Historical Review of Animal Use

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Beginnings of Modern Animal Facilitated Research: Organized experimental research involving animals began in the 19th century. Work such as that of Francois Magendie on the spinal nerves firmly established the importance of animal experiments to the study of physiology. Magendie and his student, Claude Bernard, were key figures in the development of experimental medicine in Europe. Earlier in the 19th century, Marshall Hall (Britain) employed experimental models in his studies of the physiology of reflex action. During this same time, reforms of medical school curricula opened up niches for full time experimental physiologists, thus creating a class of medical researchers separate from practicing physicians.

Experimental medicine advanced rapidly and spectacularly in Europe and Britain in the late 19th and early 20th centuries. The development of antibiotics, antitoxins and vaccines was particularly important in influencing public attitudes toward experimental medicine. Paul Erlich's efforts to identify compounds that would act as "magic bullets", destroying disease agents without harming the host, led to the first use of an organic arsenic as a treatment for syphilis in 1911. In the 1930's, sulfa drugs were discovered by Domagk (Germany). About this same time Fleming (Great Britain) made the observations that led to the discovery of the antibiotic properties of penicillin.

National Institutes of Health: Although the first National Institute of Health was founded in 1930, federal government support for medical research dates to 1878. Even so, it was after World War II before the modern interest of medical research began. Passage of the Public Health Service Act in 1944 provided the basis for increased federal funding and for the establishment of the reconstituted National Institutes of Health in 1948. The resulting rapid growth of biomedical research was accompanied by a corresponding increase in the use of research animals. It has been estimated that about 18 million animals were used annually in medical research in the mid-1950's. That total continued to rise through the 1960's, but since has begun to decline. The Congressional Office of Technology Assessment (1986) used government (USDA/APHIS) census data to estimate current annual use at around 17-22 million.

University of Colorado Health Science Center: Created by a legislative act of the state of Colorado. The collective UCHSC research effort has contributed greatly to the store of medical knowledge. Historical giants such a Dr. Tom Starzl, well known for his liver and multiple organs transplants, began their efforts within the UCHSC community.

A History of the Human-Animal Relationship: From the earliest recorded times to the present, animals have been a prominent focus of human interest. Most early religions present a somewhat mixed view of the relationship between humans and other animals. Greek philosophers debated many of the questions regarding the nature of the relationship between humans and animals which continue to concern us today. Animists believed that kindness to animals was a duty and that humans and animals had souls which were equivalent and interchangeable after death. The mechanists argued against the existence of a soul in humans or animals, except as a purely physical organ which, like other organs, died with the body. Vitalists held to a great chain of being in which the differences between animals and humans were of degree, not kind. Utilitarians held that the world had been created for man's use and pleasure and, therefore, man was entitled to use animals as he saw fit. Roman philosophers continued the debate about the nature of humans' relationship to animals; in practice the Romans generally regarded animals as property to be used as their owners saw fit.

During the mid-18th century a new, more sentimental and romantic attitude toward animals began to emerge in the art and literature of Europe. Cruel treatment of animals became a target of essayists and moralists.

The industrial age of the 19th century again saw a decline in the concern for animals. It was this climate that..

fosters the beginning of our modern day humane society's and animal protection agencies.

Now in the ebbing days of the 20th century, we note a societal attitude of concern balanced with accepted use for both human and animal good. Recent American Medical Association survey's suggest that the majority of our society believe using animals for human good is appropriate. However, many of these same people are concerned about the care animals receive in biomedical research facilities.

