

Written by Dr. Jarrod Bailey B.Sc. (HONS), Ph.D.
Science Director, Europeans for Medical Progress www.curedisease.net

**Animal Aid exposes and campaigns
peacefully against all animal abuse,
and promotes a cruelty-free lifestyle**



Animal Aid
The Old Chapel, Bradford Street, Tonbridge, TN9 1AW
Tel: 01732 364546 info@animalaid.org.uk
www.animalaid.org.uk

Published by Animal Aid, October 2005
ISBN: 1-905327-03-X



Man Or Mouse

Genetically Modified Animals in Medical Research **A Critical Review**

Written by Dr. Jarrod Bailey B.Sc. (HONS), Ph.D.



Animal Aid

The Old Chapel, Bradford Street, Tonbridge, TN9 1AW

Tel: 01732 364546 info@animalaid.org.uk

www.animalaid.org.uk

Published by Animal Aid, October 2005

ISBN: 1-905327-03-X



Man Or Mouse

Genetically Modified Animals in Medical Research A Critical Review

Written by Dr. Jarrod Bailey B.Sc. (HONS), Ph.D.
Science Director, Europeans for Medical Progress
www.curedisease.net

Table of Contents

| | |
|---|-----------|
| Executive Summary | 1 |
| Introduction | 3 |
| Why use animals in scientific research? | 3 |
| How many animals are used? | 4 |
| Genes And Proteins: A Brief Introduction | 6 |
| What's in a gene? | 6 |
| Gene Expression – An exquisitely controlled process | 6 |
| Making Genetically Modified Animals | 9 |
| What exactly are GM, mutant and transgenic animals? Some terms... | 9 |
| So how are they made? | 9 |
| 1. Virus-based transgenesis | 9 |
| 2. Pronuclear microinjection | 10 |
| 3. Embryonic stem cell method | 10 |
| Recent developments and other uses of GM technology | 11 |
| Mutations and Mutagenesis | 12 |
| The mouse mutagenesis project | 12 |
| Uses of, and Problems with, Genetically Modified Animals | 14 |
| 1. Human disease models | 14 |
| Cystic Fibrosis | 15 |
| Parkinson's Disease | 16 |
| Diabetes (Type 1, or Insulin Dependent) | 17 |
| Alzheimer's Disease | 18 |
| Other diseases | 19 |
| 2. Toxicology | 20 |
| 3. Production of organs for human transplant | 20 |
| 4. Pharmaceutical factories | 21 |
| 5. Cloning | 22 |
| Alternatives to Research involving Transgenic Animals | 23 |
| Summary and Conclusions | 24 |
| References | 25 |
| Glossary | 32 |



Executive Summary

Hundreds of millions of genetically modified (GM) animals are used in medical research and in the testing of drugs and chemicals every year. In response to their increasing utilisation, Animal Aid has commissioned this report, which examines the GM animal phenomenon, and shows that they have made no tangible contributions to human health and medicine – and, for sound scientific reasons – are never likely to do so.

In this report we show that:

- The annual total number of animal experiments in the UK was in steep decline from 1975 to 1985, during which time it fell by around 40%. The use of 'normal' (non-GM) animals has continued to decline at a similar rate since 1985, as scientists have abandoned this approach due to its lack of success and relevance to human health. The increased use of GM animals has, however, reversed the overall trend. Because of GM animals, the number of animal experiments is once again increasing, with GM animals constituting almost one third of all procedures.
- GM research has failed to live up to its promise. Rather than 'fixing' the old non-GM animal models by genetically manipulating them to resemble more closely human beings and human diseases, this process has revealed itself to be hopelessly inefficient and crude. Some 70% of the time when a GM animal intended to replicate a human disease is created, it does not 'perform' as expected. Put simply, the actions of, and interactions between, our genes are much more complex than first thought: minor genetic differences between individual humans combine to be greater than the sum of the parts. To expect to derive useful information from another species altogether is extraordinary.
- Modelling human diseases with GM animals has been disastrous. Cystic fibrosis (CF) and Alzheimer's disease (AD) have been extensively researched using GM animals, which has served only to confound our knowledge of them and to impede progress. CF affects the pancreas in almost all human sufferers, and kills via lung infections. Mice with 'identical' genetic mutations to human CF patients do not show these effects, but rather die early from intestinal blockages not seen in humans. GM animals have failed to replicate the pathology of human AD: GM mice with identical brain pathology to human AD sufferers show no or only slight effects, and have failed to shed any light on the function of genes strongly linked with human AD. Other GM animal models of human diseases such as Parkinson's and diabetes have failed similarly. Nor should we expect a different outcome from currently 'fashionable' projects, such as the new genetically manipulated mouse 'model' of Down's Syndrome. In contrast, human-specific research methods continue to make significant contributions to medical progress in these areas.
- GM animals have failed in all fields in which they have been applied. Animals have been engineered to be more predictive models in toxicology testing, but this has not been realised. Engineering animals to grow organs for human transplants has been futile and is considered highly dangerous because of the risk of 'novel' disease organisms passing from 'donor' animal to human recipient and from there into the wider population. While some animals have been created who can produce drugs in their milk, for example, there are questions surrounding the need for this. There are also serious concerns regarding the welfare of the animals involved. Cloning of various animals has been reported in the media, but continues to be extremely inefficient, with poor survival rates, significant welfare problems and a high incidence of defects and abnormalities.
- Human-specific research, utilising tried and tested methods that have



Executive Summary

contributed greatly to medical progress, along with cutting-edge technologies, are the only way to achieve safe, efficient cures and treatments for human diseases in the shortest possible time. Increasing numbers of scientists and doctors agree, and are turning to research methods involving – for example - human tissue and cells, computer modelling, DNA ‘microarray’ chips, microfluidics and advanced scanning technologies – shunning animal-based approaches.

- On balance, GM animals have made a negative contribution to human medicine. At the same time, it must be recognised that competition for medical sector funding remains acute. An important choice confronts society: the choice between more resources directed at animal-based research with a fruitless track record – or support for work that is directly relevant to the patients of today and tomorrow. Animal Aid believes that a comprehensive scientific evaluation of animal-based approaches to research is absolutely imperative to achieve real medical progress.



"There isn't a single genetically manipulated mouse that has been used yet to produce a drug that cures disease."

Kathleen Murray, Director of Transgenic Services, Charles River Laboratories Inc., USA. 2002, speaking almost 20 years after the first genetically modified animal was created.

Introduction

Animals have been used as 'surrogate humans' by scientists for many decades in an attempt to discover more about the workings of the human body, the chemicals and other substances that can harm it, the diseases that affect it, and the drugs that can potentially cure it. The history of this endeavour is not only a litany of failures, but also of disasters that have caused enormous amounts of human as well as animal suffering.

Demonstrably, the big medical breakthroughs of the 20th century have been despite, rather than because of, animal experiments. Tried and tested non-animal techniques form the foundation of medical progress, and new cutting edge technologies are accelerating it at the beginning of the 21st century.

But animal experiments continue unabated, with numbers again increasing in recent years due to the introduction of genetically-modified and transgenic animals, which allow scientists to manipulate the genetic material of animals in the hope that they can more closely resemble, for example, humans with a specific disease.

In this report we outline what genes 'do' and how genetically-modified animals are created. We discuss in some depth their uses as models for human diseases, and also briefly in the production of pharmaceutical substances and as a source of organs for human transplant. We show that all of these endeavours constitute science as poor and irrelevant to human medicine as research involving non genetically-modified animals always has been, and outline the best way forward.

The cost for humans is high, as we see transgenic research providing data that cannot be safely relied upon when applied to human medicine. Such research also

diverts substantial sums of money from the human-specific methods that are providing the answers. The cost in animal lives and welfare is also great, as a result of scientists using a hugely inefficient and wasteful process to create animals who are destined to suffer from birth.

The use of animals in biomedical research, whether genetically modified or not, is a big an exercise in futility as it always has been. A bad model is a bad model, no matter what is done to improve it. No amount of genetic manipulation can make an animal 'human' enough to provide data that is relevant, predictive, reliable and safe enough to apply to humans. Furthermore, the creation of genetically modified animals raises ethical and welfare questions more critical than ever before. These two considerations mean that any benefit that could ever stem from such research could never justify the means, or the human cost in terms of the harmful consequences of misleading animal data. Animal Aid therefore remains committed to the abolition of all animal research, and to promoting more humane medical research.

Why use animals in scientific research?

Animal experimentation as we know it has been going on for well over a century, but has proceeded with particular fervour over the last 40-50 years. Many millions of animals of many different species have been used in scientific and medical research in an attempt to elucidate biological processes and the causes and progress of human disease, to gauge the potential hazards of chemicals and foodstuffs, for example, and to determine the safety and efficacy of proposed new drugs. In short, animals have been used as 'surrogate humans' in the hope that information gleaned from experiments



involving them would be applicable to human beings, and thus lead to a safer and more pleasant world – a world in which we would be shielded from exposure to harmful substances, and where any human illness would be merely transient while myriad cures took their effects.

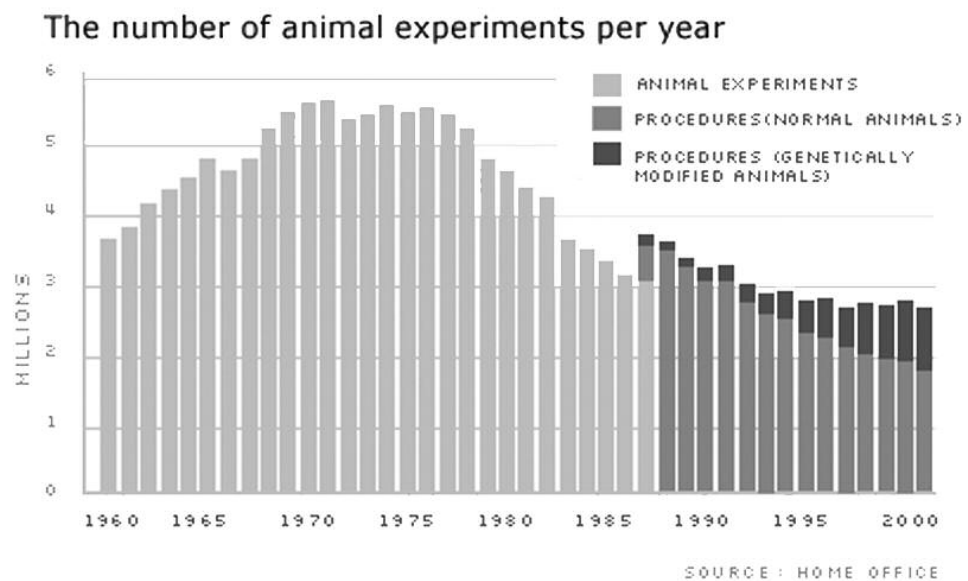
It is easy to see how, decades ago when cell biology and genetics were in their infancy and the structure and function of DNA was a mystery, animal experiments seemed like a very good idea to scientists struggling to understand and cure disease. After all, there are so many similarities between all mammalian species, including us: we all suckle our young; our hearts and lungs do the same job, etc. etc. However, it quickly became apparent that, despite superficial similarities, there existed profound differences between species. Even when scientists couldn't know the nature of them, such differences were evident because they manifested themselves in the results of their experiments. In its early days, for example, penicillin appeared to be ineffective in rabbits, and it is toxic to guinea pigs.

How many animals are used?

While animal experiments persisted, the numbers of animals used and procedures performed levelled off in the early to mid 1970s, before declining steeply over the next ten years (**Figure 1**). This coincided with the advent of alternative and groundbreaking new scientific methods including DNA-oriented and other 'test-tube' (*in vitro*) technologies, and arguably with the tacit admission that the animal model had been a failure. Optimistic predictions for a brighter future for research and the millions of people standing to benefit were premature, however. Since the late 1980s, despite the number of 'normal' animals used in research continuing to decrease, the overall trend has been reversed as scientists have rushed to work with genetically modified (GM) or 'transgenic' animals.

