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Öbrink Memorial Lecture: The Age of Biology: Opportunities and Challenges for Laboratory Animal Medicine

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Introduction

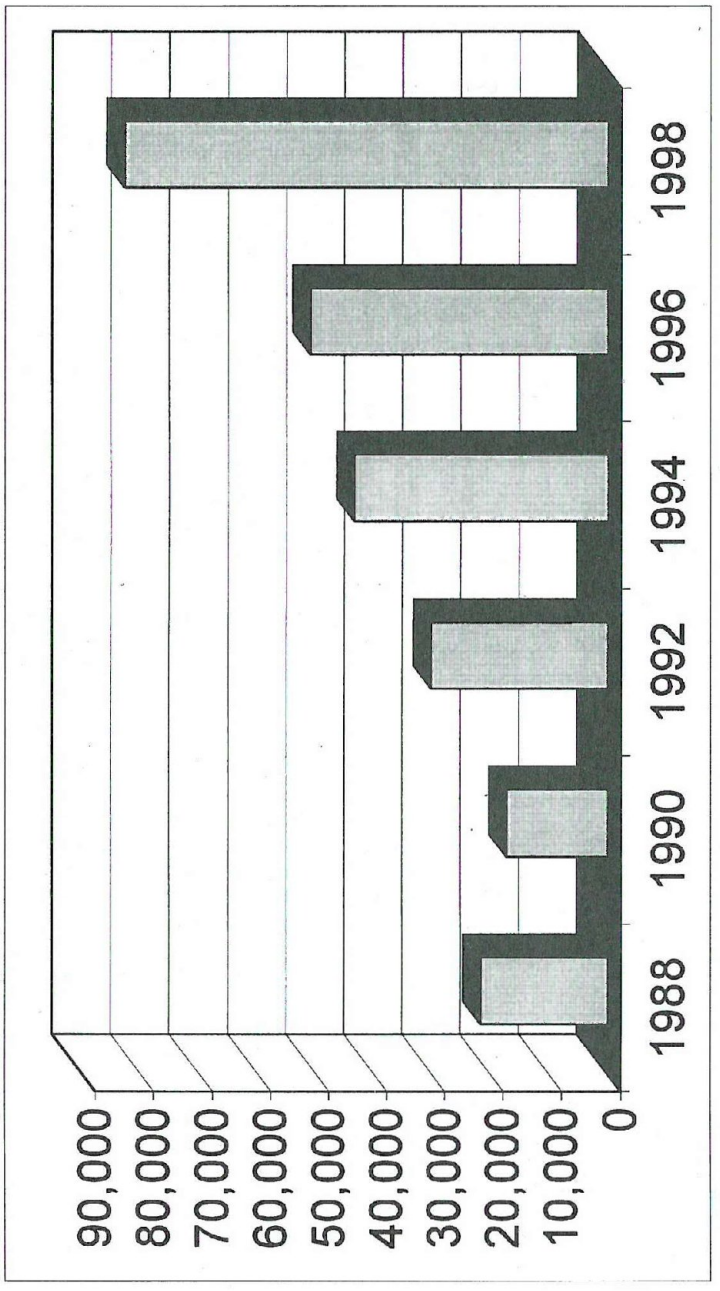
It is a special privilege to present the initial Karl J. Öbrink Lecture, honor an individual who made a difference and focus on his legacy, not the loss. His legacy in Scandinavia includes SCANDLAS, as he was an avid supporter of the organization. Dr. Öbrink's impact extends across the Atlantic as the categories of adverse effects he presented at the ICLAS Congress in Vancouver *(Öbrink and Wass, 1985)* were incorporated in the review procedures at the University of Washington. Category I involves little or no discomfort, Category II involves some distress or discomfort, Category III involves significant distress or discomfort, and Category IV involves procedures with severe pain. Projects have been classified based on their category and forwarded to members of the Institutional Animal Care and Use Committee (IACUC) for either approval, approval with modification(s), or disapproval. This classification facilitated the committee's review of approximately 1,200 protocols annually by prohibiting projects in Category IV and focusing their attention on projects in Categories II and III.

