New Focus on Replacing Animals in the Lab

Animal welfare movement is raising consciousness, but science and economics supply the main impetus for change

In the past few years the animal welfare movement has undergone both philosophical and scientific bolstering. Animal activists have long been concerned about the use of animals in scientific research, but now, instead of only attacking allegedly inhumane experiments, they are actively promoting the development of "alternatives" to the use of animals in research.

This concern has found expression in the introduction of a bill in Congress (HR 556) which calls for the establishment of a Center for Alternative Research in the National Institutes of Health (NIH) and the diversion of up to 50 percent of all appropriations for animal-related research to research using alternatives.

Members of the House Science and Technology Committee are now trying to design a more moderate measure. H.R. 556 might sound promising if the failure of scientists to shift their work massively away from the use of whole animals (usually meaning vertebrates) were owing to a combination of inertia and lack of funds. The fact is, however, as was made clear at congressional hearings in October, a massive shift away from the use of animals in research will not be possible in the foreseeable future. The technology is not just sitting around waiting to be deployed. Rather, much more basic research using animals will be necessary before major reductions are possible.

Just what are "alternatives"? H.R. 556 defines them as including "mathematical models, isolated organs, tissue and cell cultures, chemical assays, anthropomorphic dummies, simulated tissues and body fluids, mechanical models, computer simulations or lower organisms." Broadly speaking, gene splicing and work with hybridomas—which enables the creation of large quantities of antibodies in vitro—qualify as alternatives to animals. So do various newly developed technologies, such as positron emission tomography and nuclear magnetic resonance, which permit noninvasive scanning of an organism.

Many animal welfare people talk rather glibly about "alternatives" as though one-to-one substitutions of nonanimal for animal tests could be made in the foreseeable future. But others prefer a broader definition covering the "three R's"—replacement, reduction (of animals), and refinement (of tests). Some scientists balk altogether at the idea that alternatives can "replace" animals, since the only genuine substitution for a whole animal is another whole animal. They prefer to refer to nonanimal research methods as "adjuncts" to animal tests.

In fact, over the past 5 years or so, there has been considerable movement toward adoption of nonwhole-animal assays in toxicity testing—specifically tests for mutagenicity and carcinogenicity (teratogenicity, which is usually mentioned in conjunction with these, is still not amenable to detection without using live animals). One of the driving forces was the passage in 1976 of the Toxic Substances Control Act (TSCA) which, when fully implemented, will mandate premarket toxicity testing of all new industrial chemicals. With hundreds of major new chemicals entering the market each year, scientists are under great pressure to use available facilities as efficiently as possible; in vitro screening techniques offer valuable information on which chemicals are potential hazards and therefore candidates for animal screening.

Another, though lesser, impetus for reducing animal use is supplied by developments in basic biomedical research. Rapid strides in molecular and cell biology have pushed the frontiers of biology closer and closer to fundamental mysteries—to the rules that govern all life—so that many basic questions about cell behavior are best addressed through isolation of the simplest systems possible.

Despite all the options cited by animal activists—ranging from elimination of duplicative research to the substitution of "lower" animals for vertebrates—there are basically two major approaches that show promise for reducing animal use. One is generally referred to as short-term tests, involving the cultivation of living material in culture; the other is mathematical modeling.

Short-term tests have become vital to toxicology testing, thanks to TSCA and the government's concern with environmental toxins as well as the high cost of animal toxicity studies. Toxicology testing has become a huge business. About 63,000 chemicals are in common use, some 48,000 of them in commercially significant amounts, according to the National Toxicology Program of the National Institutes of Health (NIH), which tests selected chemicals. More than 500 new ones are introduced each year. Only about 6000 had been tested for carcinogenicity by 1978, according to the Environmental Protection Agency. The nation has the capacity to test only about 300 a year. It costs about $500,000 to subject rats and mice to a lifetime cancer study. A total toxicological work-up, including such things as special tests for eye and skin toxicity, can run as high as $2.5 million.

Short-term tests are most useful in determining mutagenicity (changes in DNA), which is usually an indicator of carcinogenicity. The Ames test, developed in 1971, is by far the best known of the in vitro assays and is now used in some 2000 laboratories around the country. It involves application of a chemical to a preparation of *Salmonella* bacteria, with rat liver extract added to metabolize the compound. The Ames test is about 80 percent reliable in determining mutagenicity (since it is more sensitive than an animal assay it usually errs on the side of false positives).

