Overview and Integration of Cellular Metabolism

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Lecture 48: Heme Metabolism -III (Heme Degradation, Transport and Bilirubin Metabolism)

Hello everyone, welcome back to our lecture series on Overview on Integration of Cellular Metabolism. We are continuing with heme metabolism, this is the third lecture where we will be studying the heme degradation, how heme is transport and we will be also discussing the bilirubin metabolism. So, these are the concepts that will be covering in today's class, how heme is degraded, how it leads to the formation of bile pigment, how bilirubin gets conjugated and circulated, what are the various tests by which we can detect bile pigment and we will also briefly discuss the principle of phototherapy right. So, fate of heme right. So, hemoglobin is the major source of heme, we already know hemoglobin when it is degraded it has got us where hemoglobin is present, it is present in red blood cells right and red blood cells have got a life span of 120 days after which they are degraded in the reticulo endothelial system that is the splenic and hepatic sinusoids and when they are destructed heme is released. So, globin a protein part is released and heme is released right.

So, it heme we already know during synthesis and the structure of heme, how it is a cyclical tetrapyrrole structure it is having and these are the groups right methyl vinyl, methyl vinyl, methyl propionyl and propionyl methyl right that is a very important thing to remember and already told you that you should remember this structure and not the intermediates. Anyway so, we are discussing the steps of heme degradation to this. So, heme free heme right once it has been out of the RBCs, it is acted upon by the enzyme heme oxygenase ok. Heme oxygenase the ring opens right heme was a cyclical structure.

So, the ring now opens to form a linear tetrapyrrole structure which is delivered in. So, there is a specific region where the chain needs to be opened up and this reaction also produces carbon monoxide and iron, iron is also given up. So, we already know that heme is actually a ferroproto porphyrin. So, now, again the iron is gone and it is now open structure belivardine right. So, what happens this iron has got many fades right iron binds to ferritin special in this case carbon monoxide is actually a poison it binds to

hemoglobin, but the concentration is very low carbon monoxide has got a potential to inhibit electron transport chain complex 4.

So, that is how it acts as a poison anyway we got belivardine. What happens in the next step? Belivardine is acted upon you can see this is the structure ok here. So, belivardine is reduced by belivardine reduction. So, basically this methenyl bridge ok is actually reduced to methane, methenyl is reduced to methane this is CH2 ok. So, this reduction of double bond happens with the reductase which needs NADPH ok.

So, beli heme to belivardine by oxygenation and then reduction belivardine to belirubine ok. So, there is a characteristic colour difference between these compounds mind it this is cyclical compound heme it is actually red or brownish in colour when it is out of the RBCs. When it is transformed to belivardine it is green in colour and ultimately when it is acted upon by NADPH dependent belivardine reductase it is yellowish in colour yellowish orange belirubine right. So, belivardine and belirubine are actually bile pigments you should know that belirubine specifically has got no function in body and it is excreted through bile purely excretary product ok. You should have a quantitative idea so, about 6 gram of hemoglobin is broken per day from which 250 mg of belirubine is formed right.

It is also formed in a very minute quantity that is 50 mg compared to 250 milligram from myoglobin and other heme containing proteins right. So, in total about 300 is produced and 80 percent is from the old RBCs that are destroyed. So, recirculation of that product and 10 percent from ineffective erythropoiesis and rest 10 percent from degradation of other heme containing pigment and myoglobin alright. So, this is basically the source or the calculation of total belirubine that is present in our system every day right. So, this is the roughly erythroids RBC source is 80 percent and non erythroids are 20 percent.

You should know what are these sub options because these may betaken up and given to in a multiple choice question. So, one thing from here may be shifted here in an all except type of question state the reasons of non erythroids sources of belirubine. So, be very careful right. A very easy way to remember is please remember all of these then automatically something which is not these will be from erythroids sources right. So, always be smart in learning.

So, what is shunt belirubine? Now, to study the this belirubine metabolism basically or how you know succinyl-CoA and glycine is actually united to form heme right and ultimately heme is degraded to form belirubine. So, what is shunt belirubine? When a labeled glycine molecule now glycine can be either labeled at the carbon or nitrogen. So,

when it is labeled as nitro it is either labeled by 15 n 15 or carbon by C 14. So, radio labeled or radioactive glycine is incorporated injected. So, that it is incorporated into RBC and naturally after 120 days 100 to 120 days roughly varies around 3 months the radioactive belirubine will be excreted this is normal.

