CLONES AND STEM CELLS

Dr. Chris Skidmore

22 August 2007

Part 1

Thank you very much. Let's let everyone else sit down and get comfortable. It may be a great favour for me to come back from retirement, but I only have to walk a quarter a mile across the campus, so it's hardly hard work for me. As you see, my topic today is 'Clones and Stem Cells'. And this is the outline of my lecture. I'm going to first of all talk a little about what I mean by a clone, then I'm going to talk about how multi-cellular organisms - things like us - grow, from single cells, and the role in that particular process which we call differentiation of what we call stem cells. OK? A hot topic today, particularly in the United States. Then I am going to give you a short history of cloning, using a method we call nuclear transfer, and then I'm going to talk about the possibilities of using stem cells for what we call *cell replacement therapy*. And what then what relationship that bears to the topic of cloning humans.

So, first of all, what is a clone? Well the word, clone, as we spell it in English, derives from an ancient Greek word - which is spelt differently of course - ok but said more less the same,
clone, right? which means a twig, a small branch of a tree. And the reason we get this word is because one way of propagating trees, as you probably know, growing new trees is to take a twig from one old tree and graft it on to another tree. And when you do that, the mother tree as it were, supplies the nutrient, and what grows out from the branch is not same as the mother tree, but is the same as the tree as it was taken from. And so what we're doing is we're reproducing the tree we took the twig from, but attached to another root stock, as they call it. So this way, we can produce lots of copies of our original tree by taking lots of twigs and sticking them onto new roots. And so this word clone, was originally used for a group of cultivated plants, all from one stock, which have been produced by this method of grafting.

Now, of course we are then producing new trees asexually, right? We are not using the sex organs, we are not using seeds at all, so this word then became a word which meant any group of cells or organisms which had been produced asexually. The way we use this word now, derives from the use it then began to have in microbiology, which is of course microbes as you know, grow, most of the time asexually. They don't have - many microbes don't have a sexual phase at all. And so if we plate out, single microbes in a dish, they grow. And this little thing we can see here is a group of microbes which have all derived from a single microbe and they have derived from it simply by division. The process that we call in biology,mitosis, that simple cell division. And so all the cells in this clone, because they have derived from a single cell, are genetically identical. They have inherited the same genes. So, they've, of course, reproduced asexually, so we've taken the word from meaning reproducing asexually, to mean a group of identical organisms. OK? So, all the cells in this clone here are genetically identical and therefore we call them a clone.

In the 1980s and 1990s, in the last century, when DNA manipulation or genetic engineering as it is sometimes called, with first mooted, people used bacteria like this for growing pieces of DNA. And they isolated microbes containing a particular piece of DNA, by this cloning mechanism. That is they dropped single microbes onto a plate, and waited for them to grow. And so, in such an experiment, all the cells in a particular clone would contain the same piece of added DNA, and therefore we describe this as cloning DNA. Now from this usage comes a lot of confusion, right? Because when we talk about cloning an animal, or a plant and cloning a piece of DNA, we are not quite talking about the same process. But because all these things are manipulations, people tend to lump them together. And one of the purposes of today's lecture is to try and dissect them.

So this is the last time I am going talk about DNA manipulation really. I'm just explaining to you why it is, we sometimes use this word *clone* in the context of DNA manipulation. However, if we want to clone and animal or plant, we can do that in many ways. And the basic methods particularly for animals are either taking a very small embryo and splitting it up into its individual cells and then asking those cells to start again. We can do that with embryos of 4 cells and of 8 cells. Or by the process which we are going to talk about later, called nuclear transfer.

Part 2

So that's one piece of introduction, just introducing to you the idea of a clone. A group of cells or organisms which are genetically identical. Now I want to talk about this other process. This process which takes place when multi-cellular animals grow. Now like our bacterial colonies we just looked at, our bacterial clones, all multi-cellular organisms are produced sexually from a single cell, by mitosis, that is they all start off as single cells. In our case, that would be a fertilised egg. And therefore of course, they are clones, that is we expect them to be genetically identical. But, in a multi-cellular organism like me, clearly I have different parts of my body, which appear to be different. My skin appears to be made up of cells which are different from my blood, and the cells in my heart are different from those again and the cells in my muscles are different again. Although we are, a clone, that is we have arisen from a single cell by mitosis, we look different. We have what geneticists call different *phenotypes,* cells with different sets of characteristics, OK? And that is because these cells have become different - that's the word we use 'differentiated', simply means become different. They become specialised. So, blood cells have to do a different job from skin cells and heart cells and muscle cells.
And we find in fact that this is a process - this process of specialisation involves the lack of ability to divide, so as we become more specialised, our ability to divide by mitosis goes down. Of course if it didn't go down, we would simply grow - the number of cells in our body would keep on increasing wouldn't it? But it doesn't.

