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The First Forty Years of the Alternatives Approach: Refining, Reducing, and Replacing the Use of Laboratory Animals

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CHAPTER

An updated version of "Looking Back Thirty-three Years to Russell and Burch: The Development of the Concept of the Three Rs (Alternatives)" (Rowan 1994)

Martin L. Stephens, Alan M. Goldberg, and Andrew N. Rowan

Introduction

The concept of the Three Rs—reduction, refinement, and replacement of animal use in biomedical experimentation—stems from a project launched in 1954 by a British organization, the Universities Federation for Animal Welfare (UFAW). UFAW commissioned William Russell and Rex Burch to analyze the status of humane experimental techniques involving animals. In 1959 these scientists published a book that set out the principles of the Three Rs, which came to be known as alternative methods. Initially, Russell and Burch's book was largely ignored, but their ideas were gradually picked up by the animal protection community in the 1960s and early '70s. In the '80s, spurred by public pressure, the alternatives approach was incorporated into national legislation throughout the developed countries and embraced by industry in Europe and America. Government centers devoted to the validation and regulatory acceptance of alternative methods were established during the '90s. By 2000 the use of animals in research had fallen by up to fifty percent from its high in the 1970s.

The Alternatives Approach in the Context of the Animal Research Issue

Animals have been used as experimental subjects in biomedical research, testing, and education during the last 150 to 200 years, but the practice began to burgeon in nineteenth century Europe. Alarmed by this increase, early critics of animal research challenged it from several perspectives. They argued variously that animal research was cruel and inhumane; unethical; and medically unproductive, unnecessary, or even misleading. Their criticism largely proved unpersuasive (French 1975; Turner 1980). Activism in the United States over animal research waned after World War I and remained at a low level until after World War II, when a new dimension in the animal research controversy emerged.

Spurred in part by advances in technological methods, animal protectionists began advocating *for* alternatives to laboratory animal use, not simply advocating *against* animal use or otherwise criticizing the status quo. These alternatives make up the Three Rs: methods that could *replace* or *reduce* laboratory animal use in specific procedures or *refine* such use so that animals experience less suffering. Sympathetic scientists joined in this more constructive approach; indeed, scientists themselves were the ones who first formulated the Three Rs concept. At the dawn of the twenty-first century, this approach is proving to be a powerful force in decreasing the use and distress of animals in experimental biology and medicine.

Table 1
Alternatives Chronology: 1876–1959

1876	Cruelty to Animals Act—the first law to specifically regulate animal experimentation—is enacted in Great Britain.
1927	The LD50 Test is introduced to standardize the potency of digitalis extract.
1938	The Federal Food, Drug, and Cosmetic Act is enacted, marking the first time a U.S. government agency is given the power to regulate consumer products.
1944	Eye irritancy testing is standardized as the Draize Test.
1954	Universities Federation for Animal Welfare (UFAW) establishes a committee to study humane techniques used in laboratory animal experiments.
1957	UFAW holds a symposium, "Humane Techniques in the Laboratory," at which William Russell presents a paper, marking the first time the Three Rs of replacement, reduction, and refinement are discussed in public.
1959	Russell and Burch's study is published as *The Principles of Humane Experimental Technique*, which develops the Three Rs approach at length.

Estimates of the numbers of research animals used annually in the United States and worldwide are highly speculative. The last official estimate for the United States was 17 to 22 million animals (U.S. Office of Technology Assessment 1986), but that study was conducted more than fifteen years ago. There is some evidence that this estimate was made during a period of declining animal use that began in the 1960s and continued into the '90s (Rowan et al. 1995). Consequently, the current figure could be lower. Worldwide animal use was estimated to be between 60 and 85 million animals in the early 1990s (Rowan 1995), but more conservative estimates of rodent use suggest a total of 40 million animals worldwide (D. Kawahara, personal communication with A. Goldberg 1998).

The 1950s: The Three Rs Approach Launched

The British scientists William Russell and Rex Burch formally launched the Three Rs with their book *The Principles of Humane Experimental Technique* (Russell and Burch 1959). However, hints of Russell and Burch's ideas had appeared in earlier discussions about the appropriate use of animals in research. Marshall Hall, a British experimental physiologist during the first half of the nineteenth century, proposed five principles for animal experimentation that would eliminate unnecessary and repetitive procedures and minimize suffering (Manuel 1987). Hall also recommended the use of phylogenetically "lower," less sentient, animals and praised the findings of a colleague who demonstrated that an animal that had just been killed could be substituted for a living one, thereby eliminating pain.

Fifty years after Hall set out his five principles, a short-lived research foundation—the Leigh Brown Trust—was established to promote and encourage scientific research without inflicting pain on experimental animals (French 1975). Although the Trust commissioned several publications in the 1890s, it never succeeded in developing a research program that convinced a significant proportion of the research community to adopt its principles. From 1900 to 1950, those who opposed the use of animals lost much of their political influence and were relegated to the fringes of political activity. As a result, little attention was paid to the ethical questions posed by the use of animals in research.

After World War II, interest in the animal research issue began to grow again. In the United States, newly formed animal protection groups began to criticize animal research practices. In England the Three Rs concept of alternatives began to emerge from the work of UFAW. UFAW published a handbook on the care and management of laboratory animals (Worden 1947) that was well received. This gave UFAW the confidence to address the more contentious topic of experimental techniques involving animals (as distinct from animal care). Accordingly, in 1954 Major Charles Hume (the founder of UFAW and its director at the time) established a committee to initiate a systematic examination of the progress of humane technique in the laboratory. Hume served as the committee's secretary, but it is noteworthy that the committee was chaired by Peter Medawar, a well-respected immunologist, and also included among its members William Lane-Petter, secretary of the Research Defence Society, an organization established to defend animal research. The committee employed William Russell (a zoologist) and Rex Burch (a microbiologist) to carry out the project (Hume 1962).

