Introduction to Biomolecules
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Lecture-33
Hormonal Regulation and Integration of Mammalian Metabolism

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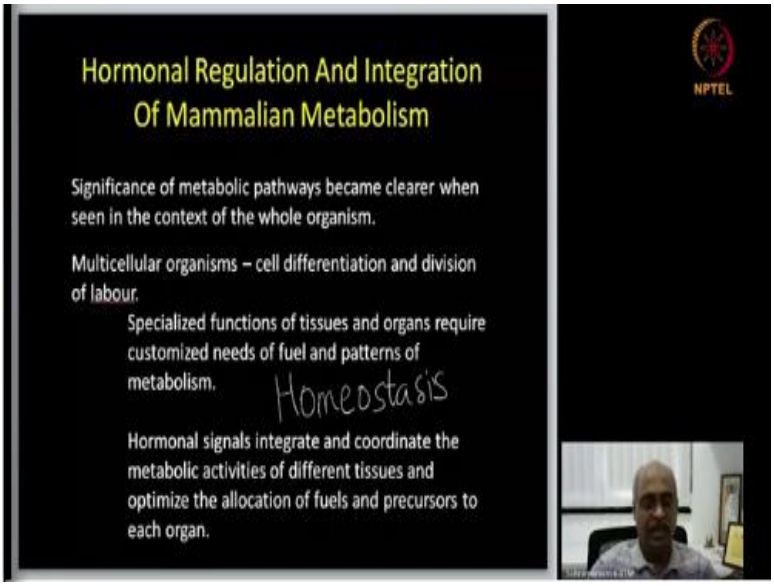

So, let us start discussion on integration of mammalian metabolism**.** So, we are focusing only on the mammals because that is what is best studied, although regulation happens obviously in all organisms, in more or less similar mechanisms**.** But here specifically we focus on the hormones and hormonal regulation is best understood in mammals. So, some of the important points are outlined in this slide, so the main point is that we need to see all the reactions that we learned so far in the context of a whole organism**.**

So, although we were talking about cytoplasm, mitochondria, chloroplast thylakoid membrane, stroma and so on, but these are not isolated cells in culture, so these are part of a whole organism. And different organs have different specialization in terms of function so it is the main point which you see in the second bullet multicellular organisms. So, you have cell differentiation, meaning initially during development you make multiple cells and then they have to become multiple kinds of cells**.**

For example the cells of your eye rods and cones sense light, whereas cells of blood like for example if you take red blood cell, it does the job of carrying oxygen from lungs to tissues**.** Skin protects you from outside environment; immune cells fight foreign bodies. And like intestinal mucosa, specializes in absorbing all the nutrients we learnt and making complex molecules and transporting to other tissues.

So, each organ has it is own kind of cells and they have their own specialized function, so that is the essence of multicellularity**.** So, which means each tissue will have a customized need for both fuel and precursors**.** So, therefore the metabolism in them will be customized too, so the main goal of regulation is to integrate and coordinate the metabolic activities of different tissues**.**

So, we already saw like for example in yesterday's discussion we saw liver and extra hepatic tissues in terms of cholesterol biosynthesis and cholesterol metabolism, cholesterol breakdown as well as biosynthesis. Similarly we saw about beta oxidation and then we saw about free fatty acid biosynthesis. So, liver has it is own specific function, pancreas for example it produces digestive enzymes and then insulin, glucagon, somatostatin hormones.

So, adipose tissue, if you take for example it specializes in making triacylglycerols and cholesteryl esters and storing them, muscle will burn them meaning beta oxidation and breakdown of cholesterol to derive energy. It will also have glycolysis when you are having vigorous exercise, where oxygen availability is less than the few rate of ATP production. So, all of this need to be coordinated and integrated, so that as an organism an individual organism exists in a normal healthy equilibrium which we call as homeostasis.

Homeostasis is essentially the equilibrium state where all supplied demand of energy precursors or raw materials all are well balanced. And the organism exists in a healthy state, for example a healthy human being maintains a constant body weight, once reaching adulthood for about on an average for about 40 years with no weight gain or loss, so that state is called homeostasis. So, all of this emphasizes the importance of regulation and integration and these are done by hormonal signals.

So, that is what today we are going to focus on. So, what are these hormones and what are the basic principles that tell us about how hormones function?

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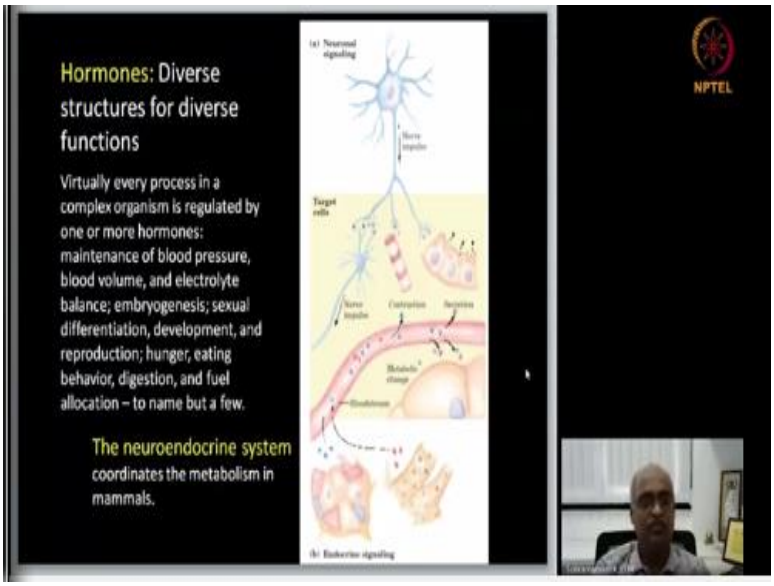

So, hormones have diverse structures and for diverse functions, so we will see one by one in some detail**.** So, the main focus in this cartoon is actually the connection between the neuronal systems and endocrine systems. So, the endocrine system is the one that produces hormones, like adrenal gland that we learnt is an endocrine gland. And the beta cells, alpha cells and gamma cells of pancreas islets of Langerhans, so that is an endocrine system.

Thyroid gland, so that is an endocrine organ, testis, endocrine organ because it does produce testosterone, ovary is an endocrine gland. So, these glands produce the hormones that are secreted into the blood and they go to other tissues to regulate function**.** So, these are therefore ductless glands, so they do not have ducts, so these are ductless glands and the transport is via blood and that is what is the definition of an endocrine organ and endocrine gland.

And the study of endocrine glands and the hormone production and the functions of hormones we call as endocrinology**.** So, for example if you do an undergraduate degree in biochemistry, endocrinology will be one paper, so you will spend about 40 classes learning only hormones. So, here the main focus is connection of this endocrine gland with the neuronal signaling, so we call this as neuroendocrine system**.**

The neuronal signaling is of essentially an electric impulse due to changes in the concentration of ions, the ions inside the axon versus outside. So, you have an active potential due to the difference in the ionic concentration and that difference rapidly travels along the length of these long cytoplasmic processes which we call as axons**.** So, this is the cell body of a neuron with the nucleus there.

And these small structures are dendrites, these dendrites few make a junction with this long structure called axons of other neurons. For example here this is the axon of this neuron and it is branches make connection with the dendrites of another neuron and that neurons axon is this, so this is how the electric impulse. Essentially active potential that transfers, quite instantaneously so rather than carrying a material in bloodstream where the rate of flow diffusion all that comes into play.

