Organic Chemistry In Biology And Drug Development
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Lecture - 50
Resistance to Beta - Lactam Antibiotics

Welcome back. Today we will continue our discussion on the antibacterial, antifungal, antiviral type of agents which are classified as antimicrobial agents or antimicrobial drugs. We have seen that penicillins were one of the first antibiotics that was discovered. Before that, there were antibacterial agents that were available; one was Salvarsan which was discovered by Paul Ehrlich and then Sir. U. N. Brahmachary discovered the urea-stibamine.

This was followed by Gerhard Johannes Paul Domagk, who discovered the sulfonamides, the name of the drug was Prontosil; but these are all antibacterial agents, they are not produced by any microorganisms. So, then came the era of microorganism derived antibacterial agents which are called antibiotics and the first one to be discovered was penicillin and I told you the history of the discovery of penicillin.

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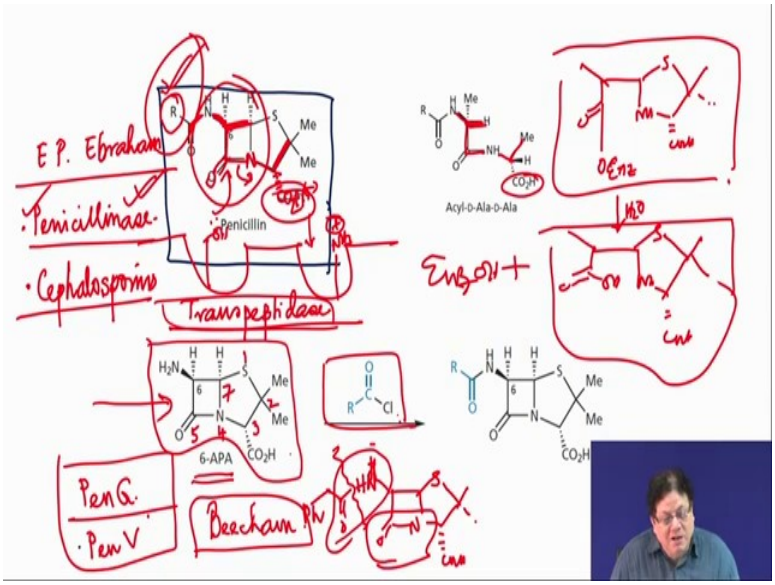

And we ended up by studying that how the penicillins killed the bacteria as it stops the biosynthesis of the cell wall that is essential for the bacterial survival. And the cell wall was composed of a peptidoglycan where the peptide chains are hanging from the glycan

units and the peptide chains are cross-linked; it is basically very similar to what is called vulcanization of rubber where you can make the soft rubber into a hard rubber by cross-linking with disulphide, trisulfide kind of bonds.

So, it is very similar that if you have peptide chains hanging, you can make the cross-links among the peptide chains and then that makes the cell wall very rigid which can hold the cytosol; that means, all the ingredients in the cell are kept intact and the cell wall does not allow free passage of molecules from outside; only when it is required, those type of external molecules can enter.

Now, the last stage of the cell wall biosynthesis involved a transpeptidation reaction and the enzyme is called transpeptidase. And, what it does, it actually catalyses the transpeptidation reaction where a glycine amine attacks a terminal D-alanine-D-alanine peptide and replaces the terminal D-alanine by the glycine; this mechanism operates in gram positive bacteria. In gram negative bacteria, that lysine arm where the pentaglycine unit is present, that is replaced by the meso-diaminopimelic acid; but the overall transpeptidation mechanism remains the same or very similar.

Why does penicillin block the cell wall biosynthesis? The reason is that the penicillin structure resembles the structure of the dipeptide D-alanine-D-alanine. So, which part is it resembling? This is the part that if you look at the structure of D-alanine, that is written on the right hand side. So, you have CO_2H here and up to that point and then you have this.

