Biochemical Engineering

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Module No. # 01

Lecture No. # 19

TCA Cycle

Good morning students in my last class I have mentioned about the glycolytic pathway. Now, in that pathway, I have mentioned that, how glucose is converted to pyruvic acid and that process I have already mentioned that; that process mainly takes place in the cytoplasmic fluid of the cell and I have also also mentioned that, one molecule of glucose is producing two molecules of pyruvic acid and pyruvate is the end product of the glycolytic pathway.

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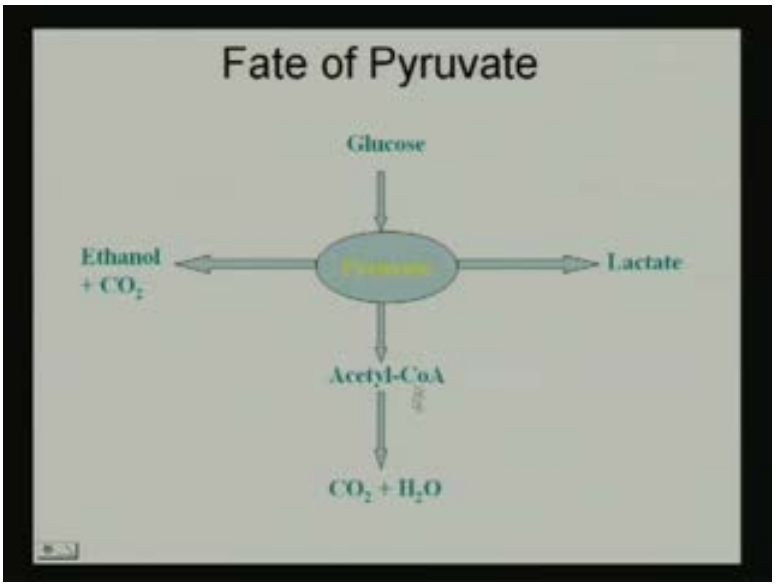

Now, if we see this picture, then we can find that, this is the glycolytic pathway and pyruvate is the end product. Now, these particular reactions are taking place in the

cytoplasmic fluid of the cell and now this is the end product, now this product is to be utilised in many many purposes.

Now, under an aerobic condition this pyruvate may get converted to ethanol and carbon dioxide, it may convert it to lactate and this pyruvic acid, when it further going for this respiratory chain and entering to the respiratory chain from the cytoplasmic fluid, that it is converted to acetyl CoA and this acetyl CoA is being utilised for respiratory process. And for this conversion of pyruvate to acetyl CoA, a molecule of carbon dioxide is being released.

Now, when it is entering to the respiratory chain, it is this particular process is taking place in the mitochondria of the cell. Now, it has to be transported from cytoplasm to these mitochondria.

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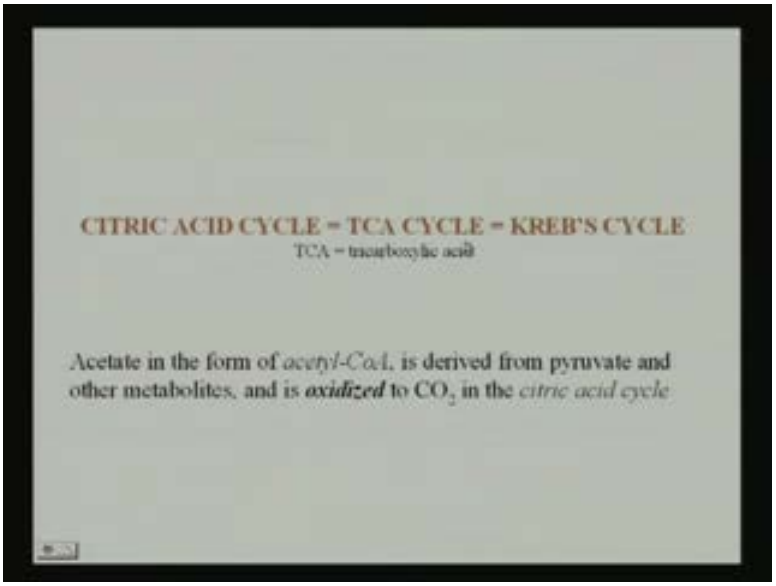

And the first product being 6 carbon compound citric acid, this particular cycle is otherwise known as citric acid cycle or the tricarboxylic acid cycle and as this particular this say this cycle has been discovered by the scientist Krebs, this particular cycle is also known as Krebs cycle. Now, whatever may be the name whether it is citric citric acid cycle or TCA cycle or Krebs cycle it matters that, that end product of glycolytic process that is the pyruvic acid is converted to acetyl CoA and then, this acetyl CoA is participating in this particular reaction.

Now, this acetate in the form of acetyl CoA is derived from pyruvic acid and other metabolites and is oxidized to carbon dioxide in the citric acid cycle.

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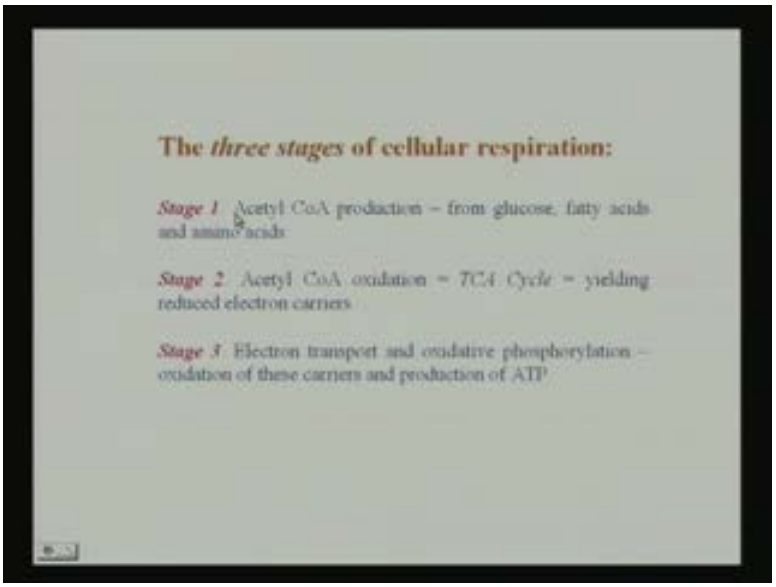

Now, the three stages of cellular respirations are: this acetyl CoA production that is from glucose, fatty acid and amino acids. So, this acetyl CoA is playing an important role in the respiratory chain of living cell. Stage 2, acetyl CoA is being oxidized and it starts that and it start the reactions for TCA cycle yielding the reduced electron carrier, now when this electron carriers are being produced, when the cycle get complete gets completes complete then, it enters to the electron transport chain and oxidative phosphorylation; that means, how this chain is getting completed; first glucose to pyruvate, not only this glucose the source is from glucose to pyruvate from beta acid oxidation also, acetyl CoA can be formed, it can also form from amino acids.

So, whatever may be the source, this acetyl CoA production is also another important factor. Then, acetyl CoA is getting oxidised and then, it starts the TCA cycle and when this TCA cycle is completed, then electron transport chain and oxidative phosphorylation starts and its it ends with the this production of ATP and that way it completes the respiratory process.

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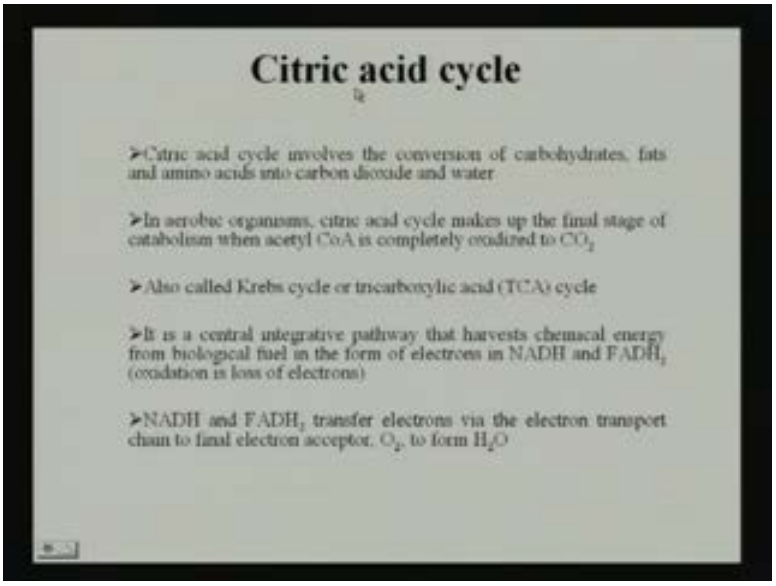

So, this citric acid cycle at a glance if we see, the citric acid cycle involves the conversion of carbohydrate, fats and amino acid into the into carbon dioxide and water. In aerobic organisms, citric acid cycle makes up the final stage of catabolic catabolism, when acetyl CoA is completely oxidized to carbon dioxide. This is also known as this Krebs cycle or TCA cycle as I have already mentioned. It is a central integrated pathway that harvests the chemical energy from the biological fuel in the form of electrons in NADH and FADH 2. NADH and FADH 2 then, transfer the electron via the electron transport chain to the final electron acceptor that is oxygen to form the water and here the total respiratory cellular respiration is getting completed.

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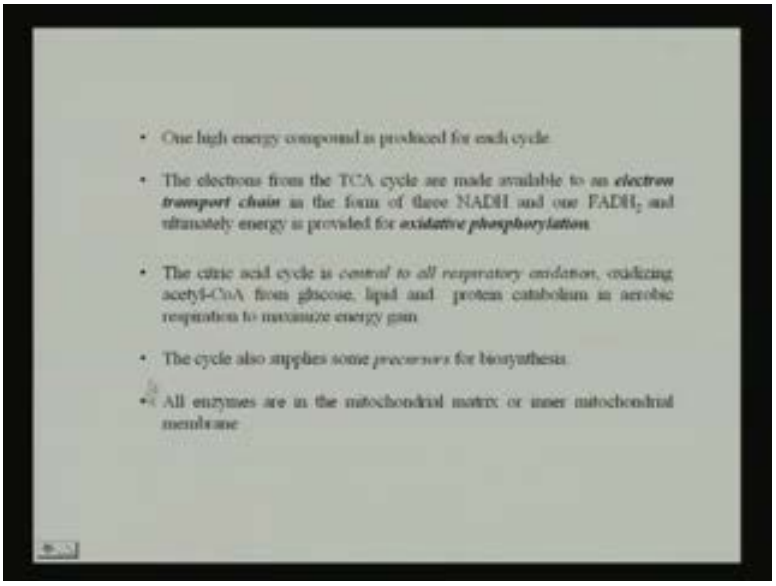

One high energy compound is produced for each cycle of citric acid or TCA. The electron from TCA cycle are made available to the electron transport chain in the form of three NADH and one FADH 2 and ultimately the energy is provided for oxidative phosphorylation, now when we will be completing TCA cycle we will just see that, how and how many molecules of NADH and FADH 2 are produced along with some by-products. The citric acid cycle is control to all respiratory oxidation, oxidizing acetyl CoA from glucose, lipid and protein catabolism in aerobic respiration to maximise the energy gain.

Now, when we will be completing this TCA cycle we will also see that, how enriched a particular cell is, when it completes this particular cycle. The cycle is supplied this this cycle also supplies some precursors for biosynthesis. All enzymes are in the mitochondrial matrix or inner mitochondrial membrane; that means, the glycolytic process though takes place in cytoplasmic feed, but in mitochondria this TCA cycle takes place.

