Biochemistry
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Lecture # 23
Bioenergetics – I

We begin our lecture on a bioenergetics which is the final part of this course. We will be speaking on certain aspects of energy of systems and mostly later on the metabolism of carbohydrates. If we consider the bioenergetics of life, we consider the thermodynamics of energy conversions in living systems.

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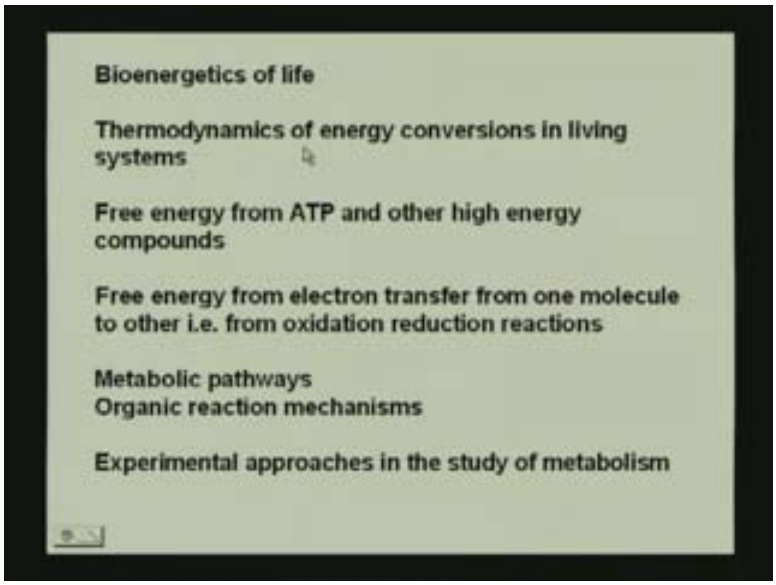

We have seen before how we look at ATP as the source of energy and it will be more apparent now when we see how this energy of the breakage of the high energy phosphate bond is actually going to give us a lot of the energy that is required to drive these processes. We have the free energy from ATP and other high energy compounds then the free energy from electron transfer from one molecule to another in ordinary oxidation reduction reactions where again we will be using some compounds that we have considered that have been derived from the vitamins. Then we will be looking at some metabolic pathways and involved in that we will be looking mostly at the break down of glucose, how glucose is broken down in the body and in that there will be certain not very many but some organic reaction mechanisms and there will be some studies based on how the energy is utilized in the processes in glycolysis or the tri carboxylic acid cycle where we have the final breakdown of glucose.

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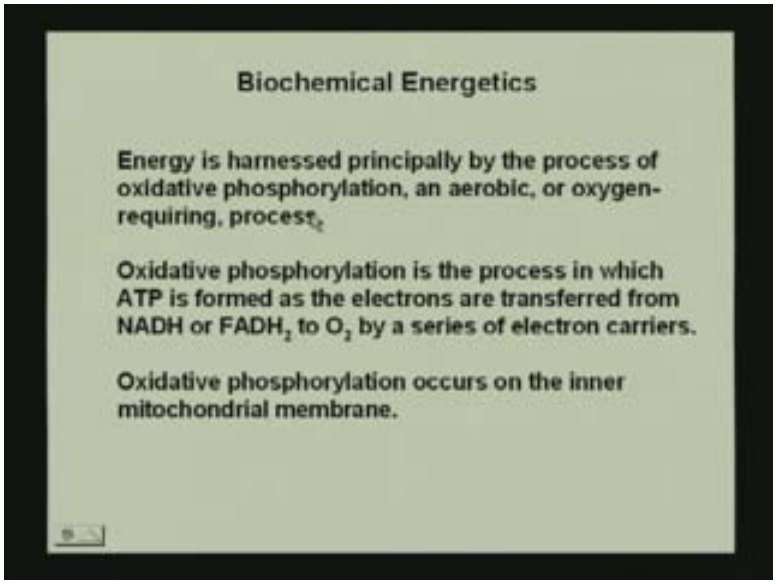

When we consider bio chemical energetics usually the energy is actually harnessed by a process that is called oxidative phosphorylation this is an aerobic process. An aerobic processes means it is a processes that requires oxygen. This oxidative phosphorylation is the process by which ATP is formed. And you realize that since ATP is the currency of energy we require its formation at a very high level as well because its breakdown is going to finally drive a lot of other processes that are going on.

Therefore the formation of ATP is extremely important and oxidative phosphorylation is the process in which it is formed as electrons are transferred from NADH or $FADH_2$ to oxygen by a series of electron carriers. Now we won't go into the details of all the mechanism but we will just look at the broad overview of how this oxidative phosphorylation system works and how actually ATP is formed. Now this process of oxidative phosphorylation occurs in the mitochondrial membrane? You have all heard or studied from your school days that mitochondria is the power house and what we look at it now is a bit more detailed that the fact that it is the power house is because it gets you the source of energy that is ATP. In its mitochondrial membrane if we look at the structure of mitochondria we have this mitochondria system. This is, you know, a picture of mitochondria and what we have here is, we have the membrane, a charge across this membrane and in here if you remember these folds are called crystane.

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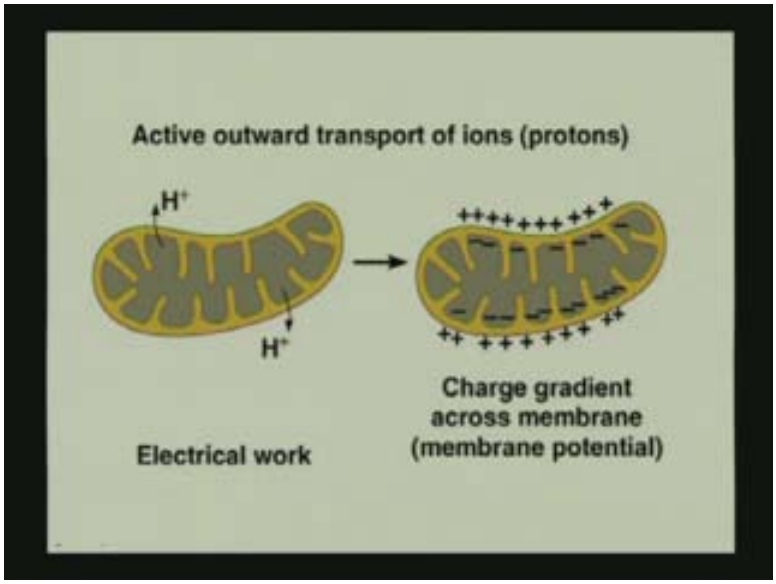

These folds actually there is this inter-membrane space and there is the inter-cellular space, the cytoplasmic space the inter membrane space and the outside of the membrane. These are the three features we are going to consider in the charge gradient across the membrane and we will see how important that is in driving what is called a proton pump. Because this proton pump is essential for the formation of ATP which occurs in the inner mitochondrial membrane. But before we get into that we will just lo at the basic aspects of energy in equilibrium and how they are related in other terms like delta G and all that. This is something you have studied before.

But in general when we consider any energetics or any equilibrium we look at a delta G factor and we know that at equilibrium this value is 0. We have here certain products and certain reactants A + B going to C + D at a definite temperature and associated with that we can get a specific equilibrium constant and with the equilibrium constant we know that when we have the negative for the delta G0 prime we are going to have a spontaneous reaction. Now the prime usually if not mentioned otherwise it refers to a biological system where the temperature is -30 ^{0}C.

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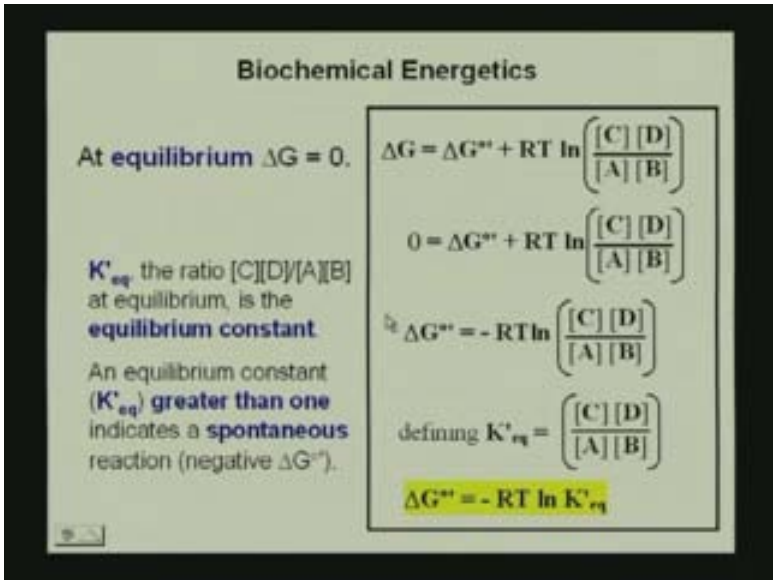

It is not your normal delta G0 where the temperature is 25 degree C. If not mentioned the delta G0 prime T has to be 37 degree C. If we look at the variations if we just consider you can see how different the variations get. now the calculation here have been done at 25 degree C for the delta G0 prime but in the normal cases when the temperature is not mentioned it means you use 37 degree C for your calculations. If we consider just one mole concentration of reactants and products what you will see here is how the order of the equilibrium constant changes more than a 100 fold for a relatively smaller value of the kJ mol^{-1} ΔG changes. You have to be very careful about this, this is due to the *ln* feature that you have here. Since it is an exponential dependence what you have is even if you have just a 12 kJ mol^{-1} difference here you are looking at a hundred fold difference in your equilibrium constant which means your reaction is going to get to equilibrium at a much different rate than obviously if you had just your energy at this power. When you consider the $\Delta G_0^{'}$ values here you understand that you are going to have a forward spontaneous reaction, this is at equilibrium and this goes in the backward direction a non spontaneous reaction.

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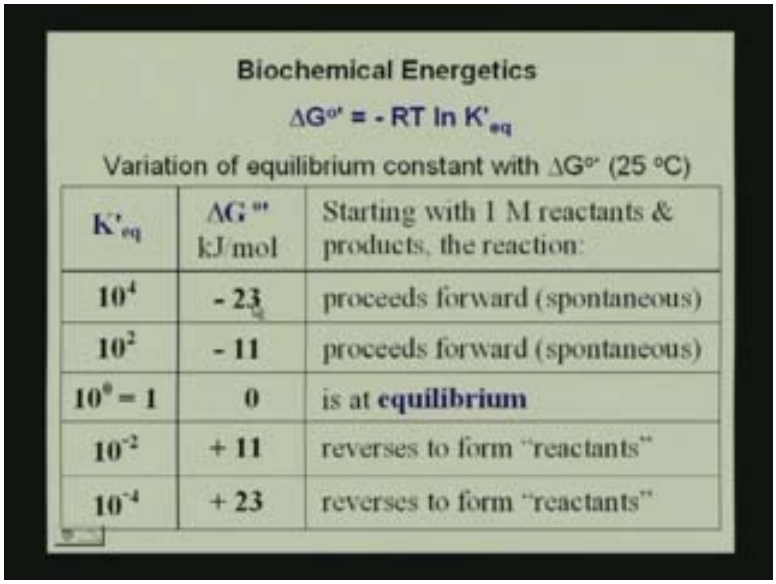

Now when we consider the energetics bio energetics requires energy coupling.

