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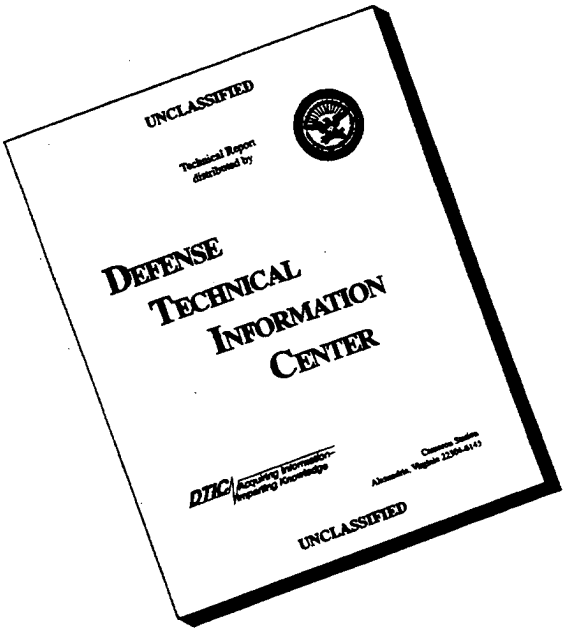
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13. ABSTRACT *(Maximum 200*

This grant provides partial core support for the Institute of Laboratory Animal Resources (ILAR), which is a component of the Commission on Life Sciences, one of the principal operating units of the National Academy of Sciences (Academy). The Academy operates as a private, non-profit institution charged with providing advice to agencies of the federal government on matters of science and technology. ILAR provides information on the selection, care, and use of biologicals and animals used in research, testing, and education. ILAR's best known report, the *Guide for the Care and Use of Laboratory Animals*, was revised in 1994-5 to be published in 1996. Oversight for the work of ILAR is provided by ILAR Council, a standing committee of 15 scientists, veterinarians, and ethicists, which meets three times each year to review all aspects of ILAR's program and develop new initiatives. ILAR has two types of programs: core and special projects. This grant supports the core program, consisting of the meetings and activities of Council and staff in the publishing of *ILAR Journal*, activities of the Animal Models and Genetic Stocks Information Program, and International Activities. Partial support is also provided for the development of special projects and convening of workshops.

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FOREWORD

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____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

T. Lamar Tisdale 3/15/96

PI - Signature / Date

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List of personnel receiving pay from the contract

APPENDIX 1 - ILAR Committee Rosters
Institute of Laboratory Animal Resources Council
Committee to Revise the *Guide for the Care and Use of Laboratory Animals*
Committee on Rodents
Committee on Occupational Health and Safety in the Care and Use of Research Animals
Committee on the Psychological Well-being of Nonhuman Primates
Committee on the Long-term Care of Chimpanzees in Biomedical and Behavioral Research
Participants: Workshop on the North American Free Trade Agreement

APPENDIX 2 - ILAR Reports
Guide for the Care and Use of Laboratory Animals, 7th ed. (In press)
Rodents: Laboratory Animal Management Series (In press)

ILAR Journal, Volume 37, Number 1. Winter 1995
ILAR Journal, Volume 37, Number 2, Spring 1995
ILAR Journal, Volume 37, Number 3, 1995 (In press, Table of Contents attached)
ILAR Journal, Volume 37, Number 4, 1995 (Table of Contents attached)
ILAR Journal, Volume 38, Number 1, 1996 (Table of Contents attached)

1995 Annual Report
Institute of Laboratory Animal Resources
National Research Council
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INTRODUCTION

This grant provides partial core support for the Institute of Laboratory Animal Resources (ILAR), which is a component of the Commission on Life Sciences (CLS), one of the principal operating units of the National Academy of Sciences (Academy). Under an 1863 congressional charter, the Academy operates as a private, non-profit institution charged with providing advice to agencies of the federal government on matters of science and technology. Since 1952, ILAR has served this role in regard to the selection, care, and use of biologicals and animals used in research, testing, and education. ILAR's best known report is the *Guide for the Care and Use of Laboratory Animals* (*Guide*), of which preparation of the seventh edition was completed during this grant year. ILAR consists of a staff of four to six depending on the nature of the work underway. Oversight for the work of ILAR is provided by ILAR Council and the CLS.

ILAR Council is a standing committee of 15 scientists, veterinarians, and ethicists, which meets three times each year to review all aspects of ILAR's program and develop new initiatives. John VandeBerg, Scientific Director, Southwest Foundation for Biomedical Research is the chairman of Council (see Appendix 1: ILAR Committee Rosters).

ILAR's work follows procedures prescribed in the charter of the Academy and operating procedures of the National Research Council (NRC), the administrative arm of the Academy. When federal agencies request the advice of the NRC, a series of events is set into motion that typically leads to published recommendations on the desired topic. The strength of this process is achieved by selecting and appointing a balanced committee of experts that produces a report in accordance with NRC operating procedures. Separately appointed committees of experts provide anonymous reviews of each report. Staff supports and enables this process behind the scenes, which is a component of ILAR's core program.

BODY

ILAR has two types of programs: core and special projects. The core program includes the work involved with supporting ILAR's advisory council, maintaining ILAR's ongoing programs, and initiating and prioritizing ILAR's special projects. This grant supports the core program. Special projects are those accomplished by NRC-appointed volunteers who serve on NRC-appointed committees. All ILAR committees work under the auspices of the NRC, and are overseen by the CLS. Committee reports, which usually take 18 to 36 months to complete, are submitted for independent peer review. Reports are normally published by the National Academy Press. In 1995, 80 scientists, veterinarians, medical ethicists, public members, and institutional administrators served on ILAR committees (see Appendix 1: ILAR Committee Rosters). Many others participated in ILAR-held workshops and public fora.

I. Core Activities

A. ILAR Council.

ILAR Council met three times in 1995 to review ongoing work and plan new activities: March 17-18 at the Arnold and Mabel Beckman Center, Irvine, California; July 20-21 at the National Academy Sciences Building, Washington, D.C.; and October 10-11 at the National Academy Sciences Building, Washington, D.C. The Beckman and J. Erik Jonsson Woods Hole Center, Woods Hole, Massachusetts are study sites of the Academy and enable greater participation of west and east coast members, respectively. ILAR Council (Roster in Appendix I) works under the general guidance of ILAR's Mission Statement:

> *The Institute of Laboratory Animal Resources (ILAR) develops guidelines and disseminates information on the scientific, technological, and ethical use of animals and related biological resources in research, testing, and education. ILAR promotes high-quality, humane care of animals and the appropriate use of animals and alternatives. ILAR functions within the mission of the National Academy of Sciences as an advisor to the federal government, the biomedical research community, and the public.*

The primary responsibility of Council is to review all ongoing activities. This is the main focus of Council meetings and innumerable conference calls between meetings.

Council reviewed the progress of the following current committees during 1995. See below for project descriptions.

Occupational Health and Safety in Care and Use of Research Animals

Psychological Well-being of Nonhuman Primates

Revision of the Guide for the Care and Use of Laboratory Animals (Guide)

Rodents: Laboratory Animal Management Series

Long-term Care and Use of Chimpanzees in Biomedical Research

Workshop on Collection and Importation of Biological Materials, Animals, and Plants

Workshop on the North American Free Trade Agreement (NAFTA)

Expansion of *ILAR News* to *ILAR Journal*

Council initiated or reviewed the activities of the following new projects during 1995. See below for project descriptions.

Workshop on Biological Resource Databases

Implementing The Science Standards: the Appropriate Use of Animals and Their Alternatives in Education

Transgenic Organisms: Benefits and Risks

The Cost of Animal-based Research

Workshop: Modernization of Laboratory Animal Management Reports

The Role of New and Emerging Models in Biomedical and Behavioral Research

In addition to these ongoing projects and new initiatives (see II. Special Projects), Council and staff concentrated on the activities of the following three core activities.

B. ILAR Journal

ILAR Journal, a quarterly, peer-reviewed publication, provides thoughtful and timely information for all those who use, care for, and oversee the use of laboratory animals. The audience of *ILAR Journal* includes more than 3,500 investigators in biomedical and related

research, institutional officials for research, veterinarians, and members of animal care and use committees. The *ILAR Journal* Editorial Board, a subcommittee of the ILAR Council, plans each issue around a chosen theme and carefully solicits authors to best present a balanced view of the topic. Each submission is assessed by the Editorial Board and then peer-reviewed prior to acceptance for publication. Margaret Landi is Editor-in-Chief, and Richard C. Van Sluyters and Charles McCarthy are members of the Editorial Board.

Volume 37, Number 1 (Winter 1995) launched *ILAR Journal* (formerly *ILAR News*) with an entire issue devoted to *Perspectives on Xenotransplantation* (Attached). Timed to coincide with a major workshop conducted by the Institute of Medicine, in conjunction with an ILAR session on *Emerging Diseases Associated with Xenotransplantation*, this issue of the *Journal* was enthusiastically received. The volume consisted of seven peer-reviewed manuscripts covering a wide range of issues associated with xenotransplantation.

The second edition (Spring 1995) addressed an issue of growing interest throughout the world. Stemming from a workshop conducted with representatives from Mexico and Canada on *The Effect of NAFTA on Biomedical Research*, this issue provides five articles describing the policies, laws, and customs regarding the use of research animals in different countries (Canada, Japan, New Zealand, United Kingdom, and the Unites States). Increasing pressures are being imposed on U.S. scientists and administrators by foreign organizations with authorities granted by NAFTA and GATT treaties. These pressures come in the form of new policy statements, principles, and standards that seek to alter U.S. policies to conform with those of other countries. These impact the ease of trade of biological products between research establishments in

different countries, impose greater restrictions on transportation of animals, and risk the competitiveness of trade of animal-research based products, such as pharmaceuticals and biologicals. This volume strives toward a better understanding of issues in other countries with the hopes that it might lead to improved dialogue among industrial countries on animal-science related issues. It is also hoped that developing countries can adopt existing policies and standards rather than continuing an escalation of new ones. The volume concludes with a summary article by James Glosser on *The Impact of International Free Trade Agreements on Animal Research* and a call for comments from individuals impacted by federal or state laws affecting the cost of animal-based research or the issuance of permits to collect, transport, or import/export animals or biological materials.

Currently in press is an issue devoted to adjuvants and antibody production, which includes review articles on polyclonal antibody production, the use of adjuvants, monoclonal antibody production, and recombinant antibody production. IACUC members will also find particularly useful an article that surveys institutional policies relating to adjuvants and antibody production. Other topics under development include husbandry and care of fish, amphibians, and reptiles; unique animal models used in research (including unusual rodent species, swine, primates, transgenic animals, and opossums); computational models of biomedical research; a guide to internet resources for laboratory animal science; animal models of aging; and comparative gene mapping of different species of animals.

Issues for IACUCs, book reviews, abstracts from relevant conferences or symposiums, as well as announcements of news items of interest, meetings, and new books will continue as features of *ILAR Journal*.

C. Animal Models and Genetic Stocks Information Program (AMGS)

Some of the most critical information needed by scientists is often the most difficult to obtain, including information that assists a scientist to select the most appropriate model for the proposed research and, if the model is an animal, to find sources of the model and provide appropriate care. For over 40 years, ILAR has conducted a program to provide such information. That program, called the Animal Models and Genetic Stocks Information Program, offers assistance in locating sources of animals, selecting appropriate animal models, using standardized nomenclature, understanding the importance of the use of animals in biomedical and behavioral research and testing, and interpreting guidelines for the humane care and use of animals. It includes two databases: one (called Animals for Research) contains commercially available and investigator-held colonies of animals for research; the other is a registry of codes used with standardized nomenclature of rodents and rabbits to identify institutions that maintain breeding colonies. To answer questions, ILAR also draws on its library of reference material, including ILAR committee reports, and has access to several medical libraries. Although staff members do not do literature searches, they often assist investigators by suggesting appropriate key words to use in a literature search. Staff also draws on its own experience and expertise to provide information or refers queries to other experts, usually NRC committee members.

Although widely known and used, the AMGS Program is not currently advertised, because ILAR does not have the resources to handle the expected increase in requests. During 1995 ILAR staff documented over 1,300 responses to questions and responded to many more by telephone, fax, and email. Most of the requests were for sources of animals (80%), with over 70% of those being for sources of mice and rats. The remainder of the inquiries were for sources of other animals and information on animal models, nomenclature, facilities, alternatives, and other topics.

Most of the inquiries were made by people in research institutions, including universities and hospitals (55%), federal research laboratories (9%), private research institutions (8%), and industry (16%); the remainder (12%) were from a variety of sources, including, architects, congressional staff, law firms, and students. During 1995, over 2,000 ILAR publications and packets of information for students were distributed free-of-charge, and another 1,200 ILAR reports were sold by the National Academy Press.

ILAR is currently developing a World Wide Web (WWW) home page, which will be available online in early 1996. This site will offer information on current and future ILAR programs and publications as well as links to related sites on the WWW. In conjunction with development of the ILAR home page, ILAR is continuing with plans to update the Animals for Research database and to make it and the Registry of Laboratory Codes available online. The databases are described below:

- *Listings of Sources of Laboratory Animals.* ILAR's in-house Animals for Research database includes commercially available and investigator-held colonies of laboratory animals in North America. ILAR plans to update this database and expand it to include international sources.

Another important area for expansion is in nonmammalian models. Scientists are increasingly making use of lower vertebrates and invertebrates in such fields as embryology, developmental biology, toxicology, carcinogenesis, physiology, and aging. Some of the most commonly used are zebra fish (*Brachydanio serio*), medakas (*Oryzias latipes*), guppies (*Poecilia reticulata*), axolotls (*Ambystoma mexicanum*), African clawed toads (*Xenopus laevis*), squid (*Loligo* spp.), sea urchins (*Echinus* spp.), and fruit flies (*Drosophila* spp.). ILAR plans to expand its listings of sources of nonmammalian models and to include some nontraditional sources.

- *Registry of Laboratory Codes*. The registry, currently maintaining as an in-house database at ILAR, will be made available online. It will allow users to assign themselves provisional codes electronically, with final approval by ILAR staff. This database is expected to be available online in early 1996.

D. International Activities

The International Subcommittee of ILAR Council met three times in 1995, preceding each meeting of Council. ILAR's international activities mission statement for the Western Hemisphere is:

> *As a national resource for science-based policy development, ILAR will seek to establish a joint partnership with Canada (linked through NAFTA) for the dissemination of educational and training materials in Mexico. The goal of this activity is to assist in the development of regional self-reliance in health research. Mexico will serve as a model for follow-on activities in Central and South America and the Caribbean.*

For Asia and the Pacific Rim:

> *ILAR seeks to further the relationships with Japan through the U.S.-Japan Non-Energy Agreement and with the International Council for Laboratory Animal Science for the further development and refinement of animal models, the sharing of information and facilities, and the education and training of young scientists in developing countries.*

For Europe:

> *ILAR seeks to work with European countries to assist with the harmonization of nontariff trade barriers, regulations, and the collection and transfer of biological materials.*

In order to facilitate these liaisons, ILAR interacts with numerous organizations and agencies in the United States and foreign countries. Among these are the NRC/CLS joint programs with the Mexican Academy of Sciences, and the Pan American Health Organization, Fogarty International Center at NIH, Department of State, Centers for Disease Control and Prevention, Interagency Research Animal Committee, U.S. Agency for International Development, Canadian Council on Animal Care, Agriculture Canada, and various Mexican departments of animal health and agriculture. In addition, ILAR maintains close contact with U.S. scientific societies, pharmaceutical companies, biomedical investigators, veterinarians, and administrators. This network serves to alert ILAR of existing or anticipated international problems affecting biomedical and biological research and biodiversity and to enable ILAR to better understand the broad needs of U.S. science in interacting with foreign organizations. Strengthening this list of international contacts, including those in other Academies of Science, is thought to be a high priority.

Beginning in March of 1996, ILAR will have a home page on the internet. In conjunction with the home pages of the National Academy of Science and the National Academy Press, full texts of all NRC reports will become easily available internationally. With support provided by this grant, and that of other ILAR sponsors, this home gage will be a significant advancement in ILAR's attempt to widely disseminate its reports. Through this international dissemination, reports such as ILAR's *Guide for the Care and Use of Laboratory Animals* will become readily available to individuals throughout the world and to organizations desiring to develop new policies and practices.

ILAR has remained involved in issues with potential impact on the use, trade, and transport of animals and biological products and interacts routinely with members of the International Council on Laboratory Animal Science and the International Council of Scientific Unions, ICSU.

In addition to interacting with organizations in other countries, ILAR's international activity involves working closely with the AMGS and *ILAR Journal* subcommittees in numerous overlapping areas of interest, including database development and electronic communication, and initiation of a "department" of international activities in *ILAR Journal.*

II. Special Projects

In addition to those areas discussed under I. Core Projects, this grant provides support for some of the activities of ILAR's Special Projects, the second primary focus of ILAR's activity. These projects normally evolve in one of two ways. The first way is when ILAR Council or another NRC component believes a workshop is needed to explore a specific topic to determine whether more in-depth study should be undertaken. The *Workshop: Modernization of Laboratory Animal Management Reports* is an example. In this workshop, ILAR Council explored with sponsors of this grant, representatives from the National Institutes of Health, and users of ILAR reports. This discussion assisted ILAR in focusing its efforts on those products most in need. *Laboratory Animal Management Series* reports on nonhuman primates, swine, and ruminants were regarded to be in highest demand and will be the next to be revised in this series of species-specific reports that serve as valuable supplements to the *Guide*

The second way in which special projects evolve is through a request of a federal agency or by Congress. Most NRC special projects are conducted in order to provide advice to one or more federal agencies, and such is the case for the following ILAR's projects: Revision of the *Guide for Care and Use of Laboratory Animals* (*Guide*); *Occupational Health and Safety in Care and Use of Research Animals*; *Psychological Well-being of Nonhuman Primates*; *Rodents: Laboratory Animal Management Series;* and *Scientific Requirements and Long-term Care of Chimpanzees.* Upon receipt of a request to provide guidance or recommendations, ILAR staff, with the assistance of ILAR Council and others, conducts a literature survey and writes a proposal. These proposals are then submitted to the sponsor(s). Upon receipt of funding, the normal method by which the NRC addresses such issues is to appoint an expert committee to author a report, the members of which serve without compensation. Much of the planning and preparation of these proposals is supported

by core funds. Through the support of activities of ILAR Council, invited advisors, and the ILAR staff, core grants enable many of the planning and developmental activities that lead to special projects. Following is a list of these special projects, including a summary of accomplishments during 1995 in each, and plans for the future.

A. Revision of the Guide for Care and Use of Laboratory Animals (Guide) (7th Edition)

This report was completed in 1995 and released to the public on January 2, 1996. In different international scientific forums throughout 1996, members of the authoring committee plan to conduct reviews of the recommendations of this report and how they differ from the 6th edition. The report is scheduled to be translated into Japanese and Spanish. It will be distributed to all PHS grantees in which vertebrate animals are used in research and accredited units of the American Association for Accreditation of Laboratory Animal Care. It will be available on ILAR's home page and in a CD-ROM being prepared by the U.S. Department of Agriculture's Animal Welfare Information Center. The *Guide* continues to emphasize performance goals and places increased importance on development of institutional programs that can ensure the humane care and use of research animals.

B. Laboratory Animal Management Series

As companions to the *Guide*, ILAR extensively revised the second species-specific report in the *Laboratory Animal Management* series. *Rodents: Laboratory Animal Management Series* was completed and submitted to the National Academy Press for publication. This report, and the 1994 *Dogs: Laboratory Animal Management Series*, provide detailed species specific information in a succinct format for use by those seeking information not contained in the *Guide* regarding the care and use of laboratory animals. *Dogs* was included in the 1994 Annual Report. *Rodents* is attached (see Appendix 2: ILAR Reports). Rosters of all committees active in 1995 are attached (see Appendix 1: ILAR Committee Rosters).

Based on the recommendations of the workshop, *Modernization of Laboratory Animal Management Reports,* ILAR will seek to initiate revision of reports on nonhuman primates, swine, and ruminants in 1996.

C. Occupational Health and Safety in the Care and Use of Research Animals

The authoring committee of this report completed its final draft in 1995, and the report was submitted to the NRC's Report Review Office. A panel of experts has been appointed to review the report. Contingent on the outcome of this review, the document should be available in prepublication copy by May, 1996. This report will provide the first comprehensive recommendations for development and oversight of occupational health and safety programs for personnel involved in animal care and use. The committee roster is attached (see Appendix 1: ILAR Committee Rosters).

D. Psychological Well-being of Nonhuman Primates

The report responds to an amendment to the Animal Welfare Act, which requires institutions to develop programs to ensure the psychological well-being of nonhuman primates. It will provide readers with a structure by which to develop a functional psychological well-being program; strategies for animal care personnel to use in developing enrichment techniques; strategies for animal care and use committees and veterinarians to use in assessing compliance with federal requirements; and strategies for animal welfare inspectors and site visitors to use in assessing the success of the program in achieving the goals of well-being. The report was reviewed by a panel of experts appointed by the NRC's Report Review Office. As is the strength of the NRC's review process, the authoring committee is required to respond to reviewer's comments. Upon completion of this response, and approval by NRC, the report will be published by the National Academy Press. The committee roster is attached (see Appendix 1: ILAR Committee Rosters).

E. A Workshop to Examine the Appropriate Use of Animals and Their Alternatives in Education

Funding for this study has not been identified. This study is being proposed as a three-day workshop during which invited science teachers and administrators, biologists, veterinarians, and others will define the objectives of animal use, examine proper treatment of animals by students and teachers. Two reports will be developed by an NRC-appointed steering committee. The first report will be a technical document that will reflect the workshop's discussions. The second will be a summary document for lay audiences, written by a popular science writer. The two reports will be of interest to teachers at the K-12 levels; school administrators; science coordinators; local, state, and federal officials; parents; and professional societies. Funding is being sought for this project. The office of Scientific Education, NIH, has agreed to take agency leadership for funding. The National Science Foundation has also expressed an interest, as have pharmaceutical companies.

F. Transgenic Animals: Benefits and Risks

Plans for a study of *Transgenic Animals: Benefits and Risks* were further elaborated in 1995. Although continuing to be of high priority, no progress was made in 1995 on this workshop due to other commitments. A workshop, led by the Commission on Life Sciences, is planned for 1996. The goal of this workshop is to explore the ethical and public policy issues involved in the development and use of biologically modified organisms. A science writer will participate in the workshop and assist in developing an informative booklet describing the risks and benefits of transgenic technology. The audience of the report is intended to be the public and Congress.

G. The Cost of Research

Plans for a study of *The Cost of Research* were further elaborated in 1995. A workshop is planned to explore the true costs of biological research, including animal costs (actual and indirect), administrative costs (oversight committees, paper work, and regulatory requirements), Office of Management and Budget (OMB) Circular A-21 (which prohibits indirect costs for animal colonies), and other issues. The audience of the report will be regulatory agencies and scientists.

CONCLUSIONS AND FUTURE DIRECTIONS

- Three NRC-appointed committees consisting of 41 volunteer members met for a total of 21 days in executive session, conducted 2 seminars at national meetings, and met with 17 consultants.
- Two NRC-appointed committees consisting of 26 volunteer members finalized their reports. [*Rodents* and the *Guide are* in press.]
- One NRC-appointed committee consisting of 11 volunteers completed its draft and submitted the report *Occupational Health and Safety in the Care and Use of Research Animals* to be reviewed.
- Three committee projects were continued in 1995, all of which have been released in prepublication copy (and in press) or in review; *Psychological Well-being of Nonhuman Primates*; revision of the *Guide for the Care and Use of Laboratory Animals;* and *Rodents: Laboratory Animal Management Series.* Forty-four volunteers served on these committees.
- ILAR Council and staff continued to revise and seek funding for Studies of *The Appropriate Use of Animals and Their Alternatives in Education*; *Transgenic Animals: Benefits and Risks*; and *The Cost of Research.*
- *ILAR News* became *ILAR Journal* with the first issue of 1995. The new *ILAR Journal* will continue and expand the tradition of presenting thoughtful and timely scientific articles, commentary, and discussions on issues that impact the laboratory animal science community.
- Two issues of *ILAR Journal* were published, one was submitted to the press, and one is being prepared. Twenty-five authors, fifty-eight reviewers, and members of the Editorial Board worked on these issues.

List of personnel receiving pay from the contract:

Eric Fischer, Director, ILAR
Thomas Wolfle, Program Director, ILAR
Mara Glenshaw, Editor, *ILAR News*
Tania Williams, Research Associate
Paulette Adams, Senior Project Assistant to the Director
Carol Rozmiarek, Project Assistant to the Program Director

1995 Annual Report
Institute of Laboratory Animal Resources
National Research Council
Grant Number DAMD17-93-J-3016

Appendix 1
ILAR Committee Rosters

INSTITUTE OF LABORATORY ANIMAL RESOURCES
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Appendix 2
ILAR Reports

Guide for the Care and Use of Laboratory Animals (In press)
Rodents: Laboratory Animal Management Series (In press)
ILAR Journal, Volume 37, Number 1, Winter 1995
ILAR Journal, Volume 37, Number 2, Spring 1995
ILAR Journal, Volume 37, Number 3, 1995 (In press, Table of Contents)
ILAR Journal, Volume 37, Number 4, 1995 (Table of Contents)
ILAR Journal, Volume 38, Number 1, 1996 (Table of Contents)

IN PRESS

GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS

Institute of Laboratory Animal Resources
Commission on Life Sciences
National Research Council

March 20, 1996

NOTE

The *Guide for Care and Use of Laboratory Animals* was released to the sponsors and the public on January 2, 1996, in a prepublication form. After that, the Institute of Laboratory Animal Resources (ILAR) received comments from users and members of the Committee to Revise the *Guide*. The *Guide* has always been characterized as a living document, subject to modification with changing conditions and new information. That characterization results in a continuing emphasis on performance goals as opposed to engineering approaches. The use of performance goals places increasing responsibility on the user and results in greater enhancement of animal well-being; but performance goals require careful interpretation, whereas engineering goals leave no room for interpretation. With that difference in mind, the National Research Council and the appointed reviewers strove for accuracy and clarity. However, some errors and ambiguities were identified by readers of the prepublication copy. Some pointed out pagination, spelling, and reference errors. Others noted that some statements were being misinterpreted. After careful consideration, some changes have been made in this edition. For example, punctuation and spelling were corrected, and wording was changed to clarify meaning. An example of changes for clarification is replacement of the word "develop" with "review and approve" in descriptions of animal care and use committee (IACUC) oversight of housing plans, sanitation, and bedding selection; these are responsibilities of animal-care personnel, not of the IACUC, as the word "develop" might have implied. The discussion of monitoring of food and fluid restriction in small animals was clarified by addition of the phrase "such as rodents." Appendix B (Selected Organizations Related to Laboratory Animal Science) of the review copy that was sent to reviewers requested advice from reviewers regarding what organizations should be listed; some were added in the prepublication copy and others later. A footnote added to page 2 and referred to in three places reminds readers that the *Guide* is written for a broad international audience some of whom are not covered by either the Public Health Service Policy on Humane Care and Use of Laboratory Animals or the Animal Welfare Regulations but that those who are covered by these rules must abide by them even when the *Guide* recommends a different approach. That admonition is provided throughout the *Guide,* but its placement in the introduction was thought important. ILAR believes that each of these changes will help users to interpret and apply the recommendations as intended. *There was no substantial change in the content of the prepublication version.*

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NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, National Academy of Engineering, and Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, National Academy of Engineering, and Institute of Medicine.

The National Academy of Sciences is a private, nonprofit, self-perpetuating society of distinguished scholars engaged in scientific and engineering research, dedicated to the furtherance of science and technology and to their use for the general welfare. Upon the authority of the charter granted to it by the Congress in 1863, the Academy has a mandate that requires it to advise the federal government on scientific and technical matters. Dr. Bruce Alberts is president of the National Academy of Sciences.

The National Academy of Engineering was established in 1964, under the charter of the National Academy of Sciences, as a parallel organization of outstanding engineers. It is autonomous in its administration and in the selection of its members, sharing with the National Academy of Sciences the responsibility for advising the federal government. The National Academy of Engineering also sponsors engineering programs aimed at meeting national needs, encourages education and research, and recognizes the superior achievements of engineers. Dr. Harold Liebowitz is president of the National Academy of Engineering.

The Institute of Medicine was established in 1970 by the National Academy of Sciences to secure the services of eminent members of appropriate professions in the examination of policy matters pertaining to the health of the public. The Institute acts under the responsibility given to the National Academy of Sciences by its congressional charter to be an adviser to the federal government and upon its own initiative to identify issues of medical care, research, and education. Dr. Kenneth I. Shine is president of the Institute of Medicine.

The National Research Council was established by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and advising the federal government. Functioning in accordance with general policies determined by the Academy, the Council has become the principal operating agency of both the National Academy of Sciences and National Academy of Engineering in the conduct of their services to the government, the public, and the scientific and engineering communities. The Council is administered jointly by both Academies and the Institute of Medicine. Dr. Bruce Alberts and Dr. Harold Liebowitz are chairman and vice-chairman, respectively, of the National Research Council.

This study was supported by the Comparative Medicine Program, National Center for Research Resources the Interagency Research Animal Committee, and the Office for Protection from Research Risks, National Institutes of Health/Department of Health and Human Services; the U.S. Department of Agriculture; and the Veterans' Administration. The grant was awarded by the Comparative Medicine Program, National Center for Research Resources, and all agency funding was provided under grant NIH RR08779-02.

Core support is provided to the Institute of Laboratory Animal Resources by the Comparative Medicine Program, National Center for Research Resources, National Institutes of Health, through grant number 5P40RR0137; the National Science Foundation through grant number BIR-9024967; the U.S. Army Medical Research and Development Command, which serves as the lead agency for combined U.S. Department of Defense funding also received from the Human Systems Division of the U.S. Air Force Systems Command, Armed Forces Radiobiology Research Institute, Uniformed Services University of the Health Sciences, and U.S. Naval Medical Research and Development Command, through grant number DAMD17-93-J-3016; and the American Cancer Society through grant number RC-1-34.

Any opinions, findings, and conclusions or recommendations expressed in this publication do not necessarily reflect the views of DHHS or other sponsors, nor does the mention of trade names, commercial products, or organizations imply endorsement by the U.S. government or other sponsors.

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The Institute of Laboratory Animal Resources (ILAR) was founded in 1952 under the auspices of the National Research Council. A component of the Commission on Life Sciences, ILAR develops guidelines and disseminates information on the scientific, technological, and ethical use of animals and related biological resources in research, testing, and education. ILAR promotes high-quality, humane care of animals and the appropriate use of animals and alternatives. ILAR functions within the mission of the National Academy of Sciences as an advisor to the federal government, the biomedical research community, and the public.

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Preface

The *Guide for the Care and Use of Laboratory Animals* (the *Guide*) was first published in 1963 under the title *Guide for Laboratory Animal Facilities and Care* and was revised in 1965, 1968, 1972, 1978, and 1985. More than 400,000 copies have been distributed since it was first published, and it is widely accepted as a primary reference on animal care and use. The changes and new material in this seventh edition are in keeping with the belief that the *Guide* is subject to modification with changing conditions and new information.

The purpose of the *Guide*, as expressed in the charge to the Committee to Revise the *Guide for the Care and Use of Laboratory Animals*, is to assist institutions in caring for and using animals in ways judged to be scientifically, technically, and humanely appropriate. The *Guide* is also intended to assist investigators in fulfilling their obligation to plan and conduct animal experiments in accord with the highest scientific, humane, and ethical principles. The recommendations are based on published data, scientific principles, expert opinion, and experience with methods and practices that have proved to be consistent with high-quality, humane animal care and use.

Previous editions of the *Guide* were supported solely by the National Institutes of Health (NIH) and published by the Government Printing Office. As an indication of its wide use, this edition was financially supported by NIH, the Department of Agriculture, and the Veterans' Administration and was published by the National Academy Press.

The *Guide* is organized into four chapters on the major components of an animal care and use program: institutional policies and responsibilities; animal environment, housing, and management; veterinary medical care; and physical plant. Responsibilities of institutional officials, institutional animal care and use committees, investigators, and veterinarians are discussed in each chapter.

In 1991, an ad hoc committee appointed by the Institute of Laboratory Animal Resources (ILAR) recommended that the *Guide* be revised. The Committee to Revise the *Guide for the Care and Use of Laboratory Animals* was appointed in 1993 by the National Research Council; its 15 members included research scientists, veterinarians, and nonscientists representing bioethics and the public's interest in animal welfare.

Before revision began, written and oral comments on the *Guide* were solicited widely from the scientific community and the general public. Open meetings were held in Washington, DC, on December 1, 1993; in San Francisco, California, on February 2, 1994; and in St. Louis, Missouri, on February 4, 1994. Comments made at those meetings and written comments were considered by the committee and contributed substantially to this revision of the *Guide*.

The committee acknowledges the contributions of William I. Gay and Bennett J. Cohen in the development of the original *Guide*. In 1959, Animal Care Panel (ACP) President Cohen appointed the Committee on Ethical Considerations in the Care of Laboratory Animals to evaluate animal care and use. That committee was chaired by Dr. Gay, who soon recognized that the committee could not evaluate animal-care programs objectively without appropriate criteria on which to base its evaluations; that is, standards were needed. The ACP executive committee agreed, and the Professional Standards Committee was appointed. NIH later awarded the ACP a contract to "determine and establish a professional standard for laboratory animal care and facilities." Dr.

Cohen chaired the ACP Animal Facilities Standards Committee, which prepared the first *Guide for Laboratory Animal Facilities and Care*.

The Committee to Revise the *Guide for the Care and Use of Laboratory Animals* expresses its appreciation to the Animal Welfare Information Center, National Agricultural Library, U.S. Department of Agriculture, for its assistance in compiling bibliographies and references. This task would have been quite formidable without their help. Appreciation is also extended to the reviewers of the volume, to Norman Grossblatt for editing the manuscript, to Carol Rozmiarek for providing exemplary secretarial assistance and preparing multiple drafts, and to Thomas L. Wolfle, who managed the process from beginning to end.

Readers who detect errors of omission or commission are invited to send corrections and suggestions to the Institute of Laboratory Animal Resources, National Research Council, 2101 Constitution Avenue, NW, Washington, DC 20418.

Derrell Clark, *Chairman*
Committee to Revise the *Guide for the Care and Use of Laboratory Animals*

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Introduction

This edition of the *Guide for the Care and Use of Laboratory Animals* (the *Guide*) strongly affirms the conviction that all who care for or use animals in research, teaching, or testing must assume responsibility for their well-being. The *Guide* is applicable only after the decision is made to use animals in research, teaching, or testing. Decisions associated with the need to use animals are not within the purview of the *Guide*, but responsibility for animal well-being begins for the investigator with that decision. Additional responsibilities of the investigator, and other personnel, are elaborated in Chapter 1.