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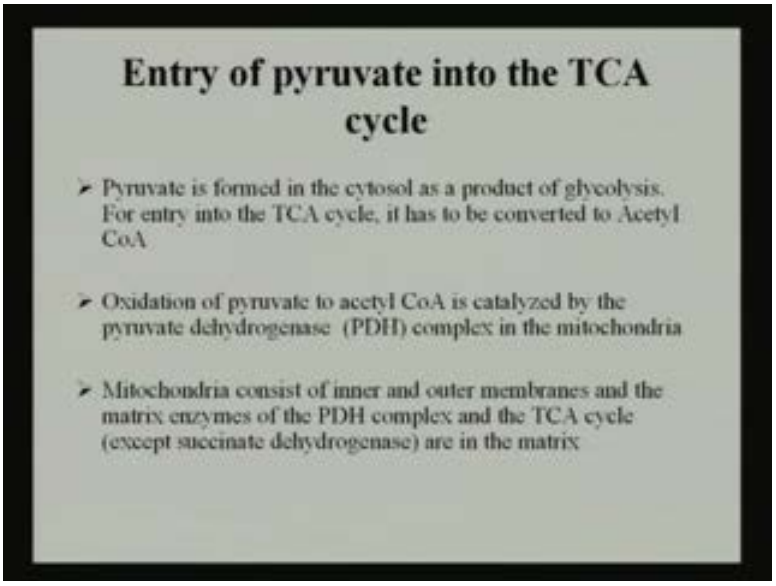

Now, it is very important to know the entry of pyruvate into the TCA cycle; pyruvate is formed in the cytoplasm and as a product of glycolysis. For entry to TCA cycle, it has to be converted to acetyl CoA. Oxidation of pyruvate to acetyl CoA is catalyzed by the pyruvate dehydrogenase enzyme complex which is present in the mitochondria of the cell. Mitochondria consist of inner and outer membrane also the matrix of these mitochondria, where most of the enzymes are present except this succinate dehydrogenase, which is present on the inner membrane of mitochondria.

Now, what we have learned that, pyruvate is to be converted to acetyl CoA and then, acetyl CoA is entering to these mitochondria and inside these mitochondria the pyruvate dehydrogenase enzyme is present. Now, we have to have this particular that enzyme what we are talking about is the pyruvate dehydrogenase and this particular enzyme is playing a very important role in conversion of pyruvate to acetyl CoA.

Now, if we see this pyruvate dehydrogenase enzyme, it is a very very complex enzyme. Now, if we see its structure we will find that, it has got 20 sided polyhedron structure and if we see actually the core moieties of this pyruvate dehydrogenase then, we can find that, the core of the this particular enzyme is made up of dihydrolipoyl transacetylase. So, this is the enzyme, it is the core enzyme of pyruvate dehydrogenase.

Now, here this enzyme has got 60 identical peptide chains. So, you can understand that, how complex these core moieties are and its molecular weight is approximately, 3.1

million dalton. So, you can you can understand that, how complex this particular molecule is, each contain covalently linked lipoic acid. So, this is a huge enzyme and it is obviously allosteric in nature, intra cellular and it plays the different various significant roles as far as the TCA cycle is concerned.

Now here, if we see this pyruvate dehydrogenase enzyme, it has got the core moieties, which is dihydrolipoyl transacetylase enzyme to which that, pyruvate dehydrogenase of 20 such molecules, this another enzyme pyruvate dehydrogenase has 20 such molecules, each molecule has the molecular weight of 1,54,000 dalton is attached to this, this is the core moieties to this core moieties that pyruvate dehydrogenase which of 20 such molecules are attached, each are having 1,54,000 dalton and to this particular moiety another enzyme called dihydrolipoyl dehydrogenase is also attached, which is 5 to 6 molecules of 1,10,000 dalton.

So, you can understand that, how complex this pyruvate dehydrogenase molecule is. So, this is the core moieties; that means, we can understand that, pyruvate dehydrogenase is present in the mitochondria, which mainly constitute, which mainly composed of 3 main enzymes, the core being that dihydrolipoyl transacetylase, where 60 identical peptide chains are present in these core moieties to which 20 molecules of pyruvate dehydrogenase is also attached to this and 5 to 6 molecules of dihydrolipoyl dehydrogenase enzyme is attached to this.

Now here, this is all about this core structure, but besides this to this pyruvate dehydrogenase enzyme 5 to 6 molecules of pyruvate dehydrogenase kinase is also attached of which are of molecular weight of 62,000 dalton and the 7 molecules of pyruvate dehydrogenase phosphatase enzyme is present of molecule molecular weight of 1,00,000 dalton.

So, you can understand that, though it has got 3 particular enzyme and these 3 enzymes has got direct role in conversion of pyruvate to acetyl CoA, but this last 2 enzymes, pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase; this 2 enzymes are playing a significant role in the regulatory process of TCA cycle. Now, we will come to this particular complex structure and we will also see the role of these 2 enzymes as far as the regulation of the TCA cycle is concerned.

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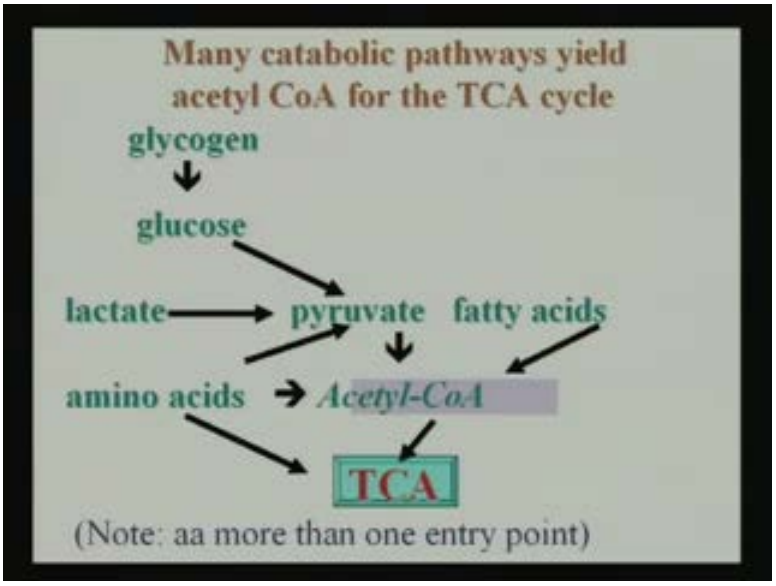

Now, as I have already mention that, this pyruvic acid can be produced from glycogen to glucose to pyruvate, lactate to pyruvate, fatty acid and this pyruvate is amino acid to pyruvate and this pyruvate is getting converted to acetyl CoA, fatty acid can also give acetyl CoA and this acetyl CoA is entering to the TCA cycle.

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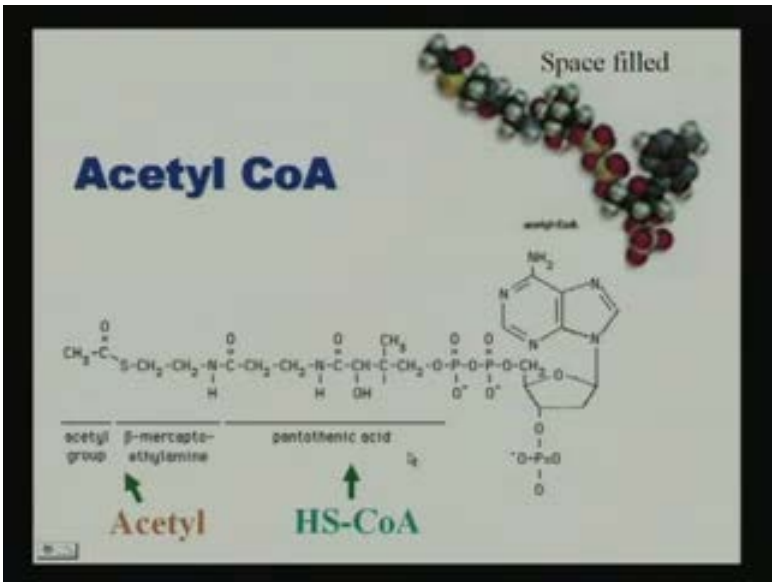

Now, acetyl CoA if we see its structure it has got 1 acetyl group and to which the CoA structure is attached to this. So, this is the structure of acetyl CoA.

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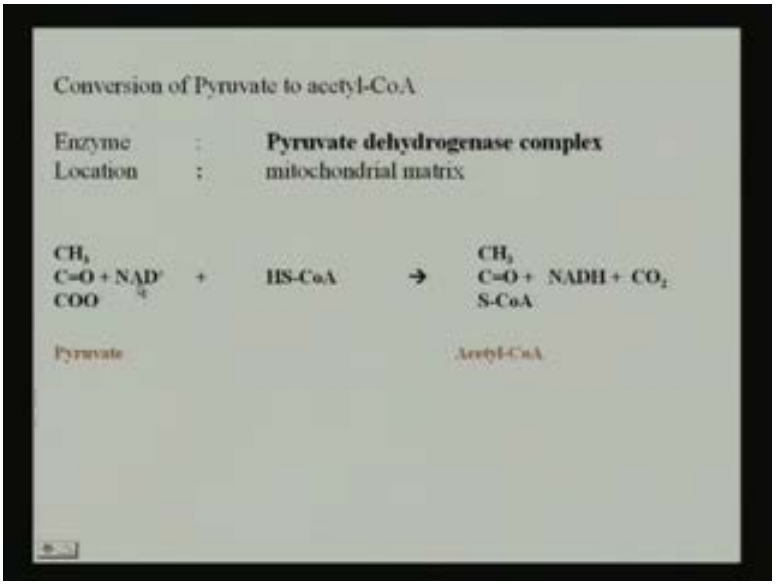

Now, if we see the conversion of pyruvate to acetyl CoA, then we can find as we have already learned that, pyruvate dehydrogenase complex is playing a significant role. So, here we can see that, this pyruvate in presence of this NAD and coenzyme A is producing acetyl CoA, NADH and carbon dioxide. Now, as we have learned that, 1 molecule of glucose is giving 2 molecules of pyruvate; this 2 molecules of pyruvate when it is getting converted we are getting 2 molecules of acetyl CoA, 2 molecules of NADH and 2 molecules of carbon dioxide.

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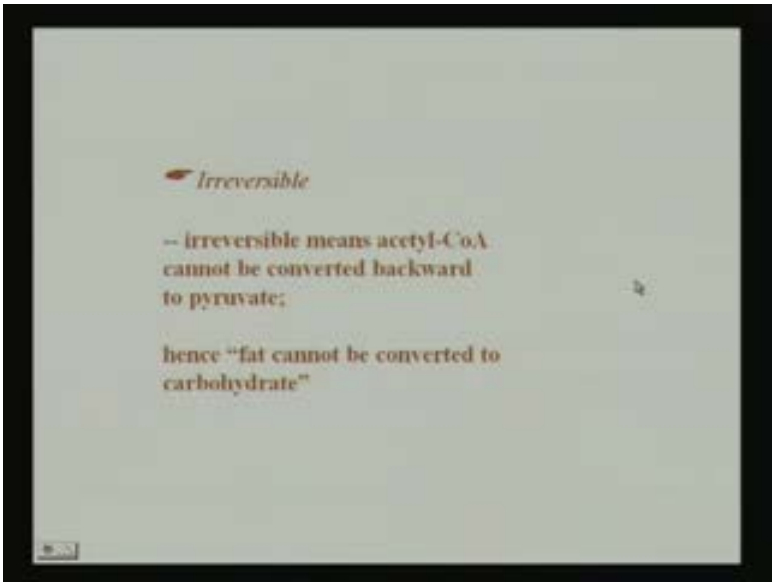

Now, this is an irreversible process it means acetyl CoA cannot be converted back to pyruvate; hence, fat cannot be converted to carbohydrate.

