ESSENTIALS FOR ANIMAL RESEARCH A PRIMER FOR RESEARCH PERSONNEL

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Second Edition

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INTRODUCTION

This manual was developed from the outlines of a course entitled Essentials for Animal Research, originally developed at the University of Illinois at Chicago for graduate students who wanted to learn more about the use of animals in research than generally covered in the training received in their chosen area of concentration. From its inception, the course has constantly evolved to remain current with ever-changing regulations and an increasing awareness by graduate students of the issues concerning the use of animals in biomedical research, teaching and testing. The course introduces those elements which have become essential requirements for using animals in research, teaching or testing programs. These requirements primarily center around the responsibilities one assumes when they intend to use animals in their work. The ultimate responsibility lies with the Principle Investigator who must have a working knowledge of the regulations, be familiar with the factors that affect the selection, acquisition and maintenance of experimental animals and be aware of the ethical and social issues involved with the use of animals in biomedical research.

The goals and objectives established for developing the class lectures are applicable to the material presented in this manual. With these goals in mind, the authors developed the ten chapters included in this manual. Remember it was not the authors' intentions to present an exhaustive treatise on key elements essential for conducting animal research in a manner which assures individual and institutional compliance with pertinent regulatory requirements, but rather an introduction to the subject matter in a manner which will hopefully encourage additional reading where appropriate.

In writing this manual it was the author's intent to provide the reader with:

An appreciation and basic understanding of the regulatory process and the means by which compliance can be assured. An overview of those factors which can affect the selection, acquisition and maintenance of animals used in biomedical research.

An understanding of the basic principles of controlling pain and distress, preventing intraoperative infection and assuring a humane death in the animals used.

An awareness of the responsibilities that one assumes when choosing to use laboratory animals. These responsibilities would include, but not be limited to, those which involve an obligation to the institution, regulatory and funding agencies, the public and the animals.

The manual has been organized into ten chapters, the first seven are intended to cover the specific objectives described above. The last three chapters contain resource information on the Animal Welfare Information Center of the National Agricultural Library, a list of organizations from which additional information can be obtained and a list of general

references covering topics of interest to the investigator who utilizes animals in research, teaching and testing programs.

The second edition of this manual has been updated to reflect recent changes in the regulations, the report of the 1993 AVMA Panel on Euthanasia and the expanded resources and services of the Animal Welfare Information Center.

The authors would like to thank Drs. James Harwell, Louis Ramazzotto and Richard Simmonds for their input and support during the review process of this project and Ms. Lisa Halliday and Ms. Doris Thomas for their assistance in preparing the second edition. To the staff of the AWIC, we send our appreciation for their enthusiastic support throughout the project and for their assistance in the final stages of transferring the electronic version of the manual to the library.

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Chapter 1
Regulations and Requirements

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INTRODUCTION

Since the ultimate responsibility for compliance with regulations that affect the care and use of animals lies with the investigator, it is important that he/she have a working knowledge of the basic regulatory requirements. In this manual, the types of regulations will be discussed under two broad general headings:

1. Involuntary

2. Voluntary

Involuntary regulations can be defined as those required by law or set forth as a condition of funding. There are four types of regulatory controls which can be considered as involuntary:

1. The Animal Welfare Act (AWA)

2. The Public Health Service Policy

3. The Good Laboratory Practices Act

4. The Requirements of Private Funding Agencies

Voluntary regulations can be defined as those that an individual or institution adheres to as part of their overall commitment to research and academic excellence. There are two types of regulatory controls which can be considered as voluntary:

1. Accreditation by the American Associa- tion for Accreditation of Laboratory Animal Care (AAALAC)

2. Requirements of Individual Users

INVOLUNTARY REGULATIONS

Animal Welfare Act

The Animal Welfare Act was first passed August 24, 1966, as PL-89-544. It was entitled the "Laboratory Animal Welfare Act" and authorized, "The Secretary of Agriculture to promulgate such rules and regulations, and orders as he may deem necessary to effectuate the purposes of this Act." The purposes of the original act were to:

1. Protect the owners of dogs and cats from theft of such pets.

2. Prevent the sale or use of dogs and cats which had been stolen.

3. Insure that certain animals intended for use in research facilities were provided humane care and treatment.

In charging the Secretary, Congress specifically prohibited the promulgation of rules, regulations, or orders which would interfere with the conduct of actual research. Determination of what constituted actual research was left to the discretion of the research facility.

The original Act covered non-human primates, guinea pigs, hamsters, rabbits, dogs and cats. Humane treatment was required while they were at the dealers or research facility and while being transported by dealers. Dealers were required to be licensed. Research facilities which used, or intended to use, dogs or cats and either purchased them in commerce or received any federal funds were required to be registered.

The Secretary also established regulations and standards for the implementation of unannounced facility inspections and for the maintenance of specific records by dealers and research institutions. Responsibility for administering the Act was delegated within the United States Department of Agriculture (USDA) to the Administrator of the Animal and Plant Health Inspection Service (APHIS). Enforcement duties are the responsibility of the APHIS Deputy Administrator for Regulatory Enforcement and Animal Care (REAC). The actual inspections are conducted by 46 Veterinary Medical Officers working under one of the four REAC Sector Supervisors. The Sector offices are located in Fort Worth, Texas, Tampa, Florida, Annapolis, Maryland, and Sacramento, California.

In 1970 the original Act was amended (PL-91-579) and renamed the Animal Welfare Act. The amended Act covered broader classes of animals and included those used in exhibitions and sold at auction and regulated anyone involved in these activities. The definition of an animal was expanded to include all warmblooded animals. The definition of a research facility was expanded to include those institutions using covered live animals and not just dogs and cats. These facilities were required to file an annual report. Civil penalties were also added for refusing to obey a valid cease and desist order from the Secretary. The term "handling" was added to the basic categories for which standards were to be created and the phrase "adequate veterinary care" was broadened to include the appropriate use of anesthetics, analgesics and tranquilizers.

The intent of the original Act to prohibit interference with research was clarified and the Secretary was enjoined from directly or indirectly interfering with, or harassing in any manner, research facilities during the conduct of actual research or experimentation. The determination of when actual research was being done was still left to the discretion of the research facility itself.

In 1976, the Animal Welfare Act was further amended to enlarge and redefine the regulation of animals during transportation and to combat the use of animals for fighting. Essentially the Act was broadened to include all forms of commercial transportation of animals and required all carriers and intermediate handlers who were not required to be licensed under the Act to register with the USDA. It also expanded the definition of a dealer and extended the record keeping requirements to carriers and intermediate handlers.

In 1976, the Secretary also promulgated regulations which specifically excluded rats, mice, birds, horses and farm animals from the definition of an animal. This exclusionary language effectively excludes over 80 percent of the animals currently used in research, teaching and testing from coverage under the Animal Welfare Act.

In 1985 the Act was further amended with the passage of the Food Security Act of 1985 (PL-99-198) which contained an amendment entitled the "Improved Standards for Laboratory Animals Act." This amendment strengthened the standards for providing laboratory animal care, increased enforcement of the Act, provided for collection and dissemination of information to reduce unintended duplication of experiments using animals and mandated training for those who handle animals.

The 1985 amendment to the AWA also included development of standards: for the "exercise of dogs," for "provision of a physical environment which promotes the psychological well-being of primates," for limitation of multiple survival surgeries, and to require the investigator to consult with a veterinarian in the design of experiments which have the potential for causing pain to insure the proper use of anesthetics, analgesics and tranquilizers. Each research facility has to show upon inspection, and include in their annual report, assurances that professionally acceptable standards for the care, treatment and use of animals are being used during the actual research or experimentation. As part of these standards, the investigator is required to consider alternative techniques to those which might cause pain or distress in the experimental animals.

The 1985 amendment required the Chief Executive Officer of each research facility to appoint an Institutional Animal Committee consisting of at least three members including a doctor of veterinary medicine and one member who is not affiliated with the institution. The regulations promulgated to implement the amendment designated this committee as the Institutional Animal Care and Use Committee (IACUC) and charged it to act as an agent of the research facility in assuring compliance with the Act. The Committee is required to inspect all animal facilities and study areas at least once every six months, and to review the condition of the animals and the practices involving pain to the animals to insure compliance with the regulations and standards promulgated under the Act. The Committee is also required to review once every six months the research facility's program to assure that the care and use of the animals conforms with the regulations and standards. The Committee must file a report of its inspection with the Institutional official of the research facility. If significant deficiencies or deviations are not corrected in accordance with the specific plan approved by the Committee, the USDA and any Federal funding agencies must be notified in writing.

The Committee must also review and approve all proposed activities involving the care and use of animals in research, testing or teaching procedures and all subsequent significant changes of ongoing activities. As part of this review, the Committee must evaluate procedures which minimize discomfort, distress and pain and that when an activity is likely to cause pain that a veterinarian has been consulted in planning for the administration of anesthetics, analgesics and tranquilizers and that paralytic agents are not employed except in the anesthetized animal. The IACUC must also determine that animals which experience severe or chronic pain are euthanatized consistent with the design of study, that the living conditions meet the species needs, that necessary medical care will be provided, that all procedures will be performed by

qualified individuals, that survival surgery will be performed aseptically and that no animal will undergo more than one operative procedure that is not justified and approved. Methods of euthanasia must be consistent with the definition contained in the regulations.

The IACUC must also assure on behalf of the research facility that the principal investigator considered alternatives to painful procedures and that the work being proposed does not unnecessarily duplicate previous experiments. To provide assurance of the former the Committee must review the written narrative description provided by the investigator. This description must include the methods and sources used in determining that alternatives were not available.

In reviewing proposed activities and modifications, the IACUC can grant exceptions to the regulations and standards, if they have been justified in writing by the principal investigator.

In addition to the above requirements, the research facility is required to provide training in the following areas to scientists, animal technicians and other personnel involved with animal care and treatment:

1. Humane practice of animal mainte- nance and experimentation.

2. Research or testing methods that mini- mize or eliminate the use of animals or limit pain or distress.

3. Utilization of the information service of the National Agricultural Library.

4. Methods whereby deficiencies in ani- mal care and treatment should be re- ported.

The regulations require that each research facility establish a program of adequate veterinary care that includes: appropriate facilities, personnel and equipment; methods to control, diagnose and treat diseases; daily observation and provision of care; guidance to personnel on the use of anesthetic, analgesic and euthanasia procedures and pre- and post-procedural care. Specific requirements for maintaining records and filing annual reports are included in the regulations along with a miscellaneous section containing a variety of requirements to which a research facility must adhere.

The most recent amendment to the AWA (PL 101-624) was passed in 1990 and was entitled the Pet Protection Act. The regulations developed to implement this amendment define the minimal holding period for animals in pounds and shelters that are sold to dealers, and establish record keeping requirements for dealers who obtain dogs or cats from these sources.

Public Health Service Policy

The Public Health Service Policy on Humane Care and Use of Laboratory Animals can be found in Chapter 4206 of the NIH Manual and Chapter 1-43 of the PHS Manual. The NIH originally initiated the Policy in 1971. It was extended to all PHS activities January 1, 1979, and was revised in the spring of 1985 with implementation to be effective January 1, 1986. With the passage of the Health Research Extension Act of 1985 (PL-99-158), the Policy was further

revised and the Director of the NIH was required by law to establish guidelines which heretofore had only been a matter of PHS policy. An additional revision was released in September 1986 which reflected the changes required by this Act.

Under the PHS policy, each institution using animals in PHS-sponsored projects must provide acceptable written assurance of its compliance with the Policy. In this Letter of Assurance the institutions must describe:

1. The Institutional Program for the Care and Use of Animals.

2. The Institutional Status.

3. The Institutional Animal Care and Use Committee (IACUC).

The Institutional Program must include a list of every branch and major component, the lines of authority for administering the program; the qualifications, authority and responsibility of the veterinarian(s), the membership of the Institutional Animal Care and Use Committee and the procedures which they follow must be stated. The employee health program must be described for those who have frequent animal contact. A training or instruction program in the humane practices of animal care and use must be available to scientists, animal technicians and other personnel involved in animal care, treatment and use. The gross square footage, average daily census and annual usage of each animal facility must be listed.

The Institutional Status must be stated as either Category one (1) (AAALAC accredited) or Category two (2) (nonaccredited). Institutions in Category two (2) must establish a reasonable plan with a specific timetable for correcting any departures from the recommendations in the *Guide for the Care and Use of Laboratory Animals* (86-23).

The IACUC must be appointed by the Chief Executive Officer and consist of at least five members; one of whom is a veterinarian with program responsibility, a practicing scientist, an individual whose expertise is in a non-biological science and an individual who is not affiliated with the institution. This Committee must use the *Guide* to review the animal facilities and the institutional program for humane care and use of animals at least once every six months and prepare reports of these evaluations for the responsible institutional official. The Committee must review and approve animal-related components of proposals and significant modifications made in ongoing activities involving the care and use of animals. The Committee is responsible for reviewing concerns involving the care and use of animals and making recommendations to the institutional official regarding any aspect of the animal program, the facilities, or the personnel training. They are also authorized to suspend activity involving the care and use of animals as set forth in the PHS Policy.

In reviewing the animal care and use component of a proposal, the IACUC must confirm that the project will be conducted in accordance with the AWA and consistent with the recommendations in the *Guide*. In addition, all procedures are reviewed to assure that pain or distress will be minimized and that (when necessary) appropriate anesthetics, analgesics and tranquilizers will be used. The living conditions and medical care available must be appropriate for the species used,

and personnel conducting the procedures must be appropriately trained and qualified. Methods of euthanasia should be consistent with the recommendations of the American Veterinary Medical Association Panel on Euthanasia.

The investigator is responsible for completing a proposal in accordance with recommendations in the PHS Policy and the instructions contained in the PHS 398 application packet. As of September 1991, the instructions for completing 398 can be found in two locations within the application package. On page 13 the research investigator's responsibilities for assuring compliance with the PHS Policy are clearly addressed. Detailed instructions for completing Section 6 of the Research Plan which describes the use of Vertebrate Animals can be found on page 23.

The institution is responsible for maintaining all the necessary records to document compliance with the PHS Policy and for filing annual reports developed by the IACUC which detail any changes in the program and indicate the dates of the semi-annual inspections and programmatic reviews.

The PHS Policy described above is intended to implement and supplement the "U.S. Government Principles for the Utilization and Care of Vertebrate Animals in Testing, Research and Training." The nine principles are published in the PHS Policy and in the Appendix of the *Guide*. All those responsible for the design, supervision and review of the animal care and use component of a proposal should be familiar with this document.

Good Laboratory Practices

In 1978 the Food and Drug Administration adopted the Good Laboratory Practices rules which applied to all regulated parties who conduct nonclinical safety assessment studies. The rules require the creation of Standard Operating Procedures for all aspects of the study including animal care and use. A Quality Assurance Unit must be established to conduct internal inspection of practices and records to insure compliance with established policies and procedures. In general the recommendations contained in the *Guide* would suffice in terms of animal care when adherence is properly documented.

Private Funding Agencies

In recent years the requirements of many private funding agencies which fund research projects involving the care and use of laboratory animals have changed. It is important to obtain the requirements from the agency before spending time preparing a proposal. Some of these agencies not only require review of the proposal by the IACUC, but require proof of accreditation by AAALAC. In many instances, the proposals must be reviewed and approved prior to submission.

VOLUNTARY

American Association for Accreditation of Laboratory Animal Care (AAALAC)

AAALAC was originally chartered April 30, 1965, as a voluntary organization that accredited institutional programs of animal care and use. AAALAC is governed by a Board of Trustees composed of representatives of 39 professional organizations. An 18-member Board-appointed Council on Accreditation along with four scientific/technical panelist make recommendations based on the results of site visits to evaluate an institution's compliance with the recommendations contained in the *Guide*. This is a peer review process in which standards are being continually upgraded to reflect current knowledge in laboratory animal medicine and science. In its accreditation program the AAALAC Council uses the *Guide* more as a compilation of regulatory "standards" and not as a set of "recommendations."

Since the AAALAC accreditation program and the *Guide* are so closely linked, a brief review of the *Guide's* history and its current contents are warranted. In 1963 the first *Guide for Laboratory Animal Facilities and Care* was published by the Institute for Laboratory Animal Resources (ILAR) under a contract from NIH. Since its original release the *Guide* has been revised in 1965, 1968, 1972 (when the title was changed to the *Guide for the Care and Use of Laboratory Animals*) 1978 and 1985. In the most recent revision, the organization of the chapters was changed to reflect the increasing role and responsibility of the institutional program in establishing acceptable standards for the care and use of laboratory animals. The first chapter is now Institutional Policies. The remaining four chapters are Laboratory Animal Husbandry, Veterinary Care, Physical Plant and Special Considerations. Prior to an AAALAC site visit, each institution is required to prepare a description of the institutional facilities and programs using the AAALAC Outline for Description of The Institutional Animal Care and Use Program, which follows the *Guide's* chapter headings.

Once accredited, an institution must submit an annual report describing changes in the program and facilities and documenting the annual usage of animals. Site visits occur at least every three years and these visits consist of an inspection and review of policies, procedures and facilities which comprise the animal care and use program inclusive of selected animal usage areas. Should deficiencies be identified in a previously accredited program, the institution is either granted a definied period in which to make specified changes, or if the deficiencies are major, accreditation could be withdrawn.

Individual Users

The instructions for completing PHS 398 clearly define the roles and responsibilities of the investigator in assuring proper care and usage of laboratory animals. In addition to this requirement, it should be understood that any type of care or use of an animal which results in the creation of nonexperimental variables can potentially compromise the integrity of an entire project. As part of their commitment to scientific excellence, the users should provide the impetus for setting and maintaining high standards for the care and use of laboratory animals within their individual and collective institutions. Failure to do so invites increased internal and external regulatory requirements which can drain limited institutional research resources. Good animal care is good science; the practice of good science should be the primary goal of all who have chosen careers in the scientific community.

SUMMARY

In summary, the regulations that affect the use of animals in research, teaching and testing programs are numerous. A working knowledge of the applicable regulations is necessary if the principal investigator is to insure that proposals for funding contain the necessary information and to assure that the conduct of all research proposals is in compliance with the requirements of the regulatory and funding agencies. While the ultimate responsibility for compliance rests with the principal investigator, institutional policies should be designed to provide those responsible for compliance with the necessary resources to do so.

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Chapter 2

Alternative Methodologies

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INTRODUCTION

In the regulations promulgated to implement the Animal Welfare Act as amended in 1985, the research facility must provide assurances that the principal investigators considered alternatives techniques to painful procedures and provide guidance concerning research and testing methods that limit the use of animals or minimize the animals' distress. In this chapter the reader will be introduced to the classical concept of alternatives with a brief discussion of each major category including a limited number of examples. For more indepth coverage of the subject, the reader is encouraged to obtain the latest bibliography on alternative techniques available from the Animal Welfare Information Center of the National Agricultural Library (see Chapter 8).

In recent years the term **alternative techniques** has come into common usage in the current controversy involving the use of animals in research, teaching and testing. It is a term that has different meanings to different people and this difference largely depends on which side of the issue one is found. To many biomedical researchers, alternative techniques refer to those which can be used in addition to the more traditional animal models. These techniques can focus on specific biological functions and in many cases reduce the numbers of animals used. Therefore these methods are an adjunct to the more commonly used animal models. To the so-called abolitionist who seeks the immediate end to all animal research, teaching and testing, the term **alternative** refers to those techniques which can entirely replace the use of animals. The dictionary, defines **alternative** as: "offering or expressing a choice." The dictionary also defines **technique** as "a method of accomplishing a desired aim." By combining these definitions, the term **alternative technique** becomes "one which offers a choice in accomplishing a desired aim."