Trends, Challenges and Opportunities

The Age of Biology--can anyone who reads a newspaper or watches television news doubt the growing importance of this megatrend or one of the broad outlines characterizing the future? Indeed, in a previous communication *(Van Hoosier, 1996)*, I proposed that The Age of Biology, along with two other megatrends -- Globalization and The Era of Women in Leadership, represent changes, challenges, and opportunities for laboratory animal scientists. In a 1999 presentation at the Panum Institute in Copenhagen, I focused on the Age of Biology and emerging diseases in laboratory mice and the analogies between factors contributing to those diseases in humans and in animals (*Van Hoosier, 1999)*. Today, I continue my focus on the Age of Biology by exploring paradigms for committee review and the monitoring of research projects that involve genetically engineered animals.

Gene splicing began in the 1970s, and the era of transgenic animals was ushered in by mighty mouse, which resulted from the injection of growth hormone genes from a rat into a fertilized mouse egg. Genetic engineering methods consist of insertion, deletion, or alteration of a segment or segments of DNA followed by observation of the effects. The DNA segment of interest may be overexpressed, knocked out, or inactivated, and then the animal and any offspring are observed to learn the effects of the genetic manipulation. The Proceedings of a workshop entitled Welfare Aspects of Transgenic Animals (*van Zutphen and van der Meer (eds.), 1995*) should be consulted for an overview of the procedures, applications and issues involved. Although many have predicted a decrease in the number of animals used in research, the techniques for generating mouse models of human genetic diseases and for gene therapy of human diseases have resulted in an annual increase of 10% to 20% in the mouse populations of many U.S. institutions, including the author's (Fig 1). John Naisbitt, the author of the book *Megatrends 2000* (*Naisbitt, 1984*), says that as we move into the next millennium, biotechnology will be as important as the computer. (As a matter of fact, biology provides the metaphors in computer science , e.g., a computer "virus" and a "mouse"). The deciphering

Fig. 1. Annual Mouse Population at UW



of the human genetic code may well prove to be the greatest scientific discovery of the next century. As true for so many facets of The Age of Biology, this topic is riddled with ethical issues. I'm a veterinarian without formal training in ethics, and I feel on thin ice when discussing ethics. But we must realize that those who may have had training in ethics--philosophers, lawyers, politicians--do not in the real world have the greatest influence over how animals are treated. Approaches to the subject of human-animal relations, including the relation of researchers with genetically engineered animals, must pay attention to the practices of the veterinary profession. As these approaches are explored and developed, one of our challenges as laboratory animal veterinarians is to bridge the worlds of the investigator, the bioethicist, and the public.

Some individuals in society today believe that we should not make transgenic or knockout animals. In 1988, there was an initiative in Switzerland to prohibit the intentional alteration of the genome of any animal unless the action could directly save the life of a human and forbid the importation of transgenic animals. The voters of Switzerland turned down the "Gene Protective Initiative" by a 2:1 margin *(Koenig, 1998; Schatz, 1998)*. If it had passed, it would have prohibited animal research that involved genetic engineering. Irrespective of how one feels about this issue, it is worthwhile to keep in mind some special issues related to the creation of genetic models used to study human diseases *(Rollin, 1995; Spinelli, 1996)*.

**Animal welfare concerns* include the identification of endpoints at which the transgene can be recognized to be causing serious disease; the development of humane methods for obtaining samples for transgene testing; and the potential for creating new infectious agents that may cause animal health problems.

**Environmental concerns* include the creation of animals with foreign genes or with altered pathogens that could cause unpredictable environmental effects if released; the use of transgenic animals in agriculture in a way that narrows the gene pool; new engineered disease states produced in the mammalian gene pool that could affect humans; and the production by genetic engineering of very rapid wholesale changes in organisms.

**Human health concerns* include the generation of byproducts during the production of food from animals that may be harmful to those consuming the animals; the possibility that animals made susceptible to human disease may get loose and pose a risk to humans; the recombination of animal and human pathogens to form super pathogens; the creation of animals resistant to one strain of pathogens giving rise to new pathogens or pathogenic strains; the creation of new infectious agents that may cause serious health problems; and the military application of transgenic technology.

**Theological concerns* include the consideration that placement of animal genes in humans or human genes in animals may violate God's moral order and even that any genetic engineering may violate God's moral order. There is also a question of the moral standing or rights of animals with human DNA.