So, this is the part which is present in D-alanine-D-alanine peptide, the stereochemistry is also similar CO_2H and CO_2H. And, because this β-lactam is very reactive and it resembles the D-alanine-D-alanine dipeptide; the CO_2H is basically present as CO_2 minus. In the active site pocket, there is a serine and there is a positively charged handle in the presence of a lysine ammonium ion and here it is the serine.

So, the serine attacks the β-lactam and breaks the β-lactam, and then it stays there for a long time. Only after a long time water can come and attack this acyl-enzyme complex and release the enzyme and by that time cell is lysed; that means, all the contents of the cell are released, it could not withstand the internal osmotic pressure for a long time. So, the cell wall has to be synthesized very quickly, it cannot wait for the enzyme to be released.

Now, what happened? Soon after the discovery of penicillin, there was one scientist, his name was E P. Ebraham. He suggested the β-lactam structure of penicillin that is the first thing that what he did, then he observed that there are some microorganisms which produce an enzyme which can hydrolyze the penicillin and release itself from the penicillin. That means, it is another enzyme which is very similar to the transpeptidase enzyme, but the difference is that it goes and attacks and then is released by water.

So, what I am saying that as soon as penicillin is attacked by the enzyme, in case of transpeptidase, this remained intact for a long time. But, in case of the enzyme that Sir Ebraham detected, penicillin is immediately hydrolyzed and you get a hydrolyzed penicillin and the enzyme is free and this enzyme then goes and attacks another penicillin molecule and then hydrolyzes it.

Now, remember this hydrolyzed penicillin does not have any activity. The hydrolyzed penicillin lacks the antibacterial activity because it lacks the β-lactam ring that is the pharmacophore of the system. So, this enzyme, Ebraham called as penicillinase, just from the fact that it hydrolyzes the penicillin; later on it turned out to be a very important discovery.

So, Ebraham proposed the structure of β-lactam which was confirmed by Dorothy Hodgkin and then he proposed the presence of penicillinase enzyme. Just right in 1945, when penicillin was introduced, first it was given to the soldiers of the Second World War who were infected with wounds. But in the same year 1945, Ebraham saw the danger; that means, there are enzymes which can destroy penicillin and he called it as penicillinase.

And then he searched whether other microbial products are there which can withstand the penicillinase activity. Because what happens, the bacteria which produces penicillinase enzyme, they are inactive they are basically resistant to the penicillin because the penicillin is made inactive by hydrolysis. So, then E P. Ebraham discovered another set of antibiotics and these are called cephalosporins.

Now before the discovery of penicillin or right after the discovery of cephalosporin; see from discovery going into the market, it takes a long time. So, what happened mean while whatever penicillins were being used, they became resistant; they were not working to kill the bacteria. The first penicillin was penicillin G that is benzyl penicillin;

and then penicillin V that was phenylmethoxy penicillin. But these were not working against the microorganism, because the microorganisms have started to produce the penicillinase.

In the meanwhile, people tried to make new penicillins. Now, how can you make new penicillins? By varying this group R. Different penicillins that we have today, starting from this penicillin G, penicillin V then ampicillin, amoxicillin, cloxacillin, methicillin, their pharmocophore; that means, this part comprised of the b-lactam ring and the thiazolidine ring remained the same, only difference is that R group of the acylated amine, because the penicillins are actually acylated, this NH_2 is acylated to NHCOR.

So, you can vary the R groups, and then it was found that if you make sterically bulky R, then it can resist the water from approaching the acyl enzyme-complex, if that be the case; that means, the penicillinase cannot work very efficiently and that is what we wanted.

However, the trouble is that if you make the R group very big, then the enzyme transpeptidase also cannot attack the penicillin because it is too bulky. So, you have to have some optimum size of R group; then people tried to make different aromatic rings with different donor, acceptor all these; different penicillins were made, again I reiterate that all depends on the variation in the R group.

And this is very easy to make because if you have this amine which is by the way called 6-aminopenicillanic acid, there is a big pharmaceutical company called Beecham pharmaceutical company; they are very famous for their antibacterial research. And usually the penicillin that we used to get in the 50's were the penicillin G or penicillin V, and that means, you already had the $COCH_2Ph$ which is the penicillin G.

