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Brian J. O'Neil

Grace/Detroit Receiving Hospitals

Jeffrey A. Kline

Carolinas Medical Center

Keith Burkhart

Pennsylvania State Medical Center

John Younger

University of Michigan - Ann Arbor

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Research Fundamentals: V. The Use of Laboratory Animal Models in Research

BRIAN J. O'NEIL, MD, JEFFREY A. KLINE, MD, KEITH BURKHART, MD,
JOHN YOUNGER, MD

Abstract. Animal research has provided important information about many aspects of the pathophysiology of human disease. Well-performed animal studies can determine the potential benefit of many proposed therapeutic interventions, and experimental results from animal studies have served as the basis for many landmark clinical trials. Many animal research models are described in the research literature, and choosing the appropriate model to answer a research question can be a daunting task. Even more challenging is developing a new model when none of the

existing systems are relevant to the proposed question. This article was prepared by members of the SAEM Research Committee to provide an overview of animal modeling. Important considerations in choosing, applying, and developing animal research models are outlined. Practical discussions of potential problems with animal models are also provided. **Key words:** laboratory; animals; animal models; animal research. *ACADEMIC EMERGENCY MEDICINE* 1999; 6:75–82

ANIMAL models of acute disease have contributed significantly to the advancement of scientific knowledge. Animal experimentation is typically initiated prior to actual human investigation, and provides the background for human research. The strengths of animal modeling are the ability to control confounding variables and perform extensive biochemical, biomechanical, and pathophysiologic assays. These techniques provide the foundation on which cause-and-effect associations are built.

The use of animal studies to study human disease assumes that animal models adequately reflect the pathophysiology of human disease and that the results obtained can be applied to humans. These assumptions are not absolutely true, because animals do not respond exactly like humans. However, well-designed animal studies still contribute to the basic knowledge of human disease. There is no ideal animal species for modeling

human response, and since no two species respond identically to the same pathologic condition, results from animal models must be carefully scrutinized prior to their clinical application. An ideal experimental model produces reproducible lesions, in a graded fashion, along a comparable temporal course, and has measurable standards.

This article is intended for those who are planning on, have already started, or are having difficulty using an animal model. We concentrate on general aspects of animal models of acute disease and discuss problems and flaws that are encountered in nearly every model.

GETTING STARTED

Many of the research questions relevant to emergency medicine (EM) can be addressed by appropriate animal-based study. Major areas of animal research within our specialty include, but are not limited to, cerebral ischemia and reperfusion, head injury, toxicology, asthma and other pulmonary diseases, preconditioning responses, and resuscitation from either cardiac arrest or traumatic hypovolemic shock.

The most important step for individuals undertaking their first animal research effort is to choose a well-focused, achievable project. Formulation of a valuable research project starts with an enlightened question that has the ability to be proven false. This research question should fill existing gaps in the knowledge base, refute or significantly strengthen previous literature, or have the ability to change the way we think or conduct medicine. In order to achieve these goals, the re-

From the Department of Emergency Medicine, Grace/Detroit Receiving Hospitals (BON), Detroit, MI; Department of Emergency Medicine, Carolinas Medical Center (JAK), Charlotte, NC; Department of Emergency Medicine, Milton S. Hershey Medical Center, Pennsylvania State Medical Center (KB), Hershey, PA; and Division of Emergency Medicine, University of Michigan—Ann Arbor (JY), Ann Arbor, MI.

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Address for correspondence and reprints: Brian J. O'Neil, MD, Detroit Receiving Hospital, Department of Emergency Medicine, 4201 St. Antoine, Detroit, MI 48201. Fax: 313-745-3653; e-mail: boneil@cms.cc.wayne.edu

TABLE 1. Commonly Encountered Animal Research Expenses

Institutional overhead: indirect costs
Physical plant/operating room costs
Principal investigator and technician salary support with fringe benefits
Animals
• Purchase and shipping costs
• Per-diem handling expenses
• Veterinary care
• Disposal fees
Equipment
• Life support and monitoring equipment
• Disposable surgical instruments
Medications
• Medical grade oxygen
• Anesthetic and analgesic agents
• Antibiotics
• Euthanasia agents
Pathology
• Blood gases and chemistry
• Research-specific assays or histopathology
Data-related expenses
• Data entry
• Data analysis
• Statistical software
• Hardware and support
Publication expenses

searcher must understand the strengths and limitations of the animal model chosen to answer the research question.

