SECTION

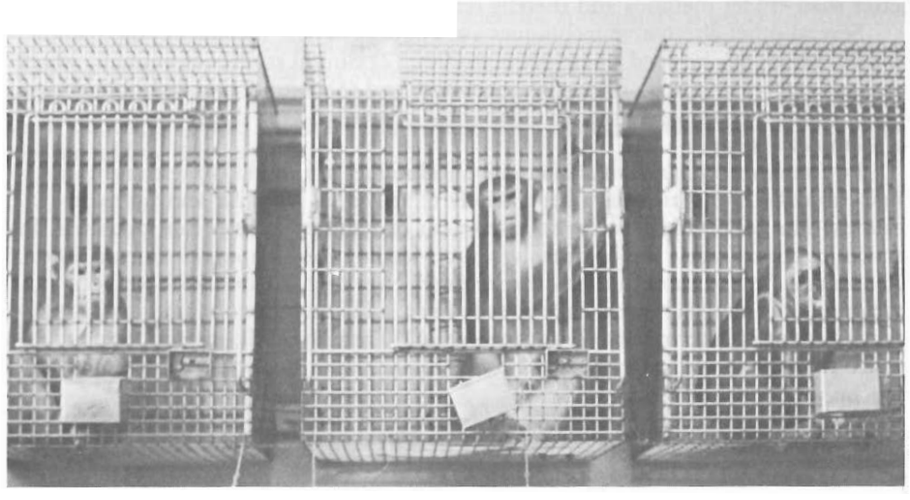
I

Overview

ANIMALS ARE USED extensively in laboratory procedures, especially in biomedical research, toxicity testing, and education. Estimates of current usage range from about twenty million to seventy million animals per year in the United States alone.[1,2] Many of these animals suffer severely. Some are deliberately sickened, injured, or killed. Others suffer from neglect, ignorance, indifference, or outright cruelty.

No one wants to see animals suffer, regardless of one's opinion of the ethics of animal research. For that reason alone, alternative methods should be developed to *replace* the use of animals in laboratory procedures, to *reduce* animal use, or to *refine* procedures so that pain or suffering is reduced. Replacement, reduction, and refinement constitute the three Rs of the "alternatives approach" to laboratory practices. The ultimate goal of this approach is the complete replacement of laboratory animals with non-animal methods.

1. Typical housing of monkeys used in research



The enormous toll in animal suffering is only one reason why the scientific and lay communities should make every effort to explore research alternatives. Others are the high cost and long duration of animal studies; potential inaccuracies in extrapolating from animals to humans; the questionable value of animal-based toxicity tests; and limitations on what can be learned from conventional animal studies.[3] Scientists are recognizing that alternatives can be more effective and practical than animal studies.

Five major types of alternatives have been developed:

(1) *Human studies* include clinical, epidemiological, and postmortem investigations. For example, most substances known to cause cancer in humans have been identified by epidemiological studies, not animal tests.

(2) *In vitro techniques* are used to study tissues, cells, or cellular components in the controlled environment of laboratory containers. Living samples can be taken from humans or animals. Even though animals may be used, one animal usually provides enough tissue for numerous samples or the tissue can be propagated indefinitely, serving study after study. *In vitro* techniques have been used in research on AIDS (Acquired Immune Deficiency Syndrome) to isolate, identify, and concentrate the AIDS virus and are now being used to screen drugs rapidly for anti-AIDS virus activity.

(3) *Mathematical models* describe a biological system under study in mathematical terms in order to predict novel features of that system. Existing information about the system is used to design the model and make predictions. For example, a model has been designed as a potential replacement for the animal-based LD50 test, which estimates the dose of a substance needed to kill fifty percent of the test animals. The model is designed to predict the lethal dose of untested chemicals by comparing them to tested chemicals on the basis of chemical structure and properties. Modeling can also identify the most fruitful avenue to pursue in an ongoing study and thereby preclude fruitless experimentation.

(4) *Less sentient organisms* are used on the premise that some organisms have less capacity for pain and suffering than do others. In general, invertebrates, microorganisms, and plants are less sentient than vertebrates, and vertebrate embryos are less sentient than the adults. The Ames test uses bacteria instead of animals to detect cancer-causing chemicals.

(5) *Physical/chemical techniques* exploit instruments and chemical procedures, not animals, to analyze the physical and chemical properties of drugs, body chemicals, and other compounds. For example, diagnostic kits made of simple materials and chemicals have replaced the use of rabbits in diagnosing pregnancy. Physical/chemical techniques can also reduce animal use if they perform their tasks better than cruder methods and thereby require fewer animals per test.

Several other alternative techniques are available. These include (1) mechanical models, which can be used in car-crash studies; (2) clinical studies of animals, which can have carry-over effects in human medicine; and (3) computer-aided drug design, which avoids the animal-based trial-and-error process of drug discovery so prominent today.

Two noteworthy targets for alternative techniques are the LD50 test and the Draize test, both of which have been widely criticized on scientific and humane grounds. The LD50 test provides an assessment of a compound's poison potential. In its most common form, the test involves force-feeding the compound to from 40 to 200 animals. Several modifications that require fewer than 20 animals have been developed and, in some cases, have already been substituted for the traditional test. Several promising alternatives do not involve LD50 testing at all; instead, they involve techniques such as *in vitro* methods, mathematical modeling, and use of less sentient species.

The Draize test assesses a chemical's potential to damage the eye. Recently developed modifications have the potential to refine the test (which now is performed on rabbits) by providing anesthetics or to reduce the number of

animals used per test from 6 to 18 to *fewer* than 6. Several substitute tests are being developed using *in vitro* techniques and less sentient organisms (e.g., chicken embryos).

Despite these promising efforts, the traditional forms of the Draize and LD50 tests stay in use, partly as a defense by industry against product-liability claims, partly the result of regulators' bureaucratic inertia, partly through fear of consumer backlash, and partly because of inconsistencies among international guidelines.

Alternatives can play a major role in education. Here are a few examples of how they can be put to use:

(1) The British system for training surgeons can replace the American system of practicing surgery on healthy animals. The former is an apprenticeship that stresses clinical experience with humans (for medical students) or animals (for veterinary students). In microsurgery, whose fine details make apprenticeship difficult, human placentas may soon replace animals in training specialists.

(2) Computer-assisted mannequins that simulate the workings of the human or animal body can demonstrate medical procedures, normal physiology, and drug effects.

(3) Computer programs can simulate surgical procedures, drug effects, and metabolic functions.

(4) Human cadavers can be used in virtually all aspects of medical training.

Progress in developing alternatives in all areas of laboratory animal use has been encouraging, especially given the limited financial investment that has, so far, been forthcoming. Much of this progress has come within the last ten years, as public concern for animals has provided greater incentive to develop alternatives. Such public concern can influence laboratory practices and benefit not only animals, but also scientific progress and public health.

Scientific innovations are making the direct study of humans (as opposed to the study of "animal models" of humans) increasingly practical and rewarding. Conventional clinical studies are being supplemented with *in vitro* studies of human tissues or modeling studies using human data. Sophisticated new imaging techniques, which can generate visual images of the body's interior without the need for invasive procedures, are being used to study the human brain in action harmlessly. In all of this research, the direct study of humans obviates the need to draw conclusions about humans from potentially misleading animal studies.

In toxicity testing, recent emphasis on alternative approaches is generating a reevaluation of routine animal tests that, in some cases, are decades old. Testing is being brought out of the Dark Ages.

Unfortunately, despite clear evidence of the importance of alternative methods in the history of biomedical research, the scientific community is generally lukewarm to the alternatives approach. If the general public became more aware of alternatives and the promise they hold *and* communicated that awareness to their legislators (who control most of the research funding), the scientific community would have to take alternatives more seriously. Our hope is that, through efforts such as this guide, we can add to that awareness.

SECTION

II

A Closer Look At Replacements, Reductions, And Refinements

THE ULTIMATE GOAL of the search for alternatives is the complete replacement of animals in all laboratory procedures. While some procedures have been replaced completely, replacement in all of biomedical research, testing, and education will take many years. Reductions and refinements can be viewed as interim steps toward the achievement of complete replacement. The practice of seeking replacements, reductions, and refinements to animal experimentation is the "alternatives approach." While some animal advocates include only replacements in this approach, such a view is unnecessarily restrictive.

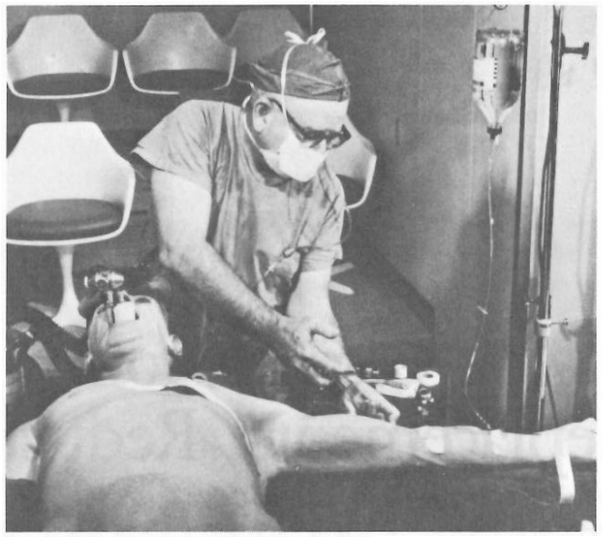
Let us look at the concepts of replacement, reduction, and refinement as they can be applied in biomedical research, toxicity testing, and education.

Replacement

In biomedical research, an alternative technique known as tissue culture has replaced, to a great extent, the use of animals in research on viruses, which cause a variety of diseases. Tissue culture involves maintaining samples of living cells or tissue from the body in laboratory containers. Studying viruses in tissue culture enables researchers to concentrate the virus and to screen drugs for anti-viral activity. Tissue-culture research on AIDS has isolated, identified, and concentrated the AIDS virus. This technique is now being used to screen drugs quickly for anti-AIDS virus activity. The results of some of these tests have been promising.[1]

In toxicity testing, tissue culture may replace the use of animals in detecting chemicals that cause photosensitivity reactions in humans. These chemicals cause inflammation or tissue damage when ingested or applied to the skin, but only in the presence of light. Existing tests for photosensitizing activity, including experiments on animals and human volunteers, are inadequate. A recently developed alternative uses a certain type of human blood cell. When these cells are incubated with photosensitizing chemicals in the presence of light, their genetic activity is slowed. The test monitors this activity. The test is quick, inexpensive, and reliable, and yields results comparable to those of ***in vivo*** tests.[2]

In education, computer-assisted mannequins are replacing some uses of animals in medical training. A mannequin known as "Sim" is used to train anesthesiologists at the University of Southern California Medical Center. Sim responds appropriately to various procedures, such as intramuscular injections of certain drugs. Students can draw blood, pass a catheter, practice intubation, and administer treatment for shock.



American Anti-Vivisection Society

II. Sim, a computerized mannequin that can replace animals as subjects on which to practice medical procedures

In tests comparing students who practiced on the first prototype (Sim One) to a group who trained on animals, the Sim One students consistently needed fewer tries and less time to master intubation techniques. A new version of the mannequin is now being mass-produced.

Reduction

Reduction alternatives are based on the premise that the number of animals used in a procedure should be the minimum necessary to achieve the desired goal. This premise can be applied to the design of experiments, as certain designs are more economical in their use of animals than are others.[3] Once a design has been chosen, one needs to determine judiciously the number of animals to be used in experimental and control groups. This planning can be aided by certain statistical methods.

In biomedical research, the potential for reduction in the number of animals used was illustrated in an unpublished analysis commissioned by The Humane Society of the United States. In a random sample of published research reports, in nearly all cases, animal use could have been reduced from twenty-five percent to more than seventy percent simply by a better choice of experimental design and statistical tests. The quality of the results or their statistical validity would not have been compromised.

In toxicity testing, the principle of reduction has been applied to the LD50 test, used to estimate the lethal dose of a substance. This test traditionally involves from 40 to 200 animals. A modification of this test uses 6 to 10 animals to determine the approximate lethal dose (ALD). In the ALD test, each animal is given a dose fifty percent higher than the one given to the previous animal. This procedure continues until a lethal (or fatal) dose is reached. The ALD test produces results that correlate well with the traditional LD50, yet it uses far fewer animals.[4]

In education, the number of animals used in high school and college dissections can easily be reduced. Instead of each student dissecting a different animal, students can view a few professionally dissected animals. These prepared specimens, known as prosections, illustrate anatomy with a clarity that few student-prepared specimens can match. Specially preserved and encased prosections can be used year after year, sparing additional animal sacrifice.

Refinement

Any modification in the care and use of laboratory animals is a refinement if it reduces pain and suffering without compromising the outcome of the intended laboratory project. Refinements can be beneficial from a scientific as well as humane view because pain and suffering lead to behavioral, physiological, and anatomical abnormalities that can distort the outcome of experiments.[5,6]

Refinements can yield the most immediate changes in the area of alternatives. Living quarters can be made more comfortable, diet improved, and anesthetics and analgesics administered more widely. The application of refinements is limited largely by the imagination and motivation of experimenters and their technicians.

In biomedical research, some procedures used for the long-term restraint of animals are being refined. Traditionally, scientists have immobilized animals for weeks or months at a time to prevent them from pulling tubes from their bodies, to facilitate the administration of frequently injected drugs, or for a variety of other reasons. Restraining devices such as primate restraint chairs are being replaced by various systems that allow freedom of movement, such as harnesses, tethers, and biotelemetry systems (devices worn externally or implanted internally that transmit information from the animal to a remote sensing device).

Several refinements have been proposed for toxicity tests. In Draize testing, chemicals are instilled in the eyes of rabbits to assess their irritancy potential. Administering anesthetics and analgesics to relieve pain and antihistamines to reduce the degree of eye damage would provide some relief to test animals. There has been concern that these substances could distort test results or even be counterproductive if anesthetics increased injury by inhibiting defensive responses such as blinking[7] or if antihistamines prolonged recovery from injury.[8] However, some anesthetics do not have these drawbacks.[9]

In education, the concept of refinement can be readily applied to laboratory exercises in rat behavior that are common in introductory psychology and behavioral biology courses. Some of these exercises demonstrate learning principles; some are opportunities for students to categorize and observe behavior; and others provide opportunities to practice using event recorders (devices that facilitate the recording of behavioral information). Several procedures commonly associated with these exercises are needlessly stressful.

For example, rats are highly social animals yet traditionally have been housed singly. They are punished in order to aid learning, when the use of reward would accomplish the same goal. They are deprived of food or water in order to enhance the performance of some tasks. They are nocturnal animals accustomed to an alternating day/night cycle, yet are often kept in perpetual daylight. If they *are* provided with a day/night light cycle, they are forced to perform during the daytime, when they would normally be inactive.

Refinements of these procedures are obvious. The animals should be housed socially and kept in an environment with an alternating day/night cycle. The cycle can be reversed so that the animals are active during the day, when classes are usually held. Ideally, the animals should be observed under red light, which facilitates student observations but does not distort nighttime activity. The rats should be coaxed to learn by reward, not punishment, and their performance enhanced through reward with favorite foods, not starvation.

Although the concepts of replacement, reduction, and refinement are distinct, they can be combined in the same alternative procedure. An example is the Limulus Amebocyte Lysate (LAL) test, used to determine whether or not therapeutic solutions will cause fever when administered intravenously to humans. The fever-producing substance, or "pyrogen," is a toxic segment of the contaminating bacteria's surface. The active ingredient in the LAL test is obtained from the blood of horseshoe crabs that are caught in oceans, handled, and then released. Certain collected red blood cells are burst to obtain a clear solution. This solution forms an easily recognized opaque gel when mixed with a pyrogenic fluid.

The LAL test was recently approved for use by the federal government and is beginning to replace the older Pyrogen test. The latter uses a minimum of three rabbits per test, each of which is administered experimental fluids that can be so damaging that the animals have to be killed after the test. The LAL test is over 100 times more sensitive than the Pyrogen test and is also more economical, convenient, and reliable.[10]

The LAL test is a *replacement* for the Pyrogen test in that laboratory animals are not required. The LAL test involves a *reduction* in that one horseshoe crab substitutes for several rabbits. It is a *refinement* in that the horseshoe crabs are only temporarily disturbed in the wild, while the rabbits live in small cages and are subjected to experimental procedures. The LAL test would be a refinement even if horseshoe crabs were subjected to the same treatment as rabbits, because horseshoe crabs, by virtue of a more primitive nervous system, probably are much less capable of experiencing pain than are rabbits.

SECTION

III

Promotion of the Alternatives Concept: History

THE EARLIER DEFINITIONS of replacement, reduction, and refinement are slight modifications of original definitions proposed by W.M.S. Russell and R.L. Burch in 1959.[1] Russell and Burch labeled these principles the three Rs of humane experimental technique. They also introduced the notions of fidelity and discrimination, which are important in assessing the relative merits of using animals and alternatives. In the context of biomedical research and testing, animals and alternatives are used as surrogates or models for humans. A surrogate is a high fidelity model to the extent that it resembles humans. In general, chimpanzees are high fidelity models; bacteria are low fidelity models.

Models can have low fidelity but nevertheless be more useful than high fidelity models in certain cases. This is because low fidelity models can be better discriminators of the response under study. For example, horseshoe crabs in the LAL test are replacing rabbits in the Pyrogen test. Horseshoe crabs happen to be better than rabbits in discriminating the human fever response, despite the fact that horseshoe crabs are lower fidelity models of humans.

A failure to consider a model's discrimination or sensitivity can lead to what Russell and Burch labeled the "high fidelity fallacy." This fallacy ignores discrimination by stating that, in general, models should have high fidelity. In practice, this fallacy leads to excessive use of mammals, given their relatively high fidelity to humans. This usage pattern can be seen in federally funded research projects, discussed later. The high fidelity fallacy must be successfully repudiated before alternatives will gain widespread acceptance.

Since publication of Russell and Burch's book in 1959, several developments in the promotion of the alternatives concept have occurred. Animal protectionists have established several organizations to finance the development of alternatives (Table I). Perhaps the most prominent of these has been FRAME, which recently began a coordinated effort to develop an alternative to the LD50 test. Other organizations are promoting alternatives by offering cash prizes.

