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The Creation of Transgenic Animal "Models" for Human Genetic Disease

Perhaps the greatest socio-ethical challenges associated with the development and use of transgenic animals in biomedical research are the problems associated with animal welfare. Whereas the issue of biosafety does, indeed, represent a major concern, the minimization of such risk is as much a prudential concern as an ethical one for investigators, as they themselves are put at risk by failure to provide adequate safeguards against the dangers of transgenic animal research. Animal welfare concerns, on the other hand, represent a far greater moral challenge, for concern about animal welfare often does not coincide with perceived self-interest and, indeed, can exact costs in terms of self-interest, in the form of money, time, extra personnel, delay in research, etc. In other words, many researchers have traditionally not equated concern for animal welfare with self-interest and are, thus, unlikely to do the right thing for reasons of self-interest. Somewhat mitigating this blanket statement is the relatively recent acknowledgement of the fact that failure to assure animal welfare can skew variables relevant to research and actually compromise research (Rollin, 1990), but nonetheless, the coincidence of the two is far from perfect. As we shall see, certain aspects of transgenic animal research do represent an area where welfare could be ignored without obviously jeopardizing the work in question. Thus moral concern must take up the slack left after prudential considerations are exhausted.

The emergence of a systematic social ethic whose purview extends to the treatment of laboratory animals is a relatively recent phenomenon, as evidenced by the fact that researchers basically enjoyed *carte blanche* in the use of animals until the mid 1980s (Rollin, 1991). For most of the 19th and 20th centuries, the only consensus ethical principle extant in society for the treatment of animals was a prohibition against overt, willful, intentional, needless, wanton cruelty, as expressed in anti-cruelty legislation. Concerned as much with ferreting out sadistic individuals who might begin with animals and move to humans as with protecting animals, these laws, therefore, did not address "normal," "necessary" or "beneficial" sources of animal suffering

such as agriculture, research, hunting, trapping or education; these are typically exempted from the anti-cruelty laws by statute, or else have been excluded by judicial decision. Rather, the laws focused on deviant behavior leading to "unnecessary" animal suffering. It is only in the past decade that society has begun to realize that a mere fraction of animal suffering is a result of overt cruelty—the vast majority of animal suffering at human hands, in fact, grows out of such decent motivations as increasing knowledge, curing disease, increasing efficiency of food production, protecting humans against toxic substances and so on. Correlative with this realization has come a demand for the control of suffering in areas of animal use which previously enjoyed *laissez faire,* notably toxicity testing, animal research and animal agriculture. First to be directly affected by this demand was animal research, with two major pieces of federal legislation designed to assure the welfare of research animals passed in the U.S. in 1985.

Perhaps the main feature of this legislation, which I have discussed at length elsewhere (Rollin, 1989; 1991), is a mandate to control pain, suffering and distress in research animals except where scientifically necessary, as in the study of pain, and even there, to minimize it as far as possible. Second, the legislation is designed to assure "enforced self-regulation" of animal research and dialogue about animal welfare concerns, through the vehicle of protocol and facilities review by animal care committees. Third, the legislation suggests that welfare concerns are not limited to controlling overt pain and suffering, but actually points towards providing some positive opportunity for animals to express their biological and behavioral natures—this is exemplified by the requirements of exercise for dogs and provision of an environment conducive to the psychological well-being of primates. This legislation has already had many salubrious effects on the welfare of laboratory animals, perhaps the most dramatic being the focusing of scientific attention on recognizing, characterizing and alleviating animal pain. It has also led researchers to far greater awareness of ethical questions in research, something which was traditionally stifled by widespread belief that science is and ought to be, "value-free" (Rollin, 1989).

Thus, we see the emergence of a new ethic for animals demanding, in essence, maximization of the interests of animals while they are being used for human benefit. The most articulate expression of this ethic thus far, has been the demand for the control of animal pain and suffering in research.