The Animal Welfare Movement: Some of the earliest efforts to achieve more humane treatment of animals concentrated on outlawing (England) such practices as bull baiting, cock fighting, and the abuse of domestic animals. In 1822, Parliament enacted a law outlawing cruelty to horses, cattle and donkeys. This legislative activity arose mainly out of a growing feeling of revulsion on the part of the upper and middle classes against the abuse of domestic animals. Great Britain's first effective humane organization, the Society for the Prevention of Cruelty to Animals (later the RSPCA), was founded in 1824. During its first few decades, the society agitated successfully against many forms of animal abuse and cruelty, and as a result, in 1835, Parliament moved to extend protection to all domestic animals and to outlaw bear, bull and badger baiting and dog and cock fights. In 1855, the use of dogs as draft animals was outlawed. In the 1860's the RSPCA turned its full attention to the vivisection issue and, in 1863, the society adopted a policy opposing all painful animal experiments. In 1871, the British Association for the Advancement of Science produced a set of guidelines intended to minimize animal suffering in legitimate experiments and to discourage illegitimate experimentation. A group specifically devoted to the cause of antivivisection, the Victoria Street Society, was founded in 1875. In response to increasing public pressure and debate, a few prominent scientists struggled to form a lobby to support research and to set standards for regulating animal experiments. In 1876, after active lobbying by scientists and antivivisectionist, Parliament passed a bill regulating use of animals in experiments. The 1876 Act included these provisions:

1. Experiments using animals could only be performed on premises licensed by the Home Office;

2. Experimenters had to apply annually for a license and these applications had to be endorsed by recognized scientific authorities;

3. Experiments were limited to those which advanced knowledge, saved or prolonged lives or alleviated suffering;

4. Painful experiments required special permission;

5. Curare was not permitted as an anesthetic;

6. Means of enforcement and penalties for violators were specified.

The early American colonists' attitudes towards animals reflected attitudes prevalent in England and Europe at the time. Colonial Massachusetts passed the earliest known humane law in 1641 which forbade cruelty to domestic animals and was enforced in court cases. In 1822, New York passed a bill forbidding cruelty or misuse of horses, cows and sheep. Massachusetts passed a similar law in 1835, followed by Connecticut and Wisconsin in 1838. It was not until 1921, however, that all U.S. states and territories had such laws.

In the mid-19th century, Henry Bergh founded the ASPCA, an American version of the Society for the Prevention of Cruelty to Animals. With assistance from society's such as the ASPCA, the first federal anti-cruelty law was enacted in 1873. This act (the '28-hour Law') regulated the number of hours during which cattle could be confined during shipment and mandated the provision of food, water and opportunity to rest. In 1906, this law was replaced with a stronger version with better provisions for inspection and enforcement. The federal humane slaughter law, finally passed in 1958, regulated meat packers which supplied the federal government.

The U.S. humane movement first took up the issue of laboratory animal welfare in the last quarter of the 19th century. It was during this time that the American Antivivisection Society was founded in Philadelphia (1883). In the 1880's and 90's, there were several unsuccessful attempts to get restrictive legislation along the lines of the British 1876 Act enacted at both the state and federal levels. The important point to remember however, was the general focus on animal welfare, not animal rights.

In the late 1940's, the National Society for Medical Research began to work for local and state laws which would require animal shelters to release unclaimed dogs and cats to research institutions for use in biomedical experiments and education. In 1949, Minnesota passed the first 'pound release' law and other states quickly followed. The enactment of the pound release laws ended a forty year period during which there was relatively little conflict between humane organizations and the biomedical research community. In response to the perception among some humane workers that established organizations were not taking a strong-enough stand, two new organizations were formed; the Animal Welfare Institute, and the Humane Society of the United States.

Throughout the 1960's, a variety of interested parties called for federal regulation of research animal use. The allegation that stolen pets were being used in research led to introduction of bills in Congress in 1965 to regulate the trade in dogs for research, and the subsequent passage of the AWA 1966. The act has since been amended and broadened (most recently in 1993).

The Animal Rights Movement: The 1970s and 1980s saw a marked increase in the number and activity of rights organizations--many of which advocate complete and equal "animal rights" to the classic rights of humans. Some of the more militant, "underground" animal rights groups have used terrorist actions against research facilities and biomedical researchers. Illegal activities have included vandalism of animal facilities and research laboratories/records (Oregon, Texas, others), stealing research animals (Washington, Michigan, others), arson (California, others), threats against researchers and their families (Pennsylvania, others), and in one case, attempted murder of the president of a large biotechnological firm (United States Surgical Corporation). To date, these militants have caused property damage totaling millions of dollars. The number of persons, responsible for these crimes who have been arrested and prosecuted has been very few.