The goal of this *Guide* is to promote the humane care of animals used in biomedical and behavioral research, teaching, and testing; the basic objective is to provide information that will enhance animal well-being, the quality of biomedical research, and the advancement of biologic knowledge that is relevant to humans or animals. The use of animals as experimental subjects in the 20th century has contributed to many important advances in scientific and medical knowledge (Leader and Stark 1987). Although scientists have also developed nonanimal models for research, teaching, and testing (NRC 1977; see Appendix A, "Alternatives"), these models often cannot completely mimic the complex human or animal body, and continued progress in human and animal health and well-being requires the use of living animals. Nevertheless, efforts to develop and use scientifically valid alternatives, adjuncts, and refinements to animal research should continue.

In this *Guide*, laboratory animals include any vertebrate animal (e.g., traditional laboratory animals, farm animals, wildlife, and aquatic animals) used in research, teaching, or testing. When appropriate, exceptions or specific emphases for farm animals are provided. The *Guide* does not specifically address farm animals used in agricultural research or teaching, wildlife and aquatic animals studied in natural settings, or invertebrate animals used in research; however, many of the general principles in this *Guide* apply to these species and situations.

REGULATIONS, POLICIES, AND PRINCIPLES

This *Guide* endorses the responsibilities of investigators as stated in the *U.S. Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training* (IRAC 1985; see Appendix D). Interpretation and application of those principles and this *Guide* require professional knowledge. In summary, the principles encourage

- Design and performance of procedures on the basis of relevance to human or animal health, advancement of knowledge, or the good of society.
- Use of appropriate species, quality, and number of animals.
- Avoidance or minimization of discomfort, distress, and pain in concert with sound science.
- Use of appropriate sedation, analgesia, or anesthesia.
- Establishment of experimental end points.

- Provision of appropriate animal husbandry directed and performed by qualified persons.
- Conduct of experimentation on living animals only by or under the close supervision of qualified and experienced persons.

In general, the principles stipulate responsibilities of investigators, whose activities regarding use of animals are subject to oversight by an institutional animal care and use committee (IACUC).

Animal facilities and programs should be operated in accord with this *Guide,* the Animal Welfare Regulations, or AWRs (CFR 1985); the Public Health Service Policy on Humane Care and Use of Laboratory Animals, or PHS Policy (PHS 1986); and other applicable federal (Appendixes C and D), state, and local laws, regulations, and policies.[1] Supplemental information on breeding, care, management, and use of selected laboratory animal species is available in other publications prepared by the Institute of Laboratory Animal Resources (ILAR) and other organizations (Appendix A). References in this *Guide* provide the reader with additional information that supports statements made in the *Guide* or presents divergent opinions.

EVALUATION CRITERIA

The *Guide* charges users of research animals with the responsibility of achieving specified outcomes but leaves it up to them how to accomplish these goals. This "performance" approach is desirable because many variables (such as the species and previous history of the animals, facilities, expertise of the people, and research goals) often make prescriptive ("engineering") approaches impractical and unwarranted. Engineering standards are sometimes useful to establish a baseline, but they do not specify the goal or outcome (such as well-being, sanitation, or personnel safety) in terms of measurable criteria as do performance standards.

The engineering approach does not provide for interpretation or modification in the event that acceptable alternative methods are available or unusual circumstances arise. Performance standards define an outcome in detail and provide criteria for assessing that outcome, but do not limit the methods by which to achieve that outcome. This performance approach requires professional input and judgment to achieve outcome goals. Optimally, engineering and performance standards are balanced, thereby providing standards while allowing flexibility and judgment based on individual situations. Scientists, veterinarians, technicians, and others have extensive experience and information covering many of the topics discussed in this *Guide*. Research on laboratory animal management continues to generate scientific information that should be used in evaluating performance and engineering standards. For some issues, insufficient information is available, and continued research into improved methods of animal care and use is needed.

[1] Users are reminded that the *Guide* is written for a diverse group of national and international institutions and organizations, many of which are covered by neither the AWRs nor the PHS Policy. On a few matters, the *Guide* differs from the AWRs and the PHS Policy; users regulated by the AWRs or the PHS Policy must comply with them.

The *Guide* is deliberately written in general terms so that its recommendations can be applied in the diverse institutions and settings that produce or use animals for research, teaching, and testing; generalizations and broad recommendations are imperative in such a document. This approach requires that users, IACUCs, veterinarians, and producers use professional judgment in making specific decisions regarding animal care and use. Because this *Guide* is written in general terms, IACUCs have a key role in interpretation, oversight, and evaluation of institutional animal care and use programs. The question frequently arises as to how the words *must* and *should* are used in the *Guide* and how IACUCs should interpret their relative priority. In general, the verb *must* is used for broad programmatic or basic aspects that the Committee to Revise the *Guide* considers are imperative. The verb *should* is used as a strong recommendation for achieving a goal. However, the committee recognizes that individual circumstances might justify an alternative strategy.

FARM ANIMALS

Uses of farm animals in research, teaching, and testing are often separated into biomedical uses and agricultural uses because of government regulations (AWRs), institutional policies, administrative structure, funding sources, or user goals. That separation has led to a dual system with different criteria for evaluating protocols and standards of housing and care for animals of the same species on the basis of perceived biomedical or agricultural research objectives (Stricklin and Mench 1994). For some studies, this separation is clear. For example, animal models of human diseases, organ transplantation, and major surgery are considered biomedical uses; and studies on food and fiber production, such as feeding trials, are usually considered agricultural uses. However, the separation often is not clear, as in the case of some nutrition and disease studies. Administrators, regulators, and IACUCs often face a dilemma in deciding how to handle such studies (Stricklin and others 1990).

The use of farm animals in research should be subject to the same ethical considerations as the use of other animals in research, regardless of an investigator's research objectives or funding source (Stricklin and others 1990). However, differences in research goals lead to fundamental differences between biomedical and agricultural research. Agricultural research often necessitates that animals be managed according to contemporary farm-production practices for research goals to be reached (Stricklin and Mench 1994). For example, natural environmental conditions might be desirable for agricultural research, whereas control of environmental conditions to minimize variation might be desirable in biomedical research (Tillman 1994).

Housing systems for farm animals used in biomedical research might or might not differ from those in agricultural research. Animals used in either biomedical or agricultural research can be housed in cages or stalls or in paddocks or pastures (Tillman 1994). Some agricultural studies need uniform conditions to minimize environmental variability, and some biomedical studies are conducted in farm settings. Thus, the protocol, rather than the category of research, should determine the setting (farm or laboratory). Decisions on categorizing research uses of farm animals and defining standards for their care and use should be based on user goals, protocols, and concern for animal well-being and should be made by the IACUC.

Regardless of the category of research, institutions are expected to provide oversight of all research animals and ensure that their pain and distress is minimized.

This *Guide* applies to farm animals used in biomedical research, including those maintained in typical farm settings. For such animals in a farm setting, the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (1988), or revisions thereof, is a useful resource. Additional information regarding facilities and management of farm animals in an agricultural setting can be obtained from the Midwest Plan Service's *Structures and Environment Handbook* (1987) and from agricultural engineers or animal-science experts at state agricultural extension services and land-grant colleges and universities.

NONTRADITIONAL SPECIES

A species not commonly used in biomedical research is sometimes the animal model of choice because of its unique characteristics. For example, hibernation can be studied only in species that hibernate. An appropriate environment should be provided for nontraditional species, and for some species it might be necessary to approximate the natural habitat. Expert advice on the natural history and behavior of nontraditional species should be sought when such animals are to be introduced into a research environment. Because of the large number of nontraditional species and their varied requirements, this *Guide* cannot provide husbandry details appropriate to all such species. However, several scientific organizations have developed guides for particular species of nontraditional animals (e.g., ILAR and the Scientists Center for Animal Welfare, SCAW). A partial list of sources is available in Appendix A.

FIELD INVESTIGATIONS

Biomedical and behavioral investigations occasionally involve observation or use of vertebrate animals under field conditions. Although some of the recommendations listed in this volume are not applicable to field conditions, the basic principles of humane care and use apply to the use of animals living in natural conditions.

Investigators conducting field studies with animals should assure their IACUC that collection of specimens or invasive procedures will comply with state and federal regulations and this *Guide*. Zoonoses and occupational health and safety issues should be reviewed by the IACUC to ensure that field studies do not compromise the health and safety of other animals or persons working in the field. Guidelines for using animals in field studies prepared by professional societies are useful when they adhere to the humane principles of the *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training* (Appendix D) and this *Guide* (see Appendix A, "Exotic, Wild, and Zoo Animals" and "Other Animals").

OVERVIEW

In an attempt to facilitate its usefulness and ease in locating specific topics, the organization of this edition of the *Guide* is slightly different from that of the preceding edition. Material from the preceding edition's Chapter 5, "Special Considerations," has been incorporated into Chapters 1-4. Genetics and nomenclature are now discussed in Chapter 2; facilities and procedures for animal research with hazardous agents and occupational health and safety are considered in Chapter 1. Recommendations for farm animals are incorporated throughout the text where appropriate.

This edition of the *Guide* is divided into four chapters and four appendixes. Chapter 1 focuses on institutional policies and responsibilities, including the monitoring of the care and use of animals, considerations for evaluation of some specific research procedures, veterinary care, personnel qualifications and training, and occupational health and safety; the latter section summarizes another National Research Council committee report (NRC In press) and includes information about facilities and procedures for animal research with hazardous agents. Chapter 2 focuses on the animals themselves and provides recommendations for housing and environment, behavioral management, husbandry, and population management, including discussions of identification, records, genetics, and nomenclature. Chapter 3 discusses veterinary medical care and responsibilities of the attending veterinarian; it includes recommendations relative to animal procurement and transportation, preventive medicine, surgery, pain and analgesia, and euthanasia. Chapter 4 discusses the physical plant, including functional areas and construction guidelines, with expanded discussions of heating, ventilation, and air-conditioning (HVAC) systems and facilities for aseptic surgery.

The appendixes in this edition remain largely the same as in the preceding edition. Appendix A contains an updated bibliography, categorized by topic; Appendix B lists selected organizations related to laboratory animal science; Appendix C presents federal laws relevant to animal care and use; and Appendix D provides the PHS endorsement of the *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training* (IRAC 1985).

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Institutional Policies and Responsibilities

Proper care, use, and humane treatment of animals used in research, testing, and education (referred to in this *Guide* as animal care and use) require scientific and professional judgment based on knowledge of the needs of the animals and the special requirements of the research, testing, and educational programs. The guidelines in this section are intended to aid in developing institutional policies governing the care and use of animals.

Each institution should establish and provide resources for an animal care and use program that is managed in accord with this *Guide* and in compliance with applicable federal, state, and local laws and regulations, such as the federal Animal Welfare Regulations, or AWRs (CFR 1985), and Public Health Service Policy on Humane Care and Use of Laboratory Animals, or PHS Policy (PHS 1986). To implement the recommendations in this *Guide* effectively, an institutional animal care and use committee (IACUC) must be established to oversee and evaluate the program.

Responsibility for directing the program is generally given either to a veterinarian with training or experience in laboratory animal science and medicine or to another qualified professional. At least one veterinarian qualified through experience or training in laboratory animal science and medicine or in the species being used must be associated with the program. The institution is responsible for maintaining records of the activities of the IACUC and for conducting an occupational health and safety program.

MONITORING THE CARE AND USE OF ANIMALS

Institutional Animal Care and Use Committee

The responsible administrative official at each institution must appoint an IACUC, also referred to as "the committee," to oversee and evaluate the institution's animal program, procedures, and facilities to ensure that they are consistent with the recommendations in this *Guide*, the AWRs, and the PHS Policy. It is the institution's responsibility to provide suitable orientation, background materials, access to appropriate resources, and, if necessary, specific training to assist IACUC members in understanding and evaluating issues brought before the committee.

Committee membership should include the following:

- A doctor of veterinary medicine, who is certified (see American College of Laboratory Animal Medicine, ACLAM, Appendix B) or has training or experience in laboratory animal science and medicine or in the use of the species in question.
- At least one practicing scientist experienced in research involving animals.
- At least one public member to represent general community interests in the proper care and use of animals. Public members should not be laboratory-animal users, be

affiliated with the institution, or be members of the immediate family of a person who is affiliated with the institution.

The size of the institution and the nature and extent of the research, testing, and educational programs will determine the number of members of the committee and their terms of appointment. Additional information about committee composition can be found in the PHS Policy and the AWRs.

The committee is responsible for oversight and evaluation of the animal care and use program and its components described in this *Guide*. Its functions include inspection of facilities; evaluation of programs and animal-activity areas; submission of reports to responsible institutional officials; review of proposed uses of animals in research, testing, or education (i.e., protocols); and establishment of a mechanism for receipt and review of concerns involving the care and use of animals at the institution.

The IACUC must meet as often as necessary to fulfill its responsibilities, but it should meet at least once every 6 months. Records of committee meetings and of results of deliberations should be maintained. The committee should review the animal-care program and inspect the animal facilities and activity areas at least once every 6 months. After review and inspection, a written report, signed by a majority of the IACUC, should be made to the responsible administrative officials of the institution on the status of the animal care and use program and other activities as stated herein and as required by federal, state, or local regulations and policies. Protocols should be reviewed in accord with the AWRs, the PHS Policy, *U.S. Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training* (IRAC 1985; see Appendix D), and this *Guide* (see footnote, p. 2).

Animal Care and Use Protocols

The following topics should be considered in the preparation and review of animal care and use protocols:

- Rationale and purpose of the proposed use of animals.
- Justification of the species and number of animals requested. Whenever possible, the number of animals requested should be justified statistically.
- Availability or appropriateness of the use of less-invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation (see Appendix A, "Alternatives").
- Adequacy of training and experience of personnel in the procedures used.
- Unusual housing and husbandry requirements.
- Appropriate sedation, analgesia, and anesthesia. (Scales of pain or invasiveness might aid in the preparation and review of protocols; see Appendix A, "Anesthesia, Pain and Surgery.")
- Unnecessary duplication of experiments.
- Conduct of multiple major operative procedures.
- Criteria and process for timely intervention, removal of animals from a study, or euthanasia if painful or stressful outcomes are anticipated.
- Postprocedure care.
- Method of euthanasia or disposition of animal.

- Safety of working environment for personnel.

Occasionally, protocols include procedures that have not been previously encountered or that have the potential to cause pain or distress that cannot be reliably controlled. Such procedures might include physical restraint, multiple major survival surgery, food or fluid restriction, use of adjuvants, use of death as an end point, use of noxious stimuli, skin or corneal irritancy testing, allowance of excessive tumor burden, intracardiac or orbital-sinus blood sampling, or the use of abnormal environmental conditions. Relevant objective information regarding the procedures and the purpose of the study should be sought from the literature, veterinarians, investigators, and others knowledgeable about the effects on animals. If little is known regarding a specific procedure, limited pilot studies designed to assess the effects of the procedure on the animals, conducted under IACUC oversight, might be appropriate. General guidelines for evaluation of some of those methods are provided in this section, but they might not apply in all instances.

Physical Restraint

Physical restraint is the use of manual or mechanical means to limit some or all of an animal's normal movement for the purpose of examination, collection of samples, drug administration, therapy, or experimental manipulation. Animals are restrained for brief periods, usually minutes, in most research applications.

Animals can be physically restrained briefly either manually or with restraint devices. Restraint devices should be suitable in size, design, and operation to minimize discomfort or injury to the animal. Many dogs, nonhuman primates (e.g., Reinhardt 1991, 1995), and other animals can be trained, through use of positive reinforcement, to present limbs or remain immobile for brief procedures.

Prolonged restraint, including chairing of nonhuman primates, should be avoided unless it is essential for achieving research objectives and is approved by the IACUC. Less-restrictive systems that do not limit an animal's ability to make normal postural adjustments, such as the tether system for nonhuman primates and stanchions for farm animals, should be used when compatible with protocol objectives (Bryant 1980; Byrd 1979; Grandin 1991; McNamee and others 1984; Morton and others 1987; Wakeley and others 1974). When restraint devices are used, they should be specifically designed to accomplish research goals that are impossible or impractical to accomplish by other means or to prevent injury to animals or personnel.

The following are important guidelines for restraint:

- Restraint devices are not to be considered normal methods of housing.
- Restraint devices should not be used simply as a convenience in handling or managing animals.
- The period of restraint should be the minimum required to accomplish the research objectives.
- Animals to be placed in restraint devices should be given training to adapt to the equipment and personnel.
- Provision should be made for observation of the animal at appropriate intervals, as determined by the IACUC.

- Veterinary care should be provided if lesions or illnesses associated with restraint are observed. The presence of lesions, illness, or severe behavioral change often necessitates temporary or permanent removal of the animal from restraint.

Multiple Major Surgical Procedures

Major surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic function. Multiple major survival surgical procedures on a single animal are discouraged but may be permitted if scientifically justified by the user and approved by the IACUC. For example, multiple major survival surgical procedures can be justified if they are related components of a research project, if they will conserve scarce animal resources (NRC 1990; see also footnote, p. 2), or if they are needed for clinical reasons. If multiple major survival surgery is approved, the IACUC should pay particular attention to animal well-being through continuing evaluation of outcomes. Cost savings alone is not an adequate reason for performing multiple major survival surgical procedures (AWRs).

Food or Fluid Restriction

When experimental situations require food or fluid restriction, at least minimal quantities of food and fluid should be available to provide for development of young animals and to maintain long-term well-being of all animals. Restriction for research purposes should be scientifically justified, and a program should be established to monitor physiologic or behavioral indexes, including criteria (such as weight loss or state of hydration) for temporary or permanent removal of an animal from the experimental protocol (Van Sluyters and Oberdorfer 1991). Restriction is typically measured as a percentage of the ad libitum or normal daily intake or as percentage change in an animal's body weight.

Precautions that should be used in cases of fluid restriction to avoid acute or chronic dehydration include daily recording of fluid intake and recording of body weight at least once a week (NIH 1990)—or more often, as might be needed for small animals, such as rodents. Special attention should be given to ensuring that animals consume a suitably balanced diet (NYAS 1988) because food consumption might decrease with fluid restriction. The least restriction that will achieve the scientific objective should be used. In the case of conditioned-response research protocols, use of a highly preferred food or fluid as positive reinforcement, instead of restriction, is recommended. Dietary control for husbandry or clinical purposes is addressed in Chapter 2.

VETERINARY CARE

Adequate veterinary care must be provided, including access to all animals for evaluation of their health and well-being. Institutional mission, programmatic goals, and size of the animal program will determine the need for full-time, part-time, or consultative veterinary services. Visits by a consulting or part-time veterinarian should be at intervals appropriate to programmatic needs. For specific responsibilities of the veterinarian, see Chapter 3.

Ethical, humane, and scientific considerations sometimes require the use of sedatives, analgesics, or anesthetics in animals (see Appendix A). An attending veterinarian (i.e., a veterinarian who has direct or delegated authority) should give research personnel advice that ensures that humane needs are met and are compatible with scientific requirements. The AWRs and PHS Policy require that the attending veterinarian have the authority to oversee the adequacy of other aspects of animal care and use. These can include animal husbandry and nutrition, sanitation practices, zoonosis control, and hazard containment.

PERSONNEL QUALIFICATIONS AND TRAINING

AWRs and PHS Policy require institutions to ensure that people caring for or using animals are qualified to do so. The number and qualifications of personnel required to conduct and support an animal care and use program depend on several factors, including the type and size of institution, the administrative structure for providing adequate animal care, the characteristics of the physical plant, the number and species of animals maintained, and the nature of the research, testing, and educational activities.

Personnel caring for animals should be appropriately trained (see Appendix A, "Technical and Professional Education"), and the institution should provide for formal or on-the-job training to facilitate effective implementation of the program and humane care and use of animals. According to the programmatic scope, personnel will be required with expertise in other disciplines, such as animal husbandry, administration, laboratory animal medicine and pathology, occupational health and safety, behavioral management, genetic management, and various other aspects of research support.

There are a number of options for the training of technicians. Many states have colleges with accredited programs in veterinary technology (AVMA 1995); most are 2-year programs that result in associate of science degrees, and some are 4-year programs that result in bachelor of science degrees. Nondegree training, with certification programs for laboratory animal technicians and technologists, can be obtained from the American Association for Laboratory Animal Science (AALAS). There are commercially available training materials that are appropriate for self-study (Appendix B). Personnel using or caring for animals should also participate regularly in continuing-education activities relevant to their responsibilities. They are encouraged to be involved in local and national meetings of AALAS and other relevant professional organizations. On-the-job training should be part of every technician's job and should be supplemented with institution-sponsored discussion and training programs and with reference materials applicable to their jobs and the species with which they work (Kreger 1995). Coordinators of institutional training programs can seek assistance from the Animal Welfare Information Center (AWIC) and ILAR (NRC 1991). The *Guide to the Care and Use of Experimental Animals* by the Canadian Council on Animal Care (CCAC 1993) and guidelines of some other countries are valuable additions to the libraries of laboratory animal scientists (Appendix B).

Investigators, technical personnel, trainees, and visiting investigators who perform animal anesthesia, surgery, or other experimental manipulations must be qualified through training or experience to accomplish these tasks in a humane and scientifically acceptable manner.

OCCUPATIONAL HEALTH AND SAFETY OF PERSONNEL

An occupational health and safety program must be part of the overall animal care and use program (CDC and NIH 1993; CFR 1984a,b,c; PHS Policy). The program must be consistent with federal, state, and local regulations and should focus on maintaining a safe and healthy workplace. The program will depend on the facility, research activities, hazards, and animal species involved. The National Research Council publication *Occupational Health and Safety in the Care and Use of Research Animals* (NRC In press) contains guidelines and references for establishing and maintaining an effective, comprehensive program (also see Appendix A). An effective program relies on strong administrative support and interactions among several institutional functions or activities, including the research program (as represented by the investigator), the animal care and use program (as represented by the veterinarian and the IACUC), the environmental health and safety program, occupational-health services, and administration (e.g., human resources, finance, and facility-maintenance personnel). Operational and day-to-day responsibility for safety in the workplace, however, resides with the laboratory or facility supervisor (e.g., principal investigator, facility director, or veterinarian) and depends on performance of safe work practices by all employees.

Hazard Identification and Risk Assessment

Professional staff who conduct and support research programs that involve hazardous biologic, chemical, or physical agents (including ionizing and nonionizing radiation) should be qualified to assess dangers associated with the programs and to select safeguards appropriate to the risks. An effective occupational health and safety program ensures that the risks associated with the experimental use of animals are reduced to acceptable levels. Potential hazards—such as animal bites, chemical cleaning agents, allergens, and zoonoses—that are inherent in or intrinsic to animal use should also be identified and evaluated. Health and safety specialists with knowledge in appropriate disciplines should be involved in the assessment of risks associated with hazardous activities and in the development of procedures to manage such risks. The extent and level of participation of personnel in the occupational health and safety program should be based on the hazards posed by the animals and materials used; on the exposure intensity, duration, and frequency; on the susceptibility of the personnel; and on the history of occupational illness and injury in the particular workplace (Clark 1993).

Personnel Training

Personnel at risk should be provided with clearly defined procedures for conducting their duties, should understand the hazards involved, and should be proficient in implementing the required safeguards.

Personnel should be trained regarding zoonoses, chemical safety, microbiologic and physical hazards (including those related to radiation and allergies), unusual conditions or agents that might be part of experimental procedures (including the use of genetically engineered animals and the use of human tissue in immunocompromised animals), handling of waste materials, personal hygiene, and other considerations (e.g., precautions to be taken

during personnel pregnancy, illness, or decreased immunocompetence) as appropriate to the risk imposed by their workplace.

Personal Hygiene

It is essential that all personnel maintain a high standard of personal cleanliness. Clothing suitable for use in the animal facility and laboratories in which animals are used should be supplied and laundered by the institution. A commercial laundering service is acceptable in many situations; however, appropriate arrangements should be made to decontaminate clothing exposed to potential hazards. Disposable gloves, masks, head covers, coats, coveralls, and shoe covers might be desirable in some circumstances. Personnel should wash their hands and change clothing as often as necessary to maintain personal hygiene. Outer garments worn in the animal rooms should not be worn outside the animal facility. Personnel should not be permitted to eat, drink, use tobacco products, or apply cosmetics in animal rooms.

Facilities, Procedures, and Monitoring

Facilities required to support occupational health and safety concerns associated with animal care and use programs will vary. Because a high standard of personal cleanliness is essential, facilities and supplies for meeting this obligation should be provided. Washing and showering facilities appropriate to the program should be available. Facilities, equipment, and procedures should also be designed, selected, and developed to provide for ergonomically sound operations that reduce the potential of physical injury to personnel (such as might be caused by the lifting of heavy equipment or animals and the use of repetitive movements). Safety equipment should be properly maintained and routinely calibrated.

The selection of appropriate animal-housing systems requires professional knowledge and judgment and depends on the nature of the hazards in question, the types of animals used, and the design of the experiments. Experimental animals should be housed so that potentially contaminated food and bedding, feces, and urine can be handled in a controlled manner. Facilities, equipment, and procedures should be provided for appropriate bedding disposal.

Appropriate methods should be used for assessing exposure to potentially hazardous biologic, chemical, and physical agents where the possibility of exceeding permissible exposure limits (PELs) exists (CFR 1984b).

Animal Experimentation Involving Hazards

In selecting specific safeguards for animal experimentation with hazardous agents, careful attention should be given to procedures for animal care and housing, storage and disbursement of the agents, dose preparation and administration, body-fluid and tissue handling, waste and carcass disposal, and personal protection. Special safety equipment should be used in combination with appropriate management and safe practices. As a general rule, safety depends on trained personnel who rigorously follow safe practices.

Institutions should have written policies governing experimentation with hazardous biologic, chemical, and physical agents. An oversight process (such as use of a safety committee) should be developed to involve persons who are knowledgeable in the evaluation

of hazards and safety issues. Because the use of animals in such studies requires special considerations, the procedures and facilities to be used should undergo review for specific safety concerns. Formal safety programs should be established to assess the hazards, determine the safeguards needed for their control, ensure that the staff has the necessary training and skills, and ensure that the facilities are adequate for the safe conduct of the research. Technical support should be provided to monitor and ensure compliance with institutional safety policies.

The Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH) publication *Biosafety in Microbiological and Biomedical Laboratories* (1993) and the National Research Council (In press) recommend practices and procedures, safety equipment, and facility requirements for working with hazardous biologic agents and materials. Facilities that handle agents of unknown risk should consult with appropriate CDC personnel about hazard control and medical surveillance.

Special facilities and safety equipment are needed to protect the animal-care and investigative staff, other occupants of the facility, the public, animals, and the environment from exposure to hazardous biologic, chemical, and physical agents used in animal experimentation. Facilities used for animal experimentation with hazardous agents should be separated from other animal housing and support areas, research and clinical laboratories, and patient-care facilities and should be appropriately identified; and access to them should be limited to authorized personnel. Such facilities should be designed and constructed to facilitate cleaning and maintenance of mechanical systems. A properly managed and used double-corridor facility or barrier entry system is an effective means of reducing cross-contamination. Floor drains should always contain liquid or be sealed effectively by other means. Automatic trap priming can be provided to ensure that traps remain filled.

Hazardous agents should be contained within the study environment. Control of airflow (such as through the use of biologic-safety cabinets) that minimizes the escape of contaminants is a primary barrier used in the handling and administration of hazardous agents and the performance of necropsies on contaminated animals (CDC 1995; Kruse and others 1991). Special features of the facility—such as airlocks, negative air pressure, air filters, and redundant mechanical equipment with automatic switching—are secondary barriers aimed at preventing accidental release of hazards outside the facility and work environment.

Exposure to anesthetic waste gases should be limited. This is usually accomplished by using various scavenging techniques. If ether is used, personnel safety should be ensured by proper use of signs and by using equipment and practices to minimize risks associated with its explosiveness.

Personal Protection

Personal protective equipment should be provided, and other safety measures should be adopted when needed. Animal-care personnel should wear appropriate institution-issued protective clothing, shoes or shoe covers, and gloves. Clean protective clothing should be provided as often as necessary. If it is appropriate, personnel should shower when they leave the animal-care, procedure, or dose-preparation areas. Protective clothing and equipment should not be worn beyond the boundary of the hazardous-agent work area or the animal facility. Personnel with potential exposure to hazardous agents should be provided with

personal protective equipment appropriate to the agents (CFR 1984c). For example, personnel exposed to nonhuman primates should be provided with such protective items as gloves, arm protectors, masks, and face shields. Hearing protection should be provided in high-noise areas. Personnel working in areas where they might be exposed to contaminated airborne particulate material or vapors should be provided with suitable respiratory protection (CFR 1984c).

Medical Evaluation and Preventive Medicine for Personnel

Development and implementation of a program of medical evaluation and preventive medicine should involve input from trained health professionals, such as occupational-health physicians and nurses. Confidentiality and other medical and legal factors must be considered in the context of appropriate federal, state, and local regulations.

A health-history evaluation before work assignment is advisable to assess potential risks for individual employees. Periodic medical evaluations are advisable for people in some risk categories. An appropriate immunization schedule should be adopted. It is important to immunize animal-care personnel against tetanus. In addition, pre-exposure immunization should be offered to people at risk of infection or exposure to such agents as rabies or hepatitis B virus. Vaccination is recommended if research is to be conducted on infectious diseases for which effective vaccines are available. Specific recommendations can be found in the CDC and NIH publication *Biosafety in Microbiological and Biomedical Laboratories* (1993). Pre-employment or pre-exposure serum collection is advisable only in specific circumstances as determined by an occupational health and safety professional (NRC In press). In such cases, identification, traceability, retention, and storage conditions of samples should be considered, and the purpose for which the serum samples will be used must be consistent with applicable state laws and consistent with the Federal Policy for the Protection of Human Subjects (Federal Register 56(117): 28002-28032, June 18, 1991).

Zoonosis surveillance should be a part of an occupational-health program (CDC and NIH 1993; Fox and others 1984; NRC In press). Personnel should be instructed to notify their supervisors of potential or known exposures and of suspected health hazards and illnesses. Clear procedures should be established for reporting all accidents, bites, scratches, and allergic reactions (NRC In press).

Nonhuman-primate diseases that are transmissible to humans can be serious hazards. Animal technicians, clinicians, investigators, predoctoral and postdoctoral trainees, research technicians, consultants, maintenance workers, security personnel, and others who have contact with nonhuman primates or have duties in nonhuman-primate housing areas should be routinely screened for tuberculosis. Because of the potential for *Cercopithecine herpesvirus 1* (formerly *Herpesvirus simiae*) exposure, personnel who work with macaques should have access to and be instructed in the use of bite and scratch emergency-care stations (Holmes and others 1995). A procedure should be established for ensuring medical care for bites and scratches.

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2
Animal Environment, Housing, and Management

Proper housing and management of animal facilities are essential to animal well-being, to the quality of research data and teaching or testing programs in which animals are used, and to the health and safety of personnel. A good management program provides the environment, housing, and care that permit animals to grow, mature, reproduce, and maintain good health; provides for their well-being; and minimizes variations that can affect research results. Specific operating practices depend on many factors that are peculiar to individual institutions and situations. Well-trained and motivated personnel can often ensure high-quality animal care, even in institutions with less than optimal physical plants or equipment.

Many factors should be considered in planning for adequate and appropriate physical and social environment, housing, space, and management. These include

- The species, strain, and breed of the animal and individual characteristics, such as sex, age, size, behavior, experiences, and health.
- The ability of the animals to form social groups with conspecifics through sight, smell, and possibly contact, whether the animals are maintained singly or in groups.
- The design and construction of housing.
- The availability or suitability of enrichments.
- The project goals and experimental design (e.g., production, breeding, research, testing, and teaching).
- The intensity of animal manipulation and invasiveness of the procedures conducted.
- The presence of hazardous or disease-causing materials.
- The duration of the holding period.

Animals should be housed with a goal of maximizing species-specific behaviors and minimizing stress-induced behaviors. For social species, this normally requires housing in compatible pairs or groups. A strategy for achieving desired housing should be developed by animal-care personnel with review and approval by the IACUC. Decisions by the IACUC, in consultation with the investigator and veterinarian, should be aimed at achieving high standards for professional and husbandry practices considered appropriate for the health and well-being of the species and consistent with the research objectives. After the decision-making process, objective assessments should be made to substantiate the adequacy of animal environment, husbandry, and management.

The environment in which animals are maintained should be appropriate to the species, its life history, and its intended use. For some species, it might be appropriate to approximate the natural environment for breeding and maintenance. Expert advice might be sought for special requirements associated with the experiment or animal subject (for example, hazardous-agent use, behavioral studies, and immunocompromised animals, farm animals, and nontraditional laboratory species).

The following sections discuss some considerations of the physical environment related to common research animals.

PHYSICAL ENVIRONMENT

Microenvironment and Macroenvironment

The *microenvironment* of an animal is the physical environment immediately surrounding it—the primary enclosure with its own temperature, humidity, and gaseous and particulate composition of the air. The physical environment of the secondary enclosure—such as a room, a barn, or an outdoor habitat—constitutes the *macroenvironment*. Although the microenvironment and the macroenvironment are linked by ventilation between the primary and secondary enclosures, the environment in the primary enclosure can be quite different from the environment in the secondary enclosure and is affected by the design of both enclosures.

Measurement of the characteristics of the microenvironment can be difficult in small primary enclosures. Available data indicate that temperature, humidity, and concentrations of gases and particulate matter are often higher in an animal's microenvironment than in the macroenvironment (Besch 1980; Flynn 1959; Gamble and Clough 1976; Murakami 1971; Serrano 1971). Microenvironmental conditions can induce changes in metabolic and physiologic processes or alterations in disease susceptibility (Broderson and others 1976; Schoeb and others 1982; Vesell and others 1976).

Housing

Primary Enclosures

The primary enclosure (usually a cage, pen, or stall) provides the limits of an animal's immediate environment. Acceptable primary enclosures

- Allow for the normal physiologic and behavioral needs of the animals, including urination and defecation, maintenance of body temperature, normal movement and postural adjustments, and, where indicated, reproduction.
- Allow conspecific social interaction and development of hierarchies within or between enclosures.
- Make it possible for the animals to remain clean and dry (as consistent with the requirements of the species).
- Allow adequate ventilation.
- Allow the animals access to food and water and permit easy filling, refilling, changing, servicing, and cleaning of food and water utensils.
- Provide a secure environment that does not allow escape of or accidental entrapment of animals or their appendages between opposing surfaces or by structural openings.
- Are free of sharp edges or projections that could cause injury to the animals.

- Allow observation of the animals with minimal disturbance of them.

Primary enclosures should be constructed with materials that balance the needs of the animal with the ability to provide for sanitation. They should have smooth, impervious surfaces with minimal ledges, angles, corners, and overlapping surfaces so that accumulation of dirt, debris, and moisture is reduced and satisfactory cleaning and disinfecting are possible. They should be constructed of durable materials that resist corrosion and withstand rough handling without chipping, cracking, or rusting. Less-durable materials, such as wood, can provide a more appropriate environment in some situations (such as runs, pens, and outdoor corrals) and can be used to construct perches, climbing structures, resting areas, and perimeter fences for primary enclosures. Wooden items might need to be replaced periodically because of damage or difficulties with sanitation.

All primary enclosures should be kept in good repair to prevent escape of or injury to animals, promote physical comfort, and facilitate sanitation and servicing. Rusting or oxidized equipment that threatens the health or safety of the animals should be repaired or replaced.