Since the 1970s, scientific conferences have been addressing the alternatives concept, with mixed results.[2] In Canada, a gathering of toxicologists recommended that the government and organizations supporting toxicological research "initiate and fund research programs with the specific objective of developing and validating non-animal models for use in the safety-evaluation process."[3]

Government action on alternatives began in Europe. In 1971, the Council of

TABLE I

Some Sources of Financial Support for the Development of Alternatives

A. Organizations established to finance development of alternatives:

Organization	Location
American Fund for Alternatives to Animals in Research	United States
Dr. Hadwen Trust for Humane Research	United Kingdom
FRAME (Fund for the Replacement of Animals in Medical Experiments)	United Kingdom
Humane Research Trust	United Kingdom
Irish Anti–Vivisection Society Humane Research Fund	Ireland
Lawson Tait Trust	United Kingdom
Lord Dowding Fund	United Kingdom

B. Organizations offering monetary awards for alternatives development:

Organization	Area of Award
Doerenkamp & Zbinden Foundation for Realistic Animal Protection in Scientific Research	Education
European Federation of Pharmaceutical Industries Associations	Research or Testing
Millenium Guild	Testing
World Society for the Protection of Animals (Marchiz Animal Welfare Award)	Research, Testing, or Education

Europe called for the establishment of a documentation and information center for alternatives and a facility to store tissue material to use in alternatives research. Unfortunately, progress on this initiative has been slow.[4] However, Holland, Sweden, and West Germany have animal–research laws that favorably mention alternatives. Sweden has even earmarked a small amount of money for alternatives research.[5] Centers for alternatives research now exist in Switzerland, West Germany, Canada, and the United States. The centers are funded by industry, animal–protection groups, and/or governments.

In the United States, several legislative initiatives on alternatives have been advanced since 1980. The one that would have been the most far–reaching, if it had passed, is the Research Modernization Act. Introduced in Congress in 1980, it would have established a national center for alternatives research, redirected a certain percentage of funds originally earmarked for live animal research into alternatives research, and coordinated training programs in alternative methods.

A legislative breakthrough came in 1985 with passage of the National Institutes of Health (NIH) reauthorization bill, which contains provisions on alternatives. Sponsored by Representative Doug Walgren, these provisions call for NIH to establish a plan for research into replacements, reductions, and refinements. The plan must also include the development of such methods that have been found to be valid and reliable and the training of scientists in their use.

Similar provisions are contained in amendments to the Animal Welfare Act that became law in December of 1985.[6] The amendments were designated the "Improved Standards for Laboratory Animals Act" and formerly constituted the Dole/Brown bill, named for its sponsors, Senator Robert Dole and Representative George E. Brown, Jr. The amendments mandate training for researchers and technicians in alternative methods for research and testing. The amendments also call for the creation of an information service at the National Agricultural Library in cooperation with the National Library of Medicine. The service would provide information on alternative research methods, including refinements such as the increased use of anesthetics and analgesics, and methods to prevent unintended duplication of animal experiments.

Alternatives in toxicity testing have been promoted by public campaigns against the use of animals in the Draize test and the LD50 test. These campaigns are spearheaded by large coalitions of animal–protection groups.

The Draize test is used extensively by the cosmetics industry. The anti–Draize campaign singled out a major cosmetic company, Revlon, which, under pressure, donated $750,000 in 1980 to Rockefeller University to develop an alternative to the Draize test. The rest of the cosmetics industry, also under pressure, contributed one million dollars in 1981 to establish the Center for Alternatives to Animal Testing at the Johns Hopkins University School of Public Health. The industry, through its representative, the Cosmetic, Toiletry, and Fragrance Association, continues to support the center and similar efforts at eight other institutions.

The target of the other campaign, the LD50 test, provides a rough estimate of the toxicity of household products and other chemicals. Several federal agencies, including the Food and Drug Administration, the Consumer Product Safety Commission, and the Environmental Protection Agency, recently announced that they no longer require the test for regulatory purposes. These agencies, however, continue to accept LD50 results, and industry has been slow to discontinue the test in the absence of an outright ban. A bill introduced in the 1985 Congress would have required each federal agency actively to discourage use of the LD50 and to recommend alternatives. (The bill may be reconsidered in the future.)

Other noteworthy events in the history of the alternatives approach were the recent establishment of a fund for alternatives to animal use in research and teaching at Texas A & M's College of Veterinary Medicine and the establishment of the United States' first professorship in humane ethics and animal welfare in 1985 at the University of Pennsylvania School of Veterinary Medicine. One of the goals of the position will be to investigate alternatives to animal experimentation in medical research.[7]

SECTION

IV

Current Uses of Animals in Education, Toxicity Testing, And Biomedical Research

THE ALTERNATIVES APPROACH is a response to the use of animals in laboratory procedures. Consequently, any thorough analysis of alternatives should discuss the nature and scope of laboratory animal use. How many and what types of animals are used and for what purposes? Unfortunately, such information is scanty and conflicting. Even estimates of total laboratory animal use differ widely. Our ignorance on these matters is a reflection of how complacent policymakers are toward the use of animals in laboratories.

Andrew Rowan of the Tufts University School of Veterinary Medicine has discussed the shortcomings of existing estimates of laboratory animal use and derived his own estimate from a variety of sources.[1] His figures are adopted here. He estimated that seventy million animals are used per year in the United States alone.* Nearly ninety percent are mice, rats, and other rodents, and the remainder, in decreasing numerical importance, are birds, frogs, rabbits, dogs, ungulates, cats, and primates. Excluded from these figures are invertebrate animals (e.g., fruit flies, squid, and earthworms) because good estimates of numbers are lacking.

These millions of animals are used in three general activities: education, toxicity testing, and biomedical research.

Education

Animals are used in a variety of procedures in biological, medical, and veterinary education. Such procedures include destroying a frog's brain to test spinal reflexes (performed in high school); dissecting cats, dogs, minks, and fish to learn anatomy (performed in college); and practicing surgery on dogs obtained from pounds (performed in medical and veterinary schools). Some high school students

*Rowan recently subdivided his estimate of animal use to reflect the fact that not all of the animals that are bred for research or acquired by laboratories are actually used.[2] A certain percentage of these animals die or are killed because they do not meet research specifications (e.g., age, sex, weight, general health). Estimates of this figure range from a few percent of those acquired to almost fifty percent.[3] Rowan's estimate of the number of animals actually used is twenty-five to thirty-five million animals per year. His earlier estimate is retained here because it better reflects the toll that laboratory practices take on animals, regardless of whether the animals are actually used.

experiment on animals and display their results at science fairs. (For a comprehensive discussion of the use of animals in high school biology classes and science fairs, see Heather McGiffin and Nancy Brownley's *Animals in Education*.[4])

In the United States, an estimated 5.7 million animals are used every year in education.[5] A detailed breakdown of this figure is unavailable, owing primarily to the fact that educational and research uses of animals are often intermixed at the undergraduate and graduate levels.

Figures are available for medical and veterinary schools. In the nation's 127 medical schools, a total of 36,700 animals is used annually, with rats and dogs making up seventy-one percent of this total (Table II).[6] The majority of these animals are used in the teaching of surgery (fifty-one percent) and physiology (sixteen percent). Most of the dogs (sixty-four percent) are used in these two disciplines.

TABLE II

Estimated Animal Use in Medical Education in the United States, 1983–84[a]

Kind of Animal	Number Used	%
Rat	14,000	38.1
Dog	12,000	32.7
Mouse	3,000	8.2
Rabbit	1,700	4.6
Cat	800	2.2
Hamster	800	2.2
Pig	200	0.5
Primate	130	0.4
Guinea pig	70	0.2
Other[b]	4,000	10.9
TOTAL	**36,700**	**100.0**

[a]Estimate is based on an extrapolation of a survey of sixteen selected medical schools evenly distributed by geographic region (Northeast, Midwest, South, or West), ownership (public or private), and research expenditures (low, medium, or high).

[b]Includes frogs, sheep, and pigeons.

SOURCE: Office of Technology Assessment, *Alternatives to Animal Use in Research, Testing, and Education* (Washington, D.C.: OTA, 1986).

Most of the surveyed medical schools expressed regret over not being able to use animals to a greater extent in education, often citing cost as a limiting factor.

Table III shows comparable data on the nation's veterinary schools.[7] The census includes only those animals that began an exercise alive and either died or were killed during the course of the exercise. The census excludes animals purchased as cadavers (presumably because the carcasses were by-products of other industries); those that were clinical patients; and those killed at the schools prior to laboratory exercises. The latter exclusion is regrettable because, as a result, the numbers in Table III underestimate the adverse impact of veterinary schools on live animals.

TABLE III

Estimated Animal Use in Veterinary Education in the United States, 1983–84[a]

Kind of Animal	Number Used	%
Dog	8,020	48.2
Mouse	2,180	13.1
Rat	2,083	12.5
Bird	1,323	7.9
Reptile	433	2.6
Sheep	423	2.5
Cat	414	2.5
Horse	378	2.3
Rabbit	195	1.2
Goat	194	1.2
Pig	140	0.8
Guinea pig	112	0.7
Cow	111	0.7
Other[b]	649	3.5
TOTAL	**16,655**	**100.1**

[a]This census of all U.S. veterinary schools does not include privately owned or pet animals used for clinical demonstrations, animals purchased as cadavers, or those subjected to euthanasia prior to the laboratory exercise. It includes only those animals that began the course alive and then either died or were subjected to euthanasia during the course of the laboratory session.

[b]Includes fish, frogs, hamsters, and exotic species.

SOURCE: Office of Technology Assessment, *Alternatives*.

In the academic year 1983–84, 16,655 animals were used in veterinary education. Dogs account for almost half, and mice, rats, and birds constitute most of the remaining animals.

Education provides fertile ground for the application of alternatives because many educational projects are repetitive exercises whose outcomes are known in advance. They are unrefined in that the students who conduct them have little knowledge of surgery, anesthesia, experimental design, or the animals' needs. Educational uses of animals seem especially ripe for replacements that simulate exercises with the use of films, videotapes, computers, or mannequins.

Toxicity Testing

Toxicity tests are attempts to determine whether chemicals are safe for human use and the limits under which hazardous chemicals can be used safely. Tests are conducted on a variety of chemical and biological substances, including drugs, vaccines, food additives, cosmetics, household cleaners, pesticides, and industrial chemicals. Test substances may be force-fed, inhaled, or applied to the skin or eyes. Routine testing of new drugs examines the general effects of a single dose (acute toxicity) or repeated doses (chronic toxicity), or specific effects, such as induction of cancer (carcinogenicity), genetic damage (mutagenicity), and congenital malformations (teratogenicity).

Toxicity testing is an enormous enterprise in the United States. It involves an estimated fourteen million animals per year.[8] The cost of testing a single substance varies from about twenty thousand dollars (using a limited range of tests) to over one million dollars for a comprehensive evaluation.[9]

In the United States, the federal government plays a major role in toxicity testing, both through laws that require or encourage testing and through guidelines that influence testing procedures.[10] The Food and Drug Administration (FDA) oversees most testing. Other federal agencies that require animal testing, either explicitly or implicitly, include the Consumer Product Safety Commission, the Department of Transportation, and the Environmental Protection Agency.[11]

Product testing is sometimes required for pre-market approval; more often, it is simply implied by requirements for safe and effective products. Federal statutes explicitly require animal testing in only a handful of instances, such as the Federal Hazardous Substances Act (administered by the Consumer Product Safety Commission) and the Hazardous Materials Transportation Act (administered by the Department of Transportation).[12]

The status quo of toxicity testing is woefully inadequate. Animal tests are too costly and time-consuming to protect the public adequately from hazardous substances. A basic toxicity study may cost half a million dollars and take two years to conduct. Consequently, only a few of the tens of thousands of commercially important chemicals have been extensively tested and most have scarcely been tested at all.[13] Furthermore, an estimated one thousand new chemicals enter the market *every year*.[14] There simply are not enough skilled personnel to evaluate the flood of new chemicals and the backlog of old chemicals, even if money were available.[15]

Not only are the duration and cost of animal testing prompting public health officials and toxicologists to consider alternatives, but so is the dubious value of many animal tests. For example, physicians have severely criticized animal tests for birth defects ("teratogenicity tests"). Their criticisms have been compiled by Dr. Robert Sharpe of the Lord Dowding Fund for Humane Research.[16,17] In the book *Drugs and Pregnancy*, physician P. Lewis of Hammersmith Hospital, London, wrote that animal teratogenicity tests are "virtually useless scientifically." Another contributor to the same book, physician D. Hawkins of the Institute of Obstetrics and Gynaecology wrote: "The great majority of perinatal toxicological studies seem to be intended to convey medicolegal protection to the pharmaceutical houses and political protection to the official regulatory bodies, rather than produce information that might be of value in human therapeutics." Physician R. Smithells of the University of Leeds characterized the extensive battery of animal teratogenicity tests as "more in the nature of a public relations exercise than a serious contribution to drug safety." Physician R. Brent of Jefferson Medical College has made similar comments.

Dr. Smithells also feared that animal teratogenicity tests might do more harm than good by screening out new drugs that induced malformations in newborn laboratory animals but could prove therapeutically useful and non-teratogenic in humans.

> The illogicality of the situation is demonstrated by the continued use of well-established drugs which are known to be teratogenic in some mammalian species (e.g. aspirin, penicillin/streptomycin, cortisone). Conversely, a new drug which comes through its animal reproductive studies with flying colours may nevertheless be teratogenic in man.

The situation is not much better in other areas of toxicity testing. According to the International Agency for Research on Cancer:

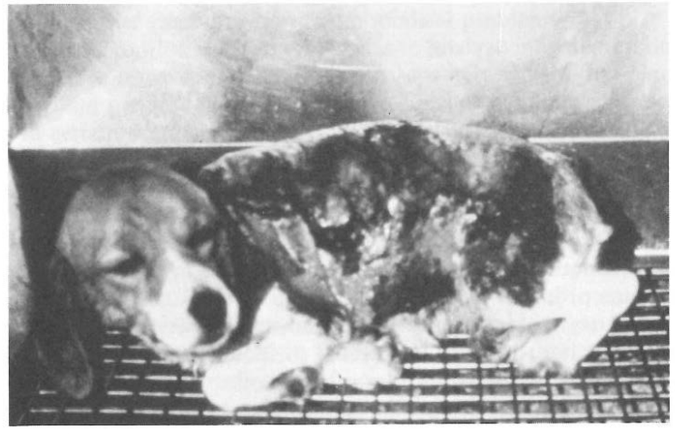
> ...At the present time, a correlation between carcinogenicity in animals and possible human risk cannot be made on a scientific basis....No objective criteria exist to interpret animal data directly in terms of human risk.[18]

Despite the obvious need for alternatives to animal testing, their promotion is not without impediments. National and international regulations that mandate animal testing are often inflexible and slow to change. Even when regulations are updated in one country, a company seeking to market its products *internationally* may still conduct the same set of traditional animal tests in order to satisfy all regulations simultaneously. Companies may also persist in conducting animal tests if they view the tests as indispensable in defending themselves from product-liability lawsuits. Also, animal testing tends to intensify in response to public outcry against newly discovered instances of harmful drugs, hygenic products, and environmental chemicals. The time has surely come to reevaluate current toxicity tests, rather than intensify their usage.

Biomedical Research

Animals are used in biomedical research as "models" or surrogates of human beings in order to understand the functioning of the healthy body; determine the effects of diseases and trauma on the body; and discover remedies for disorders, among other uses. Roughly fifty million animals per year are used in biomedical research in the United States alone.[19] The degree of their pain and suffering depends on the details of their care and use. Many laboratory animals are housed alone in small, barren cages. They are part of a variety of experiments; perhaps the least fortunate are those that are burned, frozen, poisoned, blinded, irradiated, crushed, infected, or shot. Although anesthetics and analgesics are sometimes administered to these animals, they do not provide total relief, as any dental patient knows.

Animal research is extensive and diverse enough to permit the fruitful application of alternatives, especially reduction and refinement. The rapidity and extent of this application will depend on financial backing and the imagination and motivation of researchers, as well as their perceptions of outside pressure. Certain impediments will have to be overcome, however. These include resistance to change from the research community and from industries with a financial stake in continued animal research, including suppliers of animals, cages, food, and antibiotics. Similar considerations apply to the use of animals in education and toxicity testing.



HSUS

III. This beagle was burned over a large portion of its body as part of an experiment.

SECTION

V

What Are the Alternatives?

THE SAME TYPES of alternatives can be used in both research and toxicity testing. These alternatives are in various stages of development and span a wide variety of procedures and systems, including human studies, *in vitro* techniques, mathematical and computer modeling, use of less sentient organisms, and physical and chemical techniques. We will look at each of these possibilities, then determine how they apply to the Draize and LD50 tests.

Human Studies

Humans are already used extensively in research. For example, 400,000 to 800,000 patients a year are enrolled in organized clinical investigations of drugs and other treatments in the U.S.[1] However, an even greater emphasis on human studies could reduce the demand for laboratory animals. Sick or injured persons could be studied to improve the diagnosis, treatment, and prevention of medical problems. Healthy volunteers could be incorporated into these *clinical studies* as controls. Healthy volunteers could also be useful in studies that focus on maintaining or improving health, rather than on coping with medical problems.

A second way to conduct human research is to analyze information on large numbers of people to uncover potential relationships between the incidence of disease or injury and people's habits or environments, such as smoking, drinking, and working in certain occupations. These *epidemiological studies* are helpful in identifying probable causes of health problems. Similar studies are helpful in identifying promoters of good health. These studies may not convincingly demonstrate a cause–and–effect relationship in some cases; however, they are often helpful in providing clues that focus future research efforts.

The remaining category of human research consists of *postmortem studies* of cadavers donated to science. These studies are particularly useful in anatomical and transplant research. Cadavers are also sources of transplantable organs.

Cadavers have far more potential in biomedical research than current usage suggests. In fact, postmortem studies could revolutionize research, toxicity testing, and education and thereby greatly reduce our reliance on laboratory animals. The key, according to a physician[2] and an educator and physician,[3] is to use cadavers that are brain–dead but whose physiological functions are sustained by artificial support systems. Known as "neomorts," these cadavers resemble comatose patients but have

been certified as legally dead.