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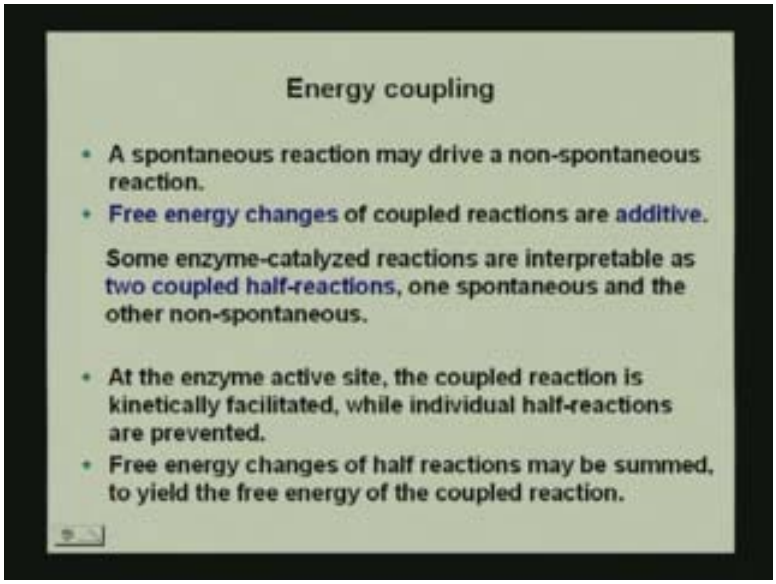

You do not have a continuous spontaneous reaction usually because that is going to form a large amount of products. You might not need all those products all the time. so the whole process of the formation of the products from the breakdown of the reactants in the enzymatic processes has to be tightly regulated. because remember we studied feedback inhibition. What was feedback inhibition? It was where you had your final product inhibit the initial enzyme. so what you are doing here in this case is you have a spontaneous reaction that drives a non spontaneous reaction. The energy is coupled in such a way that

the free energy changes obvious reactions are additive. and what we have is these enzyme catalyzed reactions they are interpreted as two coupled half reactions where you have the energy of one compensate the energy of the other more than compensate it where you are going to get a value that is going to be making the specific reaction spontaneous in nature. What happens is at the enzyme active site the coupled reactions are kinetically facilitated which means they go at a specific rate but the individual half reactions are prevented. You do not have just a certain half reaction go on but when the enzyme active site has both the reactants. There is a coupled reaction that goes on and the free energy changes of the half reactions are summed to yield the free energy of the coupled reaction which usually gets to a spontaneous reaction a negative value.

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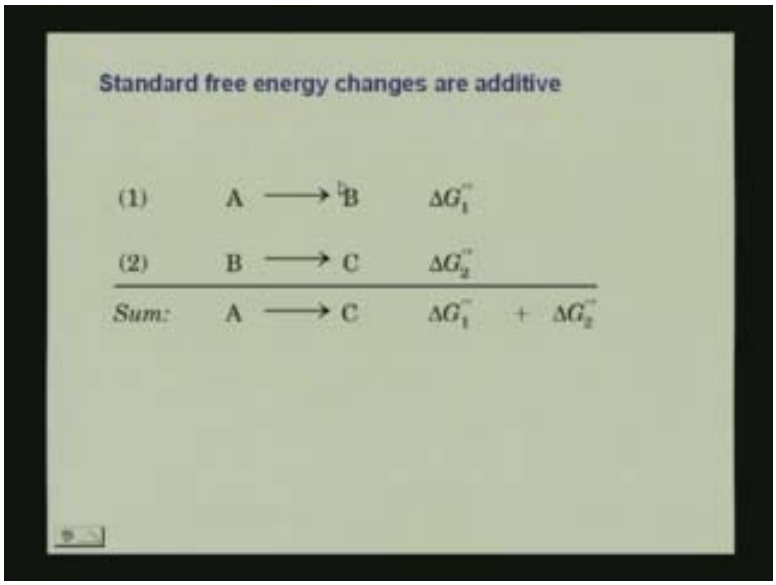

I have an example. Essentially what we are looking at? We are looking at a standard free energy change. If we have a half reaction that goes from A to B and another half reaction goes from B to C then we can have the overall reaction go from A to C and the standard free energies of both of these are going to be additive. It may so happen that you not have the B component present in both cases, this is one such case and the other case may be you have a completely different reaction that is actually going to provide the energy for this reaction to go forward. That would be a normal biochemical couple reaction and in most cases we get the energy from the ATP breakdown.

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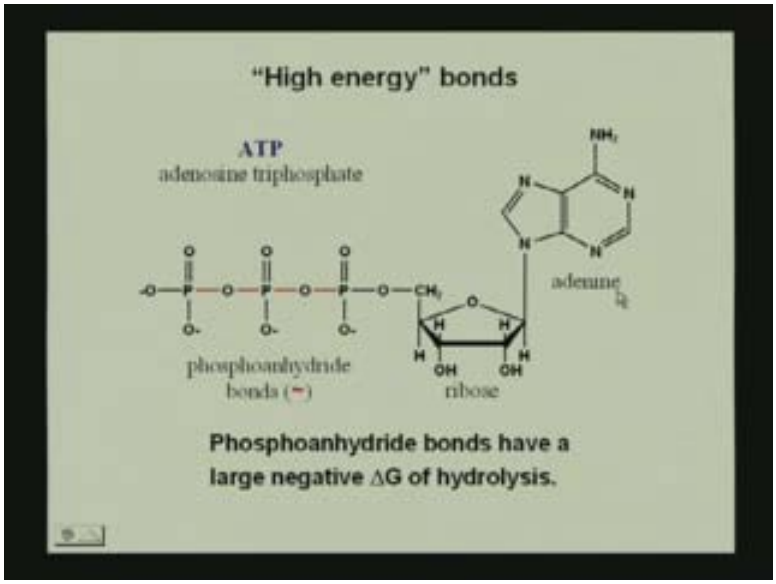

So what are we speaking about? We are speaking about this high energy bonds of ATP. This is a structure that you now recognize. And what we have here is a very large $-\Delta G_0$ of hydrolysis meaning that this breakdown is extremely spontaneous in nature. Now the basis of high energy of hydrolysis of ATP is due to the resonance stabilization of products that you get. It is also due to the electrostatic repulsion between the negatively charged oxygen atoms in ATP. Where are these negatively charged oxygen atoms? They are here. You would not have them one beside the other all the time. So it would be relatively easier for it to break of to give you ADP + P_i or AMP + PP_i.

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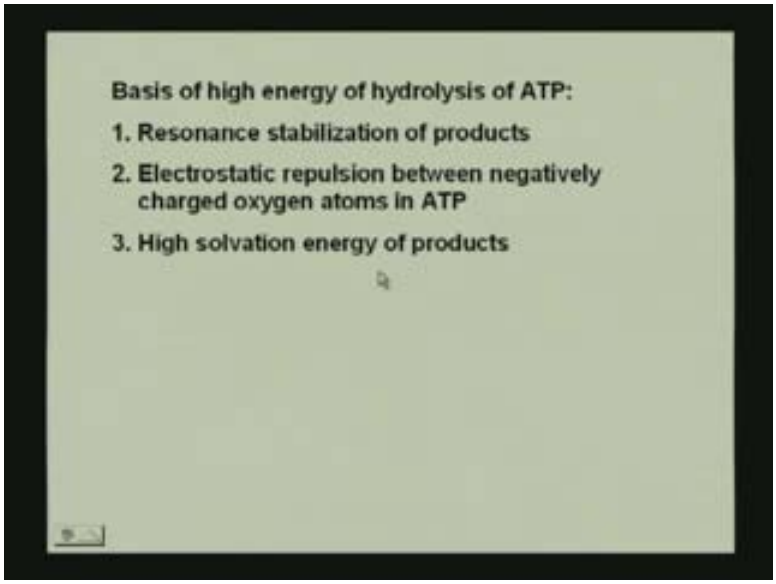

Also we have a high salvation energy of the products which amongst to the high energy of hydrolysis for ATP. The reactions that require the breakdown of ATP are such reactions that are going to have a $+\Delta G_0$ by themselves.

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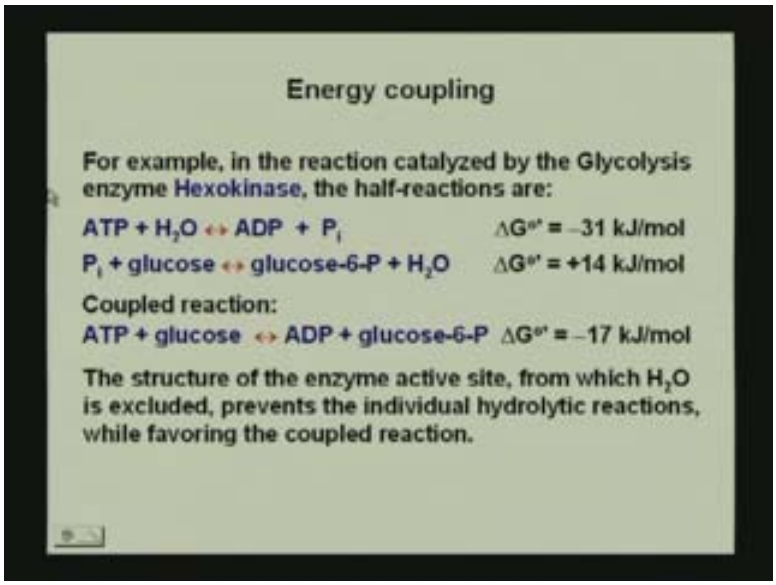

If we look at such an example the enzyme hexokinase, a kinase is a transferace enzyme that transfers a phosphate group. It is a transferace but a kinase is a specific type of transferace that transfers the phosphate group. Now, in the reaction catalyzed by the process Glycolysis the first step in the glucose breakdown is the formation of glucose 6 phosphate, this we will be studying in detail when we go to the glucose breakdown. The formation of glucose 6 phosphate from glucose + P_i is a +14 kJ mol^{-1} so by itself it is non spontaneous. so you will not have your glucose with the help of the enzyme hexokinase. This name hexo means it is working on a 6 membered carbon ring or your 6 membered glucose carbon sugar here. It is a hexose, it is the kinase working on the hexose so it is a hexokinase which means that it is going to be involved in the transfer of the phosphate ion from just the P_i in this case two glucose giving you glucose 6 phosphate.