Some housing systems have special caging and ventilation equipment, including filter-top cages, ventilated cages, isolators, and cubicles. Generally, the purpose of these systems is to minimize the spread of airborne disease agents between cages or groups of cages. They often require different husbandry practices, such as alterations in the frequency of bedding change, the use of aseptic handling techniques, and specialized cleaning, disinfecting, or sterilization regimens to prevent microbial transmission by other than the airborne route.

Rodents are often housed on wire flooring, which enhances sanitation of the cage by enabling urine and feces to pass through to a collection tray. However, some evidence suggests that solid-bottom caging, with bedding, is preferred by rodents (Fullerton and Gilliatt 1967; Grover-Johnson and Spencer 1981; Ortman and others 1983). Solid-bottom caging, with bedding, is therefore recommended for rodents. Vinyl-coated flooring is often used for other species, such as dogs and nonhuman primates. IACUC review of this aspect of the animal care program should ensure that caging enhances animal well-being consistent with good sanitation and the requirements of the research project.

Sheltered or Outdoor Housing

Sheltered or outdoor housing—such as barns, corrals, pastures, and islands—is a common primary housing method for some species and is acceptable for many situations. In most cases, outdoor housing entails maintaining animals in groups.

When animals are maintained in outdoor runs, pens, or other large enclosures, there must be protection from extremes in temperature or other harsh weather conditions and adequate protective and escape mechanisms for submissive animals. These goals can be achieved by such features as windbreaks, shelters, shaded areas, areas with forced ventilation, heat-radiating structures, or means of retreat to conditioned spaces, such as an indoor portion of a run. Shelters should be accessible to all animals, have sufficient ventilation, and be designed to prevent buildup of waste materials and excessive moisture. Houses, dens, boxes, shelves, perches, and other furnishings should be constructed in a manner and made of materials that allow cleaning or replacement in accord with generally accepted husbandry practices when the furnishings are excessively soiled or worn.

Floors or ground-level surfaces of outdoor housing facilities can be covered with dirt, absorbent bedding, sand, gravel, grass, or similar material that can be removed or replaced when that is needed to ensure appropriate sanitation. Excessive buildup of animal waste and stagnant water should be avoided by, for example, using contoured or drained surfaces. Other surfaces should be able to withstand the elements and be easily maintained.

Successful management of outdoor housing relies on consideration of

- An adequate acclimation period in advance of seasonal changes when animals are first introduced to outdoor housing.
- Training of animals to cooperate with veterinary and investigative personnel and to enter chutes or cages for restraint or transport.
- Species-appropriate social environment.
- Grouping of compatible animals.
- Adequate security via a perimeter fence or other means.

Naturalistic Environments

Areas like pastures and islands afford opportunities to provide a suitable environment for maintaining or producing animals and for some types of research. Their use results in the loss of some control over nutrition, health care and surveillance, and pedigree management. These limitations should be balanced against the benefits of having the animals live in more natural conditions. Animals should be added to, removed from, and returned to social groups in this setting with appropriate consideration of the effects on the individual animals and on the group. Adequate supplies of food, fresh water, and natural or constructed shelter should be ensured.

Space Recommendations

An animal's space needs are complex, and consideration of only the animal's body weight or surface area is insufficient. Therefore, the space recommendations presented here are based on professional judgment and experience and should be considered as recommendations of appropriate cage sizes for animals under conditions commonly found in laboratory animal housing facilities. Vertical height, structuring of the space, and enrichments can clearly affect animals' use of space. Some species benefit more from wall space (e.g., "thigmotactic" rodents), shelters (e.g., some New World primates), or cage complexities (e.g., cats and chimpanzees) than from simple increases in floor space (Anzaldo and others 1994; Stricklin 1995). Thus, basing cage-size recommendations on floor space alone is inadequate. In this regard, the *Guide* might differ from the AWRs (see footnote, p. 2).

Space allocations should be reviewed and modified as necessary to address individual housing situations and animal needs (for example, for prenatal and postnatal care, obese animals, and group or individual housing). Such animal-performance indexes as health, reproduction, growth, behavior, activity, and use of space can be used to assess the adequacy of housing. At a minimum, an animal must have enough space to turn around and to express normal postural adjustments, must have ready access to food and water, and must have enough clean-bedded or unobstructed area to move and rest in. For cats, a raised resting surface should be included in the cage. Raised resting surfaces or perches are also often

desirable for dogs and nonhuman primates. Low resting surfaces that do not allow the space under them to be comfortably occupied by the animal should be counted as part of the floor space. Floor space taken up by food bowls, water containers, litter boxes, or other devices not intended for movement or resting should not be considered part of the floor space.

The need for and type of adjustments in the amounts of primary enclosure space recommended in the tables that follow should be approved at the institutional level by the IACUC and should be based on the performance outcomes described in the preceding paragraph with due consideration of the AWRs and PHS Policy (see footnote, p. 2). Professional judgment, surveys of the literature and current practices, and consideration of the animals' physical, behavioral, and social needs and of the nature of the protocol and its requirements might be necessary (see Crockett and others 1993, 1995). Assessment of animals' space needs should be a continuing process. With the passage of time or long-term protocols, adjustments in floor space and height should be considered and modified as necessary.

It is not within the scope or size constraints of the *Guide* to discuss the housing requirements of all species used in research. For species not mentioned, space and height allocations for an animal of equivalent size and with a similar activity profile and similar behavior can be used as a starting point from which adjustments that take species-specific and individual needs into account can be made.

Whenever it is appropriate, social animals should be housed in pairs or groups, rather than individually, provided that such housing is not contraindicated by the protocol in question and does not pose an undue risk to the animals (Brain and Bention 1979). Depending on a variety of biologic and behavioral factors, group-housed animals might need less or more total space per animal than individually housed animals. Recommendations provided below are based on the assumption that pair or group housing is generally preferable to single housing, even when members of the pair or group have slightly less space *per animal* than when singly caged. For example, each animal can share the space allotted to the animals with which it is housed. Furthermore, some rodents or swine housed in compatible groups seek each other out and share cage space by huddling together along walls, lying on each other during periods of rest, or gathering in areas of retreat (White 1990; White and others 1989). Cattle, sheep, and goats exhibit herding behavior and seek group associations and close physical contact. Conversely, some animals, such as various species of nonhuman primates, might need additional individual space when group-housed to reduce the level of aggression.

The height of enclosures can be important in the normal behavior and postural adjustments of some species. Cage heights should take into account typical postures of an animal and provide adequate clearance for normal cage components, such as feeders and water devices, including sipper tubes. Some species of nonhuman primates use the vertical dimensions of the cage to a greater extent than the floor. For them, the ability to perch and to have adequate vertical space to keep the whole body above the cage floor can improve their well-being.

Space allocations for animals should be based on the following tables, but might need to be increased, or decreased with approval of the IACUC, on the basis of criteria previously listed.

Table 2.1 lists recommended space allocations for commonly used laboratory rodents housed in groups. If they are housed individually or exceed the weights in the table, animals might require more space.

TABLE 2.1 Recommended Space for Commonly Used Group-Housed Laboratory Rodents

Animals	Weight, g	Floor Area/Animal, in^{2} [a]	Height[b], in[c]
Mice	<10	6	5
	Up to 15	8	5
	Up to 25	12	5
	>25[d]	≥15	5
Rats	<100	17	7
	Up to 200	23	7
	Up to 300	29	7
	Up to 400	40	7
	Up to 500	60	7
	>500[d]	≥70	7
Hamsters	<60	10	6
	Up to 80	13	6
	Up to 100	16	6
	>100[d]	≥19	6
Guinea pigs	≤350	60	7
	>350[d]	≥101	7

[a]To convert square inches to square centimeters, multiply by 6.45.
[b]From cage floor to cage top.
[c]To convert inches to centimeters, multiply by 2.54.
[d]Larger animals might require more space to meet the performance standards (see text).

Table 2.2 lists recommended space allocations for other common laboratory animals. These allocations are based, in general, on the needs of individually housed animals. Space allocations should be re-evaluated to provide for enrichment of the primary enclosure or to accommodate animals that exceed the weights in the table. For group housing, determination of the total space needed is not necessarily based on the sum of the amounts recommended for individually housed animals. Space for group-housed animals should be based on individual species needs, behavior, compatibility of the animals, numbers of animals, and goals of the housing situation.

TABLE 2.2 Recommended Space for Rabbits, Cats, Dogs, Nonhuman Primates, and Birds

Animals	Weight, kg[a]	Floor Area/Animal, ft^{2} [b]	Height[c], in[d]
Rabbits	<2	1.5	14
	Up to 4	3.0	14
	Up to 5.4	4.0	14
	>5.4[e]	≥5.0	14
Cats	≤4	3.0	24
	>4[e]	≥4.0	24
Dogs[f]	<15	8.0	--
	Up to 30	12.0	--

Animals	Weight, kg[a]	Floor Area/Animal, ft^{2} [b]	Height[c], in[d]
	>30[e]	≥24.0	--
Monkeys[g, h] (including baboons)			
Group 1	Up to 1	1.6	20
Group 2	Up to 3	3.0	30
Group 3	Up to 10	4.3	30
Group 4	Up to 15	6.0	32
Group 5	Up to 25	8.0	36
Group 6	Up to 30	10.0	46
Group 7	>30[e]	15.0	46
Apes (Pongidae)[h]			
Group 1	Up to 20	10.0	55
Group 2	Up to 35	15.0	60
Group 3	>35[i]	25.0	84
Pigeons[j]	--	0.8	--
Quail[j]	--	0.25	--
Chickens[j]	<0.25	0.25	--
	Up to 0.5	0.50	--
	Up to 1.5	1.00	--
	Up to 3.0	2.00	--
	>3.0[e]	≥3.00	--

[a]To convert kilograms to pounds, multiply by 2.2.
[b]To convert square feet to square meters, multiply by 0.09.
[c]From cage floor to cage top.
[d]To convert inches to centimeters, multiply by 2.54.
[e]Larger animals might require more space to meet performance standards (see text).
[f]These recommendations might require modification according to body conformation of individual animals and breeds. Some dogs, especially those toward upper limit of each weight range, might require additional space to ensure compliance with the regulations of the Animal Welfare Act. These regulations (CFR 1985) mandate that the height of each cage be sufficient to allow occupant to stand in "comfortable position" and that the minimal square feet of floor space be equal to "mathematical square of the sum of the length of the dog in inches, as measured from the tip of its nose to the base of its tail, plus 6 inches."
[g]Callitrichidae, Cebidae, Cercopithecidae, and *Papio*. Baboons might require more height than other monkeys.
[h]For some species (e.g., *Brachyteles*, *Hylobates*, *Symphalangus*, *Pongo*, and *Pan*), cage height should be such that an animal can, when fully extended, swing from the cage ceiling without having its feet touch the floor. Cage-ceiling design should enhance brachiating movement.
[i]Apes weighing over 50 kg are more effectively housed in permanent housing of masonry, concrete, and wire-panel structure than in conventional caging.
[j]Cage height should be sufficient for the animals to stand erect with their feet on the floor.

Table 2.3 lists recommended space allocations for farm animals commonly used in a laboratory setting. When animals, housed individually or in groups, exceed the weights in the table, more space might be required. If they are group-housed, adequate access to water and feeder space should be provided (Larson and Hegg 1976; Midwest Plan Service 1987).

TABLE 2.3 Recommended Space for Commonly Used Farm Animals

Animals/Enclosure	Weight, kg[a]	Floor Area/Animal, ft^{2} [b]
Sheep and Goats		
1	<25	10.0

Animals/Enclosure		Weight, kg[a]	Floor Area/Animal, ft^{2} [b]
		Up to 50	15.0
		>50[c]	20.0
	2-5	<25	8.5
		Up to 50	12.5
		>50[c]	17.0
	>5	<25	7.5
		Up to 50	11.3
		>50[c]	15.0
Swine			
	1	<15	8.0
		Up to 25	12.0
		Up to 50	15.0
		Up to 100	24.0
		Up to 200	48.0
		>200[c]	≥60.0
	2-5	<25	6.0
		Up to 50	10.0
		Up to 100	20.0
		Up to 200	40.0
		>200[c]	≥52.0
	>5	<25	6.0
		Up to 50	9.0
		Up to 100	18.0
		Up to 200	36.0
		>200[c]	≥48.0
Cattle			
	1	<75	24.0
		Up to 200	48.0
		Up to 350	72.0
		Up to 500	96.0
		Up to 650	124.0
		>650[c]	≥144.0
	2-5	<75	20.0
		Up to 200	40.0
		Up to 350	60.0
		Up to 500	80.0
		Up to 650	105.0
		>650[c]	≥120.0
	>5	<75	18.0
		Up to 200	36.0
		Up to 350	54.0
		Up to 500	72.0
		Up to 650	93.0
		>650[c]	≥108.0
Horses		--	144.0
Ponies			
	1-4	--	72.0
	>4/Pen	≤200	60.0
		>200[c]	≥72.0

[a]To convert kilograms to pounds, multiply by 2.2.

[b]To convert square feet to square meters, multiply by 0.09.

[c]Larger animals might require more space to meet performance standards (see text).

Temperature and Humidity

Regulation of body temperature within normal variation is necessary for the well-being of homeotherms. Generally, exposure of unadapted animals to temperatures above 85°F (29.4°C) or below 40°F (4.4°C), without access to shelter or other protective mechanisms, might produce clinical effects (Gordon 1990), which could be life-threatening. Animals can adapt to extremes by behavioral, physiologic, and morphologic mechanisms, but such adaptation takes time and might alter protocol outcomes or otherwise affect performance (Garrard and others 1974; Gordon 1993; Pennycuik 1967).

Environmental temperature and relative humidity can depend on husbandry and housing design and can differ considerably between primary and secondary enclosures. Factors that contribute to variation in temperature and humidity include housing material and construction, use of filter tops, number of animals per cage, forced ventilation of the enclosures, frequency of bedding changes, and bedding type.

Some conditions might require increased environmental temperatures, such as postoperative recovery, maintenance of chicks for the first few days after hatching, housing of some hairless rodents, and housing of neonates that have been separated from their mothers. The magnitude of the temperature increase depends on the circumstances of housing; sometimes, raising the temperature in the primary enclosure alone (rather than raising the temperature of the secondary enclosure) is sufficient.

In the absence of well-controlled studies, professional judgment and experience have resulted in recommendations for dry-bulb temperatures (Table 2.4) for several common species. In the case of animals in confined spaces, the range of daily temperature fluctuations should be kept to a minimum to avoid repeated large demands on the animals' metabolic and behavioral processes to compensate for changes in the thermal environment. Relative humidity should also be controlled, but not nearly as narrowly as temperature; the acceptable range of relative humidity is 30 to 70%. The temperature ranges in Table 2.4 might not apply to captive wild animals, wild animals maintained in their natural environment, or animals in outdoor enclosures that are given the opportunity to adapt by being exposed to seasonal changes in ambient conditions.

TABLE 2.4 Recommended Dry-Bulb Temperatures for Common Laboratory Animals

Animal	Dry-Bulb Temperature	
	°C	°F
Mouse, rat, hamster, gerbil, guinea pig	18-26	64-79
Rabbit	16-22	61-72
Cat, dog, nonhuman primate	18-29	64-84
Farm animals and poultry	16-27	61-81

Ventilation

The purposes of ventilation are to supply adequate oxygen; remove thermal loads caused by animal respiration, lights, and equipment; dilute gaseous and particulate contaminants; adjust the moisture content of room air; and, where appropriate, create static-pressure differentials between adjoining spaces. Establishing a room ventilation rate, however, does not ensure the adequacy of the ventilation of an animal's primary enclosure and hence does not guarantee the quality of the microenvironment.

The degree to which air movement (drafts) causes discomfort or biologic consequences has not been established for most species. The volume and physical characteristics of the air supplied to a room and its diffusion pattern influence the ventilation of an animal's primary enclosure and so are important determinants of its microenvironment. The relationship of the type and location of supply-air diffusers and exhaust vents to the number, arrangement, location, and type of primary enclosures in a room or other secondary enclosure affects how well the primary enclosures are ventilated and should therefore be considered. The use of computer modeling for assessing those factors in relation to heat loading and air diffusion patterns can be helpful in optimizing ventilation of primary and secondary enclosures (for example, Hughes and Reynolds 1995; Reynolds and Hughes 1994).

The guideline of 10-15 fresh-air changes per hour has been used for secondary enclosures for many years and is considered an acceptable general standard. Although it is effective in many animal-housing settings, the guideline does not take into account the range of possible heat loads; the species, size, and number of animals involved; the type of bedding or frequency of cage-changing; the room dimensions; or the efficiency of air distribution from the secondary to the primary enclosure. In some situations, the use of such a broad guideline might pose a problem by overventilating a secondary enclosure that contains few animals and thereby wasting energy or by underventilating a secondary enclosure that contains many animals and thereby allowing heat and odor accumulation.

To determine more accurately the ventilation required, the minimal ventilation rate (commonly in cubic feet per minute) required to accommodate heat loads generated by animals can be calculated with the assistance of mechanical engineers. The heat generated by animals can be calculated with the average-total-heat-gain formula as published by the American Society of Heating, Refrigeration, and Air-Conditioning Engineers (ASHRAE) (1992). The formula is species-independent, so it is applicable to any heat-generating animal. Minimal required ventilation is determined by calculating the amount of cooling required (total cooling load) to control the heat load expected to be generated by the largest number of animals to be housed in the enclosure in question plus any heat expected to be produced by nonanimal sources and heat transfer through room surfaces. The total-cooling-load calculation method can also be used for an animal space that has a fixed ventilation rate to determine the maximal number of animals (based on total animal mass) that can be housed in the space.

Even though that calculation can be used to determine minimal ventilation needed to prevent heat buildup, other factors—such as odor control, allergen control, particle generation, and control of metabolically generated gases—might necessitate ventilation beyond the calculated minimum. When the calculated minimal required ventilation is substantially less than 10 air changes per hour, lower ventilation rates might be appropriate in the secondary enclosure, provided that they do not result in harmful or unacceptable concentrations of toxic

gases, odors, or particles in the primary enclosure. Similarly, when the calculated minimal required ventilation exceeds 15 air changes per hour, provisions should be made for additional ventilation required to address the other factors. In some cases, fixed ventilation in the secondary enclosure might necessitate adjustment of sanitation schedules or limitation of animal numbers to maintain appropriate environmental conditions.

Caging with forced ventilation that uses filtered room air and other types of special primary enclosures with independent air supplies (i.e., air not drawn from the room) can effectively address the ventilation requirements of animals without the need to ventilate secondary enclosures to the extent that would be needed if there were no independent primary-enclosure ventilation. Nevertheless, a secondary enclosure should be ventilated sufficiently to provide for the heat loads released from its primary enclosures. If the specialized enclosures contain adequate particulate and gaseous filtration to address contamination risks, recycled air may be used in the secondary enclosures.

Filtered isolation caging without forced ventilation, such as that used in some types of rodent housing, restricts ventilation. To compensate, it might be necessary to adjust husbandry practices—including sanitation, placement of cages in the secondary enclosure, and cage densities—to improve the microenvironment and heat dissipation.

The use of recycled air to ventilate animal rooms saves considerable amounts of energy but might entail some risk. Many animal pathogens can be airborne or travel on fomites, such as dust, so exhaust air to be recycled into heating, ventilation, and air conditioning (HVAC) systems that serve multiple rooms presents a risk of cross contamination. The exhaust air to be recycled should be HEPA-filtered (high-efficiency particulate air-filtered) to remove airborne particles before it is recycled; the extent and efficiency of filtration should be proportional to the estimated risk. HEPA filters are available in various efficiencies that can be used to match the magnitude of risk (ASHRAE 1992). Air that does not originate from animal-use areas but has been used to ventilate other spaces (e.g., some human-occupancy areas and food, bedding, and supply storage areas) may be recycled for animal-space ventilation and might require less-intensive filtration or conditioning than air recycled from animal-use space. The risks in some situations, however, might be too great to consider recycling (e.g., in the case of nonhuman-primate and biohazard areas).

Toxic or odor-causing gases, such as ammonia, can be kept within acceptable limits if they are removed by the ventilation system and replaced with air that contains either a lower concentration or none of these gases. Treatment of recycled air for these substances by chemical absorption or scrubbing might be effective; however, the use of nonrecycled air is preferred for ventilation of animal use and holding areas. The use of HEPA-filtered recycled air without gaseous filtration (such as with activated-charcoal filters) can be used but only in limited applications, provided that

- Room air is mixed with at least 50% fresh air (that is, the supply air does not exceed 50% recycled air).
- Husbandry practices, such as bedding-change and cage-washing frequency, and the preparation of recycled air used are sufficient to minimize toxic gases and odors.
- Recycled air is returned only to the room or area from which it was generated, except if it comes from other than animal-housing areas.

- Recycled air is appropriately conditioned and mixed with sufficient fresh air to address the thermal and humidity requirements of animals in that space.

Frequent bedding changes and cage-cleaning coupled with husbandry practices, such as low animal density within the room and lower environmental temperature and humidity, can also reduce the concentration of toxic or odor-causing gases in animal-room air. Treatment of recycled air for either particulate or gaseous contaminants is expensive and can be rendered ineffective by improper or insufficient maintenance of filtration systems. These systems should be properly maintained and monitored appropriately to maximize their effectiveness.

The successful operation of any HVAC system requires regular maintenance and evaluation, including measurement of its function at the level of the secondary enclosure. Such measurements should include supply- and exhaust-air volumes, as well as static-pressure differentials, where applicable.

Illumination

Light can affect the physiology, morphology, and behavior of various animals (Brainard and others 1986; Erkert and Grober 1986; Newbold and others 1991; Tucker and others 1984). Potential photostressors include inappropriate photoperiod, photointensity, and spectral quality of the light (Stoskopf 1983). Numerous factors can affect animals' needs for light and should be considered when an appropriate illumination level is being established for an animal holding room. These include light intensity, duration of exposure, wavelength of light, light history of the animal, pigmentation of the animal, time of light exposure during the circadian cycle, body temperature, hormonal status, age, species, sex, and stock or strain of animal (Brainard 1989; Duncan and O'Steen 1985; O'Steen 1980; Saltarelli and Coppola 1979; Semple-Rowland and Dawson 1987; Wax 1977).

In general, lighting should be diffused throughout an animal holding area and provide sufficient illumination for the well-being of the animals and to allow good housekeeping practices, adequate inspection of animals—including the bottom-most cages in racks—and safe working conditions for personnel. Light in animal holding rooms should provide for adequate vision and for neuroendocrine regulation of diurnal and circadian cycles (Brainard 1989).

Photoperiod is a critical regulator of reproductive behavior in many species of animals (Brainard and others 1986; Cherry 1987) and can also alter body-weight gain and feed intake (Tucker and others 1984). Inadvertent light exposure during the dark cycle should be minimized or avoided. Because some species will not eat in low light or darkness, such illumination schedules should be limited to a duration that will not compromise the well-being of the animals. A time-controlled lighting system should be used to ensure a regular diurnal cycle, and timer performance should be checked periodically to ensure proper cycling.

The most commonly used laboratory animals are nocturnal. Because the albino rat is more susceptible to phototoxic retinopathy than other species, it has been used as a basis for establishing room illumination levels (Lanum 1979). Data for room light intensities for other animals, based on scientific studies, are not available. Light levels of about 325 lux (30 ft-candles) about 1.0 m (3.3 ft) above the floor appear to be sufficient for animal care and do not

cause clinical signs of phototoxic retinopathy in albino rats (Bellhorn 1980), and levels up to 400 lux (37 ft-candles) as measured in an empty room 1 m from the floor have been found to be satisfactory for rodents if management practices are used to prevent retinal damage in albinos (Clough 1982). However, the light experience of an individual animal can affect its sensitivity to phototoxicity; light of 130-270 lux above the light intensity under which it was raised has been reported to be near the threshold of retinal damage in some individual albino rats according to histologic, morphometric, and electrophysiologic evidence (Semple-Rowland and Dawson 1987). Some guidelines recommend a light intensity as low as 40 lux at the position of the animal in midcage (NASA 1988). Young albino and pigmented mice prefer much-lower illumination than adults (Wax 1977), although potential retinal damage associated with housing these rodents at higher light levels is mostly reversible. Thus, for animals that have been shown to be susceptible to phototoxic retinopathy, light at the cage level should be between 130 and 325 lux.

Management practices, such as rotating cage position relative to the light source (Greenman and others 1982) or providing animals with ways to modify their own light exposure by behavioral means (e.g., via tunneling or hiding in a structure), can be used to reduce inappropriate light stimulation of animals. Provision of variable-intensity light controls might be considered as a means of ensuring that light intensities are consistent with the needs of animals and personnel working in animal rooms and with energy conservation. Such controls should have some form of vernier scale and a lockable setting and should not be used merely to turn room lighting on and off. The Illuminating Engineering Society of North America (IESNA) handbook (Kaufman 1984, 1987) can assist in decisions concerning lighting uniformity, color-rendering index, shielding, glare control, reflection, lifetime, heat generation, and ballast selection.

Noise

Noise produced by animals and animal-care activities is inherent in the operation of an animal facility (Pfaff and Stecker 1976). Therefore, noise control should be considered in facility design and operation (Pekrul 1991). Assessment of the potential effects of noise on an animal warrants consideration of the intensity, frequency, rapidity of onset, duration, and vibration potential of the sound and the hearing range, noise-exposure history, and sound-effect susceptibility of the species, stock, or strain.

Separation of human and animal areas minimizes disturbances to both the human and animal occupants of the facility. Noisy animals—such as dogs, swine, goats, and nonhuman primates—should be housed away from quieter animals, such as rodents, rabbits, and cats. Environments should be designed to accommodate animals that make noise, rather than resorting to methods of noise reduction. Exposure to sound louder than 85 dB can have both auditory and nonauditory effects (Fletcher 1976; Peterson 1980), including eosinopenia and increased adrenal weights in rodents (Geber and others 1966; Nayfield and Besch 1981), reduced fertility in rodents (Zondek and Tamari 1964), and increased blood pressure in nonhuman primates (Peterson and others 1981). Many species can hear frequencies of sound that are inaudible to humans (Brown and Pye 1975; Warfield 1973), so the potential effects of equipment and materials that produce noise in the hearing range of nearby animals—such as video display terminals (Sales 1991) should be carefully considered. To the greatest extent

possible, activities that might be noisy should be conducted in rooms or areas separate from those used for animal housing.

Because changes in patterns of sound exposure have different effects on different animals (Armario and others 1985; Clough 1982), personnel should try to minimize the production of unnecessary noise. Excessive and intermittent noise can be minimized by training personnel in alternatives to practices that produce noise and by the use of cushioned casters and bumpers on carts, trucks, and racks. Radios, alarms, and other sound generators should not be used in animal rooms unless they are parts of an approved protocol or an enrichment program.

BEHAVIORAL MANAGEMENT

Structural Environment

The structural environment consists of components of the primary enclosure—cage furniture, equipment for environmental enrichment, objects for manipulation by the animals, and cage complexities. Depending on the animal species and use, the structural environment should include resting boards, shelves or perches, toys, foraging devices, nesting materials, tunnels, swings, or other objects that increase opportunities for the expression of species-typical postures and activities and enhance the animals' well-being. Much has been learned in recent years about the natural history and environmental needs of many animals, but continuing research into those environments that enhance the well-being of research animals is encouraged. Selected publications that describe enrichment strategies for common laboratory animal species are listed in Appendix A and in bibliographies prepared by the Animal Welfare Information Center (AWIC 1992; NRC In press).

Social Environment

Consideration should be given to an animal's social needs. The social environment usually involves physical contact and communication among members of the same species (conspecifics), although it can include noncontact communication among individuals through visual, auditory, and olfactory signals. When it is appropriate and compatible with the protocol, social animals should be housed in physical contact with conspecifics. For example, grouping of social primates or canids is often beneficial to them if groups comprise compatible individuals. Appropriate social interactions among conspecifics are essential for normal development in many species. A social companion might buffer the effects of a stressful situation (Gust and others 1994), reduce behavioral abnormality (Reinhardt and others 1988, 1989), increase opportunities for exercise (Whary and others 1993), and expand species-typical behavior and cognitive stimulation. Such factors as population density, ability to disperse, initial familiarity among animals, and social rank should be evaluated when animals are being grouped (Borer and others 1988; Diamond and others 1987; Drickamer 1977; Harvey and Chevins 1987; Ortiz and others 1985; Vandenbergh 1986, 1989). In selecting a suitable social environment, attention should be given to whether the animals are naturally territorial or communal and whether they should be housed singly, in pairs, or in

groups. An understanding of species-typical natural social behavior will facilitate successful social housing.

However, not all members of a social species can or should be maintained socially; experimental, health, and behavioral reasons might preclude a successful outcome of this kind of housing. Social housing can increase the likelihood of animal wounds due to fighting (Bayne and others 1995), increase susceptibility to such metabolic disorders as atherosclerosis (Kaplan and others 1982), and alter behavior and physiologic functions (Bernstein 1964; Bernstein and others 1974a,b). In addition, differences between sexes in compatibility have been observed in various species (Crockett and others 1994; Grant and Macintosh 1963; Vandenbergh 1971; vom Saal 1984). These risks of social housing are greatly reduced if the animals are socially compatible and the social unit is stable.

It is desirable that social animals be housed in groups; however, when they must be housed alone, other forms of enrichment should be provided to compensate for the absence of other animals, such as safe and positive interaction with the care staff and enrichment of the structural environment.

Activity

Animal activity typically implies motor activity but also includes cognitive activity and social interaction. Animals maintained in a laboratory environment might have a more-restricted activity profile than those in a free-ranging state. An animal's motor activity, including use of the vertical dimension, should be considered in evaluation of suitable housing or assessment of the appropriateness of the quantity or quality of an activity displayed by an animal. Forced activity for reasons other than attempts to meet therapeutic or approved protocol objectives should be avoided. In most species, physical activity that is repetitive, is non-goal-oriented, and excludes other behavior is considered undesirable (AWIC 1992; Bayne 1991; NRC In press; see also Appendix A, "Enrichment").

Animals should have opportunities to exhibit species-typical activity patterns. Dogs, cats, and many other domesticated animals benefit from positive human interaction (Rollin 1990). Dogs can be given opportunities for activity by being walked on a leash, having access to a run, or being moved into another area (such as a room, larger cage, or outdoor pen) for social contact, play, or exploration. Cages are often used for short-term housing of dogs for veterinary care and some research purposes, but pens, runs, and other out-of-cage areas provide more space for movement, and their use is encouraged (Wolff and Rupert 1991). Loafing areas, exercise lots, and pastures are suitable for large farm animals, such as sheep, horses, and cattle.

HUSBANDRY

Food

Animals should be fed palatable, noncontaminated, and nutritionally adequate food daily or according to their particular requirements unless the protocol in which they are being used requires otherwise. Subcommittees of the National Research Council Committee on

Animal Nutrition have prepared comprehensive treatments of the nutrient requirements of laboratory animals (NRC 1977, 1978, 1981a,b, 1982, 1983, 1984, 1985a,b, 1986, 1988, 1989a,b, 1994, 1995). Their publications consider issues of quality assurance, freedom from chemical or microbial contaminants and presence of natural toxicants in feedstuffs, bioavailability of nutrients in feeds, and palatability.

Animal-colony managers should be judicious in purchasing, transporting, storing, and handling food to minimize the introduction of diseases, parasites, potential disease vectors (e.g., insects and other vermin), and chemical contaminants into animal colonies. Purchasers are encouraged to consider manufacturers' and suppliers' procedures and practices for protecting and ensuring diet quality (e.g., storage, vermin-control, and handling procedures). Institutions should urge feed vendors to provide data from feed analysis for critical nutrients periodically. The date of manufacture and other factors that affect shelf-life of food should be known by the user. Stale food or food transported and stored inappropriately can become deficient in nutrients. Careful attention should be paid to quantities received in each shipment, and stock should be rotated so that the oldest food is used first.

Areas in which diets and diet ingredients are processed or stored should be kept clean and enclosed to prevent entry of pests. Food should be stored off the floor on pallets, racks, or carts. Unused, opened bags of food should be stored in vermin-proof containers to minimize contamination and to avoid potential spread of disease agents. Exposure to temperatures above 21°C (70°F), extremes in relative humidity, unsanitary conditions, light, oxygen, and insects and other vermin hasten the deterioration of food. Precautions should be taken if perishable items—such as meats, fruits, and vegetables—are fed, because storage conditions are potential sources of contamination and can lead to variation in food quality. Contaminants in food can have dramatic effects on biochemical and physiologic processes, even if the contaminants are present in concentrations too low to cause clinical signs of toxicity. For example, some contaminants induce the synthesis of hepatic enzymes that can alter an animal's response to drugs (Ames and others 1993; Newberne 1975). Some experimental protocols might require the use of pretested animal diets in which both biologic and nonbiologic contaminants are identified and their concentrations documented.

Most natural-ingredient, dry laboratory-animal diets that contain preservatives and are stored properly can be used up to about 6 months after manufacture. Vitamin C in manufactured feeds, however, generally has a shelf-life of only 3 months. The use of stabilized forms of vitamin C can extend the shelf-life of feed. If a diet containing outdated vitamin C is to be fed to animals that require dietary vitamin C, it is necessary to provide an appropriate vitamin C supplement. Refrigeration preserves nutritional quality and lengthens shelf-life, but food-storage time should be reduced to the lowest practical period and the recommendations of manufacturers should be considered. Purified and chemically defined diets are often less stable than natural-ingredient diets, and their shelf-life is usually less than 6 months (Fullerton and others 1982); these diets should be stored at 4°C (39°F) or lower.

Autoclavable diets require adjustments in nutrient concentrations, kinds of ingredients, and methods of preparation to withstand degradation during sterilization (Wostman 1975). The date of sterilization should be recorded and the diet used quickly. Irradiated diets might be considered as an alternative to autoclaved diets.

Feeders should be designed and placed to allow easy access to food and to minimize contamination with urine and feces. When animals are housed in groups, there should be

enough space and enough feeding points to minimize competition for food and ensure access to food for all animals, especially if feed is restricted as part of the protocol or management routine. Food-storage containers should not be transferred between areas that pose different risks of contamination, and they should be cleaned and sanitized regularly.

Moderate restriction of calorie and protein intakes for clinical or husbandry reasons has been shown to increase longevity and decrease obesity, reproduction, and cancer rates in a number of species (Ames and others 1993; Keenan and others 1994). Such restriction can be achieved by decreasing metabolizable energy, protein density, or both in the diet or by controlling ration amount or frequency of feeding. The choice of mechanism for calorie restriction is species-dependent and will affect physiologic adaptations and alter metabolic responses (Leveille and Hanson 1966). Calorie restriction is an accepted practice for long-term housing of some species, such as some rodents and rabbits, and as an adjunct to some clinical and surgical procedures.