So, here we are, this is what I mean by differentiation. OK? On this slide you can see three sorts of cells. You can see these cells here, which are red blood cells, which we call erithrocytes, and they are basically bags of haemoglobin, the

pigment that we use to pick up oxygen. In these pictures we can also see two of the white blood cells in your, that you find in your blood. This is a white blood cell called a b-lymphocyte. This is a white blood cell called a granulocyte and the white blood cells can still divide a little, but red blood cells can't. But all these three cells, derived originally from the same cell. So these cells have become different. And the cell that they came from, is what we call a stem cell. And of course that word, 'stem' just you know just interestingly is another word for a part of a plant, isn't it? The main part of a plant, the part that grows out of the ground. And that shows these stem cells are meant to be a basic part of the cell system.

Now stem cells are unspecialised cells. So they are not like these ones, right, which have become specialised. But they are unspecialised. And they retain the ability to divide. And they can be induced in the body or sometimes out of it to become one or more types of differentiated cell. If we just look at how that particular thing works in your blood system. Here are some of the cells we saw in the picture, here is an erithrocyte, a red blood cell, here is a b-lymphocyte, here is a granulocyte, OK? Now all those three types derive eventually from this kind of stem cell here which is here completely undifferentiated. Now these cells don't really look like this nor are they a pretty colour like this. These colours are just there to help you look at the diagrams. What some people call false colour.

So, in your bone marrow, which is where all your blood cells come from, in the inside of your bones, there exist some of these cells, these stem cells which can divide and then give rise by various acts of becoming different, acts of differentiation into all the different cell types you find in your blood. In fact we know that there are two lineages, as we call them, two lines of descent from the stem cell via slightly more specialised stem cells. ok? the lymphoid stem cells, the myloid stem cells to give different sorts of blood cell.

So these stem cells - there we were showing them existing in the bone marrow - but they exist inside the early embryo and in small numbers in most tissues where they stay to maintain and repair the tissue. So your muscles, although there have lots of specialised cells in them, also have a few stem cells which sit there and make more muscle cells when you tear your muscle, let's say.

Part 3

Now let's just look at the embryonic stem cells, the ones that happen in the early embryo. This is a picture of the human reproductive tract, of course female reproductive tract, ok? and here is the ovary and here is the ovary releasing an egg. And the egg as you know travels down the reproductive track where it meets the sperm and is fertilised, and then the fertilised egg starts to divide here two cells, here four cells, here eight cells, then it reaches the uterus and continues differentiating and this - you see it goes from a little bundle of cells here, right, to a much more structured appearance here, and I want to look at this form of the earlier embryo which we call the blastocyst.

Here it is in an electron micrograph. This is a very early blastocyst, in fact it's a mouse blastocyst, not a human one. And you will be able to see that there are really two structures here. There's an outer circular or spherical shell which is

surrounded by few little cells, and here there is a blob. This blob here we call the inner cell mass. And it consists of about thirty cells. These cells here are the cells which are going on to become the foetus and then the adult, in this case mouse. These cells around the outside here are going to make up the placenta, and they're not going to be part of the mature mouse, so this, it's only these cells here, the inner cells, mass cells, which are going to become the foetus. And we call these cells the embryonic stem cells. So, these cells here, about thirty of them ,are going to give rise to all the cells in the mouse's body. And if we were looking at a human blastocyst, then they would give rise to all the cells in a human body. So, here we have the fertilised egg, here we have the little ball of cells, perhaps eight cells here, and here we have the blastocyst containing its stem cells.

I've no doubt - just one more thing. -we can either isolate these stem cells, and we can grow them in culture or inside the body. They will gradually differentiate to give first of all tissue specific stem cells. Here are the ones that give rise to the blood system and here are the blood cells, here are the ones which give rise to the nerves, here's a nerve cell. Here is the one which gives rise to the muscle here is a picture of a bone. Because the same cells give rise to bone and to muscle. OK?

