Biochemistry
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Lecture - 27
Metabolism III

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

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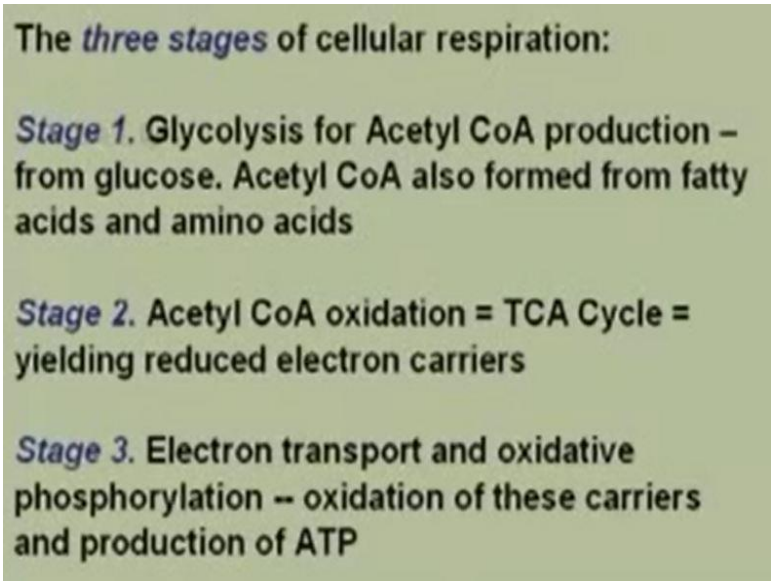

Now if we look at the three stages of cellular respiration. There are actually the first stage that we have gone through that is glycolysis for acetyl coenzyme A production. We have already seen the breakdown of glucose to pyruvate. And we will see now how pyruvate actually gets to acetyl coenzyme A. That then gets in to the tricarboxylic acid cycle which eventually leads to the production of carbon di oxide.

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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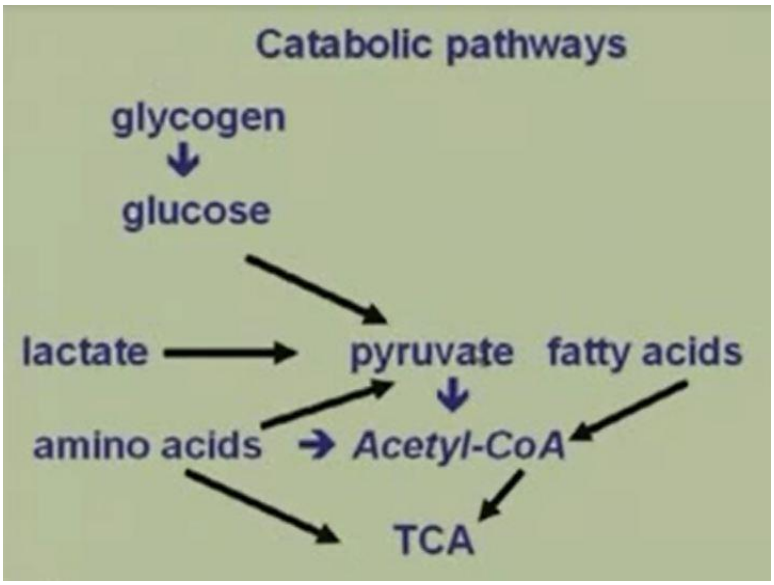

And if we consider the catabolic pathways of all the breakdown whether its amino acids or fatty acids or glucose. They ultimately lead to acetyl CoA which is the key component of the TCA cycle which we will see. So what we have studied in glycolysis is glucose getting to pyruvate. Now the reverse of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

It is not 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. 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 in to acetyl CoA okay. So we have considered so for the breakdown of glucose to pyruvate.

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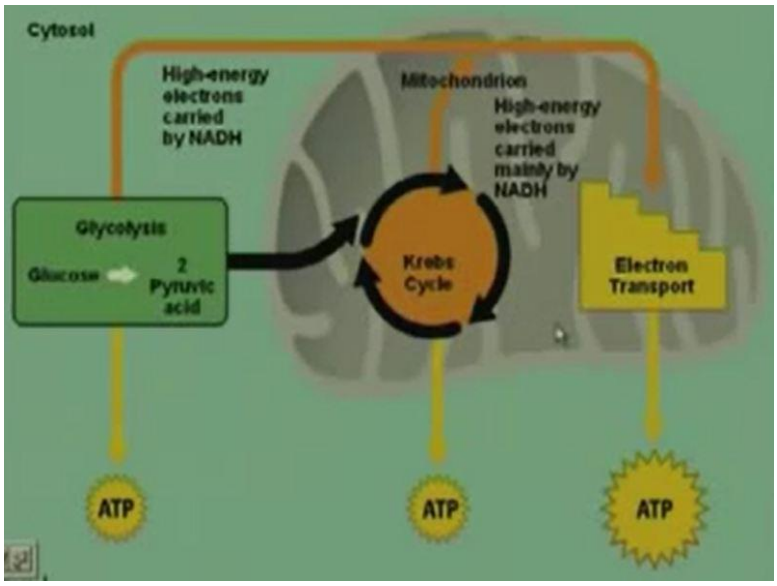

Now in general we have these three overall steps. All of them produced ATP okay. That is our major concern in the production of energy. Production of energy in the form of ATP which is ultimately going to be used for the breakdown of this high energy phosphate bonds that is going to release a large amount of energy for our bodily functions. So we have the first step glycolysis where from glucose we get two pyruvic acid molecules.

Here we have the Krebs cycle which we are going to study right now and the electron transfer 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 cristae of the Mitochondria.

At 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 the Mitochondria Krebs cycle and electron transport. The electron transport we know occurs in the inner mitochondrial membrane where we have complex as one through four and the F0F1 ATP is that creates the ATP from the proton motive force.

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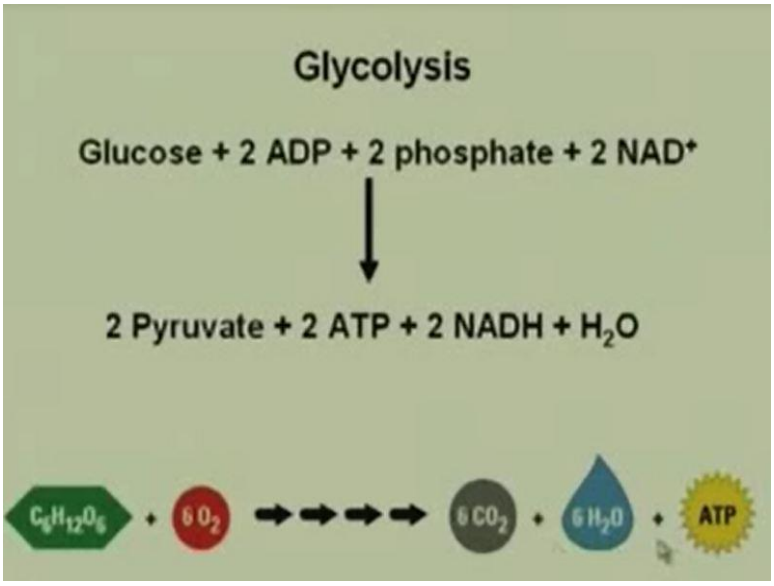

So this is what we have done previously where we consider glycolysis, the glucose getting to two pyruvates. We have gone through all these steps but ultimately glucose is going to be broken down in to carbon dioxide and water with ATP okay. So our next step now is to see how pyruvate is going to get to carbon dioxide.

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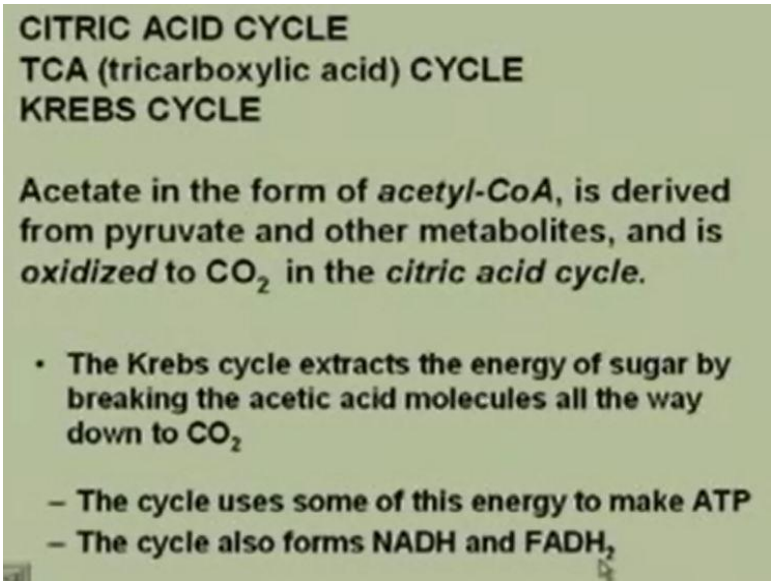

This is formed by the Krebs cycle. There are they have three different name to this cycle it is called the CAC or the Citric acid cycle or the TCA or the tricarboxylic acid cycle or the Krebs cycle since it was most of these reactions where determined by Hans Krebs. Now acetate in the form of acetyl CoA is derived from pyruvate. The key step after pyruvate is obtained in the breakdown of glucose is the formation of acetate.

This acetate is taken up by coenzyme A to form acetyl CoA and then later on it is oxidized in to Co2 in this citric acid cycle. 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. The cycle uses some of this energy to make ATP and it also forms NADH and FADH2. Later what we are going to see is we are going to see an energy balance see exactly what the amount of energy we are going to get from a single molecule of glucose?

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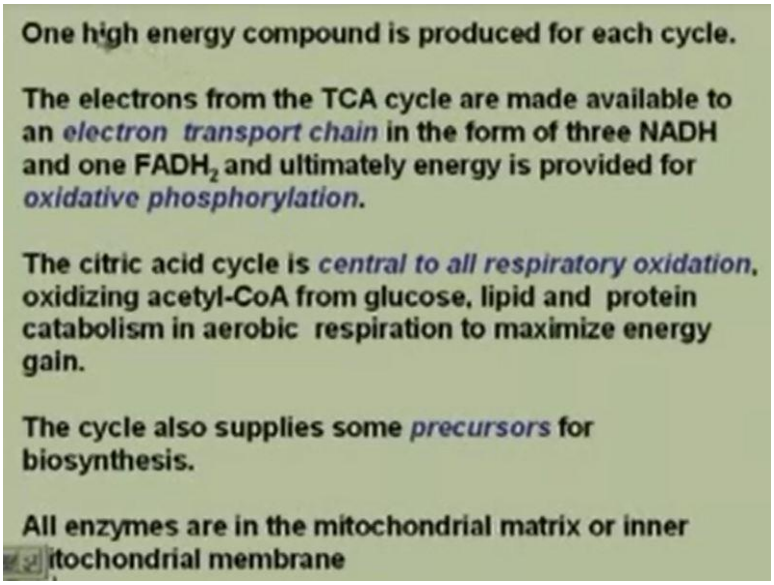

Now in the Krebs cycle, we have these are the key features of the Krebs cycle. We have one high energy component produced for each cycle. What we mean by a high energy compound? It is actually GTP. GTP is formed in this case but a triphosphate bond being formed okay like in glucose we had ATP being formed that is the formation of high energy compound. The electrons from the tricarboxylic acid cycle are made available to an electron transport chain in the form of three NADH and one FADH2 so these are also formed in the reactions in the Krebs cycle.

This NADH and FADH2 you remember is utilized in oxidative phosphorylation for the production of ATP where we require these cofactors in the complexes 1, 2 and 3 and 4 also. The citric acid cycle you have to remember is central to all respiratory oxidation okay. It oxidizes acetyl CoA that is obtained from glucose, lipid and protein catabolism. So understand that this

acetyl CoA is an extremely important component because it is formed from the breakdown not only of carbohydrates but also of lipids and of proteins okay.

And the cycle also supplies some precursors for other biosynthesis for other biosynthetic methods in the formation of proteins and other biological macro molecules. 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 the cell.

