ANIMAL ORGANS IN HUMANS:

uncalculated risks and unanswered questions

by
Dr Gill Langley and Joyce D'Silva

A report produced jointly by

British Union for the Abolition of Vivisection
and
Compassion in World Farming

October 1998

"Twelve voices were shouting in anger, and they were all alike. No question, now, what had happened to the faces of the pigs. The creatures outside looked from pig to man, and from man to pig, and from pig to man again: but already it was impossible to say which was which."

George Orwell, Animal Farm, 1945.

ISBN: 1 870356 21 7

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The organisations

Compassion in World Farming (CIWF)

Compassion in World Farming (CIWF) is the leading UK organisation campaigning for the welfare of farm animals.

CIWF campaigns have led to UK bans on cruel rearing systems such as the keeping of veal calves in narrow crates and the confinement of pregnant sows in stalls and tethers. In 1997 animals were formally recognised as "sentient beings" in the Amsterdam Treaty, largely as a result of a 10-year campaign by CIWF.

CIWF campaigns against genetic engineering of farm animals, believing it poses new threats to their welfare.

British Union for the Abolition of Vivisection (BUAV)

Founded in 1898, the British Union for the Abolition of Vivisection (BUAV) is Britain's leading organisation campaigning to end animal experiments.

BUAV campaigns have resulted in a ban on animal testing of finished cosmetic products and a virtual ban on the use of wild-caught primates for research. The BUAV has carried out undercover investigations which have revealed illegal practices in research laboratories and is leading efforts to obtain improvements to and effective implementation of UK and European law on animal experimentation. It has also campaigned consistently against animal genetic engineering, patenting, xenotransplantation and cloning.

Contents

1. Introduction

This report, produced jointly by the British Union for the Abolition of Vivisection and Compassion in World Farming, fills a number of significant gaps in the current debate about xenotransplantation.

Most xenotransplantation research has focused on overcoming the first cross-species barrier: hyperacute rejection of an animal organ within minutes or hours of it being placed in the human body. While in time this may be achieved, strategies to deal with acute vascular rejection, and cellular and humoral reactions, have not yet been perfected.

The problem of infectious risks to individual and public health remains unsolved and this report deals with the latest developments in understanding these risks.

Still, there are some critical questions about xenotransplantation which have not been acknowledged, let alone answered. This report asks those questions. The first is, even if the human body can be persuaded to accept a pig or baboon organ, will that organ be capable of sustaining human life and health?

There are innumerable species differences – physiological, biochemical and pharmacological – which could lead to fatal mismatches between a transplanted animal organ and a human patient. Knowledge of these is scattered rather sparsely across many different scientific disciplines and has not been drawn together in any one place.

Few scientists have acknowledged openly that we simply do not know if animal organs will be capable of supporting human health in the long term. This central question can no longer be avoided, but the answers cannot be definitively obtained from experiments on other animal species. This report spells out in detail the likely species incompatibilities for each organ currently being considered for xenotransplantation and what this means for the first human guinea pigs. For reasons of brevity, the report does not deal with transplantation of animal cells and tissues into humans, but only whole organs.

Another major issue has not been aired in any forum to date. This is the condition of chimerism which inevitably follows the transplant of an animal organ into a human. Animal cells are not contained solely within the organ, but migrate throughout the patient's body. Moreover an animal liver, kidney or lung will produce animal rather than human proteins and other factors, which circulate widely. Xenotransplant patients will be living embodiments of the mythical concept of chimerism, a hybrid of different species.

Chimerism obviously has physical consequences, but it will also have profound psychological and emotional effects. It will challenge a patient's sense of self and impact the way they are viewed by others – yet the existence of this condition has not even been made clear to the public.

The acceptability of xenotransplants to the public is still uncertain. The European Commission's Eurobarometer survey of 1996, which interviewed 16,246 people from all EU countries, showed that overall attitudes to xenotransplantation were negative: although it was seen as possibly useful, it was also perceived as a very risky and morally unacceptable technology which was to be discouraged [1].

In this report we also summarise the ethical and welfare issues concerning experiments on animals for xenotransplant research and their possible use as source animals for organs. Both these aspects are responsible for much pain and distress caused to many animals. We prefer the term "source

animals" to "donor animals", because animals do not choose to donate their organs for xenotransplantation.

Both CIWF and the BUAV are opposed to animal research for xenotransplantation, as well as the use of animals as a source of organs for transplantation. We believe that animals are entitled to respect and compassion, which such exploitation denies them.

An extensive public information and consultation programme is urgently needed to determine whether society is willing to accept the risks posed by xenotransplantation. This report contributes to that process. There is little point in pursuing a technology which the public rejects, particularly when any benefits will accrue only to the individual but the public will share the risks. We hope that plans by the Council of Europe to facilitate a Europe-wide public debate will come to fruition.

Despite the many critical shortfalls in scientific understanding and public policy development, some companies have lobbied extensively to try this novel technology in humans sooner rather than later. For example Imutran, a British company of the multinational Novartis Pharma group, announced as early as 1995 that it was ready to start clinical trials. In countries like the USA, sporadic experiments in animal-to-human organ transplants have been underway for several years, but success measured by patient survival has been very poor.

Commercial pressure has also resulted in undue weight being given to xenotransplantation as a potential solution to human organ shortages, in comparison with other options – to increase the supply of human organs, to complete clinical trials of artificial organs, and to prioritise research into prevention and treatment of disease.

Presumably because of the commercial need for confidentiality, many aspects of xenotransplant research have not been published and therefore have not been subjected to full scrutiny by the scientific and animal welfare communities. For an entirely novel field of medicine with unprecedented implications for public health and animal welfare, this is unacceptable.

It is not widely realised that xenotransplantation is still highly experimental. Twenty-five per cent of patients who receive a *human* heart die within a year, and half of those who receive a *human* lung do not survive two years. These less-than-perfect survival rates are built on more than 30 years of clinical experience and a century of animal experimentation.

This puts in perspective claims that successful animal-to-human transplants are imminent. In fact some artificial organs, such as the left ventricular assist device, are further advanced than xenotransplantation. It may take decades of research with laboratory animals and with human patients, before xenotransplantation is routinely successful – if ever.

2. SUMMARY OF CONCLUSIONS AND RECOMMENDATIONS

1. The use of primates or other animals as experimental recipients of xenotransplants has caused massive suffering, and we call on the UK government to halt this research (Section 10).

2. Instead, resources could be directed into improving the supply of human organs, continuing clinical trials with artificial and bioengineered organs and researching humanely the causes, diagnosis, prevention and treatment of the major diseases which create the need for organ transplantation (Section 10).

3. The production of primates and transgenic pigs as source animals for organs will involve much ongoing pain and distress. We believe that the suffering experienced by animals in surgical embryo retrieval and embryo transfer, and the specific pathogen-free method of birth followed by rearing of animals in isolators and biosecure rooms, cannot be justified. We therefore call for the xenotransplantation programme to be stopped forthwith (Section 10).

4. Failing a halt to the xenotransplantation research and production programme, and since the wider population will be put at risk of disease should a xenotransplant patient become infectious, a moratorium on clinical trials should exist at least until there has been an extensive programme of public education and consultation (Section 3).

5. A clear and logical harm-benefit threshold for human trials should be discussed and established if human xenotransplantation research is to proceed. Practical issues regarding informed consent, accountability and insurance would also need to be clarified (Section 3).

6. A national system of virus discovery, detection and diagnostics, for application to source animals as well as patients and their close contacts, is not in place. Its development would require considerable research (Section 3).

7. At present, knowledge is inadequate to permit a scientifically based decision to be made about which animal viruses pose the greatest risk, and which the least, to individual and public health (Section 3).

8. While the implications of numerous species differences in physiology, biochemistry and pharmacology remain unknown, it would be foolhardy to proceed with clinical trials of animal organs in humans (Section 4).

9. A programme of *in vitro* research should first be conducted to gain more knowledge of which cross-species mismatches will be most significant to the health and well-being of patients (Section 4).

10. Therapeutic strategies to ameliorate the significant functional incompatibilities would need to be devised in advance of clinical trials (Section 4).

11. Chimerism is an issue which must be addressed fully and urgently by regulatory bodies. As general awareness of this consequence of xenotransplantation is very low, it is important that the wider public consultations which we recommend include the implications of chimerism (Section 9).

12. With the public continuing to be divided on the acceptability of xenotransplantation, a full and open consultation is urgently needed on the ethical issues arising from the use of animals both in xenotransplantation research and as sources of organs for clinical trials (Section 10).

13. We agree with the UK Advisory Group on the Ethics of Xenotransplantation (the Kennedy committee) that primates should not be used as source animals for organs for xenotransplantation. However, we believe that there is no scientific or empirical reason for distinguishing between primates and pigs on the basis of their capacity to suffer (Section 10).

14. A statistical breakdown of all transgenic pigs produced in the UK for xenotransplantation research should be published by the government. This should include the number and proportions of heterozygous and homozygous transgenic pigs produced, together with a detailed analysis of their health records, as well as the failure rate for micro-injected embryos. These data would allow

a proper understanding of the adverse welfare effects associated with the genetic engineering of pigs (Section 10).

3. Infectious risks to patients and the public

Seldom, if ever, have we had as much knowledge to prevent a future epidemic. What is lacking is the wisdom to act upon that knowledge.

Dr Jonathan Allan,
Department of Virology & Immunology, Southwest Foundation for Biomedical Research, USA.[2]

3.1 The main issues

Xenotransplantation creates risks of transferring known and unknown animal viruses to humans, as well as the emergence of completely new infections. These risks are real and not hypothetical. What is unknown is their magnitude.

It has always been understood that patients who volunteer for new treatments or surgical operations may experience side effects of unexpected type or severity. Few medical interventions carry no risk at all. The significant difference with xenotransplantation is the higher likelihood of infectious disease, possibly of a previously unknown kind, spreading to the wider population from the patient. Major new epidemics cannot be ruled out. This makes xenotransplantation a significant public health issue, not just one of personal risk.

Many classes of pathogenic viruses could infect a xenotransplant patient, including adenoviruses, filoviruses, papovaviruses, papillomaviruses, parvoviruses, hepadnaviruses, morbilliviruses, hantaviruses, arenaviruses, arteriviruses, flaviviruses and togaviruses. It is not easy to discover which specific viruses from these families would pose a risk to public health.

Viruses that do not normally infect humans may become pathogenic if they are placed directly within the human body via a transplanted organ, since all the normal barriers posed by the skin, the stomach's acidity or the defences of the lung airways, have been avoided.

It is now known that cells from a grafted human or animal organ migrate throughout the body of a transplant patient. This migration of cells provides an opportunity for viral infection to spread too, and indeed two patients who received baboon liver transplants had persistent baboon viruses as well as baboon cells throughout their bodies.[3]

If transgenic animals are used, there is the possibility that a virus could make use of the new genes to disguise itself from the patient's immune system,[4] which will anyway be suppressed by drug treatment to prevent organ rejection. To make matters worse, the organs of some transgenic pigs are being altered to stifle the bloodborne factors called complement, normally responsible for immediate rejection of a pig organ. Complement also usually provides a defence against virus infection, but in such a case this defence would be suppressed.

Retroviruses – including endogenous retroviruses, type C and D retroviruses, bovine leukaemia-type viruses and lentiviruses – are a particular risk. The DNA of endogenous retroviruses can hide undetected within the genes of the animal, sometimes lying silently for long periods of time and not actually causing disease. They are inherited by offspring, so that it may be impossible to breed virus-free animals. When transferred into an unfamiliar host, such as a human patient, retroviruses can be activated and cause disease.

According to Dr Jonathan Stoye of the British National Institute for Medical Research and Dr John Coffin of Tufts University School of Medicine, USA, implanting an organ carrying a dormant endogenous retrovirus into a patient is equivalent to injecting the patient with live virus.[5]

When transferred to a different species (such as a human), viruses may also recombine genetically with a virus already present in the human body, leading to the formation of a novel strain.

According to Dr Ian McConnell, Department of Clinical Veterinary Medicine, University of Cambridge,[6]

"Some of the most successful viruses (eg. pox viruses) survive largely on account of the fact that they have expropriated human genes and incorporated them into their own structure to resist defence mechanisms... The transplanted organ is a reservoir of pig DNA waiting for recombination to happen, resulting in potential new viruses of unknown infectivity."

Many flu epidemics have begun this way, probably including the 1918 pandemic which killed some 30 million people, where pigs are thought to have acted as a "mixing vessel" for bird and human flu viruses, creating new, lethal strains.

It is well established that most emerging human infections originated in other species. Some recent examples in humans and other animals include:

- About 30 laboratory workers in Yugoslavia and Germany developed haemorrhagic fever and were killed by the Marburg virus after exposure to infected monkeys.
- More than a third of the Serengeti's lion population has been wiped out by canine distemper virus. Until now this virus was always restricted to canine species, but has inexplicably infected a cat population.
- The BSE/CJD crisis is believed to have arisen when an infectious agent passed originally from sheep to cows and then to humans.

- The Ebola virus, which spreads from primates to people, has caused the deaths of hundreds of people in Africa.
- The re-emergence of dengue fever in Texas is thought to have originated from non-human hosts.

The greatest fear is that an unexpected infection may develop in a human patient, and then spread to other people. The worst scenario, of an infectious epidemic for which there is no vaccine or cure, spreading through the human population, cannot be ruled out. AIDS almost certainly arose in this way: by transfer of the virus from primates to humans and thence around the world, causing the current epidemic of an estimated 4.5 million cases.[7]

Dr Louisa Chapman, an epidemiologist at the US Centres for Disease Control and Prevention, was quoted in the *New Scientist* as saying:
"Given that [AIDS may have come from primates], *you can't dismiss out of hand that using animal tissues in humans may be a very effective way to introduce another equivalent infection"*.[8]

Other recent examples of animal viruses infecting humans and then spreading from person to person for the first time include a hantavirus outbreak in Argentina in December 1996. The outbreak was reportedly the first which involved person-to-person transmission of the hantavirus. Normally the flu-like illness, which can be fatal, is spread only by contact with rodents, but in this

case the route of infection went from the first patient to his mother, his two doctors, another patient in the hospital and that patient's wife.

Similarly, 1996 saw the first cases of monkeypox being spread directly among people, rather than solely from rodents to humans, in Zaire. The number of cases of infection, which can be fatal, shot up from fewer than eight per year in the 1980s, to 163 cases in just one Zairean province in 1996.

Methods in common use to detect and diagnose viruses are not necessarily sensitive or specific enough for xenotransplantation purposes. According to Dr Frederick Murphy, professor of virology at the University of California, Davis, this is partly due to "extreme compartmentalization of diagnostics technology and shortcomings in technology transfer and training".[9]

3.2 Infectious risks from pigs

In conclusion, our studies indicate that the potential for porcine endogenous viruses to transfer from donor tissue to the human host is more plausible than a fanciful scare story.

Dr Clive Patience, Dr Yasuhiro and Dr Robin Weiss, of the Institute of Cancer Research, London.[10]

Although humans and pigs have co-existed for centuries, in the situation of a pig's organ being placed directly within the body of an immunocompromised human patient, pathogens can more easily be transferred. Pigs can carry bacterial, viral, fungal, protozoal and helminth pathogens and presently there is no assurance that they are free of prion diseases. Diseases which are known to be transmissible from pigs to humans include brucellosis, leptospirosis, listeriosis, anthrax, erysipelas, campylobacteriosis, equine encephalitis, all strains of influenza, herpes, toxoplasmosis and ascariasis.[11]

In the last decade alone, three new human herpesviruses have been discovered. No-one knows how many strains of herpes pigs harbour, and herpesviruses remain inactive for long periods of time, but can cause severe disease in a different species. Only a proportion of viruses has ever been identified, and many undiscovered strains exist.

Some pathogens can be screened for, but even raising pigs in germ-free conditions will not eliminate unknown endogenous retroviruses lying hidden in an animal's genes. Pigs can be raised free of certain specified viruses (and other pathogens), but not free of *all* viruses.

In 1997, British researchers reported that a pig C-type endogenous retrovirus called PERV-PK is capable of infecting and replicating in human cells in the test tube.[12] They found PERV in the hearts, spleens and kidneys of ordinary pigs, and estimated that there are about 50 copies of the viral DNA in each pig cell. These would be extremely difficult to remove.

The authors stated that if PERV-PK or a similar virus replicated in an immunosuppressed human patient, unwanted effects including cancer could not be ruled out – even if the virus caused no disease in pigs.

Subsequently, two related but distinct classes of PERV (A and B) were identified.[13] Both are capable of infecting human cells and both are found in and inherited by several breeds of pigs. The viruses are expected to be present in pigs which have been genetically engineered for xenotransplantation purposes.