In the future, scientists expect that batteries of short-term tests will reduce the need for many in vivo toxicity tests. David Brusick of Litton Bionetics believes "we should be able to completely replace the whole-animal [toxicity] bioassay with an appropriate set of short-term tests coupled with metabolic studies in mammals."

At the Food and Drug Administration (FDA), four mutagenicity tests are now allowed to substitute for preliminary animal cancer screens of drugs administered to food animals. And the agency is considering allowing manufacturers of human food additives to substitute several overlapping in vitro tests for an animal carcinogenicity assay on "low concern" additives. A manufacturer would then use some or all of the following: an Ames test, a *Drosophila* test (looking for mutations in multiple generations of fruit flies), a test looking for an unscheduled DNA synthesis in cells in culture, a test for a point mutation in a mammalian cell culture, and a mammalian cell transformation test. If results are negative, about $450,000 could be saved

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by bypassing an in vivo carcinogenicity study.

Although tests for genetic toxicity offer the only widely used shortcut in toxicological testing, others are on the horizon. Chick embryos, for example, may prove a cheaper, more convenient and less wasteful way to do some kinds of neurotoxicity testing. At an NIH symposium last February,* Stata Norton of the University of Kansas reported that she was able to produce the same effects from morphine injections in chick embryos as in baby rats. She suggested that the chick embryo is "a simpler system which nevertheless retains some of the complexity of the mammalian nervous system" and thus was able to provide some information that ordinarily is gained from mammals.

Cells in culture are also used in screening for new pharmaceuticals. The National Cancer Institute (NCI) is starting a $1-million-a-year program to test possible anticancer drugs with an assay on cultures of human cancer cells. Currently, the NCI drug testing program uses two banks of mouse experiments in the early stages of determining whether a new drug shows promise in anticancer activity. The human cancer cell assay will be used as a supplement, and if it does as well in predicting anticancer activity as rodent experiments, it may ultimately replace them. Bristol-Myers recently announced a similar project, in which new drugs are being tested on cultured cells from individual human cancers. The hope is that this will be a more effective way of matching chemotherapy to particular types of cancers than is offered by mouse assays.

Enormous strides in cell and organ culture have been made in recent years with the result that scientists theoretically possess the know-how to keep any type of cell culture alive in a medium for an extended period of time without their regressing to a more primitive state (as noncancerous cells are wont to do). Most cell culturing is done in the service of basic research and has not been exploited as much as it could be in toxicity testing, says Roland Nardone of Catholic University.

But currently, for the first time, several groups are involved in applied research, including the use of cell cultures, to seek nonwhole-animal bioassays to replace the notorious Draize test. The Draize test is an ocular toxicity test in which substances are placed in the eyes of rabbits. It has for several years been the target of a coalition of some 400 animal welfare groups who selected it as a cause with public appeal. Not only does it involve hurting rabbits, but it is widely used in testing of nonessential substances, namely, cosmetics. The Draize coalition has been remarkably successful at turning public pressure onto cosmetic companies, who have capitulated recently by awarding substantial sums for research on nonanimal substitutes. Thus, within the past year, four different institutions† have begun research programs. The largest is at Johns Hopkins University which got $1 million from the cosmetics industry to set up a Center for Alternatives to Animal Testing. Its head, Alan Goldberg, says the center is unusual in that its research programs—both intramural and extramural—are being designed to run the gamut from fundamental research to applications. Goldberg says they are now looking for proposals to investigate cell mechanisms, particularly how cells and tissues respond to foreign challenges.

At Rockefeller University a group headed by Dennis Stark will also be doing basic research, with particular emphasis on developing data on the inflammatory response. Stark doesn't have any idea what kinds of tests his group will come up with but speculates that in order to replace the Draize test a bank of perhaps ten tests will have to be developed. He envisages separate tests for the various parts of the eye that might be affected as well as for the inflammatory response, effects of treatment, duration of damage, and so forth.

Joseph Leighton at the Medical College of Pennsylvania, who believes that use of the Draize test can ultimately be reduced by 90 percent, is working with chick embryos. He says the vascular membrane covering the egg, called the chorioallantoic membrane, has complicated features that confer some of the benefit of working with a whole animal. Finally, at Tufts University, William Douglas is experimenting with cultures of human corneal cells.

At Johnson and Johnson Baby Products, John McCormack reported at the NIH February meeting, yet another in vitro ocular toxicity test has been developed, this one using mast cells from rat peritoneal tissue. This test, used with water-soluble surface-active substances such as shampoos, involves measuring the release of radioactively labeled serotonin by the mast cells. Serotonin is liberated in conjunction with histamine which in turn is related to inflammation. McCormack says the test has high correlation with in vivo results, and one rat peritoneum supplies findings that would require 48 whole animals in an in vivo study.