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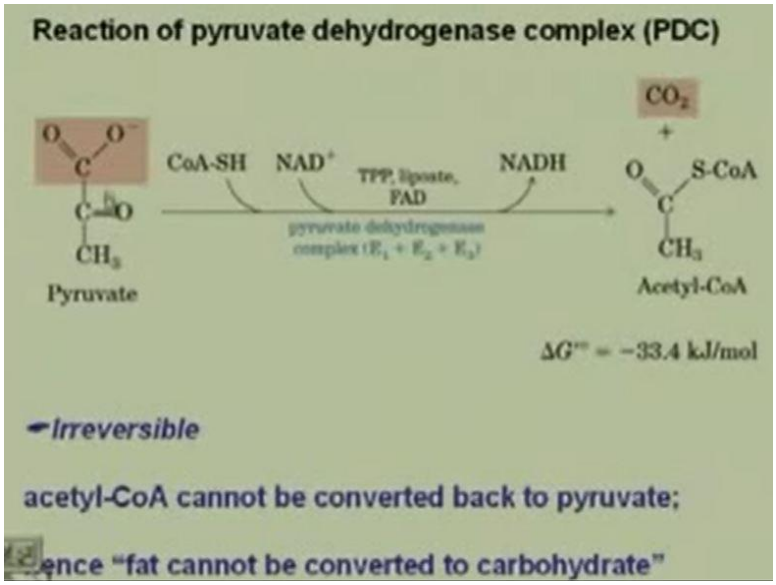

Now this is the major reaction. What we have here is? We have pyruvate. Pyruvate is CH3COCO- that is pyruvate. We have here coenzyme A that is we will look at the structure in a moment NAD+ this goes to NADH and we form acetyl CoA with the release of carbon dioxide. The enzyme for this is pyruvate dehydrogenase complex. So it is a complex of enzymes actually three enzymes E1, E2 and E3.

We will see what those components are in a moment and this is called the PDC, the pyruvate dehydrogenase complex that comprises of three enzymes. Now once acetyl CoA is formed it does not get back to pyruvate which makes this reaction irreversible which means basically that fact actually cannot be converted to carbohydrates because even though you get to the same acetyl CoA because fat breakdown will also get you to acetyl CoA okay.

But since acetyl CoA cannot get back to pyruvate you cannot get back to glucose because pyruvate can go 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 breakdown of fatty acids. We should have been able to obtain carbohydrate from fats okay. But that is not possible due to the irreversibility of this step.

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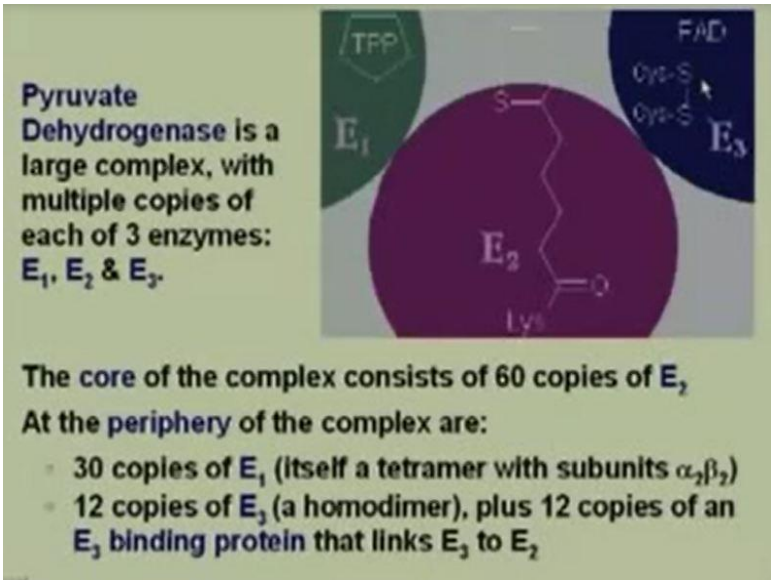

Now this has three enzymes E1, E2 and E3. Now we would not go into the details of the mechanism or how the procedure actually takes place but nevertheless we need to know something about the complex. It has as I had been mentioning E1, E2 and E3. There are 60 copies of E2 in the core of the complex, 30 copies of E1 and 12 copies of E3 okay. So you can imagine that this is a huge complex in the way it actually acts.

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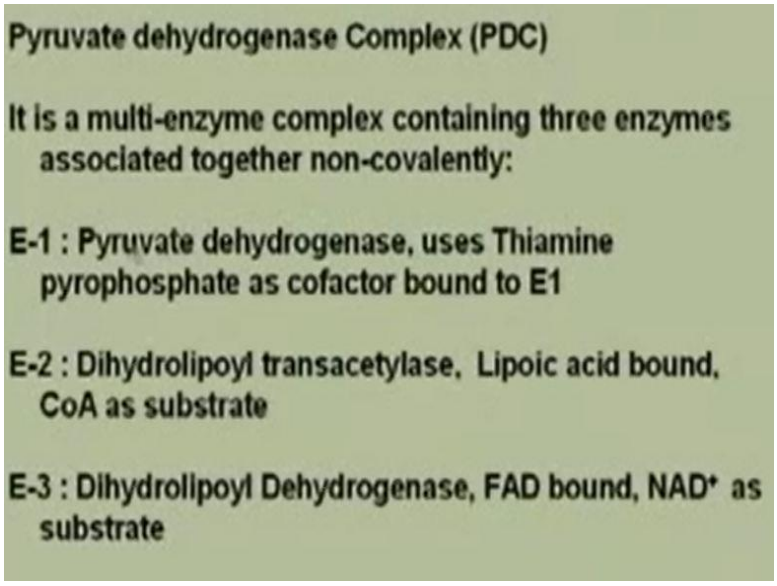

Each of these has their own cofactors associated with it okay. So E1 is actually pyruvate dehydrogenase that uses TPP as the cofactor. E2 is dihydrolipoyl transacetylase that is lipoic acid bound and uses coenzyme A as the substrate. So as soon as we have coenzyme A we know that that we are going to create now acetyl CoA the acetate coming from the pyruvate. And in the next one we have E3 were FAD is the cofactor and NAD+ is the substrate.

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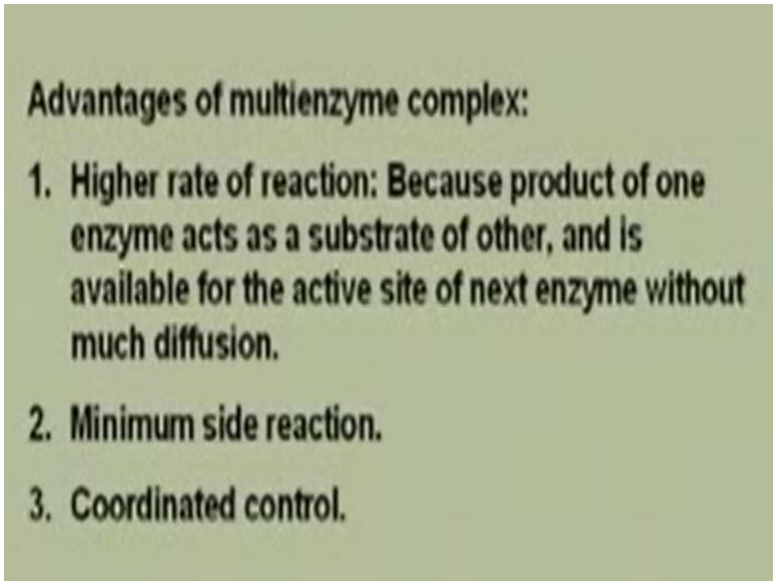

Now the advantages of having this multi-enzyme complex is that usually for other reactions that we have been seeing for glycolysis or we will see for the Krebs cycle have a single enzyme that acts on it. This complex is a multi-enzyme complex and the utility of the multi-enzyme complex

actually shows how important the formation of acetyl CoA actually is and how tightly it has to be regulated as well okay.

So what happens when we have this multi-enzyme complex is we have a higher rate reaction. Because the product of one enzyme actually acts as the substrate for the next enzyme so as soon as E1 acts E2 will come in to the picture then E2 acts and E3 comes in to the picture. 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 than in a sense.

Because the enzyme itself does not have to get back to it is original form right in 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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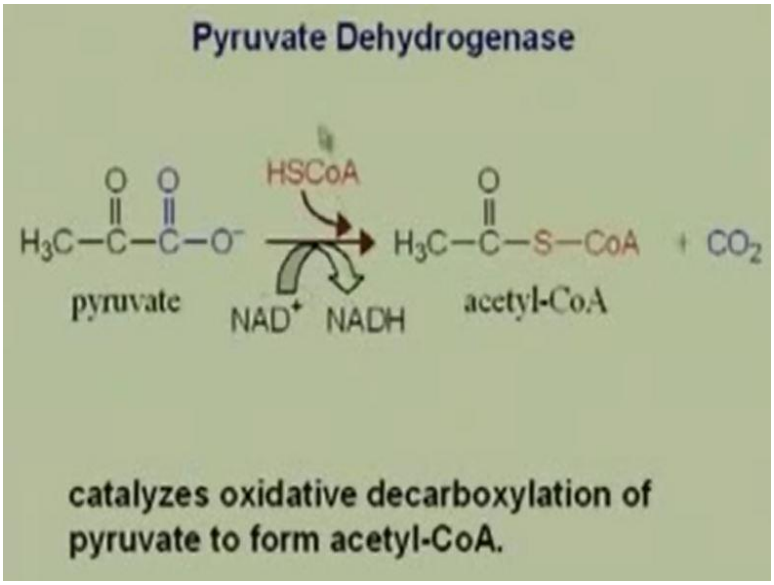

So the overall reaction is pyruvate getting in to acetyl CoA with the release of carbon dioxide with this enzyme.

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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

So these are the three enzymes E1, E2, E3 and these are the three prosthetic groups that are attached to the specific enzymes okay. TPP, Lipoamide, FAD to E1, E2, E3 that is these three enzymes that comprise the PDC the pyruvate dehydrogenase complex.

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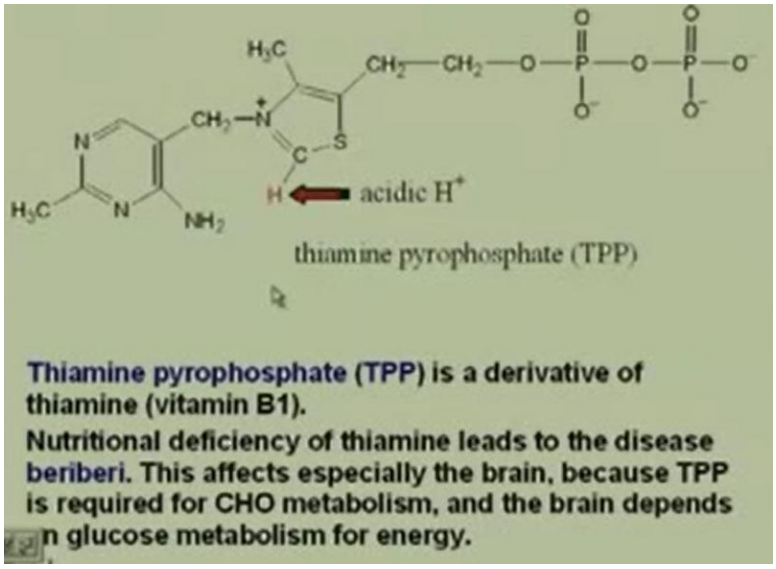

Now in E1 you all remember that when we studied vitamins. We had vitamin B1 thiamine that actually formed TPP okay. In TPP we have an acidic H+ and if you remember we spoke about this acidic H+ and how it is important in certain reactions and we will see how it is important in this one. We have this acidic hydrogen that actually dissociates from the ring and creates the carbon ion okay.

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 okay. So the acetyl is formed with the help of TPP that is part of E1 of the PDC that is the pyruvate dehydrogenase complex.

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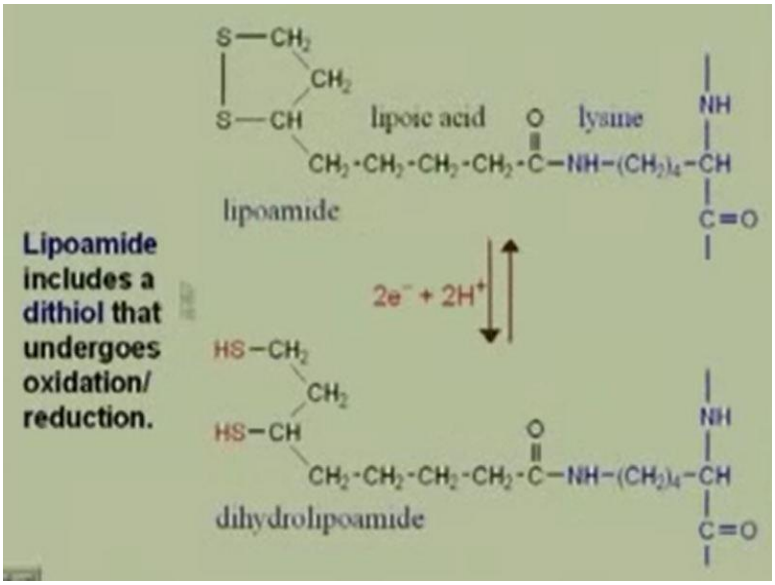

In the second part, we actually have a lipoic acid that is linked to lysine in the enzyme E2. And here we have a certain reaction which is going to get into the reduction of this divinyl disulfide here where we have SH SH that is going to be dihydrolipoamide okay. Now the reason why we are going through this is because we have to understand how acetyl CoA is actually being formed.