An exhaustive literature search must be performed to determine the usefulness of various animal models for answering the research question. Extensive understanding of what is known about the topic and existing animal models is necessary in order to devise an experiment that will add to the existing knowledge base. A thorough search of the literature should be performed on multiple databases, including alternate sources such as Vetline. Investigators should not hesitate to seek out manuscripts and monographs from literature dating as far back as the early part of the century. These dated writings often provide the only published, candid reasons why certain animals models work and why others fail.

When determining the appropriate animal model to answer the research question, first consider the models currently used by departmental colleagues. Your rigorous literature search will help determine whether the model is appropriate for answering the question that has been proposed. The value of beginning with a working model in a functioning laboratory cannot be overemphasized. If the question cannot be answered by currently used models, other avenues should be explored. The literature search might reveal similar areas of research interest in other departments within your institution. This may provide access to laboratories, consultants, and mentors, and open doors to

fruitful, long-term collaboration. However, before soliciting outside help, you should already have a relatively well-honed research question, have reviewed the literature pertaining to other research models, and have reviewed the work of the collaborator you are about to approach. By contacting senior investigators in other departments, you may gain access to unpublished data, funded laboratories willing to share specialized techniques, and working animal models suitable or adaptable to EM research. Phone conversations with published researchers, whose questions or models are similar to yours, will provide additional important information. These investigators may give you tips to ensure that you don't repeat their mistakes, and provide you with refinements, changes, or improvements they have made to their models. Hopefully, your first attempt at research will be a mentored experience that eventually fosters independence. If local mentors are not available, established researchers contacted periodically by telephone can provide necessary guidance and support.

When a workable animal model is identified, it must be carefully considered in terms of the research question. What are the strengths and weaknesses of the model? If any weaknesses exist, how can they be exploited or corrected? Is the model easily reproducible, or is there a wide variability from subject to subject? Is the model used by a number of different laboratories, or has it been used in only one laboratory? Does the model use specialized surgical techniques or specialized laboratory equipment? If so, how long will it take you to learn these techniques, who will teach them, and how will necessary equipment be acquired? How suitable is the model to prove or disprove your specific research question? Are the controls appropriate for your question, and are there positive and negative controls available? How clinically relevant is the model? If a workable model cannot be found, it is possible to develop one, but this process is long and arduous, and success is not guaranteed.

A final consideration before beginning work with an animal model is to determine whether adequate time and money are available to perform the project. Beginning researchers are frequently surprised at the amount of time actually required to undertake a seemingly straightforward animal experiment. It takes time to obtain approval for the proposed project from the animal investigation committee, to develop and perfect the necessary surgical and/or assay techniques, to perform a dose-response or receiver operating characteristic curve, and to perform the miscellaneous "debugging" required for every model. If new model development is included in the project timetable, an

additional three months to a year should be anticipated. Time, space, and economic requirements for animal experimentation must be assessed and projected before beginning the study. Table 1 lists some common expenses encountered in animal and bench research. Table 2 is a reasonable estimate of the cost of purchasing commonly used laboratory animals. Usually, young investigators can access "start-up" money from intramural or, occasionally, extramural grant sources. Early funds should be used to generate preliminary data intended to attract extramural support.