So, from there, to hydrolyze back to the amine will be very difficult. What I am saying that if you isolate the penicillin G; that means, from the fermentation medium, what you are getting is this NH CO CH_2Ph that is the pencillin G. But in order to make new penicillins, you need to hydrolyze this amide bond, make the free amine, and then acylate it again with different acylating agents; but that hydrolysis is very difficult this β-lactam amide is more reactive part as compared to the acyclic amide bond. So, that will never be successful if you want to chemically hydrolyze this, β-lactam amide is the one which will be hydrolyzed faster.

However, they could find some enzyme which can deacylate this bond and ultimately by several trial and error methods, Beecham came out with a system where the microorganism produces the free amine where the end product is the 6-aminopenicillanic acid. This was a crucial discovery by Beecham because once this was available in gram quantities, kilo gram quantities, one could acylate by any acylating agent RCOCl.

So, all the pharmaceutical companies even academic laboratories started to use whatever acylating agents they had and then started to measure the antibacterial activity and that is how ultimately different penicillins were made.

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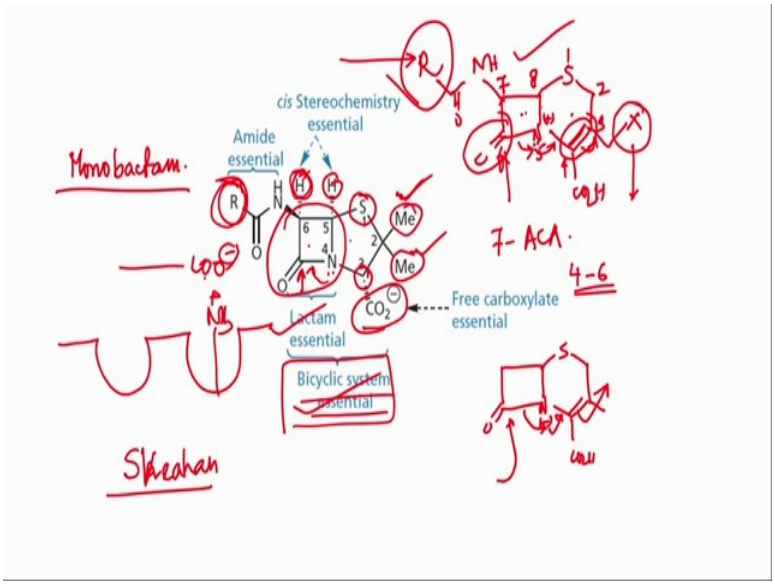

Coming back to the structure of penicillin, what type of changes you can make in the penicillin molecule so, that it retains the activity without jeopardizing the activity? First of all your β-lactam is essential; this is called structure activity relationship (SAR); that means, you slowly take off certain structures, certain groups and see whether the molecule looses the activity or not. If it loses the activity; that means, that part is essential, if it does not lose the activity, then you can strip off some of the extraneous groups.

So, if you remove the β-lactam, it loses the activity. So, lactam is essential then it was that bicyclic system is essential, all the penicillins are bicyclic, but I will tell you that today there are compounds which have a β-lactam, but they are not bicyclic, some monobactams are also now available.

So, the hypothesis that bicyclic system is mandatory is no longer true, but a majority of β-lactam antibiotics are belonging to the bicyclic frame work. But it was initially thought that only the bicyclic skeleton will work, but I will give you one example where a monocyclic β-lactam is also now being used as a an anti-bacterial agent.

Then this stereochemistry at C5 and C6 has to be *cis*. If this is *trans*, then the penicillin loses it is activity, this stereochemistry is also very important. If you change the the β-carboxylic acid, it loses the activity and the free carboxylate is also essential, you cannot remove the carboxylate. And obviously, you know the reason because I told you the active site basically has a serine and a positively charged arm and this is the one where this CO_2 minus will bind and bring the molecule towards the serine. So, that's why CO_2 minus is very important.

