Alternatives to Laboratory Animals: Definition and Discussion

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ALTERNATIVES TO LABORATORY ANIMALS

Definition and Discussion

By
ANDREW N. ROWAN

The Institute for the Study of Animal Problems
Washington, D.C.
Institute for the Study of Animal Problems

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TABLE OF CONTENTS

PREFACE

ALTERNATIVES: A DEFINITION

ANIMAL USE IN BIOMEDICAL PROGRAMS
   A) Biological and Medical Education
   B) Toxicity Testing
   C) Original and Applied Research

WHAT ARE THE ALTERNATIVES
   A) Physico-Chemical Techniques
   B) Culture of Single-Celled Organisms
   C) Mathematical and Computer Modeling
   D) Tissue Culture
   E) Clinical and Epidemiological Studies
   F) Other Techniques

NUMBERS OF ANIMALS USED

ANALYSIS OF TWO ALTERNATIVES
   A) Development and Production of Polio Vaccines
   B) The LD50 Test

CONCLUSION

REFERENCES

SELECTED BIBLIOGRAPHY
PREFACE

The origins of the concept of “alternatives” to the use of animals in research may be traced back to the 1800's and the furore about using live animals in surgical and other experiments. Some of the animal protection societies in England were prepared to accept animal experimentation provided it was performed under anesthesia. Even Dr. Marshall Hall, who championed the spread of experimental medicine in 19th century England, considered it necessary to control and prevent unwarranted, inept and cruel experimentation (French, 1975). The concept of alternatives has developed in the 20th century to encompass not only the reduction (prevention) of painful experimentation, but also the reduction and/or total replacement of animal use in research (Russell & Burch, 1959).

This booklet is a review of the scientific and technical aspects of “alternatives”. It does not attempt to debate the ethical questions surrounding animal experimentation nor provide a catalogue of cases of animal research. It provides a relatively brief introduction to the potential and limitations of the concept of alternatives—a concept which could ultimately lead to the resolution of the long-standing conflict between those opposed to painful experiments and those seeking to improve human and animal health.

ALTERNATIVES: A DEFINITION

As applied to the use of animals in laboratory programs, an alternative is any system or method which covers one or more of the following:

- **Replacing** the use of laboratory animals altogether;
- **Reducing** the number of animals required;
- **Refining** an existing procedure or technique so as to minimize the level of stress endured by the animal.

These have been described as the three R’s (Russell & Burch, 1959) and in combination make up the approach covered by the term “alternatives”. In addition, an alternative must provide information or results which allow the researcher to draw the same conclusions with at least the same degree of confidence. Thus, if one has an animal system which detects cancer-causing agents with 90% certainty, then any proposed alternative system should be able to do at least as well. Each of the three aspects is discussed in greater detail below.

A) Replacement

Complete replacement of laboratory animal use occurs relatively infrequently. The field of vitamin research is one area in which replacement has taken place. Many of the early animal assay systems (used to measure the quantity of the vitamin in the experimental sample) have now been replaced by physico-chemical methods. For example, it is now possible to replace the biological (animal) assay systems for vitamins A, D and E by physico-chemical techniques (Wiggins, 1976).

B) Reduction

There are many ways to effect a reduction in the number of laboratory animals being used and, therefore, this aspect of alternatives shows the greatest potential. First, a carefully designed experiment could reduce the demand for laboratory animals. For example, a study in 1961 by an independent statistics consultancy showed that the number of animals used in a randomly selected sample of published research reports could have been reduced by 25-43% with no loss of statistical validity (HSUS, 1962). The analysts confined their study solely to the question of sound statistical design and analysis. Second, the researcher should be assured before starting the experiment that the problem being investigated is worth solving and that the experiment will not involve unnecessary duplication of earlier work. Third, the research should ensure that the animal model proposed for the study is the most appropriate one available. The selection should be based on such factors as availability, level of sentience (where possible) and the potential relevance of the results.

Finally, the number of laboratory animals used may be reduced by carrying out
studies as far as possible in non-sentient systems. Scientists have made notable advances, enabling some research problems to be investigated in vitro (literally meaning in glass), which have led to a reduction in the demand for research animals. For example, the United States was importing approximately 150,000 rhesus monkeys a year for the development and production of the polio vaccine in the late 1950's (Conway, 1965). The demand has now been reduced to approximately 6,000 monkeys per annum (Rowan & LeCornu, 1978). (See page 16 for a more detailed discussion.)

C) Refinement
Stress (pain and suffering) is an undesirable factor (from both scientific and humanitarian view points) in animal experiments unless the research is specifically concerned with stress or its effects. In addition, stress may occur during housing of the animal before and after the actual experiment. Any refinement in research techniques or standards of husbandry which could reduce stress will, therefore, qualify as an alternative. For example, the British Pharmacopoeia Addendum (1977) specifies a paralytic rather than lethal endpoint (British Pharmacopoeia, 1973) for the tetanus antitoxin potency test. The paralytic endpoint involves only a mild paralysis of the hind-limb of the mouse and this is considerably less stressful than determination of the lethal dose.

ANIMAL USE IN BIOMEDICAL PROGRAMS

The scope for developing and using alternatives varies according to the objectives of the particular laboratory activity using animals. For example, the arguments about the use of animals in an educational exercise will have different emphasis from those for a cancer research project. Laboratory animal use is, therefore, divided into three categories—education, toxicity testing and safety evaluation, and the generation of new facts and testing of theories.