Support systems are even now being used by the biomedical community to keep cadavers functional for medical or scientific reasons. A recent example is that of an Indiana woman who died as a result of a car accident but whose body was sustained by artificial supports until her child could be born by cesarean section several weeks later. Although practical problems currently make neomort technology too expensive and complicated for widespread use, it is believed that these problems may be solved in the near future.[4] Not so likely to be resolved readily, however, are the ethical and moral considerations restraining such use.

Because the availability of neomorts will undoubtedly be limited, priorities for their use will have to be established. Likewise, a variety of technical, ethical, and legal issues will need to be resolved.[5,6] Scientists believe that such a resolution will usher in a new era in biomedicine and science.

Although the reported use of neomorts has been limited for reasons indicated above, an example of one study, as well as examples of more traditional human research, is discussed below.

• Human research has played an important role in the development of artificial heart implants. The first clinical implant of the so-called Jarvik-7 artificial heart was performed in 1982 on Barney Clark. One of the researchers involved in this operation remarked that more was learned from this single case than from all of their preceding research, which included dozens of implants in animals. Even if this remark is an exaggeration, it underscores the importance that researchers attach to clinical trials.

Beyond its role in clinical testing of the Jarvik-7 heart, human research also played an important but undervalued role in pre-clinical testing. While it is widely known that the Jarvik-7 was extensively tested in animals, it is hardly known (much less appreciated) that postmortem studies were also involved. Physicians at Temple University implanted the Jarvik-7 heart in five brain-dead humans. They experimented with three different surgical implant techniques. They wrote:

> ...We were confronted with the question of whether or not an artificial heart successfully tested in calves would fit and function in man. But how to proceed in man with some assurance of success?...Today it is possible to test the functional capabilities of intrathoraic blood pumps in brain-dead but hemodynamically stable human subjects at no risk, so that it is not necessary to learn the fundamentals of fit and function in patients....The relatives of the deceased subjects have been extremely supportive of our experiments. Their hope is that, through these studies, others may live longer and more comfortably.[7]

Although this neomort study was a follow-up of animal studies, the clear implication of neomort research is that our reliance on laboratory animals will be reduced.

• Recent progress in understanding Alzheimer's disease, or senile dementia, stems from clinical and postmortem studies of Alzheimer's patients. Anatomical and biochemical studies were conducted on brains of deceased patients, small brain biopsies, and cerebrospinal fluid from living patients.

• Most substances known to cause cancer in humans have been identified by epidemiological studies rather than by carcinogen tests in animals.[8] These hazards were identified primarily through occupational association.

• Treatments for drug overdose are being improved through clinical studies conducted at hospital poison centers.[9] These centers are designed so that patients can

be studied while given emergency treatment. One such center was established at Guy's Hospital in England, where researchers concluded:

> Whilst the data from the animal studies required by regulatory bodies provide some basic information of the mechanism of toxicity and relative toxicity, it cannot be assumed that this information will be entirely relevant for man. Furthermore, whilst these studies may give indications as to the appropriate treatment for acute overdosage, they are unlikely to indicate the efficacy of treatment. *Experience gained from a careful assessment of patients suffering from acute overdosage of drugs is potentially much more useful than that obtained from animal tests.*[10]

• Epidemiological studies have linked genetic damage to a variety of factors, including drugs, metals, industrial chemicals, radiation, tobacco smoke, and alcohol. The evidence is particularly strong for vinyl chloride, alcohol, and tobacco – the higher the dose, the greater the incidence of genetic damage.[11] Genetic damage was assessed by monitoring chromosome breakage in certain blood cells.

Further evidence of the importance of human studies comes from an analysis of Nobel Prizes awarded in medicine or physiology. These prestigious prizes are awarded for outstanding contributions in basic and applied research. Seventy-two prizes have been awarded from 1901 (the year the prizes were initiated) through 1985. Of these, twenty-two (thirty-one percent) involved human studies to some degree, including ten (fourteen percent) projects that were wholly or primarily conducted on humans.

Despite such accomplishments, not all human studies can be considered alternatives to animal studies. Instead, many human studies are follow-ups of research on animals. Researchers often turn to animals before conducting studies on humans because of ethical and practical problems of studying humans directly. However, findings from animal studies must be verified in humans because they cannot be extrapolated to humans with great accuracy. Given the uncertainties of this extrapolation, follow-up research on humans can truly be regarded as experimental and the human subjects regarded as the last in a series of "guinea pigs."

Sophisticated new techniques are helping to overcome ethical and practical restrictions that have limited the extent to which humans could be studied directly, without recourse to potentially misleading animal models. For example, remarkable new "imaging" techniques, which can generate visual images of the body's interior without the need for invasive procedures, are now being used to harmlessly study the human brain in action. One such technique is positron emission tomography (PET): tiny amounts of radioactive chemicals mark areas of interest in the brain, and a brain scanner detects these chemicals and generates pictures or "scans" that show the living brain in action.

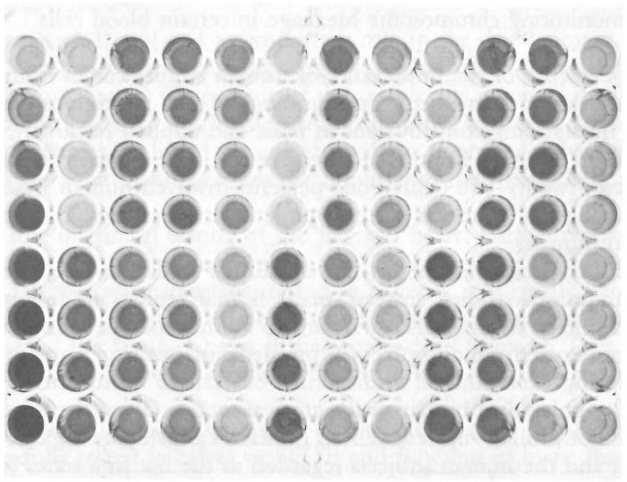
PET has recently been applied in the study of Parkinson's disease, which afflicts 400,000 Americans, mostly the elderly. Sufferers exhibit tremor, muscle rigidity and weakness, and a shuffling gait. PET scans were taken of the brains of volunteers who were known to have used a synthetic form of heroin tainted with a brain-damaging substance. The scans revealed Parkinson's-like damage to specific brain cells in the absence of overt signs of the disease. Such signs have begun to appear in persons who first used the heroin two years previously. These studies suggested that exposure to similar toxic substances may predispose people to develop Parkinson's later in life, when additional brain-cell loss occurs as a result of aging.

This application of PET scans to humans has sparked a revolution in the understanding of Parkinson's, which has baffled physicians for more than a century.[12] Such studies are pointing the way to human research on the diagnosis, treatment, and prevention of the disease.[13,14]

In Vitro Techniques

> There is virtually no field of biomedical research that has not been affected by *in vitro* technology.[15]

Some human and animal tissues can be removed from the body and studied *in vitro* (literally, "in glass," i.e., in a laboratory container). *In vitro* alternatives can be either replacements or reductions. If tissue samples are derived entirely from humans (from biopsies, autopsies, and placentas), then the research is a replacement. If animals are deliberately killed to obtain tissue samples, then the research is a reduction because tissue from a single animal often is enough to substitute for several animals. *In vitro* studies involving animal tissue can be replacements if the tissue is propagated indefinitely, providing material for study after study.



Rockefeller University Laboratory for *In Vitro* Toxicology Assay Development

IV. An example of a potential in vitro *alternative to the Draize test: it assesses the potential of chemicals to inhibit cell growth, which, for at least one class of chemicals, correlates well with eye irritancy. Cells are grown in each of the ninety-six wells, treated with test chemicals. A reagent is then added that develops color in proportion to the extent of cell-growth inhibition.*

There are several *in vitro* techniques; they differ in the type of material being cultured and the duration of the culturing. *Subcellular fractions* contain parts of cells or the entire contents of disgorged cells. *Short-term cellular systems* contain cells and tissues that are cultured less than twenty-four hours. These diverse systems contain isolated cells suspended in a fluid medium, tissue derived from biopsies, "tissue slices" from whole organs, or whole organs treated with special chemicals. *Tissue culture* contains cells and tissues that are nurtured for at least twenty-four hours.

Tissue culture is a prominent part of current research in alternatives. Living tissue is cultured in a medium that supplies nutrients. More sophisticated culturing schemes can better mimic the workings of the whole animal by supplying chemical substances that regulate cell function, such as hormones.[16]

Tissue culture includes cell culture and organ culture. In *cell culture*, a tissue fragment is dissociated into its component cells. The first generation of these cells is

a primary cell culture. If the cells grow and multiply indefinitely, a continuous cell line is established.

In *organ culture*, the emphasis is not on the growth and reproduction of isolated cells but rather on the maintenance of the tissue's three-dimensional structure and function. Organ cultures are relatively short-lived and do not propagate themselves, so fresh samples are needed each time cultures are set up. This may necessitate killing animals. However, as in cell culture, many organ cultures usually can be derived from one animal and hence these cultures qualify as reduction alternatives.

The placenta is a readily available organ that can be studied *in vitro* after it is discharged with the rest of the afterbirth. The placenta is a complex, multipurpose organ that is highly susceptible to drugs and chemicals, which makes it a suitable system for pharmacological and toxicological studies. Its potential as an alternative is conveyed in the title of a recent book, *Placenta – A Neglected Experimental Animal*.[17] A research program aimed at substituting placentas for animals in toxicity testing is being coordinated at the University of London and financed by the Lord Dowding Fund for Humane Research.[18]

In vitro techniques have several advantages over *in vivo* techniques, that is, studies of intact organisms. They enable tissue, cells, or subcellular components to be studied apart from confounding influences of other body systems. Because chemicals of interest can be added directly to the culture, much smaller amounts of chemicals are needed. This sensitivity was the main reason why the National Cancer Institute (NCI) recently launched a $2.5 million screening program for anti-tumor agents. An NCI representative noted that "the materials that we are typically looking for are trace constituents, so the *in vivo* model is inherently an insensitive one and we may miss, in most cases, our most interesting lead."[19] Cells to be cultured can first be cloned to achieve genetic homogenicity or be manipulated in other desired ways and then studied.

Although *in vitro* techniques are ideally suited to studying biological systems in isolation, they can also be designed to reflect interactions between systems. For example, tissue from one organ can be exposed to specific hormones produced by other organs, or a potentially toxic chemical can be incubated with liver cells to determine whether the liver detoxifies the chemical before it can exert any toxic effect on other cells. Although *in vitro* systems can be made more complicated in this way, the strengths of the *in vitro* approach are its simplicity and precision. While it is true that *in vitro* studies are ill-suited to model complex systems and hence will never fully replace *in vivo* studies, the converse is also true.

In vitro technology can be applied to study virtually any type of cells in the body. The practical problem of not being able to grow specialized cells has now been largely solved.[20]

Examples of *in vitro* procedures follow:

- The LAL test, described earlier, is an *in vitro* test that uses subcellular components obtained from horseshoe crabs to determine whether intravenous fluids will induce fever. This newly introduced test is already being conducted more than a million times annually.[21]

- A tissue-culture technique has been developed to standardize the potency of rabies vaccine.[22] This vaccine consists of a weakened, live form of the rabies virus. The potency of each batch of vaccine must be standardized so that it is not too strong or too weak. Potency is currently evaluated in an LD50 test on mice, but the twenty-one day test period makes this test impractical. Confounding factors, such as unrelated deaths and differential susceptibility of animals to the virus, can increase the variability in test results. The alternative, tissue-culture test is as sensitive as the mouse test but takes only twenty-four hours to conduct. The basis for the test is a sophisticated technique that involves fluorescent antibodies. When these antibodies

attach to their targets (in this case, cells infected with rabies virus), the resulting complex is easily detected and quantified under a microscope, owing to the antibody's fluorescence. The developer of the test recommends it as a replacement for the mouse test.

• A new *in vitro* test for diagnosing infant botulism is at the threshold of clinical application. Developed by M. Dezfulian of the Johns Hopkins Center for Alternatives to Animal Testing, the test probably will replace the conventional test for this disease, which requires up to 200 mice. Infant botulism results from a chronic intestinal infection by bacteria, which produce a toxin that causes extensive damage. It is now the most common form of botulism, outranking acute infection from food poisoning.

The disease is diagnosed by culturing stool samples. In the conventional test, an extract from this medium is injected into mice. It is fatal if the botulism toxin is present. In the alternative test, the toxin is detected by an *in vitro* reaction with antitoxin antibodies. In its present formulation, this procedure does use animals (rabbits) to produce the antibodies. However, this step is fairly harmless and produces enough antibody from one rabbit to substitute for hundreds of mice. The alternative test has several advantages over the conventional test. The mouse bioassay is generally considered to be cumbersome and inconvenient, while the alternative test is easily carried out in routine clinical laboratories. Its results are obtained overnight, in comparison to seven to fourteen days in the bioassay. The alternative test has the added advantage of not being confounded by any lethal substances, other than the botulism toxin, that might be present in stool.[23] In Dezfulian's testing,[24] the new procedure proved as effective—if not more so—than the mouse test.

• Tissue–culture techniques have reduced the demand for laboratory animals in virus research. Animals are no longer needed as living test tubes to culture viruses. Tissue–culture techniques can also be used to screen substances for their potential as antiviral drugs. For example, one pharmaceutical company used mice to screen for antiviral drugs. The company later added a cell culture as a primary screen and organ culture as a secondary screen and retained mice as a final screen. In 1963, the company screened one thousand substances per year using approximately sixteen thousand mice. Twelve years later, after adopting *in vitro* techniques, it screened twenty–two times more substances per year using approximately one tenth the number of mice![25]

• Tissue–culture techniques are at the forefront of basic research in biomedicine, particularly in studies of the immune system. According to the National Academy of Sciences, "Major recent advances in our knowledge of the immune system made possible by cell cultures would have been virtually impossible to achieve in intact vertebrates."[26] The same report notes the following:

> It is clear that the study of *in vitro* antibody responses has led to a major portion of our understanding of immune system responses. Using an *in vitro* system, one can make 200 to 400 cultures from a single mouse. If these same studies were to be conducted *in vivo*, they would require 200 to 400 mice to achieve the same number of observations.

Cell–culture techniques have recently been applied to behavioral research in studies of the biochemical basis of depression and mania. Human skin cells were maintained in culture and assessed for their ability to bind to various pharmacological agents.[27] The cultured cells of manic depressives and their relatives exhibited biochemical properties markedly different from the cells of persons without a family history of manic depression. One commentator characterized this research as "a step forward, applying to psychiatry the techniques of tissue sampling and cell

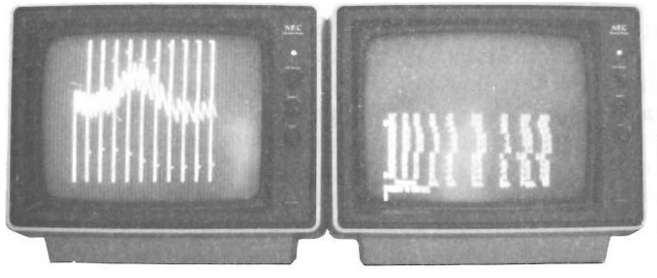
culture that have been of great value in characterizing molecular abnormalities in numerous medical diseases."[28] Imaginative research such as this expands the scope of *in vitro* studies beyond what was formerly attempted.

Mathematical and Computer Models

Modern approaches to biomedical research are increasingly incorporating the language of mathematics into their descriptions of living systems. Mathematical approaches are being applied in studies of all levels of biological organization, from interactions among molecules to interactions among organisms. In these approaches, existing information is used to describe the system under study in mathematical terms. The resulting mathematical model usually is a simplified version of reality but is, nonetheless, helpful in understanding complicated systems, especially those in which several variables influence an outcome.

As an illustration, consider the outcome to be the degree to which various chemicals are toxic. Toxicity is likely to be influenced by several factors, including the size and shape of the chemicals' molecules, the presence of certain reactive groups, the way reactive fragments are linked together, and the chemicals' affinity for fats versus water. Each of these factors can be represented mathematically by one or more variables or "parameters." In this example, toxicity would be modeled on the basis of the chemicals' structure, composition, and physical/chemical properties. Toxicity data on already–tested compounds could be used to help predict the toxicity of unknown compounds. Models such as these are known as structure/activity relationships (SARs) because chemical structure is used to predict activity, in this case, toxicity.

Once mathematical models are formulated, they must be verified to see if they accurately reflect the relationship under study. In toxicity testing, this verification procedure is known as validation. In the area of research, verification usually involves a procedure known as simulation. In a simulation, one or more parameters in the model is changed to determine if the response is similar to that seen in the living system. If dissimilar responses are obtained, the model can be refined or entirely reformulated. Because simulations usually are too complex to conduct by hand, researchers often turn to computers. Computer simulations are useful not only in validating models but also in suggesting new mechanisms and hypotheses for further study.



Dr. James Walker, University of Texas, Galveston

V. Computer modeling equipment used by Dr. James Walker in physiology exercises that have traditionally been performed on dogs. These two monitors show data in graphic and tabular form.

Modeling is now an integral part of research in many laboratories, particularly in the pharmaceutical industry.[29] Unfortunately, its more widespread application is hampered by a general lack of mathematical and computer skills among researchers and the cost of computer equipment and commercially available programs. NIH has recently taken steps to overcome these problems. It financed the creation of the

Biomedical Simulation Resource at Duke University Medical Center, which makes its facilities for building and examining mathematical models available nationally to biomedical researchers. The resource offers technical advice and access to computers and programs either at the facility or over a telephone data network.[30]

Computer models serve at least two general purposes in alternatives research. First, *they can substitute for animal tests, in some cases.* The extent to which models need to be backed up by animal tests depends on how well the models perform during validation. The better the performance, the less the need for back-up tests. In toxicity testing, models are likely to bring major reductions in animal use because existing information from animal studies on thousands of compounds can be applied toward predicting toxicity of closely related compounds that have not been tested. The outlook is not quite as bright when models are applied in new areas of research, since the results from the simplified models will have to be checked in the far more complex living system.

Second, *mathematical models can make animal research more humane* by identifying promising avenues of investigation and thereby preventing fruitless animal research or by estimating the toxicities of a closely-related series of compounds, so that only the least toxic compounds will be developed and tested on animals.

These functions of mathematical models are illustrated in the following examples and in the following chapter.