So, at the end of this long chain of neurons, one neurons axon to dendrites of another neuron then the axon of another and so on. Finally they connect to muscles, so this is neuromuscular junction. So, where the chemicals secreted from the neuron in response to the travel of the action potential activate signals cells in the muscle**.** For example acetylcholine is an important neurotransmitter. So, the chemicals produced at the tip of the axons that bind to receptors on the subsequent neuron are final ultimate tissue like muscles or they secretory gland cells of some other tissue, those chemicals are neurotransmitters**.**

So, neurotransmitters are produced by neurons in response to an axon potential that is traveling along the length of this neuronal circuitry. And so these signals are secreted, the neurotransmitters are secreted not just only in the neuromuscular junction. So, neuron to neuron junction which is called synapses, even there they are produced and the endocrine glands on the other hand they produce signals and that travel via bloodstream.

And from the blood capillaries they diffuse into the tissues and they could again do the same thing a neuronal signal, neurotransmitter or in a hormone coming from endocrine gland, both can act on similar functions, for example in this case muscle contraction in this case secretion

making another tissue to secrete a molecule. So, this is how the neuronal signaling and endocrine signaling are connected and integrated one. And in some cases the signaling molecule may very well be the same**,** the molecule produced by the endocrine gland can also be a neurotransmitter, certain some molecules function as both. So, that is the neuroendocrine connection.

And what are the processes regulated by these signaling molecules, like primarily hormones, virtually everything in the body**,** so a few are listed here. For example blood pressure maintenance, blood volume, electrolyte balance, embryogenesis, embryonic development, remember yesterday I was talking about cholesterol modified signaling and sexual differentiation development, meaning the process of embryogenesis making all the different organs and reproduction.

So, we saw the role of testosterone and estradiol yesterday, progesterone, hunger, eating behavior, digestion, fuel allocation. So, virtually everything is subject to regulation by hormones and to some extra neuroendocrine systems. Sometimes the neuronal signaling will be essential to stimulate the endocrine system or control the endocrine function. So, we will see examples of that as we go along**.**

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So, some interesting historical tidbits about the hormone discovery. So, how would you people discover hormones? Hormones since they are signaling molecules, they can act at extremely low

concentrations and their presence can be easily detected based on the function they do and that is what we call bioassay. For example insulin does it stimulate a cell to take up glucose and induce protein synthesis or lipid biosynthesis?

So, like that for every hormone you have very sensitive way of finding it is existence. But then purifying them and having enough of the pure molecule the hormone, so that you can characterize many aspects of it is structure and function, was a challenging task. Because they are producing extremely low quantities, so that is where we see couple of interesting historical events.

Like for example Roger Guillemin's group extracted this thyrotropin releasing hormone. So, this is a hormone produced by pituitary that stimulates thyroid to release the thyroid hormone thyroxin**,** so thyrotropin releasing hormone. So, we will learn about propane and this releasing hormones pretty soon as we go to. All you need to know here it to understand this name is that in the hormonal signaling we have a hierarchy**.**

So, there are certain hormones produced by glands for example like pituitary, they go and induce other glands to produce the hormones that they need to produce. And pituitary in turn is stimulated by hypothalamus which is part of our brain**.** So, I wrongly told you pituitary, it is the hypothalamus that produces the thyrotropin releasing hormone that without going to pituitary directly acts on thyroid glands to produce thyroxin.

And how did Guillemin's group get TRH, shortly for thyrotropin releasing hormone, they got it by getting it from the hypothalamus of million pigs**.** So, that is the kind of labour that was involved in getting this. And Andrew Schally's group got it from 20 tons of hypothalamus from 2 million sheep**.** These 2 groups independently purified the same hormone and they both were awarded in 1977 on Nobel prize for working on this TRH purifying and characterizing TRH.

Along with the Rosalyn Yalow who developed an important assay for detecting hormones called radioimmunoassay**,** so we will see that process in detail. So, this is the kind of heroic effort was involved in isolating pure hormones. And once isolated it is a child's play you can readily

synthesize them because their structures are not very complex. So, it is a tri peptide having 3 amino acids that is all thyrotropin releasing hormone is.

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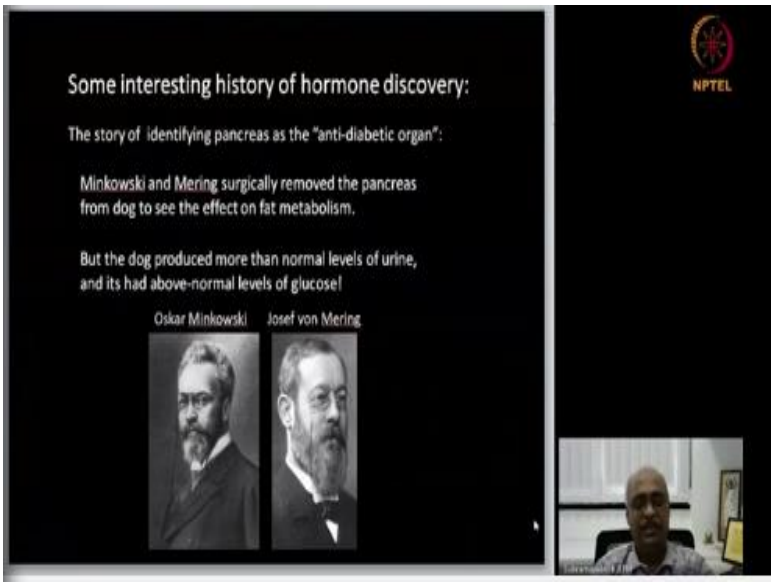

So, another interesting historical event is about the discovery of insulin**.** These 2 guys Minkowski and Josef von Mering they both had an argument they both were working in a hospital lab environment. And they were wondering what is the role of pancreas in fat metabolism. By that time pancreas is known to have lot of lipases and these 2 thought that the pancreas might be involved in fat digestion**.**

So, they wanted to find out whether it is really true and they had a debate about it and they decided to do an experiment. See these were not even scientist, they were like technicians and they surgically removed the pancreas from a dog to see the effect on the fat metabolism. But before even they proceeded to work on the fat metabolism in the absence of pancreas, they found that the dog was producing way more than normal level of urine.

And also in the routine measurements, they found it had a lot more glucose in the blood and urine than normal. This made them to think that pancreas is probably involved in controlling the diabetes**,** so that is the anti-diabetic organ. So, these 2 guys identified pancreas to be the anti-diabetic organ and then they tried to purify the active ingredient what is the molecule that pancreas produces that acts as an anti-diabetic agent.

And they did not succeed and many others did not succeed for a long time since their original finding for about 30 years or so. It is primarily because the active ingredient here or active principle is insulin and insulin is a protein. And pancreas as we have seen produces proteases, so probably when they have not taken care of inhibiting the proteases insulin was probably degraded and therefore they never succeeded in purifying insulin.

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But decade's later Frederick Banting working in the lab of Macleod with the assistant of this graduate student Charles Best, they succeeded by taking care of this proteolysis in isolating and purifying insulin**.** So, in the purification process what you do is the pancreatic extract like crushed pancreas will have the anti-diabetic effect. And now you keep purifying from that extract and each fraction that you isolate from the initial soup you test whether it has this activity or not.

So, that is how your assay for a biological activity. And then any fraction that does not have the activity then you know the molecule is not there, any fraction that has then you know it as in that. So, like that you go all the way to pure molecule and that is how Banting ended up purifying insulin. And so for that obviously he was awarded Nobel prize like Banting and his boss Macleod, they both got Nobel prize.

