Biochemical Engineering

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Module No. # 01

Lecture No. # 34

Kinetics of Receptor-Mediated Endocytosis

Welcome to this lecture on Biochemical Engineering; the next lecture in the series, and what we are going to talk about today is receptor mediated endocytosis which we have been talking about in the previous lectures, and we will continue that. Our specific interest or specific focus in today's lecture is going be, as the title says, kinetics of receptor-mediated endocytosis.

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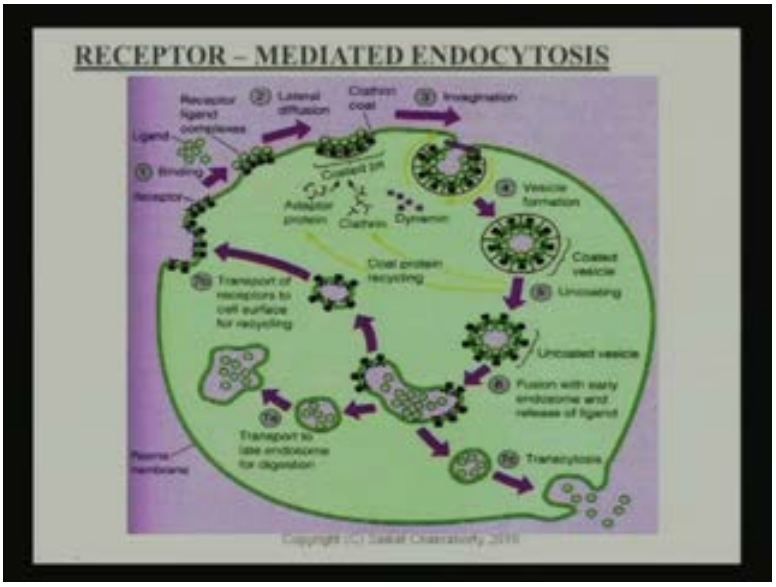

So, what do you see on the screen here is what we talked about in the previous lecture, which was total process of receptor mediated endocytosis. So, just to recap, as I said that it is very important for us to understand the process in order to be able to model the kinetics.

So, just to recap, as you can see over here, where the mouse is, so, the first step as this noted here by 1, that happens is the receptor binds to the, receptor is on the cell surface. Then, next step is the receptor binds to the ligand to form this complex over here, which is denoted by C, later on you will see. Then these complexes; this is for a certain type 1 receptor. These complexes diffuse laterally along the cell surface so that the bottom part of it or the root part of it is coated by the coated pit. So, the coated pit comes over here, and this coated pits; pits are essentially set of proteins, adapter, clathrin and dynamin proteins, which give mechanical stability to this receptor ligand binding. It allows, it is a function of this coated pit; it is essentially (()) to when the invagination takes place when the vesicle is formed.

So, this type 1 receptor as I said, in which the receptor ligand binding first takes place, followed by the formation of the coated pit as you can see can over here. And then the coated, then there is something called invagination takes place. So, part of the cell membranes, invagination essentially means as you see where the mouse is. So, the part of the cell main kind of invaginates; that is, goes in and treks the ligands.

So, the green particles that you see over here are the ligands, the black dots are the receptor, and these lines over here represents the coated pit. So, the kind of invaginate and form a neck like structure, in which the whole thing comes in. And then this neck joins each other. The two necks join each other to form a vesicle.

So, and what happens, what is the concept, what is the difference between invagination and a vesicle? Vesicle is something that is covered or sort of you know surrounded on all sides by the cell. What is invagination, its such that it is surrounded on more sides by cell, but there is an entry or exit out to the outside atmosphere.

Now what happens is that once this invagination process is over, and the vesicle has been formed, then what happens is that the whole thing, the ligands are entrapped inside the vesicle. So, then the coated pits get removed and they separate out into the different proteins and then go back into the to form coated pits again. So, and then what you end up having is an uncoated vesicle. And then, you know when the uncoated vesicle is formed, there is pH change that occurs in the system. I have talked about this and the pH change; what happens is the hydrogen ion pumps open up, and they start to pump hydrogen ions inside the system. The reason this pH change occurs or in other words, the

hydrogen acidification occurs as a result of which the pH goes down is, so that you when the receptor ligand binding, we studied in the last chapter, there is a forward and a backward rate constant. So, the forward rate is depressed or reduced and the backward rate constant is enhanced, as a result of which, the receptor and the ligand dissociate.

So, whatever was in the associated or complex form, they dissociate into two, and then separate out into two regions. The receptor goes back, remain where they are, on the membrane of the vesicle whereas, the ligands are swimming free. Then they separate out into this vesicle breaks into two sort of vesicles: one; contains the receptors on the cell surface, and the other one contains the ligands. The ligands are either metabolized or thrown out of the body, and the one that contains the vesicle; they form these kind of channels and microtubules, and the microtubules leave the cell.

So, that that is a process that happens, and the reason I am going back is because now our plan is to be able to model this entire process. And we looked at some of the things that we talked about, and we also talked about the disease; familial hypercholesterolemia, which we said is very prevalent among south Asians, and we the reason we said that we want to study this is because; there are three different aspects that are possible. So, we understand there is a receptor ligand binding.

So, from this picture for example, we understand that if you are doing, if we are to do a kinetic study of the system, so, this as this picture shows that. So, you have the receptor ligand binding; that is, association. You have the dissociation of the receptor ligand, and there is internalization, and then there is vesicle formation. So, these processes are there. So, we have study the kinetics of all of these processes.

So, the point is that, when we talked about disease such as familial hypercholesterolemia, as I said before that this disease can happen due to several mutation; different kinds of mutation. So, its, so essentially the disease is that this you have this LDL- low density lipoprotein in the blood, and these low density lipoproteins supposed to go into the cells and supposed to be metabolized. Now when you have the disease, this LDL remains in the blood. The ability of the cells to internalize it and metabolize it goes down.

Now, the question that we have to prove is why is it not being internalized, or why is it not being metabolized. Is it because the receptor ligand binding kinetics; is there something wrong with it, you know there is a retardation or there is something wrong

with dissociation association kinetics, or is it the internalization kinetics, or is it the formation; what was the third point do you remember? The three mutations that we talked about. One was the association dissociation, one was the internalization, and what was the third point, the formation on the coated pits. We said is there a problem with the formation of the coated pits or the lateral diffusion of the receptors to the form coated pits.

Now, in order to that, in order to really quantify this process and understand that when the person has a disease which is the one that is really the one that has to be taken care of, or the you know something we can do. So, what we can do? We can, the doctor might prescribe drugs which can alter. Even so, if it is a genetic disease, the doctor may be able to prescribe drugs which can alter some of this kinetics. The reaction kinetics; some of these processes. In order to be able to identify these, we need to quantify.