As I just mentioned this overall reaction has a $+\Delta G_0^{'}$. However, if you couple it with the hydrolysis of ATP, ATP hydrolysis gives you ADP + P_i and we have a very large negative value here for the breakdown of ATP and when you couple these two reactions together what is happening is you have ATP + glucose form ADP + glucose 6 phosphate giving you an overall favorable $\Delta G_0^{'}$ of the reaction. This makes this reaction spontaneous. So this is what you would call energy coupling.

The structure of the enzyme active site from which H_2O is excluded prevents individual hydrolytic reactions but it does favor the coupled reaction. You understand that the

enzyme active sites are extremely specific in the way they work. You have seen certain enzyme active sites and how they actually work. We will not go into the details of how hexokinase works but for understanding the energetics what you have to realize here is that the non spontaneous reactions are couples with other reactions that are spontaneous in nature in the case ATP hydrolysis but this together will give you a favorable energy which makes the reaction go forward.

Nature has chosen certain specific hydrolysis reactions for the specific types of non spontaneous reactions. For example, in this case we need ATP hydrolysis. but in some cases if the energy is enough to be compensated by another specific hydrolysis or another electron carrier or whatever then it may not require this amount of energy for the coupled reaction and then what is chosen, another hydrolysis is chosen to couple it so that we do not have too much extra energy.

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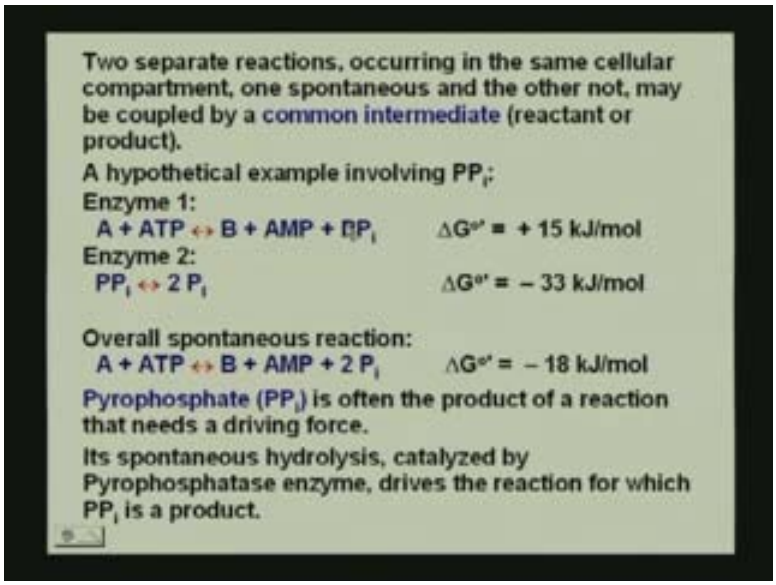

We can have this also. This is another case where we have two separate reactions that occur in the same cellular compartment one is spontaneous and the other is not that would be typical of a couple reaction and it is coupled by a common intermediate. For example, if we look at a hypothetical example that involves PP_i we have A + ATP going to B + AMP + PPi. This particular reaction that occurs in enzyme 1 has a $+\Delta G_0$. It having a $+\Delta G$ means it is non spontaneous. If we want to form B from A we have to have a corresponding compensatory reaction that is going to have a delta G that is negative then we can couple it with this reaction to give an overall spontaneous reaction. And the energy has to be such that the negative value obviously has to be more than this in magnitude.

So, if we look at enzyme two that is breaking up the PPi into 2 Pi it gives us a delta G0 prime of – 33 kilo joules per mole which more than compensates for the spontaneity of

this one. So the overall spontaneous reaction is going to give us A + ATP, B + AMP + 2 P_i. Again we are looking actually at ATP breakdown. We are not going to ADP + P_i in this case. The first reaction itself is forming ATP is breaking down into AMP + PP_i. The reaction which couples or which actually provides the energy for the first reaction to go forward is the PPi breaking down into 2Pi. So what happens is this pyrophosphate is often the product of a reaction that needs a driving force and we have this break down. If you look at both the examples that I showed you both of them are breaking down ATP which means that ATP has to be produced somewhere. If you don't have enough production of ATP obviously none of these reactions are going to be possible.

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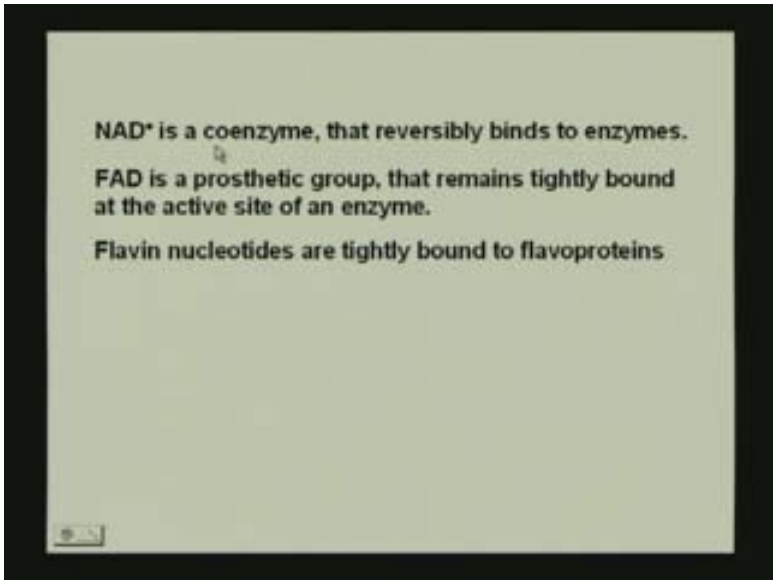

Therefore we are going to look at generally how ATP is formed in a very simplistic manner. We have looked at these molecules before. We will go into some of the details of what we looked at before. We considered these when we studied vitamins and coenzymes. NAD^+ is a coenzyme that reversibly binds to enzymes that is what is a coenzyme. FAD is a prosthetic group that remains tightly bound at the active site of an enzyme. We will see how these are utilized basically in the process of oxidative phosphorylation to actually give you in the production of ATP. And these Flavin, we will look at what these molecules are, the Flavin nucleotides are tightly bound to what are called flavoproteins. The proteins that have the FAD or the Flavin nucleotides are called flavoproteins and these are required in the reactions of oxidative phosphorylation to give you the energetics or whatever is required for the production of ATP.

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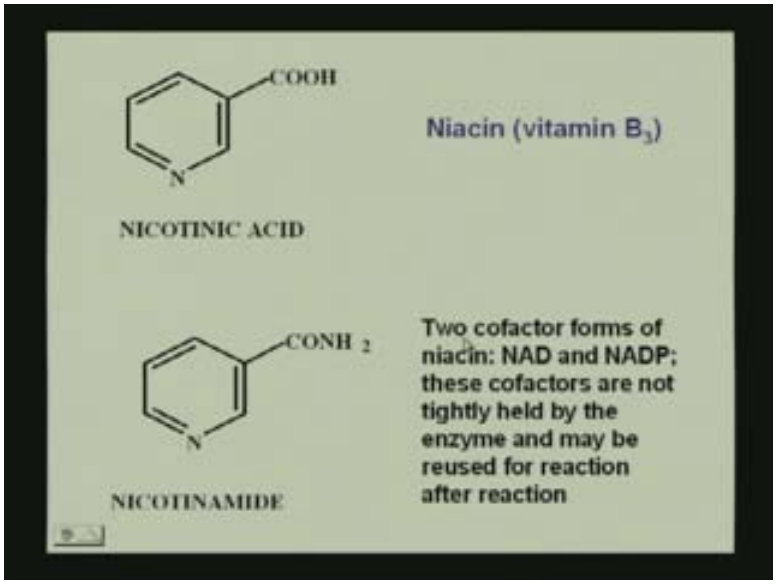

This is something we studied before where we are looking at vitamin B_3 (Niacin) where we have cofactor forms that were NAD and NADP. They are not tightly held, they are the cofactors and reused reaction after reaction. We don't use them in the raw form here. We have to transform them either to NAD^+ or NADP. What happens in these reactions is we have an oxidized form and a reduced form.

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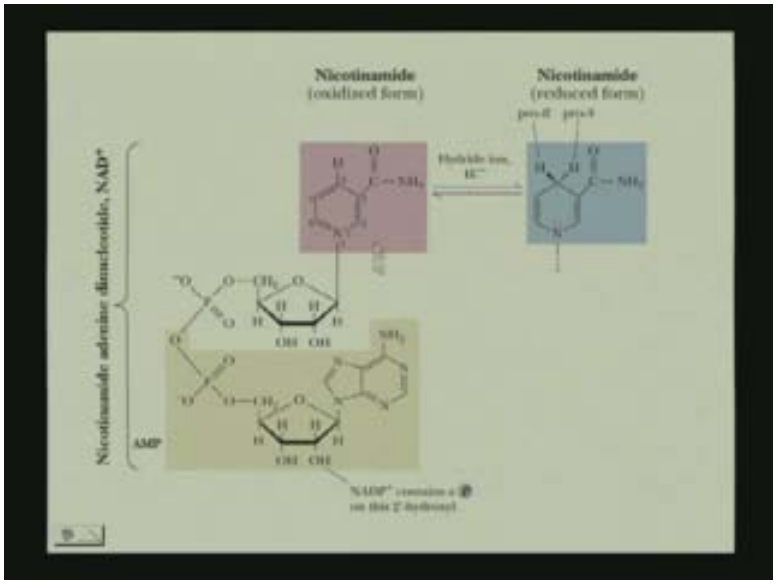

What are these forms? When we are looking at nicotinamide adenine dinucleotide we know that we have the nicotinamide adenine dinucleotide.

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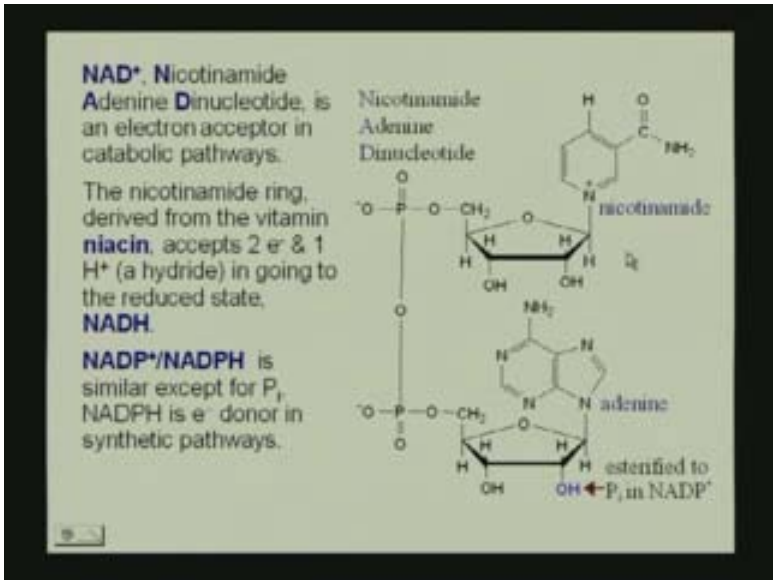

A nucleotide has a single phosphate. A dinucleotide has these 2 phosphates so we have a nicotinamide, adenine and a dinucleotide so we have NAD. It is an electron acceptor. The rest of the molecule is not going to be required, this is NAD^+ this nitrogen has a plus (+) this particular nitrogen has a +ve charge to it. The only difference that we have between NAD and NAD^+ is this $2^{'}$OH on the adenine nucleotide is phosphorylated so we have NAD^+ here, and this is $NADP^+$ here, we have the OH here and this NADP+ with the phosphate here.