And they both knew the important contribution by these 2 people and they shared their Nobel prize money with these 2 people**.** Banting with Best and Macleod with Collip shared their Nobel money when they were awarded. This was one of the discoveries where the discovery to the treatment was in about 2 months**.** So, the moment they knew insulin is the one that is doing the job then people started producing insulin from pig your slaughter pigs and isolate insulin and then provide to the human beings.

Because pig insulin works in human as human insulin, similarly cow insulin for a long time they used cow insulin. So, these meat industry, cattle or pig kind of things were used for isolating insulin initially. But in the early 80's, late 70's when the recombinant DNA technology came people were able to clone the insulin encoding human gene in plasmids and put in bacteria and make bacteria to produce lot of human insulin in industrial scale. And that is what is currently used **ok,** so this is the story of the discovery of insulin**.**

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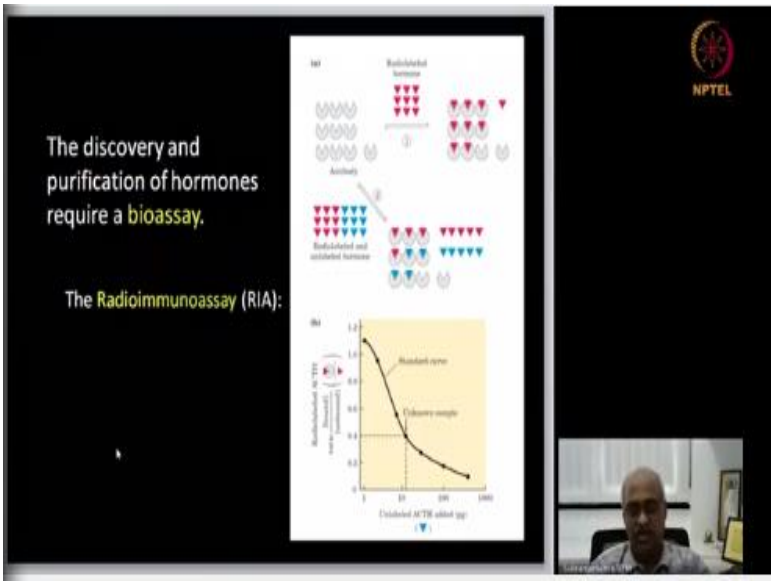

So, we were kind of along the way we were talking about assay how do you assay for the hormone? So, the assay I was kind of briefly describing particularly in this insulin purification is based on the activity of the hormone**,** based on the biological activity and that is a bioassay**.** So, in contrast to that there are other methods of detecting and quantitating the amount of hormones which is not dependent on biological activity.

But it depends on the biological specificity of receptor ligand binding or ligand antibody antigen binding**.** So, one such assay based on this specific molecular interaction is this radioimmunoassay**.** So, this was developed by Rosaline Yalow and she won Nobel prize for this. So, that is described here, it is extremely simple. So, for a hormone you inject into an experimental animal like for example you take human insulin let us say.

Inject into a rabbit and since the post translation modifications etcetera and the human insulin will be different from that in rabbit. Human insulin will be recognized as an foreign body, meaning it will be an immunogen and that will induce the immune system of rabbits to produce antibodies against it. Now you take blood from that rabbit, isolate serum and that serum will have antibodies against human insulin.

And you can actually using affinity chromatography you can purify the antibodies that are specific for the human insulin. So, let us say you have got an antibody like that shown here in this grey structure on the top left of this cartoon. So, now you incubate in the test tube with a radio labeled hormone, let us say insulin in which you have one of the atoms radio labeled, meaning radioactive atom.

Like you can have radioactive sulfur or radioactive carbon or you can have radioactive hydrogen like tritium. So, when you have radio labeled hormone, it will have radiation and that radiation can be detected using monitors, counter, scintillation counters or auto radiogram by exposing to X-ray film etcetera. So, in this particular ways a scintillation counter is what is appropriate, because this is done in liquid form, this experiment.

So, now the radio labeled hormone will bind to the antibody as shown here. So, you will have bound hormone as well as free hormone when you are mixed these 2. And how much of will be bound, how much of will remain as free depends on the concentration of the antibody used, and the affinity between these 2**.** So, if the affinity is very high, meaning the dissociation constant Kd is extremely low.

Then at the extremely low concentration itself they will readily bind. So, now and this bound and free can be separated, because this antibody is very big molecule compared to the hormone, so the antibodies can be made into a bigger complex using secondary antibody or they can be covalently attached to a solid substrate etcetera. So, the main point is the bound and unbound can be readily separated.

So, now in this scenario let us introduce unlabeled hormone. So, the binding specificity has nothing to do with radioactivity. So, both will have equal affinity for binding to the antibody. Now if you have unlabeled one and that will occupy some of the antibodies and which cannot be occupied now therefore by the labeled one. In other words the unlabeled one will compete with the labeled one.

So, how much of the labeled one is in the free form and how much is in the bound form is a function of the amount of the unlabeled one, that is what is the basic principle of radioimmunoassay and that is how you quantitate. Now for example you keep a constant amount of antibody and constant amount of radio labeled hormone. Now you keep increasing in different reactions, the concentration of the unlabeled one.

Let us say initially you use known concentrations of pure unlabeled hormone that will help you to plot a graph like this. Now you take another experimental thing where you have the same amount of antibody, same amount of radio labeled hormone and you add unknown source of this hormone. Now depending on how much is bound and unbound like for example here in Y axis you have a ratio of the bound to unbound.

Then based on that value and based on this working standard graph, you will be able to know the unlabeled one has about this much, so this is log scale, so it is roughly about 20 picogram is what is present. So, this is the principle of radioimmunoassay. Essentially you have fixed amount of antibody binding to a fixed amount of radio labeled hormone. Now you add unlabeled hormone and the amount of that impacts the labeled one binding.

And if you have used known quantities of unlabeled hormone and plotted a graph like this, then an unknown one can be easily using this graph can be measured. So, this is the basic principle of radioimmunoassay, it is readily doable and whole lot of labs around the world every day use radioimmunoassay. There is slightly more sophisticated method called enzyme linked immunosorbent assay or ELISA that is what is currently more commonly used in place of radioimmunoassay.

But still there are certain quantization where radioimmunoassay is still currently used. So, this is all about how do I detect and quantitate hormones.

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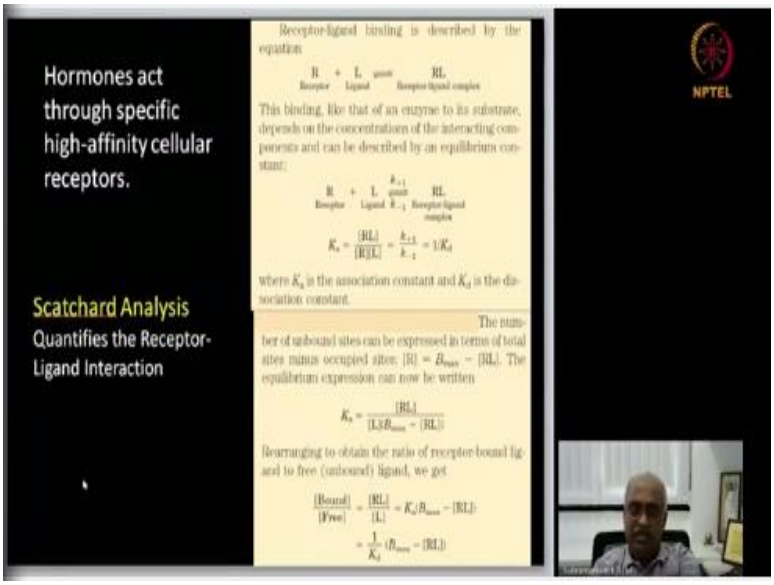

And little bit kinetics, just a couple of slides kinetics on this. So, essentially the 2 main points, one, hormones act through specific high affinity cellular receptors, 2 important keywords here, specific. They are highly specific 1 hormone that binds to a receptor may not be bound by any other hormone. Similarly a hormone that binds to a particular receptor may not bind to any other receptor such high specificity exists.