The process of quantification that will follow in today's lecture is, first we need to come up with the model of our own, and which we think is a reasonably good model. Then we need to perform experiments, and then we need to fit our experimental data to our model to be able to evaluate the kinetic data.

Now what we do? We fit, come up with our model fit, perform our experiments, fit our data and then we perform a experiment for two different kinds of cells. We perform it for normal cells; we perform it for cells having familial hypercholesterolemia. And then we can compare the rate constants that we get for people normal cells and the rate constant that we get in cells having familial hypercholesterolemia.

And may be at the end of that, we may be able to conclude something that these just these are the constants that are defective or this is a process or because its important you know you have a multiple three things we talked about, that even after three, there are other processes, but at least for the say, ligand or the LDL to get into the cell, these are the 3 basic steps: formation of the coated pits if its type 2 for example, formation of the coated pits, followed by the receptor ligand binding, followed by the internalization. And this is a sequence. These are not processes that happen in parallel. These are processes that happen in series.

So, what is a disadvantage when a process happens in series; there are advantages also, but disadvantage, advantage is that driving force you know we can use the same whole

driving force. The disadvantage is that when a process happens in series, if one of them these processes go kaput, then the entire process go kaput. So, we need to figure out which is the one. So, that is our, you know to that end, let us work.

So, these are the three mutations that we talked about; the coated pit and receptor ligand binding and the internalization. So, this is the first thing. So, we are going to do a kinetic modeling on this. So, let us let me first describe the experiments to you, then I will come back and do the modeling, and then will compare the experiments with the experimental results with the modeling data.

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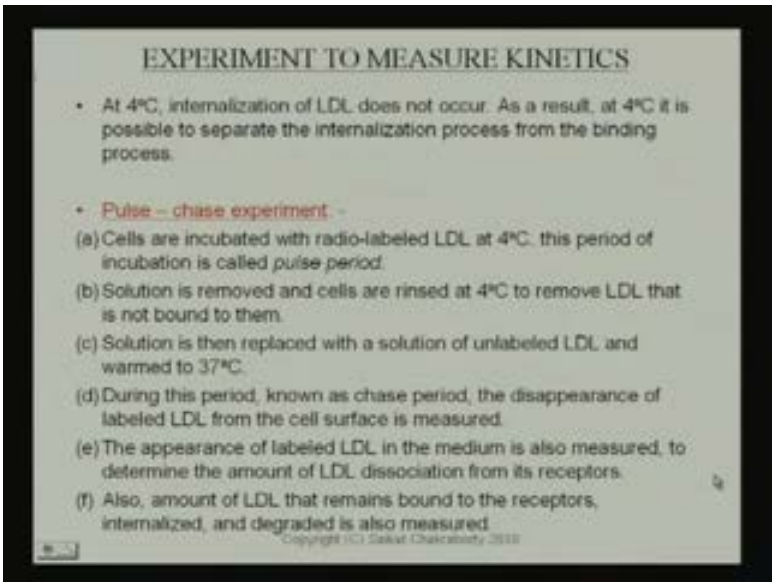

So, this is the first experiment that we do is known as a pulse chase experiment. This experiment is called as its written in red, pulse chase experiment. So, this is very common experiment that is done in case of LDL. And the fact that we exploit over here is that, at 4 degree centigrade, internalization of low density lipoprotein does not occur. The internalization part does not occur. So, what are we trying to do? We have a series of reactions that are happening. We are trying to freeze it down to a temperature where to one of the processes is cut out, and you essentially have couple of other processes staying there.

Now, the coated pit; we will come to that a little later, may be in the following lecture, how do you model the coated pit, because when we talked about kinetics, you know lets understand this. So, we can talk very clearly about kinetics of the receptor ligand binding

because these are binding processes, right. So, you can talk binding dissociation association process, we can talk about kinetics. You can about kinetics for internalization, but my question to you is, can you talk about kinetics for formation of the coated pits. These are very complex process.

First, there has to be the association of the different three different kinds of proteins, they have to come together. So, definitely you can talk about some kind of kinetics there, but the thing that is that we are worried about is not the formation of the coated pits in themselves, but the localization of the receptors on those coated pits, which has to happen through what, through lateral diffusion along the cell membrane of the receptors or the bound receptor ligand bound complex. So, you know, even if it is a type 1, type 2 whatever; its either the receptor will, if it is a type 1, then the receptor ligand bound complex will localize to the coated pit. If it is a type 2, then the receptor alone, but it has to.

So, it is not necessarily the formation of the coated pit from these three different kinds of proteins that we talked about, but that actual localization. So, how do we model that. That is a question you know I have. Do you do you think it could be modeled as a kinetic process, or is it a diffusional process? What is it?

Strictly speaking, answer is a strictly speaking its a kinetic plus diffusional process, because the things are you know moving in the lateral direction which is diffusion, but when the receptors reach the coated pit, it still has to bind to the coated pit, right. So, the binding process involved in that or reaction process involved in the. We will see later in the course how to do that, but right now we are not worried about in the pulse chase experiment. We are trying to deal with the other two processes; one which is the association dissociation of the receptor ligand and the third one; that is, the internalization.

So, in the pulse chase experiment, you have what is known as a pulse phase first. In the pulse phase, you reduce the temperature to 4 degree centigrade, and as a result of which the internalization does not occur. And therefore, it is at 4 degree centigrade, it is possible to separate out the internalization process from the binding process.

So, here the step by step mechanism of this experiment, though cells are incubated with radio-labeled LDL you know basically, you need some kind of label to be able to

identify it some fluorescence or something at 4 degree centigrade. This period is known as a pulse period. Next, cells are removed and solution is removed and cells are rinsed at 4 degree centigrade to remove LDL that is not bound to them.

So, you basically allow the cells to bind as long as possible. So, incubate means for a very long period. You allow the cells to bind to them as long as possible and during the pulse phase and when. So, essentially you presume that the binding will reach and equilibrium or a steady state. So, once this has been allowed, then you lets the take the take the labeled LDL out and rinse it out, so that why do you rinse it?

So, that if there is any extra LDL which is not bound, but it is in the vicinity, swimming in the vicinity could also be taken care of we removed because the rinsing is not going to; remember the rinsing is not going to remove the bound LDL. The LDL that has bound to the receptor; rinsing is not going to remove that. So, rinsing ensures that everything that is free, all kinds of free receptors LDL receptors that are there labeled are removed.

Then we take the system, warm the system to 37 degree centigrade. What happens when you warm the system to 37 degree centigrade? You allow for the internalization process to start. So, the system is now replaced with unlabeled LDL. So, unlabeled LDL means you can still have the LDL, but it is not going to make any difference because our measurements are not going to change. Our measurements are going to be based on 4 four degree centigrade.

Now, but still why do you think we put the at the unlabeled LDL? We are not interested in measuring those LDL, but we still want them to be there. Why? ((No audio from 14:30 to 14:41)) What happens when you know you have a system and what happens if you raise the temperature of the system.