We have acetate now we have got the rather the acetyl now right. Where did the acetyl come from? It came from E1. What happened to E1 in the previous step?

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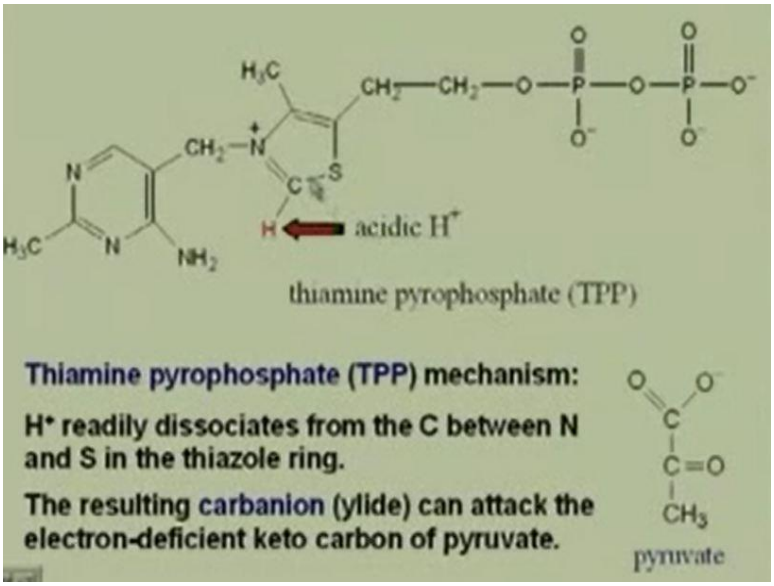

We released CO2 and we had acetyl that is now linked to this fine.

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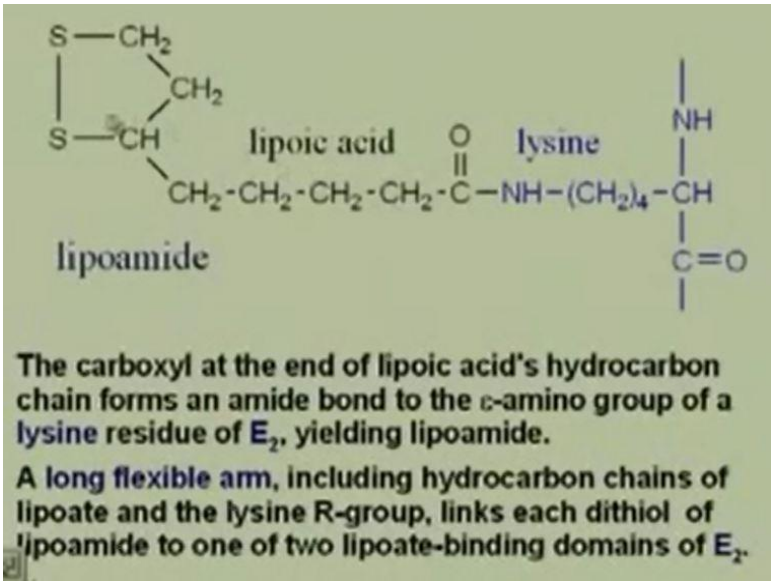

So now we have to go on in our further step. This is the part where we have the dithiol that undergoes oxidation and reduction. One important thing of this is this dithiol reacts with lipoic acid with the lysine of the Where is this lysine? It is present in E2 okay. This is the prosthetic group that 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 okay but we are not going into the mechanistic details of this.

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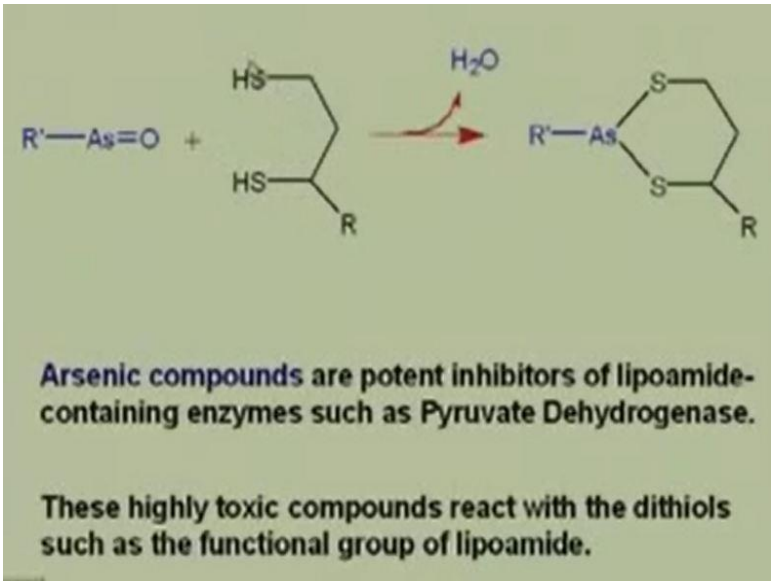

One important thing that you might want to know is about this SH SH that is the dihydro lipoamide is actually sometimes you hear about arsenic poisoning okay. What happens is? This is what gets into inhibiting this lipoamide containing enzymes. So what happens is the acetyl CoA is not produced okay because 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 certain tonic that was supposed to be made that actually sort of you know what a tonic was sort of giving you more energy in the sense and that actually let to arsenic poisoning. Charles Darwin in fact died of arsenic poisoning. This is the mechanistic thing that happened where this compound was actually formed. That prevented what prevented the dehydrogenase complex from acting, preventing the formation of acetyl CoA, preventing the complete degradation of the glucose okay.

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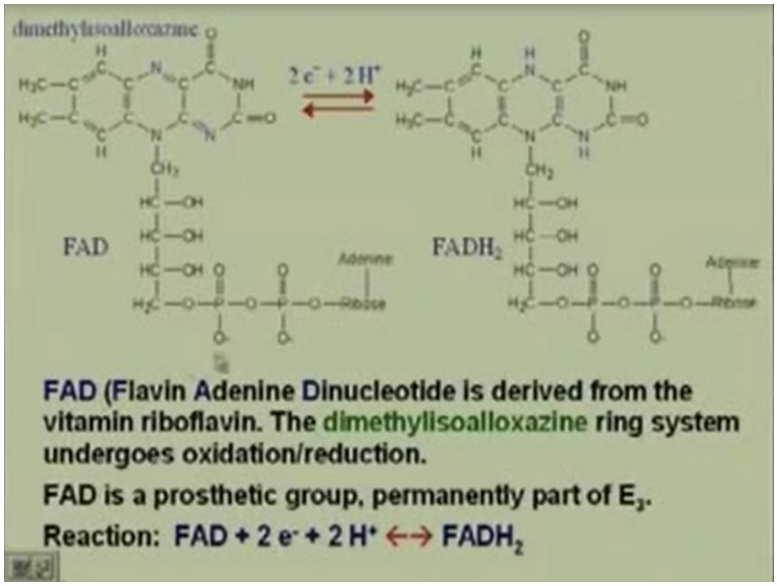

Then we also have FAD. FAD is part of enzyme part E3 okay. So we have E1 that is TPP, E2 that is the Lipoamide and E3 that is our FAD.

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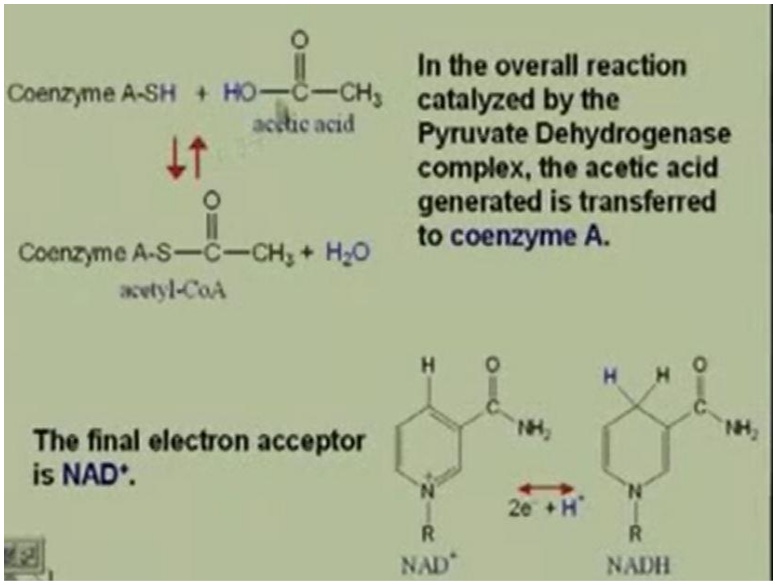

So basically what is happening? We have coenzyme A that is the thiol with the acetic acid forming acetyl CoA and the finally electron acceptor because FAD is going to get to FADH2 okay. But it has to get back to FAD. So that it can react again so the final electron acceptor is NAD+. That is the substrate for enzyme E3 that will take up the H2 that has been given to FAD and get it back to FAD okay. So FAD forms FADH2 here.

This to get this back to FAD what must happen? This has to be taken up by something. Whose is going to take it up? NAD+ is going to take it up in the third reaction. So ultimately the electron acceptor is the NAD+ which is why it is mentioned that NAD+ is a substrate for that particular mechanism.

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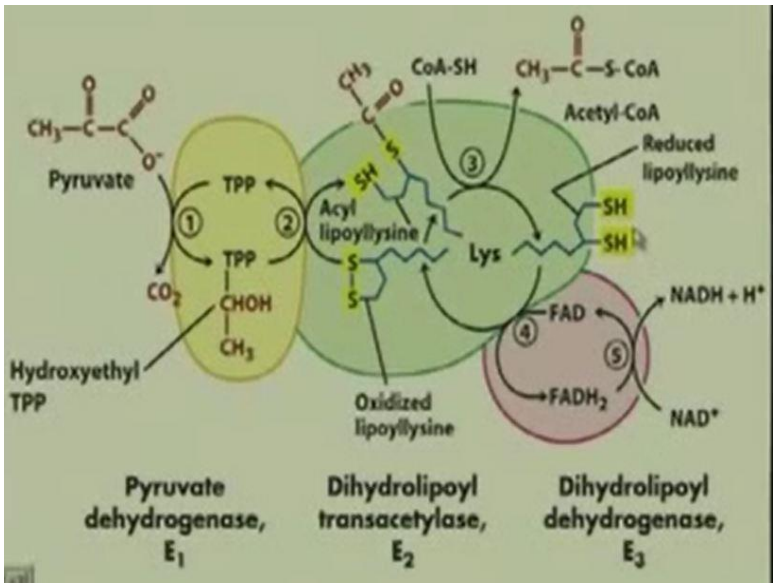

Okay this is essentially what is happening? The yellow one here is E1. This green one is E2 and the pink one here is E3. The first step as we saw is we have the TPP. So here we have the loss of this carbon dioxide. This is carbon dioxide which is coming of here right. We have now an acyl part so this acyl part is linked to where to the TPP right because you have the delayed 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. 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 right the lipoic acid part here.

Now what happens is? Coenzyme A now comes into 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 dithol there so two of these

H has to be removed. How can they be removed? They are removed by FAD. FAD then comes into the picture picks up the two hydrogens and this gets backs to your oxidized lipoyllysine.

Is that clear? So this is just a general procedure where you would have these because you release 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 another pyruvate. Eventually what happens is this gets to the oxidized part so this can now take up another acetyl, another acyl rather okay. So another acyl can now get attacked to this. FAD has now been reduced to FADH2 what must happen in this case FADH2 has to get back to FAD.

So what comes in to the picture then NAD+. NAD+ then acts as this and picks up the hydrogen right. 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 okay. Now let us see what happens?