An American study, reported in 1998, found another porcine endogenous retrovirus, PERV MSL, embedded in the genes of white blood cells of miniature pigs.[14]

Normally humans would be protected against C-type retroviruses of other animals by complement factors in the bloodstream. Complement usually attacks such viruses, but animals being developed as organ sources may be genetically engineered to suppress the activity of human complement, in order to prevent hyperacute rejection of the organ. In such a situation a pig virus, unhindered by complement and in the body of a patient with a compromised immune system, would have the opportunity to replicate (actively multiply) and spread.

The British study also showed that, once inside human cells and replicating, PERV became almost completely resistant to attack from complement. This means that any PERV released from a pig organ during surgery, which then penetrated nearby human tissues, would have the chance to adapt to human-to-human transmission – the nightmare scenario of xenotransplantation.

The British findings led the American Food and Drug Administration to put a temporary stop on pig-to-human transplants, but this was lifted after reviews of patients who had undergone exposure to living pig tissues. Confidence in the wisdom of the British company Imutran had not been bolstered by the fact that it first announced imminent human trials with transgenic pig organs in September 1995. Not until 1998 did it announce a study of patients who have already been exposed to living pig tissues, to see whether they had acquired presently detectable pig viruses.

A review of some 150 patients was launched and in August 1998 researchers announced that no pig virus DNA, or antibodies to pig viruses, had been found in the blood of 10 Swedish patients who had received transplants of pig pancreas tissue. Similarly negative findings were reported for 24 patients who had had transplants of pig fetal brain cells and for 25 traceable patients whose blood had been circulated, outside their body, through pig liver cells.[15]

While these results were seen by the biotechnology industry as encouraging, Dr Jonathan Allan, a virologist of the Southwest Foundation for Biomedical Research, USA, and Dr Robin Weiss of London's Institute of Cancer Research, were not really reassured *(op. cit.)*. They pointed out that the findings may not be strictly applicable to the higher-risk situation of whole organ transplantation.

An entire animal organ is a larger reservoir of viruses; it may be genetically engineered to suppress human complement defences and the patient's immune system would be damped down by drugs. Additionally, negative results cannot be a complete proof: they may indicate an absence of viruses, but they may also merely reflect a failure to detect them.

3.3 Infectious risks from primates

Retrospective analysis of tissues from two human transplant patients with end-stage hepatic disease who died 70 and 27 days after the transplantation of baboon livers revealed the presence of two simian retroviruses of baboon origin, simian foamy virus (SFV) and baboon endogenous retrovirus (BaEV), in multiple tissue compartments.

Dr Jonathan Allan and colleagues, of the Southwest Foundation for Biomedical Research, USA.[16]

Many scientists believe that the infectious risks posed by the use of organs from baboons or other monkeys are too great to consider using them as source animals for xenotransplantation. It seems likely that for this, as well as for ethical reasons, the UK may not approve the setting up of baboon colonies for routine xenotransplant purposes. However, in the USA and some other countries, baboons have already been used as source animals and are still under consideration for future use on a routine basis.

Screening primates for known pathogens would reduce but not eliminate the risk. There are no existing colonies of pathogen-free baboons and possibly too few individuals of the required health status even to set up such a colony. In 1993 an assessment was made of the suitability of a French colony of captive-bred baboons. For anatomical, microbiological and immunological reasons, only eight of the 30 were considered potentially suitable as sources of organs for xenotransplantation.[17]

A number of viruses which are harmless in their normal monkey hosts may cause fatal illness when passed to other primates or to humans:

- Macaque monkeys naturally carry the herpesvirus B, which causes them minor discomfort. People infected by close contact with infected monkeys suffer rapid and fatal encephalomyelitis.
- Two other herpesviruses, herpes saimuri and herpes ateles, are harmless in one monkey species but cause cancers in other species.
- Simian haemorrhagic fever virus is harmless in African baboons, but lethal in Asian rhesus monkeys. Its effect on humans is not known.
- An outbreak of malignant lymphoma in a colony of baboons in Kuwait was traced to cross-species transmission of STLV-1 from rhesus macaques to the baboons.[18]

Baboons carry several persistent viruses known to be capable of infecting human cells, including herpesviruses such as simian agent 8, herpes papio and cytomegalovirus; and retroviruses including simian foamy virus, simian T-cell lymphotropic virus (STLV), baboon endogenous virus and simian endogenous retrovirus.[19] It is also possible that there are primate prion diseases.

Any of these could be transmitted to humans by xenotransplantation and, indeed, at least one patient is known to have been infected with baboon viruses (see later). Baboon cytomegalovirus is endemic in baboon populations, and the virus can replicate in human cells. More than 90 per cent of wild-caught adult baboons imported into the USA from Kenya and South Africa, and 85 per cent

of captive-bred baboons from several US colonies, were infected with herpesvirus papio 2.[20] The Southwest Foundation for Biomedical Research in Texas found that all the 38 adult baboons in their colony were infected with simian foamy virus.[21]

As with pigs, a major risk is endogenous retroviruses whose DNA is buried in the host animal's genes and is inherited by offspring. The first human retrovirus, HTLV, was only discovered relatively recently. It causes leukemia in humans and is believed to have arisen by cross-species transmission from African monkeys.

Warning of the dangers of retroviruses to xenotransplant recipients, Dr Jonathan Stoye of the British National Institute for Medical Research and Dr John Coffin of Tufts University School of Medicine, USA, wrote:

"Such viruses are widely distributed in mammalian species including pigs and baboons, potential donors for these procedures. Since they are inherited in the germ line in the form of proviral DNA, they are impossible to remove using the usual methods for deriving pathogen-free animals".[22]

In early 1996, the American Food and Drug Administration approved a proposal by the University of California to transplant baboon bone marrow into an AIDS patient. The committee recommended the University use a retrovirus-free baboon, but a nationwide screening programme only found two baboons of the appropriate health status. Even so, the baboon used in this operation was known to have baboon endogenous virus, which can infect human cells in culture, and it may also have carried other viruses.[23]

If animals are naturally immune to viruses they carry, or there is a long latency period before symptoms develop, or the virus is dormant for long periods of time (as is the case with herpesviruses and retroviruses), it may be very difficult to know whether they are infected.

As we have seen, retroviruses may infect humans directly, or they could mutate or recombine with human pathogens to create new diseases in an immunosuppressed xenotransplant patient.

Previously unknown viruses are a major risk and cannot be screened for. New viruses are still frequently being discovered. In May 1997, researchers discovered a new retrovirus in baboons.[24] Named a D-type simian endogenous retrovirus (SERV), its DNA was intact – suggesting that it could become activated in a new host, according to its discoverer, virologist Dr Jaap Goudsmit of Amsterdam. New types of simian T-lymphotropic viruses, STLV-PH969 and STLV-PP1664, have recently been identified.

In 1994 an uncharacterised virus caused encephalitis-like illness in some individuals in a baboon colony at the Southwest Foundation for Biomedical Research, Texas.[25] Holding the largest colony in the world, the Foundation is a supplier of baboons as a source of tissues and organs for transplants into humans. The Foundation checked and eliminated 50 viruses known to cause encephalitis in humans and baboons, without identifying the culprit.

According to Dr Jonathan Allan, an expert virologist at the Southwest Foundation for Biomedical Research,
"Most new pandemics arise through inadvertent transmission of viruses from another species (which functions as a natural reservoir) to humans... Scientists do not have the luxury of a crystal ball for predicting the outcomes of these experiments. What we do have is AIDS as a reference point".[26]

Two patients with end-stage liver disease who received baboon liver transplants in 1992 and 1993 died after 26 and 70 days. In the latter case, the source baboon tested positive for simian foamy virus, and had evidence of previous infections with Epstein-Barr virus, cytomegalovirus, simian agent 8 and varicella-zoster virus.[27]

Retrospective analysis of the body tissues of both patients revealed the presence of two retroviruses of baboon origin, simian foamy virus and baboon endogneous retrovirus, in several types of tissue.[28] The authors stated that the persistence of these baboon viruses in the patients' bodies throughout the post-transplant period "underscores the potential infectious risks associated with xenotransplantation".[29]

In the spring of 1998, 41 prominent virologists sent a joint statement to the US Public Health Service, recommending that primates should not be used as source animals for xenotransplantation.

3.4 Infectious risk management

If the xenotransplantation programme goes ahead, source animals will obviously be screened for known pathogens, as and when sensitive and reliable tests are available. This will reduce risks, but no screening is foolproof.

Inactive viruses and endogenous retroviruses are particularly difficult to detect, and it is impossible to look for unknown pathogens. Screening source animals will not prevent the spread of an unknown or a new virus into the human population.

Since it is inevitable that animal organs will harbour some pathogens, it has to be decided which are likely to pose the least risk and will be "allowed" in xenotransplant tissues and organs, and which must be eliminated. The present state of knowledge does not permit such a decision to be made rationally.

There has been too little systematic thought regarding the start of clinical trials. Sensible harm-benefit thresholds have not been developed or agreed. The risk to public health could not be justified unless there were no hope of enhancing human organ supply, no hope of improving present ability to identify and exclude high-risk pathogens, and unless xenotransplantation can save many lives in the near future. None of these provisos is true.

The issue of informed consent is particularly challenging for xenotransplantation. Prospective volunteer patients are likely to be very ill indeed, but must understand unusually complex concerns about the experimental status of xenotransplantation, the risks of infection to themselves and to close contacts, behavioural modifications which may be required to reduce this risk, the genetic engineering aspects, the topic of chimerism, the uncertainty of whether animal organs will support human life, and the experimental nature of anti-rejection drug regimes.

3.4.1 The UK situation

There has been a moratorium in the UK on transplants of animal tissues to humans since the 1996 report of the Advisory Group on the Ethics of Xenotransplantation.[30]

This has effectively been lifted with the publication, in August 1998, of the UK Xenotransplantation Interim Regulatory Authority's (UKXIRA) guidance notes on applying for permission to transplant animal organs into people.[31] No detailed requirements for the health status of source animals are specified. The guidance merely tells applicants that,

"Information should be provided about the pathogens known to be of concern in the species and the measures taken to exclude these pathogens. Where it is not possible to exclude a particular pathogen, detailed information should be provided about the pathogen and its possible effect on humans."

Despite the lack of any new scientific evidence that animal organs will support human life and health, or of any significant reassurance that infectious risks can be quantified or eliminated, this document is an acceptance by the UK government that the first human volunteers may now be used in medical procedures which they inevitably will not survive.

It also indicates official thinking: the acceptability of a trade-off between a benefit which may accrue to some individuals in the future (when and if xenotransplantation becomes routinely successful) versus unknown risks to the wider population. This decision has been made without any real effort at public consultation.

Applicants for permission to conduct xenotransplants in the UK will need to "include details of the precautions to be taken" to monitor patients, and provide details of any requirements to be imposed on patients or their close contacts. At least for the moment, each application will require the specific approval of the Secretary of State.

UKXIRA is preparing codes of practice for biosecurity levels which will be needed for the production of source animals, and on surveillance procedures for patients who receive animal tissue. It is envisaged that inspectors will monitor compliance with standards and approve sites, before permission is given for xenotransplants.

3.4.2 The US situation

A number of animal-to-human organ transplants have already been conducted in the USA. In 1996 the US Department of Health and Human Sciences (DHHS) published draft guidelines, now undergoing revision, on xenotransplantation and infectious diseases.[32]

The American Medical Research Modernization Committee has submitted a long critique of the DHHS document, and deals with the topic in its own report on xenotransplantation.[33] Dr Jonathan Allan, a virologist at the Southwest Foundation for Biomedical Research in Texas, claims that the US public health services caved in under pressure from the transplant community, in producing "a set of dubious guidelines... that ultimately leaves most of the responsibility for policing xenotransplantation to transplant surgeons and the local institutional review boards".[34] In August 1998, the US Food and Drug Administration announced that it would publish revised guidelines later in the year.

A US moratorium has been called for by a group of nine American researchers, including one who is a paid consultant to Novartis Pharma, the multinational company leading xenotransplantation research.[35]

This group of experts is concerned with unresolved ethical issues centering on the need for public consent for a process which benefits an individual but could put the public at risk. They recommend that xenotransplantation should have an entirely novel method of evaluation, consultation and regulation, involving a national committee where citizens are properly represented, as well as professional ethicists, scientists and doctors.

Until these ethical issues are resolved, the American scientists recommended that all clinical work, including using animal organs as a temporary support, should be halted. However, the American Food and Drug Administration have made it clear that they are not considering a moratorium.

Since the wider population will be put at risk of disease should a xenotransplant patient become infectious, a moratorium on clinical trials should exist at least until there has been an extensive programme of public education and consultation.

A clear and logical harm-benefit threshold for clinical trials should be discussed and established if human xenotransplantation research is to proceed.

Practical issues of informed consent, accountability and insurance would also have to be clarified in advance.

A national system of virus discovery, detection and diagnostics, for application to source animals as well as patients and their close contacts, would have to be in place before clinical trials. This will require considerable research.

At present, knowledge is inadequate to permit a scientifically-based decision to be made about which animal viruses are the most and the least risky in terms of individual and public health.

3.5 Section summary – Infectious risks to patients and the public

Most medical interventions carry some risk to the patient, but with xenotransplantation there is a serious likelihood of infectious disease transmitted by an animal organ to a patient also putting the wider population at risk.

Many classes of pathogenic viruses could infect humans. Animal viruses which do not normally infect people could do so when they are placed, in an animal organ, directly within a patient's body. The cells from the animal organ migrate to all parts of the recipient's body, spreading any viruses they carry. There may also be prion diseases in pigs or primates.

Animal viruses silent in their normal host can be activated when transferred into a different species. Viruses may also mutate or recombine with existing human viruses, to form an entirely new pathogen.

Many human epidemics have arisen from cross-species transmission of viruses, such as devastating flu epidemics earlier this century (which originated in pigs) and the current AIDS pandemic (believed to have originated in primates).

Endogenous retroviruses, whose DNA hides silently within the genes of their hosts and can be inherited by offspring, are a particular concern. They are persistent and cannot be easily detected, or eliminated by raising animals in germ-free conditions.

Pigs carry many viruses, only some of which can be screened for. New pig viruses have recently been discovered, which can infect human cells in the test tube, multiply within them and become resistant to a human defence system called complement.

A recent survey of patients, who had come into contact with living pig cells, did not find evidence of infection by pig viruses. For a number of reasons, this provides little reassurance that whole animal organ transplants will not transmit viruses to humans.

The risk of viral transmission from primates, our closer relatives, is even greater. Pathogen-free colonies of monkeys do not exist and would be exceedingly difficult to establish. At least two patients who received baboon liver transplants acquired several baboon viruses.

At present, knowledge is inadequate to allow an informed decision about which animal viruses pose the greatest risks to humans. Nevertheless, xenotransplants are allowed and have been conducted in the USA. The UK's moratorium has effectively been lifted by the recent publication of guidance for applications to conduct human xenotransplantation research.

4. Can animal organs sustain human life?

This similarity of physiological function among members of the same species has allowed investigations in [human organ] transplantation to be devoted almost exclusively to immunology and the nature and prevention of rejection. This luxury will not exist in the field of xenotransplantation, where issues of comparative physiology will assume great importance. It will accomplish little to solve the puzzle of xenograft rejection if an organ cannot function in another species ... there is almost no direct experimental evidence to answer the question ...

Dr Robert Kirkman,
Department of Surgery, Harvard Medical School, USA.[36]

Most xenotransplantation research has focused on trying to overcome the several stages of organ rejection. Very little attention has been paid to the question of whether pig or primate organs are functionally capable – anatomically, physiologically, biochemically and pharmacologically - of sustaining human life. There will inevitably be incompatibilities between animal organs and the human body, many of which are currently unknown.

Dr Jeffrey Platt, a xenotransplant researcher at the Duke University Medical Center, USA, wrote, *"There is only limited information to suggest how well a foreign organ might function in a human subject, yet clearly this question is of greatest clinical importance"*.[37]

The UK's Advisory Group on the Ethics of Xenotransplantation, chaired by Professor Ian Kennedy, stated:[38]
"We therefore conclude that the evidence on transplant function, organ growth and the functioning of the recipient's immune system within the transplant is too limited, at the current time, to move to clinical trials."

These knowledge gaps still exist, yet in 1998 the UK Xenotransplantation Interim Regulatory Authority issued guidance for applications to conduct clinical trials. Has the Kennedy report been ignored, or forgotten? Or have the ethical questions proved too thorny and been abandoned?

Some dissimilarities between other animals and humans would affect any type of animal organ transplanted into a human. According to Professor Sir Roy Calne, the responses of a grafted animal organ to human pituitary, adrenal, thyroid and pancreatic and sex hormones are unpredictable.[39]

Professor Calne was quoted by the *New Scientist* as saying: *"Even proteins produced by close species such as the baboon are different from their human counterparts"*.[40]

Other species disparities relevant to transplanting any animal organ into a human, are concerned with blood circulation and coagulation.