Now therefore the features of NAD plus or the NAD plus are that we have the nicotinamide that has been derived from niacin so this is derived form the vitamin B_3 (Niacin). It accepts two electrons and one proton that means a hydride and goes to the reduced state NADH. Similarly, we have $NADP^+$ and NADPH. It is similar as I said it only has this extra phosphate at the $2^{'}$ position. So the variations that you are looking at are NAD^+ and NADH. If we look at the previous slide, the rest of this molecule is required for recognition for the enzymatic process but for the NAD^+ going to NADH it is only this part that is the nicotinamide part that is required so we refer to rest of this whole portion as nothing else but R. that is exactly what we have. R is the rest of the NAD^+.

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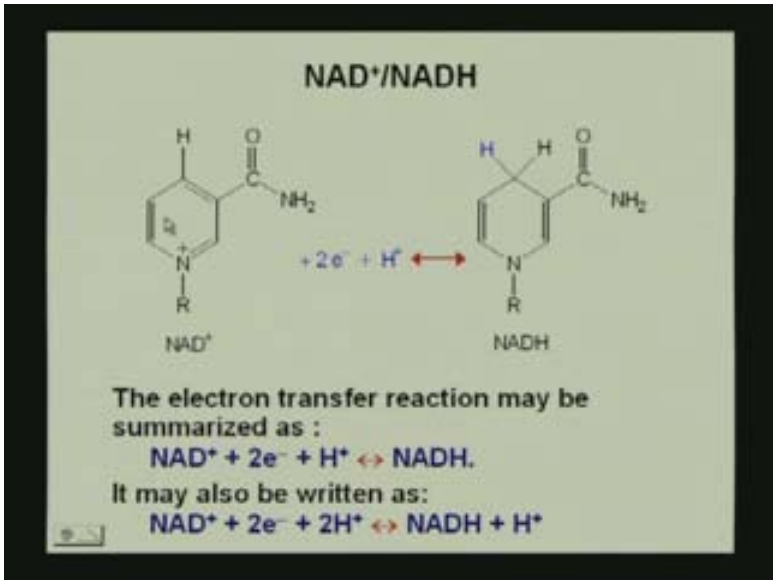

We have the N^+ here now what it does accept is two electrons and a proton. This becomes, it loses its plus and it has an additional H so it is now NADH without the positive charge. So what it accepts is two electrons and a proton. The electron transfer reaction can be summarized as $NAD^+ + 2e^- + H^+ \rightarrow NADH$. It is also written as $NAD^+ + 2e^- + 2H^+ \rightarrow NADH + H^+$. You have to recognize here that the reactions that NAD^+ or for example when I show you FMN and FAD as well these reactions are going to occur in or these co-factors or prosthetics groups are going to be required in enzymatic reactions that are going to be of what type of a redox type. Because either the hydrogen has to be taken away or the hydrogen has to be supplied so in that case we cannot have the ATP come into the picture. So, when it is a certain dehydrogenase enzyme or an oxidized type of enzyme it will require NAD^+ or FMN or FAD for the particular reaction to go forward. So you have to recognize in the energetic procedure what sort of transformation is taking place. because each of these are transformation steps breakdown steps that we are going to study and as we go through them we will see that obviously when you want to add a phosphate with the help of a kinase that is going to transfer the phosphate you cannot use any of these you have to use ATP. but when we have a redox reaction that is going to use your redox reaction that is the dehydrogenase or an oxidize you will require $NADP^+$ and NADH and depending on the enzyme that you have you will either use this or you will use FMN or FAD.

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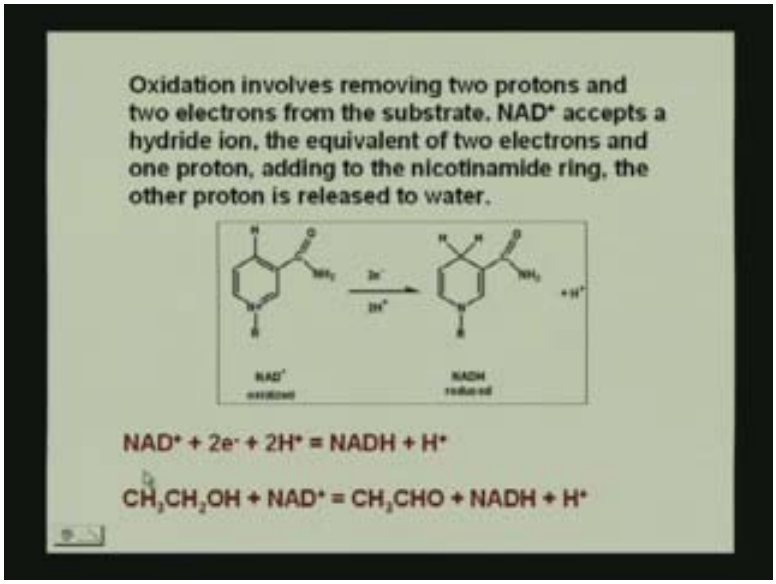

So basically what we have is this is one example where we have ethyl alcohol going to acetaldehyde where we are looking at $NAD^+ \rightarrow NADH + H^+$. Basically what happens is the oxidation involves removing 2 protons and 2 electrons from the substrate NAD^+ so what is your substrate? The substrate is going to the products where you are removing 2 protons and 2 electrons from the substrate and that is going to NAD^+ to form NADH. NAD^+ is taking up these 2 protons and 2 electrons. It accepts a hydride ion that is the equivalent of 2 electrons and a proton, it adds this to the nicotinamide ring and the additional proton is released to water. So basically any reaction that is going to require the removal of 2 protons is going to use NAD^+. NAD^+ is going to take up those 2 protons, that is simple as how it actually works.

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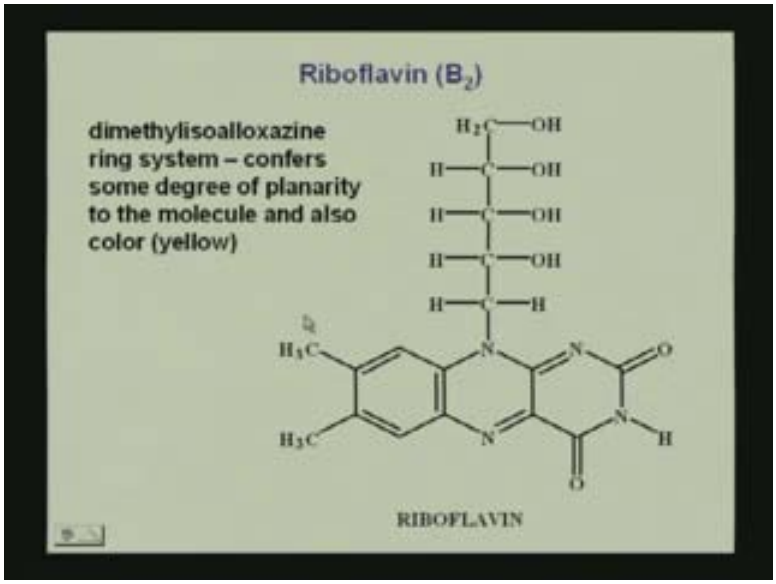

The other one that we are going to be using in oxidative phosphorylation for FAD or FMN is derived from this vitamin riboflavin vitamin B_2 and the structure there is a Flavin mononucleotide where what we have is, we basically have this isoalloxazine ring.

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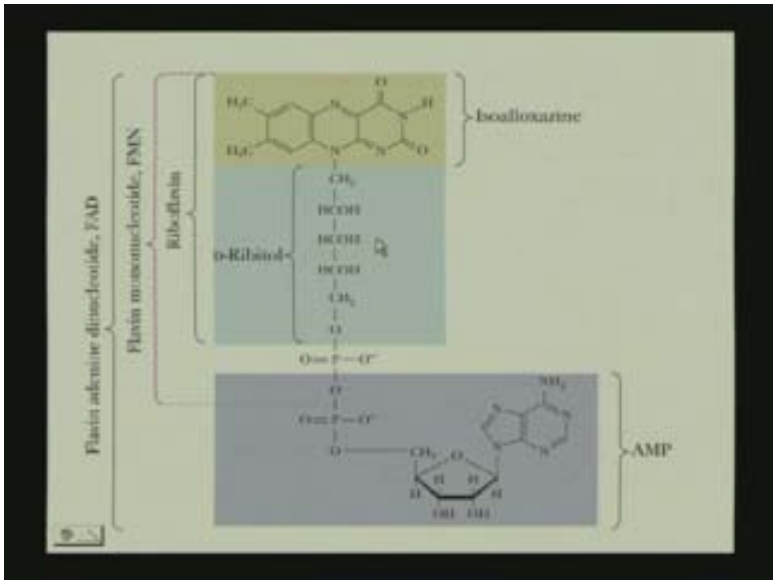

This ring isoalloxazine ring alloxazine ring is what is required here and what is going to be taking up the hydrogen. So again when we are looking at it we have this is Flavin mono nucleotide until this part, why mono nucleotide because we are talking of one phosphate. When we have Flavin adenine dinucleotide we have the adenine here and the other phosphate here. We have FMN or FAD.

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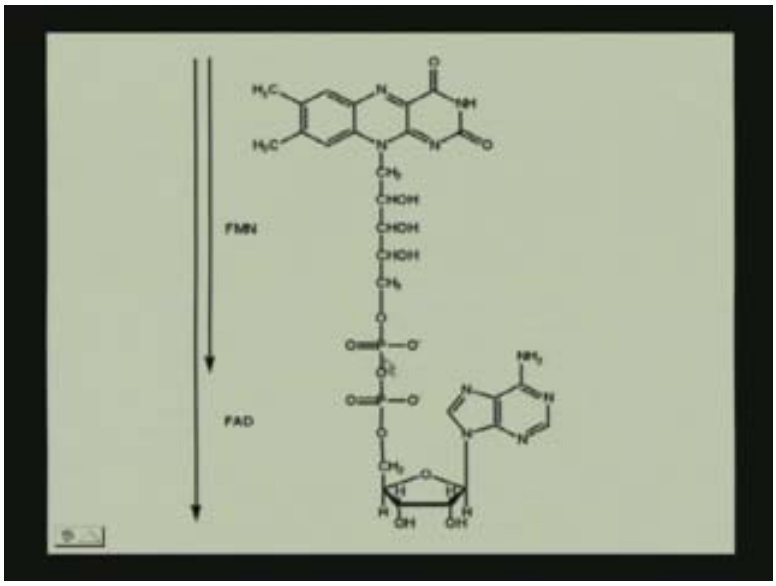

But each of these we are going to refer to this entire portion as R. Now then depending on the energetics of the process depending on what is utilized or whether it is FMN attached to the enzyme or FAD attached to the enzyme the reaction will proceed accordingly. But basically what is going to happen is this ring is going to take up the hydrogen. How is it going to do that? We have a reduced substance, we have FAD.