However, it has been found that around 15 percent of radioactive belirubine is excreted in about 10 days. Why that happens? It is because it is this fraction is actually known as shunt belirubine right that is belirubine that is already excreted without being incorporated into the RBC. So, this is actually happening due to ineffective erythropoiesis that is RBC formation or heme formation is defective and this you know can you name some diseases where heme formation is defective porphyria. So, special in erythropoietic varieties of porphyria the shunt belirubine is increased alright. So, basically shunt belirubine is that fraction of belirubine which is not I mean formation of belirubine in bone marrow without being incorporated into hemoglobin right.

So, you should be very clear right this is the 10 to 15 percent of belirubine right. So, this is a very important short note MCQ or a Viva question depending on what type of exam you are facing. Now, let us discuss the structure of belirubine. So, we already know that after belirubine has been reduced this is the central methane bridge that is connecting the two dipyrrole rings there is no confusion in that right. However, as we see that in spite of having multiple polar groups which are shown in red belirubine free belirubine is insoluble in water.

Why it happens? It is because there is an intramolecular rearrangement that leads to formation you can see the this double bond is actually getting like this right and it orients itself. So, that there is a formation of multiple internal hydrogen bonds intramolecular hydrogen bonds that leaves no free polar group to be dissolved in a polar solvent. Therefore, it is not soluble in water, water is the major polar solvent that is present in our system right. So, it is because of this reason that free belirubine is insoluble right and the central methane bridge actually becomes buried right you have I hope you have understood please note you do not need to remember all this structure is just for conceptual understanding why free belirubine is not soluble in water. You can simply answer that due to presence of intramolecular hydrogen bonding number one the central methane bridge will be buried there will be no free polar group to act with any polar solvent right.

So, this is basically the rigid tile structure of belirubine that is the name nomenclature where the central methane bridge is buried and there are multiple intramolecular hydrogen bonds in spite of presence of multiple polar groups right. So, let us see what happens once belirubine is formed right. So, once belirubine is forms you can see heme

to belivered into belirubine it is actually combining with albumin. So, this whole thing albumin belirubine complex will be then transported to the liver. So, first step is since belirubine is not hydrophilic it means it is lipophilic right therefore, it is easily bound to albumin right plasma which is the plasma protein and one molecule of albumin can bind to two molecules of belirubine right.

And in generally 100 ml of plasma can transport up to 25 mg of belirubine. So, this is the phase where albumin is bound to belirubine right. So, after combination of combination with belirubine it is then transported to the liver where albumin you should know that this combination of albumin is actually lose ok it is not very tightly bound right. So, whenever there is any need or whenever there is any excess of belirubine this albumin can actually easily dissociate from belirubine or whenever some drugs that facilitate this attachment on belirubine can easily displace the albumin. So, drugs like aspirin, penicillin etcetera they can actually displace albumin and they can lead to formation of free belirubine which being lipophilic can easily cross the blood brain barrier and get deposited in the brain leading to the formation especially in babies leading to the formation newborn babies leading to the formation of a disease that is known as carnitaurus where the whole brain is actually coated by belirubinac that is one of the very important condition that is present in newborns will be again discussing it sooner and it leads to multiple neurological symptoms and it can also lead to coma and death of the baby anyways.

So, specially in newborns these drugs administration should be taken care of if due to some need we need due to some requirement clinical requirement we need to inject these drugs precaution should be taken right. So, belirubine albumin complex it goes to the liver after going to the liver what happens the albumin. So, belirubine is albumin is actually then dissociated after it goes to the liver albumin is dissociated and now we have free belirubine which is again insoluble, but when it enters the liver with the help of a transporter the albumin the belirubin sorry will get conjugated with glucuronic acid which actually makes it water soluble. So, let us see how it happens with happens with the help of UDP glucoronosyltransferase or UDP glucoronyltransferase that it is one and the same abbreviated as UGTs is generally a group of enzymes it generally this enzyme this group of enzyme it has got multiple iso enzyme multiple isoforms which acts on multiple subjects to convert the substrates to their corresponding glucoronide form the mechanism is common to all. So, UDP glucoronic acid is converted to UDP uridine diphosphate again where have you have learned you have read UDP donor where in case of glycogen synthesis UDP glucose was the glucose donor right.

