Chapter 6

Alternatives to Animal Use in Research

***Unless we get a handle** 011 what is **happening in the** mammalian **brain, there's no way** of knowing whether any of these [invertebrate] models is right or not.*

Richard F. Thompson
Stanford University
Science 85 6(4):33, 1985

Investigators often ask statisticians how many observations they should make (fortunately, usually before the study begins). To be answerable, this question needs fuller formulation. There is a resemblance to the question, How much money should I take when I go on vacation? Fuller information is needed there too. How long a vacation? Where? With whom?

Three questions need to be answered before the sample size is determined. How variable are the data that will be collected? How precise an answer is needed? How much confidence should there be in the answer obtained? These questions can be well worth probing even if the question of sample size will foreseeably be answered by the size of the budget or the time available for the study. Sometimes a planned study is dropped because sample-size analysis shows that it has almost no chance of providing a useful answer under the constraints of time or budget that apply.

Lincoln E. Moses
Stanford University
N. ***Engl. J. Med. 312:890-897, 1985***

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Alternatives to Animal Use in Research

Alternatives to animal use in biomedical and behavioral research fall into four broad categories:

- **continued, but modified, animal use,** including a reduction in the number of animals used, improved experimental design and statistical analyses of results, substitution of cold-blooded for warm-blooded vertebrates, substitution of laboratory mammals for domestic or companion mammals, and reduction of pain or experimental insult;
- **use of living systems,** including in vitro cultures (of cells, tissues, and organs; see table 6-l), embryos, invertebrates, micro-organisms, and plants;
- **use of nonliving systems,** such as chemical or physical systems; and
- **computer simulation.**

In this chapter, various disciplines within biomedical and behavioral research are surveyed in order to focus attention on the most promising areas for development of alternatives to animal methods. Areas not amenable to the implementation of such alternatives are also identified.

As noted in chapter 5, distinctions within and among the varied disciplines of biomedical and behavioral research are artificial in one sense: Boundaries among disciplines are often blurred, and broad areas of overlap exist. Yet the examination of discrete areas of research highlights the great variability among disciplines in the potential for using alternatives to animals.

Using alternative methods in research holds several advantages from scientific, economic, and humane perspectives, including:

- reduction in the number of animals used;
- reduction in animal pain, suffering, and experimental insult;
- reduction in investigator-induced, artifactual physiological phenomena;
- savings in time, with the benefit of obtaining results more quickly;
- the ability to perform replicative protocols on a routine basis;
- reduction in the cost of research;
- a greater flexibility to alter conditions and variables of the experimental protocol;
- reduction of error stemming from inter-individual variability; and
- the intrinsic potential of in vitro techniques to study cellular and molecular mechanisms.

At the same time, these methods are fraught with inherent disadvantages, including:

- reduced ability to study organismal growth processes;
- reduced ability to study cells, tissues, and organ systems acting in concert;
- reduced ability to study integrated biochem - ical and metabolic pathways;

Table 6-1 .—Research Methods Involving Living Components

Isolated perfused organs using:	Isolated tissue or tissue sample using:	Isolated single cell using:	Subcellular constituents using:
liver	striated muscle	fat cells	nuclei
muscle	iris	liver cells	mitochondria
heart	trachea	neurons	microsomes
lung	bronchi	glial cells	lysosomes
adrenal gland	lung	striated muscle cells	synaptosomes
pituitary gland	uterus	smooth muscle cells	cell membranes
intestine	intestine	red blood cells	muscle actin/myosin
testis	seminal vesicle	leukocytes	
skin	vas deferens	platelets	
spleen	bladder	mast cells	
kidney	spleen		
	salivary gland		
	fat pads		
	liver slice		

SOURCE" Adapted from W. Paton, *Man and Mouse: Animals in Medical Research (New* York: Oxford University Press, 19S.4),

- reduced ability to study behavior;
- reduced ability to study the recovery of damaged tissue;
- reduced ability to study interaction between the organism and its environment;
- reduced ability to study idiosyncratic or species-specific responses;
- reduced ability to distinguish between male- and female-specific phenomena; and
- . a handicap to probing the unknown and phenomena not yet identified.

This general listing of advantages and disadvantages provides a framework for examining the use of alternatives in specific disciplines of biomedical and behavioral research. Many of these pros and cons are cited in this chapter's detailed description of alternatives.

CONTINUED, BUT MODIFIED, USE OF ANIMALS IN BIOMEDICAL RESEARCH

Animal use in biomedical research can be modified in a number of ways, including strengthening experimental design to use fewer animals, reducing the degree of experimental insult, and substituting one organism for another. In the case of substitution, cold-blooded vertebrates may supplant warm-blooded ones.

Reduction in the Number of Animals Used

Up to half the animals used in research protocols may be untreated, or control, animals. The importance of using parallel, internally controlled designs for experimentation may be one of the first lessons learned by science students whose results are rejected for not providing comparable data from treatment groups of animals matched for size, age, sex, and dosage. Studies with investigator-initiated, internal controls support substantially stronger inferences than those without them.

Common and Historical Controls

Fewer animals may be used in an experiment by sharing a control group with other investigators or by not using a concurrent control group. In both cases, all the physical and genetic characteristics of the treatment group(s) must be matched to those of the control group, and the conditions under which the data are collected must be as precisely duplicated as possible. There are difficulties unique to each method. Investigators may encounter constraints on their particular study when sharing controls. For example, sharing may be impossible if one group needs to extend its studies beyond the time agreed for termination and autopsy of the shared animals, or if the actions of one group adversely affect the other, as might happen by the inadvertent spreading of a parasite or pathogen. In the case of historical controls, the difficulty rests in exactly duplicating earlier conditions. Use of such controls must be carefully documented and justified (82).

Animal Sharing

Another way to use fewer animals is to share individual experimental animals or their tissues between research groups. Although this method may encounter the same types of difficulties described for the sharing of controls, it appears to be gaining in popularity among compatible groups, At the University of Virginia, investigators in endocrinology (Department of Internal Medicine) and in the molecular genetics of heme synthesis (Department of Biology) use the pituitaries and livers of the same rats even though the two departments are on opposite sides of the campus (54).

Research animals may also be shared among different sites. This is especially practicable in the case of long-lived primates. As long as sequential protocols are not deemed inhumane or scientifically conflicting, primates may be shipped from one research site to another. The Primate Research Institute (PRI) of New Mexico State University, for example, will loan chimpanzees and rhesus and cynomolgus monkeys to qualified U.S. scientists. PRI currently has 240 chimpanzees on campus, with another 150 animals on loan.

Using animals maximally in a confined area is a mandatory part of experimental design in the research program of the National Aeronautics and Space Administration (NASA). Protocols typically call for investigators to combine projects and make efficient use of one small group of animals (125).

Improved Experimental or Statistical Design

"Every time a particle of statistical method "is properly used, fewer animals are employed than would otherwise have been necessary," wrote Russell and Burch some 27 years ago (174). Since then, progress has been made both in the number of statistical tools available and in the training of investigators in the use of these tools. Yet training still lags behind the availability of tools. Insufficient information for critical evaluation and inappropriate statistical analyses appear frequently in the literature, particularly with investigators using the t-test in cases for which analysis of variance is the appropriate measure (82).

An analysis of variance simultaneously tests two or more parameters of treatment groups for indication of significant difference. When the test statistic falls in the rejection region, the researcher can be reasonably sure that a real difference exists between treatments. The t-test estimates the difference between the mean values of one parameter of two treatments. It is a powerful measure of significance when the number of comparisons is small, but it is subject to an increasingly large potential for error as the number of parameters grows. Using multiple t-tests increases the risk of finding a significant difference between treatments where there is none. Such observations are not esoteric, since poor summarization and statistical usage may reflect poor experimental design, calling into question the results of an investigation and leading to otherwise unnecessary repetition. At least one group, the Harvard Study Group on Statistics in the Biomedical Sciences, is pursuing ways to improve statistical practice and reporting (64).

Serial sacrifice, crossover, and group sequential testing are three experimental designs that can reduce animal use in laboratory research (82). In serial sacrifice, animals with induced effects are randomly selected for sacrifice and examination for the occurrence and progress of effects over time. Such studies, as in radiation oncology (22), have the dual advantage of cutting short the time some animals must spend in an affected state and providing information about changes within the animal other than those observed when it is allowed to die without further interference. The primary disadvantage is that survival information is compromised; therefore, the resulting data cannot be compared with other studies in which survival serves as an end point.

A crossover design maybe appropriate for studies in which short-term effects are expected. Each animal serves as its own control by first receiving either a drug or a placebo, and then receiving the reverse. Such a design can be highly useful in laboratory and clinical testing, but crossovers must be used judiciously. Should there be any unexpected long-term effects, the entire test is invalidated and would need to be repeated as two separate tests.

In the group sequential design, treatment groups are compared with each other in stages. For example, if two groups are given the same dosage of two different drugs, experimentation at higher dosages is undertaken only if there is no statistically significant difference between the responses of the two groups. The sooner a difference between groups is observed, the fewer the number of trials run. Both crossover and group sequential designs have potential applications in anesthesiology, endocrinology, nutrition, pharmacology, radiology, teratology, and toxicology.

A commonly mentioned method of reducing the number of animals used is smaller treatment groups. Yet within the biomedical research community a frequently heard complaint is that too few animals to yield useful estimates are likely to be included in each treatment group, particularly in fields such as radiology (95). Problems of this nature generally grow out of the extreme economic pressures being applied to investigators to control animal costs. Well-established techniques such as saturation analyses, particularly radioimmunoassays, have radically reduced the number of animals used for any one procedure, but they may have resulted in little or no reduction in overall

use, since they have made previously difficult analyses more accessible to many more investigators.

Substituting one Species for Another

In some instances, laboratory mammals (e.g., rodents) or nonmammalian vertebrates can be used in place of companion mammals (e.g., dogs), domestic species (e.g., sheep), or primates (e.g., monkeys). As more information on the physiology, biochemistry, and endocrinology of laboratory mammals and nonmammalian species accumulates and is demonstrated to be like or unlike that of humans, greater use can be made of laboratory species, which in turn can generate more information and reduce future needs for research. Comparative neuroscience is perhaps the most rapidly expanding field and is related to physiology, biochemistry, pharmacology, developmental biology, and zoology (33). Some brain components have been found to be remarkably similar between vertebrate species (69).

Economics plays a large part in the selection of how many and what kind of animals will be used in some research (see ch. 11). An investigator following upon previous work will generally begin with the species already in use, changing only if money becomes scarcer or if a better model is clearly demonstrated. Investigators starting anew are likely to seek the advice of a facility veterinarian or of colleagues as to which species best fits their needs and to begin with the smallest acceptable animal. Still other researchers deliberately begin work with a novel animal model in order to create a new research niche.

One of the principal reasons for the increased use of rodents in all areas of biomedical research has been the availability of genetically homogeneous or pathogen-free strains. For some studies, however, a further degree of genetic definition is needed. These studies require that the research animal carry some specific genetic traits that are suited to the objectives of the research, Because of their high reproductive potential, rodents are ideal for this type of “custom designing” and extensive use is being made of these animals in a variety of disciplines (109). Oncology and immunology are two of the more familiar areas of use (92). Pharmacological research using an ethanol-preferring strain of rats has prepared the way for exploration of the genetics of alcoholism (212). Further, the male Lewis rat, an animal that rapidly acquires testicular lesions and antibodies to sperm after vasectomy, is a candidate for study of the reversal of vasectomy. This research could answer questions of human concern in anatomy, physiology, immunology, endocrinology, and reconstructive surgery (100).

Chickens and their embryos play an important role in developmental biology, endocrinology, histology, and zoology. Other current uses are in molecular biology, in which embryonic chicken brain tissue is being cultured to study the neural-cell adhesion molecule (193), and biochemistry, in which the embryonic chicken liver is being used to study the acquisition of hormone responsiveness during embryogenesis (62). In cardiology, turkeys with inherited Turkey Round Heart disease serve as models of cardiomyopathy (107), and turkey erythrocytes are fused with amphibian erythrocytes to study receptors that mediate physiological functions in heart, smooth muscle, and other tissues (199).

Frogs have long been used in anatomy, biochemistry, developmental biology, physiology, and zoology. They continue to be widely used in those disciplines and, additionally, are currently being used by NASA in radiology studies (125). In dental research, frogs are used to assess digital transplants to augment tooth and jaw regeneration (101). The newt Triturus-able to regenerate its limbs, eye lens, tail, and spinal cord—is used in developmental biology to explore mechanisms of organ regeneration (90). Turtles are used in physiology to study, for example, retinal mechanisms subserving color vision. The cone cells of the turtle retina are especially conducive to such research (161).

Fish are used in research to a lesser degree than other vertebrates, considering that there are over 30,000 species and their care is relatively uncomplicated. It has been suggested that fish would make excellent subjects for nutritional research, since many are known to show specific vitamin deficiency symptoms (210). Physiologists have used goldfish to study the implications of myelin-sheath resistances in demyelinating diseases (73). Rainbow trout embryos are being used in oncology re-

search (93). Further, there has been recent interest in a specialized feature of some piscine species: the electric organ. This tissue is exceptionally rich in a single class of cholinergic synapses. Biochemists, geneticists, and molecular biologists working with this material have determined that the structure of the acetylcholine receptor protein is remarkably like a human's (39).

Reduction of Pain or Experimental Insult

Until recently, the probability of a research animal receiving the correct amount or type of anesthetic depended largely on the inclination of individual investigators. They could accept information about anesthesia available from previous research or attempt to improve on it. In some cases where little information was available, guesswork was required. Now, the enhanced presence of facility veterinarians and animal care and use committees with oversight authority (see ch. 15) has resulted in experimental animals being recognized as veterinary patients entitled to protection from as much pain and distress as possible, while maintaining the integrity of research.

Analgesics, anesthetics, and tranquilizers are the principle tools for the reduction of experimental pain and distress (see ch. 5). Terminal anesthesia and death has become the method of choice following major organ surgery on animals, even though it might be argued that observation of the healing process logically constitutes a part of surgical research (224). Where postsurgical study is considered necessary, as in cardiology, intensive postoperative control of pain can be used in lieu of maintaining the animal under general anesthesia until death (24).

Advances in Instrumentation

New types of instruments are critical to a reduction in experimental insult, as they can lead directly to the more refined or reduced use of live animals or living material. In the past decade, practically every piece of instrumentation in biomedical laboratories has been adapted to handle "micro" samples or has been replaced by new microtechnology. Some examples of microinstrumentation include:

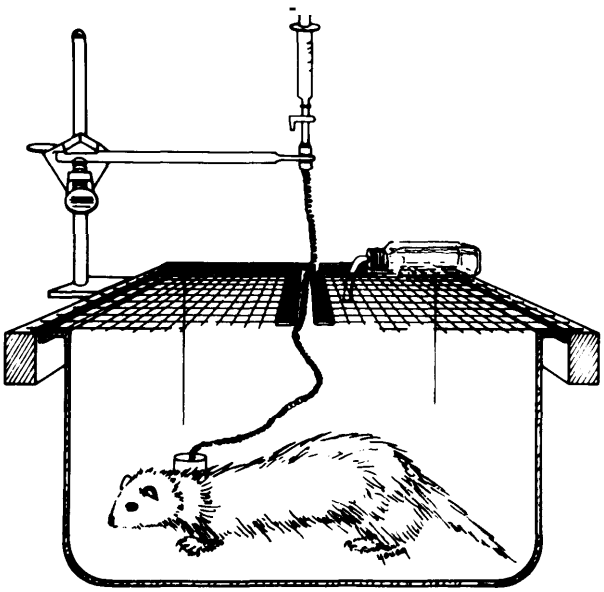
- In reproductive physiology, a 1.0 microliter sample of rat epididymal fluid collected by micropuncture can be used to examine sperm motility, determine total protein, and determine androgen-binding protein activity (207).
- In biochemistry and molecular genetics, electrophy biological techniques are being used to explore the possibility of recording the opening and closing of single membrane channels, tiny pores controlling cellular function (105).
- To study leukemias, blood diseases, and inborn errors in metabolism, a method for measuring the enzyme kinetics within a single white blood cell has been developed (134).
- A device is available that will dispense a 1.0 microliter sample as 1,000 aliquots (1 nanoliter each) for use in biochemical enzyme research or for clinical samples such as cerebrospinal fluid from infants (97),

The use of small samples for analysis by mass spectrometry (146) and by gas or liquid chromatography (86,208) exemplifies minimally invasive technology. Each year, an entire issue of Science magazine is devoted to trends in analytical instrumentation (2,3). Continued developments in analytical instrumentation, including noninvasive imaging techniques such as magnetic resonance imaging (MRI), will likely reduce the experimental insults faces by research animals.

