

Nr. 141 | August 2000

Xenotransplantation

Status and risk assessment for research into transplantation from animals to people

The risk of new diseases stops Dolly's "fathers" >

This week, the Roslin Institute in Scotland, which created the cloned sheep named Dolly, was forced to stop their research in this field. The American owners of the institute are of the opinion that the risks connected with making transplants from animals to humans are too high.

The research continues elsewhere in the world >

But the research is going to continue elsewhere in the world. This week, the cloned pig named Xena was presented to the world in Japan. This event marks the conquest of yet another barrier along the way toward transplanting organs from pigs to human beings.

Cloned pigs signal a breakthrough >

The internal organs of pigs resemble human organs to such a degree that it is anticipated that cloned pigs could be donating organs for transplantation into human beings.

The DBT assesses the risks >

This issue of "From the Board to the Parliament" attempts to provide a survey view of the current ongoing international research in the field of xenotransplantation.

Xenotransplantation is the transfer of living biological tissue from one species to another. Such tissue might consist of single cells, groups of cells or entire organs.

Progress in the field of immunology and genetic technology and also the appearance of new immune-response-inhibitory medicines have given rise to great expectations when it comes to xenotransplantation. Over the course of the last twenty years, at least 200 attempts at xenotransplantation with different kinds of living tissue from pigs are known. Moreover, for many years now, a large number of patients have been receiving dead tissue from animals, in such forms as bones and heart valves. Every year in Denmark, somewhere between 100 and 120 cardiac care patients receive heart valves removed from calves.

In contrast to earlier times, when efforts were centered primarily on transplanting entire organs, most of the attempts with xenotransplantation over the course of the past 20 years have been attempts to transplant *groups of cells* from animals to people. Nonetheless, there have also been attempts at transplanting entire organs.

General risks

Just like transplantation of organs from one person to another, a process known as allotransplantation, xenotransplantation contains a risk of transferring illness from the donor to the recipient.

When organs are transplanted from animals to people, however, there is also a risk of transferring viruses or of transferring a predisposition for viruses that might provoke new contagious diseases among human beings. Donor animals can be checked for

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the viruses that we know about, but viruses are known for being able to alter their capacity for provoking disease – their virulence. For example, certain monkeys carry a virus that is akin to herpes simplex, which causes them no harm to the monkeys other than that they sometimes break out in sores. In human beings, however, this virus provokes a particularly virulent cerebrospinal meningitis. A further risk is that hitherto unknown viruses can prove to be dangerous to people.

In this connection, researchers are focusing a considerable degree of attention on the so-called endogenous retroviruses. These are remnants of viruses which, in the course of evolution, have implanted themselves inside the genes of mammals. Inside their ordinary hosts, they do not ordinarily bring forth illness. But they *can* do so when they come in close contact with another species – as in the case of being transferred through xenotransplantation.

As of today, there are four groups of retrovirus in pigs. However, there might be more that we do not know about. Two of the four groups have shown themselves to be capable of forming viruses. And the viruses thus formed in laboratory experiments have shown themselves to be capable of penetrating their way into human cells.

One example from recent days of a virus that has migrated from animals to people is HIV. Another example is the worldwide Spanish flu of 1918-19, which destroyed the lives of around 20 million people and was thought to have originated from pigs. In the spring of last year, an epidemic of fatal encephalitis broke out in Malaysia, 100 kilometers from the capital city of Kuala Lumpur. The disease was kindled by a virus borne by the local pigs, which later came to be known as the Nipah-virus.

1. Nobody can ascertain with absolute certainty the extent to which viruses constitute a risk when organs are transplanted from animals to people. The general circumstances surrounding xenotransplantation which spell out good reasons for taking the problems very seriously can be summarized in the following points:
2. In the process of xenotransplantation, the physical barriers constituted by our skin and mucous membranes are broken down, consequently rendering possible a transfer of viruses that would have otherwise *not* been capable of penetrating their way into the human body.
3. The immune response system of virtually all transplant patients has already been weakened by medicines beforehand, be-

cause the administration of these medicines diminishes the risk of rejecting the transplanted organ. This entails that infection which would routinely be fought off under ordinary circumstances can flourish and spread inside the body of the patient.

