Medicinal Chemistry
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Lecture No 42
Finding a Lead Part - 2

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There is also the concept of multitarget drugs. So in order to understand multitarget drugs let us look at what combination therapy is. So combination therapy is used when typically in several diseases such as tuberculosis, HIV and cancer, and the reason it is done is that we give multiple drugs to the patient, Ok.

So this is normally done because what happens is that it can have these drugs since they have different targets they can form some sort of a very strong arsenal against the bacterium. And since it is hitting multiple targets it is unlikely that it will ever be able to survive this onslaught, Ok.

So that is the rationale for using combination therapy. But one of the disadvantages of combination therapy is that there are number of different medications and they are supposed to be given at different dose regimens.

So it is very difficult to follow this over a period of time because we discussed earlier that each drug has its own pharmacokinetic profile.

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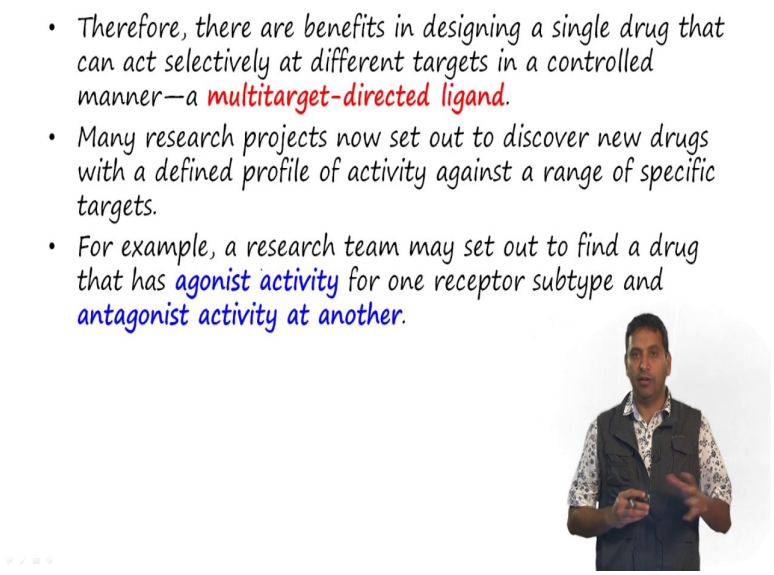

So therefore there are benefits in designing a single drug that can act selectively against different targets, Ok.

So these are known as multitarget-directed ligand or multitargeted drugs. So there are number of research projects that are now underway to discover new drugs with a defined profile of activity against a range of specific targets.

For example one may try to find out a drug that has an agonist activity for one receptor subtype and an antagonist activity against another.

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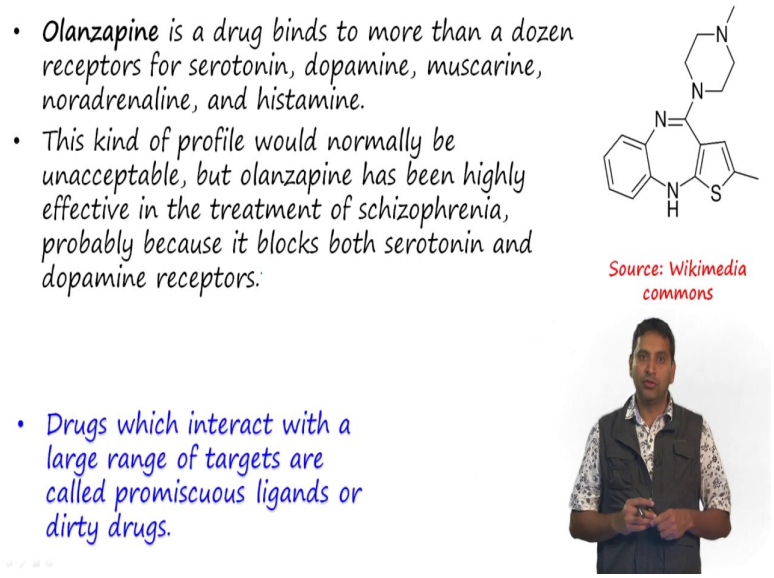

So this compound, olanzapine is a drug that binds to more than a dozen receptors for serotonin, dopamine, muscarine, noradrenaline and histamine. So usually this kind of drug is completely unacceptable for drug discovery.

But what is the reality is that this drug has been highly effective in the treatment of schizophrenia, right. And this is been attributed to its ability to block both the serotonin and

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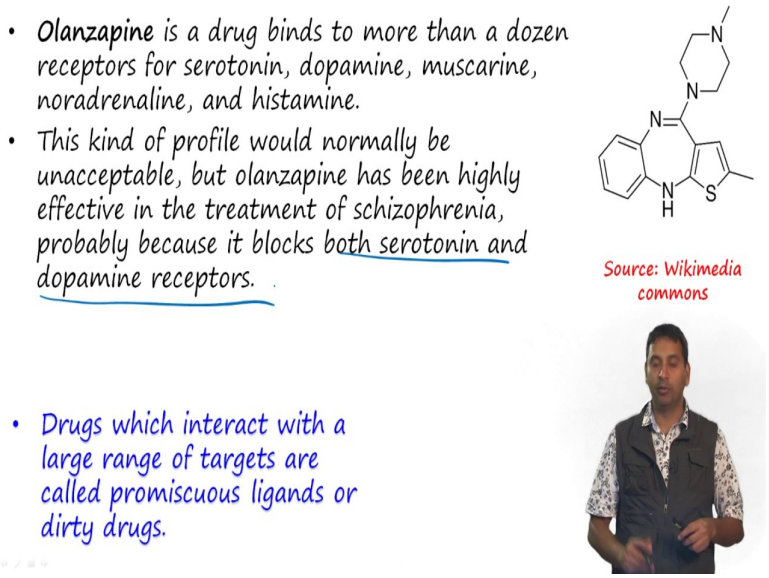

the dopamine receptor.