Now, as we've gone through these slides, there have been one or two words I haven't explained, let me explain them now. While they are on this one, this word, totipotent, and this word, pluripotent, right? Now these words come from the Latin. And this bit of the word, the 'potent' bit means 'able to do things'. Or 'powerful' Right? Possibly you know the word, omnipotent, or omnipotent, which means ' all powerful'. Like what United States would like to think itself. So, by putting 'toti', as another word meaning 'all', and toti implies that these stem cells can give rise to any cell. They are able to give rise to anything whereas this word 'pluri' means 'a few'. And this means that these stem cells can give rise only to a few types of cells. So, there is *totipotency* and *pluripotency.*

Now, let's bring these two subjects together. We talked about cloning, we talked about differentiation, cells become different. The first experiments that were done to clone plants and animals were done with an intention of asking a question about differentiated cells. So all these different cells in your body look different. Now when a cell develops, into several other sorts of cells with different characteristics, there are two things that could have happened. One, it could have changed its genes. Or two, it could have just selected some of the genes to use, and not used some of the others. How might we change the genes? Well, we might through away all the ones we don't want. In which case, a skin cell would only contain the genes that were necessary for making skin. And a blood cell would only contain the genes that were necessary for making blood, and it would have lost all the others. The alternative is that the skin cell and blood cell have exactly the same genes, but the skin cell is only using a proportion of them, and the blood cell is using a different proportion.

Now one way of telling whether these, which of these two mechanisms is the right one, which happens during differentiation, is to ask a differentiated cell, whether it can give rise to an entire organism. In other words, we can send it back to being an embryo, and ask whether it is totipotent, whether it is able to give rise to all the other cell types. Because in the first case, if it's lost some genes, it won't be able to, but in the second case, where it has retained all the

genes, it will be able to. So, if the nucleus - which is where the genes are - of a differentiated cell is placed in an embryo, we can ask the question, will it produce a normal organism? And if it produces a normal organism, then we know this first explanation, the one about chucking away some of the genes can't be right.

Part 4

And there are three classic experiments; one which was done with plants, about sixty years ago now; ome which were done with frogs and toads, about forty years ago. And finally the ones which were done with mammals which have given rise to the whole issue of human cloning, which were done in the 1990s, only about 15, perhaps 20 years ago nearly now. So, let's just talk about these three sets of experiments very briefly. An American biologist called Stewart first asked this question, 'Can I grow a whole plant from a differentiated cell?'. And this is very easy with plants. Plants are much more, as we say in biology **plastic**, that is changeable, able to be changed more easily than higher animal organisms, and we saw that, just at the very beginning of this talk when I talked about grafting. You can't take a human leg, right? cut it off and attach it to another human, can you? Right? Well you *can*, but it doesn't work, right? You can't do, can't do that grafting experiment,. So, we are human, and one of the reasons, we have very complicated, much more complicated systems, for recognising difference. But plants are much more plastic, ok, we know that they can grow by vegetative, that is grafting type methods or by sexual methods.

So, with plants it was actually a very easy experiment. Steward thought, 'what is the way I can make a plant cell think it's back in the embryo', OK?' And he thought - and his thought experiment, was 'II need to find a big seed', right? which has something in it which identifies the embryo. The seed he chose was the coconut. Some of you may be familiar with coconut milk as an ingredient in food, but coconut milks aim of course is to feed the embryonic coconut. So what Stewart did was to take this coconut milk, this embryo food, and take cells from an ordinary common or garden carrot. This carrot of course has been pictured for school children, and so it has eyes and a mouth. Now as you know, carrots don't have eyes and a mouth, so just exclude that. Right? But what - so this is the Steward experiment and you can do it in school if you want. You take a carrot, OK? You bore out a bit of carrot or you can if you want, and Stewart did originally, mash the carrot up until they were just single cells were floating around in a suspension.

And then you place that on a dish with some coconut milk, all right? And the coconut milk regresses the cells as it were, they think they are back in the embryo. And they start to do what an embryo does. First of all, they get very confused, right, and they start growing inco-ordinately. They make what in plant terms we call in plant terms, a callous. But after that, these little lumps of cells, which is all they are, work out what they are meant to be doing, and then they start growing shoots and roots And then you can take these things out of the coconut milk and put them into soil and grow them into new plants. So, technically you can take a single carrot cell from the root of a carrot, or from the leaves if you want, OK? And you can send it back, make it a new embryo and grow a new plant from it. And of course commercially that's a very powerful tool and it's been used extensively in the agriculture of trees. So, rubber plantations

are very often full of trees which all derive from a single tree. And they've been made by this cloning experiment, so they're all genetically identical.