PURSUING THE RESEARCH QUESTION WITH AN ESTABLISHED ANIMAL MODEL

The Animal Model. The scope of this article does not allow a precise detailing of the best animal models available for the study of acute disease processes. The appropriate model depends on the study objectives, the budget, and the investigator's philosophy. The best advice remains: know the literature, and seek help from seasoned investigators in your field of interest. Many texts are available to assist in this process.¹⁻⁸

The practical number of animal species available for study is limited (e.g., mice, rats, guinea pigs, rabbits, canines, swine). However, with smaller animals, a huge inventory of strains is available for each species. Vendors offer animals bred or genetically manipulated to alter virtually every organ system. Rodents and rabbits have been bred to demonstrate many modern western diseases such as hypertension, hypercholesterolemia, types I and II diabetes, and even senescent animals. The investigator should determine from the literature and from other investigators which species and strain show the disease process most accurately. For example, suppose an investigator wishes to study the propensity of a drug possessing potassium-channel-antagonizing properties to produce torsades de points in a small animal model. He or she would be well advised to choose the guinea pig instead of the rat, since rats do not show significant potassium-channel effects on heart repolarization, while guinea pig hearts do show this effect.

An animal's physiologic response to the induction of acute disease depends on endogenous and exogenous stresses. Uniform animal health and conditioning should be documented, and animals should be obtained from licensed vendors only. The vendor should guarantee the health of the animals, and the animals should undergo standard screening at your facility. For canines, screening for heartworms, rabies, and parvovirus should be considered. The investigator should ask questions

TABLE 2. Costs Associated with Commonly Used Laboratory Animals

Species	Unit Cost*	Per-diem Cost*
Mouse (CD1)	\$8.00	\$1.00
Rat (Sprague-Dawley, SPF†)	\$14.00	\$1.50
Rabbit/rabbit (SPF)	\$30.00/\$60.00	\$1.85/\$2.00
Swine (immature, mixed breed)	\$150.00	\$7.75
Dog/dog (conditioned)‡	\$125.00/\$250.00	\$6.00/\$6.00
Sheep	\$150.00	\$7.50
Baboon	\$1,200.00	\$7.00

*These costs are approximates, and vary with region and vendor.

†Specific Pathogen Free (SPF) animals are raised under more stringent conditions than other animals. SPF mice and rats are usually guaranteed by the supplier to be free of about 30 different parasitic and viral infections. SPF rabbits are screened primarily for *Pasteurella multocida*, which causes an upper respiratory tract infection.

‡Conditioned dogs have received standard canine vaccinations and have been screened for heartworms.

about quantity, content, and scheduling of the animal's diet. For instance, some animal chows are low in calcium, which could seriously alter the outcome in studies of metabolism during ischemia or shock. Consideration should also be given to light cycles and natural hormonal cycles. Most female animals have an estrus, which must be considered for experiments dependent on steroid hormones' effects. Animals are also susceptible to environmental factors, including the stress of transportation. Rodents require at least one week after transport to acclimatize to a new environment. Testing before this time may be pointless. For example, detailed cardiovascular monitoring on newly transported rats often demonstrates huge variability between animals. This variability is reduced if the animal has been allowed to stabilize (i.e., acclimatize) before the experiment. On a more subtle, and difficult-to-document scale, some species show many time-dependent biological patterns that can affect outcomes of experiments. Experiments performed on Sprague-Dawley rats in the spring may generate different results than if the same experiments were performed in late fall. Rodents, and sometimes canines, gain fat mass as they are housed. In pharmacology experiments, increased fat can alter drug distribution significantly.

These examples represent only a glimpse into the complex influence that time, environment, and animal health can impart on study outcome. In addition to the possible effects such conditions may have on the experimental results, there are other practical issues that must be considered. These include the budgetary and space concerns involved in the prolonged housing of animals or special dietary or exercise needs.

Equipment. After the species is chosen, details of

instrumentation can be formulated. Many investigators have little choice in the equipment used in their first studies. When the available equipment is assembled, the investigator must readdress the main objective and determine whether the essential question can be answered with the available tools. Can the available instruments be used in the animal species proposed for the study? If not, the objective may need to be altered, the protocol revised, or the study postponed. Resourceful researchers have found useful usable equipment from clinical engineering storage. Many disposed amplifiers, oscilloscopes, transducers, and catheters designed for clinical use can be adapted to the animal laboratory.