A) Biological and Medical Education
At least 3 million animals per annum are used in the United States for secondary school and university educational purposes (ILAR, 1970a), including frogs in high school biology classes and pound dogs for practice surgery in medical and veterinary curricula. There is little objective evidence that manipulative exercises on live animals are necessary in many of these educational courses. Certainly, all animal experimentation involving significant intervention could be excluded from high school and many undergraduate programs without jeopardizing the development of critical, yet imaginative, scientific intellects nor the flow of young scientists into research laboratories.

B) Toxicity Testing
Toxicity testing determines whether chemicals are ‘safe’ for general human use or the ‘safe’ limits of use for chemicals known to be hazardous. Tests are carried out on a wide range of chemicals and biological substances (including drugs, vaccines, pesticides, food additives, industrial chemicals, cosmetics and household products) and involve millions of animals every year. Standard toxicity tests for new drugs include procedures to determine the toxic effects of a single dose (acute toxicity), procedures to determine the effects of many repeated doses (chronic toxicity) and procedures to detect certain specialized effects such as the induction of genetic damage, the induction of cancer or the induction of deformities in the developing fetus. The design of an overall toxicity protocol will depend on the proposed use of the new substance and may incorporate only a few tests. The cost of testing varies from about $20,000 per substance (using a limited range of tests and short-term screens) to over $1 million for an in depth toxicological evaluation.

There are a number of problems involved in attempting to promote the development of alternatives for toxicity testing programs. First, relatively little is known about the mechanisms underlying toxic reactions in mammalian systems and it is, therefore, difficult to design non-animal tests which reliably mimic the human response. Second, despite the many technical shortcomings of animal testing which is crude, cumbersome and expensive, many of the techniques have become entrenched in regulatory guidelines throughout the world. The resulting ‘bureaucratic inertia’ discourages innovation. Third, consumer groups are becoming more vocal about the unknown hazards represented by the many industrial chemicals in our environment and are consequently pressuring government agencies to widen the scope of toxicity testing (usually involving more intensive testing on more animals). Partly as a result of the burden which these increased demands have placed on toxicity testing programs, steps are now being taken to develop, validate and apply alternative systems which are less expensive and more efficient.

Several years ago, a group of toxicologists estimated that a suitable battery of short-term tests, involving fewer animals than the present procedures, could result in a 10-fold reduction in cost and 5-fold reduction in time. Furthermore, they argued that these tests would involve little or no sacrifice of safety (Muul et al, 1976).

C) Original and Applied Research
A simplified description of the ‘scientific method’ used in research involves the formation of an hypothesis or theory, the deduction of certain consequences from this hypothesis, the design of an experiment to test these deductions and then the acceptance, modification or rejection of the hypothesis depending on the results of the experiment. However, the theoretical basis for and standards of critical analysis and research vary widely from one project to another. For example, Bernard Dixon, editor of New Scientist, stated:

"Where the blind empiricist will stumble from one question to the next, and the scientist with the accountant mentality will deploy a massive induction-based experiment (which may somehow throw up the result he is looking for), the top-class creative thinker designs a single, crucial
It is not possible to legislate the lack of insight out of existence or to draw up regulations which will abolish all mistakes, but more could be done to reduce the amount of wasteful research involving animals. Universities could institute lecture courses and seminars on alternatives in order to raise the general level of consciousness among students of biomedical and animal research.

The induction-based research mentioned in the quotation refers to an approach which is widespread in the pharmaceutical industry—namely, the screening of thousands of chemicals in the hope that one or more will have therapeutic properties. The researcher is guided by theoretical considerations to a relatively limited extent and will try anything which is not patently unreasonable if there is some likelihood of success. A standard technique for investigating chemicals for drug development involves designing a screening system using an animal model of the human disease. Thousands of chemicals are then tested to determine if they have any effect on the disease. In a number of cases, it is possible to develop and use a non-animal system as a preliminary screen before proceeding on to the animal model. The number of negative tests conducted in an animal system, and the overall number of animals required, will be considerably reduced. As an added advantage, alternatives are often less expensive and more efficient when used in screening systems. For example, one pharmaceutical company which has taken advantage of this feature now uses animals for only about 30% of its drug screening program as opposed to about 80% ten years earlier (Spink, 1977).

WHAT ARE THE ALTERNATIVES?

There are many techniques which can be used to reduce the demand for laboratory animal experimentation, some of which are briefly described below. Tissue culture, the most important technique for the alternatives concept, is discussed in more detail.

A) Physico-Chemical Techniques

The development of new physico-chemical techniques has played an integral role in the advancement of biomedical knowledge of the structure and function of living organisms. These techniques are applied to analyze the physical and chemical properties of biochemicals. For example, mass spectrometry and gas-liquid chromatography are very sensitive techniques for separating, identifying and measuring the amounts of individual substances in complex biological mixtures. The improvements provided by the increased sensitivity and analytic power of these new techniques means that animals can, in some cases, be replaced altogether or the numbers of animals required can be reduced. For example, until recently, the vitamin D content of biological samples was measured by the “anti-rickets” assay using young rats. Now the vitamin D content can be assessed accurately using gas chromatography and mass spectrometry (Wiggins, 1976).

B) Culture of Single-Celled Organisms

Bacteria and protozoa are free-living organisms that can be cultured in the laboratory relatively easily and used as models of basic life processes. For example, bacteria have been used extensively in the study of the basic mechanisms involved in the replication of genetic material and proteins. They have also been used in nutritional research and there are a variety of bacterial and protozoal systems which can be used to assay vitamin concentrations. Perhaps the most striking example of the potential of bacterial systems in the development of alternatives is their use in the Ames test to detect potential mutagenic (damaging the genetic material) and carcinogenic (inducing cancer) substances (McCann & Ames, 1976).