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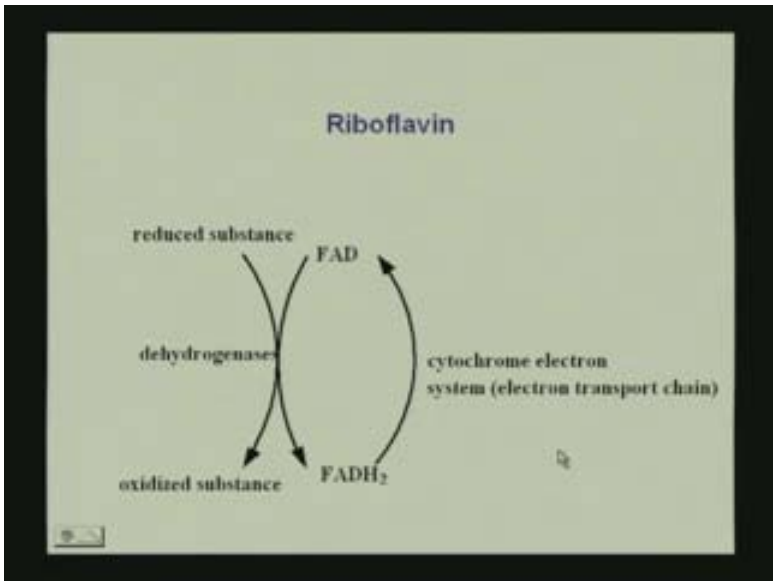

Therefore the H_2 from the reduced substance is taken up by the FAD similar to NAD^+ going to NADH so we have FAD going to $FADH_2$. This is an example of where we have

cytochrome electron system in the electron transport chain. So we have the reduced substance going to an oxidized substance with the help of enzyme a dehydrogenase in this case that is going to abstract the hydrogens from the substance from your reactant and give it to FAD forming $FADH_2$. What happens is this is the rest of the ring you now recognize,e this is just the top portion that we are interested in so we have FAD, the hydrogens are taken up by this nitrogen and this nitrogen.

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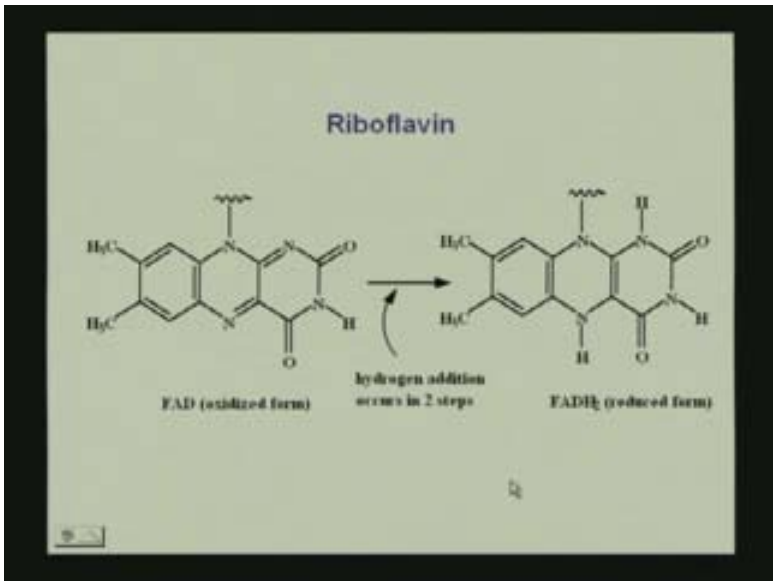

Therefore we have one here and one up there. So we have the hydrogen addition in two steps finally getting from FAD to $FADH_2$.

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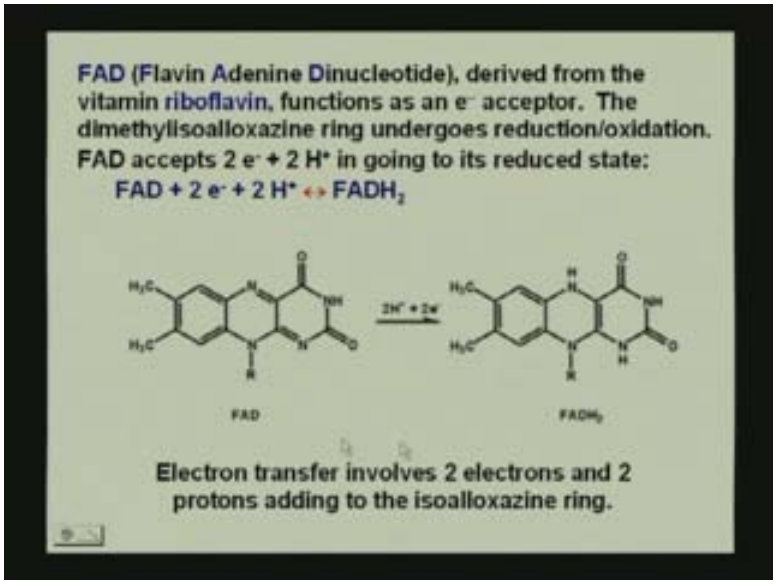

So what happens is, we have R group here, FAD and what is going to happen? We have the 2 protons taken up by the 2 nitrogens on FAD and it is going to give you $FADH_2$ so we have $FAD + 2\ e^- + H^+ \rightarrow FADH_2$. Where it is getting these protons from? It is getting them from the certain substrate that has to be converted to the product which will not have the 2 hydrogens. This is an example where we have succinate.

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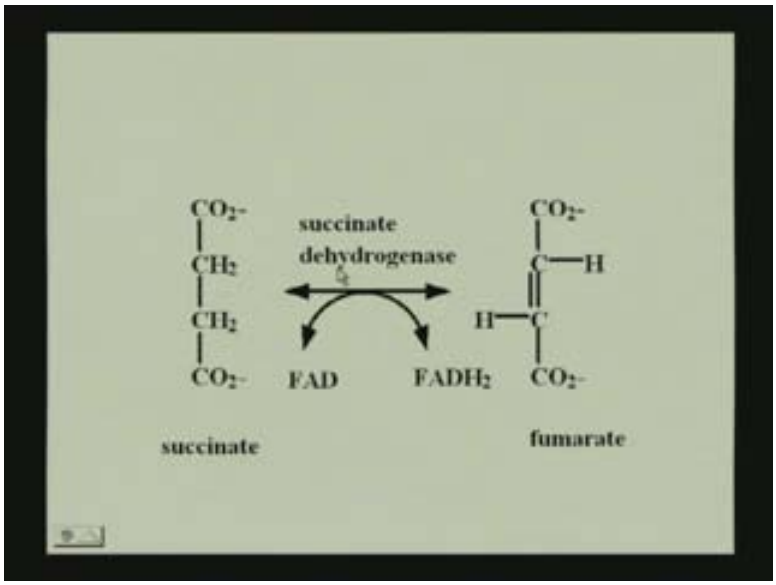

Succinate dehydrogenase what is it going to do is abstract the 2 hydrogens. In the abstraction somebody has to take it up it is as simple as that. What is going to take it up? FAD in this case is taking it up and what is happening to FAD? It is forming $FADH_2$. So this is also reaction. So succinate dehydrogenase alone will not work. First of all it has to have a place to put these two hydrogens. FAD takes up the 2 hydrogens forming $FADH_2$ and in the event you get your fumarate from succinate. We also have FMN going to $FMNH_2$.

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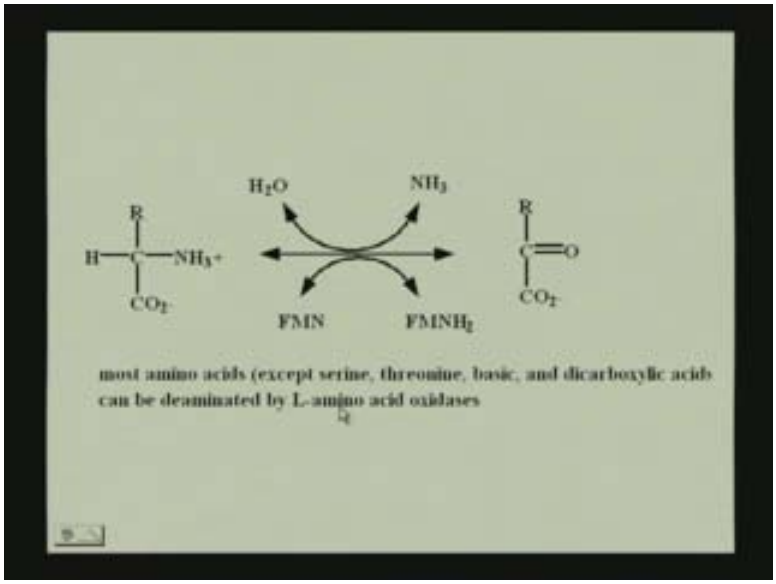

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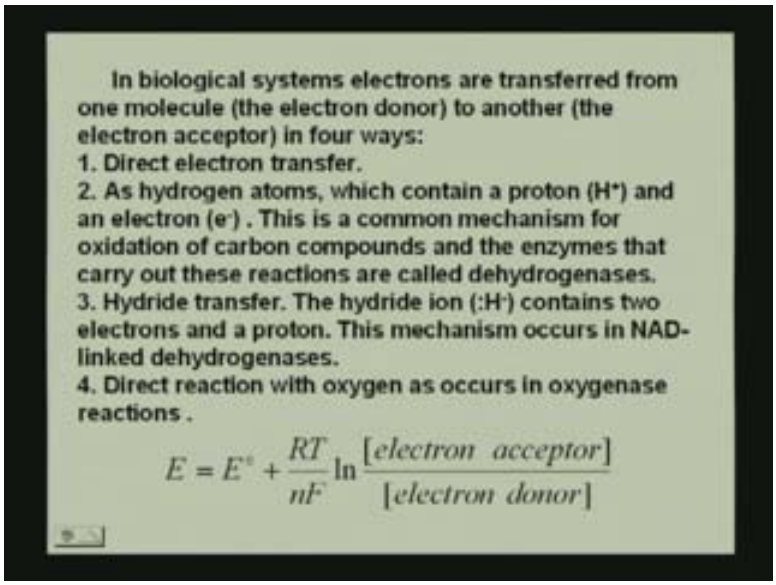

You are having a release of your NH_3. Basically the reactions are going to have proton transfers, electron transfers in your changes. Now in these systems we looked at some examples where we have NAD^+ going to NADH, FAD going to $FADH_2$ or FMN going to $FMNH_2$. Now what is happening in these electron transfer systems, all of you know Nernst equation? When we consider the biological systems the electrons are transferred from one molecule to another just in a normal electron transfer reaction. But these can occur actually in four different ways in biological systems. What are the different ways? We can have direct electron transfer that is one possibility. We can have them transferred as hydrogen atoms that contain a proton and an electron. This is a common mechanism

for oxidation of carbon compounds that we just saw using enzymes called dehydrogenases. We can have hydride transfer the hydride transfer we saw in the process of NAD where it takes up the hydride so the NAD^+ goes to NADH.

