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TRANSGENIC ANIMALS

An Interactive Qualifying Project Report

Submitted to the Faculty of

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

By:

Alex Lagadinos

Michael Scofield

Ted Toufas

August 20, 2003

APPROVED:

Prof. David S. Adams, Ph.D.
Project Advisor

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ABSTRACT

The purpose of this IQP was to investigate the controversial new topic of transgenic animals, and determine the impact of this new technology on society. Through extensive medical, scientific and ethical research, this paper documents the impact of transgenic animals on society. Topics such as the description and construction, classification and examples, ethical issues, legal issues and the impact that this newly developed technology has made are detailed in the work. Illustrations, tables and direct quotes have been utilized to convey some of the more technical aspects of the topic. The authors conclude that with cautious oversight, transgenic animals can indeed be made with minimal or no animal suffering, which have enormous potential medical benefit to society.

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EXECUTIVE SUMMARY

Transgenic animals are animals in which foreign DNA has been inserted in the animal's DNA. The purpose of making them is to create an animal that expresses a protein not normally produced by that animal. Their most frequent use is in studying human diseases. Since we cannot infect humans with diseases and try to experiment on them, animals are the closest substitute. However, almost no animals are susceptible to the same diseases that humans can get, so therefore their DNA must be altered to increase their susceptibility by either creating a genetic deficiency (gene knockout) in the animal causing it to be susceptible to a disease, or adding a gene(s) that also causes an increased susceptibility. Adding or deleting genes is how this is controlled, since genes are what proteins are encoded from.

Transgenic disease models are animals that are genetically altered to have qualities that mimic the symptoms of human pathologies. The genomes of these specific animal models were altered to exhibit qualities of a pathology consistent with the characteristic symptoms of the human disease of which they are the model for. The diseases dealt with in this IQP are HIV, Alzheimers, and a specific type of cancer. Science is struggling with research and development for all of these diseases thus these animal models are needed to gain insight into disease initiation and progression.

Transpharmers refers to transgenic animals that are genetically altered to produce a pharmaceutical compound within the animal, usually secreted into the animal's milk. The animal is used as a bioreactor for the synthesis of a therapeutic protein. Most commonly these animals produce these foreign proteins in their mammary glands. Thus the chemical is contained in the milk of the transgenic animal, and the animal only needs to be milked to obtain the therapeutic drug. This type of animal is especially useful for producing protein drugs needing a lot of cellular processing that are difficult or impossible to produce in the lab.

Xenotransplantation involves animals that are genetically altered to better prepare their organs for transplant into human recipients. The animal most commonly used for research in this field is the pig because its physiology most closely matches that of humans. However the successful xenotransplantation of an organ from a pig to a human is made problematic by several factors. There are three types of rejection that the human body undergoes when it detects a foreign compound in the body. The first is hyperacute rejection, where the immune system of the human with the xenotransplanted organ reacts violently and destroys the blood cells in the organ, and cuts off the oxygen, turning the organ black in minutes. The second is delayed xenograft rejection, where antibodies, macrophages, and natural killer cells invade the organ. The third immunological barrier to transplant is T-cell mediated chronic rejection, this can come into play months or years after transplantation. In humans this latter rejection can be controlled by immunosuppression (Butler, 2002).

"Transgenic animals and human gene sequences have enormous commercial value in agriculture, biomedical research, medicine, and the pharmaceutical industry among other fields. The social impact of these forms of biotechnology is nearly limitless" (Walter, 1998). The use of transgenic animals in science can easily be argued as a necessary technique that will create a path of amazing technology in its wake. By inserting human genes into the animals, scientists can more easily study serious human ailments without the use of human test subjects. Rudolf Jaenisch states, "transgenic technology offers exciting possibilities for generating precise animal models for human genetic diseases, and for producing large quantities of economically important proteins by means of genetically engineered farm animals" (Shannon, 1999).

However, transgenics can also be viewed as a dangerous and immoral way to better our own standard of living. James Gustafson states "A scientist has no right to intervene in the natural processes of life, because it is sacred" (Williams, 1973). Depending on the observer's point of view, this radical new technology could take either path. In our eyes, it boils down to a tug of war between the pros of making a specific animal versus the cons. In this report we used the example of Alzheimer's mouse as one with enormous medical benefit, but with no detectable animal suffering. We believe this experiment should continue. We also examined the case of the Beltsville pigs, or superpigs, who had no observable medical benefits with definite animal suffering. We agree with the self-imposed moritorium on this kind of growth hormone experiment in mammals (growth hormone fish seem to have minimal health problems).

Oncomouse^{TM} is an animal that has been genetically altered to be prone to the development of certain types of human cancer (Leder, 1999). This small animal disease

model created by Philip Leder and Timothy Stewart (Leder, 1988) is crucial in the study and understanding of cancer, and also in the screening of new and innovative cancer treatments. The controversy that surrounds this oncogenic mouse created in the 1980s stems from the struggle for Harvard and Dupont to patent the technique that is involved in its creation. Following a series of appeals in US courts, Oncomouse became the world's first patented animal.

The creation of transgenic animals has given the world possibilities that were previously thought unattainable. By utilizing these transgenic animals in research, scientists now have a much deeper understanding of human health. No new line. The creation of such animals is a tedious trial and error process which entails a plethora of different methods and techniques, including the manipulation of in vitro fertilized eggs, or embryonic stem cells.

When technology, such as transgenic science, has the potential to benefit society as greatly as it does, the arguments posed against the continuation of these experiments to our eyes become almost imperceptible. The advancement of technology is inevitable, and these techniques will only become more defined and useful as time progresses. In the future, these animals will not only save lives, but they will also improve the quality of life for all of mankind period

PROJECT OBJECTIVES

The objective of this transgenic animal IQP was to inform and educate the reader with accurate information regarding specific areas of transgenic animal research, and the impact of this new technology on society. As is the case with most new technologies, it has consequences far beyond the labs and companies practicing this new form of biology. Our research displays the positive and negative implications that transgenic research and development have bestowed upon society by showing both sides of the moral, ethical, and legal debate that has arisen from intense transgenic breakthroughs and research. The research performed in this project was also used by the authors to formulate personal conclusions regarding whether to support a continuation of this new technology.

Chapter-1: Introduction to Transgenic Animals and How They Are Made

Transgenic animals are animals in which foreign DNA has been inserted in the animal's DNA. The purpose of making them is to create an animal that expresses a protein not normally produced by that animal. Their most frequent use is in studying human diseases. Since we cannot infect humans with diseases and try to experiment on them, animals are the closest substitute. However, almost no animals are susceptible to the same diseases that humans can get, so therefore their DNA must be altered to increase their susceptibility by either creating a genetic deficiency (gene knockout) in the animal causing it to be susceptible to a disease, or adding a gene(s) that also causes an increased susceptibility. Adding or deleting genes is how this is controlled, since genes are what proteins are encoded from.

Methods for Creating Transgenic Animals

The best way to create a transgenic animal is by altering a fertilized egg during in vitro fertilization, culturing it to the blastocyst stage, and then implanting the blastocyst into the uterus of a surrogate mother. When done correctly, this process creates a transgenic animal whose DNA is altered in every cell of its body. There are several different ways of achieving this. One is through microinjection of the gene into the nucleus. First, a "donor" animal is injected with hormones to increase ovulation. Usually more than one animal is used. Then the eggs are harvested, and the male

pronucleus (larger than the female pronucleus) is injected with hundreds of copies of the desired DNA using a micropipette (see Figure-1).

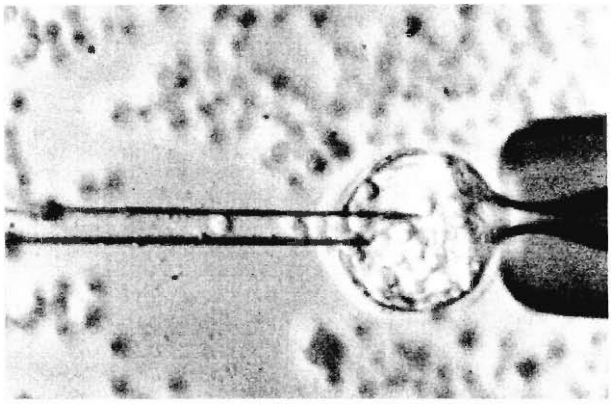

Figure 1: Microinjection of DNA into a pronucleus. (Microinjection Core Facility, 2003).

Sometimes the DNA is not incorporated, or only some of the cells of the resulting animal have the new DNA sequence. If that happens, the resulting animal is called a mosaic animal (see Figure-2) because some, not all of the cells contain the altered DNA.

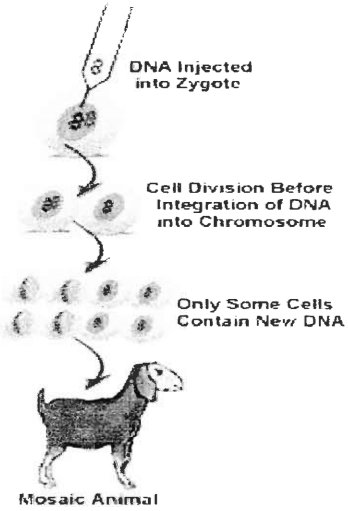

Figure 2: Example of how a mosaic animal is created (Transgenic Animals Overview, 2000) I changed the right margin here.

