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Animals and Alternatives: Societal Expectations and Scientific Need

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ABSTRACT

As Russell and Burch suggested more than 40 years ago, the most humane science is the best science. The path ahead is clear: pain and distress must be eliminated in animal experiments or reduced to an absolute minimum, and, as scientists, we must use the most humane approaches in our research. To accomplish the best science, we must train those who come after us in the principles and practice of humane science.

Introduction

The alternatives field has been marked by both change and controversy. It deals with both scientific and ethical questions. So, how are we to gauge the progress we have made since William Russell and Rex Burch first delineated *The Principles of Humane Experimental Technique* (1)?

Our progress depends on meeting both scientific need and societal expectations, i.e. the need for sound research aimed at improving health and welfare for humans and animals alike, and the expectation that we treat animals as respectfully and humanely as possible. The "Three Rs" of alternatives described by Russell and Burch -- *replacement, reduction* and *refinement* -- provide the common ground for discussion of these needs and expectations, and they offer the means through which actual progress can be made.

In a 1996 article on animal activism, *The Economist* argued that the extreme positions are minorities with views that are irreconcilably opposed: "One cannot expect discussion between those who see animal use as a holocaust and those who think animal use raises no moral issues". Those of us in the alternatives movement recognise these two positions and find ourselves clearly in the middle ground. While the two extremes may help define the needs and expectations, it is the middle path that leads to progress.

The LD50 Test

I start this lecture with the LD50 test for several reasons. This was one of the first tests that the animal activist community focused on. It was a carefully chosen attack: the test used an excessive number of animals; it was easy to explain to the public; and a member of the scientific community, Gerhardt Zbinden, had attacked it on scientific grounds. Equally important, the LD50 test also speaks to the issue of validation -- what it means now and how it was decided in the past.

The LD50 test was standardised and accepted in the 1940s. Note that there was no validation study, but the test was accepted. The process was straightforward, simple, and final. A group led by Arnold Lehman of the Food and Drug Administration decided that the LD50 test would be defined as giving a single dose to a large group of animals (60-100) and then following the animals for up to 14 days to see if they died. The test was to be carried out in two species.

For many years, this was the standard approach to the LD50. It was based on then available knowledge, professional judgement, and historical information. This level of standardisation was a major advance at the time, as it made comparisons between studies possible. We have seen much change since then -- but how much progress? Well thought-out processes and procedures have been developed for *in vitro* studies, but regulators still accept animal studies as valid for extrapolation to humans without any validation studies, despite objections from the animal protection community -- and from many in the scientific community.

Biological system predicts biological system! Is this perception correct? Does it meet either the scientific need or society's expectations? How do we develop the necessary scientific dialogue to begin to discuss these questions? And how do we make real progress?

The Word "Alternative"

Smyth (2) first used the word "alternative" to convey the Three Rs concept of William Russell and Rex Burch (1). It is an unfortunate word, in that both the scientific community and the animal protection community use it almost exclusively in the dictionary sense of "one of two mutually exclusive possibilities" -- i.e., replacement. As used in the context of Smyth and those of us in the animal protection community, however, "alternative" refers, with fidelity, to the Three Rs of Russell and Burch -- *replacement, reduction* and *refinement.*

In the early 1980s, I was invited to give a presentation to the American Association for the Advancement of Science (AAAS). The woman who introduced me stated, "Dr Goldberg is from the Johns Hopkins University Center for Complimentary, Adjunctive, and Other Methods for Testing." She was unable, truly unable, to use the word alternative. To her, it had serious negative connotations.

The word "alternative" may not stick in people's throats quite so badly these days, though it still does not sit well with many scientists. The "Three Rs" may be more palatable -- especially *refinement.* Even scientists who may blanch at the word "alternative," however, are using the methods -- be it an *in vitro* technique, non-invasive imaging, or environmental enrichment. And that is progress.

Acceptance of Animals in Biomedical Research

The use of animals for biomedical research is generally well accepted by the public at large. If the lay public are asked, "Do you accept the use of animals for biomedical research?", approximately 75% answer in the affirmative. If, however, you ask the same question, but indicate that the animals may

experience pain and/or distress, this figure drops to less than 50%. Acceptance turns to non-acceptance (3).