The function of all organs depends on adequate circulation of the blood, and the liver is especially vulnerable to hypoxia. The diameter of human red blood cells is, at 7.2 microns, larger than the 6.1 microns of pig red blood cells. This may cause microcirculation problems, compromising the viability of a transplanted organ.[41]

Incompatibilities in the blood clotting cascade could also trigger organ failure. In blood clotting, thrombomodulin is produced on the endothelial cells which line the blood vessels. The thrombomodulin binds with thrombin, itself produced from prothrombin, and with protein C in the bloodstream. This activation of protein C initiates anti-coagulation pathways, as well as anti-inflammatory activity.

In a test-tube experiment, human prothrombin was activated by pig but not human endothelium.[42] *This suggests that the blood of a xenotransplant patient may be directly coagulated by the endothelium in a transplanted pig organ, even in the absence of natural pre-formed antibodies and complement.*

Another study indicated that pig thrombomodulin, produced in blood vessels of the animal organ, will intereact differently with human thrombin and protein C in the bloodstream:

"Significant incompatibility" between pig thrombomodulin and human thrombin and protein C was observed.[43] *The results suggest that within a pig organ transplanted into a human, pig thrombomodulin would have only one per cent of its normal effectiveness in activating protein C. This could lead to graft failure through a delayed rejection, characterised by clot formation within the small blood vessels of the pig organ.*

The authors comment, "This molecular incompatibility shows the need to identify specific elements of hemostatic dysregulation between man and pigs and reveals what may be a major barrier to pig to human xenotransplantation" (op. cit.).

In a third study of pig-human interactions in the blood clotting process, the possibility was raised of human factor VII being activated by pig tissue factor, released by endothelial cells in the animal organ.[44] *This would also increase the risk of organ failure.*

Moreover factor VIIa and factor Xa are blocked by human tissue factor pathway inhibitor. However, pig tissue factor pathway inhibitor only blocks human VIIa, but not Xa.[45]

Another change in normal blood clotting which could be triggered by any pig organ involves von Willebrand Factor. This is released from the endothelial cells lining the blood vessels and binds with platelets.

In the normal human situation, binding causes platelet clumping but only in the presence of shear stress. However von Willebrand Factor from pigs can bind to human platelets and cause platelet aggregation without shear stress.

All these results indicate that abnormal blood clotting reactions could be triggered in a xenotransplant patient by mismatches with an animal organ, leading to graft rejection or death. These and other molecular incompatibilities are discussed at greater length by Dr Fritz Bach and colleagues.[46]

The enormous arsenal of enzymes possessed by humans and other animals has many physiological functions, ranging from blood coagulation (see above), through protein digestion, release of hormones and active peptides, to drug metabolism.

Differences in amino acid sequences and three-dimensional structure lead to variations in enzyme activity between species. Enzyme activators and inhibitors may also show species differences, as does tissue distribution of some enzymes. The likely mismatches between enzyme structures and substrate specificities in pigs, baboons and humans are not even fully characterised, but will have wide-ranging implications for xenotransplantation.

While ordinary patients who have a deficiency in one factor produced by an organ can often be treated, a xenotransplant patient may have to survive multiple deficiencies or over-productions of factors, as well as several aberrant interactions between animal and human substances. Therapeutic strategies for dealing with these complex mismatches have not been devised.

The following sections describe likely animal-human incompatibilities in the functions of the liver, kidneys, heart and lungs – the four organs most likely to be transplanted whole into human patients.

While the implications of species differences in physiology, biochemistry and pharmacology remain unknown, it would be foolhardy to conduct clinical trials of animal organs in humans. A programme of in vitro research should first be conducted to gain more knowledge of which cross-species mismatches will be most significant. Therapeutic strategies to ameliorate these mismatches in xenotransplant patients would need to be devised in advance of clinical trials.

4.1 Section Summary - Can animal organs sustain human life?

Xenotransplant research has primarily focused on trying to overcome the various stages of organ rejection. However, no-one knows whether pig or primate organs will be functionally capable – anatomically, physiologically, biochemically or pharmacologically – of supporting human life and health.

Many incompatibilities between species are already known and some will affect any kind of animal organ graft. For example, all organs depend on an adequate blood supply. Human red blood cells are larger than those of the pig, so that in a grafted pig organ there may be blockages in the tiniest blood vessels, which could damage or destroy the xenograft.

Incompatibilities in the complex cascade of reactions which leads to blood clotting will also tend to trigger organ failure, with possibly fatal consequences. The blood vessel walls of a pig not only activate human clotting factors by more than one process, they also suppress normal anti-clotting mechanisms. This means that as a patient's blood circulates through a pig xenograft, blood clots may form continually.

5. The Liver

What we think and feel and are is to a great extent determined by the state of our ductless glands and our viscera.
Aldous Huxley, *Meditation on El Greco*, 1931.

The liver is metabolically very active, undertaking many important roles in the body, including:

Detoxification and activation of chemicals

- Metabolism of amino acids, fats and carbohydrates
- Synthesis of bloodborne complement proteins
- Inactivation of many hormones including insulin, glucagon, cortisol, aldosterone, thyroid and sex hormones
- Storage of iron and of vitamins B12, A, D, E and K
- Synthesis of vitamin A
- Secretion of bile (digestive and excretory functions)
- Synthesis of blood clotting factors
- Synthesis of plasma proteins, including transferrin, haptoglobin and albumin.

Each one of these functions is an opportunity for mismatch between an animal liver and the needs of the human body. If the products of the animal liver are too 'foreign' and the patient generates antibodies to them, this could result in immune-complex disorders such as serum sickness type illnesses.[47]

Because of this multiplicity of essential roles, some scientists believe that an animal liver could not permanently support human life. However, others have pointed out that because the liver is relatively resistant to antibody-mediated rejection, xenografting with this organ may have the best chance of long-term success.

5.1 Research and clinical history

Liver xenotransplant experiments have been conducted extensively with a wide variety of species, including baboon and dog to pig; cynomolgus to rhesus monkey; guinea pig and hamster to rat; rabbit to dog; and pig to baboon, chimpanzee, dog and rhesus monkey.

The surgical techniques for liver transplantation were first developed in animals in 1959 and human-to-human liver transplants by Dr Thomas Starzl followed rapidly in 1963.

According to Professor Sir Roy Calne, the "early results were dismal", partly due to species differences in clotting caused by venous shunts which had, however, been used with success in dogs.[48]

The results of human-to-human liver transplantation continued to be very poor for some years afterwards. The introduction of the immunosuppressive drug cyclosporin in 1980 (although it is toxic to the kidney) led to improved survival, and today about 70 per cent of transplanted livers are expected to function for one year, and 50 per cent for five years.

Perfusing the blood of human patients externally through animal livers has been attempted many times, usually with limited, very short-term success.[49]

In 1966, Dr Starzl and colleagues transplanted a chimpanzee liver as a short-term support into a child patient in liver failure, whose own organ remained in place.[50] The animal liver survived for only 24 hours.

In the 1960s and '70s, Starzl replaced the failing livers of three children with chimpanzee organs.[51] One died immediately; there was some post-operative function in two of the children, but they died of sepsis after 9 and 14 days.

In 1992 and 1993, Starzl replaced the livers of two very sick patients with baboon organs.[52] The patients experienced several health crises before dying after 26 and 70 days. In the latter case, the enzyme alkaline phosphatase, an indicator of liver function, increased in the patient's bloodstream to more than 80 times normal levels, for unexplained reasons.[53] Other bloodstream factors related to liver function showed a shift towards baboon rather than human levels, including uric acid, cholesterol and albumin.

The deaths of both patients were due to "global dysfunction" of the liver, probably as a result of a slow humoral rejection. Starzl and his team also raised the question of species incompatibility: "...the more generic question of metabolic incompatibility has not been laid to rest".[54]

5.2 Functional incompatibilities

Because of the liver's several important roles, there are many potential incompatibilities between an animal liver's activity and the needs of a human body. Indeed, the UK's Advisory Group on the Ethics of Xenotransplantation stated in its 1996 report:[55]

"We consider it probable that a pig's liver could not carry out certain vital functions performed by a human liver, particularly in the long term... We note, moreover, that research involving the transplant of a pig's liver into a baboon may not provide the necessary information concerning its functioning in humans, as some of the biochemical and metabolic functions involved are likely to be species specific."

Some obvious species differences in liver-related blood biochemistry are shown in the Table.

Table: Species differences in blood biochemistry values related to liver function

	Human	Baboon[56]	Pig[57]
Serum albumin (g/l)	38 - 52	18 - 39	16 - 38
Alkaline phosphatase (IU/l)	50 - 125	387	60 - 269
Serum uric acid (mg/dl)	4.0 - 5.5	<0.5	0.05[58]
Serum cholesterol (mmol/l)	4.0 - 6.5	1.03 - 3.15	0.9 - 3.07

Detoxification and activation of chemicals

Variations in metabolism of drugs and other chemicals by the liver can be caused by the amount of enzymes present, the affinity of enzymes for their substrates and the availability of cofactors. Individual differences in these features *within* the human population have significant effects, on circulating concentrations of a parent drug or chemical, drug efficacy, and drug and chemical interactions and toxicity. Therefore differences *across* the species can also be expected to be substantial.

The cytochrome P450 family of liver enzymes plays a dominant role in drug metabolism, oxidising more than 80 per cent of drugs, as well as other chemicals. The enzymes may detoxify or activate, the latter route often leading to the production of toxic, particularly carcinogenic, compounds.

While there is often overall functional similarity across different species, even small changes in P450 enzymes – such as a single amino acid substitution – can cause significant changes.[59]

For example, cytochrome P4502C19 and 2C9 are not found in dogs and rats, and drugs metabolised by 2C9 in humans may instead be metabolised by 3A in rats.

Differences in drug and chemical metabolism between humans, pigs and baboons are known to occur. The metabolism of alcohol in baboons has been widely studied, as these primates are used extensively in alcohol research.

The human liver has three forms of the enzyme alcohol dehydrogenase, known as alpha 2, beta 2 and gamma 2, as well as three hybrid forms called alpha beta, alpha gamma and beta gamma. In contrast, baboon liver has only a single major version of this enzyme, the beta 2.[60]

Other significant discrepancies also exist:

The bloodstream kinetics of the drug dexfenfluramine and its metabolite were studied in baboons, cynomolgus and rhesus monkeys, because none of the other species previously examined showed sufficient similarity to humans.[61] *Despite the relatively close relationship of these primates to humans, the metabolite-to-parent drug ratio, ranging from 14 to 37, vastly exceeded the <1 seen in humans and was higher than in any other species investigated so far.*

An enzyme reaction called epoxidation occurs with aromatic compounds, such as naphthalene, with some organochlorine insecticides and with drugs such as carbamazepine.

Comparisons of the enzymes of epoxide metabolism in nine species, including baboons, led to the conclusion that "... no single species of those studied is a suitable model for the disposition of epoxides in man".[62]

Professor Roch-Ramel and Dr Anne Simmonds point out that aldehyde oxidase, an important enzyme which metabolises drugs such as cyclophosphamide and methotrexate to inactive metabolites, has markedly lower activity in humans than in non-primates such as pigs.[63]

A comparison of aldehyde oxidase activity in the livers of rabbits, guinea pigs, rats, marmosets, dogs, baboons and humans revealed that "... baboon liver contained a highly active aldehyde oxidase. Enzyme from marmoset and guinea pig liver had the closest spectrum of activity to human liver aldehyde oxidase".[64]

Drugs and other chemicals are excreted in the bile, either as the parent compound or as sulphate, glucuronide or glutathione conjugates. The rates of metabolism and excretion by an animal liver may differ from those of a human organ, potentially leading to differences in toxic or therapeutic effects.

Metabolism of amino acids, fats and carbohydrates

The liver contains many enzymes which make or break down a range of substances. As transplant scientist Professor Sir Roy Calne asked,[65] would the proteins, peptides, fats, carbohydrates and hormones produced by an animal liver be entirely compatible with the human milieu? Or would some of the animal liver products be dissimilar enough to cause an immune reaction?

A principal difference in the pig liver compared to that of a human is the complete lack of the enzyme uricase.[66] *This means that in pigs, purines are metabolised by the liver via uric acid to allantoin. In humans, however, the endpoint of purine metabolism is uric acid, and allantoin is not formed.*

How will the human body respond to the presence of allantoin in the bloodstream and to the very low levels of uric acid which an animal liver would produce?

A second difference in purine metabolism between pigs and humans, according to Dr Anne Simmonds and Professor Roch-Ramel, is that the pig "is probably the only species whose liver lacks the enzyme guanase, a key enzyme in controlling guanine ribonucleotide concentrations which regulate many cell functions – including the immune response".[67]

Complement

Complement is a family of bloodstream proteins, each with specific immune or inflammatory roles. Two of Dr Starzl's patients whose livers were replaced with baboon organs developed baboon complement C3, the major protein of the complement system.[68] Since the liver is the primary source of complement, other complement proteins would also be of animal type.

The complement proteins of eight different primates species were compared to those of humans.[69] *Old World monkeys, which include the baboon, were found to be antigenically deficient in complement components C1q, C1s and C9.*

Such differences could have important repercussions for the destruction of pathogens, and hence in combating infections, as well as for the inflammatory response.

Inactivation of hormones

Steroid hormones are made from cholesterol in the adrenal gland and gonadal tissues. The adrenal gland synthesises cortisol, aldosterone and androgen precursors. The ovaries produce estrogen and progesterone, and the testes make testosterone.

One of the roles of the liver is to inactivate these hormones. Will a pig or baboon liver interact appropriately with human adrenal and gonadal tissues, to maintain the intricate feedback systems which balance the production and inactivation of these important substances?

An obvious species difference which may have a bearing on this is the bloodstream level of cholesterol. Baboons and pigs have very low levels, between 0.9 and 3.15 millimoles per litre, compared with the normal human range of 4 – 6.5 mmol/l.

In the longest-surviving Starzl patient whose liver was replaced with a baboon organ, bloodstream cholesterol fell to 1.71 mmol/l after 45 days – that is, to baboon rather than human levels.[70] This would be yet another factor perturbing the balance of hormone synthesis and inactivation.

Vitamin B12

While some species incompatibilities would become obvious immediately after xenotransplantation, others may take some months to develop. The liver is the body's store of vitamin B12, which is also recycled by reabsorption of vitamin in the bile, from the small intestine.

Gridelli and colleagues found that a rhesus monkey whose liver was replaced by a baboon organ developed vitamin B12 deficiency after some time, probably due to a metabolic mismatch.[71]

Secretion of bile

The liver secretes bile, which passes into the small intestine where it plays a key role in fat digestion. Bile fluid is also a means of excreting metabolic end-products. In humans the primary bile acids, produced in the liver from cholesterol, are cholic and chenodeoxycholic acids. They are conjugated to form glycocholic acid, taurocholic acid, and two others, before being secreted into the bile.

Bile contains the pigment bilirubin, the waste product of red blood cell breakdown; cholesterol and phospholipids; drugs and chemicals; mineral salts, mucus and water. The presence of bile in the small intestine is required for the absorption of vitamin K. However, bile acids can also be toxic.

There are notable species variations in the composition of bile and the metabolism of bile acids:

A major bile acid, chenodeoxycholic acid and its conjugates are hydroxylated in pig liver but not in human liver. This results in hyocholic acid and its conjugates in pig bile, not seen in human bile. Glycohyocholic acid is also found in pig but not human bile, whereas glycocholic acid is the most abundant bile acid in humans, but is not found at all in pig bile.[72]

The repercussions of changes in bile acid composition for patients receiving pig livers are unknown, but toxic effects are possible.

For example, species differences in the metabolism of the bile acid chenodeoxycholic acid result in toxicity to baboons and rhesus monkeys, but not to chimpanzees or humans. In rhesus monkeys, congenital abnormalities of the liver, kidney and adrenal glands were found in the offspring of pregnant monkeys given chenodeoxycholic acid.[73]

Non-human bile acids could have other effects in a human patient, such as: modifying fat absorption; affecting the rate of absorption of fat-soluble vitamins; altering susceptibility to bowel cancer and changing the absorption of bile acids in the intestine. All or any of these would have important health consequences for xenotransplant patients.

Moreover the importance of biliary excretion of drugs and chemicals and their metabolites varies from species to species, and differences would affect the activity of some therapeutic drugs and the toxicity of some chemicals (see *Detoxification and activation of chemicals*, above).