For certain aspects of transgenic animal use, this demand will be relatively easy to satisfy. Consider, for example, the patented Harvard mouse which is disposed to the development of tumors. In the words of the patent, this is "an animal whose germ cells and somatic cells contain an activated oncogene sequence introduced into the animal...which increases the probability of the development of neoplasms (particularly malignant tumors) in the animal" (U.S. Patent Number 4,873,191). Minimizing pain and suffering

for such an animal is, in principle and in fact, no different from minimizing pain and suffering in nontransgenic animals in whom tumors are induced by other means: the establishment of endpoints for euthanasia, in terms of tumor size, so that the animal does not suffer, and the judicious use of anesthetics, analgesics and tranquilizers during operative or other procedures.

Similarly, there is no reason the second major thrust of the new social ethic cannot be applied to these transgenic animals—namely the provision of enriched environments and husbandry systems for these animals which allow them to actualize their behavioral and biological natures. In the case of transgenic mice, for instance, one should look to the recommendations outlined in literature on care of mice; for example, a recent article described a caging system for rodents that is meant to accommodate their behavioral needs (Sharmann, 1991). Indeed, the characterization of such environments and systems for a variety of animals is a primary purpose of the chapters in a book I am currently editing (Rollin and Kesel, in press). Thus, the vast majority of transgenic animals developed so far raise no additional welfare issues beyond those concerning nontransgenic laboratory animals.

Indeed, those welfare issues which are raised dramatically by transgenic animals are also continuous with analogous nontransgenic cases. I am referring to the creation and maintenance of seriously defective animals which are developed and propagated to model some human disease. This was traditionally accomplished through identification of adventitious mutations and selective breeding. Transgenic technology allows for accomplishing the same goal far more quickly and in a far wider range of areas. One can essentially replicate, in principle, any human genetic disease in animals—and therein lies the major ethical concern growing out of transgenic technology.

A recent chapter in a book devoted to transgenic animals helps to focus the concern:

> There are over 3,000 known genetic diseases. The medical costs as well as the social and emotional costs of genetic disease are enormous. Monogenic diseases account for 10% of all admissions to pediatric hospitals in North America... and 8.5% of all pediatric deaths.. .. They affect 1% of all liveborn infants. .. and they cause 7% of stillbirths and neonatal deaths. ... Those survivors with genetic diseases frequently have significant physical, developmental, or social impairment. . . . At present, medical intervention provides complete relief in only about 12% of Mendelian single-gene diseases; in nearly half of all cases, attempts at therapy provide no help at all (Karson, 1991.)

This is the context in which one needs to think about the animal welfare issues growing out of the use of transgenic animals in biomedical research.

On one hand, it is dear that researchers will embrace the creation of animal models of human genetic disease as soon as it is technically feasible to do so. Such models, which introduce the defective human genetic machinery into the animal genome, appear to researchers to provide convenient, inexpensive and—most importantly—high fidelity models for the study of the gruesome panoply of human genetic diseases outlined in the over three thousand pages of text comprising the sixth edition of the standard work on genetic disease, *The Metabolic Basis of Inherited Disease* (Scriver et al., 1989). Such "high fidelity models" may well reduce the numbers of animals used in research, a major consideration for animal welfare, but are more likely to increase the numbers as more researchers engage in hitherto impossible animal research. On the other hand, the creation of such animals can generate inestimable amounts of pain and suffering for these animals since genetic diseases, as mentioned above, often involve symptoms of great severity. The obvious question then becomes the following: Given that such animals will surely be developed wherever possible for the full range of human genetic disease, how can one assure that vast numbers of these animals do not live lives of constant pain and distress? Such a concern is directly in keeping with the emerging social ethic for the treatment of animals; as we said, one can plausibly argue that minimizing pain and distress is the core of recent federal legislation concerning animal use in research.