The Ames test employs Salmonella bacteria to detect substances which cause mutations in the genetic material. Because thousands of millions of bacteria are used to test each substance, very low mutation frequencies can be detected. The test is therefore very sensitive. In addition, the test also employs mammalian animal tissue extracts to detect “hidden mutagens”, as foreign chemicals can be altered by animal body processes (i.e., metabolized) thereby changing chemical’s biological activity. It has been shown that there is a high degree of correlation between substances which cause mutations in the Ames test and substances which cause cancer in animals and therefore a positive result in the Ames test gives a preliminary indication that a substance may be a cancer-causing agent. It should be noted that the Ames test could not alone replace the use of animals for carcinogen testing, but it is possible that a suitable battery of short-term tests (including the Ames test) could, in time, be developed “to mimic human response far more accurately than animal testing” (Hutt, 1978).

C) Mathematical and Computer Modeling

Computer modeling is a valuable tool in biomedical research and could be applied more widely than it is at present (Harrison & Harrison, 1978). However, the technique has not been fully exploited because relatively few biomedical research workers have the necessary training. Computer scientists, on the other hand, tend to use biological systems to test or develop some aspect of computer science rather than developing a representative and meaningful model of a biological process.

A model is a simplified representation of the system under investigation and is a useful device for studies that involve altering several variables at the same time. Computer and mathematical models are based on the use of equations of varying degrees of complexity to represent biological phenomena. Utilization of sophisticated modern computers permits the investigator to employ complicated mathematical functions in the construction of viable and detailed models of biological systems. These computer models can then be used to investigate
problems in a manner not possible in the living animal or to reduce the number of animals required for a particular series of experiments by identifying promising avenues of research. Computer models cannot totally replace animal experimentation since results obtained from these simplified models will have to be checked by experiments on the far more complex living systems (further details are available in Newton, 1977).

D) Tissue Culture

The terms used in tissue culture have given rise to some confusion and, therefore, before discussing its potential as an alternative, the technique is described and some of the terms defined.

An organ of the body, such as the kidney, is composed of thousands of millions of cells which are provided with nutrients and a waste-disposal system via the bloodstream. In cell and tissue culture, pieces of tissue or separated cells are grown in a suitable sterile container in an appropriate nutrient medium. This medium is replaced at regular intervals to remove waste products and to replenish the supply of nutrients.

**Organotypic culture:** An organ is cut into small pieces, usually no more than a few cubic millimeters in volume, and placed in contact with an appropriate nutrient medium. One organ can provide numerous experimental samples and, hence, a large number of studies can be conducted using material from a single animal. Since the organ segments retain many of the properties of the intact organ, the response to various external stimuli can be investigated in a meaningful manner.

**Cell culture:** Individual cells are separated by mild enzymic digestion and then grown in suitable containers. **Primary cell culture** refers to the first cultivation of cells obtained from fresh tissue. The culture will consist of a mixture of cell types which may pass through several division cycles before the cells fill the container and stop growing. At this point, if the culture is to be continued, the cells must be divided into smaller samples and seeded into new containers with fresh nutrient medium. When a primary culture is subcultured it is known as a **cell line**. Cell lines may have either a finite or an infinite life-span. Infinite cell lines are commonly derived from tumors and the research worker can produce as much material as is needed merely by continuously subculturing the cells. One cell line, known as HeLa, was derived from a human cervical tumor in 1953 and is still extensively used for cell culture research.

Cell lines with a finite life-span have been developed from a variety of sources although one of the best known is the WI38 cell line developed from human embryonic lung tissue in the early 1960's (Hayflick & Moorhead, 1961). Although, this cell line has a finite life-span, enormous quantities of material have been produced from a relatively small starting sample and extensively used in polio and other vaccine production throughout the world. Stocks are now beginning to run out and the WI38 cell line is being replaced by other similar human cell lines. Cell lines can also be developed from a single cell, a process known as cloning.

The advantages of cell culture include: 1) The ability to grow large quantities of standardized material; 2) The ability to study living material under well-defined conditions; 3) The relatively compact and inexpensive storage and handling facilities required (in comparison with laboratory animals). The disadvantages include the fact that: 1) When cells are subcultured, they tend to lose some of the specific properties which they had in the body; 2) The fact that whole body metabolism is, at best, only partially mimicked in cell culture.

Virus research is an area where tissue culture has been extensively exploited with a consequent reduction in demand for laboratory animals. For example, in 1963, a pharmaceutical company required approximately 16,000 mice per annum to screen 1,000 substances for their potential as antiviral agents. Twelve years later, after the introduction of a cell culture system as a primary screen and an organ culture system as a secondary screen, they were using approximately 1,600 mice while investigating 22,000 substances every year (Bucknall, 1979). However, tissue culture techniques have not totally replaced the use of mice which are still required for the final screening.

E) Clinical and Epidemiological Studies

Human beings are already extensively used as an experimental animal. For example, new drugs which have passed the animal testing stage are tested on selected human volunteers because one cannot extrapolate from animals with complete confidence. In addition, much valuable information on disease processes can be obtained from clinical case studies and autopsy reports. Any researcher wishing to use a human subject must satisfy strict ethical guidelines. However, it is still possible to conduct both ethical and meaningful research with human volunteers (Turner, 1975).

Another source of data on human response to disease or hazardous agents is provided by epidemiological studies. Epidemiology is based on clinical observations coupled with data on associated environmental factors to establish possible causal links between a particular environmental factor and an abnormal syndrome or disease. However, epidemiological studies cannot replace animal experiments although they can guide the research worker in promising directions and thus reduce the use of animals in fruitless projects (Alderson, 1977).