• Mathematical modeling has been used to determine the molecular characteristics of cancer-causing chemicals. One hundred and fifty structurally related chemicals were analyzed; each had been found previously to be either carcinogenic or noncarcinogenic in animal studies. The model was an attempt to distinguish between these two sets of chemicals based solely on molecular structure.[31] Using the statistical technique of pattern recognition known as *discriminant analysis*, researchers correctly classified ninety-seven percent of the compounds. Such studies should encourage further research in predicting toxicity from molecular structure. Success in these endeavors will lead to a decrease in animal use for predicting toxicity.

• The potential value of mathematical modeling to cancer research has also been illustrated by Charles DeLisi and coworkers at the National Cancer Institute. According to a recent article, Dr. DeLisi's

> computer program...analyzed the response of the immune system to cancer. From information they gave the computer about tumor growth and antibody production, it calculated that the immune system could not only fight cancer growth but stimulate it as well. Researchers know that now, says DeLisi, "but if our model had been around ten years ago, it could have predicted what it's taken scientists countless man-hours and animals to figure out. This is the value of mathematical modeling—it comes up with things that you might otherwise miss."[32]

• Mathematical modeling of malaria research illustrates the potential value of modeling in guiding research efforts. This modeling was a retrospective analysis of results from the testing of potential anti-malarial drugs. A large-scale testing program had been conducted on mice at the Walter Reed Army Institute of Research. Development of a structure/activity relationship for a certain class of chemicals synthesized early in the program showed retrospectively that further research on this class was futile, yet many other chemicals in this class were synthesized and tested in mice. This analysis suggests that prospective use of mathematical modeling will prevent much futile animal experimentation.[33]

• A computer program developed by thirty scientists at the Los Alamos

National Laboratory is an ambitious attempt to duplicate the complex physiological systems of the human body.[34] The program is known as "HUMTRN," short for human transport. It is a data bank that gives simultaneous access to ten million pieces of information on what happens when any chemically identifiable substance is taken into the human body. HUMTRN is dynamic to the point of being programmed to eat, breathe, perspire, defecate, grow, develop sexually, age, work, and die. A scientist associated with the HUMTRN project has called this program "the cutting edge of modeling technology." In one study, HUMTRN suggested that, in most kinds of nuclear accidents, teenagers and young adults would be the highest risk group in suffering long-term effects. The developers of HUMTRN refer to this mathematical model as the "research rat of the future."

Use of Less Sentient Organisms

The seventy million vertebrate animals used in U.S. laboratories every year have well-developed nervous systems and are, therefore, more likely to experience pain and suffering than are invertebrate animals and microorganisms. Invertebrates include animals without backbones, such as jellyfish, squid, earthworms, and insects. Substitution of invertebrates for vertebrates, where feasible, would constitute a refinement in virtually all cases. (In some cases, decisions about relative levels of sentience will need to be made carefully, as generalizations have exceptions.) Similarly, substitution of vertebrate embryos for the more sentient adults would be a refinement. Substitution of microorganisms such as bacteria and protozoa for vertebrates would constitute complete replacement.*

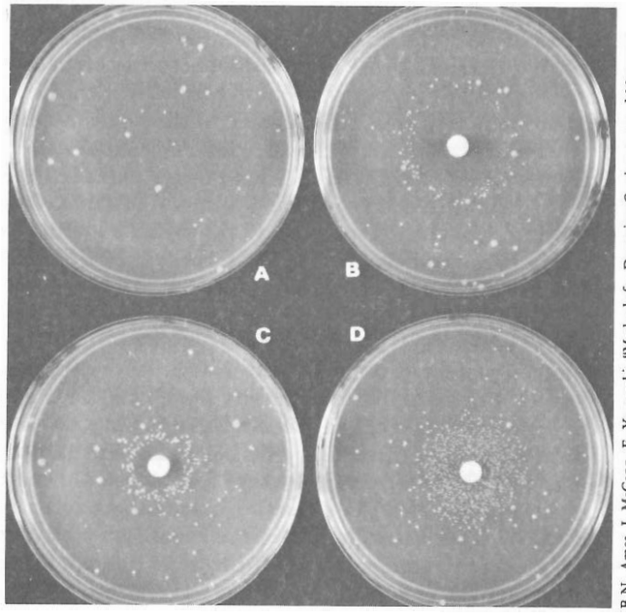
The principle of using less sentient organisms even applies to plants. Plants, as well as microorganisms and invertebrates, can be used to study basic biological processes. Two Nobel Prizes in medicine or physiology have been awarded for work in plants. Hence, plants, as well as microorganisms, invertebrates, and vertebrate embryos, can be considered "alternative organisms."

Alternative organisms are being used to develop scores of alternatives.[35,36] Several promising tests have already been developed, many in the field of toxicity testing. Some will be discussed here; others will be discussed in the sections on the LD50 test and the Draize test.

• A simple test for detecting teratogens (chemicals that cause birth defects) has been developed using hydras, tiny aquatic animals related to jellyfish. The test is based on the observation that chemicals that cause birth defects in animals also tend to disrupt normal development in hydras. This test is currently the most promising alternative screen for teratogens.[37]

• Plants may replace animals in tests to detect substances that cause skin damage in the presence of light.[38] Such substances, termed *phototoxins*, exert their effects after being ingested or applied to the skin. Laboratory animals, particularly hairless mice, are currently used routinely in phototoxicity tests. The alternative test is based on the observation that phototoxins inhibit the growth of yeast in the presence of light. The test, developed by F. Daniels, yields results that are similar to those from the mouse test when testing substances that are phototoxic when applied directly to the skin. Other alternative tests need to be developed to detect substances that are phototoxic after being ingested. Further research is needed on Daniels's test to corroborate and extend the encouraging results found to date.

*Research on microorganisms is sometimes characterized as *in vitro* because these organisms are so small they can be cultured in laboratory containers. A different classification is adopted here in order to emphasize the affinity between research on microorganisms and research on other organisms of limited or no sentience.



B.N. Ames, J. McCann, E. Yamasaki, "Methods for Detecting Carcinogens and Mutagens with the *Salmonella*/Mammalian-Microsome Mutagenicity Test," *Mutation Research*, 1975.

VI. The Ames test: each plate contains a culture medium incapable of supporting the tester bacteria unless the latter undergoes mutation. In A, bacterial colonies, seen as white dots, descended from bacteria that mutated spontaneously; in B–D, mutation was increased by adding chemical mutagens.

• The Ames test uses bacteria to detect mutagens (chemicals that induce genetic mutations). Because mutations are often associated with cancer production, the Ames test is used as a screen for carcinogenicity. This well-researched test is now a classic example of an alternative. It uses a specially prepared strain of the bacterium *Salmonella typhimurium.* The culture medium is designed so that only bacteria that have undergone certain mutations are capable of growing.

In addition to bacteria, the Ames test also makes use of *in vitro* culture of liver enzymes. Rats are the recommended source of livers, although human cadavers have potential.[39] Whatever the source, the culture contains the microsomal structures mentioned in the test's alternate title, the Salmonella/Microsome test. Potential mutagens are incubated with this culture in order to simulate a process known as "metabolic activation," which normally occurs in the liver (and to a lesser extent in other organs) of intact animals. Unless activated, mutagens might not exert their effects and would thereby escape detection.

The Ames test has been improved continually since its introduction and now gives results comparable to those of animal bioassays. It has the added advantage of being quick and inexpensive. It is widely used as an initial screen, often in combination with other short-term tests, and therefore has reduced the demand for laboratory animals in carcinogenicity testing. A considerable number of mutagens first detected by the Ames test have been shown subsequently to be carcinogenic in animal tests.[40]

About ninety percent of known carcinogens can now be detected by short-term mutagenic testing using batteries of tests.[41] These tests are inexpensive and can be conducted in one to five days.

Physical and Chemical Techniques

Physical/chemical techniques exploit instruments and chemical procedures, not animals, to analyze the physical and chemical properties of drugs, toxins, body chemicals, and other substances. For instance, high performance liquid chromatographs and mass spectrophotometers are physicochemical instruments that accurately isolate, identify, and measure the amount of a given substance in complex biological mixtures. In high performance liquid chromatography, the test substance is forced through a column of silica and different chemicals pass through at different speeds. This characteristic is used to analyze precisely the components of the substance.

Physicochemical techniques are replacements when used instead of animals to assay substances. They are reductions when they perform their analyses better than cruder methods and thereby require fewer animals per experiment. Numerous technical improvements can be considered as physicochemical reduction alternatives. For example, a device is now available that divides a one-microliter sample (which itself is tiny) into one thousand subsamples, each of which can be analyzed biochemically.[42] It is easy to see how the use of such an instrument could reduce the number of animals needed as sources of tissue samples.

• Physicochemical techniques have replaced the use of animals in assays for vitamins A, D, and E and for "biologicals" such as the hormone oxytocin. In the case of vitamin D_3, the new technique involves high performance liquid chromatography and provides a simpler, quicker, and cheaper alternative to the animal bioassay. The latter procedure involved inducing a vitamin D_3 deficiency (rickets) in rats and administering D_3-rich substances such as cod liver oil over several weeks — a laborious and time-consuming method.[43]

• Physicochemical techniques have replaced the use of rabbits in human pregnancy tests. Nowadays, one can obtain pregnancy diagnostic kits from the corner drug store. These kits contain simple materials to screen a potential mother's blood or urine for a chemical associated with pregnancy.

Other Techniques

Other techniques or systems may be used to replace, reduce, or refine the use of animals in research. These include mechanical models, veterinary patients, and computer-aided drug design.

Mechanical Models: animals are sometimes used to study effects of accidents such as vehicle crashes and specific injuries such as burns. Mechanical models are being developed that might replace animals in these studies. For example, an artificial neck developed by General Motors is being used in car-crash simulation tests, and a human simulator known as Thermoman is being used to test potential burn risks with different garments.[44]

Veterinary Patients: just as clinical studies of humans can reduce the demand for laboratory animals, so, too, can clinical studies of animals. Animals are susceptible to many of the same illnesses and injuries that plague humans. Animals that are already sick could be studied while undergoing treatment, and the resulting knowledge could benefit human health. (Of course, the primary concern in these studies should be the animals.) Clinical studies of animals could reduce the number of laboratory animals that are deliberately sickened or injured in experimental studies.

Prof. Calvin Schwabe, a respected research veterinarian, argues that both clinical and epidemiological studies of animals are being virtually overlooked as potential resources for understanding human diseases. The relevance of spontaneously occurring diseases in animals to medical research on humans is unappreciated. A consequence of this, according to Schwabe, is that most of the research in comparative medicine that is being conducted by physicians is focused upon the potentially least rewarding approach to animal diseases, namely, studying artificially induced

rather than spontaneous diseases. Veterinarian Michael Fox, in recounting Schwabe's view, calls for greater collaboration between veterinary and medical researchers.[45]

Computer-Aided Drug Design: discovering new drugs is largely a trial-and-error process, costly in terms of time, money, and animals. It takes eight years, on average, to screen a new substance from the seven thousand to eight thousand novel compounds created each year and to bring it into medical practice.[46] Fortunately, methods are being developed to replace this shotgun approach with the more directed approach of computer-aided drug design. Three-dimensional computer graphics and the theoretical field of quantum pharmacology are being used in efforts to design drugs with particular specifications. These efforts are based on the lock-and-key mechanism of drug action; that is, drugs must be the right shape and composition in order to "dock" with their targets and trigger their effects. Color graphics help visualize this process.

Although computer-aided drug design is in its infancy and is highly theoretical, there are indications that progress is being made. Several "drug designers" have been included on new drug patents for aid in discovering drugs.[47] A new drug being tested clinically for effectiveness against high blood pressure was designed with computer methods.[48] Perhaps it is not surprising, then, that several pharmaceutical companies now employ such "drug designers."

Much of the work in computer-aided drug design is apparently being conducted in Britain, where it has received some financial support from the Lord Dowding Fund. However, researchers at the University of Pittsburgh are collaborating with British researchers in attempts to use computer-aided methods to design a drug to treat sickle-cell anemia.[49] New efforts such as these hold great promise for reducing animal use by revolutionizing the process of drug discovery.

SECTION

VI

Case Studies: The LD50 Test And the Draize Test

MUCH OF THE public outcry against the use of animals in toxicity tests has centered on the LD50 test and the Draize test. It is not surprising, therefore, that much of the research into developing alternatives in toxicity testing has been directed at these two tests. Substantial progress in this research has been made during the last five years.

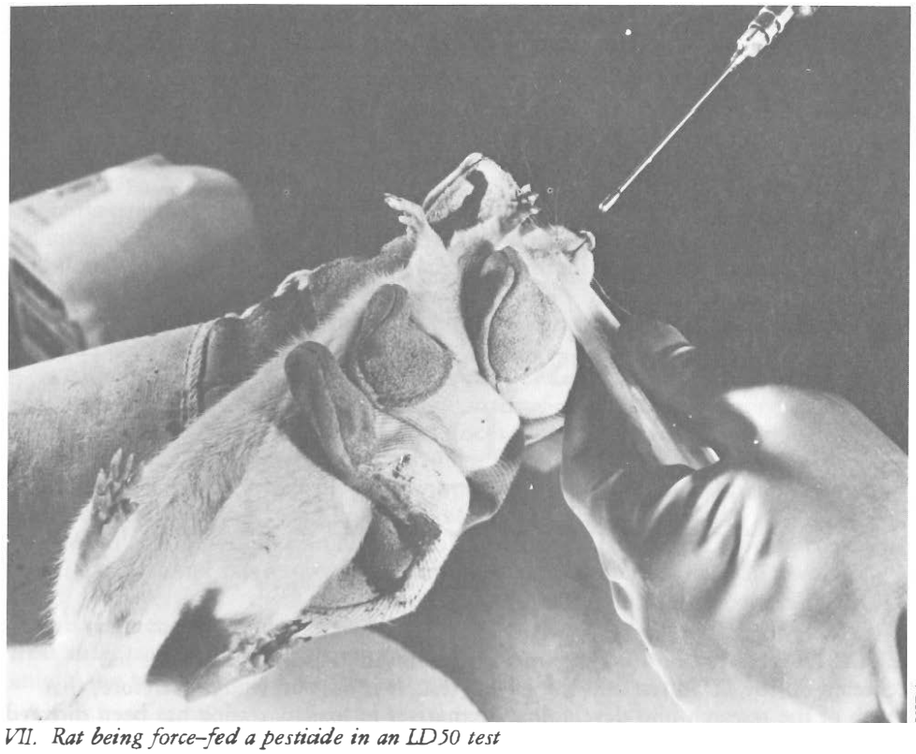
The LD50 Test

The LD50 test was developed in 1927 to standardize the potency of potentially poisonous substances destined for human use, such as diphtheria toxin, digitalis extract, and insulin. Although not originally designed to do so, the test gradually became incorporated into routine toxicity programs for testing new chemicals. Government regulations in the U.S. and abroad specified the LD50 test for evaluating new drugs, food additives, cosmetics, household products, industrial chemicals, and pesticides. Each year in the U.S., four to five million rats, mice, guinea pigs, and, less frequently, rabbits, dogs, and primates, are subjected to this test.[1]

In the LD50 test, test substances are force-fed, inhaled, injected, or applied to the skin of animals. Of these variants—the oral, inhalation, injectable, and dermal LD50 tests, respectively—the oral LD50 is the most common. It produces signs of poisoning including bleeding from the eyes, nose, or mouth; labored breathing; convulsions; tremors; paralysis; and coma.

The classical LD50 test uses large numbers of animals to derive a numerical index of toxicity (the LD50 value). This approach has two major scientific problems. First, the test is of limited value in protecting human health. This limitation stems primarily from an overemphasis on the LD50 value. Sometimes, little or no additional information (such as poison symptoms, body organs affected, and specific cause of death) is gathered. This important information could be derived from relatively few animals. According to D.V.W. Parke, the "counting of cadavers" should be replaced by full clinical and postmortem studies using fewer animals.[2]

Even when the LD50 value is supplemented with clinical and pathological information, public health officials can still be at a loss to infer the *maximum safe dose* of the test substance in humans. The LD50 provides the median *lethal* dose, not the safe dose. Moreover, the lethal dose, as ill-suited a measure as it is, still has



USDA

VII. Rat being force–fed a pesticide in an LD50 test

to be extrapolated from, say, rats, to humans. This extrapolation is nearly meaningless. According to toxicologist G. Zbinden of the University of Zurich:

> The marked species differences in acute toxicity are well recognized, making it impossible to predict the human lethal dose from the results of animal experiments. With such enormous variations between species, it is clear that the knowledge of the LD50 in a mouse or a rat does not provide much support for the prognosis in a human case of acute poisoning.[3]

A second problem with the classical LD50 test is its unnecessary precision. Large numbers of animals are used to derive a precise estimate of LD50, yet that estimate can be applied to humans in a rough manner only. According to Rowan, "If the LD50 figure of a compound is 100 milligrams per kilogram of body weight for a mouse, it could easily be anywhere between 10 and 10,000 milligrams per kilogram body weight for a human being."[4]

The illusary precision of LD50 values applies to animals as well as humans. Calculated LD50 values for the same chemical can vary substantially among laboratories (inter–laboratory variation) and among laboratory animal species (inter–species variation). For example, a study of inter–laboratory variation was conducted under the auspices of the European Economic Community.[5] Sixty–five laboratories were instructed to determine the rat oral LD50 for each of five chemicals. LD50s were determined separately for males and females, as is customary. The calculated values varied from four– to twelve–fold in males and three– to seven–fold in females. Similar inter–laboratory variation was found in another comparative study.[6]

This variation is not surprising given that the LD50 value depends on a host of biological and extraneous factors. According to Zbinden, these factors include

> ...species, strain, sex, age, and weight of the animals, abundance and composition of diet, volume and speed of administration of the test substance, vehicle, solubility, and particle size, concentration, ambient temperature, housing conditions, and even the seasons of the year. This means that the LD50, even if it is determined with high precision with a large number of animals, is not a biological constant.[7]

The precision of the classical LD50 test is also called into question by regulatory practice. Most LD50 testing is conducted according to regulatory guidelines. Ironically, the same guidelines that call for precision usually specify that LD50 values are to be lumped in limited numbers of broad categories for labeling purposes. Thus, all of that precision, gained at such cost in animal suffering, is lost in categorization!