Therefore we can have direct electron and we can have transfer as hydrogen atoms. We can have hydride transfer or we can have direct reaction with oxygen. Usually in aerobic systems where you have oxygen available y**ou can have** direct reaction with oxygen as occurs in certain oxygenase reaction. These are the four processes by which we can have electron transfer. All of these are redox processes and all of these will be using redox enzymes. Redox enzymes are either dehydrogenases or oxidases. so we can have just direct electron transfer, we can have hydrogen as hydrogen atoms that contain a proton and a electron. We can have hydride transfer or we can have a direct reaction with oxygen in the presence of oxidases.

The enzymes we are looking at are dehydrogenases and oxygenases. When we go to the breakdown or the whole metabolism of carbon hydrates as soon as you look at the reactant and the product you should first of all be able to identify what is going on. If it is loosing hydrogens you are having a redox reaction taking place, and in the process of a redox reaction is taking place the enzyme therefore will be using is either a dehydrogenase or some sort of oxidase a reverse process that is going to happen then and in that case you have a cofactor that is going to be NAD^+ or the prosthetic group FMN or FAD.

When you look at these reactions you have to recognize the type of enzyme that is involved, whether the reaction is of redox type or a transferase type, whether the reaction is in isomerization where the enzyme will be nothing but an isomerase. When we study the different processes of metabolism of the carbohydrates you should be able to recognize how each of these enzymes require a specific cofactor or a specific prosthetic group for it to work.

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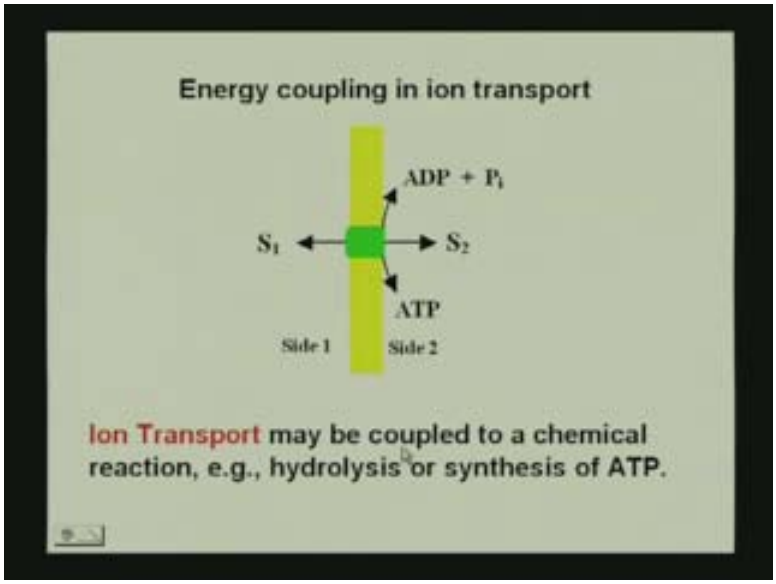

In the energy coupling in ion transport we have say the transfer of S_1 S_2. In utilizing the breakdown of ATP to form ADP + P_i we have a couple reactions. So we are coupling this usually to a certain chemical reaction when we require the energy we are using hydrolysis. We are using the hydrolysis of ATP, why is it so efficient? It is because of the resonance stabilization and certain other factors that I mentioned so the hydrolysis of ATP in the couple reaction is going to give us the possibility of ion transport. But we also have to remember that we have to produce ATP. Now you recognized this as a membrane and we have a certain enzyme that is going to act as a proton pump.

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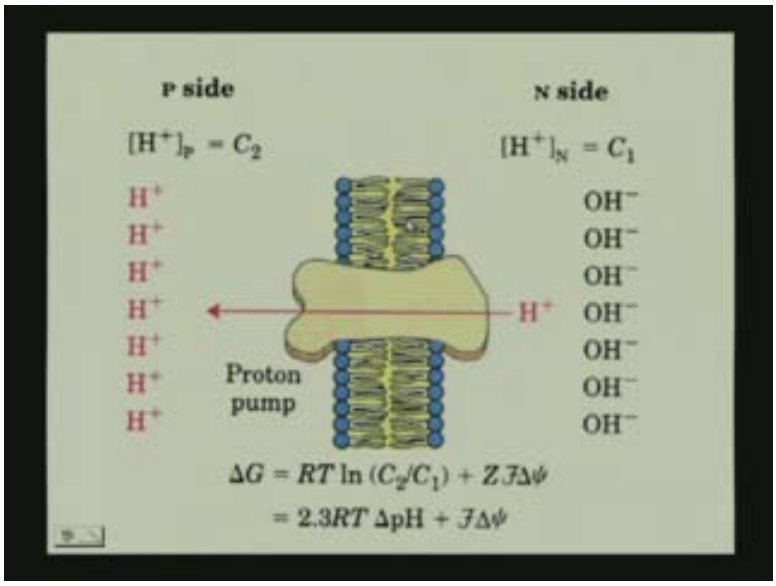

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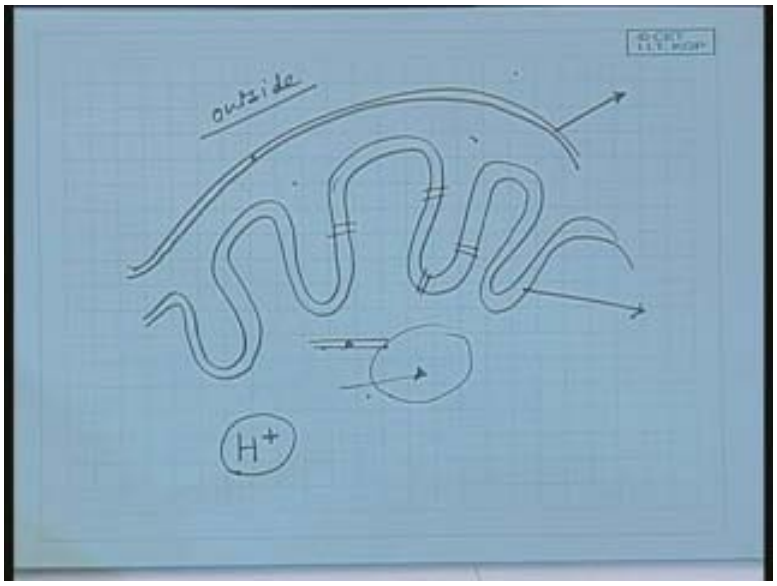

As you see this picture what happens here is this is your mitochondria the outer surface of the mitochondria so this is also a membrane so we have a lipid bilayer here we also have an inner membrane that has these folds. This is the cross section of your mitochondria. This is also a lipid bilayer. These folds are called cristae. Therefore this is the outside of the mitochondria. This is the outer membrane of the mitochondria and this is the inter membrane space. (Refer Slide Time: 36:55) This is the inner membrane of the mitochondria. So this is the inner membrane, this is the outer membrane and this is the inter membrane space; it is not outside the mitochondria. It is within the mitochondria but outside the intra cellular space of the mitochondria rather called the internal matrix. So it is away from this matrix but also away from the outside of the cell. It is this area, this is the place the inter membrane or rather the inner membrane where the process of oxidative phosphorylation occurs.

For the production of ATP we need H^+. ATP reactions occur in the mitochondria, the reactions that we just mentioned the couple reactions occur in the mitochondria so the ATP has to be present in the matrix of the mitochondria for the reaction to occur. What happens therefore is this H^+ is required for the production of ATP. Now if we go back to the slides here (Refer Slide Time: 38:25) we have what is called a positive side that is called the P side and we have a negative side called the N side.

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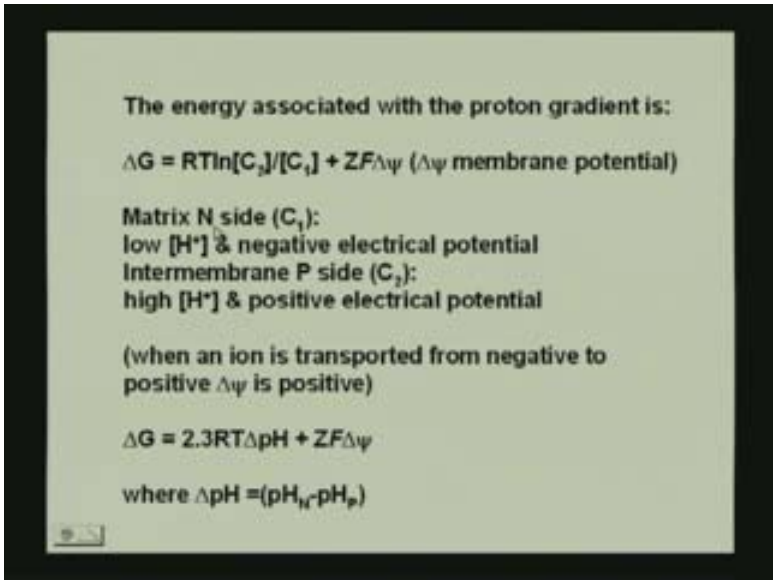

Let me just go to the next slide this one, here we have the matrix side that is the N side so in my diagram this is the N side the matrix, this is the P side that is the positive side. so what I am going to do now is I am going to blow up a part of this region so what you have to understand is we have the whole mitochondria here, we have the inner folds of the inner membrane that are called Cristae of the mitochondria (CRISTAE), we have an inter membrane space that is the P space and N space which is the matrix.