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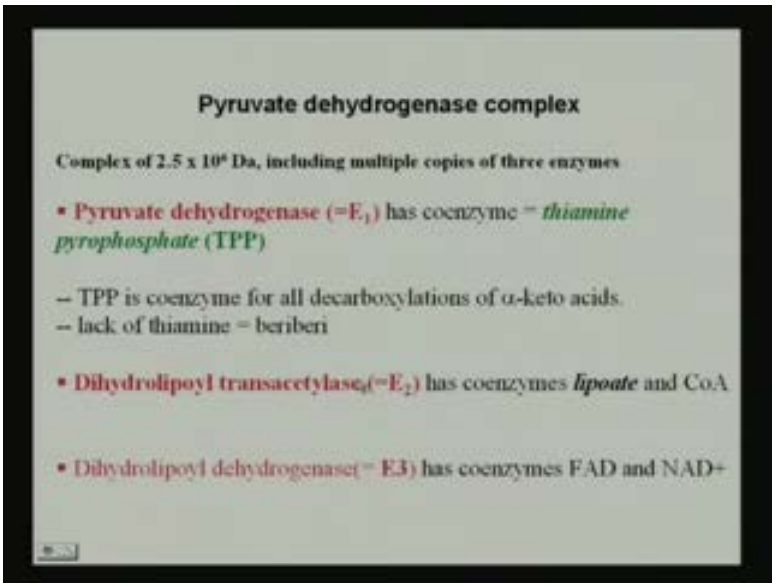

Now, if we see the mechanism of action of pyruvate dehydrogenase complex, then we can find that, this pyruvate dehydrogenase if we are symbolising it as E 1; it has it is playing a very significant role dihydrolipoyl transacetylase, if we are considering this as E 2 and dihydrolipoyl dehydrogenase, if we are symbolising as E 3.

We will find that, this main core what I have already discussed with you that, pyruvate dehydrogenase core moieties then we can find that, this E 1, E 2 and E 3 are the three component, which are participating in this reaction. Besides that, there there are 5 coenzymes, which are actively involved in this reaction and those coenzymes are thiamine pyrophosphate, which is designated as TPP, lipoate coenzyme A, FAD and NAD; these are the 5 coenzymes, which are involved in conversion of pyruvate to acetyl CoA.

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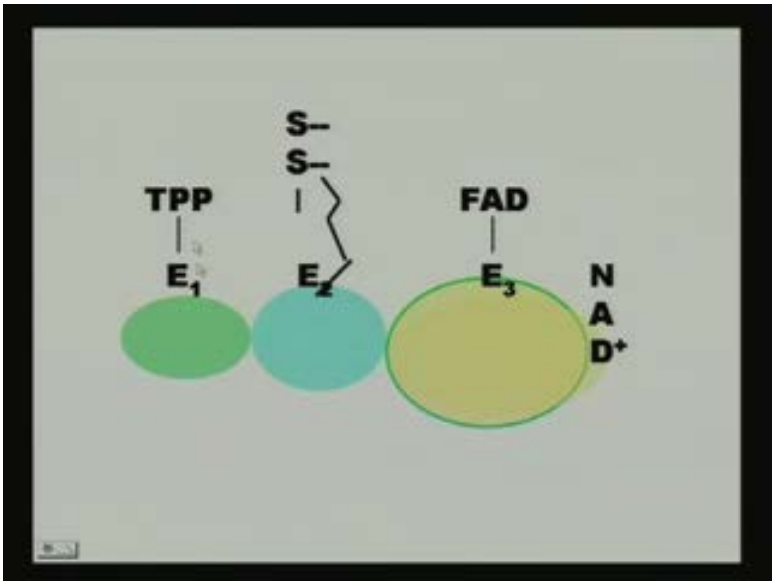

Now, if I am showing you this these are the 3 moieties E 1, E 2 and E 3; E 1 is pyruvate dehydrogenase, E 2 is dihydrolipoyl transacetylase and E 3 is dihydrolipoyl dehydrogenase; these 3 enzymes are participating with the 5 coenzymes and they are converted converting pyruvate to acetyl CoA.

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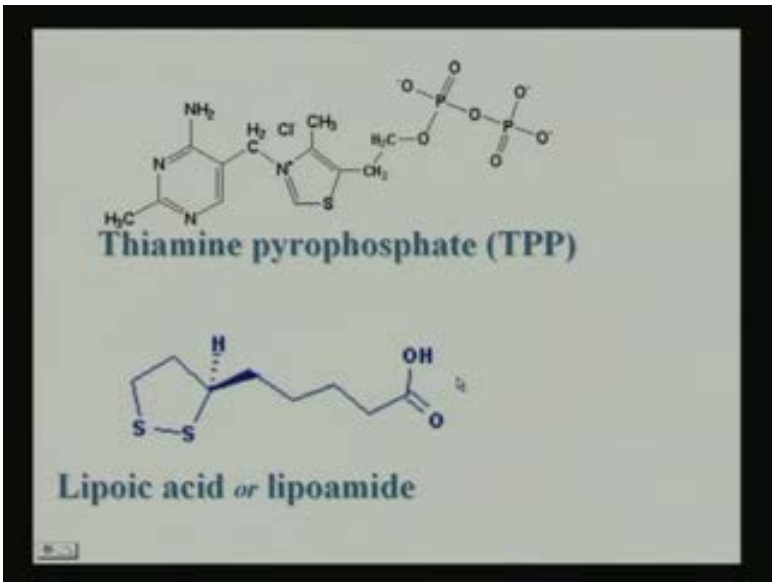

This is the structure of this TPP, this is the structure of lipoic acid, here this sulphur bonds are there.

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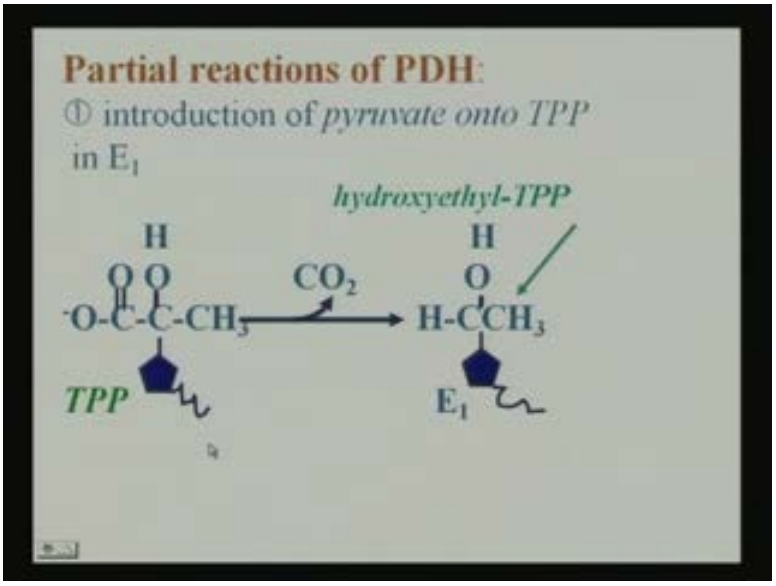

And when they are coming to this, first reaction pyruvate dehydrogenase, this introduction of pyruvate onto TPP with the enzyme E 1 is very significant. Now, this TPP content, this TPP is the cofactor and this enzyme, when it is the enzyme pyruvate dehydrogenase, when it is attached to this TPP; it release 1 molecule of carbon dioxide and hydroxyethyl TPP complex is formed.

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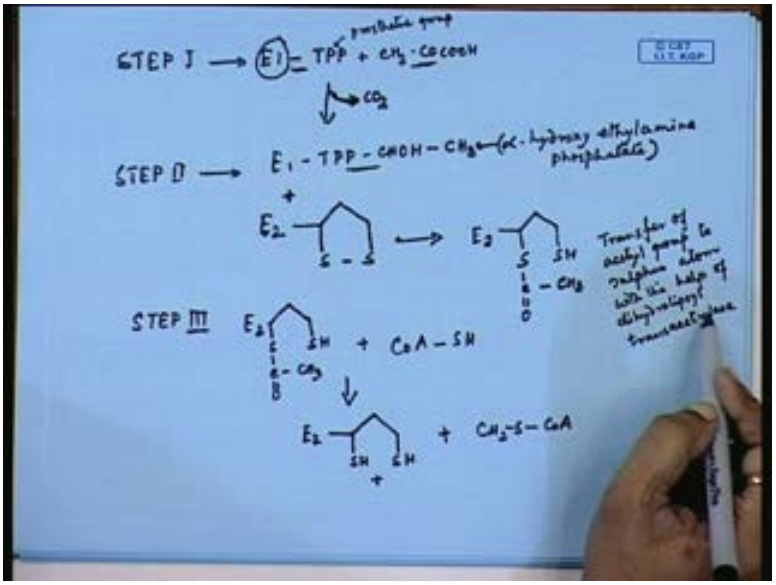

Now, if we see this reaction this TPP is this is the first reaction; E 1 is the enzyme to which this TPP is attached as the prosthetic group and pyruvate is coming in contact with

these complex and 1 molecule of carbon dioxide is released from this reaction and as a result, we are getting alpha hydroxy ethylamine phosphate, so this is the end product.

Now, when this first reaction is over then, coming to the second step, what we are getting the the end product of the first reaction is coming in contact with the second enzyme, E 2 along with the lipoyl group. And here this particular enzyme dihydrolipoyl transacetylase this enzyme is forming it is transferring the that transfer of acetyl group to the sulphur atom is taking taking place with the help of dihydrolipoyl transacetylase enzyme and when this particular reaction is going on then, we are getting the end product, this is the end product of this reaction.

And we are getting this the reaction this product, where coenzyme S H is coming in contact with this and when this coenzyme S H is coming; it the acetyl group is enzymatically transferred from this lipoyl group of dihydrolipoic acetic acid to the (( )) group of coenzyme A and this way the transfer of this this acetyl group is taking place and this enzyme is participating in this role.