So these drugs which interact with the large range of targets are called as promiscuous ligands or sometimes as dirty drugs. But this helps us to treat this disease.

And so therefore if one could sort of restrict the number of targets for this drug to 2 or 3 rather than a dozen, then it is possible that we can achieve the same result.

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- Drugs which interact with a large range of targets are called promiscuous ligands or dirty drugs.
- Such compounds would be the starting point for the design of multi-targeted drugs

So such compounds would be great starting points for the design of multitargeted drugs.

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Choice of Bioassay

- Choosing the right bioassay or test system is crucial to the success of a drug research programme.
- The test should be simple, quick, and relevant, as there are usually a large number of compounds to be analysed.
- Human testing is not possible at such an early stage, so the test has to be done *in vitro* (i.e. on isolated cells, tissues, enzymes, or receptors) or *in vivo* (on animals).

So these are the aspects that we need to consider when we are looking at what kind of a disease that we want to hit, what is the selectivity that we want to achieve and so on and so forth.

But once we get in to the program, we need to be able to test molecules, Ok. So for this we need to be able to have what is known as a bioassay,

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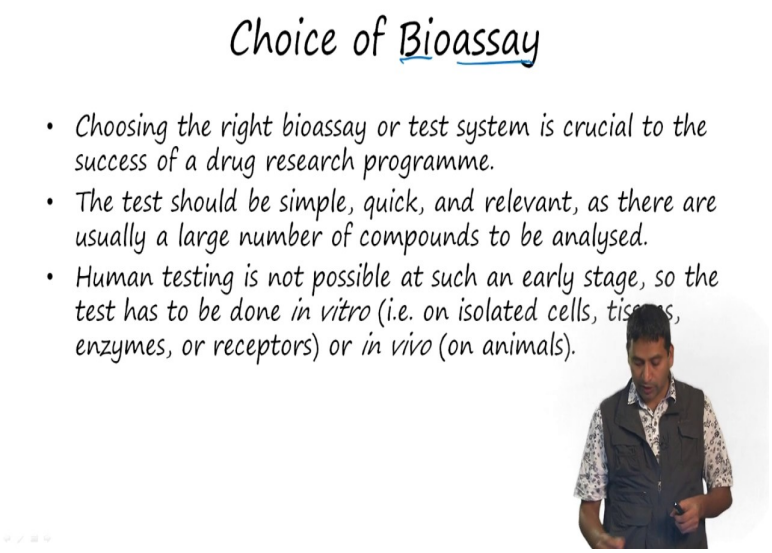

Ok.

Assay is nothing but a test system and so we need to be able to achieve the right bioassay and this, as we will figure out is very crucial to the success of a drug research program.

So there are number of challenges in designing the right kind of bioassay. The test should be simple, it should be quick, it should be relevant and there are usually a large number of compounds that can be analyzed and so it has to be scalable, Ok.

And as you will very well appreciate, human testing is not possible at a early stage, Ok. So what we need to do is something called as the

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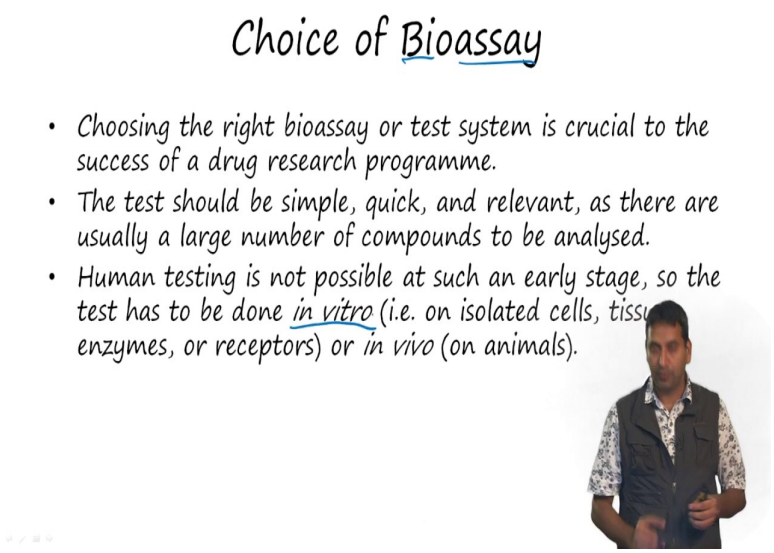

in vitro assay.