The second most frequently used technique for making a transgenic animal involves the manipulation of embryonic stem cells (ES cells). Embryonic stem cells are derived from the inner cell masses of blastocysts. A blastocyst is a hollow ball of about 140 cells that develops several days after fertilization (Itskovitz, 2003). These cells can become either somatic cells or germ line cells, so they can be very useful in creating transgenic animals because they can be incorporated into a normal blastocyst and differentiate normally. They can also be grown in culture in a lab.

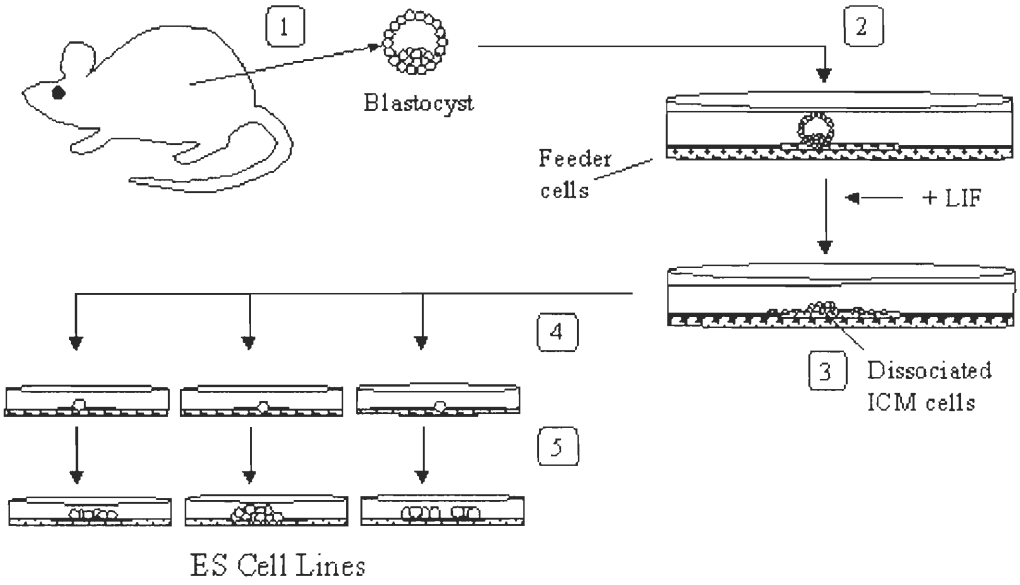

Figure 3: Culture of ES Cells. (Taconic W4/129S6 ES Cells, 2003)

The following text describes the use of ES cells to make a transgenic animal:

1. **Embryonic stem (ES) cells are obtained from pre-implantation embryos (blastocysts) collected from donor mice. The embryos are harvested either a few days after fertilization in intact female mice, or from females that were ovariectomized shortly after fertilization and given replacement steroids that prevent implantation while embryonic cell division continues.**
2. **Harvested embryos typically are cultured on a layer of embryonic fibroblast feeder cells that produce LIF (leukemia inhibitory factor) , with additional LIF often added to the medium. LIF is a cytokine that minimizes**

differentiation of the cells, and is an essential component throughout subsequent ES cell isolation steps.

3. **In culture, the inner cell mass(ICM) of harvested embryos dissociates from trophectoderm cells .**
4. **Cells from the ICM are individually selected for undifferentiated morphology, indicating their likely pluripotency, and allowed to proliferate.**
5. **Clones created from these cells that both proliferate well and retain their undifferentiated state through several passages become ES cell lines. These lines can then be evaluated for their potential as gene targeting tools, or used in studies of cellular differentiation.** (Taconic, 2003)

One way to incorporate DNA into ES cells is through electroporation. This is the process where DNA is placed at the top of a plate with the cells at the middle and an electric charge is passed through the plate. Since DNA has a negative charge due to its phosphate residues, it will migrate to the positive electrode. The electric charge enhances the absorption of DNA into the cells. DNA then migrates within the cytoplasm of the cells and integrates with the cellular DNA.

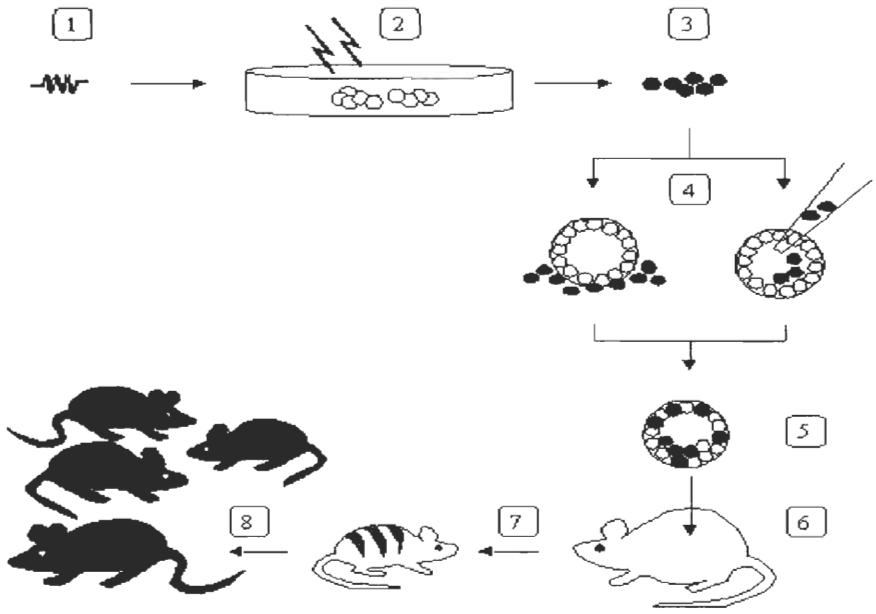

Figure 4: Electroporation of DNA into mouse ES cells.

1. **A "targeting construct" is created – a piece of DNA that carries a gene or DNA sequence of interest**

2. **The targeting construct is introduced into ES cells of a mouse by electroporation , a process that delivers an electric pulse to the cells and enhances absorption of the construct across the cell membrane. Once inside ES cells, the construct can undergo recombination with the intact genome, exchanging the construct's DNA for that of the native DNA in a specific region.**
3. **ES cells that successfully incorporate the construct are selected out and added**
4. **The ES cells are added to an early-stage embryo of the same species, either by injecting them into or co-culturing them with the "host" embryo.**
5. **Both ES cells and host embryo cells participate in growth and development of a "chimera" – an embryo consisting of dual cellular origin.**
6. **The embryo is implanted in a surrogate mother and carried to term.**
7. **The chimeric status of newborns is readily confirmed by their mixed coloring since the host embryo typically is chosen to have a different coat color from the ES cells' genetic background.**
8. **A fertile chimera that carries the construct in it germ cells is then selectively bred to establish the DNA modification permanently in a new mouse line.** (Taconic W4/129S6 ES Cells, 2003)

Microinjection is the second way to incorporate DNA into ES cells. DNA microinjection into ES cells is similar to DNA microinjection into an oocyte. The only difference is that every ES cell that you wish to deliver into the "host" blastocyst must be microinjected. It also has the drawbacks that only a mosaic animal will be formed, and not always will the animal have the transgene in its germ line cells, so its progeny will sometimes not be transgenic. The process sometimes has to be repeated to gain more transgenic animals, as opposed to just breeding more transgenic animals from two transgenic parents.

A third method for delivering foreign DNA into ES cells is to use viruses as vehicles. A retrovirus is modified to not cause disease, and foreign DNA to be expressed in the transgenic animal is added to the viral DNA. The virus is added to the culture of ES cells. The retrovirus is encoded with the transgene of choice, and it infects the ES cells. However, it again has the drawback of not being able to infect all the cells, that is, not all treated ES cells will have the retroviral DNA. Only if some of the germ line cells are successfully infected will this procedure work. The viruses frequently used to transfer DNA into ES cells are the following: Oncoretrovirus (ecotropic pseudotyped),

Oncoretrovirus (other pseudotypes), Adenovirus, Adeno-associated virus (with adenovirus), Adeno-associated virus (adeno-free), Vaccinia Virus, Herpesvirus amplicons, Foamyvirus (replication competent), Foamyvirus (replication defective), Lentivirus (non-HIV pseudotyped) (Emery, 2003).

A fourth method for introducing DNA into ES cells is through a chemical vector. DNA is first packaged into a polymeric substrate or scaffolding (frequently liposomes) which then are used to treat the ES cells. The liposomes fuse with the cell membrane delivering the DNA. Another frequently used scaffolding uses polylysine and plasmid DNA to create a powerful affinity between biotin and avidin. The created DNA complexes are then tethered to surfaces coated with neutravidin (nonglycosylated avidin). Because of the cells that were used— HEK293T (from human kidney cells) and NIH/3T3 (from mice)—grew along the surfaces, they were directly exposed to the tethered complexes and were easily transformed. Transfection was a direct function of surface DNA quantities and the number of tethers attaching to the complex. And, as determined by colorimetric assay, up to 100-fold greater transformation was observed using tethering compared with traditional bulk delivery methods. (Lesney, 2002)

All these methods are very useful in creating a transgenic animal. However, they don't always work, or have a very low percentage of success. For example, in an experiment with telomerase activity, cows were used to determine the effects of increased telomerase activity on the life span of cells. 1896 bovine oocytes were injected, and only 87 were able to be cultured to the blastocyst stage. From that 87, only 79 were able to be transferred into 32 surrogate mothers, and of those 32, only 17 became pregnant. Of those pregnancies, one fetus was removed to show development, 9 remained pregnant.