Far more knowledge gained from basic research will be required before any quantum gains can be made in replacing animals. As one investigator said, "we still don't even know how a single bacterial cell works," and until we do, it is difficult to extrapolate from cell activity in a tissue or cell culture to the same activity in a whole animal.

Aside from short-term tests, mathematical modeling is the other area that shows greatest promise for the eventual reduction of animal use. The newest development, ascribed chiefly to the work of Corwin Hansch of Pomona College in California, is called quantitative structure activity relationship analysis. This approach, using computers, occupies an even more preliminary position than in vitro tests in the hierarchy of testing that culminates in experiments with human subjects. It is being used as a method to make preliminary identification of both toxicity and efficacy of compounds. It is based on mathematical expressions of the relationship between a compound's chemical structure and its activity. Hansch explains that conversion of structural characteristics intc numbers allows for much more precision than do pictures of molecules; it also tells the investigator which differences between two compounds are significant and which trivial. Structure activity analysis relies on having a large data base containing the chemical structures of known molecules, and then comparing various molecular fragments or "keys" with those of the known chemicals.

The NCI is using structure activity analysis to screen the thousands of new chemicals sent in each year for antitumor activity. The Drug Synthesis and Chemistry Branch of the NCI's Developmental Therapeutics Program acquires samples of some 20,000 new compounds each year from its worldwide network of sources, according to Louis Hodes. The molecular structure of each is put through a computer and compared with those in their training set (data base) of 55,000 known compounds. The computer surveys each molecule, atom by atom, looking for two things: uniqueness and activity. Compounds that show activity

*Trends in Bioassay Methodology: In vivo, in vitro and Mathematical Approaches, organized by William Raub, director of extramural research at NIH.

†Tufts University has a grant of $176,000 from the American Fund for Alternatives to Animal Research; the Medical College of Pennsylvania has received $100,000 from the New England Antivivisection Society; Rockefeller University has a $750,000 grant from Revlon; Johns Hopkins has received $1 million from the Cosmetics, Toiletry and Fragrances Association and an additional $300,000 from Bristol-Myers.

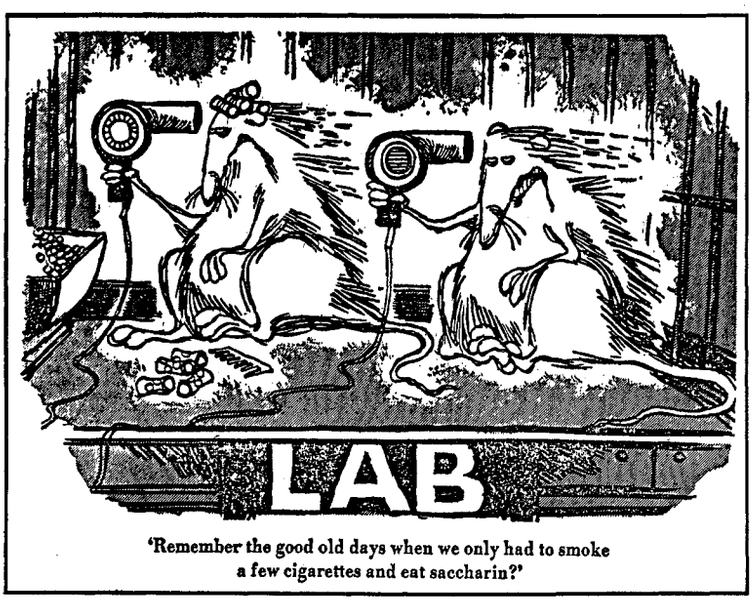
but are very similar to antitumor drugs already available are discarded; anything with an unusual structure or that has keys in common with compounds of known activity is subjected to chemical analysis and, if approved, is moved to the prescreening stage, which involves testing it on a particular type of mouse leukemia. About half the new chemicals are eliminated before they get to the mouse screen, which means the efficiency of the animal tests has doubled over the 5 years that the present screening program has been in place.

There is also growing use of mathematical models for parts of whole systems such as the cardiovascular system or the immune system. But they require enormous amounts of data—all acquired from animal experimentation—in order to be useful. Arthur Guyton of the University of Mississippi, for example, reported at the NIH meeting that mathematical modeling of high blood pressure shows that increased peripheral resistance in blood vessels cannot cause permanent hypertension—a finding that runs counter to what is taught in most medical schools. Guyton says modeling can make animal research more efficient, but it can also lead to increased numbers of animal bioassays because it raises so many new questions.