So, here with the help of UDP glucoronosyltransferase UDP glucoronyltransferase a substrate will be converted to glucuronide belirubin just replace substrate will belirubin

and you already got your reaction that is belirubin will be converted to belirubin glucuronide. So, this UDP glucoronyl or glucoronyl transferases are present in endoplasmic reticulum and they convert many internal and exogenous toxic substances to the non toxic metabolite this is a part of xenobiotic right. It is how waste products are metabolized in our system. They are a family of enzyme that are concentrated in the liver and the enzyme isoform that is specially conjugating belirubin is UGT 1 A 1 ok. It is essential for conjugation of belirubin and its eventual excretion in the system because I told you belirubin is an excretory product it needs to be thrown out of the system or excreted right and inherited UGT 1 A 1 deficiency will cause neonatal jaundice jaundice of the newborns.

So, what happens inside the cell it is conjugated glucoronic acid to make it water soluble and it is actually the first carbon of glucoronic acid that combines with the carboxyl carbon of the propionic acid side chain in the belirubin molecule. Belirubin molecule has got propionic side chain and what happens the first carbon of glucoronic acid is binding together, but how does it make it water soluble? There are actually two steps first belirubin becomes mono glucoronate then diglucoronate, but let us see how it becomes water soluble. There were multiple this is the insoluble photo of the belirubin right insoluble image where the central methane bridge is buried intramolecular hydrogen bond. Once this propionyl side chain actually combines with glucoronic acid by two sets of glucoronidation the central methane bridge is actually exposed. This increases the reactivity solubility everything.

This is why glucoronic acid or conjugated belirubin this is what this is nothing, but conjugated belirubin it is soluble. So, there occurs two subsequent conjugation first state is converted to mono glucoronate and then to diglucoronate. In most cases the reaction is complete therefore, 80 percent are formed are in diglucoronate form whereas, only 20 percent are in mono glucoronate form. There are certain drugs like primaquin, novobiosin, chloramphenicol there are multiple antibiotics and anti maladryl as well as hormonal drug like pregnant diol that actually may interfere with this conjugation and that leads to jaundice. We will be discussing jaundice in detail in our upcoming next class where we will be going through a plethora of reasons right and this is one reason if there is a problem in conjugation there will be jaundice ok.

So, conjugation happens in the liver. So, there are some differences in free and conjugated belirubin of course, solubility is an in water is an issue however, both are soluble in alcohol pole minded right inorganic solvent very important organic solvent I mean. So, plasma level in a I mean unconjugated belirubin or free belirubin this is also known as unconjugated is more compared to conjugated belirubin. In bile only conjugated belirubin is present there is no unconjugated. So, whenever by it has been

fully conjugated then only it is excreted into the bile and ultimately into the small intestine and that is the pathway of circulation of belirubin.

In urine there is no free or unconjugated belirubin whereas, conjugated belirubin may sometimes be present, but it is normally absent. So, both are actually absent, but unconjugated is never there is no possibility of being present in urine because unconjugated belirubin will not go actually I will be discussing will be showing the pathway how belirubin is coming out in urine actually. Next when we are discussing with absorption from GIT, unconjugated belirubin has been shown to be absorbed from the gut ok, but not conjugated belirubin. Unconjugated belirubin being lipophilic and diffuse and when we are discussing Vandenberg reaction it gives indirect positive and conjugated gives direct positive. So, now what happens the water soluble conjugated belirubin is excreted into the bile by an active process and this occurs against a concentration gradient right.

And this is the rate limiting step in the catabolism of heme ok. And this step is actually induced and increased by phenobarbiton. So, what is happening is the conjugated belirubin is conjugated it has transformed to diglucoronide form it will be I mean secreted in bile via the and ultimately bile is a liquid form liquid actually which is secreted in the gallbladder which is present in the gallbladder and via the bile duct it comes into the intestine via the second part of the duodenum right. It in reaches the small intestine ultimately it goes into the large intestine where inside the large intestine it is converted to a pigment that is known as urobilinogen right. So, this urobilinogen when it is formed right 20 percent of urobilinogen is actually reabsorbed from the intestine and returned to the liver via circulation that is portal circulation that is a different total different branch of circulation with blood vessels that is totally different from the systemic circulation or systemic blood vessels that are supplying the gut ok.