**Social concerns* include that possibility that small farmers may be forced out of business.

While this trend in the Age of Biology challenges us when we evaluate experimental animal protocols in genetic engineering studies, it also presents us with an opportunity to provide leadership in this area and avoid disapproving research projects that have the potential to make major contributions to progress in biology and medicine.

It is generally agreed among ethicists that ethics, rather than one's own moral point of view, be used to determine whether a particular use of animals is proper. This implies a difference between the terms *ethics* and *morals*. Because both terms are concerned with the rightness or wrongness of an action, the traditional definitions are synonymous. However, there is a distinction between the two terms. Morals connotes one's own beliefs system of what is right and what is wrong, while ethics connotes a theoretical ethics-assessment tool by which one can come to a conclusion about the appropriateness of a given act. The assessment of the ethics of an act, therefore, requires i) a basic set of principles and ii) an agreed-upon, systematic consideration of a set of circumstances rather than

the imposition of one's own moral beliefs.

The development of ethical theories pertaining to experimental animals has lagged behind the development of medical ethics. While there is no consensus about the approach to ethical assessment, most individuals would probably subscribe to either the utilitarian approach (which holds that in deciding whether the action is right, one sums up the total amount of good or benefit the action will bring about and weighs that against the total amount of harm or costs that will be caused) or the deontological approach (which holds that some acts may be judged wrong even though their consequences are, on balance, good.) I think that it is especially important that the ethical role of animals in biomedical research be considered in the context of medical research and human health. For example, one might come to different decision regarding the appropriateness of the injection of thousands of monkeys with poliovirus or poliomyelitis vaccine which could result in paralysis or death if considered in the abstract instead of the benefit associated with the control of the epidemic of poliomyelitis in humans. In addition, I would like to explore the question of whether principles and paradigms used in human medical ethics can be used as a model for the assessment of animal research.

Basic Principles

The Belmont Report.

In 1974, the U.S. Congress passed the National Research Act, which established the National Commission for the Protection of Human Subjects. The Commission was instructed to identify basic underlying ethical principles for the conduct of biomedical and behavioral research involving humans and to develop guidelines for the conduct of such research in accordance with the ethical principles identified. Their report is commonly referred to as The Belmont Report (1979) because of the location of one of their meetings, i.e., the Belmont House of the Smithsonial facility at Elkridge, Maryland.

The Commission established conditions for the use of human subjects that recognize and respect several interests:

1. respect for the autonomy of humans,
2. respect for the freedom of inquiry,
3. the avoidance of needless pain, injury, and humiliation; and
4. the maximization of and equity in the distribution of social benefits

These conditions are founded on three ethical principles:

- respect for person,
- justice, and
- beneficence (marked by performing kind or charitable acts).

It has been suggested that two of these three ethical principles make points meaningful for the rationale and justification for animal experimentation in general, including studies with genetically engineered animals. A respect for person requires a reasonable opportunity for choice, and a meaningful choice requires information. Animal research then should provide pertinent information to individuals who are trying to make a reasonable and meaningful choice. Therefore, investigations that use animals become important background information in the informed consent process. The ethical principle of beneficence requires not only doing good, but also its reciprocal--minimizing harm. Well-designed animal experiments should provide timely and sufficient information to the investigator so that he or she can minimize harm.

The Sundowner Report

While the Belmont Report identified explicit principles underlying the ethical evaluation of research involving human subjects, only implicit principles in the Guide and the Animal Welfare Act were available for animals in 1995. A committee was appointed by the National Aeronautics and Space Administration (NASA) to address questions about the use of rhesus monkeys in space for the Bion 11 and Biocosmos projects and to develop explicit principles underlying the ethical evaluation of research involving animals. The committee's report, using the Belmont Report as a model, is commonly called the Sundowner Report, after the location of their meeting in California.

The Sundowner Report was adopted by NASA to

guide careful and considered discussion of the ethical challenges that arise in the course of biomedical research using animals and provide a framework within which challenges can be rationally discussed. The passage below from the NASA document announcing adoption of the principles *(14CRF part 1232; NASA Policy Directive, 1998)* provides some context as to the basis and the use of those principles.

