Biochemical Engineering

Prof. Dr. Rintu Banerjee

Department of Agricultural and Food Engineering

Asst. Prof. Dr. Saikat Chakraborty

Department of Chemical Engineering

Indian Institute of Technology, Kharagpur

Module No. # 01

Lecture No. # 33

Receptors-Mediated Endocytosis

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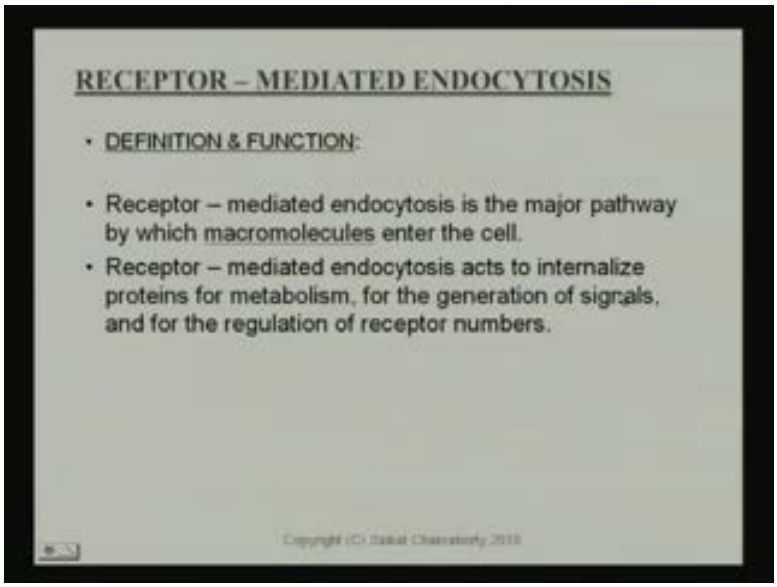
So, today we start a new chapter titled, "Receptor- Mediated Endocytosis". So, what we have been doing in the last lecture or last few lectures was receptor ligand binding.



So, we are essentially looking at the chemistry of receptor ligand binding, the kinetics and how it could be a linear kinetics, a simple linear kinetics or a bimolecular kinetics and so on; you know. So, now what we want to do is you want to study the main process of not of receptor ligand binding, but what receptor ligand binding leads to, where does it, what is its functionality in the body. And, so, we, one of the major processes that receptor ligand binding is involved in is known as receptor-mediated endocytosis. Now, I will talk about endocytosis, what it means and so on and all the details.

So, let us. So, that is a whole idea. You know apply whatever we had learnt in this new chapter and apply whatever we had learnt in the old chapter about receptor ligand binding kinetics to the process. And, the process itself that we considered, concerned with here is receptor-mediated endocytosis. So, let us go to the slides and look at it. So, this as I said, the chapter is titled receptor-mediated endocytosis. So, what is the definition and function of receptor-mediated endocytosis, we need to understand that. So, what we do in receptor in endocytosis; forget receptor-mediated, just endocytosis itself is that, it is a major pathway by which macromolecules can enter the system. And, you see I have underlined the word macromolecules here. What am I trying to say you over here, we had studied, you know previously in another course that how micro molecules… how small molecules enter the cell. right

So, there are transport processes and channels, passive diffusion, ion pumps and different kinds of, you know co-transport that is transport in different directions. One is you have a carrier mediated transports. So, the carrier will take one of these. So, these are all processes through which small molecules enter the cell; micro size, micro molecules enter the cell. And, these are natural processes, which are part of the part of the cell transport mechanism. Now, how does, how if you want to transport a macromolecule, a very large molecule inside the cell, how do you do that? Because the cell does not have an intrinsic or an inherent mechanism to do that, and the process to transport macromolecules inside the cell is known as endocytosis. You have another process which is the opposite of that and that is the process of transporting macromolecules out of the cell and that is known as exocytosis. So, receptor- mediated endocytosis. So, endocytosis is a major pathway by which macromolecules enter the cell and receptor-mediated means that endocytosis, which is mediated or facilitated by receptors. Like, you have carrier mediated transport for micro molecules, for small molecules; here, you have receptor-mediated.

So, that is a major difference between, say one of the thing that you know that similar to receptor-mediated endocytosis, this is carrier mediated transport. So, what is a major difference, receptor-mediated endocytosis is for macromolecules and carrier mediated transport is for micro molecules. That one is carrier mediated where one of the carriers, essentially binds with the protein or whatever you want to transport. And then, transport it inside and its associates. Here also, there is a similarity; almost a parallel, you know

correspondence to that. That is one of the receptor itself, you can think of the receptor as a carrier which binds to the ligands, bring the ligands in or the whatever you want to transport the macromolecule which is the ligand, brings it in and then throws it back.

Now, when do you study the process in detail, of what is this process of bringing it in and then throwing it back into the cytoplasm, you are going to study this process in detail. But, all I am trying to say over here is, there is the almost of one is to one correspondence between carrier mediated transport and in this one. Only difference, two difference is carrier mediated transport transports micro molecules; receptor-mediated transport transports macromolecules a. And, b is that carrier mediated transport is much simpler, the mechanism is lot simpler. Here, you will see that the mechanism is lot more complicated. There it was only dealing with Chemistry; here it is Chemistry and lot of physical involvement because macromolecules are large. So, you need ways to entrap those macromolecules, which is very different. You follow what I am trying to say. So, because the micro molecule is small, see you can just bring it in, but because the macromolecule itself is a volume, so there are ways to entrap this. And, what we will give special attention to is that, if what is the micro molecule is a bacteria or virus that you want to macromolecule is a virus or a bacteria that you want to kill.

So, what you want to look at. So, that is the another thing we are going to look at. So, the second point over here is that receptor-mediated endocytosis acts to internalize. This is how you kill a bacteria or virus. It internalizes the protein or whatever is the macromolecule is does not have to be necessarily protein and generation of signals and for the regulation of receptor numbers. So, let us go one by one to these three points. So, first one is internalization of protein for the metabolism. As I said that the macromolecules are larger molecules and there has to be a mechanism to bring this inside the inside the cell. It is not as simple; the mechanism of bringing this inside the cell is not as simple as it was for carrier mediated transport, where it just simply reacts. So, there is a mechanism and this mechanism is called the process of internalization. And, we will study this in much more detail, today itself.

The second one is a generation of signals; you know. As I said that receptor-mediated endocytosis, receptor-mediated transport or receptor ligand binding in itself plays a very vital role in generation of signals; you know in in neurotransmission. So, transmission

from one part of the body, say between the brain and another part of the body and it has a huge of impact; you know. And, I discussed this, in one of the earlier slides here.

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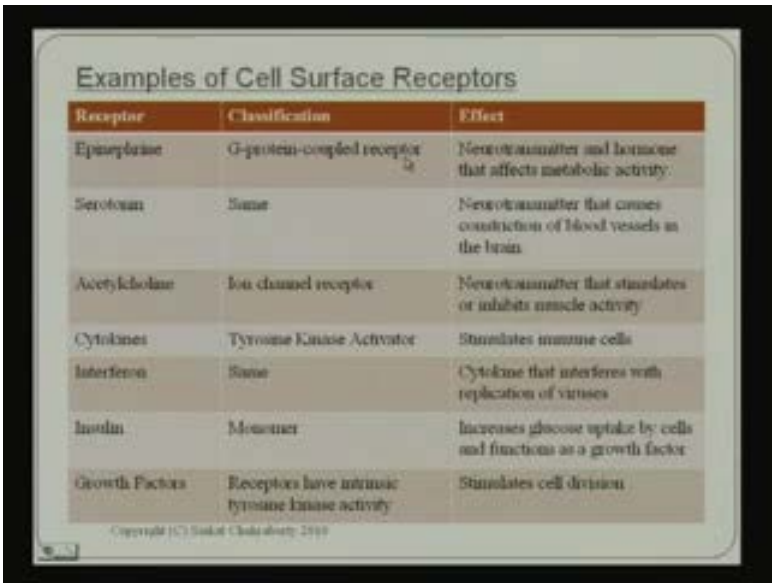

That the impact it has on or function it has in transformation in different kinds of neurotransmitters, as you see these are receptor-mediated. These are receptors of one that you see on the left, Epinephrine, Serotonin, Acetylcholine; the first three for example.

