Overview and Integration of Cellular Metabolism

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Week 11

Lecture 52: Nucleotide Metabolism – III (Pyrimidine Metabolism and Disorders)

Hello everyone, welcome back to your classes on Overview and Integration of Cellular Metabolism. We are in week 11 and class 52 and this is the third class of nucleotide metabolism where we will be covering pyrimidine metabolism and its associated disorders specifically we will be talking about pyrimidine chemistry, we will be talking about the de novo synthesis of pyrimidine, we will be talk discussing about thymidylate synthesis how nucleotides are reduced into deoxy form, we will be talking about the regulation of pyrimidine biosynthesis, the pyrimidine salvage pathway and last we will be discussing about disorders of pyrimidine metabolism, alright. So, to start with these are the major pyrimidines, we discussed about purines in our previous classes these are the pyrimidines and regarding pyrimidines the chemistry is very easy there is only one ring in purine there was a hexagonal ring followed by a pentagonal ring, ok. I hope you remember that in case of pyrimidine its there is only one benzene like ring. So, with six sides and the numbering we will start from the first in the nitrogenous carbon and go in a clockwise direction from 2 to 6, alright and different pyrimidines that is cytosine, uracil and thymine vary in their side chains. For example, here between cytosine and uracil this oxo or keto group has been changed to an amine group, right and between uracil to thymine you can see there has been addition of one methyl group.

So, these methylation amination these are things that you should keep in mind when we are dealing with inter conversion of pyrimidines, right. So, this concept will be applying later in our lectures. Now, this is the same slide that we shared in our purine metabolism classes we showed how the these are the nitrogenous bases for this class specifically we should focus on these two these three, alright. Uracil, cytosine and thymine because these are pyrimidines and these are their respective nucleoside form nucleoside means nitrogenous bases and the ribose sugar and this is the these are the nucleotides nucleotides mean ribose sugar nitrogenous base and the phosphate group.

So, all of them have got a phosphate. So, it can be monophosphate that stands for AMP or Diphosphate DP or triphosphate TPs for example, UMP, UDP, UTP those all are

nucleotides, alright and the deoxy are the one the basis that are I mean deoxy are the variety of sugar that belong to DNA and ribo in RNA that you already know, alright. So, like purine synthesis if you understood that class very well it is very easy to follow in this class because it involves the same concept just like purine synthesis. So, there are two way of synthesis number one de novo de novo means starting new. So, we assemble a car by selecting absolutely factory made new parts all the new things one by one gets assembled to make a new car this is the analogy and in case of salvage path it means we are using already used a product that are almost pre made and we just assemble that car using pre made parts from used up or other cars, alright.

So, this is the analogy of salvage process we are using basic nucleotides to synthesize or basic already synthesize pre nucleotide to synthesize their monophosphate triphosphate versions, alright. But in case of de novo synthesis the ring will be synthesized the each element will be added one by one, but in salvage process ring is already there the nitrogenous base is there we simply add the phosphate group to functionally achieve the nucleotide synthesis, right. So, in case of pyrimidine biosynthesis what are the component what are the who are the donors to form the ring, right very important MCQ question glutamine carbon dioxide in the form of bicarbonate and aspartic acid or aspartate, ok. So, these are the three components who actually donate the atoms that form a pyrimidine ring. There is a difference between pyrimidine synthesis and purine synthesis you saw in case of purine synthesis the first step was when AMP when PRPP was formed, right PRPP that is phosphoribosyl pyrophosphate.

So, basically PRPP provided the ribose 5 phosphate group on which the entire purine nucleotide was formed. Here it is completely different this approach in case of pyrimidine synthesis here first the ring is formed and only at the very last step at the later stages for ribose 5 phosphate is added to it, ok. So, it is a difference in case of purine first ribose is attached and on ribose the entire ring is formed in case of pyrimidine first ring is formed and then ribose is attached, alright. So, in case of prokaryotes the total synthesis of pyrimidine happens in 6 step this is the 6 step pathway. Why I am saying prokaryote because in eukaryote the system is much more simplified right.

