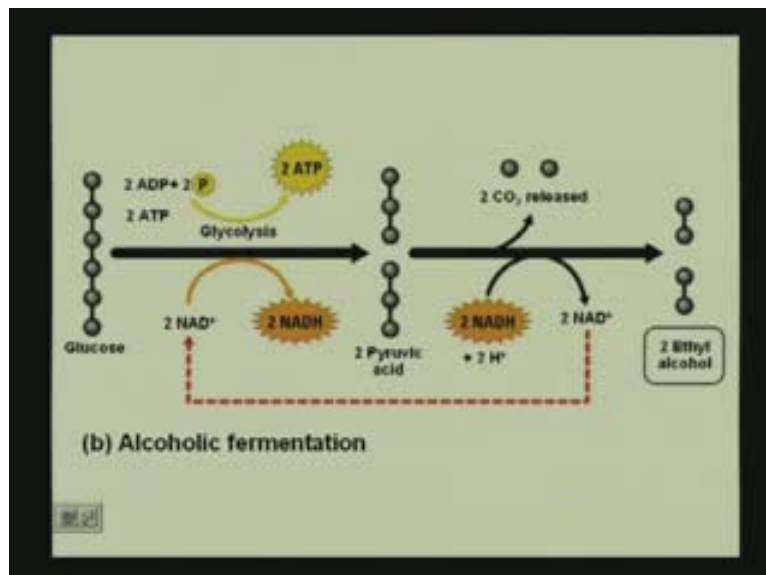
Now this is also happening where you have lactic acid formation that usually is an anaerobic form which happens a lot in the muscles, skeletal muscles.

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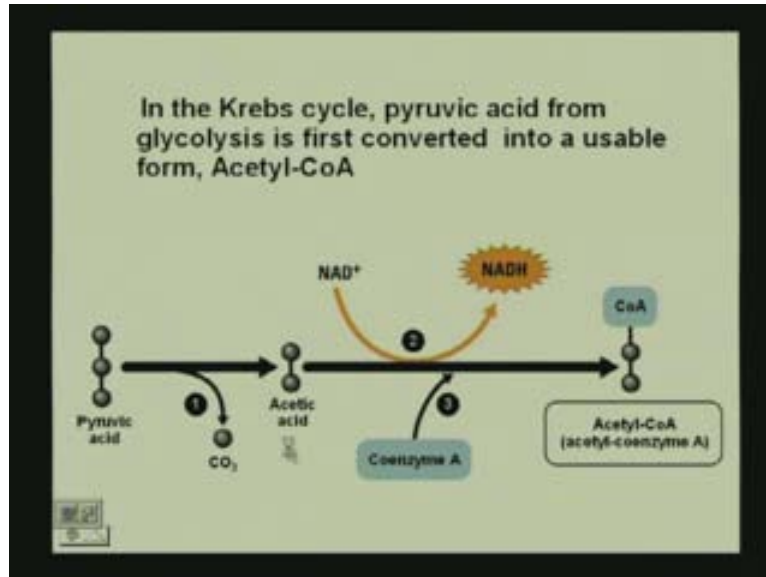
Then we also have this form where we will have alcohol fermentation in the formation of ethyl alcohol that is usually formed in yeast.

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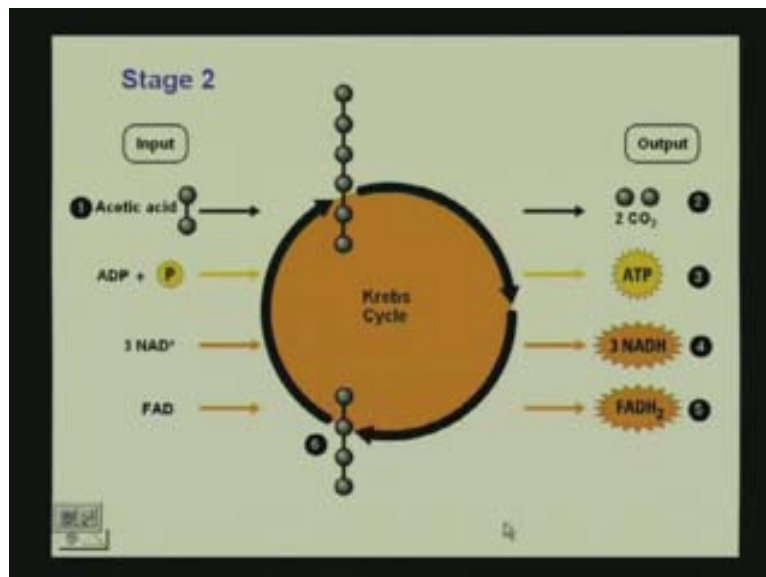
Now in the Krebs cycle we have this, so we have NAD plus going to NADH. So the pyruvic acid in this case forms acetate, this acetate then forms acetyl CoA and the acetyl CoA then from the coenzyme A we will ultimately give us acetyl CoA. This acetyl CoA is going where to the Krebs cycle.