In designing an experiment which involves the use of animals to confirm or refute a theory, one should consider all the possible techniques that could be used to gather the necessary data. From this review, choose the method which offers the best chance of generating the necessary information in the most economical manner. Economy, in this context, refers to time, actual cost and the number of animals used. By considering the choices that are available for accomplishing the desired aim of the experiment and choosing the one that offers the best chance for success, one has met the requirements of this literal definition of alternative techniques.

Since a literal definition provides a rather simplistic approach to dealing with our responsibility for reducing the potential pain and suffering of animals that must be used, it is necessary to develop a working definition of the term. In Dr. Rowan's book, *Of Mice, Models & Men*, he defines the term alternatives to refer to those techniques or methods that "replace the use of laboratory animals altogether, reduce the numbers of animals required, or refine an existing procedure or technique so as to minimize the level of stress endured by the animal." Since stress can be difficult to describe and quantitate, for the purpose of this manual it will be replaced by

the term **distress**. The working definition of alternative techniques thus evolves to "those techniques which replace the actual use of animals, reduce the numbers used, and/or refine the techniques to minimize the potential for the animal to experience pain or distress."

This concept of the 3 R's is not new. It first appeared in a book by Russell and Burch published in 1959 entitled *The Principles of Humane Experimental Technique*. In the original work, the authors defined the 3 R's as follows:

"Replacement means the substitution for conscious living higher animals of insentient material. Reduction means reduction in the numbers of animals used to obtain information of given amount and precision. Refinement means any decrease in the incidence or severity of in-humane procedures applied to those animals which still have to be used."

In this text the authors included nonrecovery techniques in anesthetized animals, as well as tissue culture, as replacement methods. Reduction included statistical techniques which were designed to reduce the actual numbers needed in the study. The use of better animals was also encouraged as a means of reducing actual numbers used. Refinement referred to techniques that reduced the potential for pain and distress. This approach still holds today. It is the principles of Replacement, Reduction and Refinement that will be covered in this chapter. To attempt to address these issues for all the uses of animals that fall under the general rubric of research, teaching and testing is far beyond the scope of this manual. Therefore the comments that follow will address only broad issues with some specific examples for the purpose of clarification.

Prior to discussing the replacement of animals with non-animal models, the word **animal** must be defined. On the surface this appears an easy task. Common sense would tell us that an animal is one of the two major kingdoms of living organisms. The dictionary defines an animal as "any of a kingdom of living beings typically differing from plants in capacity for spontaneous movement and rapid motor response to stimulation." In the Definition of Terms promulgated to implement the amended Animal Welfare Act an animal is defined as:

"any live or dead dog, cat, nonhuman primate, guinea pig, hamster, rabbit, or any other warm blooded animal, which is being used or is intended for use for research, testing, experimentation, or exhibition purposes or as a pet. This term excludes: Birds, rats of the genus Rattus and mice of genus Mus bred for use in research, and horses and other farm animals such as but not limited to livestock or poultry used or intended for use as food or fiber, or livestock, or poultry used or intended for use for improving animal nutrition, breeding, management, or production efficiency, or for improving the quality of food and fiber."

The PHS Policy defines an animal as "Any live, vertebrate animal used or intended for use in research, research training, experimentation, or biological testing or for related purposes." On the other hand the *Guide* defines an animal as "any warm blooded vertebrate animal." For the purposes of this manual, and to be consistent with most approaches to discussing alternative techniques, an animal will be any living vertebrate, with the caveat that any model system which moves down the phylogenetic scale from the generally acceptable animal model will be considered an alternative.

REPLACEMENT

Alternatives which replace animal models can be classified into the following broad general categories:

Use of Living Systems

Use of Nonliving Systems

Use of Computer Simulation

Use of Living Systems

In Vitro Techniques - The most commonly recognized nonanimal living systems are those which fall into the broad category of in vitro methods such as organ, tissue and cell culture. These techniques afford the investigator the greatest control of the "test subject's" environment. Since these systems will not work when the incorrect combination of atmosphere, humidity, temperature, pH and nutrients are provided, they tend to minimize the effects that nonexperimental variables can have on the final outcome of a study. Generally, when suboptimal environments are provided for an in vitro system, the problem becomes one of loss of all experimental results and not just the production of compromised results. The most commonly used of the in vitro methods are cell culture techniques for monoclonal antibody production, virus vaccine production, vaccine potency testing, screening for the cytopathic effects of various compounds and studying the function and make up of cell membranes. The potential uses of in vitro techniques are almost limitless and will continue to expand as more is learned about the various organs and their component tissues and cells, and as the technology of maintaining in vitro environments improves.

Invertebrate Animals - Invertebrates are another type of living system which can be used to replace the more commonly used laboratory animals. Over 90 percent of the animal species thus far identified are invertebrates. An invertebrate which has long been used in biomedical research is the fruit fly, *Drosophila melanogaster* -- a classic model for the study of genetics. This species also can be used for detecting mutagenicity, teratogenicity and reproductive toxicity. The marine invertebrates represent different species which have not been widely investigated. However in neurobiology a number of different marine species have been well characterized and used to study the physiology of the nervous system.

Micro-Organisms - The micro-organisms represent a third system which has been used to replace traditional animal models. The Ames mutagenicity/carcinogenicity test uses Salmonella typhimurium cultures to screen compounds that formerly required the use of animals. Such systems allow for an almost limitless number of compounds to be tested which can create an interesting dilemma. The more compounds that can undergo screening, the more compounds that will be potentially available to test in animals. Alternative techniques can replace the number of animals at a given step in the screening process. However, use of alternatives may increase the number of compounds that must be finally tested in intact animals.

Plants - Plants offer another alternative living system which can be used to replace animals in studies of basic molecular mechanisms. There is very little morphological and functional difference between the organelles isolated from plants and those isolated from animals. The rigid cell wall of plants, however limits their applicability for use as undisrupted cells.

Use of Nonliving Systems

Chemical Techniques - The most widely used nonliving model system involves the use of modern chemical techniques. This is particularly true of the analytical techniques which can be used to identify substances and to determine their concentration or potency. Immunochemical techniques use the binding capacity of highly specific antibodies to seek out minute quantities of antigen. A classical example of this technique can be demonstrated by the currently used techniques for identifying bacterial toxins. Toxin identification previously required the injection of as many as several hundred mice with supernatant from cultures of suspected contaminating bacteria.

These new antibody techniques save animals and speed up confirmation of a tentative diagnosis. By adding a color marker to the Enzyme Linked Immunosorbent Assay system (ELISA), the whole process becomes a commercially available test kit such as those used in home pregnancy detection. A test that previously required the use of a rabbit now can be performed using an over-the-counter test kit. There are a variety of chemical techniques that can be used to determine the presence of a particular chemical reaction or the presence of an enzyme necessary for a specific reaction. At the most basic level, the identification of a particular chemical structure in a compound can provide a great deal of insight into the potential reactivity and thus the resulting toxicity of a given substance.

Physical and/or Mechanical Systems - The use of physical and/or mechanical systems to replace living animals of even the highest order has application in teaching specific skills and/or reactions to a well defined set of predetermined circumstances. The use of computer-linked mannequins in teaching basic principles of medicine and applied techniques can be best illustrated by the mannequins used to train people in cardiopulmonary resuscitation.

Historical data can be used for analyses in a variety of databases commonly used in the field of epidemiology. However, while the body of potentially useful information that already exists in a variety of sources is immense, it may not always be in a format which permits ready accessibility for evaluation. For this reason, retrospective epidemiological studies are often the subject of fairly heated debates. Yet with the increasing access to historical data available on existing computer programs, this problem may to a large extent be overcome in the future.

Use of Computer Simulation

The standout in the alternative techniques controversy is the claim made for computer simulation as a means of virtually replacing the use of living animals. In order for a biological phenomena to be adapted to a computer model, the basic processes must be expressed in a mathematical formula. Once a formula is developed then an enormous number of variables can be introduced and swiftly processed. The key element for success is the generation of a program from the

mathematical formula. The more complete the formula, the more useful the program. The problem is that many of the questions being asked of an animal model are not defined well enough to develop the necessary mathematical model. As the core knowledge of the biological processes expands so will the opportunities to use computer simulation to replace the number of live animals being used.

REDUCTION

In discussing the ways to reduce the numbers of animals used, the definition of an animal and the principle of moving down the phylogentic scale must also be kept in mind. The four broad categories for reducing the number of animals used are:

Animal Sharing

Improved Statistical Design

Phylogenetic Reduction

Better Quality Animals

Animal Sharing

Sharing of animals can significantly reduce the number of animals used within a given institution. Between institutions, sharing is more difficult, but can be effective as demonstrated with the Primate Supply Information Clearinghouse, Regional Primate Research Center (SJ-50) University of Washington, Seattle, WA 98195. This service has reduced the total number of primates used by helping to optimize the usage of those already in facilities throughout the country.

Sharing can be as simple as allowing someone to practice a surgical approach on an animal that has been, or is to be euthanatized for other purposes, or providing organs or tissues at the time of necropsy. Sharing becomes more complicated when attempting to maximize the use of control animals, but it can significantly reduce the number used within an institution. If two studies involve the need to perform a sham operation, the administration of compounds by identical routes, the use of standard control diets or the need to condition animals to a particular environment, control animals could be shared within the institution. Animal sharing would require some form of centralized clearing process within the Institutional Animal Care Program to match the needs of the various investigators and their studies.

Improved Statistical Design

Anyone who has ever taken a course in experimental design or applied statistics has been bombarded with the importance of consulting with the statistician during the design phase of the experiment and not when the data collected needs to be analyzed. Improper design of experimental protocols and/or the failure to use appropriate statistical methods can result in the usage of an inappropriate number of experimental animals. A variety of design strategies are

available which can reduce the number of animals needed in a given study. Experimental protocols which utilize serial sacrifice, group sequential testing and crossover designs can significantly reduce the numbers of animals required.

The availability of low cost statistical packages for almost every computer on the market permits more and more investigators access to sophisticated data management and analysis. This accessibility makes possible the use of design criteria and complicated statistical analysis which heretofore have been largely confined to institutions with large statistical support units. With this ability at their finger tips, investigators should be able to maximize the analysis of the data generated from each animal used, thus reducing the total numbers of animals necessary for a particular set of data.

Phylogenetic Reduction

Projects which can be designed to use one of the myriad of invertebrate species instead of a non-human primate species represent a type of phylogenetic reduction which was discussed as a replacement technique. Such broad jumps across the phylogenetic scale are not always possible, but less dramatic shifts can significantly reduce the numbers of higher species being used in research, teaching and testing. In many instances, the theory of phylogentic reduction has been blurred by a species's use as a companion animal with little regard for phylogenetic ranking. The animals chosen for project usage should be the least advanced from a phylogenetic standpoint that will provide the necessary data.

The principle of phylogenetic reduction is generally well accepted as a way to reduce the number of animals used, but it often brings many hidden difficulties. As one descends the phylogenetic scale, the available information on the maintenance and use of these animals in a biomedical setting often becomes difficult, if not impossible, to obtain. When choosing a study model, it is critical that the principal investigator take into account the ability of the institution to provide appropriate care for the species chosen. Phylogenetic reduction is an important means of decreasing the number of animals used, but should be practiced carefully and with the full knowledge of the requirements of the species chosen.

Better Quality Animals

It is a rare study in which the initial cost of the animals to be used represents the single most expensive aspect of the study. For this reason it can often be false economy to select the source of the animal based on cost alone. When purchasing laboratory animals, it is important to keep in mind that cost and quality are usually directly correlated. By choosing the best quality animal in terms of health status, the possibility that animals will be lost or data compromised by the intrusion of a concurrent disease condition is minimized, if not eliminated. Choosing the best quality animals, in terms of genetic status, will virtually insure the consistency of animals from study to study. This requires an institutional commitment to the use of animals of defined health status and limits the investigators to the animal sources approved by the institution. Mixing of animals of different health status is a disaster waiting to happen and may negate all the benefits derived from the use of quality animals.

The role of the investigator and staff in assuring the integrity of an animal colony cannot be overemphasized. In choosing a source of animals, a veterinarian should be consulted to insure that the best animals that can be effectively maintained in the institution are purchased. Animals of different or unknown health status should never share the same environment nor common equipment in the animal facility or in the research laboratory.

REFINEMENT

Refinement refers to techniques which reduce the pain and distress to which an animal is subjected. For the purpose of this manual these techniques can be classified into the following broad categories:

Decreased Invasiveness

Improved Instrumentation

Improved Control of Pain

Improved Control of Techniques

Decreased Invasiveness

A hallmark of most of the new diagnostic and therapeutic techniques used in human medicine is the minimal degree of invasiveness that is required to successfully perform a procedure to obtain a given set of data. In many instances these techniques are applicable in the research environment and can be adopted for use in animals. A sophisticated example could be the use of Magnetic Resonance Imaging for results that formerly required euthanasia of multiple animals along a time curve to obtain assay tissue. Today one animal can provide all the information along a given curve. A less dramatic example is the vascular access device which permits repeated samples or injections in a single animal instead of using several animals. Invasiveness reduction methods are available in almost every area of biomedical research, and in project design, it is important to identify and use these methods wherever possible. Not only do they represent an alternative technique, but they generally provide much more consistent and reproducible data.

Improved Instrumentation

Monitoring Animals - In this age of microelectronics, fiber optics and laser instrumentation, the potential for refining techniques used in animal experimentation seems almost limitless. Improved instrumentation can minimize animal distress by reducing the level of restraint and/or manipulation necessary to obtain biological samples. Included in this category are the use of tethers in a variety of species to allow continuous access to the various organ systems, while permitting the animal virtually unrestricted movement within its primary enclosure. The advantages of these systems are numerous, not the least of which is minimizing a variety of nonexperimental variables associated with prolonged restraint.

Analyzing Samples - Once obtained, samples can be analyzed in very small volumes for a multitude of parameters. Examples of this can be found in the commercially available diagnostic laboratory equipment which require only microliter blood samples to perform a variety of diagnostic tests. The use of smaller sample sizes permits the use of smaller animal species and prevents the need to euthanatize many of these species to obtain the necessary volume of blood. It is now possible to obtain serial blood samples from small laboratory rodents which reduces the number of animals necessary to obtain data over the length of the study.

Improved Control of Pain

The Animal Welfare Act requires "that the principal investigator consider alternatives to any procedure likely to produce pain or distress in an experimental animal" and in any practice which could cause pain to animals that a doctor of veterinary medicine is consulted in the planning of such procedures for the use of tranquilizers, analgesics and anesthetics. Since appropriate anesthetic and analgesic agents can minimize the potential pain and distress experienced by animals, an entire chapter of this manual is devoted to the principles of using these agents. Suffice it to say, that of all the possible ways that the 3 R's can be utilized this is an area where the laboratory animal veterinarian can often be of most help to the investigator.

Improved Control of Techniques

Proficiency in the handling and restraint of animals makes it easier to perform a variety of routine procedures with minimal or no pain or distress to the animals involved. Animals are creatures of habit and when proper handling is part of their regular routine, the degree of distress caused by the procedures is minimized. Animals can be trained or conditioned to accept a variety of procedures which if suddenly forced upon them can be distressful. Almost every animal commonly used in the laboratory responds positively to a little tender loving care. It's inexpensive, readily portable, safe even at the highest doses and spreads rapidly through the staff. To develop the proper techniques and gain confidence in their use requires training by someone with appropriate experience. This can be the veterinarian, a member of the animal care staff or a fellow investigator. Whomever it may be should be sought out before a new species or technique is incorporated into the study. This will reduce the potential distress of all animals involved in the study up to and including the principal investigator.

SUMMARY

In this chapter, the use of **alternative techniques** has been defined in terms of the present regulatory requirements and the principles of Replacement, Reduction and Refinement were introduced. In summary, the reader should consider a fourth R--Responsibility. The use of animals in teaching and research brings with it a responsibility to minimize animal pain and distress. The adoption of the 3 Rs as part of the process of planning and conducting projects using laboratory animals will go a long way toward implementing Responsibility--the fourth R.

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Chapter 3

Animal Care and Use: A Nonexperimental Variable

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INTRODUCTION

The response of a laboratory animal to an experimental variable is influenced by a variety of genetic and environmental factors. An understanding of these factors is necessary to control their affects and minimize the potential influence of nonexperimental variability on the final outcome of a given experimental protocol. Minimizing nonexperimental variability can optimize the use of animals in a given study.

Since the 1930's, the concept of genetic makeup, or genotype of an animal, combining with the developmental environment to produce the phenotypic expression of the animal had been well accepted. A useful concept concerning the relationship of genetic and environmental factors--dramatype--was proposed by Russell and Burch in 1959. They defined dramatype to be the pattern of performance in a single physiological response of short duration relative to the animal's life time. It is determined by phenotype and the immediate environment in which the response is elicited. This concept distinguishes between the developmental environment, which directly interacts with genetic factors, and the proximate or immediate environment, which acts upon the combined system. Simplified, genotype plus developmental environment equals phenotype and phenotype plus the immediate environment equals dramatype. This concept stresses the interrelation of the genetic background of the animal, the environment in which it is raised and housed and the laboratory environment in which the animal is used or tested.

Genotype may be controlled through the use of genetically defined animals produced in structured breeding systems or by genetic engineering. This is easiest to accomplish through the purchase of genetically defined animals from reputable suppliers. In-house breeding programs are difficult and time consuming to maintain in a manner which assures genotypic integrity. If such colonies must be used, it is advisable to consult a geneticist to design a breeding program that produces animals of defined genetic characteristics. A genetic monitoring program might also be required to define the genetic makeup of the animals produced. This can be an expensive proposition and requires some expertise to perform. The phenotype can be influenced by regulating environmental conditions in which the animals are reared. For uniform dramatype, the environmental conditions in which the animals are tested must be controlled.

This chapter will deal with three broad categories of nonexperimental variables: physical factors, chemical factors and microbial factors. Physical factors which will be discussed include: cage design and construction, temperature, humidity, ventilation, light intensity and photoperiodicity, noise, bedding, watering systems, feeding, housing systems, shipping and handling. Chemical factors to be discussed will include contaminants of food, water, bedding, and air. Microbial factors will be discussed in terms of some of the common viral, bacterial and parasitic diseases that can affect laboratory animals. The total of all of the components included in these three

broad categories combines with the animal's genetic background to constitute Russell and Burch's concept of phenotype and dramatype. It is important to appreciate that our knowlege of the effects of nonexperimental variables is rapidly expanding and the purpose of this chapter is to introduce the reader to this subject rather than present an exhaustive or complete treatise.

PHYSICAL FACTORS

The physical environment of laboratory animals may be considered to consist of the animal room, or macroenvironment, and the primary enclosure (cage), or microenvironment. Cage design and composition influence the interaction between micro and macroenvironment. Therefore the temperature, humidity, airflow, concentration of waste gases, illumination and noise levels within the cage may be quite different from that monitored at the room level. Each of these factors represents an important nonexperimental variable that will be discussed in more detail.

Cage Design

Cage design and construction material can influence the study results. Galvanized caging material or rubber bottle stoppers can serve as a source of trace minerals which could affect the results of studies where the level of these compounds is being controlled. Other important considerations include whether contact bedding can be used or if animals must be housed on a wire floor. The type of sample collection may require the use of a metabolic cage, or observation studies may require the use of clear rather than opaque caging. The behavioral characteristics of the animal will also dictate the type of cage design used. For example, some animals require perches, nesting boxes or hiding places, and others require built-in restraint devices such as the squeeze mechanisms often found in primate caging. Reproductive needs may require specific caging features. In some species the male must have a method of escape from an overaggressive female. Many neonates have inadequate homeothermic mechanisms and will become hypothermic if not protected by contact bedding or nesting material placed in the cages.