So, portal circulation generally is a circulation shunt between the intestine and the liver. We do not need to go into the details of the anatomy, but know this some form or one fifth of this urobilinogen is reabsorbed and again resecreted. So, this is the part where it is reabsorbed and it is going to resecreted this back arrow this is actually the enterohepatic circulation right and what happens to the other urobilinogen it is excreted in the urine or kidney it is excreted via the kidney. So, since urobilinogen is passed through blood the small fraction is excreted in urine alright and this urobilinogen right that is present in urine it is due to the fact that it is reabsorbed from the gut. Otherwise once it is in the gut it is destined to go into the feces which happens for the majority because bilirubin is converted to pigments like urobilinogen and starchobilinogen that are abbreviated as UBG and SVG right.

Both are colourless compound to start with. So, bilirubin or the bile that is present in the gut the conjugated bilirubin that is present in bile in the large intestine. So, from following intestine goes to large intestine it is converted to urobilinogen and starchobilinogen that are colourless products, but they are oxidized by atmospheric oxygen or oxidation right. Oxygen is present in the in large gut there are air pockets these are air filled organs or intestines right. So, ultimately they are converted to coloured products and these are that is the reason that both urobilinogen and starchobilinogen actually impart our characteristic colours to urine and stool right.

Urine generally urobilinogen is not that much colourful right. So, it gives a tinge or straw colour. So, normally urine is very thinly coloured when there is excess water we are drinking excess water urine is supposed to be colourless, but whenever we are having dehydration that is when the pigments get concentrated and we get some dark coloured urine dark yellow coloured or light yellow coloured right. But stool characteristic coloured is imparted by starchobilinogen and it leads to the standard reddish brown colour of the stool right. So, this is the pathway where we can see the physiological route of bilirubin excretion conversion excretion and this is the simultaneous biochemical changes that are happening.

So, bilirubin is transported to the liver with albumin right where in the liver it is actually getting converted to bilirubin diglucoronide this is transported to the intestine right and intestine in as a conjugated bilirubin via bile right and where it is converted to urobilinogen. Thereafter urobilinogen is converted to starchobilinogen and then starchobilin which is converted excreted in the feces ok and some part of urobilinogen is also transported to the kidney and is excreted in the urine as urobilin ok. So, remember this is the pathway of bilirubin excretion. Now, we are discussing test for bile pigments. So, specifically you need to remember name of the tests what we use in order to detect each of these compound components of bilirubin metabolism.

So, if we need to test bilirubin it can be tested by Fuchet's test, Meilin's test, Vandenberg's test ok Vandenberg's test. The urobilinogen is detected by Arlicks test same Arlicks aldehyde test that we discussed in our class of porphyria that was detecting urobilinogen and porphyribilinogen right the same test is used over here that is it is mixture with aldehydrogen to give a pink colour. And urobilin Schleisinger's test ok this is very important this is specifically the urobilin and starchobilin it gives a positive test. It should be note that this tests are mutually exclusive means Fuchet's test, Meilin's test is positive for bilirubin and it is negative for bilinogen and urobilinogen urobilin likewise for Arlicks test and Schleisinger's test these are also positive respectively for urobilinogen and urobilin alright. So, among all this test that we have discussed we need to know about Vandenberg's test the theoretical aspect of it because other tests will be

taught to you I mean you need to perform to detect either bile pigment or bile salt eventually in your practical examination.