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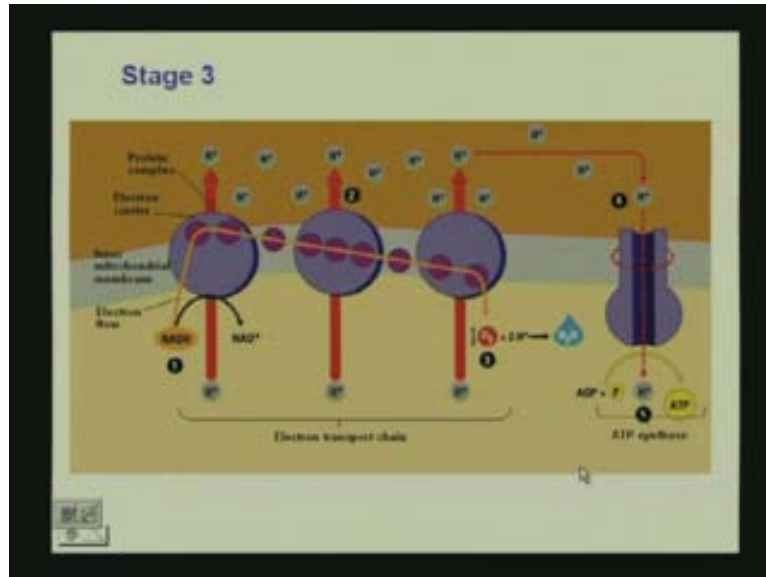
So this is where we have our krebs cycle where our input is going to be acetic acid then we have a phosphate come in, we have ADP plus phosphate plus 3 NAD plus and one FAD and the output is 2 carbondioxides, two are formed in two steps. We have ATP being formed, three NADH being formed, one FADH_2 being formed.

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So now we had 6 carbons. Where did the 6 come from? Oxaloacetate provided 4, acetyl provided 2. Then we lost CO_2 along the cycle and we came back to 4. So that is our krebs cycle. Then in stage 3 it is just our NADH and all the electron transport chain finally forming ATP in our oxidative phosphorylation step which we have done before.

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So now we have look at our balance sheet. This is what we did last time, glycolysis we have 2 ATP's that are used up, 4 ATP produced. Why? Because we have 2, 3 carbon fragments each of them are producing 2. So we have 4 ATP produced so eventually we have a net production of 2 ATP per glucose.

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Balance sheet for ~P bonds of ATP:

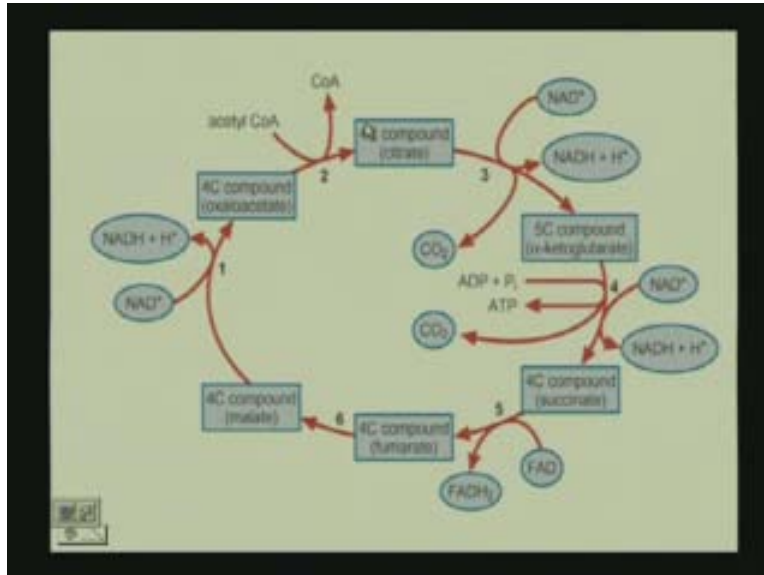
- 2 ATP expended
- 4 ATP produced (2 from each of two 3C fragments from glucose)
- Net production of 2 ~P bonds of ATP per glucose.