In vivo measurements using fiber optics now provide miniaturized spectrophotometric analysis from within the ducts and blood vessels, determine blood velocity, measure temperature changes, monitor intracranial and intracardiac pressure, measure fluorescent marker molecules in tumors, measure pH, and even determine glucose concentration (166). Fiber optics offer great promise: They can be inserted into vessels and ducts via small catheters with little discomfort and into the abdominal cavity using local anesthetics (a laparoscopy), and they can be used repeatedly within the same animal to obtain measurements without permanent damage. Chronic intravascular catheters are used in a similar way to obtain repeated blood samples for hormone measurement from freely moving, undisturbed animals (see fig. 6-1) (189).

Other minimally invasive techniques in animal research include immunoscintigraphy, amniocentesis, and use of the laser. In immunoscintigraphy,

Figure 6=1.–Apparatus for Remote Blood-Sampling via Chronic, Intravascular Catheter From Unrestrained Ferret



SOURCE: C.L. Sisk, Michigan State University.

the production of target-specific monoclinal antibodies has improved the ability of external radioimaging techniques to locate tumors and to identify certain noncancerous diseases; radiolabeled antibodies attach to the target tissue and are then visualized (59). Amniocentesis is used for the early detection of genetic diseases, teratological events, and fetal distress, particularly in domestic species (67). A new application of the laser in oncology involves its ability to initiate a lethal photochemical reaction in cancerous tissue during photoradiation therapy (41).

Some apparently new noninvasive techniques are actually adapted, miniaturized, or computerized versions of older methods. One such example is a small, inflatable tail cuff used to measure blood pressure in a rat's tail during hypertension studies (225). In another example, urine is used in some specialized methods: In physiological research, electrical impedance measures canine urinary output (1).

Tandem mass spectrometry is being used for breath analysis to screen for diabetes, cirrhosis, renal disease, and ovulation. Many diseases remain to be examined, but there is potential for use of this technique in toxicology, nutrition, metabolic diseases, endocrinology, anesthesia, physiology, and pathology (133).

A technique developed for the determination of the quality of agricultural crops (162) and the percent of fat in beef (135) uses amplified, digitized, computer-corrected diffuse reflectance spectrophotometry in the near-infrared region. It involves simply placing an appropriate sensor on the surface of the skin and it can be adapted for oncological, physiological, and nutritional research (102).

Other increasingly popular noninvasive techniques include ultrasonography -which is used in cardiology to locate vessels (145), to determine blood-flow velocity (176), and to detect early atherosclerosis (108)-and magnetic resonance imaging, used to examine the energetic of skeletal muscle in gerontological research (201), to diagnose metabolic disorders (32), and to provide details of molecular structure and dynamics in liquids and solids (130).

USE OF LIVING SYSTEMS IN BIOMEDICAL RESEARCH

In Vitro Research

In vitro biomedical research entails the maintenance of organs, tissues (or fragments of organs and tissues), and cells outside of the body. Depending on the conditions of harvesting and preparing the living material for in vitro maintenance, the cells may be grown as a population of independent cells (cell culture) or with the normal tissue or organ architecture preserved. In the former, the cells may be encouraged to proliferate, resulting innumerous descendant cell populations suitable for studies on growth, nutrition, cell division, and gene expression and regulation.

Table 6-2, which summarizes the characteristics of in vitro systems, makes it clear that as organization is disrupted or lost, the in vitro system has less and less of the kind of intercellular and intracellular interactions that characterize organs,

Table 6-2.-Properties of In Vitro Culture Systems

Preparation and consequences	Level of tissue organization	Reproducibility	Expression similar to in vivo	Genetic alteration by mutation and/or selection	Environmental control
Intact system (no consequence). . . .	+ + + +	+ + + +	+ + + +	+	+
Organ culture (remove influences of whole organism).	+ + + +	+ + + +	+ to + + +	+	+ +
Tissue culture (remove influences of whole organisms).	+ + +	+ + +	+ to + + +	+	+ + +
Primary cell cultures (disrupt intercellular relationships)	0	++	+ to + + +	+	+ + + +
Cell lines (intercellular relationships are reduced; cell proliferation is enhanced, at times with little control)	0	++++	+ to + + +	+ to + + + +	+ + + +

KEY: + + + + = High degree; + + + = Moderate degree; + + = Modest degree; + = Some degree; O = None.

SOURCE: Adapted from R.M. Nardone and L.A. Ouellette, "Scope of 'Alternatives': Overview of the State of the Art, " contract report prepared for the Office of Technology Assessment, U.S. Congress, July 1984.

tissues, 'and cells in the body. Nevertheless, improved accessibility of added chemicals and the opportunity to achieve genetic homogeneity by cloning and genetic manipulation by selection and fusion are important trade-offs. Indeed, at times cell-to-cell interaction may interfere with an experimental objective (156).

The explosive growth of in vitro research during the 1960s and 1970s is illustrated by the fact that the index ***of Tissue Culture*** had 84 pages of entries in 1965, 207 pages in 1970, 566 pages in 1975, and 636 pages in 1980, when the publication was discontinued because computerized information retrieval was warranted. There is virtually no field of biomedical research that has not been affected by in vitro technology. In vitro models for the study of cell senescence, atherosclerosis, development, growth, and immune reactions are illustrative of the diversity of applications in biomedical research (156).

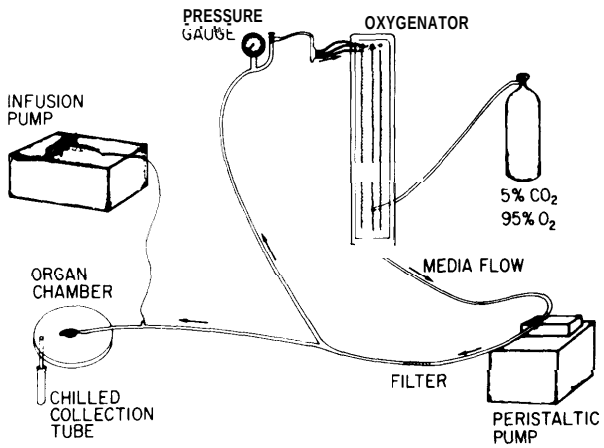
The specific conditions that best support the maintenance, growth, or differentiation of each type of culture must be determined before any useful information can be garnered. Some of the general requirements of culture systems are combinations of the proper gas atmosphere, humidity, temperature, pH, and nutrients. other culture systems may also have specific light, motion, pressure, and physical or chemical support requirements, Under the proper conditions, many can be subculture for months or frozen in liquid nitrogen for years without loss of their unique, differentiated properties.

The ability to maintain many continuous cell lines has opened the floodgates of experimentation and made the new technologies accessible to all the disciplines of biomedical research. Other advantages include ease of transport from one laboratory or country to another, the ability to culture both normal and abnormal tissue for comparison and research, the use of human cells to eliminate species variation, and the ability to expose cultures directly to exogenous molecules at specific concentrations for precise time periods. Disadvantages include the changes in structure or function observed in some cultures, and the fact that isolated systems give isolated results that may bear little relation to results obtained from the integrated systems of whole animals.

Organ Culture

At some point in the history of research, investigators have attempted with varying success to isolate and maintain every major and minor mammalian organ, for a variety of purposes. In recent years, improved techniques, such as the availability of artificial blood media, have increased the probability of successful organ culture. Blood, or artificial blood media, can be pumped through the organ to sustain it ("perfusion") (see fig. 6-2). Current applications of organ perfusion include the study of protein synthesis in lactating guinea pig mammary tissue (136) and the use of human placentas in toxicology studies, with additional potential for use in oncology and gerontology research (99).

Figure 6=2—Schematic of Experimental Organ Perfusion



SOURCE: C. Chubb, The University of Texas Health Science Center at Dallas.

Organ Perfusion: Mouse Testis With Pipette Introduced Into Artery

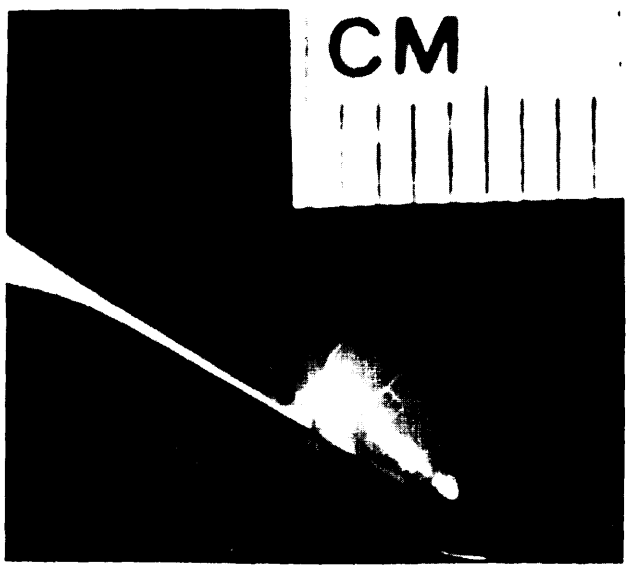


Photo credit: C. Chubb, The University of Texas Health Science Center at Dalias

whole organs are not generally amenable to long-term in vitro culture or growth. The size and complexity of whole organs make it impossible for them to receive sufficient nourishment for normal function without external support. Nevertheless, whole organs are fundamental to many types of anatomy, histology, and pathology because of their suitability for the examination of relationships between cells and tissues, and they can be sustained in culture for hours or days.

Cryostat sections (thin slices of frozen tissue cut with a microtome) through organs are maintained in vitro for oncology studies into organ-specific adhesion of metastatic tumor cells. This method closely reflects the in vivo event and therefore could eventually reduce the use of whole animals in a very active research area (159).

Whole mammalian embryos, in addition to their obligatory use in the investigation of basic developmental biology, have been cultured in vitro for other purposes. Protocols have included examinations of the effects of hormones and teratogens (42).

Tissue Culture

Many normal and pathological tissues from humans and a variety of animal species can be successfully maintained and studied in culture. Indeed, the progress that has been achieved since 1907, when R.G. Harrison first maintained frog neural tissue outside of the body for weeks, has changed the field of tissue culture from an art into a science. Keeping cultures of anything other than bacteria or viruses alive for more than a few hours was problematic until the 1950s, when investigators began to gain a better understanding of the requirements of cells and the addition of antibiotics to culture systems. The viability of cultures was extended substantially by controlling bacterial contamination.

In tissue culture, isolated pieces of a living organism are maintained with their various cell types arranged as they were in the original organism and with their differentiated functions intact. Such cultures are both “better” and “worse” than cultures of a single cell type. They are better in that the effects of manipulation can be observed in a more natural environment and different cell types can interact as they would in vivo. They are worse in that they are much more difficult to maintain. Although tissue-culture experiments require the sacrifice of an animal, they can be viewed as alternatives to animal use since numerous sections of adjoining tissue can be removed and compared. In this way, two or more treatments are administered to tissues, rather than to a number of individual animals.

Tissue culture is being successfully employed in many disciplines of biomedical research. In neurology, the use of embryonic rat mesencephalon tissues to examine the destruction of dopamine neurons has replaced the use of primates (154). Prior to this development, the monkey had served as the best model for the study of degenerative effects observed in humans. Adult rats, cats, and guinea pigs have been shown to be resistant to the destruction of dopamine neurons. Metabolic studies of an experimental antiarthritis agent have made use of the inside of hamster and rat intestinal walls (202). Physiological experiments examined the dynamics of secretion with mouse epididymis (70).

Cell Culture

Although cell culture is not a new technique, developments and applications during the past decade have come so rapidly as to create whole new research institutions and industries. Cell culture today touches every discipline of biomedical research, as well as clinical practice. The following illustrates the pervasiveness of this approach in biomedical research:

- Eggs and sperm from many species have been used by endocrinologists, physiologists, and biochemists to study the mechanisms involved in fertilization and early development (58,157).
- A hamster ovary cell line and its mutants are being used to explore the biochemistry of a membrane-associated protein essential to all animal cell function (175).
- In oncology, human interferon derived from bacterial recombinant DNA induces a transformation of human white blood cells similar to that observed during infection, cancer, and rheumatoid arthritis. This change in white blood cells provides clues to the pathology of cancer (216).
- Steroid metabolism is being studied using cultured rat epididymal cells (29).
- A monkey kidney cell line was employed to demonstrate the metabolic effects of several general anesthetics (26).
- Geneticists are developing an in vitro method of studying heme gene expression (54).
- Surgical research into the use of cultured human epitheliums for permanent coverage of large burn wounds has moved from the laboratory into clinical trials (76).

In immunology, studies on antibody synthesis and response have been bolstered by the Nobel-prize-winning elucidation of monoclinal antibodies. In its initial steps, this technique consumes large numbers of animals, as the varying immune responses of many mice are probed. Then, cloned cells from the spleen of one mouse can be exploited to produce valuable, highly specific antibodies. Antibodies so produced can obviate the need for many rabbits, sheep, and even humans in the large-scale production of antibodies. Perhaps of even greater importance for research is the high quality of the antibody produced by monoclinal cells. A comprehensive listing of current research being conducted with monoclinal antibodies from cloned cells is beyond the scope of this assessment. Some of the diagnostic potentials of monoclinal antibodies being explored in biomedical research are:

- the characterization of malignant and benign tumors;
- the identification of autoimmune antibodies in rheumatoid arthritis, systemic lupus erythematosus, myasthenia gravis, and other autoimmune diseases;
- the identification and quantification of serum proteins, hormones, and their cell-surface receptors;
- the monitoring of therapeutic drugs and identification of novel therapeutic drugs;
- the rapid diagnosis of bacterial, viral, fungal, and parasitic diseases;
- the monitoring and identification of lymphoid and hematopoietic cells in disease states; and
- pregnancy testing.

Biologists have developed techniques for the controlled disruption of cells that can leave many organelles intact or allow the harvesting of selected intracellular membranes. These fractions have proved to be invaluable in the search for information at the molecular level. For example, microsomal membrane fractions from rat and human liver have been used in comparative anesthesia research (27). In endocrinology, human placental and ovarian microsomes were used to demonstrate inhibition of steroid hormone synthesis by plant chemicals (112). In dentistry, proteins purified from unerupted fetal buds were shown to be in-

hibitory toward seeded enamel growth in culture (56). In virology, infected cell nuclei isolated from a hamster cell line were used to study influenza virion RNA replication (16). In metabolic studies, the inhibition of chick-embryo-derived collagen fibril formation by glucose suggests a direct relationship between excess glucose and poor wound healing observed in people with diabetes mellitus (126). One of the most unique uses of subcellular fractions involves the bringing together of mixed species systems in biochemical studies of protein transport across intercellular membranes. For example, researchers studying intracellular protein translocation used dog pancreatic microsomes, bovine pituitary and rabbit reticulocyte messenger RNA, bacterial nuclease, and a wheat germ cell-free system to elucidate the structure of the signal recognition particle (213).

Human Tissues and Cells

Cultured human fetal lung cells have been found to be excellent hosts to support the developmental cycle of a protozoan parasite that causes severe, persistent, life-threatening diarrhea in immunodeficient patients. There has been no effective therapy for this illness, so the in vitro system offers an opportunity to study the parasite's behavior, development, and metabolism and provides a potential method for screening therapeutic agents (50).

Virologist and oncologists have been very quick to take advantage of new human in vitro culture systems. For example, an embryonal carcinoma cell line from the stem cells of a teratocarcinoma is being used to study cytomegalovirus replication (83). Lysis of herpes simplex virus (HSV) type 2 is being investigated using human monocytes (117), and HSV latency is studied by using isolated neurons obtained from human fetuses (220).