In 2003, the last year for which detailed figures are currently available, further increases in animal use were seen. This included an 8% rise in the use of transgenic animals, representing 27% of the total

Figure 1



Forty year trend in the number of procedures performed on laboratory animals in the UK. Note the dramatic recent increase in 'procedures' on GM animals (dark shading). GM experiments have continued sharply increasing since 2000.



Figure 2

| Category | Numbers | Change 2002 - 2003 |
|-------------------------------------|-----------|------------------------|
| Total individual animals used | 2,721,599 | ↑ 2% |
| Total number or procedures | 2,791,781 | ↑ 2% |
| Procedures using transgenic animals | 764,000 | ↑ 8% (27% of total) |

Year-on-year increase in experimental procedures and animals involved from 2002 to 2003, including those involving transgenic animals.

number of procedures performed (**Figure 2**). At present, approximately 95% of these animals are mice. Without doubt, this figure will have increased considerably in the last two years; approximately 100 million mice were used in GM experiments in the US alone in 2004.¹

We are now, however, in a privileged position where we can analyse the data that has been produced, and examine the contribution that animal-based research has made to human medicine. We must ask, given the claims of those who espouse it, why was animal research in serious decline for a decade if it had been so effective in translating laboratory research to cures for disease? If this research methodology had an impressive track record and had been fundamentally and crucially involved in

medical progress, or even if it had shown some future promise, why had it been abandoned so dramatically? Application of 'The 3 Rs' (Refining, Reducing and Replacing animal experiments) cannot claim to be significantly responsible for this.

Arguably, the subsequent rise in the use of genetically modified animals after this slump was an attempt by researchers to 'fix' their failed animal models, and to make them resemble more closely the human condition. In this report we examine the current state of research involving these GM animals, cite some important examples, and discuss the problems inherent in this field and the consequences for human health and animal welfare.



Genes and Proteins: A Brief Introduction

What's in a gene?

All living creatures are made of cells; some are made of just one, others of many billions comprising hundreds of specialised types collectively organised into tissues and organs. All cells are basically small, fluid compartments containing a concentrated solution of chemicals, along with various structures that help the cell to stay alive, replicate and perform its necessary functions.

Almost all cells have in common a nucleus, a separate compartment within the cell containing DNA (deoxyribonucleic acid). This DNA, made up of building blocks known as nucleotides (of which there are four types), takes the form of the famous twisted double helix, and coils upon itself time and time again to form discrete bodies called chromosomes. Humans have 46 chromosomes (23 pairs) in the nucleus of each cell of their bodies, which together make up what is known as the human genome. The only exceptions are egg and sperm cells, which contain 23 single chromosomes not in pairs: this allows the chromosomes to 'pair up' again when the sperm and egg unite during fertilisation to make a new embryo with the normal number of chromosomes – half coming from each parent.

If one imagines the DNA strung out in a long line, it is composed of tens of thousands of units along its length known as genes. These genes serve as a template for the cell to manufacture the proteins and enzymes that make up our bodies and keep it alive. This occurs via two processes known as 'Transcription' and 'Translation.'

In transcription, pieces of 'cellular machinery' known as enzymes engage with the DNA, and track along the double helix making a copy of the DNA, but in a chemically slightly different form – known as RNA. This RNA is then used as a template for the translation process, whereby other bits of cellular machinery 'translate' the RNA code in order to assemble amino

acids into chains known as polypeptides. These, in turn, make up the all-important proteins. (Figure 3).

Gene Expression – An exquisitely controlled process

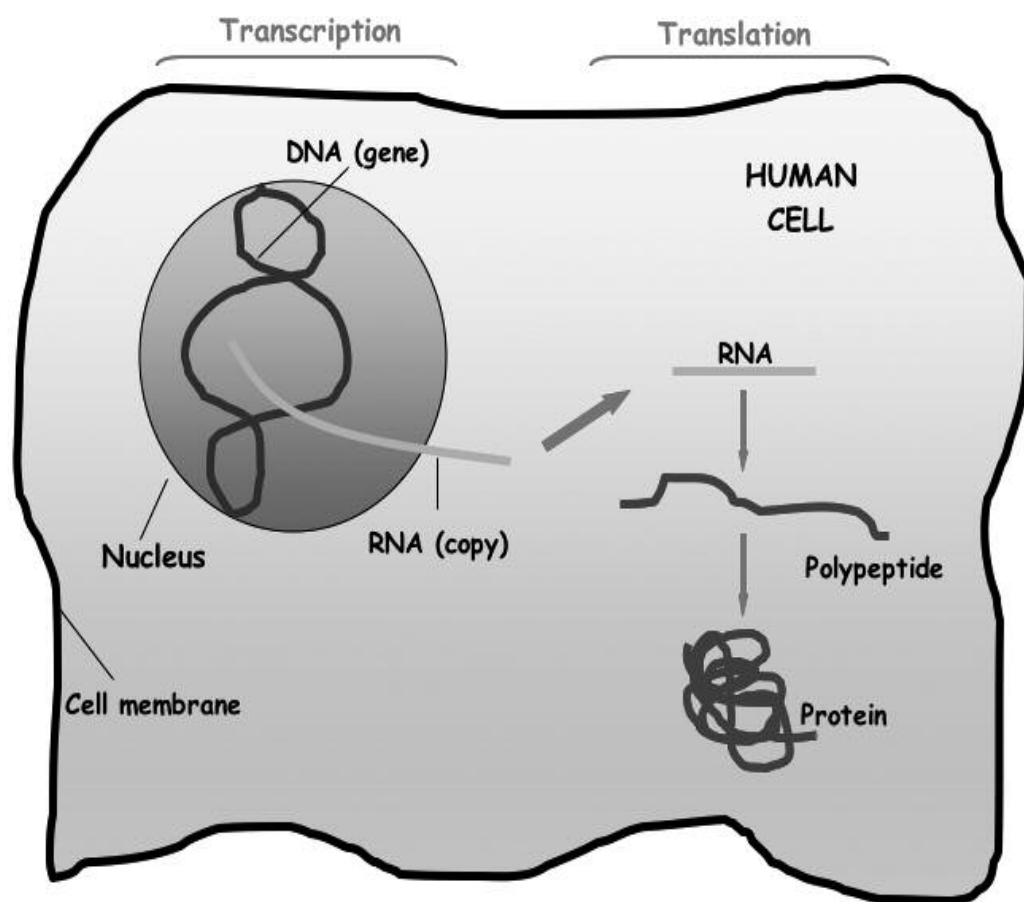
The process whereby genes serve as a template for the cell to make proteins is called gene expression. Genes are classified as 'Structural' or 'Regulatory,' depending on their function. Structural genes code for proteins that 'make up' our bodies; that build our cells and organs, and that form enzymes that carry out chemical reactions vital for life. Regulatory genes control the expression of structural and other regulatory genes, increasing and decreasing their levels of activity or turning them 'on' or 'off' completely.

This is a very tightly controlled process involving inputs from many angles, and a minor fault in any of the components or processes can have far reaching effects and be the cause of many diseases. For example, if the DNA template is damaged or altered in some way, such as by a harmful chemical, radiation – or if a damaged version is inherited from one's parents, then the protein will not form or function correctly. Sometimes, the enzymes that carry out gene expression, which are themselves products of other genes, will themselves malfunction. Pieces of DNA flanking the actual genes are also of crucial importance, acting as extra control elements – in concert with the products of the regulatory genes – to modulate gene expression. Again, a small fault or minor difference can have far-reaching consequences.

It is important to note that the protein products of genes do not go about their jobs in isolation; there is an almost infinitely complex array of interactions between them and other cellular components that can alter their functions drastically. Many of these protein complexes interact with the aforementioned DNA 'switches' to change what they do. Furthermore, one



Figure 3



A schematic of the process by which genes, made from DNA, code for proteins which make up the human body. A typical human cell is represented here, showing the process of gene transcription taking place in the cell's nucleus, and translation in the main part of the cell. Together, these processes form the basis of 'gene expression' where a gene codes for the production of a specific protein.

gene can actually give rise to many different proteins, which have varied functions and interaction partners. (Examples include 'RGS'² and 'CREM' genes³ involved in signalling between cells, tissues and nerves, responses to hormones and sensory inputs etc.). Additionally, the machinery responsible for these processes differs between species. Genetics is a very complex business!

These factors illustrate how enormously difficult it is simply to alter a gene in an animal, and then expect to see precisely what that alteration means in that particular species, and then to be able to deduce

what that means in a human context. We can also see that similar genes in different animal species can do different jobs in different ways, and that the generation of genetically modified animals, by their very nature, is a highly complicated, difficult, imprecise, inefficient and crude method (in terms of results) of determining or altering the function of a gene.

Increasingly, we are discovering that the genetic similarity between humans and chimpanzees (around 95%-98%, depending on whose estimates you believe) means little: after all, we are quite clearly *not* chimpanzees, and vice-versa. Mice are

Genes and Proteins: A Brief Introduction



Genes and Proteins: A Brief Introduction

thought to share 95% of our DNA, and tiny nematode worms about 70%. Superficially then, it seems that the small *difference* between our genes has anything but minor consequences. As scientific knowledge increases, we are learning that such minimal changes in DNA sequence lead to profound differences in biochemistry and physiology, and this occurs predominantly due to variation in the regulatory genes and DNA regions described above.

So no matter how similar our structural genes may be, if they are *regulated* differently, we're looking at a whole new scenario. One simple analogy of this is to imagine two huge, complex and almost identical church organs side-by-side. The hundreds of stops either side of the keyboards are the regulatory genes and regions of our DNA, able to exert subtle changes in the sound of the instruments individually, but also able to act in countless combinations together to alter the sounds drastically. Even if the same music is played on both organs, the sound will be entirely different unless the stops (i.e. the regulatory genes and regions) are in identical positions. Change the order and timing with which the keys are operated, and the end products are completely unrecognisable from one another.



*'The generation of transgenic animals is a cumbersome process and remains problematic in the application of technology.'*¹⁴

Making Genetically Modified Animals

What exactly are GM, mutant and transgenic animals? Some terms...

Many methods exist that can alter the genetic material of laboratory animals with differing outcomes. A brief summary of terms and techniques of production is included here, before a discussion of their uses.

Genetically modified animals are animals whose DNA has been artificially manipulated in some way. This umbrella term includes *cloned* animals and some animals in which a gene has been mutated (*mutants*) to alter its function. In addition, it includes *transgenic* animals, which have been altered to carry a 'foreign' gene ('transgene') within their natural genome.

The main focus of this report is transgenic animals, which constitute the majority of GM work undertaken in biomedical research. Most transgenesis (the term for creating transgenic animals via the insertion of a *transgene* into their DNA) involves one of two basic approaches:

Knock-outs are used to study a gene's function by halting its expression, and observing the effects of its absence. This usually involves preparing a copy of the gene in the laboratory with a segment deleted and into which a foreign segment of DNA (usually a 'marker' gene to track its presence) is inserted. This new piece of DNA is then used to replace the normal, fully functioning gene in a target animal. Because the coding sequence of the gene has been disrupted, its function will be 'knocked out' because it is unable to be expressed properly, and cannot give rise to the protein for which it codes.

Knock-ins are used to study the function of a 'foreign' gene by introducing it into a target animal to observe the effects of its expression.

So how are they made?

*'The Canadian Council on Animal Care classifies transgenic experiments in the second-most severe 'category of invasiveness,' with the potential to cause 'moderate to severe distress or discomfort.'*¹⁵

There are many different combinations of techniques using different transgene constructs that are currently used to generate transgenic animals. Broadly speaking, however, all methods fit into three categories. A concise overview of these methods is given here, along with a brief consideration of proposed improvements and other uses of GM technology.