And of course make for much more uniform rubber trees, but it does have one snag. If you have genetically identical trees or plants of any kind, once a disease comes along, which is suited to these trees, it will wipe them all out. Because one of the reasons for genetic diversity is to make sure that when a disease comes along it doesn't wipe everybody out. But of course if we deliberately do away with that genetic diversity, we open ourselves to the danger of disease. So that's the carrot cloning experiment.

Bit more difficult to do it with these things, right? This is the South African clawed toad. All the females in the audience will be very pleased to know this big one is the female, right? Clawed toads have it the right way round. Females are dominant. This is the insignificant looking little male.

Part 5

Right, so how do we do the cloning experiment with toads, and why do we do it with toads? Of course the reason is that toads and frogs have the largest eggs, some of the largest eggs in the mammalian world right? And not only that, but as you know they fertilised those eggs out in open water, and they just float around, don't they? OK, what we call in English frog spawn, or toad spawn. It happens every spring, you find it floating in your pond if you are not careful, and from it will grow toads and frogs. Well, this is a picture of an individual frog egg. So they are easily manipulatable, right? We can pick them up, with tweezers easily enough. You can see them, they are about that size. So easy to manipulate. What these experiments basically did was to take some cells, in fact in this particular experiment, they took them from the padded foot of the frog, and then they grew them in culture and then they took these individual cells and they sucked them into a little pipette , and that broke the cells open. And they injected the cells, essentially the nucleus with a bit of cytoplasm, into an egg, here is the egg, right? which they had UV radiated, so that it had no genes of its own.

UV radiation destroys DNA and DNA is the stuff of genes. So this is an egg, here, which has been UV radiated, and it's got no genes left in it. And now we've put another set of genes in, but this time, from a differentiated cell, from the footpad of the frog. And then we watch what happens to them. And the answer is that some five percent of them start dividing in the way they should and a small proportion of those will grow up to become whole frogs, Now, it doesn't work nearly as well as it does with carrots, not nearly so efficiently, and there are some of these injected eggs which will only partially start dividing some of them which won't divide at all. But basically the proof of the pudding is that some of them do.

Now, for a long time after people discovered that that worked, people tried to make it work with mammals. You can take mammalian eggs and inject things into them but you have to do it under a microscope and it is quite difficult to do. And for years and years they didn't manage to do it. Until someone threw enough money at the problem. As it often the case in science. You not only need to want

to do something, but you need to throw enough money at it. And this was back in the days when people started thinking about genetic manipulation of farm animals, that is adding genes to farm animals. And hey succeeded in doing tha,t but then they found that these farm animals did't breed terribly well. They wanted to propagate these farm animals and they could not do it. So they thought well we will have another try at cloning farm animals. Now no-one would have actually chosen the sheep as a desirable experimental model, because they are not a very desirable experimental model. They are large, they only breed once a year, they cost a lot to keep and things like that. They are very difficult to do surgery on them. But because they had already made these genetically manipulated sheep, and they were desperate to keep them going, they threw an enormous amount of money, millions and millions of pounds, at this problem and they discovered that they could do it.

It was done by a guy called Ian Wilmot in his laboratory in Edinburgh. And basically it is the same experiment as we did with the frogs. Here we have taken, in this case, an unfertilised egg, and we take its nucleus out. We don't kill the nucleus with UV irradiation. We simply take it out ,by sucking it out. And then we add another nucleus, in this case from a differentiated cell, from a mammary cell, from a breast cell. We inject that in, and we can show that there is the nucleus sitting there inside. And then - the vital thing appeared to be to take this cell and put an electric charge through it. Again I have taken this off a website which is not using very technical language. It says they zapped it with electricity. Zap is the noise you make from a gun, you know, well a gun in one of those, you know, electronic shooting studios. So an electronic game-type noise, Zapped with electricity and that makes this nucleus fuse with the cytoplasm, this is the theory. Then you take this and you put it into what we call a *surrogate mother.* You need to put it back because you can't just float it on water and wait for it to divide. You need to put it back in the reproductive tract.