F) Other Techniques

There are a number of other techniques or systems which may be used to reduce the use of animals in biomedical programs. For example, animals are sometimes used in crash studies or in studies on the effects of certain types of injury such as burns. Mechanical models can replace animals for many of these studies and a variety of systems are available. Thermoman is a human simulator to test potential burn risks with different garments (Tickner & Bendler, 1974) and General Motors Corporation has designed and developed an artificial neck for car crash simulation tests (Landkof & Distefano, 1975).
NUMBERS OF ANIMALS USED

The total number of laboratory animals used throughout the world is estimated at 200 to 225 million per annum. The majority of these are rodents, with mice and rats accounting for approximately 85% of the total. The other important laboratory animals are guinea pigs, hamsters, rabbits, chickens, frogs, dogs, cats and non-human primates.

The number of laboratory animals used throughout the world in various countries around the world is presented in Table 1. The data has been compiled from a number of sources, including Russell & Burch (1959), surveys prompted by the International Committee on Laboratory Animals (ICLA, 1959 & 1962) and Tajima (1975). As can be seen, the use of laboratory animals increased steadily during the 1970’s. In 1966, W.B. Saunders and Co., an economic consultancy, estimated that 60 million animals were used in the United States during 1965 as follows (Anonymous, 1966):

- Mice: 36.84 million
- Rats: 15.66 million
- Guinea pigs: 2.52 million
- Hamsters: 3.30 million
- Rabbits: 1.65 million
- Exotic animals: 0.12 million

Between 1970 and 1975, the laboratory animal trade expanded further and dealers now estimate the current demand at 50 million mice, 20 million rats and about 30 million other animals, including about 200,000 cats and 450,000 dogs. Apparently, the level of the trade has now stabilized.

Surveys of laboratory animal use rarely provide a figure for total demand. For example, the Institute for Laboratory Animal Resources (ILAR) conducted a number of surveys between 1965 and 1971 (Table 2) [ILAR, 1966, 1968, 1970b, 1972]. Questionnaires were distributed to both animal breeders and animal users and the number of completed forms returned varied considerably from year to year. It is not possible to provide a reliable estimate of the 100% total of animals used by employing the appropriate correction factor (for example, multiplying the total for 1967 by two).

The most reliable figures on the growth of animal experimentation come from the United Kingdom. Table 3, which is compiled from figures given in Russell and Burch (1959) and from returns to the Home Office (which controls animal experimentation in Britain), gives a breakdown of animal use from the years 1952 to 1976. The number of animals used has increased by a factor of 2.5 since 1952 and much of this increase can be accounted for by the growth in the “other” research category which does not include cancer research, animals used to diagnose diseases, and mandatory testing of products. (Mandatory testing of products only covers the testing of biologicals, such as vaccines and hormones, and a few tests on drugs and other chemicals. It does not include the toxicity testing conducted to establish the safe limits of use of a new drug.) The growth in the “other” category parallels the growth of animal experimentation in the commercial sector and this correlation is probably not entirely fortuitous.

### Table 1: Numbers of Laboratory Animals Used in Various Countries ('000's)

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Approx. Total</th>
<th>Year</th>
<th>Approx. Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>1957</td>
<td>400</td>
<td>1971</td>
<td>802</td>
</tr>
<tr>
<td>Austria</td>
<td>1957</td>
<td>120</td>
<td>1976</td>
<td>805†</td>
</tr>
<tr>
<td>Belgium</td>
<td></td>
<td></td>
<td>1969</td>
<td>583</td>
</tr>
<tr>
<td>Canada</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Czechoslovakia</td>
<td>1959</td>
<td>910</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denmark</td>
<td>1956</td>
<td>250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td>1956</td>
<td>140</td>
<td>1971</td>
<td>165</td>
</tr>
<tr>
<td>France</td>
<td>1956</td>
<td>1,250</td>
<td>1970</td>
<td>4,420</td>
</tr>
<tr>
<td>Germany (West)</td>
<td>1957</td>
<td>1,400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Holland</td>
<td>1957</td>
<td>660</td>
<td>1977</td>
<td>3,000‡</td>
</tr>
<tr>
<td>Iceland</td>
<td>1956</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>1956</td>
<td>270</td>
<td>1970</td>
<td>1,066</td>
</tr>
<tr>
<td>Israel</td>
<td>1958</td>
<td>350</td>
<td>1969</td>
<td>545</td>
</tr>
<tr>
<td>Italy</td>
<td>1959</td>
<td>320</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>1956</td>
<td>1,600</td>
<td>1970</td>
<td>13,155</td>
</tr>
<tr>
<td>Norway</td>
<td>1956</td>
<td>21</td>
<td>1971</td>
<td>93</td>
</tr>
<tr>
<td>Poland</td>
<td>1959</td>
<td>910</td>
<td>1967</td>
<td>385</td>
</tr>
<tr>
<td>Sweden</td>
<td>1956</td>
<td>170</td>
<td>1970</td>
<td>875</td>
</tr>
<tr>
<td>Switzerland</td>
<td>1956</td>
<td>800</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turkey</td>
<td>1957</td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>United Kingdom</td>
<td>1956</td>
<td>2,500</td>
<td>1977</td>
<td>5,386</td>
</tr>
<tr>
<td>United States</td>
<td>1957</td>
<td>18,000</td>
<td>1970</td>
<td>51,000</td>
</tr>
</tbody>
</table>