Temperature and Humidity

The temperature and humidity in the animal room (macroenvironment) should be monitored and maintained within published acceptable limits. The temperature and humidity in the microenvironment is more difficult to monitor and control. Variations in temperature and humidity are influenced by such factors as filter tops, hanging wire or solid bottom caging, population density, animal activity level, cage location, and temperature and humidity in the animal room itself. Variations in temperature and humidity can have a variety of effects. For example, exposure to high temperatures will frequently cause rabbits to lick their fur which can predispose them to the formation of hairballs. Very low humidity has been associated with a rodent lesion called ring tail which is characterized by annular constrictions and can result in loss of the tail. More subtle temperature and humidity effects include: altered drug metabolism, increased disease susceptibility and decreased reproductive efficiency. These examples serve to illustrate the need for controlled temperature and humidity in the animals' micro and macroenvironment and the vital role it plays in the generation of consistent, reliable data.

Ventilation

Ventilation in animal rooms can have significant impact on the health status of the occupants. Excessive odor is often the first indication of a ventilation problem in an animal room; however, the concentration of waste gases at the cage level is usually higher than those detected at the room level. Furthermore, the concentrations capable of causing pathology are much less than human sensory threshold levels. Many design features affect room ventilation including the location, number, and configuration of supply and exhaust ducts. Cage-level ventilation is further affected by the presence and/or type of filter top on the cage as well as the design and location of the cage relative to the room airflow pattern. Ventilation should be such that it keeps the concentration of waste gases to a minimum, reduces the spread of disease, provides a stable temperature and humidity and avoids drafts.

Lighting

Light intensity and photoperiodicity in animal rooms can affect reproductive function and animal vision. The recommendation of the *Guide* for light intensity in animal rooms is 75-125 footcandles (fc). However, prolonged exposure to such levels can cause irreversible retinal degeneration in albino rodents and 25 fc has been suggested as a more appropriate intensity for these species. Variable light intensity control devices such as dimmer switches or multiple bank lighting can be installed to facilitate adequate light for observation and husbandry yet provide lower intensity light for general animal housing. Cage position on a rack can be an important factor and an 80-fold difference in light intensity can exist between the upper and lower shelf locations. Photoperiods or light/dark cycles (usually given in hours as L:D) can modify reproductive behavior and circadian rhythms. A daily light cycle which has 12 to 14 hours of light is usually recommended for most species. It is important to keep the light intensity and periodicity constant. Animal rooms should be equipped with automatic light timers. The presence of windows, either to the outside or to the corridor, can affect reproduction in some animals. Corridor windows may be desirable for observational purposes, but they can provide enough light to affect circadian rhythms in nocturnal animals. As with all environmental factors, the special characteristics of the animal should be taken into consideration when planning light cycles. Duration and type of light can affect estrus behavior. Animals can have their reproductive cycles manipulated by changing the light cycle. This technique has been used in several rodent species, cats, and farm animals. Reversed light cycles can be used to accommodate circadian rhythm, sleep and breeding studies within the normal working hours in an institution. Individual room timers provide a facility with more flexibility to meet a variety of experimental requirements.

Noise

Excessive noise can also disrupt animal breeding behavior. Noise at excessive levels can cause mechanical damage to the auditory system in both animals and man. Some effects of noise in animals include audiogenic seizures, eosinophilia, increased serum cholesterol levels and increased adrenal weights. It is recommended that noise levels in animal facilities not exceed 85 decibels (db).

Caging Accessories

In addition to the microenviron- mental effects of the physical configuration of the primary enclosure as discussed above, other aspects of the cage environment should be considered. The presence or absence of bedding material is dependent on the species and situation. For example, many breeding programs utilize some form of bedding to improve neonatal survival. An ideal bedding material should be dustfree, nonpalatable, absorbent, and free of microbial and toxic contaminants. The choice of watering system depends on species, experimental design, and management factors. Automatic watering systems are expensive to install but can pay for themselves in labor savings over time. Automatic watering systems should be flushed daily when used with low flow rates, such as in rodent rooms, to avoid stagnation and minimize bacterial buildup. When the study protocol requires delivery of a compound in the water, or measurement of daily intake is needed, water bottles or pans are often used. Choice of feeder and type of food is also species and situation dependent. Some species such as the hamster are frequently fed on the floor of the cage because their broad muzzle can make obtaining food from some rodent feeders difficult. Some species such as rabbits do not readily tolerate sudden changes in diet composition or formulation. When designing a study, it is important to consult someone knowledgeable in the biology and husbandry requirements of the species to be used, so that wherever possible, species variations are taken into consideration.

Cage Size - Occupancy Standards

Consideration should also be given to the cage size. There are specific cage size requirements set forth in the *Guide for the Care and Use of Laboratory Animals* and by the Animal Welfare Act. Cage size requirements depend upon the species, weight or size of the animal(s), number of animals in the cage and breeding status. In addition to the floor space requirements the behavioral characteristics of the species, strain, and sex must be considered when group-housing animals. For very social animals, individual housing may cause stress. Even among social animals, the formation of new groups can result in fatal trauma from fighting. Male mice will often fight when group housed, whereas male rats usually do not. Aggressive behavior can be strain specific; for example, F344 male rats and C57BL mice are generally considered to be more aggressive than other commonly used strains. Even in docile animals, overcrowding can lead to fighting, cannibalism and stress. Breeding activity can be significantly modified by group housing arrangements. For example, group-housing female mice can lead to anestrus with subsequent estrus synchronization with the introduction of a male mouse.

Shipping

The effect of shipping animals can be a significant physiological stress. Studies have documented the that prolonged transport, high ambient temperatures, lack of water and the potential for microbial contamination may have on the research data collected from animals exposed to such factors. The provision of climate-controlled transport vehicles and filtered crates decreases these stresses. Even under optimal shipping conditions, it has been shown that it takes 1-5 days for the immune system and body weights to return to normal. It is also important to remember that changes in feed, water, and housing conditions can markedly affect newly arrived animals. Animals should be given an adequate period of time to equilibrate after transport.

Handling

The frequency and type of handling an animal receives is another nonexperi- mental variable. Investigators and technicians should be familiar with and skilled in the correct techniques for handling and restraining the species involved. This can prevent injury to either the animal or the handler. Daily husbandry routines may need to be scheduled around the research needs. Close communication between the investigator and the animal care staff can minimize handling stress. For example, collection of biological samples may be performed during routine cage changing. This is particularly useful when chemical restraint is required for either function. Since many animals are creatures of habit, regular handling may reduce stress.

CHEMICALS

Chemicals found in the animal's environment may be inherently toxic or their metabolism may result in the formation of toxic products. They may directly injure cells or interfere with cellular homeostasis. The possible effect of a chemical depends on the concentration, the agent's physiochemical properties, as well as the duration, frequency and route of exposure and potential interactions. These chemicals can influence various body systems. For example, it has been demonstrated that chemicals can affect hepatic microsomal enzymes which have many functions, including the biotransformation of drugs and chemicals and regulation of oxygen radical removal. Such chemical sources include: softwood bedding, room deodorizers, insecticides, and ammonia. Chemicals can also target the immune system. Some insecticides cause lymphopenia. Heavy metals can decrease resistance to disease by the reduction of antibody formation, altered phagocytic capacity of polymorphonuclear cells and macrophages, and suppression of interferon production.

Food and Water

Food and water can serve as sources for chemical contamination of research animals. Drinking water may be contaminated with synthetic organic solutes such as pesticides. Trihalomethanes are often found in water supplies as a result of the chlorination process. Some facilities hyperchlorinate or acidify water to decrease microbial contamination; however, these techniques can affect the immune response. Inorganic contaminants may include heavy metals and nitrites. Diets can also be a source of contaminants such as estrogenic compounds, aflatoxins, insecticides, and preservatives. These compounds may occur naturally in plant materials, remain as residues from agricultural use, or be the result of contamination in storage or the processing procedures. Commercial diets assayed prior to shipment are available and the results of this assay are printed on the tag attached to each bag.

Drugs

Drug therapy, prior to or during a study, can compromise the data obtained. For example, tetracycline alters the immune cell function through its ability to depress chemotaxis and phagocytosis. Aminoglycosides can have neuromuscular blocking properties, and can have negative inotropic effects on cardiac and arterial muscle. Other agents having neuromuscular depressant activity include tetracycline, lincomycin, and the polymyxins. It is important that

investigators and the animal care staff communicate about the effect that any medications may have on study animals prior to the initiation of treatment. Similarly, anthelmintics or insecticides given by the animal care staff to treat parasitism problems, could affect research results and must be considered in protocol design.

Anesthetic agents are frequently part of experimental protocols. The researcher should balance appropriate levels of analgesia, anesthesia, and chemical restraint with the possible effects of these agents on the experimental results. For example, the dissociative agent ketamine hydrochloride is widely used in anesthesia and restraint because it is easy to administer, is effective in a wide range of species and has a wide margin of safety. Besides the better known cardiovascular effects of ketamine hydrochloride, this drug also has been shown to affect intracellular cyclic AMP, cellular permeability and calcium channels. A pharmacologic knowledge of these drugs will aid in selecting those best suited for each experimental protocol and allow for more informed interpretation of results. Consultation with the institutional veterinarian regarding the use of anesthetics and analgesics during the planning of potentially painful procedures is now a legal requirement.

MICROBIAL FACTORS

Pathogenic microbial agents can affect research by causing clinical disease, lesions and death. However, in laboratory animals, infection more frequently is asymptomatic with carriers who develop overt disease when stressed by shipping or experimental manipulation. Animals with latent infection may show no overt disease but research results may be compromised through subtle physiological, biochemical or histological changes.

Bacterial Diseases

Species-specific mycoplasmal and bacterial diseases are well documented. There are a number of these pathogens associated with commonly used laboratory animal species. For example, mycoplasmosis is an endemic disease in some conventional rodent colonies. It can cause respiratory and genital tract infections thereby affecting exercise tolerance, sensitivity to anesthetic agents, increased susceptibility to other respiratory pathogens, decreased reproductive efficiency and a variety of immune system anomalies. The investigator using rabbits should be aware of the incidence and significance of pasteurellosis as a cause of acute and chronic disease. Pasteurella multocida is very common in conventional rabbit colonies and can cause upper and lower respiratory tract infections, subcutaneous abscesses, middle and inner ear infection and reproductive tract infections. Some species may serve as asymptomatic carriers of bacterial infections which can cause severe clinical disease in other species; therefore different species should not be mixed. Bordetella bronchiseptica can often be isolated from clinically normal rabbits and rarely causes disease in that species but it can be a significant cause of respiratory disease in guinea pigs. In addition to the species-specific organisms, post-operative infections can be caused by a myriad of bacterial contaminants normally present in the animal's environment. It is important that invasive surgical procedures be done aseptically to minimize the potential affects of these opportunistic organisms.

Although not experimental variables, there are several bacterial diseases of laboratory animals which can be transmitted to man and therefore are of possible concern to those using animals in research. These may include tuberculosis, salmonellosis, campylobacterosis, and shigellosis. The investigators whose studies involve substantial animal contact should be familiar with institutional guidelines and policies regarding the prevention of zoonotic disease. These should include a program of periodic physical examination, an educational program for personnel, immunization where appropriate and the use of protective clothing.

Viral Diseases

Viral infections in laboratory animals can often be asymptomatic. As with bacterial and mycoplasmal infection, clinical viral disease can occur when an animal is stressed. These viruses can be particularly devastating because the effects on research data may not be recognized, yet still be significant. The effects of these latent viruses have been best defined in rats and mice. Barrier housing of commercially available specific pathogen-free rodents will help eliminate these viruses from a colony. Contaminated tissues, particularly murine tumors, have been implicated in many outbreaks of disease. Tissues should be screened for the presence of contaminants prior to their use in a research facility. It is beyond the scope of this chapter to review all the research implications of viral pathogens currently known; however, a few examples will be briefly mentioned. There are key viral diseases of most common laboratory animals and it is important for the investigator to work with the institutional veterinarian to become familiar with those viruses and learn how they might affect a particular research project.

Sendai virus, a common viral contaminant in conventional mouse and rat colonies, can cause histopathologic changes in the respiratory tract, immunosuppression, and decreased reproductive efficiency. It can also act synergistically with other respiratory pathogens. A viral disease of mice which is often asymptomatic but serious is Mouse Hepatitis Virus (MHV). This virus has been implicated in wasting syndromes in nude mice. It can cause respiratory, hepatic, and enteric disease. Even in asymptomatic animals, it can cause profound immunological disturbances. Some diseases of laboratory animals are often associated with clinical disease and affect a research study due to high morbidity and mortality rather than the subtle effects of the latent viruses. Canine distemper, feline panleukopenia and measles in macaques are examples of these types of viral infections. Although not as prevalent as bacterial zoonoses, some viruses of laboratory animals can be transmitted to man. Examples of these include; lymphocytic choriomeningitis, Herpes virus simiae and rabies.

Parasitic Diseases

Parasites of laboratory animals have also been implicated as nonexperimental variables in research. Some parasites such as Trichosomoides crassicauda of rats are capable of causing tumors which could significantly obscure results of a carcinogenicity study. Skin mites of mice have been shown to affect immune parameters. Parasites are also capable of causing significant clinical disease such as the rectal prolapses seen with pinworms in rodents and bowel perforation seen with Prosthenorchis elegans in non-human primates. Some parasites of laboratory animals can also be transmitted to man. Examples of these parasites are Hymenolepis nana and Entamoeba histolytica.

It is important to remember that while laboratory animals may not show clinical signs of microbial infection, the infections can have profound effects on research results. Investigators studying immunological function should be particularly familiar with the potential effects of microbial agents on their research. Transmission of contaminants can occur in tumor or tissue inoculation, from direct transmission or via fomites in the laboratory. Animals of different health status should be strictly isolated from one another and all biologic material should be screened for the presence of viral and other contaminants.

SUMMARY

The concepts of Russell and Burch - refinement, replacement, and reduction are generally well accepted in the research community. Adherence to these concepts includes attempting to minimize the nonexperimental variables introduced in this chapter. The maintenance of healthy laboratory animals and the reduction of nonexperimental variables is the responsibility of the animal care facility and the investigator working together in an atmosphere of open communication and cooperation.

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Chapter 4
Principles of Anesthesia and Analgesia

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INTRODUCTION

It is important that all scientists using animals in research meet their ethical and legal responsibilities to avoid unnecessary pain and distress to the animal. Studies involving unavoidable pain and distress must be justified by the investigator in accordance with Federal regulations and institutional policies. This chapter will cover some of these legal responsibilities as well as try to help the investigator meet these responsibilities through knowledge of the basic principles of anesthesiology. Included in these principles are an understanding of some of the basic terms used in the field of anesthesiology, the types of variables that can affect an animal's response to an anesthetic agent, the effect of a given anesthetic protocol on an experiment, some general considerations and the recognition of pain. Also mentioned in this chapter are anesthetic monitoring and some fundamentals of anesthetic crisis management. Controlled drugs and their use are briefly discussed. This chapter is not meant to be a complete treatise on the subject of laboratory animal anesthesiology, but to give an introduction to stimulate further reading in areas of specific interest.

Anesthesiology is not an exact science. Recommendations and dosages given in textbooks should be taken as guidelines. An investigator contemplating a procedure requiring anesthesia, tranquilization or analgesia should not neglect the resource of a veterinarian who can often provide valuable assistance. In fact the Animal Welfare Act requires that "in any practice which could cause pain to animals . . . a doctor of veterinary medicine is consulted in the planning of such procedures."

There are many variables affecting an animal's response to anesthesia. Because the absorption and biotransformation of drugs differs between species, it is nearly impossible to develop a single anesthetic or analgesic protocol that applies to all laboratory animals. Morphine can cause profound CNS depression in the rat and rabbit, but can cause tremors and convulsions in mice and cats. The dosage of xylazine needed to sedate a ruminant is one-tenth that necessary to sedate a dog. These are but two of many examples. A common mistake is to extrapolate dosages across animal species or from man to animals. The strain of animal used is also a variable to consider. Some rat strains are sensitive to nitrous oxide. Some breeds of dogs (whippets and greyhounds) are more sensitive to barbiturates than other breeds. The size and even the sex of the animal can make a difference in the response to anesthetics. In rats, females are more sensitive to barbiturates, but in mice, barbiturate narcosis lasts longer in males. The temperament of the animal can change the way it responds to a given agent. Some tranquilizers will cause a vicious dog to become even more difficult to handle.

Fat does not play a key role in the initial absorption of an anesthetic agent, but it does affect the body weight upon which the dosage is based. Fat can later serve as a repository for the agent, thus prolonging recovery. The age of the animal also must be considered. Since very young animals require frequent feedings, prolonged recoveries can present a formidable problem. There are also age-related changes in liver enzyme functions which affect biotransformation of

anesthetic agents. Older animals can present an anesthetic challenge due to impaired renal or hepatic function.

The animal's physical condition can affect its responses. The presence of pre-existing disease will increase an animal's anesthetic risk. Respiratory diseases can often be asymptomatic in the uncompromi-sed animal even though they are endemic in many rodent populations. Even less obvious is the effect of diet and environment. Rats fed an inadequate diet are more resistant to barbiturates, yet fasted mice have an increased barbiturate sleep time. Abnormal environmental temperatures and humidity cause stress which can result in a compromised animal and variable anesthetic responses. High temperatures sensitize rats and rabbits to anesthesia.

Various factors will influence anesthetic choice. The use of concurrent drugs changes an animal's response to anesthetic agents. For example, some antibiotics potentiate barbiturates. The type of experimental procedure planned may impact on the anesthetic protocol. In an obstetric procedure, the effects on the fetus must be considered. When surgery involves the head and face, there is limited access to the animal so the anesthetic protocol should be planned to facilitate monitoring under these circumstances.

LEGAL RESPONSIBILITIES

Minimizing pain and distress in research animals is an ethical responsibility, produces better scientific results and is the law. The Public Health Service Policy on Humane Care and Use of Laboratory Animal states that "Procedures that may cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedation, analgesia, or anesthesia unless the procedure is justified for scientific reasons in writing by the investigator." The NIH further addresses the subject of anesthesia in the *Guide for the Care and Use of Laboratory Animals*. This document states that the proper use of anesthetics and analgesics is necessary for humane and scientific reasons and recommends that the veterinarian provide guidance for their usage. The Animal Welfare Act (AWA) requires standards for animal care, treatment, and practices in experimental procedures to ensure that animal pain and distress are minimized, including adequate veterinary care with the appropriate use of anesthetic, analgesic, tranquilizing drugs or euthanasia. It prohibits the use of paralytics in painful procedures without anesthesia and states "that the withholding of tranquilizers, anesthesia, analgesia or euthanasia when scientifically necessary shall continue for only the necessary period of time." Exceptions to such standards may be made only when specified by the research protocol and any such exception shall be detailed and explained in full in a report filed with the Institutional Animal Committee. And as previously noted, it further requires that if practices could cause pain to animals, a doctor of veterinary medicine be consulted in the planning of such procedures.

TERMINOLOGY

As with all branches of science, there are certain terms one needs to be familiar with in order to communicate effectively about anesthesiology.

The following is a list of the most common terms:

Analgesia - Insensibility to pain without loss of consciousness.

General Anesthesia - Temporary, controllable and reliable loss of consciousness induced by intoxication of the CNS.

Sedation - Calm state usually accompanied by drowsiness.

Tranquilization - Calmness without drowsiness or unconsciousness. Analgesia is usually not a feature.

Time to Peak Effect - Time between initial administration and onset of the maximum expected effect.

Duration of Effect - Length of time peak effect can be expected to last after a single administration of an anesthetic dose.

Time to Recovery - Time between initial administration and the ability to stand unaided.