I. Virus-based transgenesis

This is the oldest of the three main technologies, which involves engineering a virus to deliver the transgene into target sperm and eggs. It had until recent developments been overtaken by the other methods due to its inherent shortcomings. These include multiple insertions of the transgene into the host genome – making interpretation of results difficult if not impossible: a very low rate of production of viable transgenic offspring; and the creation of so-called 'mosaic' animals, where the transgene is carried and expressed only in some cells of the body. Furthermore, genetic material from the viruses used has been known to combine with DNA sequences from the host's genome to regenerate virulent viruses,⁶ and a phenomenon known as 'insertional oncogenesis' exists which can cause the host cells to become cancerous.⁷⁻⁹

Lately, the use of a different type of virus has increased the efficiency of this technique – a development that has had



some impact on animal welfare in terms of the numbers of animals surviving the procedure, and the proportion of these who express the transgene and are therefore 'useful'. There are, however, concerns surrounding size limits for the genes that can be transferred that could rule out this method for many human genes, and the other caveats described in this report still apply.

2. Pronuclear microinjection

This commonly used method became the norm due to its comparative efficiency relative to viral transgenesis, though the typical range of 'success' of between 1% and 10% still represents 90-99% wastage of the animals involved.

In this technique, female animals (mice, for example) are injected with a hormone that causes them to produce many eggs, which are fertilised by a male. The female is then killed so that her embryos can be harvested, at which point the transgenes are introduced to the embryos via injection through a small glass needle. 20 or 30 embryos are then transplanted into a number of 'pseudopregnant' female mice (these are mice that have been previously mated with castrated males to prepare their uteruses for implantation: they are clearly *not* pregnant following this mating, but their bodies act as though they are.) About three weeks following the transplantation of embryos, comes the birth of any pups that have survived the process.

Typically, 20-30% of the embryos develop to term. 20% of these, in other words 4-6% of the initial embryos, contain the transgene. Successful uptake and function of the transgene is then confirmed via DNA analysis of these offspring, usually by cutting off the end of their tails or ears.

Successful confirmation of transgenesis at this stage is not enough. To be of any use, a suitable population of transgenic animals from which breeding can take place must be generated. This is usually done by the mating of these

individuals with 'normal' mice through two generations.

There are some major caveats with this methodology. Up to 200 copies of the transgene can be randomly inserted into the host genome – an outcome with potentially disastrous consequences:

- A gene's function is highly dependent upon its environment: its site of insertion will determine if it is expressed at all and, if so, to what level.
- Multiple copies of a gene can give rise to large amounts of its protein product, which can have promiscuous effects and even be highly toxic.
- Transgene insertion can disrupt crucial host genes, rendering them useless, as well as critical 'control regions' of DNA that switch genes on and off. Again, disruption of these areas can have far reaching and catastrophic effects.
- Tissue-specific and species-specific effects can confound results. DNA molecules can be chemically modified in certain host cells of particular animals, and this has been known to result in severe developmental abnormalities, causing extreme suffering of offspring animals. Gene expression has been known to stray from its usual site, causing a gene that is usually expressed in one specific organ or tissue to be expressed in another.¹⁰⁻¹²
- The obvious welfare implications resulting from the above issues are compounded by breeding programmes subsequent to the experimental transgenesis, which can involve large numbers of animals and therefore high levels of suffering and mortality.^{13,14}

3. Embryonic stem cell method

This method utilises stem cells (cells that have the potential to develop into any kind of specialised cell later in life, such as brain, muscle, nerve etc.) from early stage embryos known as blastocysts.¹⁵ Blastocysts are embryos that have developed for only a few days



post-fertilisation, consisting of a hollow sphere of around 100 cells and that hasn't yet implanted into the wall of the womb.

Using the ubiquitous GM mouse as an example again, embryos a few days old are removed from a freshly killed pregnant animal and the stem cells are isolated and incubated *in vitro*. During this time the transgene is introduced via an engineered virus or by using an electric current. The transgene is introduced in tandem with another 'marker' gene, such as a gene that confers resistance to an antibiotic. This allows those stem cells containing the transgene to be identified and selected using the antibiotic. Only those cells containing the transgene will be resistant to it, while all cells not containing the transgene will be sensitive to the drug and will not be viable. These stem cells are combined with a new 'host' blastocyst, which is then introduced into a pseudopregnant female.^{16,17}

This process gives rise to progeny animals known as 'chimaeras' that are composed of some cells derived from the transgene-containing stem cells, and others from the blastocyst into which the original stem cells were introduced. Often, the mice used as a source of these cells are different colours, so that the proportion of each of the progeny mice that is actually transgenic can be estimated simply by observing their colouring. Subsequent breeding will give rise to offspring of different colours, and the potentially transgenic individuals can be selected and subjected to further testing.

One advantage of this technique is that insertion of the transgene into the host genome is not random, but directed, via specific pieces of DNA flanking the transgene that recombine with matching areas of the host's DNA. This lowers the chance of inducing mutations in host genes by random insertion. Unfortunately, the technique is still no more effective overall than microinjection in terms of final outcome, with unavoidably high numbers of wasted embryos and adult animals.

In addition, only a small number of strains of mice are suitable. It is doubtful whether these strains, with their limited and relatively invariable gene pools, can reflect the genetic complexity of human beings or serve as appropriate models for human disease. This is especially so when one takes into account the genetic changes that are known to occur in the stem cells themselves when they are removed from their natural environment of the blastocyst to be grown *in vitro*.¹⁸

Recent developments and other uses of GM technology

In addition to technical improvements with the viral method mentioned previously, other attempts have been made to overcome the problems associated with transgenesis, including techniques such as sperm-mediated gene transfer (SMGT)¹⁹ and transposon-based gene delivery.²⁰ The results have been variable.²¹ The former method involves simply incubating sperm cells in a solution of DNA containing the transgene, during which it is hoped that the sperm 'absorb' the transgene, thereby enabling them to transfer it during an *in vitro* fertilisation process. This promises to be very quick and easy if it can be made to be successful: unfortunately, the jury is out. Although there are reports of successful uptake of transgenes by sperm cells,^{21,22} there are many examples of results showing the opposite,²³ and some 15 years after it was first reported it has not yet been established as a reliable form of genetic modification.

As described earlier, genetic modification doesn't just comprise the transgenic techniques outlined above. Changes in DNA known as mutations occur naturally, and form the basis of evolution and natural selection as organisms change and, sometimes, by chance become more suited to their environment. Mutations have been used in genetic research for decades, both by detecting naturally-occurring gene mutations and linking them to observable traits in the



organisms affected to elucidate the functions of that gene, or by deliberately inducing mutations to see what happens. In August 2005, £9 million was earmarked by the European Commission for the production of a 'library' of 20,000 mouse embryonic stem cells, each containing a specific mutant or 'knocked out' gene. Known as the EUCOMM Project, this endeavour is ostensibly to further our understanding of uniquely human diseases.

Mutations and mutagenesis

DNA mutations occur spontaneously as a result of mistakes made by parts of a cell's machinery responsible for copying and repairing that DNA, or through external agents such as chemicals, radiation and ultra-violet light in a process known as mutagenesis. In normal circumstances such 'errors' are rare and repaired as a matter of course with no ill-effects, although the fidelity of this process is not 100%, especially when the DNA is damaged at multiple sites and/or when that damage is severe. Even the smallest error can have extreme effects: people with sickle-cell anaemia, which affects more than 6000 people in the UK alone, are the victims of a 'point' mutation that results in the substitution of just one amino-acid in the haemoglobin of their red blood cells when the gene responsible is expressed.

The detection of gene mutations and the linking of them to observable characteristics ('phenotypes') of individuals carrying them have, over time, contributed greatly to the understanding of gene function. In the past decade this led to the random mutagenesis and screening of mice in large-scale projects, in the hope that some light could be shed upon the function of many novel genes discovered in, for example, the human genome project.

The mouse mutagenesis project

Certain chemicals exist that are especially potent DNA-damaging agents, which have formed the basis of some large-scale

research programmes in recent years involving hundreds of thousands of mice. A favourite chemical of the mouse geneticist is ENU (N-ethyl-N-nitrosourea) due to its high potency. This is injected into male mice, whose mutated sperm via subsequent matings produces an array of progeny with a huge variety of genetic mutations. Specific individuals with 'interesting' phenotypes are then chosen for further study in an attempt to identify which of their tens of thousands of genes have been mutated, and which of these may be responsible for their abnormal appearance and/or behaviour.

This approach has been extensively used in an attempt to reveal gene function in the mouse, in the hope that any information will be similar to the human situation and therefore be applicable to human disease research. Applications in this collaborative 'Mouse Mutagenesis Project'²⁴⁻²⁷ range from studies into complex behavioural traits including drug and alcohol responses, circadian rhythms, epilepsy and psychiatric diseases,²⁸⁻³⁰ to studies into skull and eye abnormalities^{31,32} and indeed a whole host of human diseases.^{33,34} Many hundreds of types of mutant mice have been created via the screening of many tens of thousands of animals,^{35,36} all of which exhibit some form of deformity, behavioural abnormality and/or physiological dysfunction.

The ethical cost of these projects is unavoidably high. This is brought into clearer focus when one considers that only 1-2% of the animals are retained – these having exhibited (by the researchers' criteria) an interesting phenotypic change: 98-99% are killed immediately as a result of having nothing 'novel' to offer. Of the 1-2% 'successes', on average only one quarter will possess a new mutation for further investigation. Therefore, three quarters of the initially intriguing mice are discarded when it is discovered that their mutations are duplicates or not of sufficient interest. Factoring in additional duplicates from other institutions participating in the project, plus mutations



that kill progeny animals during prenatal or postnatal development, the extra 'non-randomly mutagenised' animals used in breeding, the males subjected to the ENU mutagenesis in the first place, and the subsequent analytical procedures the ultimate animals of interest must endure, we can see that this area of research begins to look ethically troubling by anyone's standards.

Most people, then, would surely agree that this type of endeavour ought to demonstrate promise of a huge pay-off for human health, in order to balance these troubling animal welfare aspects. In reality there is a significant level of feeling that this does not constitute good science, but poorly defined and crude research that is of limited value in furthering the understanding of human genetics and disease. Of much more relevance to comprehending the function of human genes in a human environment and discerning the genetic basis of human diseases, would be human-specific studies using tissue samples, cultured cells and DNA microarrays ('gene chips'). The latter are small pieces of glass, upon which are spotted tiny amounts of DNA representing thousands of human genes. These can be 'probed' using DNA samples from healthy and diseased people, and scanned using a computer to reveal huge amounts of comparative information about gene activity relevant to specific diseases.

Making Genetically Modified Animals



*'It is apparent from an analysis of some transgenic disease models that the **actual** benefits of using the model are rarely completely equivalent to the **potential** benefits, and that the decrease in aspects of animal welfare might be disproportionate to any benefits gained.'*
'Problems persist when extrapolating data obtained by using such transgenic animals to the disease condition in humans.'³⁷

Uses of, and Problems with, Genetically Modified Animals

If promises from those involved in their creation are to be believed, the contribution of GM animals to human life will rival that of the wheel. Manipulating the genomes of 'imperfect' animals will lead to a complete understanding of genetics and cell biology; drugs to cure all diseases; simple and reliable test protocols to determine which chemicals, drugs and foodstuffs are safe and which dangerous and in what amounts; an unlimited supply of animal organs for human transplant with no problems of rejection; animals that can act as 'drug factories,' churning out huge amounts of effective drugs in their milk.

We look at each of these areas here, but pay particular attention to GM animals in biomedical research, which constitutes the biggest area by far in which they are used.

I. Human disease models

'With the exception of basic genetic mechanisms, the mouse is a relatively poor model for the human.'³⁸

As previously mentioned, animal models of human diseases have been around for several decades. Their use declined substantially for more than ten years, arguably as scientists realised their futility, until the advent of transgenic technology enabled them to utilise extraordinary new methods to search for those elusive 'more human-like' models that promised fame and fortune. Since that time, countless GM animals have been created and offered to science as putative models of a wide range of human diseases. The difficulty for those scientists trying to

make sense of all this... is that it's just not that simple.