The use of postmortem material from humans has significance in many areas of biomedical research, but particularly in neurology. Investigators studying the unconventional slow-virus diseases use brain tissue from humans with Creutzfeldt-Jakob disease and from animals with scrapie (144). Postmortem material from schizophrenics has provided evidence for two distinct categories of that disease (181), and temporal lobe structures from Alzheimer patients have revealed specific pathological cellular patterns in the brain hippocampal formation (104).

Examples of the use of human tissue for investigations aimed at human treatment can today be drawn from every discipline of biomedical research. Advances in in-vitro culture methods are likely to increase this use further. Postmortem tissue use is likely to continue.

Invertebrates

Invertebrates represent over 90 percent of nonplant species on the earth. Although their body structure is much less similar to humans than is vertebrate body structure, invertebrate anatomy, physiology, and biochemistry offer avenues for new approaches that have been only partially explored.

Caenorhabditis elegans, a 1 millimeter roundworm, is of intense interest to developmental biologists. As they have traced this nematode's complete cell lineage, it offers an unprecedented opportunity for the study of individual living cells (38).

Other terrestrial invertebrates are used in many disciplines of biomedical research. For example, flies, bees, earthworms, and leeches are involved in various aspects of anatomy, physiology, and biochemistry (5,33). Ants and bees are used in vision research (4). Fruit flies are well known for their participation in genetic studies. Age-related metabolic changes in other insects are being investigated for possible use in aging research (184).

Marine invertebrates represent an important, largely untapped research resource. one commentator (190) has suggested that the lack of opportunity by medical scientists to learn marine biology and the failure of marine biologists to learn pathology have combined to leave marine species overlooked. A notable exception to the underuse of marine invertebrates is neurobiology. The coelenterates, including hydra, corals, anemones, and jellyfish, have helped scientists understand primitive nervous system biochemistry. Lobsters and squid have contributed to knowledge of brain anatomy and physiology, and the grazing snail and crayfish have broadened understanding of cell biology (33).

Four advantages of using invertebrates in biomedical research are:

- different phylogenetic levels of structural and functional specialization can be exploited (e.g., different types of circulatory systems, novel chemical compounds);
- invertebrates reproduce rapidly and produce numerous offspring; experiments can be executed in days and weeks instead of months and years;
- storage, upkeep, and maintenance are inexpensive; and
- invertebrates are not prone to spreading dis - ease throughout a colony;

The overwhelming disadvantage is the considerable phylogenetic distance between invertebrates and humans.

Micro-organisms

From its origins within the medical disciplines of bacteriology, pathology, and virology, the study and use of microorganisms has branched out to influence practically every area of biomedical research, as these examples indicate:

- ***Salmonella typhimurium—bacteria*** used in mechanistic studies in genetics (124) as well as the Ames mutagenicity/carcinogenicity test (see ch. 8);
- ***Escherichia*** coli—bacteria used by developmental biologists to derive theories of gene control (90) and by molecular biologists in recombinant DNA research (75);
- ***Streptococcus*** mutans—bacteria used in dental research on the metabolic activity of plaque (91);
- ***Bacillus subtilis*** (bacteria) spores, ***Artemina sa - lina*** (brine shrimp) eggs, and ***Sordaria fimil- cula*** (fungi) ascospores-all incorporated into NASA's biostack (monolayer of biological test organisms sandwiched between thin foils of different types of nuclear track detectors) radiology experiments inside Spacelab I (31);
- ***Tetrahymena pyriformis—a*** ciliate protozoan being used to study the effects of anesthetics on metabolism (44); and
- a host of microscopic protozoans, metazoans, and rotifers used to investigate the physiology and biochemistry of photoreception and vision (4).

The advantages of using micro-organisms in biomedical research are fourfold: They reproduce rapidly at body temperature; rapid division (every 20 to 30 minutes) makes them useful for short-term studies; multigenerational studies can be performed in a short period of time; and they are inexpensive in terms of storage, upkeep, and maintenance. The major disadvantage stems from the fact that these are unicellular organisms: As a consequence, the interaction of cells cannot be studied (156).

Plants

One advantage of using organisms from the plant kingdom is that they lack anything resembling a nervous system. Presumably, plants do not feel pain; they appear to be good potential alternatives to animals. Plants, like micro-organisms, are relatively easy and inexpensive to propagate (156).

Although there is some interest in the potential use of plant cells in toxicology and oncology research (191), the use of whole cells from plants is obstructed by their very rigid cell-wall structure compared with the relatively fluid animal cell membrane. This prevents their use in many disciplines where intimate cell surface contact or transmembrane communication is essential.

Once removed from the cell, comparable organelles from plants and animals (including micro-organisms) are essentially indistinguishable in both appearance and function. For example, in studies having potentially broad applications in endocrinology and immunology, yeasts have been found to contain active steroid hormone systems (118). Yeast is used in cell biology in studies of the import of proteins into mitochondria, organelles that are essentially the same whatever their source (129). An extensive literature in cell biology, genetics, molecular biology, and virology supports the use of subcellular fractions from plants and animals, separately or together, for research into basic molecular mechanisms (213).

USE OF NONLIVING SYSTEMS IN BIOMEDICAL RESEARCH

Chemical and Physical Systems

Long before the advent of modern technology, researchers were constructing chemical models of certain phenomena that occur in living systems. There is a long and rich history in biochemistry, for example, of the application of nonliving systems to experimentation (128,147).

Enzyme biochemistry continues to be a principal area of application of nonliving methodology in biomedical research. Enzyme mechanisms maybe studied in a totally chemical system. Magnetic resonance imaging is used to obtain from enzymes in solution detailed structural data and information about the mechanism of enzyme action. By combining MRI with cryoenzymology-the use of enzyme solutions held at subzero temperatures—enzymatic reactions can be slowed enough to study intermediate products that would ordinarily exist for too short a time to be detected. Investigations that had been restricted to in vivo manipulations can now be expanded into a far wider range in vitro (131).

In dental research, a chemical system mimics the mechanics of the formation of dental caries. A two-chambered diffusion cell pairs an excess amount of specific protein crystals with a chemical solution of artificial "plaque-saliva ." Dissolution of the crystals can be studied under varying chemical conditions relevant to a better understanding of the caries process (40).

In the field of membrane biophysics, the advent of synthetic membranes has proved a boon to research and stands as one of the premier examples of nonliving alternatives to animal use. Liposomes are synthetic vesicles made of protein and fatty molecules. Their structure can be dictated by the investigator, who can combine proteins and lipids of different types and in different ratios to yield an artificial membrane. As with true biological membranes, the barriers formed by liposomes are selectively permeable. These artificial membranes are particularly useful in basic studies of the transport of molecules across membranes and of membrane damage (12).

Except for the use of mannequins to simulate accident victims in the transportation industry and in trauma centers, the principal use of physical and mechanical systems today is in education (see ch. 9) rather than in biomedical research. However, it is not inconceivable that future combinations of mechanical and electronic technology could provide biomedical researchers with artificial research subjects capable of independent, unanticipated responses.

Epidemiology: Using Existing Databases

The use of existing databases to gain new information and insights in biomedical research may be a major underused resource, if the paucity of published results is any criteria. One study that relied on such information concentrated on the relationship between 17 nutrients and the potential for development of hypertension cardiovascular disease in more than 10,000 people from the database of the National Center for Health Statistics' Health and Nutrition Examination Survey (HANES I) (137). The results proved to be highly controversial, with some of the criticism aimed at the use of the database (119).

Too little information is currently available to evaluate fully the potential dimensions of the salutary use of epidemiologic databases in basic biomedical research. The possibility exists that their enhanced use may constitute an important nonanimal method.

COMPUTER SIMULATION IN BIOMEDICAL RESEARCH

Modern approaches to biomedical research describe the functions of living systems at all organizational levels by the language of science—mathematics. Knowledge is acquired by investigating relationships among cells, tissues, fluids, organs, and organ systems. By the processes of trial and error and of hypothesis and testing, relationships begin to be understood and can be described by

mathematical expressions. These can range from simple, linear functions to various types of curved functions to multidimensional surface functions and may involve kinetic data expressed by differential equations. Variables in these equations include physical terms, such as time, temperature, weight, energy, force, volume, and motion. Complex mathematical relationships may be developed to express these cause-and-effect relationships more clearly. In some instances, a relatively simple relationship may be shown to exist, but this is unusual, since living systems are highly interactive and multidimensional in nature (48).

A relationship that can be reasonably expressed in a mathematical equation maybe considered to be a candidate biological model. The limits within which the expression will hold determine the utility and validity of the model. If it is possible to change one or more parameters in the equation, and thereby obtain the same response or responses as found in live animal research, the model may be used to "simulate"a biological preparation. Simulation implies that an investigator can manipulate the parameters at will and observe the resultant effects on the model. Used in this way, computer simulation is a useful tool for research and especially for suggesting new mechanisms or hypotheses for further study (48).

At the subcellular level, information is usually gained by electron microscopic examination or by analytical methods for the sequencing of amino acids and nucleic acids. Such information tends to be of a descriptive or topological nature rather than numerical. Recent strides in genetic engineering based on increased knowledge of DNA, RNA, and protein amino acid sequencing have required computers to store and match nucleic acid and amino acid sequences numbering in the millions (163). These capabilities are not equivalent to simulation, but they share with simulation a reliance on computers for storage and processing.

At the level of one or a few cells, models are being sought for computer simulation of sliding filament systems—believed to be the basic movement of muscle fibers, cilia, and flagella (28). Modeling of the function of individual cone cells in the eye is under study at the National Institutes of Health (NIH) (167).

Most efforts toward computer simulation of biological systems are directed at higher levels of organization, such as organs and organ systems. This bias is a consequence of the need to understand numerous feedback systems within living systems. Feedback systems are the basis for an organism's ability to maintain a homeostatic, or steady, state, Feedback mechanisms involve several organs as well as communication via the bloodstream and nervous system. For a simulation to succeed, the system must be considered as a whole. In modeling the cardiovascular system, for example, a simulation must take into account the heart, brain, lungs, and kidneys.

In the 1980s, computer modeling of organ systems is progressing on many fronts. The brief sampling of simulations listed in table 6-3 illustrates the variety of organ systems under study.

One development in this field deserving particular attention was the establishment by NIH's Division of Research Resources in 1984 of the National Biomedical Simulation Resource, a computer facility at Duke University that may be used onsite or over a telephone data network. Any project in which the results are free to be published in open scientific journals and where no profit is involved can apply to use the facility. Training sessions introduce biological scientists to the concepts of modeling, and special aid is provided in the development of simulation software (120). Projects under

Table 6-3.-Some Examples of Computer Simulation of Phenomena in Biomedical Research

Kidney function:
• Transport of electrolytes, nonelectrolytes, and water into and out of the kidney (142)
Cardiac function:
• Enzyme metabolism in cardiac muscle (214)
• Cardiac pressure-flow-volume relationships (1 52)
• Malfunctions of instrumented cardiovascular control systems (9)
Lung function:
• Respiratory mechanics (150)
Sensory physiology:
• Peripheral auditory system, and single auditory nerve fiber transmission of vibrations (180)
Neurophysiology:
• Impulse propagation along myelinated axons (73)
Developmental biology:
• Shape changes i n embryonic cells that develop into mature organs (98)

SOURCE: Office of Technology Assessment,

way in **1985** involved research in cardiology, physiology, endocrinology, toxicology, and neurology. Specific simulations included:

- regulation of sodium, potassium, and calcium in heart muscle;
- electrolyte diffusion in heart muscle;
- propagation of activity in heart muscle;
- heart volume potentials;
- mathematical modeling of blood coagulation;
- regional dose responses in the human and animal lung;
- ciliary motility;
- cochlear function in the inner ear; and
- a molecular model of ion transport in nerves and muscles.

Limitations on the utility of computer simulations stem from the lack of knowledge of all possible parameters that may play a role, however slight, in the melange of feedback-mechanisms that constitute living systems. Basic biomedical research at all levels, some of it involving live animals, will continue to provide the new knowledge required to improve existing simulations and develop models where no satisfactory one exists. The development of increasingly powerful computer programs depends on the use of animals in biomedical research.

CONTINUED, BUT MODIFIED, USE OF ANIMALS IN BEHAVIORAL RESEARCH

As in biomedical research, the continued, but modified, use of animals in behavioral research encompasses reducing the number of animals used through changes in experimental design and statistical analyses, substituting cold- for warm-blooded vertebrates, and lessening the degree of pain or experimental insult in general, and in pain research in particular. Compared with biomedical research, behavioral research offers markedly fewer opportunities to substitute cold-for warm-blooded vertebrates and to use in vitro cultures, and it holds little chance of using nonliving systems.

Reduction in the Number of Animals Used

Improved Experimental Design and Statistical Analyses

Individual animals vary in their behavior both between subjects and, in the case of one subject, over time. The goal of a behavioral experiment is to identify patterns that remain when these two sources of variability have been eliminated or taken into account. An investigator attempts to conclude that observed effects are due to the conditions being manipulated in the experiment and not to extraneous factors. This decision usually rests on the outcome of statistical tests. Ensuring the validity of such tests or improving their design can mean that fewer experiments are needed. Enhanced statistical rigor, however, may lead to increases or decreases in the number of animals required in a particular protocol.

Statistical Power.—A statistical test's sensitivity in detecting experimental effects is termed its "power." The most widely recognized method of increasing power and, hence, the sensitivity of an experiment is to use a large sample of subjects. Typically, the more variable the results, the more power is needed to detect an effect and, therefore, the greater the need for large samples. Although the magnitude of variability cannot be determined prior to an experiment, the amount of variability likely to be encountered can be estimated by conducting small, pilot studies or by examining previous research in the same or related areas. Given an estimate of variability, statistical tables can be used to determine the sample size needed to attain certain levels of power (221).

In certain instances, the methods of increasing power may reduce, not increase, the number of animals needed:

- Choosing a lower level of statistical significance (i.e., the likelihood that the results were due to chance) increases power and reduces

the number of subjects needed. However, this also increases the chances of concluding that the experimental procedure produced an effect when in fact the effect was due to chance alone. By convention, researchers generally accept the probability of a chance effect of 5 percent or less as a statistically significant result.

- Greater precision in the conduct of an experiment may reduce variability and increase power. For example, highly precise behavioral measurements coupled with the elimination or control of extraneous variables would reduce the need for large numbers of subjects (198).
- The use of different statistical analyses can increase the sensitivity and power of a protocol (e.g., analysis of the data by parametric rather than nonparametric statistical tests) (198).
- Alterations in experimental designs can increase power. Factorial designs (where two or more treatments are manipulated concurrently), for example, are more powerful and can be used instead of testing the effects of different treatments in separate experiments. Not only does the use of factorial designs increase power, it requires fewer untreated, control subjects than multiple concurrent studies do. It is important to note, however, that in areas that have not been heavily researched there are inherent dangers to the use of factorial designs. For example, there may be no observed effect of treatments given in combination, as one treatment cancels the effect of another. Without sufficient background information on the effects of the treatments administered individually, this finding would be erroneously interpreted.
- Power is increased as the magnitude of the treatment effect is increased. 'Treatment effects can be maximized by choosing widely spaced levels of the treatment variables or by including conditions that are thought to maximize the appearance of the phenomenon under study (113).

Within-Subjects Design.–Many experiments on animal behavior are conducted using a between-subjects design, That is, different groups of animals are given different treatments, and the performances of the different groups are compared. However, individuals also vary in their behavior. Depending on the degree of variability, large numbers of subjects maybe needed in each group to obtain statistically significant results. Under certain conditions, however, a within-subjects (or repeated measures) design can be used that requires only one group of animals instead of many. Under these conditions all members of the group serve in all treatment conditions. The advantage of this technique is that it minimizes variability by taking into account individual differences. The major drawback, however, is the possibility of contaminating the data and nullifying the results: Treatments already received by a subject may influence, and thereby confound, performance under subsequent treatments. Carry-over effects can be partially offset by counter-balancing, wherein the experimenter ensures an equal occurrence of each experimental treatment at each stage of the experiment; this balances any effect of prior testing equally overall treatment conditions (1 13). Although within-subjects designs are effective in reducing both variability and the number of subjects needed, the inherent danger of carry-over effects in many instances may invalidate the use of such designs.