Of those nine, 2 were aborted and one was stillborn and six calves were born. Of those six only 2 were transgenic. (Lanza et al, 2000)

Assays for Screening Transgenic Animals

To test whether an animal is transgenic, several methods have been developed to detect the presence of the foreign DNA in the genome of the animal. The first and most reliable screening method is termed a "Southern blot". In this technique, DNA is digested by a restriction enzyme, meaning it is cut into pieces at specific location, and then run on an agarose gel (see Figure-5). When it is run on an agarose gel, an electric current is run through the gel. DNA is attracted to the positive electrode and migrates through the gel. Depending on the size of the DNA, it migrates only to a certain point in the gel. A marker, consisting of the transgenic DNA initially injected into the ES cells or oocyte, is run alongside the animal's cellular DNA. If bands in the cellular DNA match bands in the marker, the transgenic DNA was successfully integrated into the cell.

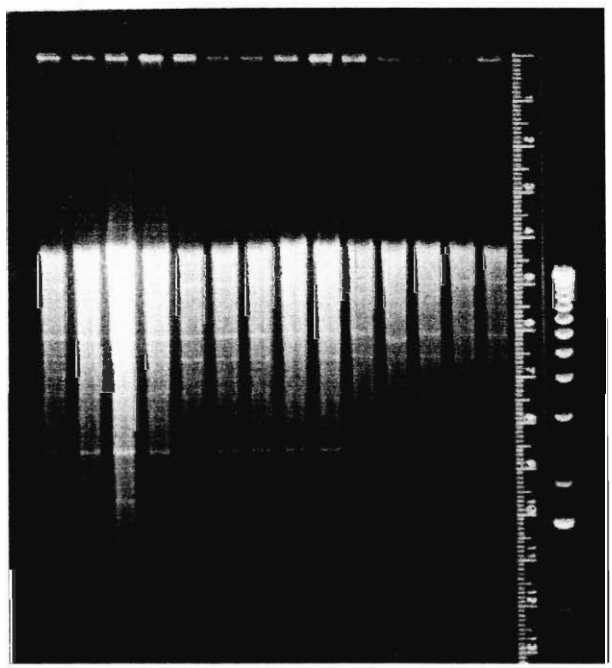

Figure 5: This figure shows an agarose gel with 14 lanes and a marker on the far right. Genomic DNA migrates as smears, with some abundant bands observable within the smears. Once the DNA is blotted to a membrane, and probed for the transgene, if the transgene band is observed in the cellular DNA, the animal is transgenic. (Southern Blot Method, 2001)

The second way to determine whether an animal is transgenic is to perform a protein blot, or Western Blot (Figure-6). If an animal is engineered to make certain proteins, like human insulin for example, an assay of proteins produced by the animal can be used instead of DNA analysis. It is run much the same way as the Southern blot, except whole cell extracts containing protein are electrophoresed and blotted. A protein will travel a distance relative to its molecular weight, which is measured in kilodaltons, or kd. After the gel is run, it is blotted to membrane, then an antibody solution for the protein to be detected is added to the gel. If the protein that the antibody binds to is present in the gel, then the antibody will bind to it and create a dark spot in the gel. If not, the lane will remain empty.

Figure 6: An example of a western blot. Here four lanes have been run, and all four lanes have the transgenic protein in them. (Western Blot Ananlysis, 2003)

An ELISA test is yet another way to determine protein expression in an animal. This powerful quantitative assay can be used to detect proteins in whole cell lysates, or to detect antibodies produced by the animal. The assay is essentially a solid-phase assay in which either antibodies or proteins are used to coat a well, then a sample is added to react

with the coat. The following example (figure-7) pertains to detecting antibodies in an animal's serum. Partially purified proteins are coated in a well on a plate dish. The serum of the animal is then added to the plate. If the animal is positive for the antibodies to the protein, they will bind to the proteins on the surface of the plate. Anti-animal (specific to the animal being used, for example anti-rat) antibodies attached to an enzyme are then added to the mixture. They will bind any active (bound) antibodies from the animal's serum. These antibodies have an enzyme attached to them, which when reacted with a chromogen substrate, cleaves the substrate to make a bright yellow. If the plate turns yellow, then the animal is positive for antibodies against that protein. If it remains clear, then the animal is negative.

Figure 7: An example of a positive ELISA test. (The Biology Project - Immunology, 1998)

Selection of Which Animal to Make Transgenic

All sorts of animals may be created transgenic, from mice to monkeys, sheep, even cows. However, scientists must take into account several external factors when picking an animal to make transgenic, including gestation time, number of progeny,

amount of milk produced per year (for transpharming) (see Table-1), and most importantly, the ability of the treatment to take effect.

Species	Generation Time (mo.)	Gestation Time (mo.)	Progeny per Pregnancy	Kg Milk per Year
Cow	24	9	1	9,000
Goat	13	5	2	2,700
Sheep	13	5	2	1,800
Pig	12	4	8-10	320

Table 1: Important factors to consider when making a transpharming animal (Adams, 2003).

As Table 1 shows, each individual species has pros and cons depending what experiment the scientist is looking to perform. If the experiment required a large number of viable offspring that contained the new gene, then a pig would be preferable to a cow because it produces more offspring at one time, and its gestation period is much lower, so the results may be seen sooner. The most common transgenic animals are mice, since they cost less, and breed in large numbers with short gestation times.

Transgenic animals are used for many different purposes which include studying diseases, producing human proteins to help mankind, and a variety of other functions to be discussed in chapter-2. They help scientists gain a better understanding of human disease, and can be used to help create cures.

CHAPTER-2: TRANSGENIC CLASSIFICATION AND EXAMPLES

Transgenic Disease Models

This section deals with animals that are genetically altered to have qualities that mimic the symptoms of human pathologies. The genomes of these specific animal models were altered to exhibit qualities of a pathology consistent with the characteristic symptoms of the human disease of which they are the model for. The diseases dealt with in this IQP section are HIV, Alzheimers, and a specific type of cancer. Science is struggling with research and development for all of these diseases thus we need these small animal models badly.

Previously these animals did not have the ability to exhibit specific human pathologies. A specific human transgene is expressed in the animal model. Expression of the *human* gene is responsible for the development of the pathology. So any research breakthroughs or therapeutic agents found while experimenting with these transgenic mice, is often directly analogous to the human disease. Expression of the human transgene, along with the expression of other endogenous mouse genes, forces the animal to exhibit qualities of the human pathology.

Animal models for diseases are highly desirable because the onset of disease is often faster than with humans, and many more individual animals can be analyzed versus patients in clinical trials. Another excellent feature of these small animal models is that these animals can be used to screen drugs that may have strong side effects.

Once a potential therapeutic agent has been discovered and thoroughly tested, it could then be tested on human cells, and then possibly used on humans. This trial and error approach is not safe enough to be performed in human research. With the creation and implementation of transgenic disease models, the possibility for advancement in research and understanding of human diseases seems imminent. In this Transgenic Disease Model section the examples given are Oncomouse, Alzheimer's mouse, and HIV/AIDS mouse.

Alzheimer's Mouse

Alzheimer's disease (AD) is a neurological disorder that affects memory. This loss of brain function is due to the build up and deposition of senile plaques in the brain. These senile plaques take the place of normally functioning brain tissue. The formation of these plaques causes the degeneration of cholinergic synaptic structures. These plaques are composed of a waxy protein called amyloid β-peptide (Aβ). What gives this Alzheimer's mouse the specific ability to exhibit this human-like AD pathology is the over-expression of human amyloid precursor protein (APP) mRNA, holo-APP, and Aβ in their brains (Games et al., 1995).

The purpose of genetically changing the mouse so that it has the ability to develop these plaques is to more accurately understand Alzheimer's disease, and possibly take actions to prevent or treat it. The specific Alzheimer's mouse discussed above over-expresses a mutant version of human APP (in which the valine amino acid at position 717 is replaced by phenylalanine), that mimics a family in Indiana (the Indiana mutation). This family develops AD with an average onset of 40 yrs old, instead of the usual 70

years old. The mouse model progressively develops many of the neuropathological hallmarks of AD in an age- and brain-region-dependant manor.

Research for the prevention of Alzheimer's disease in humans is being pushed forward by the Alzheimer's mouse model because the model allowed the development of a possible AD vaccine (Shenk et al., 1999). Mice were immunized with Aβ 42 (the 42 amino acid polypeptide protein that makes up the amyloid plaques). The immunization was either performed before the AD-like pathology begins, or at an older age once the disease is well established. Immunization of the young animals essentially prevented the development of β-amyloid-plaque formation, neuritic dystrophy and astrogliosis. Treatment of the older animals (animals where the onset of AD pathologies had already been fairly prominent) also reduced extent and progression of these AD-like neuropathologies (Schenk et al., 1999). This animal research was extended into humans by Elan Pharmaceuticals, Inc, and has lead to the conclusion that the immunization of even human patients may eventually lead to effective treatment and possibly the prevention of Alzheimer's disease.

