Introduction to Biomolecules
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Lecture-32
Cholesterol Biosynthesis and Lipid Transport (Part-2/2)

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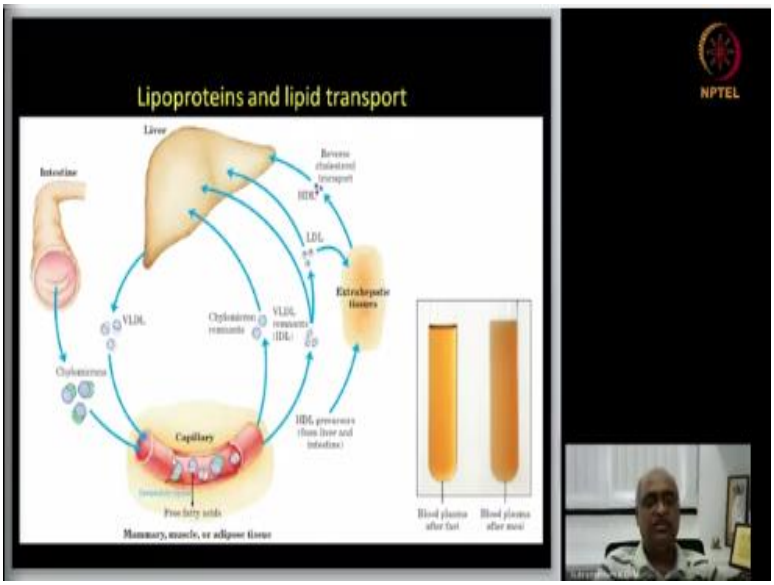

So, since there is a question about HDL and LDL, so we will just look at that slide once more and then we will go to today's class. So, yesterday we were discussing about the lipoproteins and their involvement in transporting triacylglycerols and cholesterol**.** So, primarily we looked at these 2 lipids. So, the question is let me read the question once more, I did not get the concept of HDL and LDL with respect to having high cholesterol levels and possibly having high risk of getting the disease.

So, HDL LDL and their levels and how that connects to cholesterol levels, so that is what we are going to focus now. So, the first point is we start with these 2, chylomicron and VLDL, so chylomicrons transport dietary lipids to other tissues**,** primarily to it carries in the blood and that activates a lipase. And then the free fatty acids produced by these lipases are taken by the tissues, either for beta oxidation like for example muscles or for storage, by again converting into triacylglycerol in adipose tissues**.**

And after the cargo delivery the remnants of chylomicrons go back to liver, so this is one. And second when you have plenty of carbohydrate available like carbohydrate rich diet. So, then the main dietary material here is carbohydrate, so they are not carried by chylomicrons. So, they are by glucose transporter and so on, so we did not get in to that. But anyway that excess carbohydrates in liver will be converted into triacylglycerols and from other sources.

For example amino acids after transamination the carbon skeleton will be again converted to acetyl-CoA and used for making triacylglycerols and cholesterol. And these are like instead of intestine here liver being the source of the lipid; they are packaged in VLDL**.** The primary differences are the apolipoproteins that are present and they act as signals which cells are going to take up and what cargo they carry.

So, those are identified by the lipoproteins present in them. And VLDLs again circulate to other tissues via blood through these capillaries; they again would do the same thing that happened to the dietary lipids. And from the VLDL when triacylglycerols are removed then the density slightly increases, from very low density lipoprotein they become LDL**.** So, the LDL is essentially rich in cholesterol and that should be taken up and used for storage or for energy production by other tissues, extra hepatic tissues.

So, their level in the higher level in the blood means higher levels of cholesterol that actually needs to be used. And if that is not used then that is what deposits on the blood capillaries, blood vessels leading to a disease called atherosclerosis**.** So, I think that spelling is coming in the next slide if not I will write at that point**.** So, this is what leads to cholesterol deposition, not just cholesterol even other lipids deposition on blood walls inside.

Therefore the lumen gets narrowed or constricted which increases blood pressure, because heart has to pump the blood, now through narrower tubes, so the pressure increases. And if these plugs size really increases and reduces the capillary further then the blood flow gets obstructed and then you get a cardiac arrest or heart attack**.** So, that is when the doctor says this artery is blocked and then we are clearing it up, they find that by using a process called angiogram.