If the investigator has the funding to purchase new equipment, several tenets are helpful. Make sure the company has a strong track record, and check with the technicians from experienced laboratories for equipment suggestions and specific requirements. Attempt to purchase the most recent model of any system ("state-of-the-art"), and be sure the item is not about to be discontinued. Ask about planned upgrades and improvements, and if these are scheduled soon, ask for a free upgrade or wait for its release. Confirm the company's commitment to service the equipment after the sale. Be sure the product you are about to buy is going to provide you with everything needed. For example, company literature that describes many data collection/archiving systems may outline an invincible system to store and analyze data. However, to the chagrin of many researchers, after the initial purchase, they learn that such systems often require more equipment at substantial cost to interface it with existing laboratory systems.

The technology of animal instrumentation is rapidly advancing. Every year, at major national scientific meetings, vendors display new equipment, such as flow probes, signal collecting devices, and data acquisition systems, that surpass equipment available the year before. The most relevant change in technology is the ability to achieve sophisticated instrumentation on small species. For example, it is currently possible to place newer generation transit time flow probes on the rat aorta to measure cardiac output. This method obviates the trouble of thermodilution catheters for cardiac output, freeing the jugular and carotid arteries for placement of 2-Fr micromanometers that can measure right and left ventricular instantaneous pressures (and first derivatives). More recently, ultrasonic transit time probes have been developed that are small enough to measure the cardiac output of mice. Most companies that manufacture laboratory equipment have toll-free phone numbers, and will eagerly mail literature regarding their latest developments.

Anesthesia Choices. Anesthesia alters virtually every measurable parameter in whole animal preparations. The choice of an anesthetic or model that does not require anesthesia is a critical decision, especially in central nervous system and cardiovascular research. The response of different species to individual agents is sometimes heterogeneous. For example, ketamine is not a cardiodepressant in most species, but in rabbits, ketamine is a potent negative inotrope. Volatile anesthetics produce a stable hemodynamic pattern in most animals; in swine, however, a subtle decrease in core body temperature caused by volatile anesthetics can precipitate ventricular fibrillation. A more comprehensive description of individual anesthetics for each species is available from several texts.¹⁻⁸

Defining the Controls. The ideal study design for most animal models is randomized, blinded, placebo-controlled (vehicle-only), and sham-controlled. Ensuring the presence of proper control groups is critical. Except for the interventions to be tested, the controls must be identical to the experimental subjects in all parameters, including age, weight, diet, sex, and pre- and postexperiment animal care. In some experiments several control groups are needed. For example if drug therapies are tested in an animal model, one control group would be the no-treatment group (sham treatment group), while another control group would receive the vehicle. Both control groups are needed. The sham or nonintervention group delineates the effects of anesthesia, instrumentation, and baseline mortality, and the experimental control group reveals the effect of the vehicle on the outcome measured.

The Pilot Study. In the absence of preliminary data, a limited study to gauge the workability of the model and the accuracy of predictions is essential for subsequent successful research. For instance, if the magnitude of an insult must be determined, the pilot study should be focused on this objective, using less than 25% of the final study sample size. Finding an appropriate drug dose or necessary insult duration can be a tedious process. One method to increase efficiency is to first define lower and upper boundaries of the drug dose or injury. This can be done by examining the effect of a small dose or duration of insult, then increasing to a 100-fold greater dose or insult, perhaps using the same animal. Subsequently, more moderate doses and duration of insult can be evaluated.

The pilot study is also essential for the investigator to develop technical skills, become familiar with equipment, and demonstrate the stability of

the model. Several common pitfalls can be avoided with information obtained in the pilot study. The ability to instrument and collect data with fidelity can be demonstrated. A daily experimental timeline of preparation (for drugs, buffers, equipment, and instrumentation) can be constructed. Drug solubility and stability can be confirmed. Relevant variables can be determined and controlled, including animal ventilation, anesthetic dose, infusion volumes, core temperature, necropsy, and body disposal.