This leads to two important conclusions. First, the public understand the issues. Second, the issues of concern with respect to animal use are pain, distress and humaneness.

In the United States, the *Animal Welfare Act 1966* addresses these societal concerns about the use of animals in biomedical research, and it requires policies and procedures that deal with pain and distress. The Act does not cover all three of the Three Rs, but rather focuses specifically on pain and distress.

Animal welfare, particularly the control of pain and distress, has been codified in all laws pertaining to the use of laboratory animals. In fact, the laws regarding animal welfare all discuss pain and distress as the basis for the legislation.

Do Animals have Rights?

In the United States, as in most countries (Germany excepted), animals cannot be said to possess rights. It is more accurate to state that, in some circumstances, animals enjoy certain protections -- especially protection from pain and distress in biomedical research. The *Animal Welfare Act* is the major piece of US legislation that defines the relationship between biomedical research and animals. Originally written in 1966 and amended several times since then, the Act is very well constructed and tries to balance the welfare of animals with the need for biomedical research. The Act regulates humane handling, care, treatment and transportation, BUT it cannot regulate design of the actual research or experimentation.

There are several problems with the *Animal Welfare Act.* First, it does not define animal welfare or distress. Second, rats, mice and birds – which account for approximately 90% of all research animals -- are not covered by the Act. In fact, the legislation (4) passed in 2004 specifically states that, for the purposes of this Act, rats, mice and birds are not considered animals. (It should be noted, however, that rats, mice and birds are covered by the Public Health Service and are offered full protection under that policy.)

Two very important parts of the *Animal Welfare Act* are worth highlighting. First, the Act focuses on eliminating, or at least minimising, pain and distress in experimental protocols. Second, and I quote from the Act, it "represents society's concerns regarding the welfare of animals used ... ". These two issues, I believe, should form the basis of our approach to the Three Rs.

Animal Welfare - The Five Freedoms

A curious observation is that the *Animal Welfare Act* does not define animal welfare, nor does it define distress, yet the Act is meant to promote animal welfare and reduce or eliminate distress. Fortunately, there is a body of literature (5-8) that addresses these concerns.

In practice, animal welfare can be functionally defined by the five freedoms:

1. the freedom to have access to fresh water at all times;
2. the freedom to have a nutritional diet and adequate food;
3. the freedom to be free of pain and distress;
4. the freedom to be free of anxiety and fear; and
5. the freedom to be able to express normal behaviour, for example, grooming, foraging, hiding.

In the average laboratory, some of these issues are well taken care of, while others are almost completely ignored. This is an area in need of research -- and one of the great opportunities to improve science while

also improving animal welfare. The connection between good animal welfare and good science is the foundation upon which *The Principles of Humane Experimental Technique* rest, and it is just beginning to be explored at a broader level. This linkage between good animal welfare and better science forms the basis of this presentation -- and of the online course, *Enhancing Humane Science* (see below). It is the potential driver for the scientific community to embrace these principles and practices.

Scientists readily accept that inappropriate statistical evaluation can lead to the wrong conclusions, lead others astray, and be a waste of our time and efforts. It also can soil our reputations. But do all of us recognise that poor experimental design results in the same lost value? Clearly, the answer is no. If we did, then every study would have the benefit of statistical input, and this just does not happen.

Enrichment, the fifth freedom identified above, illustrates the complexity of the issue. In a study of lead toxicity and enrichment, for example, Guilarte *et al.* (9) found that an enriched environment could prevent, and in some cases reverse, the behavioural and biochemical changes associated with lead toxicity. Which is the "normal" condition, the empty cage or the enriched cage? Which provides higher quality science? And which is the correct default position? These are not simple questions, but they are key in providing higher quality science and a more humane science.

Studies by Markowitz & Roberts (10) provide an additional example of this complexity. These authors demonstrated that enrichment actually delayed recovery following spinal surgery. The reason IS not clear. One possible interpretation is that the animals were more comfortable and moved less, and thus they recovered more slowly.

It is clear, however, that if we are to practice the most humane science and to achieve the highest quality science possible with animals, we must incorporate all of the five freedoms into our protocols and practices.