4. The transplanted organ can contain "dormant" viruses in the form of retroviruses, which can lie hidden in the donor's DNA. Therefore, it is not only the known active viruses that can give rise to problems, but also unknown viruses which might have been dwelling within one species' DNA for hundreds of years, which can be activated when they are transferred from one species to another.
5. Viruses which do not behave as pathogenic in the donor-species can develop into pathogenic viruses in the recipient-species. Furthermore, new and hitherto unknown viruses are constantly being discovered. This entails that there can be no guarantees that the donor animal is free of viruses that might have a tendency to generate disease in the body that receives the organ.

Two types of risk

It is instructive to make a distinction between the individual and the societal risks of xenotransplantation. Frequently, these will be mutually at variance. For example, the individual patient runs the risk of contracting a viral infection through the transplantation. But the patient's alternative will often be to die or to live on with an extremely extensive course of treatment. For this reason, the patient has much to gain in obtaining an organ from an animal. The societal risk, however, is that through the use of the animal organ in the transplantation, a new contagious disease might be developed, one which society is not immediately prepared to tackle. The focus on this issue of *From the Board to Parliament* is the *societal risk* of xenotransplantation.

The following assessment of the societal risk has been advanced in cooperation with, among others: Professor Peter Collignon, Infectious Diseases Physician and Microbiologist, Canberra Clinical School, Australia; Robin A. Weiss, professor of Viral Oncology, Wohl Virion Centre, London and Jonathan S. Allan, D.V.M, Southwest Foundation for Biomedical Research, Department of Virology and Immunology, San Antonio, Texas.

Since the risk presented by xenotransplantation varies according to the character of the intervention, we have chosen to classify the different types of xenotransplantation into six categories. In what

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Xenotransplantation's dilemma:

The greater the success, the greater the risk.

Today, the pig is the animal which is primarily thought to be playing the role of organ supplier. But before the pig's organs can be put to use, it is necessary to find methods of surmounting disparities between pigs' and human beings' immune systems. The greatest barrier is that people carry around antibodies against a certain carbohydrate, alpha-galactose, which is found on the surface of most other animal's cells. Alpha-galactose is also present on the surface of pigs' cells. It is regarded as being the most important reason that a so-called hyper-acute rejection occurs when, for example, a monkey is supplied with an organ taken from a pig. The foreign organ is immediately recognized as being foreign and in a matter of minutes, or at most a few hours, a violent bleeding takes place in and around the organ, which is then destroyed and rejected. In order to counter this rejection, a great many resources are being set aside these years in order to produce so-called *transgenic* animals. These are animals that have been supplied with human genes through the technique of gene-splicing.

The dilemma is, however, that the more successful we are in fabricating organs from transgenic pigs which do not call forth this hyper-acute rejection, the greater the risk there is of transferring viruses through transplantation. Among other reasons for this is the fact that the genetic modification of the pigs, which is necessary for evading the circumstances that trigger hyper-acute rejection, simultaneously entails that the human immune system has a harder time recognizing and consequently fighting off viruses in the transplanted organ.

Along with the fabrication of transgenic pigs, another effect comes into existence which can potentially play a part in advancing infections that cross the barriers between species. In transgenic pigs, human cell receptors which can render the transplanted organ susceptible to certain human viruses are manifested. Transgenic pigs can, for example, develop a susceptibility to the measles virus. And conversely, there is also the possibility that pig viruses inside these pigs can adapt themselves to human virus receptors which are not found in ordinary pigs and are consequently capable of multiplying themselves in human tissue. Experimental results indicate that viruses which have developed inside transgenic pigs do become adaptable, with the consequence that they can more easily survive and infect human organ recipients.