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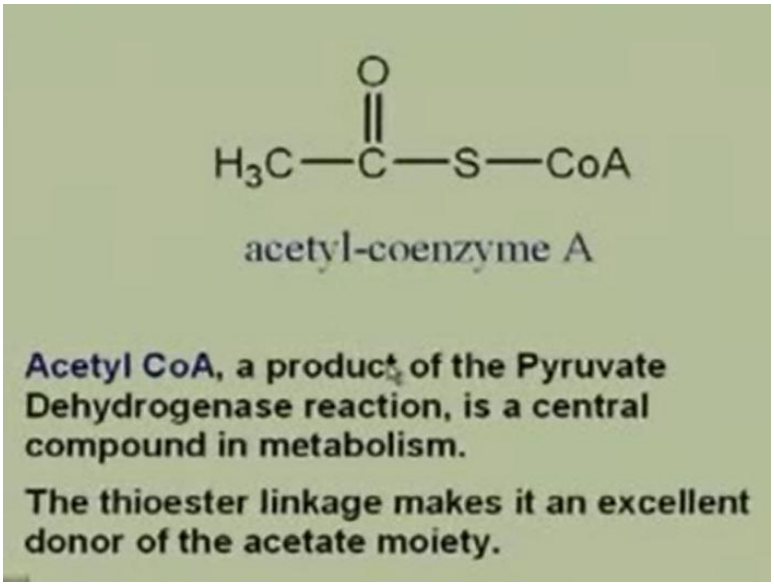

There we have Acetyl CoA which is a product of the pyruvate dehydrogenase reaction. It is a central compound in metabolism and you see how carefully nature has decided on its formation. With all these E1, E2, E3 specific cofactors acting on in and so on and so forth. Now what happens is this thioester linkage right here makes it an excellent donor for the CH3 CO group. So

whenever you have acetyl CoA come in to the picture. You know you will have a transfer of two carbon atoms because you have one carbon atom from the CH3, one from the CO okay.

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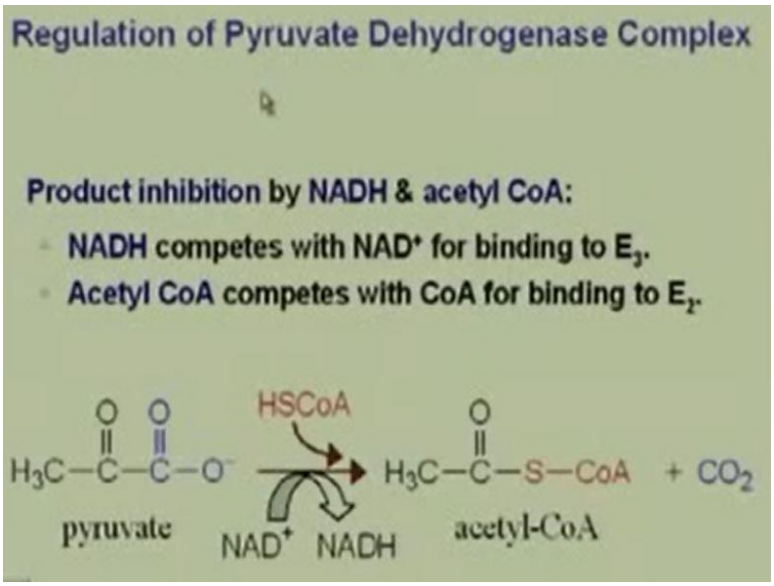

So now we have to think of there has to be a regulation of this complex as well for the complex to act correctly. Now when we have E3 NADH competes with the NAD+ for binding to E3. So it inhibited by the product. E3 where did E3 come in it the picture? We had FAD going to FADH2 right.

And since NAD+ came into the picture there to form NADH it competes with NAD+ for binding. So it is inhibited by the product an acetyl CoA competes with Coenzyme A for binding to E2. Where this acetyl CoA was a product of the enzyme E2 so we now have product inhibition by NADH and acetyl CoA and this as I mentioned is the overall reaction.

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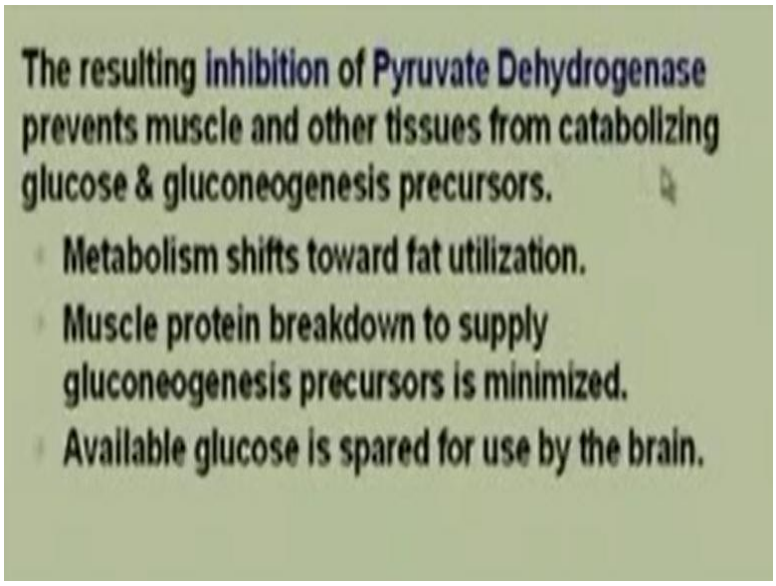

Now the resulting inhibition of the pyruvate dehydrogenase prevents muscles and other tissues from catabolizing glucose and gluconeogenesis precursors. So basically this is just a regulation of the whole system. This product inhibition you have to recognize is a regulation of the whole enzymatic procedure.

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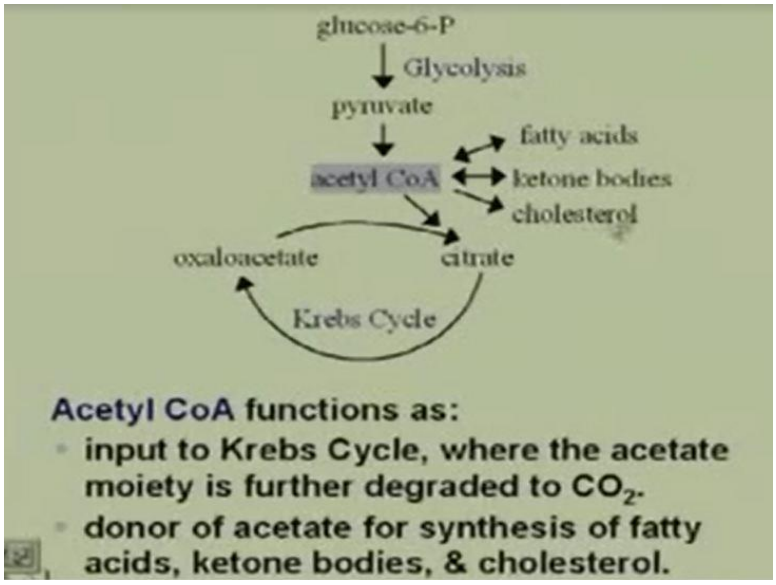

So this is what we have? We have pyruvate. Glucose getting to pyruvate and now we have acetyl CoA that is going to be the input to Krebs cycle where this acetate is going to be broken down in to carbon dioxide. And we are going to have this acetyl CoA also involved in other components where the further synthesis 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.

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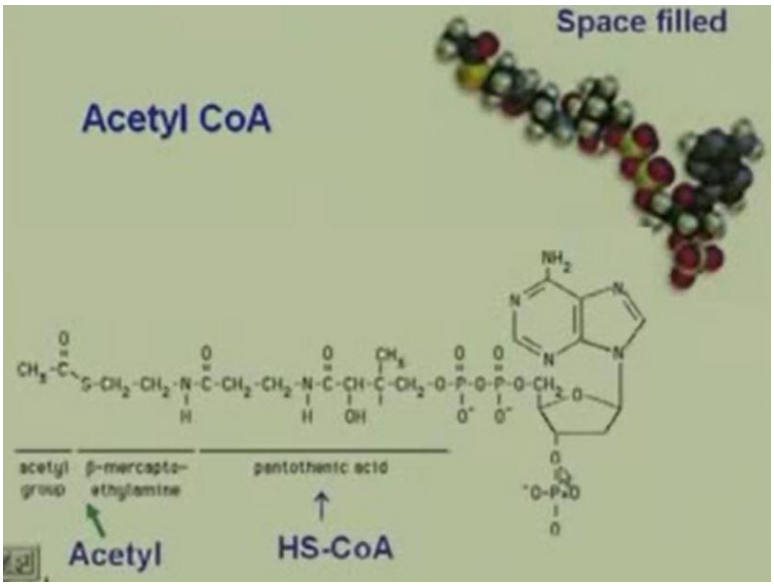

Okay this is acetyl CoA if you remember. We have the acetyl group here the beta mercaptoethylamine part, the pantothenic acid part that was derived from the vitamin and what is this part? What is this part? What part is this? We have a phosphor part here also we have to go check it up so this is acetyl CoA.

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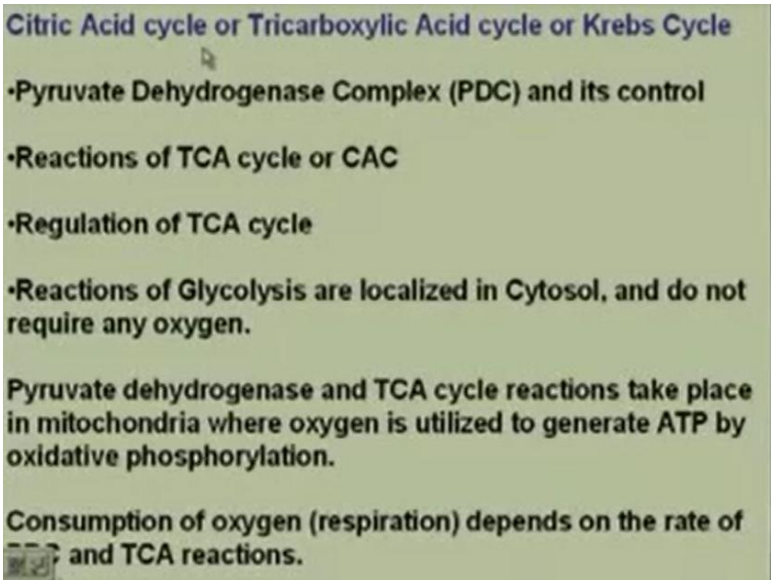

Now what do we have here? We have the basic features of the citric acid cycle where we have the PDC and it is control. Reactions of the TCA cycle and we have as I mentioned before the

reactions of glycolysis that are localized in the cytosol and these take place in the mitochondria. The matrix and the respiration the ultimate oxidative phosphorylation takes place in the inner mitochondrial membrane.

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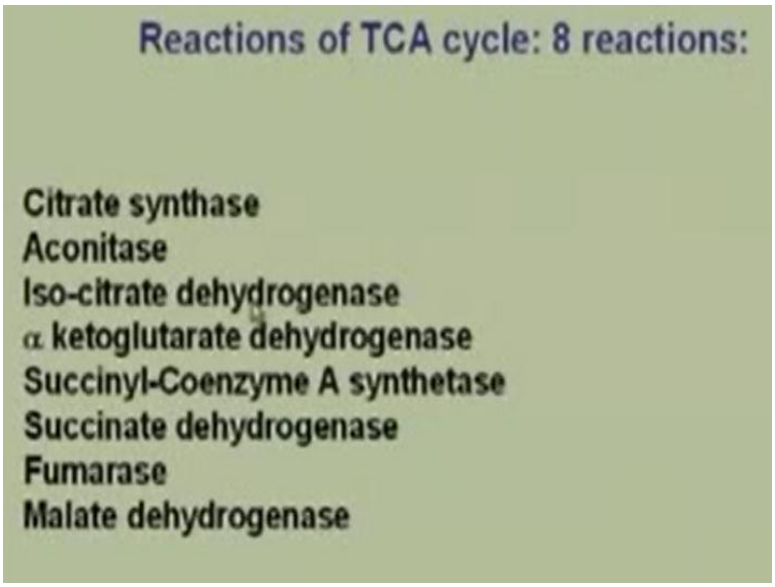

These are the enzymes. There are eight reactions going on here. We have citrate synthase, Aconitase. We will go step by step in each of these like we went for glycolysis.