So, that helps them to find where the block is and then they try to remove it up using angioplasty or surgery, mostly angioplasty. So, this is basically a balloon that goes and blows up the obstructing material. So, this is the connection of LDL to the disease higher level of LDL indicates higher level of cholesterol in the blood that has no place there. It should actually been taken up by the tissues and used up.

So, we will see that whether a disease condition we will discuss in today's class, so that is the LDL. And the difference between LDL and HDL is again apolipoproteins, so the HDLs actually on the other hand take up any extra cholesterol anywhere and they take it to the liver for metabolism**.** HDL level high meaning, you have that HDL particles plenty, so any extra cholesterol can be easily ferried into the liver.

So, high HDL means high availability of vehicle to carry of the any excess cholesterol that may be there. LDL means you have excess cholesterol loaded and that is why you have LDL level being high. So, on a healthy person should have less LDL and more HDL and there are conditions in which these levels are affected and that we will discuss in today's class. Durav did this answer your question?

(Professor Student Conversation Begins: 06:52)

Oh, yes sir but I have one more doubt, yeah go ahead, why does not the LDL combined with the HDL, like all of them why does it not like incorporated with HDL through have it gets again re-transforming? Ok, so these different apoproteins are different functions**,** so they have different receptors and then therefore they have different functions. So, HDL on the surface can combine cholesterol and triacylglycerol transported by specific transporters present on the cell membranes and LDLs do not do that. On the other hand LDLs are actually taken up by the cells by the binding of the apolipoprotein on LDL by the LDL receptors on the extra hepatic tissue.

So, that is going to be a main discussion today, so we will have a dedicated slide for that. So, it is basically the specificity and therefore the functional delegation. So, they have different functions to do, HDL's job is to pick up cholesterol and transport to liver, LDL essentially after the triacylglycerol is taken away from VLDL whatever remaining that is primarily cholesterol enriched version is what is LDL and that is to be taken up by the tissues. So, they do different

jobs and that is primarily coming from the different apolipoproteins. So, it is basically functional separation.

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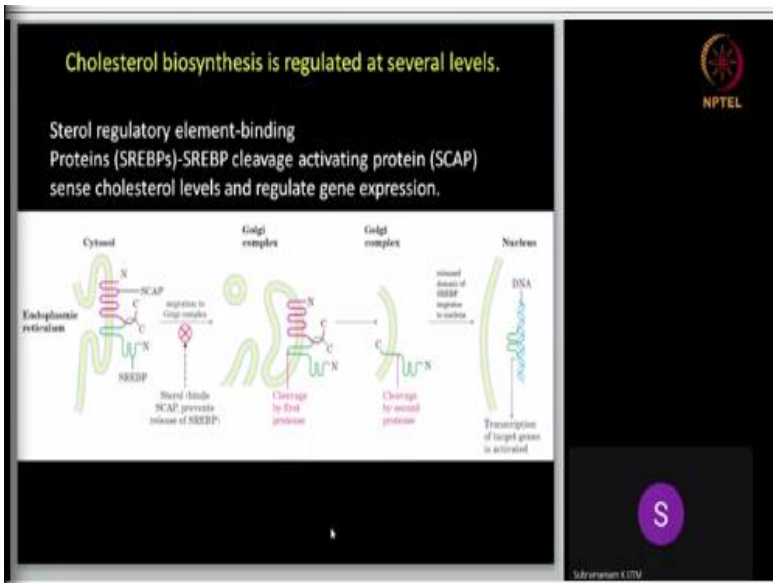

So, today we are going to focus on cholesterol biosynthesis and a little bit discussion on disease condition and then what are the other molecules synthesized from cholesterol and then we will begin our discussion on hormonal control of metabolism. So, for those of you who are interested in metabolism related diseases that is actually an entirely separate course there are books diseases of metabolism of the size as big as a campus biology book, dedicated only to those.

Because in each one of the pathways you can have defective enzymes and therefore different metabolites accumulate and each metabolite accumulation or the deficiency of the product causes different diseases. So, we did see some at when we were discussing about lipids in the molecules of life section. So, today we will see one about LDL what are the problems there? So, before that let us begin on the cholesterol biosynthesis regulation. So, as you saw yesterday cholesterol biosynthesis is really a complex synthetic process.

So, we skipped the last 20 steps, simply saying that methyl group shuffling and removal of methyl groups and shuffling of the double bonds and so on. Like that we kind of glossed over it, but whatever we learned itself is complex enough in a formation of mevalonate, formation of this

IPP and DPP and then they joining together to form squalene and then squalene cyclase in to form the steroid ring, so that itself is complex enough.