Random Block Design. -Randomized block design consists of assigning subjects to groups based on evidence of their being similar to one another in one or more characteristics known to be related to the behavior under investigation. Two or more such blocks are formed and then each block is assigned randomly to the treatment conditions. This design reduces variability by restricting the degree of individual differences within blocks, and thereby increases power (113). Although randomized block designs are effective in lowering the number of animals needed in an experiment, they are not applicable to all areas of behavioral research. The technique requires substantial prior knowledge of the behavior being investigated and is therefore limited to intensively researched areas.

Analysis of Covariance.—An analysis of covariance uses the same information as randomized block designs except that an estimate of variability is not needed beforehand. The covariance pro-

cedure is applied to data after they are collected to adjust for chance differences among groups. The analysis increases power, and fewer animals may be needed to obtain statistically significant results (113).

Single-Subject Design.—In some instances inferences can be made about populations from very small samples. This is common in psychophysical experiments in which there is a substantial prior body of evidence indicating that the behaviors under investigation do not vary appreciably within the population at large (e.g., visual sensitivity to light). Although such experiments can be conducted using just one subject, two or three are typically used to guard against the possibility of misleading results from an atypical subject (198).

In other than psychophysical experiments, the general procedure in single-subject research consists of choosing a baseline (which involves measuring the frequency of occurrence of the behavior of interest), changing one treatment variable at a time, temporarily withdrawing the experimental treatment to assess its causal effects, and repeatedly measuring the baseline behavior before and after each treatment. (More sophisticated experimental designs available for single-subject research are reviewed in ref. 96.) Single-subject designs are increasingly used in animal operant conditioning and human clinical research (187). Statistical analyses of these are reported infrequently due to the lack of many statistical techniques for handling such data, although using time-series analyses to test for changes over time is one acceptable method available (111),

A limitation to studying a single subject is the uncertainty of the generality of the findings, a problem commonly dealt with by replicating the experiment with different subjects (96). Thus the reduction in animals used may be illusory.

Inbred Strains.-One way of reducing variability (and hence increasing power) is to use highly homogeneous populations of subjects. Inbred strains of animals, produced as a result of 20 or more generations of brother-sister matings, represent one approach, though it is usually much more expensive than using randomly bred animals. Inmost inbred strains all subjects are highly identical genetically and genetically stable; they change only as a result of the slow accumulation of mutations. In contrast, outbred stocks of animals are genetically variable. They contain an unknown and uncontrollable degree of genetic variation that may obscure or mask experimental treatment effects. Inbred strains not only increase statistical power, they also reduce variability between experiments conducted indifferent laboratories or in the same laboratory at different times (68).

It can be argued that experiments should rely on animals drawn from heterogeneous, outbred populations in order to get a broad genetic basis for results that can be extrapolated, for example, to heterogeneous human populations. Yet the differences between different inbred strains are usually greater than the differences between individuals of an outbred stock. Greater generality, then, may be obtained by conducting experiments with two or more inbred strains (68).

Sharing Animal&—A team approach to research questions across biomedical and behavioral research disciplines could reduce the number of animals needed for behavioral research (173). For example, researchers studying a behavioral phenomenon by noninvasive means could, at the experiment's conclusion, give their animals to biologists investigating the anatomy or physiology of that species. Likewise, scientists from different disciplines could collaborate on research proposals: A psychologist maybe interested in studying predator-prey relations, while a biologist wants to study endocrinological changes in response to stress; ef - fective collaboration could yield two different data sets from the same animals.

Substitution of Cold-Blooded for Warm-Blooded Vertebrates

The modified use of animals in biomedical research includes the replacement of mammals and birds with fish, amphibians, and reptiles. In behavioral research, however, the often vast differences between species are likely to make such substitutions difficult. At the moment, researchers know more about why warm-blooded vertebrates **cannot** be replaced with cold-blooded ones, as this description of seven behavioral research disciplines illustrates.

Aggression

Aggressive interactions between members of the same species have been studied in a variety of fish and reptile species under both laboratory and field conditions. Considerable work has been done on intermale rivalry among sticklebacks (183) and cichlid fish (11). Aggressive interactions among cold-blooded vertebrates are frequently stereotyped and species-specific. Among sticklebacks fish, for example, full-fledged attacks can be elicited by a model that is the same color but a different shape ***(30).*** In contrast, aggression in primates can embody a variety of highly sophisticated introspective social strategies such as deception, grudging, delayed retaliation, and reconciliation (77). Thus extrapolation among all vertebrates of the results of research into aggression is difficult.

Animal Movements

Migration and homing abilities have been intensively studied in several species of fish, particularly eels and Pacific salmon. Among American and European eels, for example, the eggs hatch in the Sargasso Sea, near Bermuda. The juvenile fish make a year-long migration toward the coasts of North America and Europe. On reaching sexual maturity in 7 to 15 years, the adult eels migrate back to the Sargasso Sea to breed **(217),** a movement primarily dependent on the use of chemical cues in the water (60). In contrast, birds use solar, stellar, and magnetic cues to navigate, and whales use the topography of the ocean floor and coastline to remain on course for migratory purposes. Such dramatic differences in the way different species respond to and perceive the environment limit the use of cold-blooded vertebrates in modeling animal movements of warm-blooded ones.

Communication

Visual cues, such as changes in coloration, posture, or body appearance, have been shown to be important determinants of social interaction among fish **(3o),** which, unlike most mammals, generally have color vision. Fish also exhibit dramatic changes in appearance, such as flaring of the gill apertures, which are relatively rare among mammals. Auditory communication is marked by species differences, too. Communication among amphibians and reptiles, primarily to attract a mate, consists of simple one- or two-note utterances. Vocalization in birds and mammals consists of a wide range and variety of sounds. Moreover, unlike cold-blooded species, many birds have to learn species-specific songs. Many rodents communicate by ultrasonic vocalizations **(123)** that have no apparent counterpart among cold-blooded species.

Learning, Memory, and Problem Solving

Learning has been studied in a variety of diverse species (179, 183), and many differences are manifested. Comparing learning in goldfish and turtles with that in rats yields both similar and distinguishing features. For example, rats show a decrement in performance when an accustomed reward is changed, while goldfish and turtles do not (23). The existence of so-called biological boundaries of learning (182), apparently shaped by unique ecological pressures, precludes most substitutions of one species for another in learning paradigms.

Predator-Prey Relations

Prey-catching behavior and predator avoidance have been studied in fish, frogs, and turtles (60, 103,203). The similarity across species in behaviors used by prey to avoid being caught suggests that when a general question about reactions to predation (rather than the behavior of a given species) is of interest, cold-blooded vertebrates can substitute for warm-blooded ones (103). But there is growing evidence of neurochemical differences underlying predator avoidance behaviors even among birds and mammals **(78).**

Predators exhibit marked differences across species. Frogs, for example, sit passively and wait for an insect to come within striking distance, while some carnivores have developed sophisticated hunting strategies that often embody elements of cooperation and may even culminate in sharing foods (30).

Reproduction and Parental Care

With some notable exceptions (e.g., the African Mouthbreeder fish), parental care of offspring is absent inmost cold-blooded vertebrates, since the eggs are typically abandoned shortly after fertili -

zation. In contrast, all birds are subject to some type of parental care, and mammalian parent-offspring relations become even more complicated. Species differences in external versus internal fertilization, seasonal breeding, courtship, pair-bonding, and nest-building preclude substitutions of one species for another in this research.

Sensation and Perception

The sensory and perceptual differences among species are vast. For example, many snakes' primary mode of prey identification is chemical cues transferred from the tongue to a structure at the roof of the mouth, called Jacobsen's organ. Ingestively naive baby snakes appear to have an innate preference for prey extracts that represent species-typical foods across a variety of different snakes. Each species shows unique attack profiles that appear to be independent of maternal diet and not subject to modification by experience (e.g., baby snakes of a minnow eating species that are force-fed liver still show attack responses to minnow extracts but not to liver) (34,35).

In contrast, baby rats seem predisposed to eat the same diet as their mother, and the flavor of the maternal milk serves as a medium for the transmission of cues that rat pups use to make their initial food choices. Manipulating maternal diet during lactation has produced corresponding changes in subsequent pup food preferences. Moreover, rat pups poisoned in association with a mother's milk later avoid the types of food she had been eating (74).

Reduction of Pain or Experimental Insult

As noted earlier, a general anesthetic is preferable to a local one for surgical manipulations because it suppresses both pain and fear (114). Pain-relieving drugs should be administered to animals after surgery whenever this would not interfere with the behavior under study, and animals should be carefully monitored so that any complications that develop may be treated (197).

Transection of the spine or brain stem is recommended for surgical experiments when possible, because it renders the animal incapable of feeling pain (114). This technique has limited applicability in behavioral research, however, as postsurgi - cal behavioral assessment requires a relatively intact animal. Similarly, the nonrecovery experiments on completely anesthetized animals that were described earlier, in the biomedical research section, are rarely used in behavioral research, since most behaviors of interest do not occur when the animal is unconscious and behavioral testing is typically conducted postsurgically. Multiple surgeries on the same animal are to be avoided whenever possible, because painful consequences may be cumulative (197).

The analysis of pain in behavioral research is complicated by recent theoretical and empirical developments suggesting that fear and pain activate quite different kinds of behavior (25). Rather than being on a continuum, as might seem to be the case intuitively, data suggest that fear and pain are associated with fundamentally different motivational systems. Fear activates species-specific defensive behaviors, such as freezing, flight, or fighting, that serve to minimize encounters with pain-producing stimuli (e.g., predators). Pain, on the other hand, appears conducive to the kinds of behaviors that provide for healing and recuperation (e .g., rest, grooming, licking the affected area, and sleep). A growing body of evidence shows that fear takes priority over pain, and that fear can actually inhibit pain under some circumstances (possibly through the release of endogenous opiates). For example, soldiers who are wounded in battle frequently continue fighting and feel no pain from their injuries until after they are removed from the front lines (211). Likewise, a deer wounded by hunters may flee the scene with defensive behavior indistinguishable from that of uninjured animals, But once the deer is out of danger, pain-related recuperative behaviors predominate (25).

Brain Manipulation

In studies of the physiological bases of behavior, the recording of brain-wave patterns maybe substituted for electrical stimulation whenever possible, and brain areas may be stimulated instead of lesioning or ablating sections of the brain (121). These techniques, however, are not completely interchangeable. Recording neuronal firing as an

animal behaves allows correlational inferences to be made, but not causal ones. If the experimental goal is to determine a particular brain area that is responsible for a certain behavior, that area must be manipulated directly. Electrical stimulation of brain areas is useful in establishing causal relationships, and the most definitive and reliable results are obtained when stimulation is used in conjunction with lesioning or ablation (20).

Drug Administration

In research on the behavioral effects of experimental or currently available drugs, animals are injected either intraperitoneally (within the body cavity), intravenously, intramuscularly, or intracranially (within the skull, via an implanted cannula). Depending on whether the drug must be given repeatedly, the injection procedure can be stressful and may cause discomfort. Within the last decade alternative administration methods have been developed that may replace the need for multiple injections in some chronic drug treatment studies. Capsules of porous rubber (Silastic®, produced by Dow-Corning) implanted beneath the skin release a drug slowly into the animal's body, and stress produced by repeated injections is avoided. The method produces minimal discomfort and is well tolerated by animals (63). Small, implantable minipumps are also available to deliver drugs for days or weeks.

The use of aerosols has also been suggested (174); although this would seem to hold promise for alleviating the stress of injections, it has drawbacks. For example, animals may differ greatly in their inhalation rates, and dispersal of the drug into the air prevents adequate control of drug dosage.

Food Deprivation

It is important to distinguish between the different methods of depriving animals of food and the reasons for using any method. In most cases, animals are deprived of food to motivate them to perform various tasks or behaviors for food reward. The nature of the subject's performance of such tasks—and not the food deprivation—is the object of study.

Food deprivation is typically applied one of two ways: Animals are deprived of food for a standard period of time (e.g., 24 hours) prior to testing or they are maintained at some percentage of their normal body weight (e.g., 80 percent) (43). Each procedure has advantages and disadvantages. Food deprivation for specified intervals of time is easy to implement, but it fails to take into account species differences in metabolic rates, For example, 24 hours of food deprivation for a mammal is less severe than it would be for a bird, while for a snake it would be inconsequential. Maintaining animals at a percentage of normal body weight avoids this problem, but it requires daily handling and the delay of the trial for long periods of time to stabilize body weights.

When food deprivation is applied according to a standard time period in behavioral protocols, the most common interval is 24 hours (43). It is noteworthy that the feeding of domestic pets once a day parallels this laboratory protocol. When maintaining animals at some percentage of their normal body weight, behavioral protocols usually involve up to 20 percent weight loss (43). Experimental animals' reduced food intake is associated in some instances with enhanced longevity (172).

Several suggestions have been made to reduce, ameliorate, or eliminate food deprivation in behavioral research:

- Water deprivation, sometimes used concurrently with food deprivation, should be used to motivate behavior only if thirst or drinking is the object of study. Water deprivation affects an animal's physical condition more severely than food deprivation does, because death by dehydration occurs much more rapidly than death by starvation (121).
- The normal eating pattern of a species should be taken into account when deciding on the duration of food deprivation. For example, sparrows eat only during the light hours of the day; hamsters feed largely at night.
- In some cases, food deprivation might be avoided by using a highly preferred food as a reward (121).
- Food deprivation may also be avoided by taking the experiment into the animal's living quarters, so that it is required to perform for

food as and when it wants to eat. In this way, any deprivation would be self-imposed as under natural conditions (121,151). This technique has been used successfully in work on sensory-motor functioning in monkeys (168).

Pain Research and the Use of Electric Shock

The experience of pain is a highly adaptive capacity. It prevents organisms from engaging in behaviors that would otherwise prove maladaptive. For example, humans who are congenitally insensitive to pain become terribly scarred and mutilated, often develop a sense of being invincible, and have short life expectancies (141)143,196). Perhaps because pain plays such an essential role in regulating the behavior of organisms, pain thresholds are surprisingly consistent across a great diversity of species (115). The discovery of endogenous opiates in earthworms (5) and recent findings with spiders (61) suggest that invertebrates may also feel pain.

Pain can be induced through mechanical, thermal, electrical, or chemical stimuli (127). Of the various stimuli used for research purposes, electric shock at the levels normally used in experimentation is the only one that does not damage tissue. Most studies of pain in animals use what are called flinch-and-jump thresholds—an index of the minimal amount of electric shock or heat needed to produce a reaction. Electric shock is used as a stimulus for research into the mechanism of pain for several reasons:

- Electric shock is easily quantifiable. The parameters of shock can be manipulated and specified with a high degree of precision over a wide range.
- Electric shock can be administered so as to have a discrete or gradual onset and offset.
- Electric shock of the type most often used (i.e., a brief current of 0.001 amperes, the equivalent of a tingling sensation in the finger) does not yield physical damage, bleeding, or tissue destruction.

However, electric shock is a highly atypical stimulus (79). No contemporary terrestrial species appears to have evolved under conditions of electric shock. The question of whether data obtained this way are widely generalizable in mechanisms of pain remains unanswered.

A survey of the 608 articles appearing from 1979 through 1983 in the American Psychological Association journals that typically publish animal research (e.g., ***Journal of Comparative and Physiological Psychology*** and its successors ***Behavioral Neuroscience and Journal of Comparative Psychology)*** identified 10 percent of the studies as using electric shock. Four percent of the studies administered inescapable shocks stronger than 0.001 amperes. Most of the experiments with electric shock involved rodents; those with monkeys, dogs, and cats accounted for 0.5 **percent of the total 608 articles (43).**

Recommendations that have been made to reduce pain or discomfort in animal experiments involving aversive stimulation include:

- The lowest possible level of electric shock should be used that will at the same time maintain the behavior under study (52). However, this may reduce the statistical power and require a large sample size.
- Animals should be given predictable rather than unpredictable shock and an opportunity to control its termination (52). Rats, for example, will choose to receive more shocks at greater intensity in order to receive a warning cue prior to each shock delivery ***(10).***
- If aversive stimulation must be used, alternatives to electric shock such as loud noise or bright lights should be considered (121).
- In developing models of chronic pain, the model should closely simulate a particular chronic pain syndrome in humans (e.g., arthritis or cancer). Otherwise, there is no justification for the procedure (114).
- Animals should have an opportunity to control the intensity of the stimulus in chronic pain studies. While the objection to this might be that, given this option, the animal would "turn off" the pain stimulus, this might be circumvented by giving a preferred food reward for keeping the stimulus "on" at a given level, as in experiments with electric shock titration techniques (114).
- A reward, such as a preferred food, should be used for the correct responses instead of a punisher, such as electric shock, for incorrect response (121).