Some people believe, however, that increased use of mathematical models can lead to reduced animal use. Charles DeLisi, mathematical biologist at NCI, cites a mathematical formulation involving interactions between tumor cells and immune cells which predicts that the immune system can sometimes stimulate tumor growth. DeLisi thinks mathematics is underutilized by biologists who are often unaware when questions arise that are good candidates for mathematial solutions.

The more one learns about these new research methodologies, the clearer it becomes that very few can be realized as direct substitutes for animal bioassays. Rather, they are opening up new realms of investigation which will in many cases lead to reduction of animal use and refinement of animal experiments.

There is already evidence that lab animal use is decreasing. The National Academy of Sciences (NAS) reported in 1978 that use of research animals had gone down by 40 percent in the prior decade, from about 33 million to about 20 million among laboratories polled. Many people dispute these figures, contending that annual consumption of research animals is more like 60 million. Either way, most of the decline is attributable to the rising costs of animal purchase and care and an increase in chronic toxicity testing (which reduces turnover), according to the NAS. The reduction of animal use in education is also a significant factor.



'Remember the good old days when we only had to smoke a few cigarettes and eat saccharin?'

By Mike Peters for the Dayton *Daily News*

There is considerable debate over the best use of short-term and other nonanimal tests, which inevitably includes debate about animal tests. Animal welfare people, for example, are likely to be much more skeptical of the value of animal tests—and the extrapolation of their results to humans—than are animal toxicologists. Interestingly, the thalidomide disaster is cited by both camps, with some people claiming not enough animal testing was done, and others saying it is a perfect example of their inadequacy. Thalidomide had been tested on rodents and rabbits, but the teratogenic effect showed up only in one strain of rabbit.

Questions about validity of in vivo tests relate to growing criticism of the LD_{50}, which is the next target of the anti-Draize test coalition. The LD_{50}, developed in 1927, was originally intended as an index of drug toxicity. Now it is required for any substance—food additive, drug, household product, or industrial chemical—to which humans will be widely or heavily exposed. The test has been criticized as a crude one whose only end point is death. It is said to be of marginal usefulness in most cases because so many factors influence the outcome and extrapolation of the results to humans is questionable. Furthermore, with a substance of minimal toxicity, test populations have to be fed so much of it to get results that they may die from secondary effects unrelated to toxicity.

Government regulations are a significant obstacle to the adoption of safety tests that don't require animals, and chemical manufacturers are reluctant to take the initiative in developing nonanimal tests because of product liability fears. Thus, the most immediate gains stand to be made in the area of test validation. There are hundreds of in vitro tests available, but none are going to be widely used, or accepted for regulatory purposes, until they have been shown to be at least as sensitive as an animal system. In the meantime, there have been moves to reduce unnecessary duplication of tests by standardizing some of the ones required by four regulatory agencies: the Environmental Protection Agency, the Food and Drug Administration (FDA), the Consumer Product Safety Commission, and the Occupational Safety and Health Administration. An Interagency Regulatory Liaison Group (set up under President Carter and now defunct), headed by Victor Morgenroth of the FDA, has issued guidelines for four types of tests: acute dermal toxicity, acute oral toxicity, teratogenicity, and acute eye irritation. If the group's recommendations are heeded they could result in a significant drop in Draize tests, as the eye irritation guidelines say "substances known to be corrosive may be assumed to be eye irritants and should not be tested in the eye."

An accumulation of small changes is probably going to have more effect on the adoption of "alternatives" than a big new federal initiative. History would seem to indicate that animals are naturally replaced when scientists discover the mechanism of the purpose for which

they were used. Canaries are no longer employed to monitor the air in mines; rabbits (and later frogs) are no longer needed to discover pregnancy. As Donald Kennedy, former FDA head, said recently, "compared with most other contemporary biological techniques, animal testing is crude, cumbersome and expensive." But there is still nothing like an animal. To eliminate animals in testing, claims Hansch, "you would have to totally understand life in all its detail."

The extent to which the animal welfare movement is hastening the development of alternative methods is not clear. Certainly, the movement can claim responsibility for the new initiatives aimed at replacing the Draize test. But otherwise, it is far less of an influence than economic or scientific imperatives.