These scientific problems with the classical LD50 test provide a compelling rationale for developing and using alternatives. Equally compelling is the ethical problem— the suffering and death of millions of animals in a test of such dubious value. Several alternatives to the classical LD50 test are available. They could reduce the demand for animals, as well as save time and money, without compromising human health.

Some of these alternatives are modifications that would require fewer animals:

- One test uses six to ten animals to determine the *Approximate Lethal Dose* (ALD). This test was discussed earlier as an illustration of reduction alternatives in toxicity testing.

- The *Limit test* involves giving a small group of animals (ten to twenty) a single dose of a test substance. If no ill effects are seen, no further testing at higher doses is required. The accepted maximum dose for the test depends on the nature of the substance being tested.[8] The Limit test is especially useful for relatively harmless substances, which would necessitate unrealistically large doses in the classical test.

- In the *Up–and–Down test*, each animal receives a single dose, but that dose changes as the six or so animals are sequentially tested. The dose is lowered after signs of severe toxicity develop or is raised after an animal survives one week without such signs. The resulting information is evaluated in a commonly available computer program. The test yields a reasonable estimate of the LD50.[9]

- Other techniques also use fewer animals than the classical LD50 and yet yield LD50 figures of satisfactory precision. These include the "moving averages" technique and a graphical method suggested by Molinengo.[10]

These modifications of the LD50 test use substantially fewer animals than the classical test and, therefore, qualify as reduction alternatives. The Limit test, by its very nature, is also a refinement alternative in that it reduces exposure of animals to pain–inducing doses. All of these modifications could qualify as refinements if animals that were acutely suffering and dying were instead painlessly killed and counted among those that died or exhibited severe toxic reactions.[11] This refinement was recently recommended by the British Toxicological Society.

Many toxicologists who conduct acute toxicity tests such as the LD50 make at least some use of these alternatives, especially the Limit test.[12] The U.S. cosmetics industry substituted the Limit test for the classical LD50 test and thereby reduced animal use by seventy–five to ninety percent, according to a trade association survey.[13] And Allied Corporation has abandoned the classical LD50 test in favor of

the "Up–and–Down" test. Animal use was cut in half. This innovation and others are yielding more information, cutting costs, and reducing the stress of those animals that are used.[14]

These examples may represent just the tip of the iceberg; the consensus among participants at the second symposium of the Center for Alternatives to Animal Testing was that reduction alternatives could completely replace the classical LD50 test.[15]

Alternatives to the classical LD50 are not limited to modifications of the test itself. They also include mathematical models, *in vitro* techniques, and alternative species.

Mathematical models are being developed to predict LD50 values without using animals. The most promising of these models is that of Kurt Enslein and his colleagues at Health Designs, Inc. The model predicts the oral LD50 values for rats, based solely on a chemical's structure and properties. The model, created through an analysis of nearly two thousand chemicals that had already been tested in rats, was evaluated by generating predictions on the LD50 values of another 900 compounds that had already been tested. The predicted values were similar to the actual values obtained in animals.

The researchers concluded that their model could be used competitively with the rat LD50 test. It has many advantages: elimination of unnecessary animal testing, lower cost, faster response, and greater repeatability. K. Enslein suggested a number of applications: (1) estimating the doses to be used in animal–based LD50 tests (this application could spare animals from being tested at doses that are too small or large to be meaningful); (2) selecting least toxic compounds by obtaining estimated LD50s on similar compounds before they are synthesized, then ranking these estimates to decide which compounds to investigate further (this application could spare animals from being tested with highly toxic substances); and (3) supplying data for any acute toxicity studies as needed.[16]

The major limitation of the model is that it cannot, as yet, generate estimated LD50 values for all compounds, owing to technical problems. Enslein and his collaborators have discussed this and other limitations of their model, adequately addressed their critics, and discussed future plans to improve the model and render it more understandable to toxicologists.[17] This latter development will hasten the model's evaluation and possible application.

The model is likely to be used initially as a preliminary screen, backed up by animal testing. During this period, the model could be improved. If it then inspires confidence, it may totally replace LD50 testing in animals.

Cell–culture alternatives to the LD50 test are being developed by a research program coordinated by FRAME.[18] The program involves four laboratories in the United Kingdom and is financially supported by numerous commercial and nonprofit organizations. The aim of the program is to develop a tier approach to acute toxicity testing:

> Level 1: *In vitro* testing for gross toxic effects on fundamental properties of cultural cells,
>
> Level 2: *In vitro* testing for specific toxic effects on particular target organ cells, and
>
> Level 3: *In vivo* testing, if necessary.

Work on this program is in progress. Preliminary results on Level 1 are encouraging. The fundamental property being examined is protein synthesis by human embryonic cells. In this procedure, toxic chemicals administered to these cells inhibit protein synthesis. The test yields an LD50 value, the dose causing fifty percent inhibition of protein accumulation. LD50 values are well correlated with *in vivo* LD50 values. Although cell–culture tests such as this one may never completely

replace *in vivo* testing, their judicious use clearly has great potential.[19]

Another potential alternative to the LD50 test involves the use of less sentient organisms. Using a series of alcohol compounds as test chemicals, researchers recently obtained an excellent correlation between LD50 values in mice and inhibition of movement in tubifex worms.[20] These findings need to be extended through the testing of other compounds that have already been tested on animals but not yet tested on worms.

Given the inadequacies of, and the alternatives to, the classical LD50 test, it is not surprising that support for the test is eroding in all quarters. Even toxicologists have criticized it. Dr. S.B. deC. Baker stated that acute studies such as the classical LD50 "are of little use and are expensive in animals. The main information they give is an indication of the...dose required to commit suicide."[21] Zbinden called the LD50 "a ritual mass execution of animals."[22] Dr. D.P. Rall, director of the United States–based National Toxicology Program, called the LD50 "an anachronism. I do not think the LD50 test provides much useful information about the health hazards to humans from chemicals...."[23] The Pharmaceutical Manufacturers Association, which represents 149 research–based pharmaceutical companies in the United States, stated that "Advances in toxicity testing now make it possible to conduct most drug–safety evaluation without the Classical LD50 test."[24] Even the National Society for Medical Research, which promotes and defends the use of animals in biomedical research, has backed away from the LD50. Its new position is that "The routine use of the quantitative LD50 test is not now scientifically justified...."[25]

Despite these statements, the classical LD50 test has not been abandoned. A 1983 survey of toxicologists who conducted acute toxicity tests revealed that eighty percent used the classical LD50 test.[26]

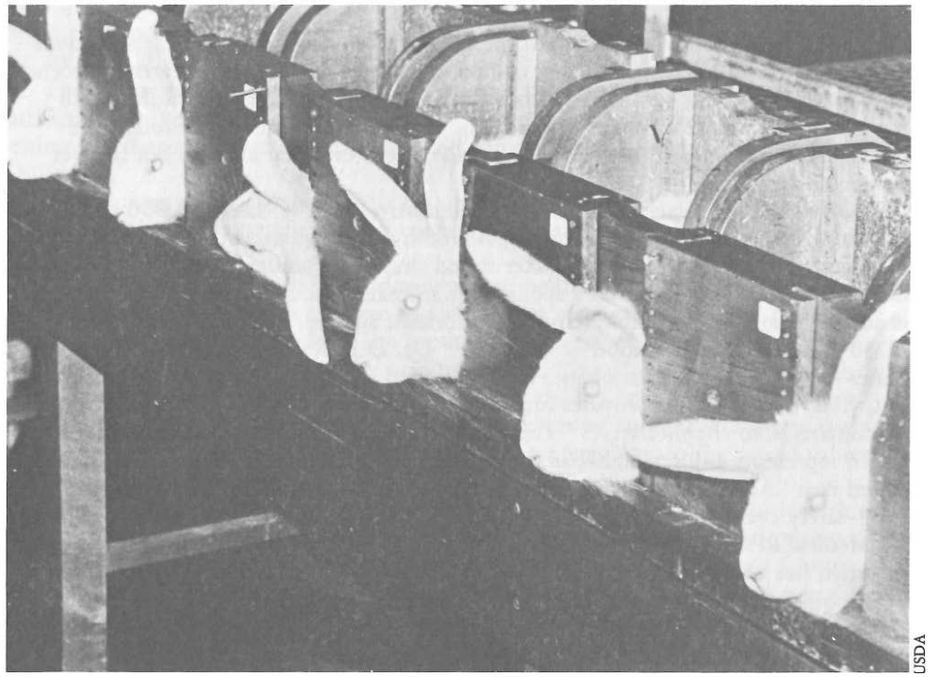
Perhaps the only scientifically legitimate use for the classical LD50 test is in rare cases in which drugs have a narrow margin of safety, so that toxic levels have to be precisely determined.[27] So why does the widespread use of the classical LD50 test persist? The main reason cited by the manufacturing and testing companies that participated in the 1983 survey was to satisfy regulatory requirements.[28] Of course, by satisfying regulations, these companies may feel better armed against damage claims brought by consumers. Perhaps these companies have difficulties in breaking an old habit. For their part, regulatory agencies also seem worried about consumer backlash and seem to be plagued by bureaucratic inertia.

A major regulatory obstacle for products marketed internationally is the Organization for Economic Cooperation and Development (OECD). OECD guidelines require that the LD50 test be conducted prior to international marketing of products. Companies whose products have any chance of being marketed overseas may automatically conduct LD50 tests, regardless of whether or not the products are eventually marketed internationally.

The Draize Eye–Irritancy Test

The Draize test is a method of assessing the eye irritancy potential of various substances including cosmetics, toiletries, household products, ophthalmic drugs, pesticides, and industrial chemicals. The test was developed following passage of the Federal Food, Drug, and Cosmetic Act of 1938, which mandated (among other things) that cosmetics be free of substances poisonous or deleterious to the user. Today, the test is a routine component of toxicology programs and regulatory evaluations worldwide. However, prospects for developing and implementing alternatives appear promising.

The Draize test is performed almost exclusively on albino rabbits. Its procedures have been modified several times since its adoption. A fixed dose (0.1 milliliters or 0.1 grams) is placed inside the lower lid of one eye of six to eighteen rabbits.[29] The lower and upper lids are then briefly held together to distribute the test substance on the eye surface. The other eye is left unused for comparison. The rabbits are restrained during the procedure and later immobilized in stocks to prevent them



USDA

VIII. Rabbits immobilized in stocks as part of a Draize test

from rubbing or scratching their eyes.

The rabbits' eyes are examined at specific times after exposure to the test substance (e.g., at 1, 24, 48, 72, and 168 hours). Damage to different parts of the eye is rated on separate scales. The maximum scores for damage to the cornea, conjunctiva, and iris are eighty, twenty, and ten points, respectively. These scores are added to yield an overall score for eye injury.

Eye irritation in the Draize test usually consists of reddening and swelling of the conjunctiva and iris and clouding of the cornea. Eye damage can be readily anticipated when substances such as hydrochloric acid, formaldehyde, alcohol, industrial solvents, drain cleaner, laundry soap, dish washing compounds, and shampoos are tested. Animals that survive the test with minor injuries are sometimes used for other laboratory studies, such as skin-irritancy testing, before they are killed.

The Draize test undoubtedly has been of some help in deciding whether or not substances are safe for human use. However, as *the* test for preventing ocular injury to humans, it leaves much to be desired. A major problem is that the *test is unreliable*. In cases in which particular substances were tested several times (either in the same laboratory or in different ones), it has not been uncommon for the same substance to be classified as an irritant in some instances and as a nonirritant in others.[30,31]

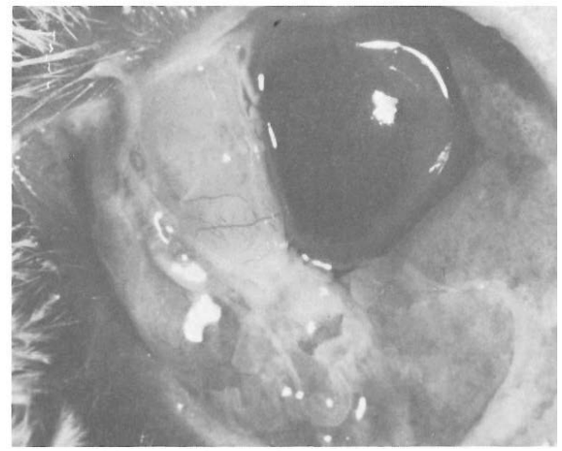
Such differing results may have been caused by variation in the scoring of similar degrees of eye damage or the haphazard distribution of the test substance on the eyeball.

The Draize test is also *crude*. It yields a score that is used to determine whether or not a test substance is an irritant—virtually a pass-fail test with an arbitrary cut-off point. For many substances, the important question for protecting human health is not whether a substance is an irritant, but how much of one it is.

Perhaps the most significant problem with the Draize test is its *questionable applicability to humans*. There have been many cases of discrepancies between the test results and human experience.[32] Rabbit eyes are, in most cases, more sensitive than human eyes,[33] partly because rabbits produce smaller quantities of tears than do humans. (Rabbits possess a nictitating membrane or "third eyelid," which may partially compensate for the reduced tear flow.)

Results of Draize tests are expressed in a way that makes the relevance of those results to human experience highly suspect. Test substances can cause different forms of eye damage to different degrees, and these are graded to yield numerical scores. These scores are weighted to reflect the purported relative importance of damage to different eye parts, then added together to yield a composite score. This score is interpreted as indicating the degree of irritant potential of the test compound. Ballantyne and Swanston have criticized this numerical Draize score as uninformative and meaningless.[34]

Several potential alternatives to the Draize test are either being developed or are already available. Some are modifications of the Draize test that would reduce either the number of animals used or the pain and suffering of the ones used. Others are new tests that are potential replacement or reduction alternatives. Eventually, a suitable battery of tests to replace the Draize test completely is likely.



U.S. Government/CPSC

IX. A rabbit's eye damaged in a Draize test

Let us first consider the modifications of the Draize test that have been proposed:

(1) *Use of anesthetics or antihistamines.*

We know that the use of certain anesthetics would not appreciably affect results of the Draize test.

(2) *Use of smaller doses.*

The volume of test substance routinely instilled in the eye is 0.1 milliliters (ml). Although this dose seems tiny, it is huge in relation to the fluid-holding capacity of the eye. Swanston has called this dose excessive and irrational.[35]

Studies have shown that a smaller dose (0.01 ml) would give better results[36] and cause less eye damage.[37] One such study was prompted by the suggestion from the National Academy of Sciences that a smaller dose could appreciably reduce the eye damage of test animals to a range more consistent with human experience.[38] Lower

doses are not only a humane refinement, but are also likely to yield results of greater relevance to protecting human health.

Using weaker dilutions of a test substance has the same effect as using smaller doses. N.J. Van Abbe recommends using dilutions when a substance is likely to cause severe reactions at the routine dose.[39] Such dilutions have the scientific advantage of enabling finer discriminations to be made from the results. According to Van Abbe, the discrimination can also be enhanced by simultaneously comparing the results with those from a reference standard.

(3) *Use of noninvasive techniques.*

One technique used to document eye damage in the Draize test involves killing the test animals and surgically excising eye tissue. However, several noninvasive and nonlethal refinements of this procedure are available. These include measuring corneal thickness using an optical device, measuring intraocular pressure using a hand-held instrument, and measuring the corneal reflex using a taut string and a simple device.[40] If these procedures were adopted, all animals would survive the test.

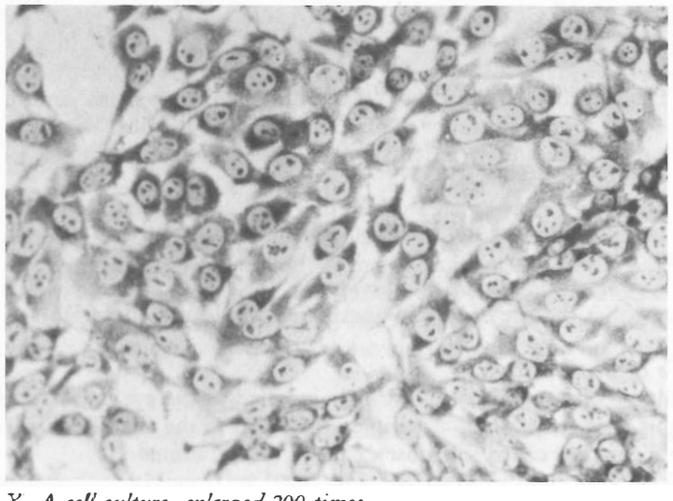
(4) *Use of fewer animals.*

The Draize test currently calls for six to eighteen rabbits. In a study investigating the effect of the number of test animals on the test's precision, increasing numbers from one up to six yielded marked improvements in precision.[41] However, increasing numbers to nine or twelve yielded "little further benefit when set against the increase in animal numbers." Hence, six animals should suffice, in most cases.

A more far-reaching reduction alternative was suggested by Koeter and van Vliet, who recommended that a preliminary Draize test be conducted with only one animal.[42] If severe irritation resulted, testing should stop. If irritation were less than severe, a few more animals could be tested, as necessary.

One can readily imagine other reduction alternatives to the Draize test. Dr. G. Flamm of the Food and Drug Administration recently recommended that any substance found to be an irritant at a low dosage should not be tested on more animals at higher doses.[43]

It is unlikely that the Draize test could be refined to the point where all pain and stress were excluded. Even if anesthesia and weak dilutions of test chemicals



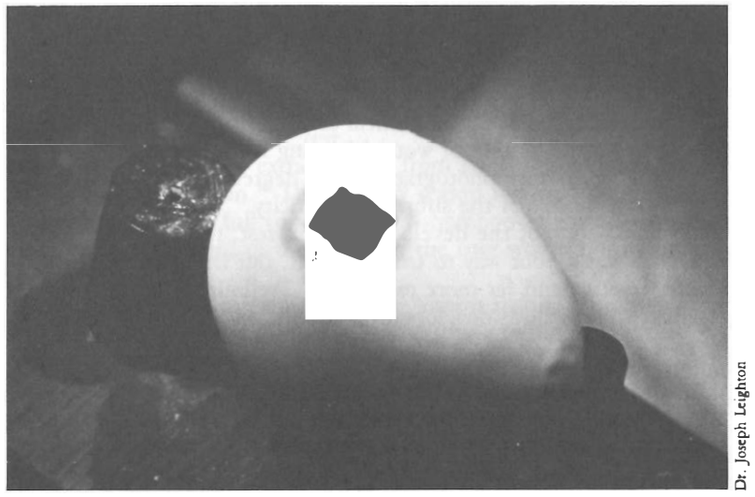
Rockefeller University Laboratory for *In Vitro* Toxicology Assay Development

X. A cell culture, enlarged 200 times

were used, the rabbits would still be living in stockades, which is undoubtedly stressful. Indeed, "rabbits not infrequently break their backs as a result of struggling to escape" from the Draize stocks.[44] This brings us to consider alternatives that would replace, or at least reduce the demand for, the Draize test.