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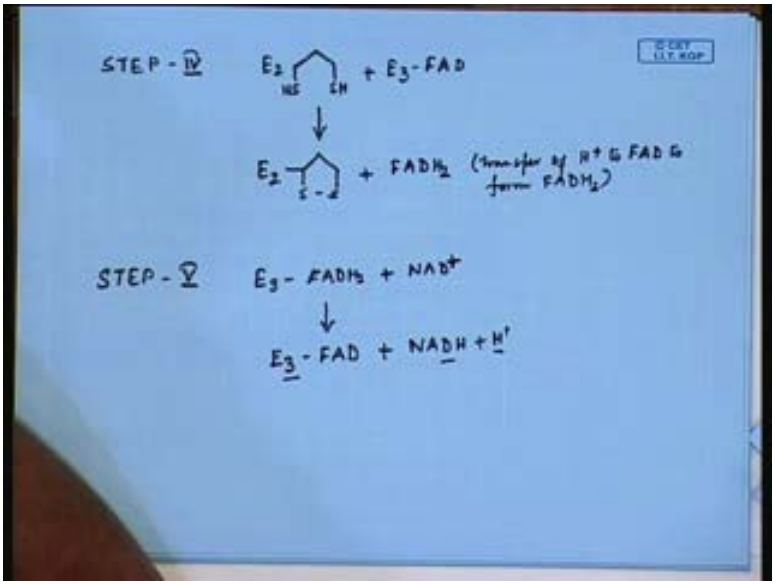

When this product is formed then, the E 3 enzyme which has 1 FAD as a cofactor is (( )) and playing a significant role, where transfer of hydrogen from FAD to FADH 2 is taking place and it forms it forms this particular E 3 FAD complex and this E 3 FAD complex in presence of this E 3 FADH 2, when it is just reacting it forms this in in

presence of another cofactor NAD; it forms E 3 FAD and NADH and H and with the release of acetyl CoA.

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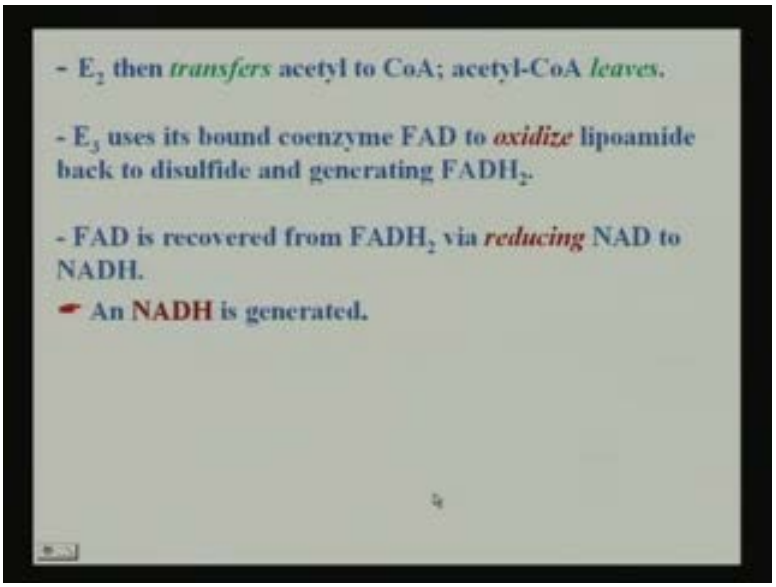

And in this way we are getting this acetyl CoA in this particular reaction. This E 2 is then transfer this acetyl to CoA and form acetyl CoA, which is the product which leaves from this complex and this acetyl CoA as I have told you this product; and this E 3 uses the bound coenzyme FAD to oxidize lipoamide back to the disulfide and and generating FADH 2. FADH 2 is recovered from FAD is recovered from FADH 2 via reducing NAD to NADH and NADH is generated as a by product in with the completion of the reaction; that means, here this coenzyme A is produced and here we have seen that, NADH is also produced after the completion of this particular cycle.

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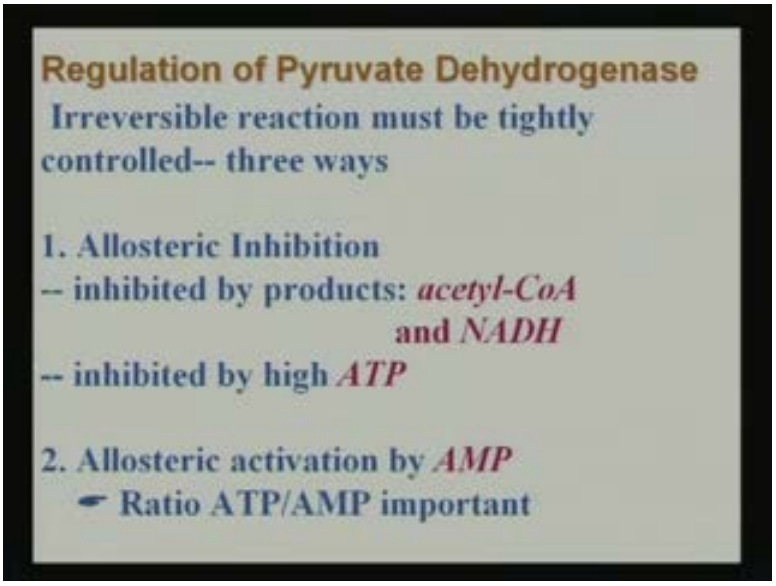

Now, this particular reaction is regulated and if we see the regulation of pyruvate dehydrogenase then we can find that, it is irreversible reaction must tightly be controlled and this control is taken place by three ways; allosteric inhibition; that means, it is inhibited by the end product of acetyl CoA and NADH, so what is there that being the allosteric enzyme the switching on and switching off process is there, if the concentration is very high acetyl CoA and NADH, it switch off the production of acetyl CoA and NADH, it is also inhibited by ATP.

The allosteric activation of AMP is also there; that means, the ratio of ATP and AMP is also playing a significant role as far as this regulation of pyruvate dehydrogenase is there; that means, when this ATP concentration is very less than AMP is boosting; it is the stimulating the reaction and reaction is taking place.

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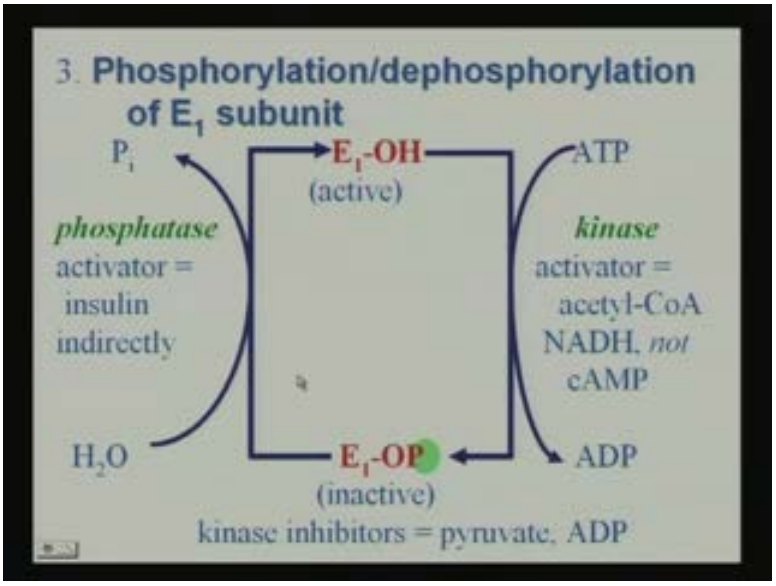

And the third way of controlling this is the phosphorylation and dephosphorylation of the E 1 that first moieties, that E 1 subunit of pyruvate dehydrogenase. Now, what is happening inside this cell this E 1, when it is phosphorylated, this phosphate is getting attached it becomes inactive and when d with the help of the enzyme, phosphatase is taking place.

Then it becomes E 1 hydroxyl group is there and it it is active and it converts this process and ATP is being utilised and ADP is produced. So, when in the cell ADP concentration is very high then what is happening? The when ADP concentration is very high, pyruvate is very high, it inhibits the this particular enzyme and total reaction is getting stopped and this is the switching on, switching off process which is controlled by this allosteric function of this enzyme.

Now, this kinase enzyme is activating here that is the transfer of phosphate group, that kinase mainly transferring the phosphate group of this ATP triphosphate and it becomes diphosphate and this enzyme is phosphorylated and when it is dephosphorylated then, it is once again active and in this way the active and inactive forms are are going on inside the cell depending upon the need and requirement of this particular product in the cell.

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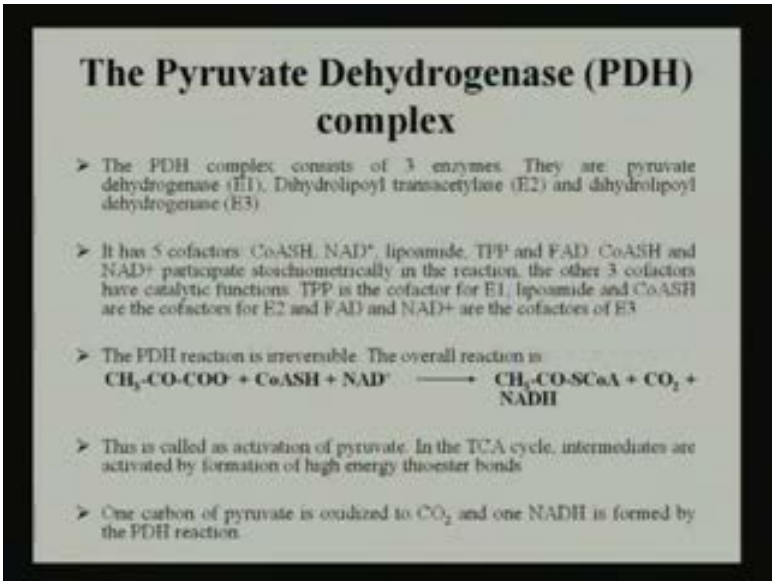

Now, if we sum up this pyruvate dehydrogenase complex then, we can till this particular thing what I have already mentioned as this pyruvate dehydrogenase complex consists of 3 enzymes that is pyruvate dehydrogenase E 1, dihydrolipoyl transacetylase E 2 and dihydrolipoyl dehydrogenase that is E 3.

It has got 5 cofactor coenzyme ASH, NAD, lipoamide, TPP and FAD; coenzyme ASH and NAD participate in the stoichiometrically in the reaction in the reaction and the other 3 cofactors have catalytic function of the particular enzymes concerned. TPP is the cofactor for E 1, lipoamide and coenzyme S H are the cofactors for E 2 and FAD and NAD are the cofactor for the sub moieties of E 3.

The pyruvate dehydrogenase reaction is irreversible and overall reaction is this pyruvic acid, coenzyme ASH and NAD gives rise to acetyl CoA, carbon dioxide and NADH this is called as activation of pyruvate in the TCA cycle, intermediates are activated by formation of high energy thioester bond. One carbon of pyruvate is oxidized to carbon dioxide and one NADH is formed by the end of this reaction.

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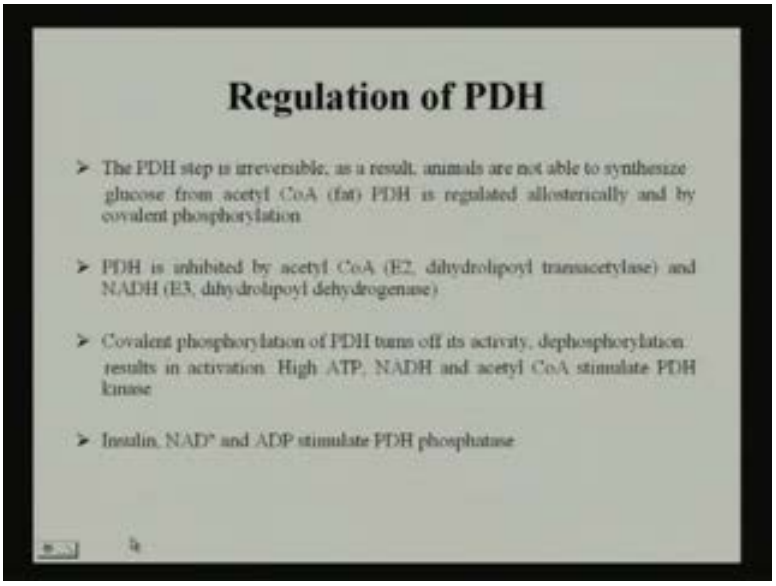

Now, if we see the regulation then, pyruvate dehydrogenase step is irreversible, as a result, animals are not able to synthesize glucose from acetyl CoA or fat, pyruvate dehydrogenase is regulated allosterically and by the covalent phosphorylation as we have already discuss this. Pyruvate dehydrogenase is inhibited by acetyl CoA that enzyme moieties dihydrolipoyl transacetylase and NADH that is E 3 moieties of dihydrolipoyl dehydrogenase being allosteric in nature.