Some scientists argue that, from an evolutionary point of view, what human beings have lost is precisely the alpha-galactose, **because the very absence of this carbohydrate** is what shields people against viruses from other species.

follows, we will take stock of the research in each category. We will also be assessing the risks connected with the implementation of transplantation on each level. The division into the six categories is linked up with a classification previously suggested by a panel of experts working under the auspices of BIOSAM. For our purposes, however, the classification has been altered in certain aspects.

The six categories are as follows:

1. Smaller-scale transference of tissue (heart valves, corneas, skin, etc.)
2. Cell transplantation of small quantities of non-gene-spliced cells, such as nerve cells used in the treatment of Parkinson's disease.
3. Cell transplantation of larger quantities of non-gene-spliced cells. For example, in the treatment of patients with diabetes, where pancreatic cells from pigs can be utilized.
4. Cell transplantation of gene-spliced-cells.
5. Links between the patient's blood vessels and animal organs, which are however maintained *external to* the patient's body. For example, the liver of a pig might be temporarily employed for replacing the liver functions in the treatment of patients suffering from an extreme form of liver disease.
6. Actual transplantation of organs.

Concerning the 1st category: Slight transference of tissue, such as heart valves removed from pigs and calves, is already being carried out on a wide scale. The risk of having transferred contagious infection from this type of material has been assessed as being very small, since heart valves are *not* composed of living tissue and can be sterilized and used in much the same way as implants of non-animal materials. In Denmark, somewhere between 100 and 120 transplantations with heart valves from calves are carried out every year.

The skin of pigs can be used to heal patients whose skin has been burned. The risk of using pigs' skin is assessed as being much greater than any risks connected with the transfer of heart valves, because in this instance we are dealing with *living tissue*. Furthermore, the risk of transferring viruses is assessed as being greater in direct proportion to the amount of animal material with which the person's system comes in contact. The risk connected with using

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pigs' skin in the healing of burns, however, is assessed as being less than that associated with the transplantation of organs into the body. This is because in the event of a skin transplant, there are more physical barriers between the transplanted tissue and the rest of the body than, for example, a case where surgeons insert a heart, which is directly linked up with the body's circulation. In Denmark, pigs' skin was formerly used for patients suffering from burn-related injuries. But about 15 years ago, this practice was largely abandoned. Now, human skin is used instead. There is no immediately accessible material in the form of a continuous observation of the consequences connected with transplanting pigs' skin to human beings.

Concerning the 2nd category: When relatively small quantities of non-gene-spliced cells are transplanted, the risk of transferring a virus is greater than in the latter case. In these kinds of procedures, living cells are being inserted into a region that is relatively shielded off from the human immune system. This makes it difficult for the immune system to fight off any development of a virus that may arise. In the United States, experiments have been carried out with transplanting cells from pig fetuses into the bodies of patients suffering from Parkinson's disease, Huntington's disease and some patients suffering epilepsy. Research of this kind is also going on in Lund, in Sweden.

Concerning the 3rd category: Generally speaking, the more animal material that is used in the procedure, the greater are the risks associated with xenotransplantation. On this account, the risks increase with this category. On an international plane, a great deal of research into the transplantation of large quantities of cells is being carried out.

In the beginning of the 1990s, ten diabetes patients at Huddinge Hospital in Sweden underwent transplantation with non-encapsulated insulin-producing **cell-islands** which had been isolated in pig embryos. However, the production of insulin proved to be insufficient for reducing the patients' needs for having insulin supplied externally.

In New Zealand, encapsulated **cell-islands** removed from newborn pigs were transplanted into six diabetes patients. The results of these experiments are not known at this time.

In Russia, **cell-islands** from the pancreases of rabbits have been transplanted. No more about the results of these experiments is known at the present time.