The control of the rate of bile flow also differs among species:

In humans, the flow of bile from the liver to the gall bladder is almost entirely induced by levels of bile acids in the bloodstream. This means that when bile acid secretion is low, there is virtually no bile flow. In contrast, in many mammals there is a much higher flow of bile which is independent of bile acid levels.[74]

Blood clotting

The liver produces most blood clotting factors and so a recipient of a pig or baboon liver will acquire mainly animal factors. This was indeed the case with the two patients who received baboon livers in the Starzl clinical trials.[75] [76]

Some of the blood-clotting factors produced by an animal liver, such as factor VII, will have to interact with human components, such as tissue factor released from endothelial cells lining the blood vessels in the rest of the patient's body. Significant molecular incompatibilities would interrupt normal blood clotting with potentially life-threatening results.

The subtle feedback mechanisms which limit clot formation to the damaged area of a blood vessel, and the processes which eventually dissolve the clot, may all be affected by mismatches between the human and animal factors.

Plasma proteins (transferrin, haptoglobin and albumin)

Two patients whose livers were replaced by baboon organs had baboon transferrin in their bloodstream.[77] This carrier protein, made in the liver, transfers iron from dying red blood cells to the bone marrow to be recycled by developing red blood cells. In delivering its iron load, transferrin actually moves into and out of the immature red blood cells.

Transferrins, like albumins, vary in structure across the species more than some other proteins.

Serum transferrin concentrations in the pig differ from that of humans. Species differences in the surrender of iron from a preferred site on the transferrin molecule, and in acid-base binding properties, have been found between rabbits and humans, and similar variations may occur in pigs and baboons.

Considering baboons as source animals for organs, Professor Hammer of the University of Munich has commented that a number of plasma proteins differ notably between humans and baboons.[78] These include haptoglobin (which carries haemoglobin to the liver in order to recycle the iron); transferrin (which transports iron to the bone marrow for developing red blood cells); ceruloplasmin (an enzyme which carries copper in the bloodstream, and may have anti-inflammatory activity); and alpha$_1$-antitrypsin (a deficiency of which leads to emphysema).

Haptoglobin is primarily made in the liver, so a patient with an animal liver may have a hybrid human-animal haemoglobin-haptoglobin complex. Such a hybrid complex may not bind normally to the specific receptors in the animal liver which usually allows clearance from the bloodstream and recycling of the iron.

Ceruloplasmin formation is stimulated when estrogen engages with receptors on liver cells – will the receptors on a pig or primate liver interact appropriately with human estrogen? Too little ceruloplasmin would affect levels of copper in the body and probably exaggerate the damaging consequences of infections or tissue injury.

Serum albumin, made by the liver, is the main protein responsible for the colloidal osmotic pressure of the blood. When this pressure falls, as it might in a human recipient of a pig or baboon liver, excess fluid moves out of the circulation into the tissues causing oedema (swelling). If uncorrected, this would lead to severe ascites caused by accumulation of fluid in the abdomen.

Albumin is present in the human bloodstream at levels of 38 – 52 grams per litre. Comparable values for the baboon and pig are considerably lower at 18-39 g/l and 16-38 g/l, respectively.

Serum albumin is an important carrier of free fatty acids and bilirubin, and a secondary carrier for thyroxine, cortisol and haem. Albumin also binds drugs, affecting their therapeutic activity. Thus any qualitative differences between human and animal albumins would have profound implications and, indeed, such disparities have been found.

Using an index of dissimilarity to demonstrate the variation in amino acid sequences in albumins, Hammer[79] states that allocating the structure of human albumin the value of 1.00, in comparison chimpanzee albumin is 1.14, baboon albumin is 2.23 and pig albumin is >35.

Studies comparing albumins from several species, including humans and pigs, show that despite broad structural similarities there are notable species variations in the molecular weight, Stokes radius and electrophoretic mobility, as well as different binding characteristics to bilirubin,[80] *methamphetamine*[81] *and other substrates.*[82]

Single amino acid differences in the serum albumin from various species, including pigs and humans, significantly altered the binding selectivity for L-tryptophan and related analogues.[83]

"Another cause of interspecies differences in response to toxicants is the extent of plasma protein binding... The rhesus monkey, rabbit and guinea-pig resembled man in having a relatively high affinity for binding salicylate, whilst the baboon, horse, dog, rat, mouse, turkey and toad had a low binding capacity".[84]

The Pfizer company found that a drug called zamifenacin was bound very differently by plasma proteins in different species: "Zamifenacin exhibited extensive plasma protein binding with human plasma showing 20 and 10-fold higher binding than that in rat and dog respectively... Oral clearance in man was low as a result of increased metabolic stability and increased plasma protein binding compared with animals".[85]

While human serum albumin can be infused into ordinary patients who lack the protein, in xenotransplant patients the problem would also be counterbalancing – both quantitatively and qualitatively – the presence of animal albumin.

5.3 Section summary - The Liver

The liver performs very many complex life-supporting functions, some of which are different in different species.

Of the important liver enzymes which activate or detoxify drugs and chemicals in the body, there are known species variations. This means that a patient with an animal liver might be more susceptible to the toxic effects of chemicals, and may seriously under- or over-react to normal doses of medicines.

The liver also makes and breaks down many natural substances found in the body. The enzyme uricase, which breaks down waste proteins called purines, simply does not exist in a pig liver. The breakdown of purines by a pig liver would produce a different, non-human substance and also lead to exceedingly low levels of uric acid in a human patient. The pig liver also completely lacks another human enzyme called guanase, possibly affecting a human patient's immune system.

The liver produces complement proteins. Baboon liver makes a different selection of complement proteins to the human liver and this may alter inflammation and the patient's ability to fight infections.

The liver regulates levels of steroid hormones such as estrogen and cortisol by interacting with the brain and with the testes and ovaries. No-one knows if an animal liver will be able to do this. Steroid hormones are made from bloodstream cholesterol, but levels of cholesterol are high in humans compared to pigs and baboons. This may affect hormone activities in xenotransplant patients.

There are species variations in the amount and composition of bile produced by the liver. This may have direct toxic effects on body tissues and may affect the activity of medicines in a patient, as well as fat absorption, the availability of vitamins such as A and D, and susceptibility to bowel cancer.

Pig and baboon livers produce a protein called albumin which is different in structure to that found in people, and they regulate bloodstream levels of this protein differently. These discrepancies could lead to painful fluid accumulation in the abdomen and disturbances in the actions of medicines.

Patients given an animal liver may suffer from disorders of blood clotting, which could be life threatening. There may also be problems with the formation of their red blood cells, since an animal liver may be unable to function correctly.

6. The Kidneys

Man is physically as well as metaphorically a thing of shreds and patches, borrowed unequally from good and bad ancestors, and a misfit from the start.

Ralph Waldo Emerson, *Beauty*, 1860.

The primary function of the kidneys – to maintain the normal volume and composition of the body fluids – involves a complex, interconnected pattern of activities. These include not just excretion and reabsorption of numerous substances, but also metabolic and hormonal activities regulating acid-base balance, electrolyte and fluid balance and thus blood pressure, as well as red blood cell production.

6.1 Research and Clinical History

The first kidney transplant operation was attempted on animals in 1902 and on humans in 1936. Today, the one-year survival rate of the transplanted human kidney (rather than the patient) is between 79 – 85 per cent.

Xenotransplant kidney experiments have been conducted between the following animal species: sheep, tiger, pig, cat, lion, wolf, fox and dingo to dog; dog to wolf; cat, hare and pig to rabbit; rabbit to cat; pig to dog, baboon, cynomolgus monkey, goat and rabbit; sheep and pig to goat; and guinea pig and mouse to rat.

Scientists from Papworth Hospital, Cambridge and from Imutran, the British company which is part of Novartis Pharma, reported in 1998 that they had replaced the kidneys of 13 cynomolgus monkeys with kidneys from normal and transgenic pigs.[86] The transgenic kidneys expressed human decay accelerating factor, which helped protect them from immediate (hyperacute) rejection. Survival of monkeys with transgenic pig kidneys was 6 – 35 days (median 13 days), and in the control group was 0.3 – 30 days (median 6.5 days). During the first eight days, six monkeys died from kidney failure. In three monkeys who received transgenic pig kidneys, the organs failed due to acute vascular rejection.

This experiment showed that transgenic pig kidneys can perform excretory functions in monkeys for up to 35 days. However, pigs and monkeys – unlike humans – are similar in having very low

levels of uric acid in their bloodstream. In a human patient, incompatibilities in uric acid levels would probably occur. Moreover, the monkeys who survived past eight days developed progressive anaemia, which became severe enough to necessitate the killing of four animals. The cause of anaemia was probably because erythropoietin secreted by the pig kidney was unable to stimulate red blood cell formation in the monkeys' bone marrow.

The transplantation of animal organs into human patients began 30 years ago. In the 1960s Professor Keith Reemtsma grafted chimpanzee kidneys into several patients.[87] One patient survived for nine months. That chimpanzee kidneys can support human life for this period of time is not, however, an indication that a pig or baboon kidney could do likewise. The routine use of chimpanzees as source animals today is impossible, due to their unavailability and because of ethical imperatives.

A contrasting result was obtained by Dr David Hume's single chimpanzee-to-human kidney transplant, undertaken at about the same time. The grafted organ produced an astounding 54 litres of urine within 24 hours, leading to the patient's rapid death from fluid imbalance.[88]

Also in the 1960s, Dr Thomas Starzl conducted six baboon-to-human kidney transplants. Exceptionally, one patient survived for two months with baboon kidneys. In the short term, some baboon kidneys achieved reasonable creatinine clearance (a standard measure of kidney activity), and active transport of glucose and potassium.[89] However, these clinical experiments are insufficient evidence that baboon kidneys could undertake, in humans, the complex array of activities required for long-term survival or health.

6.2 Functional Incompatibilities

Pig kidneys share certain fundamental characteristics with human kidneys, having broadly similar glomerular filtration rates, rate of blood flow and concentrating ability. Baboon kidney function has been less well studied.

However, apart from differences in size – a pig's kidney weighs 59 – 100 grams, a baboon's kidney is even smaller, while a human kidney weighs about 150 grams – there are numerous species differences in the specialist functions of this organ.

Excretion

Filtration of the blood is not a simple process, but one which involves selective reabsorption of substances into the bloodstream such as water, sodium, chloride, phosphate, amino acids, glucose, as well as secretion of others, such as drugs and drug metabolites, into the kidney tubules for excretion.

Transplanting pig kidneys into humans seems likely to lead to a major problem with levels of the waste product uric acid in the blood, which are very different in the two species.

Pigs, like most other mammals, have a liver enzyme called uricase which breaks down uric acid to allantoin.[90] Consequently in pigs plasma levels of uric acid are normally very low, at about 0.05 milligrams per decilitre.[91] There is also overall secretion of uric acid by the pig kidney, the amount of uric acid in the urine thus being double the amount first filtered from the blood at the glomerulus.

In humans, the situation is different. Uric acid, not allantoin, is the end-product of purine metabolism, because although the uricase gene is present it is not expressed in human liver. Levels

of uric acid in the human circulation are therefore much higher than in the pig, at approximately 4 – 5.5 mg/dl (being greater in men than in women or children).

This high level of uric acid in the human bloodstream also relates to the fact that over 90 per cent of the filtered uric acid is reabsorbed, due to the presence of a uric acid-anion exchanger in the brush-border membrane of the human kidney's proximal tubules. The situation in the pig is quite the opposite, because the brush-border exchanger is absent in the pig kidney.

What will happen when a pig kidney is flushed through with human blood containing 100 times more uric acid than the physiological level for pigs? Overloading pigs with exogenous uric acid depresses the glomerular filtration rate and leads to uric acid crystal deposition.[92]

Dr Anne Simmonds of the Purine Research Laboratory at Guy's Hospital, London, and Professor Roch-Ramel of Lausanne, believe that without adequate hydration the overload could cause uric acid stones or crystal-induced kidney damage, leading to the failure of the graft. To prevent this, patients may have to drink unusually large volumes of fluid for the rest of their lives. If they did not, or were heavy meat-eaters, or lived or travelled in hot climates, or suffered from diarrhoea, they would be at high risk of graft failure.

There is another important difference between pig and human kidneys:

In humans, the kidney's brush-border exchanger also normally co-transports a number of drugs, such as antibiotics, atropine, quinine, trimethoprim and morphine. Its absence in pig kidneys would therefore have a significant impact on the therapeutic levels of a number of drugs in the circulation of a human recipient, as well as on uric acid levels.

Red blood cell production

The kidneys regulate red blood cell production by making and releasing erythropoietin, which stimulates the formation of red blood cells from immature cells in the bone marrow. By responding to low oxygen in the blood, the kidneys thus monitor and maintain oxygen delivery to all the tissues of the body.

No-one knows whether pig kidney erythropoietin will be effective in stimulating red blood cell formation in human bone marrow. However, the indications are not good. All monkeys who survived for longer than eight days after replacement of their kidneys with transgenic pig kidneys developed progressive anaemia and had to be killed.[93] *Anaemia was thought to have occurred because of a mismatch between pig erythropoietin and red blood cell production in the monkeys' bone marrow.*

Thus it seems likely that, in addition to an already extensive treatment regime, human recipients of pig kidneys would also need to take exogenous erythropoietin. Moreover, in the case of a pig-to-human kidney transplant, there is a possibility that pig erythropoietin may be antigenic enough to induce a defence reaction in the patient.

Fluid balance

The hypothalamus and pituitary gland of the brain are involved in controlling water retention and sodium excretion, as well as the secretion of renin by the kidneys. This system possesses intricate feedback loops between the kidneys and the brain. How well will the human pituitary and hypothalamus integrate their activities with pig or baboon kidneys?

Vasopressin (antidiuretic hormone, ADH) is synthesised in the hypothalamus and secreted by the pituitary gland. Its principal role is to maintain normal fluid balance and hydration by controlling the water permeability of the kidney collecting ducts. The secretion of vasopressin itself is triggered by changes in blood concentration (osmolality) and volume which, in a xenotransplant patient, would be under the control of animal kidneys.

In all mammals except pigs, the vasopressin molecule has the amino acid arginine at residue 8. Pig vasopressin has lysine in this position. Thus pig kidneys in a human xenotransplant patient may not respond normally to human vasopressin. However, it is known that cloned pig V2 receptors for pig vasopressin differ from human V2 receptors, mainly in their N-terminal region, leading to a different relative order of ligand specificity.

Species differences (pigs versus cow) have also been measured in kidney vasopressin V2 receptors, both in density and in their affinity for several V2 ligands. The authors comment that "these findings are significant in relation to the physiological and pathological roles of renal kinins and their interaction with the neurohypophysial peptide hormone system".[94]

Renin is secreted by the kidney into the bloodstream and generates the formation of angiotensin I (and hence angiotensin II) in the bloodstream.

Angiotensin II acts on the adrenal gland and the small arteries and modulates blood pressure, involving a delicate feedback system within the kidneys.

A critical question for the xenotransplant patient is whether pig or baboon renin will effectively catalyse the formation of human angiotensin I, and how well an animal kidney will respond to the human angiotensin system. Any mismatches would have implications for fluid balance and, of course, blood pressure.

Renin is known to have an unusually stringent substrate specificity. The enzyme site for human renin is different from that of non-primate species, such as the pig. According to Wang and Liang,[95] *pig renin is very sensitive to amino acid substitutions at certain sites in its substrate. These authors state:*

"The species specificity of [pig] renin presumably arises from differing P1' – P3' residues in angiotensinogens. For example, the P1' – P3' residues from human and porcine angiotensinogens are Ile-Val-His and Leu-Val-Tyr, respectively."

It would be surprising if there were not a number of incompatibilities in enzyme reactions, receptor distributions, subtypes and affinities, between pig, baboon and human kidneys. Subtle differences may not be a problem, but more significant dissimilarities could have profound effects on organ function, fluid balance and drug responses.

6.3 Section Summary - The Kidneys

The main activity of the kidneys is to balance the volume and composition of body fluids, involving the selective recycling of some substances and excretion of others.

A human patient with pig kidneys may be especially prone to kidney stones or kidney failure, because the pig organ cannot handle the levels of uric acid found in the human bloodstream.

Unlike the human organ, the pig kidney does not have an important mechanism for controlling the levels of medicines, which could have a significant impact in a xenotransplant patient who needs to take several drugs.

The enzyme renin produced by pig kidneys differs from the human equivalent. Moreover, pig kidneys may not respond normally to a hormone (vasopressin) released from the brain of the patient. Both these discrepancies could affect blood pressure, hydration and fluid balance.

Animal experiments suggest that pig kidneys will be incapable of stimulating red blood cell formation in the bone marrow of a patient.

7. The Lungs

And when they had emptied the bowls which she had handed them, she drove them with blows of a stick into the pigsties. Now they had pigs' heads and bristles, and they grunted like pigs; but their minds were as human as they had been before. So, weeping, they were penned in their sties.

Circe transforming Odysseus' men into pigs. Homer's The Odyssey, translated and revised by Rieu, 1991.