And of course there are cross specificities, like over certain hormones bind to different kinds of receptors as well, one. And second is high affinity, these molecules are produced in very low quantities. Yesterday we learnt about this with respect to steroid biosynthesis. I was telling you

the amount of cholesterol that flows through that pathway is extremely low compared to the amount that goes through like bile salts, bile acids production.

So, hormones work at extremely low concentration and that is primarily because of the high affinity. So, the Kd values for this kind of binding is in the order of 10 power -9 to 10 power -11 molars**.** So, like for example 10 power -9 means at that concentration 50% will be bound and 50% will be unbound. So, they are specific and they have high affinity for the ligands. So, this is sort of straightforward, so this is like enzyme substrate kind of thing, receptor ligand, so you have receptor ligand complex like ES.

So, you can have a forward reverse reaction with the 2 rate constants you have like k + 1 and k - 1 for the forward and reverse. And for the binding for this direction K a will be RL, that is the product of the concentration of the 2 substrates or reactants, if you take it as a chemical reaction. And that will be equal to k+ divided by k – 1, so these 2 rate constant that will be the association constant.

And the reverse where the R and L will be on the top and this will be down, so k - 1 by k + 1 that will be like 1 by K d**.** So, that will be K d but this is 1 by K d, so equilibrium constant for the association will be 1 by the equilibrium constant for the dissociation**,** so K a is 1 by K d, so remember that. So, normally we refer in terms of K d dissociation constant, so in layman terms K d equals the concentration at which 50% are bound and 50% are unbound.

And to introduce little bit complexities such that we can actually have a straight line that can be used for quantification**,** so that is what is Scatchard analysis. So, Scatchard is the person who came up with this kind of an equation and this graph for quantification of receptor and ligand interaction. And for that we make some substitutions, so for example the free receptor will be equal to the maximum sides that are available for binding minus already bound form**.**

So, this is like the total receptor where ligand binding is possible. From that you subtract what is already bound then you get the free receptor, so R = B max by RL. And now if you introduce that into this equation, so this will be RL and here this R, now you are going to call it as B max -

RL, so that is K a. And when you rearrange it to get this bound by unbound ligand like bound and free ligand then it will rearrange into this, like K a times B max – RL. In other words K a you can substitute as 1 by K d and you can have this equation.

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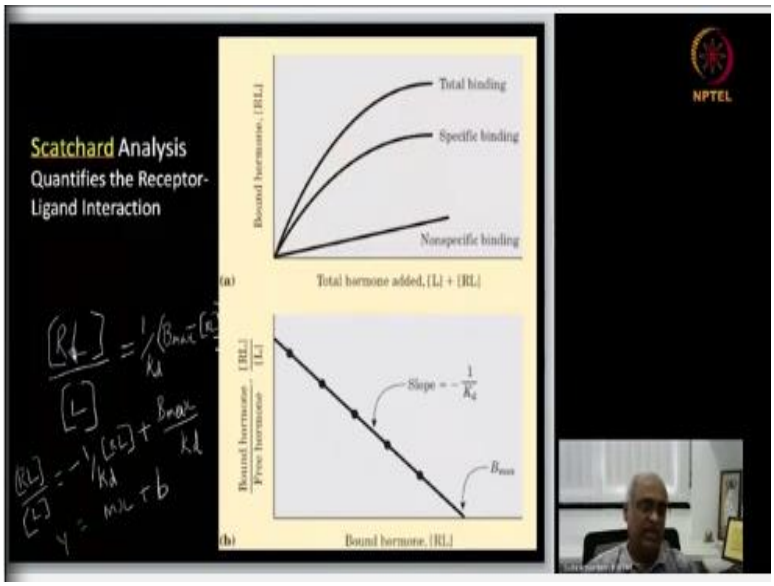

Or for plotting a straight line slope intercept kind of equation like Y = mx + b, you can rearrange this like by multiplying this it will rearrange into bound by free ligand equals -1 by K d times ligand plus total binding by the K d. So, this B max K d is like b and RL is like X and Y - 1 by K d is like m, so this equation resembles this Y = m x + b or the slope intercept equation. So, in such an equation, so then x axis intercept will EB = B max and the slope is -1 by K d.

So, you can calculate these 2 values readily by using this sort of a graph by plotting I told you these are readily obtainable and by plotting these 2 then you will be able to calculate this, so that is the scattered analysis. So, this is all the kinetics we are going to learn about receptor ligand interaction, in this case hormone receptor interaction.

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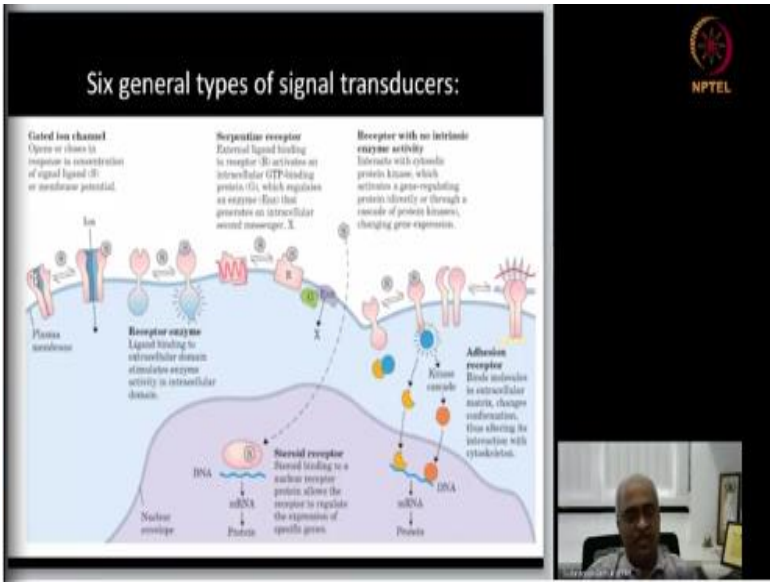

So, next we are going to look at the different kinds of signal transduction that happens with the hormone receptor binding**.** So, these are summarized in this, there are 6 types, one is ion channel mechanism where it is a channel which is normally closed, when the hormone binds conformational change in this channel, which is a protein, opens up and ions can flow through, so this is one mechanism.

Another one substrate binding on the extracellular surface activates an enzyme, part of the same peptide in the cytoplasm and that active enzyme does the activity that is required. And the third one is like the adenylate cyclase mechanism that we learnt where you have a receptor that binds the substrate and that has an associated G protein where the ligand binding leads to a conformation change such that this G protein binds GTP.

And the GTP bound form is active and that binds to an enzyme and activates that enzyme. We saw adenylate cyclase as the example where that enzyme is adenylate cyclase that produces cyclic AMP and so that is one mechanism of transduction. And fourth one is a molecule like the glucocorticoid, mineralocorticoid and other steroid hormones where they are hydrophobic. So, they do not need, they are not stopped at the plasma membrane, so they really diffuse through the plasma membrane and nuclear membrane.

And they bind to proteins which are the steroid receptors in the nucleus. And that may activate transcription of a gene or a suppressor transcription of a gene in the nucleus. So, that is how steroid receptors function and retinoic acid also functions this way, vitamin D functions this way**.** And fifth one is a receptor dimerization and activating an enzyme that is associated in intracellularly with this membrane bound receptor.

