Medicinal Chemistry
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Drug Resistance and Drug Synergism

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So before we go into case studies, let us discuss some topics on something that is very important and emerging in modern drug discovery which is the rampant observation of drug resistance. So here in today's lecture we will look at both concepts of drug Resistance as well as drug Synergy, ok.

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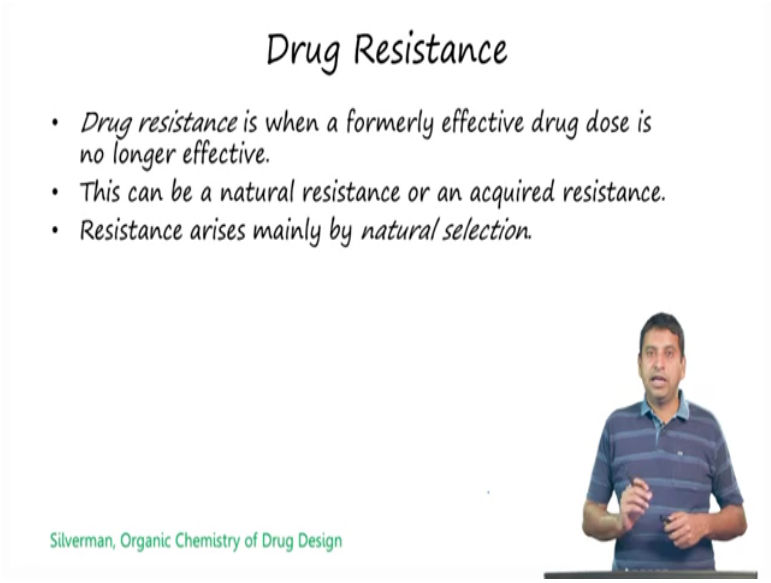

So drug resistance is observed when a formally effective drug dose is no longer effective. So this could be natural resistance or an acquired resistance, resistance mainly arises by natural selection.

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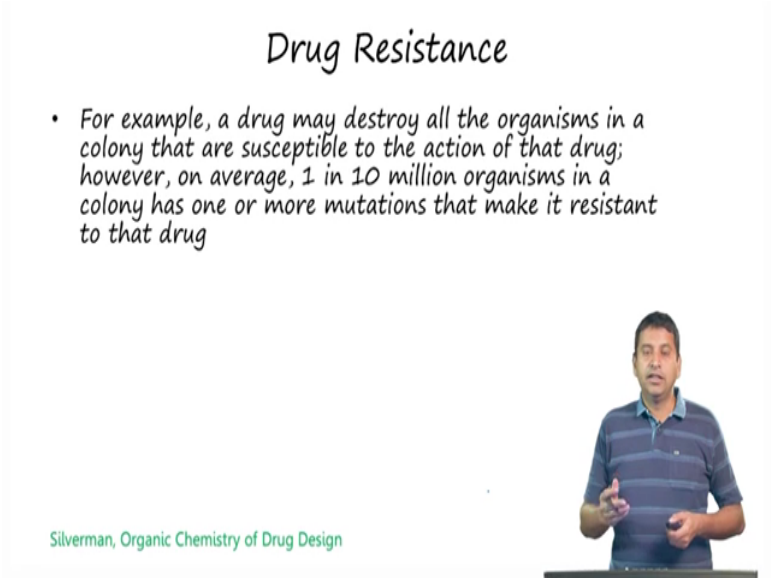

So for example a drug may destroy all the organisms in a colony, so if you have a bacteria like in a 10 million organisms you know 1 organism will survive and the rest of them will die but that 1 organism can survive and divide and perhaps have grown into a new colony, right. So you know that 1 organism which was naturally resistance to the (bio) to the anti-biotic or the drug is what is going to be causing this drug resistance, ok. So it is likely that this organism has one or more mutations that make it resistant to the drug.

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So once all the susceptible organisms have been killed, a few resistance ones can replicate and eventually become the predominant species.

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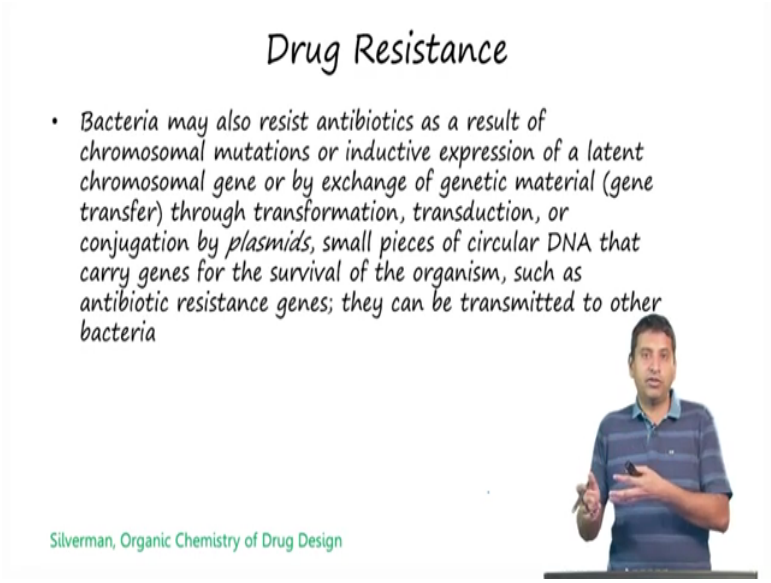

So bacteria may also resist antibiotics as a result of Chromosomal mutations or Inductive expression of a Latent Chromosomal gene or by horizontal gene transfer that is exchange of genetic material through transformation, transduction, or conjugation by plasmids. So these plasmids are nothing but small pieces of circular DNA that can carry genes for survival of the organism and if there is a group of organisms which are already resistant to the anti-biotic those genes can be transferred to another organism and that helps in the other organism to become resistant to the anti-biotic.

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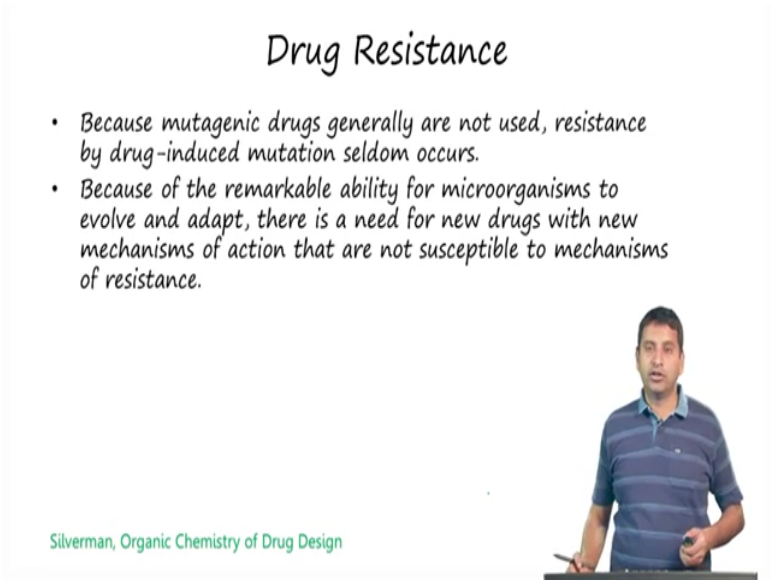

So because you know these drugs are rarely Mutagenic therefore resistance by drug-induced mutation is a very rare situation. So because of the remarkable ability for microorganisms to evolve and adapt there is a need for new drugs with new mechanisms of action that are not susceptible to the existing or prevailing mechanisms of resistance. So this is a major area of drug discovery that is going on right now including in my lab.