So we took unfertilised eggs and we in-nucleated them with a micro pipette, we took the nucleus out, we added a new nucleus and we fused the eggs and cell with an electrical pulse and after 277 attempts they produced a cloned animal. That's Dolly the sheep, perhaps the most famous sheep.

This is the methodology in real life. This is what a mammalian egg looks like as we saw before. This is the kind of nucleus size, so we have to suck up these nuclei and inject them into the middle of the egg. This thing here is what we call a holding pipette. That's a little glass tube to which we apply light suction in order to hold the egg on.

Part 6

So here is Dolly the sheep, and this is her surrogate mother. And you will notice that the surrogate mother has a black face and black feet and therefore she can't be Dolly's real mother, otherwise Dolly would have a black face and black feet. And his is one of the proofs that Dolly in fact came from the injected nucleus, because the injected nucleus came from a sheep with a white face and white feet. So Dolly comes from the injected nucleus.

Since Dolly, reproductive cloning – I call this reproductive cloning because we make a new animal - has taken place in mice, cats, dogs, horses, cattle, pigs and goats, and if you believe some people, humans. I don't believe them. This is one of the rumours. The interesting thing is the success rate is never greater than 3%, even in the best cases, and there is some evidence, particularly in farm animals, that the clones can be born abnormally large. And this seems to have something to do with the fact that after the cloning process these embryos are grown in culture for a little while before they are placed in the mother sheep. And growing in a petrie dish as it were, artificially like this, seems to have a disturbing effect on some of the control mechanisms which means these grow particularly well. And there are some indications of genetic changes taking place in some cloned animals. So we can do it, but it is not very efficient and it may be dangerous - that's the message.

So that's cloning. Now let's go back to stem cells. We'll get back to cloning again in a minute, you will work out why I am dodging around like this. We know that certain degenerative diseases can be cured by the replacement of organs. So you can have a heart transplant, or a kidney transplant, or a skin transplant. Blood diseases, because the blood derives afresh from those stem cells in your bone marrow all the time, blood cells only have a very limited life. If we have diseases of the blood we can cure that by replacement, either of the differentiated cells, that's a blood transfusion, or replacement of the stem cells that is a bone marrow transplant. We can kill off all our own bone marrow and we can add to bone marrow from somebody else.

Now that somebody else has to be a very close relative, because if not we will get our blood cells starting to attack the body. So we can only have bone marrow transplants, usually from first degree relatives, from parents or brothers and sisters. Now there are other degenerative diseases for instance Parkinson's Disease where people lose some motor control and start to tremble. Diabetes, where your pancreas starts to die and doesn't produce insulin, and muscular dystrophy where your muscles start to waste away. It is conceivable that we could cure these diseases by replacing cells rather than organs. But where would we get these cells from? – that is the problem. These cells would similarly have to be genetically very close to your own genetic make up. Your twins are probably not going to volunteer to have some of their brain cells sucked out, which is what it might need here, or the sort of surgery that would be needed to get muscle cells.

But one very good solution to this replacing cells, might be to replace stem cells. Not the differentiated cells which might not grow after all, but to replace stem cells. So where could the cells for such a therapy come from? They could come, as I said from a tissue match relative, but it would most likely be differentiated cells and they might not grow terribly well. They might come from culture of your own adult stem cells. So let's imagine I could take a piece of my own muscle out, and we could isolate the stem cells from that muscle and grow them up so there were many more of them, and then we could re-implant them so that they could grow new muscle perhaps. But of course if there were any genetic problems with your stem cells that would not work either.

However we could get these stem cells from a culture of genetically identical embryo stem cells. So we then have the problem of how would we make a

genetically identical embryo? Ad the answer to that of course is that we would clone one. So this scenario says what I am going to do. I am going to take any old differentiated cell from my body, I am going to take the nucleus and I am going to inject it into an egg , And I am going to grow from that an embryo. But only as far as that blastocyst stage that we first saw. So that I can extract the embryo stem cells and now I am going to extract those, put them into culture in a petrie dish, and grow them. Then I am going to take those cells and re-inject them into me. So this is described in the literature as *therapeutic cloning*. We're making an embryo that is identical to me let's say, and we are using it's cells to improve my health.