The exact origin of the Three Rs concept is not entirely clear (Russell 1995). In a 1959 talk, Hume indicated that Russell was the originator of the "Three Rs" concept (Hume 1962), while Russell (1995), in a retrospective paper entitled "The Development of the Three Rs Concept," credited Hume as our "inspiration and guide throughout." In that paper Russell recalled that the Three Rs concept evolved sometime between the sum-

Table 2
Alternatives Chronology: 1960–1969

1962	Lawson Tait Trust (UK) is established—the first research fund to support the scientific development of alternatives.
1963	The first edition of *The Guide for the Care and Use of Laboratory Animals*, written by the National Academy of Sciences, is published by the National Institutes of Health.
1965	Littlewood Committee Report (UK) concludes that little would be gained by paying special attention to alternatives.
1967	United Action for Animals is formed in the United States and later campaigns specifically for replacement alternatives.
1969	The Fund for the Replacement of Animals in Medical Experiments (FRAME) is formed in the United Kingdom to promote to the scientific community the idea of alternatives.
1969	Lord Dowding Fund (UK) is established to support alternatives research. Sir Peter Medawar correctly predicts the subsequent worldwide decline in animal use.

mer of 1955 and May 1957. The first recorded mention of the Three Rs was on May 7, 1957, at a meeting, "Humane Technique in the Laboratory," organized by UFAW and chaired by Medawar. Russell (1957) gave a presentation at this meeting in which he described the Three Rs. A brief proceedings (Anonymous 1957) was published later that year by the Laboratory Animals Bureau of the Medical Research Council. Many of the arguments and ideas presented by Russell and the other speakers later appeared in *The Principles of Humane Experimental Technique* (Russell and Burch 1959). See Table 1 for a chronology of these and other early developments.

It is noteworthy from an American perspective that the U.S.-based Animal Welfare Institute (AWI) provided financial support to Russell and Burch's project and that AWI's Christine Stevens made frequent visits to England to encourage their work (Russell 1995).

The 1960s: Dormancy

Although *The Principles of Humane Experimental Technique* has now become the classic text on alternatives, it received little attention when it was published in 1959 despite its promotion by UFAW in England and the AWI in the United States. There are several examples of the lukewarm reaction to the book within the scientific community. In *Nature*, a leading international science journal based in England, Weatherall (1959) commented:

> [It] is useful to have a résumé of ways which have already been adopted to make experimentation as humane as possible…[but the book] is not sufficiently informative to be used as guide either to details of experimental design or to the husbandry of experimental animals. Perhaps its chief purpose is to stimulate thought on both of these topics, and it is to be hoped it will succeed in doing so.

The British journal *Veterinary Record* (Anonymous 1959) commented that the book contained an important message and hoped that it would not be relegated "to the shelves merely for reference," but found the philosophy "somewhat difficult reading." The British medical journal *The Lancet* (Anonymous 1960) also found the book difficult going, noting that "its purpose is admirable, and its matter unexceptionable," but "it is not easy reading." It is not clear whether the tepid reviews reflected a general lack of interest in the topic or were a reaction to the book's arguments (a contemporaneous *Nature* review of a book that defended the use of animals [LaPage 1960] was, by contrast, full of praise).

LaPage's (1960) defense of animal research described the contributions of animal research to medical advance and mentioned Russell and Burch and the concept of the Three Rs only once, in a final chapter. He noted that distinguished scientists at a UFAW meeting

> discussed, among other things, how the numbers of laboratory animals used, and the numbers of experiments done on them, could be reduced, how their welfare could be improved, how the techniques used could be refined and how far, as Russell and Burch (1959) also discuss, animals could be replaced, for certain kinds of experiments at any rate.

After the initial book reviews and aside from the occasional mention of the idea of alternatives in the technical literature, the scientific community largely ignored Russell and Burch's book for nearly two decades. According to an analysis by Phillips and Sechzer (1989), the term "alternatives" did not appear in the scientific literature on the animal research issue in the 1960s, aside from a 1966 paper alluding to the concept.

During the 1960s, the animal protection community occasionally heeded Russell and Burch's 1959 call for alternatives (Table 2). In 1962 three leading antivivisection societies in the United Kingdom (the British Union for the Abolition of Vivisection, the National Antivivisection Society [NAVS], and the Scottish Society for the Prevention of Vivisection) estab-

lished the Lawson Tait Trust to encourage and support researchers who were not using any animals in their research. In 1967 United Action for Animals was established in the United States to promote alternatives, focusing on the principle of replacement. It's founder, Eleanor Seiling, spent many hours in the New York Public Library poring through scientific journals looking for examples of unnecessary animal research and of alternatives. However, she appears to have been a lone voice in the United States. By and large the animal protection literature of the 1960s did not pay much attention to the idea of alternatives.

Aside from these few examples of individuals taking up Russell and Burch's challenge in the years immediately following publication of their book, their ideas did surface directly or indirectly from time to time. In the early 1960s, the British Home Office set up a Committee of Inquiry into the workings of the 1876 Cruelty to Animals Act, chaired by Sir Sidney Littlewood. The Littlewood Committee report (1965) addressed the question of alternatives only briefly, but the mention at least indicated that the issue was beginning to be raised in public discourse. The Committee reported that it had

> repeatedly questioned scientific witnesses about the existence of alternative methods which would avoid the use of living animals. The replies have been unanimous in assuring us that such methods are actively sought and when found are readily adopted…Discoveries of adequate substitutes for animal tests have, however, so far been uncommon, and we have not been encouraged to believe that they are likely to be more frequent in the future" (paragraph 71).

The Committee accepted these arguments and concluded that the demand for the use of animals in biomedical research was likely to increase in the coming years and that the discovery of substitutes for animal tests was not likely to affect the demand for animal experimentation.

In the United States in the early 1960s, pressure from animal protectionists led to several congressional hearings on bills to regulate animal research. The printed record of the 1962 hearings is 375 pages long but apparently contains only one reference to Russell and Burch and none at all to alternatives (U.S. Congress 1962). The one reference to Russell and Burch came in testimony by Hume, still the director of UFAW, who had been flown to the United States to testify that the Cruelty to Animals Act (1876) was well regarded by British scientists. Also in 1962, The Humane Society of the United States (HSUS) published a booklet, *Animals in Research*, that alluded to the concept of reduction. The booklet reported the results of an analysis commissioned by The HSUS and carried out by Westat Research Analysts of the statistical approach used in published research papers (Anonymous 1962). The analysts concluded that the statistical design of the published studies was usually inadequate and that at least 25 percent fewer animals could have been used without altering the validity of the results.