Covalent phosphorylation of pyruvate dehydrogenase turns off its activity and dephosphorylation results in the activation high ATP, NADH and and acetyl CoA stimulate the pyruvate dehydrogenase kinase. (( )), NAD and ADP stimulate pyruvate dehydrogenase phosphatase to act.

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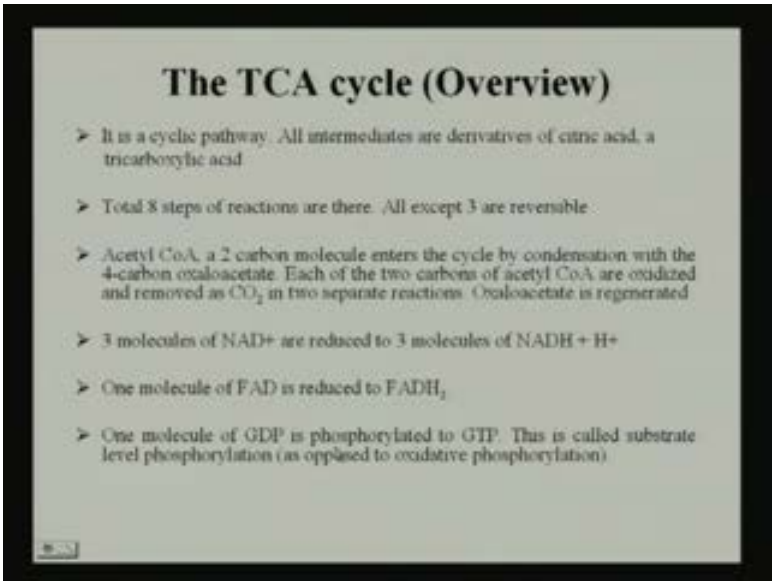

Now, if we see the TCA cycle and its overview and then, if we enter to the actual TCA cycle, then it will be a little bit easier easy it will be easy for understanding I will it is the cyclic pathway. All intermediates are derivative of citric acid, a tricarboxylic acid this is the 8 steps process; that means, glycolysis is the 10 steps process, but TCA cycle is the 8 steps process. All except 3 are reversible in nature. Acetyl CoA, the 2 carbon molecule enters the cycle by condensation with the 4 carbon oxaloacetate and each of these two carbons of acetyl CoA are oxidized and removed carbon dioxide in two separate reactions.

Oxaloacetate is regenerated and reused in this process. 3 molecules of NAD and 3 molecules of NADH and H plus are produced. One molecule of FAD is required and one molecule of FADH 2 is produced. One molecule of GDP is phosphorylated to one molecule of GTP, which is otherwise known as substrate level phosphorylation and in in case of glycolytic process I have also mention mentioned about this substrate level phosphorylation and here also we will see that, substrate level phosphorylation reactions.

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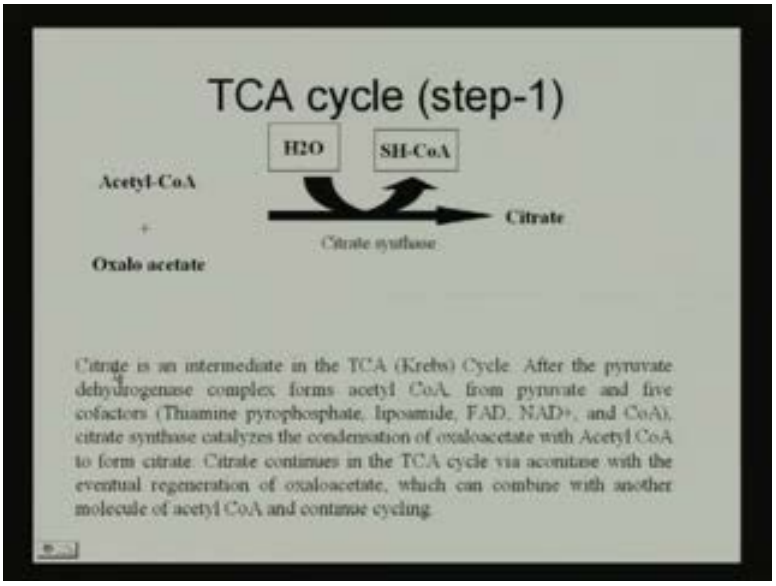

Now, coming to the actual steps, now acetyl CoA is formed now acetyl CoA is entered to the mitochondria; in the mitochondria, oxalo acetate is present, now inside this mitochondria this when this condensation reaction is going on in presence of the enzymes citrate synthase, then this synthesis of the this two different molecules; one is 2 carbon and another is 4 carbon is taking place resulting in the formation of citric acid 1 molecule of water is needed and coenzyme S H is coming out or as a by-product of the this reaction.

Now, citrate is an intermediate in TCA cycle. After the pyruvate dehydrogenase complex form acetyl CoA, from pyruvate and five cofactor thiamine pyrophosphate, TPP, lipoamide, FAD, NAD, coenzyme A, citrate synthase catalyzes the condensation of oxaloacetate with acetyl CoA to form citrate.

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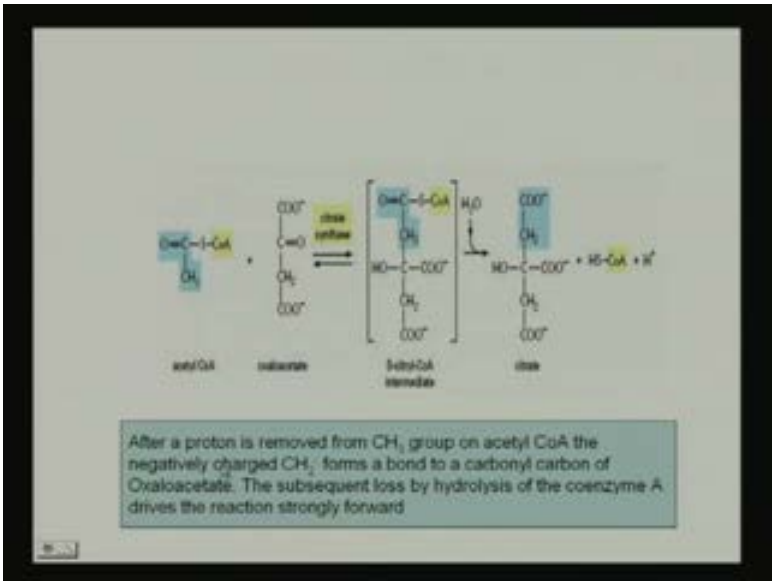

Now, here you see acetyl CoA and here oxaloacetate you see 1, 2, 3, 4 carbon moieties; oxaloacetate in presence of citrate synthase, it is producing some intermediate product called S-citryl-CoA intermediate and after a proton is removed from this C H 3 group of acetyl CoA the negatively charged CH 2; forms a bond to the carbonyl carbon of the oxaloacetate and the subsequent loss by the hydrolysis of the coenzyme A drives the reaction forward and it results in the formation of citrate.

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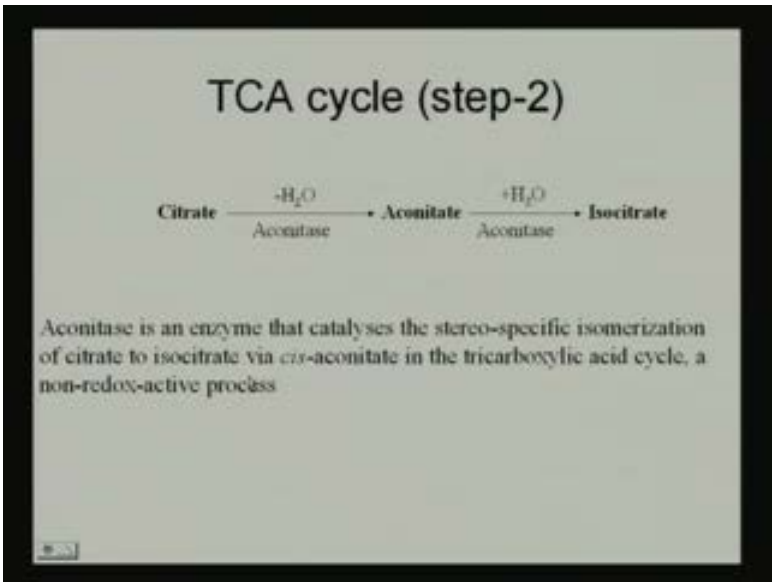

And this is the first step of citric acid cycle; in the second step what is there citrate is converted to aconitate and isocitrate. Now, acotinase is the enzyme that catalyses the stereo-specific isomerization reaction of citrate to isocitrate via cis-aconitate in the tricarboxylic acid cycle.

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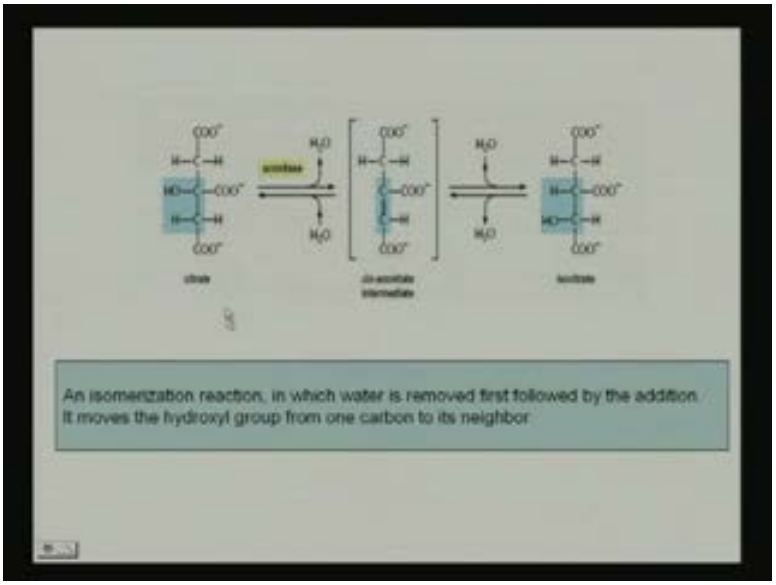

Now, if we see here, this is the isomerization reaction in which the water is removed first, 1 molecule of water is removed first followed by the addition of water; it moves the hydroxyl group, now this hydroxyl group which is there from one carbon to the another neighbouring carbon and it forms the product, which is called isocitrate.