So, let us discuss the essential first because most of the questions are framed keeping prokaryotic system in mind especially in case of pyrimidine biosynthesis, alright. So, we will break down all the steps one by one. So, let us look in the first step what happens glutamine and aspartate I am sorry glutamine and carbon dioxide in form of bicarbonate forms carbamoyl phosphate, ok. So, you have heard this name carbamoyl phosphate where I hope you remember, right because there I said carbamoyl phosphate we will again encounter during pyrimidine biosynthesis because we discussed the enzyme carbamoyl phosphate synthetase, right. That was one here we are discussing about

carbamoyl phosphate synthetase 2.

So, this is the first step that is catalyzed by the reaction carbamoyl phosphate synthetase 2 where glutamine and carbon dioxide forms carbamoyl phosphate, right and you will see that ultimately its carbamoyl phosphate extends to form a ring. So, basically we can say that immediate precursor of this nitrogen and carbon is not glutamine and carbon dioxide and bicarbonate it is actually carbamoyl phosphate, alright. So, please remember it is actually carbamoyl phosphate and aspartate that is donating all this. In reality the carbamoyl phosphate is again formed from glutamine and carbon dioxide and bicarbonate. So, you get the answer.

So, if if you get a question all of these are sources of atoms in case in pyrimidine nucleotides and you get an all except type of question. Remember there are four correct possible answers glutamine, carbon dioxide, bicarbonate, aspartate and carbamoyl phosphate. If carbamoyl phosphate is an option you cannot mark it wrong because carbamoyl phosphate is also actually a donor of the atom in case of pyrimidine ring very important from for example, point of view, right. Next step what happens this carbamoyl phosphate combines with aspartate another donor of the pyrimidine nucleotide ring and it forms carbamoyl aspartate the enzyme is aspartate trans carbamoylase which is abbreviated as ATKs or ATCAs aspartate trans carbamoylase, alright. So, carbamoyl phosphate and aspartate forms carbamoyl aspartate.

You can see the ring is almost formed. So, what else is left? Simply we can join the ring and that is what exactly happens one water molecule goes out you can easily see we can easily let go of one water molecule from here, right. So, one water molecule will leave and lead to the formation of dihydro orotic acid which remains as a salt in medium by combining with cation and also known as dihydro orotate. So, dihydro orotate dihydro orotic acid it is same, alright. Many text book will have different names and the enzyme is dihydro orotase, right.

This is the ring closure step. What happens in the next step? Dihydro orotate is converted to orotate or dihydro orotic acid is converted to orotic acid, alright and the enzyme is dihydro orotate dehydrogenase. What Q and QH2 sample? This is the reducing equivalent that is actually helping in the dehydrogenase reaction, ok. So, simply there is a double bond formation over here, ok. This is the NADH dependent dehydrogenase, ok and it leads to the removal of carbon hydrogen atoms from the single carbon atom, right.

So, we get orotate or orotic acid. The next step what will happen? So, once we got the ring as I discussed, now it will be added to the ribose 5 phosphate and we already read

who is the donor of ribose 5 phosphate in all purine and pyrimidine salve I mean de novo pathway. Yes, you are right, you have guessed it right. If you have guessed PRPP.

So, PRPP. So, the enzyme is orotate phosphoribosyl transferase and it forms OMP, orotidine 5 prime mono phosphate. So, basically what happened? A entire ribose phosphate molecule has been attached to the ring, ok and PRPP gets converted to pyrophosphate PPI which again hydrolyzes to form two molecules of organic I mean inorganic phosphate, alright. Remember you should be able to connect this to the very early class of thermodynamics because all of these reducing equivalent hydrolysis ultimately makes these reactions endorganic, alright. So, that they can spontaneously happen, ok exothermic. Next, so what happened? We got OMP, orotidine mono phosphate means we got a ring, we got a phosphate group and so what else is left? The main thing is OMP is not a familiar pyrimidine.

So, what are the familiar pyrimidines? Cytidine, uridine and thymidine. So, our main goal is this pathway can only stop if we achieve any one of those three. So, this is the main final end product in a 6 step pathway for OMP by the action of a decarboxylase enzyme simply this carbon dioxide will be replaced by a oxo group or keto group it forms UMP, uridine mono phosphate, ok. So, UMP is the final product of de novo synthesis of pyrimidine in prokaryotic system. So, why I am saying repeatedly in prokaryotic system? Mind it the system is actually same the mechanism is actually same in case of eukaryotes even in humans, right.