The best-known function of the lungs is to carry out gas exchange. Blood depleted of oxygen is pumped directly to the lungs from the heart. Oxygen from inhaled air enters the bloodstream, in exchange for waste carbon dioxide from the blood. The oxygenated blood then returns to the left side of the heart, from where it is pumped to the rest of the body.

However, the lungs are not just a pair of bellows: they also have immune and metabolic functions. In controlling the bloodstream levels of several important substances which contract or dilate the blood vessels, for example, the lungs affect not only their own milieu but also regulate some activities throughout the whole body.

Being directly exposed to the air, the lungs are susceptible to micro-organisms and have finely balanced defence systems to prevent infection. The lungs are physically rather fragile, more prone to rejection than the liver and more sensitive to low oxygen levels.

To support the life and health of a xenotransplant patient, animal lungs would have to perform these functions:

- Gas exchange
- Synthesis of the components of bronchial mucus (defence system)
- Ciliary clearance of foreign particles and pathogens (defence system)
- Secretion of immunoglobulins (defensive antibodies)
- Synthesis of angiotensin II (constricts blood vessels)
- Synthesis and inactivation of bradykinin (dilates blood vessels), prostaglandins (dilate or constrict blood vessels) and leukotrienes (mediators of inflammation and allergy)
- Uptake and inactivation of serotonin (affecting blood pressure and the kidneys)
- Synthesis of phospholipids of the lung surfactants (crucial for lung function)

7.1 Research and Clinical History

In the 1950s and '60s, lung transplant research included circulating human blood through dogs' or pigs' lungs. In the next two decades, primates, dogs and rodents were used. Research into lung xenotransplantation is not as far advanced as for the kidney, heart or liver, which is hardly surprising, since successful human-to-human lung transplantation has been achieved only relatively recently.

In the 1990s, the lungs of unmodified and transgenic pigs have been transplanted into baboons. In Japan, lungs from a baboon were transplanted into a Japanese monkey.[96] Single pig lungs were transplanted by Dr William Daggett into baboons and, in those who had been immunodepleted, the pig lungs continued to function for up to 11 hours.[97] When the remaining natural lung of each of four baboons was functionally isolated, the pig lung was able to support the anaesthetised baboons for this length of time.

With human-to-human lung transplants, international statistics show that the main causes of death are infection (45 per cent of deaths), acute rejection (10 per cent), obliterative bronchiolitis (8 per cent), operative bleeding (8 per cent) and multiple organ failure (6 per cent).

The two-year survival rate for double-lung transplants is about 50 per cent, and about 60 per cent for single lungs, depending on the original illness. The one-year survival rate after re-transplant is about 40 per cent for both types of operation as well as for heart-lung transplants.

These survival figures are well below those for heart or kidney transplants and have not improved very greatly since the late 1980s. Even though using animals as sources would enable better timing of operations, success rates with xenotransplantation would inevitably be much lower because of rejection problems and functional incompatibilities.

Some experts believe that better understanding of the diseases which at present require lung transplantation will eventually allow effective prevention and treatment, making transplants unnecessary. Given that lung xenotransplantation research is in its very early days, these other options may well be the most cost effective.

7.2 Functional Incompatibilities

With the wide range of functions undertaken by the lungs, there are a number of likely incompatibilities in physiology, biochemistry and pharmacology which have not yet been given serious consideration.

Lung size

Volume mismatch between the grafted lung and the chest cavity can cause considerable problems in human-to-human transplants. For example, a recipient with emphysema would have a very large chest volume, while a patient with idiopathic interstitial fibrosis would require a smaller organ.

The animal organ must have the right dimensions for the chest cavity and also for effective joining of blood vessels and airways. If the graft is too big, there is a risk of lung collapse and, if too small, there may be persistent leakage of liquids and gases.

This requirement rules out the use of baboon lungs, which would be too small except possibly for use in children. In this case, would baboon lungs be capable of growing to match the child's needs

in adulthood? Pig lungs are generally considered to offer the closest size match for adult humans, but organ dimensions vary greatly among different breeds and their rate of growth in the human body is unpredictable.

Gas exchange

An animal's lung would need to be capable of adequately oxygenating the blood and removing carbon dioxide in a human recipient. This capability depends on the interaction of a number of properties of the lung and its blood flow, including different lung volumes and capacities, lung elasticity, the rate of ventilation compared to the rate of blood flow to the lung, as well as the resistance and pressures in the lung's blood vessels.

In the upright human being blood flow decreases towards the top of the lung, but this distribution changes with varying postures and with exercise. The difference relates to pressure variations in the lung's blood vessels, compared to pressure in the gas sacs (alveoli) of the lung itself. Will the lung of a pig, which is a horizontal animal, adapt functionally to a vertical orientation in the human body?

Efficient gas exchange depends on an approximate match between the alveolar rate of air flow and blood flow to the lung. This is known as the ventilation-perfusion ratio, which in humans is about 1.

At rest, a pig's lungs receive a blood flow of between 1.4 and 6 litres per minute, depending on the size and breed of pig. The average figure is about 3.3 l/min, while the alveolar ventilation rate is about 2.4 l/min of air.[98] *In the resting human, the lungs receive about 5.25 l/min of air and 5-6 l/min of blood.*

In the patient with a pig's lung, the blood flow from the (human) heart will be about 5.25 l/min, while the alveolar ventilation rate in the pig lung is normally 2.4 l/min of air. This would yield an abnormally low ventilation-perfusion ratio of 0.46.

How will this mismatch, if it occurs, affect gas exchange in a xenotransplant patient? Unless a very careful choice of pig organ is made, the pig lung would be unable to provide sufficient oxygen and remove enough carbon dioxide for human needs.

Moreover, a pig's lung has evolved to receive the cardiac output of a pig's heart – often less than that of a human heart, unless large pigs are used. At what rate of human blood flow, which in a healthy person can rise fivefold to 25 l/min, would a pig's lung be damaged?

Infection and inflammation

Because the lungs are exposed directly to the outside environment as well as to the entire volume of circulating blood, their defence system must be very finely balanced. Under-activity results in repeated lung infections, while over-activity leads to inflammatory conditions of the lung tissues such as asthma, acute respiratory distress syndrome and fibrosing alveolitis.

A first line of defence against infection is the action of the cilia, hair-like projections in the airways which move a carpet of mucus up the respiratory tract to help expel foreign particles and micro-organisms.

Transplanting a lung causes a major disturbance to this mucociliary clearance, which can increase the risk of infection – especially important to patients whose immune systems are already

compromised by drug treatment. It is not known whether the mucociliary clearance rate in a pig's airways (i.e. the bronchi) would be adequate for a human patient.

There are species variations in the frequency of ciliary beating in the airways. The ciliary beat frequency in pig respiratory tract has been measured by different investigators as 5 beats per second,[99] and 11.3 – 16.9 beats per second.[100] In humans it is notably higher at 16.6 – 25 beats per second.[101]

Additionally, inflammatory mediators – which are themselves produced in the lung – have different effects on ciliary beating in different species. Varying doses of a leukotriene increased ciliary beat frequency by a maximum 75 per cent in guinea-pigs (at a dose of 10^{-9} moles per litre), by 119 per cent in rats (10^{-7} mol/l) and by 86 per cent in humans (10^{-6} mol/l).[102]

Prostaglandin E1 caused a maximum increase in ciliary beat frequency of 35.9 per cent in rabbits (at a dose of 1 microgram per millilitre), but the maximum increase seen in human tissue was 4.1 per cent (at 0.1 microgram/ml).[103]

Species variations of these kinds between humans and pigs would affect susceptibility to infections in patients receiving pig lungs – patients who are already at extra risk of infection because of the immunosuppressive drugs they must take.

The second line of defence for the lungs are the antibody- and cell-mediated systems.

White blood cells in the lung secrete antibody proteins, the immunoglobulins, which help fight infections. IgA is the principal immunoglobulin in human respiratory secretions, followed by IgG. These proportions are reversed in the pig's lung, where the major immunoglobulins are IgG followed by IgA, in the ratio of 1: 0.7.[104]

In human-to-human lung transplantation, immune system cells from the donor and recipient co-exist within the patient's body. The donor's macrophages and lymphocytes persist for up to three months, with a progressive colonisation of the lung by the recipient's cells. This probably accounts for the T-lymphocyte activation and the inflammation of the alveoli characterised by an increase in cell numbers, especially macrophages.[105]

A similar situation would be expected in animal-to-human lung transplants. The effect of defence cells of two different species mixing in the grafted organ is unknown. Pig immunoglobulins would probably be produced by the lung for at least the first several weeks, if the graft survived that long, with unpredictable effects throughout the body of the patient.

Bradykinin

Bradykinin acts locally in the lungs and also, via the circulation, in all parts of the body. It dilates the blood vessels, affecting blood flow, blood pressure, kidney function and the permeability of the capillaries. Eighty per cent of circulating bradykinin is inactivated in the lungs, probably as a way of protecting the delicate tissues from unwanted effects.

Bradykinin is inactivated by angiotensin-converting enzyme, found primarily in the capillaries of the lung. The lung also secretes carboxypeptidase M, another enzyme which inactivates a variety of peptides including bradykinin.

The level of activity of carboxypeptidase M varies between species. Activity of the enzyme in human lungs is lower than in baboon lungs, and less than half that found in dog lungs.[106] *There are also some differences in the enzyme structure between species.*

Bradykinin levels in the bloodstream need to be carefully regulated, but it is not known if an animal's lungs will be able to perform this function in a human body.

Prostaglandins

The lungs contain the enzyme prostaglandin synthase, which initiates the metabolism of arachidonic acid to prostaglandins. The different prostaglandins act throughout the body as mediators of inflammation, and as potent dilators and constrictors of blood vessels. They also affect blood clotting, and may play a part in the constriction of the airways experienced in asthma.

Prostaglandin synthase also activates some chemical carcinogens, such as the important polycyclic aromatic hydrocarbons and aromatic amines, to their toxic forms.[107] This has significance for lung toxicity, including cancer.

There are well-known species differences in levels of activity of prostaglandin synthase between rats and guinea pigs, the former having much lower activity than the latter.[108]

Similar differences between human and pig or baboon enzymes would affect prostaglandin synthesis, and alter the metabolism of toxic compounds in the lung. As the lung also inactivates some prostaglandins, with animal-to-human lung transplants the whole control system for prostaglandins could be perturbed.

7.3 Section Summary - The Lungs

As well as carrying out exchanges of oxygen and carbon dioxide according to the changing needs of the body, the lungs also have defensive and metabolic functions. Human-to-human lung transplants still have a much poorer success rate than other organs, but research is nevertheless underway to develop animal-to-human transplants.

Baboon lungs would be too small for transplant into humans, unless the patient was a small child – in which case, would they grow adequately as the child grew? Pig lungs are usually considered to be the best match for human adults, but if the organs are too small there may be persistent leakage of gases and fluid. Too large, and there is a risk of lung collapse.

Effective gas exchange depends on many features, including the rate of air movement through the lungs compared with the rate of blood circulation. In a human these rates are balanced, but in a patient with a pig's lungs the rates will be mismatched, which may be fatal. The pig is a horizontal animal, the human is designed on a vertical plane. Will gas and blood pressures in pig lungs allow full function in the vertical position? Will pig lungs be robust enough to cope with the larger blood output from a human, rather than a pig, heart?

Because the lungs are directly exposed to the outside environment, they are constantly at risk of infection. This risk is accentuated in a transplant patient whose immune system is depressed by anti-rejection treatments. The lungs have several lines of defence, starting with the mucus secretions which waft upwards through the airways, trapping and expelling particles and pathogens, driven by the beating of fine hairs called cilia.

This system operates more slowly in pig airways and responds differently to natural inflammatory messengers. Moreover, compared with humans, pig lungs produce different proportions of defence proteins called immunoglobulins. If pig lung defences are too active, a xenotransplant patient may suffer asthma, acute respiratory distress syndrome and fibrosing alveolitis. If underactive, repeated life-threatening infections would occur.

Levels of important natural chemical messengers are controlled by the lungs, and may be affected in a patient with pig lungs. In the case of bradykinin, this may alter blood flow and blood pressure throughout the body, as well as kidney function. With prostaglandins, effects would be seen on inflammation, blood flow and pressure, and possibly blood clotting.

8. The Heart

A man's heart changes his countenance, either for good or for eveil.

Apocrypha, Ecclesiasticus, 13:25.

The heart is commonly viewed as a pump for the circulation, but it performs this function in a far from mechanical manner, being constantly responsive to small changes in the demands of part or all of the body, via several feedback control systems. Although the transplanted heart is severed from its nerve supply, it still responds to hormonal control and to pressure, flow and resistance factors.

Will an animal's heart perform appropriately, or would a xenotransplant patient be unacceptably disabled by species differences in function?

8.1 Research and Clinical History

The first experiments in heart transplantation were performed in a dog and published in 1905, and the first clinical case was Dr Christiaan Barnard's unsuccessful human-to-human attempt in 1967.

Success rates for human-to-human heart transplants improved throughout the 1970s and again in the 1980s after the introduction of cyclosporin. Worldwide rates of survival after human-to-human transplantation are now about 75 per cent at one year, 70 per cent at four years, 60 per cent at eight years and 56 per cent at ten years.[109] Twenty per cent of patients die in the first three months after a heart transplant. These statistics put into perspective the long, slow process of development which was required to achieve acceptable human-to-human heart transplant survival rates.

Heart xenotransplantation research has been conducted between the following species: fox to dog; goat to calf; sheep to goat; guinea pig, mouse, hamster and pig to rat; hamster and rat to mouse; hamster, mouse, pig and rabbit to rat; monkey and pig to baboon; and pig to goat, monkey and baboon.

Compared with human transplant experience, xenotransplant research is still in its infancy. The latest developments published by the British company Imutran, part of Novartis Pharma, were relatively poor. Of ten baboons whose own hearts were replaced with decay accelerating factor-transgenic pig hearts, five died within 18 hours, four survived for 4-5 days and one was killed after nine days, with its xenotransplanted heart still functioning.[110]

To date there have been several known cases of human patients being given animal hearts, although not all have been published and none achieved long-term survival.

In 1964, Dr James Hardy and colleagues transplanted the heart of a 96-pound chimpanzee into a male patient (having obtained an unacceptably brief consent form signed by a relative). Although prior to the operation the chimpanzee's heart had an output of 4.25 litres per minute, there were signs of right heart failure almost immediately and the patient died after two hours. The chimpanzee's heart was thought to be too small to cope with the cardiac return.[111]

Four years later, Dr Denton Cooley transplanted the heart of a sheep into a patient, who died almost immediately.[112] Also in the 1960s, a pig heart was transplanted into a human but failed after four minutes; another recipient of a chimpanzee heart died shortly after the operation.[113]

In 1977, Dr Christiaan Barnard and his team transplanted the hearts of a chimpanzee and a 30-kg baboon into two patients, without removing their own organs.[114] The baboon heart was too small to sustain the circulation and the chimpanzee heart was rejected after four days.

The case of Baby Fae occurred in 1984. Dr Leonard Bailey and his team replaced the heart of a 2.2-kg baby with that of a baboon. Death occurred after 20 days, due to a progressive fall in cardiac output linked with rejection.[115]

A pig's heart was transplanted into a Polish patient in 1992. The relatively long survival time of 24 hours was considered to be due to an anti-rejection procedure, in which the patient's existing antibodies to the pig organ were reduced prior to the operation.[116] The cause of the patient's death was attributed to the pig's heart being too small to provide an adequate output.

8.2 Functional incompatibilities

The genetically engineered pig is the most likely source animal for hearts for xenotransplantation, as the baboon heart is too small except possibly for very young children. Because pigs are used widely in cardiovascular research, much is known about the function of their hearts *in situ.* Almost nothing is known about their function in a human body.

Haemodynamic characteristics

It is usually claimed that the haemodynamic characteristics of pig and human hearts are broadly similar. However, it is difficult to generalise about cardiovascular function *even between different breeds of pig.*

A study comparing the haemodynamics of three breeds of pig found significant differences in mean arterial pressure and pulmonary vascular resistance:[117]

Table: Haemodynamics of three breeds of pigs

	Hanford pigs	Yucatan minipigs	Yucatan micropigs
Mean arterial pressure (mm mercury)	89	48	53
Pulmonary vascular resistance (dyne x sec/cm x m^2)	9	60	111

Selecting the right breed of pig to suit all human needs – at the level of haemodynamic parameters, size and pharmacology – will be a difficult task when there are notable differences even between breeds of pig.