So, the receptor itself does not have enzyme activity as you saw in the second example. So, here the receptor binds but then the receptor dimerization usually activates a protein kinase. And this protein kinase may have a cascade, meaning it phosphorylates a protein and that becomes active and that phosphorylates another protein and so on. And then finally the active that is phosphorylated molecule translocates into the nucleus associates with transcription factor and regulates transcription, so this is one more mechanism.

And the last one is this addition receptor where the genes are not activated but the ligand binds to molecules in the extracellular matrix, remember we have learnt this at great length when we were learning about glycosaminoglycans in carbohydrate chapter. And those extracellular matrix molecules when they bind the ligand, they undergo conformation change. And when they undergo conformation change, they are connected to the cytoskeleton inside and they undergo changes, and that changes the cellular morphology or function and so on. So, these are the six different ways by which signal is transduced**.**

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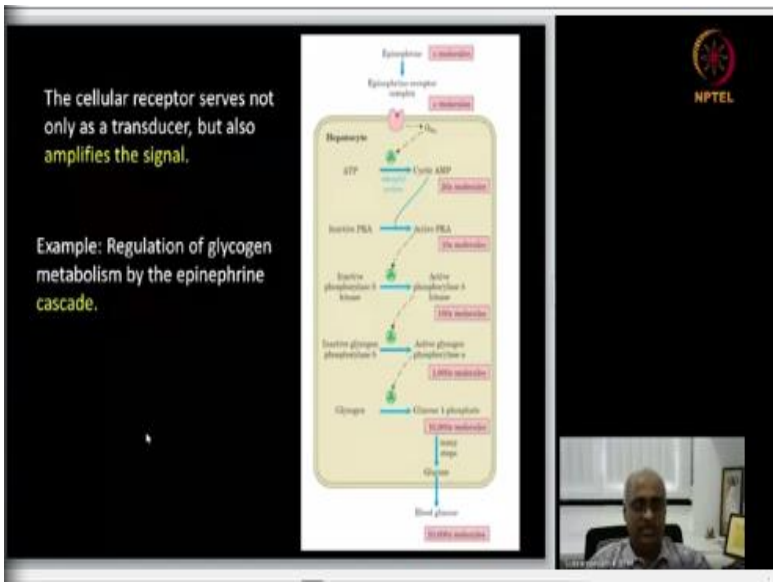

So, this is another important concept of signal transduction like hormonal signal transduction. The main point here is when you have multiple steps in the transduction as we saw with respect to this fifth mechanism, where the receptor with no intrinsic enzyme activity, when it binds the ligand it is conformation change activates a protein kinase inside the cytoplasm and that phosphorylates another protein.

And like that you could have multiple steps and those steps are called the cascade. So, in this case signal transduction cascade and this cascade has essentially cascading effect, meaning amplification of the signal**.** That is illustrated by this example of glycogen metabolism regulated by epinephrine; this is something we have already seen. So, the letters are small, so I blow it up a little bit, so that you can see.

So, you have let us say x molecules of epinephrine binding to epinephrine receptor on the cell surface. And that activates this G protein and then cyclic AMP and that cyclic AMP production is by adenylyl cyclase. And now this molecule this G protein that one single interaction, epinephrine let us say x is 1. That ends up producing 20 cyclic AMP molecules because this enzyme is constantly active, it has it is own turnover rate, adenylyl cyclase.

And that in turn each one of this cyclic AMP is going to activate a protein kinase**,** inactive to active. And let us say they end up producing 10 and they go and activate and in a phosphorylate

inactive phosphorylase b to active phosphorylase. So, this is involved in glycogen biosynthesis, so these phosphorylases are going to phosphorylate glucose molecules. And that is what was is involved in glycogen metabolism, biosynthesis, degradation, in both that phosphorylation is important.

That is the substrate phosphorylation and that is done by the enzyme which is regulated by phosphorylation by protein kinase. So, there let us say 100 molecules are done and each one of them go and activate the next enzyme in that. So, this is phosphorylase kinase, meaning the phosphorylase enzyme is phosphorylated by this and by that it becomes active. Now that enzyme goes and activates another one where you get 1000.

And finally you get for 1 molecule of epinephrine binding to receptor you end up producing 10,000 glucose phosphate molecules and then finally it becomes glucose and it gets into the blood**.** This is glycogen breakdown, because the signal we are talking about is epinephrine which is inducing gluconeogenesis and glycogen degradation**.** So, releasing glucose from glycogen is not gluconeogenesis.

Gluconeogenesis means neo that is making from non-carbohydrate source. Like amino acid metabolism producing like for example you do transamination of alanine and produce pyruvate and by pyruvate carboxylase produce phosphoenolpyruvate and that goes back to form glucose, reverse glycolysis, so that is gluconeogenesis, epinephrine induces that as well. Here in this example we are seeing glycogen breakdown, glycogen is glucose-glucose polymer.

And from the glycogen one glucose at a time is cleaved off in this glucose 1-phosphate version**.** So, this is an example of a cascade, so in extremely low concentration they can make all this possible, because of the presence of the cascade**.** So, this is an important concept in endocrinology or endocrine signaling.

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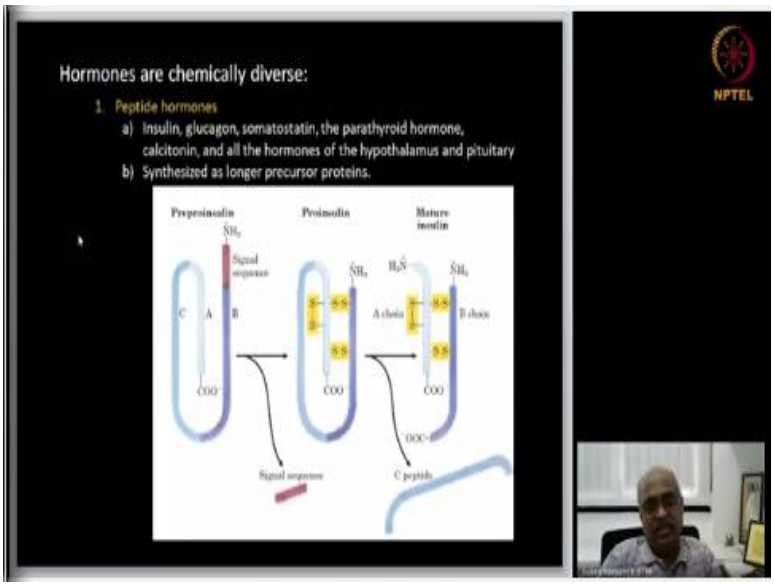

So, the remaining slides what we are actually going to talk about is the different kinds of hormones**,** they are chemically diverse. So, the first class that we are going to look at are the peptide hormones. We are already familiar with couple of them listed here, insulin and glucagon both are peptide hormones produced by the islets of Langerhans islets in pancreas. So, an important idea here is they are produced in a precursor inactive form and that undergoes proteolytic cleavage to make the active version.

We saw this with the digestive enzymes like pepsinogen then becomes pepsin by undergoing a proteolytic cleavage. So, similarly here insulin for example is produced in preproinsulin. And in that you have an n-terminal signal peptide 23 amino acids that helps in anchoring into the membrane and that is cleaved to generate proinsulin**.** And in that process these disulfide bonds are also formed.