But in eukaryotes though there are multi enzyme there the proteins have got multi enzyme domains. For example, step 1 to 3, 1 to 3 is catalyzed by a single protein that is known as dihydrorotate synthetase. So, one substrate goes on and ultimately we actually get dihydrorotate synthetase, ok. And step 5 and 6 are catalyzed by a bifunctional enzyme UMP synthetase. So, there is no separate p-r, oxo ribosyl transferase and decarboxylase.

So, step 5 and 6 are again clubbed together in case of eukaryotes. So, there are mainly two enzymes in eukaryotes which play the role of six steps, ok. Fourth enzyme is there, ok. So, in eukaryote there are total three steps. Next, so we got UMP, right.

So, our known pyrimidine UMP, uridine, cytidine and thymine. So, this is a slide that shows how we get cytidine from uridine. So, first what happens this is done in three steps. So, UMP is acted upon by kinase it is first converted to UDP by UMP kinase and then by UDP kinase it is converted to UTP, thereafter it reacts with glutamine to forms to form CTP, ok. So, it can be considered a step 7, 8, 9, ok.

Anyway, so if we just highlight this step, ok, these are 7, 8, 9 steps and if we highlight this last step, you can see that what exactly is happening here this keto group is being converted to an amino group. This is the basic difference in structure of uridine and cytidine and who actually helps in donation of this nitrogen, glutamine, alright. Again, ATP is required hence this is known as synthetase enzyme. So, CTP synthetase is the last step. So, two kinase followed by a CTP synthetase, right.

So, next what? So, we got UDP and CDP, right. So, next step before going any further we should know we did not discuss in purine synthesis because the concept is same. So, you should know that till now we actually discuss synthesis of ribonucleotides, but we for DNA synthesis we need deoxyribonucleotides. So, who does this by simply reduction. A reduction reaction it is done by the enzyme ribonucleotide diphosphate reductase that is also simply referred as ribonucleotide reductase.

Mind it nucleotides are reduced in diphosphate form only by this enzyme. So, ADP is converted to deoxy ADP, CDP to deoxy CDP, GDP to deoxy GDP and UDP to dudp, alright. This concept should be very clear. Diphosphates are the substrate for ribonucleotide reductase that is why this enzyme is also referred to as dibonucleotide diphosphate reductase or RDR, alright diphosphate very important. But this is not simply I mean this not as easy as it seems just a reduction, yes it is just a reduction, but there are many things that are going on behind the hood.

So, what is that? You see ribonucleotide diphosphate reductase actually what it does? It simply removes this oxygen from the 2 prime position and it converts this ribonucleotide diphosphate to deoxy ribonucleotide diphosphate water molecule is removed, ok. It is done by the enzyme as I told you ribonucleotide reductase. Well, ribonucleotide reductase has been shown has been studied to be active in reduced form. I mean there are disulf a sulfhydryl groups in the enzyme that catalyses the reaction that are active when they are in reduced form. So, and during the reaction that is ribonucleotide reductase reaction they are converted to their oxidized form, ok.

You can visualize this analogy with glutathione, right. There was also 2 SH bond we are reducing it to by glutathione reductase. So, much more I mean much like that. So, ribonucleotide reductase is now inactive, it is in oxidized form. So, we need to again reduce that so that action of I mean activity of ribonucleotide reductase is restored.

Who does that? It is done by another protein that is known as thioredoxin. So, thioredoxin helps in reduction of ribonucleotide reductase, alright to convert it from oxidized form to reduced form. So, by doing that thioredoxin also gets oxidized, fine, but we need reduced thioredoxin to catalyze this reaction. So, who will reduce thioredoxin?

The enzyme thioredoxin reductase, alright. So, again thioredoxin reductase is also active in reduced form, ok and when it does it acts it is inactivated it is converted to oxidized form and finally, it is also regenerated by getting converted reduced form with the help of reducing equivalent FADH2.

FADH2 gets converted to FADH ultimately who helps in regeneration of reduced FAD to FADH2 NADPH. So, it is the big chain of redox coupling going on here. So, you can just pause this video right at this moment and you can visualize think calmly you can easily understand. Reduction of this substrate, alright this gets reduced. This enzyme the first enzyme ribonucleotide reductase helps in its reduction itself gets inactivated and oxidated.

It needs to be further reduced. Who reduces it? Thioredoxin. Someone which will reduce any other substance will itself get oxidized basic redox principle. So, we got oxidized thioredoxin. Oxidized thioredoxin again needs to be reduced. It is done by the enzyme thioredoxin reductase.