In China, transplantations have been carried out on a large number of patients using tissue removed from the pancreases of calf- and pig-fetuses. But the

results of these procedures have not yet been reported internationally.

In the United States, Poland, the Czech Republic and Switzerland, one American company working in cooperation with Astra has been making clinical investigations where attempts are being made to alleviate severe pains experienced by cancer patients by using hormone-producing cells taken from calves and introducing them through the spinal canal. The cells are encapsulated in order to avoid being disturbed by the immune response system. These experiments have been carried out on more than 100 patients.

Concerning the 4th category: The risk of transferring viruses when gene-spliced cells are transplanted is greater than the risk when non-gene-spliced cells are transplanted. This is because gene-spliced cells have been altered in such a way that they *resemble* human cells. This entails that the human immune system has a more difficult time recognizing and consequently fighting back any viruses that might originate from genetically manipulated cells (cf. the box on the next page).

In Switzerland, a research project is now underway, the aim of which is to treat amyotrophic lateral sclerosis (ALS, aka Lou Gehrig's disease) with the transplantation of encapsulated gene-spliced hamsters' cells. Experiments have also been made with the transplantation of gene-spliced cells among different species of animals.

Concerning the 5th category: The use of entire organs of cells which are, however, maintained external to the patient's body and used in procedures such as dialysis is yet another kind of xenotransplantation. The risk associated with this form of xenotransplantation is assessed as being less than with the transplantation of organs or cells which are inserted *into* the body, since it is possible to make use of a membrane which is placed in between the organ and the human body. A membrane can offer a certain protection against the transference of viruses because it can stop entire cells from getting into a person's circulatory system. However, the larger the pores of the membrane, the greater the risk that viruses can penetrate their way through. And at the same time, there will always be a risk that the membrane will fall apart.

In a few instances in the United States, an entire liver removed from a pig has been used temporarily to replace the liver's function in patients suffering intensely from liver disease for the period of time that they were waiting for a transplant from another human being to become available. The blood from the patients is pumped through a pig's liver, which has been kept outside of the body. This set-up

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is known as 'extra-corporeal perfusion'. In some cases, the liver of a gene-spliced pig has been used.

Another line of development is pumping blood through a liver-dialysis apparatus which contains living liver cells taken from a pig. The American firm, Circe Biomedical, has developed such a liver-dialysis machine. Inside the machine, the patient's plasma is conveyed past encapsulated liver cells taken from a pig. Experiments are being carried out in the United States, Belgium, Germany (Hamburg), Italy, Spain and Holland. Recently, a number of similar experiments made with small patient groups have been reported in the United States. In some instances, the experiments were carried out using the organ of a gene-spliced pig.

In 1990, there was an experiment in England where a patient was set up with extra-corporeal perfusion using pig's kidneys. The patient, suffering chronic dialysis, was treated preliminarily by having **his/her** blood cleansed of antibodies. Thereafter, the patient's blood was made to circulate through a pig's kidney for six hours without showing any signs of rejection. In 1995, the experimental procedure was repeated in Gothenburg, Sweden, on two patients whose blood was similarly cleansed of antibodies, but who had not been given beforehand any immune-response-inhibitory medications. In the first of these two procedures, there was a rapid and intense rejection. The second patient reacted after fifteen minutes, with signs of anaphylactic shock. The procedure was terminated and the patient was quickly restored. The aim of the Gothenburg group experiment was to compare the results connected with kidneys taken from ordinary pigs with kidneys taken from gene-spliced pigs. However, the latter experimental procedure has not yet been carried out.

Concerning the 6th category: The greatest risk by far associated with xenotransplantation arises with the transplantation of entire organs, and especially when the organs are genetically manipulated to resemble human organs.

In the 1960s, transplantations were carried out with kidneys and hearts removed from chimpanzees, baboons and rhesus monkeys. The patients were treated with the day's immune-response-inhibitory preparations which, when used in quantities sufficient for eliciting the desired effect, kindled rather violent side effects. The individual attempts with heart transplantations were all unsuccessful. However one of the kidney-transplanted patients lived for some weeks and another one survived for nine months.