(( )) binding (( ))

Binding will

Binding will be (( ))

Why, why should binding break down at, just as soon as…

(( )).

Yeah, but you cannot say that binding will break down. Some changes will happen. It could dissociate more or associate more. You cannot say that it will necessarily dissociate yes, but to say it more simply, the answer is that, as you raise temperature, the point of equilibrium itself will change. The equilibrium rate constants as a forward and the backward rate constant; both functions of temperature in different ways, as a result, the overall equilibrium rate constant is dependent on temperature. And as you change the temperature, it will change or I cannot say a (( )) whether is going to change in the forward direction or the backward direction. Now why do I add the unlabeled LDL? So, that I make sure this is a system is at equilibrium. So, first equilibrium is disturbed, I allow next equilibrium to be established, but such that I do not count those cells because my counting is based on whatever happened at 4 degree centigrade.

So, then during this chase period, what we start to measure is remember all the measurement is only for labeled LDL because unlabeled LDL you cannot make any measurement. So, I just that that is the peripheral thing, you know that that do not worry about that, what I just said that. I adding more unlabeled LDL so that equilibrium is measured, but in our calculations, it can never come in because we are only measuring labeled one that emit fluorescence.

So, during the chase period, now what will happen during the chase period? So, the process of internalization kicks in again. So, now, in the chase period, you have binding as well as internalization, but what about the binding? Is the binding taking place? Binding is taking place but we are not counting. We are not counting them, yes.

So, just as I said, you know d at the point, d over here, binding is taking place, but binding is taking place to unlabeled LDL which is not being counted. Then internalization is the one that is now being counted. So, both binding, binding dissociation; that is, association dissociation and internalization; all these three process are happening in the chase phase at 37 degree centigrade, but because the labeled LDL has been removed, the association is not being counted. What are being counted? What are being counted? Internalization and dissociation, that also are being counted, because it can its allowed to dissociate, but the association is being removed; that is, a binding

itself is being removed because there are no label. Is it clear to all of you, the experiments step?

So, next that you know what I said just now. The appearance of labeled LDL in the medium is also measured to determine the LDL dissociation from its receptor. So, we are measuring both the associate dissociation and the internalization. Now look at d and e, tell me what you what you can figure out from these two. So, disappearance of LDL from the cell surface is being measured and appearance of LDL in the medium is being measured.

Minus (( ))

Yeah, exactly. So, d is the disappearance of the LDL from the cell surface, e is the appearance of LDL in the medium. So, d minus e equals the amount that is bound to the; that is, in being internalized. So, in this is this is more or less the thing and. so, you can, this is you know just a measurements. So, you can measure the amount of LDL; that is, being bound to the receptor and that is being internalized; bound to the receptor you can measure because it is on the cell surface. So, d gives you the… not d, but you can d gives you the disappearance, but you can also measure the amount of LDL that is there on the cell surface.

You can measure the internalization d minus e and degraded you also you can measure under the microscope because if you have if its if its emits fluorescence and if the (( )) if the LDL is degraded, then you know it will have different texture below the microscope. You can measure that just once I talked about you know how to measure remember, how to measure live cells from dead cells under the microscope and when you look at it, it is very clear. When you when you color them, when you label these and so, the dead cells and the live cells; it is not that you lose them. So, what I am trying to say is that when you label the LDL, so, LDL that is active versus LDL that is degraded. These two have two different colors which have completely different from unlabeled LDL because they are going to some unlabeled LDL is also in the system. The unlabeled LDL would not come at all under the microscope, but the labeled LDL; once its it is you know its active that one versus when it is not active or degraded will have different colors. So, you can measure them also in microscope.

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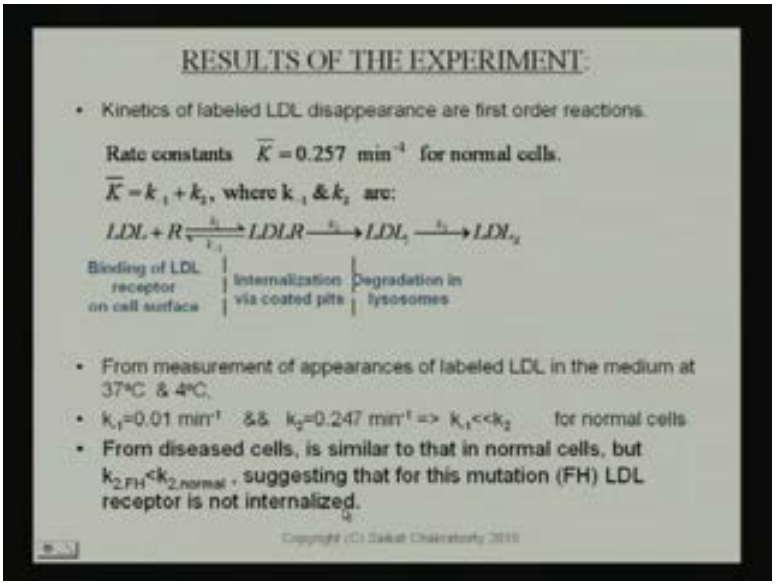

So, now let us look at the results of the experiment. So, kinetics of labeled LDL disappearance of first order and rate constant that I that I get is K bar. So, what is this? 0.257 or something I get, but let us look at this thing over here that I wrote; this arrows. So, this is K 2 is a… So, LDL is my ligand, R is a receptor, LDLR is a complex that is been formed, LDL I is a complex in internalized form, and LDL D is a complex in degraded form. That is the terminology and that is what we will continue to use for the rest of this chapter.

So, remember, LDL is for the ligand, LDR is a receptor, LDLR is a complex, LDL I is the internalized complex, and LDL D is a degraded. LDL I is a internalized complex or internalized receptor; both are same, and LDL D is a degraded ligand. So, if you look at it, so, what we are measuring in at 37 degree centigrade for that matter is a disappearance, right. So, the disappearance as we said over here, here d is essentially these two, because of the formation of the inter[nalization]- because of the internalization and because of the going back or dissociation, right. So, that is what I can measure together. Its 0.257 is what I… Then as we discussed that we can measure the amount that is in the medium and subtract that out right, and then we can isolate the internalization from the dissociation. Is that clear to everybody?

So, once we do that, we figure out that, this is what I get. So, 0.01 is the value that is of dissociation and 0.247. So, out of 0.257, 0.247 most of it actually; 90 95 percent or

whatever that number is, is because of internalization; the rate constant, and the dissociation is very slow. So, which means that internalization is the one that is that is dominating as compared to dissociation. Is that clear? These steps are clear right; that is the main step for kinetics here. I mean we will go through that again.