In some species (such as nonhuman primates) and on some occasions, varying nutritionally balanced diets and providing "treats," including fresh vegetables, can be appropriate and improve well-being. However, caution should be used in varying diets. A diet should be nutritionally balanced; it is well documented that many animals offered a cafeteria of unbalanced foods do not select a balanced diet and become obese through selection of high-energy, low-protein foods (Moore 1987). Abrupt changes in diet (which are difficult to avoid at weaning) should be minimized because they can lead to digestive and metabolic disturbances; these changes occur in omnivores and carnivores, but herbivores (Eadie and Mann 1970) are especially sensitive.

Water

Ordinarily, animals should have access to potable, uncontaminated drinking water according to their particular requirements. Water quality and the definition of potable water can vary with locality (Homberger and others 1993). Periodic monitoring for pH, hardness, and microbial or chemical contamination might be necessary to ensure that water quality is acceptable, particularly for use in studies in which normal components of water in a given locality can influence the results obtained. Water can be treated or purified to minimize or eliminate contamination when protocols require highly purified water. The selection of water treatments should be carefully considered because many forms of water treatment have the potential to cause physiologic alterations, changes in microflora, or effects on experimental results (Fidler 1977; Hall and others 1980; Hermann and others 1982; Homberger and others 1993). For example, chlorination of the water supply can be useful for some species but toxic to others (such as aquatic species).

Watering devices, such as drinking tubes and automatic waterers, should be checked daily to ensure their proper maintenance, cleanliness, and operation. Animals sometimes have to be trained to use automatic watering devices. It is better to replace water bottles than to refill them, because of the potential for microbiologic cross-contamination; however, if bottles are refilled, care should be taken to replace each bottle on the cage from which it was removed. Animals housed in outdoor facilities might have access to water in addition to that provided in watering devices, such as that available in streams or in puddles after a heavy

rainfall. Care should be taken to ensure that such accessory sources of water do not constitute a hazard, but their availability need not routinely be prevented.

Bedding

Animal bedding is a controllable environmental factor that can influence experimental data and animal well-being. The veterinarian or facility manager, in consultation with investigators, should select the most appropriate bedding material. No bedding is ideal for any given species under all management and experimental conditions, and none is ideal for all species (for example, bedding that enables burrowing is encouraged for some species). Several writers (Gibson and others 1987; Jones 1977; Kraft 1980; Thigpen and others 1989; Weichbrod and others 1986) have described desirable characteristics and means of evaluating bedding. Softwood beddings have been used, but the use of untreated softwood shavings and chips is contraindicated for some protocols because they can affect animals' metabolism (Vesell 1967; Vessell and others 1973, 1976). Cedar shavings are not recommended, because they emit aromatic hydrocarbons that induce hepatic microsomal enzymes and cytotoxicity (Torronen and others 1989; Weichbrod and others 1986) and have been reported to increase the incidence of cancer (Jacobs and Dieter 1978; Vlahakis 1977). Heat treatments applied before bedding materials are used reduce the concentration of aromatic hydrocarbons and might prevent this problem. Manufacturing, monitoring, and storage methods used by vendors should be considered when purchasing bedding products.

Bedding should be transported and stored off the floor on pallets, racks, or carts in a fashion consistent with maintenance of quality and minimization of contamination. During autoclaving, bedding can absorb moisture and as a result lose absorbency and support the growth of microorganisms. Therefore, appropriate drying times and storage conditions should be used.

Bedding should be used in amounts sufficient to keep animals dry between cage changes, and, in the case of small laboratory animals, care should be taken to keep the bedding from coming into contact with the water tube, because such contact could cause leakage of water into the cage.

Sanitation

Sanitation—the maintenance of conditions conducive to health—involves bedding change (as appropriate), cleaning, and disinfection. Cleaning removes excessive amounts of dirt and debris, and disinfection reduces or eliminates unacceptable concentrations of microorganisms.

The frequency and intensity of cleaning and disinfection should depend on what is needed to provide a healthy environment for an animal, in accord with its normal behavior and physiologic characteristics. Methods and frequencies of sanitation will vary with many factors, including the type, physical properties, and size of the enclosure; the type, number, size, age, and reproductive status of the animals; the use and type of bedding materials; temperature and relative humidity; the nature of the materials that create the need for sanitation; the normal physiologic and behavioral characteristics of the animals; and the rate of soiling of the surfaces of the enclosure. Some housing systems or experimental protocols

might require specific husbandry techniques, such as aseptic handling or modification in the frequency of bedding change.

Agents designed to mask animal odors should not be used in animal-housing facilities. They cannot substitute for good sanitation practices or for the provision of adequate ventilation, and they expose animals to volatile compounds that might alter basic physiologic and metabolic processes.

Bedding Change

Soiled bedding should be removed and replaced with fresh materials as often as is necessary to keep the animals clean and dry. The frequency is a matter of professional judgment of animal care personnel based on consultation with the investigator and depends on such factors as the number and size of the animals in the primary enclosure, the size of the enclosure, urinary and fecal output, the appearance and wetness of the bedding, and experimental conditions, such as those of surgery or debilitation, that might limit an animal's movement or access to areas of the cage that have not been soiled with urine and feces. There is no absolute minimal frequency of changing bedding, but it typically varies from daily to weekly. In some instances, frequent bedding changes are contraindicated, such as during some portions of the prepartum or postpartum period, when pheromones are essential for successful reproduction, or when research objectives do not permit changing the bedding.

Cleaning and Disinfection of Primary Enclosures

For pens or runs, frequent flushing with water and periodic use of detergents or disinfectants are usually appropriate to maintain sufficiently clean surfaces. If animal waste is to be removed by flushing, this will need to be done at least once a day. Animals should be kept dry during such flushing. The timing of pen or run cleaning should take into account normal behavioral and physiologic processes of the animals; for example, the gastrocolic reflex in meal-fed animals results in defecation shortly after food consumption.

The frequency of sanitation of cages, cage racks, and associated equipment, such as feeders and watering devices, is governed to some extent by the types of caging and husbandry practices used, including the use of regularly changed contact or noncontact bedding, regular flushing of suspended catch pans, and the use of wire-bottom or perforated-bottom cages. In general, enclosures and accessories, such as tops, should be sanitized at least once every 2 weeks. Solid-bottom caging, bottles, and sipper tubes usually require sanitation at least once a week. Some types of cages and racking might require less-frequent cleaning or disinfection; these might include large cages with very low animal density and frequent bedding changes, cages that house animals in gnotobiotic conditions with frequent bedding changes, individually ventilated cages, and cages used for special circumstances. Some circumstances, such as microisolator housing or more densely populated enclosures, might require more frequent sanitation.

Rabbits and some rodents, such as guinea pigs and hamsters, produce urine with high concentrations of proteins and minerals. Minerals and organic compounds in the urine from these animals often adhere to cage surfaces and necessitate treatment with acid solutions before washing.

Primary enclosures can be disinfected with chemicals, hot water, or a combination of both. Washing times and conditions should be sufficient to kill vegetative forms of common bacteria and other organisms that are presumed to be controllable by the sanitation program. When hot water is used alone, it is the combined effect of the temperature and the length of time that a given temperature (cumulative heat factor) is applied to the surface of the item that disinfects. The same cumulative heat factor can be obtained by exposing organisms to very high temperatures for short periods or exposing them to lower temperatures for longer periods (Wardrip and others 1994). Effective disinfection can be achieved with wash and rinse water at 143-180°F or more. The traditional 82.2°C (180°F) temperature requirement for rinse water refers to the water in the tank or in the sprayer manifold. Detergents and chemical disinfectants enhance the effectiveness of hot water but should be thoroughly rinsed from surfaces before reuse of the equipment.

Washing and disinfection of cages and equipment by hand with hot water and detergents or disinfectants can be effective but require attention to detail. It is particularly important to ensure that surfaces are rinsed free of residual chemicals and that personnel have appropriate equipment to protect themselves from exposure to hot water or chemical agents used in the process.

Water bottles, sipper tubes, stoppers, feeders, and other small pieces of equipment should be washed with detergents, hot water, and, where appropriate, chemical agents to destroy microorganisms.

If automatic watering systems are used, some mechanism to ensure that microorganisms and debris do not build up in the watering devices is recommended. The mechanism can be periodic flushing with large volumes of water or appropriate chemical agents followed by a thorough rinsing. Constant-recirculation loops that use properly maintained filters, ultraviolet lights, or other devices to sterilize recirculated water are also effective.

Conventional methods of cleaning and disinfection are adequate for most animal-care equipment. However, if pathogenic microorganisms are present or if animals with highly defined microbiologic flora or compromised immune systems are maintained, it might be necessary to sterilize caging and associated equipment after cleaning and disinfection. Sterilizers should be regularly calibrated and monitored to ensure their safety and effectiveness.

Cleaning and Disinfection of Secondary Enclosures

All components of the animal facility, including animal rooms and support spaces (such as storage areas, cage-washing facilities, corridors, and procedure rooms) should be cleaned regularly and disinfected as appropriate to the circumstances and at a frequency based on the use of the area and the nature of likely contamination.

Cleaning utensils should be assigned to specific areas and should not be transported between areas that pose different risks of contamination. Cleaning utensils themselves should be cleaned regularly and should be constructed of materials that resist corrosion. Worn items should be replaced regularly. The utensils should be stored in a neat, organized fashion that facilitates drying and minimizes contamination.

Assessing the Effectiveness of Sanitation

Monitoring of sanitation practices should be appropriate to the process and materials being cleaned; it can include visual inspection of the materials, monitoring of water temperatures, or microbiologic monitoring. The intensity of animal odors, particularly that of ammonia, should not be used as the sole means of assessing the effectiveness of the sanitation program. A decision to alter the frequency of cage-bedding changes or cage-washing should be based on such factors as the concentration of ammonia, the appearance of the cage, the condition of the bedding, and the number and size of animals housed in the cage.

Waste Disposal

Conventional, biologic, and hazardous waste should be removed and disposed of regularly and safely (NSC 1979). There are several options for effective waste disposal. Contracts with licensed commercial waste-disposal firms usually provide some assurance of regulatory compliance and safety. On-site incineration should comply with all federal, state, and local regulations.

Adequate numbers of properly labeled waste receptacles should be strategically placed throughout the facility. Waste containers should be leakproof and equipped with tight-fitting lids. It is good practice to use disposable liners and to wash containers and implements regularly. There should be a dedicated waste-storage area that can be kept free of insects and other vermin. If cold storage is used to hold material before disposal, a properly labeled, dedicated refrigerator, freezer, or cold room should be used.

Hazardous wastes must be rendered safe by sterilization, containment, or other appropriate means before being removed from the facility (US EPA 1986). Radioactive wastes should be maintained in properly labeled containers. Their disposal should be closely coordinated with radiation-safety specialists in accord with federal and state regulations. The federal government and most states and municipalities have regulations controlling disposal of hazardous wastes. Compliance with regulations concerning hazardous-agent use (Chapter 1) and disposal is an institutional responsibility.

Infectious animal carcasses can be incinerated on site or collected by a licensed contractor. Procedures for on-site packaging, labeling, transportation, and storage of these wastes should be integrated into occupational health and safety policies.

Hazardous wastes that are toxic, carcinogenic, flammable, corrosive, reactive, or otherwise unstable should be placed in properly labeled containers and disposed of as recommended by occupational health and safety specialists. In some circumstances, these wastes can be consolidated or blended.

Pest Control

Programs designed to prevent, control, or eliminate the presence of or infestation by pests are essential in an animal environment. A regularly scheduled and documented program of control and monitoring should be implemented. The ideal program prevents the entry of vermin into and eliminates harborage from the facility. For animals in outdoor facilities, consideration should also be given to eliminating or minimizing the potential risk associated

with pests and predators. Pesticides can induce toxic effects on research animals and interfere with experimental procedures (Ohio Cooperative Extension Service 1987a,b), and they should be used in animal areas only when necessary. Investigators whose animals might be exposed to pesticides should be consulted before pesticides are used. Use of pesticides should be recorded and coordinated with the animal-care management staff and be in compliance with federal, state, or local regulations. Whenever possible, nontoxic means of pest control, such as insect growth regulators (Donahue and others 1989; Garg and Donahue 1989; King and Bennett 1989) and nontoxic substances (for example, amorphous silica gel), should be used. If traps are used, methods should be humane; traps used to catch pests alive require frequent observation and humane euthanasia after capture.

Emergency, Weekend, and Holiday Care

Animals should be cared for by qualified personnel every day, including weekends and holidays, both to safeguard their well-being and to satisfy research requirements. Emergency veterinary care should be available after work hours, on weekends, and on holidays.

In the event of an emergency, institutional security personnel and fire or police officials should be able to reach people responsible for the animals. That can be enhanced by prominently posting emergency procedures, names, or telephone numbers in animal facilities or by placing them in the security department or telephone center. Emergency procedures for handling special facilities or operations should be prominently posted.

A disaster plan that takes into account both personnel and animals should be prepared as part of the overall safety plan for the animal facility. The colony manager or veterinarian responsible for the animals should be a member of the appropriate safety committee at the institution. He or she should be an "official responder" within the institution and should participate in the response to a disaster (Casper 1991).

POPULATION MANAGEMENT

Identification and Records

Means of animal identification include room, rack, pen, stall, and cage cards with written or bar-coded information; collars, bands, plates, and tabs; colored stains; ear notches and tags; tattoos; subcutaneous transponders; and freeze brands. Toe-clipping, as a method of identification of small rodents, should be used only when no other individual identification method is feasible and should be performed only on altricial neonates. Identification cards should include the source of the animal, the strain or stock, names and locations of the responsible investigators, pertinent dates, and protocol number, when applicable. Animal records are useful and can vary in type, ranging from limited information on identification cards to detailed computerized records for individual animals.

Clinical records for individual animals can also be valuable, especially for dogs, cats, nonhuman primates, and farm animals. They should include pertinent clinical and diagnostic information, date of inoculations, history of surgical procedures and postoperative care, and information on experimental use. Basic demographic information and clinical histories

enhance the value of individual animals for both breeding and research and should be readily accessible to investigators, veterinary staff, and animal-care staff. Records of rearing histories, mating histories, and behavioral profiles are useful for the management of many species, especially nonhuman primates (NRC 1979a).

Records containing basic descriptive information are essential for management of colonies of large long-lived animals and should be maintained for each animal (Dyke 1993; NRC 1979a). These records often include species, animal identifier, sire identifier, dam identifier, sex, birth or acquisition date, source, exit date, and final disposition. Such animal records are essential for genetic management and historical assessments of colonies. Relevant recorded information should be provided when animals are transferred between institutions.

Genetics and Nomenclature

Genetic characteristics are important in regard to the selection and management of animals for use in breeding colonies and in biomedical research (see Appendix A). Pedigree information allows appropriate selection of breeding pairs and of experimental animals that are unrelated or of known relatedness.

Outbred animals are widely used in biomedical research. Founding populations should be large enough to ensure the long-term heterogeneity of breeding colonies. To facilitate direct comparison of research data derived from outbred animals, genetic-management techniques should be used to maintain genetic variability and equalize founder representations (for example, Lacy 1989; Poiley 1960; Williams-Blangero 1991). Genetic variability can be monitored with computer simulations, biochemical markers, DNA markers, immunologic markers, or quantitative genetic analyses of physiologic variables (MacCluer and others 1986; Williams-Blangero 1993).

Inbred strains of various species, especially rodents, have been developed to address specific research needs (Festing 1979; Gill 1980). The homozygosity of these animals enhances the reproducibility and comparability of some experimental data. It is important to monitor inbred animals periodically for genetic homozygosity (Festing 1982; Hedrich 1990). Several methods of monitoring have been developed that use immunologic, biochemical, and molecular techniques (Cramer 1983; Groen 1977; Hoffman and others 1980; Russell and others 1993). Appropriate management systems (Green 1981; Kempthorne 1957) should be designed to minimize genetic contamination resulting from mutation and mismating.

Transgenic animals have at least one transferred gene whose site of integration and number of integrated copies might or might not have been controlled. Integrated genes can interact with background genes and environmental factors, in part as a function of site of integration, so each transgenic animal can be considered a unique resource. Care should be taken to preserve such resources through standard genetic-management procedures, including maintenance of detailed pedigree records and genetic monitoring to verify the presence and zygosity of transgenes. Cryopreservation of fertilized embryos, ova, or spermatozoa should also be considered to safeguard against alterations in transgenes over time or accidental loss of the colony.

Accurate recording, with standardized nomenclature where it is available, of both the strain and substrain or of the genetic background of animals used in a research project is important (NRC 1979b). Several publications provide rules developed by international

committees for standardized nomenclature of outbred rodents and rabbits (Festing and others 1972), inbred rats (Festing and Staats 1973; Gill 1984; NRC 1992a), inbred mice (International Committee on Standardized Genetic Nomenclature for Mice 1981a,b,c), and transgenic animals (NRC 1992b).

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3
Veterinary Medical Care

Veterinary medical care is an essential part of an animal care and use program. Adequate veterinary care consists of effective programs for

- Preventive medicine.
- Surveillance, diagnosis, treatment, and control of disease, including zoonosis control.
- Management of protocol-associated disease, disability, or other sequelae.
- Anesthesia and analgesia.
- Surgery and postsurgical care.
- Assessment of animal well-being.
- Euthanasia.

A veterinary-care program is the responsibility of the attending veterinarian, who is certified (see ACLAM, Appendix B) or has training or experience in laboratory animal science and medicine or in the care of the species being used. Some aspects of the veterinary-care program can be conducted by persons other than a veterinarian, but a mechanism for direct and frequent communication should be established to ensure that timely and accurate information is conveyed to the veterinarian on problems associated with animal health, behavior, and well-being. The veterinarian must provide guidance to investigators and all personnel involved in the care and use of animals to ensure appropriate handling, immobilization, sedation, analgesia, anesthesia, and euthanasia. The attending veterinarian must provide guidance or oversight to surgery programs and oversight of postsurgical care.

ANIMAL PROCUREMENT AND TRANSPORTATION

All animals must be acquired lawfully, and the receiving institution should make reasonable attempts to ensure that all transactions involving animal procurement are conducted in a lawful manner. If dogs and cats are obtained from USDA Class B dealers or pounds, the animals should be inspected to see whether they can be identified, as through the presence of tattoos or subcutaneous transponders. Such identification might indicate that an animal was a pet, and ownership should be verified. Attention should be given to the population status of the taxon under consideration; the threatened or endangered status of species is provided and updated annually by the Fish and Wildlife Service (DOI 50 CFR 17). The use of purpose-bred research animals might be desirable if it is consistent with research, teaching, and testing objectives.

Potential vendors should be evaluated for the quality of animals supplied by them. As a rule, vendors of purpose-bred animals (e.g., USDA Class A dealers) regularly provide information that describes the genetic and pathogen status of their colonies or individual animals. This information is useful for deciding on acceptance or rejection of animals, and

similar data should be obtained on animals received by interinstitutional or intrainstitutional transfer (such as transgenic mice).

All transportation of animals, including intrainstitutional transportation, should be planned to minimize transit time and the risk of zoonoses, protect against environmental extremes, avoid overcrowding, provide food and water when indicated, and protect against physical trauma. Some transportation-related stress is inevitable, but it can be minimized by attention to those factors. Each shipment of animals should be inspected for compliance with procurement specifications and signs of clinical disease and should be quarantined and stabilized according to procedures appropriate for the species and the circumstances. Coordination of ordering and receiving with animal-care personnel is important to ensure that animals are received properly and that appropriate facilities are available for housing.

Several documents provide details on transportation, including the AWRs and the International Air Transport Association Live Animal Regulations (IATA 1995). In addition, import of primates is regulated by the Public Health Service (CFR Title 42) with specific guidelines for tuberculin testing (CDC 1993). There are special requirements for importing and transporting African green, cynomolgus, and rhesus monkeys (FR 1990; CDC 1991).

PREVENTIVE MEDICINE

Disease prevention is an essential component of comprehensive veterinary medical care. Effective preventive-medicine programs enhance the research value of animals by maintaining healthy animals and minimizing nonprotocol sources of variation associated with disease and inapparent infection. These programs consist of various combinations of policies, procedures, and practices related to quarantine and stabilization and the separation of animals by species, source, and health status.

Quarantine, Stabilization, and Separation

Quarantine is the separation of newly received animals from those already in the facility until the health and possibly the microbial status of the newly received animals have been determined. An effective quarantine minimizes the chance for introduction of pathogens into an established colony. The veterinary medical staff should have procedures for evaluating the health and, if appropriate, the pathogen status of newly received animals, and the procedures should reflect acceptable veterinary medical practice and federal and state regulations applicable to zoonoses (Butler and others 1995). Effective quarantine procedures should be used for nonhuman primates to help limit exposure of humans to zoonotic infections. Filoviral and mycobacterial infections in nonhuman primates have recently necessitated specific guidelines for handling nonhuman primates (CDC 1991, 1993). Information from vendors on animal quality should be sufficient to enable a veterinarian to determine the length of quarantine, to define the potential risks to personnel and animals within the colony, to determine whether therapy is required before animals are released from quarantine, and, in the case of rodents, to determine whether cesarean rederivation or embryo transfer is required to free the animals of specific pathogens. Rodents might not require quarantine if data from the vendor or provider are sufficiently current and complete to define

the health status of the incoming animals and if the potential for exposure to pathogens during transit is considered. When quarantine is indicated, animals from one shipment should be separated from animals from other shipments (not necessarily from each other) to preclude transfer of infectious agents between groups.

Regardless of the duration of quarantine, newly received animals should be given a period for physiologic, psychologic, and nutritional stabilization before their use. The length of time for stabilization will depend on the type and duration of animal transportation, the species involved, and the intended use of the animals. The need for a stabilization period has been demonstrated in mice, rats, guinea pigs, and goats; it is probably required for other species as well (Drozdowicz and others 1990; Jelinek 1971; Landi and others 1982; Prasad and others 1978; Sanhouri and others 1989; Tuli and others 1995; Wallace 1976).

Physical separation of animals by species is recommended to prevent interspecies disease transmission and to eliminate anxiety and possible physiologic and behavioral changes due to interspecies conflict. Such separation is usually accomplished by housing different species in separate rooms; however, cubicles, laminar-flow units, cages that have filtered air or separate ventilation, and isolators might be suitable alternatives. In some instances, it might be acceptable to house different species in the same room, for example, if two species have a similar pathogen status and are behaviorally compatible. Some species can have subclinical or latent infections that can cause clinical disease if transmitted to another species. A few examples might serve as a guide in determining the need for separate housing by species:

- *Bordetella bronchiseptica* characteristically produces only subclinical infections in rabbits, but severe respiratory disease might occur in guinea pigs (Manning and others 1984).
- As a rule, New World (South American), Old World African, and Old World Asian species of nonhuman primates should be housed in separate rooms. Simian hemorrhagic fever (Palmer and others 1968) and simian immunodeficiency virus (Hirsch and others 1991; Murphey-Corb and others 1986), for example, cause only subclinical infections in African species but induce clinical disease in Asian species.
- Some species should be housed in separate rooms even though they are from the same geographic region. Squirrel monkeys (*Saimiri sciureus*), for example, might be latently infected with *Herpesvirus tamarinus*, which can be transmitted to and cause a fatal epizootic disease in owl monkeys (*Aotus trivirgatus*) (Hunt and Melendez 1966) and some species of marmosets and tamarins (*Saguinus oedipus*, *S. nigricollis*) (Holmes and others 1964; Melnick and others 1964).

Intraspecies separation might be essential when animals obtained from multiple sites or sources, either commercial or institutional, differ in pathogen status, e.g., sialodacryoadenitis virus in rats, mouse hepatitis virus, *Pasteurella multocida* in rabbits, for *Cercopithecine herpesvirus 1* (formerly *Herpesvirus simiae*) in macaque species, and *Mycoplasma hyopneumoniae* in swine.

Surveillance, Diagnosis, Treatment, and Control of Disease

All animals should be observed for signs of illness, injury, or abnormal behavior by a person trained to recognize such signs. As a rule, this should occur daily, but more-frequent observations might be warranted, such as during postoperative recovery or when animals are ill or have a physical deficit. There might also be situations in which daily observations of each animal is impractical, for example, when animals are housed in large outdoor settings. Professional judgment should be used to ensure that the frequency and character of observation minimize risks to individual animals.

It is imperative that appropriate methods be in place for disease surveillance and diagnosis. Unexpected deaths and signs of illness, distress, or other deviations from normal in animals should be reported promptly to ensure appropriate and timely delivery of veterinary medical care. Animals that show signs of a contagious disease should be isolated from healthy animals in the colony. If an entire room of animals is known or believed to be exposed to an infectious agent (e.g., *Mycobacterium tuberculosis* in nonhuman primates), the group should be kept intact during the process of diagnosis, treatment, and control.

Methods of disease prevention, diagnosis, and therapy should be those currently accepted in veterinary practice. Diagnostic laboratory services facilitate veterinary medical care and can include gross and microscopic pathology, clinical pathology, hematology, microbiology, clinical chemistry, and serology. The choice of medication or therapy should be made by the veterinarian in consultation with the investigator. The selected treatment plan should be therapeutically sound and, when possible, should cause no undesirable experimental variable.

Subclinical microbial, particularly viral, infections (see Appendix A) occur frequently in conventionally maintained rodents but also can occur in facilities designed and maintained for production and use of pathogen-free rodents if a component of the microbial barrier is breached. Examples of infectious agents that can be subclinical but induce profound immunologic changes or alter physiologic, pharmacologic, or toxicologic responses are Sendai virus, Kilham rat virus, mouse hepatitis virus, lymphocytic choriomeningitis virus, and *Mycoplasma pulmonis* (NRC 1991a,b). Scientific objectives of a particular protocol, the consequences of infection within a specific strain of rodent, and the adverse effects that infectious agents might have on other protocols in a facility should determine the characteristics of rodent health-surveillance programs and strategies for keeping rodents free of specific pathogens.

The principal method for detecting viral infections is serologic testing. Other methods of detecting microbial infections, such as bacterial culturing and histopathology and DNA analysis using the polymerase chain reaction (PCR), should be used in combinations that are most suitable for specific requirements of clinical and research programs. Transplantable tumors, hybridomas, cell lines, and other biologic materials can be sources of murine viruses that can contaminate rodents (Nicklas and others 1993). The mouse-antibody-production (MAP), rat-antibody-production (RAP), and hamster-antibody-production (HAP) tests are effective in monitoring for viral contamination of biologic materials (de Souza and Smith 1989; NRC 1991c) and should be considered.

SURGERY

Appropriate attention to presurgical planning, personnel training, aseptic and surgical technique, animal well-being, and animal physiologic status during all phases of a protocol will enhance the outcome of surgery (see Appendix A, "Anesthesia, Pain, and Surgery"). The individual impact of those factors will vary according to the complexity of procedures involved and the species of animal used. A team approach to a surgical project often increases the likelihood of a successful outcome by providing input from persons with different expertise (Brown and Schofield 1994; Brown and others 1993).

A continuing and thorough assessment of surgical outcomes should be performed to ensure that appropriate procedures are followed and timely corrective changes instituted. Modification of standard techniques might be desirable or even required (for instance, in rodent or field surgery), but it should not compromise the well-being of the animals. In the event of modification, assessment of outcomes should be even more intense and might have to incorporate criteria other than obvious clinical morbidity and mortality.

Presurgical planning should include input from all members of the surgical team, including the surgeon, anesthetist, veterinarian, surgical technicians, animal-care staff, and investigator. The surgical plan should identify personnel, their roles and training needs, and equipment and supplies required for the procedures planned (Cunliffe-Beamer 1993); the location and nature of the facilities in which the procedures will be conducted; and preoperative animal-health assessment and postoperative care (Brown and Schofield 1994). If a nonsterile part of an animal, such as the gastrointestinal tract, is to be surgically exposed or if a procedure is likely to cause immunosuppression, preoperative antibiotics might be appropriate (Klement and others 1987). However, the use of antibiotics should never be considered as a replacement for aseptic procedures.

It is important that persons have had appropriate training to ensure that good surgical technique is practiced, that is, asepsis, gentle tissue handling, minimal dissection of tissue, appropriate use of instruments, effective hemostasis, and correct use of suture materials and patterns (Chaffee 1974; Wingfield 1979). People performing and assisting in surgical procedures in a research setting often have a wide range of educational backgrounds and might require various levels and kinds of training before they participate in surgical procedures on animals. For example, persons trained in human surgery might need training in interspecies variations in anatomy, physiology, and the effects of anesthetic and analgesic drugs, or in postoperative requirements. Training guidelines for research surgery commensurate with a person's background are available (ASR 1989) to assist institutions in developing appropriate training programs. The PHS Policy and the AWRs place responsibility with the IACUC for determining that personnel performing surgical procedures are appropriately qualified and trained in the procedures to be performed.

In general, surgical procedures are categorized as major or minor and in the laboratory setting can be further divided into survival and nonsurvival. Major survival surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions (such as laparotomy, thoracotomy, craniotomy, joint replacement, and limb amputation). Minor survival surgery does not expose a body cavity and causes little or no physical impairment (such as wound suturing; peripheral-vessel cannulation; such routine

farm-animal procedures as castration, dehorning, and repair of prolapses; and most procedures routinely done on an "outpatient" basis in veterinary clinical practice).

Minor procedures are often performed under less-stringent conditions than major procedures but still require aseptic technique and instruments and appropriate anesthesia. Although laparoscopic procedures are often performed on an "outpatient" basis, appropriate aseptic technique is necessary if a body cavity is penetrated.

In nonsurvival surgery, an animal is euthanatized before recovery from anesthesia. It might not be necessary to follow all the techniques outlined in this section if nonsurvival surgery is performed; however, at a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean (Slattum and others 1991).

Emergency situations sometimes require immediate surgical correction under less than ideal conditions. For example, if an animal maintained outdoors needs surgical attention, movement to a surgical facility might pose an unacceptable risk to the animal or be impractical. Such situations often require more-intensive aftercare and might pose a greater risk of postoperative complications. The appropriate course of action requires veterinary medical judgment.

Aseptic technique is used to reduce microbial contamination to the lowest possible practical level (Cunliffe-Beamer 1993). No procedure, piece of equipment, or germicide alone can achieve that objective (Schonholtz 1976). Aseptic technique requires the input and cooperation of everyone who enters the operating suite (Belkin 1992; McWilliams 1976). The contribution and importance of each practice varies with the procedure. Aseptic technique includes preparation of the patient, such as hair removal and disinfection of the operative site (Hofmann 1979); preparation of the surgeon, such as the provision of decontaminated surgical attire, surgical scrub, and sterile surgical gloves (Chamberlain and Houang 1984; Pereira and others 1990; Schonholtz 1976); sterilization of instruments, supplies, and implanted materials (Kagan 1992b); and the use of operative techniques to reduce the likelihood of infection (Ayliffe 1991; Kagan 1992a; Ritter and Marmion 1987; Schofield 1994; Whyte 1988).

Specific sterilization methods should be selected on the basis of physical characteristics of materials to be sterilized (Schofield 1994). Autoclaving and gas sterilization are common effective methods. Sterilization indicators should be used to identify materials that have undergone proper sterilization (Berg 1993). Liquid chemical sterilants should be used with adequate contact times, and instruments should be rinsed with sterile water or saline before use. Alcohol is neither a sterilant nor a high-level disinfectant (Rutala 1990).

In general, unless an exception is specifically justified as an essential component of the research protocol and approved by the IACUC, nonrodent aseptic surgery should be conducted only in facilities intended for that purpose. Most bacteria are carried on airborne particles or fomites, so surgical facilities should be maintained and operated in a manner that ensures cleanliness and minimizes unnecessary traffic (AORN 1982; Bartley 1993). In some circumstances, it might be necessary to use an operating room for other purposes. In such cases, it is imperative that the room be returned to an appropriate level of cleanliness before its use for major survival surgery.

Careful surgical monitoring and timely attention to problems increase the likelihood of a successful surgical outcome. Monitoring includes checking of anesthetic depth and physiologic function and assessment of clinical signs and conditions. Maintenance of normal

body temperature minimizes cardiovascular and respiratory disturbances caused by anesthetic agents (Dardai and Heavner 1987) and is of particular importance.

The species of animal influences the components and intensity of the surgical program. The relative susceptibility of rodents to surgical infection has been debated; available data suggest that subclinical infections can cause adverse physiologic and behavioral responses (Beamer 1972; Bradfield and others 1992; Cunliffe-Beamer 1990; Waynforth 1980, 1987) that can affect both surgical success and research results. Some characteristics of common laboratory-rodent surgery—such as smaller incision sites, fewer personnel in the surgical team, manipulation of multiple animals at one sitting, and briefer procedures—as opposed to surgery in larger species, can make modifications in standard aseptic techniques necessary or desirable (Brown 1994; Cunliffe-Beamer 1993). Useful suggestions for dealing with some of the unique challenges of rodent surgery have been published (Cunliffe-Beamer 1983, 1993).

Generally, farm animals maintained for biomedical research should undergo surgery with procedures and in facilities compatible with the guidelines set forth in this section. However, some minor and emergency procedures that are commonly performed in clinical veterinary practice and in commercial agricultural settings may be conducted under less-stringent conditions than experimental surgical procedures in a biomedical-research setting. Even when conducted in an agricultural setting, these procedures require the use of appropriate aseptic technique, sedatives, analgesics, anesthetics, and conditions commensurate with the risk to the animal's health and well-being. But they might not require the intensive surgical settings, facilities, and procedures outlined here.

Presurgical planning should specify the requirements of postsurgical monitoring, care, and record-keeping, including the personnel who will perform these duties. The investigator and veterinarian share responsibility for ensuring that postsurgical care is appropriate. An important component of postsurgical care is observation of the animal and intervention as required during recovery from anesthesia and surgery. The intensity of monitoring necessary will vary with the species and the procedure and might be greater during the immediate anesthetic-recovery period than later in postoperative recovery. During the anesthetic-recovery period, the animal should be in a clean, dry area where it can be observed often by trained personnel. Particular attention should be given to thermoregulation, cardiovascular and respiratory function, and postoperative pain or discomfort during recovery from anesthesia. Additional care might be warranted, including administration of parenteral fluids for maintenance of water and electrolyte balance (FBR 1987), analgesics, and other drugs; care for surgical incisions; and maintenance of appropriate medical records.

After anesthetic recovery, monitoring is often less intense but should include attention to basic biologic functions of intake and elimination and behavioral signs of postoperative pain, monitoring for postsurgical infections, monitoring of the surgical incision, bandaging as appropriate, and timely removal of skin sutures, clips, or staples (UFAW 1989).

PAIN, ANALGESIA, AND ANESTHESIA

An integral component of veterinary medical care is prevention or alleviation of pain associated with procedural and surgical protocols. Pain is a complex experience that typically

results from stimuli that damage tissue or have the potential to damage tissue. The ability to experience and respond to pain is widespread in the animal kingdom. A painful stimulus prompts withdrawal and evasive action. Pain is a stressor and, if not relieved, can lead to unacceptable levels of stress and distress in animals. The proper use of anesthetics and analgesics in research animals is an ethical and scientific imperative. *Recognition and Alleviation of Pain and Distress in Laboratory Animals* (NRC 1992) is a source of information about the basis and control of pain (see also Appendix A).