Playing on the emotional appeal of the "animal abuse" message, the unceasing activity of animal rights groups has brought them increasing membership. They have involved the judicial system in several "animal rights" cases and have introduced many pieces of legislation at both the states and federal level. Some of this legislation ha passed and has had the consequence --direct or indirect-- of restricting how everyone performs research using animals at both the state and federal levels.

Only recently has the scientific community begun to understand the consequences of the animal rights movement. Only recently have scientist been bold enough to present its side of the issue to the public.

Ethical Principles of Animal Use: The general ethical principles of humane use of animals in research were formulated by Marshall Hall in 1831. These principals are as useful in evaluating experimental procedures today as they were then:

* Principle I: "We should never have recourse to experiment, in cases in which observation can afford us the information required."

* Principle II: "No experiment should be performed without the persuasion, after the maturest consideration, that the object will be attained by that experiment, in the form of a real and uncomplicated result."

* Principle III: "We should not needlessly repeat experiments which have already been performed by physiologist of reputation. If a doubt respecting their accuracy, or the accuracy of the deductions drawn from them, arise, it then, indeed, becomes highly important that they should be corrected or confirmed by

repetition. This principle implies the necessity of a due knowledge of what has been done by preceding physiologists."

* Principle IV: "That a given experiment should be instituted with the least possible infliction of suffering."

* Principle V: "That every physiological experiment should be performed under such circumstances as will secure a due observation and attestation of its results, and so obviate, as much as possible, the necessity for its repetition."

Hall concludes his arguments by stating: "In order fully to accomplish these objects, it would be desirable to form a society for physiological research. Each member should engage to assist the others. It should be competent to any member to propose a series of experiments, its modes, its objects. These should be first fully discussed -- purged from all sources of complication, prejudice, or error -- or rejected. If it be determined that such series of experiments be neither unnecessary nor useless...they should then be performed, repeated if necessary, and duly attested. Lastly, such experiments, with the deductions which may flow from them, may then be published with the inestimable advantage of authenticity ... Pursued in this manner, the science of physiology will be rescued from the charges of uncertainty and cruelty, and will be regarded by all men, at once as an important and essential branch of knowledge and scientific research."

Ethics and Humane Considerations: Questions concerning the ethics of animal use in research and teaching have been debated by scientists, theologians, philosophers and the lay public since the use of animals for these purposes began. Even when consideration is restricted to recent discussions of the issue, there are almost as many ethical positions as there are writers on the subject. The prevailing view is that:

* Animals can and should be used in research, which benefits humans, animals, and the ecosystem, and

* That there is no acceptable full and complete alternative to such use. Implicit in this view is the expectation that research animals will be treated humanely.

Extreme views are held by small minorities. On the one hand are those who believe that humans have no responsibility to other animals and, therefore, any use of animals is permissible. On the other hand are those who believe that all animals, human and non-human, have the same rights and, therefore, humans have no right to use animals for any purpose. There are many variants of each of these views and, even among those who hold that animals have legal rights, there is disagreement about whether all animals should be accorded the same moral or legal status.

In our society, laws and regulations at many levels require the humane treatment of animals used in research and teaching. Essential elements of humane treatment include that animals be housed in clean, comfortable quarters, that they be fed an adequate diet, and that they be maintained in good health. There is no general agreement as to what additional factors might be necessary for humane treatment. Most conscientious researchers and the agencies which regulate animal care accept that an animal's well-being is dependent on its mental state as well as its physical state. It is also recognized, however, that it is much more difficult to establish objective guidelines for the assessment of the psychological well-being of an animal than it is to monitor physical well-being. Some of the factors involved in assessing an animal's well-being are discussed in the sections which follow.

* Subjective Experience in Animals: Early views on the capacity of animals to experience pain and other sensations were often predicated more on philosophical positions than on scientific observation. Followers of the mind-body dualism of Descartes denied the existence of mental states in non-human organisms. The Romantic tradition of the 19th century attributed elaborate anthropomorphic thoughts, feelings and intentions to animals. Behaviorists of the early 20th century side-stepped the issue: because psychological states were private, they could not be characterized. Recent evidence regarding subjective experience in animals comes

from neurophysiological studies and from ethological (behavioral) studies. Physiological evidence indicates that animals which possess a central nervous system, or which show evidence of receptors for endogenous opiates, have the potential, at least, to experience pain. Behavioral evidence of pain in many higher vertebrates is similar to its manifestations in humans, including screaming, squealing and struggling, for example. Behavioral evidence of pain in species more remotely related to humans (e.g., fish), and of less obvious forms of distress like fear, frustration, exhaustion and anxiety in all nonhuman animals is harder to identify.