So, you do not want to do all this synthesis expanding a lot of NADPH and ATP unnecessarily. So, only when cholesterol is required as a precursor or cholesterol is required for new membrane synthesis, do you want to make cholesterol? So, cholesterol biosynthesis is regulated at couple of levels and both of them we will briefly touch upon. And this was worked out by the same 2 people whom we discussed yesterday their work about LDL uptake LDL endocytosis.

We saw Brown and Goldstein, so they were the ones who identified this SREBPs and SCAP and so on, so we will briefly go through that. So, these are 2 proteins sterol regulatory element binding protein SREBP**,** so this is the main regulatory molecule. So, it has an integral membrane domain and that is anchored on the endoplasmic reticulum**,** so this green line that you see here. So, it is internal part which is in not on part of the membrane this is what is the regulatory domain.

And this is in complex with another protein called SREBP cleavage activating protein or SCAP, when these 2 together or anchored on the endoplasmic reticulum they are inactive**.** And this SCAP is the one that senses cholesterol, so sense cholesterol, so it binds cholesterol essentially. So, when you have cholesterol then when a SCAP is bound with cholesterol when you have high cholesterol in the cytoplasm, then this is going to be bound with cholesterol.

And that leads to a confirmation that keeps them on the endoplasmic reticulum, presumably involving another protein that probably retains these 2 on the endoplasmic reticulum**.** But on the other hand if the cholesterol level is low and the SCAP is unbound with cholesterol then that releases these 2 from being on the endoplasmic reticulum and they migrate to golgi. So, on golgi this SREBP undergoes two proteolytic cleavages shown by these arrows, releasing the n-terminal peptide that is what is biologically active.

And this translocates into the nucleus and binds to transcription factors and this directly binds to the DNA where you have these sterol regulatory element, these are specific DNA sequences

upstream of the coding sequence of the cholesterol biosynthetic enzymes. So, essentially promote a region of those in genes. It binds there and activates transcription of those genes and ultimately by translation you are going to have the biosynthetic enzymes that then catalyze the biosynthesis of cholesterol.

So, when you have less cholesterol, then cholesterol biosynthesis is promoted by activating transcription of the genes that encode the enzymes involved in cholesterol biosynthesis, so this is how they regulate. So, I told you at the beginning when you have plenty of cholesterol then this shut off**.** SCAP binds to it and it is retained on the ER and as long as they are on ER, SREBP does not undergo proteolytic cleavage.

And this pathway is very fine controlled, because this cleaved n-terminal active version in the nucleus is very short lived, it continuously gets proteolytically cleaved. So, only as long as SCAP is not bound with cholesterol then only you have this n-terminal portion available. And this n-terminal portion should be continuously made this process, these 2 arrows must continuously be happening then only the gene transcription will be going on. Because this molecule gets constantly degraded**,** so that is how this is regulated. So, this is one mechanism of regulation of cholesterol biosynthesis.

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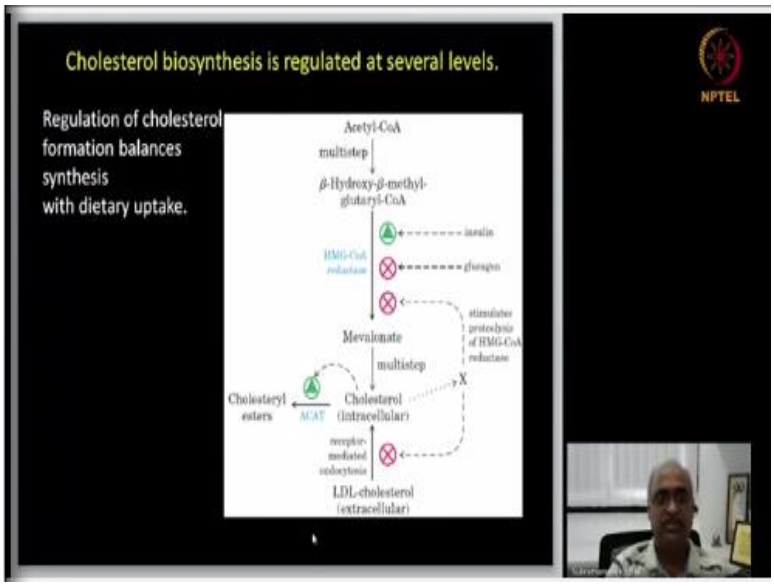

So, the other one is somewhat familiar to us. So, where; you have covalent modification by phosphorylation. So, the phosphorylated form is inactive and the dephosphorylated form is active just like we saw acetyl-CoA carboxylase for the fatty acid biosynthesis. So, the enzyme that is subject to such a regulation is HMG-CoA reductase. So, yesterday I told you this is the one that catalyzes the committed stepping cholesterol biosynthesis in general steroid biosynthesis that is the formation of mevalonate.