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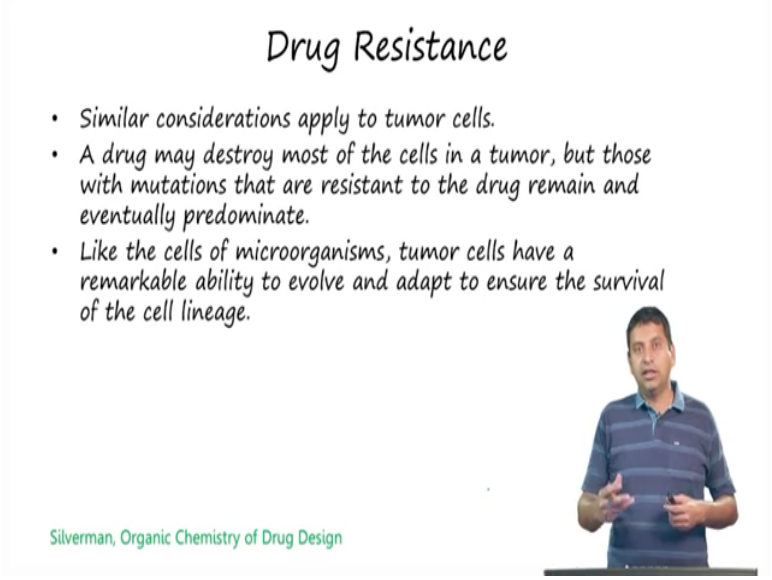

So a much like bacteria you also have cancers or tumors which have similar considerations, so once we expose the tumor to over drug then it is possible that a large number of those actually are killed but some of them which have existing mutations which can (dr) which can help with resistance are likely to predominate. Now then these will evolve and adapt and to ensure this survival of the cell lineage.

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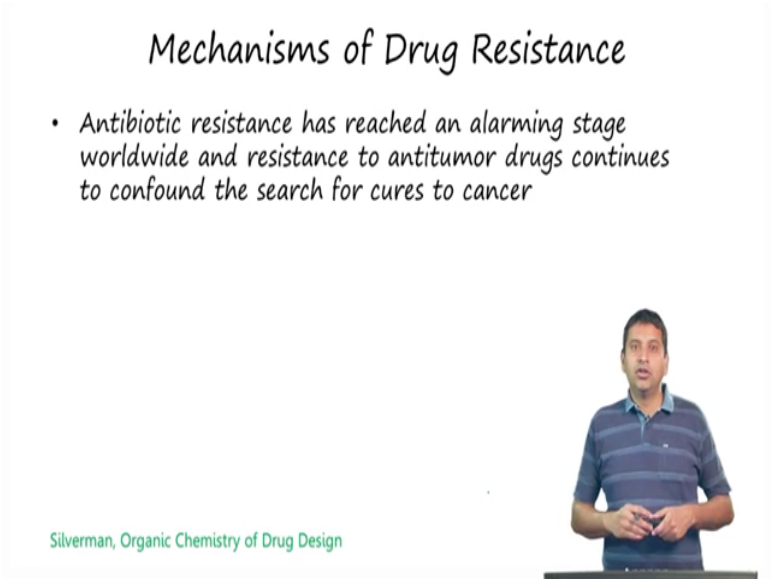

Anti-biotic resistance is now such a huge problem across the world that it is quite likely that in the next decade or two that even simple infections will difficult to cure. Similarly resistance to antitumor drugs is becoming a huge problem.

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So the mechanisms of drug resistance are classified into two major classes the first one is Exogenous resistance is when new proteins are developed by the organism or cell to protect it from the drug. The other way to develop resistance is by Endogenous resistance, this occurs by mutation or even single point mutation that is 1 amino acid changes in a protein and that can result in resistance.

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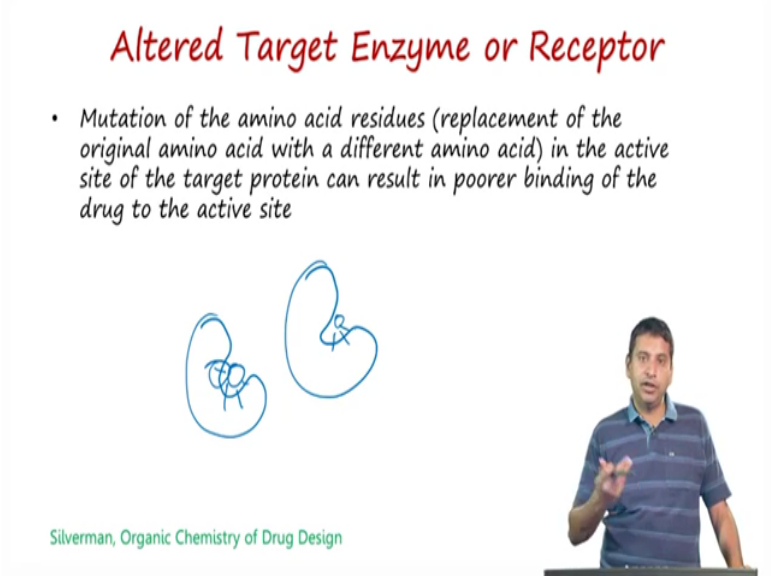

So now let us look at each of these in fair bit of a detail, so the first one is Altered Target Enzyme or Receptor here mutation of the amino acid occurs, so here the important amino acid that was originally present in the active site has now been replace by another amino acid. So once you have the binding site you know so here is a your drug and here is the binding interaction.

Now it is possible that one of these binding interactions becomes weaker, right so let us say the mutation occurs here and this becomes weaker, now if this becomes weaker than the dissociation can actually a predominate and therefore the efficacy of the drug goes down.

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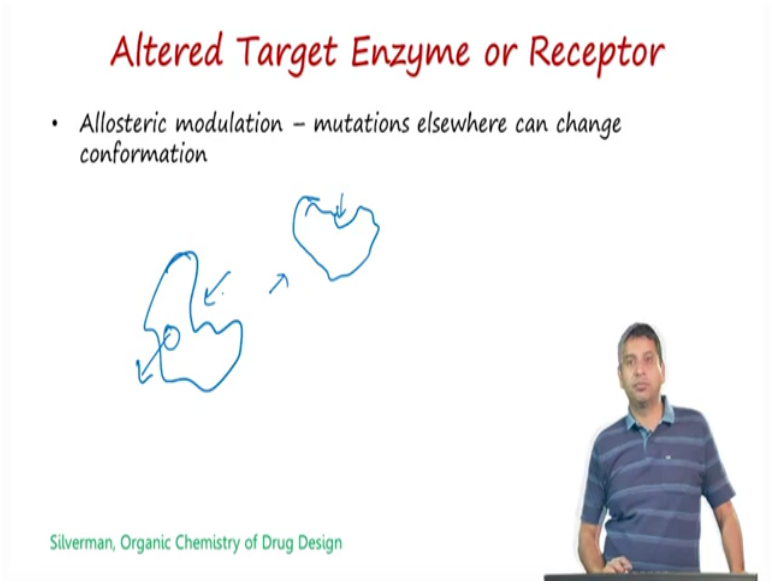

You can also have Allosteric modulation where mutations elsewhere in the conformation. So let us say you have a protein, so the mutation can occur here and this results in conformational change which changes the binding pocket of the enzymes, so this is the now this is not very good at binding anymore.