EFFECTS OF ANESTHESIA ON RESEARCH

When anesthesia, analgesia, or chemical restraint is used, it may be advisable to ascertain any distortion of results by anesthetics through limited trials. Check the literature and package inserts for the effect of the agent on the systems being experimentally evaluated. These changes need to be taken into consideration when evaluating the effect of an experimental manipulation. Choose the agent which has the least effects on the systems under investigation. General anesthetics often depress the cardiovascular and respiratory systems, alter blood gases, lower metabolism, decrease body temperature, and alter tissue perfusion. Anesthetics can also produce histopathologic changes.

GENERAL CONSIDERATIONS

Whenever possible, try a new anesthetic protocol in a limited number of animals before depending on it for surgical or painful procedures involved in an experiment. This allows determination of suitability for the anticipated protocol and allows necessary changes to be made before it effects the data being collected. It also facilitates familiarization with the anesthetic method to minimize problems later, when attention is often focused on surgical procedures or data collection.

Pay particular attention to the health of the animal before using it in an experiment. A preanesthetic checkup is a good idea. To minimize anesthetic risks, only use healthy animals and allow them to acclimate to the facility before an anesthetic procedure. Consider the general adaptation syndrome: alarm increases basal metabolic rate which may increase the amount of anesthetic needed; however, this is often followed by an exhaustion phase when less anesthetic is required.

Use the minimal degree of CNS depression necessary for the procedure that is compatible with the animal's welfare. The degree of depression required for procedures such as radiographs or blood withdrawal is not the same as that needed for a thoracotomy or orthopedic procedure. Remember, during painful procedures, the use of paralytics without anesthesia is prohibited by law.

Consider if, and to what extent, the anesthetic protocol will affect the validity of experimental results and how it will react with other drugs being used. For example, if studying catecholamine effects, halothane should be avoided since its combination with catecholamines can cause severe cardiac dysrhythmias.

Even in the absence of sophisticated equipment, try to have some basic items available to insure adequate ventilation. This includes a source of oxygen, the use of endotracheal tubes when feasible, and aspiration suction to remove excessive oral secretions, and/or vomitus.

Regard the conservation of heat as an integral part of anesthetic management. This is particularly important in small or young animals. A rectal thermometer can help monitor the animal's body temperature. More sophisticated thermal monitors are also available. Maintenance of body temperature is enhanced through the use of external heat sources such as hot water bottles, thermal blankets and heating pads. Care should be taken to avoid thermal burns from external heating sources; i.e., electric heating pads.

Administer warm, balanced salt solutions by continuous I.V. drip whenever possible. This is not always possible in very small animals but is especially important for prolonged procedures or when significant blood loss is expected. Fluids often come in bags which are easy to handle and when warmed can double for hot water bottles.

Pay particular attention to post-anesthetic care. The anesthetist's responsibility does not end when the animal is taken off the table. Allow animals to recover in an environment approaching the normal body temperature of the species. Maintain intravenous fluid infusions when possible and have an endotracheal tube in place until the swallowing reflex is recovered. Be sure the animal is protected from injury, either self-inflicted or by other animals, during recovery.

Consider the implications for laboratory safety. Scavenging systems should be used with gaseous agents. Avoid carcinogens such as urethane and chloroform. Consider flammability when using ether.

RECOGNITION AND TREATMENT OF PAIN

In the Definition of Terms developed to implement the amended Animal Welfare Act, a painful procedure is defined as, ". . . any procedure that would reasonably be expected to cause more than slight and momentary pain or distress in a human being. . . ." In both humans and most animals the total pain experience results from an interaction between sensory pathways and the affective system, which provides the motivational and emotional component of pain. This varies considerably between species and individuals within a species.

Understanding the degree of pain involved in various experimental procedures allows a prediction of animal pain or distress. Physiological responses to pain can include increased blood pressure and heart rate, pupillary dilation, increased respiration, and an arousal response on the electroencephalogram. If baseline values are known for these variables, they can be monitored for changes.

To detect behavioral signs of pain, one must be familiar with the animal's normal behavior. Behavioral responses to pain vary between species, within species, and even within the same animal. General behaviors to evaluate include: sleeping, feeding, drinking, locomotion, grooming, exploration, performance in learning and discrimination tasks, mating behavior, social interactions, and dominance/subservience responses within the social system.

Typical behavioral signs of acute pain include:

protecting the painful area
vocalizing (especially when handled or moving)
licking, biting, scratching, or shaking the painful area
restlessness
lack of mobility
failure to groom
abnormal postures
lack of normal interest in surroundings.

Unless there is evidence to the contrary, assume that a procedure that causes pain in humans will cause pain in animals. Points to remember are:

Abdominal surgery appears to be less painful in animals than humans, probably because most animals do not use their abdominal muscles for postural support.

Lumbar and thoracic spine surgery in animals also appears to be less painful than in man, probably due to man's postural requirements. However procedures involving the cervical spine seem to be more uncomfortable in animals.

In animals, chest surgery involving the sternum appears to be more painful than surgery using a lateral intercostal approach.

Surgery on the eye, ear or surrounding structures seems to distress most animals. Signs such as head tilt or shaking, or pawing or rubbing the area may be seen. Perirectal procedures also seem to produce discomfort. In addition to analgesia, protection of the affected areas is indicated.

Surgery of the femur or humerus also seems to be painful to most animals, which may be due to large muscle mass trauma.

Pain perception can be influenced by drugs and/or environmental and behavioral factors. Recovery in familiar surroundings may help to relieve pain and distress. Acclimatization prior to a procedure may also facilitate recovery. The environment should be kept stable, minimizing

stimuli that evoke a fearful response in the animal. When appropriate, interact with the animal through talking or petting. Always handle the animal in an appropriate manner.

Various analgesics are available to the investigator. These can be divided into two main categories: the centrally acting agents such as morphine, butorphanol and buprenorphine; and the peripherally acting agents such as the anti-inflammatories, aspirin and phenylbutazone. The short half-lives of many of these agents may cause a labor-intensive analgesic protocol for the investigator, but creative delivery systems (such as the osmotic minipumps and tethering systems) and the development of new drugs such as buprenorphine with longer half-lives (12 hours) should facilitate meeting the analgesic needs of most laboratory animals. When designing an analgesic protocol, the investigator should consult with a veterinarian who is experienced in laboratory animal medicine. This will help avoid problems with species specific responses such as morphine sensitivity in cats and mice or the unusually short duration of meperidine in the dog. Interaction of the analgesic with concurrently used drugs and the effect of the agent on study results (such as the effect of aspirin on healing or clotting time) must be taken into consideration when choosing the best agent for a given situation. Although there is much information available on the use of various agents in animals, it is not always easily referenced and may be difficult to find without some guidance.

ANESTHETIC MONITORING

During an anesthetic procedure, the physiologic state of the animal and the depth of anesthesia should be monitored. This allows the anesthetist to adjust the depth of anesthesia and to anticipate impending complications. The degree of jaw tone is an indication of muscle relaxation. This is easily monitored by trying to open the animal's mouth -- taking care to avoid the animal's teeth.

Pulse quality is an indication of cardiovascular function. It can be checked in several areas but is commonly felt in the inguinal region. This "hands on" evaluation of the animal also gives the anesthetist a crude indication of the animal's body temperature so that hypo- or hyper-thermic states can be detected. Capillary refill is also an indication of cardiovascular function. This is checked by pressing firmly on the mucous membranes of the gums until they blanche and then releasing the pressure and noting the time it takes the normal color to return. Full color should return in less than two seconds. A slow capillary refill time is suggestive of sluggish blood flow and may be an early indicator of shock. While checking capillary refill, also note mucous membrane color. White may indicate shock, while blue may indicate poor oxygenation. In small rodents, the foot pads or ears offer other areas to check for color.

Another method for monitoring cardiovascular and respiratory function is through auscultation of the chest. This takes more experience and is difficult in small rodents. Electrocardio-graphic monitors are also available to aid in anesthetic monitoring.

Keeping written records of your anesthetic monitoring and administration is important for several reasons. They serve as a permanent record of the procedure and of any complications and when they occurred. This can help explain unexpected experimental data later. Written records

also help to visualize significant trends which could lead to anesthetic complications. In addition, written records represent the best method to clearly document compliance with the AWA.

The aim of anesthesia is to prevent the perception of painful stimuli without undue depression of physiologic functions. One of the criteria used to monitor the depth of anesthesia is the animals' response to stimuli or their reflex responses. Responses vary with the type of anesthetic used, the species and health status of the animal, and the use of concurrent drugs, particularly paralytics.

The first reflex lost is usually the righting reflex. This reflex may be checked by turning the animal over on its back and watching to see if the animal rolls back over onto its sternum. Obviously an animal that can right itself is not at a surgical level of anesthesia!

The next reflex usually lost is the swallowing or laryngeal reflex. It is the loss of this reflex that allows placement of an endotracheal tube after induction. Once in place, slight manipulation of the tube will cause the animal to swallow, if it is waking up. With some commonly used anesthetics such as the dissociative, ketamine, the laryngeal reflex may be present even when a surgical level of anesthesia is obtained.

The palpebral or eyelid reflex is an easy one to monitor. A light touch to the medial canthus or brush of the eyelashes will cause eyelid movement if the reflex is present. It may be as obvious as a blink or just a slight muscle movement. An overly aggressive touch may cause movement that is not induced by the animal and can lead to erroneous interpretation.

The reflex most commonly used to determine if the animal is feeling deep pain is the pedal or paw pinch reflex. The toe is firmly pinched between the fingers to elicit a withdrawal response by the animal. A forcep may also be used but care must be taken not to cause tissue damage. Pinching the ear can also be used especially in rodents and rabbits. If the animal draws its head away or shakes its ear, it is still capable of feeling deep pain and is not ready for any surgical manipulations.

The pupillary reflex can also be monitored but it can be affected by many things. Common preanesthetic agents often make the pupil unresponsive to light. A dilated pupil can indicate either very light anesthesia and the perception of pain or dangerously deep anesthesia if the pupil is fixed and dilated.

The corneal reflex is usually the last to go and it is usually not necessary to get to this depth of anesthesia. This reflex is checked by very gently touching the animal's cornea and watching for movement of the eyelid.

STAGES AND PLANES OF GENERAL ANESTHESIA

General anesthesia is divided into stages and planes. Stage one is characterized by analgesia. In stage two, excitement can be seen. Signs include struggling and erratic movement. It is preferable to avoid this stage. Stage three is a surgical level of anesthesia. It is further divided into planes. Plane one is characterized by a loss of the palpebral reflex. In plane two, eyeball movement ceases and the animal exhibits deep, regular respirations. This is usually a good level

at which to do surgery. With plane three comes paralysis of the intercostal muscles and short, jerky, gasping diaphragmatic efforts. Artificial ventilation is essential at this plane. Stage four is one to avoid as it is characterized by total loss of respiratory movements, cyanosis and cardiac arrest.

SPECIFIC AGENTS

It is not within the scope of this chapter to give a detailed pharmacologic description of all the anesthetic agents and regimes used in research animals. However, a brief description of the advantages and disadvantages of some of the most commonly used agents will be given. The reader is referred to the list of references and a veterinarian when help is needed to design an appropriate anesthetic protocol for a given research project.

Preanesthetics

Preanesthetics are usually given as an anesthetic agent adjunct to ameliorate some of the deleterious side effects and/or to decrease the required dose of the primary anesthetic agent. Atropine or its analogs are commonly given. They depress secretory activity making them especially useful in animals with profuse oral secretions such as ruminants and guinea pigs. These agents also help maintain heart rate by counteracting the vagal slowing of the heart rate induced by some anesthetic agents and some surgical procedures. Atropine causes pupillary dilation, therefore this reflex cannot be used to monitor anesthetic depth in the atropinized animal.

Other commonly used preanesthetics are tranquilizers and sedatives. Use of these agents helps provide a stress-free subject for the induction of anesthesia. Acepromazine produces good tranquilization, indirectly suppresses the emetic center, potentiates the analgesic effects of other agents and provides muscle relaxation. Hypotension can be a serious side effect of this agent. It is often used in combination with the dissociative anesthetic agents such as ketamine. Xylazine is a potent hypnotic, muscle relaxant, and analgesic. Use of this agent can reduce the necessary barbiturate dose by 50 percent. Like acepromazine, xylazine is often used in combination with ketamine. Bradycardia and hypotension can be seen with xylazine. Premedication with atropine can help prevent cardiac dysrhythmias. Respiratory rate can be decreased, but increased tidal volume usually maintains normal blood gases. Xylazine can cause abortion in late pregnancy in ruminants. Diazepam is a potent tranquilizer which also has muscle relaxant and anticonvulsant properties. It is useful in combination, particularly with Innovar-VetR in rodents. Although diazepam can cause some respiratory depression, it has little effect on cardiac output or blood pressure. Morphine is a narcotic analgesic sedative. Anesthetic doses can be decreased as much as 50 percent after morphine administration. Morphine depresses the central nervous system, particularly the respiratory center, as well as peristalsis. In dogs, morphine frequently causes emesis. Morphine is generally contraindicated in the cat and mouse.

General Anesthetic: Injectable

General anesthesia is delivered by two basic methods: injection and inhalation. It is usually preferable to give injectable agents by the intravenous route (I.V.) -- given to effect; however,

intraperitoneal (I.P.), subcutaneous (S.C.) or intramuscular (I.M.) techniques are sometimes necessary or even preferable. The advantages of injectable anesthetic agents are ease of administration, low cost and lack of need for sophisticated equipment. The major disadvantage is that once the drug is given, it is in the body until it is metabolized or excreted.

Innovar-VetR is a veterinary drug which combines fentanyl, a morphine derivative, and droperidol, an alpha adrenergic blocker. Because it is a combination drug, doses are usually given in ml/kg rather than mg/kg. It is a potent analgesic. The cardiac depressant effects can be counteracted with atropine and the respiratory depressant effects can be reversed with naloxone. Innovar-VetR is a poor muscle relaxant. It is not recommended for use in horses, ruminants, or cats.

Ketamine is a commonly used dissociative anesthetic. It is short acting and produces variable analgesia. It is often combined with other agents to improve its muscle relaxation and analgesic properties as well as provide a smoother recovery. It can be given I.V., S.C., I.M or I.P. It does not cause cardiac depression and may even stimulate the cardiovascular system; however, mild respiratory depression may be seen. The swallowing reflex is maintained making intubation under ketamine alone difficult. The palpebral reflex is lost, so it is necessary to use ophthalmic ointment to prevent corneal drying.

The most commonly used injectable anesthetic agents are the barbiturates. There are two classes of barbiturates: oxybarbiturates of which pentobarbital or nembutal is the most common; and thiobarbiturates, such as thiopental, which is much faster acting. Barbiturates are potentiated by acidosis such as that which can be seen with respiratory depression or diarrhea. Many drugs potentiate the effect of barbiturates. Glucose or epinephrine cause prolonged recovery times. Barbiturates are controlled substances as defined by the Drug Enforcement Agency. Therefore a license is required for purchase and records must be kept. If possible, barbiturates should be given to effect which is difficult when administered I.P. They have an accumulative effect, which means two subsequent doses combined have a greater effect than the two doses given alone. Barbiturates are considered poor analgesics. Respiratory depression can lead to hypercarbia. Cardiovascular effects include bradycardia, hypotension, myocardial depression, and increased peripheral vascular resistance. Use of barbiturates is contraindicated in animals with liver or kidney disease. Lower doses should be used in young animals. When small doses must be given, it is often helpful to dilute stock barbiturate solution. Preanesthetics should be used when possible to decrease the amount of barbiturate needed.

General Anesthetics: Inhalation

Inhalation anesthesia has the advantages of rapid induction and recovery. Depth of anesthesia can be rapidly changed. Typically animals are initially anesthetized with an I.V. injection of an ultrashort acting barbiturate, or administered the inhalation agent by mask or by use of an induction chamber. When using gaseous anesthetic agents particular attention must be paid to provide an adequate oxygen source and for the removal of carbon dioxide. This can be done through the use of a properly maintained gas anesthesia machine. If possible, it is preferable to intubate the animal for the most efficient delivery system and to help assure a patent airway. This takes practice, especially in rodents. If the anesthesia is administered by mask, avoid placement

of the mask over the entire face as these agents are irritating to the eyes. Also avoid direct contact of the liquid form of the agent with the animal's skin or mucous membranes. Scavenging systems should be in place to minimize personnel exposure.

Nitrous oxide is often used in conjunction with an anesthetic gas due to its potentiating effect. It is always used in combination with oxygen, usually at a 50:50 or 60:40 ratio. It is quite safe, since it is neither flammable nor explosive, allows rapid induction and causes little cardiovascular disturbance. It is also a very good analgesic. It enters air-filled cavities much faster than it leaves them which could be a problem with a pneumothorax or a large gas-filled bowel. Oxygen should be administered alone for a few minutes at the end of a procedure to prevent diffusion anoxia.

A commonly used gaseous anesthetic agent is ether. Ether has a slow induction and recover period. It is highly flammable and forms explosive mixtures with oxygen and nitrous oxide. It is a potent CNS depressant and analgesic. It is extremely irritating to the mucosal lining of the respiratory tract and may induce laryngospasms, especially in cats and rabbits. Respiratory secretions are stimulated which can predispose or exacerbate respiratory infections. The respiratory depression caused is usually only a problem in guinea pigs and chinchillas. Ether causes some myocardial depression. Since ether is inexpensive and can be administered without the use of sophisticated equipment, it is very popular. To minimize explosive hazards and personnel exposure, ether should be used under a fume hood.

Three other commonly used inhalation agents are halothane, isoflurane and methoxyflurane. Halothane is nonflammable and nonexplosive. It is a good muscle relaxant and adequate analgesic. It allows rapid, smooth induction and recovery. Halothane depresses the cardiovascular system and sensitizes the heart to dysrhythmias. It also depresses the respiratory system which can lead to acidosis. Halothane requires special vaporizers and equipment. Isoflurane is also a stable, nonflammable agent. Induction and recovery are rapid. Arterial blood pressure is decreased due to lowered peripheral vascular resistance; however, perfusion is maintained. Other cardiovascular functions are well maintained, but respiratory function is depressed. Isoflurance also requires special vaporizers and equipment. Methoxyflurane is very stable and because it does not reach high concentrations at room temperature, it has a good margin of patient safety. It is a good muscle relaxant and an excellent analgesic. Like the other inhalation agents, it does cause some respiratory depression and hypotension can also be a problem. Induction and recovery are slower than with the other agents which may be an advantage by keeping the animal quieter immediately postoperative as well as providing longer acting analgesia.

SPECIES-SPECIFIC CONSIDERATIONS

When anesthetizing small rodents, particular care must be taken to avoid hypothermia. The airway is easily obstructed so be sure the neck is adequately extended and secretions are aspirated as necessary. Fasting is not necessary unless gastrointestinal surgery is planned and even then only a 6-hour fast is necessary. Water should not be restricted. Loss of the toe pinch reflex indicates surgical anesthesia in the mouse. In the rat and guinea pig, the ear pinch is more

sensitive. Rodents are difficult to intubate. If they are intubated, care must be taken to minimize dead space in the tubing.

Rabbits are probably the most difficult laboratory animal to anesthetize. Their respiratory center is particularly sensitive to anesthetics and a lot of individual variation in response exists. Rabbits should be fasted 6 hours prior to anesthesia. Water should not be restricted. The rabbit trachea is very delicate and rabbits are predisposed to pulmonary edema with prolonged inhalation administration. A normally small lung capacity combined with enzootic pulmonary disease further complicates the situation. The best indicator for surgical anesthesia is the loss of the ear pinch reflex. Intubation in rabbits is difficult due to lack of visualization of the larynx, but it can be mastered with practice.

Dogs are usually not difficult to anesthetize. Large, easily accessible veins make I.V. injection of agents quite easy. Intubation is not difficult due to the easily visible larynx. Administration of preanesthetics, particularly in large dogs, may make induction easier. Dogs should be fasted for 12 hours prior to anesthetic administration.