The most widely known alternative replaces rabbits with chicken eggs. A portion of the eggshell and adhering membranes is removed from a fertilized egg when the embryo has developed for two weeks. This procedure exposes the "chorioallantoic membrane" (CAM), which surrounds the embryo. A small amount of a potential irritant is applied to a section of the CAM. A positive response can include cloudiness, inflammation, and proliteration of blood vessels, but since the CAM has no demonstrable nerve supply, the embryo feels no pain.

Initial results from the *CAM test* show a good correlation with results from the Draize test.[45] This promising alternative is now in the "validation stage" and is being funded by various animal-welfare organizations in the United States.



Dr. Joseph Leighton

XI. The chorioallantoic membrane (CAM) test: test chemicals are placed on a portion of an insensitive membrane of a fertilized chicken egg to test for irritancy

Many potential alternatives to the Draize test involve *in vitro* systems. One is an organ culture of isolated eyes. Eyes can be obtained from human or animal cadavers, especially from sources such as slaughterhouses. An example of this organ-culture method is the *Enucleated Rabbit Eye test,* which has yielded promising results. The strengths and limitations of this type of test have been discussed by D.W. Swanston[46] and M. York.[47]

Another organ-culture system involves isolated corneas, as distinct from entire eyes.[48] Rabbit or bovine corneas are incubated with suspected irritants. Irritancy is inferred from changes in corneal thickness, ratio of wet weight to dry weight, microscopic anatomy, and corneal enzymes.

Other potential *in vitro* alternatives to the Draize test involve cell-culture systems. The cells for these tests are derived from a variety of sources, including the human cornea, mouth lining, and blood (leucocytes); rabbit cornea; rat abdominal cavity; and mouse embryo and connective tissue. In these tests, chemical irritancy is inferred from a variety of end points, including cell death, cellular release of substances associated with irritation, cell membrane damage, changes in cell

movement or metabolism, and the rate of wound healing. Examples of these cell–culture alternatives include the *Rat Mast Cell assay*, the *Fluorescin Diacetate test* the *Haemolytic Activity test*, and a variety of as–yet–unnamed tests.[49,50,51]

The Rat Mast Cell assay is already in limited use by the Johnson and Johnson Company. Mast cells are derived from connective tissue and are involved in inflammatory responses. The assay monitors the cells' release of the chemical serotonin. J. McCormack reported that "the procedure has a high degree of correlation with *in vivo* test results. In addition, it is easy to perform, accurate, and repeatable, and it limits the scope of *in vivo* testing."[52] The assay is used as a screen to eliminate severe irritants from *in vivo* testing. However, only a single class of compounds was evaluated, so it remains to be seen whether the Rat Mast Cell assay has wider applicability as a substitute for the Draize test.

Cell–culture tests funded by Revlon are producing encouraging results. Two such tests monitor either anatomical changes in cells or inhibition of cellular uptake of an important chemical constituent.[53] Rockefeller University researchers obtained excellent correlations between the results of the two tests and the Draize test.

Perhaps the most promising cell–culture alternative to the Draize test is being developed at the Eye Research Institute (Boston) and Harvard Medical School. The test is based on the observation that when the surface of a rabbit or human eye receives a minor injury, healthy cells migrate over the wound and proliferate to heal it. Irritating chemicals slow this healing process. To investigate this inhibitory effect, researchers injure two types of rabbit corneal cells *in vitro*, normal cells and those treated with an irritant. The rate of wound healing is measured by staining the wounds and using time–lapse photography. The degree to which a substance slows the response is an indicator of the substance's toxicity.

Dr. A. Neufeld, one of the developers of this test, recently commented, "Not only is the Draize test a poor way to treat animals, but the *in vitro* method appears to be far more sensitive and far more relevant."[54] Preliminary results using human cells suggest that the methods developed for rabbit cells can be successfully applied to human cells.

A promising tissue–culture alternative to the Draize test utilizes excised strips of rabbit intestine.[55] Some sixteen pieces can be isolated from a single animal. When suitably cultured, these strips will contract spontaneously for hours unless chemically poisoned. The test determines the concentration of test chemicals necessary to block fifty percent of the contractions. This test is based on the premise that some damage in the Draize test occurs when chemicals penetrate cells on the eye's surface and damage cells at lower levels. The surface cells can be viewed as a penetration barrier to chemicals; intestinal cells mimic this barrier effect. The results of this test have compared very favorably with *in vivo* data. One rabbit could provide enough material for thirty experiments, and the technique could be used for two–thirds of the Draize tests currently performed.[56]

The outlook for major changes in routine eye–irritancy testing is bright. Research on alternatives to the current Draize test is active and varied, thanks largely to public outcry over the treatment of animals in this test. Refinements and reduction alternatives to the Draize can be implemented immediately. Implementation of replacement alternatives probably will be gradual, as alternatives are incorporated into a suitable battery of tests.

While the Draize test is still in use, alternatives can be used in a supplementary manner to screen out highly irritating substances and to determine doses that will yield mild reactions in the Draize test. Before considering any form of eye–irritancy testing, investigators should ask whether a particular substance needs to be tested at all. Certain substances need not be tested because they are almost certain to cause eye irritation. These include substances that are highly acidic or alkaline and those that are already known to be severe skin irritants.[57]

As with the LD50 test, efforts to make eye–irritancy testing more humane should be directed to government regulators as well as to product manufacturers and

their testing laboratories. Such efforts should focus on eliminating variables in regulations that result in unnecessary replication and on circumventing bureaucratic inertia to accepting proven alternatives.

Recall that one reason for the persistence of the classical LD50 test is the claim that it is necessary to protect manufacturers against untoward legal action. A similar claim has been made with regard to the Draize test. However, in one legal action taken against a manufacturer of a shampoo that damaged someone's eye, rabbit–eye testing was a minor part of the case. This case (*United States v. An Article of Cosmetic...Beacon Castile Shampoo...*) merits discussion here because both supporters and opponents of the Draize test claim that it supports their arguments.

The case was a civil suit brought by the Food and Drug Administration (FDA) against the manufacturer in the wake of an eye injury sustained by a young girl. The girl dropped a container of shampoo, and the contents splashed up in her eye. To support its case that the shampoo was dangerous and therefore should not have been marketed, FDA commissioned a Draize test in rabbits and a study of human volunteers.

Despite the fact that the shampoo injured the rabbits' eyes, the court ruled against FDA and in favor of the manufacturer. In a discussion of this decision, the General Accounting Office emphasized that FDA failed to show that "the results of test on rabbit eyes can be extrapolated to humans...."[58] This statement is significant because it apparently undermined the manufacturer's defense–against–liability argument for conducting the Draize test.

Unfortunately, the issue of extrapolating from rabbits to humans was not the keystone of the judge's decision.[59] The primary reason for the ruling was that the FDA failed to show that the full concentrate of shampoo might get into the user's eye under the usual conditions of use and that the user would not automatically flush out the eye.[60]

Nevertheless, the judge did state that the "rabbit studies, standing alone, do not warrant condemnation of this product." The judge refused to accept extrapolations from rabbit–test results to human response without confirmatory data from research on human volunteers. In this case, FDA submitted conflicting and incomplete results of human studies. Although a complicated and multi–faceted case, the Beacon Castile decision does provide evidence that a court did not find rabbit–eye testing particularly helpful in determining the extent of human hazard.

Given the judge's comments, it is rather surprising that a spokesperson for The Cosmetic, Toiletry, and Fragrance Association (CTFA), a manufacturer's trade group, asserted that the case "provides support for use of the Draize test as a reliable method of substantiating that a product is safe for eye–area use."[61] According to the CTFA, the judge reasoned that rabbit eyes are more sensitive than human eyes in that the former have less capacity to tear and flush away an irritant; therefore, any chemical that does not injure rabbits' eyes is not likely to injure human eyes.

The CTFA supersensitivity–as–an–asset argument is unsupported not only by the Beacon Castile case, but also by toxicological principles. Although supersensitive species are well–suited for confidently identifying *harmless* substances, the strength of a toxicity test should be its ability to detect *harmful* substances. If a test is supersensitive, it will overclassify substances as harmful. This would be the toxicological equivalent of "crying wolf." The test's results could easily be explained away, much as studies identifying cancer–causing substances in laboratory animals sometimes are dismissed because the huge doses utilized may cause cancer by overwhelming the body's metabolism. Use of a less sensitive species or system could be more valuable in protecting human health, as well as more humane.

SECTION

VII

Alternatives in Education

Introduction

Education accounts for less than ten percent of the current level of laboratory animal use in the United States,[1] but this figure belies the importance of applying the alternatives approach to education. Scientists of tomorrow will be more likely to adopt the approach if they are exposed to it as students. Recently educated, young scientists are likely to play an important role in the development and implementation of alternatives.

Even students who have no desire to become scientists may nonetheless benefit from exposure to the alternatives approach. These students will come away with a better appreciation of animals and a more positive view of scientists' activities.

According to biology teacher G. Russell, the power of science without the control of compassion and admiration for life is too immense to be applied merely for the satisfaction of scientific curiosity. If biology were taught in a manner that developed a sense of wonder and of reverence for life, and if students felt inwardly enriched from their study of life, these students would formulate as a life-long goal the steadfast determination to protect and preserve all life and would bring healing to a world desperately in need of it.[2]

These philosophical changes might even motivate some students to consider careers in science.

Recognizing the importance of reaching young students, the American Fund for Alternatives to Animals in Research supports annual training sessions in *in vitro* toxicology for students planning a biomedical career.

This summary of alternatives in education applies primarily to the college and graduate levels, where the challenge for the alternatives approach is the greatest. Discussions of alternatives that focus on the pre-college level can be found in several compilations of humane biology projects.[3] The challenge for alternatives at the post-secondary level is not in devising projects that convey the general principles of biology, but in devising ones that convey specific information or confer specific skills. Examples include learning surgery without practicing on animals, learning comparative anatomy without killing large numbers of animals, or learning the effects of common drugs without giving those drugs to animals.

Alternatives

There is a wide variety of alternatives to educational uses of animals.

(1) *Surgical Apprenticeships*

In the United States, medical students practice surgical techniques on animals. This custom accounts for half of all animals used in medical education.[4] An alternative is the British system: medical students in Britain gain their initial experience in surgery by observing demonstrations on cadavers. Then comes a clinical apprenticeship: students observe experienced surgeons operating on sick humans, gradually begin to take part along with the surgeons, and finally carry out operations under their supervision.

The using of animals solely to gain surgical dexterity is prohibited in Britain by the Cruelty to Animals Act of 1876. According to the Royal College of Surgeons of England, the prohibition "has not proved an obstacle to the effective training of young surgeons in the United Kingdom."[5] This view is supported by a recent study of British and American surgeons, which indicated that practice surgery on animals made no difference to long-term competency.[6]

A similar prohibition applies to the use of animals in training veterinary students in Britain. These students train with experienced veterinary surgeons and use animals that need the operations for therapy. According to the British Veterinary Association, "The idea of making healthy animals sick for purposes of training is totally repugnant to the [veterinary] profession in this country."[7]

Unfortunately, this is not the case in U.S. veterinary schools, where healthy animals – primarily dogs and sheep – are subjected to practice surgery. Such procedures account for a significant percentage of the animals used in veterinary education.[8]

(2) *Placentas for Microsurgery*

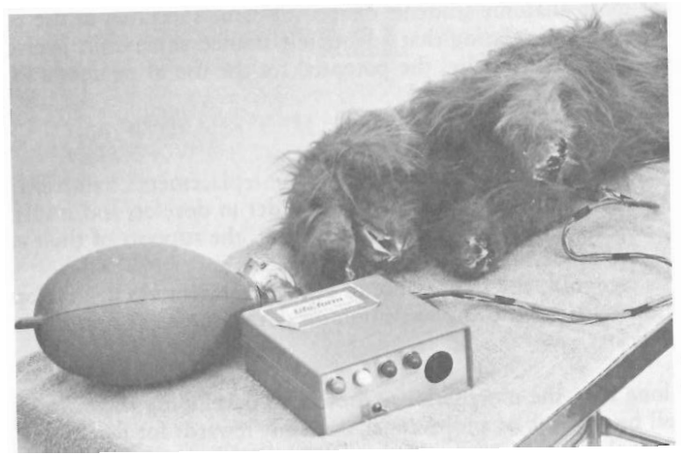
Apprenticeships work well for practicing most types of surgery but are ill-suited for the new field of microsurgery. Used primarily to reconnect severed fingers or hands or to reconstruct badly damaged tissue, microsurgery involves, among other things, reconnecting tiny blood vessels. It is not the sort of operation a trainee can readily learn at the shoulder of an accomplished microsurgeon.

For this reason, Britain is considering lifting its ban of practice surgery on animals for microsurgery. However, a promising alternative using human placentas, funded by the Lord Dowding Fund for Humane Research, is being developed by Dr. Paul Townsend, a plastic surgeon at the Frechay Hospital in England. The surface of the placenta has blood vessels of various sizes that can provide opportunities to practice microsurgery. A pump simulates blood flow through the vessels. Unfortunately, the pumped blood cannot clot, and clotting is a primary consideration in clinical microsurgery. Dr. Townsend thinks that this limitation can be overcome and that placentas can be a replacement for animals in microsurgery training.[9]

The British newspaper *The Guardian* has suggested that the British government should encourage development of the placenta alternative rather than relax controls on animal use in practice surgery.[10]

(3) *Computer-Assisted Mannequins*

Carefully designed mannequins can simulate the appearance and selected responses of humans or animals and, therefore, can play an important role in education. Widely cited examples include Sim, the mannequin discussed earlier, and "Resusci-Dog," a canine mannequin that teaches cardiopulmonary resuscitation to students at the New York State College of Veterinary Medicine. Resusci-Dog, whose development was supported by the Geraldine R. Dodge Foundation, is one component of a computerized cardiopulmonary emergency simulator that confronts students with various "emergencies." It also evaluates the students' diagnoses and



Dr. Charles Short

XII. Resusci-Dog is a canine mannequin used in teaching cardiopulmonary resuscitation at New York State College of Veterinary Medicine.

treatments and causes "patients" to respond realistically. The latest version costs $1,200 [11] and has replaced 100 dogs per year in veterinary classes at the New York school.[12]

(4) *Computer Simulations*

Some learning exercises can be conducted entirely on computers, without live animals or even mannequins. Computer programs can simulate dissections, metabolic functions, drug responses, and so on. The realism of these simulations can be increased by use of sophisticated interactive videodiscs, which display television-quality images on computer monitors.

Dr. J. Walker of the University of Texas uses computers to simulate physiological responses for medical students. Two inexpensive computers substitute for experiments that demonstrate a dog's cardiovascular regulation, digestive system, and drug responses. A recent article entitled *The Electronic Guinea Pig* describes two specific examples:

> During the cardiovascular experiment...one computer screen displays a chart that tracks blood pressure, heart rate, cardiac output, and similar information, updated every three seconds; the other screen provides a continuous reading of the most vital data. If a student wished to see the effects of the drug epinephrine – a standard medical school experiment – he presses a key marked E. Immediately the screen registers a jump in blood pressure and heart rate.
>
> Another standard experiment involves slitting open a dog's throat and pinching off the arteries. A student can simulate this on Walker's machine by pressing the O key: immediately the blood pressure indicators rise, the cardiac output drops, and some lucky dog lives.[13]

A wide variety of such computer simulations is now available.[14]

(5) *Other Procedures or Systems*

Other alternatives to traditional educational uses of animals include replacing dogs with videotapes to demonstrate the effects of poisons to veterinary students;

having two or more anatomy students dissect the same specimen or use prosections as demonstrations; and stipulating that adequately trained supervisors oversee students working on live animals. Finally, the potential for the use of neomorts in medical education should not be overlooked.

Discussion

Educators can have a profound effect on the replacement, reduction, and refinement of educational uses of animals. In order to develop and implement alternatives, educators need the proper motivation, the support of their colleagues, and financial and academic incentives.

Scientists recognize the importance of academic incentives in developing educational alternatives, as this observation on development of computer-based simulators indicates:

> In the long run, the most serious problem to developing these simulators may well be the lack of professional academic rewards for faculty members working in this area. Promotion, tenure, and salary increments are awarded predominantly for productivity in the research laboratory, not for efforts to develop innovative teaching techniques and materials. With essentially no external grant support for computer-based education activities and with few refereed high-quality journals in which to publish, two of the measures by which rewards are apportioned are not available to developers of novel educational software. This is a particular problem for junior faculty members, who often must devote their major efforts to climbing the academic ladder. Computer-based education seemingly fails to meet the perception of an academically valid and credible enterprise.[15]

Although lack of funding may impede the development of alternatives in some cases, it may actually dictate the adoption of non-animal methods in others. For example, the expense of procuring and housing dogs in medical schools may force these schools to implement computer programs instead.

Money, therefore, is not all that's needed to foster widespread application of the alternatives approach in education. Concerned instructors, educational administrators, funding agencies, students, and parents must be involved as well.

But no amount of effort will succeed unless the existing alternatives have merits in their own right. Does each alternative get the job done as well as or better than its animal-related counterpart? If not, is each alternative still adequate? Educators should clearly spell out the goals of their animal projects and determine whether or not alternatives meet those goals.

SECTION

VIII

General Discussion

PROGRESS IN IMPLEMENTING the alternatives approach has been encouraging, especially in light of the modest investment of money and effort. In toxicity testing, the first generation of alternative screening tests is being developed, validated, and implemented. In biomedical research, investigators are applying alternative techniques to answer questions in diverse fields. In education, technological innovation is yielding new alternatives, such as robot-like mannequins and computer simulators.

Much of this progress has occurred within the last ten years, as the animal-rights movement has infused the search for alternatives with an ethical imperative. Prior to this, alternatives were pursued primarily for economic, public health, and scientific reasons but rarely as a reflection of a sense of moral duty or compassion. Even today, when specific alternatives are introduced to the scientific community in research reports, concern for animals is not necessarily cited as a reason for their development or possible implementation. Nevertheless, the introduction of new alternatives, for whatever reason, is still good news.