Ok, so the in vitro assay means it is usually a test that is carried out on isolated cells, tissues, enzymes or receptors. You can also have animal models which are known as *in vivo*

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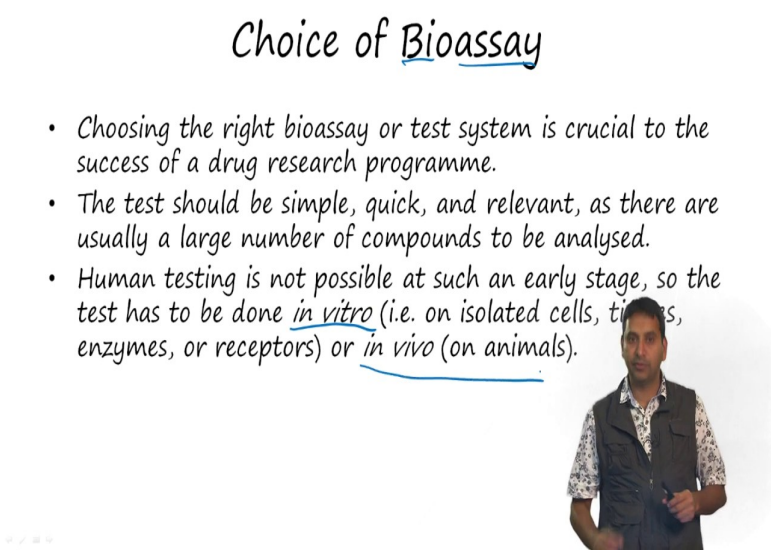

assays.

Ok, so both of these are commonly used to test for new drugs.

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- In general, *in vitro* tests are preferred over *in vivo* tests because they are cheaper, easier to carry out, less controversial and they can be automated.
- However, *in vivo* tests are often needed to check whether drugs have the desired pharmacological activity and also to monitor their pharmacokinetic properties.

In general *in vitro* assays are highly preferred over *in vivo* tests, Ok because they are, it is much cheaper and it is much easier to carry out and less controversial, because animal studies are always associated with some controversy.

And the *in vitro* test can actually be automated, Ok. So therefore *in vitro* assays are very highly preferred. However *in vivo* tests are needed because sometimes when we have a lead compound we need to be able to test whether it can show the desired activity in an animal.

Furthermore we need to be able to monitor the pharmacokinetic properties. Ok,

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- In general, *in vitro* tests are preferred over *in vivo* tests because they are cheaper, easier to carry out, less controversial and they can be automated.
- However, *in vivo* tests are often needed to check whether drugs have the desired pharmacological activity and also to monitor their pharmacokinetic properties.

so again since we cannot do these tests on humans we need to be able to do these on appropriate animal models.

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- A variety of tests are usually carried out both *in vitro* and *in vivo* to determine not only whether the candidate drugs are acting at **the desired target**, but also whether they have activity at other **undesired targets**
- The best balance of good activity at the desired target and minimal activity at other targets

So a variety of tests are usually carried out both *in vitro and in vivo* to determine not only whether the candidate drugs are acting at the desired target but also whether they have activity at other undesired targets. So we need to achieve a good balance between the best activity and minimal side effects.

So something that really hits very hard at very low concentration the desired target may not always be useful because that may have number of other side effects which are not desirable. So we need to balance these two aspects.

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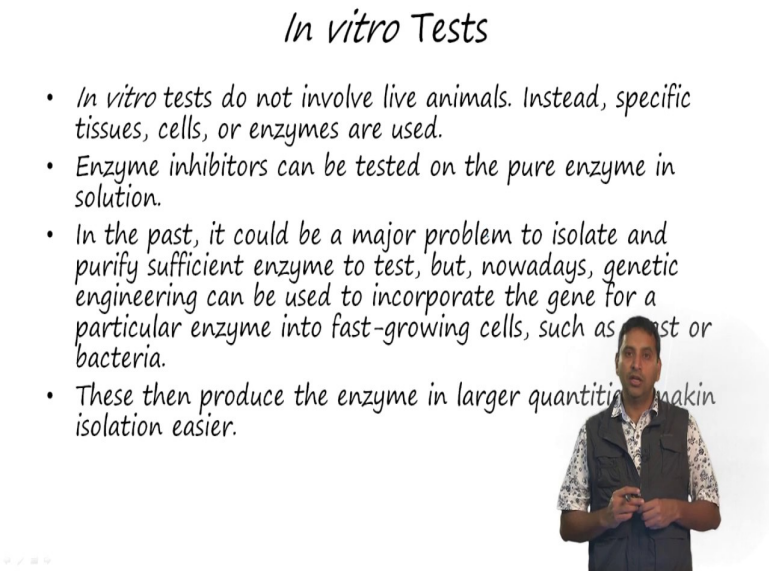

So now let us look at some *in vitro* tests. As we discussed earlier, in vitro tests do not involve live animals but instead they only have tissues, cells or enzymes Ok.

So if we decide that we want to inhibit an enzyme. So let us say we have identified an enzyme that we want to target. Now what we need to do is we need to be able to purify the enzyme.

So in the past this was a major problem because it was very difficult to isolate and purify sufficient enzyme for us to carry out the test.

But nowadays because of genetic engineering what people do is that they usually incorporate the gene which codes for the enzyme into fast growing cells such as yeast or bacteria.

And then this enzyme is produced in large quantities and this makes isolation quite easy.