So, they are neurotransmitters. So, they help in connecting two distant parts of the body through cell signaling. So, cell signaling is something like, telephoning; you know. So, each one part of the body, which is far away from the other part of the body, is sort they are sort of telephoning each other and talking to each other and telling that I need this, more of this it is like two, you know two business peoples talking to each other…So, I need more of this supplier, this chemical and somebody saying, now I am, I have got too much of it and I need to throw out. So, there is this whole talking that is going on. In the central nervous system is sort of nodal points there; you know. So, the central nervous systems is, so, if one part of the body, which is very distant from another part of the body has to talk to each other, then it has to go to the central nodal point which is like the telephone exchange out there, which is a central nervous system.

So, it sends the neurotransmission. It neurotransmits to the brain and that the brain will neurotransmit back to another. So, these neurotransmitters and hormones, these are these are some of the examples. And, so this is the cell signaling part. So, this, so, the receptor

ligand binding plays a very vital role in cell signaling. Another thing that I had talked to in the previous lecture or the lecture before I think was about t cells; that immune cells, for example. So, when there is immune attack on your lung or some other part of the body, how would that part of the body inform that to the brain that there has been an any immune attack on the system? right. So, there also cell signaling is important. So, there are there is certain cells. For example, in the lung there are t cells, which will relay this message. So, there are these messengers. So, these, there they will relay this message. But, where does receptor ligand binding play a role? It is that, when they whenever, say for example, there is an immune attack. So, certain ligands, in this case say a bacteria or a virus is going to bind to the receptor. And, as soon as the receptor those see the, those receptors that are that are there.

So, for that particular virus or bacteria, so those are sensor receptors that are there. So, they will bind specifically to a bacteria or virus. And, when they bind specifically to a bacteria or virus, then they immediately, so these are like you know spikes out there in the lung. So, immediately they send a signal back to the central nervous system or the brain that some bacterial virus has been caught here and it is very likely that, there is an immune attack on the system. Then, the brain sends signal back to the lung saying that, yes there has been an immune attack. So, if not just one may be two or three of these t cells send these message across that there is an immune attack on the system. Then, what happens is that the lungs and the brain says, yes there has been believed that there has been a total immune attack on the system and it needs to shut, starts the immune response; which means that you can talk about that little later. Immune response means essentially that the scene would be geared up; the whole immune system would be geared up. Or, in other words, what it means is that it goes from the resting state to the activated state. These are biological terms that I have used. And, the comparison is like, as I said between from peace state to the war state.

So, when enemy has attacked the lung and or the or the lung and the system needs to go from a war state. So, whenever you know that the nation is in a war, so the war state mentality is different from peace state mentality. In biological terms, these are called not peace and war state. They are called resting state and activated state. So, the system goes from the resting state to the activated state. So, this activation process is also done through cell signaling. And, everywhere there is a cell signaling. And, cell signaling has

become a very major path of part of biological studies. These just including in chemical engineering. So, everywhere there is a cell signaling receptor ligand binding plays a very important role.

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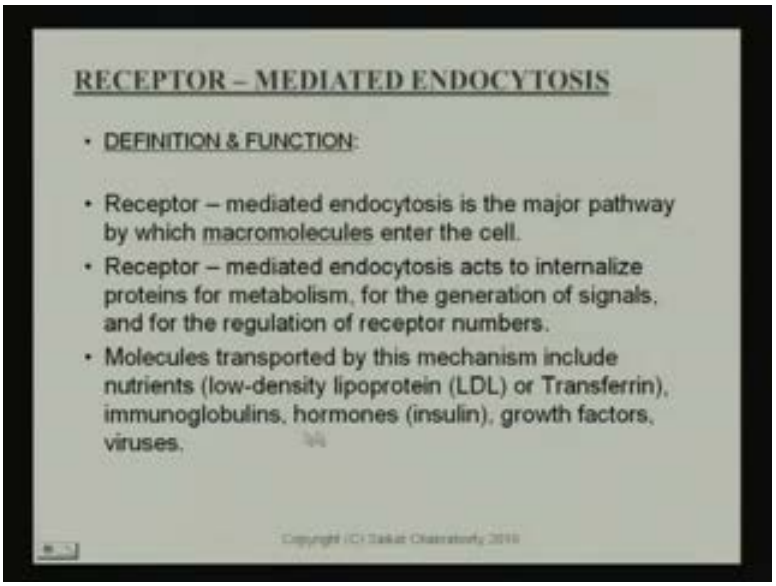

So,.. So, these are the three points I think that we are talking about; the protein, internalization of the protein, explain that, generation of signaling, which is cell signaling and the third one is the regulation of the receptor number. And, this is equally important. what happens is that the receptor number in the cell can change. So, till now in our earlier calculations, if you remember in the calculations that we did in the last chapter, we always assume that the receptor number to be a constant and n… t to be a constant. But, in reality that is not true because within a cell or within any particular organ, the receptor numbers are changing. The reason they are changing is they are dying or they are they are you know they are being produced, whatever is the case.

So, receptor numbers are changing because of that. Now, when these receptors numbers are changing, there is a necessity for the cell to regulate the receptor number. Say for example, receptors are dying and they are continuously decreasing, now what will happen? Two things will happen; the ability of the cell to do a process like endocytosis will decrease; that is metabolisms. So, endocytosis is very important, say for example, you want to you have a very large macromolecule, a protein or a something, a carbohydrate, large macromolecule, which cannot enter the cell. Glucose can enter the

cell, of course through carrier mediated transport. But, much larger than that you wanted to, you forget the immune part of it; just the fact of metabolic part of it. So, you want that macromolecule to enter the cell. It is not possible for the macromolecule to enter the cell. And, the example that we will do is life of proteins; through the rest of this chapter that these life of proteins are very large molecules and you can have large sugars also; which you know, if you cannot internalize and then it can lead to diabetes.

So, the one that we are going to look at is life of protein. And, these are large molecules; there is no way to internalize these large molecules without being able to, without the physical internalization. There is no other way to of bringing this transporting this very large molecules because hand pumps these are not...you know molecular channels are not going to work. You cannot do carrier mediated transport because this is too large for a carrier mediated transport. So, the only way you can get this inside the cell is through the process of internalization. And, the process of internalization is therefore important for metabolic activity. Right.

So, that that is one part and again the cell signaling is a is the other part. So, the regulation of the receptor number, as you see over here the last point is important for both these activities. So, if you, if the receptor number decreases beyond a point, then what will happen? That, internalization of the proteins that you want to do is not going to happen a. And, if the receptor number decreases a lot then you cannot have cell signaling also. So, the, both these, it is important to regulate. Now, what happens if the receptor number goes up a lot that is also no good either. All right. So, if the receptor number decreases, we understood what would happen; if the receptor number goes up a lot that is no good either. The reason being that you will have too much internalization of proteins. You know, it will be beyond the levels at the that a cell can take or your cell, your system could be too sensitive to any kind of cell signaling.

So, you have a message, just one message and you are not supposed to… send that message back to the brain. And, you send that message back to the brain and our whole immune response could be triggered off; where it is not important. Then, you have the examples for this is people, who have problems with tonsils. So, what are tonsils? Do you know what are tonsils? Tonsils are essentially the immune cells; they are a bunch of immune cells. So, lymph nodes, lymphoids and their essential function is immune action. So, there is essentially the first level of immunity, even before it enters the lung. So,

when you say that somebody has a problem with tonsils. So, what is meant is that you have a hypersensitive tonsil. So, tonsils, main job of the tonsils is to sense that there has been an immune attack on the system and send that message to the brain. That is all the tonsil is supposed to do.