Cats are also relatively easy to anesthetize; however, they are easily stressed when restrained so preanesthetics become even more important. The larynx is easy to visualize, but laryngospasms can make intubation difficult. Cats should be fasted for 12 hours prior to an anesthetic procedure; however if necessary, xylazine can be given to induce vomiting and serve as a tranquilizer. As noted previously, narcotics can cause severe convulsions in cats and should be avoided.

There are several considerations when anesthetizing swine. The pig heart is smaller in proportion to body size than is the heart of other domestic animals, which is a disadvantage during periods of anesthetic stress. Size and temperament can make restraint difficult and the use of preanesthetics essential. Pigs are predisposed to ventricular fibrillation and some breeds exhibit malignant hyperthermia when exposed to halothane. The anatomy of the larynx and soft palate predispose pigs to respiratory distress if not intubated; however, this same anatomy combined with laryngospasms can make intubation difficult. The ear vein is the most readily accessible for I.V. injections. Pigs should be fasted 12-18 hours prior to anesthetic administration.

The temperament and size of ruminants present a challenge to the anesthetist. Again, preanesthetics are desirable; however, ruminants are very sensitive to xylazine, so only small doses are needed. They are also very sensitive to barbiturates. Food should be withheld for 24-48 hours with water withheld for 6 hours prior to the procedure. Gastric bloat can be minimized by passing a stomach tube in the anesthetized animal once it is on the table. Avoiding prolonged procedures and recoveries will also help minimize bloat as well as decrease the incidence of pressure myositis. Thick pleura and extensive pulmonary supportive tissue necessitate the use of high ventilation pressures. Excessive salivation is difficult to control even with the generous use of atropine. The jugular vein is the easiest to use for the I.V. anesthetic administration.

The use of sedatives and tranquilizers as preanesthetics in nonhuman primates presents a risk to the handler because the animal may present a false appearance of sedation in the cage and become quite active when aroused! Ketamine, given I.M. in a monkey restrained in a squeeze cage, is the most common form of preanesthesia. This is often followed with an I.V. injection of

an additional anesthetic agent with maintenance accomplished with additional injectable agents or inhalation anesthetics. This procedure minimizes the hazards of bites or scratches to personnel or escape by the patient. Monkeys should be fasted for 12 hours prior to an anesthetic procedure; however, ketamine given alone usually does not cause emesis. Monkeys are usually not difficult to intubate after a little practice.

ANESTHETIC EMERGENCIES

Anesthetic emergencies are usually caused by human error. This may be due to inappropriate selection of agents or doses, failure to recognize and treat inadequacies of respiration or circulation before collapse, neglect in checking equipment or the use of unhealthy animals.

Respiratory failure is often caused by airway obstruction or barbiturate overdose. Airway obstruction can occur because of positioning of the animal, secretions in the trachea, or misplacement of the endotracheal tube. Barbiturates are particularly potent respiratory depressants and they must be used with care and "to effect." Signs of respiratory failure include gasping, exaggerated chest movements and cyanosis. Gasping movements can be misinterpreted to be voluntary movements indicating inadequate anesthesia causing the inexperienced anesthetist to actually give more anesthetic agent.

When respiratory failure occurs, the first thing to do is discontinue anesthetic administration. Then check for airway patency. Artificial ventilation can be performed through the nostrils or through the endotracheal tube by compressing the rebreathing bag on the anesthetic machine or the use of a manual resuscitator bag. An ear syringe can make a good rodent resuscitator, as it fits right over the nose of larger rodents. If the failure was caused by a narcotic, reversal agents may be used. Other drugs such as doxapram can be used to stimulate the respiratory system.

Causes of circulatory arrest include drugs, hypoxia, hypercapnia, changes in the vascular volume or bed, deleterious reflex responses, obstruction of venous return, severe electrolyte imbalance, and primary cardiac pathology. Careful maintenance of ventilation is one way to avoid hypoxia and hypercapnia. Changes in vascular volume can be minimized through the use of good hemostasis by the surgeon and adequate I.V. fluid volume replacement by the anesthetist. Surgeons must be careful when moving abdominal contents around, not to place too much pressure on the posterior vena cava and thus impede blood flow return to the heart. Electrolytes can be monitored and imbalances corrected during surgery before they get to the life threatening stage. In some cases the presence of primary heart pathology may be identified in a routine presurgical physical exam.

Signs of cardiac failure are white or cyanotic mucous membranes, no pulsation in major arteries, no wound bleeding and no palpable heart beat.

Treatment of cardiac arrest begins the same way as that for respiratory arrest and includes discontinuation of anesthetic administration, checking for a patent airway, and the administration of oxygen. If possible, also lower the cranial end of the animal by 30 percent. Closed chest massage can be done by compressing the thorax by one third to one half its width or depth at a ratio of 5 compressions to each ventilation. Fluid replacement should occur as rapidly as

possible. Drugs such as epinephrine, sodium bicarbonate, prednisolone sodium succinate, calcium chloride and lidocaine can be used but vary with different situations which may be hard to define without the use of an electrocardiogram.

Anyone performing frequent anesthetic procedures should have a well-stocked emergency kit handy with such items as endotracheal tubes, a manual resuscitator bag, syringes and needles, and some or all of the drugs mentioned above. It is helpful to have a card in this kit which list all the dosages for these drugs to insure proper usage during the rare occasion when they are needed. Frequent emergencies are an indication of improper anesthetic or surgical techniques and should be reviewed with the veterinarian to ascertain a possible cause and implement a potential solution.

CONTROLLED SUBSTANCES

Many anesthetics, analgesics and tranquilizers are controlled substances. They are divided into five schedules based upon their abuse potential. Schedule I drugs are those with a very high abuse potential for which there is no medical use. Schedule II drugs also have a high abuse potential but are accepted for medical use. This schedule includes agents with narcotic, stimulant or depressant actions such as morphine, codeine, meperidine, oxymorphone, pentobarbital, cocaine and opium. Schedule III includes some of the barbituric acid derivatives. Schedule IV has phenobarbital, chloral hydrate, and diazepam. Schedule V agents are those with narcotics in limited quantities such as antitussives and antidiarrheals. This is only a partial list of the drugs in each schedule. For a more complete list refer to the Drug Enforcement Administration.

Controlled substances can only be purchased by someone with a narcotics license which is obtained from the Drug Enforcement Administration. Controlled substances must be stored under lock and key, preferably in a safe. Permanent records must be maintained and should not be stored with the drugs.

SUMMARY

This chapter reviewed the principles of anesthesiology and highlighted examples of animal and anesthetic variations. The need to carefully choose and evaluate an anesthetic protocol cannot be overemphasized. Improper use of anesthetic agents can result in loss of valuable research data at the very least, and misuse of the animal at the very worst. Prior to using an anesthetic protocol, ascertain its species-specific effects, interactions with other agents and effect on experimental data. When the use of analgesics is indicated, the animal's response to potential painful stimuli must be evaluated in terms of its normal behavior and the effect of the agent on the species. When using anesthetics and analgesics in laboratory animals, advice from a veterinarian should be obtained during the planning stages of the projects. In designing an anesthetic or analgesic protocol, remember if it would hurt you, it will probably cause pain to an animal. When in doubt, don't proceed without carefully evaluated trial runs.

It is the principal investigators' legal responsibility to minimize pain and distress in the animals they use, and a key element in meeting this responsibility is the proper use of anesthetics, analgesics and tranquilizers.

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Chapter 5
Principles of Aseptic Technique

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Note: Figures 1-4 only available in original hardcopy of this publication.

INTRODUCTION

The regulations promulgated to implement the amended Animal Welfare Act require that all survival surgery be performed using aseptic procedures. This includes the use of surgical gloves, masks, sterile instruments and aseptic technique.

In this chapter, the Principles of Aseptic Technique will be discussed with the emphasis on the practical application of these principles in the laboratory setting. In centralized experimental surgeries, a well-trained staff should be available to advise those who use such facilities and oversee its operation to ensure the maintenance of an aseptic environment for survival surgery. When survival surgery is conducted outside such an environment, it is the principal investigator's responsibility to ensure that appropriate aseptic conditions and practices are maintained. This chapter will provide the necessary information to carry out this responsibility.

Prior to discussing the specific principles of aseptic surgery a brief review of pertinent terminology is necessary.

TERMINOLOGY

Antimicrobial - An agent or action that kills or inhibits the growth of micro-organisms.

Antiseptic - A chemical agent that is applied topically to inhibit the growth of micro-organisms.

Asepsis - Prevention of microbial contamination of living tissues or sterile materials by excluding, removing or killing micro-organisms.

Autoclave - A steam sterilizer consisting of a metal chamber constructed to withstand the pressure that is required to raise the temperature of steam to the level required for sterilization. Early models were termed "autoclaves" because they were fitted with a self-closing door.

Bactericide - A chemical or physical agent that kills vegetative (non-spore forming) bacteria.

Bacteriostat - An agent that prevents multiplication of bacteria.

Commensals - Non-pathogenic micro-organisms that are living and reproducing as human or animal parasites.

Contamination - Introduction of micro-organisms to sterile articles, materials or tissues.

Disinfectant - An agent that is intended to kill or remove pathogenic micro-organisms, with the exception of bacterial spores.

Pasteurization - A process that kills nonspore-forming micro-organisms by hot water or steam at 65-100oC

Pathogenic - A species that is capable of causing disease micro-organism in a susceptible host.

Sanitization - A process that reduces microbial contamination to a low level by the use of cleaning solutions, hot water or chemical disinfectants.

Sterilant - An agent that kills all types of micro-organisms.

Sterile - Free from micro-organisms.

Sterilization - The complete destruction of micro-organisms.

Since the pioneering work of such surgeons as Joseph Lister, who introduced the use of carbolic acid antiseptics in 1865, and William Halstead, who advocated the use of surgical gloves in 1898, surgeons have strived to eliminate surgical infections through the use of aseptic technique. Potential sources of contamination are well defined. They include the patient and the surgical environment: the surgeon and support staff, the instruments, sutures, drapes and all other equipment which can have contact with the surgical field.

FACILITIES

The basis for this discussion about facilities will be the recommendations for Aseptic Surgery contained in the *Guide for the Care and Use of Laboratory Animals*. The *Guide* states:

"Functional areas for aseptic surgery should include a separate support area, a preparation area, the operating room or rooms and an area for intensive care and supportive treatment of animals. The interior surfaces of this facility should be constructed of materials that are impervious to moisture and easily cleaned. The surgical support area should be designed for storing instruments and supplies for washing and sterilizing instruments. Items that are used on a regular basis, such as anesthetic machines and suture materials, can be stored in the operating room."

"There should be a separate surgical preparation area for animals. An area equipped with surgical sinks should be close to, but apart from, the operating room. A dressing area should be provided for personnel to change into surgical attire."

The surgical facility should be located outside normal facility traffic patterns. This can help to minimize the potential for surgical suite contamination by the movement of personnel and equipment. Personnel access to these areas should be restricted to essential surgical support staff.

Ideally, the operating room ventilation system should provide a net positive pressure with respect to the surrounding facilities. The system should be regularly monitored. Maintenance work

should be performed when the surgery is idle. Ventilation filters should be inspected and cleaned or replaced at regular intervals. If explosive anesthetics agents are to be used, the *Guide* recommends that floors should be conductive and electrical outlets should be explosion-proof and located not less than 5 feet off the floor. Dedicated surgical facilities should be used for aseptic surgeries and the storage of essential surgical equipment, not as general storage space.

EQUIPMENT

The equipment in areas used for aseptic surgery should be easy to clean and portable to simplify sanitization of the area. The operating table should be constructed with a durable surface material impervious to moisture which can be readily cleaned. Plastic or stainless steel is frequently used for this purpose. Other useful table design features which assist patient positioning include height and tilt adjustments, V-trough configuration and restraint strap cleats. A disadvantage of stainless steel construction is that it predisposes animals to hypothermia. This can be corrected by the routine use of a heating pad placed under the surgical patient. Reusable, easy to clean vinyl heating pads which recirculate hot water are frequently used for this purpose. Inexpensive short-term alternatives include hot water bottles or heat lamps. Any heat source should be used with caution to prevent patient burns.

Instrument tables provide the surgeon ready access to the surgical instruments and minimize the risk of sterilized instrument contamination by contact with non-sterile fields. Commercially available instrument tables, such as Mayo stands, consist of a stainless steel tray supported by a pedestal base with a foot-operated height adjustment device, but any tray arrangement may be used for this purpose. The unit should be easy to clean and simple to operate. The drapes in an instrument pack frequently include impervious table covers which can minimize instrument contamination and allow the surgeon to reposition the table without breaking aseptic technique during the procedure. Surgical buckets on wheels (kick buckets), which can be readily positioned with the feet, are another recommended piece of equipment. They should be easy to clean and lined with a plastic bag which should be changed at the end of the procedure.

Adequate lighting is essential for performing surgical procedures. A variety of fixtures can be used to provide sufficient light. The commercially available surgical light fixtures may be ceiling or wall-mounted or free standing. Surgical lights are often positioned above the operative area and should be regularly wiped with a moist towel prior to use to minimize potential contamination of the sterile field below. Light fixtures designed with detachable sterilizable handles allow the surgeon to adjust the beam during surgery. Wheeled, height-adjustable intravenous drip stands should be available when conducting major surgery. Care should be taken to assure that the I.V. tubing does not contaminate the sterile fields. Positioning the I.V. tubing along the heating blanket helps warm I.V. solutions before infusion.

Surgical suction is another useful accessory. Sterilized tubing and suction tips are provided for use in the aseptic field. The tubing is connected to a non-sterile suction bottle which in turn is connected to a built-in vacuum line. If built-in vacuum lines are not available, portable electric vacuum pumps are commercially available.

Ancillary equipment such as respirators, electrosurgical units and ECG monitors should be portable and included with the light fixtures in a routine equipment cleaning schedule. Specific details on such devices could be obtained from an institutional veterinarian or surgical supervisor.

Surgical instrumentation and pack preparation will vary with the type and complexity of surgery to be performed. Consultation with an institutional veterinarian or surgical supervisor could be helpful when selecting the appropriate surgical instruments necessary to perform a proposed procedure. Instrument packs should be double wrapped. Various commercial materials are available for this purpose. Although pack instrument preparation will be discussed later, as many sterilizable items as possible should be included. These might include prepackaged surgical blades, sponges, saline bowls and miscellaneous catheters.

Personnel

Aseptic technique requires careful attention to a series of steps which begins with patient and instrument preparation and ends at final wound closure. Failure at any one step may result in wound infection which could compromise the animal's health and the experimental data derived from the animal. Aseptic technique designs all actions and motions to protect the sterile field from contamination. The surgeon and surgical support staff must be adequately trained to perform each step correctly. Acquiring and developing the necessary skills to maintain aseptic technique requires practice. Personnel should receive instruction on the indications for aseptic technique, the sources of potential contamination, patient, instrument and equipment preparation, sterilization systems, gowning and gloving techniques, and intraoperative aseptic management. Once this theoretical knowledge is gained, trainees can rapidly learn by observing the aseptic management techniques of a well-trained surgical support staff. Trainees should practice each step until correct techniques become second nature.

Assistance with employee training may be available from the institutional veterinarian, a member of the animal care staff and/or a member of a hospital surgery staff.

STERILIZATION

Sterilization is the process that is intended to kill or remove all types of micro-organisms. There are two principal sterilization methods:

1) Physical (dry heat or saturated steam)

2) Chemical (ethylene oxide gas or chemical liquids).

Factors which determine the method to be used are the type of micro-organisms involved, the nature of the article to be sterilized and the time available for sterilization.

Physical Methods (Steam)

Steam sterilization (frequently referred to as autoclaving) depends on the use of steam above 100oC. Temperatures ranging from 121-134oC at pressures of 15-30 psi are generally recommended. The biocidal action of moist heat is a denaturation of major cell constituents. Many sterilizers are designed to provide an automatic sterilization cycle. In the first stage of the cycle, air is evacuated and the chamber brought to the pre-set sterilizing temperature, which is maintained for a holding period sufficient to kill all microbial contaminants. Minimum holding times for the sterilization of medical equipment are 15 minutes at 121oC, 10 minutes at 126oC, and 3 minutes at 134oC. The steam is then removed and instrument packs are allowed to dry or liquids cool. The drying stage may be adjusted to suit the load. The chamber is then restored to atmospheric pressure by the introduction of filtered air.

The recommended periods of exposure vary with the nature of the article to be sterilized and the method used to wrap the article. Specific details are available from the references at the end of the chapter.

Steam sterilization has the advantage of rapid penetration of wrapped materials with the destruction of all viruses and bacteria, including the most resistant spores. The sterilization of different supplies is more readily controlled than in other types of sterilizers. However oils, grease and powdered substances cannot be sterilized by this method. The steam autoclave must be maintained in good repair and operated correctly in order to perform to specifications. Sterilization failure can occur when machines are not regularly serviced.

Steam autoclave function should be monitored continuously using one or more of several commercially available indicator systems. The color change on a chemical dye impregnated indicator strip placed within the pack can provide a convenient and rapid visual check that the appropriate sterilization conditions were reached. Function should also be monitored on a regular basis using commercially available biological indicators. Spore strips of Bacillus stearothermophilus are placed within the wrapped article prior to sterilization. After sterilization the strip is incubated at 57oC for 48 hours. The absence of growth indicates effective sporicidal autoclave action.

Chemical Methods (Gas)

Ethylene oxide gas is effective against all types of micro-organisms. The biocidal action of this gas is considered to be alkylation of nucleic acids. It is non-corrosive and safe for most plastic and polyethylene materials. However, it is not applicable to liquids or to articles in impervious packaging material. It cannot be used to sterilize animal diets due to the potential toxic effects of this gas. It can also be a toxic hazard for animals receiving prosthetic implants which have been sterilized by this gas. The operating pressures and temperatures (45-60oC and 10-12 psi) of ethylene oxide sterilizers are considerably less than for steam units. Articles should be well aerated prior to use to minimize the potential for tissue toxicity. Aeration should be done in a manner which minimizes exposure of personnel. This can be accomplished through the use of self-aerating sterilizers or separate aeration cabinets.

Ethylene oxide gas is a potential carcinogen and mutagen and represents a potential occupational health hazard for personnel operating sterilizers. Operation of gas sterilizers and aerators should

be in strict conformance with manufacturers' recommendations and institutional policies. Personnel exposure should be minimized by appropriate ventilation of exhaust gas. A regular monitoring program for personnel should be in place.

Gas sterilizer function should be monitored continuously using one of several commercially available indicator systems. The color change on a chemical dye-impregnated indicator strip placed within the pack can provide a convenient and rapid visual check that the appropriate sterilization conditions were reached. Function should also be monitored on a regular basis using a commercially available biological indicator such as spore strips of Bacillus subtilus which are placed within the wrapped article prior to sterilization. After sterilization the strip is incubated at 37oC for 24 hours. The absence of growth indicates effective sterilization.

Temperature-sensitive adhesive tape used to secure packages prior to sterilization only indicates that the package has been exposed to the sterilizer; this tape does not monitor sterilizer function.

Chemical Methods (Liquids)

The use of chemical solutions as a sterilization technique for surgical equipment is frequently employed, but it should be stressed that most solutions only disinfect and do not guarantee sterility. When the necessity for maintaining sterility is a critical factor, as in the implantation of prosthetic devices, indwelling catheters or vascular access ports, disinfection in chemical solutions is not recommended. Such prostheses should be thoroughly sterilized by either gas or steam. Chemical solutions, however, offer the advantages of safety for delicate and thermolabile plastics.

Other limitations of chemical solutions should also be appreciated. Equipment must be thoroughly cleaned before immersion, as chemical action is ineffective in the presence of proteins or fats. There are currently no indicators commercially available to monitor the effectiveness of this sterilization method.