AIDS Mouse

This mouse is a small animal disease model for the human immunodeficiency virus (HIV-1). This transgenic animal has a transgenic gene encoding HIV-1 nef, or the entire HIV-1 genome. The latter animal has the ability to synthesize all of the proteins associated with the HIV virus. In addition to the HIV-1 genome, these mice also contain the gene for the human CD4 promoter flanked by the mouse CD4 gene for expression in T cells of monocyte/microphage lineage. The inventors of this small animal disease

model are Paul Jolicoeur, Zaher Hanna, and Dennis G. Kay (Hanna et al, 1998). The assignee of this invention is the Institute of Clinical Research in Montreal, Canada (Institut de Recherches ..., 1999).

The above mentioned genes were delivered into a newly fertilized mouse zygote, then the zygote was tranfered into the uterus of a female recipient mouse. Once this animal gave birth, the pups were selected for their phenotypic correlation with human HIV pathology.

This specific invention (HIV/AIDS mouse) was designed to exhibit specific human HIV infection qualities (wasting, atrophic lymphoid organs, atrophic kidneys, and early death) so that scientists doing research on HIV/AIDS could more closely understand how the virus works. In conjunction with being beneficial for research, the AIDS mouse can also provide a possible method to screen for potential therapeutic agents.

Oncomouse

The people who originally created Oncomouse are Philip Leder and Timothy Stewart, in conjunction with the President and Fellows of Harvard College (Leder and Stewart, 1984). This invention features a transgenic a rodent, such as a mouse, whose germ cells and somatic cells contain an activated human oncogene sequence introduced into the animal at an embryonic stage. This is done preferably at the one-cell, or fertilized oocyte stage, and generally not later than about the 8-cell stage. An "activated oncogene sequence", means an oncogene (cancer causing gene) which, when incorporated into the genome of the animal, increases the probability of the development of neoplasms,

particularly malignant tumors (Leder, 1988). The introduction of the oncogene sequence at the fertilized oocyte stage ensures that the oncogene sequence will be present in all of the germ cells and somatic cells of the transgenic animal. This means that all of the original animal's descendants will carry the activated oncogene sequence in all of their germ cells and somatic cells (Leder, 1988).

This small animal model has many positive implications for cancer research. Mice had not previously been able to exhibit behavior that mirrors human cancer. Not only can we now understand more about the way the disease affects the animals and ourselves through research and observation, but we can also experiment with any number of supposed anti-tumor compounds thought to confer protection against neoplasms. Most importantly we can experiment with any anti-tumor agent and if the animal dies the loss would be considerably less than if a human test subject were used in the experiment. If this foreign compound reduces the incidence of neoplasm development compared to untreated animals, this data can be taken as an indication for protection against tumor activities.

It is also true that in human research, the patients are sometimes fragile, and cannot be subjected to harsh chemical treatments that may or may not have anti-tumor qualities. Using this animal model for research will certainly help scientists to understand things about cancer that they never could previously (Leder, 1988).

Transpharmers

This classification of transgenic animal refers to individuals that are genetically altered to produce a pharmaceutical compound within the animal, usually secreted into the animal's milk. The animal is used as a bioreactor for the synthesis of a therapeutic protein. Most commonly these animals produce these foreign proteins in their mammary glands. Thus the chemical is contained in the milk of the transgenic animal. This type of animal is incredibly useful due to the fact that the compound that they produce may be difficult or impossible to produce in the lab.

As an example, the first genetically altered goat to produce a pharmaceutical in its milk was Tracy a transgenic sheep. Tracy was used to synthesize Alpha-1-antitrypsin (AAT), also known as alpha-1-protease inhibitor, and was created by PPL Pharmaceuticals in 1991. It is a human blood protein whose prime physiological target is neutrophil elastase (Edwards, 1991).

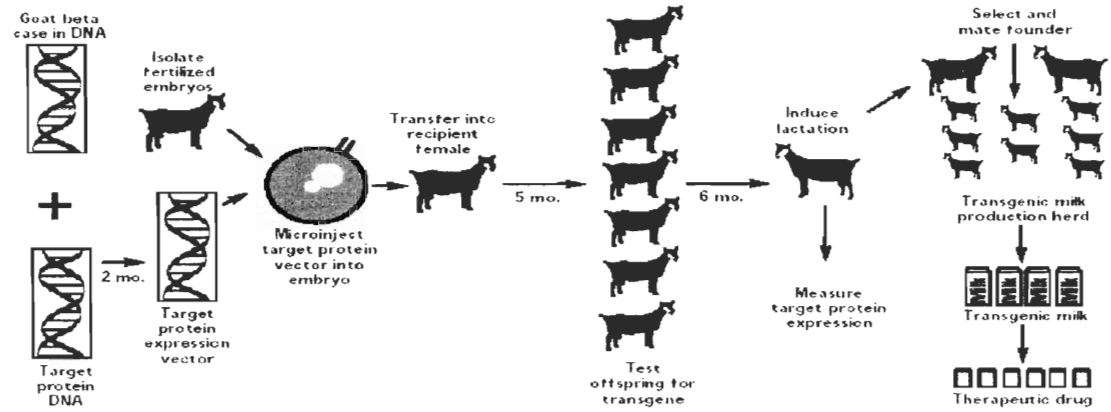

Figure-1. Production of Transpharming Goats. This diagram shows the method in which the transgenic pharming goats are produced so that they express the transgene that specifies the production of a human pharmaceutical compound in their milk (Gavlin 1995).

This first transgenic transpharmer was produced in the manor suggested by the diagram above. An embryo is fertilized in vitro, then once fertilized the appropriate DNA is microinjected into the embryo along with the mammary directing signal. This is then placed in a surrogate mother and then the transgenic animal is born producing the pharmaceutical compound in its milk.

These bioreactors for pharmaceutical proteins could dramatically reduce the amount of time and effort it takes to produce a sufficient amount of these compounds used for medicine. Tracy produced human protein at approximately 40g/l. PPL was clearly established as a leader in 1991 for the transgenic production of human proteins.

Xenotransplanters

This classification of transgenic animal involves animals that are genetically altered to better prepare their organs for transplant into human recipients. The animal most commonly used for research in this field is the pig because its physiology most closely matches that of humans. However the successful xenotransplantation of an organ from a pig to a human is made problematic by several factors. There are three types of rejection that the human body undergoes when it detects a foreign compound in the body. The first is hyperacute rejection, where the immune system of the human with the xenotransplanted organ reacts violently and destroys the blood cells in the organ, and cuts off the oxygen, turning the organ black in minutes. The second is delayed xenograft rejection, where antibodies, macrophages, and natural killer cells invade the organ. The third immunological barrier to transplant is T-cell mediated chronic rejection, this can

come into play months or years after transplantation. In humans this latter rejection can be controlled by immunosuppression (Butler, 2002).

A transgenic animal that has been created in an attempt to alleviate some of these rejection troubles is the "Knockout" pig (Lai et al, 2000). By knockout they mean that each pig has an inactivated gene, in this case for α-1,3-galactosyltransferase, an enzyme that adds the sugar residue α-1,3-galactose onto the surface of pig cells. The presence of this sugar residue on pig cells is a major signal designating the pig cells as foreign, and is an obstacle for successful xenotransplantation. The immune system of humans and Old World primates reacts violently to this sugar which was phased out in humans by evolution. The bodies of human patients with xenotransplantated pig organs recognize the sugar as foreign and attack and kill the pig organs in minutes. It is this hyper-acute rejection that keeps the human body from being able to use these xenotransplantated organs. If the inactivated gene for α-1, 3-galactosyltransferase successfully stops the production of this sugar in the pigs, without intense pathologies due to the genetic modifications, then possibly the organs may not be rejected by the human recipients (Butler, 2002).

There are some negatives to this approach. If these organs make it past hyper-acute rejection, then they could still fall victim to the other types of rejection. In addition, even if the organs are not rejected, they can possibly transfer porcine viruses to human recipients. Although transplanted organs are currently screened for known viruses, the transplants could potentially transfer unknown (and thus unscreened) viruses. It is the conclusion of this IQP team however that the potential medical benefits for

saving human lives with this xenotransplant technique far outweigh the negatives, especially if all best attempts are made to screen for known viruses. This research could push forth the possibility of using pigs as a way to farm organs for humans, which could alleviate the trials of organ shortages.

Food Sources

This section deals with transgenic animals that have been modified to be more suitable for consumption by humans. Some of these animals have had the gene for human growth hormone spliced into their genome to allow more rapid growth, and be better suitable for consumption earlier in their life cycle. When this experiment is done with fish, this is an interesting concept because these fish never reach sexual maturity.

Superfish

Specifically a gene construct encoding growth hormone has been incorporated into the genome of several commercial salmonid species. The resulting transgene affected the salmonids by giving a 3-11 fold weight gain (Devlin et al, 2001).