And, if you have a hypersensitive tonsil, what happens is that like I got, you know, so, what happens is that it sends a message back to the brain. You do not have a problem essentially, there is no not much of an infection attack. You know, infections are there everywhere. You know you are breathing in infection all the time. So, may be a very trace amount of infection by this tonsils senses that and thinks that… use in infection attack, immune attack on the system and it sends the message to the brain and that brain starts the whole immune response. You start to get fever, your immune, you know system is enhanced; you go from the, immune system goes from the resting state to the activated state and so on. All this entire process starts off in the in the tonsils. So, why, what would happen? You know essentially that, what will happen is that, when you have a hypersensitive tonsil; on the surface of the tonsil, the cells of the tonsil, you probably have an excess of. So, when somebody has that so-called tonsil problem, so it means that probably there will be an excess of these receptors, signaling receptors; so, which will trigger off the entire signaling mechanism.

So, as the result, what you need to do is, you need to regulate the number of receptors. You do not need it too high, we do not need it too small. So, this is the thing that I just mentioned that the macromolecules. So, the molecules transported by these mechanisms, so include nutrients. So, as I said that, it is not just immune, you know saving the system from immune attacks and virus and bacteria, but they include nutrients. And, this example I gave. Low density lipoprotein(L D L) and immunoglobulins and insulin; you know. So, that is why it is so, receptor ligand binding is so important for diabetes and growth factors as well as virus and bacteria.

So, this is one part of it. The immune system part of one part of it, and then these nutrients, these are all important for the growth and metabolism in the in the body; the L D L, the insulin, and so on. The example that we will take in this chapter is an example of L D L, low density lipoprotein.

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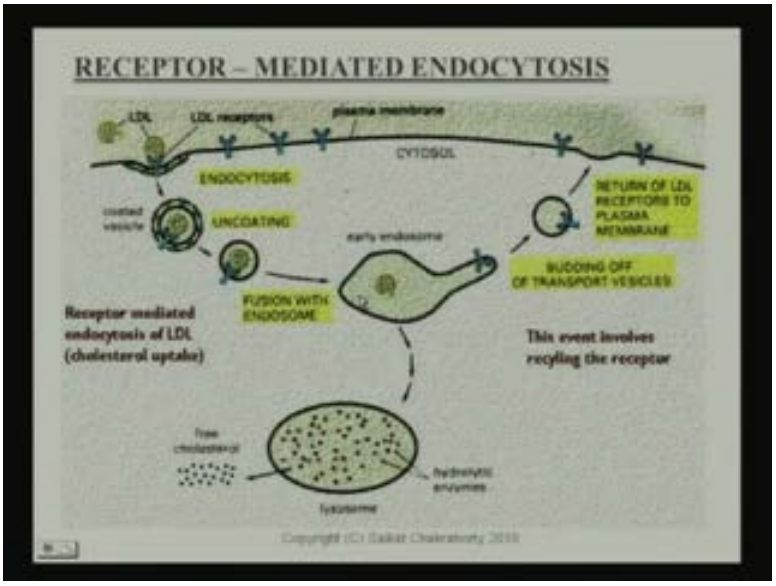

So, this is a picture, I think you can see it properly. So, can you see this properly? So, this is the picture for L D L, the low density lipoprotein what happens. So, I have better picture. This is, let us just have a quick look at this. So, this is a L D L molecule, low density lipoprotein molecule, which is a large macromolecule, which cannot enter the cell otherwise. And, you have these L D L receptor result here. So, these blue things are the receptors. So, this is how you always represent a receptor; a cup like structure, which is stem out there and ligands are always represented by dots.

So, when they combine, you represent them as the, so this is the ligand with the dot and this and then, combined form would be represented as the cup like structure plus with the dot on it. So, even when you draw your pictures, this is how you are supposed to draw. So, this is the plasma membrane. And, as I said before that the receptor is such that a part of it. The stem is inside the cytoplasm and the cup like structure is portraying out of the cytoplasm. And, this is for to capture. And, this is, this called a coated pits. And, I will come to that little later. This this this protein structure that you see over here is called a coated plate. Let us not worry about that at this point of time. Let us look at the simple thing.

So, this lipoprotein comes and binds in the ,with the receptor. Then, this coated pits that you have over here, forms what is known as a vesicle. And, I will tell, talk about that how this vesicle is formed, and all that stuff we will do this in details. So, forms what is

known as a vesicle, then the vesicle coating goes away and you have these uncoated vesicle. And, you know, so this this this thing grows a little bit and you have this endosome. Now, in the endosome, I will discuss this that what happens is that, there is a pH change in the endosome, as a result.

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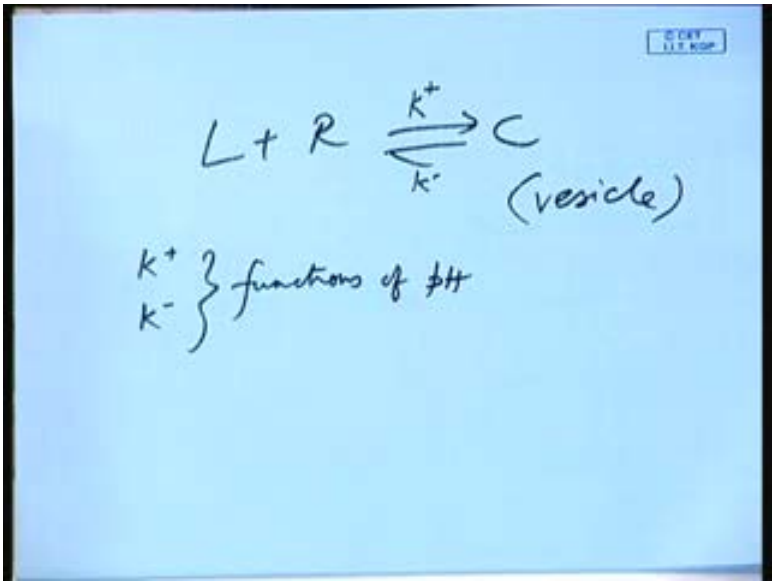

So, whenever the receptor ligand binding process is taking place, how is, what is the reaction L plus R giving. So, this receptor ligand binding, for example, L plus R giving complex. Right.

So, in the vesicle you have this complex. Right. So, the coated vesicle that you have in that case I am coated, you have the complex. But, remember, you have this k plus, let us call this and let us call this k minus. So, you have these two rate constants. And, k plus and k minus are functions of pH; of temperature also and pH. But, here pH is called as... So, what happens is that the pH, the endosome, so, if I can go to the screen now, inside the endosome here, you have here the L in the complex form. Now, inside the endosome the pH changes. Typically, they decrease the pH, they acidify the endosome and decrease the pH. Why they do it is if you decrease the pH, then the backward reaction is facilitated. You have the k plus a forward reaction rate constant if the backward reaction rate constant.

So, the forward reaction rate constant is facilitated or you know enhanced that higher pH; whereas the backward reaction rate constant increases that lower pH. If the pH in the

system is lower, then the backward reaction happens. Right. So, then what happens the complex dissociates preferentially into the ligand and the receptor. So, this is what has happened. So, you see inside this in the picture. So, the complex is dissociated into the ligand and the receptor. And, then you form this, you know lysosome. What is basically, so, you have internalized now, what has happened? This L D L was outside the cell, now you have been able to internalize this. And, this is, you know broken down and all these things and the receptor is sent back. Why is the receptor sent back because the body is a very major thing. You know, it does not want to waste anything. So, as I said it needs to regulate the number of. So, this receptor sitting inside the cell cytoplasm makes no senses. It is not going to function. It is not going to do its job.

So, it has to be sent back to the cell surface where it will continue doing, what it is doing. So, I hope this is clear. Right. So, we will come back to it. I have better pictures and more detailed discussion.