So, first here, binding of this LDL receptor on cell surface. This is a second order reaction that happens. Formation of the complex LDL R and then it dissociates back. This is a first order reaction. Then this is again taken LDL R which is a complex and the LDL I is the ligand in the internalized form.

So, it could be either in bound because within internalization, there are two different parts; remember what is it called, early endosome and late endosome or early vesicle late vesicle. So, what is the difference between the early and the late? What is the difference between the… lets go back to the picture again. What is the difference between the between the early and the early and the late vesicle or the early and…

(( ))

No, coated pit is not the point. Coated pit; it could be uncoated so, but here right here, I mean why are you (( ))

(( ))

Yeah, the pH. The pH is the system is a difference between the between the early vesicle and the late vesicle because of early endosome and late endosomes. So, to say, because as you decrease the pH of the system, they dissociate, and I have said this many times already. So, whatever is the case, whether it is a early or late, the total amount of ligand in the system is still the same, right; between these two, say this one and this one, if you see on the picture, the ligand is still the same because ligands have been trapped in there.

So, really it does not matter. So, when I say LDL I, what I mean is ligand in the internalized form here which does not matter whether it is bound to the receptor or free from the receptor. So, and that forms a degraded LDL or the endosome in the degraded in the lysosomes which is LDL D. And this is the total process that we are interested in.

Now what are we missing over here? Is there something that we are missing over here? We are missing the localization to the coated pits, and that is a little more complex thing.

So, what we will do is first we will write the entire kinetic model, write our equations, write our solution and everything, and then add the coated pit part at the proper position. What do I mean by saying that a proper position?

Two types of (( ))

Yeah, because we have two types of class 1 and class 2 receptors. So, for class 1 kind of receptor, it will come at a certain point in the model. Another kind of receptor, it will come before. So, that is why I said. So, basically what we are doing is writing the kinetic model as it is, and then we write this model for the localization in the coated pit added in. So, we will do that as the last thing. So, this is for normal cells.

Now what you have to tell me that you know into it, what do you think that you will, what kind of results if you get if you do it for the diseased cells? The cells with familial hypercholesterolemia. So, that was the point right. I said that we will do the experiment for both kinds of cells; normal and diseased, and then we will have a model and we compare that. We already have a model as you see the picture over there. So, as soon as you write the equation you essentially; not the equation, reaction itself, you already thought of a model. So, these rate constants are based on that model. So, what do you think, what will happen if you take a disease cell.

(( )).

Let us talk about first K bar that you have. Was that going to be higher or smaller? That is what you are going to measure. That should be an easy thing to answer. I think there are other one that you trying to answer is hard for you to answer. What you can easily answer is what will happen to K bar, will it increase or decrease. ((No audio from 26:17 to 26:26))

Increase.

Increase. Anybody else? Why do you think it will increase? What is the…

K minus 1 will increase

That is very hard. I do not I do not think you should try to answer whether K minus 1 will increase or K 2 will increase. That K minus 1 will increase you are saying.

(( ))

That is hard to say, I mean I would ask you not to venture that path, but what you can talk about is whether K bar will increase, over all K bar because there is something you can only measure experimentally. Even I cannot tell I mean whether you know which one of these two is going to be higher, but over all K bar is going to… what will happen, decrease or increase?

Decrease.

Decrease. So, K bar is essentially gives you the disappearance of the rate constant. Disappearance from where?

Over the surface (( ))

From the cell surface. Yeah, that in the bound form you know that yeah here itself. So, this, on the cell surface in the bound form. So, if this increases, then its disappearing more. There are several possibilities; if its increases and its disappearing more in which case, with of out of K minus 1 and K 2, which has to be higher?

Let us talk of the two possibilities yeah. So, let us talk about the two possibilities; one is you talk in a disease cell, you perform the experiments and you find that K bar is higher. So, twice or something. So, in that case, you tell me that which one will be higher; out of K minus 1 and K 2. K minus 1 has to be higher. Is it clear? Is that say intuitive way of looking at it, but the hopefully that is clear, if not I will explain. K minus 1 has to be higher because if it is increasing which means that this whole thing is you know being not being internalized, just leaves the cell and if it is decreasing then,

K 2 is smaller

K 2 is smaller.

Smaller, yeah. So, that is… So, let us see now what really happens. So, this is what really happens. So, for a diseased cell, it turns out that everything is similar and its a second option. So, K 2 for the diseased cell is smaller, suggesting that the mutation of the LDL is (( )) for that for this mutation, LDL receptor is not internalized.

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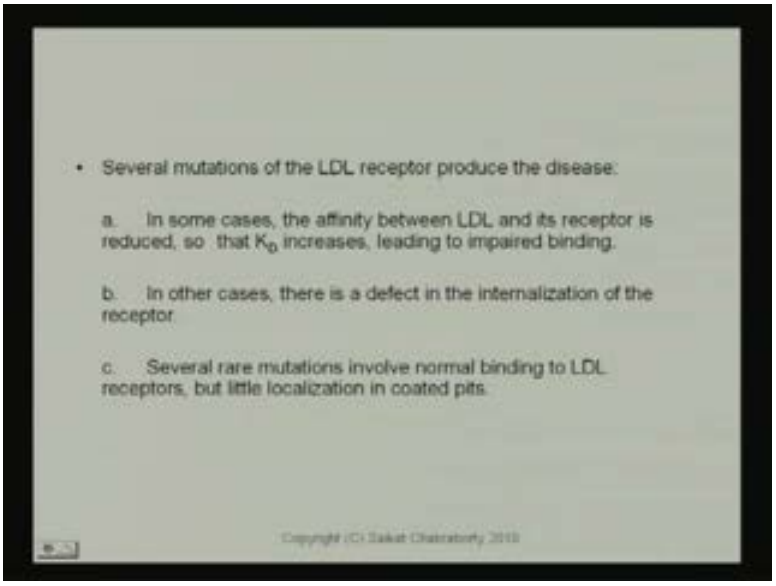

So, what is the (( )), you know the all these possibilities were there. Remember you know we talked about the three different possibilities. So, forget about C because we do not have C in our model yet. So, there are two possibilities right. So, that is why I said there are two ways of looking, there are two things that can happen; one is that K d increases or in other words K minus 1 increases; that is the possibility. Though it is binding, but the dissociation rate constant in the diseased cell suddenly goes up, as a result, though its binding, its leaving the leaving know its dissociating again.

Another possibility is that yes its binding that dissociation rate constant is not going up, but what is happing is the internalization rate constant is going down. And there, this is something that you and I cannot predict without doing the experiment that which of it is actually happening.

So, but my job was what I was trying to tell you is that let us try and convert those possibilities in terms of mutations to kinetics because that is the way you know when you have to look at physiological system, that is what you have to do. What is first those three things that we said over here, that is called this is a, you know, this is a slightly different way when you model up physiological system.