USE OF LIVING SYSTEMS IN BEHAVIORAL RESEARCH

In behavioral research, using living components derived from whole animals or living nonanimal systems as alternatives to animals could conceivably involve embryos; cell, tissue, and organ cultures; invertebrates; and plants. The greatest potential in this area, however, appears to rest with the use of invertebrates.

Several factors limit the use of embryos (used here to refer to the conceptus, embryo, and fetus prior to birth) as an alternative or complement to young or adult animals:

- Some studies involving embryos may be conducted when the subject is very close to birth or hatching. The advanced developmental status of the organism at this point raises the same kinds of ethical considerations that would apply to the use of postnatal animals (see ch. 4).
- In behavioral studies involving mammalian embryos, the mother is necessarily involved in most experimental manipulations performed on the embryo. As a consequence, embryological manipulations on mammals cannot logically avoid the use of adult mammals.
- Behavioral studies using embryos may involve testing for effects later in adult life (e.g., behavioral teratological studies). In these instances, embryos are not being used as alternatives, since the procedures also require postnatal assessment.
- Only a limited number of behaviors can be studied in embryos, partly because of practical problems associated with access to the embryo.
- Embryos live in a dramatically different environment than fully developed adult animals. This difference constrains the generalizabil - ity of behavioral data obtained from them.

Cell, tissue, and organ cultures do not figure prominently in the equation of alternatives to animal use in behavioral research. In isolation and in culture, cells, tissues, and organs exhibit few activities that fall among the disciplines of behavioral research.

A rare example of the use of cell culture in behavioral research comes from studies of the biochemical basis of depression and manic mood changes. Skin fibroblast cells obtained from humans and maintained in culture for several months were assessed for their ability to bind a variety of pharmacologic agents. The cultured cells of patients and relatives of patients with manic-depressive illness exhibited markedly different biochemical properties than the cultured cells of persons without a history of manic depression (155). One commentator characterized this as ‘(a step forward, applying to psychiatry the techniques of tissue sampling and cell culture that have been of great value in characterizing molecular abnormalities in numerous medical diseases” (192). Continued development of this line of research could reduce the use of animals in such investigations.

Invertebrates

Few behavioral studies use invertebrates as subjects (139). As a consequence, relatively little is known about invertebrate behavior. In behavioral research, invertebrates offer a fertile testing ground for any theory that claims to be broadly based across the phyla of the animal kingdom (138). Certain groups of invertebrates are promising subjects for behavioral research.

The brains of octopuses and squid approach those of vertebrates in relative size and complexity (178). Visual discrimination learning has been studied extensively in the octopus. Octopuses can discriminate between pairs of geometric shapes that differ with respect to vertical, horizontal, and oblique orientations. The octopus and squid show learning performance on a par with mammals on such tasks as detour problems, reversal learning, delayed response, and delayed reinforcement (178,218).

Among all the invertebrates, the only species with a neuroanatomy and learning ability comparable to vertebrates are the octopus and squid. Practical problems in obtaining, transporting, and housing these marine species have always precluded their widespread use as alternatives in behavioral research (178), However, recent advances in the laboratory culture of octopuses make them promising research candidates, although providing live food (e.g., shrimp) on a consistent basis

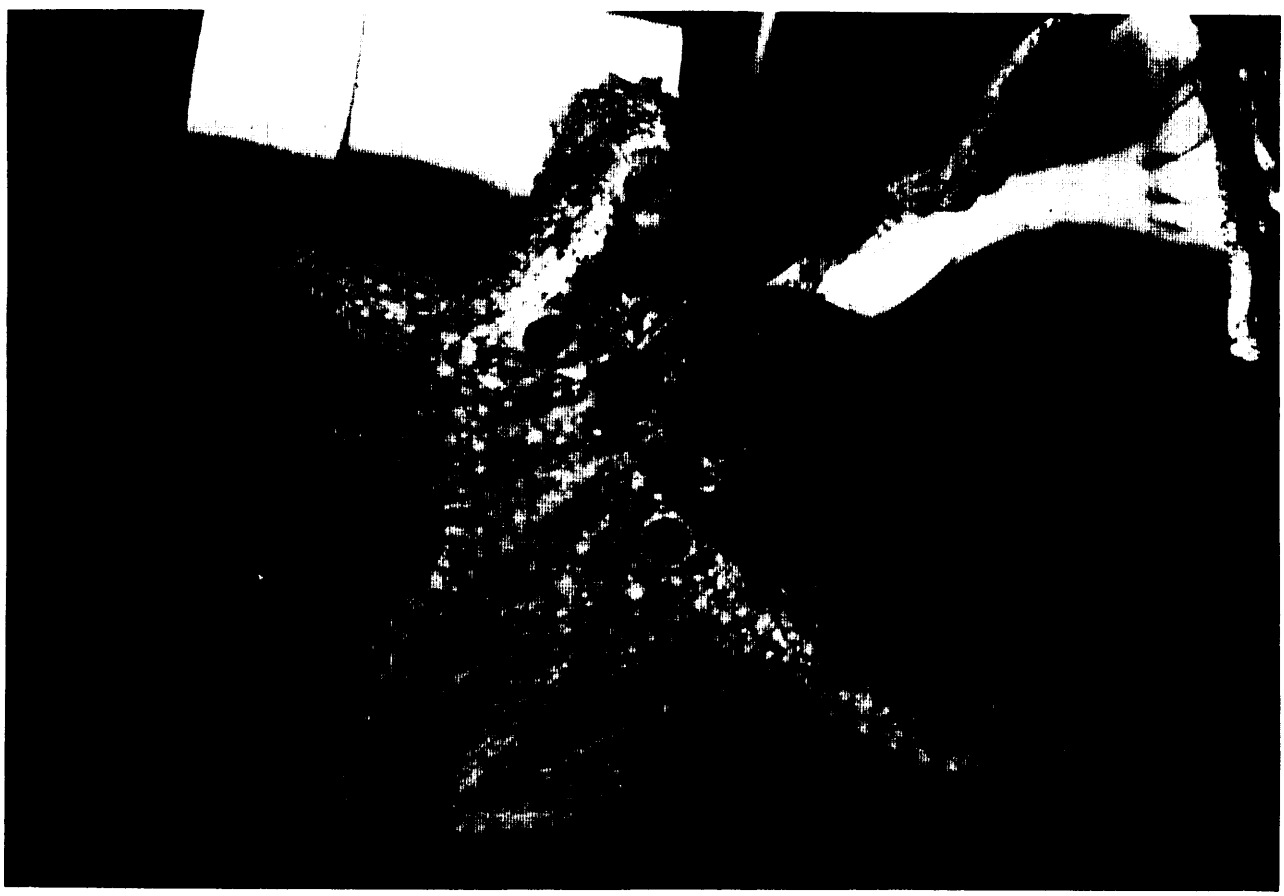
remains a major logistical obstacle. The development of a dead or artificial food ration is currently a high priority in octopus culture (88). The same highly developed nervous system that makes the octopus and squid desirable replacements for vertebrates may cause some ethical objections to use of these invertebrates. In addition, their adaptation to a completely aquatic existence would also make tenuous any extrapolations to the behavior of terrestrial mammals.

Starfish and sea urchins exhibit habituation—the waning of a response to stimuli, as a result of repeated elicitation of that response—and they can learn escape behaviors in response to a cue paired with aversive stimulation (46).

Earthworms exhibit habituation (45), can learn to associate light with a food reward (66), and can learn to travel a maze to receive darkness and moisture as reinforcing stimuli (85). Flatworms are also of considerable interest, since they represent a bilateral body form, as do mammals. Flatworms exhibit a concentration of nervous tissue and sensory organs in the anterior, or head, portion of their bodies, and they have refined internal organ systems (47). Flatworms exhibit habituation, can be conditioned to avoid alight after it has been paired with shock, and can learn to approach an area for food reward. There are also claims that such learned events are remembered after these worms undergo regeneration, and that learning can be transferred from one animal to another by cannibalism (reviewed in ref. 47).

Insects are valuable behavioral models in communication, navigation, learning and memory, and behavioral genetics.

Oc opus *Oc opus bimaculoides* Cu u ed F om he Egg n he Labo a ory



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- **Communication. Honeybees recruit others to a new food source through a dance performed at the hive that conveys distance and directional information (209). Many species of insects (e.g., moths and ants) communicate chemically by pheromones, which serve sex attractant, repellant, and/or trail-marking functions (reviewed in ref. 30). Other species, such as the cricket, communicate by songs produced by rubbing body parts together (19).**
- **Navigation. Honeybees have demonstrated extraordinary abilities to locate and return to a food source "mapped out" for them by other bees. They can also return to an artificial feeding source designed for experimental purposes to test their navigation abilities (37, 209)215).**
- **Learning and Memory. Habituation has been demonstrated in a variety of insect species (46). Honeybees also appear capable of more advanced forms of learning, such as learning to associate a specific color with a food reward, and they can remember this association after a 2-week interval (219). Cockroaches can learn to leave their preferred dark retreats and stay in the light to avoid being shocked; ants have been trained to travel a maze to receive food rewards (85).**
- **Behavioral Genetics. Because of the relative** ease with which their chromosomes and individual genes can be identified, fruit flies have been used extensively to elucidate the genetic basis for a variety of behaviors (132).

Habituation has been demonstrated in a variety of spiders (45), and spiders are capable of learning and remembering the location of prey in their webs (85). They can also be trained to associate food dipped in quinine or sugar with different tones (46).

Even though protozoa possess both plant- and animal-like characteristics and lack nervous tissue, some forms of learning have been demonstrated in these single celled organisms. Habituation has been demonstrated in paramecia (45). Although the results generated much controversy (reviewed in ref. 45), one investigator claimed to have trained paramecia to enter a specific area of their water container in order to receive food reinforcement (81). It has also been reported that paramecia show spontaneous alternation in a T-maze, a phenomenon also observed in rodents (139).

A recent study of learning ability in paramecia has demonstrated classical conditioning of an escape movement (94). This study also found that paramecia develop memory for the training event, since significantly fewer trials were needed 24 hours later to relearn the response. Data such as these challenge the widely held assumption that learning is a property of synaptic interactions between nerve cells—absent in protozoa—and not of individual cells themselves.

Plants

From a behavioral perspective, plants differ from animals in two principal ways. First, plants lack the means of achieving rapid intra- and inter-organismal communication and coordination due to the absence of a nervous system. However, plants do regulate intra-organismal activities occurring at different sites through the use of hormones. Plants and animals thus share the basic principles of endocrine function. Second, plants differ from animals in that they are stationary. They must wait for energy to come to them, while most animals move about to obtain different sources of energy.

Despite these differences, plants do show rudimentary forms of behavior (188). Plants can grow and move in response to light, and some plants have achieved the capacity for relatively rapid movement to exploit certain animals as prey (e.g., the venus fly trap). The mimosa plant, which can fold its leaves when touched, has been a subject of particular interest. Certain of its cells appear to generate primitive action potentials-electrical activity that may be analogous to neuronal functioning in animals (186). There have also been reports that the folding response of the mimosa plant shows habituation (6) and even some of the rudiments of classical conditioning (8). Although some claim evidence of feelings, emotions, and even thinking in plants based on polygraph recordings (206), others contend these are artifactual (80).

A number of plants defend themselves from predators via thorns, stickers, or toxic chemicals that produce sickness, irritation, or even death if touched or consumed. It has been demonstrated

that some plants, under attack by insects and micro-organisms, develop highly sophisticated defenses involving the emission of antibiotic-like substances and chemicals that inhibit insect digestive enzymes. Indeed, some plants can apparently communicate chemically with as-yet-unaffected neighboring plants to induce leaf-chemistry changes in advance of infestation (reviewed in ref. 164).

These impressive features of the botanical world notwithstanding, it is unlikely that plants will make an important contribution to behavioral research. The lack of a central nervous system, and in particular a brain, renders the plant an inappropriate model for use among the disciplines of behavioral research.

USE OF NONLIVING SYSTEMS IN BEHAVIORAL RESEARCH

Inanimate chemical or physical systems are unlikely to prove useful in behavioral research, for reasons intrinsic to the nature of behavior. A dynamic, emergent process, behavior functions to allow organisms to adapt to moment-to-moment changes in the environment. In a sense, all behavior ultimately functions to aid and abet survival and reproduction (50. Adaptation, survival, and reproduction are not properties of nonliving systems. And behavior involves information processing and a continuous series of choices among an array of alternatives (140). Although chemical or physical systems may change in response to certain environmental stimuli, the nature of such changes does not involve decisionmaking or information-processing.

Behavior is a byproduct of interactions between sensory, neural, hormonal, genetic, and experiential factors. As such, it is influenced by the situation at hand, the developmental history of the organism, and prior experience with similar and related situations. It appears inappropriate to imbue inanimate chemical or physical systems with the capacity for experience. Devoid of such a capacity for experience, nonliving systems are unlikely alternatives to using animals in behavioral research.

Examples of the application of chemical or physical systems to behavioral research are sparse. One involves the use of chemical reagents to mimic the properties of rhythmic behavioral phenomena in animals. Certain chemical reagents exhibit changes in state that oscillate periodically in a fashion similar to some biologically based rhythms. However, the chemical reactions themselves remain poorly understood (222).

COMPUTER SIMULATION IN BEHAVIORAL RESEARCH

A computer simulation is an operating model that depicts not only the state of a behavioral system at a particular point in time but also changes that occur in that system over time. Because dynamic processes are of quintessential importance in behavioral research, computer simulation stands as a potentially useful tool for the behavioral scientist.

In order to simulate a living system, a computer programmer must have information about that system. The more information at hand, the better the simulation (53). In the strictest sense, a completely accurate simulation presupposes that everything that there is to know about the system in question is known. To construct a computer simulation that would fully replace the use of a live organism in behavioral research would require knowing everything about the behavior in question, which in turn would preclude the need for a computer simulation for research purposes.

Yet, if computer simulation cannot fully replace living organisms, it can and does contribute to behavioral research. Although the fundamental behavioral qualities of adaptation, survival, and reproduction do not pertain to computer programs, computer soft ware does, for example, embody information processing and decisionmaking. Exam-

pies of recent attempts toward computer simulation in behavioral research are listed in table 6-4.

Computer simulations are used in behavioral research in a number of ways. Statistical simulations, in particular, are increasingly frequent. For example, one computer program simulates random-choice behavior in mazes (194), and two programs simulate random movements of animals under various conditions (17,49). The output generated by these kinds of simulations is compared with animal-generated data to see if factors other than pure chance are influencing the animals' behavior.

Statistical simulations are also used to test hypotheses that may not be subject to empirical confirmation. One investigator used a computer simulation to test the proposition that "if enough monkeys were allowed to pound away at typewriters for enough time, all the great works of literature would result" (21). The larger objective in this study was to determine if the extreme cases of human genius could be accounted for through chance processes.

Table 6-4.—Some Examples of Computer Simulation of Behavioral Phenomena

Spacing mechanisms and animal movements:
• Space use and movement patterns (17,49)
• Movements of juvenile Atlantic herring (110)
• Animal spacing (153)
• Mosquito flight patterns (165)
• Foraging of the honey eater bird (169)
• Random choice in radial arm mazes (194)
Learning, memory, and problem solving:
Ž Classical conditioning (15,18)
• Learning in neural systems (177)
• Habituation (195)
• Behavior in a psychoecological space (84)
• Mechanisms for reducing inhibition (223)
Sensation and perception:
• Visual pattern analysis (13)
• Landmark learning by bees (37)
• Chemical recruitment in ants (106)
Communication:
• Bird song (55,185)
• Animal vocalizations (57)
Sensation and perception:
• Neuron models (122)
• Neural basis for pain and touch (148)
Body maintenance:
• Food intake (14)
Ž Control of drinking behavior (204)
Reproduction and parental care:
• Sexual behavior of the male rat (72,205)
Ž Infanticide in langurs (89)
• Evolution of reproductive synchrony (116)
• Mating behavior of *Spodoptera littoralis* (200)

SOURCE: Office of Technology Assessment.