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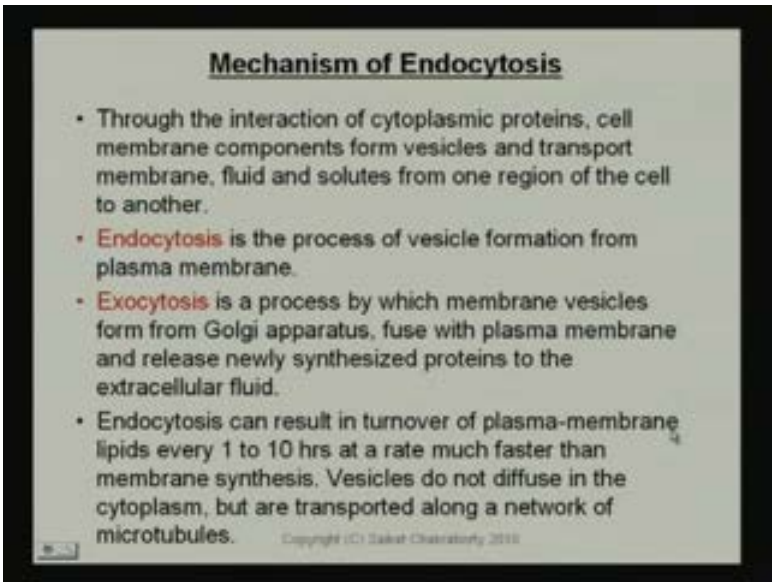

So, let us look at the mechanism of endocytosis. This is I have already explained that. It is just going point by point. Everything that I said is what is written here. So, through the interaction of the cytoplasmic protein, cell membrane components form vesicles and transport membrane form vesicles and transport membrane fluid and solutes from one region of the cell to another. Fine. This is no no big deal out here.

So, endocytosis; there is a definition of endocytosis. It is a process of vesicle formation from the plasma membrane. So, you have, this process of vesicle formation out here is known as endocytosis. So, outside it was there, it entered and that step is known as endocytosis

Exocytosis is a process by which the membrane vesicles form from Golgi apparatus, fuse with a plasma membrane and release newly synthesized proteins to the extracellular fluid. We are not studying exocytosis in this chapter, but this is just to inform you what the definition is. It is the reverse process. So, if you synthesize something inside and you want it into the blood stream, so this is the process. Just the opposite process of endocytosis. And, endocytosis can result in turnover of plasma membrane lipids for every one to ten hours at a rate much faster than membrane synthesis. Vesicles do not diffuse in the cytoplasm, but are transported along a network of microtubules.

So, what this first sentence means is that if you go back to the picture, this process of the membrane turnover that is a membrane being recycled in and out; that can happen over ten hours. So… with endocytosis, the membrane itself over a period of ten hours can be recycled in and then out. So, the whole membrane recycle process. And, vesicles do not diffuse in the cytoplasm, but are transported along a network of microtubules; which means that once a vesicle has been formed over here, it is not going to diffuse from one part of the one part of the cytoplasm. But, there there are some microtubules that are formed inside, through which the channel like structures, through which it can flow.

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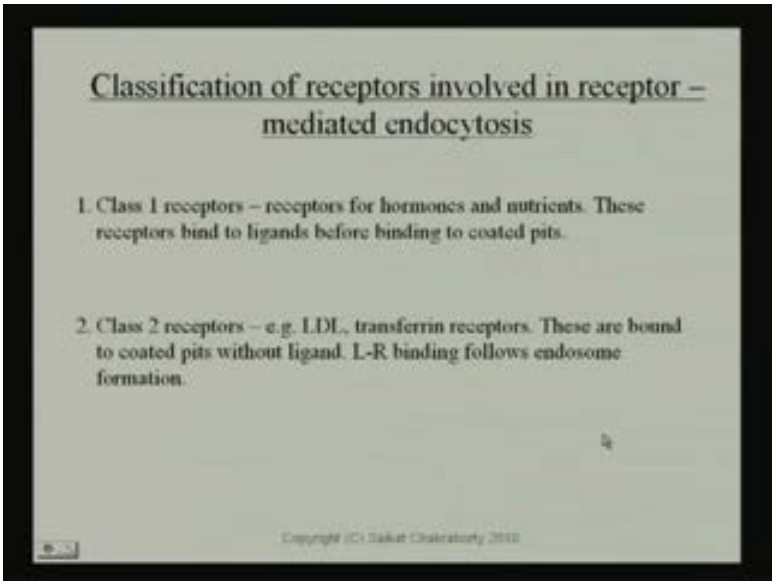

So, this is I think important and this will be necessary what is up there now, on for our analysis. So, classification of membranes involving involved in in receptors, involved in receptor-mediated endocytosis. So, there are two kinds of receptors that we typically talk of. One is called a class 1 receptor and the other one is called a class 2 receptor. Let us go to the definitions and I will explain in the next few minutes. So, class 1 receptors are receptors for hormones and nutrients. And, these receptors bind to the ligands before binding to the coated pits. Let us just go through the definition now and then I will explain in a minute. And, class 2 receptors are receptors like L D L. These bind to the coated pit without the ligand and the ligand receptor binding follows that.

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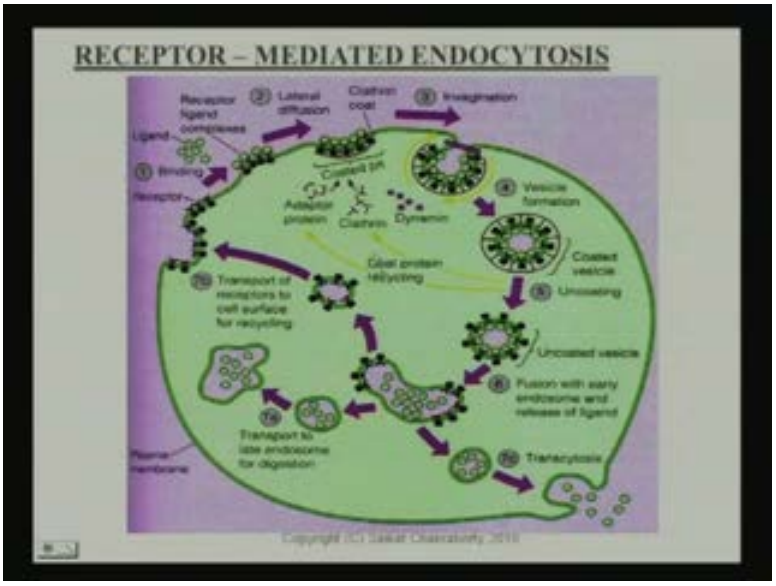

Now, let us see what this is. So, this picture that you see over here may be I have another picture. Yes. So, this picture that you see over here, see this is a receptor. These are the receptors that is there and then if this receptor binds to the ligand, you see that where the arrow is.

So, this is this is these black dots are my receptor and my receptor goes and binds to the ligands. The green things are the ligands here. And then, the coated pits are formed. And, let us see, maybe we have the other way around here. Oh! it is the same kind actually. I am sorry, but anyhow.

So, this is this receptor you know, binds to the ligands before binding to the coated pits. So, this is my class 1 receptor and what will be my class 2 receptor? would be varied first, forms the coated pit and then it, so, there is a very little difference, but up to the, so whatever difference we have is up to this point two, you know number two, beyond that it is all the same the receptors. And, the class 1 and class 2 receptors are all the same.

So, you have the binding process over here. So, it forms a receptor ligand complex. And then, these complexes then diffuse laterally. And, this is one thing that is slightly different. And, we will come to that in the next lecture of the lecture after that. If the diffusion of these coat of these bound things, a complexes; so, when one of the major difference is that, when the system binds to the, when this receptor… complex the in case of class 2 receptors, when the coated pit is first formed and then the complex is

formed. And then, the lateral diffusion will take place for the complex itself at the whole; whereas in this case, the lateral diffusion would be, sorry, the lateral diffusion would take place of for the coated pit with the complex. Ok.

So, the coated pit with the complex will move laterally. Fine. Whereas, here it will be the individual complexes that have to move together, if there is to be a lateral diffusion followed by the formation of the coated pit. And, this is the, you know these proteins come together. And, we will study that later. These proteins come together, the adapter protein and clathrin and dynamin, they come together to form the coated pit. So, the coated pit is essentially like, a thicker part of the, membrane like kind of thick ends. You know, another layer of, you can think of that that another layer of protein adds to the membrane and thick ends of membrane. Why is that, why is that done, why do you think that is done…

Yes. So, not, yes. That is one part of it. Essentially that you, when you form the vesicle when you form the vesicle, you want to make sure that that it is mechanically stable. there is mechanical rigidity of for the vesicle. And, the vesicle does not rupture. And, as we said that if the vesicle ruptures, then what will happen if there is a virus, if there is the, these are nutrients, then no problem at all. But, if there is a virus, then if the cell vesicle ruptures just like that before the bacteria is killed, then the bacteria will actually go and infringe into the cell. And, whatever you wanted to do you will have the reverse effect of; you wanted to kill the bacteria, but you were in a way… facilitating the bacteria to enter the cell. So, that is why you form a structure, a protein structure just to give stability to this entire vesicle; so that, it is not ruptured at any point of time, unless the cell wants it to rupture.