Glycolysis - total pathway, omitting H^+ :

$$\text{glucose} + 2 \text{NAD}^+ + 2 \text{ADP} + 2 \text{P}_i \rightarrow 2 \text{pyruvate} + 2 \text{NADH} + 2 \text{ATP}$$

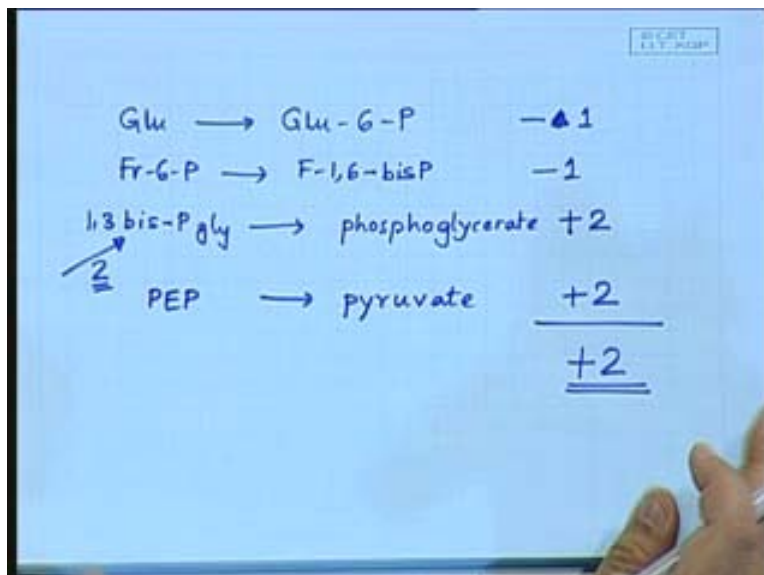
This is what I showed you last time, so this is basically now our balance sheet. So this is the carbon so we have now in our first steps we have glucose going to glucose 6 phosphate. What did that to? It took away an ATP.

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If we are talking in terms of ATP it is minus one. What was our next step? Fructose 6 phosphate to fructose 1, 6 bisphosphate, that also took away another one. Then this is all the ATP we are considering now, then we will get back to our.

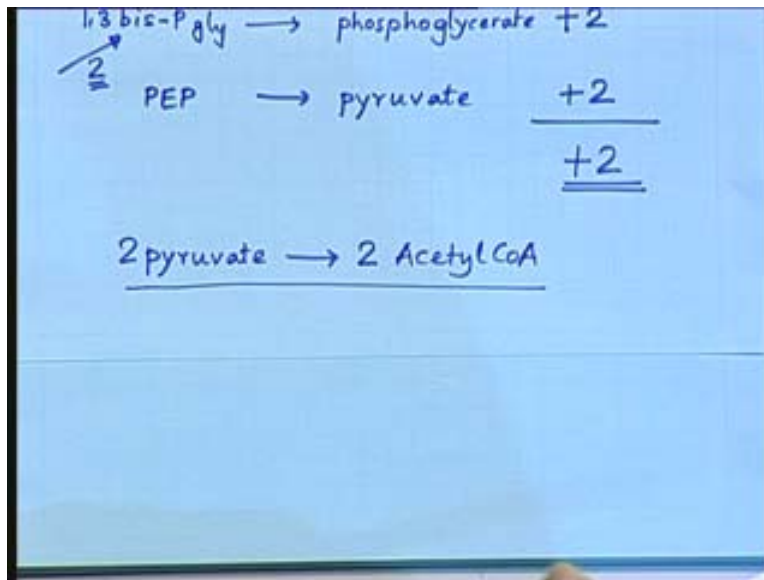
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Then we have the bisphospho that is the 1, 3 bisphosphoglycerate. What did that form? That forms the phosphoglycerate. And what happened there? It was plus 2 actually because we have 2 of the 3 carbons now. Then from the phosphoglycerate we form the phosphoenol pyruvate, the phosphoenol pyruvate gave you the pyruvate. So we have the phosphoenol pyruvate give you the pyruvate plus 2. So eventually we have plus 2 that is the amount of ATP from glycolysis that is what we figure out.

Now when we have 2 pyruvate because we have to remember that one glucose is giving 2 pyruvate, you cannot forget that. Two pyruvate to two acetyl CoA in that step we have 2 NADH. In this step remember when we have pyruvate go to acetyl CoA in the complex, what happen to NAD plus? The last step in E₃ where FADH₂ had formed, we had NAD plus go to NADH₂.