Thioredoxin reductase itself gets oxidized. We again need to reduce it with the help of FADH2. FADH2 will also get reduced. So, NADPH helps in reduction of FAD2 FADH2, alright. So, if ribonucleotide reductase comes as a short note in any descriptive exam, be prepared to draw this diagram and you should practice it on your own so that you can reproduce it in the exams, ok. So, now we will discuss how we can generate or produce UBUMP in our system.

We discussed ribonucleotide reductase. So, whenever we are discussing about deoxy sugars, the ribonucleotide reductase is producing deoxy sugars, but it is dinucleotide, but we need mononucleotide. Why we will see, but right now we are focusing in production of mononucleotide that is deoxymononucleotide which can be done in two way. Number one, an ADP molecule can combine with dUDP. It forms dUMP plus ATP, alright. The second way where dUDP combines with ATP to forms dUTP and ADP, right.

And in the second step, dUTP gets hydrolyzed to form dUMP and pyrophosphate. So, these are the two ways how we can generate dUMP in our system. Why are we so much focused regarding dUMP? It is because we need dUMP to form thymidylate, right. The last pyrimidine, the last piece of puzzle that was unsolved.

So, we got UDP, we got CDP, now we get thymidine. Thymidine was not formed, right. Thymidine to form thymidine, we need dUMP because this is the main compound component which is needed for DNA synthesis, ok. Now, this dUMP is acted upon by the enzyme thymidylate synthase, ok. We will break it down.

So, this is the reaction in a nutshell, ok. Why this reaction is important? Because once you have studied the whole reaction, we need we can answer the question that we I mean the situation that we studied when we are discussing nucleotide analogs. We studied nucleotide analogs like 6-mercaptopurine, 5-fluoro uracil, methotrexate and I told you methotrexate was inhibiting the enzyme dihydrofolate reductase, right. 5-FU or 5-fluoro uracil is inhibiting thymidylate synthase. So, these are the enzymes that are the target areas for chemotherapeutic drugs or anti-cancer drug, right. So, just remember dihydrofolate reductase and thymidylate synthase.

So, now, let us break the break this reaction I mean let us magnify the steps. So, we got dUMP from two ways in last slide. So, dUMP how can we convert dUMP uridine to thymine? I showed you during the I mean in the very first slide of pyrimidine by simply adding a methyl group. So, this is nothing but a methylation reaction done by the enzyme thymidylate synthase dUMP to dTMP. Who is the methyl donor? Common methyl donor we came across this molecule multiple in multiple area N5N10 methylenetetrahydrofolate.

It is often also abbreviated as 510 or often it is just N5N10. N5N10 methylenetetrahydrofolate itself it is converted to dihydrofolate. You can track the structure if you are interested to see what molecules are getting transferred from where exactly the methyl group is getting transferred is this one and this hydrogen they are getting transferred to form dTMP hours job is done. So, what is what do we need more? We need more N5N10 methylenetetrahydrofolate. So, that multiple dUMP can be converted to multiple dTMP right.

So, we need regeneration of N5N10 methylenetetrahydrofolate. So, who does that? So, we got dihydrofolate in the previous reaction. This is acted upon by the enzyme dihydrofolate reductase to form tetrahydrofolate the reducing equivalent who donates the hydrogen group in order to convert dihydrofolate to tetrahydrofolate is again NADPH ok. And I told you this dihydrofolate reductase inhibitor for example, methotrexate is an anti chemotherapeutic drug right or chemotherapeutic drug or anti cancer drug alright.

So, we got tetrahydrofolate. Now this reaction you know tetrahydrofolate to methylenetetrahydrofolate again there is a transfer of methyl group. Basically this accepts a methyl group to degenerate N5N10. Who donates the methyl group? If you can connect to your previous class where we discuss synthesis of serine and glycine SHMT serine hydroxy methyl transferase. Over there we emphasized on this part and we just mentioned these molecules right and we I told you when you finally, know this reaction of pyrimidine synthesis the entire one carbon reaction chain will be completed. So, I

hope you can now open that class or go back to that class after you have after you are done with this class and now you can fully relate this entire one carbon reaction cycle.