Outcome Measures. While the study hypothesis will dictate the primary outcome measure, literature reviews and pilot studies may identify important secondary outcome parameters that the study design can also measure. When possible, outcome measures should be validated by more than one method (e.g., quantitative neurologic outcome and brain histopathologic changes during postischemic reperfusion), because this will validate and strengthen the results.

Data collection and data entry are tedious and time-consuming, and can be expensive. It is crucial that the database is set up before data collection, that it is user-friendly, and that the data are easily extractable from the database. Computer-based statistical and database programs are readily available timesaving assets. Continuous data recording of laboratory data is also available, such as with MacLab. This is very useful in animal studies that require frequent sampling of physiologic data.

COMMON PROBLEMS IN ANIMAL RESEARCH

Animal models of human disease usually fail because of physiologic instability or the failure of the model to reflect human pathobiology. The first problem often involves technical difficulties, such as hemodynamic instability during basal conditions, the inability to instrument the animal without producing undue stress, catheter or equipment failure, wide metabolic fluctuations during the experiment, and data storage disorganization. Some technical difficulties are salient (e.g., uncontrolled hemorrhage during instrumentation, death from anesthesia) and others are more subtle (e.g., drug binding to the delivery tubing, overhead lights producing ultraviolet-induced oxidant damage in tissue preparations). When an experimental animal or tissue preparation fails to produce a stable baseline or reproducible pathobiology, the investigator should critically examine the system. Is the animal being ventilated and oxygenated? Is the anesthesia being delivered accurately? Is the drug soluble in the vehicle used for its preparation? Are drug de-

livery, distribution, metabolism, stability, and solubility homogeneous between animals? Several personal vignettes from one of the authors (JAK) help illustrate how simple mistakes can confound experiments for months:

1. In one study, investigators concluded that tachyphylaxis caused a reduction in positive inotropy of catecholamines during an experimental study of cardiogenic shock. Later, the researchers learned that catecholamines are unstable in saline unless the saline is acidified and light-shielded.

2. One model of septic shock generated widely different hemodynamic responses: on one day, IV endotoxin produced rapid death, but on another day, the same dose from the same drug lot produced little hemodynamic effect. It was later discovered that the endotoxin preparation is a suspension, and must be agitated to a homogeneous state before injection.

3. Doppler flow probes often show weak signals in chronically instrumented canines. Usually, experiments are performed in overnight fasted animals. Fasting can cause mild dehydration, and blood flow to vital organs can be diminished at the outset of the experiment. Robust signals often return if a standard protocol of IV rehydration is incorporated before data collection begins.

4. Ventilated, pentobarbital-anesthetized rabbits often die suddenly, just when the experiment seems stable. However, without positive end-expiratory pressure (PEEP) during ventilation, rabbits quickly lose lung functional residual capacity, develop hypoxia, and die. Animals therefore show sudden instability if PEEP has not been applied.

When animal models fail to mimic human physiology, the problems are often related to protocol insufficiencies or genetic differences that produce disparate physiology. Examples of protocol failures are illustrated in some toxicity studies. Because most human overdoses occur by ingestion, first-pass hepatic metabolism can profoundly alter the course of drug toxicity. Specific enantiomers of some toxins are preferentially metabolized, leading to accumulation of the other isomer, which may be more or less biologically active. In most animal studies of overdose, drugs are given intravenously. Parenteral infusion may produce a different effect than if the drug were delivered in the gut or via the portal vein. Genetic differences are often evident in studies of receptor pharmacology and cellular metabolism. For instance, rats make poor models for the study the cardiac effect of glycosides, because their hearts do not show the typical response of other species. Additionally, either rats possess a great density of ATPase in their sarcolemma, or the enzyme is resistant to glycoside binding; this also interferes with the experimental results. Problems of interspecies genetic and phys-

iologic differences can often be discovered during a compulsive literature search.