* Captivity and Suffering: Some opponents to the use of animals in research have suggested that captivity alone causes suffering for animals. They argue that distress is indicated whenever an animal shows behavior that deviates from the behavior exhibited by wild conspecifics. This concept of suffering is based on unfounded assumptions about the relationship between behavior of wild and captive animals. Behavior of wild and captive or domestic members of the same species may differ for a number of reasons. For example, many species commonly used in research have been subject to many years (in some cases, thousands of years) of artificial selection by humans. The genetics and the normal behavior of these animals may now be very different from those of their wild progenitors. In addition, many ethological studies, such as the pioneering work of Konrad Lorenz, have shown the importance of early experience on late behavior. Animals of wild parentage, which are born and raised under captive conditions, may behave differently than wild born and raised conspecifics. Such genetic and environmentally determined behavioral differences do not automatically indicate suffering. Is it legitimate to classify all behavioral differences between wild and captive animals as negative ones? Many would argue no. Animals in captivity are free from the need to watch for and to escape from predators. It is difficult to interpret this alteration in the animal's environment as a negative one, or to conclude that animals suffer by not having to avoid predators. It has been argued that safety alone does not constitute psychological well-being, especially for normally social animals housed alone. This is an important consideration. Knowledge of the behavior of wild animals may be useful in designing research environments which promote psychological well-being. It is often assumed that wild animals live in a kind of natural paradise and that it is only the appearance and intervention of human agencies that bring about suffering. This essentially Rousseauian view is at odds with the wealth of information derived from field studies of animal populations. Scarcity of food and water, predation, disease and intra-species aggression are some of the factors which have been identified as normal parts of a wild environment and which cause intense suffering in wild animals on a regular basis.

Recognizing Signs of Distress in Lab Animals: As is the case with pain, physiological parameters and behavioral responses provide important cues to distress in animals, although distress is harder to define and identify than pain. Physiological parameters include hormonal responses (changes in the levels of adrenal hormones, for example), increased susceptibility to disease (which may indicate an impaired immune system), or weight changes. Any unusual behavior in an animal which shows physiological signs of stress may be such a cue. Some behavioral changes, however, may be normal adaptive responses which help the animal cope with a new environment or moderate stress, so behavioral changes should not automatically be considered pathological. Behavioral changes which occur in the absence of physiological signs of stress may also indicate suffering. For example, conflict behavior, in which an animal exhibits conflicting tendencies to perform different or incompatible behaviors, may indicate fear or frustration. Conflict behavior can, however, result from positive stimuli also, such as conflict between the desires to eat and mate. Finally, the appearance of abnormal or stereotypic behavior, such as the pacing of big cats in zoo cages or the self-mutilation of monkeys housed in restricted environments, can signal distress. Even clearly abnormal behaviors may help animals cope with the conditions of captivity, however. For instance, chimpanzees which throw feces at zoo visitors may be enhancing the psychological condition of their captivity; thus, this might be considered an adaptive behavior.

Selecting an Animal Model for Research: The use of animals in research and instruction generally occurs in one of two contexts:

1. The animals serve as model systems for the investigation of phenomena and processes which cannot be studied directly, or

2. The animals are being studied to investigate a problem specific to the particular species.

Most biomedical research falls into the former category. Examples of the latter include field studies of behavioral and ecological adaptations of animal species, studies of taxonomic relationships among species, or captive studies of physiological or behavioral processes which form an important part of the adaptations of one or more species.

Ethical and humane considerations should be viewed as compatible with good scientific practice. There is a body of literature that supports the premise that animals which are humanely cared for are healthier (both physically and psychologically) and, therefore, make better, more predictable subjects. Similarly, unless a research project is intended to study pain itself, pain on the part of the animal subject will not contribute anything positive to the experimental procedure. Thus, ethics and humane considerations can be viewed as integral parts of the process of experimental design and model selection.