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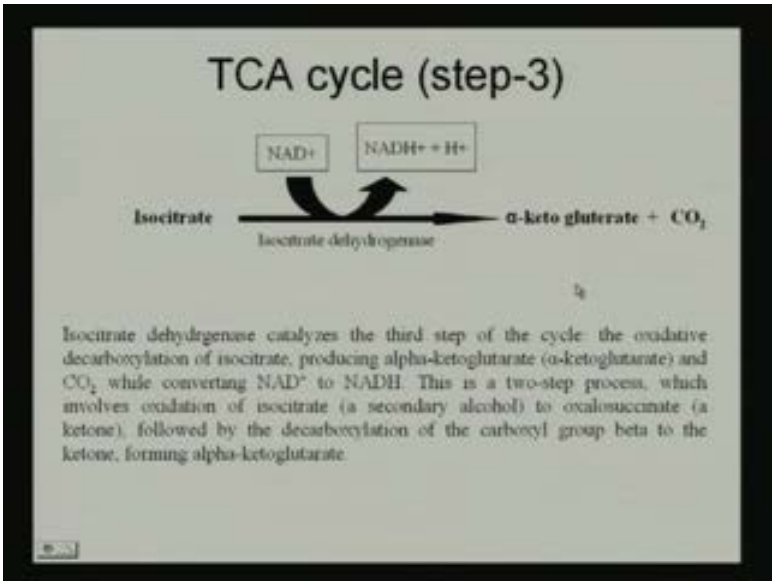

This is the second step; in the third step of TCA cycle isocitrate is converted to alpha keto gluterate.

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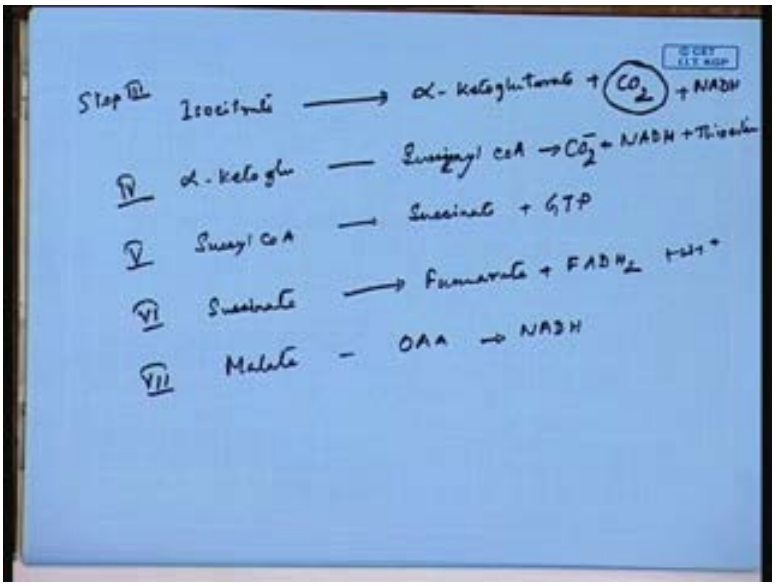

And here in this particular reaction, what we can see that, product is that isocitrate to alpha keto glutarate and it release 1 molecule of carbon dioxide, when 1 molecule of isocitrate is utilised it produce 1 molecule of carbon dioxide.

Here you see this isocitrate dehydrogenase enzyme is needed is actively participating in the catalytic activities and here 1 molecule of NAD is converting converted to NADH;

that means, we can also get 1 molecule of NADH in this particular reaction; that means, as a result what we are getting this is that, decarboxylation reaction is taking place that oxidative decarboxylation of isocitrate, producing alpha-ketoglutarate and carbon dioxide is being released in the this process, while NAD is getting converted to NADH and this is a two-step reaction, which involves the oxidation of isocitrate, a secondary alcohol to oxalo succinate, a ketone followed by the decarboxylation of carboxyl group beta to ketone, forming alpha-ketoglutarate.

So, this is the isocitrate here, isocitrate dehydrogenase NAD to NADH is produced oxalo succinate intermediate is there and here 1 molecule of carbon dioxide is being released and we are getting alpha ketoglutarate. The first of the four oxidation steps in this cycle the carbon carrying the hydroxyl group is converted to carbonyl group and immediate the immediate product is unstable, losing a molecule of carbon dioxide while still bound to the enzyme.

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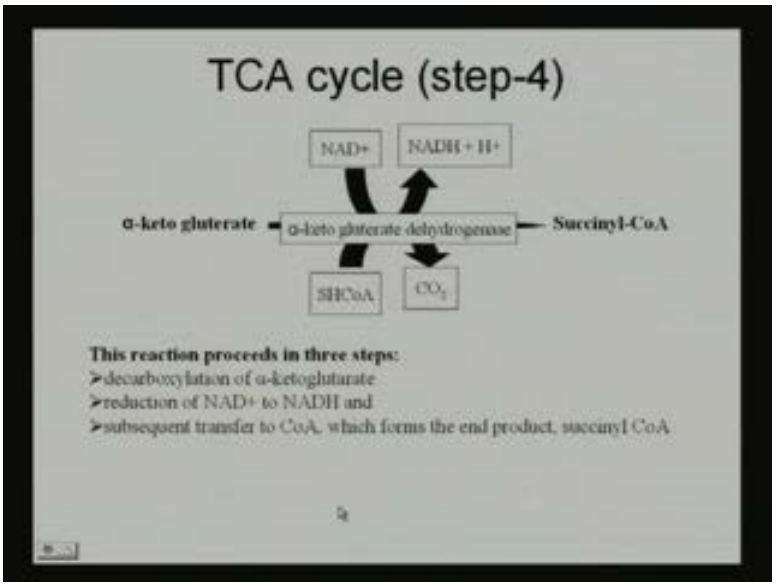

And this is the third step of TCA cycle. In the fourth step you see alpha ketoglutarate is converted to succinyl CoA. Now, this reaction is mainly takes place in three steps: now decarboxylation of alpha ketoglutarate, reduction of NAD to NADH and subsequent transfer of coenzyme A, which forms the end product of succinyl CoA.

Now; that means, here alpha ketoglutarate here, alpha ketoglutarate dehydrogenase inside is here, 1 molecule of NAD is also; that means, in step four that is alpha

ketoglutarate to succinyl CoA succinyl CoA here also we can find that, 1 molecule of carbon dioxide is released and 1 molecule of NADH is released at coenzyme S H is required to form succinyl CoA.

Now, if we see that, the reaction then we can find that, this is alpha ketoglutarate, this is a one, two, three, four and five carbon moieties here, when 1 molecule of carbon dioxide is going and coenzyme is coming and getting bound with this particular succinyl and form this succinyl CoA this is this becomes one, two, three, four carbon compound. So, this is the reaction the alpha ketoglutarate dehydrogenase complex closely resembles to the large complex that converts pyruvate to acetyl CoA. It catalyzes the oxidation that produces NADH, Co2 and high energy thioester bond to coenzyme A. So; that means, thioester bond is also produce with this, which is linked with this coenzyme A.

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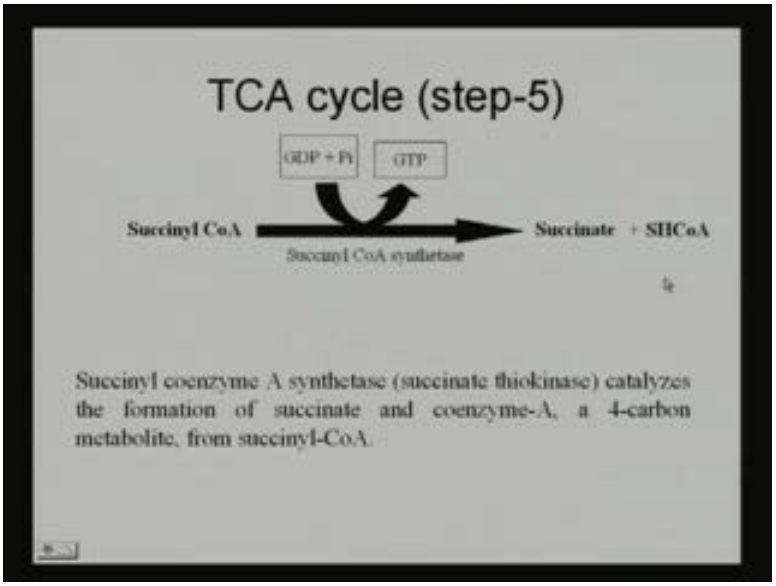

In an fifth step of this TCA cycle, succinyl CoA is converted to succinate and coenzyme A is coming out of this process and here in this process in step five, this succinyl CoA to succinate here; 1 molecule of GTP is produced and this GTP in in case of bacteria and plant; it is in the form of ATP. Now, succinyl CoA synthetase enzyme is releasing that coenzyme A and it forms this succinic acid.

Succinyl coenzyme a synthetase that, it is a thiokinase enzyme catalyze, catalyzes the formation of succinate and coenzyme A, 4 carbon metabolite that and form a coenzyme A, this is the 4 carbon compound and coenzyme A is getting released and this is succinic

acid is produced and here 1 molecule of GTP is is produced and this is otherwise GDP is converted to GTP in the presence of inorganic phosphate, 1 molecule of water is also needed to carry out this reaction.

A phosphate molecule from solution displace the coenzyme a forming a high-energy phosphate linked succinate and this is also the example of substrate level phosphorylation what I have already mentioned you in case of glycolytic process and this GTP which is there in the in animal system and it is form of ATP in case of bacteria and plant.

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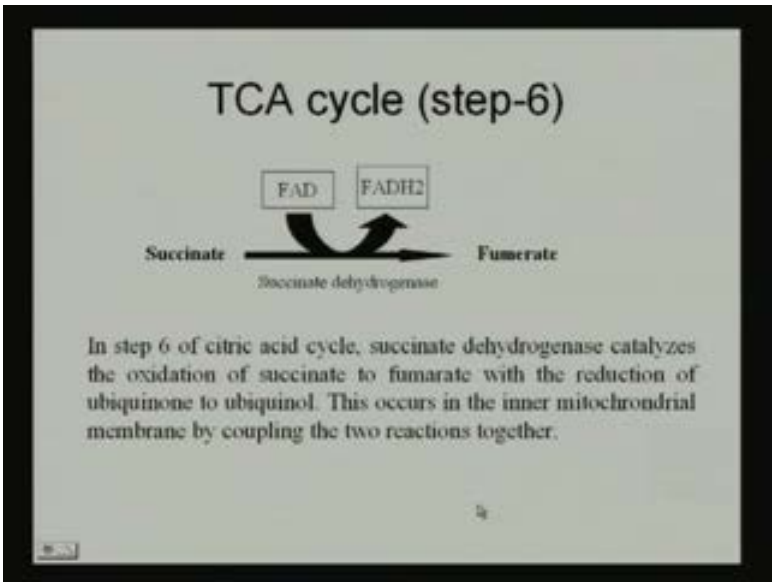

In the sixth step, succinate is converted to fumarate; that means, in the sixth step succinate is converted to fumarate and here we are getting 1 molecule of FADH 2, 1 FAD molecule, this particular succinate dehydrogenase is the enzyme, which is not present in the mitochondrial matrix, but this is a membrane bound enzyme, which is found in the inner membrane of mitochondria and here 1 FAD molecule is getting converted to FADH 2.

Now here, if we see the reaction you see this is the succinate and in places of FAD, it is forming the fumarate, this is the third oxidation step in this cycle, where FAD removes two hydrogen from and form the succinate. So, here this hydrogen is also produced in this reaction, 2 molecules of hydrogen are produced in this reaction.