PURSUING THE RESEARCH QUESTION IN THE ABSENCE OF AN ESTABLISHED ANIMAL MODEL

On occasion an exhaustive literature search and multiple discussions with the experts in the field will not produce information on an established animal model or one that can be modified to answer the proposed research question. It may then be necessary to develop a new model. The first step is to review the literature and determine which species are best suited to answer the research question. Once this is determined, contact an expert in that species and discuss the problem with him or her. A visit to the expert's laboratory may be very useful, since some techniques are difficult to describe over the phone. The expert will be able to assist in model development, or warn of the major difficulties or flaws that may be encountered as you attempt to establish a new model. Hopefully, the expert may be able to suggest ways to avoid or anticipate problems. If no appropriate model exists, take the best parts of those that do, and combine them into a model that should address the research question. This will take considerable manipulation, deletion, and revision. This will be possible only if you have an understanding of the anesthesia, instrumentation, sampling techniques, assays to be performed, outcomes to be measured, and the effect each of these will have on the model. Each aspect of the new model must be evaluated for its potential to affect the outcome of interest. For example, the choice of anesthesia is critical not only to rates of resuscitation, but also to final outcome measures (e.g., ketamine and pentobarbital are neuroprotectants and may influence neurotrauma outcome). Even the slightest factor, such as fasting or not fasting the animal prior to experimentation, can change outcome. Maintaining body temperature is also an important consideration in animal experimentation, since temperature fluctuations significantly affect metabolic rates. The importance of the method you choose to produce the desired experimental state cannot be overemphasized. For example, the choice of method to produce cardiac arrest such as potassium injection, electrical shock, or asphyxia will have a significant effect on neurologic outcome and histopathology.^{9,10}

Time and effort can be reduced if the experimental protocol is critically reviewed and pilot-tested prior to its implementation. Assume that anything that can go wrong will, and plan ways to correct or circumvent these anticipated problems. The initial application of the new model without

any intervention will establish the baseline and may delineate problems that can easily be corrected. If problems are identified, only one protocol change at a time should be attempted, until the problem is resolved.

In drug intervention studies, the next step is evaluating drug toxicity and dose-response. Previous literature may give an estimate of the therapeutic dose; a response curve that incorporates this level somewhere in the middle is a good starting point for future work. If no reasonable estimate is available from existing sources, a wide range of doses should be initially tested, with subsequent narrowing of the range in future trials. Positive and negative controls, whenever possible, should be used to establish the LD50 and dose-response. Once these have been established, the outcome measure or assays to be used must be evaluated. Again, you must ensure that you have a working and reproducible model by resolving problems in a systematic manner. The best way to avoid significant loss of time, money, and enthusiasm is thorough planning and preparation prior to commencing work with a newly developed model.

There are many invaluable references available for those considering the use of different animal species for experimental models.¹¹⁻¹⁶ The advantages and disadvantages of each of the four major species used in most animal studies are outlined in Table 3. Rodents are an excellent choice in studies where small effects are expected and therefore large sample sizes are required. It has been suggested that, when possible, rodents be used for pilot studies to define and refine a model or hypothesis. You can then advance to higher species to confirm outcomes and potential human benefits.¹⁷

ANIMAL INVESTIGATION COMMITTEE (AIC) PREPARATION

The AIC is the approval body that reviews animal protocols. Its main goal is to ensure that animal research is conducted ethically. Ethical concerns in animal research include proper housing, anesthesia, sterile surgical procedures (if a survival study), correct medication dosing and ventilatory settings, proper and assured euthanasia techniques, and, in outcome and long-term survival studies, insurance that survivor animals do not become hypothermic, dehydrated, or malnourished and will not suffer pain or discomfort after the experiment. A useful reference regarding these issues is the NIH guide to animal models.¹⁸ The AIC evaluates the potential value of your study with respect to the advancement of human or animal health, knowledge, education, or training. Justification of the species