All diseases have a genetic component to some degree. Some diseases are 'caused' largely by external factors such as diet, lifestyle and pollution, in which our genes merely predispose us to a degree of resistance or susceptibility. With lung cancer, for example, one individual could smoke heavily into old age yet never suffer significantly, whereas another non-smoker could contract the disease early in life from passive exposure. Other diseases have a firm genetic cause: sometimes only one gene is responsible, sometimes several. Some identical genes and mutations can cause disease in some individuals but not in others.

It's also important to bear in mind here that humans have 23 pairs of chromosomes in each of their cells; we have two copies of each of our genes, one is passed from the mother and the other from the father. Sometimes, a faulty gene inherited from one parent will be of no consequence providing we have a normal copy from the other; here, the faulty gene is termed 'recessive'. Only when an individual has two copies of the faulty gene is there an effect. But in some cases, certain mutations can be 'dominant', meaning that if we inherit one faulty copy we also get the disease, despite the presence of a normal copy.

GM animals have been used to model a number of human diseases from each of these categories. As an introduction to their use and their relevance to the human diseases for which they are models, a brief summary of some of the



most interesting is given here. We begin with the genetically 'simple' and perhaps the longest running GM disease investigation for which there is plentiful data: cystic fibrosis.

Cystic Fibrosis

As the most common inherited fatal disease in much of the world, affecting 1 in every 2500 children, cystic fibrosis (CF) has always attracted a great deal of research attention. Its cause in humans has been known since the 1980s when *in vitro* research culminated in the discovery of the gene responsible, known as CFTR. This gene encodes a protein that functions as a 'channel' in and out of various cells of the body. When faulty, this gene gives rise to a channel that doesn't form properly and this results in the build up of very thick mucus – predominantly in the lungs and pancreas but also sometimes in the liver. Pancreatic problems affect most CF sufferers but by far the main cause of death is chronic lung infection, rooted in the sticky secretions that form a breeding ground for harmful bacteria.

Instead of proceeding at full pace with human-based research, scientists turned to mice and began to look for a similar gene to see if, when mutated, it caused anything resembling human CF. This persistence with animal use occurred despite a previous record of abject failure with non-GM CF animal models. Despite huge expense and effort centred around the injection of bacteria into these 'conventional' animals' lungs and the induction of allergic airway disease, the project consistently failed to provide any useful information.

Presumably the hope had been, if successful results were forthcoming, to use the GM model to probe further the progress of the disease and also to test potential treatments and cures. A gene was finally found that was 78% similar to the human CFTR gene, and this was the signal to create transgenic mice with mutations. Because CF is a recessive condition, mice had to be engineered and bred with two mutant CFTR genes, termed (-/-) mice.

Some observations and excerpts from the scientific literature reveal the true extent of the disaster that has unfolded since this time. It is important to keep in mind the progression of CF in human beings: 95% of sufferers die from lung infections; pancreas disorders affect 85%, and liver disorders up to 43%. Intestinal obstruction is not a human factor:

- CFTR (-/-) mice 'do not have the mucus-clogged lungs and persistent lung infections that plague human CF sufferers. That could be because the lungs of mice are fundamentally different from those of humans – they have fewer mucus-secreting glands and cells overall ... our CFTR mice die early in life of gastrointestinal obstruction, and not from pulmonary [lung] infections.'³⁹
- 'Symptoms of the CF mice are hardly identical to those of human CF... there are just too many subtle physiological differences between the two species.'⁴⁰

Different teams began to argue about the merits of their own particular mice. None of them, however, could argue that its model had been a success:

- 'The apparent differences in [the mouse models] underscore the complexities of modelling human diseases in animals.'⁴¹
- In all mouse models pancreatic blockages, if they occurred at all, did so at a late stage, only occasionally, and were much less severe than the human equivalent.^{39,42,43}
- Many CF mice suffer from intestinal blockages (not seen in humans), and die in early life from them.⁴³⁻⁴⁶
- Almost all male CF sufferers are infertile due to reproductive tract problems: there is no such evidence in the mouse models.^{39,47}

The unavoidable conclusion is that animal models of CF have been an unqualified failure. This was always going to be the case: chronic lung infections, brought about by thick mucus secreted by the serous glands, kill human CF patients

Uses of, and Problems with, Genetically Modified Animals: I. Human Disease Models



Uses of, and Problems with, Genetically Modified Animals:

I. Human Disease Models

...and mice don't have these. Their lungs are different, their cells are different and their gene expression is different.⁴⁸⁻⁵⁰ What use is an animal model of CF in which many animals die of unrelated complications before lung and pancreatic problems have a chance to manifest, and where those animals that survive don't suffer the pancreatic problems and lung infections that are the main targets of the disease in humans anyway?⁵¹⁻⁵⁴

The history of CF research had been an immensely promising list of achievements without using animals. Autopsies, clinical observation and *in vitro* studies were responsible for revealing the biochemical nature of the disease, its mechanisms, the pancreatic problems and the development of diagnostic tests.⁵⁵⁻⁵⁸ Yet still researchers persisted with and obsessed about an animal model. Even in the face of the information above and with successful laboratory-based culture of human CF-affected cells a reality, scientists are *still* consuming valuable funds and time using animals, presumably out of habit, hubris and intransigence... and it still isn't working. Studies in mice showed that an alternative channel to CFTR could compensate for it when it is 'knocked out,' resulting in healthier tissue. In human tissue, this is not the case.⁵⁹⁻⁶¹ In mice, the activity of curcumin (a component of the spice turmeric) can correct CF defects, a finding that caused great excitement but that was not replicated in human cell cultures.^{62,63} 'Gene therapy' trials that were successful in mice^{64,65} have led to no less than 29 failed human trials.⁶⁶

Evidence that scientists are moving away from transgenic mice CF research might be encouraging, if that movement wasn't towards other animal models. The mouse may have proved to be futile to many researchers, but it seems their problem is not with the animal model; it's with the species. Cue efforts to investigate CFTR activity in sheep, ferrets, and non-human primates.⁶⁷⁻⁶⁹ The inability of many scientists to act upon the facts in front of them is astounding.

The contribution of non-animal research, however, continues, despite attracting less

funding than animal models.⁷⁰ This is evidenced by the development of effective treatments to protect against killer infections and thin the lung mucus⁷¹⁻⁷³ such as dietary measures, physiotherapy and infection control, that have increased life expectancy by decades to over 40 years.⁷⁴

Parkinson's Disease

Parkinson's Disease (PD) currently affects 1 in 500 people in the UK, representing 120,000 people in total or about 1-2% of the population above the age of 65.⁷⁵ It manifests as tremors, muscular stiffness and slowness of movement, and is characterised by the loss of cells in a part of the brain called the substantia nigra that produces a chemical known as dopamine, which is involved in the transmission of signals between nerve cells (neurons).

The causes and progression of PD are poorly understood, but there are thought to be genetic and environmental factors involved. In addition to loss of cells producing dopamine (dopaminergic or 'DA' neurons), a hallmark of the disease is the presence of objects called 'Lewy Bodies' in those DA neurons that do survive. These Lewy Bodies are primarily composed of three proteins, called alpha-synuclein, ubiquitin and parkin.

Genetic studies of human populations and PD sufferers have revealed that mutations in the genes that produce these three proteins, amongst a number of other candidate genes, can actually cause the disease.⁷⁶⁻⁸³ Naturally, since these human-based discoveries, transgenic knock-out mice have been created with defects in these genes based on the assumption that they would reveal a whole host of useful information about human PD.

Rather predictably though, they have served to confound the situation, producing results that are unclear, inconsistent and contradictory to human PD:

- In mice, a lack of alpha-synuclein results in neither the Parkinsonian phenotype nor alterations in DA pathways. They do not exhibit any obvious pathological



features, and their development is normal.⁸⁴

- Transgenic mice overexpressing either normal or mutant alpha-synuclein have generated inconsistent results.⁸⁵⁻⁸⁷
- Recent gene targeting strategies have generated several mouse lines with mutations in the DA system, which serve only to highlight the crudeness of this approach. Mice lacking genes involved in DA production die at a late embryonic stage or shortly after birth.^{88,89}
- Transgenic rats with altered alpha-synuclein expression have been created that did show substantial dopamine loss. However, their motor behaviour was only marginally affected,⁹⁰ or not affected at all.⁹¹
- Mice with the parkin gene knocked out show no clinical or pathological problems.⁹²

So what can we expect to learn from models that show marked and fundamental differences to human PD? On the whole they don't reproduce the loss of DA neurons in the substantia nigra of the brain or even show any pathological changes in them; they reveal no difference in the release and re-uptake of dopamine; they lack typical Lewy Body formation; they show changes in the motor neurons of the spinal cord not seen in human PD; they suggest alpha-synuclein is not essential for DA neuron function. In fact, there is a good chance that they don't represent PD at all, but a different type of neurodegenerative disease altogether.⁹³

Yet again, GM animals have failed to further medical progress, just as their non-GM predecessors always have. The latter are based on monkey and rat models in which poisons are injected that affect their brains: overwhelmingly, clinical trials that have stemmed from the data produced have shown negative or, at best, 'unsatisfactory' results. A review of these strategies was compelled to state, 'The results of all these studies raise several questions about the true reliability and validity of animal data, the

adequacy of the current working hypotheses, and the presently used tools to evaluate a specific effect.'⁹⁴

As always, a critical examination of the literature shows that we have non-animal research to thank for the great discoveries surrounding PD. Autopsies revealed the importance of the brain's substantia nigra; *in vitro* research indicated dopamine deficiency; epidemiology is uncovering the genetic basis of the disease and susceptibility to it; clinical studies and serendipity were the foundation of others.

Diabetes (Type I, or Insulin Dependent')

Type I diabetes occurs when part of the pancreas is destroyed by the body's own immune system, meaning that it cannot produce insulin. Without insulin entering the bloodstream from the pancreas, the glucose in our blood derived from the food we eat cannot be delivered to the cells of our bodies that need it for energy. Instead of getting into our cells, this glucose builds up in the blood, with extremely dangerous consequences.

Diabetes research followed the course of all other areas of medical research by turning towards animal models. In this instance, modern transgenic technologies have not been entirely responsible for this: simple breeding of rodents gave rise to the 'Non Obese Diabetic' (NOD) mouse and the BioBreeding (BB) rat, although transgenesis has been employed in an attempt to determine the role of the immune system.

Unfortunately for people suffering from diabetes all over the world, animal models have succeeded only in impeding and misleading researchers. Some quotes from a recent comprehensive review on the subject⁹⁵ are extremely candid:

'Animal models... have led to misconceptions and erroneous extrapolations, as well as false expectations with regard to the efficacy of immunotherapy. We argue that animal models have limited value when it comes to teaching us about Type I diabetes in humans.'

'The Type I diabetes research community

Uses of, and Problems with, Genetically Modified Animals: I. Human Disease Models



Uses of, and Problems with, Genetically Modified Animals: I. Human Disease Models

has developed a 'selective blindness' as evidenced by its failure to recognise a number of shortcomings associated with animal models of the disease.'

'Studies of transgenic animals or gene knock-out mice represent case reports that could suffer from cell biological and immunological artefacts unrelated to and incompatible with Type I diabetes in humans, or even in rats and mice.'

'Animal models have, over time, proved to be more often inaccurate than accurate, especially with regard to therapeutic interventions.'

Some of these reported successful therapeutic interventions in NOD mice include bee venom, castration, 'emotionality' and nicotine. Conflictingly, other examples are cold exposure and elevated temperature, and solitary housing and overcrowding. Almost 200 interventions have been shown to prevent or delay Type I diabetes in NOD mice... with no success in humans.

There are profound differences between the immune systems of mice and humans, amounting to more than 80 examples,⁹⁶ a fact that surely invalidates any attempt to infer useful information from mouse models. Even so, the differences are generally ignored, despite them being the reason why the results from transgenic mouse studies into Type I diabetes have been difficult to understand.^{97,98} In fact, NOD mice lack many immune system components present in humans that render them resistant to diabetic ketoacidosis – the most serious manifestation of diabetes, leading to coma and often death.