Arguably the most significant development on the alternatives front during the 1960s was the establishment in 1969 of the U.K.-based charitable organization FRAME (Fund for the Replacement of Animals in Medical Experiments) to promote the concept of alternatives among scientists. Although small in size and influence in its early years, FRAME has become a powerful force for advancing alternative methods. Also in 1969 the U.K.-based NAVS set up the Lord Dowding Fund to support alternatives research. Both FRAME and the Dowding Fund were relatively well received by some popular science magazines (both the *New Scientist* and *World Medicine* praised the new, more scientific approach represented by the two organizations). Attitudes in the United States were more negative. A 1971 editorial in the *Journal of the American Medical Association* (Anonymous 1971) criticized FRAME in scathing terms, commenting that FRAME might be better named FRAUDS (Fund for the Replacement of Animals Used in the Discovery of Science).

By the close of the 1960s, Peter Medawar, the British scientist who had encouraged UFAW to undertake the Russell and Burch project, had won a Nobel Prize for his work in immunology and had been knighted by the British Crown. In a 1969 essay published a few years later, Medawar commented presciently on the prospects for alternatives and a decrease in animal use:

> The use of animals in laboratories to enlarge our understanding of nature is part of a far wider exploratory process, and one cannot assay its value in isolation—as 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"* (Medawar 1972, emphasis added).

Table 3
Alternatives Chronology: 1970–1979

1970	FRAME publishes *Is the Laboratory Obsolete?*, which outlines replacement methodologies such as computer modeling, tissue culture studies, and the use of lower organisms.
1971	Council of Europe Resolution 621 suggests that an alternatives database be established, the first significant government recommendation on alternatives.
	Bruce Ames of the University of California at Berkeley introduces a nonanimal test for detecting mutation-causing substances, later known as the Ames Test, using a bacterium.
1972	The Felix Wankel Prize (now 50,000 deutsche marks) for advancing the field of alternatives is offered for the first time.
1973	FRAME begins to publish *ATLA* (Alternatives to Laboratory Animals).
1975	The U.S. National Academy of Sciences holds the United States' first major scientific meeting on alternatives.
1977	The Netherlands Animal Protection Law includes a specific section on alternatives that has grown into a program in which the government provides the equivalent of hundreds of thousands of dollars to support alternatives research.
1978	FRAME hosts "Alternatives in Drug Development and Testing" at the Royal Society—Europe's first big scientific meeting on alternatives.
	David Smyth, president of the United Kingdom Research Defense Society—established to support animal research—publishes the first book examining alternatives since the publication of Russell and Burch's 1959 work.
1979	At the urging of United Action for Animals, the Research Modernization Act (H.R. 4805), which would redirect 30–50 percent of animal research funding to alternatives, is introduced in Congress.
	The Swedish government allocates $90,000 in funding for alternatives—the first government funding for alternatives.
	The Dutch Minister of Health states that the government supports the use of alternatives.

The 1970s: Animal Protectionists Heed the Call

During the 1970s, the alternatives approach became a key theme for the animal protection movement, which was growing in both size and political clout (Rowan 1989). The HSUS established a committee of experts on alternatives in the early '70s and later in the decade published a twenty-five page booklet on the subject (Rowan 1979). The political and scientific establishments also began to be drawn into the debate, as indicated by some selected events (Table 3). The first major political initiative on alternatives came in 1971 when the Council of Europe passed Resolution 621. This proposed, among other things, the establishment of a documentation and information center on alternatives and tissue banks for research. Deliberations on Resolution 621 did not begin until the late '70s, and the ensuing final Council of Europe Convention dropped some of the specific recommendations on alternatives. Instead, the Convention reflected the broad concern over animal research and made some rather general recommendations on alternatives.

In Europe a number of countries (for example, Denmark, the Federal Republic of Germany, the Netherlands, Sweden, and Switzerland) enacted animal research legislation that included specific support for alternatives. In Sweden the government established an advisory Central Committee on Experimental Animals to develop and promote alternatives and allocated the equivalent of $90,000 annually for the support of research on alternatives. This represented the first government funding for alternatives. In 1977 the Netherlands Animal Protection Law included a specific section on alternatives that has grown into a program in which the government provides the equivalent of hundreds of thousands of dollars to support alternatives research.

In the United Kingdom, FRAME began publishing *ATLA Abstracts* to identify articles in the scientific literature that focused on alternatives. While the journal had little impact when it was simply publishing abstracts, it started to include review articles in 1976 and then, early in the '80s, dropped the abstracts altogether and adopted its current format, which is centered on original articles. *ATLA* (Alternatives to Laboratory Animals) is now well enough established to be covered by the Science Citation Index.

In the United States, interest in alternatives grew slowly. By the mid-'70s, the term had entered the

Table 4
Alternatives Chronology: 1980–1989

1980 American activist Henry Spira launches the Draize campaign against the rabbit-based eye irritancy test.

As a result of the Draize campaign, Revlon gives a $750,000 grant to Rockefeller University to establish an alternatives research program.

The New England Antivivisection Society gives $100,000 for alternatives research on tissue culture, and a second animal-welfare consortium provides $176,000 for Chorio-Allantoic Membrane (CAM) test development.

1981 As a result of the Draize campaign, the cosmetics industry gives $1 million to Johns Hopkins University to establish the Center for Alternatives to Animal Testing (CAAT) (Avon and Bristol-Myers Squibb were the leading donors).

Swiss animal legislation specifically requires consideration of alternatives.

Zbinden and Flury-Roversi publish a critique of the LD50 Test.

1982 Colgate Palmolive provides $300,000 to investigate the CAM system.

CAAT holds its first symposium.

1983 Switzerland provides two million Swiss francs over two years for alternatives research.

The Food and Drug Administration (FDA) formally announces that it no longer requires data from the classical LD50 Test.

Utrecht University in the Netherlands establishes research and education programs directed towards further implementation of the Three Rs.

1984 FRAME receives £160,000 from the Home Office—the first UK government funding for alternatives research.

1985 The Health Research Extension Act is passed, requiring the NIH to develop a plan for alternatives.

Animal Welfare Act amendments are passed, requiring greater attention to alternatives to research techniques that cause pain and distress.