The most exciting alternatives in the areas of research and testing are based on the development of techniques such as tissue culture and computer modeling. Such breakthroughs in technique have been extremely important in the history of science, as Rowan[1] has emphasized. Technical innovations are used to answer old questions and address new ones. A historical example is the application of tissue culture to the prevention of polio. The development of a polio vaccine required that large amounts of polio virus be readily available. This was impractical using mice and monkeys, which were used extensively in polio research. Enders and coworkers discovered that the virus could be cultivated *in vitro*. This paved the way for Salk, Sabin, and others to develop effective vaccines. A testament to the importance of the tissue-culture work in combating polio is that Enders and coworkers, not Salk or Sabin, were awarded the Nobel Prize for their polio research!

Some new techniques are not alternatives in themselves but can, nonetheless, decrease reliance on laboratory animals by creating new possibilities for studying humans without recourse to questionable animal models. An example is positron emission tomography, discussed earlier in relation to human studies of Parkinson's disease.

Techniques such as positron emission tomography, which decrease reliance on

animal models by facilitating the direct study of humans, are sorely needed in biomedical research. Consider the remarks of Stephen Suomi, himself an advocate of animal models:

> The primary rationale for creating most animal models lies not so much in any obvious and impressive strengths of such models as it lies in the problems inherent in conducting research with humans as subjects.[2]

The primary problem in conducting research on humans is avoiding undue risk or harm to the subjects. Consequently, new, powerful techniques that are relatively harmless should be eagerly embraced by animal modelers.

Biomedical research is not the only area of laboratory animal use that has benefited from the application of new techniques. Alternative techniques, especially tissue-culture and computer modeling, are transforming toxicology from an empirical exercise into a predictive science. Tissue-culture techniques can not only determine whether or not chemicals are toxic but also uncover how toxic chemicals exert their effects. Modeling can help identify the structural features of chemicals that are likely to cause toxicity. Current methods treat each new chemical as a complete unknown and use whole animals to determine whether or not a substance is toxic.

The need for a transformation in toxicology was forcefully underscored by Nobel Laureate Joshua Lederberg, president of Rockefeller University:

> I think the testing of substances could be greatly improved above all by better understanding of the mechanisms by which these substances work. The one or two or three hundred millions of dollars a year that we're now spending on routine animal tests are almost all worthless from the point of view of standard-setting. It is simply not possible with all the animals in the world to go through new chemicals in the blind way that we have at the present time, and reach credible conclusions about the hazards to human health.[3]

To a substantial degree, our continued need for animals in testing is a function of our ignorance rather than our knowledge.[4] In 1972, Nobel Laureate Sir Peter Medawar predicted that the use of laboratory animals on its then-current scale would decrease as biomedical knowledge increased. This should hold especially for toxicity testing, given that its goals and methods are much more limited than those pertaining to research.

Toxicity testing does comprise a diverse array of tests. The replacement of all of these tests with alternative techniques will take a long time. In the meantime, toxicologists should exploit existing alternatives to the fullest extent. For example, if a comprehensive evaluation of a new chemical requires both alternative tests and traditional tests, the former should be conducted first; in this manner, chemicals that "fail" the alternatives tests need not be tested further on animals.

Another way of reducing animal use in toxicity testing is to make results of these tests public. Many manufacturers of drugs, cosmetics, pesticides, and other compounds make extensive use of animals not only in toxicity testing but also in product development. The results of these investigations are sometimes regarded as trade secrets; thus, competing companies may be inadvertently investigating the same compounds, resulting in a waste of animals. Although competition among companies requires that these investigations be kept confidential to some degree, such secrecy exacts a toll in animal life and suffering. A compromise solution would be to require companies to divulge the results of their investigations after a specified time.[5]

Some companies are taking steps to avoid unintentional repetition of toxicity tests. The Chemical Industry Institute of Toxicology, for example, earmarks contributions from member companies for toxicological testing and distributes the

results widely.[6]

We have seen that poor and inhumane animal tests persist despite their faults. As Sharpe noted, "we cannot delegate our responsibilities onto other animals, who only reward us with illusions of safety."[7] The remedy for this sorry state of affairs in toxicity testing is not only better tests, especially alternative tests, but also the realization that the proper measure of man *is* man. The United States should consider adopting a post-marketing surveillance scheme patterned after the one used in Britain. Early detection of problems with new products in actual use should be an essential component of safety programs. This was recommended by a 1977 European convention on drug monitoring. The scientists in attendance concluded, in part:

> *Only by the careful study of medicines in every day use* can greatest benefits be obtained from their administration, the untoward rare potential disaster recognised at the earliest possible moment, and the ill effects minimised. Absolute safety is unattainable and its pursuit, regardless of other considerations, is achieving more harm than good [emphasis added].[8]

Future progress on alternatives will depend, in part, on the extent to which the alternatives approach is embraced by biomedical scientists. While a few of these scientists view the approach favorably, the response of others has been lukewarm.[9] Researchers seldom target their work toward alternatives as ends in themselves. Some dismiss the approach altogether.[10,11] Recent progress on alternatives suggests that these naysayers are fighting a losing battle.

Several factors probably contribute to the scientific community's resistance to the alternatives approach. First, alternatives tend to be viewed in the narrow sense of replacements. Because replacements for some types of laboratory animal procedures will take many years to develop, this narrow view of alternatives engenders unnecessary pessimism. Adoption of the broader definition of alternatives as reductions and refinements as well as replacements should make the alternatives approach seem less quixotic.

A second reason for scientists' resistance to alternatives may be that alternatives are promoted by (among others) the opponents of animal research, namely, anti-vivisectionists. Animal researchers may not want to be seen as giving in to their opponents or they may view anti-vivisectionists as zealously promoting alternatives that are ineffective in order to save animals.

A third and related reason is that advocates for alternatives may be viewed as irrational and anti-research. However, the target of these advocates is not research in general, but animal research. Given the extent to which groups advocating alternatives are funding research on alternatives, the anti-research charge seems to be a smoke screen.

Fourth, researchers who were trained to use animals may be hesitant to learn new techniques.

Perhaps the most cynical suggestion for the resistance to alternatives is that alternatives pose a threat to the multi-billion-dollar industry of animal research. Thousands of people make a living from animal research. Scientists do so by conducting the research; veterinarians by administering to research animals; dealers by selling animals; and manufacturers by supplying cages, food, antibiotics, etc. Research institutions also profit by receiving a hefty percentage of the money awarded to their individual researchers. Some of these people or institutions undoubtedly would rather maintain the status quo than make the adjustment to an alternatives-based research industry.

Some money is given specifically for alternatives research. Though the amount is small compared to funding for animal research, it may lure some animal researchers into alternatives. T.D. Overcast and B.D. Sales claim that some animal researchers are pursuing alternatives for another reason, namely, to protect themselves from the impact of future regulations on animal research.[12] However, this claim may have

been made more for its alarmist effect on regulators and its portrayal of animal researchers as beleaguered than for its reflection of a realistic trend.

Of course, it would be foolish to suggest that all biomedical scientists oppose the alternatives approach and all have questionable motives, even those who pursue alternatives.

Whatever the motivations and beliefs of researchers, the case for alternatives must ultimately be judged on its own merits. There is a surprising amount of historical information on which to base this judgment. In the following analysis, consider alternatives in the narrow sense of techniques that avoid the use of intact animals altogether. Actually, this is too narrow a definition because we want to include the use of "less sentient organisms" (invertebrates, microorganisms, plants, and vertebrate embryos) as an alternative technique. And, of course, we are also including human and *in vitro* studies, mathematical modeling, and physical–chemical techniques.

Most of these techniques have existed for decades, although they have not been discussed much in the context of alternatives until the last ten years or so. Sometimes, these techniques were used in projects that could have been conducted on intact vertebrate animals; today, we'd categorize these as alternative projects. In other instances, "alternative" techniques were used in projects that were beyond the capabilities of vertebrate studies.

Nobel Prize awards in medicine or physiology can be used as an index of the importance of alternative techniques in the history of biomedical research. These awards are generally believed to recognize research "of the highest calibre, the most enduring influence, and the most importance to biomedical science" according to the National Academy of Sciences (NAS).[13]

Awards that were made for research whose success depended wholly or primarily on alternative techniques were identified. The remaining awards were for projects that were successful owing primarily to *in vivo* studies of vertebrates, labeled non–alternative techniques. Sufficient information was available to classify all but two awards. Although most of the other seventy–four awards were readily classified, some proved difficult. These were generally assigned to the non–alternative category. When the same award was divided among two or more projects, the award was classified in the alternative category as long as at least one project depended wholly or primarily on alternative techniques.

About fifty (or two–thirds) of the Nobel Prizes were awarded to projects using alternative techniques (see Appendix A). This finding clearly documents the importance of these techniques in the history of biomedicine. The techniques advocated in this report have been used to conduct first–rate biomedical research and can continue to do so.

Those projects that used alternative techniques were further classified as to whether the projects themselves can be considered alternatives to research on whole vertebrates or whether the projects investigated topics that could not have been investigated using whole vertebrates. Although there were several equivocal cases, the fifty awards for projects using alternative techniques fell about equally in both categories (twenty–four and twenty–six, respectively).

The techniques advocated in this report have been the cornerstone of some of the twentieth century's most significant biomedical research. In some cases, they have substituted for the use of vertebrates; in other cases, they have added to our biomedical knowledge in ways that were not feasible using vertebrates.

Two considerations are important in interpreting the results of the Nobel analysis. One is that most of the award–winning projects were conducted before the alternatives approach was first articulated (1959). This increases expectations of what can be achieved if biomedical researchers actively pursue alternatives as ends in themselves.

The second consideration is that more awards would have gone to projects that used alternative techniques if not for the traditional emphasis on *in vivo* vertebrate

studies in biomedical research. For example, many animal researchers were skeptical of tissue–culture systems in the early days of this technique's existence. According to NAS, if not for this skepticism, tissue culture "might have been used to discover many of the vitamins, amino acids, and hormones."[14] Tissue culture could have been used to discover the hormone insulin, for instance. Even human studies could have yielded this discovery. Yet the researchers who discovered insulin used traditional *in vivo* methods, with dogs. They were awarded the Nobel Prize in 1923. This by–product of tradition is often regarded as a triumph of animal research, yet other techniques could have done the job.

Twentieth century Nobel Prizes in medicine or physiology were analyzed from a somewhat different perspective by NAS, which focused on the types of organisms used in the award–winning research, instead of on techniques. The results were compared to funding patterns of NIH to assess whether funds were allocated among types of organisms according to their representation in award–winning research (which presumably is some indication of the relative value of these organisms in biomedicine).

NAS concluded that research on mammals was being overfunded in relation to its representation in the Nobel awards and in other outstanding research:

> Considering the great strides in our understanding of biology and medicine that have resulted from the study of microorganisms, invertebrates, and lower vertebrates, the proportion of NIH resources that supports research in this area may be small in comparison to the resources dedicated to research with mammals.[15]

This suggested misapplication of funds may result from what was described earlier as the high fidelity fallacy — that mammals are of exceptionally high fidelity as models of humans and therefore should be used as often as possible. NAS recommended resisting this perspective:

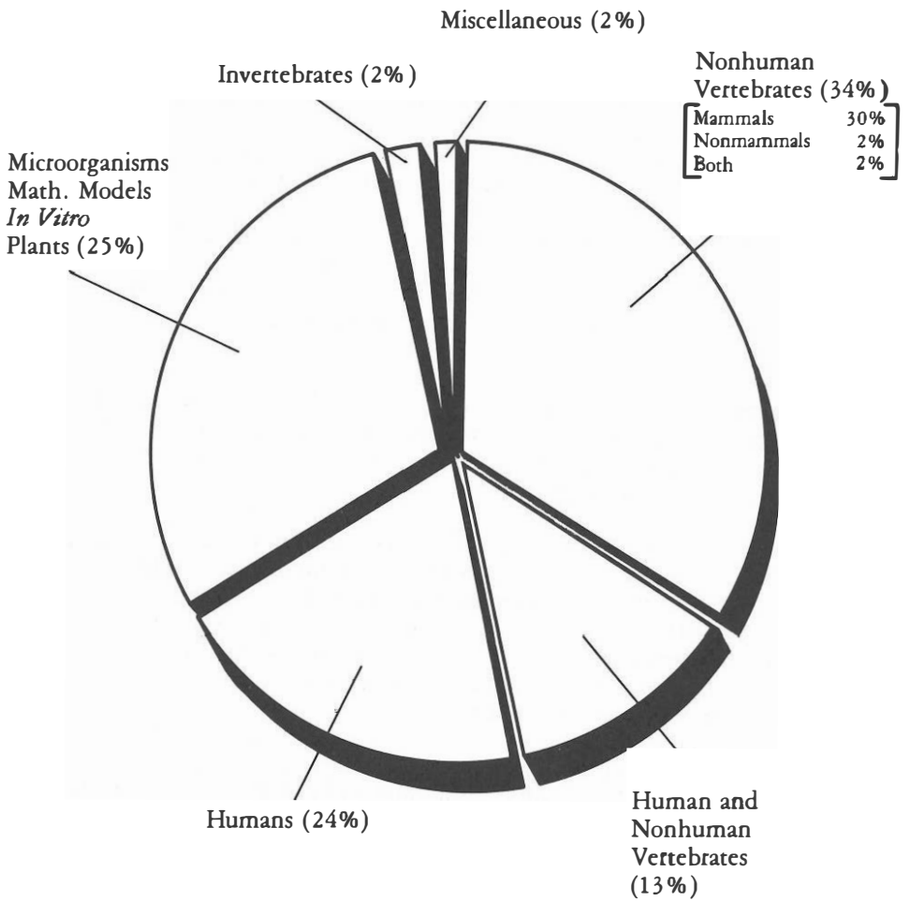
> Proposals for the study of invertebrates, lower vertebrates, microorganisms, cell– and tissue–culture systems, or mathematical approaches should be regarded as having the same potential relevance to biomedical research as proposals for work on systems that are phylogenetically more closely related to humans. Support should be given to good research without taxonomic or phylogenetic bias on the part of the sponsor and should include comparative and phylogenetic studies.[16]

NAS's recommendation is directed at NIH funding patterns such as the one depicted in Figure 1. The figure displays information for 1983, the most recent year for which information is available. Information from previous years (1980–1982) shows similar trends. Note that research on vertebrate animals, especially mammals, was the highest funded category. It exceeded the combined funding for research using *in vitro* techniques, mathematical modeling, and less sentient organisms (invertebrates, microorganisms, and plants). Funding for research on nonhuman mammals alone exceeded funding for human research, despite the fact that the mission of NIH is to protect human health.

Funding decisions are influenced to a certain extent by the interests and perspectives of the scientific community. Hence, alternatives research undoubtedly would be a higher priority if scientists supported the alternatives approach. In issues that involve the use of animals in research, such as alternatives, scientists are often portrayed as being engaged in polarized battles with anti–vivisectionists. In a sense, we are all anti–vivisectionists because none of us *wants* animals to have to suffer or die in laboratories. The alternatives approach can provide a common ground for both researchers and animal advocates to demonstrate their humane concern. This view is reflected in the policy of The Humane Society of the United States on the use of

FIGURE 1

NIH Funding, 1983[1]



TOTAL: $3,234,737,694

1. Distribution of support from the National Institutes of Health (NIH) for research on various organisms during 1983. Figures are for extramural research (i.e., research not conducted at NIH) only. "Miscellaneous" includes projects on invertebrates in combination with various other organisms.

SOURCE:
Adapted from Table 4-3 in National Academy of Sciences, *Models for Biomedical Research* (Washington D.C.: National Academy Press, 1985).

laboratory animals (see Appendix B).

Enthusiastic support for the alternatives approach, not only by researchers and animal-rights advocates but also by funding agencies, regulatory agencies, educators, and the general public, will hasten the day when laboratory animals are spared from their regrettable plight.

The alternatives approach is part of a more inclusive approach toward animal research that is characterized by concern for animals. This humane concern can be expressed in various ways in addition to seeking replacement, reduction, and refinement. For example, researchers contemplating the use of animals should determine, first, whether their topic is worth investigating and, second, whether their research would involve unnecessary duplication. Such duplication can be reduced by searching through computerized bibliographies of published research reports and by determining whether relevant research published in foreign languages has been translated. The John Crerar Library at the University of Chicago is a clearinghouse for such translations. Third, researchers should determine whether the chosen animal species is the best (or at least an adequate) subject of study. Research conducted on poor or invalid "animal models" is a waste of animals and effort.

Albert Schweitzer was a prominent exponent of this perspective. He wrote:

> Those who carry out scientific experiments with animals, in order to apply the knowledge gained to the alleviation of human ills, should never reassure themselves with the generality that their cruel acts serve a useful purpose.
>
> In each individual case they must ask themselves whether there is a real necessity for imposing such a sacrifice upon a living creature. They must try to reduce suffering insofar as they are able.[17]

A humane approach to research goes beyond asking questions about specific projects. It calls for a reappraisal of the entire biomedical research paradigm, which emphasizes the development of treatments for people who are already sick. The application of this paradigm has exacted a heavy toll in animal suffering and death. Many people have cogently argued that this paradigm is misguided even from the point of view of human health.[18] Human health would be better served if prevention were emphasized over treatment. A biomedical research program that emphasized prevention would shift research away from animal studies and direct it more toward screening programs and alternative techniques, especially epidemiological and clinical studies on humans.

The case for prevention over treatment was recently made by John Cairns of Harvard University's School of Public Health[19] in a discussion of cancer research. About one hundred different kinds of human cancer are recognized. Because these cancers have their own characteristics, each should be considered as if it were a separate disease. Unfortunately, fewer than fifty percent of cancer patients can be cured by surgery. Supplementary treatments involve administration of hormones, radiation, and chemotherapy. The success rate of these supplementary treatments has been disappointing; they avert only about two to three percent of the 400,000 deaths from cancer each year in the U.S., and they can have serious, sometimes lethal, side effects. Cancer specialist Dr. H. Bush notes that some treatments are so physically and psychologically degrading that some patients wonder whether the treatment is more disabling than the disease.[20] Although some cancers can be effectively treated, these are not the major forms of cancer.