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- For example, **HIV protease** has been cloned and expressed in the bacterium *Escherichia coli.*
- A variety of experiments can be carried out on this enzyme to determine whether an enzyme inhibitor is competitive or non-competitive, and to determine IC_{50} values

So for example the HIV enzyme HIV protease has been cloned and expressed in *E coli*.

And so once you have the purified enzyme then you can use this enzyme to carry out tests. We will again look at some of these details later on in the course. But this forms a very good starting point to look for inhibition of an enzyme.

So developing a bioassay *in vitro* with an enzyme will involve purification of the enzyme, typically cloning and purification from a model organism such as *E. coli* and then we need to be able to develop the experiment such that we can monitor for inhibition.

Not only that we have already discussed in detail about how to plot the Lineweaver-Burk plot, for example and we can determine whether it is a competitive inhibition, non-competitive inhibition and so on. And of course the important parameter that we wish to

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- For example, **HIV protease** has been cloned and expressed in the bacterium *Escherichia coli.*
- A variety of experiments can be carried out on this enzyme to determine whether an enzyme inhibitor is competitive or non-competitive, and to determine IC_{50} values

look at is the IC_{50} which is nothing the inhibitory concentration 50 percent.

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- Receptor agonists and antagonists can be tested on isolated tissues or cells which express the target receptor on their surface.
- Sometimes these tissues can be used to test drugs for physiological effects.
- For example, bronchodilator activity can be tested by observing how well compounds inhibit contraction of isolated tracheal smooth muscle.

Similarly if we decide that receptor is a target then receptor agonist and antagonist can be tested on isolated tissues or cells. So these tissues or cells obviously will express the target receptor on the surface. So what we would do is we would add the drug candidate to this tissue and look for activity, right.

So sometimes these tissues can be used to test drugs for physiological effects. So for example this bronchodilator, dilatory activity can be tested by observing how well compounds inhibit the contraction of isolated tracheal

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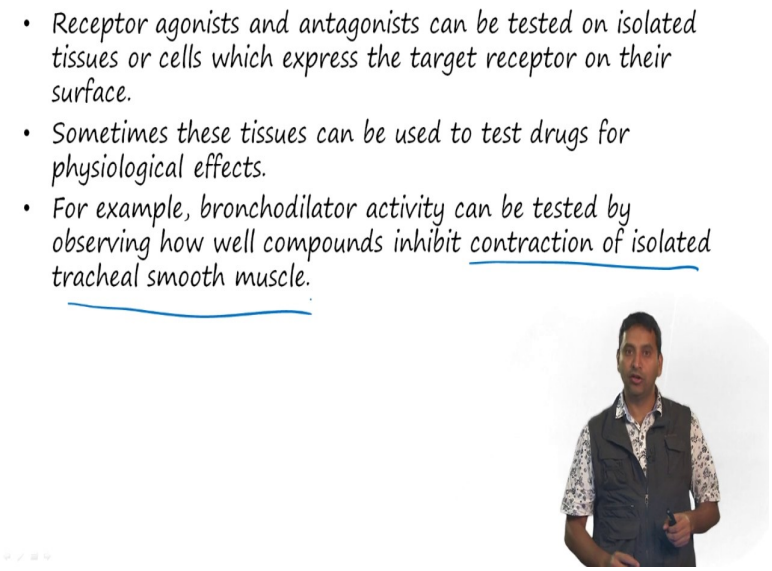

smooth muscle.

So you can isolate this tracheal smooth muscle and figure out how well the compound acts in contracting it.

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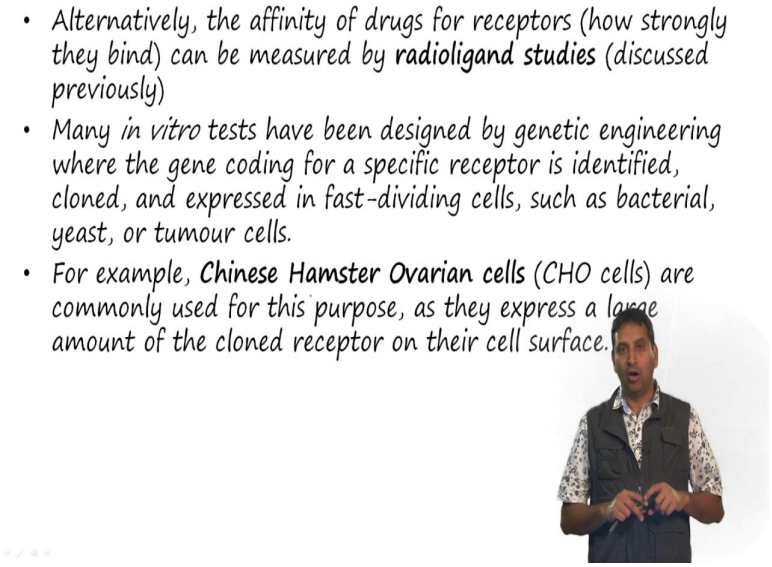

Alternately the affinity of drugs for receptors, that is how well they can bind can be measured by radioligand studies.

So we have already looked at this previously and we have derived plots on this. Many *in vitro* tests have been designed by genetic engineering where the gene encoding for a specific

receptor is identified, cloned and expressed in fast dividing cells such as bacteria, yeast or tumors.