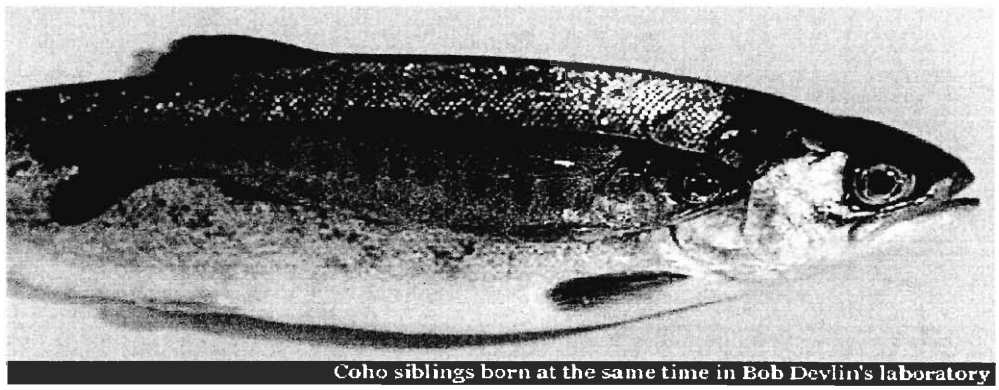

Figure-2. Transgenic Fish. Pictured above are two sibling fish, one containing the Aquatic Advantage™ growth gene insert, while the other is a non transgenic sibling (Devlin 2001).

The most interesting finding in the production of these "superfish" is that the growth response is strongly influenced by the intrinsic growth rate and genetic background of the host strain. Inserting growth-hormone transgenes into a highly domesticated fish does not necessarily result in further growth enhancement. In some experiments, rainbow trout eggs were microinjected with a salmon gene construct over expressing growth hormone construct OnMTGH1 (Devlin et al, 2001).

However all transgenic trout died before sexual maturity, and there were various cranial abnormalities that are not present in the non-transgenic control fish. Some transgenic fish over-expressing growth hormone have been successful, however these fish cannot be let out into the wild to breed with regular fish, and the fish usually do not reach sexual maturity, so the species of fish itself does not posses the ability to flourish. It goes without being said that it is a possibility to simply breed more fish specifically for size, and meat yield with traditional breeding methods, rather than changing their genetics. It is possible that the need for this particular application of transgenic technology is to small to warrant extensive research in "superfish."

Superpig

Another example of an animal modified to better suit human consumption is "The Beltsville pig." This pig was genetically modified to over-express a transgene for human growth hormone. This "superpig" created in Beltsville Maryland (1989) suffered several pathological conditions due to the over-expressed hGH. The pig suffered from lethargy, lameness, uncoordinated gait, exopthalmos, and thickened skin (Ewing ,1990).

These pigs grew so large that they couldn't support their own weight, and often suffered from arthritis and other deformities undoubtedly linked to its severely quickened growth rate. The terrible tale of the Beltsville experiment was a disaster both for biotechnology and for the public image of GM (Boomer 1989).

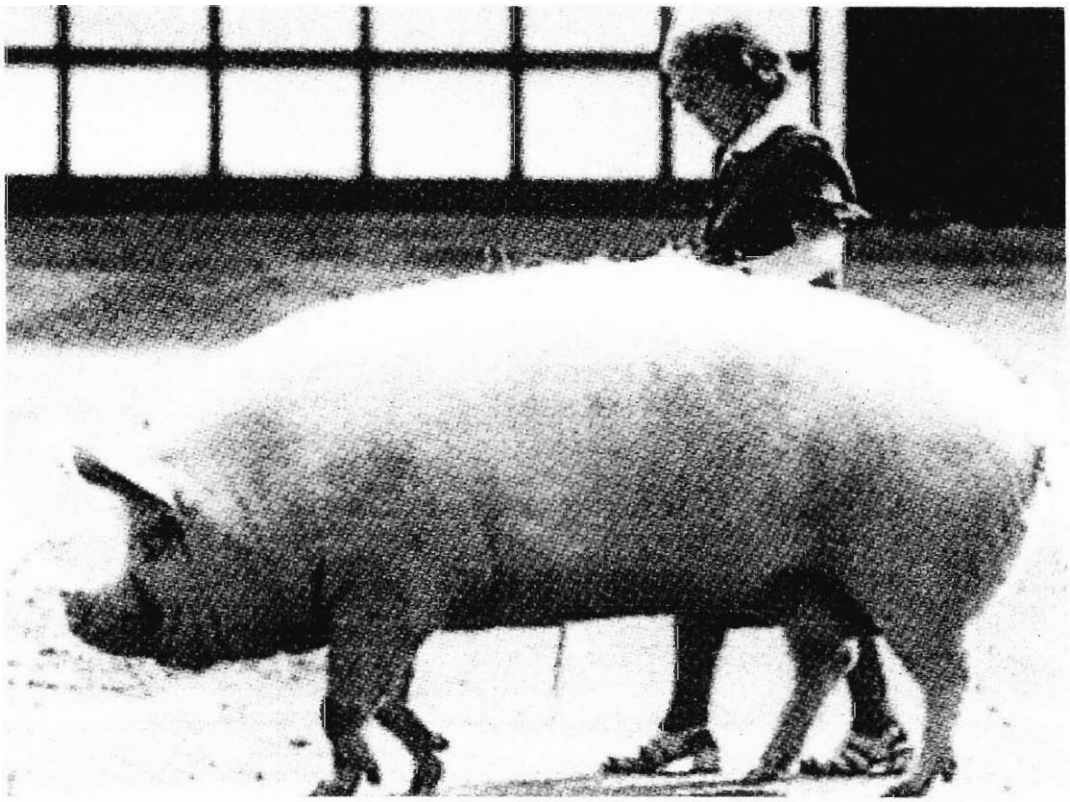

Figure-3. Beltsville Pig. Pictured above is the monstrous super pig next to a normally sized woman. These pigs grew so fast that their organs swelled up, the oversized immobile pig eventually died prematurely (Boomer 1989).

Chapter 3: Transgenic Ethics

Intro: Benefits vs. Detriments

"Transgenic animals and human gene sequences have enormous commercial value in agriculture, biomedical research, medicine, and the pharmaceutical industry among other fields. The social impact of these forms of biotechnology is nearly limitless" (Walter, 1998). The use of transgenic animals in science can easily be argued as a necessary technique that will create a path of amazing technology in its wake. By inserting human genes into the animals, scientists can more easily study serious human ailments without the use of human test subjects. Rudolf Jaenisch states, "transgenic technology offers exciting possibilities for generating precise animal models for human genetic diseases, and for producing large quantities of economically important proteins by means of genetically engineered farm animals" (Shannon, 1999). However, it also can be viewed as a dangerous and immoral way to better our own standard of living. James Gustafson states "A scientist has no right to intervene in the natural processes of life, because it is sacred" (Williams, 1973). Depending on the observer's point of view, this radical new technology could take either path.

There are cases, such as the "Alzheimer's Mouse" and the technique known as Transpharming, in which the use of these animals is extremely beneficial as well as safe. In these instances the animals suffer little or no observable pain at all, and their use in research could easily be argued by both parties as of extreme medical importance and

humane. There are also cases, such as the food animal model "super pig", in which the knowledge gained from those specific experiments with the animals is futile, and the specimens were subjected to much physical pain. In this latter case it can easily be seen that the use of the animals was both inhumane and unnecessary. There are also "grey area" cases, such as "Oncomouse" and a new technique known as xenotransplantation, where the specimen must sometimes undergo a great deal of physical pain or sacrifice, but the knowledge gained from the use of the animals is indeed phenomenal. This grey area is where the controversy of this new experimental technology is born.

Alzheimer's Mouse Ethics

To more fully understand these different cases, one must look carefully into these specific examples in more detail. An animal model known as "Alzheimer's Mouse" best defines the first scenario described above where the animal subject undergoes no observable suffering, while the medical benefits gained from its use seem to be endless. Created by Professor David S. Adams (WPI) and his colleagues at the former Transgenic Sciences, Inc. (Games et al, 1995), this animal was the world's first Alzheimer's animal model. Alzheimer's disease is characterized by memory loss, disorientation and the loss of moral judgment. Autopsies have proven that the cause of these symptoms result from the creation of holes in the brain composed of a protein termed amyloid. To prevent this spongiform pathology, one must find a way to prevent the amyloid plaques from developing. Newly developed untested drugs cannot be tested in humans since we don't know what side effects they may have, and animal testing for these drugs is not possible since animals do not get Alzheimer's disease (with the exception of 60 year old

Orangutan monkeys). The solution proposed by Professor Adams et al was to clone the human gene for amyloid and insert it into a plasmid DNA delivery vector. The DNA was microinjected into in vitro fertilized mouse eggs, and the eggs implanted into a foster mother. Newborn pups were screened for the presence of the transgene by Southern blotting. This led to the creation of a living transgenic animal model to study the amyloid plaque formation. "The most notable feature of these transgenic mice is their alzheimer-like neuropathology, which includes extracellular amyloid-beta deposition, dystrophic neuritic components, gliosis and loss of synaptic density with regional specificity resembling that of AD" (Games et al., 1995). In this model the animal undergoes no observable physical pain, and the only difference in the specimen's behavior was its slower learning rate on a maze test. This mouse has since been patented by Elan Pharmaceuticals Inc., and has been used to create the world's first Alzheimer's vaccine (Schenk et al., 1999). The vaccine is designed to prevent the formation of the amyloid plaques which should inevitably eliminate the symptoms of Alzheimer's disease. The vaccine consists of an injection of an amyloid-beta protein, which will create antibodies that will coat the plaques in the brain so as to bring them to the attention of the immune system. Microglial cells then appear in the place of the remaining plaques that have been identified by the amyloid-beta antibodies, and proceed to eliminate the plaques. Tests showed that seven out of nine mice who received the vaccine had no detectable amyloid-beta deposits in their brains (Schenk et al., 1999). This newly developed vaccine is now in human clinical trials.