Cairns contrasted the disappointments with our national cancer policy, which emphasizes treatment, with a potential policy that emphasizes screening and prevention. He wrote:

> Thanks to the cigarette, the U.S. now suffers a completely unnecessary

> additional 100,000 deaths per year from lung cancer. These numbers dwarf the 5,000 to 10,000 lives that are being saved by chemotherapy. Some countries have banned all tobacco advertising, and this has had an almost instant effect on tobacco sales. The failure of the U.S. Government to take such a step far outweighs all the advances made in the treatment of cancer since the advent of modern surgery.

Cairns also turned to the history of modern medicine to support his case for a prevention-based cancer policy:

> None of the important causes of death has been primarily controlled by treatment. The death rates from malaria, cholera, typhus, tuberculosis, scurvy, pellagra, and the other scourges of the past have dwindled in the U.S. mainly because humankind has learned how to prevent these diseases, not simply because they can be treated. There are many grounds for believing that when any major disease is tackled on a national scale, the chief effort should be to prevent its occurrence. To put most of the effort into treatment is to deny all precedent.

The so-called war on cancer is just one example of limited gains resulting from animal research. Millions of dollars have been spent searching for elusive cures to various other diseases, while support for diagnostic programs and preventive measures pales in comparison. Humans as well as animals are the losers.

While defenders of animal research are quick to point out the successes of animal research, they fail to add that the advancement of medicine and human health has been hindered by an overemphasis on this form of research. In addition to cancer research, examples include research on cocaine abuse,[21] depression,[22,23] and cardiovascular diseases.[24]

Our inflated hopes for animal-based treatments are undoubtedly fueled by researchers' self-aggrandizing pronouncements and the resulting media hype. As physician Bush noted with respect to cancer research, "Cures seem to happen more in press releases than in patients."[25] Dr. P. Goldhaber, the dean at Harvard's School of Dental Medicine, argued that researchers are "boasting prematurely about the advances and triumphs" of their work and are "extrapolating prematurely from...animal studies to humans."[26] He cited the fields of dentistry, cancer and tuberculosis research to support his conclusion.

An emphasis on cure detracts not only from prevention but also from the physical and psychological care of the sick. Bush wrote:

> As many cancer clinicians have found, a diagnosis of cancer can so demoralize a patient that the debilitating effects are far worse than the early physical effects of the disease....
>
> It is time that more of the research dollars now devoted to cure be diverted to finding new and more humane ways of caring that will make a cancer patient's remaining years happier, more comfortable, and more productive. My experience suggests that in the patient's eyes good care aimed at improving the quality of life may be just as important as cure.[27]

There are signs that our national cancer policy is beginning to reflect the importance of prevention.[28] The National Cancer Institute is financing the establishment of "cancer prevention research units" around the country to discover cancer-preventing strategies, including dietary changes. Instead of using laboratory animals, this research will test "likely cancer preventives in the most persuasive way possible—in the real world, over periods of years, on thousands of healthy human beings."[29]

A reorientation of research toward prevention need not entail a total abandonment of research aimed at treatment. However, treatment-based research should take advantage of new applications of alternative techniques in a wide variety of areas, including cancer[30] and AIDS.

The paradigm shift from treatment to prevention can be translated into our everyday lives. On the basis of numerous human studies, physician J. Scharffenberg concluded that personal health and salvation from disease are largely a matter of personal choice. He described a life-style that promotes health and dramatically reduces the risk of disease. It includes, among other things,

> a good diet of fruits, whole grains, nuts, and vegetables while avoiding the meat and high animal fat products and eggs, adequate sleep, good exercise in the open air, abstinence from harmful things such as tobacco, alcohol, coffee, tea, and other drugs, drinking plenty of water, moderation in all things including the amount of food eaten....[31]

Taking greater personal responsibility for our own health would lessen our reliance on animal-based treatments. In the event that we become sick, we should think twice before taking drugs that were developed or tested on animals. Are treatments available that are not animal-based? Will rest and relaxation be sufficient for recovery?

In a similar vein, we should keep laboratory animals in mind when shopping. First, we should buy products whose development and testing did not involve animals. A list of companies that sell "cruelty-free" cosmetics and toiletries is available from The Humane Society of the United States. Second, we should avoid buying household products that are "new and improved," as these modifications probably necessitated further animal testing.

SECTION

IX

Conclusions

RECENT PROGRESS in the development and implementation of alternatives is encouraging, especially given the relatively small investment of money and effort. Technical advances are already being translated into tangible results, not only in terms of animal welfare, but also in public health and cost savings. The public's concern for animals is partly responsible for this progress.

The application of the alternatives approach is still far from the ultimate goal of eliminating the use of laboratory animals. What is needed is a more concerted effort among researchers, toxicologists, educators, funding agencies, and regulatory agencies.

Whether or not such an effort is made will depend in large part on public enthusiasm. As a first step, people should familiarize themselves with the alternatives approach. This will enable them to recognize wild exaggerations made by animal–research defenders; for example, that biomedical research would collapse without the traditional use of animals or that the only alternative to using animals is to use ourselves.

Researchers probably would take alternatives more seriously if more people became knowledgeable on the topic. Researchers and laypersons eventually may share the goal of replacing animals in laboratories. If this goal is met, our mental image of biomedical research as an animal huddled in a cage will be replaced by more heartening images.

· APPENDIX A ·

Nobel Prizes in Medicine or Physiology Awarded for Research Whose Success Depended Primarily or Wholly on Alternative Techniques.

YEAR	WINNER	TECHNIQUE[1]	TOPIC
1902	R. Ross	LSO	Discovered insect vector of malaria and other aspects of this disease
1903	N. Finsen	IV	Treatment of diseases, especially lupus melgaries with concentrated light radiation
1907	C. Laveran	H	Role of protozoa in causing diseases
1908	E. Metchnikoff[2]	LSO, IV	Immunity
1909	T. Kocher	H	Physiology, pathology, and surgery of the thyroid gland
1910	A. Kossel	IV	Protein chemistry of cells, including nucleic substances
1911	A. Gullstrand	H, MM	Dioptrics of the eye
1914	R. Bárány	H	Physiology and pathology of the vestibular apparatus
1915–1918, 1921, 1925: No Prizes Awarded			
1927	J. Wagner–Jauregg	H	Malaria inoculation in treatment of dementia paralytica
1928	C. Nicolle	H	Work on typhus
1930	K. Landsteiner	IV	Discovery of the human blood groups
1931	O. Warburg	IV	Nature and mode of action of the respiratory enzyme in yeast
1933	T. Morgan	LSO or IV	Role of the chromosome in heredity (fruit flies)
1935	H. Spemann	LSO	Organizer effect in amphibian embryonic development
1937	A. von Szent–Györgyi	IV	Biological combustion process, with special reference to vitamin C and the catalysis of fumaric acid
1940–1942: No Prizes Awarded			
1944	J. Erlanger H. Gasser	IV	Differentiated functions of single nerve fibers
1945	A. Fleming E. Chain H. Florey	IV	Penicillin and its curative effect on various infectious diseases
1946	H. Muller	LSO	Production of mutations by X ray
1948	P. Müller	LSO	Efficiency of DDT as a contact poison against several arthropods
1949	E. Moniz[2]	H	Therapeutic value of a psycho–surgical procedure in certain psychoses

YEAR	WINNER	TECHNIQUE[1]	TOPIC
1951	M. Theiler	IV	Vaccine against yellow fever
1953	H. Krebs F. Lipmann	IV	Citric acid cycle and coenzyme A and its role in intermediary metabolism
1954	J. Enders F. Robbins T. Weller	IV	Cultivation of poliomyelitis viruses in tissue culture
1955	H. Theorell	IV	Nature and mode of action of oxidizing enzymes
1956	A. Cournand W. Forssmann D. Richards	H	Heart catheterization and pathological changes in the circulatory system
1958	G. Beadle E. Tatum	LSO	Genes regulate chemical processes (bread mold)
	J. Lederberg	LSO	Genetic recombination and the organization of the genetic apparatus of bacteria
1959	S. Ochoa A. Kornberg	IV	Mechanisms of the biological synthesis of RNA and DNA
1962	F. Crick J. Watson M. Wilkins	IV	Molecular structure of nucleic acids and its significance for the transfer of information in living material
1963	J. Eccles A. Hodgkin A. Huxley	IV	Ionic involvement in the excitation and inhibition of nerve cell membranes
1964	K. Bloch F. Lynen	LSO, IV	Mechanism and regulation of cholesterol and fatty acid metabolism
1965	F. Jacob A. Lwoff J. Monod	LSO	Genes that control activity of other genes
1966	C. Huggins[2]	H	Hormonal treatment for cancer of prostate and breast
1967	G. Wald[2] K. Hartline	IV LSO, H	Chemical and physiological visual process in the eye
1968	M. Nirenberg R. Holley H. Khorana	IV IV IV	Interpretation of the genetic code and its function in protein synthesis
1969	M. Delbruck A. Hershey S. Luria	LSO/IV LSO/IV LSO/IV	Replication mechanism and genetic structure of bacterial viruses
1970	B. Katz[2]	IV	Transmitters in nerve terminals and the mechanism of their storage, release, and activation
1971	E. Sutherland, Jr.	IV	Mechanisms of the action of hormones

YEAR	WINNER	TECHNIQUE[1]	TOPIC
1972	R. Porter G. Edelman	IV IV	Chemical structure of antibodies
1973	K. von Frisch K. Lorenz N. Tinbergen	Mi Mi Mi	Organization and elicitation of individual and social behavior patterns
1974	A. Claude G. Palade C. de Duve	IV IV IV	Structural and functional organization of the cell
1975	R. Dulbecco D. Baltimore H. Temin	IV IV IV	Interaction between tumor viruses and the genetic material of cells
1976	B. Blumberg[2]	H	New mechanism for the origin and dissemination of infectious disease
1977	R. Yalow	IV	Development of radioimmuno-assay and the principles underlying it
	R. Guillemin A. Schally	Mi	Hypothalamic hormones
1978	W. Arber H. Smith D. Nathans	LSO/IV LSO/IV LSO/IV	Discovery and application of restriction enzymes
1979	A. Cormack G. Hounsfield	MM, H MM, H	Development of the X ray diagnostic technique, computer-assisted tomography
1981	R. Sperry[2]	H	Functions of the cerebral hemispheres
1982	S. Bergstrom B. Samuelsson J. Vane	IV, PC IV, H IV	Biochemistry and physiology of prostaglandins
1983	B. McClintock	LSO	Discovery of mobile genetic elements (in corn)
1984	C. Milstein G. Kohler[3]	IV	Development of a technique for monoclonal antibody formation
1985	M. Brown J. Goldstein	IV, H	Cholesterol biochemistry and familial hypercholesterolemia

[1]H = Human Studies, IV = *In Vitro* Studies, MM = Mathematical Modeling, PC = Physicochemical Techniques, LSO = Studies of Less Sentient Organisms (Vertebrate Embryos, Invertebrates, Microorganisms, and Plants), and Mi = Miscellaneous.

[2]Award shared with researcher(s) who used non-alternative methods.

[3]Award shared with N. Jerne for his theoretical contribution.

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· APPENDIX B ·

Animals in Biomedical Research and Testing, from Statement of Policy, The Humane Society of the United States (1984).

The Humane Society of the United States recognizes that benefit for both animals and mankind has been achieved through some scientific research and testing on animals, but that the advancement of medicine and human health has also been hindered by an overemphasis on such animal research. It recognizes that uses of animals in biomedical research, safety testing, and other programs are many and varied, and that this research and testing is not likely to end in the immediate future.

While some of the animals used in research and testing are subjected to procedures that result in only momentary discomfort, The HSUS believes that millions of laboratory animals do suffer severely and needlessly in painful experiments, resulting from exposure to noxious substances and pathogenic organisms, or from cruelty, carelessness, ignorance, and indifference. The HSUS also contends that toxicity testing on live animals, as now required by government agencies to test the safety of serums, drugs, cosmetics, and other chemicals, is often unreliable, inaccurate, and unnecessary and should be replaced as soon as possible by new methods not involving animal suffering. Existing measures intended to ensure humane treatment, including the Animal Welfare Act and its enforcement, have proven inadequate. The Animal Welfare Act should be strictly enforced. Coverage should be expanded to include all vertebrates used, protect animals undergoing the actual research and experimental process, and require prohibition of specific painful invasive procedures.

The HSUS believes that scientists and facilities using experimental animals should be held strictly accountable for their care and use and should keep animals in a manner fulfilling both physical and behavioral needs. Experiments should be rigorously planned, with proper statistical design, so as to minimize the number of animals necessary to be used to achieve reliable results and, through the administration of anesthesia and analgesics and other appropriate medication and veterinary care required, to preclude animal suffering. The HSUS believes that government agencies and relevant professional organizations should encourage and actively support efforts to eliminate animal suffering in the laboratory.

Therefore, The HSUS strongly advocates the development and application of alternative methods of research and testing, which could reduce the number of animals required, refine existing techniques and procedures so as to minimize the level of stress endured by an animal, and replace the use of laboratory animals. Refinement and reduction are interim steps toward the ultimate goal of complete replacement of animals in biomedical research and product testing.

Therefore, it is the policy of The Humane Society of the United States to use every means in its power to reduce and end the suffering of animals in biomedical research and testing laboratories by advocating the attitudes and approaches set forth in this statement.

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27. Bush, "Cure," 35.

28. G. Blonston, "Prevention," *Science 84* (September 1984):36.

29. Ibid.

30. J.L. Petricciani, I. Levenbook, and R. Locke, "Human Muscle: A Model for the Study of Human Neoplasia," *Progress Without Pain* (Autumn 1984):11–18. First published in *Investigational New Drugs 1* (Boston: Martinus Nijhoff, 1983), 397–402.

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Acute toxicity: Toxic effects produced by a single dose of a compound. Dose is often large relative to the body weight of the animal. The test usually is limited to one to two weeks in duration.

Analgesic: Substance used to induce insensitivity to pain without loss of consciousness.

Anatomy: The structure of an organism.

Animal model: A particular type of animal used as a surrogate for humans in a research project. The animal is defined by its species and perhaps additional features, such as genetic uniformity.

Assay: A procedure or system used to determine the potency or concentration of a compound.

Bioassay: A procedure or system used to determine the potency or concentration of a compound by its effect upon animals, isolated tissues, or microorganisms, as compared with a standard preparation.

Biological: Biologically active substances such as hormones, antibodies, vaccines, and antiserum.

Biopsy: A specimen of tissue obtained from living patients for diagnostic examination; or the process of removing this tissue.

Carcinogen: Any agent that produces cancer.

Carcinogenic: Causing cancer.

Cardiopulmonary resuscitation: Restoration of breathing and heartbeat after apparent death.

Chronic (long–term): Multiple doses or continual exposure in feed, water, or atmosphere to determine safety thresholds and long–term toxic effects. Duration of exposure can be two years or longer.

Chorioallantoic membrane: A membrane found in the avian egg.

Clinical: Relating to observations of a patient or the course of his or her symptoms; often used in contradistinction to experimental, as in experimental study of animals intentionally made sick.

Conjunctiva: The membrane that lines the inner surface of the eyelids and connects to the forepart of the eyeball.

Congenital: Existing at birth; referring to certain mental or physical traits or peculiarities, malformations, etc.

Cornea: The transparent, front part of the eye that covers the iris and pupil.

Dissection: The act of cutting apart the tissues of the body in the study of anatomy (or in a surgical operation).

Enzyme: A protein secreted by cells that acts as a catalyst to induce chemical changes in other substances.

Epidemiology: The study of the prevalence and spread of disease in a community, especially infectious and epidemic diseases.

Genetic: Referring to the hereditary (or genetic) material.

Hormone: A chemical substance formed in one organ or part of the body and carried in the blood to another organ or part. Hormones can alter functional activity, and sometimes even the structure, of one or more organs.

Immune system: The body's system that is involved in combating infectious diseases, rejecting foreign tissue, and inducing hypersensitivity (allergic reaction) to specific substances.

Invertebrate: The taxonomic name for multi-cellular animals without backbones; for example, worms, crayfish, flies, and beetles.

Invasive: Referring to procedures that involve penetrating the body, as in surgery.

***In Vitro*:** In a test tube or other laboratory container; referring to bodily tissue, cells, or cellular components studied in isolation. See *in vivo*.

***In Vivo*:** In the living body; referring to processes studied in the intact organism, as opposed to *in vitro*.

Iris: The part of the eye that controls the amount of light hitting the lens; the iris is variously colored in different individuals.

Median: The middle value in a set of measurements; an LD50 value is the median lethal dose.

Metabolism: The sum of the processes by which a particular substance is handled biochemically by the body.

Microsome: One of the small, spherical vesicles derived from a cell structure (the endoplasmic reticulum) during isolation of cell-free extracts. It does not exist as such in the undisrupted cell.

Microsurgery: Surgical procedures performed under the magnification of special surgical microscopes.

Mutagen: Any agent that induces mutations.

Mutagenic: Causing mutation.

Mutation: A change in the genetic material that is perpetuated in subsequent divisions of the cell in which it occurs.

Neomort: The body of a recently deceased person.

Nervous system: The body's system involved in transmitting nerve impulses from receptor organs, such as the eye, to the brain (or spinal cord), where they are interpreted; this may result in a response that consists of a nerve impulse being transmitted to an effector, such as the hand.

Ophthalmic: Relating to the eye.

Pathological: Resulting from disease.

Physiology: The study of the normal, vital processes of animals and plants, such as respiration.

Postmortem: After death, referring to examinations of corpses.

Replication: The act of repeating a process or observation; in genetics, referring to the duplication of genetic material that precedes cell division.

Spinal reflex: An involuntary movement in response to stimulation to the body's surface and transmitted to the spinal cord.

Statistics: A discipline that deals with techniques for designing research and for analyzing and drawing conclusions from the resulting data.

Structure/activity relationship (SAR): A mathematical model that relates the structure of a series of chemicals to their activity, such as toxicity.

Teratogen: Any agent that induces abnormal development, particularly malformations.

Teratogenic: Causing abnormal development.

Toxicity: The state of being toxic or poisonous.

Vertebrate: The taxonomic name for animals with backbones, namely, fishes, amphibians, reptiles, birds, and mammals.