Alcohols are neither sporicidal nor viricidal. They are not stable and lose effectiveness through evaporation. Alcohols cannot be used for instruments that have plastic or cemented parts.

The chlorine compounds exert their biocidal action by oxidization. The formulations which require the mixing of acid and base components with water to generate chlorine dioxide, offer the advantages of wide spectrum biocidal action and a safe alternative to the more hazardous phenols or formaldehydes. The active shelf life of mixed chemicals is reported to be 24-48 hours.

If chemical sterilization of instruments is the method to be used, it can be performed in covered trays containing fresh solutions. A two-tray system, one each for even-numbered and odd-numbered days, will ensure that instruments have a full 24-hour contact time.

PREPARATION OF THE ANIMAL

The animals should be prepared in an area separate from where surgery will be performed. Preparation is facilitated by first inducing anesthesia. The stomach, rectum and urinary bladder

can then be evacuated as required at this stage. Hair is then removed from the surgical site using electric clippers equipped with a fine blade. A liberal area is clipped to anticipate any enlargement of the initial surgical incision and minimize wound contamination from adjacent unclipped areas. In rodents the need to minimize the loss of heat during surgery and recovery must be balanced against the need to provide an adequate aseptic field when clipping the animal. Animal hair, particularly rabbit hair, tends to clog clipper blades. This can be minimized by frequent cleaning of the blades and regular lubrication with a commercial aerosol product between use. A vacuum can be used to clean up after clipping. Depilatory creams may be applied to the surgical site, but they may cause contact dermatitis which may interfere with the healing process.

Initial skin cleaning can be done prior to moving the animal to the operating area. When the animal is moved to the operating area, it should be positioned on a heating pad on the surgical table. To avoid burns heating pads should be wrapped to prevent direct contact with the animal. Inclined positioning with a tilt table is indicated for some procedures and some species. The surgical approach will dictate actual animal position; however, some guidelines to consider are:

a. The animal's respiratory function should not be compromised by overextension of forelegs stretched towards the head, or by excessive body tilt which causes pressure from the abdominal organs on the diaphragm.

b. Limbs should not be extended beyond their normal range of motion and restraint straps should be padded as needed to prevent impaired venous return in extremities.

c. After the animal has been secured, any monitoring devices such as ECG electrodes and esophageal stethoscopes should be placed and their function tested.

d. Ruminants are frequently positioned on a slight incline with the head dependent, to minimize the potential for aspiration of rumen fluids. After intubation with a cuffed endotracheal tube, a large bore stomach tube is also frequently placed down the esophagus to remove rumen fluids and gas.

The animal is now ready for final preparation of the surgical site. Personnel who perform the presurgical skin preparation should wear a cap and mask when preparing the surgical scrub supplies and when opening pre-sterilized sponge and drape packs. Skin preparation solutions may be applied with a sterile sponge held by a pair of sterile forceps or by a hand wearing a sterile glove. A sterile surgical glove is put on one hand, while the other hand is used to hold and manipulate non-sterile bottles of surgical scrub solution. A sterile sponge held in the gloved hand is saturated with surgical scrub solution and the surgical area is scrubbed beginning with the central incision site and working progressively in a circular fashion to the margins of the shaved area (see Fig. 1). The sponge is then discarded and the process repeated, working from the center to the outside to minimize contamination of the surgical site.

Some of the most frequently used chemical solutions for preoperative surgical skin preparation are: chlorhexidine, iodophors and povidone-iodine surgical scrubs. Recommended contact times vary from 2 to 4 minutes.

Following removal of the scrub solution with a 70 percent alcohol solution using the same technique, an iodine skin solution is painted on the site using the above technique and left to dry.

Drapes serve to isolate the surgical site and minimize wound contamination. Drapes should be positioned without the fabric dragging across a non-sterile surface. There are two basic types of drape systems used: fenestrated and four corner.

Fenestrated drapes have a hole in them which is placed over the surgical site. Frequently used for smaller species, these drapes are utilized for routine elective procedures. The fenestration should be just slightly larger than the intended incision.

The second alternative is the four corner drape system in which a drape is placed at each of the four margins of the surgical site. Four corner drapes are applied one by one in a clockwise or counterclockwise direction. Each drape should be carefully positioned with a 6 to 8 inch edge folded underneath at the incision site (see Fig. 2 A to D). Small adjustments in position can then be made without contaminating the underside of the drape. Drapes can be secured in place with towel clamps at the four corners or aerosol adhesive applied to the margins of the surgical site prior to draping.

Some surgeons prefer to secure four corner drapes, then apply a fenestrated drape as a second layer of protection (see Fig. 2, E and F). Ideally, the patient and entire surgical table should be draped, and the drape extended to the instrument table. The need to monitor the draped patient should always be considered. The surgeon who has to work alone often has to assess eye and jaw reflexes, mucous membrane or tongue color; therefore the head should not be entirely covered by drape material.

Self-adhesive backed paper drapes and clear plastic drape material with one adhesive surface are also commercially available.

PREPARATION OF A SURGICAL PACK

A well-organized and consistent surgical pack preparation system can avoid errors and facilitate surgery. Instruments can be cleaned by hand or with an ultrasonic cleaning unit. After cleaning, each instrument should be inspected to ensure that all debris has been removed. After physical cleaning, instruments can be dipped in a commercial protective lubricant solution and allowed to drain dry. Items should be assembled on a tray and arranged in a consistent order. Materials should be placed in sequential order so that items used first are placed on top (see Fig. 3). Packs should not be too densely packed in the autoclave to allow for adequate steam or gas penetration. Indicator test strips can be placed deep within the pack. Packs should be double wrapped, and the outer wrap should be secured with adhesive indicator tape on which is recorded the date of sterilization. When applicable, the type or contents of pack (e.g., laparotomy, thorocotomy) can also be noted on the tape.

Note the following points when opening a sterilized surgical pack. The sterilization date should be checked; the shelf life of wrapped instruments is generally considered to be up to 6 months. The adhesive indicator tape should be noted for the appropriate color change and the pack

description should be checked, when applicable. Packs should be placed on a dry instrument tray and the outer wrapping carefully unfolded by touching only the corners of the outside drape surface. The operator should avoid reaching over the pack. The packs should not be opened too early. The surgeon working without assistance should open the pack immediately before scrubbing. Any other sterilized supplies which can be opened onto a sterile field should be made ready at this time.

PREPARATION OF THE SURGEON

In a laboratory setting, the extent of surgeon preparation will depend on the facilities and the need for strict attention to aseptic technique. Well-equipped surgical facilities, in which sophisticated survival procedures are performed, generally require surgeons to wear appropriate surgical clothing and to scrub, gown and glove. Instruction in such procedures should be done on a one-to-one or small group basis in appropriately designed scrub rooms. To augment the actual hands-on approach or when necessary a video tape demonstration or pictorial diagrams can be used. Readers are advised to consult the references quoted at the end of the chapter for instructional details.

To minimize wound contamination potential, the surgeon should change into surgical scrubs and shoes or wear shoe covers. Head covers and face masks should cover all facial hair. Remove all rings, jewelry and wrist watches before scrubbing. Finger-nails should be trimmed short and cleaned with a disposable nail cleaner. Scrub sinks equipped with leg or foot-operated faucets are ideal. Regular faucets must be turned on, adjusted and not touched again. The hands and forearms are washed for 30 to 60 seconds with a surgical scrub soap. Then a sterile brush is used to methodically scrub all surfaces of the hands, fingers and forearms down to the elbows. Both arms are rinsed and the process repeated starting with fingertips working down to the elbows. The definition of a "complete surgical scrub" is controversial. However, contact times of 3 to 15 minutes and/or 5 to 20 strokes per surface are frequently recommended.

After rinsing, the hands are held together high and rinse water allowed to drip from the elbows. This minimizes the contamination of hands by water dripping from the non-sterile upper arm areas. The surgeon should avoid touching anything at this stage except to dry the hands with a sterile towel. Next the sterile gown is carefully removed from the pack to avoid touching the outside of the gown. It is held away from the body and shaken out. The sleeve hole is located and each arm inserted in turn. Correct gowning requires an assistant to tie the back of the gown at the neck and waist (being careful to touch only the inner gown surface).

Sterile surgical gloves are packaged with the cuff of each glove turned down. This allows the gloves to be put on without the bare hands ever touching the outside surface of the glove. One glove is picked up by the turned-down cuff and pulled onto the hand with the cuff left turned down (see Fig. 4 - 1 and 2). Using the gloved hand, pick up the remaining glove by inserting the fingers into the cuff and pulling it onto the opposite hand (see Fig. 4 - 3). Then the glove cuff is lifted over and onto the gown cuff and the process repeated on the other hand (see Fig. 4 - 4,- 5,- 6). This technique is known as "open gloving." An alternative and more difficult method is closed gloving, descriptions of which can be found in general surgical texts. Remove the powder on the outer glove surface by wiping the gloved hands with a damp sterile gauze. Arms and

hands should be held above the waist at all times. Aseptic technique is maintained when the gowned and gloved surgical team only touches sterilized equipment within the sterile field.

The surgeon working alone faces logistical problems when attempting rigid aseptic protocol as defined above. A proposed practical sequence of steps to minimize errors is presented as follows:

1. Assemble all sterilized supplies.
2. Change into scrubs.
3. Set up table, heat pads and gas machines, check equipment.
4. Weigh animal, induce anesthesia. Prepare animal by hair clip and shave, catheters placed as required.
5. Position and secure animal on the table.
6. Connect to gas machine, connect accessory monitors. Start I.V. lines as required.
7. Make certain that a stable anesthetic plane is attained.
8. Put on cap, mask. Open sterile instrument and prep packs.
9. Using one sterile glove, prepare surgical site with scrub solutions.
10. Put on new sterile glove and drape patient.
11. Remove gloves. Recheck stable anesthetic state. Open glove and gown packs if not included in instrument pack.
12. Perform surgical scrub.
13. Put on gown and gloves.
14. Start surgery.

SUMMARY

The practice of aseptic technique, when performing survival surgical procedures, minimizes the chances that animal health or experimental data will be compromised by post-surgical infections. Aseptic techniques require that appropriate facilities and equipment be available and that the personnel involved be adequately trained. The key element in maintaining an aseptic environment is well-trained personnel who understand the principles of aseptic technique and utilize this knowledge on an ongoing basis.

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Chapter 6

Perioperative Care

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INTRODUCTION

Effectively managed perioperative care improves the animals' recovery by minimizing their pain and distress thus improving the well-being of the animal and the quality of research data which can be derived from that animal. For the purpose of this discussion, the development of an effective perioperative care program will be broken down into three overlapping phases; preoperative planning, intraoperative management and postoperative support. Since the nature of the surgical activity in an institution will largely determine the type of perioperative program required, evaluation of this activity should be an ongoing part of the institution's overall animal care program.

The investigator, animal care staff and institutional veterinarian are all essential members of the perioperative care team. Communication between these team members is essential to minimize patient distress and to create an environment in which a perioperative care program, tailored to the institution's needs, can be effectively managed.

The following discussion will review some of the general principles that should be considered in the establishment and management of an effective perioperative care program. Since an effective perioperative program must be tailored to each institution's needs, the three-phase approach to developing such a program will be discussed in general terms. For more specific details, the reader is referred to the references included in this manual.

PREOPERATIVE PLANNING

Personnel who will be involved with perioperative management and care should be identified with particular attention to assure that they are appropriately trained. These individuals need to be able to identify problems immediately and be familiar with their management. The preplanning inclusion of the support staff, animal care technicians, research technicians and veterinarians helps assure timely treatment of complications. The responsibilities of those involved with perioperative care need to be well defined to assure effective care. Anticipated complications, such as pain, vomiting, and paresis, or special maintenance requirements (e.g., special diets and dressing changes), need to be thoroughly discussed to facilitate the development of an effective perioperative management plan. A secondary plan to handle the unexpected or less likely complications should also be established.

Surgical success is optimized, and more reliable research data is economically generated when animals in good physical condition are used. This starts with the purchase of disease-free laboratory animals. Some latent or enzootic diseases in laboratory animals include: mycoplasmosis in rats, pasteurellosis in rabbits, distemper in dogs, or Sendai or Mouse Hepatitis

Virus in mice. Investigators can consult with the institutional veterinarian or animal care supervisor to identify the most appropriate source of healthy animals for their study.

A presurgical physical exam is often appropriate and serves to identify potential problems. This may identify animals which should be rejected from the study or which need some treatment or special considerations prior to inclusion in the study. Special anesthetic or surgical support requirements may also be determined at this time. This exam should include visual observation, and may also include palpation, auscultation, body temperature, diagnostic laboratory and/or radiographic tests.

The next step in the preoperative planning process is to design the most appropriate anesthetic protocol. Factors such as species, type of surgery, duration, effect on parameters to be measured during surgery, etc., should all be considered. Minimal central nervous system depression consistent with adequate analgesia will hasten postoperative recovery and still provide humane care for the animal. Well planned anesthetic monitoring performed by appropriately trained individuals will help avoid complications. The Animal Welfare Act requires that the institutional veterinarian be consulted when designing a study which has the potential for causing pain to the laboratory animal. This consultation can be of great assistance when designing an anesthetic protocol.

The necessity for presurgical fasting is species specific. For example, rabbits should be fasted for 6 hours prior to intra-abdominal surgery whereas ruminants should be fasted for 48 hours.

The actual surgical procedure, including the following of aseptic techniques, should be planned with a goal to avoid postoperative complications. When selecting a surgical approach, the anatomy and normal body posture should be considered. For example, the dog tolerates lateral thoracotomy with minimal evidence of discomfort whereas a sternal approach is likely to cause significant postoperative pain and slower recovery. In larger animals such as ruminants, a paracostal approach to the abdomen is frequently used instead of a ventral midline due to the high incision tension caused by the heavy abdominal viscera and the sternal resting position favored by these species. The type of suture and suture pattern should also be planned with the species in mind. Many animals will either bite at or rub an incision line, so appropriate wound closure techniques should be used. For example, subcuticular sutures are often used in nonhuman primates who frequently pick at exposed sutures. The inherent difficulty in keeping a wound clean and the capillary action of some uncoated braided fibers which can combine to cause infection of the surgical site should be considered when selecting external suture materials. Planning for the use of intraoperative analgesics and/or long-acting local anesthetics should be considered to minimize postoperative pain.

Cadaver practice and nonsurvival trials can help train investigators in the sophisticated surgical procedures planned. This practice can minimize anesthetic and surgical time thereby promoting uneventful recovery during the actual experiment.

Plans to monitor the animal for signs of postoperative infection should be made. The humane and economic constraints of research make preventable morbidity and mortality from sepsis unacceptable. If the use of antibiotics is anticipated, they should be administered preoperatively

to provide maximum blood levels during the perioperative period. Dosages for antibiotics should be appropriate for the species, with consideration given to species-specific drug toxicity. For example, penicillin is contraindicated in guinea pigs.

The actual location for postoperative recovery needs to be predetermined. Recovery in the laboratory may be adequate for minor procedures; however, major surgeries may require a fully equipped and staffed postoperative recovery room. Transportation to the recovery area also needs to be considered. Care should be taken to avoid injury to the animal during anesthetic recovery (whether by cage mates or by self-inflicted trauma). Position the animal in the transport cage to prevent obstruction of the airway. Just as in the surgery and the postoperative recovery cage, maintenance of body temperature is an important consideration during transport.

INTRAOPERATIVE CARE

To maintain homeostasis during anesthesia, the physiological condition of the animal should be regularly monitored. Cardiovascular function can be monitored using mucous membrane color, auscultation or with electrocardiogram and blood pressure monitors, depending on the situation and resources available. In addition, basic monitoring requires close attention to respiratory function. Mucous membrane color can also give an indication of oxygenation. Respiratory volume and rate can also be observed. Some situations may require the use of a blood gas analyzer. A source of oxygen should be available in case of emergencies even when short, simple procedures are performed. For longer procedures, periodic manual inflation of the lungs will help prevent atelectasis. Adequate cardiopulmonary function during the operative procedure will facilitate a more rapid and uneventful recovery.

Body temperature should also be monitored during surgery, and maintained through the use of heated water blankets, drapes and underpads, hot water bottles, etc. Warming intravenous fluids prior to administration can also aid intraoperative thermoregulation. Hypothermia can be a major problem in animals, particularly small animals whose larger surface area in relation to body mass results in quicker relative heat loss. Hyperthermia is generally a species-specific phenomenom seen in some breeds of pigs and families of dogs.

The small total blood volume of some of the laboratory animal species necessitates careful attention to hemostasis during surgery, to prevent hypovolemic shock. Prolonged surgical procedures or those procedures with significant relative blood loss, may require the use of intravenous fluids to maintain blood pressure and prevent shock. The use of blood transfusions may be a useful adjunct in some situations. Blood type matching is generally not a practical consideration in many of the laboratory animal species.

Positioning of the animal on the table should be done to avoid compromising cardiovascular or respiratory function. Improper positioning can lead to other complications such as aspiration pneumonia, tissue necrosis at pressure points or edema.

Strict adherence to the principles of aseptic technique is necessary to avoid postsurgical infection. These principles can be reviewed in Chapter 5.

Careful handling of tissues during the surgery is another factor that will help minimize postsurgical complications. Traumatic handling of tissue with hands or instruments will delay healing and may lead to such complications as paralytic ileus. Careful replacement of viscera will help avoid complications such as intestinal torsion. Attention should be given to insuring that exposed tissues do not become dessicated. A sterile moist gauze sponge placed over tissues is often used for this purpose. Wound closure techniques with either staples or suture material should be performed in a manner which minimizes tissue damage. Skin sutures should allow for some tissue swelling or necrosis may result. Suture material should be chosen to minimize tissue reaction and should be of the size appropriate for the location and species. It is important to remember that since most laboratory animals are quadrapeds, the full weight of their abdominal viscera is on a midline abdominal incision; therefore, it is usually prudent to use an interrupted pattern in the abdominal wall. A subcuticular pattern in the skin may prevent self-mutilation of skin sutures at the surgical site.

POSTOPERATIVE SUPPORT

The postoperative period can be divided into three phases. The first phase is that of **anesthetic recovery.** This may be the most critical time as it is usually the time of greatest physiologic disturbance and crises can arise quite rapidly. For that reason frequent observation is required. The second phase is that of **acute postoperative care** when the animal is usually maintained in the recovery area until adequate stabilization allows removal to a more standard husbandry situation (i.e., eating and drinking has resumed and critical physiological parameters are within acceptable ranges for the model created). The third phase, and one most often neglected, is that of **long-term postoperative care**. This long-term management is important to return the animal to as normal a physiological and behavioral state as possible. During this phase, routine postoperative procedures such as regular observation of the surgical site, suture removal, observation for return to normal motor function, dressing changes, physical therapy if indicated, etc., should be followed.

Careful observation by trained personnel is the key to good postoperative care. Frequency of monitoring is determined by the nature of the surgical procedure and the stage of recovery. Immediate attention needs to be given to the animal's vital signs. Cardiovascular and respiratory function must be checked and maintained. Specific details about monitoring can be found in Chapter 4, Principles of Anesthesia and Analgesia. Until the animal has recovered from anesthesia, it should be rotated or turned over every 30-60 minutes to facilitate respiration and avoid dependent edema.