And, you, as you see into this whole process, you will see that there is, you know that is an inherent wisdom of the cell. So, the cell is doing things, in a way that it really wants to do. So, so look here. So, then the vesicle is the, vesicle is formed. And, then the coated pit is thrown out and recycled, so that it can go back to the membrane because a coated pit does not have to be there all through. And, this goes back over here and forms on the surface. Why, so that it can be there to trap in the next set of, you know to facilitate the next set of receptor ligand binding. fine. So, this is now the uncoated vesicle, just as we showed. And then, this process of transformation happens; that is the pH of this system changes. Now, how do you think the pH of the system would change this this vesicle for.

So, from here to here, the pH is obviously changed. Otherwise, what you see over here is that the dissociation has occurred. The receptors have unbound themselves from the ligands. And, that cannot happen, as I just now explained that cannot happen, unless the pH of the system changes. So, how do you think the pH of the system changes?

Yes. Exactly. So, what happens is this, on these vesicles they open up hydrogen pumps, hydrogen ion pumps. And, these hydrogen ion pumps that are opened up pump in hydrogen ion from the outside from the cytoplasm inside the vesicle. And, when these hydrogen ions are pumped in from inside from the cytoplasm, inside the vesicles the pH L system goes down. And, the reaction is facilitated or favored in the backward direction. And, the complex now dissociates into the ligand and the receptor. So, here after this you know sort of dissociation takes place. Then, now it is time for the ligand and receptor to separate out. And, as you can see over here, the ligands go back and stick to the membrane of this vesicle; while the receptor are encapsulated within. And, then it forms these vesicles. And, the ligand form forms a separate part of the, so, receptors form a separate vesicle; the ligand forms a separate vesicle. And, the ligands go back to the cell membrane to do its next cycle of transport; because you have a limited number of receptors, then you do not have the ability to waste them. And, these ligands as you can see here, they could be used for different kind. They could be used for metabolism if their proteins and nutrients that you need, if they are not needed, then they could be thrown out and we will do in a detail.

So, I hope this process, step by step process is clear to all of you. The reason am I saying is because you have to do a detailed modeling of all these steps of the process. And, unless the physical mechanism is not clear to us, it is not possible.

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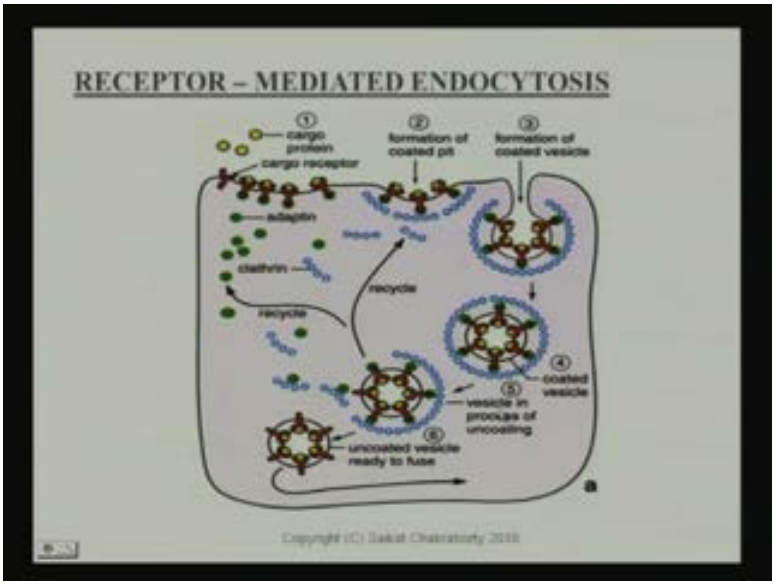

So, if there is any question on this, you can ask. This is a same thing, you know essentially the same kind of picture that the receptor ligands bind to the receptor and then the coated pit is formed. So, plus 1 and exactly you know, very similar kind of thing; the coated goes away. And, I do not think there is any point in repeating this one. Right.

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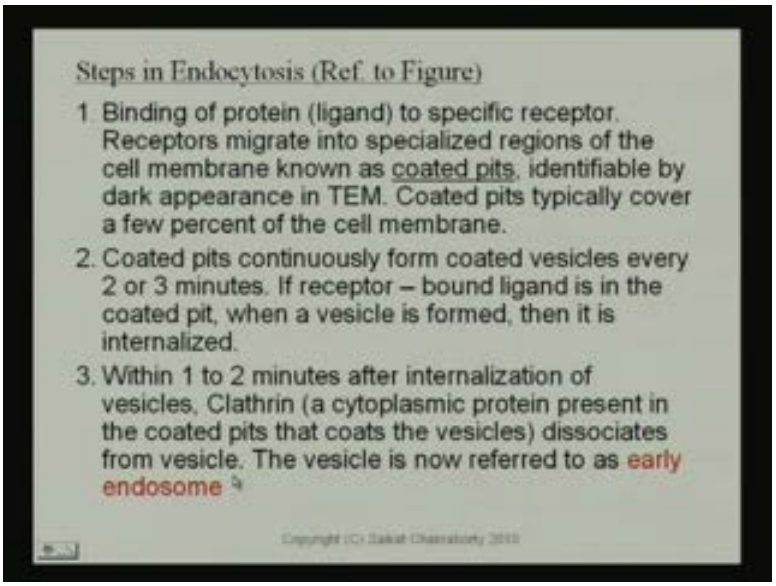

So, now let us go step by step to the process. I think I explained most of them, but it is still, walk through this. So, step one is this; because each of these steps within, when we model the system, we have to understand and quantify each of these steps. So, that is the

whole idea. So, in receptor-mediated endocytosis, first is the binding of the protein, which is a ligand to the specific receptor. So, these are specific receptors. And, we had discussed this at the beginning of the receptor ligand binding chapter that there is a whole issue of specificity. So, one kind of receptor is supposed to bind to one or another; 2 kinds of different ligands. So, the binding of the receptor to specific receptor, protein to the specific receptor. And, the receptors migrate into specialized regions in the cell membrane known as coated pits and identifiable by dark membrane in the T E M. So, coated pits typically cover a few percentage of the cell membrane. So, this again you know, if you should realize that, here we are again talking of which class of receptors? Class 1.

Class 1 is what I showed you. Where the ligand binding, receptor ligand binding takes place before the coated pits are formed. But, here we are talking of the coated pits being formed first. So, this is class 2. So, because we, the reason we are looking at class 2 is, essentially we are planning to model the class 2 kind of receptor. And, I discuss this. So, specific example that we are going to talk of is low density lipoprotein. And, that is the class 2 kind of receptor. So, just small changes at the beginning of the, so the entire process is more or less same; just a small change. So, accordingly you have to vary your model initially. So, if we, if I ask you to do the class 1 kind of modeling, so just a small change at the beginning and then the rest of the process is going to be the model as same.

So,. So, this is my picture. So, these are the, this is the coated pit and this shows this dark, you know dark edges in the transmission electron microscope and they cover a few percentage; up to five percentage of the cell membrane, not a very large amount of the cell membrane. So, coated pits form continuously, pits continuously form coated vesicles for every two or three minutes. So, once to a, this is the time scale of the system. So, once a coated pit is formed, the total receptor ligand formation and the vesicle formation that total process takes around two or three minutes a cycle of that the internalization process. Then, internalization has already happened within a minute or two. After the internalization has happened, this protein and the cytoplasmic protein that is present, clathrin dissociates from the vesicle and the vesicle is now referred to as a early endosome. Why is it called early endosome, how is it different from the late endosome is the pH factor. And, as we just discussed.