Selection of an appropriate model must be based on extensive familiarity with the problem or system to be studied, so that the researcher can determine the range of biological responses necessary to the experimental design. Once this familiarity is developed, either by extensive review of the published literature or from pilot studies, the researcher can proceed to select an appropriate model--whether a live animal, an animal--derived tissue/cell, or a inanimate model.

Live animal models are usually chosen when the system being studied can best (or only) be understood in the context of its interactions with other systems in the organism (e.g., sexual differentiation in embryonic development) or when it is the organism, as a whole, which is to be studied (e.g., the ontogeny of aggressive behavior). Some systems are better studied in isolation using animal cells, tissue or organs. For example, a number of biochemical and cellular processes can be studied in tissue or organ culture derived from animal material. For other kinds of studies, biostatistical or computer models may be appropriate. It should be obvious, however, that the data generated from such non-animal models are only as good as the data upon which the models are based; thus, animal studies of some kind are a prerequisite for developing and verifying all models.

Factors Affecting Choice of Species: An animal model is a living organism in which normal biological processes can be studied, or in which a spontaneous or induced pathological process can be investigated. To be effective, the process being modeled should closely resemble the analogous process in human beings or some other species in one or more ways. Some important criteria of animal models are:

1. Relevance to the problem being studied;

2. Accuracy with which the model reflects all or some important aspects of the problem,

3. Model reproducibility, and

4. Model availability to researchers, and

5. General species characteristics such as life history parameters, behavior and diet.

* Life history parameters: Aspects of life history patterns (which may influence species choice) include developmental rate, age of puberty, frequency and timing of reproduction, gestation length, physical size and life-span. These intrinsic parameters can be important. For example, species with relatively short life spans are sometimes the most useful choice for models of the long term effects of early ontogenetic factors. Life history parameters may also combine with other species' characteristics to influence appropriateness of model

choice: for example, litter size is an important consideration when studying a naturally-occurring genetic condition since a large litter will increase the likelihood that some offspring will carry the trait.

* Behavior: The normal behavior patterns of a species can be important to model choice whether or not the researcher is interested in the animal's behavior, per se. The normal social organization of a species affects such variables as how animals must be housed or fed and under what circumstances they will reproduce successfully. Social and individual behavioral characteristics may influence research variables in both obvious and subtle ways. An example of an obvious behavioral constraint is that, with many species, placing more than one adult in a social group under the relatively confined conditions of captivity will result in serious aggression that may disrupt research. A more subtle, and in this case positive, effect of social variables is the finding that female macaques with irregular menstrual cycles can be induced to cycle normally and thereby improve their breeding performance when housed with conspecific females with regular cycles. Even more subtle effects have been observed. For example, as a result of behavioral sex differences in mice, females, but not males, show enhanced immune response when housed in groups. Such behavioral effects may introduce uncontrolled variables into experiments if not anticipated by the researcher.

* Diet: Knowledge of the normal nutrient requirements of a species is an important factor in selection of an appropriate model. Potential effects of diet on experimental variables can be readily evaluated for common laboratory species whose nutrient requirements are well studied, but this may not be possible for rarely-used or little-studied species whose normal diet may be unknown. Use of poorly nourished animal subjects which lack some critical dietary component may introduce extraneous, uncontrolled variability into experiments.

* Genetics: Genetic factors are important in model selection in several respects. The species selected should have a well-known background, since some species may develop naturally-occurring genetic diseases or conditions which can provide useful spontaneous models. In research areas where no spontaneous animal model has been identified, extensive knowledge of a species' genetic properties is essential for the selection of a likely candidate for an induced model. In some common laboratory species, mutant or inbred strains have been developed with well-documented and often highly specific genetic properties. Catalogues and bibliographies of such models provide a useful source of information for researchers.

Alternatives to Using Animals: Regulations developed to implement the AWA, require the research facility to provide assurances that principal investigators have considered alternative techniques to painful procedures and provide guidance concerning research and testing methods that limit the use of animals or minimize the animals' distress. This section introduces the concept of alternatives with a brief discussion of each major category including a limited number of examples.