"Introduction: A strong allegiance to the principles of bioethics is vital to any discussion of responsible research practices. As reflected in the considerations of the National Commission for the Protection of Human Subjects, "scientific research has produced substantial social benefits... [and] some troubling ethical questions" *(The Belmont Report,1979)*. The Belmont Report identified the key fundamental principles underlying the ethical evaluation of research involving human subjects. Similarly, the principles governing the ethical evaluation of the use of animals in research must be made equally explicit."

"It is generally agreed that vertebrate animals warrant moral concern. The following principles are offered to guide careful and considered discussion of the ethical challenges that arise in the course of research, a process that must balance risks, burdens, and benefits. NASA will abide by these principles as well as all applicable laws and policies that govern the ethical use of animals. It is recognized that awareness of these principles will not prevent conflicts. Rather, these principles are meant to provide a framework within which challenges can be rationally addressed."

"Basic Principles: The use of animals in research involves responsibility, not only for the stewardship of the animals but to the scientific community and society as well. Stewardship is a universal responsibility that goes beyond the immediate research needs to include acquisition, care and disposition of the animals, while responsibility to the scientific community and society requires an appropriate understanding of and sensitivity to scientific needs and community attitudes toward the use of animals."

"Among the basic principles generally accepted in our culture, three are particularly relevant to the ethics of research using animals: respect for life, societal benefit, and non-maleficence".

"1. Respect for Life

Living creatures deserve respect. This principle requires that animals used in research should be of an appropriate species and health status and that the research should involve the minimum number of animals required to obtain valid scientific results. It also recognizes that the use of different species may raise different ethical concerns. Selection of appropriate species should consider cognitive capacity and other morally relevant factors. Additionally, methods such as mathematical models, computer simulation, and in vitro systems should be considered and used whenever possible."

"2. Societal Benefit

The advancement of biological knowledge and the improvements in the protection of the health and well being of both humans and other animals provide strong justification for biomedical and behavioral research. This principle entails that in cases where animals are used, the assessment of the overall ethical value of such use should include consideration of the full range of potential societal goods, the populations affected, and the burdens that are expected to be borne by the subjects of the research."

"3. Non-maleficence

Vertebrate animals are sentient. This principle entails that the minimization of distress, pain, and suffering is a moral imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in humans may cause pain or distress in other sentient animals."

I submit for your consideration that fundamental principles underlying the ethical evaluation of research with animals have been developed. The Sundowner Principles are an extension and modification of the Belmont Principles for human medical ethics and provide a foundation for the ethical evaluation of research involving animal subjects.

Committee Review and Decision Paradigm.

"Casuistry" - the application of general principles of ethics in the determination of right and wrong.

Casuistry has its origins with Catholic moral theologians from the fourteenth century onward and their use of moral rules to address ethical issues. In *Clinical Ethics*, the Jonsen casuistry grid for reviewing human medical ethics (Table 1) is applied as an assessment tool to complement the principles in the Belmont Report for medical interventions with patients *(Jonsen et al., 1998)*. Is there a single assessment tool that can be applied to the use of laboratory animals in biomedical research? It is my perception that most, if not all, members of committees evaluating research projects using animals would answer "no." However, at a workshop in Seattle, Jonsen presented a casuistry grid that could be applied to the use of animals in research (Table 2).

Initial Project review

To explore the Belmont Report and the Jonsen's Casuistry Grid for Human Medical Ethics as a model for the application of the Sundowner Report and an analogous Grid (Table 2) for biomedical research using animals, a research project entitled Genetic Engineering Approaches to Neurobiology *(Szczypka et al., 1999)* is summarized below.

Background: Parkinson's disease, one of the most devastating disorders of the nervous system, results when most dopamine-producing cells in a certain area of the brain die. Dopamine is a neurotransmitter, a substance that conducts cell-to-cell signals in the brain. Symptoms of the disease include muscle rigidity, tremors, slowness of movement, poor balance, and problems with walking. Treatment with levodopa, which converts to dopamine in the brain, becomes ineffective in most patients over time and often causes side effects; brain surgery is used to treat some patients.

Objectives: To determine what dopaminergic pathways are essential for normal activity, eating, and drinking behaviors and to correct the effects of dopamine deficiency by gene therapy.