Heart size and cardiac output

Studies with transplant patients demonstrate that, even though some size adaptation may occur in the months after the operation, if the donor human heart is too small it does not function well. In such cases the output of the transplanted heart at rest may be maintained in terms of volume of blood pumped per minute, but only at the cost of the heart working harder, for example by an increased number of beats per minute.[118] Transplant patients with hearts from smaller donors were less able to tolerate exercise.[119]

As pointed out by Dr Julia Greenstein, Chief Scientific Officer at the US company BioTransplant, which is researching xenotransplantation,

"The problem with domestic pigs is that they can grow to be 1000 lb in weight so no-one knows whether, if you transplant a pig heart into a 150 lb man, it will be constrained by the physical size of the recipient or will continue to grow until it is the size it would have become in the pig".[120]

Size disparities with xenotransplanted hearts from chimpanzees, pigs and baboons have already caused patients' deaths, as the organs failed to cope with the demands of a human body (see *Research and clinical history*, above). Because there are no medium- or long-term data, we do not know whether a transplanted animal heart would grow to an appropriate size in the human body.

The effect of transplanting a heart which is not carefully matched for size can be quickly fatal: two out of 10 baboons whose hearts were replaced with transgenic pig organs died within hours, because size mismatch led to blood clots forming in the pulmonary artery leading to the lungs.[121]

The hearts of some pigs are approximately the size of some human hearts, so size matching would theoretically be possible.

However, heart weight-to-body weight ratios vary even between different breeds of pig. In the Hanford miniature pig, this ratio is 4.6, significantly different from the ratio of 5.7 seen in the Yucatan miniature pig.[122]

The fact that a pig is a horizontal animal, while a human is a vertical animal, poses a question about the ability of a pig heart to circulate blood adequately to the upper regions of the human body, especially the brain. When asked this question at a conference in 1996, [123] Mr John Dunning, consultant cardiothoracic surgeon at Papworth Hospital, Cambridge, UK, replied that pigs could be trained on a treadmill to increase the efficiency of their hearts prior to being killed for their organs.

At rest, the output of a pig's heart is between 1.4 and 6 litres per minute (averaging about 3.3 l/min) depending on the size and breed of pig. In the resting human, the equivalent cardiac output is generally 5-6 l/min of blood.

Due to the loss of its nerve supply, the cardiac output of a transplanted human heart is generally poorer than normal. For the same reason, any shortfall in output from an animal's heart would also be accentuated after transplantation. Indeed, in research published in 1998, some transgenic pig hearts transplanted into baboons failed to produce even a low cardiac output.[124]

In human-to-human heart transplants, multiple organ failure is a serious problem. A reduction in cardiac output can cause a comparatively larger drop in the splanchnic circulation of the digestive tract, activating a cascade of inflammation leading to organ failure.[125]

Thus the output of a transplanted animal heart is crucially important for a patient's survival and quality of life, but at present there is very little information about the capability of an animal organ to fulfil these requirements.

The heart's response to drugs

The loss of nerve supply which occurs in transplant surgery causes a depletion of natural neurotransmitters – the catecholamines – in the heart muscle cells, and probably alters the densities of adrenergic receptors. The transplanted heart becomes super-sensitive to catecholamines.

Adrenaline is secreted by the adrenal gland into the bloodstream and its levels in the circulation thus continue to affect a transplanted heart. Moreover, adrenaline or noradrenaline are given therapeutically for a number of cardiovascular conditions. There are notable species differences in the density of adrenergic receptors in the heart and in its response to such drugs, and an animal's heart in a human body may act unpredictably and inappropriately.

For example, the heart cells of the dog have far fewer alpha 1-adrenergic receptors and far more beta-adrenergic receptors than the heart cells of the rat or rabbit, and the receptors of the different species show varying selectivities for drugs.[126] *Moreover, the effect of noradrenaline in causing stronger heart contractions is nearly two orders of magnitude higher in the cat than in the human.*[127]

Levels of adrenaline in the bloodstream are very different in pigs and humans. In humans, the mean level is 380 picograms per millilitre (range 150-820 pg/ml), while Hannon measured adrenaline levels of only 69 pg/ml (20-132 pg/ml) in the pig – less than one-fifth the concentration found in the human bloodstream.[128]

How would a pig heart respond to this higher level of adrenaline in the human? The question, like so many, remains unanswered. However in very recent research, disorders of heart rhythm killed within hours a baboon whose own heart was replaced with a transgenic pig organ.[129]

There are species differences in the response of the heart to other drugs, whether produced naturally by the body, or taken by the patient. Astonishingly, a drug may have opposite effects on the hearts of different breeds of the same animal:

The hearts of two different breeds of pigs reacted disparately to a dose of the drug cocaine.[130] *In the Yorkshire pig, cocaine had a depressant effect on the heart, decreasing mean arterial pressure as well as rate pressure (blood pressure x heart rate). In the Yucatan miniature pig, cocaine increased both these parameters.*

This kind of variability would make selection of an appropriate breed of pig as a source animal for xenotransplants exceedingly difficult.

Blood clots

A substantial problem in ordinary human heart transplants is the abnormal behaviour of the platelets, found in the bloodstream. These cells tend to clump together spontaneously, especially in the transplanted organ, which can lead to life-threatening blood clots.

This problem could be exacerbated in patients receiving a pig heart: In the healthy human body, a substance called von Willebrand Factor released from blood vessel linings causes platelet aggregation – but only in the presence of shear stress. However von Willebrand Factor from pigs

can bind to human platelets and cause platelet aggregation even without shear stress.[131] This may increase the likelihood of platelet build-up in the blood vessels of a transplanted pig heart, with a higher risk of fatal blood clots for the human recipient.

Loss of nerve supply

The transplanted heart, whether human or other animal, has lost its nervous connections – both incoming (efferent) and outgoing (afferent). The consequences of this denervation of transplanted human hearts have been known for many years. One effect is a more rapid resting heart rate, which in patients with a transplanted human heart is in the range of 88-104 beats per minute, compared with 60-80 beats/min of the normal human heart.

This effect of transplantation may be exaggerated with a pig heart. The normal resting heart rate of these animals is reported to be 111-123 beats per minute, [132] *notably higher than the comparable rate of a resting human heart. After denervation and inside the xenotransplant patient, a pig heart may well be beating at the rapid rate of 160 per minute, even at rest.*

Exercise capacity

In transplant patients with a human heart, the grafted organ is rather slow to adjust to the onset and cessation of exercise. Patients with hearts from smaller donors adapted even more poorly to exertion.[133] The precise causes are not known, but are linked to the loss of nervous supply and supersensitivity to catecholamines such as noradrenaline in the bloodstream.

However, as we have already seen, there are notable species differences in the density and type of adrenergic receptors in the heart, as well as the magnitude of the heart's response to catecholamines. It is possible that a patient with an animal's heart may be more restricted in the amount of exercise they can manage, and hence in their quality of life.

8.3 Section Summary - The Heart

It is essential for transplant success that a grafted heart matches in size the needs of the recipient. This may be difficult with animal organs, since even within different breeds of pigs, heart weight-to-body weight ratios can vary significantly.

Several patients have already died because the baboon, pig or chimpanzee hearts they were given could not cope with the demands of the human body. If an animal's heart is too small, a xenotransplant patient would be seriously disabled, and perhaps little better off than before the operation. Size mismatches can also cause life-threatening blood clots.

An animal heart will need to pump the right volume of blood round a human body, at the right pressure. Despite a faster heart beat, a pig's heart normally pumps lower amounts of blood per minute than required by a human patient. One specialist has proposed that source pigs would need to train on a treadmill to boost their heart performance – after all, a pig's heart has developed to pump blood around a horizontal pig body, rather than a vertical human one.

If the output of the heart is too low, multiple organ failure and death would result. Moreover in pigs, normal blood pressures vary surprisingly even between different breeds. Selecting a breed which fulfils all the necessary requirements may be difficult.

There are species differences in the sensitivity of the heart to natural chemical transmitters such as adrenaline, whose levels in a pig's circulation are also much lower than in that of a human. Such

discrepancies may lead to difficulties in the heart adapting to changing conditions, or to disorders of heart rhythm.

Compared with humans, animals' hearts respond differently to a number of drugs. Drugs sometimes even have completely opposite effects in different breeds of the same species. For example, in the Yorkshire pig cocaine depresses the heart, but in the Yucatan miniature pig the heart is stimulated. Since xenotransplant patients would be taking a cocktail of medicines, the consequences are unpredictable. Is there one breed of pig whose heart is the right size and reacts in a human way to all the medicines a patient will need to take?

The lining of the blood vessels in a pig heart releases a substance called von Willebrand Factor. Because of a mismatch with cells called platelets in human blood, blood clots will be prone to forming in the organ, causing heart damage or death.

9. Human-animal Hybrids - The New Chimeras

What a chimera is man! What a novelty! What a monster, what a chaos, what a contradiction, what a prodigy! Judge of all things, imbecile worm of the earth.

Blaise Pascal, 1623-1662, Pensées.

A chimera is a mythical beast – such as the lion-eagle chimera called the griffon – combining features of different animal species. Modern science has brought the myth to life, for example by the creation in the 1980s of the geep, a sheep-goat chimera. A scientific definition of a chimera is an organism which contains cells or tissues of more than one individual or species. The geep is only one of many other human-created, but in most cases unpublicised, chimeras.

It is not well known to the public that with human-to-human transplants, cells from the donated organ disperse throughout the body of the recipient and persist for many years. In the early 1990s it was found that people who had received human kidney transplants 30 years before had white blood cells (leucocytes) from the donor, which had spread throughout their tissues.[134] The leucocytes were "passengers" in the donated organ, having derived originally from the donor's bone marrow.

Following this discovery, human donor cells were found in other organs in transplant recipients, especially the liver. Thus a transplant patient with a human organ is a chimera of two individuals.

When animal organs are transplanted into patients, the same process occurs. In this case the recipients are chimeras of two different animal species. This was shown in the 1992 experiment by Dr Thomas Starzl, in which a patient received a baboon liver to replace his own failing organ. For the 70 days of his survival, this man was a baboon-human chimera, formed by a process which Starzl refers to as "baboonization" (*op.cit.*). Baboon DNA, representing traces of baboon white blood cells, was found in every tissue of the patient's body which was tested, including the heart, lungs, kidneys and lymph nodes.

Dr Starzl, along with many other scientists, believes that widespread chimerism is more than an inevitable outcome of xenotransplantation; they see it as a necessary prerequisite for success. The view is based on many animal xenotransplant experiments, because the animals who survived for the longest periods of time had cells from the source animal species spread widely through their bodies.

The theory is that the existence of the source animal's bone marrow cells in the recipient may provide some long-term protection from graft rejection. For this reason, Starzl's second baboon-liver transplant patient was actually given an infusion of baboon bone marrow cells, with the intent that these cells should establish themselves permanently in the patient's bone marrow.

Dr Starzl's two liver xenotransplant patients not only had baboon cells distributed throughout their body tissues, but the grafted organs naturally produced baboon products. These included the following:[135]

- The grafted liver produced baboon blood-clotting factors in the human patients.
- Transferrin, a bloodstream protein which carries iron from dying red blood cells to the bone marrow for recycling, became baboon transferrin.
- The liver enzyme alkaline phosphatase became baboon enzyme.
- The patients developed baboon complement protein C3 rather than human C3, because the liver is the primary source of this protein.
- The patients' serum albumin fell to the very low bloodstream levels seen in baboons, less than half the normal human levels.
- The amount of uric acid in the patient's blood also dropped to the baboon range – only one-tenth normal human levels.
- Bloodstream cholesterol fell to baboon levels, about one-third of the normal human amount.

There are two obvious concerns arising from the chimerism which is bound to occur in animal-to-human transplants. Firstly, what will be the physical effects? This section has covered in some detail the possible problems arising from the production, by an animal organ, of animal rather than human proteins and other factors.

The overall effect of these functional incompatibilities on a person's health and wellbeing, let alone survival, is almost impossible to judge. The physical implications of animal white blood cells migrating to all the tissues of the recipient's body is not easy to assess either, but certainly it means that cells carrying the DNA of animal viruses will spread those viruses to every corner of the patient's body.

However, chimerism may also pose enormous psychological problems for the xenotransplant patient. Many patients may be prepared to accept an animal's organ into their bodies on the understanding that its 'animal presence' is strictly confined to one organ, in one place. Most people do not realise that this animal presence cannot be prevented from spreading to every other organ and tissue.

This will fundamentally challenge some people's concept of themselves as a human being and their notions of selfhood, as well as affecting how others see them. If fully informed, some will turn down an animal organ transplant for these reasons, but others may accept only to find it very difficult to come to terms with.

We know, moreover, that the state of mind of a transplant patient with a human organ is critical for their subsequent recovery and continuing health and survival. This is illustrated by the fact that the 15-25-year-old group of human-heart transplant patients has a higher death rate than recipients aged 50 or over, due mostly to higher rates of suicide and refusal to comply with drug regimes.

Chimerism is an issue which should be addressed fully and urgently by regulatory bodies. As general awareness of this consequence of xenotransplantation is very low, it is vitally important that the wider public consultation which we recommend includes the implications of chimerism.

9.1 Section Summary - Human-animal hybrids, the new chimeras

It is not widely known that xenotransplant patients will have animal cells (pig or baboon, for example) dispersed in every organ in their bodies, including their hearts, lungs, kidneys, bone marrow and lymph nodes. Effectively, they become a scientifically-created, living embodiment of the once-mythical concept of the chimera – a creature which combines features of different animal species. Indeed, transplant specialists believe that this spread of animal cells is a prerequisite for success in xenotransplantation.

Most people wrongly imagine that, following a xenotransplant, the animal 'presence' is discrete, controlled and contained within the animal organ in only one part of the body. Chimerism inevitably has physical effects, which have been discussed in detail in other sections. However, it will also have powerful psychological and emotional consequences, challenging a patient's sense of self and affecting how others see them. A transplant patient's state of mind is known to impact on their health and survival.

The little-known fact of chimerism must be discussed openly, and addressed fully before any clinical trials are approved.

10. Animal Welfare and Ethics

An animal can be viewed as a set of genetic potentials.

Dr Caird Rexroad Jr, of the Agricultural Research Service, United States Department of Agriculture, Beltsville, USA.[136]

10.1 Ethics

That renowned medical journal *The Lancet* may not be regarded as a radical publication - and certainly not an animal rights mouthpiece. So what led them to publish an editorial in January 1997 which ran "Xenotransplantation with transgenic animals involves an entirely new form of exploitation: manipulating the genes, not by selective breeding, but by insertion of genetic material from another species"? [137]

The science of genetic engineering has indeed posed new ethical dilemmas:

- Is it ethical to add human genes to other species in order that we humans may utilise their genetically manipulated organs as spare parts when our own organs fail us?
- From where do we get the right to change the genome of another species?
- If the procedures involved inevitably cause animals to suffer, can we justify this suffering?
- Is it 'good' for one species to endure new forms of imposed suffering so that another species may (possibly) enjoy a prolonged lifespan?
- Is xenotransplantation speciesism taken too far?

At the core of these questions lies the ultimate question of the traditionally accepted primacy of the human. That primacy appears to be rooted in the Christian concept of the soul, which apparently has been designated only to the human, and which puts our status way above that of genetically similar but allegedly soul-less creatures, such as primates or pigs.

The other source for human primacy appears to be the assumption that as evolution has progressed our intellectual and communicative abilities over time, so that we truly appear to 'control the earth and the creatures in it', this technological superiority carries with it an innate worthiness - we *matter* more than other species.

Both these widely-held views would probably lead to an acceptance of xenotransplantation. Yet an honest look at them surely reveals that they are assumptions - one based on a religious belief, the other on a basically fascist interpretation of the role of various species of living creatures - to put it crudely, might is right.

However, the public is apparently already very divided on the acceptability of xenotransplantation. In a letter to *The Lancet*, Mohacsi and colleagues published the results of a survey of acute-care nurses and patients with kidney failure. Sixty-six per cent of acute-care nurses were opposed to xenotransplantation, and of the patients (who represented the group most likely to benefit from xenotransplantation) only 48 per cent believed it to be appropriate to breed animals to provide organs for humans. Only 42 per cent said they would accept such an organ.[138]

We believe that before xenotransplantation goes any further, there needs to be a full, honest and open debate of the ethical issues raised. With an ever-increasing number of people expressing belief in some sort of 'animal rights' and with the public apparently already very divided on the acceptability of xenotransplantation, this is an ideal opportunity to air these fundamental issues before any final decisions are taken.

10.2 Welfare

The current state of knowledge in the neurosciences amply demonstrates that animals are provided with cognitive abilities which, without reaching the degree of development of human beings, are sufficient for us to acknowledge that animals can feel not only physical but also psychic suffering.

Dr R Dantzer, INSERM, France.[139]

The report entitled *Animal Tissue into Humans*[140] by the UK's Advisory Group on the Ethics of Xenotransplantation (hereafter referred to as the Kennedy Report) admitted that animals involved in the xenotransplantation programme will suffer.