Where you can vary the groups is the R as I told you other is these methyls. If you change the methyls, nobody will know what will happen, if you change the methyls or if you change the sulfur if you put oxygen, if you put carbon whether that will retain the activity or not, or if you can make a bigger bicyclic compound like a beta lactam and 6-membered ring. So, all these possibilities were there.

So, people started looking at it, fortunately always Mother Nature helps us in showing the pathway, like I told you that cephalosporins were discovered by E. P. Ebraham. Now, what are cephalosporins? It is a very similar compound with the β-lactam here, with a sulfur here, and it is a 6-membered compound and then you have X here, you have a double bond here, you have a CO_2H here and you have the very similar acyl group.

Now, here this will be a 7 position, like 1 2 3 4 5 6 7 and this is 8. So, the starting point for this compound to make different varieties of R is what is called 7-ACA; 7aminocephalosporanic acid; earlier it was 6-APA; 6-aminopenicillanic acid, this is 7-aminocephalosporanic acid..

But in cephalosporin, you have little bit more option like you vary the X, you can vary the R. So, there are two handles where you can change very easily. Now, coming back to penicillin, I said what will happen, if you remove the methyls or put certain different groups, whether it has been tried. The problem was that this molecule was made by the microorganism, if you want to synthesize this molecule chemically, that was tricky.

In fact, it was chemically synthesized by John Sheehan.

John Sheehan at MIT reported the first total synthesis of penicillin but the problem is that it was a laborious process involving long steps. Sheehan's synthesis was to confirm the structure of penicillin basically like of many of the synthesis are so, that will never be tried in the company to make penicillin.

So, it is really very difficult to replace these methyls and see what happens to the biological activity; it is very difficult to change this stereochemistry here because these are all generated by the microorganism and microorganism makes only one chiral form ; it does not make the other form. So, to make the other form is very difficult you have to do synthesis.

This one is little bit easier, you can epimerize the hydrogen because it is adjacent to a carbonyl and then possibly with base, you can epimerize this hydrogen. So, those types of studies are easier and changing R is also easier, but changing the methyls are very difficult. But, I will tell you today that how the entire penicillin molecule has been changed and in the process, lot of new molecules have been discovered.

So, let us come back to this cephalosporin; look at the structure; it is now a 4-membered ring and 6-membered ring. Now in the 6-membered ring, it has a double bond here; in penicillin you do not have a double bond. Now you know that if you can increase the steric strain on the β-lactam ring that will increase the reactivity of this carbonyl.

Now, how to increase the reactivity of the carbonyl? Why people were saying that bicyclic framework was essential? Because when you put another ring on the top of a 4-membered ring then that increases the strain in the 4-membered ring, and this can be easily detected by taking the IR frequency the carbonyl stretching frequency. So, that has been done.

So, it was found that penicillin carbonyl stretching frequency is very high; that means, whatever little conjugation was going on, that is just cut off by putting this 5-membered ring. Now, if you have a larger ring here, a 6-membered ring; so, what will happen the reactivity of the carbonyl will go down? Now, what nature has done? So, nature knows that a 5-4 combination is very good; you do not need any double bond or anything and this carbonyl is very reactive, but when you have a 4-6 system like in cephalosporin.

So, you have to now activate the carbonyl little bit more because this is a 6-membered ring that cannot impose the same kind of steric strain that the 5-mebered ring can do. So, nature has put a double bond here, why? Because, now the nitrogen lone pair, instead of going to that side containing the carbonyl, the lone pair can conjugate on this side having the double bond. If it conjugates on this side, that creates a positively charged nitrogen, that activates the carbonyl, because the more positive charge on this nitrogen, the more reactive will be the carbonyl.

You can think it in another way by thinking of the leaving group character of the nitrogen; that is also possible, you can also say that this nitrogen, when it leaves, creates an N minus which is delocalized; that also you can say, but the leaving group character of this β-lactam nitrogen is more if you have double bond here. Now to prove this hypothesis, people have saturated this molecule; that means, removed the double bond and then found that the activity drastically reduced. So, the double bond is very essential.