Methods: Knockout mice will be made that cannot synthesize dopamine because of the inactivation of tyrosine hydoxylase (TH) the rate limiting enzyme required for its synthesis. (It is estimated that100 to 200 mice will be required to make the knockout strain and that 20 to 40 pairs of breeding mice will be required for back crossing or for the propagation of the strain on a continuing basis.) A non-replicating adenovirus (AAV) carrying the TH gene will be injected by a cannula inserted in the appropriate region of the brain; the virus should infect non-dividing neurons. (It is anticipated that the treatment of about 50 dopamine-deficient mice would be required to determine whether the injection procedure is successful and where adenovirus delivery is most efficacious.)

Anticipated adverse effects: It is predicted that dopamine deficient (DA-/-) mice will be aphagic, adipsic, and akinetic and may expire without intervention either with daily injections of L-DOPA. or with gene therapy.

Contributions to scientific knowledge: The experiments will open the door to a better understanding of how the brain uses dopamine, explore new strategies for treating Parkinson's disease in humans and provide an experimental animal model for viral gene therapy, a technology being attempted in a variety of diseases.

As in the consideration of medical ethics, circumstances will dictate which issues need to be explored in greater detail as the review group applies the Grid (Table 2) and weighs the costs and benefits of a project. In the project summarized above, there are no Alternatives for the objective. The potential benefits associated with the Contributions to Scientific Knowledge are highly significant as the project has a high priority based on peer review, the procedural questions have satisfactory answers, and the animal model should be useful for exploring the treatment of Parkinson's disease in humans. However, the review group should keep in mind that there can never be a guarantee or certainty on the usefulness or importance of any one particular experiment as scientific advancement is not always a linear endeavor. Regarding External Factors, the potential benefit can be assigned heavy weight versus the costs because of the importance of the condition under study as summarized under the background above. The principal costs are associated with the Quality of Life part of the grid.

Table 1. Jonsen's Casuistry Grid for Human Medical Ethics

MEDICAL INDICATIONS	PATIENT PREFERENCES
1. What is a patient's medical problem? history? diagnosis? prognosis?	1. What has the patient expressed about preferences for treatment?
2. Is problem acute? chronic? critical? emergent? reversible?	2. Has patient been informed of benefits and risks, understood, and given consent?
3. What are goals of treatment?	3. Is patient mentally capable and legally competent? What is evidence of incapacity?
4. What are probabilities of success?	4. Has patient expressed prior preferences, e.g., Advance Directives?
5. What are plans in case of therapeutic failure?	5. If incapacitated, who is appropriate surrogate? Is surrogate using appropriate standards?
6. In sum, how can this patient be benefitted by medical and nursing care, and how can harm be avoided?	6. Is patient unwilling or unable to cooperate with medical treatment? If so, why?
	7. In sum, is patient's right to choose being respected to extent possible in ethics and law?
QUALITY OF LIFE	**CONTEXTUAL FEATURES**
1. What are the prospects, with or without treatment, for a return to patient's normal life?	1. Are there family issues that might influence treatment decisions?
2. Are there biases that might prejudice provider's evaluation of patient's quality of life?	2. Are there provider (physicians and nurses) issues that might influence treatment decisions?
3. What physical, mental, and social deficits is patient likely to experience if treatment succeeds?	3. Are there financial and economic factors?
4. Is patient's present or future condition such that continued life might be judged undesirable by them?	4. Are there religious, cultural factors?
5. Any plan and rationale to forgo treatment?	5. Is there any justification to breach confidentiality?
6. What plans for comfort and palliative care?	6. Are there problems of allocation of resources?
	7. What are legal implications of treatment decisions?
	8. Is clinical research or teaching involved?
	9. Any provider or institutional conflict of interest?

Table 2. Jonsen's Casuistry Grid as a Model of Decision Paradigms Applied to Experimental Animal Issues