Part 7

So this is the difference between reproductive, that is Dolly- producing, producing a new whole organism and therapeutic cloning. And we've got here a mouse example, all right? So here is my adult cell, differentiated cell, I take the nucleus and I put it into an egg which does not have a nucleus and here is my early embryo. But this time I take my early embryo, I put it into the uterus of a surrogate mother and I get a whole animal, reproductive cloning.

OK, but I can start off in exactly the same way – here is a differentiated cell, here is an egg without a nucleus. I combine the two, I get my early embryo but this time I do not put my early embryo into a surrogate mother. I put these cells onto a petrie dish, I allow them to differentiate and I put these cells back – isn't technology wonderful!And I put them back into an animal to replace damaged cells.

As you see, the top part of the diagram is entirely the same. So in order to do this therapeutic cloning we have to produce a new embryo. So the question can humans be cloned? – the probable answer of course is 'yes'. If you had the time and the money, you could probably, certainly develop the methods, and here is no doubt that the methods could be developed. The reasons why no-one recommends it, at present, and of course it's banned in most countries in the world, is that as I said it has a very low success rate. And since every pregnancy for a human female is potentially life threatening the risk to the child and the mother, if the success rate remains at 3%, that means on average every mother would have to go through 30 pregnancies in order to get a cloned child. Not anything you would look forward to and what most human females do not achieve in their current life times.

And of course the child might be born disabled in some way, we don't know enough about cloned mammals to say that they wouldn't. So my opinion is that the current reports which are largely from a fairly secretive Italian, and possibly the Koreans, they have not been scientifically verified and I don't think they've happened.

There is an important point which in answering this question – 'why is a genetically identical child a good thing?'. Why would you want a genetically identical child? Well, people often say OK if you have lost a child under tragic circumstances, let's say a small child, two, three years old has been lost in a car accident, you know you which thought was your fault or something, you can be

so devastated that it would be comforting to be able, as it were, to recreate that child. The problem with this is, that the child will not be identical. Now you probably know some people who are genetically identical, twins are genetically identical are they not? Yes. And they have the advantage of growing up at the same time in the same household. And even twins are different. If you know some twins, you may even be able to tell them apart. They may look superficially identical but underneath they prefer different music, they wear slightly different clothes, they marry different people which is a good thing. Very often at any rate. So they are not identical. That is because of the influence of the environment on us all. If you took, let us say, cells from a dead child and you tried to recreate it of course you would be bringing it up then, if you were successful, well it would be in the same family, comprised of the same people but they would be, let's say, 5 years older, the parents would be a different age, the world would have moved on. But these parents would have gone through a traumatic event. We are postulating the death of a child and the creation of another one. They would think about their child completely differently from the one that they had originally. They would treat it completely differently. So the environment would be changed. It's most unlikely that they would end up with the same child.

However, people think that this cell replacement therapy is such a good idea, that we should try that. Despite the fact that at the first stage it is exactly the same as reproductive cloning. Because we are producing a new human embryo, and the cells taken from a new embryo can be stored to replace damaged cells later in life as we have said. Research to look at this was given the go-ahead in the UK in 2004. And people have in fact produced the first stage that is the ES cells. But they haven't gone on to go any further as yet.

Now I think, and you may think the same, or you may think different but I think that this creation of a new embryo and then using it as a source of spare parts is, I feel that this is not a good thing. Because in general we think of embryos as having the potential to become whole human beings. This is an embryo which is going to be created *not to* become a whole human being. So it has an entirely different moral status and I don't believe that that's a good thing. I don't know what you think about that. Because by concentrating on this human embryo cell therapy, we are not thinking about developing adult stem cell therapy. I said we could get, these replacement cells by using the stem cells in our own body, OK? And we are not developing that because we think the other way would be easier, but I think the other way is morally problematic, and therefore I'm not particularly happy about it whereas the adult stem cell would have no moral problems because you would be taking your own cells and using them to create new cells for you.

So, we've talked about cloning, creating new organisms. I talked about why that happened and we've looked at whether we might want to do it for humans. I have talked about stem cells and I've shown you why they're important, and we thought a little about how we might use them for therapeutic purposes, for what sorts of diseases. The way that people are, researching at the moment to produce those stem cells involves the creation of new embryos, a cloning again, OK? And I'm a little worried about that, perhaps you are, the American government are so worried about that they won't even let people try and make embryonic stem cells. So, it's a problem, a moral problem and one I'll leave with you. If you have been, thank you for listening.