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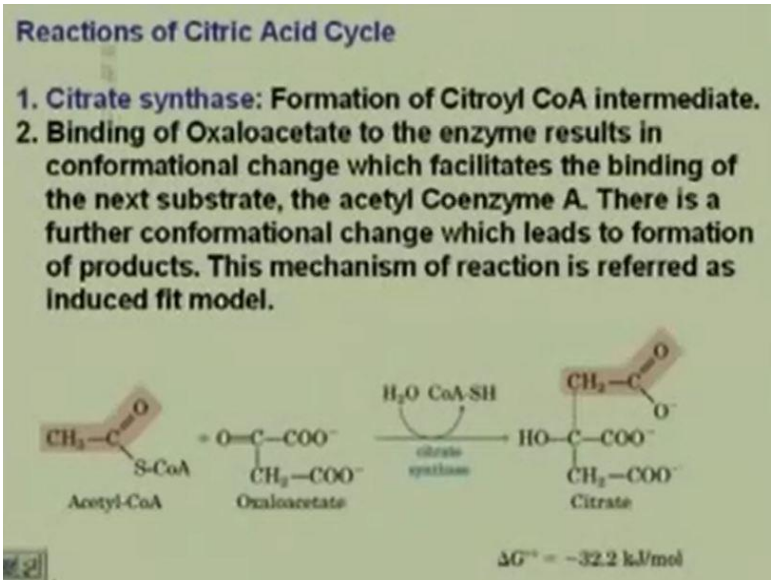

In the first step, we have Acetyl CoA, oxaloacetic acid. In the presence of citrate synthase what is citrate synthase mean? It is going to form citrate. So we have Oxaloacetate here that has now one thing when you consider or study the citric acid cycle. You have to keep count of the carbon

atoms and you have to keep count of what is going where. If you label any of this just how these reactions were actually deciphered.

If you labeled say the Acetyl, this carbon which carbon dioxide it was forming can be determined. So when we have this acetyl CoA and we have oxaloacetate. Oxaloacetate has four carbon atoms. We are adding acetyl to it so now this has six carbon atoms. Four from your oxaloacetate and two from the acetyl part of acetyl CoA. So now we have synthesized citrate using citrate synthase.

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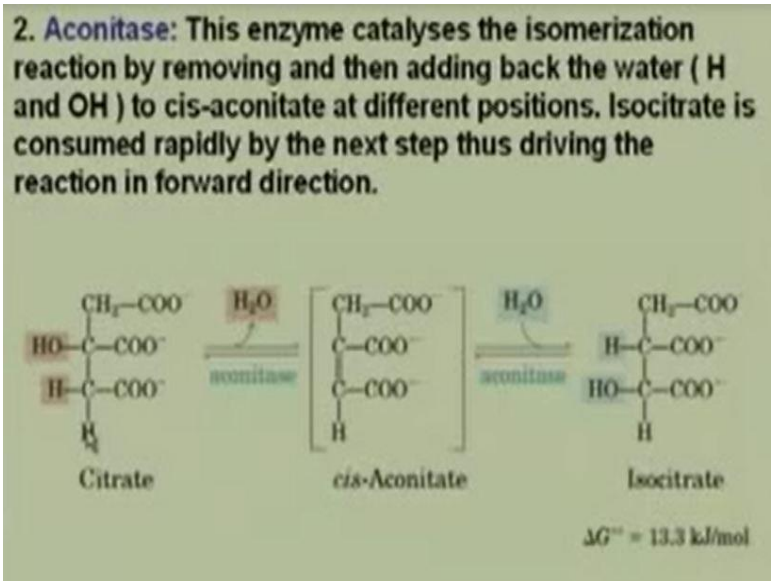

Now we have citrate. We have Aconitase that is nothing but an isomerize that forms isocitrate where you have just the interchanging of the H and OH on carbon atoms one and two here. Okay so you have citrate to isocitrate. Now actually the delta G0 prime if you see here is a positive quantity but since there is some equilibrium any little bit of this that is formed is pushed in to 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 okay. So that is our second step. Our first step was the formation of citrate by citrate synthase the next step is the formation of isocitrate.

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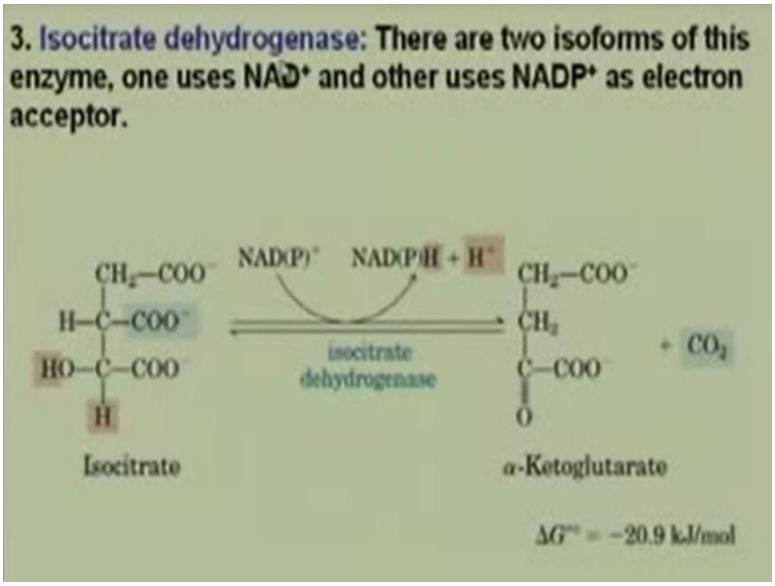

The third step is isocitrate dehydrogenase. What is that means? It means it is going to remove now water we have H plus removed here in NAD and in some case there are actually two forms of the enzyme that are called two isoforms of the enzymes one of them prefers NAD+ as the cofactor, one of them prefers NAD+ as the cofactor okay. But both of them were ultimately result in the removal these two hydrogens and the COO here.

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

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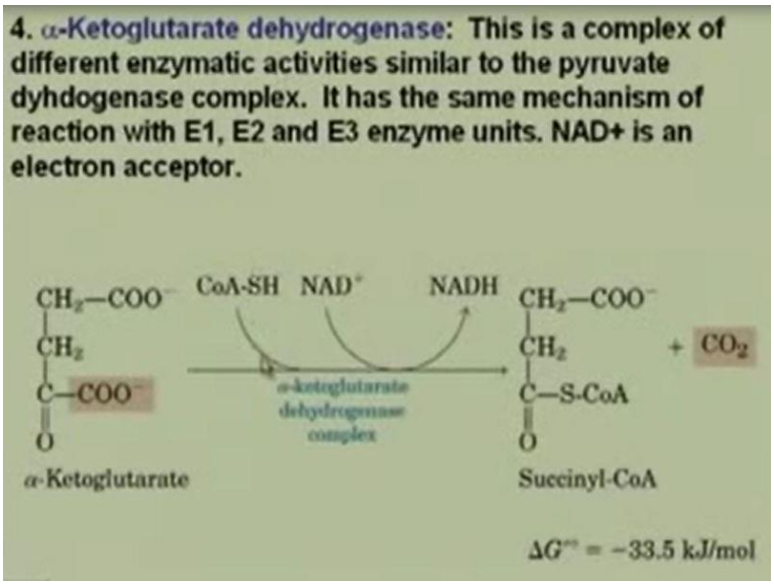

The fourth step is alpha ketoglutarate dehydrogenase. Again we have NAD+ and NADH. Now NADH is being formed here and will later on when I show you the whole cycle. We have to keep count of how many NADH are being formed? How many FADH2 are being formed? How many ATP are being formed because that is going to tell us how much energy we are going to get. It is not as difficult or as complicated as seems. It is pretty simple actually.

If you just follow the steps round the cycle so what we have here? We have alpha ketoglutarate going to succinyl CoA. If these are just cycles this has less steps that the glycolysis cycle eight compared to ten.

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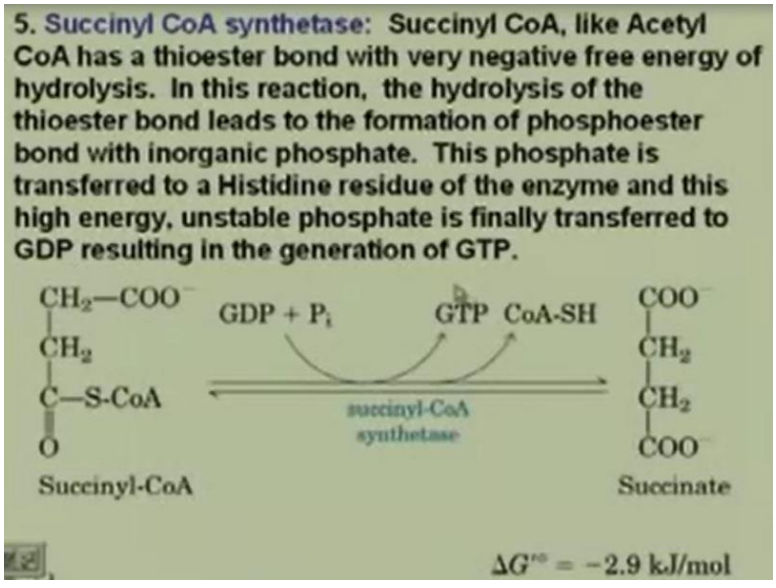

Then we have Succinyl CoA go to succinate and this is the step that results in the production of GTP, the high energy bond okay. So it is the step succinyl CoA synthase where it has a thioester bond a high energy thioester bond that actually forms GTP from GDP plus PR. So we have succinyl CoA going to succinate.

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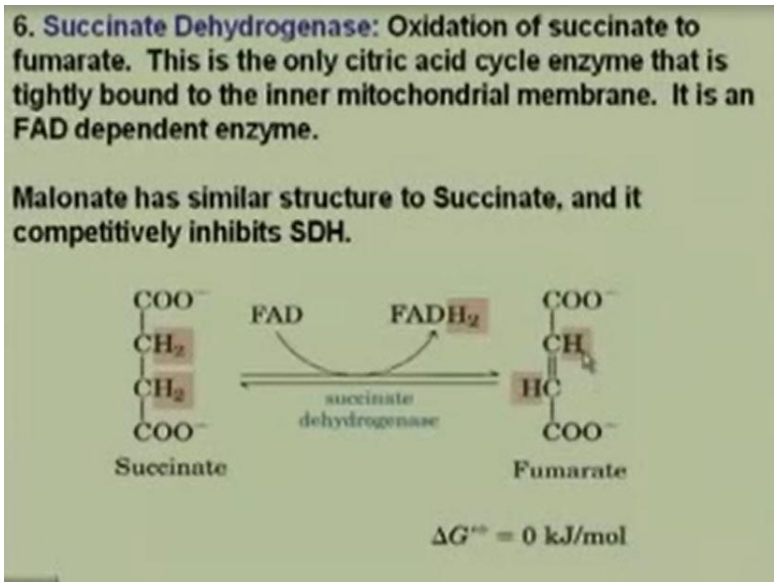

Step number six is succinate dehydrogenase where you see how we are getting smaller and smaller the succinate is now we have looked at this enzyme before when we looked at FAD going to FADH2. If you remember when I mentioned how FAD goes to FADH2. We consider succinate going to fumarate and in this case we have the removal of two hydrogen atoms here. This is in the inner mitochondrial membrane and it happens spontaneously okay.

So we now have formed fumarate. How many carbons do we have now? Four. How many did citrate have? Six. The citrate that we consider anyway I will show you. The citrate had 1, 2, 3, 4, 5, and 6. Oxaloacetate that we started off with had four. We added two carbons to make it six. Then we still had six because we have gone to just an isomeric form of it then we lost a carbon here so 1, 2, 3, 4, 5 now okay.

Then we lost a carbon here in alpha keto in forming succinyl CoA we lost another carbon. So we are now down to four. So now we still have four from succinyl CoA to succinate. We still have four because we have just gone for a dehydrogenase.

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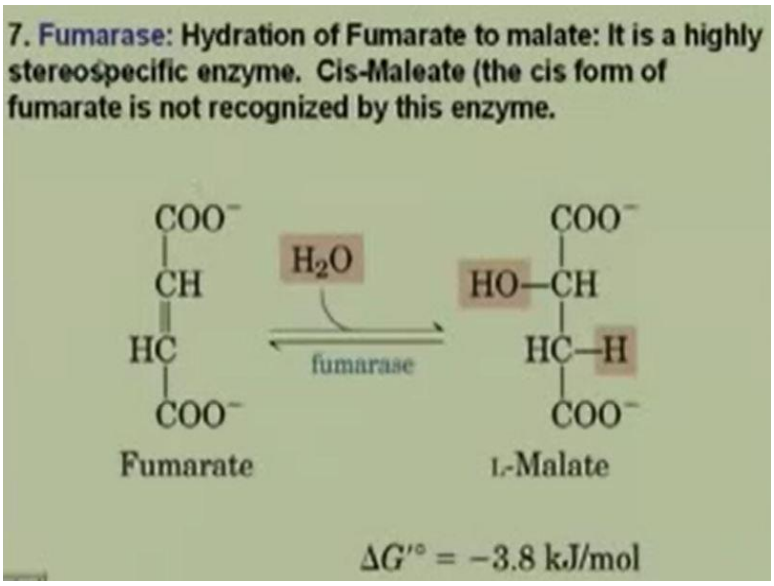

Fumarate to Malate this is fumarase where we have H2O forming malate.