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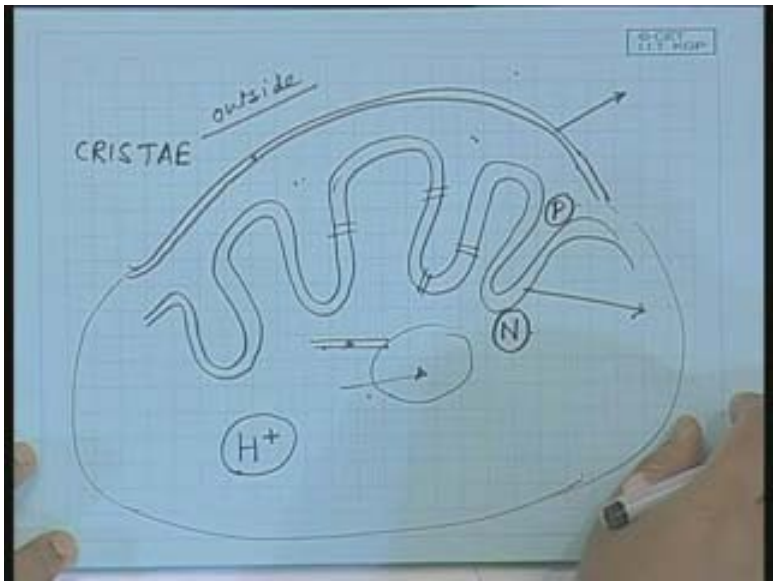

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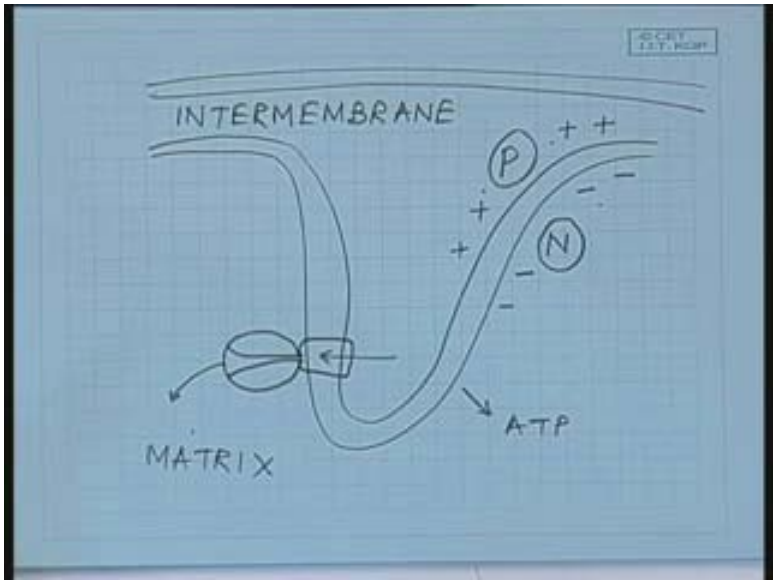

When we look at therefore a single fold so this becomes a single Cristae and we have our outer membrane. This is my matrix and this is my inter membrane space. What happens here is here I have a positive charge which means I have a larger number of protons here and outside or rather in the matrix I have what is called the N side. It is very difficult to sort of mention an inside and outside here because all of this is actually inside but this is inter membrane and this is matrix side. We have a relatively less part or the N side is more negative.

For the production of ATP or first of all we have to realize that we need ATP inside the matrix. The ATP production has to be inside. But for the ATP production we need protons. The protons are on at a higher concentration in the inter membrane space. So what you have to do is we have a certain protein that is called ATP synthase for which we will look at the structure in a moment, where we have the protons that have to get in here and ATP is produced here.

The ATP has to be produced in the matrix because all the reactions are going on in the matrix but it requires a large amount of protons for it to occur. That means what has to happen is protons have to be pumped from the inside to the outside against a proton gradient because there is a positive charge on the outside and a negative charge on the inside. But since we require the protons for the ATP to be produced protons have to be pumped to the inter membrane space, that is what we have here. Therefore we have a P side and an N side. The N side is the negative, where is this N side? It is the matrix of the mitochondria and where is this P side? It is the inter membrane space of the mitochondria.

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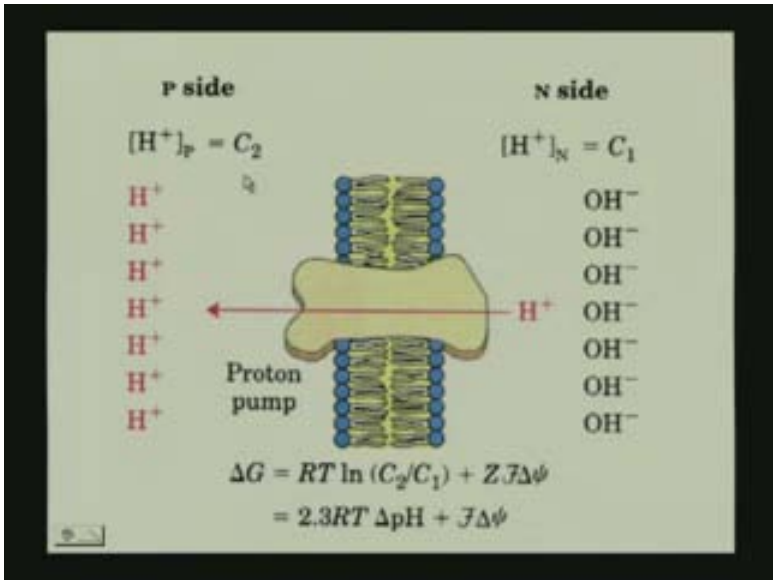

This is where a higher concentration of protons exists but we require an even more amount of protons for ATP to be produced. Hence what you have to do is protons have to be pumped from this to that side. That is essentially what is happening. Now, because of this negative and positive side here you have a membrane potential developed.

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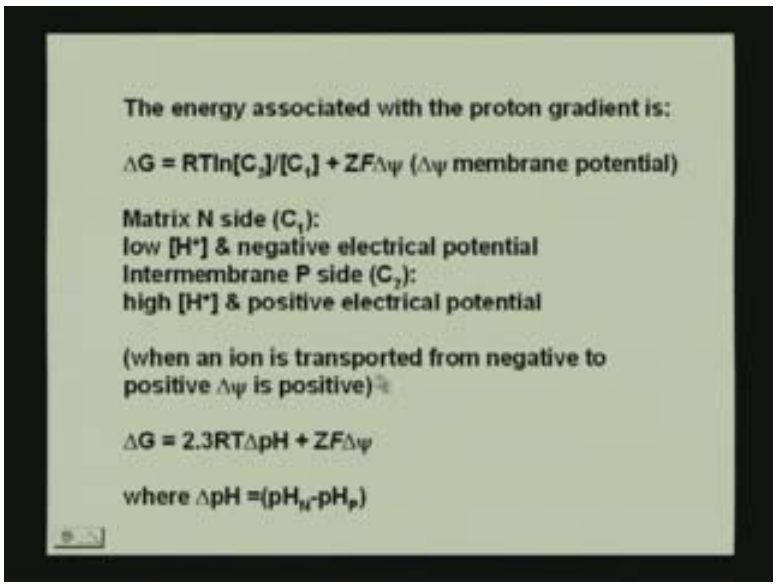

When an ion is transported from negative to positive $\Delta\psi$ which is the membrane potential is positive. Because you are going from a negative value to a positive value what is $\Delta\psi$? It is a positive value. Now, when you have the matrix side the N side that has a low proton or low hydrogen ion concentration a low proton concentration a

negative electrical potential. The inter membrane side which is the C_2 concentration in this case has a high proton concentration and has positive electrical potential. So when you are looking at the ΔG values you have a RT *ln* what is your product in this case? It is going to the inter membrane side so it is C_2/C_1. You have a Z F $\Delta\psi$ which is nothing but your NFE it is the potential. Now, when you consider the low H^+ concentration and the high H^+ concentration you can link the logarithm of the hydrogen ion concentration with the pH. So if we just work this out I have my ΔG i.e. $\Delta G = RT \ln \frac{C_2}{C_1}$. Where is my C_2? My C_1 is low H^+ and my C_2 is high H^+ and you have to remember that the high H^+ is in the inter membrane space and you are still pumping in H^+ to that space because you have to make ATP. This is plus your $ZF\Delta\psi$.

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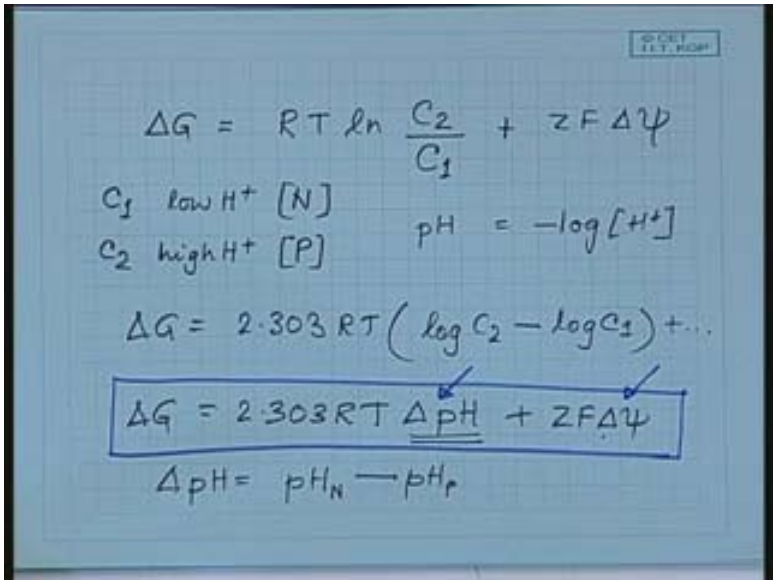

Now if you want to convert we know that the pH = -log[H^+]. So all we have to do is relate this with the pH. So we can write this $\Delta G = 2.303RT \log(C_2 - C_1)$ 2.303RT other part. Now what we have here is, it is going to be $\Delta G = 2.303RT\ \Delta\text{pH}$ where ΔpH is going to be equal to then the pH_N going to pH_P side i.e. $\Delta\text{pH} = pH_N - pH_P$. Now where is this low H +? it is on the N side and where is the high pH? The high H+ is on the high H^+ means low pH so we have this relation. This is your ΔG.

So basically what you are doing is you are using this relation (Refer Slide Time: 47:12) based on the delta pH, what is this delta pH? It is the difference of the hydrogen ion concentrations between the matrix and the inter membrane space. This is the membrane potential, what membrane potential? it is the inner membrane potential. Therefore we have the energy associated with the proton gradient. Now, when we look at the energy available from electron transfer it is conserved as a proton gradient and what happens is when we have this reaction of NADH going to NAD+ you recognize that if NAD+ is

going to form NADH there has to be a reaction that is going to get it back to NAD+ so it can be reutilized just like you would have your enzymes.