Europe Versus the USA

The USA and Europe clearly differ in their approaches to the issues of animal research. It is this author's impression that Europe has a political will, which is backed by significant financial resources. Because of this, the use of animals in biomedical research is widely discussed in Europe, and the Three Rs of alternatives are broadly supported. The formation and development of ECVAM and the establishment of the UK's new National Centre for the Replacement, Refinement and Reduction of Animals in Research provide but two examples.

The Three Rs of alternatives are integral to documents produced by the European Science Foundation and to the white papers for the future chemicals policy in Europe (the Registration, Evaluation and Authorisation of Chemicals [REACH] policy). These documents include statements outlining the requirements for alternatives. Such is not the case in the USA.

The USA and Europe also differ in how we deal with education regarding the use of animals in research. In The Netherlands, for example, each person who is going to work with animals must take a 3-week, 5-day-a-week, 8-hour-a-day course. In the USA, the requirement for ordering and using animals in research may be as simple as a two-hour online course covering the procedures for filling out an institutional animal care and use committee (IACUC) form.

Painful procedures, such as the mouse ascites method to produce monoclonal antibodies (mAbs), have been outlawed in Europe for more than 10 years. In the USA, *in vitro* methods for mAb production are the recommended default position, but this recommendation is not always followed. We know that many individuals and institutions still use the ascites method, \\ithout giving thought to cell culture alternatives.

In the USA, the animal activist community has been the main driver in the discussion of these issues. Animal advocates and their organisations have generated what little political will there is in this country. The moderate animal protection world has had positive and impressive successes, including the passage of the *Animal Welfare Act* in 1966 and its subsequent amendments. These groups have contributed to improved animal welfare conditions and to improved science.

The more extreme animal rights groups, on the other hand -- those that demand immediate cessation of all animal experiments and use violence and/or calumny (purposeful deception; the use of a partial truth to encourage the drawing of an incorrect conclusion) as their approach to communication -- have done more to harm animals than they can possibly understand. The scientists' rejection of animal rights, and their continuous fight against animal welfare legislation and activities, are direct responses to the calumny and violence perpetrated by the extremists in the animal rights movement. Honest dialogue, discussion and understanding will lead to a more humane and more open approach to animal welfare in the laboratory.

IACUCs in the USA and ethics committees in Europe function quite differently. Each approaches the use of animals through different criteria. In the USA, the quality of science is evaluated first; then welfare considerations are addressed. In Europe, a risk-benefit analysis is required. The US IACUC has surrogate responsibility for the animals - i.e. the same responsibility that a parent has for a young child -- whereas, in Europe, the animal is viewed as having intrinsic value. In the USA, each protocol is evaluated as a stand-alone proposal. In Europe, each protocol is evaluated separately, but as part of a research programme. In my experience, despite their differences, the end results of these two approaches have been remarkably similar. Caution is necessary, however. This is from very limited sampling, and we know that different committees can review the same protocol very differently (11).

All of the above examples point to differences in personal understandings, interpretation, and standardization of process. These issues can be clarified by education and knowledge; then standards can be developed. My goal is to enhance the standard of animal care by scientific researchers. The laboratory animal science course Bert van Zutphen developed in The Netherlands (see below) has been the leader and inspiration in this field. It is our challenge and responsibility to find ways to achieve equivalent or even better results in this critical area.

Enhancing the Standard of Care

Most US scientists (and scientists in many other places as well) have not been taught the principles of humane science or the concepts embodied in the Three Rs of alternatives. In order to practice humane science, we must change the culture -- and the education -- of our scientists. We have to provide the tools that will allow the scientist to meet not only the letter of the law, but the intent of the law as well. To truly address societal concerns about the use of animals in biomedical research, we must train scientists in the Three Rs and, most importantly, in the recognition and elimination of pain and/or distress.

Some scientists in both the USA and Europe fully practice humane science already. It is time, however, for scientists who do not yet have the background or knowledge, to recognise that NOT practicing humane science can compromise their research. Scientists are not cruel; they have devoted their lives to making the world a better place. They are missing a skill set and/or a set of tools, however, that will allow them to enhance their approach to the use of research animals in ways that will eliminate pain and/or distress.