Index Medicus, an index of published biomedical studies, adds the subject heading "Alternatives to Animal Testing."

The European Research Group into Alternatives to Toxicity Testing (ERGATT) is formed.

The Soap and Detergent Association (USA) initiates the In Vitro Alternatives Program.

1986 CAAT and Bausch and Lomb sponsor a workshop on alternatives and acute ocular irritation testing.

The UK's Animals (Scientific Procedures) Act replaces the 1876 act.

The U.S. Congress's Office of Technology Assessment issues a landmark report, "Alternatives to Animal Use in Research, Testing and Education."

The Council of Environmental Ministers of the European Community enacts EC Directive 86/609, requiring that member countries develop legislation promoting the Three Rs.

An FDA survey reports a 96 percent decrease in the use of the classic LD50 tests in 1985 compared with the period 1975–1979.

Two new cell toxicology journals, *Toxicology In Vitro* and *Molecular Toxicology*, are established.

The Organization for Economic Cooperation and Development (OECD) announces changes in its guidelines for acute oral and dermal toxicity and starts to discuss alternatives.

British Industrial Biological Research Association (BIBRA) increases funding of alternatives research to £700,000 per annum.

The Industrial In Vitro Toxicology Society (IVTS) is established in the United Kingdom.

Federal Republic of Germany enacts new laws on animal protection requiring consideration of alternatives in animal research.

1987 The HSUS publishes an analysis of the historical importance of alternative methods in biomedical research awarded Nobel Prizes.

The Dutch Alternatives to Animal Experiments Platform is established with participation from government, industry, and animal welfare organizations.

In Vitro Toxicology: A Journal of Molecular and Cellular Toxicology is established.

The Swiss Foundation "Finanzpool 3 R" is established to support alternatives research with one million Swiss francs.

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Table 4
Alternatives Chronology: 1980–1989

1988	A government/industry workshop is held on alternatives in ocular irritancy testing, to review the Soap and Detergent Association's Alternatives Program.
	The Industrial In Vitro Toxicology Group holds its first meeting.
	The U.S. Republican presidential platform encourages the implementation of alternatives to animal testing.
	The J.F. Morgan Foundation for Alternatives Research is established in Canada.
	The Swiss government's Office for Animal Experiments and Alternatives is established.
1989	The Center for the Documentation and Evaluation of Alternative Methods to Animal Experiments, known by its German acronym ZEBET, is established in Germany.
	Procter and Gamble announces that it is contributing $450,000 per year for three years to its University Animal Alternative Research Program.
	Avon Products announces that it will no longer use the Draize Test.
	The Scandinavian Society for Cell Biology establishes the Multicenter Evaluation of In Vitro Cytotoxicity (MEIC) to assess alternatives to LD50 testing for acute toxicity.
	The Second International Conference on Practical In Vitro Toxicology is held in the United Kingdom.
	The Swedish Fund for Scientific Research without Animal Experiments invests 700,000 Swedish crowns in alternatives projects.
	The Clonetics Corporation begins to market cells and cell testing methods.
	The American Anti-Vivisection Society establishes the Demeter Fund (later known as the Alternatives Research and Development Foundation) in order to support nonanimal research, funding up to $50,000 annually for one or more projects.

vocabulary of the animal movement on a large scale and had begun to find its way into the scientific literature (Phillips and Sechzer 1989). The National Academy of Sciences (NAS) organized a meeting on alternatives in 1975 (NAS 1977), but the broader scientific community was not happy about the idea of alternatives, and there was much criticism of the Academy for providing a platform for "antivivisectionists" by organizing the meeting. In the late '70s, Seiling of United Action for Animals managed to persuade a New York congressman to introduce the Research Modernization Act, which called on the National Institutes of Health (NIH) to reallocate 30 to 50 percent of all money spent on animal research to "alternatives" (in the narrow sense of replacement, not the full Three Rs). The Act caught the imagination of the animal protection movement in spite of its vague language and lack of contact with political realities. This public pressure then forced Congress to start to pay attention to alternatives.

The 1980s: Government and Industry Begin to Heed the Call

The growing pressure from the animal protection community for alternatives paid dividends in the '80s, as industry in Europe and America began to embrace the alternatives concept and governments played an increasingly important role (Table 4).

In 1983 Switzerland enacted a legislative requirement for consideration of alternatives and the government earmarked two million Swiss francs over two years for alternatives research. Five years later the Swiss government established an office for animal experiments and alternatives.

In 1986 the Council of Environmental Ministers of the European Communities passed EEC Directive 86/609, which required member countries to develop enabling legislation promoting the Three Rs. The Animals (Scientific Procedures) Act of 1986, replacing the 1876 Cruelty to Animals Act, was passed in the United Kingdom. It required greater attention to the issue of animal suffering (refinement). Also in 1986 the Federal Republic of Germany enacted new laws on animal protection requiring consideration of alternatives in animal research. Three years later Germany established the Center for the Documentation and Evaluation of Alternative Methods to Animal Experiments, known by its German acronym ZEBET, which spearheaded several government initiatives

to validate alternative tests. In the Netherlands government officials began collecting data on the extent of the suffering experienced by laboratory animals, and the Organization for Economic Cooperation and Development (OECD), driven by representatives from Europe, began to address the Three Rs in their guidelines for toxicity testing.

Worldwide, probably the most significant event in the '80s was the launching of campaigns in many of the developed countries against animal testing of cosmetics, toiletries, and household products. These campaigns built on the efforts and publications during the late '70s by scientists and organizations such as FRAME, which laid out the scientific challenges to the routine use of animals in toxicity testing (Balls et al. 1983; Zbinden and Flury-Roversi 1981). The main actor in the U.S. animal protection campaign was labor and civil rights activist Henry Spira, who turned his attention to animals after reading an article by Australian philosopher Peter Singer (1973). Spira contacted with activists in England (such as Jean Pink of Animal Aid, who had been targeting cosmetics testing since 1977), Europe, and Australia and helped to focus and coordinate protests against the eye irritancy testing (the Draize Test) of cosmetics worldwide.