† Adamiker (1977)
TABLE 2: Laborator y Animal Use in the United States

<table>
<thead>
<tr>
<th>Year</th>
<th>Questionnaires Distributed</th>
<th>Returned</th>
<th>(%)</th>
<th>Total No. of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1965</td>
<td>2323</td>
<td>918</td>
<td>(39.5)</td>
<td>36,537,407</td>
</tr>
<tr>
<td>1967</td>
<td>2718</td>
<td>1366</td>
<td>(50.3)</td>
<td>42,875,443</td>
</tr>
<tr>
<td>1969</td>
<td>3240</td>
<td>2258</td>
<td>(69.7)</td>
<td>52,096,390</td>
</tr>
<tr>
<td>1971</td>
<td>3000</td>
<td>1834</td>
<td>(61.1)</td>
<td>44,160,192</td>
</tr>
</tbody>
</table>

The United Kingdom has just revised the forms on which animal users send in their returns and now requires a far more detailed breakdown of the procedures carried out on laboratory animals and the broad purpose of these studies (Tables 1 and 2) [Home Office, 1978].

TABLE 3: Number of Laboratory Animals Used in the UK (000's): Research Areas and Research Facilities

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL</td>
<td>2,120</td>
<td>4,042</td>
<td>5,327</td>
<td>5,475</td>
</tr>
</tbody>
</table>

A) Research Area

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>202</th>
<th>203</th>
<th>216</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandatory Testing of Products</td>
<td>811</td>
<td>1,214</td>
<td>1,073</td>
<td>1,263</td>
</tr>
<tr>
<td>Cancer Research</td>
<td>96</td>
<td>259</td>
<td>397</td>
<td>444</td>
</tr>
<tr>
<td>Other</td>
<td>1,011</td>
<td>2,366</td>
<td>3,641</td>
<td>3,678</td>
</tr>
</tbody>
</table>

B) Research Facilities

<table>
<thead>
<tr>
<th>Government</th>
<th>400</th>
<th>815</th>
<th>1,012</th>
<th>996</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other Non-Commercial</td>
<td>520</td>
<td></td>
<td></td>
<td>944</td>
</tr>
<tr>
<td>Commercial</td>
<td>1,200</td>
<td></td>
<td></td>
<td>3,535</td>
</tr>
</tbody>
</table>

TABLE 4: Laboratory Animal Use in the United Kingdom in 1977

<table>
<thead>
<tr>
<th>Animal</th>
<th>Study of Normal &amp; Abnormal Function</th>
<th>Development and Testing of Substances</th>
<th>Therapeutic Agents</th>
<th>Other Purposes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>710,316</td>
<td>1,892,073</td>
<td>25,097</td>
<td>8,407</td>
</tr>
<tr>
<td>Rat</td>
<td>356,744</td>
<td>903,895</td>
<td>9,133</td>
<td>1,423</td>
</tr>
<tr>
<td>Other Rodents</td>
<td>39,133</td>
<td>117,999</td>
<td>23,621</td>
<td>7,029</td>
</tr>
<tr>
<td>Rabbit</td>
<td>25,097</td>
<td>130,652</td>
<td>25,097</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>5,188</td>
<td>2,537</td>
<td>5,188</td>
<td>1,021</td>
</tr>
<tr>
<td>Dog</td>
<td>3,423</td>
<td>8,407</td>
<td>3,423</td>
<td>1,021</td>
</tr>
<tr>
<td>Primate</td>
<td>1,820</td>
<td>5,122</td>
<td>1,820</td>
<td>1,021</td>
</tr>
<tr>
<td>Other Vertebrates</td>
<td>144,255</td>
<td>271,903</td>
<td>144,255</td>
<td>58,724</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1,286,016</td>
<td>2,832,587</td>
<td>1,286,016</td>
<td>76,424</td>
</tr>
</tbody>
</table>

* Includes experiments in which the animal was used for more than one of the purposes specified in the original tables.
** Includes 81-301 experiments for which a detailed analysis is not available.
**TABLE 5: Laboratory Animal Use in the United Kingdom in 1977: Animals in Toxicity Testing.**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Study of Normal &amp; Abnormal Structure &amp; Function</th>
<th>Development and Testing of: Therapeutic Products</th>
<th>Other Substances</th>
<th>Other Purposes*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Toxicity Tests</td>
<td>53,647</td>
<td>290,629</td>
<td>121,984</td>
<td>93,657</td>
<td>559,917</td>
</tr>
<tr>
<td>Sub-acute &amp; Chronic Toxicity Tests</td>
<td>12,547</td>
<td>134,799</td>
<td>58,460</td>
<td>22,310</td>
<td>228,124</td>
</tr>
<tr>
<td>Distrib. &amp; Metabolism Studies</td>
<td>44,896</td>
<td>90,392</td>
<td>11,560</td>
<td>13,911</td>
<td>160,759</td>
</tr>
<tr>
<td>More than one of Above</td>
<td>5,643</td>
<td>7,756</td>
<td>26,795</td>
<td>14,161</td>
<td>54,355</td>
</tr>
<tr>
<td>TOTALS:</td>
<td>116,733</td>
<td>523,576</td>
<td>218,799</td>
<td>144,039</td>
<td>1,003,155</td>
</tr>
</tbody>
</table>

*Includes experiments in which the animal was used for more than one of the purposes specified in the original tables.