So for example the Chinese Hamster Ovarian cells are very commonly used to over-express some of the cloned receptors on the surface. And then we look for how the cell responds to it.

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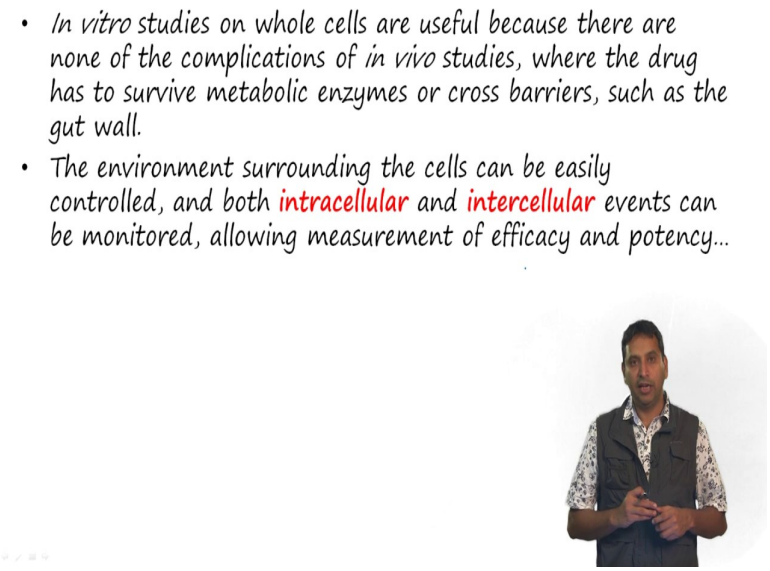

In vitro studies on whole cells are useful because they are not associated with the complications that are typically found with *in vivo* studies.

So for example when we are looking at an *in vivo* model we need to figure out how to administer the drug. And this has to survive the metabolic enzymes and cross barriers such as the gut wall and so on. So all of these can be somewhat bypassed when you are looking at *in vitro* studies.

However as you can imagine, *in vitro* studies also have very similar limitations because we do not know whether the drug is going to reach the target of interest or not. But the environment surrounding the cells can be easily controlled. So we can look at both intracellular as well as intercellular events and it allows us to measure the efficacy and potency of the compound.

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- Primary cell cultures (i.e. cells that have not been modified) can be produced from embryonic tissues; transformed cell lines are derived from tumour tissue.
- Cells grown in this fashion are all identical.

There are also some things known as primary cell cultures which are basically cells which have been isolated from animals. And these are not transformed or modified cell lines. And they can be produced from embryonic tissues or from other transformed cell lines which are derived from tumour tissues, Ok.

So cells which are grown in this fashion are all identical and so they are very useful in testing out our compounds.

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- Antibacterial drugs are tested *in vitro* by measuring how effectively they inhibit or kill bacterial cells in culture.
- It may seem strange to describe this as an *in vitro* test, as bacterial cells are living microorganisms.
- However, *in vivo* antibacterial tests are defined as those that are carried out on animals or humans to test whether antibacterial agents combat infection.

So if you have set out to develop antibacterial drugs they can easily be tested by measuring how effectively they inhibit or kill bacterial

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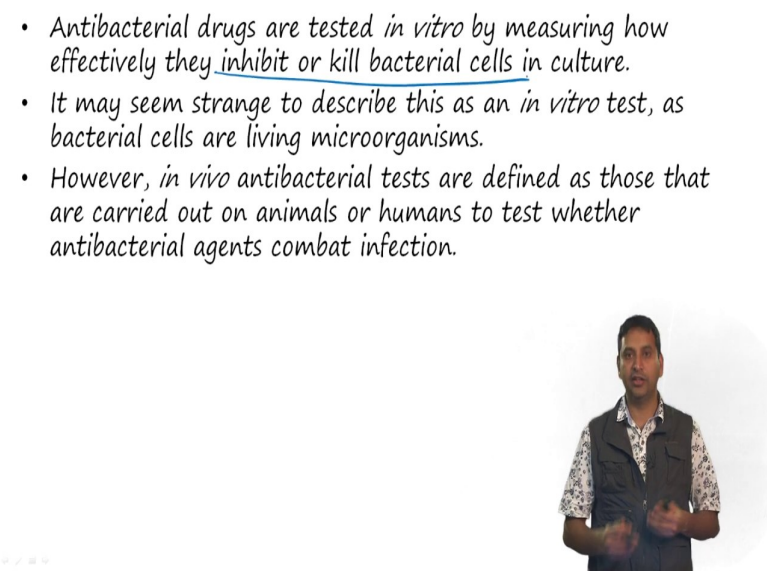

cells in culture. So what we can do is we can grow *E. coli* or Streptococci or any other bacteria in a safe environment.

And then once they are in culture then we can add a drug and measure how well they inhibit the growth of this bacteria. It may seem strange to describe this as *in vitro* test but bacteria, since bacteria are actually living organisms. But just the fact that they are able to inhibit does not mean that it is going to work in a animal model.

So *in vivo* antibacterial tests are actually defined as those that are carried out on animals. So there is a distinction here which we need to appreciate. Of course the *in vivo* test can also be done on humans at a much later stage and whether the compound is able to combat infection or not.