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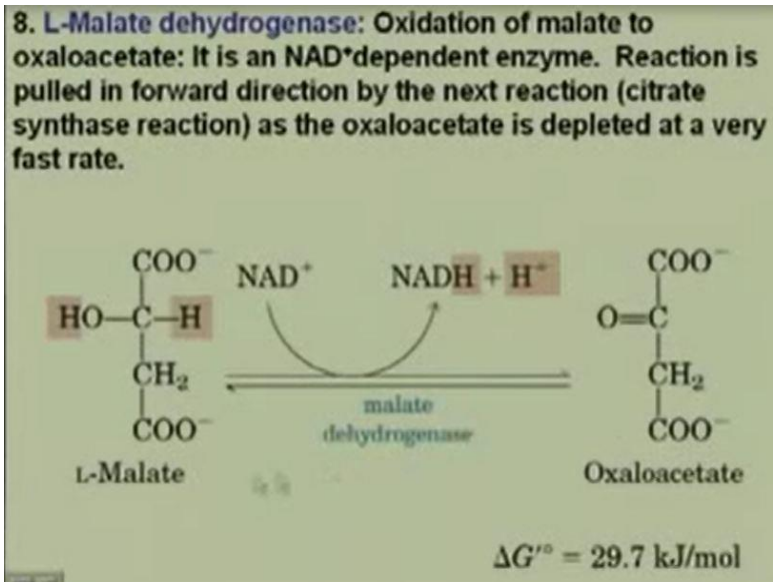

Then we have malate back to Oxaloacetate finished. So you have Oxaloacetate that is ready to pick on another acetyl CoA to form what citrate. So that is the whole cycle okay. So eventually what we have is if we look at the overall steps, we have to now figure out where the NADH was formed. So let us go back and in the first step we do not have any production of NADH okay. So in step number one we have formed citrate.

Step number two, we still do not have NADH formed we have just an isomerized reaction going to isocitrate. Step three we have lost Co2 we have produced one NADH so we keep track of that okay so we have formed one NADH. In the fourth step, we have another NADH so two. In this step we have one GTP that has to be count in another counter. So we have another counter for GTP and ATP production because this GTP is ultimately forms ATP okay.

We have one FADH2 okay. So two NADH, one FADH2 and one GTP then another NADH so how many do we have now. We have three NADH let us just keep track of it.

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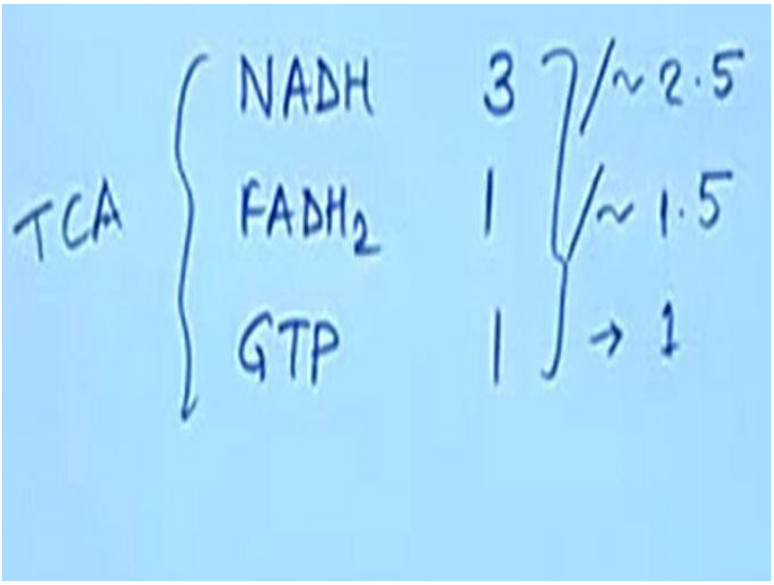

We have NADH three, FADH2 we have one and GTP we have one. So these are all coming from our TCA cycle. Now this NADH and FADH2 where is it going to go? It is going vote for oxidative phosphorylation and the production of ATP and when we looked at the overall reactions of oxidative phosphorylation what 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. We will get back to the energy calculation once we complete this. So now we figure out in the eighth so because we are back to oxaloacetate now so we have three NADH produced, one FADH2 produced and one ATP.

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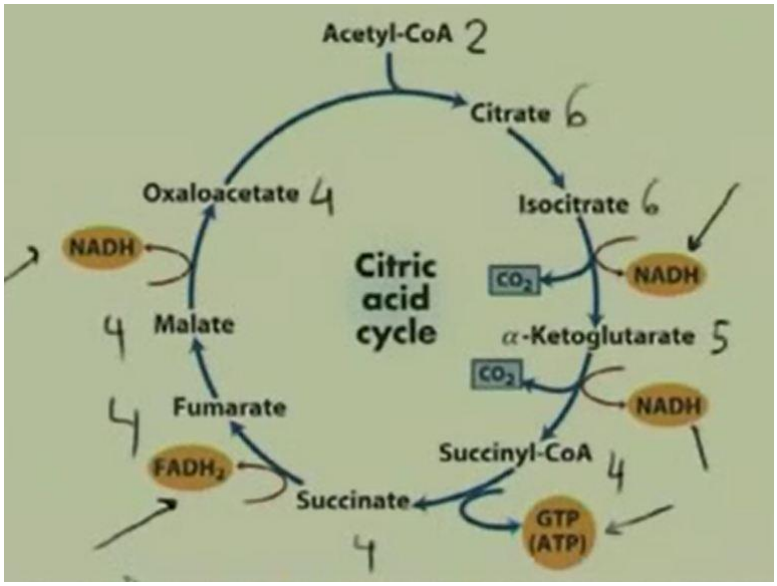

So here is our whole cycle. Acetyl CoA comes in to the picture form citrate, oxaloacetate. From citrate isocitrate, NADH produced okay. So here is one NADH. So we have oxaloacetate. How many carbons here? Four. How many carbons here? Two, six right. We still have six. We have lost one five, lost another one four four four four okay. How many NADH will we have? One NADH, two NADH, three NADH.

How many ATP? One. GTP is it is converted to ATP because go (()) (35:30). And we have one FADH2 okay. So that is our count. What else do we have to remember? That from glucose I got two of these so the whole thing is going to happen twice because glucose gave me two pyruvate and this is happening to each pyruvate. When I had glucose well I have the steps for you just in simple carbon atom steps.

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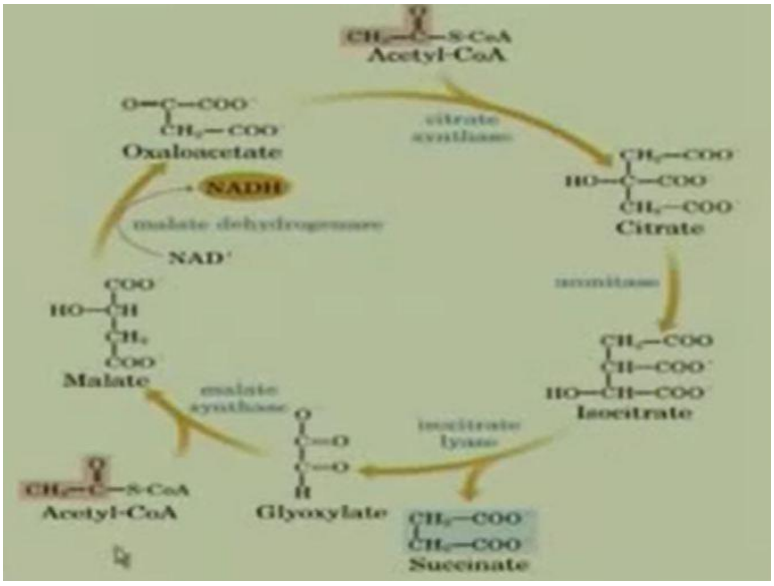

So this is just the same thing just showing where the reactions or where what are happening?

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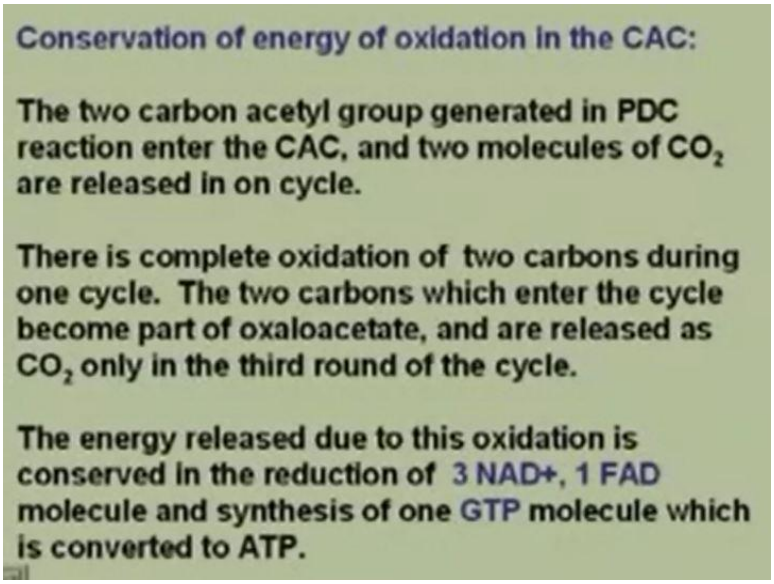

Now we figure out what we have? So now we have to look at the conservation of energy of oxidation in the citric acid cycle. We have two carbon acetyl groups generated in the pyruvate dehydrogenase complex. There is complete oxidation of two carbons during once cycle. You saw that. We have two Co2 been released okay the two carbon atoms which enter the cycle become part of the oxaloacetate for the next cycle.

The two carbon atoms that have entered in the top the Co2's lost in from the middle what was existing in the oxaloacetate. Remember, I showed you where the Co2s are coming off okay.

Those Co2s are not part of the acetyl CoA. The acetyl CoA shifts in the formation the two carbon atom which enter the cycle become part of the oxaloacetate and they are released in the third round.

So you have 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+, one FAD and the synthesis of one GTP molecule which is converted to ATP.

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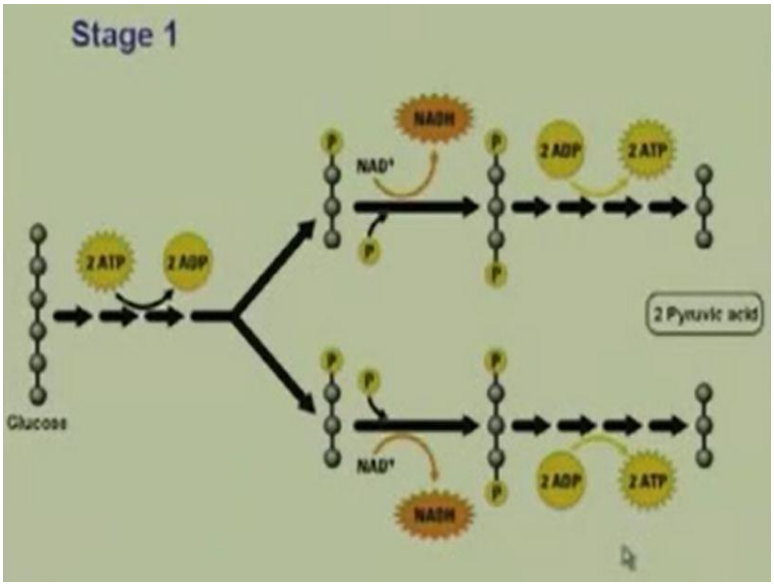

So this is our stage-1, six carbon atoms we had two ATP remember that were taken up in the formation of two steps there for the glucose six phosphate and for the one six bisphosphate. So we had two ATP broken down in to two ADP. Now so we now have this were these two dihydroxyacetone phosphate and glyceraldehyde 3 phosphate right so both of these still have the phosphate then we had NADH being produced and we had two ATP being produced.

Remember then we said that since we had four ATP here and two ATP being used up we eventually had two ATP to consider. So that is our stage-1 of our glycolysis.