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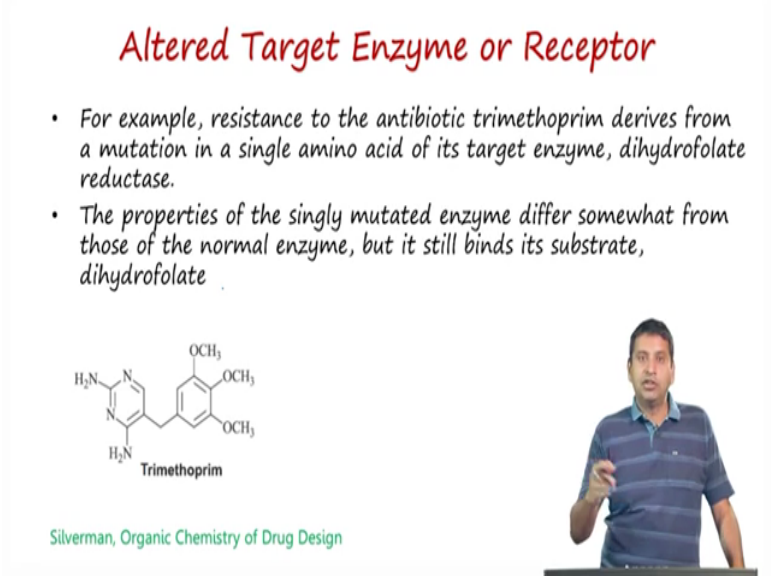

For example resistance to the anti-biotic trimethoprim derives from a single mutation of a single amino acid of the target enzyme which is Dihydrofolate reductase. So the properties of the singly muted an enzymes differ somewhat from those of normal enzyme but it is still binds to the substrate Dihydrofolate.

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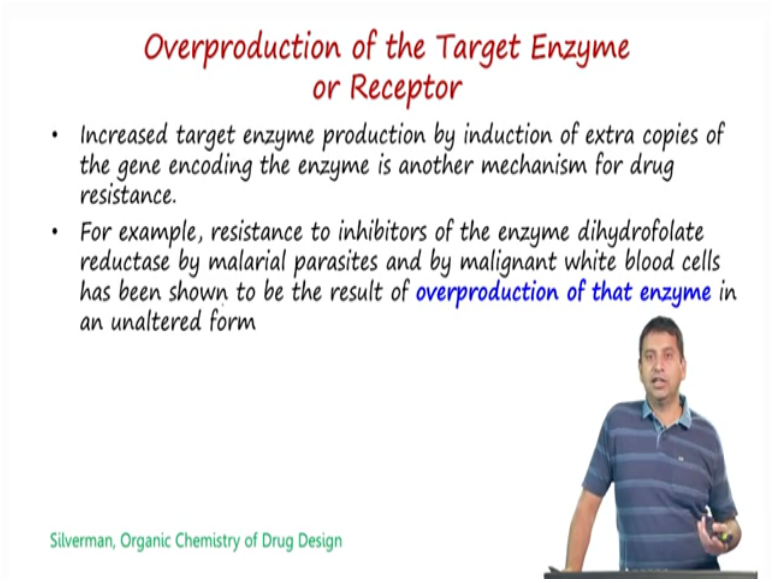

So now the next way in which you can have the resistance is over production of the target enzyme or receptor. So let us say we have a excellent reversible inhibitor of an enzyme or an agonist for a receptor, now if you have a large number of these enzymes that is if the protein expression goes up then it is you need a much higher concentration of the inhibitor to actually go and inhibit the enzyme.

So one way in which the cell can counter this drug is to increase the enzyme production by induction of extra copies of the gene encoding the enzyme, ok. So for example the enzyme Dihydrofolate reductase by malarial parasite the inhibitors of this has over production of that enzyme and if the unaltered form and this results in resistance.

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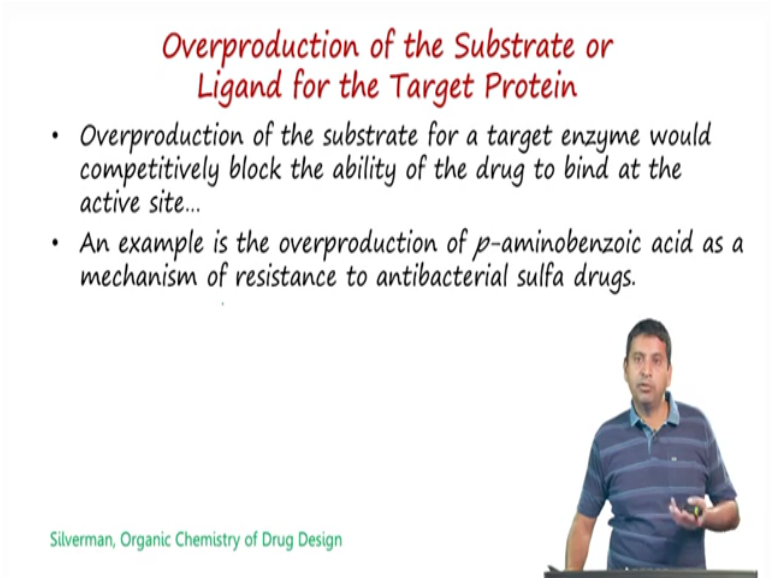

You can also have over production of the substrate or ligand for the target protein. So like we discussed earlier a number of the inhibitors that we develop are reversible inhibitors, so therefore if the substrate concentration goes up the efficacy of the inhibitors goes down therefore this could be one way in which the organism is able to acquire to become resistant. So an example here is overproduction of Para Aminobenzoic acid which is the substrate for the enzyme and sulfa drugs acts on this enzyme and therefore by increasing the concentration of Para Aminobenzoic acid the bacteria becomes resistant to sulfa drug.