The simulation was based on an initial assumption that monkeys typing at random— or a computer simulation using random numbers–would generate huge volumes of nonsense. Statistical properties of the English language (e.g., the relative frequencies of individual characters or sequences of characters) were added to the simulation. As higher-order properties of English (i.e., the relative frequencies of three- and four-letter sequences) were incorporated into the algorithm, the rate of generation of intelligible words, phrases, and sentences increased. These results led to a hypothesis that genius could be simulated by a process of random choice with a weighting procedure, subject to a prior preparatory process in which an individual absorbs the necessary operational patterns that characterize the discipline. In studies such as this, it is not merely the output generated by the computer model that is of interest, but the simulation process as well.

Computer simulations have considerable heuristic value (65): They may yield insight about the system or phenomenon being modeled (71) and, as a consequence, stimulate additional research. The value of computer simulation as a heuristic device has been summarized as follows (158):

> Simulation gives a means of exploring the plausibility of models in which theoretical sophistication exceeds the state of the art in empirical testing. Simulations provide tools for empirically analyzing theories in order to better understand their implications and predictions. Simulations are a means of exploring interactions between components of complex models. They pose a practical challenge to operationalize theoretical constructs, which can lead to incidental discoveries about related processes. Finally, they engender a concern with issues of process control that contributes to the development of general principles with broad applications.

Computer simulation holds promise for understanding complex cognitive processes. For example, the computer is often considered analogous, at least in some ways, to the human brain (7)—both process large amounts of information, and their respective outcomes are a consequence of

multiple, relatively simple operations. On the other hand, there are major differences:

- **computers have larger and many fewer components than the brain;**
- **computer operations occur with much greater speed than neural operations;**
- **computers operate through sequential processing; and**
- **computers attend to all input, while the brain** is selectively attentive.

Such differences do not, however, preclude the use of computers as functional models (170). Moreover, one advantage of computers is that they demand rigorous and exact description. And an investigator need not invoke a variety of hypothetical or mentalistic variables (e g., hope, fear, desire, and intention) to describe their functioning (170.

It is important to note that any predictions generated by a computer simulation must be tested and verified using the system the computer was designed to replace (149). In this sense, the use of animals in behavioral research is likely to continue in lockstep with the development of computer simulation software.

One commentator summarized the use of computers as an alternative to animals in behavioral research in this way (114):

> **At the present time and for the foreseeable future it seems clear that the computer will not be a feasible substitute for experiments on animals. The fundamental reason is that a computer cannot acquire data other than those that are generated by carefully designed experimental studies in animals. What the computer does provide is** a superb technique for processing vast amounts of data with great speed and accuracy and for presenting them in almost any manner the investigator desires. To suggest that enough data are already available from previous work, so that from them programs can be generated and subjected to a variety of permutations that would lead to new insights, overlooks an important fact. In any animal experiment there are numerous variables over which we have little control, and there are virtually always as many more about which we as yet know nothing but which may have very significant influences on the phenomenon under investigation. In real life, which after all is what matters in biologic research, these variables may be crucial and may give important clues to entirely unsuspected phenomena that are sometimes far more important than the original subject of the study. In a word, computers do not generate new concepts or acquire new data. They process data and permit the investigator to view it in more manageable or novel ways, and this may facilitate new hypotheses or insights.

In summary, computer simulation can serve to facilitate behavioral research. The need for certain protocols may be precluded, or protocols may be refocused by computer simulation before they commence. Modeling techniques using computer simulation lead to the refinement of experimental protocols to be conducted on animals (36). Yet as the preceding quote implies, in facilitating behavioral research computer simulation may actually increase, rather than decrease, the use of animals because data can be analyzed more quickly and in much greater detail, leading to proportionately more hypotheses to investigate (87,160).

SUMMARY AND CONCLUSIONS

Animal use in research can be modified in a number of ways, including strengthening experimental design to minimize the number of animals used, reducing the degree of experimental insult, and substituting one species for another. The outright replacement of animals with nonanimal methods in research is not at hand, and, because of the nature of biomedical and behavioral research, in many instances it is not likely to become feasible.

Advances in instrumentation are critical to the more refined or reduced use of live animals or living material. In the past decade, practically every piece of instrumentation in biomedical laboratories has been adapted to handle ‘(micro” samples or has been replaced by new microtechnology. The use of small samples for analysis by mass spectrometry and by gas or liquid chromatography leads to less invasive technology. Fiber optics, for

example, can be used to perform analyses inside ducts and blood vessels with little discomfort and no permanent damage to the animal. Continued developments in analytical instrumentation, including noninvasive imaging techniques such as magnetic resonance imaging, will likely ameliorate the degree of experimental insult faced by research animals.

In vitro technology has affected virtually every field of biomedical research. This technology entails the maintenance of organs, tissues, and cells outside of the body and may affect research animal use in two important ways. First, when organs, tissues, or cells are removed from animals and cultured, experiments may be conducted with fewer animals than would be necessary in whole-animal experiments and, of course, without pain. Cells from one animal, for example, may be divided among a dozen experimental cultures and a dozen control cultures, replacing 24 animals that might be used in a comparable whole-animal experiment.

Second, when cells proliferate in culture, commercially available cell lines can completely eliminate animal use in some experiments. Such cell cultures are derived directly from preexisting cell cultures—not animals, Researchers have used, for example, a monkey kidney cell line to study the metabolic effects of general anesthetics.

In vitro experiments are not equivalent to whole-animal experiments. In in-vitro systems, as organization is disrupted or lost, the in vitro system has less and less of the kind of interactions that characterize cells in the body. This can be an advantage and a disadvantage. Interactions may cause extraneous phenomena that obscure the process under study. Conversely, the absence of interactions may produce results that are at variance with what actually occurs in the live animal. Conclusions drawn from in vitro studies must eventually be validated by comparison with results of whole-animal experiments.

In some areas of biomedical research, invertebrates and micro-organisms can be used, and even plant parts can yield information about animal systems. Structures within plant and animal cells, when removed from the cells, are essentially indistinguishable in both appearance and function. Cell biologists, for example, use yeast cells as a source of mitochondria—the energy-generating structures present in all plant and animal cells.

Hard and fast distinctions between biomedical and behavioral research are difficult to sustain. Nevertheless, it is apparent that there is even less potential for replacement of animals in behavioral than in biomedical research. In vitro techniques and nonliving systems are not viable alternatives to the use of animals in behavioral research.

Invertebrates are used extensively in behavioral research. Octopuses and squid are studied because of their highly developed brains. In addition, the discovery of learning and memory in single-cell organisms has challenged the widely held assumption that these phenomena are properties of the interactions of nerve cells rather than of individual cells, and has opened new avenues of research with nonanimal methods.

Computer simulation is a valuable tool in biomedical and behavioral research, but it does not stand as a replacement for animal use. The development of new and more sophisticated computer simulations in biomedical and behavioral research is predicated on data derived from animals. Increased use of computer simulation may have the paradoxical effect of leading to an increase in the number of animals used in experimentation, as more hypotheses and experimental questions are generated more rapidly.

CHAPTER 6 REFERENCES

1. Abbey, J., Close, L., and Jacobs, M., "Pilot Study: Use of Electrical Impedance to Measure Urinary **Volume in Canines,** *"Communic. Nurs. Res.* 11:22, **1977.**
2. **Abelson, P. H., "Analytical Instruments," Science** 226:249, **1984.**
3. **Abelson, P.H., "Instrumentation, "Science** 230:245, **1985.**
4. **Ali,** M.A. **(cd.),** ***Photoreception and Vision in Invertebrates (New*** **York: Plenum Press, 1984).**
5. Alumets, J., **Hakanson, R., Sundler, F., et al.,** "Neuronal **Localisation of** Immunoreactive **Enkepha-**

lin and B-endorphin in the Earthworm," *Nature* 279:805-806, *1979.*

6. Applewhite, P.B., "Behavioral Plasticity in the Sensitive Plant, Mimosa," *Behav. Biol.* 7:47-53, 1972.

7. Apter, M. J., *The Computer Simulation of Behaviour* (London: Hutchinson, 1970).

8. Armus, H. L., "Conditioning of the Sensitive Plant, *Mimosa pudica,*" *Comparative Psychology: Research in Animal Behavior,* M.R. Demy and S.C. Ratner (eds.) (Homewood, IL: Dorsey Press, 1970).

9. Attinger, E. O., Chairman, Department of Biomedical Engineering, University of Virginia Medical Center, Charlottesville, VA, personal communication, Aug. 14, 1984.

10. Badia, P., Culbertson, S., and Harsh, J., "Choice of Longer or Stronger Signaled Shock Over Shorter or Weaker Unsignaled Shock," J. *Exper. Anal. Behav.* 19:25-33, *1973.*

11. Baerends, G. P., and Baerends-Van Roon, J.M., "An Introduction to the Ethology of Cichlid Fishes," *Behaviour* [Suppl.] 1:1-243, 1950.

12. Bangham, A.D., Hill, M. W., and Miller, N. G., "Preparation and Use of Liposomes as Models of Biological Membranes," *Methods in Membrane Biology, Vol. 1,* E.D. Kern (cd.) (New York: Plenum Press, 1974).

13. Barlow, H. B., Narasimham, R., and Rosenfeld, A., "Visual Pattern Analysis in Machines and Animals," *Science* 177:567-575, 1972.

14. Barnwell, G. M., and Stafford, F. S., "Mathematical Model for Decision Making Neural Circuits Controlling Food Intake," Bull. *Psychonom. Soc. 5:473-476, 1975.*

15. Barto, A. G., and Sutton, R. S., "Simulation of Anticipatory Responses in Classical Conditioning by a Neuron-like Adaptive Element," *Behav. Brain Res.* 4:221-235, *1982.*

16. Beaton, A. R., and Krug, R. M., "Synthesis of the Templates for Influenza Virion RNA Replication In Vitro," *Biochemistry* 81:4682-4686, *1984.*

17. Bekoff, M., and Wieland, C., "SPACE-OUT: Graphics programs to Study and to Simulate Space Use and Movement Patterns, "*Behav. Res. Meth. Instru.* 14:34-36, *1982.*

18. Benedict, J. O., "CLASCONISM: A Computer Program to Simulate Experiments in Classical Conditioning," *Behav. Res. Meth. Instru.* 11:603-604, *1979.*

19. Bennet-Clark, H. C., "A New French Mole Cricket, Differing in Song and Morphology From *Gryllotalpa gryllotalpa L.* (Orthoptera: Gryllotalpidae)," *Proc. Royal Entomolog. Soc. London (B) 39:125-132, 1970.*

20. Bennett, T. L., *Introduction to Physiological Psychology* (Monterey, CA: Brooks, Cole, 1982).

21. Bennett, W. R., "How Artificial Is Intelligence? "Am. *Sci.* 65:694-702, 1977.

22. Berlin, B., Brodsky, J., and Clifford, P., "Testing Disease Dependence in Survival Experiments With Serial Sacrifice," *J. Am. Stat. Assoc.* 74:5-14, *1979.*

23, Bitterman, M. E., "The Comparative Analysis of Learning," *Science* 188:699-709, 1975.

24. Bloom, S., "Alternative Method for Heart Attack Studies Reduces Animal Pain," *Sci. Center Newsletter* 6(2):4, 1984.

25. Belles, R. C., and Fanselow, M. S., '(A Perceptual-Defensive-Recuperative Model of Fear and Pain," *Behav. Brain Sci.* 3:291-323, 1980.

26. Brabec, M.J., Bedows, E., Davidson, B. A., et al., "Effect of General Anesthetics and Pressure on Aerobic Metabolism of Monkey Kidney Cells," *Anesthesiology* 61:43-47, *1984.*

27. Bradshaw, J.J., and Ivanetich, K. M., "Isoflurane: A Comparison of Its Metabolism by Human and Rat Hepatic Cytochrome P-450," *Anesth. Analg.* 63:805-813, *1984.*

28. Brokaw, C.J., "Models for Oscillation and Bend Propagation by Flagella," *Symp. Soc. Exp. Biol.* 35:313-338, 1982.

29. Brown, D, V., and Amann, R. P., "Inhibition of Testosterone Metabolism in Cultured Rat Epididymal Principal Cells by Dihydrotestosterone and Progesterone, " *Biol. Reprod.* 30:67-73, *1984.*

30. Brown, J. L., *The Evolution of Behavior (New* York: W.W. Norton & Co., 1975).

31. Bucker, H., Horneck, G., Facius, R., et al., "Radiobiological Advanced Biostack Experiment, "Science 225:222-224, 1984.

32. Budinger, T.F., and Lauterbur, P. C., "Nuclear Magnetic Resonance Technology for Medical Studies)" *Science* 226:288-297, 1984.

33. Bullock, T. H., "Comparative Neuroscience Holds Promise for Quiet Revolutions)" *Science* 225:473-478, 1984.

34, Burghardt, G, M., "Chemical-Cue Preferences of Inexperienced Snakes: Comparative Aspects," *Science* 157:718-721, 1967.

35. Burghardt, G.M., "Intraspecific Geographical Variation in Chemical Food Cue Preferences of Newborn Garter Snakes (*Thamnophis sirtalis*)," *Behaviour* 36:246-257, *1970.*

36. Carson, E. R., "The Role of Mathematical Models in Biochemical Research," *Animals in Scientific Research: An Effective Substitute for Man?* (London: Macmillan, 1983).