Fundamental to the relief of pain in animals is the ability to recognize its clinical signs in specific species (Hughes and Lang 1983; Soma 1987). Species vary in their response to pain (Breazile 1987; Morton and Griffiths 1985; Wright and others 1985), so criteria for assessing pain in various species differ. Some species-specific behavioral manifestations of pain or distress are used as indicators, for example, vocalization, depression or other behavioral changes, abnormal appearance or posture, and immobility (NRC 1992). It is therefore essential that personnel caring for and using animals be very familiar with species-specific (and individual) behavioral, physiologic, and biochemical indicators of well-being (Dresser 1988; Dubner 1987; Kitchen and others 1987). In general, unless the contrary is known or established it should be assumed that procedures that cause pain in humans also cause pain in animals (IRAC 1985).

The selection of the most appropriate analgesic or anesthetic should reflect professional judgment as to which best meets clinical and humane requirements without compromising the scientific aspects of the research protocol. Preoperative or intraoperative administration of analgesics might enhance postsurgical analgesia. The selection depends on many factors, such as the species and age of the animal, the type and degree of pain, the likely effects of particular agents on specific organ systems, the length of the operative procedure, and the safety of an agent for an animal, particularly if a physiologic deficit is induced by a surgical or other experimental procedure. Such devices as precision vaporizers and respirators increase the safety and choices of inhalation agents for use in rodents and other small species.

Some classes of drugs—such as sedatives, anxiolytics, and neuromuscular blocking agents—are not analgesic or anesthetic and thus do not relieve pain; however, they might be used in combination with appropriate analgesics and anesthetics. Neuromuscular blocking agents (e.g., pancuronium) are sometimes used to paralyze skeletal muscles during surgery in which general anesthetics have been administered (Klein 1987). When these agents are used during surgery or in any other painful procedure, many signs of anesthetic depth are eliminated because of the paralysis. However, autonomic nervous system changes (e.g., sudden changes in heart rate and blood pressure) can be indicators of pain related to an inadequate depth of anesthesia. If paralyzing agents are to be used, it is recommended that the appropriate amount of anesthetic be first defined on the basis of results of a similar procedure that used the anesthetic without a blocking agent (NRC 1992).

In addition to anesthetics, analgesics, and tranquilizers, nonpharmacologic control of pain is often effective (NRC 1992; Spinelli 1990).

Neuromuscular blocking drugs, as noted earlier, do not provide relief from pain. They are used to paralyze skeletal muscles while an animal is fully anesthetized. They might be used in properly ventilated conscious animals for specific types of nonpainful, well-controlled neurophysiologic studies. However, it is imperative that any such proposed use be carefully evaluated by the IACUC to ensure the well-being of the animal because acute stress is

believed to be a consequence of paralysis in a conscious state and it is known that humans, if conscious, can experience distress when paralyzed with these drugs (NRC 1992; Van Sluyters and Oberdorfer 1991).

EUTHANASIA

Euthanasia is the act of killing animals by methods that induce rapid unconsciousness and death without pain or distress. Unless a deviation is justified for scientific or medical reasons, methods should be consistent with the *1993 Report of the AVMA Panel on Euthanasia* (AVMA 1993 or later editions). In evaluating the appropriateness of methods, some of the criteria that should be considered are ability to induce loss of consciousness and death with no or only momentary pain, distress, or anxiety; reliability; nonreversibility; time required to induce unconsciousness; species and age limitations; compatibility with research objectives; and safety of and emotional effect on personnel.

Euthanasia might be necessary at the end of a protocol or as a means to relieve pain or distress that cannot be alleviated by analgesics, sedatives, or other treatments. Protocols should include criteria for initiating euthanasia, such as degree of a physical or behavioral deficit or tumor size, that will enable a prompt decision to be made by the veterinarian and the investigator to ensure that the end point is humane and the objective of the protocol is achieved.

Euthanasia should be carried out in a manner that avoids animal distress. In some cases, vocalization and release of pheromones occur during induction of unconsciousness. For that reason, other animals should not be present when euthanasia is performed (AVMA 1993).

The selection of specific agents and methods for euthanasia will depend on the species involved and the objectives of the protocol. Generally, inhalant or noninhalant chemical agents (such as barbiturates, nonexplosive inhalant anesthetics, and CO_2) are preferable to physical methods (such as cervical dislocation, decapitation, and use of a penetrating captive bolt). However, scientific considerations might preclude the use of chemical agents for some protocols. All methods of euthanasia should be reviewed and approved by the IACUC.

It is essential that euthanasia be performed by personnel who are skilled in methods for the species in question and that it be performed in a professional and compassionate manner. Death should be confirmed by personnel who can recognize cessation of vital signs in the species being euthanatized. Euthanatizing animals is psychologically difficult for some animal-care, veterinary, and research personnel, particularly if they are involved in performing euthanasia repetitively or if they have become emotionally attached to the animals being euthanatized (Arluke 1990; NRC 1992; Rollin 1986; Wolfle 1985). When delegating euthanasia responsibilities, supervisors should be aware of this as a potential problem for some employees or students.

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4
Physical Plant

A well-planned, well-designed, well-constructed, and properly maintained facility is an important element of good animal care and use, and it facilitates efficient, economical, and safe operation (see Appendix A, "Design and Construction of Animal Facilities"). The design and size of an animal facility depend on the scope of institutional research activities, the animals to be housed, the physical relationship to the rest of the institution, and the geographic location. Effective planning and design should include input from personnel experienced with animal-facility design and operation and from representative users of the proposed facility. Computational fluid dynamics (CFD) modeling of new facilities and caging might be beneficial (Reynolds and Hughes 1994). An animal facility should be designed and constructed in accord with all applicable state and local building codes. Modular units (such as custom-designed trailers or prefabricated structures) should comply with construction guidelines described in this chapter.

Good animal management and human comfort and health protection require separation of animal facilities from personnel areas, such as offices and conference rooms. Separation can be accomplished by having the animal quarters in a separate building, wing, floor, or room. Careful planning should make it possible to place animal-housing areas next to or near research laboratories but separated from them by barriers, such as entry locks, corridors, or floors. Animals should be housed in facilities dedicated to or assigned for that purpose and should not be housed in laboratories merely for convenience. If animals must be maintained in a laboratory area to satisfy a protocol, the area should be appropriate to house and care for the animals; if needed, measures should be taken to minimize occupational hazards related to exposure to animals.

Building materials should be selected to facilitate efficient and hygienic operation of animal facilities. Durable, moisture-proof, fire-resistant, seamless materials are most desirable for interior surfaces. Surfaces should be highly resistant to the effects of cleaning agents, scrubbing, high-pressure sprays, and impact. Paints and glazes should be nontoxic if used on surfaces with which animals will have direct contact. In the construction of outdoor facilities, consideration should be given to surfaces that withstand the elements and can be easily maintained.

FUNCTIONAL AREAS

Professional judgment should be exercised in the development of a practical, functional, and efficient physical plant for animal care and use. The size, nature, and intensity of an institutional animal program will determine the specific facility and support functions needed. In facilities that are small, maintain few animals, or maintain animals under special conditions—such as facilities used exclusively for housing gnotobiotic or specific-pathogen-free (SPF)

colonies or animals in runs, pens, or outdoor housing—some functional areas listed below might be unnecessary or might be included in a multipurpose area.

Space is required for

- Animal housing, care, and sanitation.
- Receipt, quarantine, and separation of animals.
- Separation of species or isolation of individual projects when necessary.
- Storage.

Most multipurpose animal facilities also include the following:

- Specialized laboratories or space contiguous with or near animal-housing areas for such activities as surgery, intensive care, necropsy, radiography, preparation of special diets, experimental procedures, clinical treatment, and diagnostic laboratory procedures.
- Containment facilities or equipment, if hazardous biologic, physical, or chemical agents are to be used.
- Receiving and storage areas for food, bedding, pharmaceuticals, biologics, and supplies.
- Space for washing and sterilizing equipment and supplies and, depending on the volume of work, machines for washing cages, bottles, glassware, racks, and waste cans; a utility sink; an autoclave for equipment, food, and bedding; and separate areas for holding soiled and clean equipment.
- Space for storing wastes before incineration or removal.
- Space for cold storage or disposal of carcasses.
- Space for administrative and supervisory personnel, including space for training and education of staff.
- Showers, sinks, lockers, toilets, and break areas for personnel.
- Security features, such as card-key systems, electronic surveillance, and alarms.

CONSTRUCTION GUIDELINES

Corridors

Corridors should be wide enough to facilitate the movement of personnel and equipment. Corridors 6-8 ft wide can accommodate the needs of most facilities. Floor-wall junctions should be designed to facilitate cleaning. In corridors leading to dog and swine housing facilities, cage-washing facilities, and other high-noise areas, double-door entry or other noise traps should be considered. Wherever possible, water lines, drainpipes, electric-service connections, and other utilities should be accessible through access panels or chases in corridors outside the animal rooms. Fire alarms, fire extinguishers, and telephones should be recessed or installed high enough to prevent damage from the movement of large equipment.

Animal-Room Doors

For safety, doors should open into animal rooms; however, if it is necessary that they open toward a corridor, there should be recessed vestibules. Doors with viewing windows might be preferable for safety and other reasons. However, the ability to cover viewing windows might be considered in situations where exposure to light or hallway activities would be undesirable. Doors should be large enough (approximately 42 x 84 in) to allow the easy passage of racks and equipment. Doors should fit tightly within their frames, and both doors and frames should be appropriately sealed to prevent vermin entry or harborage. Doors should be constructed of and, where appropriate, coated with materials that resist corrosion. Self-closing doors equipped with recessed or shielded handles, threshold sweeps, and kickplates are usually preferred. Where room-level security is necessary or it is desirable to limit access (as in the case of the use of hazardous agents), room doors should be equipped with locks. Doors should be designed to be opened from the inside without a key.

Exterior Windows

Windows are acceptable in some animal rooms and can constitute a type of environmental enrichment for some species, especially nonhuman primates, dogs, some agricultural animals, and other large mammals. The effects of windows on temperature, photoperiod control, and security should be considered in design decisions. Where temperature cannot be regulated properly because of heat loss or gain through the windows or where photoperiod is an important consideration (as in breeding colonies of rodents), exterior windows usually are inappropriate.

Floors

Floors should be moisture-resistant, nonabsorbent, impact-resistant, and relatively smooth, although textured surfaces might be required in some high-moisture areas and for some species (such as farm animals). Floors should be resistant to the action of urine and other biologic materials and to the adverse effects of hot water and cleaning agents. They should be capable of supporting racks, equipment, and stored items without becoming gouged, cracked, or pitted. Depending on their use, floors should be monolithic or have a minimal number of joints. Some materials that have proved satisfactory are epoxy aggregates, hard-surface sealed concrete, and special hardened rubber-base aggregates. Correct installation is essential to ensure long-term stability of the surface. If sills are installed at the entrance to a room, they should be designed to allow for convenient passage of equipment.

Drainage

Where floor drains are used, the floors should be sloped and drain traps kept filled with liquid. To minimize humidity, drainage should allow rapid removal of water and drying of surfaces (Gorton and Besch 1974). Drainpipes should be at least 4 in (10.2 cm) in diameter. In some areas, such as dog kennels and farm-animal facilities, larger drain pipes are recommended. A rim-flush drain or heavy-duty disposal unit set in the floor might be useful for the disposal of solid waste. When drains are not in use for long periods, they should be

capped and sealed to prevent backflow of sewer gases and other contaminants; lockable drain covers might be advisable for this purpose in some circumstances.

Floor drains are not essential in all animal rooms, particularly those housing rodents. Floors in such rooms can be sanitized satisfactorily by wet vacuuming or mopping with appropriate cleaning compounds or disinfectants.

Walls

Walls should be smooth, moisture-resistant, nonabsorbent, and resistant to damage from impact. They should be free of cracks, of unsealed utility penetrations, and of imperfect junctions with doors, ceilings, floors, and corners. Surface materials should be capable of withstanding cleaning with detergents and disinfectants and the impact of water under high pressure. The use of curbs, guardrails or bumpers, and corner guards should be considered to protect walls and corners from damage.

Ceilings

Ceilings should be smooth, moisture-resistant, and free of imperfect junctions. Surface materials should be capable of withstanding cleaning with detergents and disinfectants. Ceilings of plaster or fire-proof plasterboard should be sealed and finished with a washable paint. Ceilings formed by the concrete floor above are satisfactory if they are smoothed and sealed or are painted. Generally, suspended ceilings are undesirable unless they are fabricated of impervious materials and free of imperfect junctions. Exposed plumbing, ductwork, and light fixtures are undesirable unless the surfaces can be readily cleaned.

Heating, Ventilation, and Air-Conditioning (HVAC)

Temperature and humidity control minimizes variations due either to changing climatic conditions or to differences in the number and kind of animals in a room. Air-conditioning is an effective means of regulating temperature and humidity. HVAC systems should be designed for reliability, ease of maintenance, and energy conservation. They should be able to meet requirements for animals as discussed in Chapter 2. A system should be capable of adjustments in dry-bulb temperatures of ±1°C (±2°F). The relative humidity should generally be maintained within a range of 30-70% throughout the year. Temperature is best regulated by having thermostatic control for each room. Use of a zonal control for multiple rooms can result in temperature variations between the "master-control" animal room and the other rooms in the zone because of differences in animal densities within the rooms and heat gain or loss in ventilation ducts and other surfaces within the zone.

Regular monitoring of the HVAC system is important and is best done at the individual-room level. Previously specified temperature and humidity ranges can be modified to meet special animal needs in circumstances in which all or most of the animal facility is designed exclusively for acclimated species with similar requirements (for example, when animals are held in a sheltered or outdoor facility).

Brief and infrequent, moderate fluctuations in temperature and relative humidity outside suggested ranges are well tolerated by most species commonly used in research. Most HVAC systems are designed for average high and low temperatures and humidities

experienced in a geographic area within ±5% variation (ASHRAE 1992). When extremes in external ambient conditions that are beyond design specifications occur, provisions should be in place to minimize the magnitude and duration of fluctuations in temperature and relative humidity outside the recommended ranges. Such measures can include partial redundancy, partial recycling of air, altered ventilation rates, or the use of auxiliary equipment. In the event of a partial HVAC system failure, systems should be designed to supply facility needs at a reduced level. It is essential that life-threatening heat accumulation or loss be prevented during mechanical failure. Totally redundant systems are seldom practical or necessary except under special circumstances (as in some biohazard areas). Temporary needs for ventilation of sheltered or outdoor facilities can usually be met with auxiliary equipment.

In some instances, high-efficiency particulate air (HEPA) filters are recommended for air supplied to animal-holding, procedural, and surgical facilities. Also, consideration should be given to the regulation of air-pressure differentials in surgical, procedural, housing, and service areas. For example, areas for quarantine, housing, and use of animals exposed to hazardous materials and for housing of nonhuman primates should be kept under relative negative pressure, whereas areas for surgery, for clean-equipment storage, and for housing of pathogen-free animals should be kept under relative positive pressure with clean air. Maintaining air-pressure differentials is not the principal or only method by which cross contamination is controlled and should not be relied on for containment. Few air-handling systems have the necessary controls or capacity to maintain pressure differentials across doors or similar structures when they are opened for even brief periods. Containment requires the use of biologic-safety cabinets and exhausted airlocks or other means, some of which are described in Chapter 1.

If recirculated air is used, its quality and quantity should be in accord with recommendations in Chapter 2. The type and efficiency of air treatment should be matched to the quantity and types of contaminants and to the risks that they pose.

Power and Lighting

The electric system should be safe and provide appropriate lighting, a sufficient number of power outlets, and suitable amperage for specialized equipment. In the event of power failure, an alternative or emergency power supply should be available to maintain critical services (for example, the HVAC system) or support functions (for example, freezers, ventilated racks, and isolators) in animal rooms, operating suites, and other essential areas.

Light fixtures, timers, switches, and outlets should be properly sealed to prevent vermin from living there. Recessed energy-efficient fluorescent lights are most commonly used in animal facilities. A time-controlled lighting system should be used to ensure a uniform diurnal lighting cycle. Timer performance and timer-overriding systems should be checked regularly to ensure proper cycling. Light bulbs or fixtures should be equipped with protective covers to ensure the safety of the animals and personnel. Moisture-resistant switches and outlets and ground-fault interrupters should be used in areas with high water use, such as cage-washing areas and aquarium-maintenance areas.

Storage Areas

Adequate space should be provided for storage of equipment, supplies, food, bedding, and refuse. Corridors used for passage of personnel or equipment are not appropriate storage areas. Storage space can be minimized when delivery is reliable and frequent. Bedding and food should be stored in a separate area in which materials that pose a risk of contamination from toxic or hazardous substances are not stored. Refuse-storage areas should be separated from other storage areas (see Chapter 2). Refrigerated storage, separated from other cold storage, is essential for storage of dead animals and animal-tissue waste; this storage area should be kept below 7°C (44.6°F) to reduce putrefaction of wastes and animal carcasses.

Noise Control

Noise control is an important consideration in an animal facility (see Chapter 2). Noise-producing support functions, such as cage-washing, are commonly separated from housing and experimental functions. Masonry walls are more effective than metal or plaster walls in containing noise because their density reduces sound transmission. Generally, acoustic materials applied directly to the ceiling or as part of a suspended ceiling of an animal room present problems for sanitation and vermin control and are not recommended. However, sanitizable sound-attenuating materials bonded to walls or ceilings might be appropriate for noise control in some situations. Experience has shown that well-constructed corridor doors, sound-attenuating doors, or double-door entry can help to control the transmission of sound along corridors.

Attention should be paid to attenuating noise generated by equipment. Fire and environmental-monitoring alarm systems and public-address systems should be selected and located to minimize potential animal exposure. The much-higher frequencies that are capable of being discriminated by some species make it important to consider the location of equipment capable of generating sound at ultrasonic frequencies.

Facilities for Sanitizing Materials

A dedicated, central area for sanitizing cages and ancillary equipment should be provided. Mechanical cage-washing equipment is generally needed and should be selected to match the types of caging and equipment used. Consideration should be given to such factors as

- Location with respect to animal rooms and waste-disposal and storage areas.
- Ease of access, including doors of sufficient width to facilitate movement of equipment.
- Sufficient space for staging and maneuvering of equipment.
- Provision for safe bedding disposal and prewashing activities.
- Traffic flow that separates animals and equipment moving between clean and soiled areas.
- Insulation of walls and ceilings where necessary.
- Sound attenuation.
- Utilities, such as hot and cold water, steam, floor drains, and electric power.

- Ventilation, including installation of vents and provision for dissipation of steam and fumes from sanitizing processes.

FACILITIES FOR ASEPTIC SURGERY

The design of a surgical facility should accommodate the species to be operated on and the complexity of the procedures to be performed (Hessler 1991; see also Appendix A, "Design and Construction of Animal Facilities"). For most rodent surgery, a facility may be small and simple, such as a dedicated space in a laboratory appropriately managed to minimize contamination from other activities in the room during surgery. The facility often becomes larger and more complex as the number of animals, the size of animals, or the complexity of procedures increases, for instance, large-volume rodent procedures, the need for special restraint devices, hydraulic operating tables, and floor drains for farm-animal surgery, and procedures that require large surgical teams and support equipment and thus large space. The relationship of surgical facilities to diagnostic laboratories, radiology facilities, animal housing, staff offices, and so on should be considered in the overall context of the complexity of the surgical program. Surgical facilities should be sufficiently separate from other areas to minimize unnecessary traffic and decrease the potential for contamination (Humphreys 1993). Centralized facilities provide important advantages in cost savings in equipment, conservation of space and personnel resources, reduced transit of animals, and enhanced professional oversight of facilities and procedures.

For most surgical programs, functional components of aseptic surgery include surgical support, animal preparation, surgeon's scrub, operating room, and postoperative recovery. The areas that support those functions should be designed to minimize traffic flow and separate the related, nonsurgical activities from the surgical procedure in the operating room. The separation is best achieved by physical barriers (AORN 1982) but might also be achieved by distance between areas or by the timing of appropriate cleaning and disinfection between activities. The number of personnel and their level of activity have been shown to be directly related to the level of bacterial contamination and the incidence of postoperative wound infection (Fitzgerald 1979). Traffic in the operating room itself can be reduced by the installation of an observation window, a communication system (such as an intercom system), and judicious location of doors.

Control of contamination and ease of cleaning should be key considerations in the design of a surgical facility. The interior surfaces should be constructed of materials that are monolithic and impervious to moisture. Ventilation systems supplying filtered air at positive pressure can reduce the risk of postoperative infection (Ayscue 1986; Bartley 1993; Bourdillon 1946; Schonholtz 1976). Careful location of air supply and exhaust ducts and appropriate room-ventilation rates are also recommended to minimize contamination (Ayliffe 1991; Bartley 1993; Holton and Ridgway 1993; Humphreys 1993). To facilitate cleaning, the operating rooms should have as little fixed equipment as possible (Schonholtz 1976; UFAW 1989). Other features of the operating room to consider include surgical lights to provide adequate illumination (Ayscue 1986), sufficient electric outlets for support equipment, and gas-scavenging capability.

The surgical-support area should be designed for washing and sterilizing instruments and for storing instruments and supplies. Autoclaves are commonly placed in this area. It is often desirable to have a large sink in the animal-preparation area to facilitate cleaning of the animal and the operative site. A dressing area should be provided for personnel to change into surgical attire; a multipurpose locker room can serve this function. There should be a scrub area for surgeons, equipped with foot, knee, or electric-eye surgical sinks (Knecht and others 1981). To minimize the potential for contamination of the surgical site by aerosols generated during scrubbing, the scrub area is usually outside the operating room.

A postoperative-recovery area should provide the physical environment to support the needs of the animal during the period of anesthetic and immediate postsurgical recovery and should be so placed as to allow adequate observation of the animal during this period. The electric and mechanical requirements of monitoring and support equipment should be considered. The type of caging and support equipment will depend on the species and types of procedures but should be designed to be easily cleaned and to support physiologic functions, such as thermoregulation and respiration. Depending on the circumstances, a postoperative recovery area for farm animals might be modified or nonexistent in some field situations, but precautions should be taken to minimize risk of injury to recovering animals.

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Appendix B
Selected Organizations Related to Laboratory Animal Science

American Association for Accreditation of Laboratory Animal Care (AAALAC), 11300 Rockville Pike, Suite 1211, Rockville, MD 20852-3035 (phone: 301-231-5353; fax: 301-231-8282; e-mail: accredit@aaalac.org).

This nonprofit organization was formed in 1965 by leading U.S. scientific and educational organizations to promote high-quality animal care, use, and well-being and to enhance life-sciences research and education through a voluntary accreditation program. Any institution maintaining, using, importing, or breeding laboratory animals for scientific purposes is eligible to apply for AAALAC accreditation. The animal-care facilities of applicant institutions are visited and the program of animal care and use thoroughly evaluated by experts in laboratory animal science, who submit a detailed report to the Council on Accreditation. The council reviews applications and site-visit reports, using guidelines in the *Guide for the Care and Use of Laboratory Animals*, to determine whether full accreditation should be awarded. Accredited institutions are required to submit annual reports on the status of their animal facilities, and site revisits are conducted at intervals of 3 years or less. The Council on Accreditation reviews the annual and site-revisit reports to determine whether full accreditation should continue.

Fully accredited animal-care facilities receive a certificate of accreditation and are included on a list of such facilities published by the association. Many private biomedical organization strongly recommend that all grantees be supported by an AAALAC-accredited animal program. Full accreditation by AAALAC is accepted by the Office for Protection from Research Risks of the National Institutes of Health as strong evidence that the animal facilities are in compliance with Public Health Service policy.

American Association for Laboratory Animal Science (AALAS), 70 Timber Creek Drive, Suite 5, Cordova, TN 38018 (phone: 901-754-8620; fax: 901-753-0046; e-mail: info@aalas.org; URL: http://www.aalas.org/).

AALAS is a professional, nonprofit organization of persons and institutions concerned with the production, care, and study of animals used in biomedical research. The organization provides a medium for the exchange of scientific information on all phases of laboratory animal care and use through its educational activities and certification. AALAS is dedicated to advancing and disseminating knowledge about the responsible care and use of laboratory animals for the benefit of human and animal life. AALAS publishes *Laboratory Animal Science* (bimonthly journal), *Contemporary Topics* (bimonthly journal), training manuals for laboratory animal technicians, an annual membership directory, a directory of certified

technologists, and occasional pamphlets on special subjects. AALAS answers inquiries; conducts certification program for laboratory animal technicians; conducts annual scientific sessions at which original papers are presented, with seminars and workshops on laboratory animal science; distributes publications; lends film and slide sets; and makes referrals to other sources of information. Services are available to anyone.

American College of Laboratory Animal Medicine (ACLAM), Dr. Charles W. McPherson, Executive Director, 200 Summerwinds Drive, Cary, NC 27511 (phone: 919-859-5985; fax: 919-851-3126).

ACLAM is a specialty board recognized by the American Veterinary Medical Association (AVMA). It was founded in 1957 to encourage education, training, and research; to establish standards of training and experience for qualification; and to certify, by examination, qualified laboratory animal specialists as diplomates. To achieve these goals, the college seeks to interest veterinarians in furthering both training and qualifications in laboratory animal medicine.

The annual ACLAM Forum is a major continuing-education meeting. ACLAM also meets and sponsors programs in conjunction with the annual meetings of AVMA and the American Association for Laboratory Animal Science. It emphasizes and sponsors continuing-education programs; cosponsors symposia; cosponsors about 30 autotutorial programs on use, husbandry, and diseases of animals commonly used in research; and has produced 14 volumes on laboratory subjects, such as *The Laboratory* Rat and *The Mouse in Biomedical Research.*

American Humane Association (AHA), 236 Massachusetts Avenue, NE, Suite 203, Washington, D.C. 20002 (phone: 202-543-7780; fax: 202-546-3266).

AHA is a professional, nonprofit organization of organizations and individuals concerned with the exploitation, abuse, and neglect of children and animals. AHA was founded in 1877 and was the first national organization to protect children and animals.

AHA supports the 3 R's in biomedical research: refinement, reduction, and replacement where possible. AHA informs its members of issues in biomedical research through its magazine, *Advocate*, which is published quarterly.

American Society of Laboratory Animal Practitioners (ASLAP), Dr. Bradford S. Goodwin, Jr., Secretary-Treasurer, University of Texas, Medical School-CLAMC, 6431 Fannin Street, Room 1132, Houston, TX 77030-1501 (phone: 713-792-5127; fax: 713-794-4177).

ASLAP, founded in 1966, is open to any graduate of a veterinary college accredited or recognized by the American Veterinary Medical Association (AVMA) or Canadian Veterinary Medical Association (CVMA) who is engaged in laboratory animal practice and maintains membership in AVMA, CVMA, or any other national veterinary medical association recognized by AVMA. Its purpose is to disseminate ideas, experiences, and knowledge among veterinarians engaged in laboratory animal practice through education,

training, and research at both predoctoral and postdoctoral levels. Two educational meetings are held annually, one each in conjunction with the annual meetings of AVMA and the American Association for Laboratory Animal Science.

American Society of Primatologists (ASP), Regional Primate Research Center, University of Washington, Seattle, WA 98195 (URL: http://www.asp.org).

The purposes of ASP are exclusively educational and scientific—specifically, to promote and encourage the discovery and exchange of information regarding primates, including all aspects of their anatomy, behavior, development, ecology, evolution, genetics, nutrition, physiology, reproduction, systematic, conservation, husbandry, and use in biomedical research. The ASP holds an annual meeting, sponsors the *American Journal of Primatology*, and publishes the ASP Bulletin quarterly. Any person engaged in scientific primatology or interested in supporting the goals of the society may apply for membership. Membership and information about the International Primatological Society can be obtained from ASP.

American Veterinary Medical Association (AVMA), 1931 North Meacham Road, Suite 100, Schaumburg, IL 60173-4360 (phone: 800-248-2862; fax: 708-925-1329; URL: http://www.avma.org/).

AVMA is the major national organization of veterinarians. Its objective is to advance the science and art of veterinary medicine, including its relationship to public health and agriculture. AVMA is the recognized accrediting agency for schools and colleges of veterinary medicine. It promotes specialization in veterinary medicine through the formal recognition of specialty-certifying organizations, including the American College of Laboratory Animal Medicine. The AVMA Committee on Animal Technician Activities and Training accredits 2-year programs in animal technology at institutions of higher learning throughout the United States. A list of accredited programs and a summary of individual state laws and regulations relative to veterinarians and animal technicians are available from AVMA.

Animal Welfare Information Center (AWIC), National Agricultural Library, 5th floor, Beltsville, MD 20705-2351 (phone: 301-504-6212; fax: 301-504-7125; e-mail: awic@nal.usda.gov; URL: http://netvet.wustl.edu/awic.htm or http://www.nalusda.gov).

AWIC, at the National Agricultural Library, was established by the 1985 amendments to the Animal Welfare Act. It provides information on employee training, improved methods of experimentation (including alternatives), and animal-care and animal-use- topics through the production of bibliographies, workshops, resource guides, and *The Animal Welfare Information Center Newsletter*. AWIC services are geared toward those who must comply with the Animal Welfare Act, such as researchers, veterinarians, exhibitors, and dealers. Publications and additional information are available from AWIC.

Animal Welfare Institute (AWI), P.O. Box 3650, Washington, DC 20007 (phone: 202-337-2332; fax: 202-338-9478; e-mail: awi@igc.apc.org).

AWI is a nonprofit educational organization dedicated to reducing the pain and fear inflicted on animals by humans. Since its founding in 1951, AWI has promoted humane treatment of laboratory animals, emphasizing the importance of socialization, exercise, and environmental enhancement. The institute supports the "3 R's": replacement of experimental animals with alternatives, refinement to reduce animal pain and suffering, and reduction in the numbers of animals used. Educational material published by AWI includes the *AWI Quarterly*, *Comfortable Quarters for Laboratory Animals*, *Beyond the Laboratory Door*, *and Animals and Their Legal Rights* and is available free to scientific institutions and libraries and at cost to others. The institute welcomes correspondence and discussion with scientists, technicians, and IACUC members on improving the lives of laboratory animals.

Association of Primate Veterinarians (APV), Dr. Dan Dalgard, Secretary, Corning Hazleton, 9200 Leesburg Turnpike, Vienna, VA 22162-1699 (phone: 703-893-5400 ext. 5390; fax: 703-759-6947).

APV is a nonprofit organization whose missions are to promote the dissemination of information related to the health, care, and welfare of nonhuman primates and to provide a mechanism by which primate veterinarians can speak collectively on matters regarding nonhuman primates. The organization developed after an initial workshop on the clinical care of nonhuman primates held in 1973 at the National Institutes of Health. Six years later, bylaws were adopted to formalize the missions and operation of the group. Members of APV are veterinarians who are concerned with the health, care, and welfare of nonhuman primates. The association meets annually, publishes a quarterly newsletter, and contributes to other scholarly and regulatory efforts and issues concerning nonhuman primates.

Australia and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART): ANZCCART Australia, The Executive Officer, PO Box 19, Glen Osmond, South Australia 5064, (phone: +61-8-303-7393; fax: +61-8-303-7113; e-mail: anzccart@waite.adelaide.edu.au; URL: http://www.adelaide.edu.au/ANZCCART/); ANZCCART New Zealand, The Executive Officer, C/- The Royal Society of New Zealand, PO Box 598 , Wellington, New Zealand (phone: +64-4-472 7421; fax: +64-4-473 1841; e-mail: anzccart@rsnz.govt.nz; URL: http://www.adelaide.edu.au/ANZCCART/).

ANZCCART was established in 1987 in response to concerns in both the scientific and the wider communities about the use of animals in research and teaching. ANZCCART is an independent body that has been developed to provide a national focus for these issues. Through its varied activities, ANZCCART seeks to promote effective communication and cooperation between all those concerned with the care and use of animals in research and teaching. ANZCCART's missions are to promote excellence in the care of animals used in research and teaching and thereby minimize their discomfort, to ensure that the outcomes of the scientific uses of animals are worth while, and to foster informed and responsible

discussion and debate within the scientific and wider communities regarding the scientific uses of animals.

Canadian Association for Laboratory Animal Medicine/L'Association canadienne de la médecine des animaux de laboratoire (CALAM/ACMAL), Dr. Brenda Cross, Secretary-Treasurer, 102 Animal Resources Centre, 120 Maintenance Road, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5C4.

CALAM/ACMAL is a national organization of veterinarians with an interest in laboratory animal medicine. The association's missions are to advise interested parties on all matters pertaining to laboratory animal medicine, to further the education of its members, and to promote ethics and professionalism in the field. The association is committed to the provision of appropriate veterinary care for all animals used in research, teaching, or testing. The association publishes a newsletter, *Interface*, four times a year.

Canadian Association for Laboratory Animal Science/L'association canadienne pour la technologie des animeaux laboratoire (CALAS/ACTAL), Dr. Donald McKay, Executive Secretary, CW401 Biological Science Building, Bioscience Animal Service, University of Alberta, Edmonton, Alberta, Canada T6G 2E9 (phone: 403-492-5193; fax: 403-492-7257; e-mail: dmckay@gpu.srv.ualberta.ca).

CALAS/ACTAL is composed of a multidisciplinary group of people and institutions concerned with the care and use of laboratory animals in research, teaching, and testing. The aims of the association are to advance the knowledge, skills, and status of those who care for and use laboratory animals; to improve the standards of animal care and research; and to provide a forum for the exchange and dissemination of knowledge regarding animal care and research. CALAS/ACTAL maintains a Registry for Laboratory Animal Technicians, publishes a newsletter six times a year, and hosts an annual national convention.

Canadian Council on Animal Care (CCAC), Constitution Square, Tower II, 315-350 Albert, Ottawa, Ontario, Canada K1R 1B1 (phone: 613-238-4031; fax: 613-238-2837; e-mail: ccac@carleton.ca).

CCAC, founded in 1968 under the aegis of the Association of Universities and Colleges of Canada, became an independently incorporated, autonomous organization in 1982. Through its development of guidelines, assessment visits, and educational/consultation programs, the CCAC is the main advisory and review agency for the use of animals in Canadian science. Compliance with CCAC guidelines, published in two volumes, is a requirement for the receipt of grants or contracts. CCAC is currently funded by the Natural Sciences and Engineering Council of Canada, the Medical Research Council of Canada, and some federal departments.

Center for Alternatives to Animal Testing (CAAT), Johns Hopkins University, 111 Market Place, Suite 840, Baltimore, MD 21202-6709 (phone: 410-223-1693; fax: 410-223-1603; e-mail: caat@jhuhyg.sph.jhu.edu; URL: http://infonet.welch.jhu.edu/~caat/).

CAAT was founded in 1981 to develop alternatives to the use of whole animals for product development and safety testing. Although CAAT's mission focuses primarily on the development of alternatives for testing, the center also works with organizations seeking to implement the 3 R's in research and education. These organizations are throughout the world, primarily in North America, Europe, Australia, and Japan.

CAAT is an academic research center based in the School of Hygiene and Public Health at Johns Hopkins University in Baltimore, whose programs encompass laboratory research, education/information, and validation of alternative methods.

CAAT's primary outreach to scientific and lay audiences its newsletter, which is published three times a year. A new newsletter for middle-school students, *CAATALYST*, is published three times a year.