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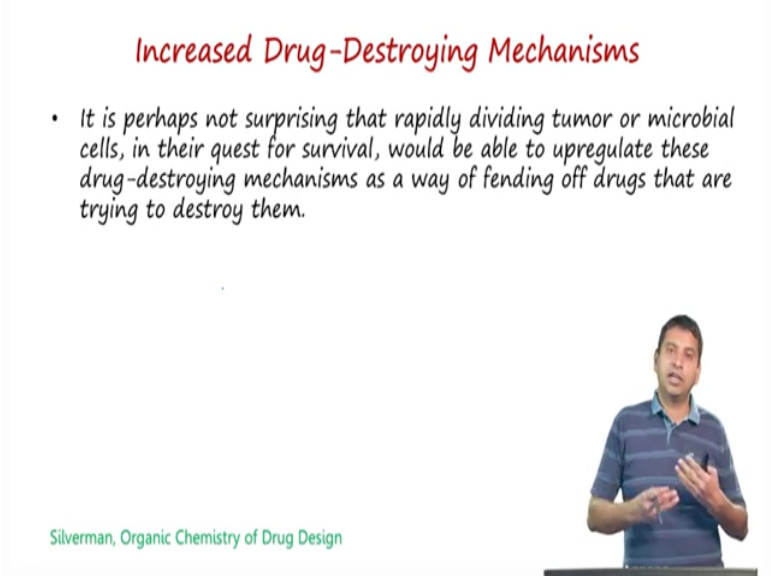

You can also have increased drug destroying mechanisms, so here in when you have a rapidly dividing tumors or microbial cells then in they want to survive and so they would up regulate these drug-destroying mechanisms as a way to fend off the drugs that are trying to destroy them. So this is something that again can occur in these organisms.

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So you know resistance can occur by induction of genes to produce new enzymes increase the quantities of existing enzymes or produce other substances to degrade or sequester the drug. So all of these will may result in destroying the drug.

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So within 10 years of introduction of Penicillin for example half of the strains of Staphylococcus aureus had become resistant and by 1990s more than 90 percent of the strains were resistant. The principal cause of resistance to penicillin is the generation of this enzyme called as Beta lactamase, here Beta lactamase destroy the penicillin and they make the drug ineffective.

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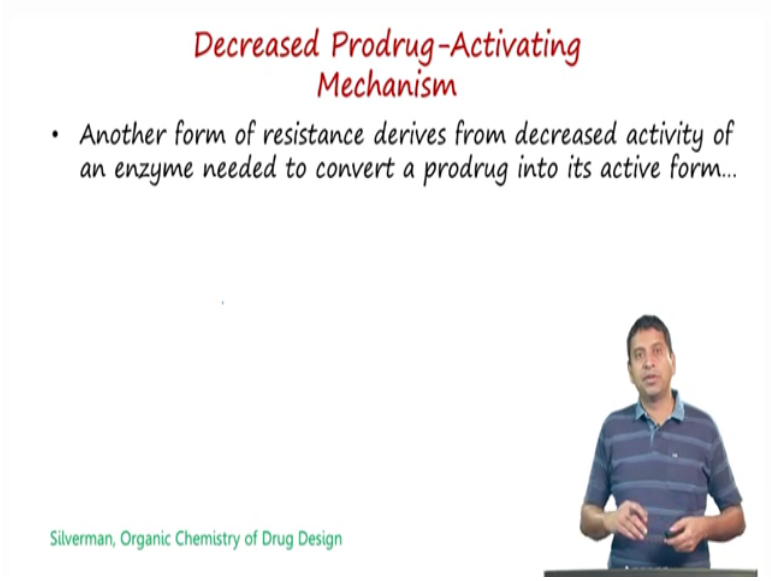

We have looked at in detail about pro drugs and pro drugs the concept in pro drug is that you need a metabolism for the pro drug to be activated, so one other form of resistance is that if the enzyme that is activating the pro drug is going down in quantity, so the activity of the enzyme can go down and then that can result in drug resistance.

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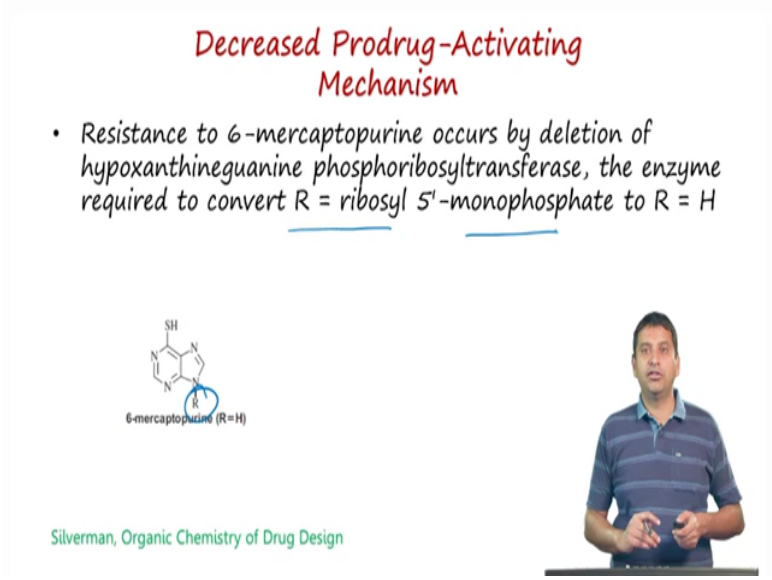

So an example here is the resistance is 6 Mercaptopurine which occurs by deletion of hypoxanthine-guanine Phosphoribosyltransferase, the enzyme which converts this ribosyl form to the hydrogen R equals H.

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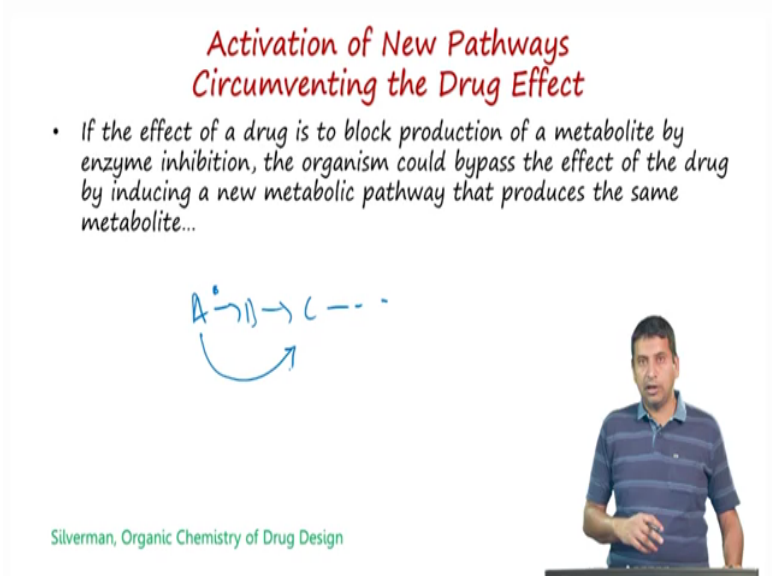

Another major way in which resistance can occur is activation of new Pathways that is Circumvent the drug effect. So what this means is that if you can have the drug (effay) affecting a particular production or a particular enzyme then the organism could bypass these effect of the drug by inducing a new metabolic pathway, ok. So let us say you have A going to B going to C and so on and now if you inhibit this enzyme here then it is possible that the

organism bypasses this enzyme and forms finds a different way to carry out the same metabolic transformation.