In the United States, Spira's campaign built a coalition of four hundred animal protection organizations that targeted the use of the Draize Test by cosmetic companies in general and Revlon in particular. Within twelve months, the coalition's activities resulted in more than $1.75 million of funding for alternatives research. The Rockefeller University received $750,000 from Revlon to establish a laboratory for *in vitro* toxicological assay development, and the Johns Hopkins University Center for Alternatives to Animal Testing (CAAT) was established with $1 million from the Cosmetic, Toiletry, and Fragrance Association. Avon Products, Bristol-Myers Squibb, and other companies provided the bulk of the funds for CAAT and also provided funds for FRAME programs in the United Kingdom.

The effectiveness of Spira's campaign was based on several factors. First, he engaged in extensive preliminary planning and preparation. For example, Spira acquired numerous copies of the government Draize Test training film and slides (showing inflamed and damaged rabbit eyes) *before* the campaign started. (By late 1980 these materials were no longer being handed out for free by the government to anyone who asked.) Second, he did not shy away from the hard-nosed street politics he had learned in the labor and civil rights campaigns; he made skillful use of demonstrations and the media. Third, he was always willing to negotiate with the opposition and he avoided *ad hominem* attacks and insults. This earned him the respect of his opponents. Fourth, he engaged in a constant search for solutions in which everyone could feel he or she had won something. (Importantly, he did not boast to the media about victories over corporate targets.) When Revlon finally negotiated a settlement with Spira that set up the Rockefeller alternatives research program, Spira not only stopped his campaign, but he also praised Revlon for its innovative program and invited other cosmetic companies to take similarly progressive steps.

The Draize campaign initiated enormous changes in the field of alternatives in toxicity testing. From 1981 to 1991, there was a tremendous shift in attitude toward alternatives in toxicity testing within industry. Corporate toxicologists who had gone along with the initial grants for alternatives research in 1980 and 1981 because they felt such actions were necessary for public relations reasons, became excited by the technical and scientific challenge of alternatives by the end of the decade. Colgate-Palmolive began to fund research into the Chorio-Allantoic Membrane (CAM) test in 1982 (to the tune of $300,000) and within three years had set up an alternatives program in its in-house laboratories. Procter and Gamble and Bristol-Myers Squibb made the search for alternatives part of their corporate culture; they currently provide millions of dollars annually for intramural and extramural alternatives programs. Industrial *in vitro* toxicology associations have been started in both Europe and the United States, and several toxicology journals specializing in *in vitro* approaches were established in the late 1980s. For-profit companies that develop and market *in vitro* tests, such as the Clonetics Corporation and the National Testing Corporation, later known as In Vitro International, were established.

Despite all the interest, however, scientists were still cautious about relying too heavily on the new *in vitro* techniques. Toxicological risk evaluation is a difficult art, and the transformation of alternative methods from screening tools for preliminary decision-making to their use as replacements for whole animals did not begin to come to fruition until the 1990s. However, a widespread consensus emerged during the '80s that toxicology testing needed to move in a different direction. Thus, at CAAT's first symposium (in 1982), the participants mostly wondered *if* an alternative to the Draize Test could be found (Goldberg 1983), but within five years, participants at CAAT symposia were discussing *when* such an alternative would be available.

While similar developments were evident in Europe, there were large segments of scientists outside industry that resisted the concept of alternatives in the United States. In fact, important research institutions such as the NIH avoided use of the term "alternatives" whenever possible. For example, the Health Research Extension Act of 1985 required the NIH to establish an alternatives program, to which the NIH gave the awkward title "Biomedical Models and Materials Resources." A few years later, a Public Health Service draft document on animal welfare commented that "efforts have led to the discovery of research

methods that are useful as 'adjuncts' to animal research, in that they complement animal models but rarely replace them. Thus, these adjuncts are not true 'alternatives'—even the use of this latter term can be misleading" (Public Health Service 1989).

A more balanced approach to the issue was evident in the U.S. Office of Technology Assessment's landmark report, "Alternatives to Animal Use in Research, Testing and Education," which was produced by a government office outside the orbit of the NIH and Public Heath Service. In fact, in drafting the Animal Welfare Act, Congress stipulated that the U.S. Department of Agriculture, and not the Public Health Service or its parent agency (the Department of Health, Education, and Welfare, as it was then known), oversee animal use in biomedical research.

The 1990s: Alternatives Begin to Be Validated and Accepted for Regulatory Use

If the 1970s were marked by an increase in interest in alternatives and the '80s by an increase in activity on this front, the 1990s was a period of maturation for the alternatives approach. The field already had a few academic centers, high-technology companies, and journals dedicated to the cause, as well as backing from national laws. What it needed was a better sense of when a new alternative test was qualified to replace an animal test; in other words, What constituted adequate "validation" (Goldberg 1987)? The field also needed more government-based centers not only to partner with industry and others in validating alternative tests, but perhaps more importantly, to give their stamp of approval to adequately validated tests, which would then allow for regulatory acceptance.

In Europe both needs were addressed by the establishment in 1992 of the European Centre for the Validation of Alternative Methods (ECVAM), headed by Michael Balls of FRAME (Table 5). ECVAM took an active role in establishing validation criteria and in funding and managing validation programs for promising alternative methods, and it was the European Union's (EU) primary authority for approving alternative tests.

ECVAM's counterpart in the United States is the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), established in 1994. ICCVAM was the successor to the informal Interagency Regulatory Alternatives Group and was an outgrowth of the NIH Revitalization Act of 1993. This legislation directed the National Institute of Environmental Health Sciences (NIEHS, one of the NIH institutes) to establish criteria for the validation and regulatory acceptance of alternative testing and to outline a process for regulatory review of potential alternative methods. To accomplish these tasks, the NIEHS asked the various federal regulatory and research-oriented agencies to appoint representatives to an ad hoc interagency committee to draft a report. The ICCVAM report, "Validation and Regulatory Acceptance of Toxicological Test Methods," was issued in 1997 (ICCVAM 1997). With ICCVAM's original mission accomplished, the participating federal agencies decided to change ICCVAM's status from an ad hoc entity to a standing committee to facilitate the ongoing regulatory review and acceptance of alternative methods. ICCVAM is staffed by employees who have other responsibilities to their parent agencies, so to facilitate ICCVAM's new role, the NIEHS established a support center, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) in 1998.