Center for Animals and Public Policy, Tufts University, School of Veterinary Medicine, 200 Westboro Road, N. Grafton, MA 01536 (phone: 508-839-7991; fax: 508-839-2953; e-mail: dpease@opal.tufts.edu).

The center is a unit of Tufts School of Veterinary Medicine that deals with all aspects of human-animal interactions. The center publishes two newsletters (*The Animal Policy Report*, quarterly; *The Alternatives Report*, bimonthly) and other reports and related items, including *The Animal Research Controversy*, a 200-page report that includes an appendix on the animal-protection movement. The center also has established an MS program in animals and public policy, a 1-year program directed at persons with a graduate degree or equivalent life experience.

Foundation for Biomedical Research (FBR), 818 Connecticut Avenue, NW, Suite 303, Washington, DC 20006 (phone: 202-457-0654; fax 202-457-0659; e-mail: nabr-fbr@access.digex.net; URL: http://www.fiesta.com/fbr).

FBR is a nonprofit, educational organization dedicated to promoting public understanding and support of the ethical use of animals in medical research. The Foundation has a wide range of educational materials available for students as well as the general public, including brochures, booklets, videotapes, speaker's kits, posters, and is a source of information on education and training materials related to laboratory animal science. FRB hosts press events and assists members of the media in locating researchers to address issues regarding animal research.

The Humane Society of the United States (HSUS), 2100 L Street, NW, Washington, DC 20037 (phone: 202-452-1100; fax: 301-258-3082; e-mail: HSUSLAB@ix.netcom.com).

HSUS is the nation's largest animal-protection organization. The society is active on a wide variety of humane issues, including those affecting wildlife, companion animals, and animals in laboratories and on farms. HSUS publishes a quarterly magazine (*The HSUS*

News), a newsletter (*The Animal Activist Alert*), and a variety of reports, brochures, and other advocacy materials. The society works actively on issues involving the use of animals in research, safety testing, and education. This work is spearheaded by the HSUS Animal Research Issues Section, with the aid of a Scientific Advisory Council. The aims of this research are to promote the 3 R's of replacement, reduction, and refinement; strong regulations and their enforcement; openness and accountability among research institutions; and an end to egregious mistreatment of animals. HSUS pursues these aims through educational, legislative, legal, and investigative means. Staff are available to give presentations and write articles on these topics.

Institute of Laboratory Animal Resources (ILAR), National Research Council, National Academy of Sciences, 2101 Constitution Avenue, NW, Washington, DC 20418 (phone: 202-334-2590; fax: 202-334-1687; e-mail: ILAR@nas.edu; *ILAR Journal* e-mail: ILARJ@nas.edu; URL for National Academy of Sciences: http://www.nas.edu/).

ILAR develops guidelines and disseminates information on the scientific, technologic, and ethical use of animals and related biologic resources in research, testing, and education. ILAR promotes high-quality, humane care of animals and the appropriate use of animals and alternatives. ILAR functions within the mission of the National Academy of Sciences as an adviser to the federal government, the biomedical research community, and the public. *ILAR Journal* is published quarterly and is distributed without charge to scientists, biomedical administrators, medical libraries, and students.

International Council for Laboratory Animal Science (ICLAS), Dr. Steven Pakes, Secretary General, Division of Comparative Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX (phone: 214-648-3340; fax: 214-648-2659; e-mail: spakes@mednet.swmed.edu).

ICLAS is an international nongovernment scientific organization that was founded in 1961 under the auspices of UNESCO and several scientific unions. The aims of ICLAS are to promote and coordinate the development of laboratory animal science throughout the world, to promote international collaboration in laboratory animal science, to promote the definition and monitoring of quality laboratory animals, to collect and disseminate information on laboratory animal science, and to promote the humane use of animals in research, testing, and teaching through recognition of ethical principles and scientific responsibilities.

ICLAS has programs addressing microbiologic and genetic monitoring and standardization, assisting developing countries in pursuing their objectives in improving the care and use of laboratory animals, and improving education and training in laboratory animal science. ICLAS accomplishes its goals through regional scientific meetings, an international scientific meeting held every 4 years, the dissemination of information, and expert consultation with those requesting assistance.

ICLAS membership is composed of national members, scientific union members, scientific members, and associate members. The Governing Board is responsible for implementing the general policy of ICLAS and is elected by the General Assembly every 4 years.

Laboratory Animal Management Association (LAMA), Mr. Paul Schwikert, Past-President. P.O. Box 1744, Silver Spring, MD 20915 (phone: 313-577-1418; fax: 313-577-5890).

LAMA is a nonprofit educational organization. Membership includes individuals and institutions involved in laboratory animal management, medicine, and science. The mission of the association, founded in 1984, is to "enhance the quality of management and care of laboratory animals throughout the world." The objectives of LAMA include promoting the dissemination of ideas, experiences, and knowledge in the management of laboratory animals, encouraging continued education, acting as a spokesperson for the field of laboratory animal management, and assisting in the training of managers. The organization conducts a midyear forum on management issues and topics of interest to the general membership an and annual meeting in conjunction with the American Association of Laboratory Animals Science national meeting. *LAMA Review* is a quarterly journal on management issues published by the organization, and *LAMA Lines* is a bimonthly newsletter on topics of general interest to the membership.

Massachusetts Society for the Prevention of Cruelty to Animals/American Humane Education Society (MSPCA/AHES), 350 South Huntington Avenue, Boston, MA 02130 (phone: 617-522-7400; fax: 617-522-4885).

The Center for Laboratory Animal Welfare at MSPCA/AHES was formed in 1992 to bring thoughtful analysis to the complex issues surrounding the use of animals in research, testing, and education. Its work involves researching issues related to the welfare of laboratory animals, creating educational materials, and developing programs on issues of interest to the public.

Founded in 1868, MSPCA/AHES is one of the largest animal-protection organizations in the world. It operates three animal hospitals, seven animal shelters, and a statewide law-enforcement program in Massachusetts. It is widely recognized for national leadership in humane education, publications, legislative issues, and veterinary medicine.

National Association for Biomedical Research (NABR), 818 Connecticut Avenue, NW, Suite 303, Washington, DC 20006 (phone: 202-857-0540; fax 202-659-1902; e-mail: nabr-fbr@access.digex.net; URL: http://www.fiesta.com/nabr).

NABR is a nonprofit organization of 350 institutional members from both academia and industry whose mission is to advocate public policy that recognizes the vital role of laboratory animals in research, education and safety testing. NABR is a source of information concerning existing and proposed animal welfare legislation and regulations at the national, state, and local level.

Office for Protection from Research Risks (OPRR), National Institutes of Health, 6100 Executive Blvd., Suite 3B01, Rockville, MD 20892 (phone: 301-496-7163; fax: 301-402-2803).

The Division of Animal Welfare of OPRR fulfills responsibilities set forth in the Public Health Service (PHS) Act. These include developing and monitoring, as well as exercising compliance oversight relative to, the PHS Policy on Humane Care and Use of Laboratory Animals (Policy), which applies to animals involved in research conducted or supported by any component of PHS; establishing criteria for and negotiation of assurances of compliance with institutions engaged in PHS-conducted or PHS-supported research using animals; directing the development and implementation of educational and instructional programs with respect to the use of animals in research; and evaluating the effectiveness of PHS policies and programs for the humane care and use of laboratory animals.

Primate Information Center, Regional Primate Research Center SJ-50, University of Washington, Seattle, WA 98195 (phone: 206-543-4376; fax: 206-865-0305).

The Primate Information Center's goal is to provide bibliographic access to all scientific literature on nonhuman primates for the research and educational communities. Coverage spans all publication categories (articles, books, abstracts, technical reports, dissertations, book chapters, etc.) and many subjects (behavior, colony management, ecology, reproduction, field studies, disease models, veterinary science, pharmacology, physiology, evolution, taxonomy, genetics, zoogeography, etc.). A comprehensive computerized database is maintained and used to publish a variety of bibliographic products to fulfill this mission. The collection of materials on primate research is fairly comprehensive. However, the center is an indexing service and not a library, so materials generally do not circulate. It will make individually negotiated exceptions for items that researchers are not able to acquire otherwise.

Primate Supply Information Clearinghouse (PSIC), Cathy A. Johnson-Delany, Director, Regional Primate Research Center, SJ-50 University of Washington, Seattle, WA 98195 (phone: 206-543-5178; fax: 206-685-0305; e-mail: cathydj@bart.rprc.washington.edu).

The goal of PSIC is to provide communication between research institutions, zoologic parks, and domestic breeding colonies for the efficient sharing of nonhuman primates and their tissues, equipment, and services. PSIC also publishes *New Listings* and the *Annual Resource Guide*.

Purina Mills, Inc., 505 North 4th and D Street, Richmond, IN 47374.

Purina Mills, Inc. offers a correspondence course, called Laboratory Animal Care Course, for everyone working with small animals. The course includes the following six lessons: introduction to laboratory animals; management of laboratory animals; housing, equipment, and handling; disease and control; glossary; and housing supplements and miscellaneous.

Scientists Center for Animal Welfare (SCAW), 7833 Walker Drive, Suite 340, Greenbelt, MD 20770 (phone: 301-345-3500; fax: 301-345-3503).

SCAW is an independent organization supported by individuals and institutions involved in research with animals and concerned about maintaining the highest standards of humane care. SCAW publishes resource materials, organizes conferences, and supports a wide variety of educational activities.

Universities Federation for Animal Welfare (UFAW), 8 Hamilton Close, South Mimms, Potters Bar, Herts EN6 3QD, United Kingdom (phone: 44-707-58202; fax: 44-707-49279).

UFAW was founded in 1926 as the University of London Animal Welfare Society (ULAWS). Its work expanded, and in order to allow a wider membership, UFAW was formed in 1938 with ULAWS as its first branch. UFAW publishes the *UFAW Handbook on the Care and Management of Laboratory Animals* and other publications.

United States Department of Agriculture, Animal and Plant Health Inspection Service, Regulatory Enforcement of Animal Care (REAC), 4700 River Road, Unit 84, Riverdale, MD 20737-1234 (phone: 301-734-4981; fax: 301-734-4328; e-mail: sstith@aphis.usda.gov).

The missions of the Animal Care Program are to provide leadership in establishing acceptable standards of humane animal care and treatment and to monitor and achieve compliance through inspections and educational and cooperative efforts. Copies of the Animal Welfare Regulations and the Animal Welfare Act are available from REAC.

Wisconsin Regional Primate Research Center (WRPRC) Library, University of Wisconsin, 1220 Capitol Court, Madison, WI 53715-1299 (phone: 608-263-3512; fax: 608-263-4031; e-mail: library@primate.wisc.edu; URL: http://www.primate.wisc.edu/WRPRC).

The library supports research programs of WRPRC and aids in the dissemination of information about nonhuman primates to the scientific community. Books, periodicals, newsletters, and other documents in all languages related to primatology are included. Special collections include rare books and audiovisual materials.

Appendix C
Some Federal Laws Relevant To Animal Care and Use

ANIMAL WELFARE

The Animal Welfare Act of 1966 (P.L. 89-544)—as amended by the Animal Welfare Act of 1970 (P.L. 91-579); 1976 Amendments to the Animal Welfare Act (P.L. 94-279); the Food Security Act of 1985 (P.L 99-198), Subtitle F (Animal Welfare File Name: PL99198); and the Food and Agriculture Conservation and Trade Act of 1990 (P.L. 101-624), Section 2503, Protection of Pets (File Name: PL101624)—contains provisions to prevent the sale or use of animals that have been stolen, to prohibit animal-fighting ventures, and to ensure that animals used in research, for exhibition, or as pets receive humane care and treatment. The law provides for regulating the transport, purchase, sale, housing, care, handling, and treatment of such animals.

Regulatory authority under the Animal Welfare Act is vested in the secretary of the U.S. Department of Agriculture (USDA) and implemented by USDA's Animal and Plant Health Inspection Service (APHIS). Rules and regulations pertaining to implementation are published in the Code of Federal Regulations, Title 9 (Animals and Animal Products), Chapter 1, Subchapter A (Animal Welfare). Available from: Regulatory Enforcement and Animal Care, APHIS, USDA, Unit 85, 4700 River Road, Riverdale, MD 20737-1234. File Name 9CFR93.

ENDANGERED SPECIES

The Endangered Species Act of 1973 (P.L. 93-205; 87 Statute 884) became effective on December 28, 1973, supplanting the Endangered Species Conservation Act of 1969 (P.L. 91-135; 83 Statute 275). The new law seeks "to provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved, to provide a program for the conservation of such endangered species and threatened species, and to take such steps as may be appropriate to achieve the purposes of the treaties and conservation of wild flora and fauna worldwide."

Regulatory authority under the Endangered Species Act is vested in the secretary of the U.S. Department of the Interior (USDI) and implemented by USDI's Fish and Wildlife Service. Implementing rules and regulations are published in the Code of Federal Regulations, Title 50 (Wildlife and Fisheries), Chapter 1 (U.S. Fish and Wildlife Service, Department of the Interior), Subchapter B, Part 17 (Endangered and Threatened Wildlife and Plants). Copies of the regulations, including a list of species currently considered endangered or threatened, can be obtained by writing to the Office of Endangered Species, U.S. Department of the Interior, Fish and Wildlife Service, Washington, DC 20240.

Appendix D
Public Health Service Policy and Government Principles Regarding The Care and Use of Animals

PUBLIC HEALTH SERVICE POLICY ON HUMANE CARE AND USE OF LABORATORY ANIMALS

The *Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals* was updated in 1986. In the policy statement, the PHS endorses the *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training* (reprinted below), which were developed by the Interagency Research Animal Committee. The PHS policy implements and supplements these principles. Information concerning the policy can be obtained from the Office for Protection from Research Risks, National Institutes of Health, 6100 Executive Boulevard, MSC 7507, Rockville, MD 20892-7507.

PRINCIPLES FOR THE CARE AND USE OF ANIMALS USED IN TESTING, RESEARCH, AND TRAINING

The principles below were prepared by the Interagency Research Animal Committee. This committee, which was established in 1983, serves as a focal point for federal agencies' discussions of issues involving all animal species needed for biomedical research and testing. The committee's principal concerns are the conservation, use, care, and welfare of research animals. Its responsibilities include information exchange, program coordination, and contributions to policy development.

U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training

The development of knowledge necessary for the improvement of the health and well-being of humans as well as other animals requires *in vivo* experimentation with a wide variety of animal species. Whenever U.S. Government agencies develop requirements for testing, research, or training procedures involving the use of vertebrate animals, the following principles shall be considered; and whenever these agencies actually perform or sponsor such procedures, the responsible Institutional Official shall ensure that these principles are adhered to:

I. The transportation, care, and use of animals should be in accordance with the Animal Welfare Act (7 U.S.C. 2131 et seq.) and other applicable Federal laws, guidelines, and policies.[2]

II. Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.

III. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and *in vitro* biological systems should be considered.

IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.

V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

VI. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.

VII. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.

VIII. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals.

IX. Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned but should be made, with due regard to Principle II, by an appropriate review group such as an institutional animal care and use committee. Such exceptions should not be made solely for the purposes of teaching or demonstration.

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[2] For guidance throughout these Principles, the reader is referred to the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animals Resources, National Academy of Sciences.

LABORATORY ANIMAL MANAGEMENT SERIES

RODENTS

IN PRESS

Committee on Rodents
Institute of Laboratory Animal Resources
Commission on Life Sciences
National Research Council

February 1996

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, National Academy of Engineering, and Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, National Academy of Engineering, and Institute of Medicine.

The National Academy of Sciences is a private, nonprofit, self-perpetuating society of distinguished scholars engaged in scientific and engineering research, dedicated to the furtherance of science and technology and to their use for the general welfare. Upon the authority of the charter granted to it by the Congress in 1863, the Academy has a mandate that requires it to advise the federal government on scientific and technical matters. Dr. Bruce M. Alberts is president of the National Academy of Sciences.

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The Institute of Laboratory Animal Resources (ILAR) was founded in 1952 under the auspices of the National Research Council. A component of the Commission on Life Sciences, ILAR develops guidelines and positions and disseminates information on the scientific, technological, and ethical use of laboratory animals and related biological resources. ILAR promotes high-quality, humane care of laboratory animals and the appropriate use of laboratory animals and alternatives in research, testing, and education. ILAR serves as an advisor to the federal government, the biomedical research community, and the public.

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Preface

Biomedical and behavioral research, product testing, and many aspects of science education rely heavily on the use of animals. Quality care of these animals is essential, not only for the animals' welfare, but also for obtaining valid data. Environmental and biologic factors can influence experimental results by exerting subtle influences on an animal's physiologic characteristics, behavior, or both. Although there is a tendency to feel more concern for species to which humans develop an attachment (e.g., dogs and cats) and species that are biologically "closer" to humans (nonhuman primates), the same attention to environmental control for and good care of every laboratory species is necessary to ensure the high quality of both science and ethical practice.

Rodents are, by far, the largest group of animals used in research and testing. In 1986, the Office of Technology Assessment estimated that 17-22 million animals were being used each year in the United States, of which about 13.2-16.2 million were rodents (*Alternatives*

to Animal Use in Research, Testing, and Education; Pub. No. OTA-BA-273; U. S. Congress Office of Technology Assessment; Washington, D.C.; 1986). In the 15 years since the last Institute of Laboratory Animal Resources report on the general management of rodents was published, important advances in biomedical research and increased public awareness have created a new environment for animal research. Modern technology—such as insertion of functional genes from other species into mice or rats, elimination of a single selected gene or function in mice, and the recreation of elements of the human immune system in mice—has greatly expanded the usefulness of rodents in drug development and as models of human diseases. The technologic requirements of such advanced systems have led to improved understanding and implementation of environmental requirements for the care and use of rodents in research.

The intent of this report is to provide current information to laboratory animal scientists (including both animal-care technicians and veterinarians), investigators, research technicians, and administrators on general elements of rodent care and use that should be considered both for optimal design and conduct of research and to meet current standards of care and use. We emphasize that this report provides guidelines and should not be used as a substitute for good professional judgement, which is essential in the application of the guidelines. Where possible, we refer to other documents that provide more detail on specific aspects of rodent care and use.

Bonnie J. Mills
Chairman, Committee on Rodents

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Laboratory Animals and Public Perspective

REGULATORY ISSUES

In recent years, virtually every aspect of biomedical research has been increasingly subjected to public scrutiny. A major concern is the justification of public funding. In addition, heightened public awareness and pressure have resulted in increased oversight in such areas as the health and safety of workers, the state of the environment, and the welfare of animals used in research, teaching, and testing. Design and review of protocols involving the use of animals should include consideration of applicable regulations and public accountability in each of those areas.

Two federal laws govern the use of animals. The Health Research Extension Act (PL 99-158), passed in 1985, amended Title 42, Section 289d, of the U.S. Code and gave the

force of law to the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (PHS, 1986; hereafter called *PHS Policy*). *PHS Policy* applies to all activities conducted or funded by the Public Health Service (PHS) that involve any live vertebrate animal used or intended for use in research, training, or testing. It requires compliance with the Animal Welfare Regulations (AWRs), and it specifies minimal components of an institution's animal care and use program, oversight responsibilities, and reporting requirements. Programs for animal care and use must be based on the *Guide for the Care and Use of Laboratory Animals* (NRC, 1985 et seq.), hereafter called the *Guide*; any departure from its recommendations must be documented and justified. *PHS Policy* stresses institutional self-regulation and gives responsibility for oversight to an institutional animal care and use committee (IACUC). The Office for Protection from Research Risks (OPRR) is responsible for the general administration and coordination of *PHS Policy*. OPRR responsibilities include reviewing and approving (or disapproving) institutional assurances, communicating with institutions concerning implementation of *PHS Policy*, investigating allegations of noncompliance by PHS-funded institutions, reviewing and approving (or disapproving) waivers to *PHS Policy*, and making site visits to selected institutions.

Title 7, Sections 2131 et seq., of the U.S. Code, popularly called the Animal Welfare Act and most recently amended in 1985 by PL 99-198, was originally written in 1966 to protect pets. Its focus has since shifted to protecting laboratory animals. In addition to requiring that the U.S. Department of Agriculture (USDA) establish minimal standards for animal husbandry, care, treatment, and transportation, the act now includes provisions to reduce animal use by eliminating unnecessary duplication and mandates consideration of

alternatives to procedures that are likely to cause pain or distress in live animals. The amended act applies to most warm-blooded animals used or intended for use in research, teaching, or testing in the United States. Like *PHS Policy*, it emphasizes institutional self-regulation and gives oversight responsibility to an IACUC. Regulatory Enforcement and Animal Care (REAC), a part of the USDA Animal and Plant Health Inspection Service, administers and enforces the regulations (9 CFR 1-3) and carries out inspections of facilities to determine compliance. Laboratory mice (genus *Mus*) and rats (genus *Rattus*), which make up more than 90 percent of the animals used in research, are not covered by the AWRs and are not subject to REAC inspection. However, there is a movement to include them; the decision on this issue is likely to be made in federal court.

Other regulations, policies, and guidelines address animal-care issues, although they are not specifically directed at animal research. They include the Good Laboratory Practice rules promulgated by the Food and Drug Administration (21 CFR 58) and the Environmental Protection Agency (40 CFR 160 and 40 CFR 792), which provide standards for the care and housing of test animals, and *Biosafety in Microbiological and Biomedical Laboratories* (Richmond and McKinney, 1993), which provides guidelines for containment of animals and animal wastes during and resulting from animal experimentation with pathogens.

For reviews and discussions of the various regulations and guidelines, refer to *Education and Training in the Care and Use of Laboratory Animals: A Guide for Developing Institutional Programs*, Part III, Chapter 1 (NRC, 1991); *Use of Laboratory Animals in Biomedical and Behavorial Research*, Chapter 5 (NRC, 1988); *The Biomedical Investigator's Handbook for Researchers Using Animal Models*, Chapter 6 (Foundation for Biomedical

Research, 1987); and *The Institutional Animal Care and Use Committee Guidebook (IACUC Guidebook)* (ARENA/OPRR, 1992).

In addition to the regulations noted above, animal experimentation with hazardous agents is subject to regulations that govern handling, use, and disposal of hazardous agents, such as radioisotopes and toxic chemicals. Likewise, protection of workers from a variety of potential workplace hazards is mandated by occupational safety and health agencies at the federal level and, in many cases, at the state level. It is the responsibility of each investigator using animals to know and comply with relevant regulations, guidelines, and policies (federal, state, local, and institutional).

ETHICAL CONSIDERATIONS

The laws, regulations, policies, and guidelines discussed above establish common standards for the humane care and use of laboratory animals. Recent revisions have refined earlier standards and improved the well-being of laboratory animals. Nevertheless, it is the obligation of every investigator who uses animals to ensure that the highest principles of humane care and use are applied. These principles are summarized in the U.S. government "Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Education" (published in NRC, 1985, pp. 81-83, and PHS, 1986, pp. 27-28), which was prepared by the Interagency Research Animal Committee, a group whose main concerns are the conservation, use, care, and welfare of research animals. The principles address such issues as the value of the proposed work; selection of appropriate models; minimization of

pain and distress; use of sedation, analgesia, or anesthesia when painful procedures are necessary; euthanasia of animals that might suffer severe or chronic pain or distress; provision of appropriate housing and veterinary care; training of personnel; and IACUC oversight of exceptions to the principles. The principles emphasize the role of the IACUC in determining the appropriateness and value of proposed work in which animals are likely to be subjected to unalleviated pain or discomfort. Some kinds of research should be especially carefully reviewed and periodically re-evaluated by IACUCs, including studies that involve unalleviated pain or distress (such as those in which death is the end point) and studies that involve food or water deprivation.

Some people and groups question the value of using animals in biomedical research and suggest that the knowledge gained is not sufficiently applicable to human disease to justify the pain, distress, and loss of life suffered by laboratory animals. However, Nicoll and Russell (1991) point out that animal research has contributed in an important way to 74 percent of 386 major biomedical advances made from 1901 to 1975 and that 71 percent of the 82 Nobel prizes for physiology or medicine awarded from 1901 to 1982 were given for research that depended on studies with animals. The regular occurrence of new infectious diseases of humans and animals—such as Legionnaire's disease, AIDS, Lyme disease, and canine parvovirus disease—and the existence of diseases that kill hundreds of thousands of people and animals a year—such as cancer, cardiovascular disease, and stroke—make research in living systems imperative if we wish to continue to make medical progress.

Most of the public are rightly concerned with the elimination of unnecessary animal suffering and the protection of pets, and it is an obligation of scientists to educate the press,

the legislature, and the public about the efforts made by the scientific community to minimize animal pain and suffering, the extensive review to which animal research is subjected, and the great benefits we and our pets derive from animal research. These benefits include the development of antiviral vaccines (e.g., vaccines against poliovirus, canine parvovirus, and feline leukemia virus), advances in tissue transplantation (e.g., of kidneys, corneas, skin, heart, liver, and bone marrow), and the development of new treatments for cardiovascular disease (e.g., open-heart surgery, valve replacement, and artery replacement). The educational process should stress that scientists and most of the public agree that the use of animals in research is necessary, that animals should be cared for and used as humanely as possible, and that unnecessary suffering should be prevented. Results of such educational efforts are beginning to appear in the form of state and federal legislation to protect animal-research facilities and laboratories from vandalism. The educational process should continue, and all scientists should be committed to it.

Useful discussions of the ethical issues related to animal research can be found in *Use of Laboratory Animals in Biomedical and Behavioral Research* (NRC, 1988); *The Biomedical Investigator's Handbook for Researchers Using Animal Models* (Foundation for Biomedical Research, 1987); *Mozart, Alexander the Great, and the Animal Rights/Liberation Philosophy* (Nicoll and Russell, 1991); and *Education and Training in the Care and Use of Laboratory Animals: A Guide for Developing Institutional Programs*, Part III, Chapter 2 (NRC, 1991).

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2

Responsibilities of Animal Care and Use Committees

PROGRAM OVERSIGHT

The Animal Welfare Regulations, or AWRs (9 CFR 2.31), mandate that each institution in which warm-blooded animals other than birds, rodents of the genera *Mus* and *Rattus*, and farm animals are used in research, testing, or education have an institutional animal care and use committee (IACUC) to oversee the institution's animal care and use program. *Public Health Service Policy on Humane Care and Use of Laboratory Animals*, or *PHS Policy* (PHS, 1986), has the same requirement for each PHS-funded institution that uses live vertebrates. Program oversight is more than semiannual facility inspections and protocol reviews; it places a more global responsibility on the IACUC for general oversight of the animal program. In a quality program, the highest standards of science and ethics are

understood and supported at every level of animal use, from the animal-care technician to the program administrator.

Program oversight should include consideration of all institutional functions, policies, or practices that directly affect the care and use of laboratory animals. It might include training; occupational health and safety; the veterinary-care program; use of animals in teaching; consistency of institutional policies with local, state, and federal regulations; interactions with other internal groups, such as those responsible for space allocation, research administration, and biosafety; interactions with external groups, such as funding agencies; specific concerns or complaints about animal use; investigation of unauthorized activities involving the use of animals; and effective communication between investigators, animal-care staff, and administrators.

An IACUC customarily reviews programs at the same time that it conducts semiannual facility inspections. It is important to document that both the program and the facilities have been reviewed by the IACUC and to note program improvements, as well as program deficiencies. Results of semiannual reviews must be provided to the institutional official and must include a plan for correcting deficiencies and minority views (9 CFR 2.31c3; 9 CFR 2.35a3; PHS, 1986).

PROTOCOL REVIEW

One of the many important responsibilities of an IACUC is to review the protocols for research, testing, or teaching projects in which any species covered by the AWRs or *PHS*

Policy will be used. The protocol-review mechanism is designed to ensure that investigators consider the care and use of their animals and that procedures comply with federal, state, and institutional regulations and policies. In addition, the review mechanism enables an IACUC to become an important institutional resource, assisting investigators in all areas involving the use of animals.

Each research protocol should include the following information, much of which is required by the AWRs, *PHS Policy*, or both:

- the purpose of the study;
- the rationale for selection of the species and the numbers of animals to be used;
- the strain, sex, and age of the animals to be used;
- the living conditions of the animals, particularly special housing and husbandry requirements;
- the experimental methods and manipulations;
- justification of multiple major survival surgeries on any individual animal;
- preprocedural and postprocedural care and medications;
- procedures that will be undertaken to avoid or minimize more than momentary discomfort, pain, and distress, including, where appropriate, the use of anesthetics, analgesics, and tranquilizers;
- if experimental manipulation is likely to cause more than momentary or slight pain or distress that for scientifically valid reasons cannot be relieved by appropriate drugs, the process undertaken to ensure that there are no appropriate alternatives (some types of

research, such as trauma studies and studies in which death is the end point, are particularly sensitive in this regard);

- procedures that will be used to monitor the animals in studies in which close monitoring is required, for example, those involving food or water deprivation and tumor growth (studies that require close monitoring should include specific end points);
- procedures and justification for long-term restraint;
- the euthanasia method, including a justification if it is not consistent with the recommendations of the American Veterinary Medical Association Panel on Euthanasia (AVMA, 1993 et seq.);
- assurance that the protocol does not unnecessarily duplicate previous work; and
- the qualifications of personnel who will perform the procedures outlined.

Protocol submission and review formats differ widely from one institution to another and depend on a number of variables, including the size and mission of the institution, other levels of scientific review to which the protocol will be subjected, and past experiences of the IACUC. Thorough and careful preparation of a protocol will facilitate the review process and reduce delay. One review approach used by IACUCs, particularly in large institutions, is to assign a knowledgeable committee member to each protocol as the primary reviewer. The primary reviewer deals directly with the investigator to clarify issues in question. Changes or clarifications in the protocol that result from the reviewer's discussions with the investigator are submitted to the IACUC in writing. Later, at an IACUC meeting, the primary reviewer presents and discusses the protocol and relates discussions with the

investigator. After the reviewer's presentation of the protocol. the reviewer recommends a course of action, which is then discussed and voted on by the IACUC. Another kind of protocol review (which is especially effective in small institutions with few protocols) is initial review by the entire IACUC. Many committees rely on additional review by experts (either on or outside the committee) in specific subjects; for example, a veterinarian should review protocols for appropriateness of the proposed anesthesia and analgesia, and a statistician might review statistically complicated study designs. In some institutions, such as pharmaceutical companies, some kinds of studies (e.g., pharmaceutical development and toxicology screening) are based on standard operating procedures. Nevertheless, IACUC review and approval are required before study initiation.

Several outcomes of protocol review are possible: approval, approval contingent on receipt of additional information (to respond to minor problems with the protocol), deferral and rereview after receipt of additional information (to respond to major problems with the protocol), and withholding of approval. If approval of a protocol is withheld, an investigator should be given the opportunity to respond to the critique of the IACUC in writing, to appear in person at an IACUC meeting to present his or her viewpoint, or both. It is also important that expedited review be possible; however, the use of expedited review does not negate the requirement (9 CFR 2.31; PHS, 1986, Section IV.C.2) that each IACUC member be given the opportunity to review every protocol and to call for a full committee review before approval is given (McCarthy and Miller, 1990).

The question of protocol review for scientific merit has been handled in a variety of ways by IACUCs. Many protocols are subjected to extensive, external scientific review as

part of the funding process; in such instances, the IACUC can be relatively assured of appropriate scientific review. For studies that will not undergo outside review for scientific merit, many IACUCs require signoff by the investigators, department chairmen, or internal review committees; this makes signers responsible for providing assurance that the proposed studies have been designed and will be performed "with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society" (NRC, 1985, p.82; PHS, 1986, p.27). Occasionally, IACUC members and scientists differ as to the relevance of proposed studies to human and animal health and the advancement of knowledge. Each institution should develop guidelines for dealing with this potential conflict.

It is important that the IACUC document the protocol-review process, so that it is clear that all aspects of a project, especially aspects that might seriously affect animal well-being, have been thoroughly considered by the IACUC; minority views must be included (9 CFR 2.31). IACUCs should keep accurate records, pay careful attention to semantics, and be familiar with local, state, and federal "freedom of information" laws that make records available to the general public on request.

PERSONNEL QUALIFICATIONS AND TRAINING

Job applicants for positions that require access to an animal facility should be carefully screened. Checks for records of criminal activity might be warranted. Potential employees should understand clearly the nature of the work. Education of animal-care and research personnel regarding proper security procedures is critical to ensuring facility security. This training should be part of new-employee orientation and should be reinforced frequently.

Both *PHS Policy* (PHS, 1986) and the AWRs (9 CFR 3.32) require that institutions provide training on the care and use of animals. It is the responsibility of the IACUC to ensure that animal-care and research staff are appropriately trained (PHS, 1986). As part of program oversight, the IACUC must ensure that procedures for providing and documenting training are in place; however, the responsibility for design and implementation of training programs varies. Responsibilty for course objectives and format is frequently shared by staff from various functional units, such as veterinary staff, employee-health personnel, safety officers, and IACUC members.

People for whom it is required that training be made available (9 CFR 2.32) include those who provide animal husbandry (caretakers), those who perform technical procedures on animals (research staff and animal technicians and technologists), those who provide veterinary medical care and treatment (veterinarians and veterinary technicians). The National Research Council has recommended that training also be provided to other personnel, including administrative and housekeeping staffs). Training is also important for those who are responsible for oversight (IACUC members and administrators). The varied

backgrounds and responsibilities of the people for whom training is provided, the size and nature of the institution, the variety and numbers of animals used, and the nature of animal use (i.e., research, teaching, and testing) are important in the design of an institutional training program. The program should be tailored to meet the institution's specific needs and designed with ease of use and convenience in mind. Although the format and content might vary considerably between institutions, there is some agreement on minimal information that should be provided. The following topics are considered by the National Research Coucil to be essential elements of a basic training program (NRC, 1991):

- laws, regulations, and policies that affect the care and use of animals;
- ethical and scientific issues;
- alternatives to the use of animals;
- responsibilities of the IACUC and the research and veterinary staffs;
- pain and distress;
- anesthetics, analgesics, tranquilizers, and neuromuscular blocking agents;
- survival surgery and postsurgical care;
- euthanasia;
- husbandry, care, and the importance of the environment; and
- resources for additional information.

For each of those elements, all personnel should be provided a general overview that is designed to promote understanding of and facilitate compliance with regulations and policies.

Depending on the audience and the topic, it might not be necessary to provide a high degree of detail. For example, the discussion of survival surgery should familiarize the audience with regulations and acceptable standards for surgical procedures and postsurgical care, but it need not provide details of specific surgical methods, which would be important only to those performing or assisting with the surgery or postsurgical care.

In contrast, substantial detail should be provided to people in direct contact with animals, and the content should be appropriate to their responsibilities for animal care or use. For example, detailed information on species-specific housing methods, husbandry procedures, and handling techniques should be provided to animal caretakers; research staff should be specifically qualified through training or experience for each approved procedure in the designated species; and veterinary staff should be appropriately trained in relevant aspects of laboratory animal medicine.