Type I diabetes is a complex, multifactorial disease in which there is no straightforward relationship between particular genes and the condition itself. If valuable and promising findings from human-oriented investigations had been built upon without recourse to animal models, we would undoubtedly be better poised in our search for a cure. Contrary to popular belief, animal experiments were not necessary for the discovery of insulin or its production to help diabetics; this much can be easily established by examining any full and

objective account of the history of the disease. As ever, a more in depth study of the course of medical progress reveals that autopsies, clinical studies, *in vitro* work and medical serendipity were at the core of our understanding of and progress towards treating and curing diabetes. Patient studies revealed that the basis of the disease is an autoimmune attack on the insulin producing cells of the pancreas, and also resulted in the discovery of oral drugs that obviate the need for insulin injections in some patients. *In vitro* techniques allowed the discovery, purification and mass production of insulin, while human based studies continue to elucidate the genetics behind and the causes of the disease.

Alzheimer's Disease

Alzheimer's Disease (AD) represents 70% of all dementias, affecting just under 1% of the population. It is characterised by progressive cognitive and memory decline, with the associated presence of distinctive hallmarks of AD in the brain, known as amyloid plaques and neurofibrillary tangles.

These plaques and tangles were discovered via autopsies of affected patients, and have been extensively characterised since then. Plaques are composed largely of a protein called A β (beta-amyloid), which is 'cut' from a naturally-occurring precursor protein known as APP. Tangles are made up of another 'normal' protein known as 'tau' that is present in all ageing human brains.

Transgenic mice have been used in various ways to investigate the formation of these plaques and tangles. Mice with mutant tau genes were created in the hope that they could shed some light upon tangle formation in the human brain affected by AD, but they failed to show any AD-like symptoms or even any sign of altered neurological function. These findings suggested that tau may be a simple effect of the disease rather than a cause, though it is now generally thought that tau is highly species-specific: tau pathology in the ageing brain is unique to human beings with the exception of a few old baboons, for example.⁹⁹ Transgenic



animals expressing large amounts of APP protein, mutant APP, or with APP completely knocked out have failed to indicate the function of the protein or its role in AD:¹⁰⁰ those animals accumulating A β plaques display only subtle effects, and do not develop neurofibrillary tangles or suffer from significant neurodegeneration.¹⁰¹ The same is true for other animals engineered with genetic mutations found from screens of human AD patients, such as in the 'presenilin 1' and 'presenilin 2' genes.^{102,103} In contrast, human presenilin mutations resulting in the overproduction of Ab and plaque formation¹⁰⁴ are a major cause of AD, although the exact mechanisms are not known.

In summary, animal models of AD have failed to replicate the pathology of the human disease and to shed any light upon its true causes: despite a huge investment in terms of funding, human effort and sacrificed animals, we are no further forward in knowing if AD-associated plaques and tangles are a cause or an effect of the disease. Meanwhile, animal-based research continues to mislead and cause harm. A proposed AD vaccine, for example, developed using transgenic mice (which incidentally also tested 'safe' in monkeys, rabbits and guinea pigs), had to be withdrawn after it caused serious brain inflammation in clinical trials.¹⁰⁵ And, of course, human based research is continuing to pay dividends: genetic screens, clinical research and *in vitro* studies are forging ahead, unravelling the course of the human disease, revealing new genes and drug targets.

Other diseases

Available space precludes a more comprehensive précis of GM animal models of other human diseases; suffice to state that a common theme runs through them all. The disastrous consequences of modelling even a 'simple' (in genetic terms) disease such as cystic fibrosis should have resulted in all signs pointing away from the road towards GM animal research, yet it continues to be

pursued with more vigour than ever. And CF is not the only such example; take, for instance, two other single-gene diseases that have been 'modelled' using transgenic mice: Tay-Sachs Disease and Lesch-Nyhan Syndrome. Tay-Sachs Disease results when a faulty gene causes the accumulation of a fatty material called 'ganglioside' in nerve cells throughout the body, leading to deafness, blindness, dementia and, ultimately, paralysis. Transgenic mice mirrored Tay-Sachs in humans by accumulating gangliosides. Unlike humans, this occurred only in specific nerve cells, and crucially the mice failed to show any Tay-Sachs symptoms.^{106,107} Mice carrying similar genetic mutations to those found in humans with Lesch-Nyhan Syndrome showed none of the self-harming behaviour or mental retardation seen in the latter – a difference subsequently revealed to be due to a simple biochemical disparity between the species.^{108,109} If models of a one-gene disease fail so miserably, how can we expect 'stab-in-the-dark' models of immensely complex human diseases involving many unknown genes to bear fruit?

Uses of, and Problems with, Genetically Modified Animals

I. Human Disease Models



Uses of, and Problems with, Genetically Modified Animals

2. Toxicology & 3. Production of Organs for Human Transplant

2. Toxicology

In recent years, transgenesis has been used in what can only be considered to be a last-ditch attempt to derive some form of useful information from animals used in toxicity testing. For decades, the assessment of which chemicals, drugs, food additives and so on might pose a hazard to human health has relied heavily upon administering them to mice and rats, and examining their tissues for damage. It is now universally accepted that the correlation between results from these investigations are in the region of 5-30%, a statistic that belies claims that these tests can be in any way predictive of human response.^{110,111}

And so these mice have been transformed into new, improved transgenic animals that are now more susceptible to the harmful effects of various substances – and, it is hoped, be more predictive of which substances will poison and/or cause cancer in human beings. The reality is that transgenic animals are continuing to produce inconsistent results and be of no predictive value in such assessments, and that no single transgenic animal or combination of transgenic animals performs nearly well enough to be considered sufficiently reliable for regulatory use.^{112,113} For example, genetically engineered mice manipulated to investigate genes involved in cancers of the nervous system in children showed that some genes and mutations clearly associated with specific human tumours produced very different effects in mice, and that one cancer-causing genetic pathway in rats did not operate in any human tumours.¹¹⁴

As ever, non-animal techniques are showing much more promise in this field. Toxicity and carcinogenicity screens using batteries of tests involving human cells, DNA chips, in vitro methods and computer modelling are paving the way. Only stubborn and dogmatic resistance to them in favour of the 'tried and tested' approach (no matter how badly this has failed) is standing in the way of their adoption.

3. Production of Organs for Human Transplant

Due to the perennial shortage of human organs available for transplant, attempts to use animal organs in a process known as 'xenotransplantation' have been made for many years. All of them have failed due to the rejection by the host's immune system of the 'foreign' transplanted organ. This also occurs in human to human transplants, though any reaction is minimised via careful tissue matching prior to transplant followed by the prescription of immunosuppressive drugs to dampen this response. When the donor organ is from a different species, however, the host immune response can become a violent 'hyperacute rejection' in which the organ can be destroyed within minutes.

In recent times, pigs have been genetically manipulated in an attempt to overcome this problem. Pigs were chosen because they possess organs that are roughly the same size as their human counterparts. Additionally, because they are used as food animals in their billions, they do not pose ethical difficulties for most people. They have been engineered so that their organs for transplant should be less prone to rejection, by either removing surface proteins that reveal them as 'alien' in a new host, or by adding a human protein that can inhibit the molecular mechanisms responsible for rejection.¹¹⁵⁻¹¹⁷

Unfortunately for the companies that have invested tens of millions of pounds in xenotransplantation in the hope of realising the speedy and massive financial gains promised by its proponents, things have not gone according to plan. Organ rejection has proved to be a more complex process than originally thought, involving many more genes and molecular pathways that must be intercepted.¹¹⁸ The scale of the problem posed by viruses carried by pigs was clearly underestimated: there are high profile examples of infectious agents crossing species barriers, such as the Ebola and Marburg viruses from monkeys, the possible evolution of HIV from a monkey virus, BSE/CJD from cows, and recent avian flu epidemics in Asia.



Perhaps more worryingly, instances of viruses crossing to humans from pigs make grim reading: the 'Nipah' virus crossed from pigs to humans and killed more than 100 people in Malaysia in 1999;¹¹⁹ the Spanish Flu that killed up to 50 million people in 1918 is thought to have been a mutant pig flu virus,¹²⁰ and other agents have been identified that normally infect pigs but that can also infect humans.^{121,122} Clearly, the danger of infection here is not only limited to potential recipients of xenotransplants: everyone is at risk. One particular hazard is a type of pig virus known as 'Porcine Endogenous Retrovirus' (PERV), and experiments have shown that these viruses can and do infect human tissue. PERVS cannot be eliminated from donor pig organs, a fact that prompted a renowned virus expert to state, 'Public health officials should resist the transplant community's clamour for animal organs in light of this data.'¹²³

A substantial question mark has been placed over the suitability of pig organs for human transplant even if these rejection problems and infection risks could be surmounted, based on basic but major physiological and biochemical differences between the organs^{124,125} that mean the organs simply may not function in a human environment. Another factor that has not escaped the attention of even the most ardent supporter of xenotransplantation is the cost in terms of animal welfare: not only do the animals involved (that have included sheep, tigers, pigs, cats, lions, wolves, foxes, dingoes, dogs, hares, rabbits, baboons, monkeys, goats, guinea pigs, mice and rats) suffer from the genetic modification process as detailed earlier, but they then undergo invasive surgery, the side-effects of immunosuppressive drugs, and invariably suffer the consequences of organ failure leading to death.

As ever, there are alternatives to this exercise in futility that have been demonstrably successful where they have been implemented. Simple strategies such as encouraging lifestyle changes that prevent the need for many transplants; encouraging the 75% of people who express a willingness to carry donor cards to do

so; improving organ supply and debating the introduction of an 'opt-out' scheme such as that which means organ transplants occur at almost three times the rate in Spain compared with the UK. Stem cell technologies are improving too, with the promise that they could eventually result in the production of tissues and organs for 'self-transplant'; already, they have been used to repair the hearts of heart-failure patients who no longer need transplants.¹²⁶

4. Pharmaceutical Factories

Human proteins are used therapeutically in the treatment of a wide range of diseases, such as multiple sclerosis, hepatitis, cancer, cystic fibrosis and malaria. These proteins have been successfully produced via a number of methods for some years, including GM bacterial and yeast cultures, cultures of mammalian and plant cells, and entire GM plant crops, with each method having distinct advantages and disadvantages. Transgenic animals have been added to this list more recently, not due to necessity, but mainly because companies producing the therapeutic proteins believe that, once developed, pharmaceutical-producing GM animals can be scaled up to a huge degree and will then generate almost limitless amounts of product very cheaply.¹²⁷⁻¹²⁹

Cows, chickens, goats, pigs, rabbits and sheep have been genetically engineered to produce therapeutic proteins in an industry known as 'pharming' or 'biopharming.' The animals are manipulated so that they produce these products in their milk, mostly, but also in their urine, blood, or even sperm.^{130,131} Large amounts of these proteins are then purified and processed into a final product. Animal welfare concerns include all that has been mentioned previously in this report regarding the production of transgenic animals, but there are some additional problems specific to pharming. In principle, transgene expression is intended to be confined to, for example, the mammary gland in those animals engineered to produce the transgene protein product in their milk. However, 'leaky' gene



Uses of, and Problems with, Genetically Modified Animals

5. Cloning

expression is often detected in other tissues, and the proteins are often found in the animals' blood.^{132,133} This can have severe negative health consequences,¹³⁴ causing animals to suffer from 'pathologies and other severe systemic effects', as reported by the National Academy of Sciences in the USA.

Scientific and medical concerns surrounding these endeavours include, in common with xenotransplantation, the risk of cross-species disease transmission. This risk, of course, is real, though it may be considered minor by patients relying upon a transgenic therapeutic protein to ameliorate their suffering and/or disease. In addition, it is a statement of fact that many if not most human proteins will not be 'as they should' structurally, functionally and biochemically unless they are produced in a human milieu, i.e. in cultured human cells. Some proteins are absolutely fine being produced in bacteria, for example, but others show marked differences – ranging from ostensibly inconsequential, superficial changes, to massive and catastrophic ones. Proteins in the latter class need to be produced in 'higher' cells... so why produce them in cow's milk instead of cultured human cells? The only answer is: profit. And to produce such therapeutic proteins in transgenic animals, with all that the process entails, when this is not strictly necessary, can be regarded as ethically abhorrent and unjustifiable, especially in cases where they could be efficiently produced using plants and other means.