Well let me tell you this is an opportunity or this is a must this is a time where you should go back to all of your classes because very soon the integration of metabolism classes are coming up and for that you need to have all of the metabolic cycles at the back of your mind. So, that you can easily connect and relate to that class without pausing that video 1000 times right. Moreover the upcoming class will be about inborn error of metabolism which will also require you to revise various disorders of metabolism that was discussed in throughout the various weeks all right. So, this is a very good time to start revising if you have not started it already ok.

So, let us move on. So, the let us discuss about carbamoyl phosphate synthetase I told you where did you remember did you remember carbamoyl phosphate synthetase 1. If you remembered urea cycle I give you 10 upon 10 because urea cycle is the one where carbamoyl phosphate synthetase 1 was discussed. Over there CPS 1 was utilizing an ammonia bicarbonate and two ATP molecule to form carbamoyl phosphate. Whereas, in case of CPS 2 as we studied it is using carbon dioxide or bicarbonate glutamine and forming carbamoyl phosphate for carbamoyl phosphate by using ATP right ATP is used because these are synthetase enzyme this is variety 2.

Now, how these these are regulated ok. CPS 2 is allosterically regulated right. So, who are the allosteric activators PRPP and IMPR allosteric activators and several pyrimidines UTP and CTPR allosteric inhibitors right. You should know that this enzyme aspartate trans carbamoylase is often although this is the second step of the pathway this is actually the first committed step of the pathway all right and multiple regulators also affect this step. So, positive regulators are ATP and negative regulators are UTP and CTP.

So, can you I mean relate how UTP basically mainly UTP right. So, UTP is acting as a feedback inhibitor and by default CTP because we get CTP from UTP how it is acting simply you just visualize if this reaction happens we got multiple UTP in our system. So, if our body has got more and more UTP we do not need to synthesize anymore UTP this is simply step of or concept of feedback inhibition ok. So, here is a actually more schematic diagram if this regulation of pyrimidine nucleotide comes in a comes as a long question you can practice the diagram and represent. So, that we can see CTP and UTP are inhibiting aspartate trans carbamoylase ATP is actually helping or promoting the action of aspartate trans carbamoylase also you should keep in mind CTP inhibits its own synthesis by feedback inhibition and GTP actually helps its own synthesis allosterically ok. So, the basic difference between pyrimidine nucleotides as I told you

they are synthesized on PRPP ribose molecule on top of it it is added whereas, it is first synthesized and then added to PRPP it is regulated by reciprocal regulation I hope you remember GTP and ATP it is mainly regulated by UTP it generates IMP here it generates UMP and CMP and both requires energy right this is not a difference we are just comparing and contrasting.

Also one important information regarding ribonucleotide I mean reductase that is formation of deoxy ribonucleotides this is a chart if you actually want to score very good in your exam this is something you need to remember because it is difficult number one it is difficult and this is a very nice to know area specifically because you need to remember so many permutation combination of molecules. So, who is actually acting as a positive regulator and inhibitor there is no easy way of remembering this you just have to write this over and over again to commit it to your memory right. So, we now we will now discuss the breakdown of nucleic acids concept same just like purine we discuss nucleic acids will be first broken down by nucleases to mononucleotides right by and then it will be broken down by nucleotides and phosphatases to nucleoside which will be further broken down to nitrogenous bases. Now this nitrogenous bases can be degraded by direct catabolic pathway or they can be reutilized using salvage reaction to form 5 prime mononucleotides right. So, in salvage of pyrimidine you can we can see basically here a case where uridine has been converted to uracil that is the base and ribose 1 phosphate and in this salvage reaction uridine is directly combining with I mean uracil is combining with ribose 1 phosphate to form uridines is a reversible reaction all right it can occur in both ways.

Here we can see inter conversion of multiple pyrimidine by cytidine uridine kinase here also deoxy cytidine kinase thymidine kinase. So, all of them convert these bases to their monophosphate forms and I mean this is very obvious all right inter conversion of pyrimidine nucleotides you can see first we are forming uridine product then by reducing it we can form deoxy d UMP actually and by methylation we are forming thymidine right. Again by transfer of amino group from glutamine we are getting cytidine and then we can again reduce it to form deoxy cytidine and similarly they can be converted to their mono and di and simply by kinase and phosphoryl enzymes into their phosphorylated less and higher phosphorylated forms ok. And just as a revision I hope now you can easily understand the conversion of multiple purine nucleotides in a similar way you can just place the central synthesis of IMP by de novo synthesis and just you can relate how those are inter converted into each other ok. So, finally, we come to the last phase of the discussion that is pyrimidine catabolism.