Then it undergoes 2 more proteolytic cleavages, one here and one here and this blue colour portion is released as a C-peptide which has it is own function. And this A chain, B chain 2 chain containing final product is what is active mature insulin**.** So, this is an important point, the peptide hormones are synthesized as longer precursor proteins and they are cleaved into active form, so that is one.

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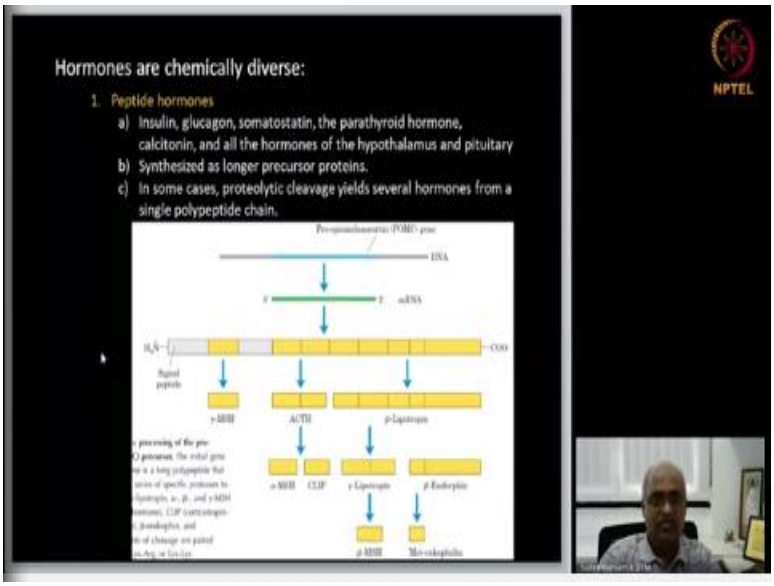

And some of them are actually from the long primary polypeptide; the proteolytic cleavage produces multiple hormones as shown in this example here**.** This proopiomelanocortin POMC gene. So, that produces this long peptide and that is cleaved into 1, 2 this itself is functional too, 2, 3, 4, 5, 6, 7, 8 different hormones are produced from this cleavage**.** So, for example good vigorous physical exercise produces this endorphin and that makes you feel happy and positive and active, so that is by this peptide.

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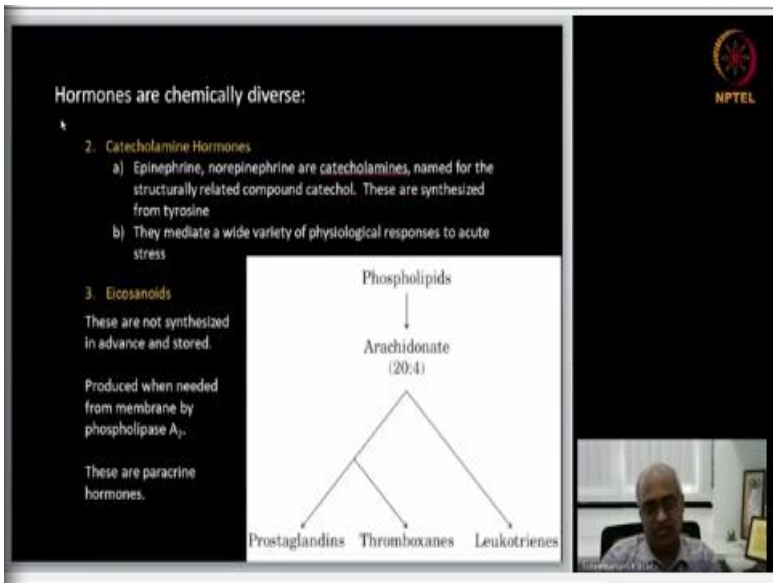

And so that is the protein hormones. So, 2 things we saw there, one they are produced as large peptides which are cleaved into active version, one. Second is sometimes such proteolytic cleavage may produce multiple hormones from a single original polypeptide. So, the second one

is catecholamine hormones because they have a structurally related compound catechol and they are related to it and that is why they are called catecholamines.

We already know them, epinephrine we know very well, just now we saw it is role in glycogen metabolism. We have seen it is role in fatty acid biosynthesis as well as cholesterol biosynthesis II. And these are synthesized from the amino acid tyrosine, so these are essentially modified tyrosines. And so their function is they mediate wide variety of physiological responses to acute stress.

And third one is eicosanoids, these are produced from poly unsaturated fatty acid arachidonic acid, this is a 20 carbon fatty acid with 4 double bonds. We have already seen them and their functions, prostaglandin, smooth muscle contraction, thromboxanes blood clotting, leukotriene allergy response, so all this we have already seen. So, these act in neighboring cells and therefore these are called paracrine hormones, they actually do not travel too far in the blood.

These are not produced and stored, like for example the beta cells really store high concentration of insulin in secretory vesicles and that is secreted in response to glucose in the blood. But eicosanoids are not produced and stored, so they are made from the membrane phospholipase can cleave and release this fatty acid from the phospholipid of the membrane**,** so these are produced locally and act locally.

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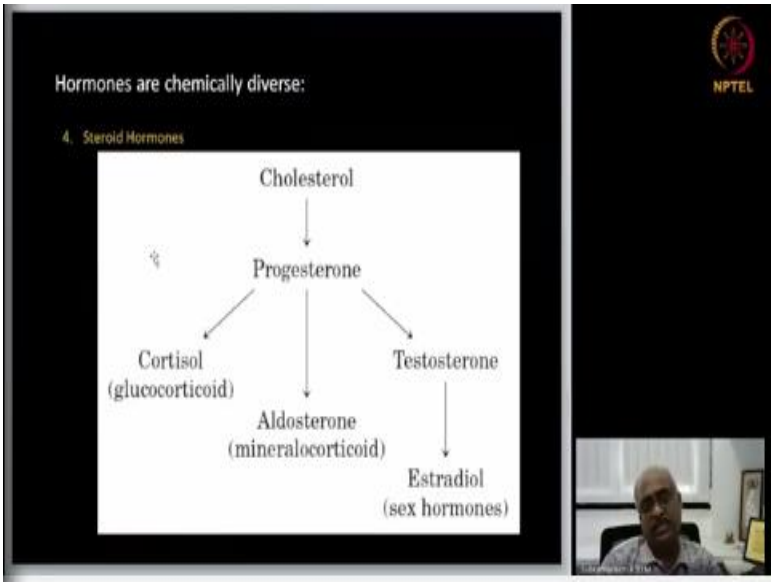

Fourth one steroid hormones, we know all of them, so we have just seen them yesterday. They have produced from cholesterol and we also know what each one of them do.

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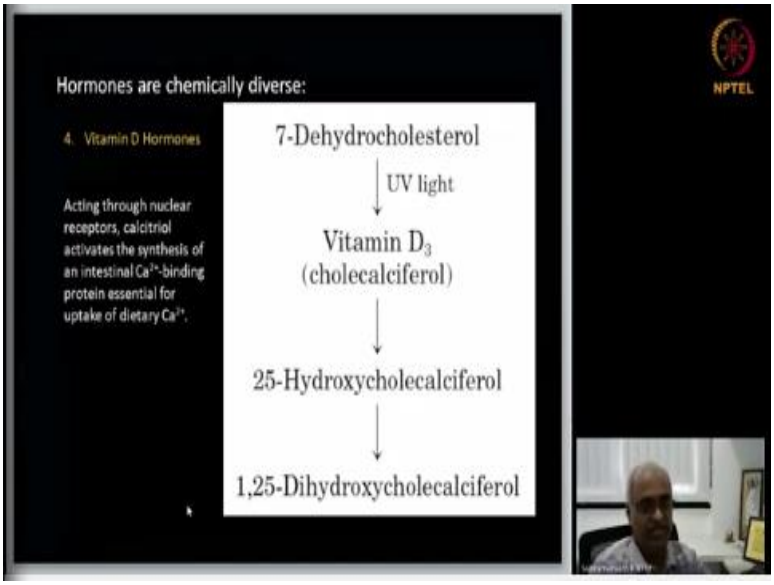

And vitamin D one of you Ashwin, you asked lost at least twice, so I did not need to separately study about it, fortunately it is part of our hormone discussion itself. So, this is produced by you can have them from 2 different sources, one is from 7-dehydrocholesterol on our skin UV light can induce the formation of the active version which is this 125-dihydroxycholecalferol. And the first reaction 7-dehydro to cholecalciferol formation is done by UV light on the skin.