5. Cloning

Although cloned animals are not all strictly, by definition, 'genetically modified' or 'transgenic,' a brief consideration is given here because the cloning process is extremely important to transgenic research.

The technique, which seeks to produce a physical replica of another individual organism, involves fusing a cell from the individual to be cloned (which may or may not be GM) with an egg cell from a donor individual that has had its nucleus (containing its DNA) removed. The egg is then free to develop, but uses the DNA from the other

individual as its 'programming,' thereby resulting in a copy of that individual.

The main scientific reasons for this activity are to develop so-called 'therapeutic cloning' and 'reproductive cloning' technologies. It is hoped that therapeutic cloning could serve to produce body parts, organs and cells that are an identical match to one's own, paving the way to safer and more effective transplants and treatments for all kinds of diseases. Reproductive cloning, meanwhile, is currently being developed to allow scientists to replicate transgenic animals of interest.

It may be that exponents of cloning, like those of transgenesis and those of many other scientific disciplines who believe in the promise of their work, overstate the potential impact of it. It may be that therapeutic cloning could be the answer to the prayers of many people suffering from a variety of diseases. But that should not overshadow considerations of the amount of work involved to achieve success, or the significant caveats intimately associated with it, or the ethical concerns of the processes involved, or the fact that, what works in one species bears no relation to what will or will not work in another.

Suffice to say here that cloning is an immensely inefficient process, with poor survival rates and a high degree of associated suffering for the animals involved. Typically, only one animal reaches adulthood for every 100 manipulated eggs; a 99% failure rate. Of these 'successful' clones, almost all animals will display some form of defect or abnormality, and many will die prematurely.

The intentions of some of its most vociferous advocates must also be suspect, with human cloning, agricultural animal cloning and companion animal cloning never far from the headlines. Just recently (in August of 2005) it was reported that the world's first canine clone, an Afghan hound, had been created in South Korea. The team responsible transferred 1095 embryos into 123 surrogate mothers – but just three pregnancies resulted.



Alternatives to Research involving Transgenic Animals

There is an abundance of evidence that, if examined objectively, illustrates in no uncertain terms that animal experiments have been incidental to medical progress, and it seems that GM animals aren't going to change things one bit. We have little to thank them for in the history of medicine. But even if they had been central and crucial to many areas of medical progress over the years, we'd still have to ask if they are necessary or even helpful in the year 2005. It's high time that a proper assessment of the roles and contributions of various scientific approaches was undertaken.

*'The mouse-wrangling business is booming... a \$200 million affair. The leading commercial ranch... raked in \$140 million in rodent sales last year.'*¹³⁵

We live in an era with a great potential for medical advancement. The tools and technologies available to scientists to unpick the secrets of nature and to get a real handle on the terrible diseases that afflict mankind are truly mind-boggling, and are showing us the way ahead. It is distressing to realise that a small yet significant and immensely powerful group of researchers, backed by the industries that stand to profit from their work, cling to animal research with the same tenacity with which people cling to their livelihoods and their life savings; and if they were honest, they'd admit that this is exactly what they're doing.

Fortunately, many scientists are genuinely objective, questioning, analytical and impartial. And an overwhelming majority of them pursue career paths that do not involve animal research – actively shunning it. The reason is simple: animal research is not the answer. Goal-oriented scientists working to get to the bottom of human diseases know that animal research will tell them nothing of any use, but that working with better, more predictive and more relevant methods will.

This is why the majority of research is *in vitro*, using human cells and tissues obtained from 'tissue banks'. This is why the use of computer modelling, bioinformatics (using computers to analyse biological data), DNA chips and human stem cells is increasing rapidly. It is why clinical studies and patient registers are forging ahead, and why massive-scale genetic screening programmes are planned, using cutting edge technology to unravel the fundamental basis of so many diseases. Ultimately, we want new, effective and safe drugs to treat our ailments, and we don't need animals biochemically far-removed from us to tell us how to do this.

We live in an age with PET (Positron Emission Tomography) and MRI (Magnetic Resonance Imaging) scanners that can trace miniscule amounts of new drugs through a *human* body to see where they go, what they do, how they are got rid of and how quickly. They can look at what's happening inside the human brain and other organs. Robotic devices can scan our genes in hours to detect abnormalities and subtle differences that can cause problems. We have no need whatsoever to resort to animal-based studies. They have always confounded what we have learnt from human studies, and never more so than GM animals. If science doesn't move onwards and upwards, away from this paradigm, we will impede medical progress by years.

**Alternatives to Research
involving Transgenic Animals**



Summary and Conclusions

In assessing the uses of genetically modified animals as we move into the 21st century, the considerations and deliberations of society boil down to one salient question: do they make a positive contribution to human health and wellbeing? For if they don't, then all other factors are irrelevant. If, on balance, they do more harm than good, there can be no argument for persisting with their creation and development, especially when they are destined to suffer from the moment of their birth.

In each case, we must ask if the time, money and effort being invested in improving transgenic techniques are worthwhile. Might the claims being made by the scientists involved be, to some degree, overblown, as is often the case with relatively new technologies? Do GM animals *really* promise to cure disease? Do they have a track record of promoting medical progress since their introduction 20 years ago? Might the resources dedicated to them be better allocated elsewhere, to more relevant research? Can any amount of genetic engineering ever make animals 'human enough' to give meaningful results?

In light of the facts and figures summarised in this report, the inescapable conclusion must be that the 'balance of trade' for GM animals is severely in the red. The harm that has been caused by their use is so widespread and comprehensive that an inordinate number of tangible successes would have to be realised even to begin to redress the balance.

Any correlation of data from research involving GM animals with the human situation is an exception, not the rule. Any future successes must be weighed against all past failures. Such data must be predictive of human diseases and responses to be of any use. There is no merit in claiming, as some do, that we must now make more use of GM animals to catalogue the differences between 'them' and 'us,' in the hope that this will enable us to sidestep the obstacles caused by species differences and make some sense of the

animal models, of cancer for example.¹³⁶ If we want cures for human cancers, the bitter experience of the past 40 years tells us to look at cancers in human beings.

Transgenesis may well be scientifically intriguing and challenging, but it is also a folly. We have shown in this report the reckless nature of transgenic research, whether ostensibly to find cures for disease or to supply organs for transplant. The price we pay is incalculable human suffering as medical progress is impeded by years, and the immense suffering of millions of animals.

It is not for those who object to animal experimentation for whatever reason to defend their stance: it is for those who support and pursue it to defend what they do with substance and hard evidence. Precious little evidence has so far been offered in defence of animal experiments that can withstand scrutiny, and GM animals are not set to change this.

There is increasing demand from the general public, as well as from the scientific and medical communities, for an independent, objective, comprehensive and transparent scientific evaluation of animal research. Such an evaluation has never taken place, despite the impressive evidence calling into question the scientific value of the 'animal model'. As more substantiation of the scientific objections to animal research put forward by groups such as Animal Aid and Europeans for Medical Progress comes to light, it becomes imperative. We call upon the government to rise above the propaganda issued by those with vested interests in the continuance of animal experimentation, and make good their erstwhile promise of an inquiry into its scientific validity.

"These models do not represent the basic essence of human diseases... Prestigious journals, however, appear more fascinated with the modern mythology of transgenic and knock-out mice than the humble reality of human disease."

Dr. Francesco M. Marincola, Editor-in-Chief of the Journal of Translational Medicine.



References

1. Mukerjee M. Speaking for the Animals. *Scientific American* Aug 2004. p. 96.
2. Hollinger S and Hepler JR. (2002) Cellular regulation of RGS proteins: modulators and integrators of G protein signaling. *Pharmacol Rev* 54(3):527-59.
3. Behr R and Weinbauer GF. (2001) cAMP response element modulator (CREM): an essential factor for spermatogenesis in primates? *Int J Androl* 24(3):126-35.
4. Dyck MK, Lacroix D, Pothier F, Sirard MA (2003) Making recombinant proteins in animals--different systems, different applications. *Trends Biotechnol* 21(9):394-9.
5. Canadian Council on Animal Care (CCAC) guidelines on Transgenic Animals. (1997).
6. Munk C, Lohler J, Prassolov V, Just L, Stockschlader M and Stodking C. Amphoteric murine leukemia viruses induce spongiform encephalo-myelopathy. *PNAS* 1997; 94: 5837-5842.
7. Cornetta K, Morgan RA, Anderson WF. Safety issues related to retroviral-mediated gene transfer in humans. *Human Gene Ther.* 1991; 2(1):5-14.
8. Gunter KC, Khan AS, Noguchi P. The safety of retroviral vectors. *Human Gene Ther.* 1993; 4(5):643.
9. Temin HM. Safety considerations in somatic gene-therapy of human-disease with retrovirus vectors. *Hum Gene Ther.* 1990; 1(2):111-123.
10. Murphy D, Bishop A, Rindi G, Murphy MN, Stamp GW, Hanson J, Polak JM, Hogan B. (1987) Mice transgenic for a vasopressin-SV40 hybrid oncogene develop tumors of the endocrine pancreas and the anterior pituitary. A possible model for human multiple endocrine neoplasia type 1. *Am J Pathol*;129(3):552-66.
11. Palmiter RD and Brinster RL. (1986) Germ-line transformation of mice. *Annu Rev Genet*;20:465-99.
12. Dean RG. (1998) Testing promoter/gene constructs for transgenic animals. University of Georgia Animal and Dairy Science, annual report, pp104-108.
13. Rink L and Wenzel BE (1996) Transgenic mice models in autoimmunity – discussion. *Exp Clin Endocrinol Diabetes* 104 Suppl 3:46-8. Review.
14. Van der Meer M, Baumans V, Hofhuis FM et al. (2001) Consequences of gene targeting procedures for behavioural responses and morphological development of newborn mice. *Transgenic Research* 10;399-408.
15. Shuldiner AR (1996) Transgenic animals. *N Engl J Med.* 334(10):653-5. Review.
16. Galli-Taliadoros LA, Sedgwick JD, Wood SA, Korner H (1995) Gene knock-out technology: a methodological overview for the interested novice. *J Immunol Methods* 181(1):1-15. Review.
17. Bradley A, Evans M, Kaufman MH, Robertson E (1984) Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. *Nature* 309(5965):255-6.
18. Humpherys D, Eggan K, Akutsu H et al. (2001) Epigenetic instability in ES cells and cloned mice. *Science* 293(5527):95-7.
19. Smith K and Spadafora C (2005) Sperm-mediated gene transfer: applications and implications. *Bioessays* 27(5):551-62. Pagan Westphal S (2002) Designer animals made easy. *New Scientist* 2336;6-7.
20. Pagan Westphal S (2002) Designer animals made easy. *New Scientist* 2336;6-7.