So, pyrimidine nucleotides are hydroxylated just as we discussed these are no exception to purines by nucleo to their nucleosides and inorganic phosphates. So, nucleoside means

ribose and nitrogenous bases and phosphate once we detach nucleotide becomes nucleoside. On further degradation thymine, uracilamine those individual nitrogenous bases will be produced. So, our goal is to determine how this thymine and uracil bases are degraded and upon degradation they will produce intermediates of central metabolism what is central metabolism basically the integration of metabolism and we will be able to identify certain common products by which you can easily say where those common products can be diverted ok.

So, this is actually the pathway. So, again let us break it down magnify each and every pathway. So, we start with uracil and thymidine they are first acted upon by a dehydrogenase enzyme NADPH dependent dehydrogenase enzyme that will be converted into dihydro version. So, dihydro uracil and dihydro thymine which will be acted upon by the enzyme dihydropyriminidase simply a water molecule is coming and it is converting them to uridopropionetan, uridoisobutyrate ok. The structures are not important the names are important of course, the structure is important for you to understand conceptually, but it is not important for you to remember and draw all these structures because it is difficult to remember so much ok. But if you can it is best to remember because otherwise you would not need to simply mindlessly memorize each and every metabolic step.

Lastly what happens this uridopropionet and uridisobutyrate are acted upon by the enzyme uridopropionase and they form beta alanine and beta amino isobutyrate. These are successively degraded to acetyl coenzyme A substrate for many things and ultimately succinyl-CoA. So, both of them can enter into the carbohydrate metabolism or there are multiple fates of acetyl-CoA and succinyl-CoA that have already been discussed. So, finally, we are discussing about disorders of pyrimidine metabolism and there is only one that you need to remember due to the deficiency of. So, why there are three alternatives because in procarriers these are two different enzyme and in eukaryotes this is basically single enzyme that forms the performs these two steps.

So, what happens orotic aciduria means orotic acid is accumulated. In the last two step orotic acid is converted to OMP and finally, to UMP this is not done. So, this is autosomal recessive disorder what are the characteristic feature definitely orotic aciduria means excess orotic acid will be extracted in urine. Even crystals of orotic acid have been found to be extracted in urine and those may cause urinary tract infection. Apart from that what are the systemic symptoms growth retardation and anemia megaloblastic variety of anemia. What is the treatment? Treatment is actually administration of UMP alright or uridine triacetate which is converted to UMP in vivo.

So, how actually UMP administration treats orotic aciduria simple if we have got excess

UMP ultimately it forms UTP in system. If there is excess UTP I told you UTP inhibits the pyrimidine biosynthesis and if UTP in fact, it inhibits synthesis of carbamol phosphate. So, if the committed step or the earlier step is actually inhibited there will be no production of intermediate. So, there will be less and less production of orotic acid or orotate and hence you this will be treated I mean there will be no substrate to excrete fine.

So, UMP will act as a treatment for orotic aciduria. And what are the varieties of orotic aciduria? They are they can be found I mean they have been I mean elaborated as multiple types. One is a major type in which both enzymes activities are deficient that is known as type one is much more serious. Whereas, there is a milder variety in which only the decarboxylase function is deficient. So, there will be OMP ok and in the first majority variety there will be in formation of excess orotic acid. Mind it we also mentioned orotic aciduria in urea cycle due to the enzyme deficiency or anything trans carbamol.

If this enzyme is deficient what will happen that will lead to a situation of hyperammonemia along with orotic aciduria because urea cycle disorder leads to hyperammonemia. But there will be no inborn orotic aciduria mind it these are all inborners the first two that presents very early. And a very minor non symptomatic disorder is beta iso I mean beta amino iso butyric aciduria there this is a transaminase deficiency and basically this is non symptomatic and do not need much treatment to start with ok. So, this is the conclusion we have discussed pyrimidine chemistry, we have discussed de novo synthesis of pyrimidine, we have discussed how it is synthesizing prokaryotes and eukaryotes, we have discussed how it is regulated, we have discussed the reduction how it is reduced by ribonucleotide reductase, we have discussed synthesis of thymidylate the salvage pathway and inter conversion of pyrimidine fine pyrimidine catabolism and the disorder of pyrimidine biosynthesis that is orotic aciduria. Well these are my references for the today's class and I thank you all for your patient hearing.