Postoperative recovery is best accomplished in a dedicated postoperative recovery room, ideally located adjacent to the operating area and close to those persons responsible for postoperative monitoring. As in all animal rooms, this room should be easy to sanitize, equipped with cages designed to avoid injury to occupants and of appropriate size for the species involved. Usually animals should be individually housed during recovery in cages that have been sanitized between usage. Care should be taken to physically separate species which could transmit disease to one another. Depending on the procedures, this room should be equipped with a variety of items designed to assist with maintenance of homeostasis. Thermometers should be available to monitor body temperature. Hypothermia can be managed with the use of heat lamps, heating

pads, hot water bottles, increased ambient room temperature, or heated cages. Intravenous stands and fluids should be available. Maintenance of adequate respiratory function is imperative to good recovery; therefore, a source of oxygen, endotracheal tubes and laryngoscopes, resuscitation breathing bags and suction should be available. Emergency drugs, miscellaneous dressings and supplies also should be readily available. An additional light source may assist in examination and treatment of postoperative patients. A place to write and maintain individual postoperative records should be present.

Pain, an undesirable aftereffect of surgery, can be difficult to detect due to species and individual variation. Therefore, the investigator must be familiar with the animal's normal posture and behavior. Typical behavioral signs of pain include: guarding the painful area, vocalizing, licking, biting, self-mutilation, restlessness, lack of mobility, failure to groom, abnormal posture, failure to show normal patterns of inquisitiveness, and failure to eat or drink. Understanding the degree of pain involved in various experimental procedures allows a prediction of pain to the animal. Unless there is evidence to the contrary, assume that a procedure or a condition painful for humans will also be painful for animals. When in doubt as to an animal's pain status, analgesics should be given. Subsequent improvement in the animal's condition suggests the previous existence of pain. In addition to the administration of analgesics, parenteral fluids may be continued during the postoperative period. Administration of antibiotics may also be initiated or continued.

Food and water intake is usually restricted during the immediate postoperative period. When food and water are reintroduced to the animal, special diets may be indicated. Intake should be monitored as it is very important to the success of the recovery that the patient maintain an anabolic state. Oral or parenteral supplementation may be necessary in some cases.

Quantity and quality of urine and feces should also be monitored because changes may indicate one of several postoperative complications such as paralytic ileus, renal shutdown or irritation hypermotility. Appropriate treatment can then be initiated. Body temperature should be regularly monitored for signs of hypothermia or infection. The wound site should also be observed for signs of infection, incision breakdown, or self-inflicted trauma. Elizabethan collars and/or bandages can be used to protect the surgical site from self-inflicted trauma. If Elizabethan collars are used, the staff should assure that the animal can reach food and water. Drains, collars and dressings need to be checked and changed regularly.

Long-term postoperative maintenance may include continued observation of incisions, dressing maintenance, suture removal, regular checks to monitor weight loss, and observation for decubital ulcers or edema. Physical therapy may also be needed in some cases for postoperative paresis or paralysis.

PROGRAM EVALUATION

After a procedure and the subsequent postoperative periods, the perioperative plan and its implementation should be evaluated and changes initiated where indicated. This review should have the input of the investigator/surgeon, the research technicians, the veterinarian and the animal care staff. Modifications that result from this evaluation need to be reviewed with all

personnel involved including the Institutional Animal Care and Use Committee, where appropriate. An investigator needs to be prepared to make appropriate changes in a procedure to prevent reoccurrence of avoidable perioperative complications.

SUMMARY

An effective, comprehensive perioperative care program includes: preplanning involving all appropriate personnel; careful performance of the operative procedure in accordance with the predetermined plan; careful postoperative observation by trained personnel during all phases of recovery; and regular evaluation of the postoperative program in light of the institution's overall animal care program. It should be understood that such a program is tailored to the research being conducted within the institution and individualized to the well-being of each animal involved. The investigator, animal care staff and institutional veterinarian are all essential members of the perioperative care team. Communication between these team members is essential to minimize patient distress and to create an environment in which a perioperative care program can be effectively managed.

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Chapter 7
Euthanasia

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INTRODUCTION

A chapter on euthanasia was included in this manual for several reasons. The first was to remind investigators of their responsibilities in assuring institutional compliance with the regulations and requirements of the various regulatory and accrediting agencies as they relate to euthanatizing laboratory animals. The second was to make them aware of the *Report of the American Veterinary Medical Association Panel on Euthanasia* (AVMA) which is recognized by all regulatory agencies as the accepted published guidelines for selecting and evaluating euthanasia techniques. The final reason was to provide the investigator with a summarized version of the AVMA document for quick reference and easy reading.

The term euthanasia is included in the Definition of Terms (9 CFR Part 1) of the Animal Welfare Regulations:

"Euthanasia means the humane destruction of an animal accomplished by a method which produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death."

The AVMA Panel defined euthanasia in terms of the original greek terms "*eu*" meaning good and "*thanatos*" meaning death. The panel goes on to state:

"A "good death" would be one that occurs without pain and distress. In the context of the report euthanasia is the act of inducing humane death in an animal. Euthanasia techniques should result in rapid unconsciousness followed by cardiac or respiratory arrest and ultimate loss of brain function. In addition, the technique should minimize any stress and anxiety experienced by the animal prior to unconsciousness."

The *Guide for the Care and Use of Laboratory Animals* is the basis for complying with the Public Health Science Policy. The *Guide* defines euthanasia as: "the procedure of killing animals rapidly and painlessly."

When selecting a euthanasia technique, remember that death should be accompanied by no pain, no fear and no significant stress.

The key issue then in providing this "good death" is to minimize the pain and distress experienced by the animal. The issue of pain is specifically addressed in some depth in the AVMA Panel report which indicates that for pain to be perceived the nerve impulses stimulated by various noxious stimuli must reach a functional cerebral cortex. A method which causes rapid loss of consciousness would then, by definition, produce a painless death.

The issue of distress is discussed in terms of the continuum represented between stress and distress with particular emphasis on the role that handling and restraint play in minimizing

distress. Fear and stress in the animals to be euthanatized can be minimized or eliminated entirely when they are handled in a humane manner and the individuals charged with this task are well trained in handling the species involved and cognizant of the importance of their role in providing the animal a "good death."

Training of personnel who will be performing euthanasia should include an understanding of the normal behvior of the species involved and how restraint affects that behavior. Personnel should also understand the mechanism by which a euthanasia technique produces unconsciousness and death. Prior to being given ultimate responsibilty for euthanasia personnel should have demonstrated their proficiency under closely supervised conditions.

REGULATIONS AND REQUIREMENTS

The regulations promulgated to implement the amended Animal Welfare Act require that the euthanasia methods used be in accordance with the definition of the term as detailed above, except when scientifically justified in writing by the principal investigator. In addition, the program of adequate veterinary care must contain a mechanism whereby investigators or other personnel receive guidance concerning the euthanasia of the animals they care for and use.

The *Guide* requires that personnel performing euthanasia be trained to use acceptable techniques which should follow the guidelines established by the AVMA. When methods recommended in these guidelines cannot be used, the *Guide* indicates they be reviewed and approved by the institutional veterinarian.

The Public Health Service Policy on Humane Care and Use of Laboratory Animals generally indicates that the recommendations contained in the *Guide* should be those used to establish acceptable animal care and use programs. The use of euthanasia techniques is an exception to this general rule. The Institutional Animal Care and Use Committee is specifically charged with reviewing the methods of euthanasia to assure compliance with the recommendations of the AVMA. Methods deviating from these recommendations must be "justified for scientific reasons in writing by the investigator."

In addition to the requirements contained in the PHS Policy, the PHS Grant Application Form PHS 398 requires the investigator to address the method of euthanasia in Section 6. The fifth point in this section is: "Describe any euthanasia method to be used and the reasons for its selection. State whether this method is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. If **not**, present a justification for not following the recommendations."

Regardless of which set of regulations and/or requirements the use of animals falls under, the key issue in assuring compliance, as it relates to euthanasia of the animals, is adherence to the recommendations of the AVMA Panel on Euthanasia. Responsibility for this compliance begins with the Principal Investigator in designing the project, continues with the Institutional Animal Care and Use Committee in reviewing the project and with the veterinarian in monitoring the program. All those involved should have a working knowledge of the fundamental principles contained in the AVMA document. The remainder of this chapter is designed to provide this

knowledge by summarizing the document and providing an easy-to-follow table applicable in most incidences.

1993 REPORT OF THE AVMA PANEL ON EUTHANASIA

Introduction

In the introduction emphasis is placed upon the need to define and recognize pain in animals and to be able to separate what may be a response to pain from a reflex response. For pain to be experienced, the cerebral cortex and subcortical areas must be functional and any technique which renders these areas nonfunctional would eliminate an animal's ability to feel pain. Emphasis is also placed on the importance of proper restraint in euthanatizing animals to minimize stress to the animals and prevent injuries to the personnel involved. The panel also defined the criteria for training personnel who will be performing euthanasia.

Criteria for selection of an appropriate euthanasia method are listed and include: species involved, means of restraint available, skill of personnel, numbers to be euthanatized and other considerations. While not discussed in this section, the importance of considering the effect of the euthanasia technique on the experimental data must also be of primary concern. Techniques which potentially compromise data could result in more animals being used.

This section of the report concludes with a brief overview of the tables included with the report and introduces a classification scheme for euthanasia techniques: Acceptable, Conditionally Acceptable and Unacceptable. The basis for this classification is the potential for the animal to experience pain or distress when the technique is the sole means of producing death. The techniques classified as Conditionally Acceptable all require performing procedures which could be subject to operator error and thus would have a potential for creating pain and distress. When performed correctly they are humane techniques, but since there are other techniques which do not have the same potential for operator error, they are Conditionally Acceptable. The conditions are that the procedure must be scientifically justified and approved by the IACUC. The approval by the IACUC should be based upon a review of the technical skills of the personnel performing the technique.

General Consideration

This section of the report covers a variety of issues begining with the criteria used by the panel to evaluate the methods discussed in the report. The key issues were the rapid, reliable induction of unconsciouness without pain or distress in a manner that was safe for the personnel performing it. A section also addresses the steps that should be taken when circumstances arise that are not clearly covered by the report and the importance of excercising professional judgment in selecting an appropriate euthanasia method. The importance of verification of death prior to disposal of animals is also emphasized.

Behavioral Considerations

There are two sections on Behavioral Considerations. In the first section, the need to understand the behavior of the animals in order to accurately evaluate the presence of pain and/or distress is emphasized. The need to consider the effect that performing euthanasia can have on staff involved with these procedures is discussed in the second section. This factor is one that must be considered by all those who supervise animal care and use personnel. Performing euthanasia can represent a significant stress for many individuals and can result in job dissatisfaction and/or failure to correctly perform the technique. This is particularly true when physical methods of euthanasia are being used or large numbers of animals are routinely euthanatized.

Modes of Action

Euthanatizing agents terminate life by three basic mechanisms: (1) hypoxia, direct or indirect; (2) direct depression of neurons for vital life functions; and (3) physical damage to brain tissue.

Euthanatizing agents which produce death by hypoxia can act at various sites and the time of onset of unconsciousness can be variable. In some cases, unconsciousness may occur prior to cessation of motor activity. Hence, even if animals demonstrate muscular contractions, they are not perceiving pain.

Euthanatizing agents acting by direct neuronal depression depress nerve cells first, blocking apprehension and pain perception; this is followed by unconsciousness and death.

The use of physical methods for euthanatizing animals places an added responsibility on the principal investigator to insure that those who perform euthanasia are knowledgeable, well-trained individuals, because appropriate application of these methods is essential to produce a painless death.

Inhalant Agents

In this section the use of anesthetic and nonanesthetic gases which either produce hypoxemia or directly depress the CNS is discussed. Of key importance in the use of these agents is properly operating equipment which assures that the appropriate concentration of gas is obtained thus minimizing the potential stress on the animals and the time necessary to produce unconsciousness. Of equal importance is the need to protect personnel from these gases. Many gases such as carbon monoxide and the anesthetic gases can cause serious health problems, while others such as ether must be used in designated areas.

When using gases to euthanatize animals, it important that the stress to the animal be minimized. Stress can result when the animal comes into contact with the liquid forms of these agents, when the animal is placed into a chamber devoid of enough oxygen to create a suffocating environment or when the gas is forced into the chamber under pressure in a manner which upsets the animals. Since neonatal animals appear to be resistant to hypoxia, the use of inhalant agents in puppies and kittens under 16 weeks of age is not recommended.

Whereas many of the gaseous agents require highly sophisticated equipment and are expensive or difficult to obtain or use in an institution, CO2 is inexpensive to use, poses little risk to

personnel, is quite effective and does not interfere with most types of research. If CO2 is not available in your institution, ask the veterinarian about the possibility of acquiring the necessary equipment for use as a centralized resource.

Noninhalant Pharmacological Agents

The majority of agents included in this group are barbituric acid derivatives which have the advantage of producing a rapid loss of consciousness but have the disadvantage of being controlled drugs for which a Drug Enforcement Agency (DEA) number must be provided at purchase and special records of usage must be maintained. Whenever possible these drugs should be administered intravenously. In animals under 7 kg the intraperitoneal route is acceptable.

T-61 is an injectable noncontrolled drug which has been used in the United States but is no longer commercially available in this country. Should it still be available in your institution, it must be administered intravenously and in accordance with the labeled instructions. For this reason the use of this drug is discouraged except in the hands of highly skilled personnel.

Physical Methods

The methods included in this section produce unconsciousness by direct damage to the brain. With the exception of the focused beam microwave, all of these methods are classified as Conditionally Acceptable means of euthanasia. Their use is generally recommended only when other acceptable means have been excluded, when the animals are sedated or unconscious and when their use has been scientifically justified. The key to their use is that they must be performed correctly to produce the "good death" described earlier in this chapter. Since these techniques require the most skill to perform, they are most likely to be affected by human error. To minimize the chance of human error, the personnel performing these techniques must be properly trained and the responsibility for this training lies with the principal investigator. For those techniques commonly employed in research, the AVMA Panel charges the IACUC with reviewing those protocols using physical techniques to assure that their use has been scientifically justified and that those performing the procedures are appropriately trained.

When the 1993 Panel was formed, it was charged with addressing contemporary issues including the use of decapitation. This charge was in response to the discussions that arose within the biomedical community following the release of the recommendations for the use of the decapitation and cervical dislocations contained in the 1986 report. The recommendations contained in the 1993 Panel Report are consistent with the recommendations for all of the Conditionally Acceptable methods. Investigators who must use these techniques should adequately justify their use scientifically to their IACUC and the various funding agencies and insure that those performing these techniques are adequately trained.

Ongoing Evaluation of Euthanasia Methods

Once a method of euthanasia has been selected and approved by the IACUC, it should be evaluated by the principal investigator on an ongoing basis to assure that it is indeed meeting the goal of producing a "good death," by rapid loss of consciousness and a painless death. The

procedures should also minimize the potential psychological stress to the animals and personnel involved. The cost of the procedure, the compatibility with the research goals and the safety of the personnel performing the techniques should also be monitored. Where controlled drugs are used the potential for abuse must be considered, but the use of commercially available euthanasia solutions would almost eliminate this concern.

Summary of Recommendations for Euthanasia

The table included with this chapter is an attempt to summarize the 1993 Report of the AVMA Panel on Euthanasia. It is intended to be an easy-to-use reference source. When questions arise concerning the method of euthanasia to be used, the institutional veterinarian should be consulted for additional information.

SUMMARY

The use of animals in biomedical research is a privilege. That privilege places a great deal of responsibility with the supervising scientist to assure compliance with the highest scientific, regulatory and societal values. At no time is this compliance more subject to review and scrutiny than when it becomes necessary to kill the animals that have been involved in a study. The importance of this final step is emphasized by the prominence of the issue of euthanasia in the regulations, policies and guidelines of the various regulatory, accrediting and funding agencies. If the "good death" definition is employed as the standard for technique evaluation, then one should be able to proceed with the confidence of carrying out the responsibility that comes with the privilege of using animals in research, teaching and testing.

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SUMMARY OF RECOMMENDATIONS FOR EUTHANASIA

METHODS OF EUTHANASIA	SPECIES	REMARKS ON SUITABILITY
Inhalant Agents:a **Anesthetics**	**Because in the liquid state most inhalant anesthetics act as topical irritants, animals should be exposed to the vapors of the anesthetic only. Air or oxygen must be provided during the induction period.**	
Ether	Cats, young dogs, birds, rodents, amphibians, reptiles and other small animals	Acceptable - Should be used in approved hood according to institutional guidelines.
Halothane	Cats, young dogs, birds, rodents, amphibians, reptiles and other small animals	Acceptable - Considered the most effective inhalant anesthesia for euthanasia.
Methoxyflurane	Cats, young dogs, birds rodents, amphibians, reptiles and other small animals	Acceptable
Isoflurane	Cats, young dogs, birds, rodents, amphibians, reptiles and other small animals	Acceptable
Nitrous Oxide (NO)	Cats, young dogs, birds, rodents, amphibians, reptiles and other small animals	Conditionally Acceptable - when used with other inhalant anesthetics.
Enflurane	Cats, young dogs, birds, rodents, amphibians, reptiles and other small animals aShould not be used in dogs and cats < 16 weeks of age	Acceptable

Inhalant Agents:	**Most agents in this category require the use of special equipment.**	
Non-Anesthetics		
Carbon Dioxide (CO2)	Dogs, cats, rodents, rabbits, amphibians, reptiles and other small animals	Acceptable - Bottled gas preferred, requires special equipment.
Carbon Monoxide (CO)	Dogs, cats, rodents, rabbits and amphibians	Acceptable - Bottled gas only, requires special equipment.
Nitrogen (N2)/Argon (AR)	Dogs, cats, rodents, and rabbits	Conditionally Acceptable - Animals should be anesthetized or heavily sedated.
Non-Inhalant Pharmacologic Agents	**Use of these agents requires adequate restraint and mastery of appropriate injection techniques**.	
Barbituric Acid	Most species acceptable alternative in small animals (<7 kg).	Acceptable - Should be administered by IV whenever practical. IP is an

SUMMARY OF RECOMMENDATIONS FOR EUTHANASIA

METHODS OF EUTHANASIA	SPECIES	REMARKS ON SUITABILITY
T-61	Mammalian species	Acceptable - When administered IV. Not available in U.S.
Tricaine methanesulfate (MS 222)	Fish and amphibians	Acceptable
Benzocaine Hydrochloride	Fish and amphibians	Acceptable
Physical Methods	**These methods require that the user have complete mastery of the techniques to be used**.	
Electrocution	Mammalian species	Conditionally Acceptable - Two step procedure, requires special equipment.
Penetrating Captive-Bolt Pistol	Large animals, dogs and rabbits	Conditionally Acceptable - Requires special skills and equipment.
Cervical Dislocation	Small mammals, birds, rats (200 gm or less) and rabbit under 1 kg	Conditionally Acceptable - Proper technique is essential . Larger rats and rabbits require special equipment and demonstrated proficiency.
Decapitation	Small mammals, birds, amphibians, fish and reptiles	Conditionally Acceptable - Should be followed by pithing in poikilotherm.
Pithing	Some poikilotherms	Conditionally acceptable - Death not immediate unless double pithed.
Microwave	Small rodents	Acceptable -Requires special restraint and focusing equipment. Microwave ovens are absolutely condemned for use.
Adjunctive Methods When properly done these methods induce unconsciousness, but do not ensure death.		
Stunning	Small mammals, fish, amphibians and reptiles	Single blow to head followed by a method that ensures death.
Exsanguination	Most species	Must be rendered unconscious by some other method first.

Chapter 8
The Animal Welfare Information Center of The

National Agricultural Library

Jean Larson, M.A., B.A., A.A.S

INTRODUCTION

The Animal Welfare Information Center (AWIC) was established at the National Agricultural Library (NAL) in 1986 as a result of the amended Animal Welfare Act (PL 99-189). In the act, Congress mandated that:

"The Secretary [of the U.S. Department of Agriculture] shall establish an information service at the National Agricultural Library. Such service shall, in cooperation with the National Library of Medicine, provide information:

(1) pertinent to employee training;

(2) which could prevent unintended duplication of animal experimentation as determined by the research facility;

(3) on improved methods of animal experimentation, including methods which could -

(A) reduce or replace animal use; and

(B) minimize pain and distress to animals, such as anesthetic and analgesic procedures.