The course, *Enhancing Humane Science* (see below), provides this knowledge, as well as the necessary tools, to fully practice humane science. As the Chinese proverb says, "it is in the doing."

Alternatives: The Three Rs - *Reduction, Refinement, Replacement*

I put them alphabetically and out of the order suggested by William Russell and Rex Burch, only to emphasise that all Three Rs are critically important to good science. My working hypothesis is that humane science is better science. The proof follows.

Reduction is not just about using fewer animals. Michael Festing has demonstrated clearly that the use of improper statistical design and methods can result in data that are misleading, wrong, and wasteful of resources and animals (12-14).

Not only are appropriate statistical approaches necessary for reduction, but experimental design is critical for refinement and replacement as well. Among some segments of the scientific community, reduction seems to be the only "R" considered. While it is a worthy goal in and of itself, it is only part of a fully humane approach.

Refinement is the process of eliminating or reducing any potential pain and distress in a protocol. Newer, more-advanced methods, such as non-invasive imaging, allow one to eliminate and/or minimise pain and distress, while reducing the numbers of animals by as much as 80%. In noninvasive techniques, each animal acts as its own control, further enhancing the quality of the science.

The use of MRI, PET scanning, biophotonics, and other non-invasive techniques, clearly has proven their value in the practice of humane science. Papers by Guilarte *et al.,* Stokestad, and Contag *et al.* (15-18) amply demonstrate these principles. In biophotonic imaging, for example, cancer growth rate, metastatic potential, and chemo-effectiveness have been measured and evaluated in animals that do not have palpable tumours -- even though, if allowed to grow and become solid, the tumour would produce considerable discomfort. The quality of the science IS enhanced, as each animal serves as its own control, providing better quality control and better statistical evaluation, while dramatically reducing the number of animals.

Replacement goes well beyond *in vitro* methods, and different replacement alternatives are needed for different purposes, for example, in basic science, product development and discovery, or regulation.

Basic science. In vitro methodologies and other non-animal methods are a routine part of basic science studies. At most biology-related meetings, *in vitro* methods have dominated the proceedings for more than a decade, with probably more than 80% of the papers involving *in vitro* methods. Basic scientists do not think of *in vitro* as an alternative or complementary approach; they think of it as *the* approach. *In vitro* methods also are a key element of the Three Rs, but science clearly is the driver for these "alternative" methods. Thus, good science and humane science go hand in hand.

Even within the world of tissue culture, further refinement is possible. Some *in vitro* methods require fetal calf serum, some systems use primary cells (raising many animal welfare issues), and still others use biologically derived materials. Each of these examples offers further opportunities to enhance humaneness.

Product development and discovery. Here, *in vitro* methods and other short-term tests are standard. Industry uses *in vitro* and other short-term, nonmammalian tests to determine which products will be developed for commercial use. From this set of tests, companies make their basic decisions as to whether a product will be developed and put through regulatory-required protocols (19).

Regulatory testing. Things have changed substantially since the acceptance of the LD50 in the 1950s. The regulatory community now requires a test to be validated by a well-defined process, such as those

described by ECVAM or the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). This process must be complete prior to regulatory acceptance. To date, ICCVAM has validated two methods and ECVAM thirteen. Only a few of the *in vitro* methods have been accepted by the regulatory community, however, and no single method has been accepted by all agencies. The process is slow, expensive, and in need of re-evaluation and refinement. ECVAM has begun to examine ways to make the process more flexible and user-friendly (20), but this still does not address the regulatory communities' acceptance in a timely fashion.

An approach that may speed the process is a criterion-driven approach to validation. Specific criteria that a developer must meet could be established, thus allowing for self-directed validation. Any agency then could evaluate a method against the criteria and determine whether the method was acceptable. These are issues for other papers, however.

Humane Sciences Course

The issues raised in this lecture all focus on enhancing the standard of care for laboratory animals. The question to be addressed is how to provide the research scientist with the necessary tools and knowledge to fully practice humane science.