TABLE 3. Some Advantages and Disadvantages of Animal Species

Species	Advantages	Disadvantages
Rodents	Well-established models in cardiac and cerebral resuscitation Anatomy and physiology well delineated Cerebrovascular responses similar to humans Good homogeneity within strains ^{19*} Low cost of animals, supplies, and care Minimal laboratory facilities and personnel needed	Does not always reproduce human anatomy or physiologic responses ^{20,21} (e.g., spontaneous defibrillation, resistance to infection, cranial anatomy) CPR mechanics different than from humans Small blood volume and tissue volumes Assessment of cortical function difficult unless preexperimental learning strategies are used
Canines	Relatively inexpensive Extensive availability Models well established Neurologic scoring well established	Cardiac physiology different, with no coronary artery disease Increased collateral flow Left coronary artery dominance Higher cardiac output and regional blood flow vastly different, chest wall gross anatomy different from humans ²²
Swine	Excellent thoracic, anatomic, and cardiac physiologic similarity to humans Can be bred with atherosclerosis ^{6,7,8,15} Closed chest blood flow similar to humans	Limited model and outcome scoring experience
Primates	The best model for reproduction of the human condition Anatomy, physiology, and cortical functions more like humans than other species Can be bred to reproduce some human disease states such as hypertension and coronary artery disease ^{16,17,18,23,24}	Expensive to buy and maintain High genetic variability (although this reflects actual clinical situation, it is not desirable when trying to establish models) Few centers with model expertise

*For complete citations, see the reference list.

used and the biological characteristics that make this species appropriate and/or essential to the proposed study is required by the AIC, as well as an estimate of the number of animals that will be used. Explanation of why alternatives to the animal model, such as mathematical models, computer simulations, or in-vitro studies (cell culture) are not acceptable or are unsatisfactory, and the rationale for these conclusions should be outlined for the AIC. The AIC will request a copy of the detailed experimental protocol, with the surgical procedures described in detail. The AIC will also request a summary of the study in layman's terms, since not everyone on this review committee has a medical or research background. Some AICs require that a veterinarian be consulted prior to submission of the proposal to the AIC to evaluate those procedures that will involve pain or distress. The proposed anesthetics, analgesics, paralytics, antibiotics, or other drugs and the dosage, route, and frequency of administration should be described. Other methods used to reduce or lessen potential suffering should also be detailed. The projected or expected clinical conditions or abnormalities that will result from the experimental procedures must be outlined. Postoperative care must describe any condition that may cause pain and distress (e.g., from restraints or handling), and the proposed plan to alleviate these discomforts. A

specific delineation of handling procedures, drug dosing schedules, the length of postoperative care and monitoring, and assessment schedules will also be required. Investigators are required to verify that they have read and will comply with the provisions of the AIC Standard Operating Procedure Guidelines for animal care and record keeping.

If the conditions of the protocol subject the animal to pain or distress without anesthetic, significant justification is required. Alternative methods, and why these are not usable, should be detailed. The method of euthanasia and how death will be assured must be documented, and if the animal is not to be euthanized, its final disposition must be described. Survivor animals must be frequently examined after the experiment. Criteria for early euthanasia must be established for survivors that fare poorly and are unable to care for themselves after the experiment. The investigator's qualifications and experience in animal handling and care must be documented. The AIC will ask for identification of potential chemical, radiation, and biological hazards that staff, bystanders, and animals may be exposed to, and will require a completed Hazardous Agents Form. You will need to certify that you and coworkers assume responsibility for the work described in the proposal, and are qualified at handling and restraining animals and per-

forming the experimental techniques required. Those personnel responsible for animal husbandry and housing must be identified.

CONCLUSION

In all research, advance planning and proper preparation save significant time and resources, and ultimately yield greater productivity. Keys to this preparation include rereviewing the experimental design and analyzing the performance and reproducibility of preliminary studies. Prior to actual experimentation, your design should undergo a thorough peer-review process, either internally or externally.

When establishing an animal model, review the literature thoroughly, talk with other researchers to learn from their previous experiences, and note strengths and weaknesses of previous models. Consider the ethical questions involved in animal studies, and address all institutional requirements. Choose the animal model very carefully, considering all aspects of the project that may impact the outcomes to be measured. Refine the study design and verify the model using the proper positive, negative, and sham controls.