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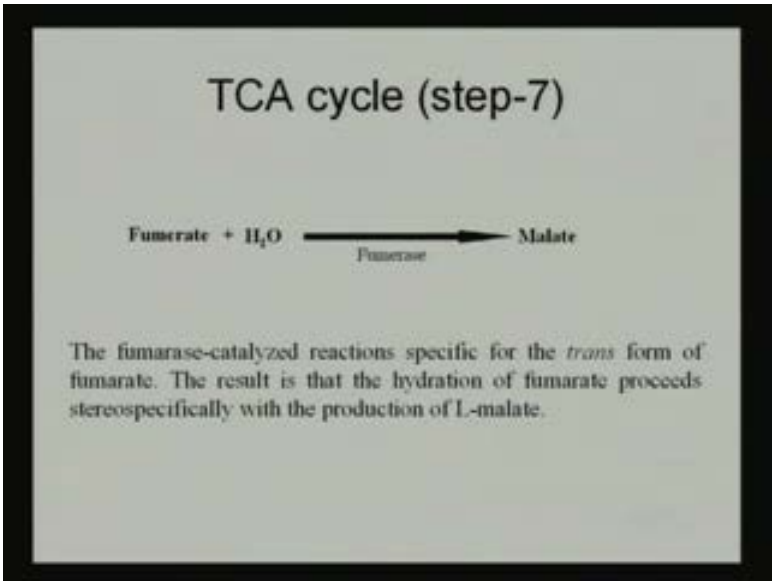

Now, when fumarate in the next step that step seven; that fumarate is getting converted to malate and in the presence of enzyme fumarase. And here, 1 molecule of oxygen water is needed and it forms the malate you see the addition of water to the fumarate places the hydroxyl group next to the carbonyl carbon and it forms the malate.

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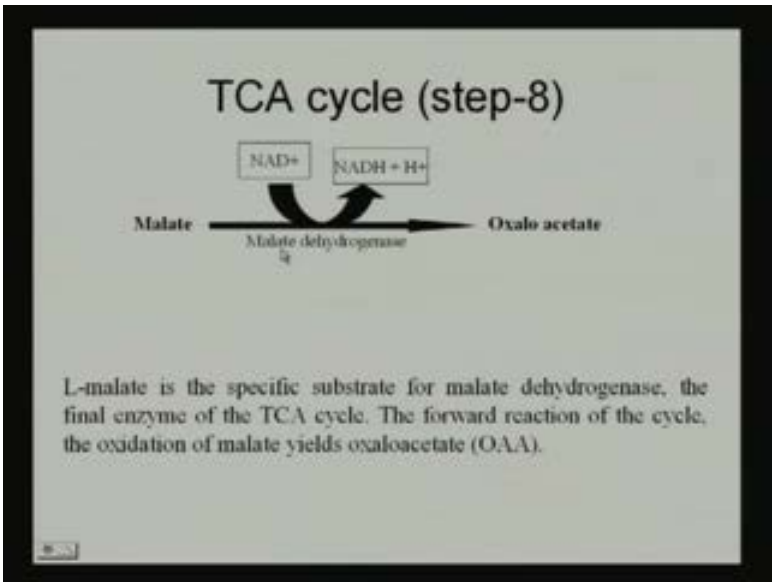

And when this malate is converting like this taking part and it converted to oxaloacetate the, first product of TCA cycle in presence of the enzyme malate dehydrogenase; that

means, in step eight that, malate is converted to oxaloacetate and here we can get 1 molecule of NADH and oxaloacetate.

So, if we see this reaction then we can find that, you see increases of malate dehydrogenase in the last four oxidation steps in the cycle, the carbon carrying the hydroxyl group is converted to the carbonyl group regenerating the oxaloacetate needed for the first step of this reaction. And this is once again the 4 carbon compound and in this way, the cycle completes.

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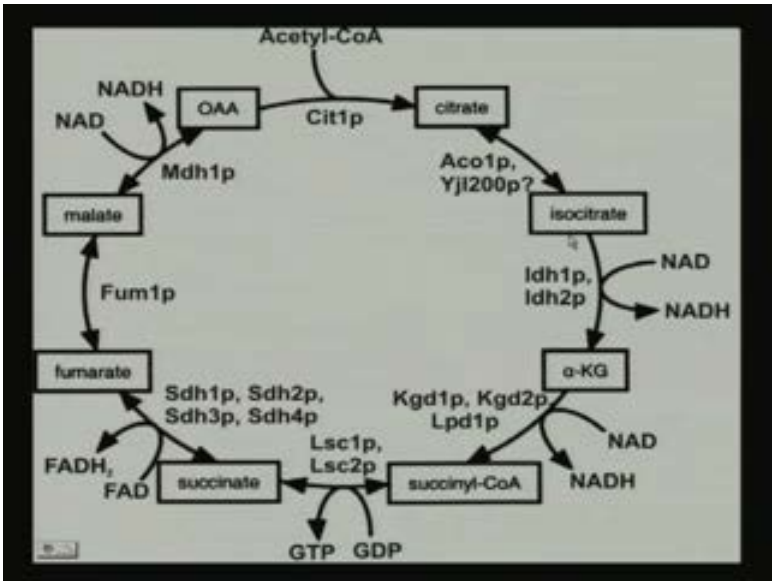

Now, if we see that, total TCA cycle then we can find that, you see acetyl CoA is coming and it is it is coming in contact with this oxaloacetate condensation reactions are going on and it forms the citrate, the 6 carbon compound, then citrate to isocitrate; isocitrate to alpha ketoglutarate, alpha ketoglutarate to succinyl CoA followed by succinate, fumarate, malate and oxaloacetate and in this way the total cycle gets complete completed and when this cycle is getting completed we are calling back yes this cycle is continuing and cellular respiration is going on inside the mitochondria of the cell.

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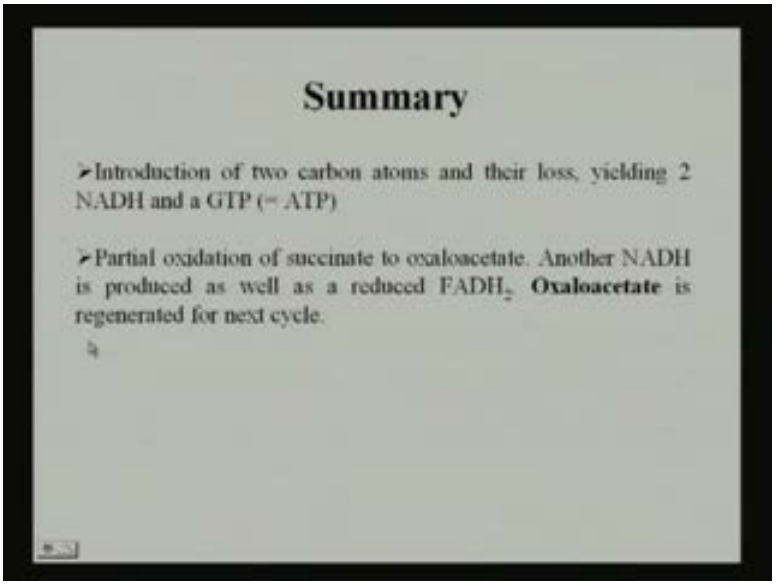

Now, if we see the summary of this particular reaction then we can find that, that introduction of two carbon atom and their loss, yielding 2 NADH and a GTP or ATP equivalent to ATP. Partial oxidation of succinate to Oxaloacetate, another NADH is produced as well as a reduced FADH 2. Oxaloacetate is regenerated for the next cycle.

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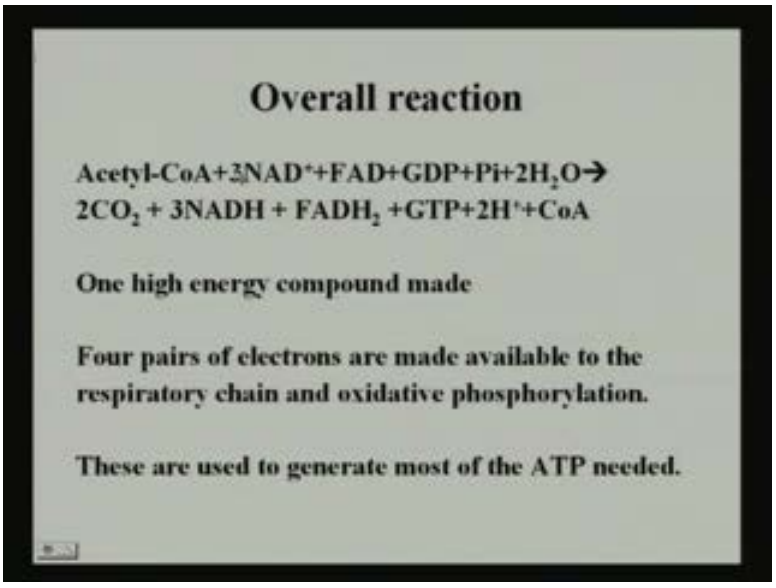

And if we see the overall reaction then, we can write this reactions like this, acetyl CoA and 3 molecules molecules of NAD because, we can see that, this is 1, this is 2 and this is 3; that means, 3 molecules of NAD is needed, 1 molecule of FAD 1 molecule of FAD,

1 molecule of and GTP and P i and in presence of 2 molecules of water gives rise to 2 molecules of carbon dioxide, see 1 molecule and another molecule, this is 1 carbon dioxide and this is another molecule of carbon dioxide.

So, it results 2 molecules of carbon dioxide, 3 molecules of NADH, 1 molecule of FADH 2, 1 molecule of GTP, 2 H plus 2 H plus and 1 coenzyme A which is produced in this 1 molecule of pyruvate and when 1 glucose is producing 2 molecules of pyruvate then, this entire thing is getting double. One high energy compound is made. Four pairs of electrons are made available to the respiratory chain of oxidative phosphorylation. These are used to generate most of this ATP, which are needed.

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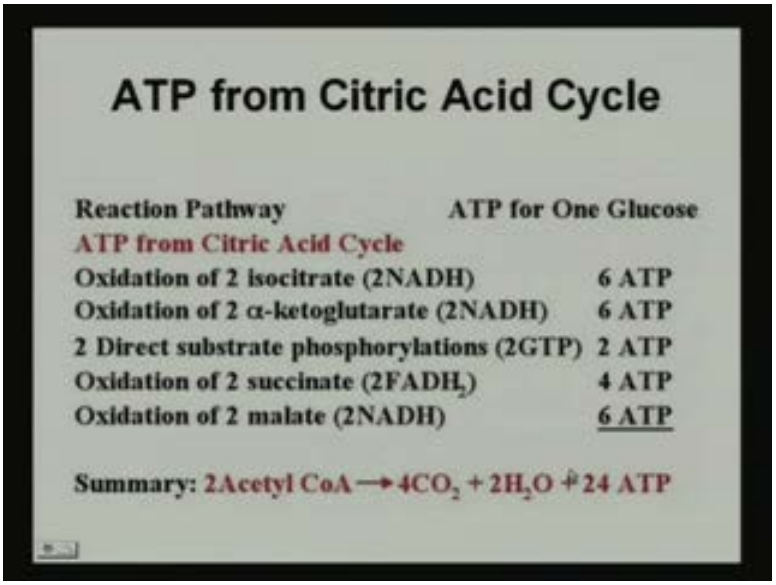

And if we see the ATP from the citric acid cycle we can find that, that ATP from citric acid cycle that oxidation of 2 isocitrate, the 2 molecule of molecules of NADH is there; that means, 1 molecule of NADH is equivalent to 3 molecules of ATP; that means it is 6 molecules of ATP equivalent.