Professor Bert van Zutphen of The Netherlands recognised the importance of this question long ago; his answer was to develop an intensive course on laboratory animal science. Over the past 20-plus years, he has trained more than 6,000 scientists in the principles and practice of humane science. As a consequence, Dutch scientists are not only conversant with the Three Rs -- they bring these concepts to bear in all stages of planning and conducting their research. This is not the case in most countries.

I believe that providing such a course is one way -- possibly the only way -- to train future generations of scientists in humane science. To this end, I have worked with faculty members at Johns Hopkins to develop a course that offers the basic materials necessary to begin the practice of humane science. This free online course, entitled *Enhancing Humane Science/Improving Animal Research,* consists of 12 audio lectures, each about 30 minutes long, with accompanying slides, resource lists, and study questions. Table 1 shows the topics covered.

These lectures provide information that shows how practising the most humane science also improves the quality of science. Conversely, the lectures demonstrate that NOT practising humane science wastes both time and resources, and can lead the scientific community astray with inaccurate results. The course asks the necessary questions that allow individual investigators to question themselves. For example, animals in barren cages may display stereotypical behaviour (21). Furthermore, mice that are housed in barren cages and that have not been trained to accept handling, show increased heart rates (22). What are the consequences of using an animal that is not "normal" as a research subject? What are the implications for the data that result from these experiments?

This course makes it eminently clear that the principles and practices of humane science are essential to the conduct of sound animal research.

The Troubled Middle/The Silent Middle

The Economist article on animal activism clearly identified the extreme positions -- those who see no moral issue in using animals for biomedical research and those who see animal use as a holocaust. Straughan Donnelley of the Hastings Center subsequently described those who hold the majority public position as the "troubled middle" (personal communication). The troubled middle understands the need

for animal-based research, but wants to make sure that pain and distress are eliminated or at least minimised.

Table 1: Outline for humane science course

Week	Title	Speaker
1	Opening	Alan M. Goldberg, James Owiny
2	Introduction to humane science: informed decisions, responsible use of animals, ethics, pain, distress, suffering	Andrew Rowan, Alan M. Goldberg
3	Rodent surgery -- quality matters	Randy Brown
4	Post-operative and post-procedural care	Sylvia Singletary, Christian Newcomber
5	Measurement, relief (prevention) of pain and distress	Norman Peterson
6	Impact of stress on quality of data	Andrew Rowan
7	Humane endpoints	James Owiny
8	Welfare, the Five Freedoms, housing	Julie Watson
9	Enrichment	Tom Guilarte
10	Non-invasive techniques	Kathy Gabrielson
11	*In vitro* and other replacement approaches	Alan M. Goldberg, James Yager
12	Experimental design, statistical concepts, role of pilot studies	Karl Broman

In my experience, the scientific community supports humane research and, for the most part, would prefer not to have to use animals. Animal research still is necessary, however, and individual scientists do not want to stand out and be identified as animal advocates. Thus, they remain silent on the issue - even when they themselves practice humane science. I refer to these scientists as the "silent middle."

The two extreme positions described in *The Economist* reject either societal expectations in the first case or scientific need in the second. The "troubled middle" and the "silent middle" accept both expectations and need, though the two groups may differ in their specific concerns.

The issues for the general public are transparency, accountability and humaneness. The public want to know how, why and when animals are used. They also want to know that, when animals are used, the research protocol will cause no pain or distress to the animal -- or at least, no more than a level that would be acceptable if the subject were human.

The issues for the scientific community include enhancing the standard of care for all animals, doing the best science possible, increasing the effectiveness of IACUCs without increasing administrative burden, and dealing with the calumny of the extremist animal rights community.

Conclusions

So, have we made progress? Yes, most definitely. Are we there yet? No, not by a long shot. We continue to be dependent upon animal use in safety testing and biomedical research. We continue to disagree over the nature and extent of our responsibilities and obligations to the animals we use. What has become increasingly clear, however, is that humane science is indeed the best science -- as Russell and Burch so cogently demonstrated more than 40 years ago. This is where scientific need and societal expectations converge. And this is where the path ahead is clear: pain and distress must be eliminated in animal experiments or reduced to an absolute minimum; we as scientists must use the most humane

approaches to our research; and we must train those who come after us in the principles and practice of humane science.

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