References

1. Rollin BE, Kesel ML (eds). *The Experimental Animal in Biomedical Research*. Boca Raton, FL: CRC Press, 1990.
2. Swindle MM. Swine as replacements for dogs in the surgical teaching and research laboratory. *Lab Anim Sci*. 1984; 34: 383-5.
3. Stanton HC, Mersman HJ. *Swine in Cardiovascular Research*. Boca Raton, FL: CRC Press, 1986.
4. Harmison LT. *Research Animals in Medicine*: National Heart and Lung Institute. Washington, DC: DHEW Publication No. (NIH) 72-33, 1972.
5. Mitruka BM, Rawnsley HM, Vadehra DV. *Animals for Medical Research: Models for the Study of Human Disease*. New York: John Wiley and Sons, 1976.
6. Gad SC, Chengelis CP. *Among Models in Toxicology*. New York: Marcel Dekker, 1992.
7. Farris EJ. *The Rat in Laboratory Investigation*. Philadelphia: J. B. Lippincott, 1949.
8. Swindle MM, Moody DC, Phillips LD (eds). *Swine as Models in Biomedical Research*. Ames, IA: Iowa State University Press, 1992.
9. de Garavilla L, Babbs CF, Tacker WA. An experimental circulatory arrest model in the rat to evaluate calcium antagonists in cerebral resuscitation. *Am J Emerg Med*. 1984; 2: 321-6.
10. von Planta I, Weil MH, von Planta M, et al. Cardiopulmonary resuscitation in the rat. *J Appl Physiol*. 1988; 65: 2641-7.
11. Mitruka BM, Rawnsley HM, Vadehra DV. *Animals for Medical Research: Models for the Study of Human Disease*. New York: John Wiley and Sons, 1976.
12. Bishop SP. Cardiovascular research. In: Baker HJ, Lindsey JR, Welsbroth SH (eds). *The Laboratory Rat*, Vol. II. San Diego, CA: Academic Press, 1980, pp 162-79.
13. Foster HL, Small JD, Fox JG (eds). *The Mouse in Biomedical Research*. New York: Academic Press, 1982.
14. Kaplan HM, Timmons EH. *The Rabbit. A Model for the Principles of Mammalian Physiology and Surgery*. New York: Academic Press, 1979.
15. Shifrine NM, Wilson ED (eds). *The Canine as a Biomedical Research Model: Immunological, Hematological and Oncological Aspects*. Washington, DC: Technical Information Center, U.S. Department of Energy, 1980.
16. Dukelow WR (ed). *Nonhuman Primate Models for Human Diseases*. Boca Raton, FL: CRC Press, 1983.
17. Yealy D. How much "significance" is significant? The transition from animal models to human trials in the resuscitation research. *Ann Emerg Med*. 1993; 22:11-6.
18. *Guide for the Care and Use of Laboratory Animals*. Bethesda, MD: HHS, NIH Publication No. 86-23, 1985.
19. Gross DR. *Animal Models in Cardiovascular Research*. Boston: Kluwer Academic Publishers, 1985.
20. Zicha J, Karen P, Krapata V, et al. Hemodynamics of conscious brattleboro rats. *Ann N Y Acad Sci*. 1982; 294:409-13.
21. Malik AB, Kaplan JE, Saba TM. Reference sample method of cardiac output and regional blood flow determinations in the rat. *J Appl Physiol*. 1976; 40:472-5.
22. Halperin HR, Tsitlik JE, Guerci AD, et al. Determinants of blood flow to vital organs during cardiopulmonary resuscitation in dogs. *Circulation*. 1986; 73:539-50.
23. Malinow MR. The role of nonhuman primates in research on atherosclerosis regression. *Artery*. 1981; 9:2-11.
24. Mahley RW. Development of accelerated atherosclerosis. Concepts derived from cell biology and animal model studies. *Prog Cardiovasc Dis*. 1983; 26:109-32.