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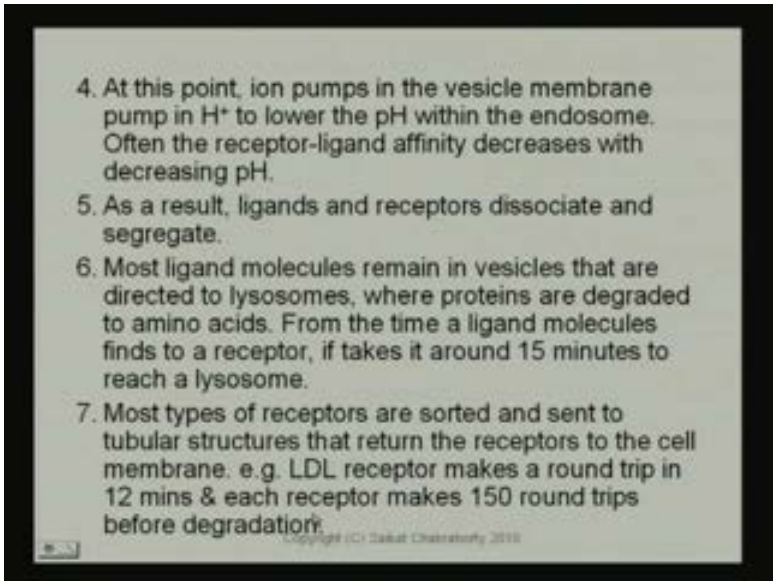

Now, the whole question of pH comes in. So, early endosome as you see over here in the picture, early endosome will have the receptor bound to the ligands; whereas, this is the late endosome where there will be a dissociation. And, how that dissociation is effected is through the hydrogen ion pumps. So, at this point, what happens is ion pumps in the vesicle membrane, pump in hydrogen ion to lower the pH within the endosome. So, the ion pumps open up and hydrogen ion gets streams into the vesicle and the pH of the system is lowered. And, because of the lowering of the pH the receptor ligand affinity decreases with the decrease in of pH or the forward rate constant decreases and the backward rate constant increases. And, the complex now starts to dissociate into the receptor and the ligand. And, that leads to the formation of the late endosome. This is a late endosome.

So, five was the formation of the late endosome where the receptors and the ligands are dissociated and segregated; not just dissociated and also segregated. Segregation how would it help because now this have to split into two different parts. So, if they are segregated, specially segregated, it will, splitting would make sure that one of the endosome contains all the ligands, one of the endosome contains all the receptors. So, most ligand molecules to remain in vesicles that are directed to the lysosomes, where proteins are degraded, if these are proteins they would be degraded to amino acids. And, from the time a ligand molecule finds, binds to a receptor or finds a receptor, it takes around fifteen minutes to reach the lysosomes. So, this whole process that what we are

talking of. Let us see from here, from here all the way to here, this process is around fifteen minutes. And, this entire process is around twenty minutes. That is the time scale of the entire receptor ligand binding process. And, the formation what I said, from here to the form formation of the endosome where which contain. So, from the vesicle, the late endosome and then the formation of the endosome, which only contains the ligand that processes, takes around fifteen minutes.

So,. So, most types of receptors are sorted and sent to the tubular structures that lead, return the receptors to the cell membrane. So, you know let us go back to the picture. So, these receptors that are there in these are microtubules that are there, that are formed. And, these microtubules, so together send the receptors back to the cell membrane, where they will be ready for the next set of receptor ligand binding. And, L D L receptors, this is L D L as I said twenty minutes. But, you know it is not strict for each kind of thing, it will be different, but that is a time scale. So, L D L receptors make this whole round trip, the receptors for in twelve minutes and each receptor makes hundred and fifty round trips before degradation. So, this is a receptor, twelve minutes. The endosome and the ligand will take a little bit more time because you know the ligand formation because the receptors will leave a little early, if you remember.

So, may be five minutes more or something like that further it is ligands. But, the receptor makes a round trip in twelve minutes and each receptor go does up to hundred and fifty round trip. So, what happens after hundred and fifty round trip, these receptors essentially a degraded and they die. Fine. So, this is the, this is the process.

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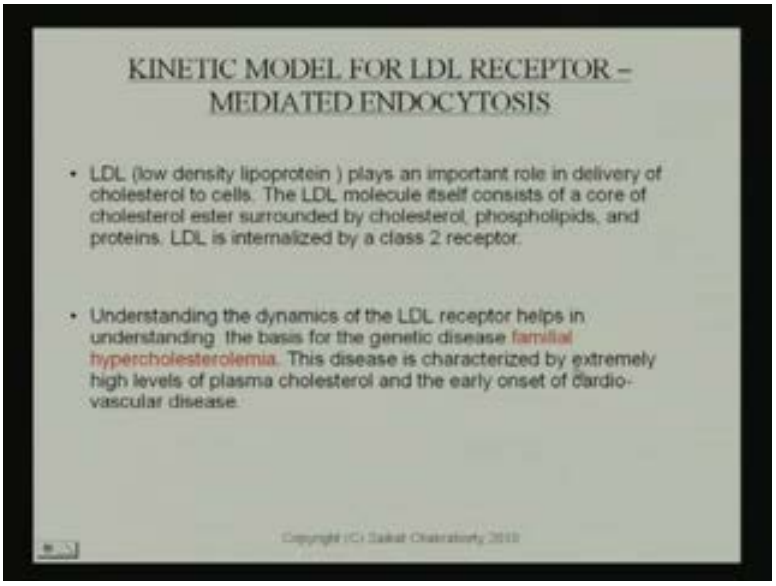

Now, next thing we are going to do is, is there any doubt or question on the process itself. So, the next because if we understand the process, a next thing we are going to look at is the kinetic studies on the process.

So, the reason and the thing that we are going to focus on is receptor is low density lipoprotein. As I said, L R binding for low density lipoprotein. And, the reason we are doing this, you know. So, L D L receptor, so here few words on L D L low density, lipoprotein.

So, these are the ligands that would bind to the L D L receptors. So, when we talk of, I think L D L R is going to be the symbol that we will use for L D L receptors. And, L D L for the protein, I think that is what we use. So, L D L low density lipoprotein plays what is, why do we want it, plays an important role in the delivery of cholesterol to the cell. The L D L molecule itself consists of a core of cholesterol ester surrounded by cholesterol, phospholipids, and proteins. I think, you you know, I do not have that slide with me now, but we had in some point of time shown a picture earlier, of how this looks like with the cholesterol sticking in the phospholipids and the proteins and the cholesterol molecules sticking out. If you remember that was in the previous course. Anyhow, maybe I will get that slide and show it to you in the next class.

So, L D L, I know this is something we had already discussed. L D L, the internalization process is a class 2 by class 2 receptors; which means that the coated pits are formed first

before the binding occurs. And, what we need to understand is a dynamics of the L D L receptor because this dynamics of L D L receptors forms the basis for the genetic disease, which is known as familial cholesterol hypercholesterolemia. This disease is characterized by extremely high levels of plasma cholesterol and the early onset of cardio-vascular diseases. So, what happens in this disease, you know familial hypercholesterolemia, as I said we can, you know there are several diseases which have to do with L R binding. you know diabetes as I just talked about, you know insulin, which is also class 2 thing. What happens in this case, for example, this familial hypercholesterolemia. So, let us look at the terms, each of the terms familial hypercholesterolemia.

So, when we said these two, it consists of three words familial, hyper, cholesterolemia. So, first thing that we are talking about is we are dealing with cholesterol molecule. That is why this a word cholesterolemia, hypercholesterolemia; excess of cholesterol. And, familial means it is a genetic disease. So, three points; it is a genetic disease which involves excess of cholesterol, but my question to you is excess of cholesterol where? In the blood stream. yes.