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**TABLE 6: Laboratory Animal Use in the United Kingdom in 1977: Organizations and Objects of Experiments**

<table>
<thead>
<tr>
<th>Organizations</th>
<th>Diagnosis</th>
<th>Dev. of Therapeutics</th>
<th>Legislative Requirements</th>
<th>Other Testing</th>
<th>Other Exper.</th>
<th>Unclassified</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univ., Med. Sch. &amp; Polytechnics</td>
<td>66,710</td>
<td>110,235</td>
<td>5,874</td>
<td>3,808</td>
<td>696,259</td>
<td>25,463</td>
<td>908,349</td>
</tr>
<tr>
<td>Govt. &amp; Quasi-Govt. Organ.</td>
<td>43,958</td>
<td>185,237</td>
<td>45,650</td>
<td>12,630</td>
<td>343,243</td>
<td>9,583</td>
<td>640,301</td>
</tr>
<tr>
<td>Hospitals &amp; Publ. Hlth. Labs.</td>
<td>53,709</td>
<td>28,677</td>
<td>7,378</td>
<td>13</td>
<td>124,524</td>
<td>3,975</td>
<td>218,276</td>
</tr>
<tr>
<td>Other Non-Profit Organ.</td>
<td>4,828</td>
<td>248,602</td>
<td>440,165</td>
<td>5,614</td>
<td>145,193</td>
<td>13,859</td>
<td>858,261</td>
</tr>
<tr>
<td>Commercial Organ.</td>
<td>1,790</td>
<td>1,513,995</td>
<td>943,055</td>
<td>103,042</td>
<td>170,055</td>
<td>28,451</td>
<td>2,760,388</td>
</tr>
<tr>
<td>TOTALS:</td>
<td>170,995</td>
<td>2,086,746</td>
<td>1,442,122</td>
<td>125,107</td>
<td>1,479,274</td>
<td>81,331</td>
<td>5,385,575</td>
</tr>
</tbody>
</table>

---
Tables 4 to 6 provide a relatively detailed breakdown of the types of experiments conducted on animals and the organizations involved. The various categories are as follows. The study of abnormal and normal structure and function would mainly fall under the category of basic research. The development and testing of therapeutic products would include efforts to discover new drugs, the subsequent development of such drugs and the testing of drugs and biological therapeutics such as vaccines and insulin. The development and testing of other substances includes cosmetics (a total of 11,695 animals in 1977), household products (9,463 animals in 1977) and industrial chemicals. In Table 5, the category of acute toxicity tests includes determinations of the LD50, the lethal dose killing 50% of the target population (see pg. 17 for more details of this test). About 20% of the animals used in 1977 were for toxicity testing. In Table 6, it is not clear which institutions qualify as non-profit organizations because Wellcome Laboratories, a major pharmaceutical entrepreneur in the United Kingdom, could possibly be classified as a non-profit organization under the terms of its incorporation. However, their animal experimentation is the same as that conducted by the commercial pharmaceutical companies.

The ‘diagnosis’ category in Table 6 is probably the same as that in Table 3. The development of therapeutics would refer almost solely to the discovery and development of new drugs and other therapeutic products. The safety testing of such substances would be included in the category of ‘testing to satisfy legislative requirements.’ Other testing refers to the safety testing of products such as cosmetics which are not regulated by a legislative document.

There are no comparable figures for the United States although the 1967 ILAR survey reported that government laboratories accounted for 35%, commercial laboratories for 39%, and non-profit organizations for 26% of the total laboratory animal demand (ILAR, 1970a). In Canada, government laboratories accounted for 12%, commercial laboratories for 27% and non-commercial laboratories for 61% of the total demand in 1972-1973 which totaled 2.84 million animals (CCAC, 1974). The differences between the UK, USA and Canada reflect the differences in commercial investment and government support for biomedical research in each country.

The use of laboratory animals has increased steadily throughout the world since 1945 but there have been some signs of a reversal of this trend. Alternative techniques are being used more widely and interest in them will not doubt continue to grow as technical difficulties are overcome. For example, in the late 1960's, Sir Peter Medewar (a Nobel Laureate in Medicine) stated:

"The use of experimental animals in laboratories to enlarge our understanding of nature is part of a far wider exploratory process, and one cannot assess its value in isolation - if it were an activity which, if prohibited would deprive us only of the material benefits that grow directly out of its own use. Any such prohibition of learning or confinement of the understanding would have widespread and damaging consequences, but this does not imply that we are forevermore, and in increasing numbers, to enlist animals in the scientific service of man. I think that the use of experimental animals on the present scale is a temporary episode in biological and medical history, and that its peak will be reached in ten years time, or perhaps even sooner. In the meantime, we must grapple with the paradox that nothing but research on animals will provide us with the knowledge that will make it possible for us, one day, to dispense with the use of them altogether" (Medewar, 1972).

There are signs that we have reached the peak mentioned by Medewar, but it is not clear what the effects will be of the new laws on regulating toxic substances (e.g., the Toxic Substances Control Act in the USA and the Health and Safety at Work Act in the UK). These Acts could result in a large increase in the use of animals to test the 65,000 chemicals in common use already and the 1,000 being added every year. On the other hand, increased consumer pressure for safer products could provide a stimulus to the development of more efficient (and less expensive) testing systems which would also reduce the number of laboratory animals required.

**ANALYSIS OF TWO ALTERNATIVES**

This booklet concludes with a discussion of the potential for alternatives in two areas—polio vaccine production and acute toxicity testing, specifically the LD50 test. A vast reduction in animal use has already been effected in polio vaccine production, but many animals are still being used needlessly for the determination of the LD50 of drugs and other chemicals.