The movement is unquestionably affecting how many scientists view their work. Some see this as consciousness-raising for scientists who work with animals—just as physicists developed a new awareness about the implications of their work after the bomb, and more recently clinicians developed a new sensitivity toward the rights of human subjects. What frightens some scientists is that the current movement is gaining added force from America's streak of anti-intellectualism, which lends a flavor to the extreme wing of the animal rights movement reminiscent of right-to-life and creationist zealotry.

Leaving out the extremists on both sides of the question, scientists and animal welfare people do not appear to be much in conflict. Franklin M. Loew of Johns Hopkins University, head of the NAS laboratory animal group, believes there is really only a difference in priorities: the animal people see reduction of animal use as a desirable goal in itself; while to scientists, the goal is secondary to that of doing good science. There is greater disagreement over means, with one group pressing for more money while the other contends that development of alternatives is progressing as fast as the science will allow.

There are few who believe that all animals can some day be eliminated from research. In many areas, including disease modeling, experimental surgery, and many behavioral studies, the only substitute for an animal would be a human being. Otherwise it is difficult to predict the future since both the science and the ethics are in flux. Says William Raub of NIH: "There is the possibility that 10 years from now our current views of the ethics and morality of research will be labeled as being biologically naïve."—CONSTANCE HOLDEN

Small Business R & D Bill Approved 90 to 0

A bill designed to channel almost 1 percent of the federal government's R & D budget into a new program to spur innovation by small businesses swept through the Senate last month by a vote of 90 to 0. The bill's chief sponsor, freshman Senator Warren Rudman (R–N.H.), is understandably pleased with his first legislative triumph. But he says he is so disturbed by the "avarice" displayed by university officials in opposing some aspects of the bill that he is planning an investigation of the way basic research is funded.

Undaunted, critics of the legislation, who argue that it will divert funds from basic to applied research at a time when basic research budgets are already under stress (*Science*, 27 November, p. 1003), are planning a major lobbying effort in the House. Although a similar bill, sponsored by Representative John J. LaFalce (D–N.Y.), has been approved unanimously by the House Committee on Small Business, there is still time for opponents to get a hearing. Three other committees—Armed Services, Energy and Commerce, and Science and Technology—have been granted jurisdiction and they have until 1 March to propose amendments. This will be the first time that committees directly concerned with science budgets have had a chance to consider the impact of the legislation.

Although the Rudman and LaFalce bills differ in some important respects, they would both require federal research agencies to set aside a portion of their R & D funds for so-called Small Business Innovation Research (SBIR) programs. The focus of the lobbying effort, which is being spearheaded by the Association of American Universities (AAU), will be to exempt basic research budgets from this proposed set-aside. Another objective will be to secure an exemption for the National Institutes of Health (NIH).

The argument is that since the SBIR programs will involve mostly applied research and development, the money should not be taken from support of academic science. But the bills' sponsors do not agree. Rudman, for example, argues that "if basic research gets its exemption, it will give bureaucrats a chance to emasculate the bill." They would simply classify an unwarranted fraction of their programs as basic research, he says. Moreover, the universities have benefited from "the grandest set-aside of all," Rudman claims, because until recently businesses have been excluded from competing for NIH grants.

In any case, Rudman argues that basic research is sufficiently protected by an amendment, proposed by Senator Harrison Schmitt (R–N.M.), which was included in the Senate bill. It simply states that basic research funds cannot be reduced by more than 1 percent to pay for SBIR programs.

Lobbyists for the universities are not appeased by the amendment, however. "It doesn't stop the highway robbery, but it limits the damage," says Newton Cattell of the AAU. Support for a total exemption for basic research has also come from one of the legislation's most prominent backers, the Federation of American Scientists (FAS).

Testimony by a former FAS official, Philip Speser, in support of both the Rudman and LaFalce bills has been widely publicized by the bills' backers as evidence of approval by the scientific community. But in a statement issued on the eve of Senate passage of the legislation, FAS executive director Jeremy Stone urged that basic research funds be exempted. "It would be an ironic and counterproductive effect of the bill if it were to encourage innovation in small businesses only at the cost of depleting the new ideas which small businesses might apply," he argues. Stone explains the FAS's apparent change of mind by asserting that he had always assumed that the money for SBIR programs would come from applied research and development budgets.

In the meantime, Rudman, a man who does not mince his words, says he is disgusted with the university lobbyists, who he says are simply out to protect their turf. "I have encountered greed and avarice from the basic research community that I would have expected from the oil companies," he told *Science* in an interview. "The ivory towers are getting gray in my opinion," he said. Suggesting that "there is an old boy network in the basic research community" that influ-

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