So, that is why you should go out in sunlight at least for half an hour a day, not surprisingly 95% Indians are deficient in vitamin D. And the function of the vitamin D the active form this long chemical name or this common calcitriol. Because it has a 2 dihydroxy group in addition to the cholesterol's alcohol group, so that is why it is called triol, calci because it is involved in calcium metabolism**.** So, it is important for the synthesis of intestinal calcium binding protein, which is essential for uptake of dietary calcium.

So, and it works in conjunction with parathyroid hormone as well, PTH. So, PTH and vitamin D work in conjunction in activating the transcription of the gene that encodes this calcium binding protein which is essential for uptake of the calcium. So, if you do not have vitamin D, you do not produce this protein enough and you have problem uptake of calcium and that leads to weak bones and osteoporosis and problems with that kind of a situation.

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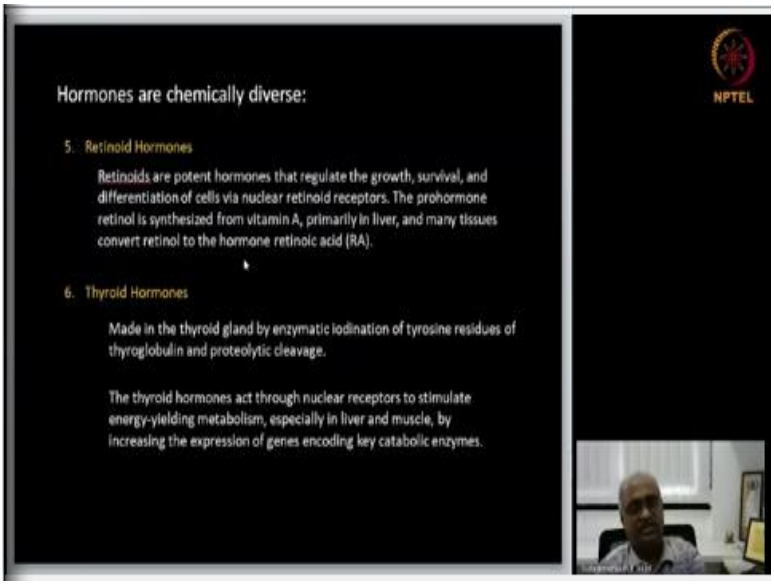

So, 5, retinoid hormones, so, we have seen retinoic acid, beta carotene breaks into 2 retinol the alcohol version and when that is oxidized you have retinoic acid or it can become aldehyde and then you have the cis-trans isomerism involved in light sensing. So, the acid version is the retinoic acid, which translocates into the nucleus and binds to retinoic acid receptor and that binds to retinoic acid response elements and regulate gene expression**.**

And that what kinds of genes are regulated, genes involved in growth, survival and differentiation of cells these processes are regulated by receptors bound by retinoic acid. And these are present in the nucleus, because retinoic acid can readily go through the plasma membrane and nuclear membranes. And the last class that we are going to learn about are thyroid hormones, these are produced by the thyroid glands by iodinating tyrosine residues, triodothyronine is one of the active versions.

And where these tyrosine moieties come from? They come from thyroglobulins produced by the thyroid gland and thyroglobulin is hydrolyzed by like proteolytic cleavage to produce the iodinated tyrosines. And these again are hydrophobic and directly go through the membranes and bind to nuclear receptors to stimulate metabolism, essentially energy yielding, like catabolism in liver and muscle. And they do it by since it is in the nucleus; it is by activating gene expression of genes that encode these catabolic enzymes.

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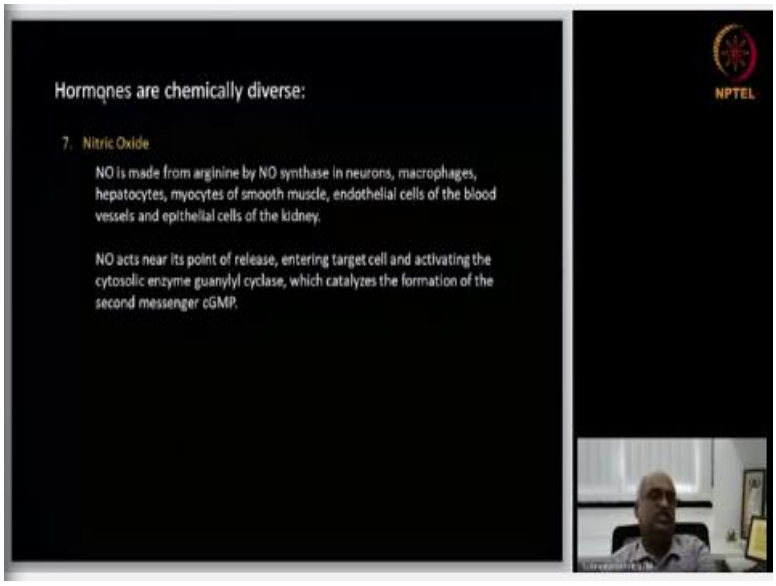

So, one more I forgot, nitric oxide it is an interesting molecule. So, this is a gas, an extremely simple gas NO**.** So, this is produced from arginine by nitric oxide synthase, this work locally**.** So, these are produced by neurons, macrophages these are cells involved in body defense, hepatocytes liver cells, myocytes of smooth muscle, endothelial cells of blood. So, they are involved in blood vessels or dilation, constriction regulation, epithelial cells of kidney.

So, they are very popular for their role on smooth muscle relaxation, but they play a very important role in endothelium as well in blood vessel normal function. So, these act near the point of release, because this is a gaseous molecule, now another gaseous hormone molecule exists in plants and that is called ethylene. So, ethylene again is gas that is required for fruit ripening.

So, these enter target cells and activate cytosolic guanylyl cyclase. Like adenylyl cyclase, this is guanylyl cyclase producing cyclic GMP**.** And cyclic GMP has it is own set of downstream effects.