21. Maione B, Lavitrano M, Spadafora C, Kiessling AA (1998) Sperm-mediated gene transfer in mice. *Mol Reprod Dev*;50(4):406-9.
22. Zani M, Lavitrano M, French D, Lulli V. (1995) The mechanism of binding of exogenous DNA to sperm cells: factors controlling the DNA uptake. *Exp Cell Res*. 1995; 217(1):57.
23. Smith KR (2004) Gene Therapy: The Potential Applicability of Gene Transfer Technology to the Human Germline. *Int. J. Med. Sci.* 2004 1(2): 76-91.
24. Nolan PM, Peters J, Strivens M et al. (2000) A systematic, genome-wide, phenotype-driven mutagenesis programme for gene function studies in the mouse. *Nat Genet*;25(4):440-3.
25. Nolan PM, Peters J, Vizor L et al. (2000) Implementation of a large-scale ENU mutagenesis program: towards increasing the mouse mutant resource. *Mamm Genome*;11(7):500-6.
26. Hrabe de Angelis MH, Flaswinkel H, Fuchs H et al. (2000) Genome-wide, large-scale production of mutant mice by ENU mutagenesis. *Nat Genet*;25(4):444-7.
27. Jackson IJ (2001) Mouse mutagenesis on target. *Nat Genet*;28(3):198-200.
28. Goldowitz D, Frankel WN, Takahashi JS et al. (2004) Large-scale mutagenesis of the mouse to understand the genetic bases of nervous system structure and function. *Brain Res Mol Brain Res*;132(2):105-15.
29. Ohl F and Keck ME (2003) Behavioural screening in mutagenised mice--in search for novel animal models of psychiatric disorders. *Eur J Pharmacol*;480(1-3):219-28.
30. Keays DA and Nolan PM (2003) N-ethyl-N-nitrosourea mouse mutants in the dissection of behavioural and psychiatric disorders. *Eur J Pharmacol*;480(1-3):205-17.
31. Nishimura I, Drake TA, Lusk AJ et al. (2003) ENU large-scale mutagenesis and quantitative trait linkage (QTL) analysis in mice: novel technologies for searching polygenetic determinants of craniofacial abnormalities. *Crit Rev Oral Biol Med*;14(5):320-30.
32. Thaug C, West K, Clark BJ et al. (2002) Novel ENU-induced eye mutations in the mouse: models for human eye disease. *Hum Mol Genet*;11(7):755-67.
33. Nolan PM, Hugill A, Cox RD (2002) ENU mutagenesis in the mouse: application to human genetic disease. *Brief Funct Genomic Proteomic*;1(3):278-89.
34. Abbott A (2000) A mutant mouse menagerie. *Nature* 406;559.
35. Abbott A (2002) Geneticists lay plans for rationalized rodents. *Nature* 417;211.
36. Jenkins ES, Gray S, Combes RD (2001) Mutagenesis screens: can they be justified? *Altern Lab Anim*;29(1):63-8.
37. Mephram TB, Combes RD, Balls M et al. (1998) The use of transgenic animals in the European Union:the report and recommendations of ECVAM workshop 29. *ATLA* 26;21-43.
38. Petters RM and Sommer JR (2000) Transgenic animals as models for human disease. *Transgenic Res* 9(4-5):347-51; discussion 345-6.
39. Snouwaert JN, Brigman KK, Latour AM et al. (1992) An animal model for cystic fibrosis made by gene targeting. *Science*. 1992;257(5073):1083-8.
40. Barinaga M (1992) Knockout mice offer first animal model for CF. *Science*;257(5073):1046-7.



41. Wilson JM and Collins FS (1992) Cystic fibrosis. More from the modellers. *Nature*; 359(6392):195-6.
42. Dorin JR, Dickinson P, Alton EW et al. (1992) Cystic fibrosis in the mouse by targeted insertional mutagenesis. *Nature*;359(6392):211-5.
43. Ratcliff R, Evans MJ, Cuthbert AW et al. (1993) Production of a severe cystic fibrosis mutation in mice by gene targeting. *Nature Genetics* 4, 35 - 41.
44. Ameen N, Alexis J, Salas P (2000) Cellular localization of the cystic fibrosis transmembrane conductance regulator in mouse intestinal tract. *Histochem Cell Biol*;114: 69-75.
45. Hardcastle J, Harwood MD, Taylor CJ (2004) Small intestinal glucose absorption in cystic fibrosis: a study in human and transgenic DeltaF508 cystic fibrosis mouse tissues. *J Pharm Pharmacol*;56: 329-38.
46. Coates SW Jr, Hogenauer C, Santa Ana CA et al. (2004) Inhibition of neutral sodium absorption by a prostaglandin analogue in patients with cystic fibrosis. *Gastroenterology*;127: 65-72.
47. Santis G and Geddes D (1994) Recent advances in cystic fibrosis. *Postgrad Med J*; 70: 247 - 251.
48. Engelhardt JF, Yankaskas JR, Ernst SA et al. (1992) Submucosal glands are the predominant site of CFTR expression in the human bronchus. *Nat Genet* 2: 240-8.
49. Pack RJ, Al-Ugaily LH, Morris G, Widdicombe JG. (1980) The distribution and structure of cells in the tracheal epithelium of the mouse. *Cell Tissue Res* 208: 65-84.
50. Kelley KA, Stamm S, Kozak CA. (1992) Expression and chromosome localization of the murine cystic fibrosis transmembrane conductance regulator. *Genomics* 13: 381-8.
51. Rogers DF, Alton EW, Dewar A et al. (1990) Tracheal potential difference in the reserpine and isoproterenol rat models of cystic fibrosis. *Exp Lung Res*;16:661-670.
52. Pier GB, Small GJ, Warren HB (1990) Protection against mucoid *Pseudomonas aeruginosa* in rodent models of endobronchial infection. *Science*;249:537-540.
53. Koller BH, Kim HS, Latour AM et al. (1991) Toward an animal model of cystic fibrosis: Targeted interruption of exon 10 of the cystic fibrosis transmembrane regulator gene in embryonic stem cells. *Proc Natl Acad Sci USA*;88:10730-10734.
54. Rosenfeld MA, Yoshimura K, Trapnell BC et al. (1992) In vivo transfer of the human cystic fibrosis transmembrane conductance regulator gene to the airway epithelium. *Cell* 68(1);143-155.
55. Kessler WR and Andersen DH (1951) Heat prostration in fibrocystic disease of the pancreas and other conditions. *Pediatrics* 8(5): 648-656.
56. Di Sant' Agnese PA, Darling RC, Perera GA, Shea E (1953) Abnormal electrolyte composition of sweat in cystic fibrosis of the pancreas; Clinical significance and relationship to the disease. *Pediatrics* 12(5): 549-563.
57. Yamaya M, Finkbeiner WE, Widdicombe JH (1991) Altered ion transport by tracheal glands in cystic fibrosis. *Am J Physiol Lung Cell Mol Physiol*; 261: 491 - 494.
58. Cozens AL, Yezzi MJ, Chin L et al. (1992) Characterization of Immortal Cystic Fibrosis Tracheobronchial Gland Epithelial Cells. *PNAS* 89: 5171-5175.
59. Clarke LL, Grubb BR, Yankaskas JR et al. (1994) Relationship of a non-cystic fibrosis transmembrane conductance regulator-mediated chloride conductance to organ-level disease in *Cftr(-/-)* mice. *Proc Natl Acad Sci USA* 91:479-483.



60. Rozmahel R, Wilschanski M, Matin A et al. (1996) Modulation of disease severity in cystic fibrosis transmembrane conductance regulator deficient mice by a secondary genetic factor. *Nat Genet* 12:280-287.
61. Bronsveld I, Mekus F, Bijman J et al. (2001) Chloride conductance and genetic background modulate the cystic fibrosis phenotype of Delta-F508 homozygous twins and siblings. *J Clin Invest* 108: 1705-15.
62. Egan ME, Pearson M, Weiner SA et al. (2004) Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects. *Science* 304: 600-2.
63. Song Y, Sonawane ND, Salinas D et al. (2004) Evidence against the rescue of defective DeltaF508-CFTR cellular processing by curcumin in cell culture and mouse models. *J Biol Chem* 279(39):40629-33. Epub 2004 Jul 26.
64. Hyde SC, Gill DR, Higgins CF et al. (1993) Correction of the ion transport defect in cystic fibrosis transgenic mice by gene therapy. *Nature* 362: 250-255.
65. Alton EW, Middleton PG, Caplen NJ et al. (1993) Non-invasive liposome-mediated gene delivery can correct the ion transport defect in cystic fibrosis mice. *Nat Genet* 5: 135-142.
66. Griesenbach U, Geddes DM, Alton EW. (2003) Update on gene therapy for cystic fibrosis. *Curr Opin Mol Ther* 5: 489-94.
67. Li Z and Engelhardt JF. (2003) Progress toward generating a ferret model of cystic fibrosis by somatic cell nuclear transfer. *Reprod Biol Endocrinol* 1(1):83.
68. Williams SH, Sahota V, Palmari-Pallag T et al. (2003) Evaluation of gene targeting by homologous recombination in ovine somatic cells. *Mol Reprod Dev* 66(2):115-25.
69. Flotte TR, Zeitlin PL, Reynolds TC (2003) Phase I trial of intranasal and endobronchial administration of a recombinant adeno-associated virus serotype 2 (rAAV2)-CFTR vector in adult cystic fibrosis patients: a two-part clinical study. *Hum Gene Ther* 14(11):1079-88.
70. Coates AL and Bush A. (2003) Basic science research vs. clinical research in cystic fibrosis: Has the pendulum swung too far? *Pediatr Pulmonol* 36: 175-7.
71. Shak S, Capon DJ, Hellmiss R et al. (1990) Recombinant Human DNase I Reduces the Viscosity of Cystic Fibrosis Sputum. *Proc Natl Acad Sci USA* 87: 9188-9192.
72. Hubbard RC, McElvaney NG, Birrer P et al. (1992) A preliminary study of aerosolized recombinant human deoxyribonuclease I in the treatment of cystic fibrosis. *N Engl J Med*;326(12):812-5.
73. Zabner J, Seiler MP, Launspach JL et al. (2000) The osmolyte xylitol reduces the salt concentration of airway surface liquid and may enhance bacterial killing. *Proc Natl Acad Sci USA* 97: 11614-11619.
74. Cystic Fibrosis Foundation Annual Report, 2003. <http://www.cff.org/publications/files/2003%20Annual%20Report.pdf>
75. de Rijk MC, Launer LJ, Berger K et al. (2000) Prevalence of Parkinson's disease in Europe: A collaborative study of population-based cohorts. *Neurologic Diseases in the Elderly Research Group. Neurology* 54(11 Suppl 5):S21-3.
76. Kitada T, Asakawa S, Hattori N et al. (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 392(6676):605-8.
77. Kruger R, Kuhn W, Muller T et al. (1998) Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nat Genet* 18(2):106-8.



78. Kruger R, Eberhardt O, Riess O, Schulz JB (2002) Parkinson's disease: one biochemical pathway to fit all genes? *Trends Mol Med* 8(5):236-40.
79. Polymeropoulos MH, Lavedan C, Leroy E et al. (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 276(5321):2045-7.
80. Bonifati V, Rizzu P, van Baren MJ et al. (2003) Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science* 299(5604):256-9. Epub 2002 Nov 21.
81. van Duijn CM, Dekker MC, Bonifati V et al. (2001) Park7, a novel locus for autosomal recessive early-onset parkinsonism, on chromosome 1p36. *Am J Hum Genet* 69(3):629-34. Epub 2001 Jul 2.
82. Foltynie T, Sawcer S, Brayne C, Barker RA (2002) The genetic basis of Parkinson's disease. *J Neurol Neurosurg Psychiatry* 73(4):363-70.
83. Gwinn-Hardy K, Farrer M (2002) Parkinson's genetics: an embarrassment of riches. *Ann Neurol* 51(1):7-8.
84. Abeliovich A, Schmitz Y, Farinas I et al. (2000) Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. *Neuron* 25(1):239-52.
85. Beal MF (2001) Experimental models of Parkinson's disease. *Nat Rev Neurosci* 2(5):325-34.
86. Vila M, Wu DC, Przedborski S (2001) Engineered modeling and the secrets of Parkinson's disease. *Trends Neurosci* 24(11 Suppl):S49-55.
87. Masliah E, Rockenstein E, Veinbergs I et al. (2000) Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders. *Science*;287(5456):1265-9.
88. Zhou QY, Quaife CJ, Palmiter RD (1995) Targeted disruption of the tyrosine hydroxylase gene reveals that catecholamines are required for mouse fetal development. *Nature* 374(6523):640-3.
89. Zhou QY, Palmiter RD (1995) Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. *Cell* 83(7):1197-209.
90. Kirik D, Rosenblad C, Burger C (2002) Parkinson-like neurodegeneration induced by targeted overexpression of alpha-synuclein in the nigrostriatal system. *J Neurosci* 22(7):2780-91.
91. Klein RL, King MA, Hamby ME, Meyer EM (2002) Dopaminergic cell loss induced by human A30P alpha-synuclein gene transfer to the rat substantia nigra. *Hum Gene Ther* 13(5):605-12.
92. Goldberg MS, Palacino JJ, Meloni EG, Shen J (2002) Generation and characterisation of parkin knockout mice. Program 118.1 Washington DC: Society for Neuroscience.
93. Goedert M (2001) Alpha-synuclein and neurodegenerative diseases. *Nat Rev Neurosci* 2(7):492-501.
94. Linazasoro G (2004) Recent failures of new potential symptomatic treatments for Parkinson's disease: causes and solutions. *Mov Disord* 19(7):743-54.
95. Roep BO and Atkinson M (2004) Animal models have little to teach us about type 1 diabetes: 1. In support of this proposal. *Diabetologia*;47(10):1650-6. Epub 2004 Oct 13.
96. Mestas J, Hughes CC (2004) Of mice and not men: differences between mouse and human immunology. *J Immunol* 172(5):2731-8.
97. Lohmann T (1998) Limitations of animal models for studying type 1 diabetes. *Diabetes Metab Rev* 14(2):192.