This is an approach that you need to take. First is you make a hypothesis, this is called the hypothesis. So, that is any physiological system modeling, you have to start with a hypothesis unlike in an engineering modeling, why because the systems are very

complex. So, you have to come up with some hypothesis, simplifying hypothesis of your own. So, just like we came up with these three hypotheses here, and then you perform.

So, this is the one and only way of doing it, you know, there is no other way. Then you write a model or create a model, then you perform experiments, and then you check with your experimental data to your fit your experimental data to your modeling or whatever, and then check through finding out the rate constants whether your hypothesis was correct or not. Then, if it is not correct, then what you do? You get rid of those hypotheses, come out with a fresh set of hypothesis. That is a way you know people do when we do for the logical modeling, then you have go in circles; there is a do loop out there.

So, you make a set of hypothesis, write your model, perform your experiments, get your kinetic data out of it, and then check if your hypothesis is correct or not. If it is not, then you start with a fresh set of hypothesis and go in this circle till your hypothesis and your solution matches, ok.

So, this is the very you know the best way of doing physiological modeling unlike in chemical engineering modeling where you can just write an equation, and then solve it, in physiological systems, you cannot do that. So, that that is what we did we did. We had this couple of hypothesis. Now we had these two possibilities based on this hypothesis. So, possibility one confirms hypothesis one, and possibility two confirms hypothesis two.

When we actually got the data, we found that it is not hypothesis one, but it is hypothesis two that is correct, which means that in this process of you know, let me go back, in this process of this entire process, so, the part that is not working, the part that is not working when a person has familial hypercholesterolemia is the internalization process. It is not the binding or the dissociation; they are fine you know when I say the south Asians are genetically pre-disposed to familial hypercholesterolemia; what its meant is that it is not that their binding or dissociation of the receptor ligand that is a problem, but it is an internalization process of the receptor ligand complex that is not happening.

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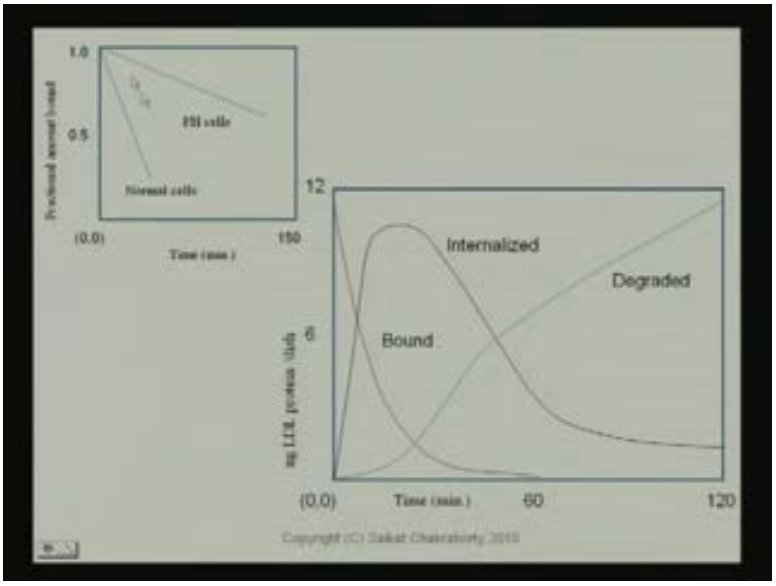

So, now, I will show you the experimental results some here. Yeah here, this is a experimental result. So, as you can see in this tiny picture out here, on the top, this one I will come back later, but let us look at this picture out here on the top.

So, what is seen is that, normal cells have fractional amount bound is much less with time. So, at t equals 0, excuse me at t equal 0, you have say you know this is fractional amount bound, if total amount bound the same for normal and FH familial hypercholesterolemia cells, but as time increases, the normal cell starts to internalize. So, which means what? That is, if these two are same at t equal 0, t equal 0 is the end of the you know pulse phase in the beginning of the chase phase, that is my time, that is when the clock starts ticking.

So, excuse me. So, if these two are same at t equal 0, what does this mean? It means that there is absolutely no problem with the binding. Same amount has bound both to the normal cell and the disease cell. Now when you look this thing on the on the top, the familial hypercholesterolemia the thing it sees same point it starts, but the slopes are completely different. As a result, the slopes have to do with the kinetics you know the rate constant of internalization, which means that slopes are different slopes as… So, it means that, the internalization rate constant for familial hypercholesterolemia is much lesser than the internalization rate constant for normal cells. As a result, what it means is whatever is being taken in, is not being internalized, fine.

Now, this is the overall picture with time. So, with the amounts that form this generally you know this is both for normal and for FH; this holds this picture that you see over here. So, what happens is this is the bound form, and I will show you, we will do the modeling in a minute; maybe I will do that modeling first and then come back and talk about this. So, just a second, you know this is a full model. Yeah let us do the modeling for the…

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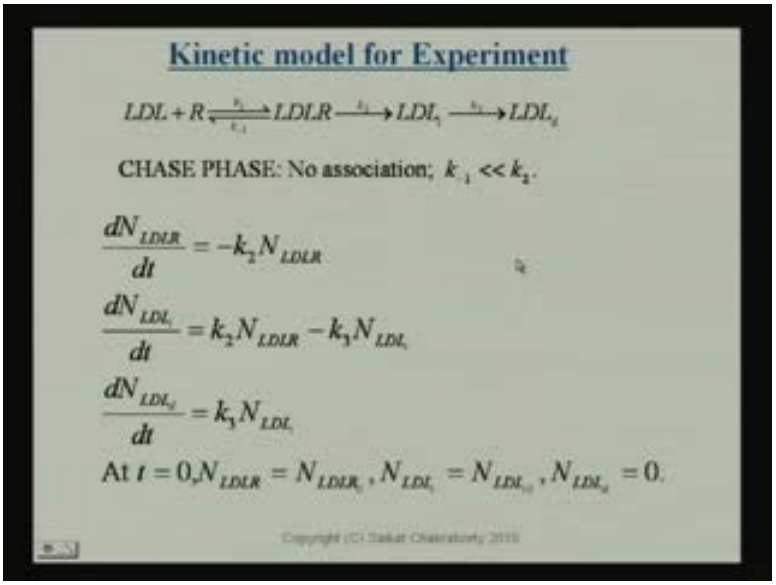

So, I will come back to this picture after we do the modeling. You will be able appreciate this a little better because we three. So, when we do the modeling, so, this is a kinetic model as you can see on the screen. LDL plus R giving LDLR, LDL I and LDL D. So, that is the thing. So, what kind of simplifications can you make for the… So, right now we are doing the as you can see its written there, kinetic model for the experiment. This is not the full model. The full model; I will come to in a few minutes.