With appropriations of $750,000 per year directed to the Library through the Animal and Plant Health Inspection Service (APHIS) for fiscal years 1987 and 1988, AWIC was established as an information center within NAL.

NAL AND AWIC

Information centers at NAL exist in an interdependent relationship within the NAL physical and administrative structure. The information center concept was developed within the Library in order to better serve the diverse but often narrow-focused subject requirements of much of the NAL clientele. A dozen such centers similar to AWIC serve unique user groups in areas such as food and nutrition, aquaculture, biotechnology, etc. Each center's staff members have subject area expertise, participate in outreach and networking activities and develop publications and projects tailored to the needs of the user groups. Due to differing needs of the user groups, unique activities, programs, support services and responsibilities have developed. To provide the AWIC patron with a better understanding of the unique aspects of the AWIC program and how to access AWIC and NAL services, a more detailed exploration of the activities, products and projects is provided below.

National Agricultural Library

The Library facility is located on the grounds of USDA's Agricultural Research Center in Beltsville, Maryland. Currently NAL houses over 2 million items including books, journals, newsletters, proceedings, reports, maps, microforms, slides, video recordings, films, posters and rare manuscripts. The scope of the collection reflects published materials that have supported the activities, research and regulatory responsibilities of the U.S. Department of Agriculture. Obviously, the collection is strong in what is traditionally considered agricultural subjects--food and fiber production. It is also strong in applied veterinary science, animal and human nutrition, forestry, natural resources, etc.

Much of the collection is available for use by the U.S. public, and some activities support an international community. Documents and audiovisual materials are made available to non-USDA patrons via interlibrary loan. Interlibrary loan requests are honored from any established library--corporate, academic, organizational, public, etc.-- in the United States. Photocopies of articles (according to U.S. copyright laws) are available on a worldwide basis for a standard charge per page. USDA personnel are serviced either through field libraries, the land-grant universities or by direct request.

There are several fact sheets available from NAL that explain in detail what services are available, who is served and how to request service. They are listed below:

1. *Document Delivery Services to Individuals.*
2. *Document Delivery Services Available to Foreign Libraries, Information Centers and Commercial Organizations.*
3. *Availability of Documents.*
4. *Guidelines for Requesting Materials.*
5. *Guide to Services.*

The facility is open to the public from 8:00 a.m. to 4:30 p.m., Eastern time, Monday through Friday, and closed on Federal holidays. Formal tours are available to visitors on request.

Animal Welfare Information Center

As an information center within NAL, AWIC's role is to provide reference services, bibliographies and listings of relevant documents, establish the subject scope for acquisitions and indexing, conduct outreach activities and interact with user groups. In turn, AWIC relies on NAL for the purchase and maintenance of the subject relevant part of the collection, lending services and other technical services that ensure user access. Because of these cooperative efforts, the substantial resources of the Library enable the AWIC staff to supply information on a broad array of subjects, even though the main thrust of AWIC's subject responsibilities are determined by the Animal Welfare Act (AWA).

Materials commonly accessed for AWIC's clientele cover important technical, ethical, political and legal issues related to the welfare of animals. The publication *Animal Welfare Information Center Scope Notes for Indexers,* which has served as an internal policy document, outlines the animal and subject areas considered to be within the scope both for acquisition of published

materials for the NAL collection and for indexing these materials for the AGRICOLA database (AGRICOLA will be discussed below).

Briefly, subjects indexed include: anesthesia, analgesia, euthanasia, training and education of technicians and investigators, transportation and acquisition of animals, species husbandry, animal behavior, environmental factors affecting animals, laboratory animal management, Institutional Animal Care and Use Committees, regulations and legislation concerning the humane treatment of animals, philosophies of animal welfare/rights and alternatives to the use of animals in research, testing and education.

NAL subject coverage overlaps somewhat with the National Library of Medicine (NLM), but there are many types of non-referred materials being added to the NAL collection that are not collected by NLM (training materials, reports, course syllabi, etc). This division of effort expands the resources available to the user group.

AGRICOLA

One result of computer technology has been the advent of computer databases in general and the bibliographic database in particular. These have become important repositories referencing the world's scientific literature. Databases enable information providers to develop customized bibliographies for the patron's specific information needs. To access as well as disseminate the extensive information resources in the NAL collection, NAL staff generate an internationally available database called AGRIcultural On-Line Access (AGRICOLA). Established in 1970, AGRICOLA contains over 3 million citations to books, articles and audiovisuals covering agriculture and related subjects. Contrary to public opinion, there is no database specifically for animal welfare generated by AWIC. Many published books, journals, videotapes, reports, etc. relevant to AWIC are included in the AGRICOLA database. Approximately one-fifth of the AGRICOLA database is devoted to citations on animal production, laboratory animal science, veterinary medicine and animal welfare.

AGRICOLA is currently available through DIALOG Information Retrieval Service (in files 10 and 110). AGRICOLA may be accessed from the above commercial vendors using standard dial-up computer terminals. The publication *Searching AGRICOLA for Animal Welfare* details thesaurus terms, strategies and techniques for efficiently searching the database for animal welfare topics on DIALOG. The database is also available commercially on compact disc through Silver Platter. (For further information regarding DIALOG services call 800-334-2564.)

AWIC SERVICES AND ACTIVITIES

Reference

Reference services are available to anyone who calls the Center. However, most AWIC users are biomedical researchers, veterinarians, animal technicians and caretakers, USDA regulatory staff, facility managers, academics, organization personnel, curators in zoological parks, librarians and students.

These services may be a quick answer, a suggested general resource, reference to an article and/or a database search. In the event that an extensive database search is suggested, the patron has the option of a free abbreviated search or to purchase the more comprehensive online DIALOG database search on a cost recovery basis.

Databases routinely utilized by the AWIC staff include the DIALOG files (numbers in parentheses are the file numbers in the DIALOG system), AGRICOLA (10, 110), MEDLINE (154, 155), EMBASE (72, 172, 173), BIOSIS PREVIEWS (5, 55) CAB ABSTRACTS (50, 53) and Life Sciences (76). Under some circumstances, computer, legal or other peripheral subject-matter databases are utilized.

Since many organizations and institutions have full-service libraries with the capability of multi-database searching, AWIC staff are a back-up resource, providing materials, other information resources and advice to librarians. For those organizations/individuals with limited information resources, AWIC can provide more comprehensive services.

AWIC staff regularly maintain and use a variety of subject-related vertical files that include: selected articles, copies of Federal bills and legislation, published materials from a variety of organizations, subject files of acquired books and audiovisuals, and clippings from newspapers and magazines. These files provide a source of personal contacts, information about related organizations, and serve as a quick reference to current events and popular animal-related topics.

AWIC Publications

Several types of publications are generated by the AWIC staff. Most publications fall into the following five NAL publication series: *Quick Bibliography (QB), Special Reference Brief (SRB), AWIC Series, Fact Sheet,* or *The Animal Welfare Information Center Newsletter*, a free quarterly newsletter published by the staff. The various publication series and their unique aspects are discussed below.

Quick Bibliography (QB). *QB's* are downloaded from the most current file of AGRICOLA, therefore, they contain approximately 300 recent, bibliographic references to a portion of the topical literature. The citations are listed in alphabetical order by title and may have abstracts. Author and subject indices are provided as additional access points. *QB's* on topics of continuing interest are updated on a yearly basis. Topics include animal disease models, issues regarding the use of animals, welfare issues of livestock animals, and many other subjects.

Special Reference Brief (SRB). Several of the AWIC publications fall into this series due to the subject limitations of AGRICOLA. *SRB's* are very labor-intensive because they are produced from multiple sources, both electronic and manual. They are more comprehensive than *QB*'s and include carefully selected bibliographic citations on the topic. The *SRB* format includes a brief introduction to the topic, a selected listing of references organized by category and an author listing. They can contain additional non-bibliographic information such as relevant organizations, other information resources, etc. There are no limits on either numbers of citations or age of the cited documents. All *SRB's* are reviewed by a highly respected expert in the field.

Topics covered in this series are euthanasia, exercise for dogs, various toxicity testing methods, animal models of disease, etc.

AWIC Series. Feedback from user groups indicated that some non-bibliographic information such as listings of the audiovisuals, Federal legislation, computer simulation models for teaching, etc. was needed. Since these types of publications did not fall into an established series, the *AWIC Series* was started to accommodate the diverse nature of the information. These publications are generated from many sources.

Fact Sheets. *Fact Sheets* contain information intended to help a patron use the Center more effectively. They are usually limited to one or two pages and designed to answer questions that are often asked about the services of AWIC. Currently, *Fact Sheets* are available through various electronic and non-electronic ways of contacting AWIC, tips on searching for alternatives, information products in electronic format, etc.

Animal Welfare Information Center Newsletter. This free quarterly newsletter contains articles by guest authors on topics related to various animal care and use issues. Each issue includes a listing of newly introduced Federal legislation, recently released AWIC publications and upcoming national meetings. In the past there have been articles on environmental enrichment for gum feeding animals, the psychological issues of people who use animals in research, and how to scientifically determine animal well-being, etc.

Efforts continue to address new and old welfare issues through either new publications or updates of old publications.

At the present time, all AWIC publications are supplied without charge. It should be noted that AWIC-produced publications are not copyrighted and may be photocopied without permission. For a current listing of publications available, please contact the AWIC.

Referrals

AWIC staff have developed an extensive network with subject experts and organizations active in the area of animal care and use. In order to adequately answer some patrons' questions, AWIC staff may recommend that a patron tap into the expertise of respected individuals and groups. In all cases, the recommended expert/organization has agreed to be a resource.

OUTREACH ACTIVITIES

To ensure that all of the regulated users know of the products, services and other program activities, the AWIC staff engage in a variety of outreach-oriented activities.

Staff members:
are available for presentations at seminars and conferences.
exhibit at a variety of major conferences and annual meetings. While exhibiting, publications are distributed, questions are answered, and demonstrations of topical electronic documents, or software programs are given.

share information and establish linkages with other groups.
participate on various USDA and/or non-governmental committees.
provide articles for a variety of publications on request.
conduct workshops on information science and meeting the mandate of the AWA.
host visitors and/or visiting scholars.
provide meeting space for USDA and non-USDA groups engaged in Center-related activities.

Cooperative Projects

From its inception AWIC has supported projects that promote the mandates of the Animal Welfare Act. Center support for these projects has been of various sorts--financial in the form of grants and cooperative agreements, staff time and expertise, and the absorption of printing and/or distribution costs.

The types of projects that have been completed include the collaborative production of bibliographies, manuals and handbooks, conference proceedings and training audiovisuals. A complete listing is available on request.

A 12-minute tape entitled *"Resources Today for the Research of Tomorrow"* is available on loan. This video provides a brief overview of the organization and resources of AWIC. It can be used as part of an institution's training resources as an introduction to AWIC for faculty and staff.

For your convenience there are various ways that you can contact the Center. Staff members are available to take your calls between 8:00 a.m. and 4:30 p.m., Eastern time.

1. Direct line via telephone - (301) 504-6212. (There is a telephone answering machine on this line.)
2. Coordinator - (301) 504-5215.
3. FAX machine - (301) 504-6409.
4. INTERNET - awic@nalusda.gov

A table-top exhibit describing the purpose and functions of the Center is available for loan to interested groups. The display is sent via overnight express mail and copies of AWIC publications may be included with the exhibit. Return shipment must be arranged and paid for by the requestor.

Patrons are welcome to visit AWIC and other NAL offices on weekdays from 8:00 a.m. to 4:30 p.m. A tour of the Library facility is available by appointment. The AWIC mailing address is:
Animal Welfare Information Center
National Agricultural Library
10301 Baltimore Blvd.
Beltsville, MD 20705-2351

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Chapter 9
Organizations, Associations and Societies

Marilyn J. Brown, D.V.M., M.S.
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and
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ORGANIZATIONS, ASSOCIATIONS AND SOCIETIES

AAALAC
American Association for Accreditation of Laboratory Animal Care
Voluntary accrediting body for demonstrating achievement of certain standards for an animal care and use program.
Albert E. New, Executive Director, 11300 Rockville Pike, Suite 1211, Bethesda, MD 20852-3035. (301) 231-5353.

AALAS
American Association for Laboratory Animal Science
A professional association for veterinarians, animal care workers, managers and manufacturers involved in laboratory animal science. Publisher of *Laboratory Animal Science* and *Contemporary Topics*.
Michael Sondag, Executive Director, 70 Timber Creek Drive, Suite 5, Cordova, TN 38018. (901) 754-8620.

AAMC
Association of American Medical Colleges
Through its ad Hoc Group for Medical Research Funding published recommendations and guidelines on the use of animals in research. 2450 N. Street NW, Washington, DC 20037. (202) 828-0455.

ACLAM
American College of Laboratory Animal Medicine
Certifies veterinarians (Diplomates) who achieve certain standards in Laboratory Animal Medicine.
Charles W. McPherson, Secretary-Treasurer, 200 Summerwinds Drive, Cary, NC 27511. (919) 851-3126.

AMA
American Medical Association
A professional association of physicians. Published a White Paper on the *Use of Animals in Biomedical Research*. 515 North State St., Chicago, IL 60610. (312) 464-5000.

APA
American Psychological Association
An association founded to advance the understanding of basic behavioral principles. Publishes a

detailed statement on the care and use of animals entitled *Guidelines for Ethical Conduct in the Care and Use of Animals*. 750 Firt St. NE, Washington, DC 20002-4242. (202) 336-6000.

APHIS
Animal Plant and Health Inspection Service
That division of the U.S. Department of Agriculture that administers the federal Animal Welfare Act. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, REAC, 6505 Belcrest Rd., Room 268-FB, Hyattsville, MD 20782. (301) 436-7833.

APS
American Physiological Society
First scientific society to adopt a written statement on the prevention of cruelty to research animals. Distributes the *Guiding Principles in the Care and Use of Animals* to members for signing and posting. 9650 Rockville Pike, Bethesda, MD 20814. (301) 530-7164.

ASLAP
American Society of Laboratory Animal Practitioners
An organization of veterinarians engaged or interested in the practice of laboratory animal medicine.
Brad Godwin, Secretary-Treasurer. 6431 Fannin, Room 1.132, Houston, TX 77030- 1501. (713) 792-5127.

AVMA
American Veterinary Medical Association
A professional association of veterinarians. In 1993, the AVMA published recommended standards for euthanasia procedures which are accepted as national guidelines. 1931 Meacham Rd., Schaumburg, IL 60173- 4360. (708) 925-8070.

AWI
Animal Welfare Institute
A national organization active in laboratory animal welfare issues. Its sister organization, the Society for Animal Protective Legislation, is a major lobbying force. The AWI encourages lay persons to serve on IACUC's and has a number of publications pertinent to laboratory animal welfare.
Mrs. Christine Stevens, P.O. Box 3650, Washington, DC 20007. (202) 337-2332.

AWIC
Animal Welfare Information Center
The information center of the National Agricultural Library established as result of the 1985 amendment to the Animal Welfare Act. See Chapter 8. Animal Welfare Information Center, National Agricultural Library, Beltsville, MD 20705. (301) 504-6212.

CAAT
Center for Alternatives to Animal Testing
Established in 1981 to encourage and support the development of non-animal testing methods. The center supports grants, sponsors symposia and publishes a variety of materials.

Johns Hopkins School of Public Health, 615 North Wolfe St., Baltimore, MD 21205. (410) 955-3343.

CALAS
Canadian Association of Laboratory Animal Science
A professional association for veterinarians and technicians involved with laboratory animal science.
Donald G. McKay, Executive Director, BioScience Animal Services, M524 Biological Sciences Building, The University of Alberta, T6G 2E9 Canada. (403) 432-5193.

CCAC
Canadian Council on Animal Care
The national body that establishes policy on the care and use of laboratory animals in Canada. Has many useful publications. 1000-151 Slater Street, Ottawa, Ontario, K1P 5H3. (613) 238-4031.

DEA
Drug Enforcement Administration - United States Department of Justice
The regulatory agency responsible for the enforcement of laws pertaining to controlled substances. Licenses to use controlled substance are obtained from this agency.
DEA Central Station, Washington, DC 20037. (202) 307-7250.

FASEB
Federation of American Societies of Experimental Biology.
A federation of leading professional associations, including, physiologists and pharmacologists and other major disciplines involved with animal experiments. 9650 Rockville Pike, Bethesda, MD 20814. (301) 530-7075.

FBR
Foundation for Biomedical Research
A nonprofit educational organization established to inform the American public about the proper and necessary role of animal models in biomedical research and testing. 818 Connecticut Ave., N.W., Suite 303, Washington, DC 20006. (202) 457-0654.

FDA
U.S. Food and Drug Administration, Office of Animal Care and Use
The federal agency responsible for enforcement of the Good Laboratory Practices (GLP) regulations. 7500 Standish Place, Room 485 Rockville, MD 20855. (301) 295-8798.

IASP
International Association for the Study of Pain
Publishes the journal *Pain* and has developed **"Ethical Standards for Investigators of Experimental Pain in Animals."** 909 NE, 43rd St., Suite 306, Seattle, WA 98105-6020. (206) 547-1703.

ILAR
Institute of Laboratory Animal Resources
That part of the National Academy of Sciences which has responsibility for laboratory animal issues. ILAR is responsible for preparing part of the Public Health Service Policy entitled *Guide for the Care and Use of Laboratory Animals*. 2101 Constitution Ave., NW, Washington, DC 20418. (202) 334-2590.

ICLAS
International Council for Laboratory Animal Science
Osmo Hanninen, University of Kuopio, SF-70211, Kuopio 10, Finland.

NABR
National Association for Biomedical Research
An association of biomedical facilities concerned with legislation on laboratory animal welfare and with presenting information about the benefits to human health resulting from animal experiments.
Frankie Trull, Executive Director, 818 Connecticut Ave., NW, Suite 303, Washington, DC 20006. (202) 857-0540.

NAL
National Agricultural Library
(See AWIC)

NAS
National Academy of Sciences
Established the National Research Council in 1916 for the purpose of furthering knowledge and advising the federal government. The *Guide for the Care and Use of Laboratory Animals* was reviewed and approved by the Governing Board of the National Research Council. See ILAR.

NIH
National Institutes of Health
A federal agency which disburses funds for biomedical research and sets policy on laboratory animal welfare, (Public Health Service Policy). Office of Animal Care and Use, 9000 Rockville Pike, Bethesda, MD, 20892. (301) 496-5793.

NSF
National Science Foundation
A federal agency responsible for disbursement of funds in support of non-biomedical research, i.e., zoological and wildlife research. 1800 G. Street N.W., Washington, DC 20550. (202) 357-9854.

OLAW
Office of Laboratory Animal Welfare, National Institutes of Health
The office which oversees compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. 9000 Rockville Pike, Building 31, Room 4809, Bethesda, MD 20892. (301) 496-7041.

PHS
Public Health Service
Comprises several federal agencies that are involved with either medical research or provision of medical health services. The National Institutes of Health is the major agency within the PHS relevant to laboratory animal issues.

SCAW
Scientists Center for Animal Welfare
A nonprofit educational organization of scientists that upholds justifiable animal research and conducts programs to help ensure compliance with federal policies, introduction of alternatives where feasible, and sensitivity to humane issues among scientists.
Lee Krulisch, Executive Director, Golden Triangle Building One, 7833 Walker Dr., Suite 340, Greenbelt, MD 20770. (301) 345-3500.

USDA
United States Department of Agriculture
The federal agency responsible for enforcement of the federal Animal Welfare Act (see APHIS).

Chapter 10
General References

John C. Schofield, B.V.Sc., M.R.C.V.S.
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