So, this enzyme is dephosphorylated when you have insulin signal. So, insulin will be high when you have glucose high. So, when you have high level of glucose you want to take up the glucose and convert into storage version, that is cholesterol, triacylglycerol etcetera. So, insulin by stimulating AC carboxylase acetyl-CoA a carboxylase is going to stimulate free fatty acid biosynthesis and ultimately triacylglycerol.

In addition by stimulating HMG-CoA reductase by dephosphorylating this enzyme, it stimulates cholesterol biosynthesis as well. So, essentially glucose is being taken up and anabolic reactions of this biosynthesis of lipid are stimulated. On the other hand if glucose is not available then you want to mobilize lipids for energy sources and producing the precursors for gluconeogenesis and ketone bodies in the blood.

And that is done by blocking the biosynthesis by glucagon. So, the same thing that; would block the malonyl-CoA synthetase in free fatty acid synthesis. So, this via cyclic AMP and protein kinase A would end up phosphorylated this enzyme and that inactivates it. So, this is how phosphorylation, dephosphorylation regulates HMG-CoA and this is integrated to hormone signals again.

And then we know from mevalonate, multiple steps you go to cholesterol. And cholesterol itself has a feedback kind of regulation, one it suppresses LDL uptake. Because LDL is the one that is going to bring cholesterol and when you have a lot of cholesterol already in the cytoplasm you do not want to take more LDL into the cell. And therefore the LDL proteins are apolipoprotein transcription those gene transcriptions is inhibited by cholesterol.

So, that is one role it does and it also stimulates proteolysis by an unknown protein of the degradation of HMG-CoA reductase. A third thing cholesterol does is it stimulates this acylating enzyme this acyl-CoA acyl transferase which adds to the hydroxyl group of cholesterol, it adds the fatty acid acyl moiety making cholesteryl ester and that is storage form this is strongly hydrophobic which is inside the chylomicron that we saw yesterday.

So, these are the ways in which the cholesterol biosynthesis is regulated, essentially you balance the synthesis with the dietary uptake. When you have plenty of dietary cholesterol available then you do not synthesize, you turn it off. And instead you take it up and then convert into cholesteryl ester and store it. So, these are the 2 important ways in which cholesterol biosynthesis is regulated.

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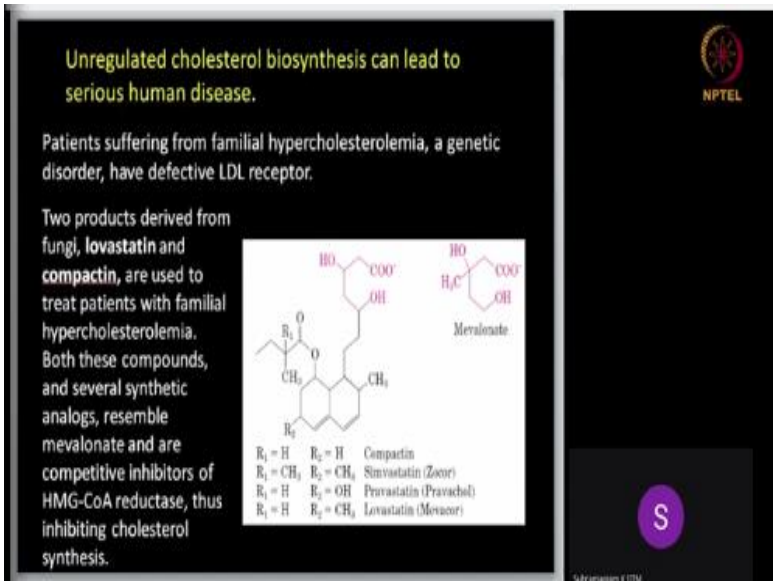

So, now let us see what are the different**,** so I told you we will discuss a disease. So, in all these discussions you definitely heard me say several times these apolipoproteins. And these apolipoproteins being specific they are characteristic for chylomicrons, LDL, VLDL and HDL**.** So, when you have deficiencies with them then you can have problems. And another thing is yesterday we saw a cartoon probably the last slide where I showed you that this LDL is taken up by endocytosis.