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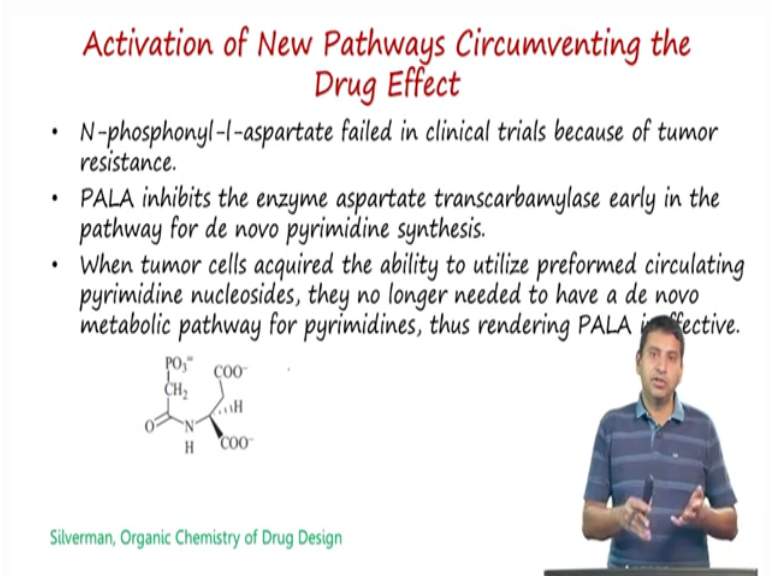

So here is an example, so N-phosphonyl, L-aspartate was a very successful in treating cancer but it failed in clinical trials. So this PALA as this is called it inhibits the enzyme Aspartate Transcarbamoylase, ok and this is a very important in the de novo pyrimidine synthesis. So when tumor cells acquired the ability to utilize preformed circulating pyrimidine nucleosides then they no longer needed to have a de novo metabolic pathway for pyrimidine is, so therefore this drug becomes ineffective.

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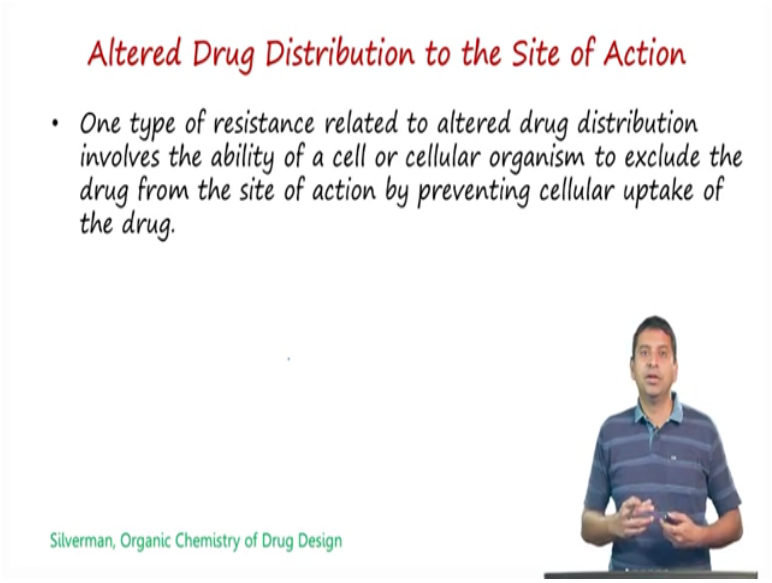

So another way in which resistance can occur is to have altered drug distribution to the site of the action. So this happens you now when the cell or cellular organism excludes the drug from the site of the action by preventing cellular uptake of the drug.

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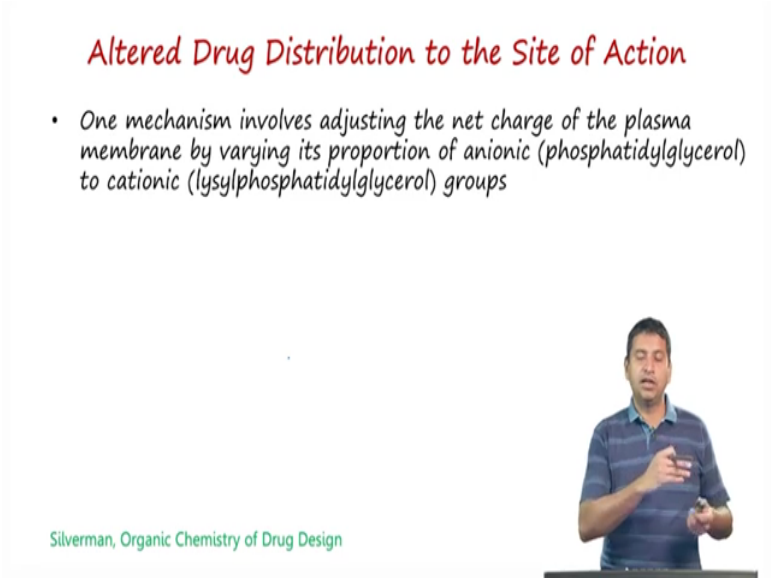

So one of the ways in which this can occur is by you know changing the net charge on the membrane, so if you can for example if there is a Phosphatidylglycerol you know membrane it can be changed to Lysyl phosphatidylglycerol groups then it converts anionic group to a cationic group, so this change can affect the permeability of the drug. So if the permeability of the drug goes down then clearly it is efficacy will go down.

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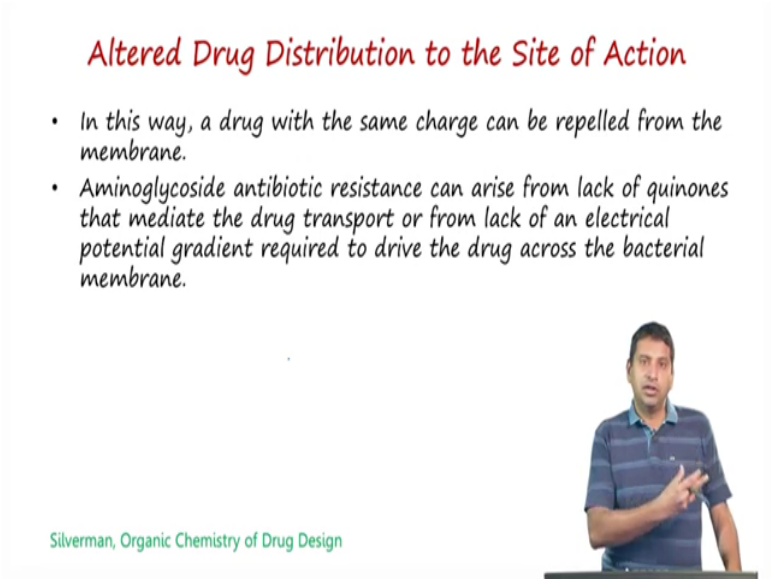

In this way the drug with the same charge can actually now be repelled, so aminoglycosides antibiotics for example become ineffective because the Quinones that mediate the drug transport or from lack of Quinones that mediate the drug transport or from the lack of an electrical potential gradient required to drive the drug across the bacterial Membrane, so these two can result in decrease up take of aminoglycosides which is going to result in resistance.