37. Cartwright, B. A., and Collett, T. S., "Landmark

Learning in Bees," *J. Comp. Physiol.* 151:521-543, 1983.
38 Chalfie, M., "Neuronal Development in *Caenorhabditis elegans*, "*Trends in Neuroscience*, June *1984.*
39 Changeux, J., Devillers-Thiery, A., and Chemouilli, P., "Acetylcholine Receptor: An Allosteric Protein)" *Science* 225:1335-1345, 1984.
40. Chow, L. C., and Brown, W .E., "A Physiocochemical Bench-Scale Caries Model,")J. *Dent. Res. 63:868-873,* 1984.
41. Christensen, C. P., "New Laser Source Technology," *Science* 224:117-123, 1984.
42. Cline, E.M., Randall, P. A., and Oliphant, E. E., "Hormone Mediated Oviductal Influence on Mouse Embryo Development, "*Fert. Steril.* 28:766-771, *1977.*
43. Coile, D. C., and Miller, N. E., "How Radical Animal Activists Try to Mislead Humane People, "Am. *Psychol.* 39:700-701, *1984.*
44. Conklin, K. A., and Lau, S., "Halothane Effects on AT Content and Uridine Uptake and Phosphorylation in *Tetrahymena pyriforrnis*," *Anesthesiology* 61:78-82, *1984.*
45. Corning, W. C., Dyal, J. A., and Willows, A.O.D. (eds.), *Invertebrate Learning, Vol. 1: Protozoans Through Annelids (New* York: Plenum Press, 1973).
46 Corning, W. C., Dyal, J. A., and Willows, A.O.D. (eds.), *Invertebrate Learning, Vol. 2 Arthropods and Gastropod Mollusks (New* York: Plenum Press, 1973).
47. Corning, W. C., and Kelly, S., "Platyhelminthes: The Turbellarians,' 'Invertebrate *Learning Vol. l: Protozoans Through Anne/ids,* W.C. Corning, J.A. Dyal, and A.O.D. Willows (eds.) (New York: Plenum Press, 1973).
48. Craig, P. N., "Overview of Computer Use in Research, Testing, and Education, " contract report prepared for the Office of Technology Assessment, U.S. Congress, August 1984.
49 Cubiciotti, D., "DOPPELGANGER: A FORTRAN Program for Simulating Time-Sampled Spatial Locations of ' Phantom' Animals Performing Discrete Pseudorandom Walks, " *Behav. Res. Meth. Instru.* 14:485-486, *1982.*
50. Current, W .L., and Haynes, T. B., "Complete Development of *Cryptosporidium* in Cell Culture, " *Science* 224:603-605, *1984.*
51. Daly, M., and Wilson, M., Sex, *Evolution, and Behavior* (Boston, MA: Willard Grant Press, 1983).
52. Davis, H., "Ethical Considerations in the Aversive Control of Behavior," Soc. Sci. *Med. 15* F:61-67, *1981.*
53. Dennett, D. C., "Commentary/Pylyshyn: Computational Models and Empirical Constraints, " *Behav. Brain Sci.* 1:103-104, *1978.*
54. Dierks, P. M., Associate Professor, Department of Biology, University of Virginia, Charlottesville, VA, personal communication, Aug. 21, 1984.
55. Dobson, C.W., and Lemon, R. E., "Markov Sequences in Songs of American Thrushes," *Behaviour* 68:86-105, *1979.*
56. Doi, Y., Eanes, E.D., Shimokawa, H., et al., "Inhibition of Seeded Growth of Enamel Apatite Crystals by Amelogenin and Enamelin Proteins In Vitro," *J. Dent. Res.* 63:98-105, *1984.*
57. Dooling, R.J., Clark, C., Miller, R., et al., "Program Package for the Analysis and Synthesis of Animal Vocalizations," *Behav. Res. Meth. Instru.* 14:487, *1982.*
58. Dukelow, W .R., Chan, P.J., Hutz, R.J., et al., "Preimplantation Development of the Primate Embryo after In Vitro Fertilization, "*J. Exp. Zool.* 228:215-221, 1983.
59. East, I.J., Keenan, A. M., Larson, S. M., et al., "Scintigraphy of Normal Mouse Ovaries With Monoclonal Antibodies to ZP-2, the Major Zona Pellucida Protein, " *Science* 225:938-941, 1984.
60. Eibl-Eibesfeldt, I., *Ethology: The Biology of Behavior (New* York: HoIt, Rinehart & Winston, 1975).
61. Eisner, T., and Camazine, S., "Spider Leg Autotomy Induced By *Prey Venom* Injection: An Adaptive Response to 'Pain '?" *Proc. Nat]. Acad. Sci. USA* 80:3382-3385, *1983.*
62. Elbrecht, A., Lazier, C. B., Protter, A. A., et al., "Independent Development Programs for Two Estrogen-Regulated Genes, "Science 225:639-641, 1984.
63. Ellison, G., Nielsen, E. B., and Lyon, M., "Animal Models of Psychosis: Hallucinatory Behaviors in Monkeys During the Late Stage of Continuous Amphetamine Intoxication," *J. Psychiat. Res. 16:13-22, 1981.*
64. Emerson, J. D., and Colditz, G. A., "Use of Statistical Analysis in The New England Journal of Medicine," *N. Engl. J. Med.* 309:709-713, 1983.
65. Estes, W. K., "Some Targets for Mathematical Psychology," *J. Math. Psycho].* 12:263-282, *1975.*
66. Evans, S.M., *Studies in Invertebrate Behavior* (London: Heinemann, 1968).
67. Ewald, B. H., and Gregg, D. A., "Animal Research for Animals) "Ann. *N.Y. Acad. Sci.* 406:48-58, *1983.*
68. Festing, M.F.W., *Inbred Strains in Biomedical Research (New* York: Oxford University Press, 1979).
69. Field, D.J., Collins, R. A., and Lee, J. C., "Heterogeneity of Vertebrate Brain Tubulins," *Biochemistry* 81:4041-4045, 1984.
70. Flickinger, C. J., "Radioautographic Analysis of the Secretory Pathway for Glycoproteins in Principal Cells of the Mouse Epididymis Exposed to Tritiated Fucose," *Biol. Reprod.* 32:377-390, *1985.*

71. Freeman, L., "Two Problems in Computer Simulation in the Social and Behavioral Sciences," Soc. *Sci. Info.* 10:103-109, 1971.
72. Freeman, S., and McFarland, D., "Towards a Model for the Copulatory Behavior of the Male Rat: II. RATSEX-An Exercise in Simulation, "*Motivational Control Systems Analysis,* D.J. McFarland (cd.) (London: Academic Press, 1974).
73. Funch, P.G., and Faber, D. S., "Measurement of Myelin Sheath Resistances: Implications for Axonal Conduction and Pathophysiology," *Science* 225: 538-540, 1984.
74. Galef, B.G., Jr., and Sherry, D. F., "Mother's Milk: A Medium for Transmission of Cues Reflecting the Flavor of Mother's Diet, "*J. Comp. Physiol. Psycho].* 83:374-378, *1973.*
75. Gall, J., "Recombinant DNA Research, "Laboratory *Animal Use in Basic Biomedical Research* (draft) (Bethesda, MD: American Society for Cell Biology, 1984).
76. Gallico, G. G., 111, O'Connor, N.E,, Compton, C. C., et al., "Permanent Coverage of Large Burn Wounds With Autologous Cultured Human Epithelium," *N. Engl. J. Med.* 311:448-451, *1984.*
77. Gallup, G.G., Jr., "Toward a Comparative Psychology of *Mind, "Animal Cognition and Behavior,* R.L. Mellgren (cd.) (New York: North Holland, 1983).
78. Gallup, G. G., Jr., Boren, J. L., Suarez, S.D., et al., "The Psychopharmacology of Tonic Immobility in Chickens)" *The Brain and Behavior of the Fowl,* T. Ookawa (cd.) (Tokyo: Japan Scientific Societies Press, 1983).
79. Gallup, G.G., Jr., and Suarez, S.D., "On the Use of Animals in Psychological Research, "Psycho]. *Rec.* 30:211-218, 1980.
80. Galston, A. W., and Slayman, C. L., "The Not-So-Secret Life of Plants, "Am. *Sci.* 67:337-344, 1979.
81. Gelber, B., "Investigations of the Behavior of *Paramecium aurelia:* I. Modification of Behavior After Training With Food Reinforcement, "*J. Comp. Physiol. Psycho].* 45:58-65, *1952.*
82. Geller, N.L., "Statistical Strategies for Animal Conservation," *Ann. N.Y. Acad. Sci.* 406:20-31, *1983.*
83. Gonczol, E., Andrews, P. W., and Plotkin, S. A., "Cytomegovirus Replicates in Differentiated But Not in Undifferentiated Human Embryonal Carcinoma Cells," *Science* 224:159-161, 1984.
84. Goude, G., "A Theory of Behavior in a Two-Balanced Psychoecological Space," *Scand. J. Psycho].* 22:225-242, *1981.*
85. Grier, J.W., *Biology of Animal Beha vior* (St. Louis, MO: Times Mirror/Mosby, 1984).
86. Gross, M.L., and Rempel, D.L.,"Fourier Transform Mass Spectrometry," *Science* 226:261-267, 1984.
87. Guyton, A.C., "Physiological System Modeling as an Alternative Model Procedure," in U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, *Trends in Bioassay Methodology: In Vivo, In Vitro and Mathematical Approaches,* NIH Pub. No. 82-2382 (Washington, DC, 1981).
88. Han.ion, R.T., and Forsythe, J.W., "Advances in the Laboratory Culture of Octopuses for Biomedical Research," Lab. *Anim. Sci.* 35(1):33-40, 1985.
89. Hausfater, G., Aref, S., and Cairns, S, J., "Infanticide as an Alternative Male Reproductive Strategy in Langurs: A Mathematical ModeI, "J. *Theor. Biol.* 94:391-412, 1982.
90. Hay, E. A., "Development of Systems Which Do Not Focus on Whole *Animals, "Laboratory Animal Use in Basic Biomedical Research* (draft) (Bethesda, MD: American Society for Cell Biology, 1984).
91. Hayes, M. L., "The Effects of Fatty Acids; nd Their Monoesters on the Metabolic Activity of Dental Plaque," J. *Dent. Res. 63: 2-5, 1984.*
92. Held, J.R., Doherty, J., and King, N.B., "Societal Benefits Derived From Animal Research, "*J. Am. Vet, Med. Assoc.* 185: 268-269, 1984.
93. Hendricks, J. D., Meyers, T. R., Casteel, J. L., et al., "Rainbow Trout Embryos: Advantages and Limitations for Carcinogenesis Research," in U.S. Department of Health and Human Services, Public Health Service, National Cancer Institute, *Use of Small Fish Species in Carcinogenicity Testing,* Monograph 65, NIH Pub. No. (PHS) 84-2652 (Bethesda, MD, May 1984).
94. Hennessey, T. M., Rucker, W .B., and McDiarmid, C .G., "Classical Conditioning in Paramecia, "*Anim. Learn. Behav.* 7:417-423, 1979.
95. Herbert, D.E., Associate Professor, Department of Radiology, University of South Alabama, Mobile, AL, personal communication, July 24, 1984.
96. Hersen, M., and Barlow, D.H. (eds.), *Single Case Experimental Designs (New* York: Pergamon Press, 1976).
97. Hieftje, G.M., "New Techniques and Tools in Clinical Chemistry)" *Clin. Chem. 29: 1659-1644,* 1983.
98. Hilfer, S. R., and Hilfer, E. S., "Computer Simulation of Organogenesis: An Approach to the Analysis of Shape Changes in Epithelial Organs," *Dev. Biol.* 97:444-453, *1983.*
99. Hodgkinson, L., "Tree of Life," *Progress Without Pain* (Lord Dowding Fund, National Anti-Vivisection Society, Ltd., London) 21:20-25, 1984.
100. Howards, S. S., Professor, Departments of Urology and Physiology, University of Virginia, Charlottesville, VA, personal communication, Oct. 10, 1984.
101. Howes, R. I., and Eakers, E. C., "Augmentation of

Tooth and Jaw Regeneration in the Frog With a Digital Transplant,'' J. *Dent. Res.* 63:670-674, 1984.
102 Hruschka, W. R., Instrumentation Research Laboratory, BARC, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD, personal communication, July 26, 1984,
103. Huntingford, F. A.: Some Ethical Issues Raised By Studies of Predation and Aggression, " *Anim. Behav.* 32:210-215, 1984.
104. Hyman, B.T., Van Hoesen, G. W., Damasio, A. R., et al.,"Alzheimer's Disease: Cell-Specific Pathology Isolates the Hippocampal Formation," *Science* 225:1168-1170, 1984.
105. Iverson, L. L., "Molecular Neurobiology," *Science* 225:1468, 1984.
106. Jaffe, K., "Theoretical Analysis of the Communication System for Chemical Mass Recruitment in Ants," *J. Theor. Biol.* 84:589-610, 1980.
107. Jankus, E. F., Staley, N. A., and Noren, G. R., "Turkey Round Heart Disease: A Model Cardiomyopathy," ***Research Animals in Medicine,*** Lowell T. Harmison (cd.) (Washington, DC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, 1984).
108. Johnston, K. W., Hager, M., and Cobbold, R. S. C., "Detection of Early Atherosclerosis Using the Systolic Pressure Slope ," ***Non-Invasive Clinical Measurement,*** D.E.M. Taylor and J. Whamond (eds.) (Baltimore, MD: University Park Press, 1977).
109, Jonas, A. M., "The Mouse in Biomedical Research," *Physiologist* 27:330-346, *1984.*
110. Jovellanos, C. L., and Gaskin, D. E., "Predicting the Movements of Juvenile Atlantic Herring *Clupea harengus* in the Southwest Bay of Fundy Using Computer Simulation Techniques," ***Can. J. Fish Aquat. Sci.*** 40:139-146, 1983.
111. Kazdin, A. E., "Statistical Analyses for Single-case Experimental Designs, ''Single ***Case Experimental Designs,*** M. Hersen and D.H. Barlow (eds.) (New York: Pergamon Press, 1976).
112. Kellis, J.T., Jr., and Vickery, L.E., "Inhibition of Human Estrogen Synthetase (Aromatase) by Flavones," ***Science*** 225:1032-1034, 1984.
113. Keppel, G., ***Design and Analysis: A Researcher's Handbook*** (Englewood Cliffs, NJ: Prentice-Hall, 1973).
114. Kerr, F. W. L., "The Investigator's Responsibilities in Research Using Animals," ***Scientific Perspectives on Animal Welfare,*** W.J. Dodds and F.B. Orlans (eds.) (New York: Academic Press, *1982).*
115 Kitchen, R. L., and Erickson, H.H. (eds.), *Animal Pain: Perception and Alleviation* (Bethesda, MD: American Physiological Society, 1983).
116 Knowlton, N., "Reproductive Synchrony, Parental Investment, and the Evolutionary Dynamics of Sexual Selection," *Anim. Behav.* 27:1022-1033, *1979.*
117. Koff, W.C., Fidler, I.J., Showalter, S. D., et al., "Human Monocytes Activated by Immunomodulators in Liposomes Lyse Herpesvirus-Infected But Not Normal Cells," ***Science*** 224:1007-1009, 1984.
118. Kolata, G., "Steroid Hormone Systems Found in Yeast," ***Science*** 225:913-914, 1984,
119. Kolata, G., "A New Kind of Epidemiology, ''Science 224:481, 1984.
120. Kootsey, J.M., Director, National Biomedical Simulation Resource, Duke University Medical Center, Durham, NC, personal communication, Oct. 11, 1984.
121. Lea, S.E.G.: Alternatives to the Use of Painful Stimuli in Physiological Psychology and the Study of Animal Behaviour," *ATLA Abstr.* 7:20-21, *1979.*
122. Lee, M. H., and Marudarajan, A. R., "A Computer Package for the Evaluation of Neuron Models Involving Large Uniform Networks, " *Int. J. Man-Machine Stud.* 17:189-210, *1982.*
123. Leshner, A. I., ***An Introduction to Behavioral Endocrinology (New*** York: Oxford University Press, 1978).
124 Levin, D. E., Marnett, L. J., and Ames, B. N., "Spontaneous and Mutagen-Induced Deletions: Mechanistic Studies in *Salmonella* Tester Strain TA 102," ***Genetics*** 81:4457-4461, *1984.*
125 Lewis, C. S., "NASA's Use of Animals in Research," prepared for the Life Sciences Division, National Aeronautics and Space Administration, Washington, DC, Sept. 28, 1983.
126. Lien, Y., Stern, R., Fu, J. C. C., et al., "Inhibition of Collagen Fibril Formation In Vitro and Subsequent Cross-Linking by Glucose," ***Science*** 225:1489-1491, 1984.
127. Lineberry, C .G.: Laboratory Animals in Pain Research, ''*Methods of Animal Experimentation,* W.1. Gay (cd.) (New York: Academic Press, *1981).*
128. Lineweaver, H., and Burk, D., "Determination of Enzyme Dissociation Constants, "*J. Am. Chem. Soc.* 56:658-666, *1934.*
129 Maccecchini, M., Rudin, Y., Blobel, G., et al., "Import of Proteins Into Mitochondria: Precursor Forms of the Extramitochondrially Made F1-ATPase Subunits in Yeast, " *Proc. Natl. Acad. Sci. USA* 76:343-347, *1979.*
130. Maciel, G. E., "High-Resolution Nuclear Magnetic Resonance of Solids," ***Science*** 226:282-287, 1984.
131. Mackenzie, N. E., Malthouse, J. P.G., and Scott, A. I., "Studying Enzyme Mechanism by Carbon-13 Nuclear Magnetic Resonance, " ***Science 225*** :883-889, *1984.*

132 Manning, A., "The Effects of Artificial Selection for Mating Speeds in *Drosophila melanogaster," Anim. Behav.* 9:82-92, *1961.*

133 Manolis, A., "The Diagnostic Potential of Breath Analysis," *Clin. Chem.* 29:5-15, *1983.*

134. Markovic, N. S., Markovic, O. T., and Young, D. S., "Simplified Methods for Measuring Enzy-me Kinetics in a Single Cell," *Clin. Chem.* 29:1578-1581, 1983.

135. Massie, D. R., "Fat Measurement of Ground Beef With a Gallium Arsenide Infrared Emitter," ***Quality Detection in Foods*** (St. Joseph, MI: American Society of Agricultural Engineers, 1976).

136. Mather, I. H., Jarasch, E., Bruder, G., et al., "Protein Synthesis in Lactating Guinea-pig Mammary Tissue Perfused In Vitro)" ***Exp. Cell Res.*** 151:208-223, 1984.

137. McCarron, D. A., Morris, C. D., Henry, H.J., et al., "Blood Pressure and Nutrient Intake in the United States," ***Science*** 226:386-388, 1984.

138. McConnell, J. V., "Comparative Physiology: Learning in Invertebrates, " ***Ann. Rev. Physiol. 28:107-136, 1966.***

139. McConnell, J. V., and Jacobson, A. L., "Learning in Invertebrates)" ***Comparative Psychology: A Modern Survey,*** D.A. Dewsbury and D.A. Rethlingshafer (eds.) (New York: McGraw-Hill, 1973).