Training is provided in various ways. Many people are qualified in animal care, use, or specific procedures by having formal training in degree or certification programs (e.g., veterinarians certified in laboratory animal medicine, certified animal technologists and technicians, and physicians with surgical specialties). Others might be qualified by having previous experience (e.g., investigators who have research experience with a particular animal model). Regardless of the extent of previous training, it is wise for each institution to provide information about the standards, requirements, and expectations of the institution and an updated overview of key issues to all personnel involved with animal care or use.

Institutions often need to provide extensive training to staff that provide daily care and observation of animals or to research personnel without previous or recent experience in a

particular technique or species. Various methods can be used, including lectures and seminars, videotaped lectures and demonstrations, and observation by experienced personnel. Continuing-education courses are available in many areas, particularly at or near large institutions or universities, and attendance can be encouraged by tuition-reimbursement programs. Each method has advantages and disadvantages, and each institution should select the format that serves the needs of its staff best.

Resources for developing training programs include qualified institutional staff, formal courses by recognized organizations (e.g., the American Association for Laboratory Animal Science), and written and audiovisual training aids (see NRC, 1991, part IV, chapter 3).

It is important not only to ensure or provide appropriate training, but to document that all personnel who care for or use animals are appropriately trained. Training and education can be documented in a variety of ways. For example, previous training can be documented by records, publications, and signed statements of experience, and training provided by the institution can be documented by attendance records, signed statements, and notes to personnel files. A powerful method for documenting or monitoring the qualifications of personnel is observation of animal procedures by a qualified person. This method provides an accurate assessment of the expertise of the person performing the procedure, as well as information about the health status of the animal during the procedure. Such observation is usually considered to be an appropriate component of veterinary oversight.

OCCUPATIONAL HEALTH AND SAFETY

An occupational health and safety program is an important component of the operation of any institution in which animals are used (NRC, 1995). This program should seek to safeguard the health of employees that work with laboratory animals by developing standard operating procedures to minimize the chance of exposure to zoonotic diseases and providing the necessary training so that employees will understand the risks associated with working with animals and the importance of complying with institutional procedures. The program can also serve the animals being maintained by screening employees for zoonotic diseases and, where appropriate, providing immunizations that will minimize the likelihood of introduction of zoonotic agents into the animal facility.

The design of an occupational health and safety program should be based on a careful review of the potential hazards that exist in the animal facilities. The program must comply with Occupational Safety and Health Administration (OSHA) standards (29 CFR 110-114) and should be designed with the aid of medical personnel who are knowledgeable in occupational medicine and familiar with zoonotic diseases. Each aspect of the program should be carefully and realistically evaluated with respect to the magnitude of risk involved, the legal and practical enforceability of mandated components of the program, and the costs relative to the likelihood of detecting or preventing a problem. A legal review of the final proposed program is advisable because local, state, and federal laws might preclude adoption or enforcement of some of its components.

Oversight of occupational health and safety programs varies among institutions. It is frequently assigned to employee-health staff, but in some institutions it is the reponsibility of personnel, human-resources, veterinary, or other administrative staffs. Generally, an IACUC verifies during its semiannual review that the occupational health and safety program is in place and that its components are appropriate to the institution's animal care and use program.

Few general rules can be applied to occupational health and safety programs for rodent facilities. Only a few rodent diseases pose a threat to humans, and many of these have a very low prevalence (e.g., the diseases caused by Hantaan virus, lymphocytic choriomeningitis virus, some *Salmonella* species, *Hymenolepis nana*, and *Streptobacillus moniliformis*). In most cases, prophylactic immunizations do not exist for rodent zoonotic organisms; if immunizations do exist, the risks associated with them should be balanced against the likelihood of contracting the disease. Personnel should be instructed to notify their supervisors of bite wounds, unusual illnesses, and suspected health hazards. Facilities often maintain records of individual work assignments and of employee-reported problems. That information, if kept accurately and evaluated regularly, can be of value in alerting both the institution and employees to unusual patterns of illness that could indicate an animal-related disease.

Other occupational hazards, including allergies, should be recognized, and methods should be developed for minimizing the risks and treating problems if they occur. Animal-care personnel are generally at greater risk of contracting tetanus than other segments of the workforce because the greater frequency with which they handle animals puts them at greater

risk of being bitten. Therefore, it is important that immunization against tetanus be offered to animal-care personnel and that a record of prophylactic immunizations be kept.

Exposure to potentially toxic materials and ergonomic practices associated with lifting and moving equipment and materials are also of concern in rodent facilities. The animal facilities and related support areas should be evaluated for the need for protective devices (e.g., respirators, lifting-support belts and gloves, and ear and eye protection) and for the need to develop safety measures peculiar to the tasks being conducted. If animal-care, research, and maintenance personnel could be exposed to potentially hazardous biologic, chemical, or physical agents, the exposure to such agents should be monitored. Specific safety procedures designed to minimize the risk of exposure should be developed in consultation with appropriate health and safety professionals.

The gathering of pre-employment health information—by questionnaire, physical examination conducted by a physician, or both—might be deemed appropriate, provided that such information is related specifically to evaluating the employee's potential for carrying zoonotic organisms or having predisposing conditions (e.g., allergies, immunosuppression, pregnancy, and heart disease) that would make exposure to animals hazardous to his or her health. All medical records must be kept confidential, should be reviewed by a competent health care professional, and must not be used to gather information on non-animal-related health matters that could be used to prevent hiring the employee. Conditions identified that might affect the animal care and use program (e.g., a positive result of a test for tuberculosis) or might put an employee at increased risk (e.g., pregnancy) should be communicated to appropriate personnel to minimize unnecessary risk to employees, animals,

or both. The conditions for employment and use of employee-health information should be precisely defined in advance by the institution and should comply with local, state, and federal requirements.

Periodic physical examinations might be offered to some employees in some job categories. In some institutions, programs have also been established to obtain and store individual serum samples taken before hiring and during employment for future diagnostic purposes. In general, such serum-banking procedures are seldom undertaken in rodent facilities and, when offered, are usually voluntary. In institutions in which research involving the use of zoonotic agents in rodents is conducted and in which there is a substantial risk of infection, prophylactic vaccinations, if available, should be offered to employees at risk; in such cases, it is important that employees be informed by trained medical personnel of both the benefits and the risks associated with the vaccinations.

An important component of the occupational health and safety program is employee education. Each institution should have in place a course of study consisting of lectures or seminars, self-help materials, or both to instruct personnel who work with animals about zoonoses, allergies to animals, the importance of personal hygiene, special risks associated with pregnancy, and other appropriate topics. This course of study should also include information on hazardous materials that are used in the facilities, including those regulated by the Environmental Protection Agency and the Nuclear Regulatory Commission and those used in procedures evaluated by OSHA. Of particular importance are chemical agents used in routine animal-care operations, including disinfectants, cage-cleaning solutions, and sterilizing agents.

USE OF HAZARDOUS AGENTS

Biomedical experimentation frequently involves the use of hazardous agents, which can be classified as chemical (e.g., chemical carcinogens and chemotherapy agents), physical (e.g., radioisotopes), or biologic (e.g., infectious agents and recombinant DNA). In addition to the common concerns associated with handling and storage, the use of these agents in animals introduces unique concerns, including hazards associated with administration of the agents to the animals, the mode and quantity of excretion of the agents by the animals, contact with contaminated animal tissues, and disposal of carcasses, bedding, and excrement.

It is the responsibility of the IACUC to ensure that the procedures for use and monitoring of hazardous agents have been reviewed and are appropriate (NRC, 1985 et seq.). That is commonly and most readily accomplished by requiring that any use of hazardous agents be approved by an appropriate institutional safety committee (e.g., radiation-safety committee, infectious-agents committee, biosafety committee, or recombinant-DNA-use committee) before IACUC consideration. Formal programs should be in place to review the procedures, facilities, and staff competence for the proposed studies and to monitor compliance with federal, state, and local regulations and institutional policies during the conduct of the research. Requirements of both hazard containment and good animal husbandry should be met. Areas in which hazardous agents are approved for use should be visited as part of the IACUC semiannual inspection. Review should include assurance that

there are universal warning signs where hazardous agents are contained and used and that all involved personnel are familiar with and are using approved procedures.

In addition to hazardous agents for which regulations or guidelines are well established—such as radioisotopes (10 CFR 20), infectious agents (NCI, 1974; Richmond and McKinney, 1993; NIH, 1984), and human-blood products (29 CFR 1910)—it is important that there be equal oversight of the use of experimental agents not usually thought of as hazardous, such as some categories of agents for human therapy, fresh tissue from humans or animals, cultured cell lines that might harbor pathogens, and volatile anesthetics. A list of publications pertaining to regulations and guidelines for the use of hazardous agents can be found in the *Guide* (NRC, 1985 et seq.).

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3

Criteria for Selecting Experimental Animals

SPECIES AND STOCKS

Choosing a Species for Study

For a scientific investigation to have the best chance of yielding useful results, all aspects of the experimental protocol should be carefully planned. If animal models will be used, an important part of the process is to consider whether nonanimal approaches exist. If, after careful deliberation and review of the existing literature, the investigator is satisfied that there are no suitable alternatives to the use of live animals for the study in question, the next question that should be addressed is what species would be most appropriate to use.

In choosing a species for study, it is important to weigh a variety of scientific and operational factors, including the following:

- In which species is the physiologic, metabolic, behavioral, or disease process to be studied most similar to that of humans or other animals to which the results of the studies will be applied?
- Do other species possess biologic or behavioral characteristics that make them more suitable for the planned studies (e.g., generation time and availability)?
- Does a critical review of the scientific literature indicate which species has provided the best, most applicable historical data?
- Do any features of a particular species or strain—including anatomic, physiologic, immunologic, or metabolic characteristics—render it inappropriate for the proposed study?
- In light of the methods to be used in the study, would any physical or behavioral characteristics of a particular species make the required physical manipulation or sampling procedures impossible, subject to unpredictable failure, or difficult to apply?
- Does the proposed study require animals that are highly standardized either genetically or microbiologically?

Those and other considerations often lead to the selection of a laboratory rodent species as the most appropriate model for a biomedical research protocol. Rodents are generally easy to obtain and relatively inexpensive to acquire and maintain. Other advantages of laboratory rodents as research models include small size, short generation time, and availability of microbiologically and genetically defined animals, historical control data, and well-documented information on physiologic, pathologic, and metabolic processes.

The order Rodentia encompasses many species. The most commonly used rodents are

laboratory mice[1], laboratory rats (*Rattus norvegicus*), guinea pigs (*Cavia porcellus*), Syrian hamsters (*Mesocricetus auratus*), and gerbils (*Meriones unguiculatus*). All those rodents have been extensively studied in the laboratory, and information about them can be found in the peer-reviewed literature and in a number of texts (e.g., Altman and Katz, 1979a,b; Baker et al., 1979-1980; Foster et al., 1981-1983; Fox et al., 1984; Gill et al., 1989; Harkness and Wagner, 1989; Van Hoosier and McPherson, 1987; Wagner and Manning, 1976).

Rodent Stocks

The same factors used in selecting a species for study can be used in selecting a rodent stock. Rodents have been maintained in the laboratory environment for more than 100 years. Some, such as the mouse, have been very well characterized genetically and have undergone genetic manipulation to produce animals with uniformly heritable phenotypes. A hallmark of good scientific method is reproducibility, which is accomplished by minimizing and controlling extraneous variables that can alter research results. In studies that are mechanistic, genetic uniformity is highly desirable. In contrast, genetic uniformity might be undesirable in studies that explore the diversity of application of a phenomenon over a range of phenotypes, such as product-registration studies, including safety evaluation of compounds that have therapeutic potential. In many such studies, a varied genetic background might be appropriate, as long as the range of variation can be characterized and is to some degree

[1]Laboratory mice are neither pure *Mus domesticus* nor pure *Mus musculus*; therefore, geneticists have determined that there is no appropriate scientific name (International Committee on Standardized Genetic Nomenclature for Mice, 1994a).

reproducible (Gill, 1980).

Genetically Defined Stocks

Inbred Strains. The mating of any related animals will result in inbreeding, but the most common and efficacious method for establishing and maintaining an inbred strain is brother x sister (i.e., full-sib) mating in each generation. Full-sib inbreeding for 20 generations will result in more than 98 percent genetic homogeneity, at which point the members of the stock are isogenic, and the stock is considered an inbred strain. Many inbred strains of mice and rats have been developed (Festing, 1989; Festing and Greenhouse, 1992), and they are widely used in biomedical research. Many of the commonly used strains have been inbred for over 200 generations. A few inbred strains of guinea pigs, Syrian hamsters, and gerbils have also been developed (Altman and Katz, 1979b; Festing, 1993; Hansen et al., 1981).

The isogeneity of the members of an inbred strain provides a powerful research tool. Although some genes might remain heterogeneous, most metabolic or physiologic processes, as well as their phenotypic expression, will be identical among individuals of an inbred strain, thereby eliminating a source of experimental variation. Isogeneity also allows exchange of tissue between individuals of an inbred strain without rejection.

F1 Hybrids. F1 hybrid animals are the first filial generation (the F1 generation) of a cross between two inbred strains. They are often more hardy than animals from either of the

parental strains, having what is called hybrid vigor. F1 hybrids are heterozygous at all genetic loci at which the parental strains differ; nevertheless, they are *uniformly* heterozygous. Because of the heterogeneity, F1 hybrids will not breed true; to produce them one must always cross animals of the parental inbred strains. Reciprocal hybrids are developed by reversing the strains from which the dam and the sire are taken. Reciprocal male hybrids will have Y-chromosome differences. Reciprocal female hybrids will have identical genotypes but might have differences caused by inherited maternal effects. F1 hybrids will accept tissue from either parental strain, except in the case of a Y-chromosome incompatibility (e.g., a skin graft from a male of either parental strain will be rejected by a female F1 hybrid).

Special Genetic Stocks. The effects of specific genes or chromosomal regions can be studied by using various breeding or gene manipulation methods to create a new strain that differs from the original strain by as little as a single gene.

- *A segregating inbred strain* is an inbred strain maintained by full-sib matings; however, male-female pairs are selected for mating so that one pair of genes will remain heterozygous from generation to generation. This method of mating permits well-controlled experiments because a single sibship contains both carriers and noncarriers of the gene of interest, and all the animals are essentially identical except for that gene.
- A *coisogenic strain* is an inbred strain in which a single-gene mutation has occurred and has been preserved; it is otherwise identical with the nonmutant parental strain. If the

mutation is not deleterious when homozygous, the strain can be maintained by simple full-sib matings. If the mutation adversely affects breeding performance, the coisogenic strain can be maintained by one of several special breeding systems (Green, 1981; NRC, 1989). To avoid subline divergence between the coisogenic strain and the nonmutant parental inbred strain, periodic back-crossing (see next paragraph) with the parental strain is recommended.

- A *congenic strain* is a close approximation to a coisogenic strain. It is created by mating an individual that carries a gene of interest, called the differential gene, with an individual of a standard inbred strain. An offspring that carries the differential gene is mated to another individual of the same inbred strain. This type of mating, called back-crossing, is continued for at least 10 generations to produce a congenic strain. Back-crossing for 10 generations minimizes the number of introduced genes other than the differential gene and its closely linked genes. Details on developing congenic strains have been published (Bailey, 1981; Green, 1981). Both coisogenic and congenic strains can be maintained by full-sib matings if the differential gene is homozygous; however, to avoid subline divergence between the congenic strain and the standard inbred strain, periodic back-crossing with the standard strain is recommended.

- A *transgenic strain* is similar to a coisogenic or congenic strain in that it carries a segment of genetic information not native to the strain or individual (Hogan et al., 1986; Merlino, 1991). The introduced genetic material can be from the same or another species. Transgenic animals are described in more detail in Chapter 8.

- *Recombinant inbred (RI) strains* are sets of inbred strains produced primarily to study genetic linkage. Each RI strain is derived from a cross between two standard inbred

strains. Animals from the F1 generation are then bred to produce the second filial generation (the F2 generation), members of which are randomly selected and mated to produce a series of RI lines. Members of the F2 generation are used to found RI lines because, unlike the F1 generation, they are not isogenic. The mice derived from any parental pair will be genetically homogeneous when inbreeding is complete; however, each line in a set will be homozygous for a given combination of alleles originating from the two parental inbred strains. Alleles that are linked in the parental strains will tend to remain together in the RI lines; this is the basis for their use in genetic-mapping studies.

- *Recombinant congenic strains* are like recombinant inbred strains except that each strain of a series has been derived from a back-cross instead of an F2 cross (Demant, 1986). The number of back-crosses made before full-sib inbreeding is started determines the proportion of genes from each of the parental inbred strains. Series of recombinant congenic strains are particularly useful in the genetic analysis of multiple- gene systems, such as that responsible for cancer susceptibility.

Nongenetically Defined Stocks

The terms *noninbred*, *random-mated*, and *outbred* are all used to refer to populations of animals in which, theoretically, there is no genetic uniformity between individuals. Nongenetically defined stocks make up the majority of rodents used in biomedical research and testing, and they are generally less expensive and more readily available than genetically defined stocks.

Noninbred refers to a population of animals in which no purposeful inbreeding system has been established. *Random-mated* refers to a group of animals in which the selection of breeding animals is random. It assumes an almost infinite population with no external selection pressures. In practice, such a colony probably does not exist. *Outbred* refers to a colony in which breeding is accomplished by a purposeful scheme that minimizes or eliminates inbreeding. Animals produced by these breeding systems have varied genotypes, and characterizing the range and distribution of phenotypes requires a large sample of the population.

The degree of heterozygosity in any nongenetically defined stock is continuously varying, so two populations developed from the same parental stock will show differing degrees of heterozygosity at any loci at any time. Spontaneous mutations can occur and become fixed because no purposeful selection is imposed on the population to eliminate the mutant genes. Outbred populations are always evolving and therefore are more variable than inbred strains. For that reason, large sample numbers are needed to account for phenotypic variation that could have an impact on the charactersitics being studied. If outbred animals are used, treatment and control groups in a study will not necessarily be identical, nor will the population of animals necessarily be identical if the study is repeated. The genetic variation in outbred stocks, which can be magnified by sampling error, can make results from different laboratories difficult to compare. Background data on stock characteristics will vary over time, so concurrent controls are needed to allow useful interpretation of data.

STANDARDIZED NOMENCLATURE FOR RODENTS

Standardized nomenclature allows scientists to communicate briefly and precisely the genetics of their research animals. The International Committee on Standardized Genetic Nomenclature for Mice and the International Rat Genetic Nomenclature Committee, which are affiliated with the International Council for Laboratory Animal Science, are responsible for maintaining the nomenclatures for genetically defined mice and rats, respectively, and modifying them as necessary. The sections below briefly describe the nomenclature for inbred, mutant, and outbred mice and rats. The complete rules for mice can be found in the third edition of *Genetic Variants and Strains of the Laboratory Mouse* (Lyon and Searle, in press). Those rules are regularly updated, and updates are published in *Mouse Genome* (formerly called *Mouse News Letter*; Oxford University Press) and are available on-line in MGD, the Mouse Genome Database. Information on MGD can be obtained from the Mouse Genome Informatics Group, The Jackson Laboratory, Bar Harbor, ME 04609 (telephone, 207-288-3371; fax, 207-288-5079; Internet, mgi-help@informatics.jax.org). The rules for rats have been published as an appendix to the report *Definition, Nomenclature, and Conservation of Rat Strains* (NRC, 1992a), and updates will be published in *Rat Genome*, Heinz W. Kunz, Ph.D., editor, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261. Investigators using other laboratory rodents should follow the rules for mice or rats.

Inbred Strains

An inbred strain is designated by capital letters (e.g., mouse strains AKR and CBA and rat strains BN and LEW). The mouse rules, but not the rat rules, allow the use of a combination of letters and numbers, beginning with a letter (e.g., C3H), although this type of symbol is considered less desirable. Brief symbols (generally one to four letters) are preferred. Exceptions are allowed for strains that are already widely known by designations that do not conform (e.g., mouse strains 101 and 129 and rat strains F344 and DONRYU).

Substrains

An established strain is considered to have divided into substrains when genetic differences are known or suspected to have become established in separate branches. These differences can arise either from residual heterozygosity at the time of branching or from new mutations. A substrain is designated by the full strain designation of the parent strain followed by a slanted line (slash) and an appropriate substrain symbol, as follows:

- *Mice.* The substrain symbol can be a number (e.g., DBA/1 and DBA/2); a laboratory code, which is defined below (e.g., C3H/He, where He is the laboratory code for Walter E. Heston); or, when one investigator or laboratory originates more than one substrain, a combination of a number and a laboratory code, beginning with a number (e.g., C57BL/6J and C57BL/10J, where J is the laboratory code for the Jackson Laboratory, Bar

Harbor, Maine). Exceptions, such as lower-case letters, are allowed for already well-known substrains (e.g., BALB/c and C57BR/cd).

- *Rats*. The substrain symbol is always a number when genetic differences have been demonstrated. The founding strain is considered the first substrain, and the use of /1 for it is optional (e.g., KGH or KGH/1). A laboratory code (e.g., Pit for the University of Pittsburgh Department of Pathology and N for the NIH Genetic Resource) is used to designate a substrain when genetic differences are probable but not demonstrated (e.g., BN/Pit and BN/N).

Laboratory Codes

Each laboratory or institution that breeds rodents should have a laboratory code. The registry of laboratory codes is maintained by ILAR, National Research Council, 2101 Constitution Avenue, Washington, DC 20418 (telephone, 202-334-2590; fax, 202-334-1687). The laboratory code, which can be used for all laboratory rodents, consists of either a single roman capital letter or an initial roman capital letter and one to three lower-case letters.

- *Mice*. A particular colony is indicated by appending an "@" sign and the laboratory code to the end of the strain or substrain symbol (e.g., SJL@J, the colony of strain SJL mice bred at the Jackson Laboratory; C3H/He@N, the He substrain of strain C3H bred at the NIH Genetic Resource; and CBA/Ca-*se*@J, the Ca substrain of strain CBA carrying the *se* mutation and bred at the Jackson Laboratory). If the substrain symbol and

laboratory code are the same, the @ symbol and the laboratory code can be dropped for simplicity (e.g., SJL/J@J becomes SJL/J). The laboratory code is always the last symbol used and is meant to indicate that the environmental conditions and previous history of a colony are unique. When a strain is transferred to a new laboratory, the laboratory code of the originating laboratory is dropped, and the code of the recipient is appended; laboratory codes are not accumulated.

- *Rats*. Normally, a rat strain is designated by the strain name, a slash, the substrain designation (if any), and the laboratory code (e.g., BN/1Pit). When a strain is established in another laboratory, the new laboratory code is appended (e.g., BN/1PitN). In general, more than two laboratory codes are not accumulated. Intermediate codes are dropped to avoid excessively long designations.

For both mice and rats, a strain's holder is responsible for maintaining a strain history.

F1 Hybrids

An F1 hybrid is designated by the full strain designation of the female parent, a multiplication sign, the full strain designation of the male parent, and F1 (e.g., the hybrid mouse C57BL/6J × DBA/2J F1 and the hybrid rat F344/NNia × BN/RijNia F1). If there is any chance of confusion, parentheses should be used to enclose the parental strain names [e.g., (C57BL/6J × DBA/2J)F1 and (F344/NNia × BN/RijNia)F1]. The correct formal name should be given the first time the hybrid is mentioned in a publication; an abbreviated

name can be used subsequently [e.g., C57BL/6J × DBA/2J F1 (hereafter called B6D2F1) and F344/NNia × BN/RijNia F1 (hereafter called FBNF1)].

Coisogenic, Congenic, and Segregating Inbred Strains

In mice, a coisogenic strain is designated by the strain symbol, the substrain symbol (if any), a hyphen, and the gene symbol in italics (e.g., CBA/H-*kd*). When the mutant or introduced gene is maintained in the heterozygous condition, this is indicated by including a slash and a plus sign in the symbol (e.g., CBA/H-*kd*/+). A congenic strain is designated by the full or abbreviated symbol of the background strain, a period, an abbreviated symbol of the donor strain, a hyphen, and the symbol of the differential locus and allele (e.g., B10.129-*H12*^{b}). Segregating inbred strains are designated like coisogenic strains; however, indication of the segregating locus is optional when it is part of the standard genotype of the strain (e.g., 129/J and 129/J-c^{ch}/c mean the same thing, and either can be used).

In rats, a coisogenic strain (except for alloantigenic systems—see NRC, 1992a) is designated like a coisogenic strain in mice, except that the laboratory code follows the substrain symbol and the gene symbol is not italicized (e.g., RCS/SidN-rdy). A congenic rat strain (except for alloantigenic systems) is designated like a coisogenic strain (e.g., LEW/N-rnu). For segregating inbred strains developed by inbreeding with forced heterozygosis, indication of the segregating locus is optional.

Recombinant Inbred (RI) Strains

The symbol of an RI strain should consist of an abbreviation of both parental-strain symbols separated by a capital X with no intervening spaces (e.g., CXB for an RI strain developed from a cross of BALB/c and C57BL mouse strains and LXB for an RI strain developed from a cross of LEW and BN rat strains). Different RI strains in a series should be distinguished by numbers (e.g., CXB1 and CXB2 in mice and LXB1 and LXB2 in rats).

Genes

The rules for gene nomenclature are very complicated because they apply not only to mutant genes, but also to gene complexes, biochemical variants, and other special classes of genes (e.g., transgenes). This description will cover only a small portion of the gene nomenclature. The full rules can be found in the references given previously.

The symbols for loci are brief and are chosen to convey as accurately as possible the characteristic by which the gene is usually recognized (e.g., coat color, a morphologic effect, a change in an enzyme or other protein, or resemblance to a human disease). Symbols for loci are typically two- to four-letter abbreviations of the name. For mice, the symbols are written in italics; for rats, they are not. For convenience in alphabetical lists, the initial letter of the name is usually the same as the initial letter of the symbol. Arabic numbers are included for proteins in which a number is part of the recognized name or abbreviation (e.g., in mice, *C4* and *C6*, the fourth and sixth components of complement, respectively; in rats,

C4 and C6). Except in the case of loci discovered because of a recessive mutation, the initial letter of the locus symbol is capitalized and all other letters are lower-case. Hyphens are used in gene symbols only to separate characters that together might be confusing. This rule was adopted for mice in 1993, and hyphens should be deleted from all gene symbols except where they are necessary to avoid confusion. Gene designations are appended to the designation of the parental strain, and they are separated by a hyphen.

Loci That Are Members of a Series

A locus that is a member of a series whose members specify similar proteins or other characteristics is designated by the same letter symbol and a distinguishing number (e.g., *Es1*, *Es2*, and *Es3* in mice and Es1, Es2, and Es3 in rats). For morphologic or "visible" loci with similar effects (e.g., genes that cause hairlessness), distinctive names are given because the gene actions and gene products can ultimately prove to be different (e.g., *hr* and *nu* in mice and fz and rnu in rats).

Alleles

An allele is designated by the locus symbol with an added superscript. For mice, the superscript is written in italics; for rats, it is not. An allele superscript is typically one or two lower-case letters that convey additional information about the allele. For mutant genes, no superscript is used for the first discovered allele. When further alleles are found, the first

is still designated without a superscript (e.g., *nu* for nude and nu^{str} for streaker in mice and fa for fatty and fa^{cp} for corpulent in rats). If the information is too complex to be conveyed conveniently in the symbol, the allele is given a superscript (e.g., $Es1^{a}$ and $Es1^{b}$ in mice and $Es1^{a}$ and $Es1^{b}$ in rats), and the information is otherwise conveyed. Indistinguishable alleles of independent origin (e.g., recurrences) are designated by the gene symbol with a series symbol, consisting of an Arabic number corresponding to the serial number of the recurring allele plus the laboratory code, appended as a superscript in italics. To avoid confusing the number "1" and the lower-case letter "l," the first discovered allele is left unnumbered, and the second recurring allele is numbered 2 (e.g., *bg*, beige; bg^{J}, a recurrence of the mouse mutation *bg* at the Jackson Laboratory; and bg^{2J}, a second recurrence of the mutation *bg* at the Jackson Laboratory).

A mutation or other variation that occurs in a known allele (except for alloantigenic systems in the rat) is designated by a superscript *m* and an appropriate series symbol, which consists of a number corresponding to the serial number of the mutant allele in the laboratory of origin plus the laboratory code. The symbol is separated from the original allele symbol by a hyphen (e.g., $Mup1^{a\text{-}m1J}$ for the first mutant allele of mouse $Mup1^{a}$ found by the Jackson Laboratory). For a known deletion of all or part of an allele, the superscript *m* may be replaced with the superscript *dl*. This nomenclature is used for naming targeted mutations (often called "knockout" mutations), as well as spontaneously occurring ones.

Transgenes

Nomenclature for transgenes was developed by the ILAR Committee on Transgenic Nomenclature (NRC, 1992b). A transgene symbol consists of three parts, all in roman type, as follows:

TgX(YYYYYY)#####Zzz,

where TgX is the mode, (YYYYYY) is the insert designation, and #####Zzz represents the laboratory-assigned number (#####) and laboratory code (Zzz).

The mode designates the transgene and always consists of the letters Tg (for "transgene") and a letter designating the mode of insertion of the DNA: N for nonhomologous recombination, R for insertion via infection with a retroviral vector, and H for homologous recombination. The purpose of this designation is to identify it as a symbol for a transgene and to distinguish between the three fundamentally different organizations of the introduced sequence relative to the host genome. When a targeted mutation introduced by homologous recombination does not involve the insertion of a novel functional sequence, the new mutant allele (the knockout mutation) is designated in accordance with the guidelines for gene nomenclature for each species. The gene nomenclature is also used when the process of homologous recombination results in integration of a novel functional sequence, if that sequence is a functional drug-resistance gene. For example, *Mbp*^{m1Dn} would be used to denote the first targeted mutation of the myelin basic protein (*Mbp*) in the mouse made by

Muriel T. Davisson (Dn). In this example, the transgenic insertion, even if it contains a functional neomycin-resistance gene, is incidental to "knocking out" or mutating the targeted locus (see also International Committee on Standardized Genetic Nomenclature for Mice, 1994b).

The insert designation is a symbol for the salient features of the transgene, as determined by the investigator. It is always in parentheses and consists of no more than eight characters: letters (capitals or capitals and lower-case letters) or a combination of letters and numbers. Italics, superscripts, subscripts, internal spaces, and punctuation should not be used. Short symbols (six or fewer characters) are preferred. The total number of characters in the insert designation plus the laboratory-assigned number may not exceed 11 (see below); therefore, if seven or eight characters are used, the number of digits in the laboratory-assigned number will be limited to four or three, respectively.

The third part of the symbol is a number and letter combination that uniquely identifies each independently inserted sequence. It is formed of two components. The laboratory-assigned number is a unique number that is assigned by the laboratory to each stably transmitted insertion when germline transmission is confirmed. As many as five characters (numbers as high as 99,999) may be used; however, the total number of characters in the insert designation plus the laboratory-assigned number may not exceed 11. No two lines generated within one laboratory should have the same assigned number. Unique numbers should be given even to separate lines with the same insert integrated at different positions. The number can have some intralaboratory meaning or simply be a number in a series of transgenes produced by the laboratory. The second component is the laboratory code. Thus,

the complete designation identifies the inserted site, provides a symbol for ease of communication, and supplies a unique identifier to distinguish it from all other insertions [e.g., C57BL/6J-TgN(CD8Ge)23Jwg for the human CD8 genomic clone inserted into C57BL/6 mice from the Jackson Laboratory (J) and the 23rd mouse screened in a series of microinjections done in the laboratory of Jon W. Gordon (Jwg)]. The complete rules for naming transgenes have been published (NRC, 1992b).

TBASE, a database developed at Oak Ridge National Laboratory, Oak Ridge, Tennessee, as a registry of transgenic strains, is maintained at the Johns Hopkins University, Baltimore, Maryland. Information on TBASE can be obtained from the Genome Database and Applied Research Laboratory, The Johns Hopkins University, 2024 East Monument Avenue, Baltimore, MD 21205 (telephone, 410-955-1704; fax, 410-614-0434).

Outbred Stocks

An outbred-stock designation consists of a laboratory code, a colon, and a stock symbol that consists of two to four capital letters (e.g., mouse stock Crl:ICR and rat stock Hsd:LE). The stock symbol must not be the same as that for an inbred strain of the same species. As an exception, a stock derived by outbreeding a formerly inbred strain may continue to use the original symbol; in this case, the laboratory code preceding the stock symbol characterizes the stock as outbred. An outbred stock that contains a specified mutation is designated by the laboratory code, a colon, the stock symbol, a hyphen, and the gene symbol (e.g., Crl:ZUC-fa).

The transfer of an outbred stock between breeders is indicated by listing the laboratory code of the new holder followed by the laboratory code of the holder the stock was obtained from (e.g., HsdBlu:LE for rats obtained by Harlan Sprague Dawley from Blue Spruce Farms). To avoid excessively long designations, only two laboratory codes should be used.

QUALITY

In selecting rodents for use in biomedical research, consideration should be given to the quality of the animals. Quality is most commonly characterized in terms of microbiologic status and of the systems used in raising animals to ensure that a specific microbiologic status is maintained. However, the genetics of an animal, as well as the genetic monitoring and breeding programs used to ensure genetic consistency, clearly also play an important part in defining rodent quality.

Microbiologic Quality

Rodents can be infected with a variety of adventitious pathogenic and opportunistic organisms that under the appropriate circumstances can influence research results at either the cellular or subcellular level. Some of those agents can persist in animals throughout their lives; others cause transient infections and are eliminated from the animals, leaving lasting serologic titers as the only indicators that the organisms were present. The types of organisms that can infect rodents include bacteria, protozoa, yeasts, fungi, viruses, rickettsia,

mycoplasma, and such nonmicrobial agents as helminths and arthropods.

Many of the common organisms that infect laboratory rodents have been studied extensively, and some of their research interactions have been characterized (see NRC, 1991; Bhatt et al., 1986, for review). Unfortunately, information about the effects of many other organisms is incomplete or is not available. There is no general agreement on the importance of many organisms that latently infect rodents, especially opportunistic organisms that cause disease or alter research results only under narrowly defined conditions and even then usually affect only a very small proportion of the population. Any decision on the quality of rodents to be selected for a particular research project should include a realistic assessment of the organisms that have a reasonable probability, as determined by documentation in the peer-reviewed literature, of producing confounding effects in the proposed study.

It is commonly assumed that animals for which the most extensive health monitoring has been done and to which the most rigorous techniques for excluding microorganisms have been applied are the most appropriate for use in all studies. However, for both scientific and practical reasons, that assumption is not always valid. Rodents that are free of all microorganisms (axenic rodents, see definition below) or axenic rodents that have purposely been inoculated with a few kinds of nonpathogenic microorganisms (microbiologically associated rodents) can have altered physiologic and metabolic processes that make them inappropriate models for some studies. They can also rapidly become contaminated with common microorganisms unless they are maintained with specialized housing and husbandry measures, which are expensive and can fail. The commercial availability of such rodents is

limited, and they are more expensive than rodents in which the microbial burden is not so restricted. For those reasons, the rodents most commonly used in research are ones that are free of a few specific rodent pathogens and some other microorganisms that are well known to have confounding effects on specific kinds of research.