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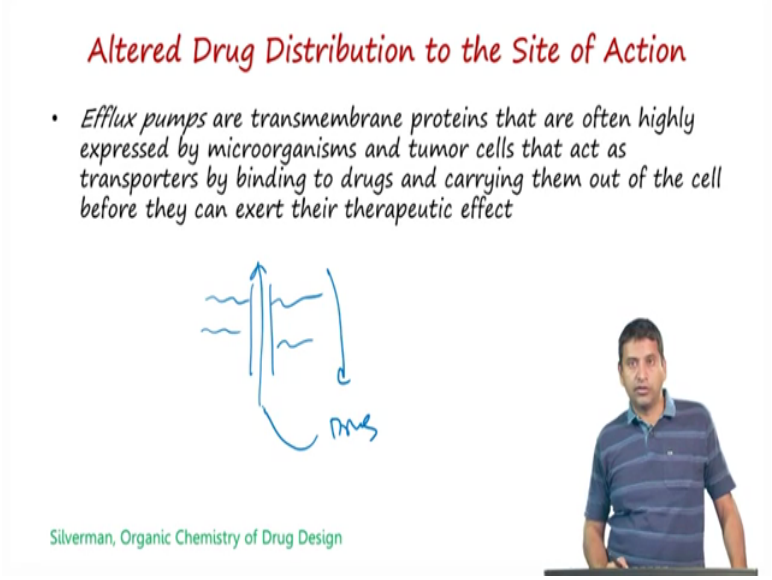

There are also what are known as Efflux pumps which are commonly expressed in many of these highly resistant cells, Efflux pumps are located basically on the protein surface and what they do is that they pull stuff out from the cell and kick it out, ok. So if drug gets in but if the Efflux pumps are very active then the drug is going to get in here but it will be kicked out, so Efflux pumps are major problem in drug resistance.

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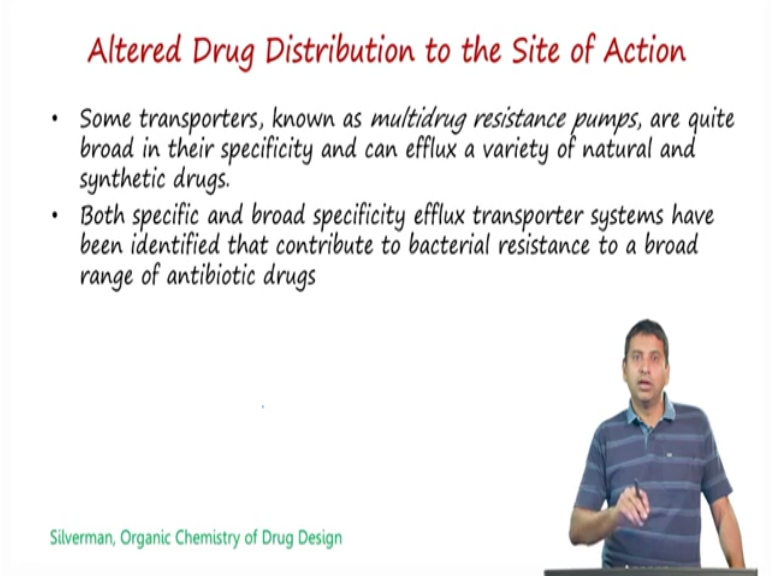

So some transporters which are known as multidrug resistance pumps are quite broad in their specificity and they can Efflux a variety of natural and synthetic drugs. So both specific and broad specificity Efflux transporter systems have been identified that contribute to bacterial resistance and not just one or two but these have a really broad range of antibiotics to which it is resistant to.

So these now become target for us to develop new antibiotics.

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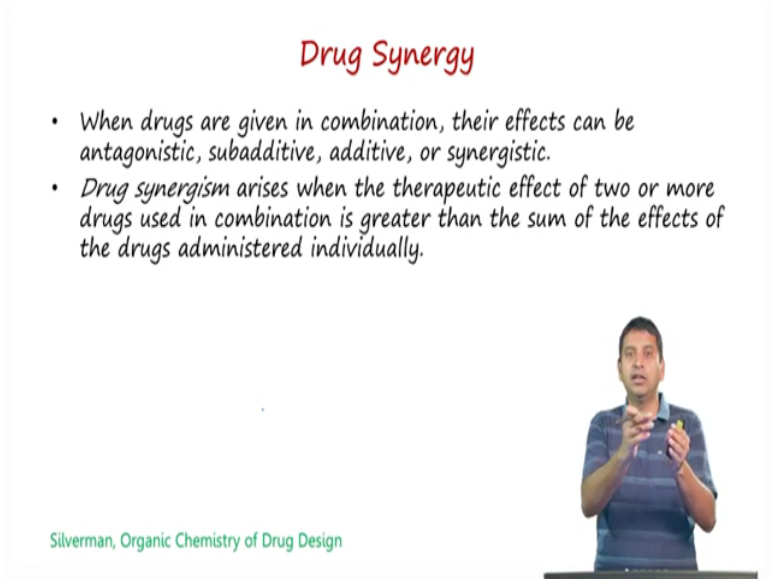

The next topic that we are going to look at is that is drug Synergy. So we have already looked at a part of this when we looked at drug (())(12:21) but here we are just going to look at in a

little bit more formally. So drug synergy when two drugs are given in combination they can be antagonist that means that one drug is going to cancel out the effect of the other or it can be sub additive that means there is going to be a little bit of addition in efficacy or it can be additive that means that the effect of A can be added to the effect of B and that is going to result in the combination therapy.

But more importantly what we look for it is synergy, so here the synergy will refers to when the therapeutic effect of these two drugs or more used in a combination is greater than the sum of the effects of the drugs administered individually. So the difference between additive and synergy is that when I add these two drugs and the concentration at which their efficacy is the net effect does not go up but in synergy the sum of the effects is larger than the individual drugs.

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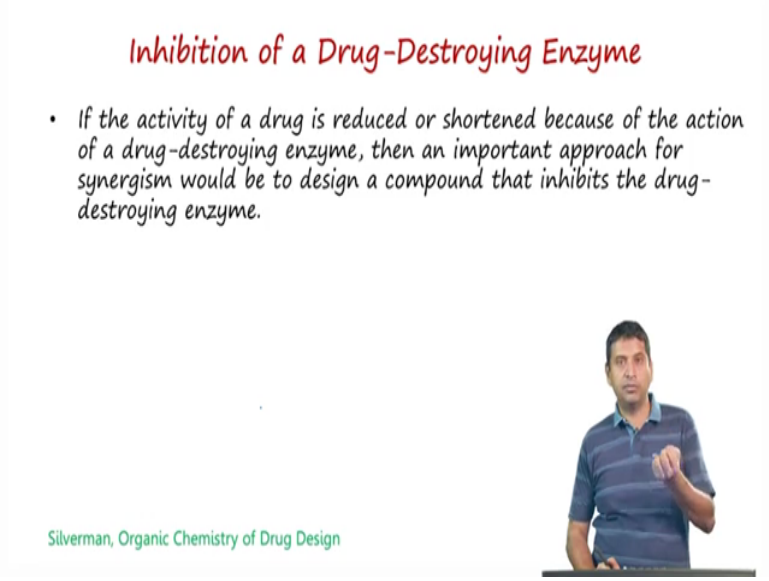

So one can think about a synergy by inhibiting a Drug-Destroying enzyme, so we have already looked at this previously so for example you can have a inhibitor of enzyme that is that destroys the drug and this will result in synergy.