In fact the Kennedy committee was so certain that suffering would be involved that they recommended against using primates as source animals for organs, saying *"We therefore conclude that it would be ethically unacceptable to use primates as source animals for transplantation, not least because they would be exposed to too much suffering"* (op.cit.).

However the Kennedy committee took this strong view on primates due to a belief that primates "have close affinities with humans ... not least by virtue of their greater self-awareness and mental capacity". The committee declared "these features increase their capacity for suffering".

We agree with the Kennedy Report that primates should not be used as source animals for organs for xenotransplantation. However we believe that there is no scientific or empirical reason for distinguishing between primates and pigs, as the Kennedy committee has done.

10.3 Pigs

Pigs are known to be highly intelligent creatures. They naturally live in family groups; the pregnant sow will build a nest, maybe a metre high, in which to give birth and nurse and protect her piglets. When sows are closely confined, they display characteristic states of psychological distress, i.e. severe escape reaction followed by inactivity and unresponsiveness, and then the development of stereotypic behaviours such as bar-chewing.

These states are similar to those recorded in the development of affective disorders in humans: conflict, learned helplessness and psychoses or neuroses. People suffering from obsessive-compulsive neuroses usually develop stereotyped behaviour such as hand-wringing. These behaviours are reckoned to reduce anxiety in humans, in much the same way as stereotypies in sows are believed to reduce stress. Pig behaviourist Dr Mike Baxter says the two may provide to be "etiologically identical", and concludes that the way sows adapt to confinement "resembles in many respects the development in humans of chronic psychiatric disorders".[141]

Donald Broom, Professor of Animal Welfare at Cambridge University, England, wrote (with Johnson),

"It would be surprising if animals such as our domestic animals, all of which have an elaborate social organisation, did not have feelings similar to many of those of man. A significant consequence of this is that if the various regulatory system components which are manifested as feelings are present in a species, there is a potential for suffering, and that is clearly of great importance both biologically and when considering moral questions" .[142]

At the time of writing, a Bristol University team is studying the theory of mind of pigs, in an attempt to assess the pig's level of self-awareness. Published papers are expected in late 1999.

Whilst the Kennedy Report [143] accepted that all animals have a 'right' to consideration, it went on to base the extent of this right on the animals' capacity to suffer. Without any further justification, the Report then listed the hierarchy of their capacity to suffer as: humans, higher primates and lower animals.

If we look at the types of suffering involved it may make it easier to decide about the acceptability of imposing such suffering on animals.

Looking at the types of suffering involved may make it easier to decide about the acceptability of imposing such suffering on animals.

10.3.1 Genetic engineering of pigs

Currently, the usual method for achieving genetic modification of pigs is micro-injection of the fertilised egg with many copies of the desired gene. The genetically engineered embryo is then surgically implanted into a surrogate mother, who carries it to term.

In the case of xenotransplantation, the aim is for the pig to acquire genes which make its organs less likely to be quickly rejected by the human recipient. Alternatively, the pig could have genes deleted for the same reason.

10.3.2 Can genetic engineering itself cause harm?

The scientific literature carries several examples of the genetic engineering of pigs. Many of the transgenic pigs have had health problems and have suffered.

In 1985, scientists at the US Department of Agriculture's Beltsville Research Center genetically engineered pigs with human or bovine growth hormone genes, to produce leaner, faster growing pigs. Many of the resulting transgenic animals suffered severe health problems such as damaged vision, deformed skulls, inability to walk and increased susceptibility to gastric ulcers and pneumonia. [144]

In 1992, Beltsville scientists put a chicken c-Ski gene into pigs, again to increase muscle. Five transgenic pigs showed increased muscle growth. Five other transgenic piglets developed floppy and weak muscles in their hind legs.[145]

In 1997, Beltsville scientists put a sheep growth hormone gene into pigs. This gene was to be 'turned on' by zinc in the diet. One pig showed a 20-fold increase in growth hormone levels. The day after the zinc supplement was withdrawn, she died from acute gastric haemorrhage due to ulceration. The scientists concluded "The development of a controllable transgene expression system that functions in farm animals is still greatly needed".[146]

These experiments show that genetic engineering is still an extremely hit-and-miss technology. Transgenic piglets differ in the effects they display, and many display unexpected effects. Many obviously suffer. There is no reason to believe that the experiments to breed transgenic pigs for xenotransplantation are likely to have a better record.

In 1992, Dr David White introduced a reconstructed human gene into pig embryos in the hope that the gene would synthesise the complement-blocking decay accelerating factor. White's company Imutran (part of Novartis Pharma) have now bred many more transgenic pigs which have the complement-blocker in their tissues and organs. In none of the published papers associated with this work have any protocols for developing the animals or statistics of failure rates or adverse effects, been described.

Another approach to producing transgenic pigs whose organs are suitable for xenotransplantation is to eliminate antigens on the pig organs. One such antigen is the Gal epitope gene, which leads to hyperacute rejection if the organ is transplanted into a different species. When the Gal epitope gene was eliminated from mice, it was shown that less human antibody bound to their cells. However the transgenic mice all developed cataracts and were blind.[147]

This raises the question: would society find it ethically acceptable to breed blind transgenic pigs as source animals for organs?

As is usual in transgenic research, only a small proportion of piglets born from micro-injected embryos carry the human gene. To date, only 0.1 – 4 per cent of modified embryos from livestock species are successfully transgenic.[148] Many other micro-injected embryos fail to survive implantation. So, apart from the unpredictable effects of the micro-injection technique itself, there is also a large amount of wastage - wastage in terms of animal lives and wasted operations on female pigs who have ova extracted and embryos implanted, all of which cease to be viable. The whole procedure therefore inevitably involves much unnecessary suffering.

10.3.3 Adverse Health Effects - the uncharted area

The genetic engineering of pigs for xenotransplantation has not produced scientific papers which deal with unexpected and adverse effects in the transgenic pigs, nor has the 'success rate' been published. These questions can only be inferred from the literature.

The UK company Imutran has been breeding transgenic pigs for several years, and in a 1997 paper Dr David White and Dr Johan van den Bogaerde described the placing of hearts from transgenic pigs into cynomolgus monkeys.[149] They used organs from heterozygous pigs, that is, pigs with the transgene on only one of a pair of chromosomes. They go on to explain that the ideal would be to use pigs bred to homozygosity (with the transgene on both of a pair of chromosomes) - but admit,

"Of all the pigs produced to date, only one line fulfils these stringent criteria. This illustrates the difficulties inherent in the production of transgenic animals for possible clinical use".

In other words, after all these years, Imutran has only successfully bred one line of homozygous transgenic pigs. It can only be conjectured that the number of such homozygous transgenic pigs is severely limited, otherwise they would presumably have been used in the xenotransplantation research programme. It could be surmised that in fact they may have encountered severe health problems with the homozygous line, as they say, "In addition, breeding to homozygosity might cause undesirable effects to the stability and health of the pigs".

A complete statistical breakdown of all transgenic pigs produced in the UK for xenotransplantation research should be published by the government. This should include the number and proportions of heterozygous and homozygous transgenic pigs produced, together with a detailed analysis of their health records, as well as the failure rate for micro-injected embryos. These data would allow a proper understanding of the adverse welfare effects associated with the genetic engineering of pigs.

10.3.4 Breeding

It is important to evaluate the level of suffering caused by the reproductive technologies used to obtain the founder transgenic animals for a xenotransplantation programme.

To obtain ova for micro-injection, female pigs will be given hormone injections to induce super-ovulation - the production of many more ova than usual. They will then be artificially inseminated. The sows will then usually undergo a laparotomy operation - a cut through the side to extract the fertilised ova. The operation tends to result in adhesions.

After micro-injection in the laboratory the tiny embryos are then surgically implanted into surrogate mother sows, whose oestrus cycles have been synchronised with hormone injections. Sometimes in genetic engineering experiments a temporary surrogate (possibly a rabbit, or another pig) may be used, killed after a week or so and the embryos checked for viability before being implanted - again by surgery - into the final surrogate.

For the xenotransplantation programme it is certain that natural birth will be rejected in favour of the specific pathogen-free (SPF) method. This entails surgical removal of the entire uterus (hysterectomy) with the piglets being placed immediately into a sterile bubble and transferred to an isolated rearing pen, where the environment is kept as pathogen-free as possible.

Piglets naturally suckle from their mothers for 9-20 weeks. In intensive farming the norm is around

3-4 weeks. In the case of piglets bred for the xenotransplantation programme, the piglets' mothers will have been killed after the SPF hysterectomy. The piglets will therefore be artificially fed from the outset and kept in isolators for two weeks.

According to the Kennedy Report [150] on the ethics of xenotransplantation, the source animal herd would be established as an SPF herd, with all piglets born via hysterectomy or hysterotomy (caesarean), taken from the sow and reared in isolation. After two weeks on sterilised food they would be placed in biosecure rooms and weaned onto a sterilised pig diet. When mature they would be the foundation stock for a new SPF high-health status herd or for xenotransplantation. From that point on, once the herd was established, the source pigs would be born to sows allowed to farrow and the piglets weaned early at 10-21 days, and then removed to biosecure rooms.

It is quite possible that the breeding sows for this herd could be kept in narrow stalls or tethers, unable to turn round throughout their 16½ week pregnancy.

However, the use of sow stalls and tethers will become illegal on agricultural land in the UK as from 1st January 1999. It would be completely wrong if these systems could still be used after that date in respect of sows bred for xenotransplantation.

In addition, it is suggested that sows would farrow in farrowing crates, common practice in intensive pig farming. However the farrowing crate is an even more restricted device into which sows are placed shortly before giving birth, and are kept there whilst the piglets are suckling. Farrowing crates have severe adverse effects on the welfare of sows. The crate is so narrow that the sow cannot even turn round. Under normal, unrestrained conditions, shortly before farrowing (giving birth), sows show a general increase in activity and a strong pre-natal instinct to nest-build. Research shows that in a period of about 20 hours of pre-natal activity, the sow can travel a distance of up to 30 kilometres.[151] All this activity is denied to a sow who is placed in a farrowing crate.

Research has also shown that alternatives to the farrowing crate - if well-designed and well-managed - can be just as effective in reducing piglet mortality and will give a much higher standard of welfare for the sow.[152]

10.3.5 Cloning

With micro-injection being so unreliable and viable homozygous lines of transgenic pigs being so hard to develop, it is not surprising that xenotransplantation researchers are apparently leaping at the chance of using the new cloning technologies to clone their 'successful' transgenic animals. Already scientists at Roslin Institute in Edinburgh, Scotland, have produced Polly, the first sheep to be both cloned and genetically engineered. The UK company PPL Therapeutics is said to be planning to clone pigs for xenotransplantation.

But cloning too appears to have inherent welfare problems. Referring to calves, a leading American cloning expert, Dr George Seidel, reckons that abnormalities occur in one per cent of calves produced by natural mating, 10-15 per cent of calves from embryos cultured *in vitro* and close to 50 per cent of calves cloned by nuclear transplantation.[153]

This type of failure rate was seen when the first two cloned lambs, Megan and Morag, were produced at Roslin Institute. Three other cloned lambs were born in the same batch, two died shortly after birth, the other at 10 days. The published paper in *Nature* failed to mention that the three lambs who died all had malformed internal organs. It also failed to mention the abnormally large birth weights of the lambs.[154]

Abnormal birth weights seem to be an inherent problem with cloned animals. A calf-cloning experiment in the US produced 40 clones, many with abnormal birth weights.[155] The heaviest calf could not stand without external support, six others had limb contraction problems. Thirty-four of the calves required veterinary therapy for conditions ranging from weakness to hypothermia to hypoglycaemia. Eight died.

To obtain clones, all the paraphernalia of ova retrieval, surgical embryo transfer to surrogates (and often the use of temporary surrogates) have still to take place. On welfare grounds, cloning is a disaster.

We believe the levels of suffering experienced by sows in surgical embryo retrieval and embryo transfer, necessary to establish a pig herd for xenotransplantation, cannot be justified.

10.3.6 Rearing

The piglets born to these sows - or those who turn out, after testing, to be transgenic - will be reared in biosecure rooms, where surfaces are likely to be of materials such as stainless steel. Such an environment will undoubtedly prove stressful to the pigs. Research has shown that pigs would normally spend over half their daylight time rooting in the soil with their long, sensitive snouts, looking for roots or insects to eat. They spend around another quarter of their time in exploratory behaviour. [156]

Pigs also choose to live in family groups. Keeping them in barren, biosecure rooms where any type of bedding or rooting material is unlikely, possibly in individual isolation (to minimise any risk of transmission of infection), will of necessity cause intense deprivation and frustration of natural instincts and social behaviour. Contact with humans would also be strictly limited to avoid sources of infection or contamination.

The SPF method of birth followed by rearing of piglets in isolators and biosecure rooms will cause grave mental and physiological suffering, and cannot be justified.

10.3.7 Monitoring

All piglets born after micro-injection at the embryo stage will have to be tested to see if they are transgenic. In addition, they will be monitored for pathogens by blood tests and swabs of the nose and tonsils. They are likely also to be vaccinated and given prophylactic medication to prevent possible infection. They will also need to be transported in sterilised vehicles to centres for retrieval of tissue for testing. Some ‘sentinel’ pigs will be killed for microbiological screening.

There is also the possibility of tissue or solid organs being removed sequentially from the same pig, with all the inherent distress of anaesthesia and surgery on a repeated basis.

Finally, of course, the pigs will be operated on for removal of organs and be killed. Perhaps, in view of this long list of practices, many invasive, others imposing psychological and social deprivation, it is not surprising that the Kennedy Report concluded: [157] “We regret that animal suffering is caused”.

10.3.8 Training

In addition to the isolated rearing conditions and repeated blood and tissue sampling of transgenic pigs, there is a real possibility that pigs whose hearts are intended for xenotransplantation will be

forced to exercise on a treadmill, so that their hearts become trained to generate an output necessary for an upright human body as opposed to a horizontal four-legged pig body. This may be distressing to the pigs.

We believe that suffering which will be experienced by transgenic pigs used as source animals in the xenotransplantation programme is unjustifiable, and that the programme should be halted forthwith.

10.4 Primates

Whilst the Kennedy Report recommended against the use of primates as source animals for organs for xenotransplantation into humans, the previous UK government disagreed with any absolute ban on their use, saying such a ban would be premature .[158]

The new government has again not taken up an absolutist position, simply saying that whilst the great apes (chimpanzees, gorillas and orang-utans) will not be used as source animals, "We ... take the view that there should be a strong presumption against the use of any other primates as sources for xenotransplantation".[159]

10.4.1 Breeding

The establishment of a specific pathogen-free (SPF) colony of primates would be extremely difficult, because of a likely lack of suitable founder animals and because primates breed slowly, especially in captivity, and take a long time to mature. If this was, nevertheless, to be attempted there is no doubt that breeding methods would cause great distress.

Young primates form a close bond with their mothers and under natural conditions are not weaned until over six months old. Indeed, in the wild, mother and young may remain together for a much longer period. Separation at an earlier age, if early weaning techniques were used, or even at birth if the SPF method involving hysterectomy were used, would clearly cause intense deprivation. An international workshop held to review laboratory animal accommodation recommended against separation of mother and infant primates earlier than six months.[160]

In addition, the establishment of a new breeding colony in the UK would involve importation of primates from countries such as Kenya. Primates imported for research travel packed in small crates in the cargo holds of aeroplanes, where they suffer from cramped conditions, inadequate ventilation, noise and extreme fluctuations in temperature. Total journey times are often over 48 hours including travel by air, road and sea, and deaths in transit are not uncommon.

If wild, rather than captive-bred, primates were imported for a breeding programme, further suffering would be caused due to the trapping and holding processes. It has been estimated that eight out of ten primates trapped in the wild do not survive to reach the laboratory.[161]

10.4.2 Rearing

The Kennedy Report recommended against the use of primates as source animals for xenotransplantation on the grounds that they would be caused too much suffering. Certainly, the suffering endured by primates when held in captivity in comparable conditions is widely recognised.[162]

Primates are highly intelligent, social and very active animals. It is difficult, if not impossible, to provide living conditions in captivity which even approach satisfying their varied and complex

basic needs. If kept in barren cages with no environmental enrichment, often alone, they experience enormous suffering. Unable to practise their natural behaviour, they develop psychological damage evidenced by pacing, endless head-weaving, self-mutilation and abnormal social behaviour. As with pigs, primates used for xenotransplantation would be caused additional suffering due to limited contact even with humans, and by monitoring for infection.