ALTERNATIVES 1. 3-R's - refinement, reduction, replacement. 2. PI search failed to identify non-animal methods. 3. *In vitro* pilot studies	**CONTRIBUTION TO SCIENTIFIC KNOWLEDGE** 1. Adherence of animal procedures to standards. a. Does PI justify departures from standards? b. Are sample sizes appropriate? c. Is survival surgery aseptic? d. Antibody production conform to guidelines? e. Proper blood sampling protocol? f. Proper facilities & equipment etc. 2. Payoff and benefit to society 3. Will scientific understanding of problem studied be advanced? 4. Training and qualifications of personnel 5. Presence or absence of peer review
EXTERNAL FACTORS 1. Distress of procedures to personnel 2. Symbolic or sentimental value of species to people 3. Sentience and scarcity of species 4. Status of transgenic animals 5. Importance of condition studied	**QUALITY OF LIFE** 1. Pain, distress, discomfort, & suffering 2. Monitoring for signs of pain 3. Anesthesia, analgesia 4. Deprivation (food, water, social) 5. Euthanasia, death as an end point 6. Application of noxious stimuli (behavior testing, toxicity) 7. Provision of adequate housing, veterinary care. 8. Treatment of controls

Although the anticipated adverse effects on the mice, e.g., aphagia, are significant, the investigator proposes the use of L-DOPA or gene therapy to treat the animals, the frequent observation of the animals and euthanasia of moribund animals and the review group is assured of adequate housing and veterinary care.

As the committee moves through the decision-making steps of the paradigm, it is in effect reviewing the consistency of the proposed research with the Sundowner Principles presented previously, i.e. respect for life (the species are appropriate, and *in silico* or *in vitro* systems are not applicable); societal benefit (the potential for the advancement of biological knowledge and improvement in the health of humans is high); and non-maleficence (procedures are in place to minimize distress, pain, and suffering). In reaching a decision to approve the Genetic Engineering Approaches to Neurobiology project presented in the initial review, the committee weighs the probability of medical benefit and/or the potential contribution to scientific knowledge against the likely costs, mainly adverse effects.

Unpredictable Outcomes and Continuing Project Review

Van der Meer and van Zutphen point out the limited amount of data published on the effects of transgenesis on the welfare of the animals *(van der Meer and van Zutphen, 1997)*. In a subsequent study, van der Meer, et al. studied the indicators of discomfort in four groups of animals of the same strain which differed in their transgenic background (no treatment, integration of a functional transgene construct, integration of a nonfunctional gene construct and mice with no treatment) by applying a protocol for assessing the effects of transgeneis, *per se (van der Meer et al., 1999)*. There was an increase in pup mortality in animals with a microinjected DNA construct and a lower rate of growth in pups with a functional DNA construct; no differences in morphological characteristics or behavioural development were observed. However, no general conclusions can be drawn from the study cited above as the effects of genetic engineering may vary between projects depending upon the strain of animals used, the DNA construct, the site(s) of DNA incorporation and number of copies integrated. It might be predicted that unanticipated effects occur more frequently during the initial or discovery phase of the field of genetic engineering than in the later stages of hypothesis based research. The two studies, or cases, summarized below illustrate the difficulty of predicting side effects or adverse outcomes for genetic engineering studies and the limitations of asking the investigator to list them in advance.

Case 1: Mice were transfected with a drosophila heat shock gene (hsp70) and a herpesvirus thymidine kinase gene. The F1 heterozygotes appeared phenotypically normal, but the F2 homozygotes had loss of hind limbs, malformed forelimbs, facial clefts, and olfactory lobe defects. *(McNeish et al., 1988)* This case points out the need for ongoing monitoring of succeeding generations of genetically altered progeny.

Case 2: A group had been making transgenic mice using an *lck* -IL-4 gene construct for a couple of years without any problems *(Lewis et al., 1993)*. Then, the researchers observed that a new line (1315) became progressively humpbacked starting between 3 and 6 months of age. The animals had decreased bone mass and kyphosis caused by a profound decrease in osteoblast activity. The line is now proposed as a good model for studying the previously unrecognized role of interleukin 4 in osteoporosis.

Morbidity and Mortality Surveillance.

Cases 1 and 2 illustrate the inadequacy of confining IACUC review to the initial approval process prior to the start of a study. Since outcomes are often unpredictable, the IACUC should consider surveillance or monitoring of ongoing studies in order to ensure adequate review of welfare considerations. At the University of Washington we began our monitoring of ongoing studies with a morbidity and mortality surveillance program. It resulted in identification of transgenic and knockout lines with many unexpected outcomes, including increased tumor incidence, diabetes, allergic encephalomyelitis, hydrocephalus, epilepsy, osteoporosis, anasarca, malocclusion, arterial wall calcification, and many

others.