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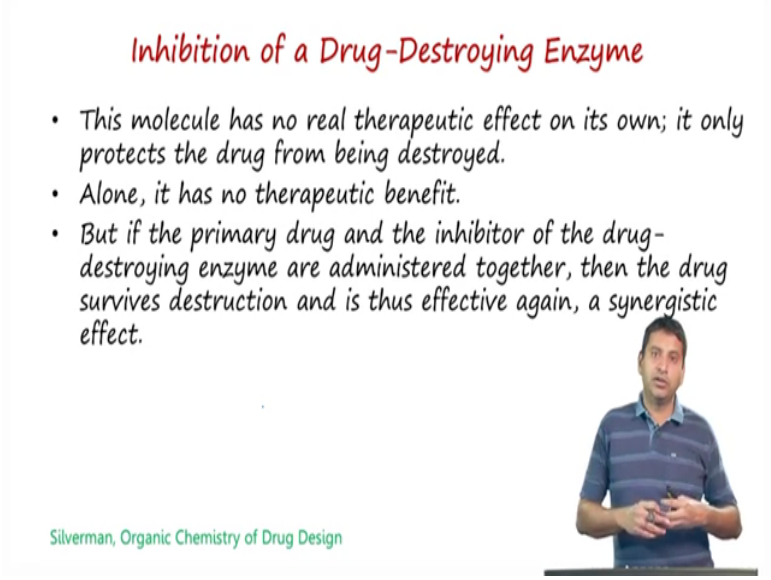

This particular molecule may not have any Therapeutic effect on it is own but it only protects the drug for being destroyed, so this is also called sensitizing the drug to or sensitizing the pathogen to the drug but if the primary drug and the inhibitors the destroying enzymes are administered together then together they can have a Synergistic effects.

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So we have already seen this previously in the case of Ampicillin and Clavulunate, so they are administered together so that you can protect Ampicillin from Beta lactamases.

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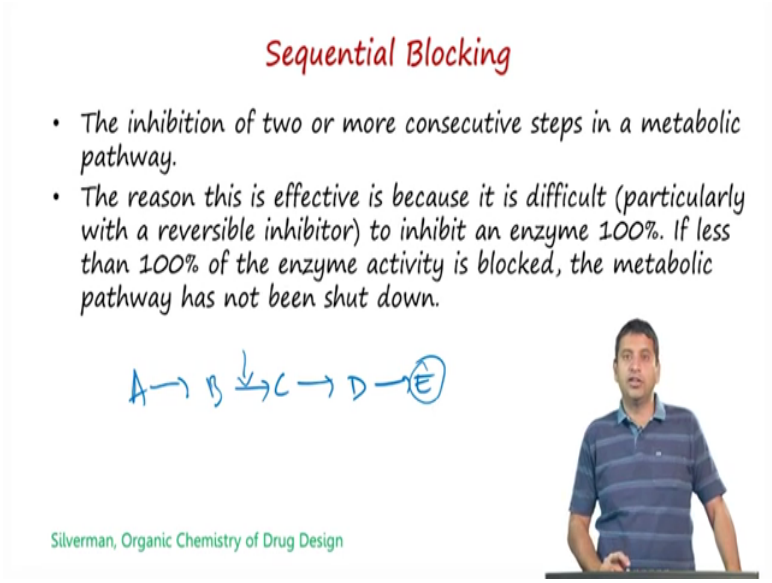

You can also do what is known as sequential blocking, so here even when we look at metabolic pathway that is important and we wanted to develop inhibitors, so here is what we look at let us say A goes to B goes to C goes to D goes to E, so this is a metabolic pathway that we want to inhibit. So let us say we develop an inhibit of for this step, ok. So here because D does not get converted into C by inhibition you do not get to the formation of E, right but if less than 100 percent if of course because we are looking at reversible inhibition it is possible that you would never get 100 percent inhibition.

So if then less than 100 percent inhibition of the enzyme activity is blocked then the metabolic pathway has a really not truly been shut down, there is always going to be some amount of (())(14:58) that is produced.

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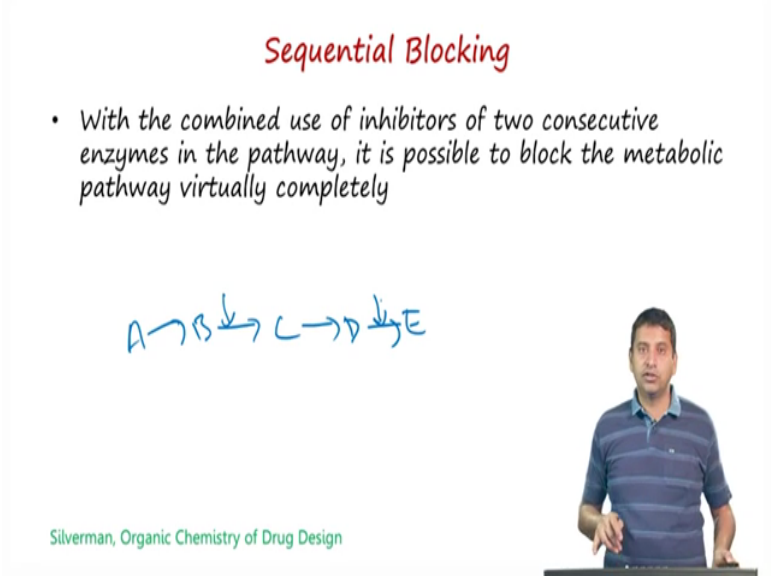

But when you used inhibitors of two consecutive enzymes in the pathway, so here what we would do is an this A going to B going to C going to D going to E what we would do is? We would not just block this but perhaps you are also blocked this, ok. So here you would develop two different inhibitors, so that they can work in Synergy.

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We have already looked at you know combinations of drugs that you know we have previously discussed in details, so I am not going to take this up again.

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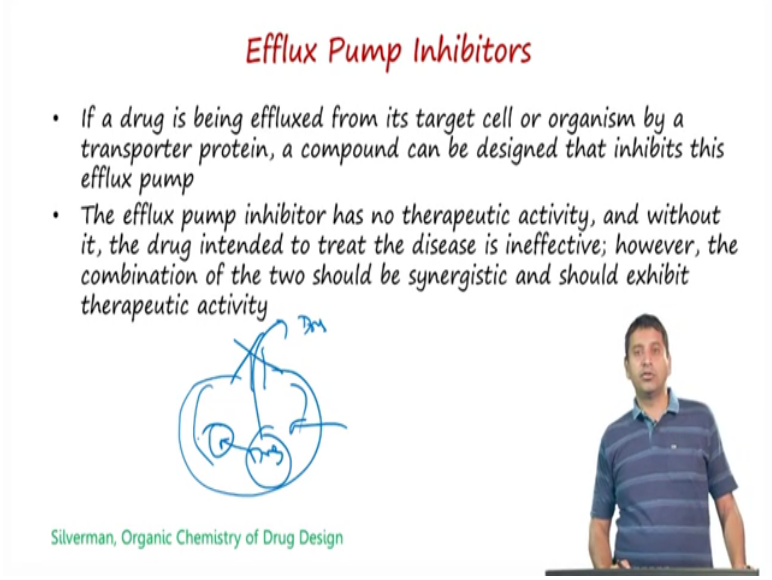

As I mentioned earlier Efflux pumps are a major problem in not in just cancer but also in bacteria and these can act very broad spectrum pumps which can kick out many drugs. So what large numbers of groups are now working on as to develop inhibitors for this Efflux pumps. So once you have inhibitor of the Efflux pumps then the concentration of the drug inside the cell, so let us say you have a cell and you have a your Efflux pump, so the drug gets in but it is Efflux out, ok.