Several large-scale validation efforts were launched during the 1990s, and ECVAM played a role in many of these through coordination, participation, or funding. The establishment of ECVAM and ICCVAM gave industry the confidence to invest in new tests and their validation, knowing that regulatory authorities were available to give advice on validation and acceptance criteria and foster the administrative process of regulatory acceptance. The efforts of ECVAM, ICCVAM, industry, and others began to bear fruit in the late 1990s. In 1998 ECVAM endorsed the 3T3 Neutral Red Uptake Phototoxicity Test for assessing phototoxicity and the Transepithelial Electrical Resistance Test and Episkin (and similar bioengineered skin constructs) for assessing skin corrosivity. The same year ECVAM also endorsed *in vitro* methods as alternatives to the asciites (mouse-based) method for producing monoclonal antibodies. The following year, ICCVAM recommended Corrositex® for assessing skin corrosivity and the Local Lymph Node Assay (a reduction and refinement alternative) for assessing allergic contact dermatitis. ICCVAM's recommendations are not binding on the individual regulatory agencies (for example, the Food and Drug Administration), but may be accepted (or not) according to agency needs; so far the agencies have acted favorably on ICCVAM's recommendations.

In addition to ICCVAM and ECVAM, the OECD has emerged as a significant authority in the acceptance of alternative methods. The OECD, an international organization that facilitates trade, formally accepted the Fixed Dose Procedure (in 1991), the Acute Toxic Class Method (1993), and the Up and Down Method (1997) as reduction alternatives to the LD50 Test for acute toxicity (the Fixed Dose Procedure is also a refinement alternative). In 1996 the OECD hosted a workshop to develop internationally harmonized criteria for the validation and regulatory acceptance of alternative methods (OECD 1996).

The "internationalization" of the alternatives field has also been aided by the establishment of the triennial World Congress on Alternatives and

Table 5
Alternatives Chronology: 1990–1999

1990 CAAT and ERGATT hold a workshop on validation of alternative methods.

The University of California Alternatives Center is established at UC–Davis.

The Platform for Alternatives to Animal Experiments in the Netherlands allocates the equivalent of $700,000 annually for the promotion and validation of research into the Three Rs and the improvement of housing and care systems.

The HSUS establishes the Russell and Burch Award for scientists who have made outstanding contributions to alternative methods.

The Japanese Society for Alternatives to Animal Experimentation begins publishing the journal AATEX (Alternatives to Animal Testing and Experimentation).

1991 The Interagency Regulatory Alternatives Group holds a workshop, "Eye Irritation Testing Alternatives: Proposals for Regulatory Consensus," in Washington, D.C.

The HSUS presents Alan Goldberg, director of CAAT, with the first Russell and Burch Award.

The OECD accepts the Fixed Dose Procedure as an alternative to the LD50 Test.

Representatives of regulatory agencies in Japan, Europe, and the United States agree to drop the classic LD50 Test as a required measure of acute toxicity.

The UK Home Office announces a grant program for the funding of alternatives research.

The Second Report of the FRAME Toxicity Committee is published in *ATLA*.

The Swiss Institute for Alternatives to Animal Testing (SIAT) is established in Zurich.

1992 The European Centre for the Validation of Alternative Methods (ECVAM) is established.

The European Parliament amends the Cosmetic Directive 76/768 to ban the marketing of cosmetics tested on animals after January 1, 1998 (a decision on the ban is later postponed until June 30, 2000).

CAAT hosts a tenth anniversary conference in Baltimore, Md., giving Founders' Awards to Dr. D.A. Henderson, the CTFA, and Henry Spira.

1993 The NIH Revitalization Act of 1993 directs the NIEHS to establish criteria for the validation and regulatory acceptance of alternative testing and to outline a process for regulatory review of potential alternative methods; it also directs the NIH director to establish an alternatives program and to report on its progress annually.

The first World Congress on Alternatives and Animal Use in the Life Sciences: Education, Research, and Testing, takes place in Baltimore.

Member states of the European Union agree on the goal that everything possible should be done to achieve a reduction of 50 percent in the use of vertebrate animals for experimentation and other scientific purposes by the year 2000.

The Interagency Regulatory Alternatives Group holds its second meeting on alternatives, in Washington, D.C.

Dr. Michael Balls of FRAME is appointed director of ECVAM.

1994 The U.S. federal government establishes the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), co-chaired by William Stokes of NIEHS and Richard Hill of EPA, in response to the 1993 NIH Revitalization Act.

The Netherlands Centre for Alternatives to Animal Use (NCA) is established as a national information center on alternatives.

1995 The Gillette Company and The HSUS launch a program to fund research and development of alternative methods; two grants of $50,000 each are awarded annually.

1996 The second World Congress on Alternatives and Animal Use in the Life Sciences is held in Utrecht, the Netherlands.

The OECD holds a workshop to develop internationally harmonized criteria on validation and regulatory acceptance.

CAAT, The HSUS, Procter and Gamble, and other organizations establish Altweb, a website devoted to information on alternative methods.

1997 ICCVAM issues guidelines on criteria for validation and regulatory acceptance of alternative methods.

The Institute for In Vitro Sciences is established in Gaithersburg, Md.

(continued on next page)

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Table 5
Alternatives Chronology: 1990–1999

1998 The HSUS presents the FDA's Neil Wilcox and ICCVAM's William Stokes with the Russell and Burch award for their contribution to the development of alternatives.

ECVAM accepts the following alternative methods: 3T3 NRU PT test as an alternative for assessing phototoxicity, Episkin and similar methods for assessing skin corrosivity, and TER (transepithelial electrical resistance) test for assessing skin corrosivity.

ECVAM endorses *in vitro* methods as alternatives to the ascites method for the production of monoclonal antibodies.

The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) is established to provide support to ICCVAM.

1999 The third World Congress on Alternatives and Animal Use in the Life Sciences is held in Bologna, Italy.

The HSUS presents Procter and Gamble scientist Dr. Katherine Stitzel with the Russell and Burch award for her contribution to the development of alternatives.

CAAT holds TestSmart (a humane and efficient approach to regulatory toxicity data) workshops in order to discuss alternatives to animal testing in the Environmental Protection Agency's High Production Volume (HPV) chemical testing program.