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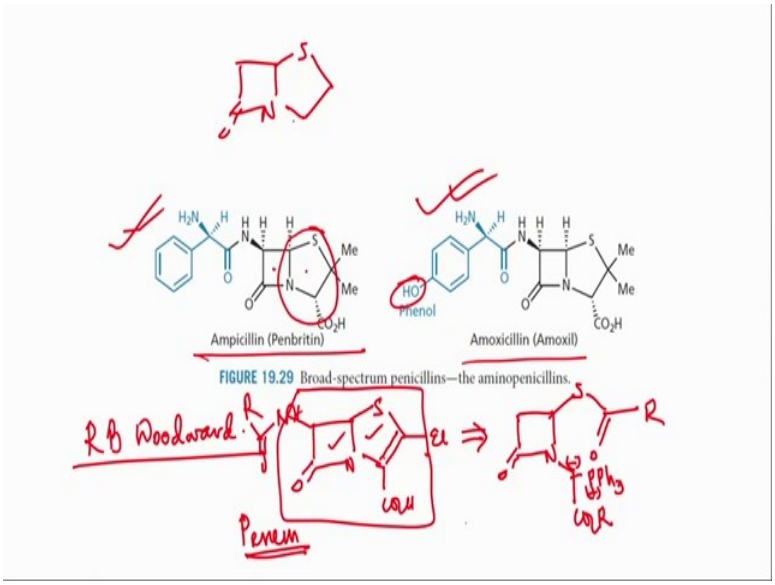

Here there are two very well-known penicillin molecules; this is ampicillin and this is amoxicillin. Only difference is that you have a hydroxy in amoxicillin, but you do not have the hydroxy in ampicillin; that means, people are modulating the hydrophobicity and actually amoxicillin and ampicillin in the 60s and 70s were very good, they could kill many of the microorganisms; but now, the they are rarely used alone; seldom they can kill the bacteria. Now, what happened, I told you that if it is 4-5 system, you do not

need any double bond or anything because the 5-membered produces enough strain on the 4-membered ring, if it is a 6-membered ring you put a double bond here.

Famous organic chemist of the last century, R B Woodward; was also trying to analyze these things and what he proposed at that time that suppose we know that a 5-membered ring is sufficient to activate the β-lactam ring. So, that it can show the biological effect and for a 6-membered ring, you need a double bond so that the nitrogen lone pair is pushed on the right side conjugated with the double bond.

So, he said that if we combine these 2 hypothesis, then we should be able to make a compound which is 5-membered and also containing a double bond. So, if you look at this skeleton now, it has got a 5-membered ring like penicillin, and it has got the double bond like cephalosporin. So, this was a remarkable compound that Woodward synthesized and showed that it is extremely potent antibiotic.

However, about one point you have to be very careful; if you make it too reactive then what will happen? First of all whether you can take it orally or not because what about the stability of this type of molecules. If it is very unstable, then you run the risk of hydrolyzing, the molecules just simply by water.

But anyway Woodward made many molecules and I think these are approved as a as very good antibiotics. It was possibly the company Mark, they synthesized, took the Woodward's concept and made these molecules by combining the two, cephalosporin structure and the penicillin structure and his idea was that this will be better than penicillin, this will be better than cephalosporin; in fact, they are extremely good antibiotics.

If you are interested in synthesis, I can just tell you that retro-synthesis is like this. How Woodward could make the double bond here, not very difficult; of course now everything looks simple, but during Woodward's days it was not that easy, but he was considered the father of organic synthesis. So, he would do an intramolecular Wittig reaction and that will give you what is called penem, I will also tell you about some of the nomenclature system.