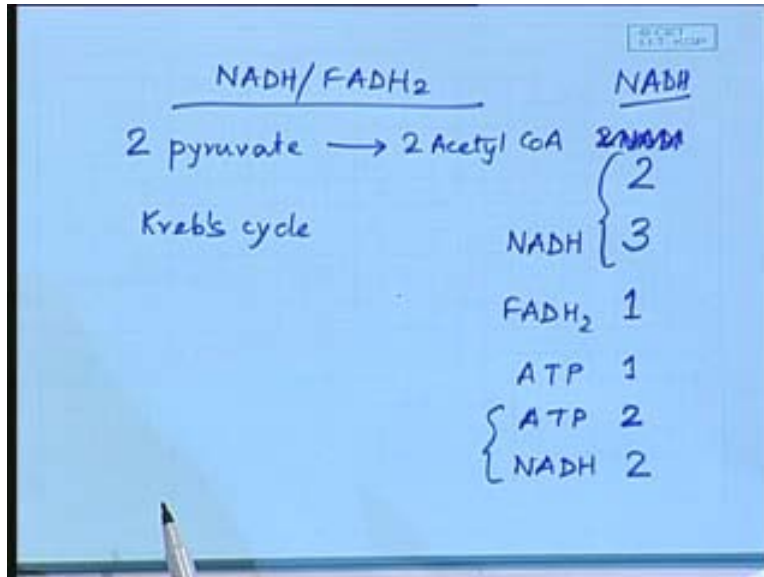
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So we have let us keep track of our NADH now. So NADH and FADH₂ so before we enter the Krebs cycle we have the 2 pyruvate going to 2 acetyl CoA because remember 2 pyruvate comes from the glucose. So how many NADH does that mean? 2 NADH. So let us write NADH here because all are NADH set one. So we have two then we have in our other steps we consider how many NADH do we finally get in the Krebs cycle? I am not writing the steps, the Krebs cycle or the TCA cycle gives us 3 NADH then FADH₂ we have one FADH₁.

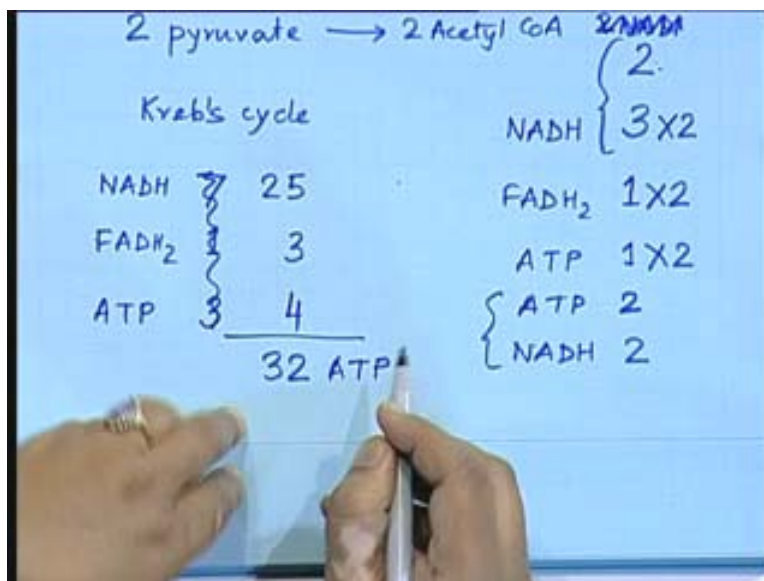
So this is actually NADH, one FADH₂ so this is for NADH, this is one FADH₂ and one ATP. And what do we get from glycolysis? **We got and what about NADH?** There is one step that provided here exactly you get 2 NADH it was the glyceradehyde 3 phosphate going to the bis phopho glycerate. So we got ATP from glycolysis two and NADH from gylcolysis two.

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We just have to go and look at the series of steps. So what else do we know from oxidative phosphorylation, that each NADH is going to give me 2.5 ATP. Each FADH₂ is going to give me 1.5 ATP. So calculate how many ATP you are supposed to get from this information, tell me. So NADH I have 5, NADH actually I have 5 and 2, 7. FADH₂ I have one, ATP I have 3. Pyruvate to acetyl CoA we have 2, Krebs cycle we have 3 but we are missing out something here.

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This has to be multiplied by 2 and this has to be multiplied by 2 and this has to be multiplied by 2 (Refer Slide Time: 47:45). Why? They are two each so how many do we have? So we have 2, 8 here and 2 10 so 10 into 2.5, 25. Two into 1.5 and 4 ATP. How many? 32 so I have 32 ATP. How much energy is that? Do you know how much energy it is? So usually they say we have **30 to 32**.