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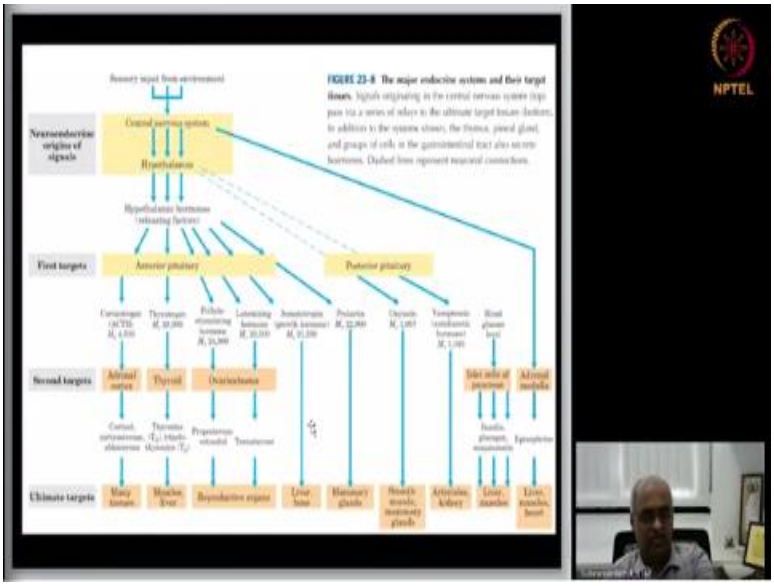

This last slide is nothing new information, whatever we saw till now is all put together in their who is the boss, who is the middle manager, who is the floor worker relationship**.** So, the main point alone I will highlight, I told you there is a hierarchy**.** Like for example we talked about hormones I am looking for the thyrotropin releasing hormone in this. Like for example thyrotropin, let me blow it up big.

So, thyrotropin is produced by pituitary, anterior pituitary that goes and stimulates thyroid to produce thyroxin**.** So, therefore this pituitary is like the middle manager here. And this in turn responds to hormones produced by hypothalamus. So, these are the releasing factor

hypothalamic hormones, they act on anterior pituitary and posterior pituitary to activate them to produce hormones is tropic hormones that go and act on the endocrine plants.

And this hypothalamus is the one that integrates then neuronal signals the endocrine signals**.** So, sensory input from environment, sensed by the central nervous system activates the hypothalamus to release these hormones, hypothalamus hormones that go on act on the pituitary that produces the trophy hormones that act on the endocrine glands. So, you have 1, 2, 3 steps in this hierarchy, but sometimes it is not strictly this.

For example if you see here, so some of the hypothalamus hormones skip the pituitary and directly act on the hormones, the endocrine glands. Like for example adrenal medulla responds to hypothalamus produces epinephrine. And there are some situations where you do not have this hypothalamus pituitary connection. For example blood glucose level directly induces the islets of Langerhans produce insulin or if glucose goes down glucagon.

And so this is a third way. So, you have one, where hypothalamus directly acts on endocrine gland and another one which is the major one where hypothalamus acts on pituitary and then pituitary acts on these endocrine glands. And the hormones produced go and work on the concerned organs, the really where the biological effect of this signaling it has to happen. Like for example prolactin, this is fourth variety I should highlight. So, here this is a hormone that is produced in response to hypothalamus by anterior pituitary.

But it does not go and act on any other endocrine gland. This prolactin produced by pituitary directly goes and stimulates the mammary glands to produce milk**,** so that is fourth variety. So, this is the way this signaling is integrated. And the final functions, meaning the organs on which these hormones act are listed at the bottom**,** so this groups all the hormones together. So, remember the whole discussion we have had till now in today's class is like as brief introduction to endocrinology.

Primarily focusing on the main concepts and that is it. We are not getting into the details of each one of them and if you are interested then you can read endocrinology chapter at least which is

there in any biochemistry book, including Lehninger. I just thought that I should throw out this idea of hormonal regulation and integration of the metabolism at an organism level as a closing point for this course. So, with this I stop and so the course is complete with this class**.** So, if you have any questions feel free to ask.

(Professor Student Conversation Begins: 00:01:40)

So, in the radioimmunoassay, yeah, so the rabbit produces antibodies to the human insulin that you mentioned, right. So, how is that useful for studying in human insulin as in how is it useful for us? Sorry, how radioimmunoassay is useful for studying insulin, yeah for humans**.** So, see from any source like for example you want to find out whether the patient has enough insulin in the blood or not, how are you going to find out right?

A patient comes with some symptoms like says I have giddiness or I am having problems, I have some diabetic final visible symptoms. And you want to find out what is the problem with the patient, then you check for glucose level in the blood, so how do you do that? So, we learned about the glucose oxidase reaction, so when you find the glucose level is high, then you want to know why the glucose level is high. So, you want to find out whether the patient has problem producing insulin. So, how will you know the patient is producing insulin or not?

You need to measure insulin, so this radioimmunoassay helps you to detect that**.** From any unknown source, like see the dashed line here is an unknown sample. So, the unknown sample when added to this equilibrium of fixed amount of antibody and fixed amount of radio labeled and how that affects this ratio is a function of the quantity of insulin present in that sample, so that is how you will find**.**

Ok sir and sir in the association comes in that you defined for receptor ligand said K a = RL upon R into L and you defined it as k + 1 by k – 1, in that equation. So, are you talking about this one in this slide, the previous one, yeah? So, here in the, so which is your question this one the K a equals yes sir 1 by K d. Sir K a = k + 1 by k - 1**,** so this one how we get to this? Yes sir, yeah this is simple weight constant equation only.

So, you have see the rate of the forward reaction will be proportional to the product of the concentration of the reactants**.** So, R 1 let us say this forward reaction R1 will equal R times L the concentration. And similarly so breakdown will be if you say R - 1 that will be equal to or not equal to the product of proportional to this and equal to k - 1 if you use rate constant times RL. And K a equilibrium constant will be, this is the same as the CD by AB, so let me write this down.

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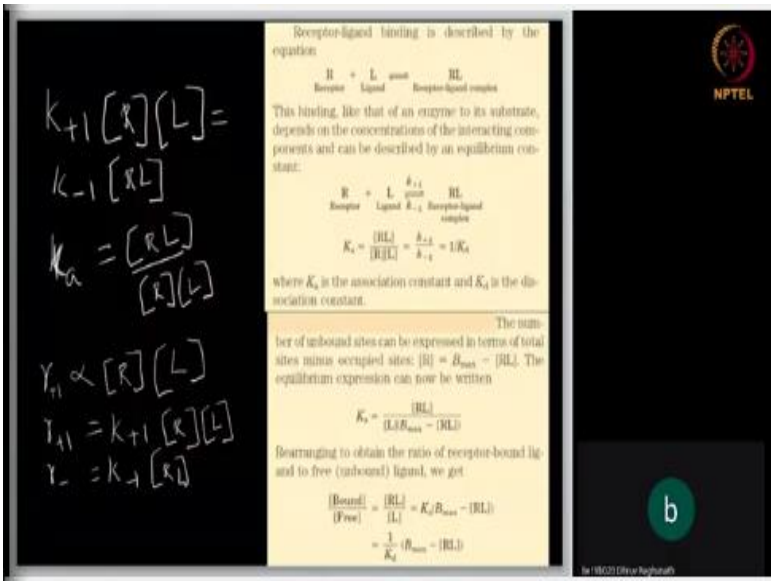

So, the forward reaction, let us say forward reaction, so the rate of the forward reaction. So, I am going to call it as r + 1, this will be proportional to, this is law of mass action. And if you introduce proportionality constant, this and for the reverse similarly**,** this will be the reactant. So, are with me till now? Yes sir, so the equilibrium constant therefore for this will be, so at equilibrium.

So, you will have this equals this, let me go fully since we are doing this, I have not done this for a while, but let us see how it goes. Ok sir, wait, let us finish this, I am trying to create space for me to write**.** So, now at equilibrium K + 1 this will be equal to RL**.** And if these 2 constants if you take out and they are that is going to be equilibrium constant, the capital K**.** So, these are should be small k, would be for the forward I am skipping one step here, it will be this.

For the reverse for the dissociation the numerator will become denominator, denominator will become numerator, because you will write it in the reverse way. And that is why it is 1 by K d, so is this clear? Yes sir, I know that, thank you, ok.

(Professor Student Conversation Ends: 08:17)