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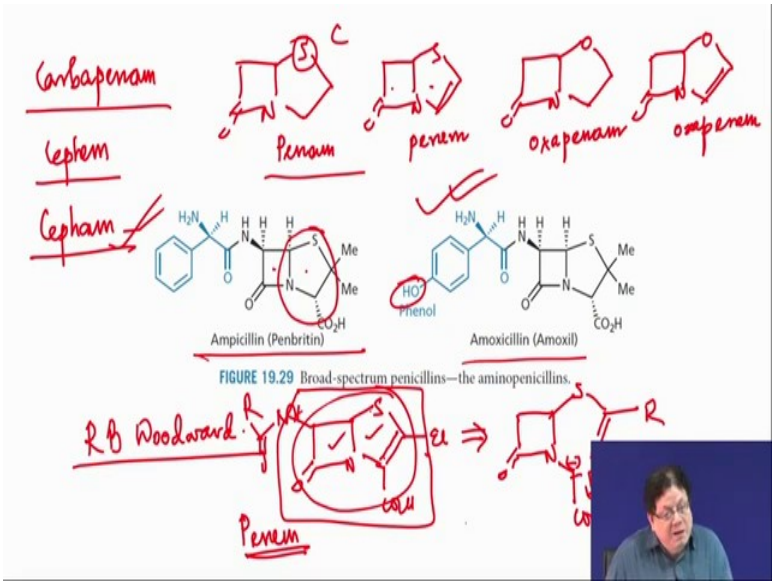

See, when you have a β-lactam attached to a sulfur, this is the skeleton, that is called penam, if you have a double bond now, that is called penem, because you have a double bond now. Suppose you do not have the sulfur, but it is replaced by oxygen so, this will be oxapenam; and then if you have a double bond that will be oxapenem. If the sulfur is replaced by a carbon that will be called carbapenam and then if there is a double bond that will be carbapenem.

So, that is how the nomenclature system goes. Like in the biologically active cephalosporin, where there is a double bond, that will be called a cephem nucleus; because you already have the double bond. If there is no double bond then you have cepham; then you can have oxacepham or oxacephem, you can have carbacepham carbacephem; that is the nomenclature system. So, these penems are extremely potent antibiotics.

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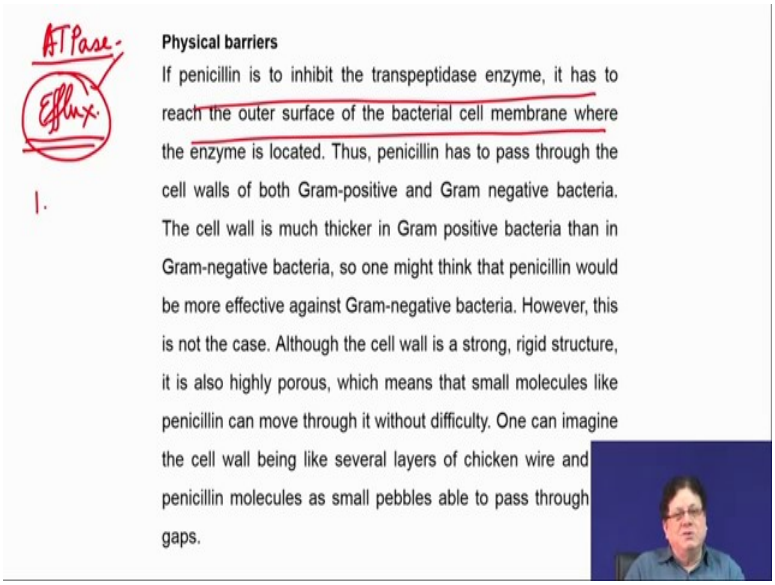

Now, we have said right from the introduction of penicillin, within 5 years; first of all E. P. Ebraham 1945, he published a paper where he showed that existence of penicillinase where that is an enzyme which hydrolyzes the penicillin and make it ineffective.

But then in subsequent years, around 1950, what happened, the hospitals started reporting that penicillin is not working. Most of the infections specially the resistant infections occur in the hospital because in the hospital you have patients coming from different backgrounds.

Also you apply the drug there. So, what happens that hospitals are the most dangerous place for staying for a long time; it is not like good hotel that you are staying there. So, they actually harbor lot of these resistant organisms; in 1950, I think Saint Mary's hospital reported the existence of these microorganisms where penicillin fail to act.