Transpharming Ethics

A technique known as transpharming is another example of a transgenic case where the results of the experiments are extremely beneficial medically, and the animals suffer no observable physical pain. The concept of transpharming involves altering an animal's genetic makeup to enable it to secrete certain medically therapeutic proteins in its milk. The milk can then be given to patients who are in need of that protein thus resulting in a painless method, for patients and animals, of treating certain ailments. The animals from which the milk is being taken do not need to be sacrificed or bled to obtain the treatments, so arguing that this technique is cruel and inhumane is irrelevant. Although early transpharming experiments were performed on mice (Archibald et al, 1990), this new technology has expanded to include other animals such as sheep (Schnieke et al, 1997), goats (Archer et al, 1994) and cows.

Followers of the Hindu religion, however, would argue that altering the genetic makeup of a cow would desecrate something sacred. Cattle "play important roles in Hindu village life. Protecting them brings spiritual merit to humans" (Sager, 2003). To most Hindu sects the cow is seen as something sacred, and tampering with it in any way is considered sacrilegious. When the Hindu position is taken into consideration, this view is somewhat insignificant; the authors do feel that this is a valid religious view, but when they take into account the strong medical benefits accompanied by the minimal amount of suffering endured by the animal, those factors far outweigh the Hindu position.

One of the most recent transpharmers that has been created is a sheep that secretes the human protein alpha-1 antitrypsin in its milk (Wright et al, 1991). This protein is one

that is mutated in patients with familial emphysema. The process is performed by injecting the gene that codes for the protein into the nucleus of a fertilized egg. When the sheep matures, it should then able to produce the viable protein in its milk. There is, however, a setback in this experiment. "DNA integrates randomly into the sheep genome. Often the injected DNA does no land in a site in the genome that allows the foreign gene (transgene) to be expressed in the desired tissue or at the appropriate level. Moreover, the sheep's endogenous genes cannot be specifically altered using this technique" (Suraokar & Bradley, 2000). As stated, this procedure is not 100% effective, but the treatment with the altered sheep's milk has been proven to effectively treat patients with the devastating ailment.

Superpig Ethics

A case often discussed by the population opposed to transgenic science involves the food animal model superpig. In this example, the animals undergo an unnecessary amount of physical pain, and the experiments have little or no obvious beneficial results. An animal known as "Superpig" was created (Nottle et al, 1999) in an effort to produce leaner and more copious amounts of meat for the modern day consumer. A DNA construct containing a human growth hormone (hGH) under the control of a strong metallothionein promoter was used to create a much larger and leaner pig for farmers. The experiment was foiled, however, when scientists realized that the pig suffered a variety of medical problems including rheumatoid arthritis. Needless to say, the pig endured a great amount of pain and was eventually put to rest.

If you now weigh superpig's medical benefits versus detriments, it is easily argued that the creation of this "super-pig" was unnecessary, and that if we truly needed to create more meat for consumers, then all we would have to do is breed more pigs (Adams, 2003). The detriments of this experiment include a long list of medical ailments which the animal suffered. In fact, biologists have created a voluntary moratorium on conducting such experiments. This moratorium on mammal growth hormone experiments is also supported by the authors of this paper.

Another, more successful, growth hormone experiment was conducted with fish (Devlin et al, 2001). Similarly, a population of "rainbow-trout eggs from a very slow-growing wild strain were genetically modified with a salmon gene construct overexpressing growth hormone (construct OnMTCH1). Like coho salmon, the transgenic trout grew much faster than non-transgenic sibling controls, achieving a 17.3-fold difference in weight by 14 months post-fertilization" (Devlin et al., 2001). However, fish taken from a fast-growing domesticated population did not cause further growth enhancement. "The effect of introducing a growth-hormone gene construct into fish to increase growth rates appears to be dependent on the degree to which earlier enhancement has been achieved by traditional genetic selection (Devlin et al., 2001). Although the desired effects (large fish) were achieved in some cases, there were also some detrimental effects to the animal's health. None of the transgenic fish survived to reach sexual maturation, and cranial abnormalities were detected in some of the specimens. Overall, this experiment also seemed to be somewhat of a failure. The consequences were not as extreme as the case of superpig, but the results had few observable uses or benefits.

Animal rights activists also pose concerns to theses newly developed food models such as the super-pig and the super-fish. In agriculture, the genetically modified animals will, inevitably, be quite expensive in comparison with the traditional livestock. This poses the threat that the cost of these animals will far surpass the available funds of most small family farms (Walter, 1998). They also pose the threat that if the animals were to interact with their natural environments, they could cause dangerous problems within their habitat. They propose threats such as: if a pig containing a human growth hormone was to breed with another animal, in an environment that was not controlled, or if it was to be eaten by another animal, then it could cause a catastrophic situation involving the mutation of unsuspecting animals genomes in the environment unbeknownst to anyone. To these activists, risks such as this one are not worth taking which would infer that they view transgenic science as very unsafe and as something that should not be performed (ANZCCART, 1999).

Oncomouse Ethics

The previous cases were chosen due to their clear positive or negative results. Not all transgenic experiments, however, have strictly positive or strictly negative results. Possibly the most interesting and intriguing case involves a certain grey area that incorporates characteristics from both of the previous cases. What if the animal subject suffers, yet the results of the experiments were extremely valuable medically? Would it be right to continue with the experiments regardless? A transgenic disease model known as "Oncomouse" was developed by Philip Leder of Harvard University and Timothy Stewart now of Genentech (Leder & Stewart, 1984), and "was the first genetically altered

animal to be patented" (Anderson, 1988). Scientists use mice, such as this one, to remove a substantial amount of guesswork from toxicological studies. To do this, they color code the gene that they insert into the mouse and that color shows up when any mutation occurs. The activation of this color shows the harmful nature of a chemical. (Walter, 1998). This specific mouse's line was made to carry the human *ras oncogene* which is commonly activated in a variety of human cancers. These mice were created in an effort to give scientists a living, breathing model to work with in the demanding search for a cure for this deadly disease. In this transgenic example, the mice undoubtedly suffer as the tumors begin to form, but the resulting knowledge gained from using them in cancer research is extremely valuable and beneficial. Would the sacrifice of a few mice compensate for the saving of thousands of human lives? One point that is frequently overlooked by detractors of such experiments is that the animal suffering can be substantially minimized in these experiments. IACUC animal care committees at various universities and labs require the sacrificing of the animal specimens before substantial suffering occurs, and pain medication is used before suffering gets too high. In these instances there is no prolonged amount of suffering before the desired results are obtained. One could argue that the sacrifice of the specimen would be equal to the human suffering in some sense, and that the death of the animal should not be brought about to purely benefit the human race. However that is not the position of the authors of this paper.

Xenotransplantation Ethics

A new technique that is also contained within this "transgenic ethics grey area" is xenotransplantation. This concept involves engineering animals that carry organs compatible with humans, then harvesting these organs for transplant purposes. Pigs have been chosen to develop such technology due to their physiological similarity to humans. Pigs are considered to be the prime subjects in this newly developed technology. "A major barrier to progress in pig-to-primate organ transplantation is the presence of the terminal alpha-1-3-galactosyl (Gal) epitopes on the surface of pig cells" (Lai et al, 2002). This sugar is naturally produced on the ends of glycoproteins in pigs, and it elicits a powerful immuno-rejection response in the patient recipient. It makes the transfer of normal organs from pigs to humans impossible. Without it, the body would reject the newly introduced organ, and the patient would inevitably die.

This transplanting technique brings up the argument of whether it is ethical to sacrifice an animal so to save a human life. Of course the animal donor will inevitably die as a result of the procedure, but a human life will be saved. Do the ends justify the means in this situation? Another proposed risk involves the passing of viral diseases from non-humans to humans. "After all, for as long as humans have domesticated animals or used them as a source of food, diseases have passed between animals and humans" (Carnell, 2000). Activists argue that this viral transmission is an unnecessary risk that should not be taken. An important point to consider here is that when a procedure of this nature is being performed, scientists use specially bred populations of animals which are considered "disease free" by PHS guidelines. Theses donor animals are kept under strict supervision in a laboratory environmental and are pre-tested for

known viruses to minimize the risks being taken. "Thirteen people in the United States die every day while waiting for an organ transplant and any advance that utilized animal tissues or organs would save many lives" (Carnell, 2000). Does that justify xenotransplantation? The incorporation of this newly developed technique into our modern day practice would greatly assist us in the growing demand for organ donors. In the view of the authors of this paper, in both the oncomouse and the xenotransplantation case, the medical benefits gained from the experiments far outweigh the detriments.