The very first attempt to produce an animal "model" for human genetic disease by transgenic means, as mentioned earlier, was the development, by embryonic stem cell technology, of a mouse which was designed to replicate Lesch-Nyhan's disease, or hypoxanthine-guanine-phosphororibosyl transferase (HRPT) deficiency (Hooper et al., 1987; Keuhn et al., 1987). Lesch-Nyhan's disease is a particularly horrible genetic disease, leading to a "devastating and untreatable neurologic and behavioral disorder" (Kelley and Wyngaarden, 1983). Patients rarely live beyond their third decade and suffer from spasticity, mental retardation and choreoathetosis. The most unforgettable and striking aspect of the disease, however, is an irresistible compulsion to self-mutilate, usually manifesting itself as biting fingers and lips. The following clinical description conveys the terrible nature of the disease:

> The most striking neurologic feature of the Lesch-Nyhan syn - drome is compulsive self-destructive behavior—between 2 and 16 years of age, affected children begin to bite their fingers, lips and buccal mucosa. This compulsion for self-mutilation becomes so extreme that it may be necessary to keep the elbows in extension with splints, or to wrap the hands with gauze or restrain them in some other manner. In several patients, mutilation of lips could only be controlled by extraction of teeth.
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> The compulsive urge to inflict painful wounds appears to grip the patient irresistibly. Often he [sic] will be content

> until one begins to remove an arm splint. At this point a communicative patient will plead that the restraints be left alone. If one continues in freeing the arm, the patient will become extremely agitated and upset. When completely unrestrained, he will begin to put the fingers into his mouth. An older patient will plead for help and if one then takes hold of the arm that has previously been freed, the patient will show obvious relief. If help is not forthcoming, a painful and often severe injury may be inflicted. The apparent urge to bite fingers is often not symmetrical. In many patients it is possible to leave one arm unrestrained without concern, even though freeing the other would result in an immediate attempt at self-mutilation.
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> These patients also attempt to injure themselves in other ways, by hitting their heads against inanimate objects or by placing their extremities in dangerous places, such as in between the spokes of a wheelchair. If the hands are unrestrained, their mutilation becomes the patient's main concern and effort to inflict injury in some other manner seems to be sublimated (Kelley and Wyngaarden, 1983).

At the present, "there is no effective therapy for the neurologic complications of the Lesch-Nyhan's syndrome"(Stout and Caskey, 1988). Thus Kelley and Wyngaarden, in their chapter on HRPT-deficiency diseases, boldly suggest that "the preferred form of therapy for complete HRPT-deficiency (Lesch-Nyhan's syndrome) at the present time is prevention," i.e. "therapeutic abortion" (Kelley and Wyngaarden, 1983). This disease is so dramatic that I predicted almost a decade ago that it would probably be the first disease for which genetic researchers would attempt to create a model by genetic engineering.

Researchers have sought animal models for this syndrome for decades and have created rats and monkeys that will self-mutilate by administration of caffeine and other drugs (Boyd et al., 1965). Thus, it is not surprising that the first disease genetically engineered by embryonic stem cell technology was, indeed, Lesch-Nyhan's disease (Hooper et al., 1987; Keuhn et al., 1987). However, these animals were phenotypically normal and displayed none of the metabolic or neurologic symptoms characteristic of the disease in humans. The reasons for this are unknown (Stout and Caskey, 1988).

This case provides us with an interesting context for our animal welfare discussion. Although the animals were, in fact, asymptomatic, presumably at some point in the future researchers will be able to generate a symptomatic model transgenically. Let us at least assume that this can occur—if it cannot, there is no animal welfare issue to concern us! Whether one ought to create such animals is a question I have addressed elsewhere (Rollin, 1986). The practical moral question that arises is clear: Given that researchers will certainly generate such animals as quickly as they are able to do so, how can one assure

that the animals live lives that are not characterized by the same pain and distress which they are created to model?