And for that the apolipoprotein of LDL binds to specific receptors on the cell surface. And binding of the LDL to it is receptor leads to endocytosis, and the LDL gets taken into the cytoplasm in the form of endosomes which fuse to lysosome. So, now if you have defects in LDL receptor, like for example due to a genetic condition, like you have a mutation. As a result the LDL receptor, the protein produced from such a defective gene probably is defective in binding LDL.

And there is a known such a disease condition genetically inherited disease, and that is called familial hypercholesterolemia**,** familial means usually refers to runs in the family, meaning genetically inherited disease. In other words inborn error, meaning the defect is in the gene and when you have a defect in LDL receptor and where LDL is not taken up by the cells, then LDL accumulates in the blood and you have then cholesterol also in the blood.

So, when LDL is not taken up then what will happen? Two things, one, dietary cholesterol accumulates in the blood in the form of LDL. And second because LDL is not coming into the cytoplasm this cartoon here there is no LDL coming in and therefore the assumption by the cell will be, we need to make cholesterol there is not enough cholesterol. So, the cholesterol biosynthesis gets stimulated and then you make cholesterol in the cytoplasm as well.

So, due to this you have really excess cholesterol and that is why it is called hypercholesterolemia, this emia sound usually refers to in blood, something hypoglycemia, meaning glucose level in blood is low, anemia iron is less in blood, ischemia low oxygen in blood. So, this emia refers to some things level in blood that is what it means.

So, here hypercholesterolemia means cholesterol level being very high in blood that is what this term means. And in such a situation what you want to do is, you want to intervene therapeutically blocking this enzyme therefore you do not make cholesterol**.** And here are those inhibitors, so the common inhibitor originally found in fungus and so they are the lovastatin and compactin found in fungi and they are used to treat.

Now we have many synthetic versions as well. So, the important point is shown here in the red colour here. So, this part of this molecule**,** so when it is R 2, it is compactin, so the basic structure remains the same and when by varying these two R 2 groups you have different statins. So, these are the statins that the cardiologist prescribes to patients having higher cholesterol or higher LDL levels**.**

And taking statins have been shown to have very little side effects and lot of people having high cholesterol level or take this drug on a daily basis forever. And there was finding that while these drugs reduce the cholesterol level, triacylglycerol levels and make your blood biochemical parameters really look textbook perfect. They do not necessarily reduce the plug formation and people did not know why is that.

But then now we know, the reason is these patients are diagnosed very late, so their arteries are already loaded with plugged with lipids and it is already narrow. So, now reducing cholesterol biosynthesis only will have in a marginal effect not a substantial effect. So, currently the thinking in the field is people prone to such diseases must be taking these statins very early in life.

Like for example these are not prescriptions, I am talking about discussions in the research literature. That probably these people should take drugs starting in their mid-20s if they really want to have benefit of these statins. So, you look at the structural similarity here, so these molecules as we learned in our enzyme inhibition they function as competitive inhibitors, meaning they compete with the mevalonate**.**

And so that is how they you block this HMG-CoA reductase. So, this is the action of statin. So, statins essentially block the biosynthesis of steroids and the condition in which steroid accumulation can happen is when LDL is not internalized due to defective LDL receptor, so those are the 2 main points from this discussion.

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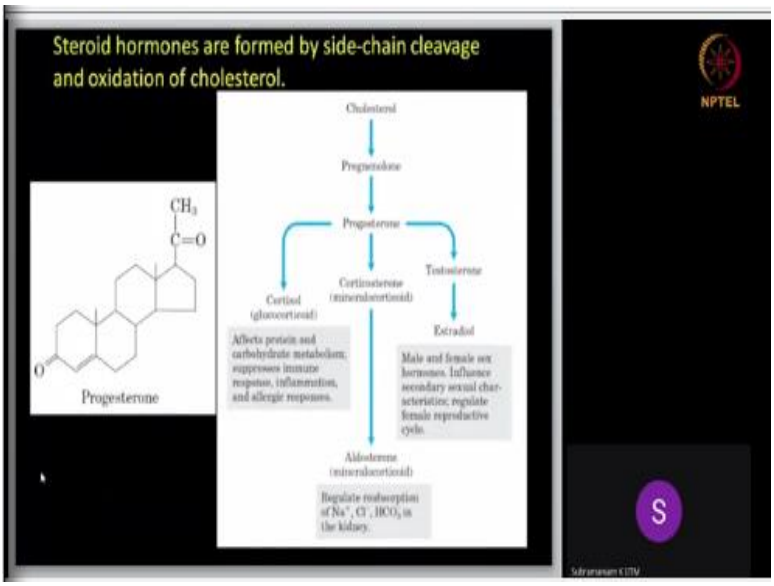