**A) Development and Production of Polio Vaccines**

In the 1940's a major research effort was launched in the United States to develop an effective vaccine against poliomyelitis. The rhesus monkey was extensively used in initial studies on the polio virus as well as in production and testing once the vaccine had been developed. Initially, monkeys were used to determine the number and distribution of different polio virus types and in other basic research on the virus. Then in 1949, it was demonstrated that the polio virus would grow in cell cultures (Enders et al, 1949). As a result, many of the studies which previously had been conducted on live monkeys, could now be done using cell culture. This discovery also opened the way to the production of a vaccine and the demand for monkeys climbed spectacularly (Table 7) when large scale vaccine production started in 1955.

Between 1956 and 1960, the majority of the imported rhesus monkeys were utilized in the polio vaccine program and following this, increasing numbers were used in other research and testing areas. In 1977, the Interagency Primate Steering Committee (IPSC) estimated that 14,000 rhesus monkeys were required every year for biomedical programs, 6,000 of which were destined for vaccine production and testing (IPSC, 1977). This represents a considerable fall in demand from the 1956-1960 period. This fall has been caused (in part) by the development of improved production techniques. Since these improvements have reduced the demand for laboratory animals (in this case, rhesus monkeys) they can be termed 'alternatives' under the definition given at the beginning of the booklet.
The development of alternative techniques has contributed to the considerable reduction in the use of rhesus monkeys in polio vaccine production and testing. Although, further reductions are possible, such reductions will depend more on regulatory requirements and economics than on scientific factors.

B) The LD50 Test

LD50 is an acronym for Lethal Dose-50% and it indicates the amount of a substance which, when administered in a single dose to a group of animals, will result in the death of half of the group within 14 days. The LD50 of a wide variety of substances is determined as a matter of course, including drugs, industrial chemicals, household products and cosmetics. The measure is used by a number of regulatory authorities, including transport agencies who employ the LD50 to decide what set of safeguards should be applied to the transport of a particular chemical. The more toxic the substance, the more rigorous the standards demanded by the relevant agency. Determination of an LD50 figure is also the usual starting point for toxicological studies on drugs or other chemicals.


<table>
<thead>
<tr>
<th>5-year Period</th>
<th>Total Number</th>
<th>Number/Annunm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1951–1955</td>
<td>100,000</td>
<td>20,000</td>
</tr>
<tr>
<td>1956–1960</td>
<td>600,000</td>
<td>120,000</td>
</tr>
<tr>
<td>1961–1965</td>
<td>250,000</td>
<td>50,000</td>
</tr>
<tr>
<td>1966–1970</td>
<td>140,000</td>
<td>28,000</td>
</tr>
<tr>
<td>1971–1975</td>
<td>105,000</td>
<td>21,000</td>
</tr>
</tbody>
</table>

The improvements include the following: First, although the vaccine is still produced on primary kidney cell cultures obtained from the rhesus monkey, technical improvements over the year have led to a 5-fold increase in virus yield from one pair of kidneys. Second, human diploid cell lines have been developed to the stage where they are suitable for polio vaccine production and this has led to the elimination of the demand for monkey kidneys in some instances. Third, quality control measures and transport conditions have been steadily improved and this has reduced trapping, transport and quarantine losses and consequently reduced the number of monkeys imported (Rowan & LeCormu, 1978).

Further improvements could be made. For example, in most countries, each batch of vaccine is tested by both the manufacturer and the regulatory authority (the tests require 40 to 70 monkeys per batch of vaccine). It should be possible to establish quality control procedures which would require only one set of testing either by the manufacturer or the control authority and this would immediately reduce the demand for rhesus monkeys by one-half. In addition, the batch sizes could be increased by a factor of two to three which would further reduce the number of monkeys required to test a standard quantity of vaccine.

The LD50 test was developed by Trewan in 1927 as a system to assay the potency of various drugs which were prepared from natural substances (e.g. digitalis) and which could not (at that time) be assayed by other means. In order to produce a statistically valid figure for the LD50, large numbers of animals had to be used due to the all-or-nothing nature of the endpoint (the animal is either dead or alive). Today, the test usually involves a minimum of 60 animals, primarily mice or rats. As a bioassay, the LD50 test has some scientific rationale although great care has to be taken to ensure that the testing conditions do not change from one day to the next because even slight variations can have marked effects on the value. However, the LD50 is now widely used as a general indication of the toxicity of the chemical, a purpose for which the test is unnecessarily precise. This is the main criticism against the test—it uses too many animals in order to determine a precise estimate of the lethal dose and yet this figure can only be extrapolated to the human situation in a very rough manner. For example, if the LD50 figure of a compound is 100 mg (per kilogram bodyweight) for a mouse, it could easily be anywhere between 10 and 1,000 mg (per kilogram bodyweight) for a human being.

Detailed criticisms of the test can be found in an article by Morrison et al (1968). Given that the LD50 test is wasteful of animals, what can be used to replace it? In modern society, consumer pressure requires that the regulatory authority has some knowledge of both the acute (single dose) and chronic (repeated dose) toxicity of new chemicals. There are, at present, no non-animal systems which provide a reliable indication of the acute toxicity of a substance for man although a report on some interesting work in this area has recently been published (Autian & Dillingham, 1978). However, it is possible to determine the acute toxicity of a substance to a satisfactory degree of precision by the method developed by Deichmann & LeBlanc in 1943. They used six to ten animals to measure the Approximate Lethal Dose (ALD) and this is satisfactory for most of the needs currently filled by the LD50 determination (see Deichmann & Mergard, 1948 for a comparison). In addition, there is little need to conduct acute toxicity tests above a dose of about 2 gm/kg bodyweight because, if no toxic reactions can be detected at this dose, then the substance is likely to be 'safe' (that is, it will have an LD50 above 20 gm/kg).