Authors View

There are many obvious detrimental affects of transgenic science. There is, in some cases, apparent suffering of the animals and there are also some realistic risks that are being taken when performing experiments of this nature. This author, however, believes that the benefits of this technology far outweigh the detriments. If a chance to save thousands of human lives was created with the suffering of a few animals, then that chance should be exploited. This world is plagued by many things such as disease that could be purged by the use of transgenic animals in research. In the case of xenotransplantation, for example, as stated previously "Thirteen people in the United States die every day while waiting for an organ transplant and any advance that utilized animal tissues or organs would save many lives" (Carnell, 2000). This statement alone should convey the message that the utilization of transgenic animals is far too beneficial to overlook. Or consider the highly controversial "Oncomouse". Cancer is a disease that has marked almost all of its victims with certain death. There still is no cure and only a limited amount of treatments. With the use of this simple disease model, drugs to treat

cancer patients can be tested and observed much more thoroughly. This has the potential to put the battle against this incurable disease into remission. How can one ignore that fact? You may say that transgenic science is considered playing God and that we should not interfere with the sanctity of life, but imagine that someone close to you was crippled by a disease such as cancer, emphysema or AIDS and the use of transgenic animals would greatly benefit the search for a treatment. Would you then say that the use of these animals is wrong? Or would you see that employing theses animals is extremely beneficial, and the number of lives saved due to this research is far to vast to ignore. The advancement of technology is inevitable, and techniques such as this one in science will only become more defined and complex. In the future, we will not only be able to more successfully treat a plethora of different diseases, but we will also be able to relieve entire germ lines of families of any chance of contracting similar diseases as well. Transgenic science is the wave of the future.

Closing

The utilization of transgenic animals in research could lead to amazing advancements in the fields of agriculture, biomedical research, medicine, and the pharmaceutical industry. This newly developed technique has showed that it has potential to create an endless contribution of medical benefits and advancements. When weighed against the detrimental effects, the benefits produced from this technology easily surpass them. Considering the number of human lives that could be saved and the vast improvement for the quality of life of every man that could result from the use of such

technology, there should be little or no opposition posed against the incorporation of these animals into research.

Chapter 4: Transgenic Legalities

Patenting Life: ONCOMOUSE

Oncomouse™ is an animal that has been genetically altered to be prone to the development of certain types of human cancer (Leder, 1999). This small animal disease model created by Philip Leder and Timothy Stewart (Leder, 1988) is crucial in the study and understanding of cancer, and also in the screening of new and innovative cancer treatments. The controversy that surrounds this oncogenic mouse created in the 1980s stems from the struggle for Harvard and Dupont to patent the technique that is involved in its creation.

Oncomouse Patent Timeline
(Thompson, 2002)

United States (3 patents issued):

- (1988) Transgenic non-human mammals

  The US Patent and Trade Office issues patent No. 4,736,866 to Harvard Medical School in 1988 for a mouse developed by Philip Leder and Timothy Stewart, it is the first US patent issued for a vertebrate.

- (1992) Method for providing a cell culture from a transgenic non-human mammal

  February 11, 1992. Philip Leder, US patent number 5, 087,571

- (1999) Testing method using transgenic mice expressing an oncogene

  July 20, 1999. Philip Leder, US patent number 5,925,803

Europe

- (1988) Application for European patent
- (1990) European patent office (EPO) rejects application arguing that animals are not patentable by the European patent convention.
- (1991) Harvard appeal EPO decides that some animals may be patentable and consider whether morality is a reason to bar patent rights.
- (1993) Oncomouse is granted European patent

Canada

- (June 1985) Harvard College files patent application for Transgenic Animals in Canada.
- (March 1993) Canadian Patent Office rules it will allow patent for the oncogene but not the mouse itself. Harvard requests a review by commissioner of patents.
- (1995) Canadian Commissioner of Patents upholds the examiner's decision(see note one), and Harvard appeals to the Canadian Federal Court Trial Division. The court upholds the previous decisions, (see note two) and Harvard files appeal to the Federal Court of Appeal.
- (September 1999) Government of Canada establishes the Canadian Biotechnology Advisory Committee to provide policy advice on biotech-related matters, like genetic patents. This association is called BIOTECanada and is run by President Janet Lambert no period (Mayer, 2002).

- (August 2000) Canadian Federal Court of Appeal rules to grant patent for Oncomouse!! In its ruling, the court said the Oncomouse is a composition of matter, thus qualifying it for a patent under law
- (October 2000) Government of Canada files appeal of August 2000 ruling to the Supreme Court of Canada. (see note three)
- (Dec. 5, 2002) Case File Number 28155, Harvard College vs. Canada (Commissioner of Patents). Supreme Court of Canada rules the Harvard mouse cannot be patented. In a 5-4 judgment, the court said the mouse does not qualify as an invention under the federal Patent Act of 1869. This specific act protects any new useful art, process, machine, or composition of matter. (see note four)

Note 1: When asked in 1995 if the modified mouse could be patented the Canadian Commissioner of Patents had answered *no*: this living creature is not an "invention" within the meaning of the Canada Patents Act. (Mitchell, A. 2002)

Note 2: Judge Nadon upheld the Commissioner of Patents and wrote, *On even the broadest of interpretation I cannot find that a mouse is "raw material" which was given new qualities from the inventor. Certainly the presence of the myc gene is new, but the mouse is not new, nor is it "raw material" in the ordinary sense of that phrase...A complex life form does not fit within the current parameters of the Patent Act without stretching the meaning of the words to their breaking point, which I am not prepared to do* (Mitchell, 2002).

Note 3: The Commisioner on Justice and Peace of the Canadian Council of Churches along with the Evangelical Fellowship of Canada request intervener status in the case.

The lawyer that represents the previously stated groups, is one William J. Sammon of Barnes, Sammon, Ottawa. Mr. Sammons brief points out that when the Canada Patent act was passed in 1869, the framers of the legislature never dreamed that the act would be used to patent an animal, or even parts of an animal. Furthermore Mr. Sammons brief detailed the ambiguous relationship between patenting and the openness of research, suggesting that under today's circumstances, patents seem to have a restrictive effect, "privatizing" results that would be otherwise shared freely among scientists. (Mitchell, 2002). The sole question that remains, if the Patent Act should be interpreted to cover higher life forms (mice) where can the line be drawn, could this be interpreted to cover humans or primates??

Note 4: Writing for the majority Justice Michael Bastarache went on to say "*The best reading of the words of the Act supports the conclusion that higher life forms are not patentable...Higher life forms cannot be conceptualized as mere 'compositions of matter' within the context of the Patent Act.*" (Mitchell, 2002).

The Supreme Court based its no patent ruling only on the meaning of the existing Canada Patent Act. But the Judges noted that Canadians, through their parliament, must think about these issues much more broadly (Mitchell, 2002).

The struggle for Harvard and Dupont to obtain a Canadian patent for the "myc" (gene construct causing cancer-like pathology) enhanced tumor susceptible mouse has lasted 18 years in countries across the globe. The patent granted to Dupont and Harvard in America has allowed Dupont to charge hundreds of thousands for the rights and licensing to the use and study of this incredible cancer research tool. Recently the

National Institute of Health and Dupont have come to an agreement about the use of the Oncomouse, granting permission for all nonprofit institutions to use the Oncomouse without cost under special written agreements, so as to push forward the current understanding of cancer, how it works, and how it can be treated. (Memorandum ...., 1999).

Patenting Life: Cons

So as it currently stands, Oncomouse is patented in the U.S. but not in Canada. The arguments against life patents are commonly based on moral and religious grounds that regard the sanctity of life and oppose its commodification. The Canadian religious communities fear the consequences of a worldview in which everything may be assigned a price for which it may be bought or sold. This wide scale commodification of all things is seen as a parallel to the biblical Babylon (Crossman, 2002).

A group of Canadians deemed the Canadian Christian Coalition (CCC) believes also that there are some things that should not have a commercial value, and these certain things should not be able to be sold because selling these objects shows a lack of respect for the objects themselves and their creator. They feel most strongly about not having a price attached to life. It is with this attitude that the CCC objects to the Oncomouse patents, and the Oncomouse existence itself. They find it perverse to doom each Oncomouse to the cancer pathology only to push forth the possibility of mans livelihood. They even went so far as to say that the Oncomouse is like a martyr, and likened its existence to Jesus, in that the mouse suffers and dies to show the world something about why it was there, and teach them something through its presence (Crossman, 2002).

Along with wide scale commodification the CCC warns against the pursuit of monetary gain, arguing that patenting should be used as a method for pushing forward technology, but however it is much like expressing ownership, and used mainly for instituting commercial revenue.

The main problems that the religious community of Canada has with the Oncomouse, is the suffering that the mouse goes through, and the monetary gain and potential for commercial revenue that the Oncomouse patents represent. They deem these qualities to be a higher negative than the positive that can be found in using the Oncomouse as a cancer research tool.

Patenting Life: Pros

The most compelling argument for these patents is based on the benefits that they deliver through medicinal advances and commercial exploitation (Deftos, 2001). Patents on life are truly a touchy subject. It is important whether the claim is for a rodent, versus a primate. Most of the public is against awarding patents on primates or humans, but where does one draw the line, should the life of rodents be patentable? It is this controversy that has brought so much attention to this small animal model for a disease that is one of top ten killers in our society.