Now let us look at what are the other molecules produced from cholesterol, I told you cholesterol is an essential molecule, without cholesterol you would not have even developed into a complete a newborn child, essential development itself requires cholesterol, embryogenesis itself means cholesterol. So, it is an extremely important molecule, so it is notoriety in modern world is not due to cholesterol being bad, it is actually we being bad.

Because our metabolism evolved to store food when it is available, remember the hunter gatherer version of living which happened for 99% of our existence on earth in terms of evolutionary time. We did not have guaranteed 3 meals sitting on an armchair, or couch potato sitting on the couch and watching TV and the only exercise being playing with the TV remote control.

So, they were actually running, jumping, climbing trees and getting low calorie fruits and vegetables and tubers and so on, occasionally an animal kill some protein from it. They did not farm lentils and rice and wheat and crystallized sugar and so on. So, therefore whenever meal was plenty, the metabolism learnt to store it**.** And when you were not finding any food or you are crossing a patch of dry land with nothing then it used the stored food.

And as a result everything was totally fine and there were no issues. But you fast forward to 21st century what you are having is 3 guaranteed meals every day apart from snacks that some people munch uncontrollably, you can see some people incessantly eat snacks. And all of that are

calories; people hardly eat non calorie food, so they are all being stored in the adipose tissue. And on top of it there is no utilization of the stored fat because they hardly get out of the couch.

And that is why you have all the problems that we have now. So, the molecules have nothing to do with it**.** So, if you have lot of physical work no amount of cholesterol is going to harm you, it will all get used up, you saw how the regulation works. So, anyway now we move on to other molecules produced from cholesterol as well as molecules coming from the intermediates of cholesterol biosynthetic pathway, so that is what we are going to look at it.

A famous molecule is shown here progesterone, so this progesterone is what regulates the estrous cycle in female reproductive system. So, this is a derivative from cholesterol, so we will see in some detail how this is made. So, cholesterol an important intermediate pregnenolone, this is the one from which the steroid hormones are produced. And the pregnenolone to progesterone and from progesterone you get in adrenal gland in adrenal cortex you have 2 hormones.

Glucocorticoids which regulate glucose level and suppress immune system, and that is why excessive use of steroid drugs will end up having immune suppressant problems. And this is the reason also why steroid drugs are given to control allergy and inflammation**.** So, naturally in our system these cortisols do that, the glucocorticoid versions. So, the name itself tells you what is this? Gluco means it senses and regulates glucose metabolism.

Cartico meaning it is produced by adrenal cortex, or it refers to steroid**.** So, it is a steroid produced by adrenal cortex functions in regulating carbohydrate metabolism**.** So, that is what you get from this name. And another adrenal cortex steroid hormone is these corticosterone version mineralocorticoids. So, these mineralocorticoids produce one of the important mineralocorticoids is aldosterone, what it does is?

It regulates the uptake of the salts in kidney reabsorption in kidney. So, therefore this hormone regulates electrolyte balance which is again very critical**.** So, these are important hormones. And you need to remember the amount of cholesterol needed to produce these steroid hormones is extremely low. Because these hormones act at extremely low concentrations, so the cholesterol

flowing through this pathway is really negligible compared to the cholesterol that goes to membrane all in bile acids and bile salt production.

And the second set of hormones, this is the adrenal hormone I am calling these 2 together as one. And the second one are the; hormones produced by the gonads, in the first intermediate is testosterone and that is converted in estradiol in ovary. And testosterone in males and estradiol in females act as the sexual hormones and these influence the secondary sexual characteristics. And also they are also along with progesterone is involved in regulation of female reproductive cycle. So, therefore the steroid hormone biosyntheses are derived from cholesterol as the precursor. And how are they produced from cholesterol? That we will see in the next slide. Oops yeah here.