Let us get back to the slides here and this is actually the whole balance sheet. The number of ATP or reduced coenzymes that are directly formed from each step and the number of ATP that are ultimately formed, so we have a count of exactly what we did where we considered all the NADH, all the FADH₂, all the possible ATP and we finally got 32. They say 30 to 32 because they have considered this as 3 to 5 but 32 ATP is fine.

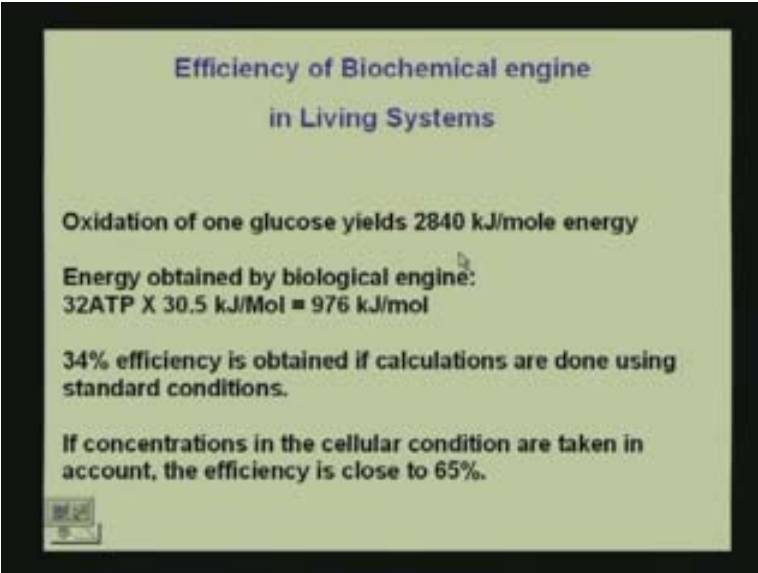
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Reaction	Number of ATP or reduced coenzymes directly formed	Number of ATP ultimately formed ¹
Glucose → glucose 6-phosphate	-1 ATP	-1
Fructose 6-phosphate → fructose 1,6-bisphosphate	-1 ATP	-1
2 Glyceraldehyde 3-phosphate → 2 1,3-bisphosphoglycerate	2 NADH	3-5
2 1,3-Bisphosphoglycerate → 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate → 2 pyruvate	2 ATP	2
2 Pyruvate → 2 acetyl-CoA	2 NADH	5
2 Isocitrate → 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate → 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA → 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate → 2 fumarate	2 FADH ₂	3
2 Malate → 2 oxaloacetate	2 NADH	5
Total		30-32

So if we consider the efficiency of the biochemical engine in the living systems, the oxidation of one glucose yields 2840 kilo joules per mole energy. That is a huge amount of energy and the energy obtained by this biological engine is 32 ATP into 30.5 kilo joules per mole that is the ATP hydrolysis, remember 31 kilo joules. So that is the amount of energy we can get for the 32 that are generated. Where? From your glycolysis, from your krebs cycle and your oxidative phosphorylation.

so in all cellular respiration the whole three steps that we considered stage one, stage two stage 3 ultimately leads to the production of 32 ATP. This 32 ATP then gives us the energy for the functioning of the cell, the functioning of all the reactions that are actually going on in the body. Now if we consider the efficiency, we actually get 34% efficiency if we consider the calculations done in standard conditions.

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**Efficiency of Biochemical engine
in Living Systems**

Oxidation of one glucose yields 2840 kJ/mole energy

Energy obtained by biological engine:
 $32\text{ATP} \times 30.5 \text{ kJ/Mol} = 976 \text{ kJ/mol}$

34% efficiency is obtained if calculations are done using standard conditions.

If concentrations in the cellular condition are taken in account, the efficiency is close to 65%.

Standard conditions meaning that we would have 25 degree centigrade but if we actually consider the cellular conditions where we are going to consider the overall efficiency in terms of a temperature of 37 degree centigrade. We will get an efficiency of 65%. So from all the enzymatic reactions that go on in the body, we get a large amount of energy based on the production of ATP that is coming from the metabolism of the carbohydrates that is the formation of glucose from where, from glycogen that is stored in the liver glucose going into pyruvate.

Pyruvate forming acetyl CoA, acetyl CoA getting into the kerbs cycle producing NADH and FADH_2 that is then going to go into the oxidative phophorylation in the whole electron transport system where complexes one through four are going to utilize these cofactors into creating the proton pump that is going to pump protons into the inter membrane space in the mitochondria. Ultimately leading to the production of ATP. So we found out that this the amount of energy that we can get and this completes our discussion on the break down of glucose to carbondioxide and water. So that will complete our metabolism and basically the essence of what we were supposed to for this course.

Thank you.