In recent years the term alternative techniques has come into common use. It is a term that has different meanings to different people. Russel and Birch, in a landmark treatise titled Principles of Humane Technique provided the definition of alternatives to include those commonly know 3 R's of Research. Rowan, writing in the text: Of Mice, Models & Men, further defines alternatives as those techniques or methods that "replace the use of laboratory animals altogether, reduce the numbers of animals required, or refine an existing procedure or technique so as to minimize the level of stress endured by the animals." In 1993, Banks adds to the 3R's of research with a fourth R known as responsibility by arguing that the biomedical investigators must acknowledge responsibility to institutional integrity, to public trust, and to animal participation. This means providing the best in humane care for the aniamls under study. The concept of alternatives now includes REPLACING the actual use of animals, REDUCING the numbers of aniamls used, REFINING the techniques to minimize the potential for the animal pain or distress, and RESPONSIBILITY to the community at large.

Replace:

* Replace by in vitro techniques: The most commonly recognized non- animal living systems are those which

fall into the broad category of in vitro methods such as organ, tissue and cell culture. The most commonly used of the in vitro methods are cell culture techniques for monoclonal antibody production, virus vaccine production, vaccine potency testing, screening for the cytopathic effects of various compounds and studying the function and make-up of cell membranes. The potential uses of in vitro techniques are almost limitless and will continue to expand as more is learned about the various organs and their component tissues and cells, and as the technology of maintaining in vitro environments improves.

* Replace vertebrates with invertebrates: Invertebrates are another type of living system which can be used to replace the more commonly used laboratory animals. Over 90 percent of the animal species thus far identified are invertebrates. An invertebrate which has long been used in biomedical research is the fruit fly, Drosophila melanogaster -- a classic model for the study of genetics. This species also can be used for detecting mutagenicity, teratogenicity and reproductive toxicity. In neurobiology a number of different marine species have been well-characterized and used to study the physiology of the nervous system.

* Replace by using micro-organisms: Micro-organisms represent a third living system which has been used to replace traditional animal models. The Ames test for mutagenicity / carcinogenicity uses Salmonella-typhimurium cultures to screen compounds that formerly required the use of animals.

* Replace by using plants: Plants offer another alternative living system. There is very little morphological and functional difference between the organelles isolated from plants and those isolated from animals. The rigid cell wall of plants, however, limits their applicability for use as undisrupted cells.

* Replace by using nonliving systems: The most widely used non-living model system involves the use of modern chemical techniques. This is particularly true of the analytical techniques which can be used to identify substances and to determine their concentration or potency. Immunochemical techniques use the binding capacity of highly specific antibodies to seek out minute quantities of antigen. A classical example of this technique can be demonstrated by the currently used techniques for identifying bacterial toxins. Toxin identification previously required the injection of as many as several hundred mice with supernatant from cultures of suspected contaminating bacteria. These new antibody techniques save animals and hasten confirmation of a tentative diagnosis. By adding a color marker to the Enzyme Linked Immunosorbent Assay system (ELISA), the whole process becomes a commercially-available test kit such as those used in home pregnancy detection. A test that previously required the use of a rabbit now can be performed using an over-the-counter test kit. The use of physical and/or mechanical systems to replace living animals of even the highest order has application in teaching specific skills and/or reactions to a well-defined set of predetermined circumstances. The use of computer-linked mannequins in teaching basic principles of medicine and applied techniques can be best illustrated by the mannequins used to train people in cardiopulmonary resuscitation.

* Replace by using computer simulation: The standout in the alternative techniques controversy is the claim made for computer simulation as a means of virtually replacing the use of living animals. In order for a biological phenomenon to be adapted to a computer model, the basic processes must be expressed in a mathematical formula. Once a formula is developed, then an enormous number of variables can be introduced and swiftly processed. The key element for success is the generation of a program from the mathematical formula. A more complete formula makes for a more useful program. The problem is that many of the questions being asked of an animal model are not defined well enough to develop the necessary mathematical formula. As the core knowledge of the biological processes expands, so will the opportunities to use computer simulation to replace the number of live animals being used.