The EPA announces major changes in its HPV program, including funding for alternative methods, following the TestSmart workshops and negotiations with animal protection organizations.

ICCVAM endorses Corrositex® for the assessment of skin corrosivity and the Murine Local Lymph Node Assay for the assessment of allergic contact dermatitis.

Animal Use in the Life Sciences, the first of which was held in Baltimore in 1993 (Goldberg and van Zutphen 1995); the second in Utrecht, the Netherlands, in 1996 (van Zutphen and Balls 1997); and the third in Bologna, Italy, in 1999. The international exchange of information on alternatives was also given a boost in 1996 with the establishment of Altweb, an Internet web site spearheaded by CAAT, Procter and Gamble, The HSUS, and others.

Political pressure played a significant role in moving the alternatives issue during the 1990s, more directly in Europe than in the United States. The issue had some momentum of its own, but outside pressure spurred progress. In Europe, for example, the European Parliament amended the Cosmetic Directive 76/768 to ban the marketing of cosmetics tested on animals after January 1, 1998, regardless of whether such testing was conducted in Europe. Although a decision on the marketing ban was later postponed until June 30, 2000, the Cosmetic Directive amendment led to the formation of ECVAM and encouraged research and development of alternatives by the European cosmetics trade association (COLIPA) and others. The marketing ban would have affected companies in the United States as well as in Europe, so the amendment also kept some political pressure focused on the issue in the United States.

Since the most recent postponement of the marketing ban, a new amendment (the seventh) has been proposed. It calls for: (1) a ban on animal testing of finished products in the European Union as soon as the directive comes into force, (2) a ban on animal testing of cosmetic ingredients where alternatives are available, and (3) a complete ban on animal testing of cosmetic ingredients within three years of implementation of the directive, regardless of the availability of alternatives. The European Commission has stated that only one two-year postponement of the ingredients-testing ban would be considered. Consequently, an absolute ban on ingredients testing could become effective within five years of implementation of the directive. Finally, the directive states that a marketing ban, which would have affected countries outside of the European Union, will not occur due to potential problems with World Trade Organization rules; this effectively "kills" the proposed sixth amendment.

Alternatives legislation in the United States in the 1990s was largely a cooperative venture between industry and animal protection. The alternatives language in the NIH Revitalization Act of 1993, which led to the creation of ICCVAM, was the product of efforts of several industry and animal protection representatives working with Rep. Henry Waxman. A similar coalition led to the introduction of the ICCVAM Authorization Act in the Senate (1999) and House (2000) in an effort to strengthen ICCVAM and make it a permanent entity. As of October 2000, this legislation was pending.

Discussion

Many animal protectionists are frustrated with the pace at which the use of animals in research and testing is being replaced, reduced, and refined. However, the growth of the alterna-

Abbreviations

ATLA	Alternatives to Laboratory Animals
CAAT	Center for Alternatives to Animal Testing
CAM	Chorio-Allantoic Membrane
ERGATT	European Research Group into Alternatives in Toxicity Testing
FRAME	Fund for the Replacement of Animals in Medical Experiments
HPLC	High Pressure Liquid Chromatography
IACUC	Institutional Animal Care and Use Committee
NAS	National Academy of Sciences (USA)
NAVS	National Anti-Vivisection Society (UK)
NIH	National Institutes of Health (USA)
OECD	Organization for Economic Cooperation and Development
UFAW	Universities Federation for Animal Welfare
ZEBET	Zentralstelle zur Erfassung und Bewertung von Ergänzungs und Ersatzmethoden zum Tierversuch

tives field since the publication of Russell and Burch's seminal book in 1959 has been remarkable, especially considering that the animal protection community itself did not embrace the alternatives issue in a significant way until the late 1970s. During the 1980s cosmetics and consumer products companies began investing millions of dollars into research and development of alternatives, national governments incorporated the alternatives approach into their animal protection legislation and, in some cases, began funding research and development of alternatives, some companies began developing and marketing alternative test kits, academic centers devoted to the issue began to be established, and the field of *in vitro* toxicology blossomed. During the 1990s government centers devoted to the validation and regulatory acceptance of alternative methods were established in Europe and the United States, the triennial World Congresses on Alternatives began, and alternative tests began to be formally approved and accepted by regulatory agencies.

Have these developments translated into a decrease in the use of laboratory animals and in their levels of pain and distress? Most countries that keep records on the use of research animals report a fall in laboratory animal numbers during the 1980s and 1990s, in some cases a dramatic fall (Rowan et al. 1995). The statistics from the United Kingdom show a decline in annual animal use from around 5.5 million in 1976 to 2.7 million in 1998. Sir Peter Medawar, who predicted in 1969 that such a decline would begin in 1979 or even earlier (Medawar 1972), was obviously more far-sighted than the Littlewood Committee, which reported in 1965 that animal use would not be influenced by the development of new (alternative) technology.

However, a key question is this: How much of the decline in research animal use in the United Kingdom and in other countries has resulted from pressure to develop and use alternative methods? The available data is not adequate to provide an unequivocal answer. While other factors such as the cost of research animals and the increased sensitivity and specificity of new techniques have no doubt been important, it is also likely that pressure from animal groups (and progressive scientists) calling for the development and use of "alternative" techniques has played a role in reducing animal use. Animal protectionists certainly increased awareness of the Three Rs and humane issues within the scientific community.

Technical developments over the past thirty years have, for example, reduced the demand for animals in the production and testing of polio vaccine and insulin (Hendriksen 1988; Trethewey 1989). Hendriksen describes how the number of monkeys used in the production and testing of polio vaccine in the Netherlands was reduced from 4,570 in 1965 to 30 in 1984 by a series of technical improvements, even though the actual amount of polio vaccine produced was about the same in the two years. The technical improvements were the result of advances in molecular techniques and cell culture biology.

Trethewey describes a similar process in insulin testing that reduced the demand for mice by 95 percent between 1970 and 1986. The major technical advance was the introduction of a mouse blood glucose test in place of the mouse convulsion test. This relatively nonstressful assay permitted the re-use of the same mouse for more than one assay leading to a further reduction in the number of animals required. High Pressure Liquid Chromatography (HPLC) techniques have been developed and introduced, and it is now possible to standardize insulin preparations using only a handful of mice to ensure that each batch is biologically active. A life-time supply of insulin for one diabetic now requires testing on the equivalent of only a single mouse and it is possible that mice will be eliminated altogether as further technical advances are made.