Oxidation of 2 alpha ketoglutarate is also giving those 6 molecules of ATP. 2 direct substrate level phosphorylations are giving 2 molecules of ATP. Oxidation of 2 succinate is giving 2 molecules of FADH 2 and 1 molecule of FADH 2 is equivalent to 2 molecules of ATP; that means, it is 4 molecules of ATP, those 2 molecules of FADH 2 is there. And oxidation of 2 malate is also producing 2 molecules of NADH and it is giving 6 molecules of ATP and all together we can if we sum up we are getting 24 molecules of

ATP. And if we summarize the reaction 2 molecule of molecules of acetyl CoA gives rise to 4 molecules of carbon dioxide, 2 molecules of water and 24 molecules of ATP.

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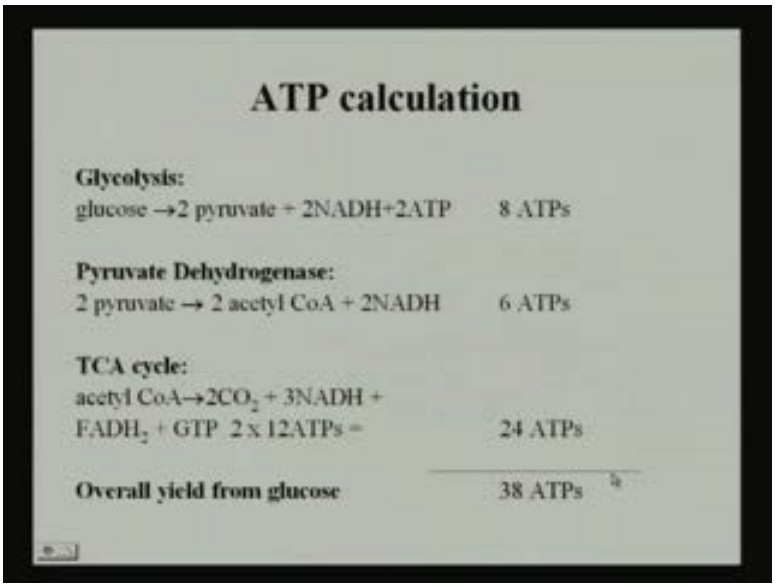

And if we see the overall ATP generation in this process starting from glycolysis to TCA cycle then, we can find that as we have already learnt that 8 molecules of ATP is produced in glycolytic pathway and we have also seen that, when pyruvate dehydrogenase is participating in conversion of pyruvate to acetyl CoA 1 that, 1 pyruvate is giving 1 NADH and 2 pyruvate is releasing 2 molecules of NADH equivalent to 6 molecules of ATP.

TCA cycle in TCA cycle we have already seen that, 24 ATP molecules are formed. So, if we overall sum off that, from glucose to the completion of TCA cycle we can find that, 38 ATP molecules are formed.

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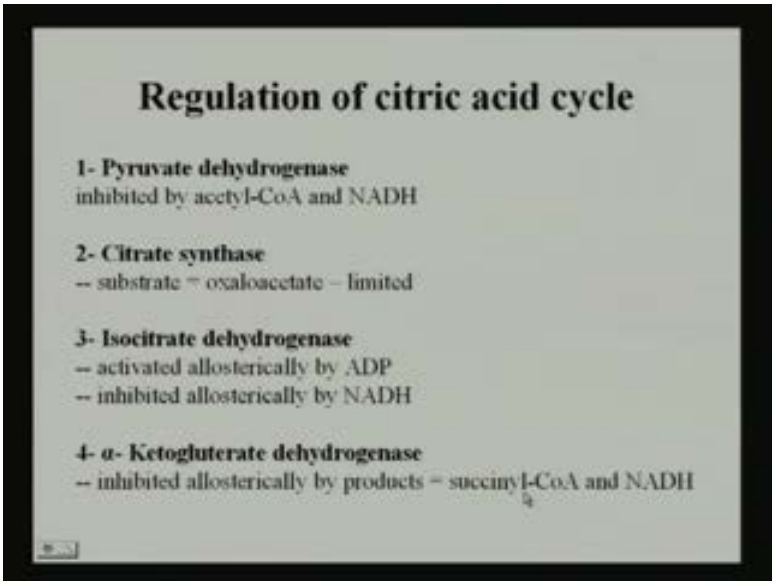

Now, as I have already mentioned that, it is the regulation of citric acid cycle. Now, pyruvate dehydrogenase is the enzyme. Citrate synthase is the enzyme. Isocitrate dehydrogenase and alpha keto ketoglutarate dehydrogenase are the enzymes, which are regulating which are playing a significant role on the regulation of citric acid.

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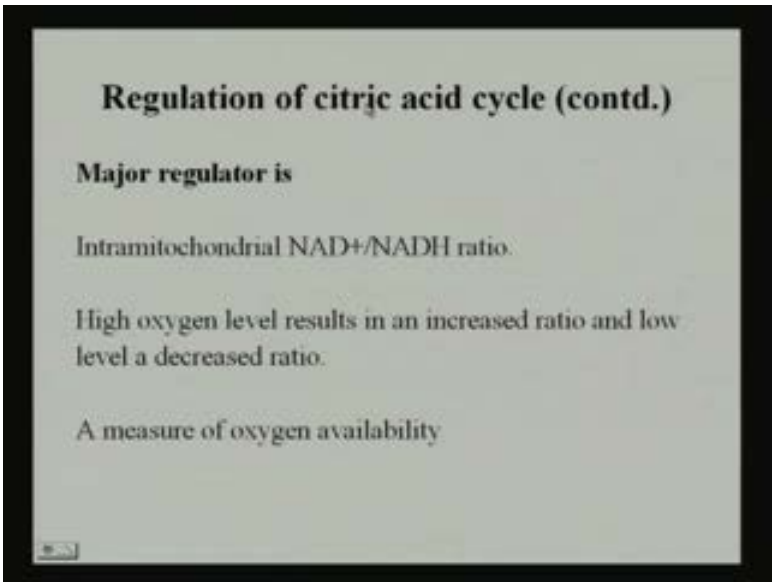

Now, when we are talking about the regulation, we we can find that, the intra mitochondrial NAD NADH ratio. High oxygen level results in the increased ratio and

low level of and low levels a decreased ratio. A measure of oxygen availability is playing a major regulatory role in citric acid cycle.

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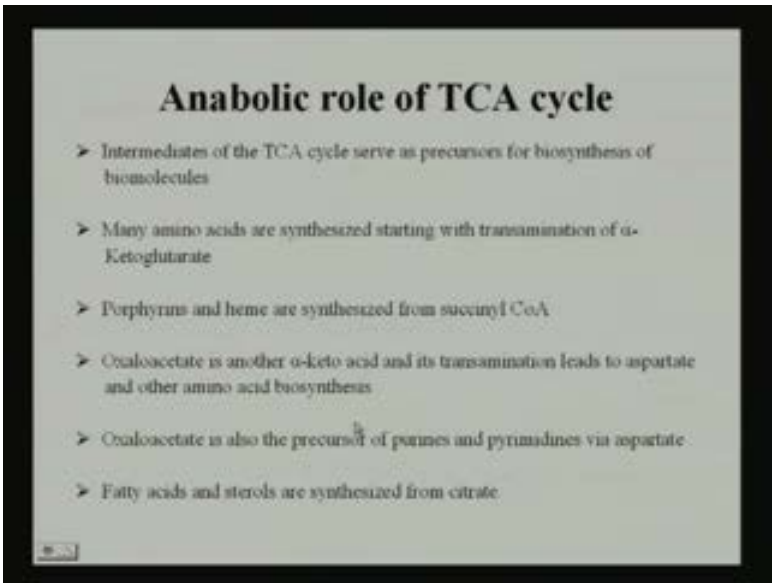

Now, coming to this anabolic role of TCA cycle we can find that, the intermediates of TCA cycle serves as the precursors for biosynthesis of biomolecules. Many amino acids are synthesized starting with transamination of alpha ketoglutarate. Porphyrins and heme are synthesized from succinyl CoA. Oxaloacetate is another alpha keto acid and its transamination leads to aspartate and other amino acid biosynthesis. Oxaloacetate is also the precursor for purines and pyrimidines via aspartate. And fatty acids and sterols are also synthesized from citrate and these are the anabolic role of TCA cycle.

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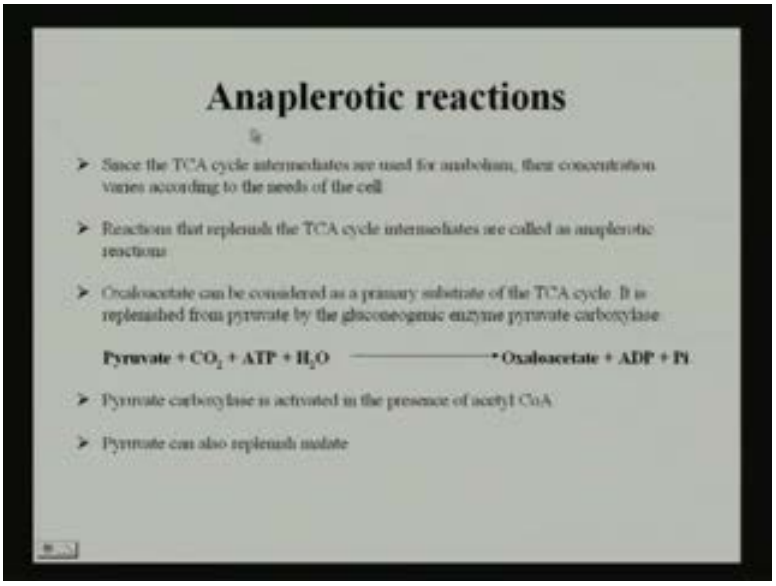

If we see the anaplerotic reactions we will find that, since the TCA cycle intermediates are used for anabolism their concentration varies according to the need of the particular cell. Reactions that replenish the TCA cycle intermediates are also called as anaplerotic reactions. Oxaloacetate can be considered as the primary substrate for TCA cycle, it is replenished from pyruvate by gluconeogenic enzyme, pyruvate carboxylase, pyruvate plus carbon dioxide ATP and water gives rise to oxaloacetate, ADP and P i.

Pyruvate carboxylase is activated in the presence of acetyl CoA. Pyruvate can also replenish malate and in this way the anaplerotic reactions are going on in TCA cycle and I had tried to give you the overview of this catabolic process as I have told you earlier that, the reactions are of two types; one is the anabolism anabolic reactions, another the catabolic reactions.

Now, we are discussing the respiratory process which is an, which is a catabolic reaction and what I am discussing that from glycolytic pathway how TCA cycle it starts and how pyruvate is converted to acetyl CoA and acetyl CoA enter to this mitochondria and it completes the cycle and this cycle continues in the living system and this way, this TCA cycle is taking place in mitochondria of any cell.

So, thank you very much students and in that next class I will be telling talking about this the electrons, which are produced which are there how they are being carried out in this particular reaction to complete the cellular respiration, thank you very much.