* Reduce by animal sharing: Sharing of animals can significantly reduce the number of animals used. Sharing can be as simple as allowing someone to practice a surgical approach on an animal that has been or is to be euthanitized for other purposes, or providing organs or tissues at the time of necropsy. Sharing becomes more complicated when attempting to maximize the use of control animals, but it can significantly reduce the number used. If two studies involve the need to perform a sham operation, the administration of compounds

by identical routes, the use of standard control diets or the need to condition animals to a particular environment, control animals could be shared within the institution.

* Reduce by improving statistical design: Improper design of experimental protocols and / or the failure to use appropriate statistical methods can result in the use of an inappropriate number of experimental animals. A variety of design strategies are available which can reduce the number of animals needed in a given study. Experimental protocols which utilize serial sacrifice, group sequential testing and crossover designs can significantly reduce the numbers of animals required.

* Reduce by phylogenetic reduction: Projects which can be designed to use one of the myriad of invertebrate species instead of a non-human primate species represent a type of phylogenetic reduction which was discussed as a replacement technique. Such broad jumps across the phylogenetic scale are not always possible, but less dramatic shifts can significantly reduce the numbers of higher species being used in research, teaching and testing. In many instances, the theory of phylogenetic reduction has been blurred by a species' use as a companion animal with little regard for phylogenetic ranking. The animals chosen for project usage should be the least advanced (from a phylogenetic standpoint) that will provide the necessary data.

* Reduce by obtaining better quality Animals: When purchasing laboratory animals, it is important to keep in mind that cost and quality are usually directly correlated. By choosing the best quality animal in terms of health status, the possibility that animals will be lost or data compromised by the intrusion of a concurrent disease condition is minimized. Choosing the best quality of animal, in terms of genetic status, will help maintain the consistency of animals from study to study. This requires an institutional commitment to the use of animals of defined health status and limits the investigators to the animal sources approved by the institution.

* Refine by decreasing invasiveness: A hallmark of most of the new diagnostic and therapeutic techniques used in human medicine is the minimal degree of invasiveness necessary to perform successfully a procedure to obtain a given set of data. In many instances, these techniques are applicable in the research environment and can be adopted for use in animals. A sophisticated example is the use of Magnetic Resonance Imaging for results that formerly required euthanasia of multiple animals along a time curve to obtain assay tissue. Today one animal can provide all the information along a given curve. A less dramatic example is the vascular access device which permits repeated samples or injections in a single animal instead of using several animals.

* Refine by improved instrumentation: In this age of microelectronics, fiber optics and laser instrumentation, the potential for refining techniques used in animal experimentation seems almost limitless. Included in this category are the use of tethers in a variety of species to allow continuous access to the various organ systems, while permitting the animal virtually unrestricted movement within its primary enclosure.

* Refine by improving control of pain: The Animal Welfare Act requires "that the principal investigator consider alternatives to any procedure likely to produce pain or distress in an experimental animal" and, in any practice which could cause pain to animals, that a doctor of veterinary medicine is consulted in the planning of such procedures.

* Refine by improving control of techniques: Proficiency in the handling and restraint of animals makes it easier to perform a variety of routine procedures with minimal or no pain or distress to the animals involved. Animals are creatures of habit and when proper handling is part of their regular routine, the degree of distress caused by the procedures is minimized. Animals can be trained or conditioned to accept a variety of procedures which, if suddenly forced upon them, can be distressful. Almost every animal commonly used in the laboratory responds positively to careful, gentle handling.

* Refine by assuring appropriate numbers for statistical validity: It is important that principal investigators use the appropriate number of animals in their experiments. The use of too many animals results in wasting of

animal resources and unnecessary expense. The use of too few animals may result in inconclusive results. Principal investigators are encouraged to consult with biostatisticians in the planning of their experiments.

Responsibility (4th R) to the animal (proper care), to scientific integrity (using neither too many nor too few aniamls to properly answer the question), and to the public (assuring competent and qualified animal care professionals and caring investigators effectively and efficiently use aniamls for the public good.