So, this is the model for the experiment. So, what kind of simplifications can you make to this model? ((No audio from 35:11 to 35:22)) What kinds of simplifications can you make to this model for the experiment? We should be able to tell this. I mean this is very easy. We already went through the steps of the experiment. ((No audio from 35:32 to 35:45)) What should be the step, what should be the simplification?

(( )) last one

Last one, last one I can leave out, but that is not what I am hinting at. What is this pulse chase? In chase phase, there is no association because we have done it then that way right. We removed all the LDL that was nearby, all the labeled LDL that that was nearby through rinsing. As a result, thing is not there. So, in the chase phase, the K 1 is 0. The pulse phase, the K 1 was the only thing that was there and a certain amount of LDL was formed. So, essentially have to real R being the initial condition is that LDL R is not 0 at t equals 0. LDL R has a certain amount just as you saw over here.

So, LDLR is not 0. So, this is a beginning of the chase phase. The LDLR is not 0 at t equal 0, it has a certain value. If you did full model, then what will happen? LDLR would be 0 at t equal 0, but because we are separating out in the pulse and chase phase, and in the value of LDLR is whatever has bound, fine, whatever is bound.

So, there is no association. We are also aware because we already performed the experiment that K minus 1 is much less than K 2 so that the backward rate constant is much smaller than K 2. So, essentially what we can do? We can chop off this part itself because first is K 1 is 0 being the chase phase, and K minus 1; I have assigned 3 percent; I do not know 4 percent maybe, 4 percent of K 2. So, you can neglect that and essentially you just have this part.

So, this is my model now. let us look go through it one by one. So, N d d t. So, essentially I am writing because there is no these are… why is there no diffusion because these are fixed to this localization the lateral diffusion will coming as during localization to the coated plates. So, d d t f LDLR equals minus K 2 and N LDLR. d d t of LDL I equals K 2, here formation K 2 and LDLR minus K 3 and LDL I, and d d t of K 3 LDL I equals d d t of N LDL d; this is from the formation equals K 3 and LDL I. And at t equals 0 as I said, N d LDL d l R is not 0, it is given by some initial value which is the value that is obtained at the end of the pulse phase.

And excuse me, the internalization also is given some value, but it could be 0. You can you can assume it to be 0 because you know you have taken the cells to be at 4 degree, but the reason that is put over here is in case there is some residual internalization; that is, before use you froze it 4 degree, if there are something that had already internalized and it is there. So, that you can measure, if that is possible, otherwise this could be 0 and of course, N LDL d equal 0. This is the initial at the initial conditions clear. So, this one

is very important and these two could be 0, both of them could be 0, fine. So, how will you solve this now; set of equations?

We can solve first (( ))

Yeah, that is the one possibility. The other one is you can solve all three together also using the matricial method yeah, whichever, but what you saying is lot easier. So, this is you know, this a very classical; these set of equations that we have is a very classical equation in physiological systems and also in some chemical engine systems. Can you predict what kind of just look at the equations there? Once you solve equation 1, put it into equation 2, what kind of solution you would get; especially let us say for equation 2. What kind of response you would get for N LDL I?

(( ))

What is that called? What is that kind of solution called? Biphasic solution, this called biphasic solution. It is a very classical thing. In most physiological systems, we will find and even in most many chemical engineering systems also, when you have this series kind of you have an essentially series kind of reaction, that is what you find and a going to b go into c; it is a kind of thing.

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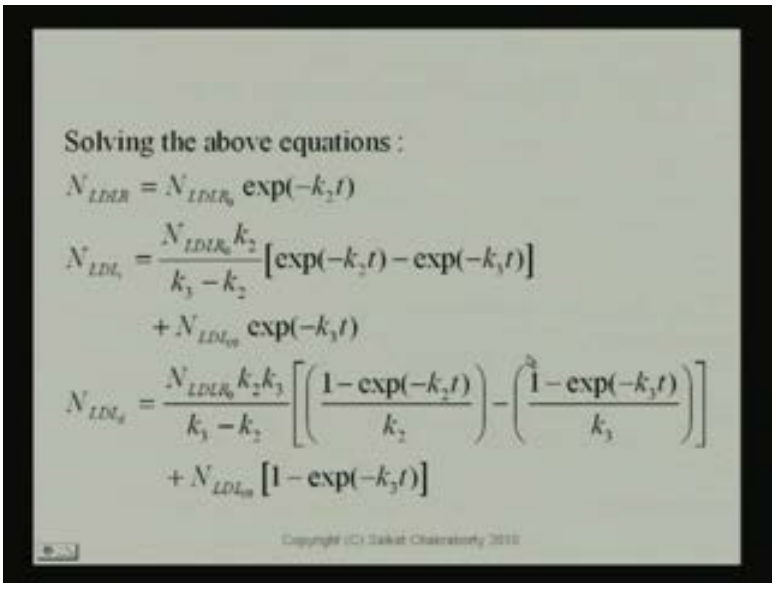

So, I think yeah, this is a solution that you get. So, the first one is exponential. Now, once you put this exponential into the second one, you get this exponential minus K 2

minus K 3. So, this is the one; these two terms you know this negative term over. So, N plus N LDL I not exponential minus K 3. Now this could be 0. N LDL I could be 0 and despite that, you will get those peaks because there are two terms which are opposing each other and that happens because you plug in the first one into the second one. So, these two exponential oppose each other. So, when this one leads, it increases. So, first term K minus K 2 leads, it increases, it reaches 0 when these two are equal that is and then it goes down again. So, that is how you get the biphasic response.

And N LDL d; you integrate this one more time, then you will get this. Now what kind of response do you expect for this one, for N LDL d? Plus C 1 by 1. So, this one, t going to 0? N LDLR; t going to 0, its N LDLR naught, and t going to infinity is 0. So, it decreases from a certain value down to 0. This one; t going to 0, it is 0, leave this term out N LDL I naught is assume it to be 0. So, this is 0 at t going to 0. t going to infinity yes what, 0 again. So, essentially it starts at 0, goes to a peak and gets down to goes down to zero.

So, here the again this term is 0, do not worry about this term. Let us talk about the rest of the terms here. So, at t going to 0, this is 0, right, and t going to infinity, this is minus 1 over K 2 minus 1 over K 3. So, essentially it flattens out. It saturates. It starts at 0, its saturate.