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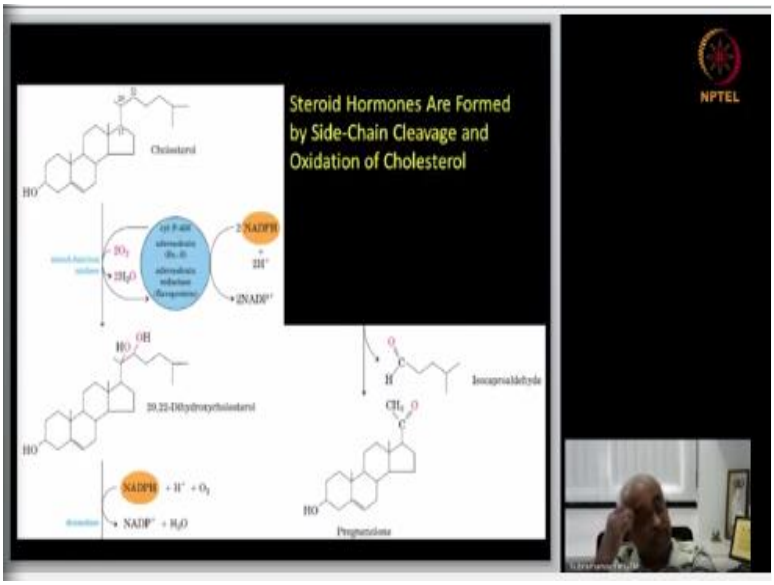

So, essentially this side chain is removed and that is removed by an interesting biochemistry**.** So, 2 adjacent carbon this 20 and 22 get oxygenated by enzymes called mixed function oxidases. And these are cytochrome P450 dependent enzymes and we need not go into the details of these electron donor transfer proteins. So, you forget about it, all you need to remember is mixed function oxidases are involved in oxygenating adjacent carbons**.**

And you get these 2 hydroxyl groups and then decimal is the next enzyme ends up cleaving this bond generating a carbonyl group**.** So, this is the primary reaction involved in the formation of all the steroid hormones, the side chain cleavage. Then you have additional steps where the other

carbon atoms may be oxygenated making hydroxyl groups. So, where which hydroxyl group depends on which hormone you are making, and that detail we need not worry for this introductory class.

If you remember this that is steroid hormones are formed by side chain cleavage and how the side chain cleavage happens on this point that adjacent carbons are oxygenated, that is they add an oxygen to make a hydroxyl group by a class of enzymes called a mixed function oxidases. And such enzymes are involved in later oxygenation steps as well in the pregnenolone precursor for the other hormones**.** So, this is starting from cholesterol. Now I told you the intermediates in the cholesterol biosynthetic pathway also serve as precursors to make other molecules.

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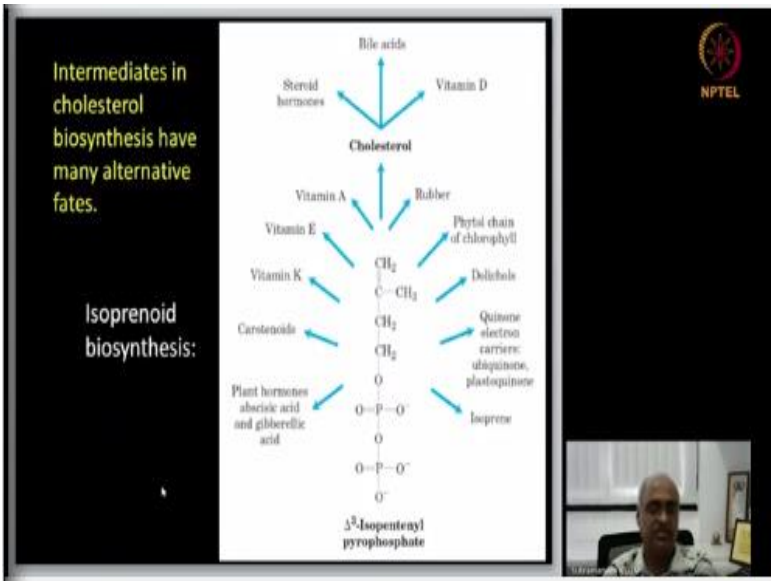

And that is in this slide**,** this whole lot of molecules are made, depending on what kind of organism you are. Like for example this isopentenyl pyrophosphate, this is the active isoprene group I told you right yesterday. And from that molecule you make all of this, let us start somewhere familiar, we will start here. So, the isoprene themselves are active molecules, this quinones that we saw ubiquinone and in plants plastoquinone.