98. Hanson MS, Cetkovic-Cvrlje M, Ramiya VK (1996) Quantitative thresholds of MHC class II I-E expressed on hemopoietically derived antigen-presenting cells in transgenic NOD/Lt mice determine level of diabetes resistance and indicate mechanism of protection. *J Immunol* 157(3):1279-87.
99. Schultz C, Hubbard GB, Tredici KD (2001) Tau pathology in neurons and glial cells of aged baboons. *Adv Exp Med Biol* 487:59-69.
100. van Leuven F (2000) Single and multiple transgenic mice as models for Alzheimer's disease. *Prog Neurobiol* 61(3):305-12.
101. St George-Hyslop PH and Westaway DA (1999) Alzheimer's disease: Antibody clears senile plaques. *Nature* 400, 116-117.
102. Hardy J and Israel A (1999) Alzheimer's disease. In search of gamma-secretase. *Nature* 398(6727):466-7.
103. Wolfe MS, Xia W, Ostaszewski BL et al. (1999) Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and -secretase activity. *Nature* 398, 513 - 517.
104. St George-Hyslop PH (2000) Piecing together Alzheimer's. *Scientific American*, Dec. 2000; p82.
105. Marwick C (2000) Promising Vaccine Treatment for Alzheimer Disease Found. *JAMA* 284:1503-1505.
106. Yamanaka S, Johnson MD, Grinberg A et al. (1994) Targeted disruption of the Hexa gene results in mice with biochemical and pathologic features of Tay-Sachs disease. *Proc Natl Acad Sci U S A* 91(21):9975-9.
107. Huang JQ, Trasler JM, Igdoura S et al. (1997) Apoptotic cell death in mouse models of GM2 gangliosidosis and observations on human Tay-Sachs and Sandhoff diseases. *Hum Mol Genet* 6(11):1879-85.
108. Kuehn MR, Bradley A, Robertson EJ, Evans MJ (1987) A potential animal model for Lesch-Nyhan syndrome through introduction of HPRT mutations into mice. *Nature* 326(6110):295-8.
109. Wu CL and Melton DW (1993) Production of a model for Lesch-Nyhan syndrome in hypoxanthine phosphoribosyltransferase-deficient mice. *Nat Genet* 3(3):235-40.
110. Meijers JM, Swaen GM, Bloemen LJ (1997) The predictive value of animal data in human cancer risk assessment. *Regul Toxicol Pharmacol* 25(2):94-102.
111. Gottmann E, Kramer S, Pfahringer B, Helma C (2001) Data quality in predictive toxicology: reproducibility of rodent carcinogenicity experiments. *Environ Health Perspect* 109(5):509-14.
112. Robinson DE and MacDonald JS (2001) Background and framework for ILSI's collaborative evaluation program on alternative models for carcinogenicity assessment. International Life Sciences Institute. *Toxicol Pathol* 29 Suppl:13-9.
113. Pettit SD (2001) Panel discussion on the application of alternative models to cancer risk assessment. *Toxicol Pathol* 29 Suppl:191-5.
114. Rice JM (2004) Causation of nervous system tumors in children: insights from traditional and genetically engineered animal models. *Toxicol Appl Pharmacol* 199(2):175-91.
115. Miyagawa S, Murakami H, Takahagi Y et al. (2001) Remodeling of the major pig xenoantigen by N-acetylglucosaminyltransferase III in transgenic pig. *J Biol Chem* 276(42):39310-9. Epub 2001 Aug 2.



116. Byrne GW, McCurry KR, Martin MJ (1997) Transgenic pigs expressing human CD59 and decay-accelerating factor produce an intrinsic barrier to complement-mediated damage. *Transplantation* 63(1):149-55.
117. Kulick DM, Salerno CT, Dalmaso AP et al. (2000) Transgenic swine lungs expressing human CD59 are protected from injury in a pig-to-human model of xeno transplantation. *J Thorac Cardiovasc Surg* 119(4 Pt 1):690-9.
118. Butler D (2002) Xenotransplant experts express caution over knockout piglets. *Nature* 415, 103-104.
119. Chua KB (2003) Nipah virus outbreak in Malaysia. *J Clin Virol* 26(3):265-75.
120. Day M (1997) Pigs caused Great War flu deaths. *New Scientist* 29 March 1997, issue 2075, p20.
121. Weiss RA (1998) Transgenic pigs and virus adaptation. *Nature* 391(6665):327-8.
122. Yoo D and Giuliivi A (2000) Xenotransplantation and the potential risk of xenogeneic transmission of porcine viruses. *Can J Vet Res* 64(4):193-203.
123. Allan J (1997) Silk purse or sow's ear. *Nat Med* 3(3):275-6.
124. Crick SJ, Sheppard MN, Ho SY et al. (1998) Anatomy of the pig heart: comparisons with normal human cardiac structure. *J Anat* 193 (Pt 1):105-19.
125. Langley, Dr. G. and Joyce D'Silva (1998) *Animal Organs in Humans: Uncalculated Risks and Unanswered Questions*. ISBN: 1 870356 21 7. British Union for the Abolition of Vivisection and Compassion in World Farming. http://www.ciwf.co.uk/Pubs/Reports/Animal_organs_in_humans.pdf
126. Perin EC, Dohmann HF, Borojevic R et al. (2004) Improved exercise capacity and ischemia 6 and 12 months after transendocardial injection of autologous bone marrow mononuclear cells for ischemic cardiomyopathy. *Circulation* 110(11 Suppl 1):II213-8.
127. Young MW, Meade H, Curling JM et al. (1998) Production of recombinant antibodies in the milk of transgenic animals. *Res Immunol* 149(6):609-10.
128. Rohricht P (1999) Transgenic Protein Production. *BioPharm*; March 01, 1999.
129. Wall RJ (1999) Biotechnology for the production of modified and innovative animal products: transgenic livestock bioreactors. *Livestock Production Science* 59(2-3); 243-255.
130. Meade H and Ziomek C (1998) Urine as a substitute for milk? *Nat Biotechnol* 16(1):21-2.
131. Dyck MK, Gagne D, Ouellet M (1999) Seminal vesicle production and secretion of growth hormone into seminal fluid. *Nat Biotechnol* 17(11):1087-90.
132. Stromqvist M, Houdebine M, Andersson JO et al. (1997) Recombinant human extra cellular superoxide dismutase produced in milk of transgenic rabbits. *Transgenic Res* 6(4):271-8.
133. Meade M et al. (1999) Expression of recombinant proteins in the milk of transgenic animals; in 'Gene Expression Systems: Using Nature for the Art of Expression.' Eds. Fernandez and Hoeffler. Academic Press.
134. Houdebine LM (2000) Transgenic animal bioreactors. *Transgenic Res* 9(4-5):305-20.
135. Osborne L (2000) Fuzzy little test tubes. *New York Times magazine*, July 29th 2000.
136. Green JE, Desai KV, Ye Y, Kavanaugh C, Calvo A, Huh JI. (2004) Application of gene expression profiling for validating models of human breast cancer. *Toxicologic Pathology*. 32 Suppl 1:84-9.



Glossary

Amino Acid

The building blocks of polypeptides and proteins.

Blastocyst

An early stage embryo (typically a few days after fertilisation), taking the form of a hollow ball of cells.

Cell

The smallest unit of living matter that can function independently. Microscopic fluid compartments containing a concentrated solution of chemicals, along with various structures that help the cell to stay alive, replicate and perform its necessary functions.

Chimaera

An individual organism whose constituent cells do not have an identical genetic constitution.

Chromosome

A long and continuous thread of DNA containing many genes.

Clone

An individual grown from a single cell of another, and genetically virtually identical to it.

Cloning

The process whereby identical individual molecules (e.g. DNA) or virtually identical organisms (clones) are produced.

DNA

Deoxyribonucleic acid. The chemical constituent of chromosomes, made up of long chains of four different 'nucleotides,' in the form a double helix.

Embryonic Stem Cell

Cells from an early-stage developing embryo, that are able to differentiate into any type of specialised cell (e.g. liver, muscle, nerve etc).

Enzyme

Any protein that affects the rate at which a biochemical reaction is carried out.

Gene

A specific section of DNA on a chromosome, coding for a particular chain of amino-acids known as a 'polypeptide.' Can be 'Structural' or 'Regulatory.'

Genetically Modified Animals

Animals whose DNA has been artificially manipulated. This includes *clones*, *mutants* and *transgenic* animals.

Genome

The entire genetic complement of a living organism.

In Vitro

Literally, 'in glass.' In research, this term refers to experiments conducted in test-tubes and plastic flasks etc., rather than '*In Vivo*' or in living animals.

Knock-outs

Organisms with one or more genes disabled via the insertion and/or deletion of segments of 'foreign' DNA.

Knock-ins

Organisms containing a fully functioning 'foreign' gene.

**Mutagenesis**

The process of causing genetic changes in individuals that alter or disrupt gene function.

Nucleotide

One of the four molecules that form the basic structural units of DNA.

Nucleus

Defined and bounded region of a cell, containing the cell's genetic material in the form of chromosomes made from DNA.

Phenotype

The observable properties of an organism resulting from the function of its genes, and their interaction with the environment.

Polypeptide

A chain of amino-acids linked by 'peptide' chemical bonds (hence the name), encoded by a gene. See 'Protein.'

Protein

Composed of a chain (or chains) of amino-acids, i.e. one or more polypeptides, with a defined '3D' complex structure that defines its biological function.

Regulatory Gene

Regulatory genes control the expression of structural and other regulatory genes, increasing and decreasing their levels of activity or turning them 'on' or 'off' completely.

RNA

Ribonucleic acid. Similar in structure, but not identical to, DNA. It is responsible for many biological functions, including a role as an intermediary in the production of proteins from DNA.

Structural Gene

Structural genes code for proteins that 'make up' our bodies; that build our cells and organs, and that form enzymes that carry out chemical reactions vital for life. See also 'Regulatory genes.'

Transcription

The first part of 'gene expression.' This is the process whereby an RNA 'copy' of DNA is produced, acting as an intermediate molecule in the production of a polypeptide/protein.

Transgene

A 'foreign' gene introduced into a new 'host' organism.

Transgenesis

The process whereby a foreign 'transgene' is assembled and introduced into a new 'host' organism.

Translation

The second main part of 'gene expression,' after transcription. This is the process whereby an RNA copy of a gene is used as a template to produce a polypeptide/protein made from amino-acids.

Virus

A sub-microscopic infectious agent, composed of a protein 'coat' containing DNA or RNA, that can 'infect' living cells and reproduce inside them.

Xenotransplantation

A process whereby organs from one species are transplanted into the bodies of another species, for example pig hearts into baboons.