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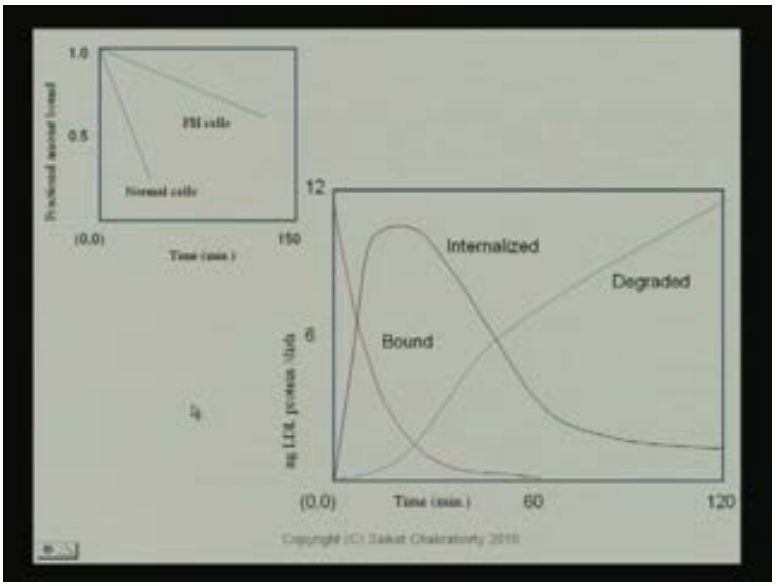

Now, let us go back to the plots and this will make more cells. So, as you can see, this is a bound 1. As we said that, it starts at a certain value, and these are values from the experiment. So, this correspond. So, the model we did, and then we measured the values for the experiment, and then you can fit this. So, the thing that is important over here is to have the model which has a sort of a model which fits the experiment because what I am trying to say over here; these are not model results by the way.

What you see on the screen here, these are not model solution. These are obtained from the experiment directly. And now the good part is the lucky part is rather is that, we have a model which fits this qualitative nature. So, whenever you know when you doing this kind of physiological modeling, the first thing that you have to look at is that see why you know how is physiological modeling different from normal chemical engineering modeling this. The reason it is different is because the levels of complexity is very high in physiological modeling.

So, in a chemical engineering modeling, you can nail it down to the last diffusion term or the convection term or the reaction term. So, you can write an equation, a diffusion convection reaction equations, say some kind of reaction equation and be happy about it because you can write an equation say I think I put in all the phenomena in there, and if I simulate this equation, I can get the real thing. What is in the physiological system, it is so complex and it is so complex that we do not even understand these systems completely physiologically.

So, the problem that we have over here is, it is so complex that we can forget about modeling this from the first principles. You can put in, you can say I think there is some diffusion in there, you can say that I think there is some reaction in there, and put these in, but host of things are going on. You know even this system that I talked about, a huge set of things are going on in these systems that we not even accounting.

You know what about for example, the reactions between the different proteins that form the coated pits, what about the reaction of the ligand to the coated pit as it is binding, what about the reaction that happens when the coated pit you know sheds its I mean if the vesicle shades the coated pit, what about the what kind of reactions that happen dissociation that happens when the pH is decreases.

So, if you want to do full modeling, then what you have to do is, you have to model for the ion pump set up there; the hydrogen ion pump set up there, and model that process itself; the process of pumping in the hydrogen ion. So, what I am trying to say is that process is very complex, and you cannot even dream of actually doing it completely completely, right. So, what you have to do, you have to be able to pick out the basic things correctly a, and the b more importantly what we have to do, we have to look at the experiment and see that what kind of qualitative model will fit the experiment; that is another thing you know. That is something that you have to do, excuse me.

So, here as you see that in the bound system, it starts at a certain value and goes to 0, as we said. This one starts at 0 and, so it starts around 12 nanogram per of LDL protein per dish per petri dish. It starts at 12, goes to 0 in about an hour, and this one starts at 0 and reduces to very small value in couple of hours, but it will still take another probably another couple of hours to actually go to 0, and the degraded one as I said, its starts at 0 and it should saturate out and in 2 hours, it still has not saturated at out as you see; that little still continue.

The reason being that the what is the reason the process of this all this means that the rate constants of degradation is very small or in other words, the process of degradation is small is slow as compared to the process of internalization or the process of bindings. As you can see here, the process of binding is the fastest actually. The process of internalization follows that. It is not as fast as the process of binding, but one of the limb is almost parallel to this. So, it is reasonably fast. And then the process of degradation is a slowest. So, this is the model we have for the experiment.

So, next thing that we are going to do now is do the full kinetic model. So, the full kinetic model is going to be slightly more complicated because you we have checked out you know here we had completely removed this section. So, we need to add that section in and let us see what happen.

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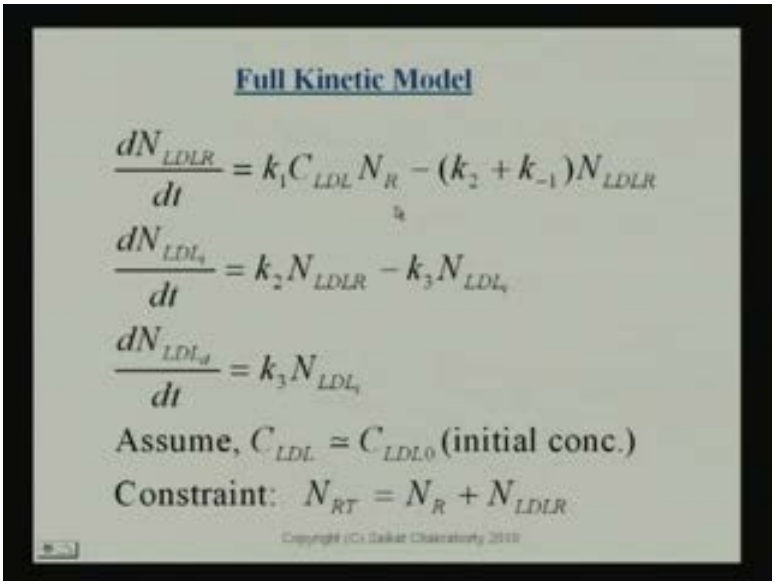

So, this is what you get, and what comes in here is the N R which is the amount of free receptors out there. Here as you see, N R being here, and this is the amount of free receptor that is there. So, and C LDL is also there. Now what as we made an assumption remember, in the LDL you know receptor ligand binding chapter that you can assume that ligands are in plenty which is something you can more or less assume because ligand is in your hand, you know you you put the system; even in the body. So, if you take a lot of (( )) fats, the ligand is obviously is in excess. So, if we if you want to do this experiment on live patient you know it is not possible, but if you still want to do, you can still pump in lot of ligands. So, the ligand is in excess. So, you make that assumption here, down there. So, C LDL equals C LDL naught. There is a initial concentration is remains as the initial concentration because its in excess, and you have N R over here.

Now, this equation as it is, is not solvable, but what we do is, we use the constraint N R T equals N R plus N LDL R. So, if you use a constraint, then you can replace N R over here, N R T minus N LDL R. So, essentially this first equation is a second order equation with three unknowns.

So, what we have done, we have converted the second order to a first order by assuming that the ligand is in excess a, and b we have got rid of the… So, you got rid of one unknown and we got rid of the other variable by using the constraint equation, but remember, these two; both the constraint equation and the and I have not said this in

other cases before, but its lot more important to note that here, both the constraint equation and this assumption are essentially assumptions. Even the constraint equation is an assumption and we will do that in the next lecture, why that is an assumption and what things we can do.