From that day onwards, people started looking for newer antibacterial agents; they know that penicillin is not the final answer; but I remember that when I talked to my father who was there at that time, he told me that when that news of discovery of penicillin came, everybody thought that all bacterial infections now are gone because penicillin is there, but within 5 years, that myth was busted. Now, the question is how the bacteria acquire resistance?

So, there are the many ways; I will talk about only the organic chemistry part by which the bacteria acquires the resistance and that is the most important part. One way that bacteria can provide resistance is through a kind of pump in their membrane where the molecule enters. See, I told you about specially the gram negative bacteria, first there is a lipid bilayer then there is a space and there is a thin cell wall then another lipid bilayer.

So, if penicillin has to work, it has to enter through the first barrier, that lipid bilayer. So, if it enters; although entering is a difficult thing, and if it enters then there are basically pumps that will again pump it out from the system, these are called efflux pumps. So, efflux pumps are one of the main reasons for many drugs, many anticancer drugs which work initially, but then it cannot kill the cells, the cancer cells because of these efflux pumps.

This is a very common mechanism by which the molecule is thrown out of the cell if it tries to enter. However, these efflux pumps need energy to run. So, these are also called ATPase, the pump actually gets the energy by hydrolyzing the ATP and then it operates these efflux pumps, because you need energy. The other survival strategy adobted by the bacteria is that it can actually produce much more concentration of this transpeptidase enzyme.

So, that it can out-number the penicillin means whatever penicillin you are giving, the bacteria produces more molecules of this transpeptidase. So, if some of the enzymes are inhibited by penicillin, but still there will be some enzymes which are left so, they can do the job. So, that is the second option, but still you can bypass that by by increasing the amount of penicillin so, that will continue; the bacteria produces more transpeptidase you also increase the dose of penicillin.

Interestingly penicillin is very harmless because of the fact that it does not target any enzyme system which is required for the host; it only targets an enzyme system that is required only by bacteria. So, I think that there is no such other example where a molecule only touches the bacterial machinery and does not touch the human machinery. That is why still today, these β-lactam antibiotics are the drugs of choice for any bacterial infection.

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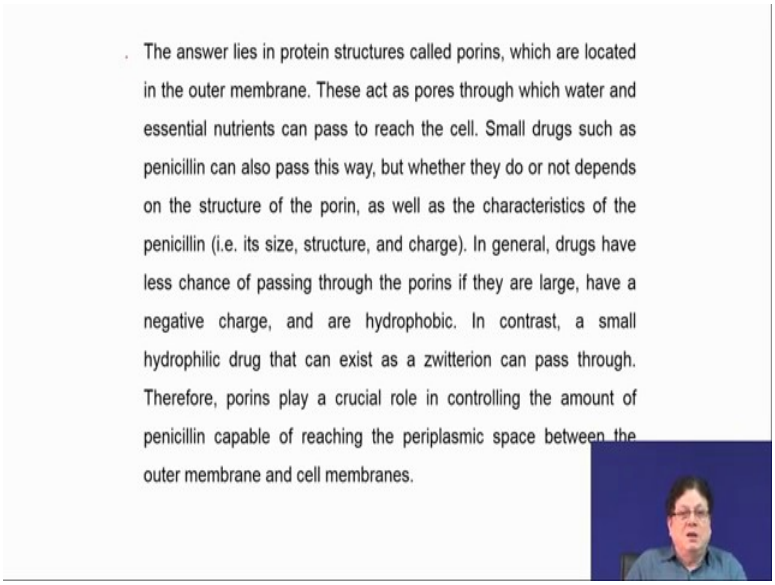

Now, after these two mechanisms, the third one is the interesting one. What it does, I told you about penicillinase. So, bacteria started producing this another type of enzyme which are penicillinase, hydrolyzes penicillin, but because penicillin is very specific, it is the 4-5 system, but you can have carbapenams, you can have penems, you can have cephalosporins. Since it is hydrolyzing the β-lactam, so these group of enzymes which hydrolyzes the penicillin are called β-lactamase.

So, this bacteria started producing this β-lactamase enzymes and in the next session we will tell you about the problem of β-lactamase, and how to tackle this problem.

Thank you.