So, what is happening, it is not that people who, and this is a genetic disease that we have already said. So, people who suffered from this disease, say familial hypercholesterolemia ,it is not that they are in taking a lot of fat fatty things, even if they reduce their fat and keep it to a very normal level or even below that normal level, still then, it is not going to make much of a difference. What is happening is that that these fat, these low density lipoproteins which are important for the cell, they have to be internalized. There is no other way. These are macromolecules and they are only way they can enter is through this internalization process. And, this internalization mechanism is something that has gone caput in these people. You know that is not, what is not working this internalization mechanism.

So, as a result whatever cholesterols it intake, end up being in the blood stream. And, that is why, when I think sometimes we do not understand, when we say high cholesterol we do not understand where it the cholesterol is. The point that I am trying to make is, this cholesterol is in the blood, the cholesterol remains in remains in the blood; whereas, it should actually be go going to the cell. And, going through the inside the cell, it should go through a metabolic process. So, that is being hampered. And, it remains inside the

cell, inside the blood. Sorry. And, what happens when it remains inside the blood? It leads to cardiac diseases. Can any of you think of why it leads to cardiac diseases?

It reduces… How would you do that?...if it settles…

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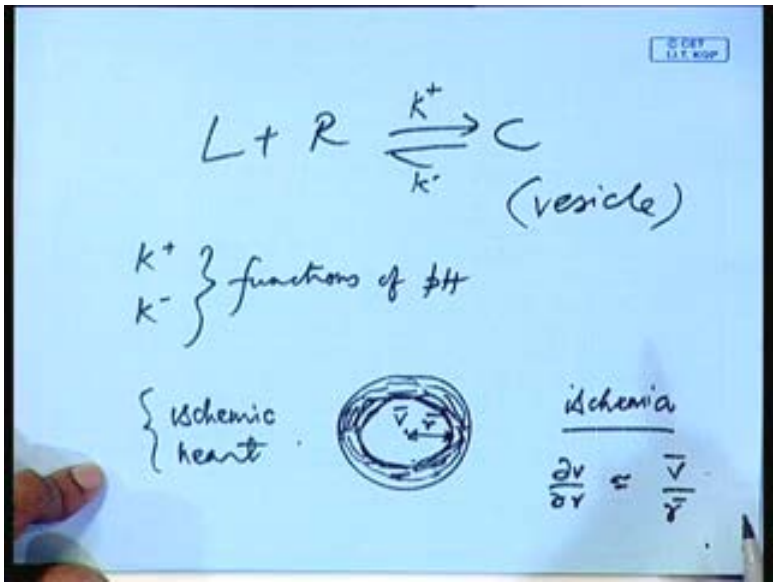

Yes. So, basically what happens is that you know. So, this my arteries, say if you looking at the artery from the top. So, you know, these are some membrane of the artery. These cholesterol molecules, they are sticky. they are sticky. So, what happens is, they start sticking to this, sticking to these arteries like this. So, they are, actually they are suppose to go inside the cell, but because they cannot go inside the cell they are roaming around and they start sticking to the membrane of the cardiac vessels arteries, capillaries or whatever; especially the arteries. And, then they reduce the diameter.

Now, when you reduce a diameter, what would happen? Blood pressure will increase. Blood pressure will increase velocity blood, velocity will also increase and the pressure will also increase. So, essentially the, all kinds of, you know, kinetic form of energy that is there in the blood stream will increase. And, as the result, you have what is known as ischemia. Right. So, ischemia. So, you have what is known as an ischemic heart. So, ischemia is basically small heart attacks that happen and this can lead to large heart attacks; the when large arteries. So, small heart attacks are like, when small arteries are being ruptured. And then, you can have a bunch of these small arteries rupturing at the

same point of time. That is one possibility or a large artery rupturing. So, whenever these arteries, this ischemia would happen; inferior ischemia, this is called in medical terms.

So, whenever in in inferior ischemia would happen, this arteries would rupture. And, because there is extreme high pressure, the blood will put too much pressure on the and this is go, this goes back to your entire Fluid Mechanics and everything that you have studied. Right. So, what will happen is, the shear stress will increase on the surface. Right. Because you have velocity is high and the and the distance between the, distance, what will happen if the velocity itself is increasing? And, if you look at this, so let us say, yes, this itself say, let us say the velocity here is something v bar and the velocity here has to be 0 from right. So, and this is your r. So, del v del r is your gradient. Let us say this is r bar. So, in this case a del v del r would be an average, approximately v over r bar. Right. So, what is happening is, your velocity is increasing and your radius is decreasing. Both ways, as a result of which your gradient is increasing. Velocity gradient is increasing both because the velocity itself the mean maximum velocity is increasing and also the… So, this is actually half of, you know average velocity is half of that. So, gradient is half of the v over r bar; average gradient. So, both your average velocity is increasing and your radius is diameter is decreasing.

So, because of these, both these effects having occurring simultaneously as shear stress is increasing. So, shear stress when it is increasing, it leads to enormous stress on the on the membrane. So, you can ask in one of the questions, you can ask me that is that, it might be the harder for the vessel to rupture. Right; because you are thickening it. So, that is the, that is there, but the point is that. So, much the shear stress increases so much on the membrane that, at one point of time the membrane ruptures and heart attack happens. So, that is a whole mechanism of heart attack. I am not sure if you have studied this before, but this is this is the whole mechanism of heart attack, when we talk of it. And, so it is important. So, that is why this disease becomes so important because it is very, you know and I think that somewhere that Indians are genetically predisposed to this disease. South Asians, not just Indians. You know that is why South Asians are asked not to eat lot of fatty material because if you compare with people in the west or people in down in south, say Africa or in the western countries they eat a lot more fatty material than Indians do. But, Indians have a huge, you know hugely prone to heart attack risk.

South Asians, I think more than fifty percentage of all the heart attacks that happened are actually of south Asians. And, that does not have to do with the fact that we have, we eat a lot of cholesterol. We actually eat lot less cholesterol than other people do. That has to do with the fact that this is a genetic disease and we are genetically predisposed to this disease because the receptor ligand binding mechanism that is there for this low density lipoprotein or lipoprotein in general for us; it is not good for south Asians. And, that is something will, when we do the kinetic study, we will try and understand that what makes us genetically predisposed to this particular disease.

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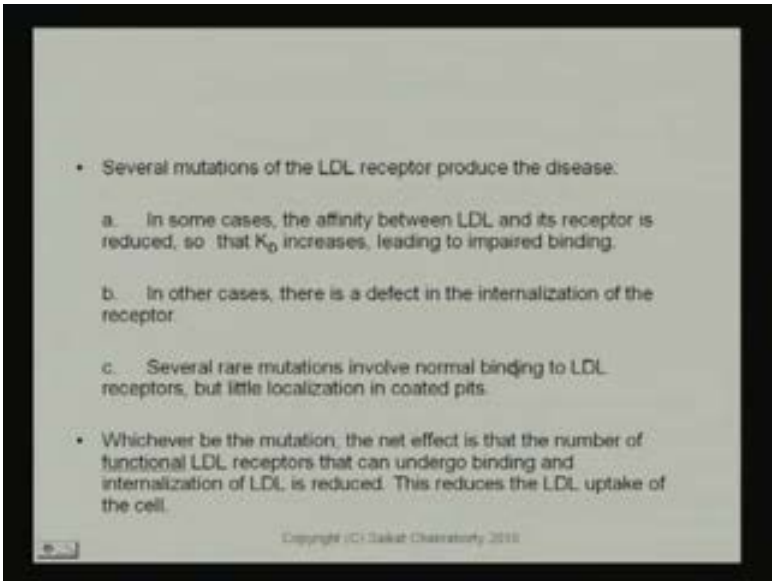

So, several mutations, so that, that brings directly into the point, that I am talking about that several mutations of the low density lipoprotein receptor can produce this disease. And, we will, let us look at what these mutations are and then later on, we will try and connect this with the kinetics of the process.