Phenotyping Protocol.
In addition to listing anticipated adverse effects in association with the initial review and morbidity and mortality surveillance, it may be helpful to ask investigators to describe phenotypes and unanticipated outcomes in their applications for protocol renewals and the continued breeding of a genetically altered line. A phenotyping protocol has been drafted for this purpose (Table 3) *(Dennis, 1999)*. The protocol is a list of suggestions or possibilities for basic phenotyping data; an investigator would not be required to provide data in all areas. S/he could submit data relevant to the particular line being studied. Results from in-depth testing in specialized areas that would help the IACUC assess the scientific importance of the strain could also be submitted. These are suggestions of data that an investigator can provide to make his/her case for continuing to breed a line with compromised welfare. Investigators may submit additional data relevant to the committee's review (e.g., immune competency or disease model data). Once it receives the completed form with any additional information, the IACUC's task is to weigh animal welfare considerations and any potential utility of the line and to decide whether to allow continued breeding of the line. In some cases, embryo or embryonic stem cell cryopreservation may be a useful alternative to continued breeding of animals whose welfare may be compromised. An article by Costa is also relevant to surveillance; standardized housing and care procedures, health control and screening from birth to identify innate deficits to assist in welfare assessment are proposed *(Costa, 1995)*.

Discussion
While the "3Rs" have been a useful concept in the past, it does not appear applicable to many genetic engineering studies with animals; for example and as mentioned previously, genetic engineering experiments in animals have resulted in an increase in the numbers of animals used in many institutions instead of a reduction. Advantages of the explicit principles in the Sundowner Report in conjunction with a casuistry grid include the precedence of the paradigm in addressing ethical problems in human medicine and the balance it brings into the equation by including the contribution to scientific knowledge of the project. In a presentation by Spinelli, minor modifications to the grid proposed by Jonsen were made *(Spinelli, 1996)* and it is anticipated that further modifications will be made as experience with the grid is acquired in the review of protocols for experimental animals. A decisional model has also been described by Stafleu for use by committees evaluating animal experiments which embodies the principles in the Belmont Report. To solve ethical dilemma scenarios, decision making rules and numerical values are assigned, compiled and the scores used as a basis for approving or disapproving a research proposal *(Stafleu et al., 1999)*. The quantitative aspects of this paradigm, or modifications of the strategy, may be useful for individuals on a review committee who have a difficult time deciding on approval or disapproval of a specific project.

Summary
As the world wrestles with the fact of genetic engineering and specifically with research involving genetically engineered animals, laboratory animal scientists are in a position to identify the issues and propose ways to address them. One of the issues raised by such research is the greater unpredictability of adverse effects. Because assessment of adverse effects is critical to evaluation of the cost and benefit of proposed research, development of a paradigm for review and monitoring of such research is of high priority. The paradigm developed at the University of Washington that applies Jonsen's casuistry grid in conjunction with surveillance systems for early identification of unanticipated adverse effects is based on established principles and provides a structured approach to evaluation of the factors involved. Application and further development of this paradigm give laboratory animal scientists yet another opportunity to use their training, experience, and position to address the concerns of animal research, bioethics, and the public good.

Table 3. Sample Phenotyping Protocol
The following can be evaluated and data submitted for each line for which continued breeding is requested.

1. Morbidity
   A. Fetal death
   B. Lifespan
2. Fertility (Litter size at birth and weaning)
3. Development:
   A. Birth weight
   B. Growth rate
   C. Hair growth
   D. Development of neonatal reflexes
   E. Age at incisor eruption
   F. Age eyes & ears open
   G. Age at standing and walking
4. Clinical parameters:
   A. Physical exam for malformations
   B. Coat condition
   C. Nasal or ocular discharge
   D. Hemogram
   E. Serum chemistry profile
   F. Tumor development
5. Simple behavioral parameters:
   A. Posture, climbing, and locomotion
   B. Eating and drinking
   C. Grooming
   D. Activity level, exploration
   E. Alertness
   F. Aggression
   G. Twitches, tremors
   H. Stereotypic behaviors
   I. Righting
   J. Auditory startle
   K. Seizures
   L. Reflexes
6. Necropsy and Histology
7. Specialized testing:
   A. T and B cell function
   B. Cytokine profile
   C. Pathogen susceptibility
   D. Complex behavioral testing
   E. Learning testing

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