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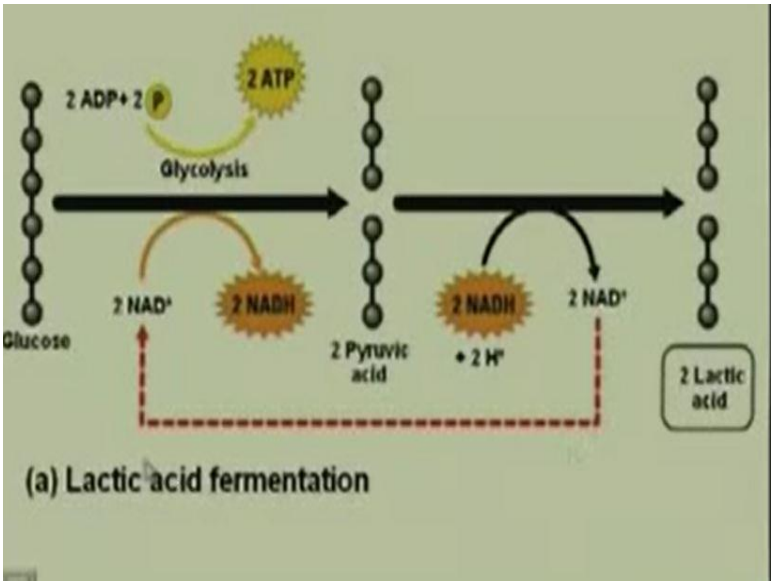

Now there are two other possibilities here. Where we can have lactic acid fermentation in that case remember we said that we do not have that NAD+ because this NAD+ is being utilized again in the reaction. This is what we did in our last class.

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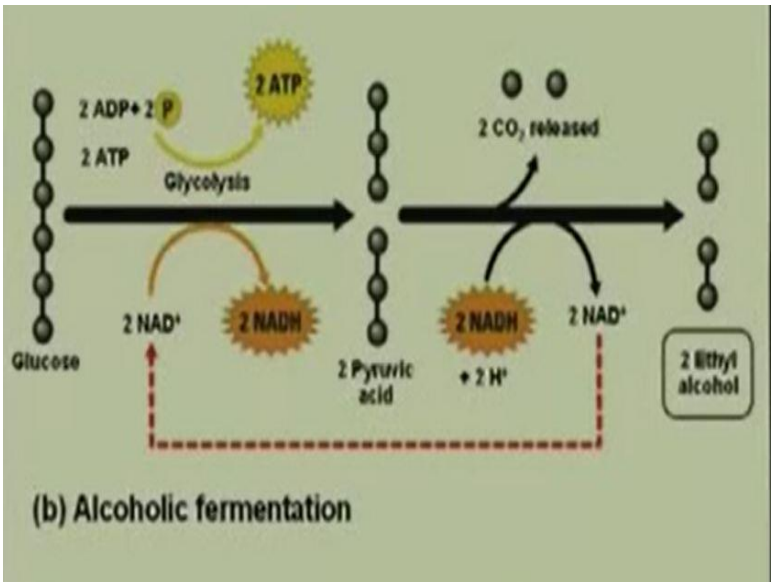

We also have alcohol formation that happens in yeast. In the fermentation where we alcoholic fermentation where the pyruvic acid so we have to look at the fate of pyruvic acid okay. So what is happening? Say in this case glucose is forming pyruvic acid but the pyruvic acid can get in to forming acetyl CoA that is going to be part of the tricarboxylic acid cycle.

Now this is also happening where you have lactic acid formation that usually is an anaerobic form which happens a lot in the muscles, the skeletal muscles. We also have this formed where we will have alcoholic fermentation in the formation of ethyl alcohol that is usually formed in yeast.

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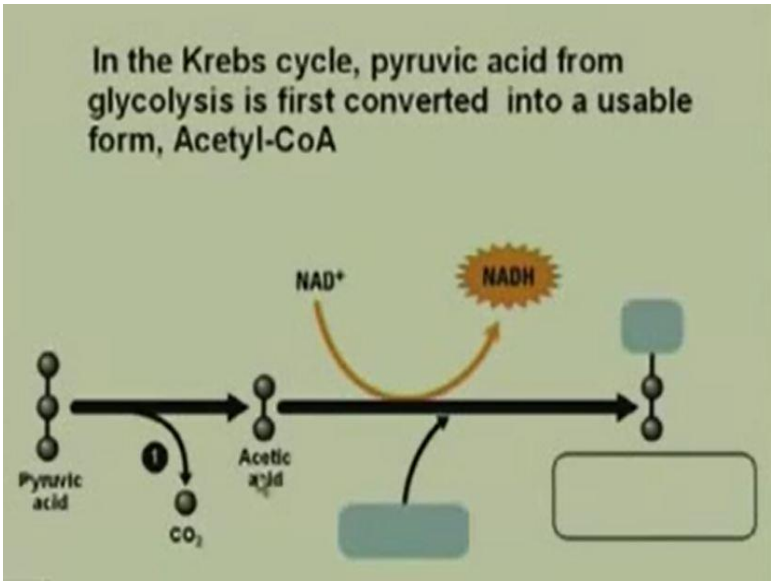

Now in the Krebs cycle, we have this NAD+ going to NADH so the pyruvic acid in this case forms acetate. This acetate then forms acetyl CoA and the acetyl CoA then from the coenzyme A will ultimately give us acetyl CoA. This acetyl CoA is going where to the Krebs cycle. So this is where we have our Krebs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

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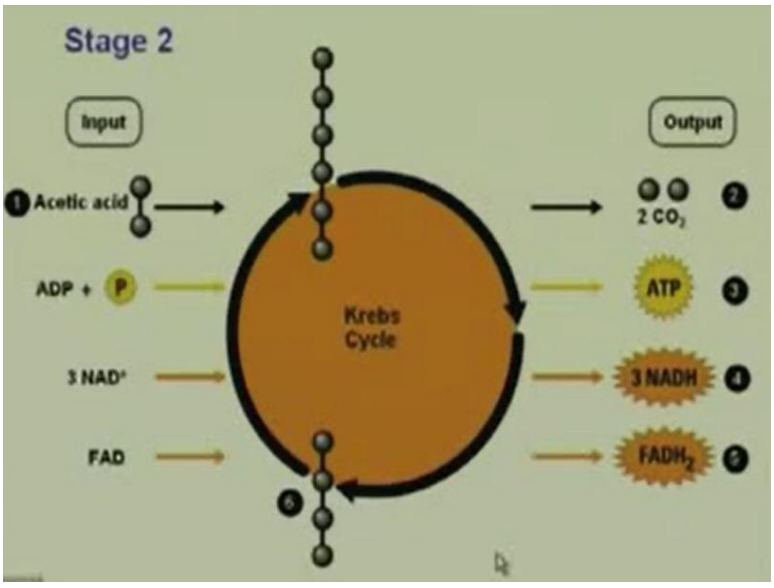

Where our input is going to be acetic acid and then have phosphate come in. We have ATP plus phosphate three NAD+ and one FAD and the output is two Co2, two are formed in two steps. We have ATP being formed three NADH being formed one FADH2 being formed. So now we have six carbons where does this come from now.

Oxaloacetate provided four, Acetyl provided two okay then we lost Co2 along the cycle and we came back to four so that is our Krebs cycle.

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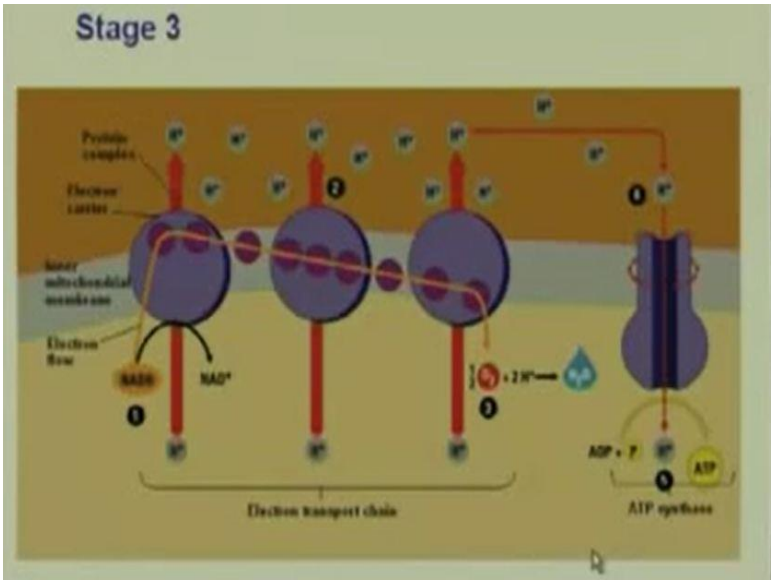

Then in stage three it is just our NADH and all the electron transport chain finally forming ATP in our oxidative phosphorylation steps which we have done before. So now we have to look at our balance sheet.

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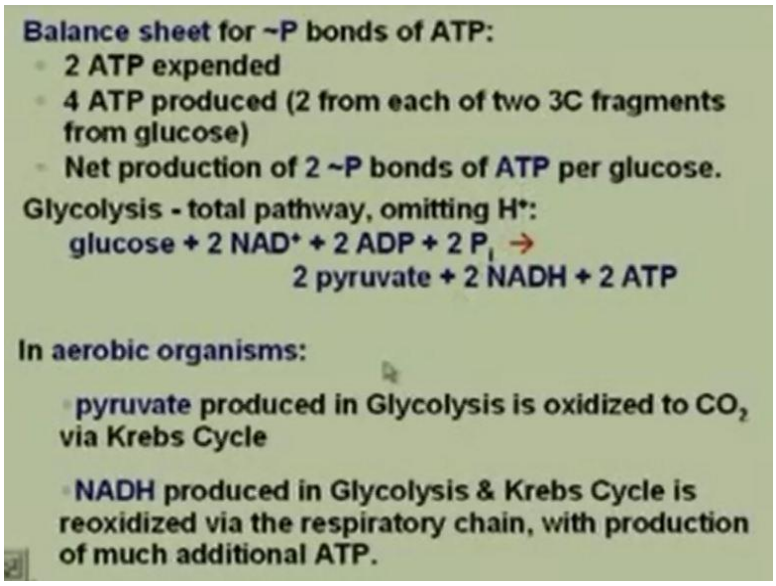

This is what we did last time. Glycolysis we have two ATP that are used up four ATP produced. Why? Because we have two three-carbon fragments each of them are producing two so we have four ATP produced. So eventually we have a net production of two ATP per glucose. This is what I showed you last time.

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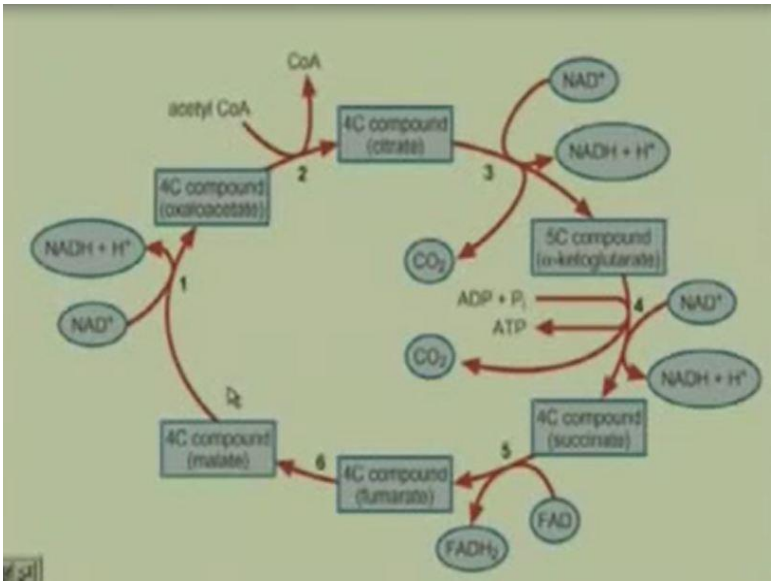

So this is basically now our balance sheet.