So, essentially it is an assumption. Why is it an assumption, because as I said I think I discussed this little bit in the previous. So, the number of receptors is being regulated. And it is not possible to assume that the number of receptors is a constant on a cell for at all points of time.

So, once we you know do this, then again you get a similar sort of similar sort of thing similar sort of equation. So, 1 minus exponential K k K t and, this should have been K 1 K is K minus 1 plus K 2 over K 1. So, basically you substitute that over here, and you know filled around with this a little bit and so, then you will get this K 2 plus K minus 1 over K 1; that would be your rate constant.

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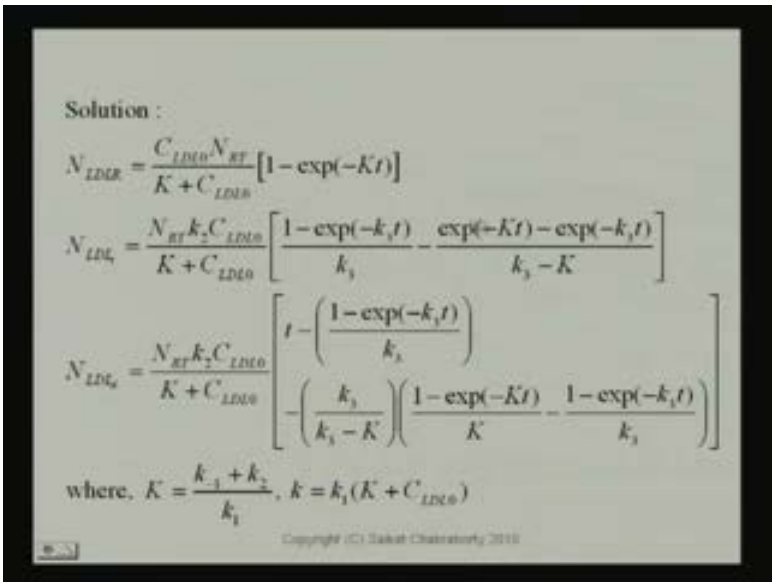

And this is what you get for LDL I. And this is what you would get for the degradation because see slightly yeah, slightly this is different from let us go back to what we got over here. Look at the LDL I think that you have over here, and here its slightly different because this expression is you know that the expression that you get over here is different and when you put plug that back over here, I mean it is you can go through the calculations at home. So, sorry.

So, this is what you had before is the same expression out here, but because this expression itself is different, so then you get a slightly different expression. And I think the limits are still the same because as let us see, as t goes to 0, this is know; the limits also have changed I think. This goes to this goes to 0, and this goes to 1 over K 3, right. And as t and this one because there is a 1 out here, when you integrate that, you get the t minus 1 minus exponential part.

So, let us look at the limit. So, a limit of this is as t goes to 0, this is a certain value, and t goes to infinity, this goes to 0 that is fine. This one is as t goes to 0, these two balances out and you get a 0 out here, and then you get 1 over K 3 out here at the initial point. And then you get, see one of the differences that you had and it is not mentioned out here is that, there we had taken an initial value for all of these, but what would be my initial value over here. So, here for example, N LDLR is 0 at t equals 0, and there we had a initial value for LDLR; the reason being that that value was what we got at the end of the pulse phase right so, but here that is not the case. We start with a clean slate almost. So, you have the 0 value and because you have that change, all the rest of the thing kind of keeps changing, and this one is still the same, and then this one will change a little bit. So, t going to 0, let us see this goes to 0, this term goes to 1 over K, this term goes to 1 over K 3, this term goes to 1 over K 3. So, definitely it is not 0 at t going to 0. At t going to infinity,

(( )) 0 (( ))

Zero t equals zero

(( ))

This is 0, this yeah this 0 at t equal to 0, but at t going to infinity, it saturates out like last time to a certain value.

So, now let us look at the plot. So, what is you know the difference between this and this is that as I said, that it does not go to this one for example, at t going to infinity, this is not going to 0. Earlier if you look compare here, t going to infinity, this went to that LDL I am talking about LDL I went to 0, but this is not going to 0.

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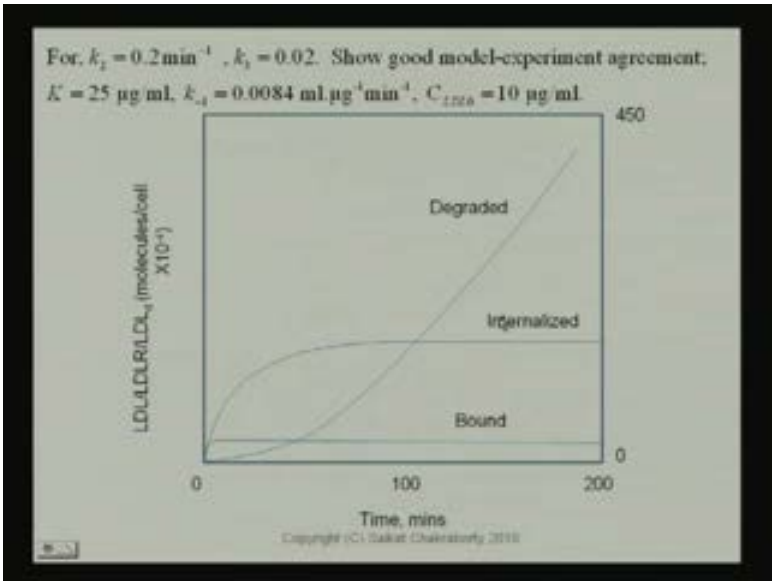

So, one of the things that happens is, even though this continues to be a biphasic response, but the biphasic thing is not similar to the biphasic that we had before or in other words, it increases and then it does not go to 0. The internalization does not go to 0. The binding saturates out because the why does the binding saturate out, because this system the equation reaction attains equilibrium, yeah. So, the binding saturates out. The degradation keeps increasing, but can you comment on why. So, these are for example, experimental value; this over here, K 2 is 0.2 minute inverse K 3 0.02 and so on. Can you comment on why the internalization does not decrease?

Because we have a plenty of (( )) assume. So, bound is always there because of bound already there, so, internalization (( ))

Yeah whether we have plenty of ligands are not, that is not the question, but the point is that the bindings unlike in the last case, where we stopped the (( )) stop to the pulse phase and stop prevented all kinds of binding to happen beyond the pulse phase. Here the binding is a continuous process, right. So, the binding goes on, and because the binding goes on the internalization goes on.

So, we have run out of time today. Next class may be the… so first slide I will show and then we will continue to do more complicated modeling about the coated pits and the general modeling and we will finish this chapter in the next lecture. So, thank you.