In 1985, a transplantation of a baboon's heart was carried out on a girl born prematurely, who was named Baby Fae. The transplantation was techni-

Need for organs is increasing

The wish to transplant organs from animals to people has come into being largely because the supply of ordinary sources for transplantation organs is not sufficient for meeting the demands. The use of organs from recently deceased or brain-dead people often runs into problems on account of objections from the nearest relatives. In Denmark, and also in the rest of the world, there has not been a great deal of success in increasing the amount of assents to using organs from deceased or brain-dead people, despite a number of committed information campaigns widely disseminated among the population.

Every year, for every one million citizens in the Western world, about one hundred contract a chronic kidney ailment that is so serious that it calls for dialysis or transplantation. In 1998, it was necessary to have 587 people in Denmark on dialysis. In addition to this, around five percent of the already transplanted patients can be expected to develop chronic kidney malfunction in the new organ and will be needing a new transplantation. All in all, estimates run that at the end of 1998, there were around 700 Danes waiting for a kidney transplantation. In the year 1998, one hundred and forty-five kidneys were transplanted in Denmark.

cally successful. However, despite the administration of modern immune-response-inhibitory preparations, the girl survived for only twenty days. The transplantation was surrounded with a great deal of attention in the media and led to an intensive debate on ethics. No further experiments with the xenotransplantation of hearts has been carried out in the United States.

In 1992, in Poland, a heart was transplanted from a pig to a human being, who survived for 24 hours. Therefore, delaying the hyper-acute rejection was actually *successful*. The explanation for this is that prior to the transplantation, the patient's antibodies against the pigs' cells were reduced.

In Pittsburgh, in 1992-93, two attempts with transplanting livers removed from baboons and transferring them into the bodies of persons were carried out. The two patients survived, respectively, for 26 and 70 days, at which time they perished, due to infection complications. Even though permission had been granted to carry out three additional transplantations, the choice was made to desist from doing so until further pre-clinical research could be carried out.

As of yet, no experiments have been made with transplanting entire organs from transgenic pigs. Just last week, the people who stood behind the financing of one of the leading projects in the devel-

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opment of transgenic pigs, The Roslin Institute, who among other accomplishments managed to create Dolly and who also stood behind the creation of five cloned pigs in March of this year, decided to cut off the financing of the project on account of the current assessment that the risk of transferring new kinds of disease to human beings is too great.

The United States, England and Sweden are held to be the three nations which have proceeded the furthest in the field of xenotransplantation.

Alternatives to xenotransplantation

Today, research is being made into the artificial cultivation of tissue as a replacement for damaged cells in the body. If human tissue and organs could be cultivated on the basis of one cell taken from a sick individual, it might be possible to vanquish one of the great problems connected with the transplantation from other people and from animals, namely the problem of *rejection* of the foreign tissue. This is because the cells are cloned; consequently they are genetically identical with the cells of the patient, where they are being put to use.

Should it become possible to cultivate human organs from embryonic stem cells, which are human embryonic cells that have not yet managed to specialize themselves in performing any one specific task inside the body, another possible source for transplantations will present itself.

These methods will not be discussed further in the present issue. However, the Danish Board of Technology has just recently published 'From the Board to the Parliament', no. 140 which deals with the subject of cloning.

Legislation in Denmark

As of today, there is no legislation in Denmark that specifically regulates xenotransplantation. Experimental procedures in this area are subject to the ordinary approval procedures for all medical experiments.

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www.tekno.dk

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The latest five issues of "From the Board to the Parliament" are entitled:

140: Alternatives to cloning

139: Uncertain gains for net-municipalities

138: The nation would do well to stake its bets on urban ecology

137: Ssshhh ... you're making a racket! – on the Technology Board's project about noise

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136: Gene-spliced foodstuffs

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