Again, this question does not differ in kind from the moral questions associated with developing traditional chronic animal models of human disease, be it by breeding, pharmacological manipulation or tissue destruction. The difference is in degree—transgenics provides the potential for generating vast numbers of animals modeling genetic diseases with devastating symptoms. A second difference lies in the fact that transgenic technology is developing at precisely the same time that social/ethical demand for controlling pain and suffering in research animals is at its historical peak and seems to be increasing.

Regrettably, researchers in the past have been cavalier in controlling pain and suffering in animals used as chronic disease models. Though many of the animals have required extraordinary amounts of care and husbandry, such efforts have been directed, for the most part, at keeping the animals alive and scientifically functional rather than at controlling pain and suffering. Given our current social ethic, it is increasingly imperative that pain and suffering be controlled in all animals used for research. Thus, concern for this dimension of animal care needs to be a fundamental principle which guides those contemplating the transgenic creation of animals which replicate human genetic disease. Such an issue is a true moral challenge for researchers, as concern for the animals' quality of life will undoubtedly make things more difficult and expensive for researchers. At the same time, it is patent that such concern is both morally and socially obligatory. Furthermore, failure to assure the public that animal suffering is being minimized could well accelerate major political constraints on all areas of biotechnology (Rollin, 1986).

Unfortunately, because the research community traditionally ignored this moral component of animal research, there is no vast literature on controlling pain and suffering in chronically defective animals. There has probably been more scientific attention to such questions during the six years following the passage of the aforementioned federal legislation than in the entire previous history of animal research (Rollin, 1989). Doubtless such attention will continue to grow at a significant rate. Researchers undertaking work with animals which model human genetic disease should, therefore, vector these concerns into protocol planning and budgeting; funding agencies should demand such planning, and animal care and use committees should not approve projects until they have evidenced that pain, suffering and distress are controlled.

In many cases—perhaps in a symptomatic Lesch-Nyhan's animal—management of suffering may require a far more radical approach than the standard uses of anesthesia, analgesia and tranquilization, which are, by and

large, used for short periods of time. If a defective animal is to be kept alive for long periods and is likely to experience pain and suffering during that period, researchers should consider the possibility of effecting total elimination of consciousness. One such approach could involve surgically rendering an animal decerebrate, so that, while vegetative functions are extant, the animal's subjective experience has been shut down. Alternatively, and perhaps more viably, one could render an animal irreversibly comatose so that it was effectively anesthetized throughout its life. Unfortunately, virtually no literature exists on induction of coma.

I have galvanized a team of researchers at Colorado State University to explore this drastic possibility. We utilize animals scheduled to be euthanized for other reasons and attempt to induce irreversible coma in these animals by induction of cerebral hypoxia. We hope to find a clear EEG criterion which signals coma. If the method is successful, perhaps the method could be taught to veterinarians at institutions planning to utilize animal models of genetic disease so that the animals will not needlessly suffer.

Obviously, such methods of controlling pain and suffering are very drastic and their effective application is fraught with difficulties. For example, they could, presumably, only be employed where higher brain function is essentially irrelevant to the study of the disease. Whether this is the case or not with Lesch-Nyhan's disease, for example, once it was established that the transgenic animal, indeed, showed all signs of the disease, is unclear. I believe it is. Certainly, at least some metabolic genetic diseases could be studied in this way.

Equally significant, there is something aesthetically, at least and perhaps morally as well (I am not clear on this), about deliberately creating such animals. At the very least, it dramatically perpetuates the notion that society is seeking to transcend—that animals are simply tools for human expedient use. It is, in my view, the lesser of two evils.