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- *In vitro* tests are also used to test for the pharmacokinetic properties of compounds
- For example, the **Caco-2 cell monolayer absorption** model is used to assess how well a drug is likely to be absorbed from the gastrointestinal tract.

in vitro tests are also used for understanding pharmacokinetic properties. So for example this Caco-2 cell monolayer absorption

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So this Caco-2 cell is derived from colon cancer cells and so this would be a good mimic of how the gastrointestinal tract functions and how well the drug is absorbed.

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- Microsomes and hepatocytes extracted from liver cells contain cytochrome P450 enzymes, and can be used to assess the likely metabolism of drug candidates, as well as identifying possible **drug-drug interactions**.

There are also microsomes and hepatocytes which can be extracted from liver cells and these contain cytochrome P450

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- Microsomes and hepatocytes extracted from liver cells contain cytochrome P450 enzymes, and can be used to assess the likely metabolism of drug candidates, as well as identifying possible **drug-drug interactions**.

enzymes which we have discussed very extensively previously, that these can be used to assess the likely metabolism of drug candidates.

And they are also used possibly

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- Microsomes and hepatocytes extracted from liver cells contain cytochrome P450 enzymes, and can be used to assess the likely metabolism of drug candidates, as well as identifying possible **drug-drug interactions**.

to identify drug-drug interactions.

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- Another *in vitro assay* using artificial membranes has been developed as a simple and rapid measure of how effectively drugs will cross the blood-brain barrier.

So another *in vitro* assay using artificial membranes has been developed to measure how well drugs will cross the blood brain barrier.

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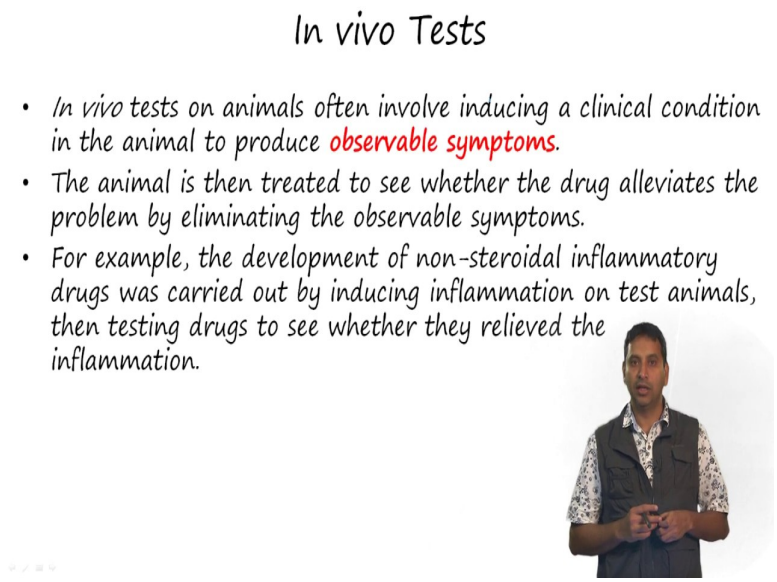

The next class of bioassays would be *in vivo* tests. So *in vivo* tests as we have looked earlier are on animals and they often involve inducing a clinical condition in the animal and then we observe for symptoms. So the animal is then treated with the drug or drug candidate to see whether the problem is alleviated.

So this forms the foundation for *in vivo* test. So for example inflammation is a huge problem. And so the development of non-steroidal anti-inflammatory drugs was carried out by inducing inflammation.

So this should be anti-inflammatory

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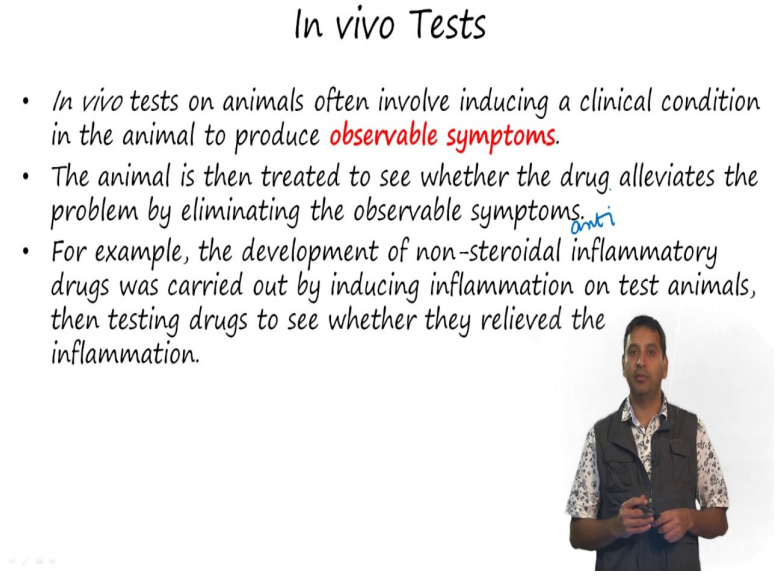

drugs was carried out by inducing inflammation on test animals. And then testing drugs to see whether they relieve the inflammation.

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- **Transgenic animals** are often used in *in vivo* testing.
- These are animals whose genetic code has been altered.
- For example, it is possible to replace some mouse genes with human genes. The mouse produces the human receptor or enzyme and this allows in vivo testing against that target.