So, first case is that the affinity between the low density lipoprotein and its receptor itself is low. So, that K D increases, leading to impaired binding. K D is what the dissociation constant or in other words, the backward rate constant over the forward rate constant. Now, one possibility. So, why are we, you know south Asians genetically predisposed. And, as I said it has to do with the kinetics as a receptor ligand binding not because of the fact that we eat too much fat, which we do not not because of the fact that our arteries are all smaller than everybody else. No. Which is not, but because of the fact that the

receptor ligand binding process is not favorable for us. And, it is not favorable because of some kinetic issues. So, the first issue is that the binding rate constant between the receptor and the ligand for people who are genetically predisposed is low. Or, in other words, the dissociation rate constant is large. The second possibility is that, no the binding is actually all right, but it is the internalization process or may be the coated pit is formed, may be the binding occurs as it should, but the internalization of the coated pit is not happening properly; may be the coated pit is not favoring the internalization or the internalization mechanism, whatever is the kinetics of the internalization process is not favorable towards the process of internalization. Right.

So, this is a completely distinct possibility. The first possibility is that the binding is not happening properly and the second possibility is, no, the binding is happening all right, but the internalization is not happening all right. The third possibility is that this internalization is not a problem, the binding is not a problem either, but what is a problem is that several rare mutations involve normal binding to L D L receptors, but little localization in coated pits. You see what I am saying.

So, let us go back to the picture. Yes. Sorry. though this is the first class 1 receptor, but still, I will try to explain with this picture. See, what is happening is that the, may be the receptors are binding all right to the coated pit, may be the internalization is all right, but within the coated pit, not enough of those bound receptor ligands are localizing because unless they localize, see, if there is receptor bound receptor ligand is here or there or somewhere else, it makes no difference. It has to localize on the coated pit for it to be for a vesicle to be formed. Is it clear all of you? clear to everybody. It has to localize on the coated pit for the vesicle to be formed because if it does not localize on the coated pit, the vesicle is not going to be formed. So, those are coated pit formation process might be all right, but the localization itself is not right.

So, excuse me, these are the three major mutations that can happen. One is affinity, second one is a defect in internalization and third one is that the coated pit itself is not is forming all right, but the, but the, but the localization is not happening all right. Now, why do you think that the localization of these receptor ligands to the coated pit might be hindered? See, think of a class 2 receptor, do not think of a class… this is where the differences come in.

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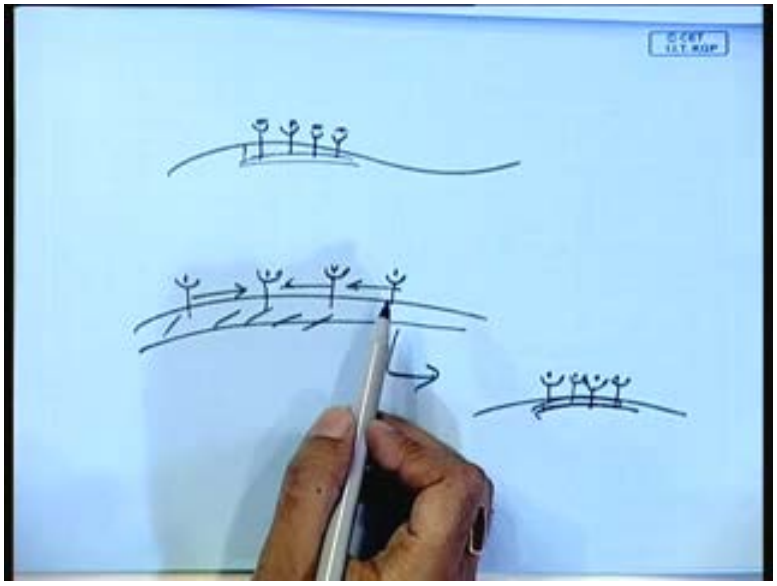

Do not think of a class 1 receptor. See, class 1 receptors are easier because what happens is that, you have these surface and you have these things over here and then, once you know sorry this is not bound yet, and then the coated pit forms forms over here and then the binding occurs. So, now, but here, you have the bound things over here and then the coated pit is going to form the lateral movement.

Right. Exactly the lateral diffusion. So, for a coated pit to be formed, they have to come in close proximity to each other. These bound ligand receptors have to come in close proximity to each other. And therefore, it is necessary that they move close to each other. So, from here, I need to go to a place where it is something like this. And then, the coated pit can form because there is not, it is not possible for the, for it to form a coated pit as long as this, a because the proteins necessary for formation of such a long, large coated pit is not available. And, b, if you form such a large coated pit, then the vesicle is going to be really huge. You know you, the cell cannot afford to have such a huge vesicle because you have to provide the environment, you have to increase the pH and all the decrease the pH and all that stuff.

So, all these mutations together or anyone of them or any two of them; some permutation combination of these mutations provide a net effect that the number of functional L D L receptors that can undergo binding. And, internalization is reduced, and this is reduces the L D L uptake. As you can see that these, all these mutations that we are talking of are

generally, you know these are mutations, genetic mutations. And therefore, the disease is genetic. So, it is not. So, one of the things we understand is that, you know when you got to go to a doctor, anybody who has a high cholesterol or the diabetes, what is the first question the doctor asks you? That, did anybody from your father side or your mother side has this disease; both for diabetes and for L D L cholesterol, high cholesterol.

So, that is the first question they ask. Did any of your parents or grandparents have diabetes or cholesterol? same with, you know anybody who has cancer with one of the questions that doctors ask is that oh! what you know, whether anybody there is a family history of cancer in this, in the cancer. So, because these releases, you know an especially the tool, the diseases that we are considering hypercholesterolemia or say diabetes, these are diseases which are resulting from receptor ligand binding, which is exactly a genetic process. So, that is why it can happen and the possibilities are these three.

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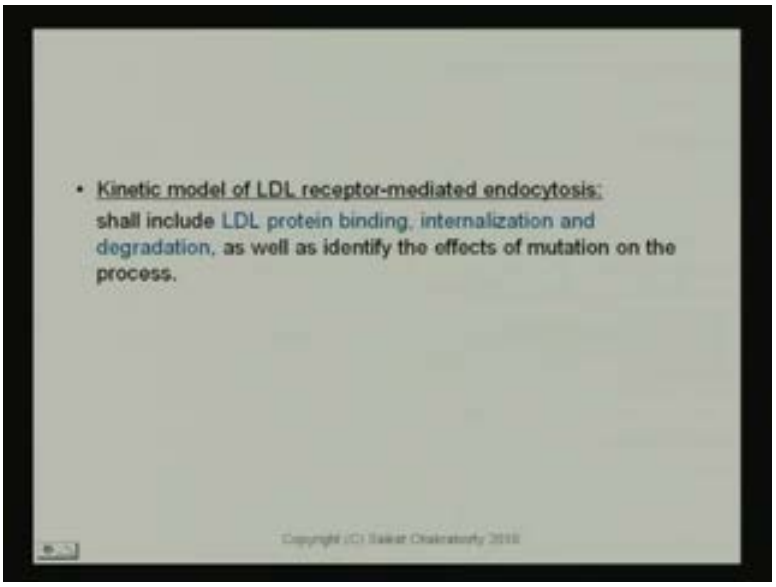

Now, we will do this in the next class. But just to give you an overview that, so what we want to do is, why do we need to quantify, why do we need to do this? Yes, as I said that, this is a genetic process and there are three mutations possible, which can lead to three kinds of defects. Now, if I want to, if I want to understand or quantify these three defects, then I have to do a total kinetic study. And then, look at the kinetic constants for each of these processes, kinetic or transport, whatever is the case. May be the lateral diffusion

could be a transport process and we will talk about that later. So, these three processes that are there and we have to quantify that. And, there, then we will be able to quantify the disease in general. So, the kinetic process includes L D L protein binding, internalization, degradation, as well as identify the effects of mutation on the process.

So, these are, I think I will stop here. But, just let me brief you. So, these are the three things that we are going to look at; so, the binding, internalization and degradation. And added to that, would be the thing that you know, he just mentioned, which is the formation of the coated pit and then lateral movement because we have to make sure that, when we make a model, all these three genetic mutations that cause this disease are included in the model; which is the dissociation and the internalization and the migration of the receptor, bound receptor ligands to a localized or localization. So, to say, so that the coated pit is formed; so, all these three, we will try to incorporate into our model and we will do the model in the next lecture.