Therefore, the number of animals required for acute toxicity testing could be reduced by determining the ALD instead of the LD50. The amount of stress suffered by the animals could be reduced by placing an upper limit of 2 gm per kilogram bodyweight on routine toxicity testing.

CONCLUSION

The present paper has not really addressed the ethical questions surrounding animal experimentation nor has it attempted to provide a catalogue of horror stories from the laboratories. Instead, it has attempted to introduce the reader to the concept of alternatives and its potential and limitations. This concept provides a constructive and realistic approach towards resolving the conflict between animal and human welfare in the laboratory.

A Selected Bibliography is included which lists a number of books and articles (with short critiques) for those interested in pursuing the subject further.
REFERENCES


Selected Bibliography

A selected bibliography has been included as an aid to those who wish to study the subject of animal experimentation and the alternatives in greater depth. The books and articles vary considerably in their approach and point of view. Some are written by academics for academics while others are written in a more popular style. All points of view are represented and the reader is advised to sample both the book by Ruesch (abolitionist) and the articles by Visscher (defending animal experimentation). Some of the books are available (or could be ordered) from the local bookstore, but others are out of print and can only be found in libraries. Most universities allow members of the public to use their library facilities for reference purposes and this may be the only means of obtaining copies of some of the references, especially if there is no convenient major public library in the area.

A) Ethics (cited alphabetically)


This book presents a detailed philosophical argument in favor of a more humane ethic. It is an excellent reference resource for the philosophy student.


This book contains essays from a number of contributors on man's ethical responsibilities toward man and animals.


This book contains a collection of writings from the Old Testament up to the present which argue for and against the position that animals have rights. One or two chapters specifically address the question of experimenting on animals.


The author discusses animal rights and uses animal experimentation as one of his examples. It is a lucidly written book and an excellent introduction to the subject.


This article defends the use of experimental animals.
B) History (cited according to historical chronology)

An article discussing Bernard's work and society's response to it (19th century).

This is an excellent history of the struggle over the 1876 Cruelty to Animals Act in the United Kingdom. Many of the insights developed by the author are still relevant today.

The book contains a chapter on vivisection and the humane movement's response to it.

This document is a record of the first testimony on animal experimentation heard by the U.S. Congress. It provides some insight into the perspectives of both the humane movement and the research community at the time.

This document provides additional details of the debate between the humane movement and the research community just before the passage of the Animal Welfare Act in 1966.

See also: Leavitt, E.S. in section C and Ryder, R. in Section D.

C) Legal and Legislative Aspects (cited alphabetically)

This book is an excellent reference and includes a chapter on the history of the Animal Welfare Act as well as outlines of important animal experimentation laws in foreign countries.

The author discusses 'alternatives' legislation.

This publication contains a description of the laws governing the use and care of laboratory animals in Canada, England, Holland and Scandinavia in addition to other useful reference material.

D) Animal Experimentation and Alternatives (cited according to publication chronology)

This was the first detailed exposition of 'alternatives'. Although the book is now out of print, the Animal Welfare Institute still has a few copies for sale. The authors discuss the three R's—replacement, reduction and refinement—and the concept of 'modeling'.

This book is out of print but it is worth trying to find a copy in the library since it is very well written. As the title implies, the author defends the use of animals in research.

This publication is a compilation of papers describing the benefits that animal experimentation has brought in the fields of nutrition, drug and vaccine development, surgery and animal care.

UFAW (1969) The Use of Animals in Toxicological Studies. UFAW: Potters Bar (see above for full address).
This publication contains a number of papers criticizing and justifying toxicity testing on animals.
UFAW (1971) *The Rational Use of Living Systems in Biomedical Research.*
UFAW: Potters Bar (see above for full address).
This publication contains several useful articles discussing drug development, vaccine production, cancer research, organ transplantation and ethical issues.

This book argues strongly against the use of experimental animals (especially in 'non-medical' research) and contains a lengthy chapter on the history of vivisection.

The author has compiled a wealth of useful information on painful experimentation and legislation concerning laboratory animals. It is lucidly written and is a recommended source book.

ATLA Abstracts, Volume 4 (1976) et seq. This publication appears twice a year and contains news items and review articles on alternatives in addition to abstracts of papers from the biomedical literature. It is published by FRAME, 312a Worple Road, London SW20 8QU and costs 40 US dollars a year. FRAME also produces other technical literature on the subject and should be contacted for additional information.

This book contains the proceedings of a symposium organized by the Institute for Laboratory Animal Resources in 1975 and contains useful information on cell culture and computer simulation. It is recommended as a reference work.

This is a well-written book discussing biomedical research and testing and the potential of alternatives. The author defines the term 'alternatives' as incorporating the elements of replacement, reduction and refinement but often uses it only in the complete 'replacement' sense.

This book caused widespread protest in Europe when it was published there. The author catalogues case after case of painful animal experimentation but does not place each experiment in its correct time-frame. Many of the cases report research conducted in the 19th century but the reader is left with the impression that such practices are still widespread today. Animals are still subjected to unwarranted experimentation, but Ruesch's case falls under its own weight.

E) Use of Animals in Schools (cited according to publication chronology)


Kelly, P.J. and Wray, J.D., Editors (1975) *The Educational Use of Living Organisms.* The English Universities Press: London [ca. $10.00].
This publication contains chapters on all aspects of the use of animals in schools.

Contains numerous ideas for school biology projects plus an extensive bibliography.

The article criticizes the use of animals in science fairs and is followed by a response from Dr. Thurmond Grafton of the National Society for Medical Research.