10.4.3 Research

Primates have been used as experimental animals in xenotransplantation research in the UK, and this has exposed them to extreme suffering and distress. Cynomolgus monkeys have had hearts from transgenic and control pigs transplanted into their abdomens. Those receiving control hearts suffered hyperacute rejection within three hours. Monkeys receiving transgenic hearts lasted up to 62 days, but received such high doses of immunosuppressive drugs that they were 'sacrificed' due to gastrointestinal complications.[163]

A report [164] of another experiment with similar monkeys referred to "Daily brief sedation ... allowed for assessment of wound integrity and graft function". These monkeys were kept on drips. Tissues and blood were regularly removed from them for analysis. One monkey suffered hyperacute rejection; another rejected on day 11. Two others survived to 21 and 32 days but "were killed due to systemic infections". Another developed a septic condition and another severe cytomegalovirus pneumonitis. Both died.

The paper [165] by van den Bogaerde and White described similar hyperacute rejection of control hearts from non-transgenic pigs. Five animals who had received transgenic hearts had to be killed "due to gastrointestinal toxicity, resulting in severe diarrhoea". This was apparently caused by the immunosuppressive drugs.

All these animals - and they represent the tip of the iceberg - have suffered acutely. It is surely time to ask if such experiments are justifiable.

The use of primates or other animals as experimental recipients of xenotransplants has caused massive suffering, and we call on the UK government to halt this research. Instead, resources could be put into improving the supply of human organs; continuing clinical trials with artificial and bioengineered organs; and researching humanely the causes, diagnosis, prevention and treatment of the major diseases which create the need for organ transplantation.

10.5 Section Summary - Animal Welfare and Ethics

Xenotransplantation, including the genetic engineering of animals, is a new form of animal exploitation which poses novel ethical dilemmas. It raises questions about the purported primacy of the human species and the basis on which this has been traditionally accepted. However, an increasing number of people express belief in some form of animal rights, and public opinion appears to be divided on the acceptability of xenotransplantation.

It is clear that developing herds of transgenic pigs as source animals for organs for transplantation involves much suffering. Many inherently distressing processes are involved, including genetic engineering, cloning, reproductive manipulations, surgical operations, the separation of sows and piglets, close confinement in unnatural indoor conditions and invasive health status monitoring.

The UK's Advisory Group on the Ethics of Xenotransplantation recommended against using primates as source animals for clinical xenotransplantation. However, successive governments have not accepted this recommendation. The processes and conditions required to produce primates for this purpose would cause severe distress and deprivation.

Primates are used as experimental animals to develop techniques of xenotransplantation, in which the organs of other animal species have been grated into them. Many of these experiments have caused illness and death. It is time to ask if the continued suffering of these animals can be justified.

11. Conclusions

The option of xenotransplantation as a potential solution to the shortage of human organs has been promoted widely in the scientific community and the media, despite the unanswered questions and uncalculated risks.

Society has been led to believe that problems of organ rejection have been solved; that pig organs have been generally 'humanised'; that extensive animal research suggests that xenotransplants in humans would be successful; that the production and selection of suitable source animals present little technical problem and few compromises in animal welfare; that the risk of an infectious disease of epidemic proportions is understood and can effectively be minimised; that animal organs are known to be capable of supporting human life and health; and that potential transplant patients are informed and willing to participate in clinical trials.

In fact, none of these premises is entirely true. The genetic engineering of pigs is not fully developed and only addresses the first stage of the rejection process, i.e. hyperacute rejection. It does not solve the problems of antibody- and T-cell-mediated rejection, which remain outstanding. Appropriate immunosuppressive drug treatments have not even been devised in animal experiments – primate recipients of pig organs have died from the toxicity of immunosuppressive drugs, and from infections. Even if a suitable drug regime could be devised for pig-to-primate transplants, it would not be directly transferable to the human xenotransplant patient.

There is still no international consensus on whether primates or transgenic pigs would be the best source animals – each species has major technical disadvantages. Moreover, critically important factors such as cardiovascular dynamics; organ weight relative to body weight; and responses to drugs, can all vary even between different strains of pigs. Any choice of species or strain of source animal will therefore be a very difficult compromise.

The likely methods of production, breeding, rearing and monitoring source animals would impose enormous costs in welfare terms on either pigs or primates, both of which are highly intelligent, active and social animals.

The risks of transmission of an existing pathogen from source animals to human recipients, and of a new disease arising from recombination or mutation, are presently unquantifiable. Insufficient research has been done, and we simply do not know enough about existing or undiscovered animal viruses, or prions, or the effects of placing an animal organ directly within the immunocompromised human body, or the results of genetic engineering on viral transmission, to predict the outcome.

The further risks of an infectious disease passing from a xenotransplant patient into the population are also uncalculated, because scientific knowledge is lacking. Pig endogenous retroviruses –

possibly the most high-risk type of virus – were only discovered for the first time in 1997. New, previously unknown human herpesviruses are identified every few years. Novel baboon endogenous retroviruses have been discovered in the last few years. It is inevitable that many other unidentified viruses exist in animals, and they cannot be screened for or eliminated.

There is scant evidence that pig or monkey organs are capable of sustaining human life and health. Apart from an isolated case of a patient receiving a chimpanzee's heart who survived for nine months, no xenotransplant patient with an animal organ has lived longer than 70 days. Chimpanzees cannot be used as source animals, for ethical and pragmatic reasons. All patients who have so far received a transplanted organ from a pig, baboon, sheep or any other species of animal have remained very sick until death intervened.

Apart from unsolved problems of animal organ rejection, there are other species barriers which have not been addressed. The innumerable variations in the physiology, biochemistry and pharmacology of organs from different species are quite likely to pre-empt successful xenotransplantation. Will pig liver blood-clotting factors cause fatal blood clots in human patients? Would a baboon kidney be able to regulate the formation of red blood cells in human bone marrow? How quickly will a pig kidney be damaged by the high levels of uric acid found in the human bloodstream? Would gas and blood pressures in an animal lung allow normal breathing in the human? Could the heart of a pig generate enough output to sustain circulation in a human?

These questions of species incompatibilities in organ function have not only not been answered, but they have hardly even been asked – and yet the UK Xenotransplantation Interim Regulatory Authority, like regulatory bodies in many countries, is forging ahead with devising a system to allow clinical trials to proceed.

Meanwhile, the full significance of chimerism – the spreading of animal cells throughout the body of a xenotransplant patient – is not appreciated by the public or by patients' groups. Public acceptability of the morality and safety of the process is highly uncertain. The public at large would be put at risk of infection, but will not generally benefit, if clinical trials of xenotransplantation were to go ahead; therefore public opinion should carry considerable weight in the decision-making process.

Compassion in World Farming and the British Union for the Abolition of Vivisection believe that xenotransplantation research has already caused, and will further cause, unacceptable suffering to sentient animals. We consider that the science of xenotransplantation is so poorly developed that clinical trials cannot reasonably be considered. We are concerned that commercial pressures are forcing the pace of a technology which is under-developed. We expect that it may take many decades of research, with animals and people, before success could be achieved with xenotransplanted organs – if ever. In the meantime, crucial resources may be diverted away from other, probably more fruitful, options.

For all these reasons, we call for a halt to the xenotransplantation programme.

"Seldom, if ever, have we had as much knowledge to prevent a future epidemic. What is lacking is the wisdom to act upon that knowledge."

Dr Jonathan Allan (Nature Med. 2:18-21)

Glossary of terms

Acute vascular rejection
Organ rejection, over a period of days, due to the presence of foreign molecules (antigens) on the lining of the blood vessels in the transplanted organ.

Albumin
Serum albumin is a plasma protein, made by the liver, which carries free fatty acids and bilirubin, as well as drugs, in the bloodstream. Serum albumin is the main protein responsible for the colloid osmotic pressure of the blood.

Alveoli
The tiny air sacs in the lungs where oxygen and carbon dioxide are exchanged.

Amino acid
One of a family of molecules which combine to form different proteins, including enzymes. Sometimes a difference of only one amino acid in the structure of an enzyme can affect its activity.

Angiotensin
Angiotensin I and angiotensin II play roles in altering blood flow through the kidneys (and hence fluid balance), as well as blood pressure throughout the body.

Antibody/antibodies
A protein (immunoglobulin) produced in the blood by the white blood cells, in response to the presence of a foreign molecule called an antigen. It is part of the body's defence or immune system, and plays a key role in the rejection of transplanted organs.

Antigen
A foreign molecule, for example on a virus or a transplanted organ, which stimulates production of an antibody. This is part of the body's immune system.

Ascites
An abnormal build-up of fluid in the abdominal cavity, which can be painful.

Bradykinin
A potent vasodilator, i.e. it causes the blood vessels to relax or dilate, thus affecting blood flow and blood pressure as well as the glomerular filtration rate of the kidneys. Bradykinin is inactivated mainly by the lungs.

Cardiac output
The volume of blood pumped by the heart into the main circulation per minute.

Chimera
An organism which contains cells, tissues or organs of more than one individual or species.

Chromosome
An inherited structure in the nucleus of most cells, made mainly of DNA and protein, which carries the genes.

Cilia
Microscopic hair-like projections of the cells lining the airways. The beating of the cilia helps expel foreign particles and pathogens by mucociliary clearance.

Cloning
The cloning of animals is the artificial production of two genetically identical individuals.

Colloid osmotic pressure
A pressure exerted by a solution, which draws fluid molecules through a semi-permeable membrane which the particles in the solution cannot cross. In the blood, colloid osmotic pressure is largely created by the protein albumin and affects fluid balance between the bloodstream and the body tissues.

Complement
One of a family of proteins found in the blood. Mainly produced in the liver, complement proteins have specific roles in inflammation and immune function. The triggering of complement activation by antigens in organs transplanted from other species is the primary cause of hyperacute rejection.

Cytochrome P450
An important and large group of liver enzymes, which catalyse the oxidation of substances, e.g. the detoxification of drugs and chemicals.

Decay accelerating factor
A complement regulating protein found on the surfaces of human cells, which prevent complement from attacking the body's own cells. Pigs have been genetically engineered so that their cells bear human decay accelerating factor. This helps to prevent the destruction by hyperacute rejection of pig organs when transplanted into a primate species (including, scientists hope, in humans).

DNA (deoxyribonucleic acid)
The inherited molecule which encodes genetic instructions required for the structure and function of living organisms. A length of DNA which encodes for a specific function is called a gene.

Endogenous retrovirus
A retrovirus whose DNA is more or less permanently incorporated into the chromosomes of host animals, and may be inherited by offspring. Endogenous retroviruses often do not cause illness in their normal host animals.

Endothelium
The lining of the blood vessels, formed by active cells called endothelial cells.

Enzyme
One of many proteins, produced by living cells, which acts as a catalyst in metabolic reactions. An enzyme combines selectively and specifically with its natural substrate/s, upon which it acts. Even a tiny change in the structure of the enzyme or its substrate can affect the efficiency of the reaction.

Erythropoietin
A hormone produced by the kidney in response to insufficient oxygen in the bloodstream. Erythropoietin stimulates the formation of new red blood cells in the bone marrow.

Gal epitope
An antigen present on the surface of some animals' cells (including pigs). It is considered foreign by human antibodies, and stimulates the process of hyperacute rejection.

Gene
An inherited functional unit of DNA within the chromosomes, which instructs a specific function such as the production of a particular protein.

Genetic engineering
The technology of changing artificially the DNA of a living organism, for example by adding genes of the same or different species, or by removing genes.

Genome
The full set of an organism's genetic instructions, i.e. all its genes.

Glomerular filtration rate
The rate of filtration of fluid and dissolved substances from the blood into the kidneys. This filtration occurs through a tiny coil of capillaries called the glomerulus and is a measure of normal kidney function.

Guanase
An enzyme in the liver and spleen which catalyses the metabolism of proteins, called purines, from cell nuclei.

Haptoglobin
A bloodstream protein which carries the used blood pigment haemoglobin to the liver, to recycle the iron.

Humoral rejection
Rejection of transplanted organs by the activity of antibodies circulating in the bloodstream.

Hyperacute rejection
Very rapid rejection of a transplanted organ from an animal which is not closely related to humans (e.g. a pig). Hyperacute rejection is mediated by pre-existing antibodies in the human bloodstream, and leads to blood clotting and failure of the transplanted organ within minutes or hours.

Hypoxia
Low levels of oxygen in the body's tissues.

Immune function or system
The body has a complex system of immunity – a defence against pathogens which includes the actions of antibodies and white blood cells.

Immunocompromised
A condition in which the body's immune function is repressed, for example by anti-rejection drugs or the selective removal of certain antibodies.

Immunoglobulins
Proteins, also called antibodies, produced by white blood cells, which play a part in immune function.

Immunosuppressive
The action of suppressing the body's immune system, for example with immunosuppressive or anti-rejection drugs which are taken by transplant patients.

Inflammation
The reaction of living tissues to injury or infection, characterised by pain, redness or swelling.

Leucocyte
Any of various white blood cells, including lymphocytes and monocytes, found in the bloodstream and active in the immune system.

Leukotriene
One of a number of related substances, released from white blood cells, which play important roles in mediating inflammation and immune reactions.

Macrophage
A type of leucocyte or white blood cell found in most tissues and organs, including the lungs. Functions include destruction of certain pathogens, and production of mediators of the immune system and of blood cell formation.

Metabolism
All the biochemical reactions in living organisms, for example the enzyme-induced breakdown of drugs and foods, or the synthesis of complex substances such as natural hormones.

Metabolite
A product of the metabolism of a substance in the body.

Micro-injection
As part of the process of genetic engineering, the injection of DNA into eggs using a very fine needle, viewed under the microscope.

Mucociliary clearance
The removal of micro-organisms and foreign particles from the airways by trapping them in mucus, which is then wafted upwards by the beating of fine hairs called cilia.

Neurotransmitter
One of a number of chemicals which transmits electrical nerve impulses between nerve cells.

Pathogen
An infectious organism, such as a virus or bacterium, which causes illness.

Plasma
The fluid component of the blood.

Plasma protein
One of many proteins, such as transferrin or albumin, found in the plasma (bloodstream). Many are carrier proteins for transporting substances from one part of the body to another.

Platelet
A type of blood cell which is important in blood clotting.

Prion disease
An illness, such as scrapie in sheep or Creutzfeldt-Jakob disease (CJD) in humans, which is believed to be caused by a rogue protein, called a prion. Prion diseases can sometimes be transmitted to other individuals or species although the process is not well understood.

Prostaglandin
One of a family of fatty acids found in many body tissues. Each prostaglandin has a specific function, ranging from effects on blood flow, blood clotting, kidney function, and dilation or constriction of blood vessels.

Protein C
An enzyme in the bloodstream which, when activated, suppresses the blood-clotting process.

Proximal tubule
One of many tiny tubules in the kidney where, after filtration of the blood, essential substances are reabsorbed into the bloodstream, while others, such as drugs, are transported into the urine for excretion.

Pulmonary
Relating to the lungs.

Receptor
One of many types of molecular structures on the surface of cells, which interacts specifically with substances such as hormones and drugs, triggering the cells' response.

Renal
Pertaining to the kidneys.

Renin
An enzyme released from the kidney into the bloodstream where it catalyses the conversion of angiotensinogen to angiotensin I. This reaction is triggered by loss of sodium from the body.

Retrovirus
A type of virus which contains genetic material in the form of RNA, rather than DNA. Within the infected cell, viral DNA is produced using the cell's machinery, so that the virus can multiply. Examples of retroviruses include HIV and certain viruses which cause cancer.

Serum
The fluid component of clotted blood, containing antibodies.

Specific pathogen-free (SPF)
Animals derived by caesarean section or hysterectomy who are free of stipulated, but not all, major pathogens. SPF animals are kept in barrier units to minimise the risk of contamination. Food, air and water are monitored for sterility, and entry by human personnel is controlled and restricted.

Substrate
A substance with which an enzyme specifically and selectively interacts, and which is modified by the enzyme's catalytic activity.

Thrombomodulin
A substance in the bloodstream which interacts with protein C and suppresses blood clotting.

Transferrin
A plasma protein, made in the liver. Transferrin carries iron from dying red blood cells to the bone marrow, for re-use by developing red blood cells.

Transgenic
An organism, including an animal, which has had a heritable foreign gene artificially inserted into its chromosomes.

Uric acid
A waste product formed by the breakdown of proteins from the nuclei of cells. Uric acid is filtered from the bloodstream by the kidneys and excreted in the urine. Excessive amounts of uric acid can cause kidney stones.

Uricase
A liver enzyme found in most mammals but not in humans. It breaks down the waste product, uric acid, to allantoin.

Vasopressin (also known as antidiuretic hormone)
A hormone, released from the brain, which controls fluid balance and hydration by acting on the kidneys.

Ventilation
The flow of air into and out of the lungs so that carbon dioxide and oxygen can be exchanged in the alveoli.

von Willebrand Factor
A factor released by the endothelial cells which line the blood vessels. It interacts with platelets in the formation of blood clots.

Xenotransplantation
The transplantation of cells, tissues or organs from one species to another.

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