140. McFarland, D.J., "Decision Making in Animals)" ***Nature*** 269:15-21, ***1977.***

141. McMurray, G .A., "Congenital Insensitivity to Pain and Its Implications for Motivation Theory, " ***Can. J. Psychol,*** 9:650-667, 1955.

142 Mejia, R., "Computer Simulation of Renal Function-Project 1Z01HL03202-11," in U.S. Department of Health and Human Services, Public Health Service, National Heart, Lung, and Blood Institute , *CRISP Report, 1982* (Bethesda, MD, 1982).

143. Melzack, R., ***The Puzzle of Pain (New*** York: Penguin, 1973).

144. Merz, P, A., Rohwer, R. G., Kascsak, R., et a]., "Infection-Specific Particle From the Unconventional Slow Virus Diseases," ***Science*** 225:437-440, 1984,

145. Metz, S., Horrow, J. C., and Balcar, I., "A Controlled Comparison of Techniques for Locating the Internal Jugular Vein Using Ultrasonography, ''Anesth. *Analg.* 63:673-679, ***1984.***

146. Meuzelaar, H. L, C., Windig, W., Harper, A. M., et. al., "Pyrolysis Mass Spectrometry of Complex Organic Materials," ***Science*** 226:268-274, 1984.

147. Michaelis, L., and Menten, M. L., "Kinetics of Invertase Action," ***Biochemistry*** 49:333-369, ***1913.***

148. Minamitani, H., and Hagita, N., "A Neutral Network Model of Pain Mechanisms: Computer Simulation of the Central Neural Activities Essential for the Pain and Touch Sensations)" *IEEE* ***Transactions on Systems, Man, and Cybernetics*** 11:481-493, ***1981.***

149. Mirham, G. A., "Simulation Methodology," ***Theory and Decision*** 7:67-94, ***1976.***

150. Moffatt, D. S., Guyton, A.C., and Adair, T. H., "Functional Diagrams of Flow and Volume for the Dog's Lung," ***J. Appl. Physiol.*** 52:1035-1042, ***1982.***

151. Moran, G., "Severe Food Deprivation: Some Thoughts Regarding Its Exclusive Use,"***Psycho], Bull.*** 82:543-557, ***1975.***

152. Moses, R. D., "Computer Simulation of the Cardiovascular System-Project 1Z01HL02719-01," in U.S. Department of Health and Human Services, Public Health Service, National Heart, Lung, and Blood Institute, *CRISP Report, 1982* (Bethesda, MD, 1982).

153. Murai, M., Thompson, W. A., and Wellington, W. G., "Simple Computer Model of Animal Spacing," *Res. Pop. Ecol.* 20:165-178, 1979.

154, Mytilineou, C., and Cohen, G., "1-Methyl-4-Phenyl-l,2,3,6-Tetrahydropy ridine Destroys Dopamine Neurons in Explants of Rat Embryo Mesencephalon," ***Science*** 225:529-531, 1984.

155, Nadi, N. S., Nurnberger, J. I., and Gershon, E. S., "Muscarinic Cholinergic Receptors on Skin Fibroblasts in Familial Affective Disorder," N. *Engl. J. Med.* 311:225-230, ***1984.***

156. Nardone, R.M., and Ouellette, L. A., "Scope of 'Alternatives': Overview of the State of the Art)" contract report prepared for the Office of Technology Assessment, U.S. Congress, July 1984.

157. Naz, R. K., Alexander, N,J., Isahakia, M., et al., "Monoclinal Antibody to a Human Germ Cell Membrane Glycoprotein That Inhibits Fertilization," ***Science*** 224:342-344, 1984.

158. Neches, R., "Simulation Systems for Cognitive Psychology," *Behav. Res. Meth. Instru.* 14:77-91, ***1982.***

159. Netland, P. A., and Zetter, B.R. "Organ-Specific Adhesion of Metastatic Tumor Cells in Vitro, " ***Science*** 224:1113-1115, 1984.

160. Newton, C, M., "Biostatistical and Biomathematical Methods in Efficient Animal Experimentation, " in National Research Council / National Academy of Sciences, ***The Future of Animals, Cells, Models, and Systems in Research, Development, Education, and Testing*** (Washington, DC: National Academy of Sciences, 1975).

161 Normann, R. A., Perlman, I., Kolb, H., et al., "Direct Excitatory Interactions Between Cones of Different Spectral Types in the Turtle Retina, ''Science 224:625-627, 1984.

162 Norris, K. H., "Near Infrared Reflectance Spectroscop—The Present and Future," ***Cereals '78: Better Nutrition for the World's Millions,*** Proceedings of the Sixth International Cereal and Bread Congress, May 1978.

163. Orcutt, B. C., George, D.G., and Dayhoff, M. O., "Protein and Nucleic Acid Sequence Data Base Systerns, " ***Ann. Rev.*** *Biophys. Bioengin.* 12:419-441, ***1983.***
164. Patrusky, B., "Plants in Their Own Behalf ," *Mosaic* 14(2):32-39, 1983.
165. Peterson, E. L., "The Temporal Pattern of Mosquito Flight Activity, " *Behaviour* 72:1-25, 1980.
166. Peterson, J. I., and Vurek, G. G., "Fiber-Optic Sensors for Biomedical Applications," ***Science*** 224: 123-127, 1984.
167. Pottala, E. W., Colburn, T. R., Covacci, R., et al., "Hardware Cone Cell Model: Operational Characteristics, ''Med. *Biol. Engin. Comput.* 20:437, ***1982.***
168. Preilowski, B., Reger, M., and Engele, H., "Combining Scientific Experimentation With Species Adequate Housing in Laboratory Studies of Non-Human Primates," ***Am. J.*** *Primatol.* 5:374, ***1984.***
169. Pyke, G. H., "Honeyeater Foraging: A Test of Optimal Foraging Theory, "*Anim. Behav.* 29:878-888, 1981.
170. Pylyshyn, Z. W., "Computational Models and Empirical Restraints, "*Behav. Brain Sci.* 1:93-99, ***1978.***
171. Reed, S. C., "Problems in Assessing Intelligence in Artifacts, "*IEEE* ***Transactions on Systems, Man, and*** *Cybernetics* 4:97 -100, 1974.
172. Ross, M. H., "Nutrition and Longevity in Experimental Animals, " ***Nutrition and Aging,*** M. Wineck (cd.) (New York: John Wiley & Sons, 1976).
173 Rowsell, H. C., "The Ethics of Biomedical Experimentation," in National Research Council / National Academy of Sciences, ***The Future*** *of Animals,* ***Cells, Models, and Systems*** *in* ***Research, Development, Education, and Testing*** (Washington, DC: National Academy of Sciences, 1975).
174 Russell, W .M.S., and Burch, R. L., ***Principles of Humane Experimental Technique*** (Springfield, IL: Charles C. Thomas, 1959).
175 Sahagian, G.G., and Neufeld, E. F., "Biosynthesis and Turnover of the Mannose 6-Phosphate Receptor in Cultured Chinese Hamster Ovary Cells, " ***J.*** *Biol. Chem.* 258:7121-7128, ***1983.***
176. Sainz, A., Roberts, V. C., Pinardi, G., et al., "Blood Flow Velocity and Acceleration Measurement by Doppler-shift Ultrasound," ***Non-Invasive Clinical Measurement,*** D.E.M. Taylor and J. Whamond (eds.) (Baltimore, MD: University Park Press, 1977).
177. Salu, Y., "Computer Simulations of Learning in Neural Systems," *Comput. Biomed.* ***Res. 16:176-189, 1983.***
178. Sanders, G. D., "The Cephalopods," ***Invertebrate Learning, Vol. 3: Cephalopods and Echinoderms,*** W.C. Corning, J.A. Dyal, and A.O.D. Willows (eds.) (New York: Plenum Press, 1975).
179. Schwartz, J. M., and Cogan, D. C., "Position Discrimination in the Salamander, *Ambystomatigrinum, " Devel. Psychobiol.* 10:355-358, ***1977.***
180, Schwid, H. A., and Geisler, C. D., "Multiple Reservoir Model of Neurotransmitter Release by a Cochlear Inner Hair Cell," *J. Acoust. Soc.* ***Am.*** 72:1435-1440, 1982.
181. Seeman, P., Ulpian, C., Bergeron, C., et al., "Bimodal Distribution of Dopamine Receptor Densities in Brains of Schizophrenics, " ***Science*** 225:728-731, 1984.
182. Seligman, M. E. P., "On the Generality of the Laws of Learning," *Psychol.* ***Rev.*** 77:406-418, 1970.
183. Sevenster, P., "Motivationand Learning in Sticklebacks *(Gasterosteus aculeatus* ***L.), " The Central Nervous System and Fish Behavior,*** D. Ingle (cd.) (Chicago: University of Chicago Press, 1968).
184. Sharma, S. P., Jit, 1., and Rai, N., "Age-Related Changes in Nucleic Acids and Protein in *Callosobruchus maculatus* Fabr. (Coleoptera)," ***Gerontology*** 30:26-29, 1984.
185. Shiovitz, K. A., and Lemon, R. E., "Species Identification of Song by Indigo Buntings as Determined by Responses to Computer Generated Sounds, ''Behaviour 74:167-199, 1980.
186. Sibaoka, T., "Action Potentials in Plant Organs, " ***Nerv.*** *Horm. Mech. Integr.* 20:40-73, 1966.
187. Sidman, M., ***Tactics of Scientific Research (New*** York: Basic Books, 1960).
188. Simon, A., "A Theoretical Approach to the Classical Conditioning of Botanical Subjects, " ***J.*** *Biol. Psychol.* 20:35-43, 1978.
189. Sisk, C. L., and Desjardins, C., "Changes in LH Pulse Amplitude and Frequency as a Function of Time After Castration of Male Ferrets, " Soc. *Neurosci. Abstr.* 9:708, ***1983.***
190. Smith, A. C., "Marine Animals in Medical Research," ***JAMA*** 242:2847, ***1979.***
191. Smyth, D. H., ***Alternatives to Animal Experiments*** (London: Scholar Press Ltd, 1978).
192. Snyder, S. H., "Cholinergic Mechanisms in Affective Disorders," *N. Engl. J.* ***Med. 311*** :254-255, ***1984.***
193. Sorkin, B. C., Hoffman, S., Edelman, G. M., et al., "Sulfation and Phosphorylation of the Neural Cell Adhesion Molecule, N-CAM," ***Science*** 225:1476-1478, 1984.
194. Spetch, M.L., and Wilkie, D. M., "A Program That Simulates Random Choice in Radial Arm Mazes and Similar Choice Situations, " *Behav.* ***Res. Meth.*** *Instru.* 12:377-378, ***1980.***
195. Stanley, S. M., *Macroevolution:* ***Patterns and Process*** (San Francisco: W.H. Freeman, 1979).
196. Sternback, R.A., *Pain: A* ***Psychophysiological*** *Analysis (New* York: Academic Press, 1968).

197. Stevens, C.G., "Human Perspectives," in National Research Council/National Academy of Sciences, *The Future of Animals, Cells, Models, and Systems in* Research, Development, Education, *and Testing* (Washington, DC: National Academy of Sciences, 1975).
198. Still, A. W., "On the Number of Subjects Used in Animal Behaviour Experiments," *Am"m. Behav.* 30:873-880, *1982.*
199. Strulovici, B., Carione, R. A., Kilpatric, B.F., et al., "Direct Demonstration of Impaired Functionality of a purified Desensitized B-Adrenergic Receptor in a Reconstituted System," *Science* 225:837-840, *1984.*
200. Symmons, P. M., and Rosenberg, L.J., "A Model Simulating Mating Behavior of *Spodoptera littoralis," J. Appl. Ecol.* 15:423-438, *1978.*
201. Taylor, D.J., Crowe, M., Bore, P.J., et al., "Examination of the Energetic of Aging Skeletal Muscle Using Nuclear Magnetic Resonance," *Gerontology* 30:2-7, *1984.*
202. Tepperman, K., Finer, R., Donovan, S., et al., "Intestinal Uptake and Metabolism of Auranofin, A New Oral Gold-Based Antiarthritis Drug," *Science* 225:430-432, 1984.
203. Tinbergen, N., *The Study of Instinct (New* York: Oxford University Press, 1951).
204. Toates, F., "Computer Simulation and the Homeostatic Control of Behavior" *Motivational Control Systems Analysis,* D.J. McFarland (cd.) (London: Academic Press, 1974).
205. Toates, F., and O'Rourke, C., "Computer Simulation of Male Rat Sexual Behavior," *Med. Biol. Engin. Comput.* 16:98-104, *1978.*
206. Tompkins, P., and Bird, C., *The Secret Life of Plants (New York:* Harper & Row, 1973).
207. Turner, T. T., Plesums, J.L., and Cabot, C.L., "Luminal Fluid Proteins of the Male Reproductive Tract," *Biol. Reprod.* 21:883-890, 1979.
208. Vestal, M. L., "High-Performance Liquid Chromatography-Mass Spectrometry," *Science* 226:275-281, 1984.
209. Von Frisch, K., *The Dance Language and* Orientation *of Bees* (Cambridge, MA: Harvard University Press, 1967).
210. Waddell, C. A., and Desai, I. D., "The Use of Laboratory Animals in Nutrition Research, " *World Rev. Nutr. Diet.* 36:206-222, 1981.
211. Wall, P.D., "On the Relation of Injury to Pain, ''Pain 6:253-264, 1979.
212. Waller, M.B., Bride, W.J., Gatto, G.J., et al., "Intragastric Self-Infusion of Ethanol by Ethanol-Preferring and Nonpreferring Lines of Rats," Science 225:78-80, 1984.
213. Walter, P., and Blobel, G., "Signal Recognition Particle Contains a 7S RNA Essential for Protein Translocation Across the Endoplasmic Reticulum)" Na*ture* 299:691-698, *1982.*
214. Waser, M.R., Garfinkel, L., Kohn, M.C., et al,. "Computer Modeling of Muscle Phosphofructokinase Kinetics)" *J. Theor. Biol.* 103:295-312, *1983.*
215. Wehner, R., "Spatial Vision in Arthropods, ''*Handbook of Sensory Physiology Vol. VII/6C,* H. Autrum (cd.) (New York: Springer, 1981).
216. Weinberg, J,B., Hobbs, M.M., and Misukonis, M.A., "Recombinant Human Interferon Induces Human Monocyte Polykaryon Formation," *Proc. Natl. Acad. Sci USA* 81:4554-4557, *1984.*
217. Weisz, P. B., *The Science of Biology (New* York: McGraw-Hill, 4th cd., 1971).
218. Wells, M.J., *Octopus: Physiology and Behaviour of* an *Advanced Invertebrate* (London: Chapman & Hall, 1978).
219. Wells, P. H., "Honeybees," *Invertebrate Learning, Vol. 2: Arthropods and Gastropod Mollusks, W.C.* Corning, J.A. Dyal, and A.O.D. Willows (eds.) (New York: Plenum Press, 1973).
220. Wigdahl, B., Smith, C.A., Traglia, H. M., et al., "Herpes Simplex Virus Latency in Isolated Human Neurons, " *Proc. Natl. Acad. Sci. USA* 81:6217-6221, *1984.*
221. Wirier, B.J., *Statistical Principles in Experimental Design (New* York: McGraw-Hill, 1971).
222. Winfree, A .T., "Rotating Chemical Reactions," *Sci. Am.* 230:82-95, *1974.*
223. Woodward, W. T., and Bitterman, M. E., "Asymptotic Reversal Learning in Pigeons: Mechanisms for Reducing Inhibition," J. *Exp. Psychol.* (Animal Behavior Processes) 2:57-66, 1976.
224. Wright, E. M., Jr., Vice-Chairman, Department of Comparative Medicine, University of Virginia, Charlottesville, VA, personal communication, Aug. 16, 1984.
225. Zanberg, P., "Animal Models in Experimental Hypertension: Relevance to Drug Testing and Discovery," *Handbook of Hypertension, Vol. 3: Pharmacology of Antihypertensive Drugs,* P.A. van Zweiten (cd.) (Amsterdam: Elsevier Science Publishers B. W., 1984).