So here if you inhibit this process then the concentration of the drug goes up, so there it can hit the target of a choice.

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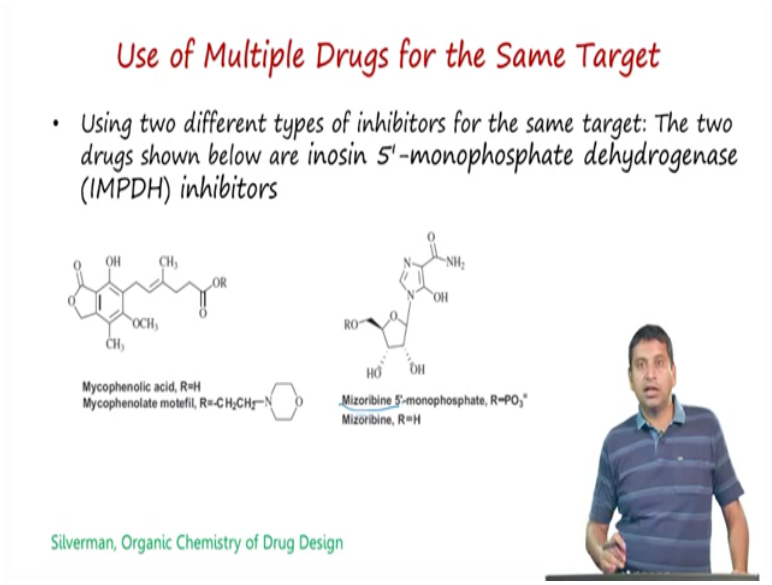

So using two different types of inhibitors for the same target it can also be used. So here is example so you have Mycophenolic acid and Mizoribine which both are inhibitors of inosine-5'-monophosphate dehydrogenase which is IMPDH, right.

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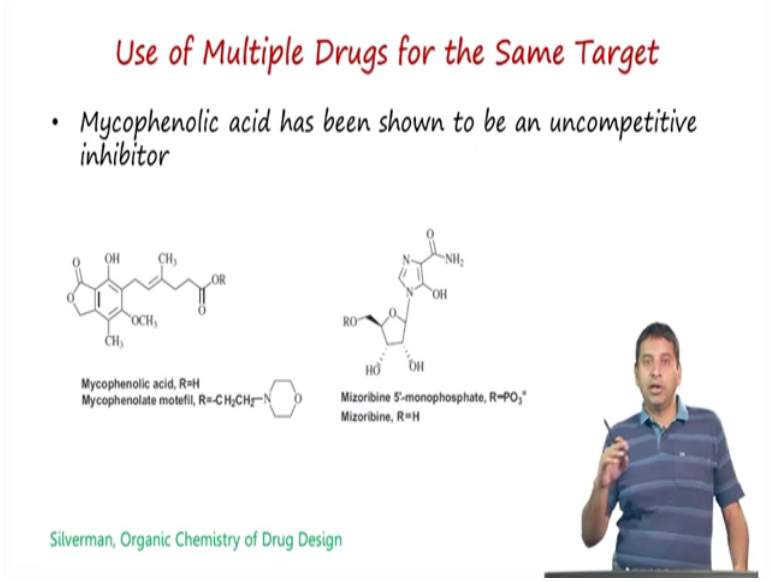

So Mycophenolic acid has been shown to be an uncompetitive inhibitor of this enzyme.

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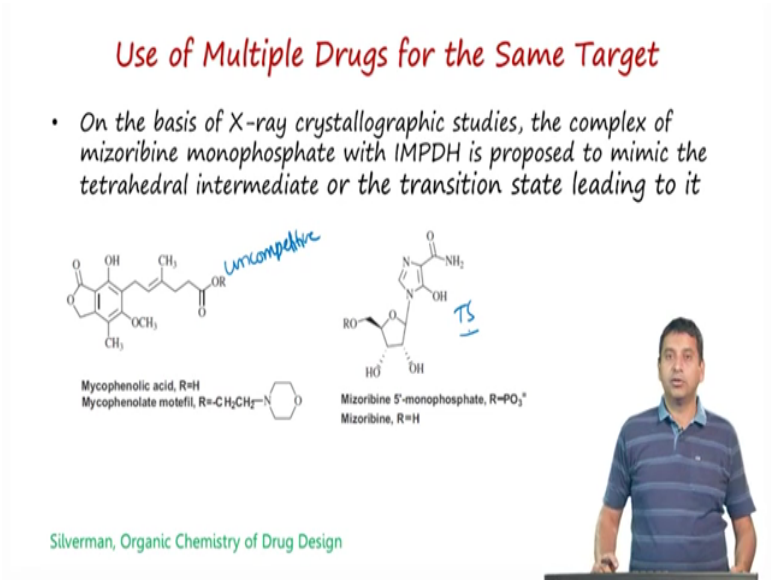

And on the basis of R-ray crystallographic studies the complex of Mizoribine, Monophosphate with IMPDH is proposed to mimic the Tetrahedral intermediate or the transaction state leading to it. So this is uncompetitive inhibitor while this is a transition state inhibitor, ok.

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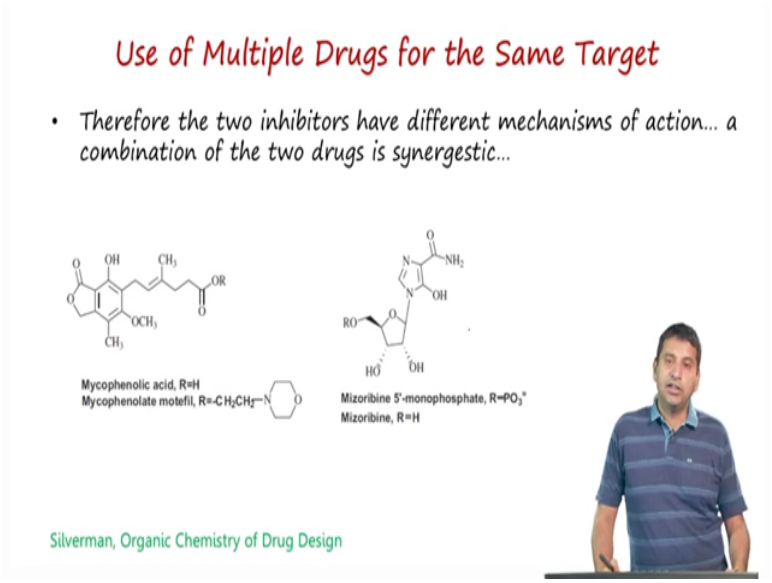

So, therefore these two inhibitors have different mechanisms of action and so if we can give a combination of these two drugs then we would expected to be synergistic inhibitors.