So, the theoretical aspect of Vandenberg's test is very important which is actually very easy that is in acidic medium bilirubin reacts with a diazo compound that diazo types sulfanilic acid to produce a purple coloured azo pigment right. And it is the conjugated variety which actually gives the reaction immediately right and hence this test is called direct reaction or direct positive and hence conjugated bilirubin is also known as direct bilirubin it is synonymous the same thing. So, what actually happening is here we already know conjugated bilirubin the central methane bridge is actually exposed and accessible and this is where the diazo compound reacts with leads to the formation of purple colour whereas, in case of unconjugated bilirubin what happens if we directly treat with the diazo reagent there will be no reaction where whereas, if we need to treat with alcohol if we treat this unconjugated bilirubin or free bilirubin with alcohol then alcohol will disrupt this intramolecular hydrogen bond because it is a much stronger it has got a much stronger affinity to form polar groups with these polar bonds polar I mean residues and then the central methane bridge will be the buried bridge will be exposed and then it will give a positive reaction. So, unconjugated bilirubin gives indirect positive or indirect reaction therefore, unconjugated bilirubin is also called indirect bilirubin right. So, one thing you should know that bile pigments that is biliverdin and bilirubin and bile salts are totally different you have been taught how bile salts are actually produced from cholesterol and how by conjugating with tauridone glycine they are forming toracolic acid and glycolic acid they help in emulsification of fat those are totally different right they are detected by HACE sulphur test.

So, the test I mean bile salt and bile pigment students have got a tendency to mix them up they are not the same bile salts are useful beneficial compound or bile pigments are actually useless excretory product. So, finally, why are we studying all the synthesis we already know due to diagnosis of porphyria why we are discussing heme metabolism degradation of heme and excretion of heme and bilirubin because there is a condition where there is an excess amount of bilirubin in blood. So, we need to know the normal value the normal value of unconjugated bilirubin we already told you 0.2 to 0.6 mg per dl whereas, conjugated is much lower 0 to 0.

2 it depends on textbook to textbook, but generally conjugated is much much lower or even negligible in some cases than unconjugated bilirubin is a bit more, but totally it is always less than 1 ok. Whereas in a situation where it exceeds 1 mg per dl this will be referred to as hyper bilirubinemia means more bilirubin right. When the value crosses 2 mg per dl what happens the bilirubin gets deposited in the yellow white sclera and skin and this is also known as jaundice alright and the ichterus is actually the Greek term for

jaundice right. So, you can see in cases of hyper bilirubinum jaundice the sclera that is the white part of the eye as well as the nail bed skin etcetera they get yellowish discolored and with more and more value of bilirubin even the whole body may get yellowish discolored, but what about the value between 1 and 2 it is known as latent jaundice full fledged clinical jaundice more than 2 mg per dl. And lastly phototherapy in some cases specially newborns is used to treat jaundice right.

Since we are discussing structure of jaundice let us discuss phototherapy what happens I already told you during discussion of Soret band bilirubin can actually I mean the intermediates of heme synthesis since it is a pyro rings were actually absorbing various wavelengths and spectrum near blue light. He bilirubin also has got a tendency to absorb ultraviolet light in the spectrum between 460 to 490, but the beauty of it is when it absorbs bilirubin gets converted to a water soluble form that is known as lumirubin that can be easily extracted in via urine. So, this is the again the insoluble bilirubin right when we are exposing it to UV light there is intramolecular readjustment this molecule is again becoming like this and the carbon atom that is present over here this actually creates a steric hindrance due to the steric hindrance the intramolecular hydrogen bonds are disrupted and these are the transient phenomena and transiently the bilirubin becomes soluble the polar rubes become exposed and it is dissolved in water. So, lumirubin in spite of being unconjugated is soluble in water and it can be excreted via urine and the solubility is increasing. This we can see is a phototherapy bed this is a blue light the newborn baby has been placed in the bed and his eyes have been covered because ultraviolet rays can I mean damage the retina.

So, prevent retinitis this is a phototherapy bed we will be again discussing jaundice in much details in the upcoming class, but you should know if you are considering the colors the beautiful colors of bilirubin metabolism hemoglobin when released it is red and brown bileverdine is green bilirubin is yellow urobilinogen actually gives yellow color to the urine right starchobilinogen gives red brown color to the feces and bilirubin yellow color normally does not give any color, but it if it is raised due to some reason that is either impaired liver function or block in excretion it rises in blood leading to the hyperbilirubinemia and jaundice. So, to conclude in this class we have discussed the pathway of degradation of heme how bile pigments are formed how bilirubin undergoes an antihepatic circulation shunt bilirubin we have discussed the difference between conjugated and unconjugated bilirubin what are the test Vandenberg test what are the mechanism of action and the structural changes of hemoglobin during conjugation and phototherapy. So, these are my references and I thank you all for your kind attention.