There are other animal models which we can develop which are known as transgenic animals. And they are very often used. So these are animals whose genetic code has been altered.

So it is possible to replace some of the mouse genes for example,

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- **Transgenic animals** are often used in *in vivo* testing.
- These are animals whose genetic code has been altered.
- For example, it is possible to replace some mouse genes with human genes. The mouse produces the human receptor or enzyme and this allows in vivo testing against that target.

with human genes. And now mouse produces the human receptor or enzyme in it and this allows for in vivo testing against the target.

So if we have convincing data, *in vitro* data to show that our compound is hitting a particular target then one would develop the, these transgenic mice and test them to see whether the target is really being affected or not.

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- Alternatively, the mouse's genes could be altered such that the animal becomes susceptible to a particular disease (e.g. breast cancer).
- Drugs can then be tested to see how well they prevent that disease.

Alternatively the mouse genes can be altered such that the animal becomes susceptible for a particular disease. So for example if we are able to induce

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- Alternatively, the mouse's genes could be altered such that the animal becomes susceptible to a particular disease (e.g. breast cancer).
- Drugs can then be tested to see how well they prevent that disease.

breast cancer in animals then we would be able to test the drug in a fairly relevant model.

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- There are several problems associated with *in vivo* testing.
- It is slow and expensive, and it also causes animal suffering.
- There are the many problems of pharmacokinetics, and so the results obtained may be misleading and difficult to rationalize if *in vivo* tests are carried out in isolation.

Of course there are several problems associated with *in vivo* testing. It is slow, it is very expensive, and it also causes animal suffering which is unacceptable to many. There are many problems associated with pharmacokinetics, for example.

So some of the results that we obtain from animal studies can be misleading. And also difficult to rationalize if the *in vivo* tests are carried out in isolation.

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- For example, a negative result may be due to the drug failing to bind to its target or not reaching the target in the first place?
- Thus, *in vitro* tests are usually carried out first to determine whether a drug interacts with its target, and *in vivo* tests are then carried out to test pharmacokinetic properties.

So for example if you get a negative result *in vivo* model and then the drug fails to bind its target we do not know whether the drug has actually reached the target or whether it is not able to bind to the target.

So *in vitro* tests are usually carried out first to determine whether the drug interacts with its target and then *in vivo* tests are then carried out to test the pharmacokinetic properties.

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- Certain *in vivo* tests might turn out to be invalid.
- It is possible that the observed symptoms might be caused by a different physiological mechanism than the one intended.
- For example, many promising anti-ulcer drugs which proved effective in animal testing were ineffective in clinical trials

There are also cases where certain *in vivo* tests might be invalid. It is possible that the observed symptoms might be caused by a different physiological mechanism than the one intended.

So there were a number of promising anti-ulcer drugs which proved to be ineffective in clinical trials.

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- Different results may be obtained in diff erent animal species. For example, **penicillin methyl ester prodrugs** are hydrolysed in mice or rats to produce active penicillins, but are not hydrolysed in rabbit, dogs, or humans.

Now it is also possible that different results may be obtained in different animal species. So the example that we can look at is this penicillin methyl ester prodrugs which are hydrolysed in mice or rats to produce the active antibacterial penicillin.

But they are not hydrolysed in rabbit, dogs or humans.

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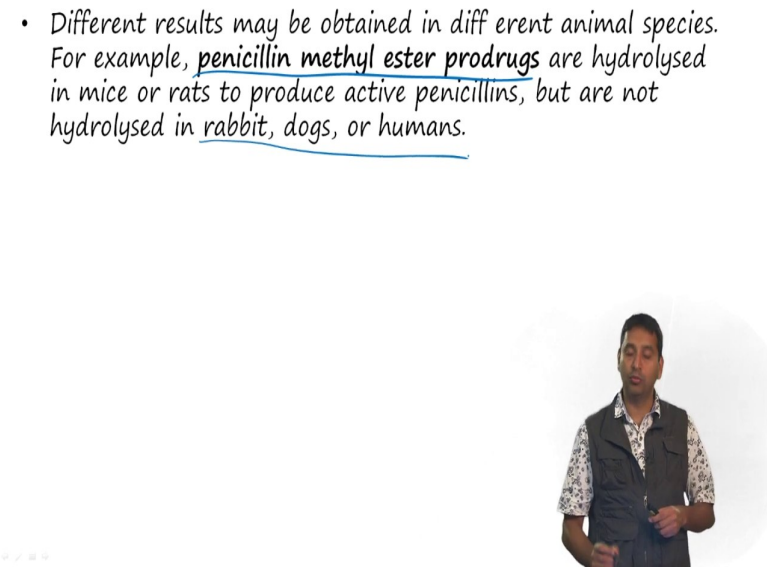

So if we have a case such as this, you would find that perhaps that the drug is not active in certain animal model but active in another animal models.

What is worse is that if we use this as a basis for human testing and if the particular hydrolysis or activation does not occur in humans then it would be major issue.