And these are electron carrier proteins, they are synthesized from this dolichols we saw that they carry carbohydrates for making lipopolysaccharides on the membrane. So, they anchor on the membrane help in transfer of groups from one molecule to the other on the membranes, so we

saw that and phytol chain in chlorophyll, so we saw phytol in chlorophyll, so there that biosynthesis all long chains where you have isoprene the 2 methyl butadiene portion.

So, that is this portion is repetitively involved in biosynthesis of those rubber and cholesterol itself. Then we learned from cholesterol, steroids, bile acids and vitamin D, so this we have already learned. Then vitamin A, that again has a long chain, vitamin E related to dolichols it is an antioxidant on the membrane, vitamin K involved in blood clotting. Carotenoids, we saw the accessory pigments in chloroplasts in the light harvesting antenna.

Then these we have not heard of abscisic acid and gibberellic acid, these are plant growth hormones. So, abscisic acid is involved in leaf senescence and falling, in the seasons where leaf fall happens and fruits ripening and falling. And for the separation of that part leaf or the fruit that is promoted by abscisic acid, gibberellic acid is involved in germination of seeds. So, many molecules are produced from this isopentenyl pyrophosphate and they all together called the isoprenoids and isoprenoid biosynthesis**.** So, these are the variety of molecules that produced from the isoprene that is produced as part of cholesterol biosynthesis. And many fragrances as I told you are derived from this**.**

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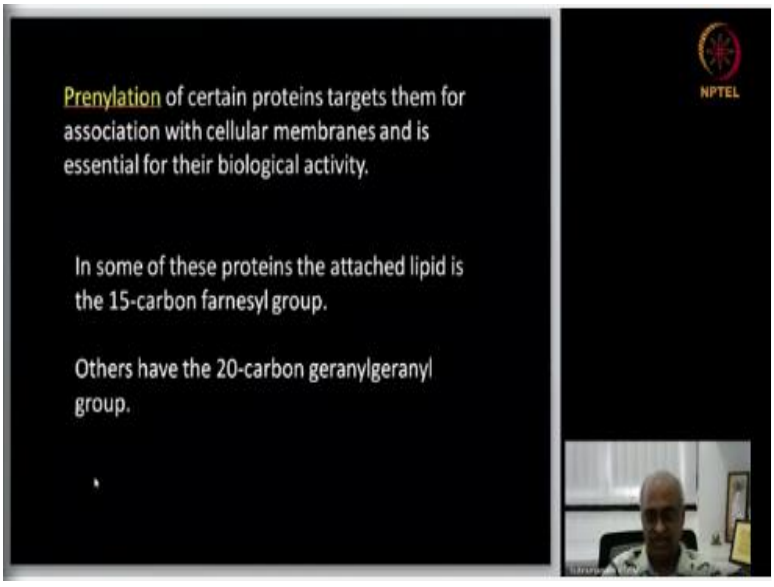

So, the last point in our metabolism discussion is this covalent modification of proteins, we revisit. So, very familiar with; phosphorylation and dephosphorylation as a covalent

modification. You have also seen here and there AMP being attached to an enzyme and that becomes an active or you have also seen what else you have seen on the way I am trying to recollect. Primarily phosphorylation, dephosphorylation, and we have seen this UDP, CDP attachments in activating but I did not discuss that in detail.

So, anyway for sure you remember the phosphorylation, dephosphorylation being a regulatory covalent modification to enzymes. Similarly there are other groups that can be added, one of them is isoprene group addition to proteins, covalent attachment and that is called prenylation. And this can be sometimes a form a cell group attachment, so that is called prenylation as well, if you want to be very specific farnesylation and sometimes it is geranylgeranyl group attachment.

So, geranyl is IPP DPP joining is geranyl group and then you have another IPP that became farnesyl. But instead if you have 3 geranyl group joining that gives you this 20 carbon geranylgeranyl group. So, these are hydrophobic molecules, so they help in anchoring some of the proteins to cell membranes, and that often is important for regulating their biological activity.

So, the membrane bound form will have an activity or sometimes it is sequestered on the membrane and taken away from activity. And those, sort of regulation is done by covalent addition of these prenyl groups, so that is prenylation. So, now you will not be able to think cholesterol in a bad way, after hearing all of this.