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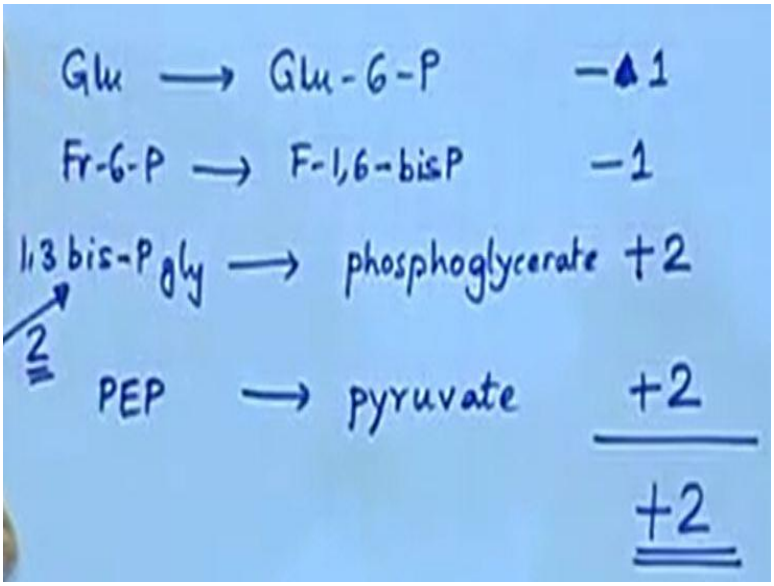

So we have in our first steps, we have glucose going to glucose six phosphate. What did that do? It took away an ATP. If we talk in terms of ATP it is -1 fine. What was our next step? Fructose six phosphate to Fructose 1, 6 Bisphosphate that also took away another one fine. Then we have, this is all the ATP we are considering now.

Then we will get back to our then we have that is the one three bisphosphoglycerate. One three bisphosphoglycerate what did that form? That forms the phosphoglycerate. Phosphoglycerate and what happen there? It was plus two actually because we have two of the three-carbons now fine. Then from the phosphoglycerate we form the phosphoenolpyruvate.

The phosphoenolpyruvate gave you the pyruvate. So we have the phosphoenolpyruvate give you the pyruvate plus two. So eventually we have plus two that is the amount of ATP from glycolysis that is what we figure out. Now when we have two pyruvates because we have to remember that one glucose is giving us two pyruvates we cannot forget that.

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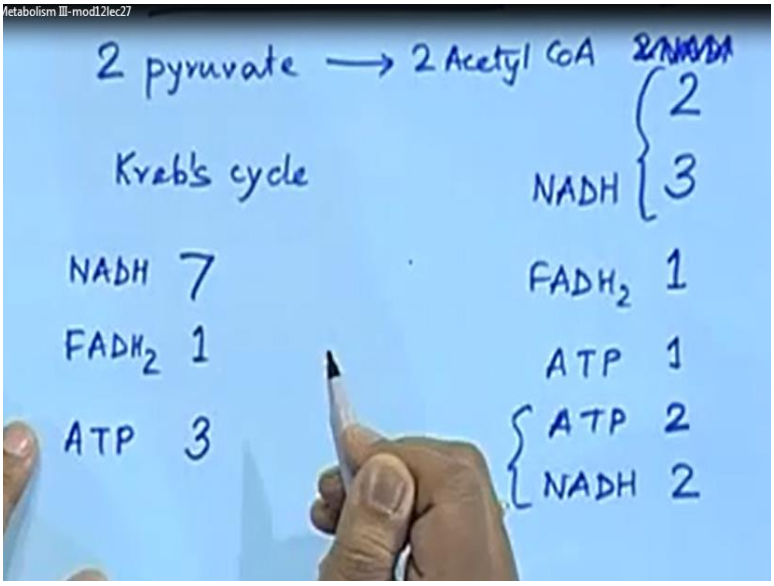

Two pyruvate to two acetyl CoA, In that step we have two NADH okay. In this step remember when we have pyruvate go to acetyl CoA in the complex. What happen to NAD+? The last step in E3 where FADH2 had formed we had NAD+ go to NADH2 remember. So let us keep track of our NADH now.

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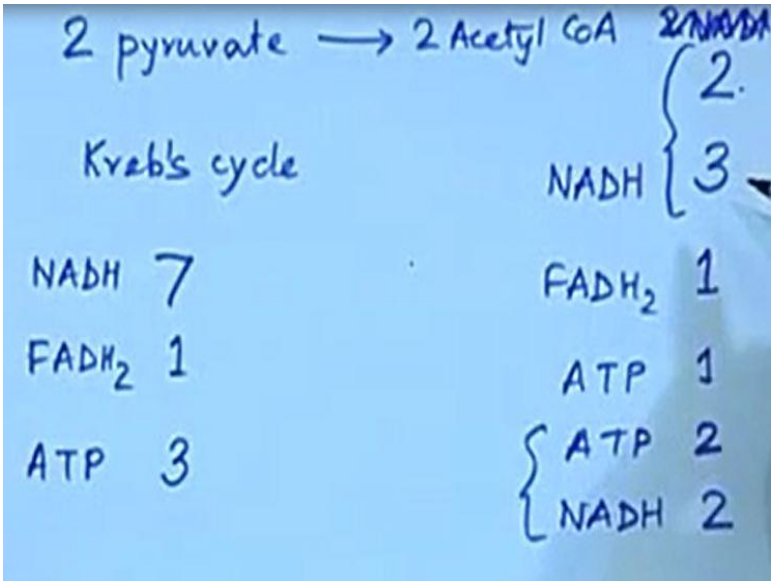

So NADH and FADH2 before we enter the Krebs cycle we have the two pyruvate two pyruvate going to two acetyl CoA because remember two pyruvate comes from the glucose. So how many NADH does that mean? Two NADH. So let us write NADH here because all are NADH except one. So we have two right. Then we have in our other steps we consider how many NADH do we finally get in the Krebs cycle? I am not right writing the steps. The Krebs cycle or the TCA

cycle gives us three NADH. Then FADH2 we have one FADH2 so this is actually we should have probably 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? Why? There is one step that provided you exactly you get two NADH it was the glyceraldehyde-3-phosphate going to the bisphosphoglycerate. So we got ATP from glycolysis two and NADH from glycolysis two. You just have to look at the series of steps okay.

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 one 1.5 ATP. So calculate how many ATP you are supposed to get from this information? Tell me. So NADH I have five. NADH actually I have five and two so seven. FADH2 I have one. ATP I have three. Pyruvate to acetyl CoA we have two, Krebs cycle we have three but we are missing out something here.

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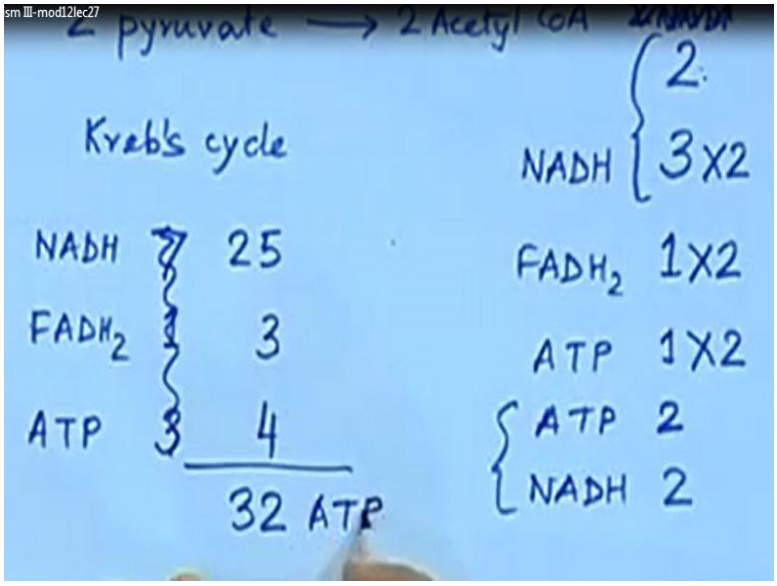

This has to be multiplied by two. This has to be multiplied by two and this has to be multiplied by two. Why? They are two each. So how many do you have. So we have two eight and two ten so ten in to 2.5 is 25, two in to 1.5 and four ATP. How many? 32. So I have 32 what? ATP. How much energy is that? 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.

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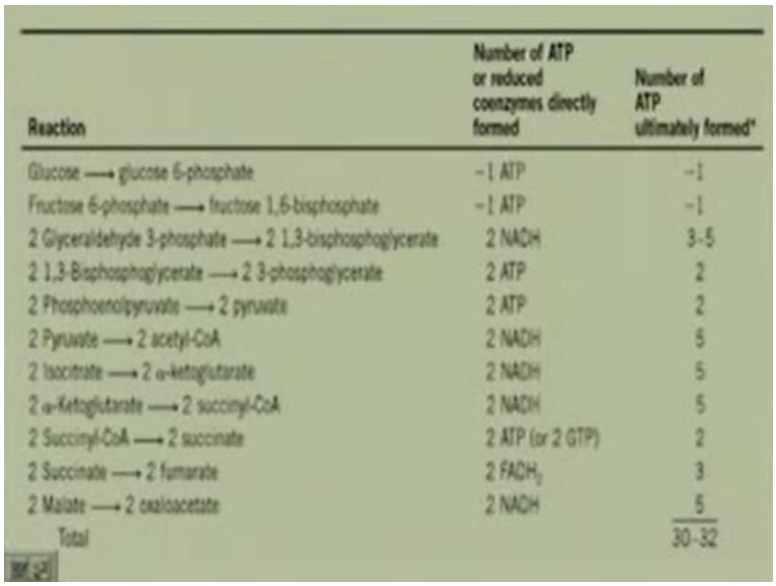

Reaction	Number of ATP or reduced coenzymes directly formed	Number of ATP ultimately formed*
Glucose ⟶ glucose 6-phosphate	−1 ATP	−1
Fructose 6-phosphate ⟶ fructose 1,6-bisphosphate	−1 ATP	−1
2 Glyceraldehyde 3-phosphate ⟶ 2 1,3-bisphosphoglycerate	2 NADH	3-5
2 1,3-Bisphosphoglycerate ⟶ 2 3-phosphoglycerate	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

Then this is actually the whole balance sheet okay. We have the number of ATP are 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 consider 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 three to five but 32 ATP is fine okay.

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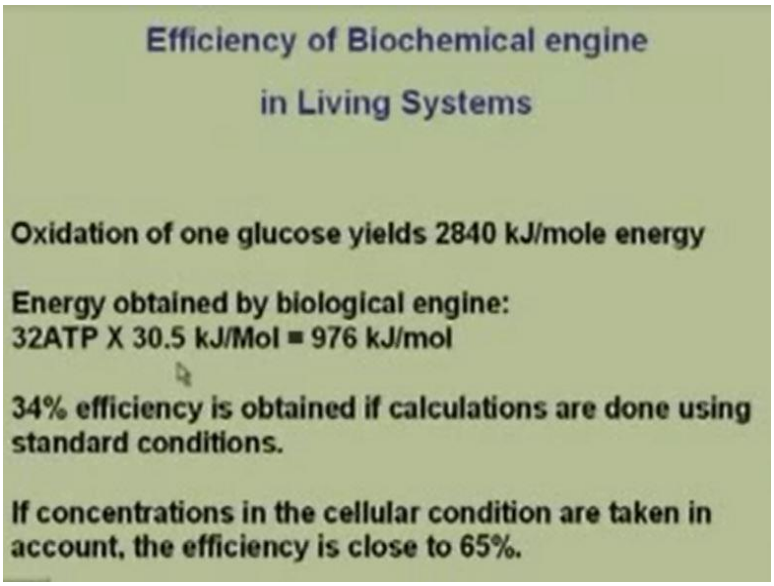

Okay so if we consider the efficiency of the biochemical engine in the living systems. The oxidation of one glucose yields 2840 kilojoules per mole of energy. That is a huge amount of energy and the energy obtained by this biological engine is 32 ATP in to 30.5 kilojoules per

mole. That is the ATP hydrolysis remember? 31 kilojoules. So that is the amount of energy we can get for the 32 ATP 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 consider at stage 1, stage 2, stage 3 ultimately leads to the production of 32 ATP okay. 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 percent efficiency if we consider the calculations done in standard conditions.

Standard conditions meaning that we would have 25 degrees 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 degrees centigrade we will get an efficiency of 65 percent okay. So from all the enzymatic reaction 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 store in the liver. Glucose going in to pyruvate, pyruvate forming acetyl CoA, acetyl CoA getting in to the Krebs cycle producing NADH and FADH2. That is then going to go in to the oxidative phosphorylation and the whole electron transport system where complex is one through four is going to utilize these cofactors in to creating the proton pump that is going pump protons in to the inter-membrane space in the mitochondria ultimately leading to the production ATP.

So we found out that this is the amount of energy that we can get and this completes our discussion on the breakdown of glucose to carbon dioxide and water so that will complete our metabolism and basically the essence of what we were supposed to do for this course. Thank you.