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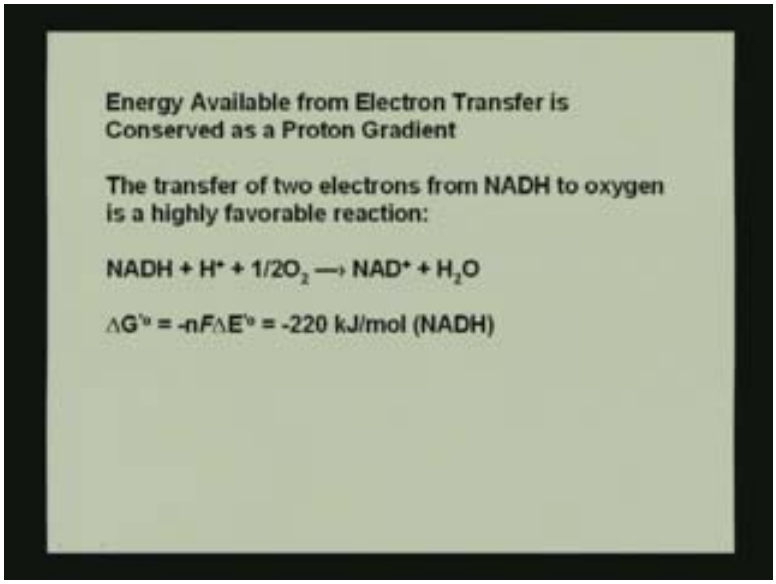

So similarly as we are breaking down the ATP we have to produce ATP and we will we will look at the production of ATP also. So there are certain reactions for example this reaction that will more than compensate for the amount of the $\Delta G_0^{'}$ that you need for this proton pump. You see what a very large amount of energy this is -220 kJ mol^{-1} and this energy will be utilized for your proton pump to maintain the proton gradient. Why do we have to do that is because we have this proton pump, we have the H^+ to produce the ATP that is why we require this.

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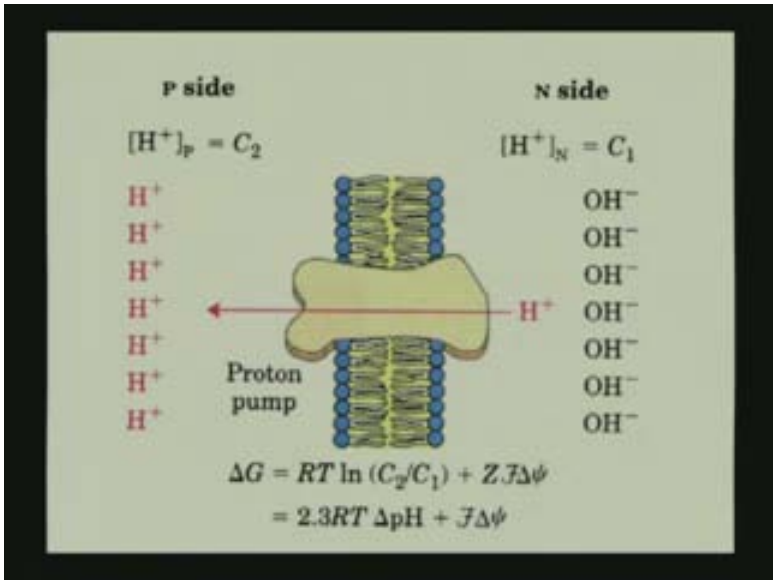

So this is what is the proton pump. We have our specific requirement where the protons have to be pumped from the negative side to the positive side for the production of ATP.

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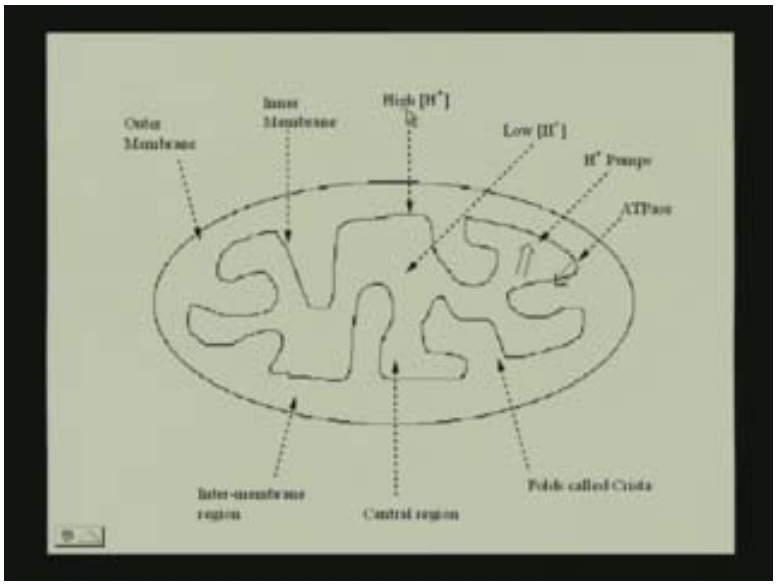

So we have basically the picture of the mitochondria here. This is the outer membrane and both of these are liquid bilayers. We have an outer membrane, we have an inner membrane and within the inter membrane space is high H+ ion concentration. We have a low H^+ here the hydrogen H^+ is pumped from the lower H^+ concentration to the high H^+ concentration because **excuse me** ATPase there is a protein called F_1 F_0 F_1 ATPase, ATPsynthase that does nothing but synthesize ATP.

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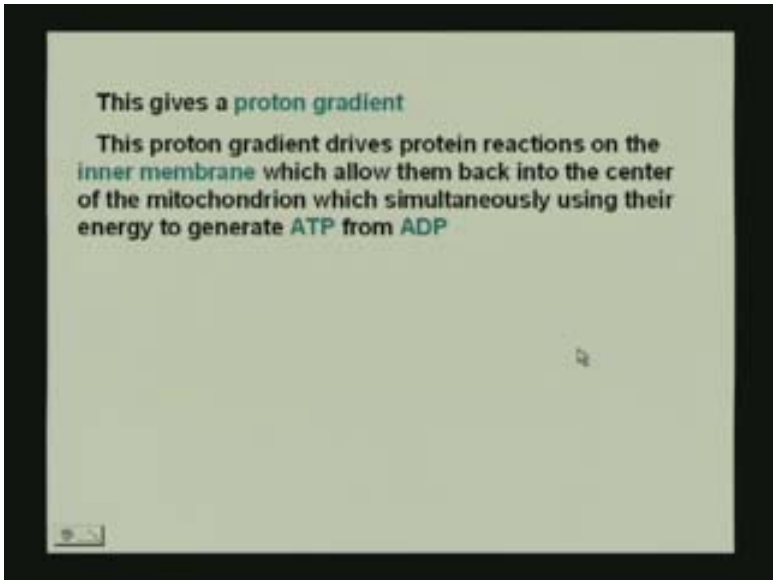

And this proton gradient drives protein reactions on the inner membrane which allows them back into the center of the mitochondria which uses this to generate ATP from ADP. Essentially what you want to do is you want to produce ATP which is why you are pumping all the protons into the inter membrane space for the production of ATP. This ADP the purpose of this oxidative phosphorylation is to use the energy to make ATP that is accomplished in two steps.

First we have the proton gradient then we have a transfer of electrons through a series of systems. We are not going into the details of the systems but this is the essential reaction that takes place where n is about three so it means that we have to pump in three protons for the production of one ATP. So three protons have to go from where, we have to get the ATP, we have to go from ADP + P_i + n H + P that is the positive end to ATP + H_2O in the negative. Therefore in the matrix side the ATP is produced. Why is it produced there? It is because all the reactions that are taking place do not happen in the inter membrane space and all of the enzymatic reactions occur in the matrix space.

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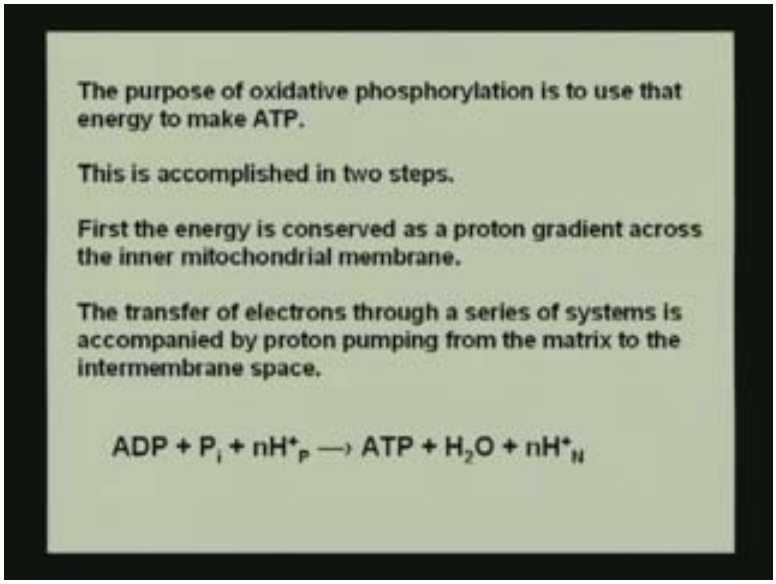

Actually I will just show you this.

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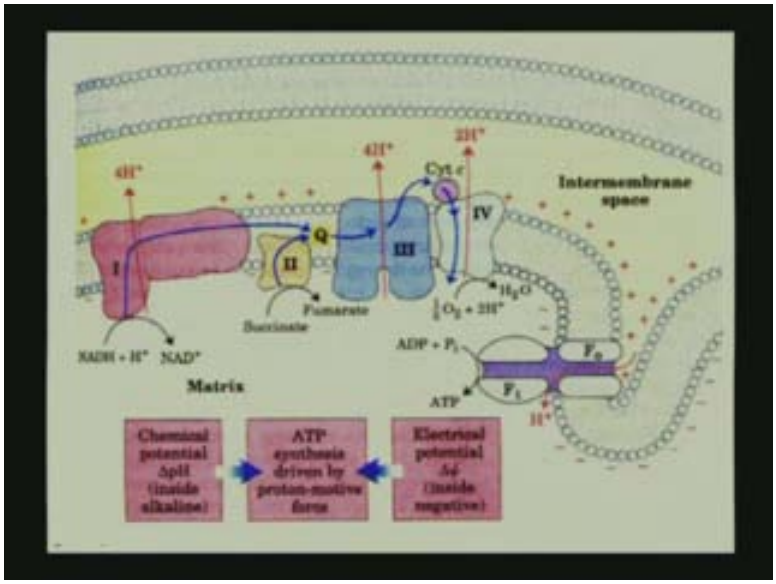

This is where we have the inter membrane space where we have a large number of positive. We have a protein that is called the ATP sythase the details of which we will do in the next class where we are looking at a series of reactions that are actually going to get us into the formation of ATP. What we learnt today was how we can actually derive from vitamins specific cofactors and coenzymes that are going to result in coupled reactions. We have ATP, the hydrolysis of ATP is going to give us enough energy to couple with another non spontaneous reaction to give us a spontaneous reaction like the

example I showed you the hexokinase where we are going from glucose to glucose six phosphates and we are getting the phosphate from the breakdown of the ATP.

In the dehydrogenase or oxidase reaction we are using NAD^+ or FMN or FAD that are going to be coupled with enzymes such as dehydrogenases or oxidases because the compounds have to lose their hydrogens and these are going to be utilized or in the redox reaction they are going to be taken up so either you have to have a reduction or an oxidation and based on what reaction you have the enzyme is going to have as a cofactor either NAD^+ or FAD. What we will see in the next class is how this ATP is actually formed and we will then go on to the metabolism of carbohydrates.