Other Transgenic Legalities

Some transgenic animals are created to be larger and faster in maturing. These animals are referred to as "super" animals. Fish, pigs, cows, and other livestock have been engineered to produce more growth hormone so they can reach maturity faster and

can be bigger than normal. Most of this research began by scientists studying the effects of the growth hormone, but now it is being examined as a possiblilty of a new food source.

Though currently illegal to distribute genetically altered food, but the Food and Drug Administration (FDA) is currently looking to expand on that. Their main concerns are on ecological factors of these animals, that is, how will they affect the environment if they get out, and if they could be potentially harmful to consumers. Tests are currently underway for fish, speciafically salmon, tilapia, channel catfish.

The project for Super-pig was abandoned due to the detrimental effects on the animal. It developed arthritis and other delapitory diseases. Most terran animals seem to have that problem since their increased mass causes more strain on their skeleton, but fish seem to have no adverse effect on them.

Breeding Laws

Breeding laws vary from state to state, and mostly deal with pedigrees of animals. No laws have been passed to the keeping of records of pedigree transgenic animals because they are not household pets, or at least not yet. If transgenic animals are made legal to be distributed as food sources or pets, records will be kept on them to be able to keep track of them. Owners of pedigree animals established the guidelines of pedigrees mostly for the status of the pet, or for competition. Those revolve around groups or organizations privately run. All states have liscencing laws so that the owner of an animal, whether it be livestock or pet, is linked with their animals. With transgenic animals, it seems that similar laws will be passed.

Chapter 5: Conclusions

The creation of transgenic animals has given the world possibilities that were previously thought unattainable. By utilizing these transgenic animals in research, scientists now have a much deeper understanding of human health.
The creation of such animals is a tedious trial and error process which entails a plethora of different methods and techniques, including the manipulation of in vitro fertilized eggs, or embryonic stem cells.

The most commonly used technique for inserting a specific gene into the genome of an animal is by microinjecting the DNA into an in vitro fertilized animal egg, and then implanting that egg into a surrogate mother. Another technique utilized involves the manipulation of embryonic stem cells (ES). These cells are first isolated from the inner cell mass of the blastocyst and then manipulated to insert foreign DNA. The cells are then re-implanted in a blastocyst, which is implanted in the uterus of a surrogate mother. Several techniques can be used for inserting foreign DNA into ES cells, including microinjection, viral transfection, liposomes, and DNA delivery through a chemical transfection. Unfortunately, these techniques do not always work to the desired extent. The percentage of actual transgenic animals born is usually very low, and the experiments must be performed multiple times to achieve the desired results.

In addition to the techniques used to create a transgenic animal, several techniques are often used to screen for transgenic offspring. Southern blots or PCR are used to detect the presence of the transgene in the animal's genome. Western blots or

ELISAs are used to screen for foreign protein production in the animal. Scientists must also take into account, the type of animals being used, and the success rate for each animal given its unique qualities. For example, qualities considered in transpharming experiments include the number of offspring per pregnancy, generation time, gestation time, and kilograms of milk produced by the animal per year. Variables like those presented in Table 1 of chapter 1 of this paper can be used as a guide in the selection of animals for such experiments.

Disease models, such as Alzheimer's mouse, HIV mouse, and oncomouse which were previously discussed in this report, have been constructed in an effort to find possible treatments for diseases that were previously dubbed incurable. Since animals are not susceptible to the same diseases as humans, their DNA must be altered "by either creating a deficiency in the animal causing it to be susceptible to a disease, or by adding a gene which causes an increased susceptibility" ~ *Theodoros Toufas.* "The genomes of these specific animals were altered to exhibit qualities of a pathology consistent with the characteristic symptoms of the human disease of which they are the model for...With the creation and implementation of these transgenic disease models, the possibility for advancement in research and understanding of human diseases seems imminent." ~ *Michael Scofield.*

Transpharming is a technique created in an effort to solve problems related to the high demands for pharmaceutical proteins. This process involves the alteration of an animal's genome to induce the secretion of needed therapeutic proteins in that animal's milk. This process greatly facilitates the production of these therapeutic proteins, some of which can not be artificially created. Tracy, a goat created by PPL Pharmaceuticals in

1991, was the first genetically altered goat to produce a pharmaceutical in its milk. She was used to create Alpha-1-antitrypsin (AAT), a human blood protein whose prime physiological target is neutrophil elastase (Edwards, 1991).

Food models, such as the "superpig" and "superfish" who were previously discussed in chapter 2, were created to produce a leaner and more plentiful meat supply for the modern day consumer. The "Beltsville Pig", created in 1989, was genetically modified to over-express a transgene for a human growth hormone. This unique trait gave the pig enormous size, which unfortunately lead to the formation of a series of harmful pathological conditions such as arthritis and deformities. A similar experiment was also performed on several different commercial salmonid species. The experiments with the "superfish" had much more success than the previous experiments with the "superpig". The results from both experiments, however, have produced no exploitable benefits.

Xenotransplanters have been created to facilitate the transfer of porcine organs to humans waiting for organ transplants. Xenotransplantation is a newly developed technique which involves the cultivating of organs inside of animal hosts, so that they may act as a source of available organs to be transplanted into human patients. The animal most commonly chosen as the host in these experiments is the pig, due to the fact that its physiology most closely matches that of humans. The transfer of organs from animals to humans poses the threat of rejection of the newly introduced organ by the recipient's body. As a response to that threat, scientists have attained the ability to genetically modify a pig so as to make the cultured organs compatible with humans.

Many people die every day waiting for donor organs, and this process could assist the medical community greatly.

However, with the creation of any of these animals discussed above comes ethical arguments and concerns. Just because scientists now know how to make transgenic animals, the question arises as to whether we *should* make transgenic animals. Such ethical arguments require a careful examination of various pros (i.e. medical benefits) and cons (i.e. potential animal suffering) for each experiment. In some cases like "Alzheimers Mouse" and the Transpharmers, the animals appear to suffer no physical harm and the medical benefits that result from their creation is extremely valuable. Arguments against, in this case, are very weak in the authors' eyes, and pose little opposition to the creation of animals of this nature. There are also converse cases, such as the food animal model "superpig", in which the pigs endured an observable amount of physical pain, and the authors perceived no medical benefits gained from these experiments. In this situation, the arguments against performing these kinds of experiments seem viable to the authors, and the use of such animal models seems futile. In fact, the scientific community placed a moratorium on performing such growth hormone experiments on pigs in the future. Similar experiments with superfish appear to not have the same detrimental effects.

The greatest transgenic ethical conflict in the authors' opinions, however, is raised in the discussion of a certain "grey area" where the animal subjects do suffer, but the benefits gained from the experiments are phenomenal. Disease models such as the "Oncomouse" and the "AIDS mouse", and the technique of xenotransplantation are included in this area. In the case of oncomouse, the animal develops human tumors

which do indeed cause some suffering. This suffering, however, can be minimized with pain medication, or by sacrificing the animals before the terminal stages of cancer. In our opinions, when the medical benefits are weighed against the amount of "controlled" suffering endured by the animal, the sacrifice of that animal seems to be justified. The potential remunerations that these animal models present to medical society are far too valuable to overlook.

The conflict surrounding the creation of these animals becomes much more confined and technical when discussing the legalities of the subject. Should animals be patented? This is a question that has been asked and interpreted in the courtroom time and time again. The United States and Europe unanimously decreed that this should and can be done with the patenting of "Oncomouse". This Harvard mouse was the first genetically altered animal ever to be patented, and it sparked an endless slew of controversy, specifically in Canada, regarding whether it is morally right to patent a life form, or if it is even legal to do so. Canada originally granted a patent for the Harvard Oncomouse ruling that it was, in fact, a composition of matter. The patent, however, was later appealed and revoked on the grounds of the Federal Patent Act of 1869, which states that "higher life forms" are not patentable, and that they cannot be conceptualized as mere 'compositions of matter' within the context. So to this date, oncomouse is not patented in Canada.

The question of patenting life itself has also been proposed. "The arguments against life patents are commonly based on moral and religious grounds that regard the sanctity of life and oppose its commodification" ~ *Michael Scofield.* The Canadian Christine Coalition (CCC) believes that there are certain things that should not be sold,

and that selling certain items, such as life, would be disrespectful to the objects themselves (the transgenic animals) and their creator (God). Their main argument disputes the idea that "patenting should be used as a method for pushing forward technology, it is much like expressing ownership, and used mainly for instituting commercial revenue" ~ *Michael Scofield.* Alongside this standpoint arises the contradiction that most of the public is against placing patents on primates or humans. Following our research, we concluded that allowing transgenic rodent patents for new compositions is a valid way to stimulate medical research. We also feel that transgenic primates should only be created when very strong medical benefits would result, with minimal suffering.

When technology, such as transgenic science, has the potential to benefit society as greatly as it does, the arguments posed against the continuation of these experiments to our eyes become almost imperceptible. The advancement of technology is inevitable, and these techniques will only become more defined and useful as time progresses. In the future, these animals will not only save lives, but they will also improve the quality of life for all of mankind.

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