The key point is that this dimension of genetic engineering of animals cannot be ignored. There is, as we saw, every reason to believe that transgenic animals will be created to study human genetic disease as soon as the technological capability exists to do so. Extant laws permit such animals to be created. The mindset of the research community makes it inevitable. It is also clear that such diseases can cause enormous amounts of pain and suffering. In the face of this development, responsible researchers need to explore all possible avenues for controlling such pain and suffering. These approaches should include such established methods as the liberal use of anesthetics, analgesics and tranquilizers, and by making as much of the research as possible acute. But these methods are unlikely to be effective in the case of those diseases where suffering begins at birth or is chronic after a certain stage of development. (Lesch-Nyhan's patients, as we mentioned, do not show symptoms from birth, but do exhibit them chronically after their onset.) Thus,

methodologies need to be developed which will control pain and suffering over extended periods of time. There is, thus far, no reason to believe that the research community has yet engaged this issue *vis d vis* animals used in other chronically painful work, let alone in genetically engineered animals. The development of such methodologies for controlling pain and suffering is likely to be exportable to numerous areas of animal research, not only transgenic creation of disease. Only in this way can research attempt to stay in harmony with the ethical stance of the society which allows and supports it.

REFERENCES

Boyd, E.M., M. Dolman, L.M. Knight and E.P. Sheppard. 1965. The Chronic Oral Toxicity of Caffeine. *Can. J. Physiol, and Pharm.* 43:95.

Hooper, M., K. Hardy, A. Handyside, S. Hunter and M. Monk. 1987. HPRT-Deficient (Lesch-Nyhan) Mouse Embryos Derived from Germline Colonization by Cultured Cells. *Nature.* 326:292.

Karson, E.M. 1991. Principles of Gene Transfer and the Treatment of Disease. In *Transgenic Animals.* N. First and F.P. Haseltine, eds. Butterworth-Heinemann, Boston.

Kelley, W.N. and J.B. Wyngaarden. 1983. Clinical Syndromes Associated with Hypoxanthine-Guanine Phosphororibosyltransferase Deficiency. In *The Metabolic Basis of Inherited Disease, fifth edition.* J.B. Stanbury, J.B. Wyngaarden, D.S. Fredrickson, J.L. Goldstein, and M.S. Brown, eds. McGraw-Hill, New York.

Kuehn, M.R., A. Bradley, E.J. Robertson and M.J. Evans. 1987. A Potential Model for Lesch-Nyhan Syndrome Through Introduction of HPRT Mutations into Mice. *Nature.* 326:295.

Rollin, B.E. 1986. The Frankenstein Thing. In *Genetic Engineering of Animals.* J.W. Evans and A. Hollaender, eds. Plenium Press, New York.

Rollin, B.E. 1989. *The Unheeded Cry: Animal Consciousness, Animal Pain and Science.* Oxford University Press, Oxford.

Rollin, B.E. 1990. Ethics and Research Animals-Theory and Practice. In *The Experimental Animal in Biomedical Research, Volume I.* B.E. Rollin and M.L. Kesel, eds. CRC Press, Boca Raton, FL.

Rollin, B.E. 1991. Federal Laws and Policies Governing Animal Research: Their History, Nature and Adequacy. In *Biomedical Ethics Reviews 1990.* J.M. Humber and R.F. Almeder, eds. Human Press, Clifton, N.J.

Rollin, B.E. and M.L. Kesel, eds. in press. *The Experimental Animal in Biomedical Research, Volume II.* CRC Press, Boca Raton, FL.

Scriver, C.R., A. L. Beaudet, W. S. Sly and D. Vale, eds. 1989. *The Metabolic Basis of Inherited Disease, Volumes I and II.* McGraw Hill, New York.

Sharmann, W. 1991. Improved Housing of Mice, Rats and Guinea Pigs: A Contribution to the Refinement of Animal Experiments. *Atla.* 19:108.

Stout, J.T. and C.T Caskey. 1988. Hypoxanthine Phosphororibosyltransferase Deficiency: The Lesch-Nyhan Syndrome and Gouty Arthritis. In *The Metabolic Basis of Inherited Disease, Volume one.* C.R. Scriver, A.L. Beaudet, W.S. Sly and D. Vale, eds. McGraw-Hill, New York.

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