Innovations in toxicity testing and the standardization of therapeutics such as insulin have reduced the demand for animals in some procedures. However, the most significant reductions have come in the search for new drugs. As pharmaceutical companies have switched from animal to *in vitro* screens for agents with potential therapeutic activity, they have recorded dramatic decreases in animal use. Hoffman–La Roche, for example, reduced its annual animal use from one million to about 300,000 without changing the number of new drug entities under investigation (Anonymous 1990). A switch by the National Cancer Institute from

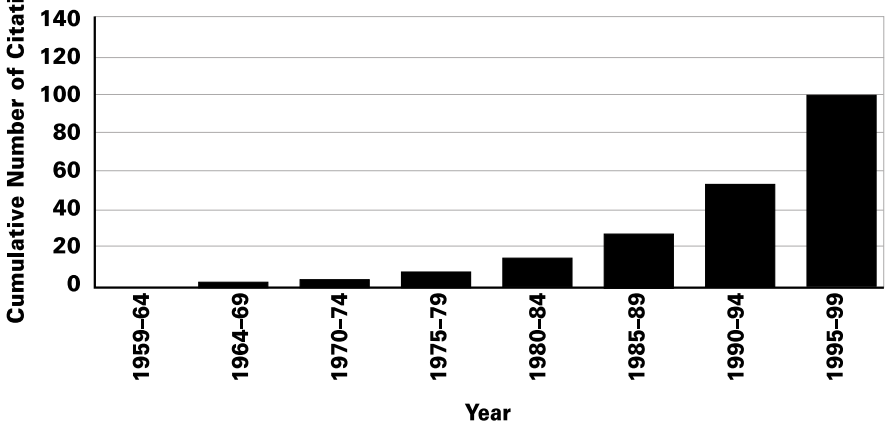
a mouse screen for potential anticancer agents to human cancer cell culture screens has resulted in a saving of several million mice per year (Rowan and Andrutis 1990).

Russell (1995) attributes the development of replacement technology, and the consequent decreases in laboratory animal use, to the waning influence of what he and Burch (1959) called the "high fidelity" fallacy—that models had to look like the organism being modeled, no matter what the power of the model to "discriminate" or elucidate the process under study. Thus mammals such as mice, dogs, and primates have historically been preferred as models of humans because they have high fidelity to humans, not necessarily because they have high discrimination. The high fidelity fallacy has lost its currency as the power of low fidelity–high discrimination techniques, such as tissue culture and use of invertebrate species (for example, *C. elegans*) has been demonstrated.

The impact of refinements on animal pain and distress is even harder to gauge than the impact of replacements and reductions on animal numbers. While animal numbers declined during the 1980s and 1990s, increasing attention was being paid to the neglected "R"—refinement—thanks in part to new legislation in Europe and the United States. In the United Kingdom, the passage of the 1986 Scientific Procedures (Animals) Act focused more attention on animal distress and led to a virtual doubling (from 21 percent to 36 percent of all procedures) in the rate of anesthetic use in animal research in six years (Anonymous 1990). In the United States, protocol review by Institutional Animal Care and Use Committees is increasingly focusing on reducing animal pain and distress.

Two technical advances that will significantly decrease pain and distress in laboratory animals are noninvasive imaging and telemetric approaches to animal data (Stokstad 1999). These approaches not only reduce or eliminate pain and distress, they also allow for a 75 to 80 percent reduction in animal numbers by increasing the reliability of the data and improving experimental design.

Figure 1
Citations to Russell and Burch's *Principles of Humane Experimental Technique* in the Published Scientific Literature



Source: Science Citation Index

One of the major challenges in making further progress in alternative methods is the indifference, if not the antagonism, to the alternatives approach on the part of many academic researchers worldwide. While the NIH no longer automatically characterizes alternatives as mere "adjuncts" of animal research, and the NIEHS actively promotes alternative methods, some biomedical research advocates have argued that use of the term "alternatives" implies that one needs to apologize for using animals in research and that this gives the public the wrong impression (Goodwin 1992). While such hostility to the alternatives approach is abating, the field of alternatives would progress much faster if academic researchers were more sympathetic to the approach.

Another challenge in implementing alternatives and in decreasing animal use is the growth of genetic engineering, particularly in mice. The NIH's in-house use of mice reached a low of about 300,000 in 1991 but has more than doubled since then, according to NIH Annual Reports and NIH Reports to the USDA. Genetic engineering can sometimes be harnessed to reduce (and refine) animal use (Gordon 1991). It can also be argued that the increasing numbers of genetically engineered mice are at least somewhat offset by a corresponding decrease in the use of other mice or species, thereby nullifying any increase in overall numbers. This seems to be what is happening in the United Kingdom, where the use of genetically modified mice has gone up tenfold, to around 500,000, but total mouse use has fallen slightly. At the very least, the impact of genetic engineering on animal use should be carefully monitored, given its potential to reverse the decreases in animal use seen during the 1980s and 1990s.

Conclusion

The program that UFAW set in motion in 1954 has born significant fruit. Although Major Hume would no doubt be surprised at the scope and potential of biomedical science today, he would be pleased at the growing recognition accorded to Russell and Burch (1959). The number of citations to Russell and Burch's book in the scientific literature increased dramatically during the 1980s and, especially, the 1990s (Figure 1).

In 1959 Hume spoke to an Ani-

mal Care Panel meeting in Washington, D.C.:

> A more recent event has been the publication of a remarkable book by Russell and Burch entitled *The Principles of Humane Experimental Technique.* This deserves to become a classic for all time, and we have great hopes that it will inaugurate a new field of systematic study. We hope that others will follow up the lead it has given, and that a generalized study of humane technique, as a systematic component of the methodology of research, will come to be considered essential to the training of a biologist (Hume 1962).

This has indeed come to pass in the Netherlands and other parts of Europe (van Zutphen, Baumans, and Beynen 1993), and we are hopeful that the Three Rs will become fully incorporated into the training of biologists in the United States.

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