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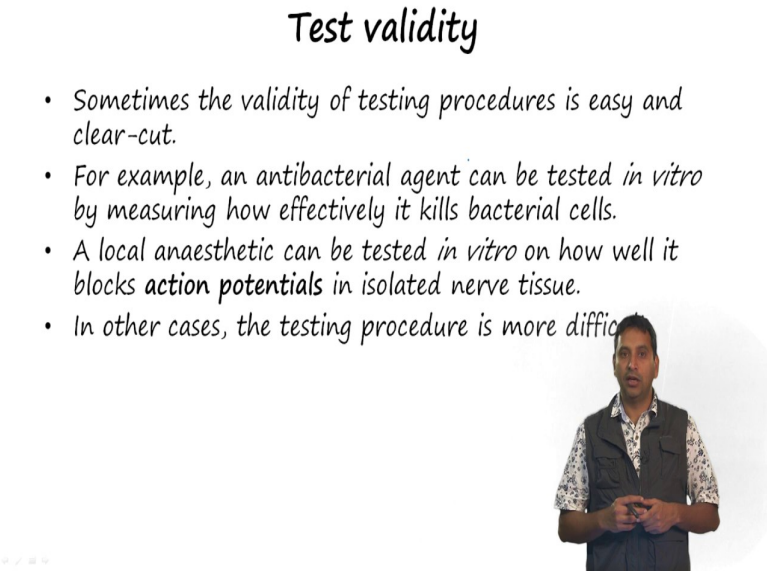

We also need to be able to look at the validity of testing, Ok. So the testing procedures will have to be easy and clear-cut. So for example when we are looking at antibacterial agents we can easily test them *in vitro* by measuring how it can kill bacterial cells.

We can also look at local anaesthetic *in vitro* to look at how it is able to block action potentials in isolated nerve tissues. But there are number of other cases where the testing procedure is actually more difficult.

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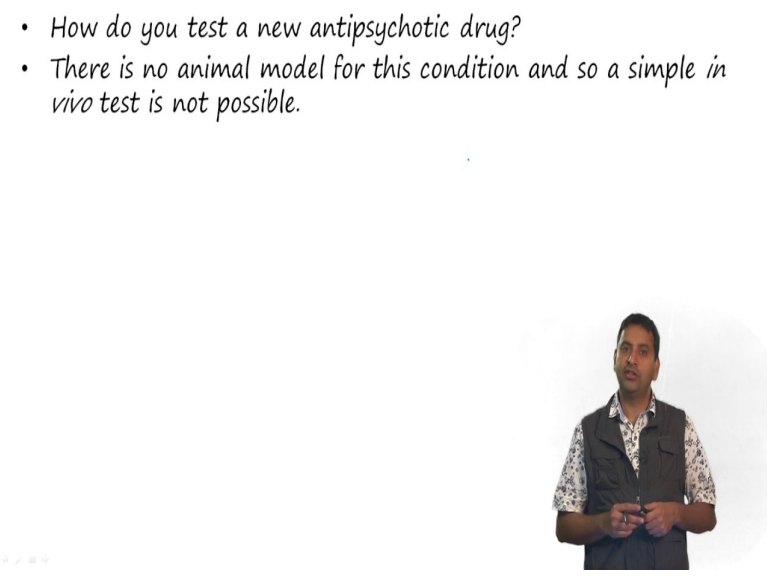

So let us consider that you want to develop a new anti-psychotic drug. So what kind of animal model would we develop? Will we able to induce psychosis in an animal? All these are very complicated and probably impossible to solve, right.

So we would not be able to look at a simple *in vivo* test to test for anti-psychotic drugs.

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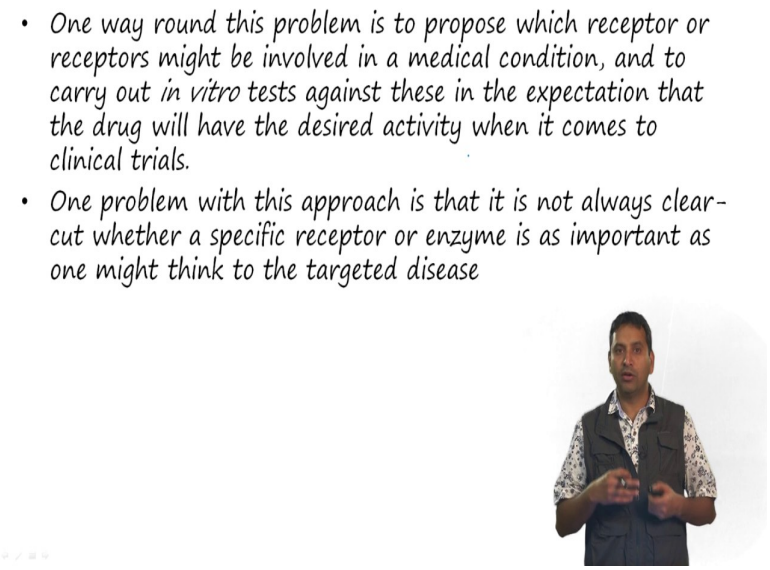

So what we would have to do is to work with the receptor or receptors which are involved in the medical condition.

So then we could carry out a battery of *in vitro* tests and if these are in expectation, in line with our expectation then we would be able to administer these and test their efficacy in some model system which is then taken forward.

Of course one problem with this approach is it is not always clear-cut whether a specific receptor or enzyme is as important as one might think in the targeted disease.

So a lot of clinical data that is obtained suggests that there could be hyperactivation of a receptor or depression of a receptor for example. Or a particular enzyme in terms of its expression levels. But this does not always translate to increased or decreased activity.