The quality of laboratory animals is generally related to the microbiologic exclusion methods used to breed and maintain them. There are three major types of maintenance: isolator-maintained, barrier-maintained, and no- containment or conventionally maintained animals. An isolator is a sterilizable chamber that is usually constructed of metal, rigid plastic, vinyl, or polyurethane. It usually has a sterilized air supply, a mechanism for introducing sterilized materials, and a series of built-in gloves to allow manipulation of the animals housed within. All materials moved into the isolator are sterilized, and animals raised within the isolator are generally maintained free from contamination by either all or specified microorganisms.

Barrier-maintained animals are bred and kept in a dedicated space, called a barrier. For barrier facilities, personnel enter through a series of locks and are usually required to disrobe, shower, and use clean, disinfected clothing. All body surfaces that will potentially make contact with animals are covered. All equipment, supplies, and conditioned air provided to the barrier facility are sterilized or disinfected. Barrier facilities can be of any size and can consist of one or more rooms. They are designed to exclude organisms for which rodents are the primary or preferred hosts but generally will not exclude organisms for which humans are hosts.

Barrier maintenance can also be achieved at the cage or rack level with equipment that

can be sterilized or otherwise disinfected. This type of maintenance depends heavily on providing large volumes of filtered or sterilized air to the animal cages. Such systems can be used successfully to maintain animals with a highly defined microbiologic status; the success of such systems depends on the techniques used and is difficult to monitor because microbiologic status might differ from cage to cage.

No-containment, or conventionally maintained, animals are raised in areas that have no special impediments to the introduction of microorganisms. This method of maintaining animals cannot ensure stability of the microbiologic status, because unwanted organisms can be introduced at any time.

Several classifications have been developed to define the microbiologic quality of laboratory animals, as follows (see also NRC, 1991):

- *Axenic* refers to animals that are derived by cesarean section or embryo transfer and reared and maintained in an isolator with aseptic techniques. It implies that the animals are demonstrably free of associated forms of life, including viruses, bacteria, fungi, protozoa, and other saprophytic or parasitic organisms. Animals of this quality require the most comprehensive and frequent monitoring of their microbiologic status and are the most difficult to obtain and maintain.

- *Microbiologically associated, defined flora, or gnotobiotic* refers to axenic animals that have been intentionally inoculated with a well-defined mixture of microorganisms and maintained continuously in an isolator to prevent contamination by other agents. Generally, a small number (usually less than 15) of species of microorganisms are used in the inoculum,

and it is implied that these organisms are nonpathogenic.

- *Pathogen-free* implies that the animals are free of all demonstrable pathogens. It is often misused, in that there is no general agreement about which agents are pathogens, what tests should be used to demonstrate the lack of pathogens and with what frequency, and how the populations should be sampled. Use of this term should be avoided because of the lack of precision of its meaning.

- *Specific-pathogen-free* (SPF) is applied to animals that show no evidence (usually by serology, culture, or histopathology) of the presence of particular microorganisms. In its strictest sense, the term should be related to a specific set of organisms and a specific set of tests or methods used to detect them. An animal can be classified as SPF if it is free of one or many pathogens.

- *Conventional* is applied to animals in which the microbial burden is unknown, uncontrolled, or both.

In addition, the term *clean conventional* is sometimes used to describe animals that are maintained in a low-security barrier and are demonstrated to be free of selected pathogens. This term is even less precise than *pathogen-free*, and its use is discouraged (NRC, 1991).

Commercial suppliers have coined various terms to indicate SPF status. All the terms are related to specific organisms of which the animals are stated to be free and for which they are regularly monitored. In some cases, the terms (e.g., *virus-antibody-free* and *murine-pathogen-free*) imply a quality of animals beyond the actual definitions of the terms. Virus-antibody-free animals, for example, are animals that are free of antibodies to specific

rodent viruses. The term is a variation of SPF, in that it relates to *specified* viruses. The implied method of detection is serology. Animals might not be free of viruses other than those specified and might not be free of other microorganisms.

Genetic Quality

In spite of diligent maintenance practices that are required in any breeding colony to identify animals properly and house them securely, people can make mistakes. In addition, loose animals, including animals that escape their housing unnoticed and wild rodents, can enter cages, mate with the inhabitants, and produce genetically contaminated offspring. Good husbandry practices carried out by trained personnel, including keeping a pedigree and clearly identifying animals and cages, can help to reduce the occurrence of such events. Nevertheless, to avoid devastating consequences of genetic contamination, a good program of genetic monitoring is warranted. Genetic monitoring consists of any method used to ensure that the genetic integrity of individuals of any particular strain has not been violated. Several commercial sources provide genetic monitoring services for inbred mouse and rat strains.

Personnel should be alert to phenotypic changes in the animals, such as unexpected coat colors or large changes in reproductive performance. In a pedigree-controlled foundation colony (see Chapter 4), it is important to monitor the breeding stock at least once every two generations so that a single erroneous mating can be detected quickly. Retired breeders or some of their progeny can be tested. In an expansion or production colony, in which it might not be cost-effective or practical to monitor so closely, sampling is recommended.

The extent of such sampling can be as broad as resources and need permit. If genetic contamination occurs outside the foundation colony, contamination will eventually be purged by the infusion of breeders from the more rigorously controlled foundation colony.

The extent of necessary testing depends on the number and genotypes of neighboring strains. A testing system should be capable of identifying the strain to which the individual belongs and differentiating it from other strains maintained nearby. Most strains can be identified with a small set of any genetic markers for which an assay is available. Newer DNA-typing methods that use multilocus probes, minisatellite markers, and "DNA-fingerprinting" analysis are powerful tools for distinguishing strains, especially strains that are closely related, but electrophoretic methods that type isoenzymes are generally more cost-effective for genetic monitoring (Hedrich, 1990; Nomura et al., 1984), in that such monitoring is most commonly done to detect mismatings. Immunologic methods are also used, and the exchange of skin grafts between individuals of a strain is a particularly effective method for screening a large number of loci in a single test. DNA from representative breeders of a strain can be stored for future use in identifying suspected genetic contaminations.

Genetic monitoring is used primarily to verify the authenticity of a given strain; new mutations are rarely detected by this means. It is impossible to monitor all loci for new mutations, given the large number of unknown loci and known loci that do not produce a visible phenotype. A good breeding-management program, as described in Chapter 4, will help to reduce unwanted genetic changes caused by mutations.

SELECTED ASPECTS OF EXPERIMENTAL DESIGN

An experiment in which laboratory animals are used should be designed carefully, so that it produces unequivocal information about the questions that it was designed to address. The two most important requirements of proper experimental design in that connection are as follows:

- Animals in different groups should vary only in the treatment that the experiment is designed to evaluate, so that the experimental outcome will not be confounded by dissimilarities in the constitution of the groups or in how they are treated or measured.
- Each treatment should be given to enough animals for the experimental outcome to be attributed confidently to treatment difference and not merely to chance.

The best way to ensure that groups of experimental animals are comparable is to draw them from a single homogeneous pool and to assign them randomly to treatment groups. Choosing animals of the same age, sex, and inbred strain for all treatment groups and even assigning littermates randomly to different treatment groups can eliminate factors that might partially account for group-to-group differences in experimental outcome.

Once animals are assigned to groups, they should be handled identically, except for the treatment differences that the experiment is designed to evaluate. Food, water, bedding, and other features of animal husbandry should be the same. For long-term experiments, cages should be rotated to minimize group differences caused by cage position. For invasive

experimental treatments, sham or placebo procedures should be performed in comparison groups; for example, animals given treatment by gavage should be compared with controls given the vehicle by gavage, animals treated surgically should be compared with animals that undergo sham surgical operations, and animals exposed to treatment by inhalation should be compared with animals placed in inhalation chambers that circulate only air. Following those precautions will ensure that differences in outcome between groups can be attributed to the experimental treatment itself and not to ancillary differences associated with the administration of the treatment.

Finally, wherever possible, the outcome of interest should be measured by people who are unaware of which treatment each animal received, because such knowledge can magnify or even create observed treatment differences. It is particularly important to carry out "blind" studies when the outcome is to be evaluated subjectively (e.g., by grading of disease severity), rather than measured quantitatively (e.g., by measuring concentrations of serum constituents).

The number of animals needed in each group will depend on many features of the experimental design, including the following:

- the goals of the study;
- the primary outcome measure that will be compared;
- the number of groups that will be compared;
- the expected number of technical failures or usable end points;
- the number and type of comparisons that will be made;

- the expected animal-to-animal and measurement variability in the outcome;
- the statistical design and analysis that will be used;
- the magnitude of the differences between control and treatment groups that it is desirable to detect;
- the projected losses; and
- the maximal tolerable chance of drawing erroneous conclusions.

The more variable an outcome measure is, either because outcomes in identically treated animals vary substantially or because there is a high degree of measurement variability, the more animals will be needed in each group to distinguish between group differences caused by treatment and those caused by chance. How outcome measurement variability, treatment difference to be detected, and tolerable chance of drawing an erroneous conclusion affect the required sample size depends on the measurement to be made, the type of group comparison to be made, and the statistical analysis to be used. Tables and formulas for comparing proportions among two or more groups have been published (Gart et al., 1986), as has useful information for other types of outcomes (Mann et al., 1991). For most experiments, it is highly desirable to collaborate with a statistician throughout, beginning with the design stage, so that appropriately defined groups of sufficient size will be available for a proper statistical analysis.

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4

Genetic Management of Breeding Colonies

Different breeding systems and genetic-engineering methods have been used to produce strains and stocks of rodents for particular experimental purposes—inbred strains; coisogenic, congenic, and transgenic strains; recombinant inbred strains; hybrid strains; and outbred stocks. Outbred stocks are used primarily when genetic heterogeneity is desired and are not useful when a controlled genotype is required. However, the loss of heterozygosity cannot be completely avoided in propagating outbred stocks, because the breeding population is necessarily finite.

GENETICALLY DEFINED STOCKS

Regardless of the breeding system or genetic manipulation used to produce a particular strain, some practices are recommended to maintain high genetic quality. Details of breeding systems used to develop various types of strains can be found elsewhere (Bailey, 1981; Green, 1981a). Here we describe the management of breeding colonies of already-developed strains.

Pedigrees

Using a pedigree method allows the parentage of individual experimental animals to be traced; aids in selection of parental pairs to avoid the inadvertent fixation of unwanted mutations, especially mutations that would affect reproductive performance; and maximizes genetic uniformity within a strain.

Traceability

Mutations occur continually in any breeding stock. Many of these mutations are recessive and, when homozygous, will be expressed as undesirable traits. When such a mutation is expressed, it is necessary to rid the breeding colony of copies of the mutation that might be carried as a heterozygous gene by individuals that are normal in phenotype. Use of a pedigree system that records the parents of each individual makes it possible to

identify relatives of the affected individual, and they can be tested for the presence of the mutation or eliminated from the colony. It is also desirable to mark the animals with their pedigree identification.

Selection of Parental Pairs

Reproductive performance, even within a highly inbred strain, can vary greatly. Environmental factors undoubtedly cause much of that variation, but spontaneously occurring mutations that adversely affect breeding performance are also contributing factors. To avoid extinction of a strain, the individuals selected for propagating it should be those with the best reproductive performance. Reproductive performance can be evaluated retroactively by examining a pedigree, that is, the reproductive performance of several generations of offspring can be used in evaluating the breeding performance of the original pair and can aid in avoiding the accidental incorporation or accumulation of deleterious recessive mutations. To ensure continuation of a strain, several families or lines should be maintained for two to three generations until one pair in each generation is retroactively chosen as the pair from which breeders in all subsequent generations will be derived. This practice not only ensures selection of reproductively fit individuals to propagate the strain but also maximizes genetic uniformity, as described below.

Genetic Uniformity

The purpose of producing an inbred strain is to achieve genetic uniformity among individuals. That allows a greater degree of reproducibility in experiments than is possible if heterogeneous individuals are used. However, total genetic uniformity is never achieved, because new mutations occur. Each new mutation has a 25 percent chance of becoming fixed in an inbred strain (Bailey, 1979). The gradual accumulation of such mutations and the resulting genetic changes are called *genetic drift*. Because of the random occurrence of mutations, genetic drift will involve different genes in two separately maintained sublines of a strain. Over time, the sublines will become increasingly different from each other; this tendency is called *subline divergence*. Bailey has estimated that separately maintained sublines will diverge at the rate of approximately one new mutation every two generations (Bailey, 1978, 1979, 1982). Even within one breeding colony, subline divergence can occur if the propagation of family branches is allowed to continue indefinitely.

Another source of subline differences is the genetic heterogeneity present in a strain at the time of subline separation. Many of the early substrains of common inbred strains were separated before the strain had been highly inbred; for example, mouse substrains C57BL/6 and C57BL/10 were separated from the C57BL strain when it had been inbred for only about 30 generations. That is more than the 20 generations conventionally accepted as the definition of an inbred strain, but the amount of heterogeneity, although small in comparison with the total number of genes, is still sufficient to result in subline differences. For example, according to Bailey's estimates, one would expect about 14 fixed differences between substrains C57BL/6 and C57BL/10 caused by the presence of unfixed genes at the time of separation. Bailey also showed that the probability of there being no heterogeneity

within an inbred strain does not reach 0.99 until after 60 generations of brother × sister inbreeding (Bailey, 1978). The practical consequence of subline divergence for research is that animals from different sublines might respond differently in identical experiments, and the difference in responses could lead to misinterpretation of the experimental results. A corollary is that no subline (or substrain) can be considered a reference standard, because all sublines undergo changes with time. Cryopreservation might offer the only means to arrest such changes. Nevertheless, it is wise to obtain breeders periodically from the original source colony, to maximize homogeneity between two colonies. A general practice is to do that after 10 generations of separation.

Within a breeding colony, pedigree management can be used to maximize genetic uniformity. One pair in each generation can be selected on the basis of breeding performance, to be the common ancestral mating for all progeny. So that all animals at any time can be traced to a single ancestral pair, the number of generations of any branch other than the common ancestral branch is limited, depending on the number of animals that are produced for experimental use, the productivity or the average number of breeding pairs of progeny expected from a single mating, and the reproductive life span of breeders.

Because most commonly used inbred strains today are highly inbred, breeding selection is not effective in increasing reproductive performance. Rather, selection is made to avoid deleterious mutations that would cause a decrease in reproductive performance. The prevalence and rate of such mutations are unknown, but distinct reductions in reproductive performance within family branches have been observed in large breeding colonies. Because

increases in reproductive performance are rare, mutations that are advantageous to reproduction are probably extremely rare.

Pedigree identification of animals used as parents for the production of hybrids is advised so that mutations or irregularities can be traced. However, pedigree management is not necessary, because there is no propagation of lines beyond that of the F1 generation.

Foundation or Nucleus Colonies, Expansion Colonies, and Production Colonies

In large breeding operations, it is often practical for management purposes to subdivide the breeding colony of each strain into separate groups—a foundation colony (sometimes called a nucleus colony), an expansion colony, and a production colony—that are maintained in separate facilities. A foundation colony is a breeding colony of sufficient size to propagate the strain (following the selection procedures described previously) and to provide breeding stock to an expansion colony. The purpose of an expansion colony is to increase the number of breeding pairs to a quantity adequate to support a production colony. A production colony is made up of breeders from an expansion colony; offspring are distributed for research, not used for breeding.

It is more practical to be rigorous about selection practices and genetic monitoring in a foundation colony, which is relatively small, than in the larger expansion and production colonies. It is also more important to carry out those activities in the foundation colony because all the stock in the expansion and production colonies is ultimately derived from it and any change occurring in the foundation colonies will eventually be propagated throughout

the entire strain. An advantage of using a separate facility for foundation colonies is that it permits microbiologic status of the foundation colony to be maintained with fewer pathogens than the other colonies. Often, foundation colonies are maintained in a separate building from expansion and production colonies to protect against loss of a strain due to disease outbreak or other catastrophe. Cryopreservation and storage of embryos can also fulfill that security requirement.

In an expansion colony, it might not be practical or cost-effective to maintain detailed pedigree records or devote much time to selection. It is relatively easy, however, to keep track of the number of generations that a family or subline has been separated from the foundation stock by making a notation on the cage card each time a new mating group is made up. By limiting the number of generations outside the foundation nucleus, maximal genetic uniformity can be achieved. Unnoticed mutations (e.g., those affecting reproductive performance) that occur in either an expansion or a production colony will ultimately be purged because of the constant infusion of highly scrutinized breeding stock from the foundation colony. Trio matings (i.e., two females mated to a sibling male) are often used in expansion colonies for efficiency.

In a production colony, especially a large one, the use of non-sib matings increases efficiency. The probability that recessive, mutated alleles will come together and be expressed in an individual is much decreased when non-sib matings are used. However, it is also less likely that such mutations will be detected and eliminated; therefore, it is not recommended that strains be propagated for more than a few generations by non-sib matings.

Normally, breeders in a production colony represent the last generation of family lines created in enlarging the colony.

NONGENETICALLY DEFINED STOCKS

The goal of breeding programs for nongenetically defined stocks is to maintain the diversity in genotypes that is present in the founding animals of that stock. Ideally, no selection pressures should be placed on the population; however, in practice, there is often a conscious or unconscious selection for reproductive performance, and great care should be taken to eliminate this bias. Ideally, a purely random mating structure should be used so that each animal has an equal chance of participating in the breeding program and of mating with any of the animals of the opposite sex within the colony with no attention to relationship, genotype, phenotype, or any other characteristic; this requires accurate identification of individual animals, extensive record-keeping, and structured randomization in which randomization tables or computer-generated randomized numbers are used to select breeding pairs.

An important limitation on any random breeding program is the size of the population that can be maintained within a facility. Even for commercial breeders, populations are limited in size; therefore, without a systematic method for ensuring that inbreeding does not occur, chance matings between relatives will gradually cause a decrease in heterozygosity within the population. The rate of decrease of heterozygosity is proportional to the population size; very small populations experience a more rapid decrease. For example, a

population of 50 will undergo a decrease in heterozygosity at the rate of about 1 percent per generation. After 20 generations, this population will have only 82 percent of the heterozygosity with which it started (Green, 1981b).

To minimize that loss of heterozygosity, one can use a structured system of mating that is not completely random but is designed to avoid inbreeding. Several such systems exist. In very small populations (up to 32 animals), systematic mating of cousins can be used to avoid brother × sister mating. When the number of animals exceeds 32, that system becomes too cumbersome to use. In larger colonies, either a circular or circular-paired mating system can be used effectively to minimize inbreeding; both systems slow the loss of heterozygosity and require regular pairing of progeny from individual cages or groups of cages with animals in adjacent cages or groups. Detailed descriptions of these systems are available (Kimura and Crow, 1963; Poiley, 1960). Alternatively, a computerized system of tracking the coefficient of inbreeding of all breeders can be used to set up matings of the least-related animals.

Loss of heterozygosity by inadvertent inbreeding and acquisition and fixation of spontaneous mutations can cause considerable genetic divergence between populations of the same nongenetically defined stock maintained at different locations. To minimize the process, there should be a regular exchange of breeding stock between populations. The number of animals that are transferred and the frequency of transfer will depend on many factors, including colony size, breeding system used, and rate at which divergence is anticipated to occur. The success of such measures can be assessed with population-genetics techniques to calculate the degree of residual heterozygosity in individual populations. These

methods usually entail surveying a large number of biochemical or immunologic markers that display polymorphism in a relatively large sample of the population.

In addition to the classic nongenetically defined populations maintained by random breeding or outbreeding, populations of rodents with substantial genetic diversity, as evidenced by heterozygosity at a large number of loci, can be developed by making systematic multiple inbred-strain crosses. In such a system, four or more inbred strains are regularly crossed in a circular fashion to yield F1 progeny that are systematically mated with a rotational system to provide F2 animals for use in experimental procedures. F2 animals will show greater genetic diversity than most common nongenetically defined stocks that have been maintained for many years as closed colonies (Green, 1981b).

Overall, the maintenance of nongenetically defined stocks is complex if inbreeding is to be minimized. These populations are unique, dynamic, and diverse and require regular characterization unless they are linked by exchange of breeding stock.

CRYOPRESERVATION

Cryopreservation, in the form of freezing of cleavage-stage embryos, offers a means to protect a stock or strain against accidental loss or genetic contamination. It also provides a genetic advantage in retarding genetic changes caused by accumulated mutations and an economic advantage in lowering the costs of strain maintenance. In some circumstances, as when quarantine regulations impede the importation of adult animals, the transportation of frozen embryos, which do not have to be quarantined, is effective. Cryopreservation of

embryos has been possible since 1972 (Whittingham et al., 1972; Wilmut, 1972) and has now been successfully carried out for at least 16 mammalian species, including mice and rats (Hedrich and Reetz, 1990; Leibo, 1986; Whittingham, 1975; Whittingham et al., 1972).

Not all stocks warrant cryopreservation. If a strain is preserved with scant information on its characteristics, for example, it is unlikely that it will be of much use in the future. The ILAR Committee on Preservation of Laboratory Resources has recommended the following criteria for identifying valuable laboratory animals: the importance of the disease process or physiologic function, the validity or genetic integrity of the stock, the difficulty of replacing the stock, versatility of the stock, and current use (NRC, 1990).

To obtain embryos of a predetermined stage for freezing, exogenous gonadotropins are administered to induce synchronous ovulation and permit timed matings. Exogenous gonadotropins also often induce superovulation (i.e., the production of more eggs than normal). A combination of pregnant mares' serum, which contains follicle-stimulating hormone, and human chorionic gonadotropin, which contains luteinizing hormone, is commonly used (Gates, 1971). Freezing eight-cell embryos generally produces the most reliable results, at least in the mouse, but other preimplantation embryo stages can also be used.

There are many methods for cryopreserving embryos (Mazur, 1990; Leibo, 1992). Generally, they are in two categories: equilibrium methods and nonequilibrium methods; the distinction depends on the osmotic forces encountered in the presence of cryoprotectant during the freezing process (Mazur, 1990). Equilibrium methods use low concentrations (1.5M) of cryoprotectants and slow, controlled cooling (approximately 0.5°C/min).

Nonequilibrium methods generally use a higher concentration of cryoprotectants (about 4-5 M) and fast cooling (more than 200°C/min). The two kinds of methods are equally successful, but nonequilibrium methods have the advantage of not requiring controlled-rate freezers.

In mice, 500 is generally considered a safe number of embryos to store. Mouse embryos show no deterioration with time when stored at -196°C, and their viability is not affected by the equivalent of 2,000 years of exposure to background radiation (Glenister et al., 1984, 1990). Mice have been born from embryos stored for 14 years with no observable differences in rates of birth from recently frozen embryos. An advantage of liquid-nitrogen storage systems is that electricity and motors are not required; only a periodic, and preferably routine, replenishment of liquid nitrogen is necessary. Alarms and automatic filling devices need electricity, but all maintenance and monitoring of liquid-nitrogen storage containers can be carried out manually if necessary.

To recover animals from frozen embryos, the embryos are thawed and transferred to pseudopregnant females, that is, females in which the hormones required to support implantation and pregnancy are induced by mating them to vasectomized or genetically sterile males. The overall rate of live births from frozen mouse embryos of inbred and mutant strains is 20 percent. The rate is usually higher for hybrid and outbred embryos, but there is extreme variability, and the rate from a given attempt can range from 0 to 100 percent.

For security, embryos from one strain would ideally be stored in separate cities; at a minimum they should be stored in two containers. Before a strain is considered safely

cryopreserved, it should have been re-established at least once from frozen embryos by recovering live born, raising them to maturity, and breeding them to produce the next generation. To avoid genetic contamination of a strain, genetic monitoring procedures should be used to verify that animals born from frozen embryos have the expected genotype.

RECORD-KEEPING

In maintaining pedigrees, the most critical records are those of parentage. One should be able to identify and trace all relationships through these records. In addition to parental information, which might include individual identification numbers and mating dates, it is useful to record the generation number, birthdate, number born, weaning date, number weaned, and disposition of progeny. The latter information is useful in evaluating the reproductive performance of a colony. A bound, archive-quality pedigree ledger or a secure computer system might be used for recording information. A computer program for colony record-keeping has been described (Silver, 1993). If ledgers are used in a colony that includes many strains, it is useful to maintain a separate book for each strain. Each book should identify the book that preceded it or, if it is the first pedigree record for its colony, the origin of the animals. In colonies that have only a few strains, it might be more practical to maintain one general ledger. In this case, it is important to identify each entry accurately according to its strain, as well as its parental and other information. For pedigree management, it is also useful to maintain a pedigree chart, at least for foundation

breeders; this helps to avoid unnecessary proliferation of family branches by allowing visualization of individual animal relationships.

Marking of each animal with its pedigree identification will preserve identity throughout its lifetime (see Chapter 5). That can be useful when animals from different sibships are housed in the same cage. The advantage of recording individual identifications of animals used in research is that retrospective analysis of such characteristics as age and family relationship can sometimes help to explain unexpected results.

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5

Husbandry

HOUSING

Caging

Caging is one of the primary components of a rodent's environment and can influence the well-being of the animals it houses. Many types of caging are available commercially. Those used to house rodents should have the following features:

- They should accommodate the normal physiologic and behavioral needs of the animals, including maintenance of body temperature, normal movement and postural adjustments, urination and defecation, and, when indicated, reproduction.
- They should facilitate the ability of the animal to remain clean and dry.

- They should allow adequate ventilation.
- They should allow the animals easy access to food and water and permit easy refilling and cleaning of the devices that contain food and water.
- They should provide a secure environment that does not allow animals to become entrapped between opposing surfaces or in ventilation openings.
- They should be free of sharp edges or projections that could cause injury to the animals housed.
- They should be constructed so that the animals can be seen easily without undue disturbance.
- They should have smooth, nonporous surfaces that will withstand regular sanitizing with hot water, detergents, and disinfectants.
- They should be constructed of materials that are not susceptible to corrosion.

In selecting caging, one should pay close attention to the ease and thoroughness with which a cage can be serviced and sanitized. In addition to smooth, impervious surfaces that are free of sharp edges, cages should have minimal corners, ledges, and overlapping surfaces, because these features allow the accumulation of dirt, debris, and moisture. Cages should be constructed of durable materials that can withstand rough handling without chipping or cracking.

Sanitizing procedures, such as autoclaving and exposure to ionizing radiation, can alter the physical characteristics of caging materials over time and can greatly shorten useful life. Rodent cages are most commonly constructed of stainless steel or plastic (polyethylene,

polypropylene, or polycarbonate), each of which has advantages and disadvantages. Galvanized metal and aluminum have also been used but are generally less acceptable because of their high potential for corrosion.

Most rodent cages have at least one wire or metal grid surface to furnish ventilation and permit inspection of the animals in the cage. Inspection of animals can be further facilitated by the use of transparent plastic cages. Opaque plastic or metal cages might provide a more desirable environment for some studies or breeding programs; however, adequate inspection of animals will usually require manipulation of each cage.

The bottoms of rodent cages can be either solid or wire. The floors of solid-bottom cages usually are covered with bedding material that absorbs urine and moisture from feces, thereby improving the quality of the cage environment and allowing for easy removal of accumulated wastes. Solid-bottom cages provide excellent support for rodents' feet, minimizing the occurrence of pododermatitis and injuries. Wire-bottom cages are equipped with a wire-mesh grid, the spaces in which are large enough to allow the passage of feces. Generally, there are two to four wires per inch (2.5 cm) in the grid. These cages are normally mounted on racks that suspend them over waste-collection pans filled with absorbent material. This caging type minimizes contact with feces and urine and is thought to improve cage ventilation. However, careful consideration should be given to the size and species of rodents to be housed in wire-bottom cages because if their feet and legs can be entrapped in the wire grid, they can suffer severe trauma, including broken bones. In addition, older, heavier rodents can develop pododermatitis if the wires in the grid are too far apart or too small in diameter to provide adequate support for the feet.

Specialized types of caging that serve specific functions are available for rodents, including caging designed to collect excreta, monitor physiologic characteristics, test behavioral responses, control aspects of the physical environment, and permit enhanced microbiologic control of the environment. Such caging can pose special cleaning and sanitation problems.

Various racking systems, both fixed and mobile, are available to hold either solid-bottom or wire-bottom cages. Racks should be constructed of durable, smooth-surfaced, nonporous materials that can be easily sanitized. Mobile racks are most commonly used because they allow greater flexibility of room arrangement and are easier to clean than fixed racks. If fixed racks are used, adequate steps should be taken to protect floors or walls from damage caused by the weight of the racks and to provide for cleaning under and between the racks. Some racks incorporate devices that automatically supply water directly to the cages they hold.

Housing Systems

Many types of housing systems with specialized caging and ventilation equipment are available for rodents. Generally, the purpose of these housing systems is to minimize the spread of airborne microorganisms between cages; but they often do not prevent transmission of nonairborne fomites. The most frequently used of these systems is the filter-top cage, which has a spun-bound or woven synthetic filter that covers the wire-mesh top of a solid-bottom cage, thereby preventing the entry or escape of airborne particles that can act as

fomites for unwanted microorganisms. The use of filter tops restricts ventilation and can alter the microenvironment of the rodents housed in the cages; therefore, to maintain a healthful environment, it might be necessary to change the bedding and clean the cages more often (Keller et al., 1989).

A cubicle (also called an Illinois cubicle or a cubical containment system) is an enclosed area of a room capable of housing one or more racks of cages. It is separated from the rest of the room by a door that usually opens and closes vertically. The cubicle is supplied by air that moves under the door from the room and is exhausted through the ceiling, or a separate air supply is provided to the cubicle through an opening in a wall, the base, or the ceiling. Cubicles have been used to reduce airborne cross contamination between groups of animals housed in conventional plastic or wire-bottom cages (White et al., 1983). They provide better ventilation than many housing methods, but they do not protect against fomite transmission of microorganisms. Strict adherence to sanitation and other husbandry procedures is required if cubicles are to be used effectively.

In some housing systems, cages are individually ventilated with highly filtered air. In some, exhaust air is also filtered or controlled in a way that greatly minimizes the risk of contaminating animals in other cages and personnel in the animal rooms. Such systems can overcome the disadvantages of using nonventilated filter-topped cages while minimizing airborne cross-contamination.

A housing system that is particularly useful for maintaining the microbiologic status of rodents has isolators made of rigid or flexible-film plastic that are designed to enclose a group of rodent cages. Built-in gloves allow the manipulation of animals and materials in the

isolators. Isolators are supplied with filtered air and have a filtered exhaust; at least one transfer device is provided for moving sterilized or disinfected materials into the isolator. To maintain the microbiologic status of an isolated group of animals, it is necessary to sterilize or otherwise disinfect all the interior surfaces of the isolators, and all materials introduced into the isolators should be first sterilized or otherwise disinfected.

Space Recommendations

It is generally assumed that there are critical measures of cage floor area and cage height below which the physiology and behavior of laboratory rodents will be adversely affected, thereby affecting the well-being of the animals and potentially influencing research outcomes. However, there are very few objective data for determining what those critical measures are or even whether such interactions exist. A number of studies designed to evaluate the effects of space on population dynamics have been conducted on wild and laboratory rodents housed in a laboratory environment (e.g., see Barnett, 1955; Christian and LeMunyan, 1958), but some of them used caging systems different from those generally used in laboratory animal facilities (e.g., see Davis, 1958; Joasoo and McKenzie, 1976; Thiessen, 1964). Changes in behavior, reproductive performance, adrenal weights, glucocorticoid and catecholamine concentrations, immunologic function, numbers of some kinds of white blood cells (usually lymphocytes), and cage-use patterns have been assessed in those studies and suggested as indicators of stress and compromised well-being (e.g., see Barrett and Stockham, 1963; Bell et al., 1971; Christian, 1960; Poole and Morgan, 1976; White et al.,

1989). However, there has never been general agreement as to which physiologic and behavioral characteristics are indicative of well-being in rodents or what magnitude of change in them would be necessary to compromise the well-being of the animals.

With few objective data available, cage space recommendations have been based on the results of surveys of existing conditions and professional judgment and consensus. The *Guide* (NRC, 1985 et seq.) provides minimal space recommendations for rodents but makes no distinction in space allotment between individually housed and group-housed rodents. Space recommendations have also been developed in other countries (CCAC, 1980; Council of Europe, 1990), but they are not totally compatible with those in the *Guide*. It is important to remember that space recommendations in the *Guide* serve only as a starting point for determining space required by rodents and might need adjustment to fit the needs of the animals and the purposes for which they are housed.

Although comprehensive studies involving all the characteristics associated with housing rodents are not available, sufficient information does exist to suggest that individually housed rodents and group-housed rodents have different space requirements. For the most part, laboratory rodents are social animals and probably benefit from living in compatible groups (Brain and Bention, 1979; NRC, 1978; White, 1990). Although more study is needed, rodents maintained for long periods, as in lifetime studies, appear to survive longer when housed in large, compatible social groups than when housed in small groups or individually (Hughes and Nowak, 1973; Rao, 1990). Individual housing is sometimes necessitated by the nature of the experimental protocol; in such instances, adequate space should be allotted to allow the animals to make normal postural adjustments, which will depend on the body size

attained by the animals during the course of the experiment. Under those circumstances, current space guidelines might not be sufficient, especially if an animal's size exceeds the scope of the recommendations.

Conversely, group-housed rodents would be expected to need less space per animal than individually housed rodents because each animal can also use the space of the other rodents with which it is housed. Studies have found that compatible social groups of rodents do not use all the available space recommended in current guidelines and probably do not require it for well-being (White, 1990; White et al., 1989). Rodents housed in compatible groups share cage space by huddling together along walls and under overhanging portions of the cage, such as feeders, as well as piling up on top of each other during long rest periods. The center of the cage is used infrequently.

Even if individually housed, rodents appear to prefer sheltered areas of the cage, especially if those areas have decreased light and height. Providing such a confined space within a cage might be one way to enrich the environment of rodents.

Sexually mature male rodents often fight when housed in groups for breeding or other purposes, but this behavior has never been shown to be a function of the amount of available floor space in the cage. Rather, the incidence of fighting appears to be related more to combining males into groups when they are sexually mature (especially if females are housed in the same room) or have participated in mating programs. Increasing the cage space is not effective in preventing the development of such behavior or in eliminating it once it has occurred. Only separation of the animals into individual cages or into smaller, compatible groups is effective in eliminating fighting.

In determining adequate cage space, it is important to consider the conditions of the experimental procedure and how long the animals will be housed. Animals that become debilitated during the course of an experimental procedure might require increased cage space or an alteration in caging to accommodate limitations in motion, recumbent positions, and the need for alternative food and water sources. Older animals are less active than younger animals and use less of the cage space or available activity devices.

The *Guide* (NRC, 1985 et seq.) and other guidelines also recommend cage heights. The recommendations do not appear to be related to the body size of rodents nor to their normal locomotion patterns. Laboratory rodents exhibit some vertical exploratory behavior when put into a new cage, and it has been suggested that relatively high cages be provided to accommodate this occasional behavior (Lawlor, 1990; Scharmann, 1991). However, there is no good evidence to suggest that rodents require tall enclosures. On the contrary, as described previously, they tend to seek shelter under objects lower than recommended in existing guidelines. Depending on the caging type, existing height guidelines can be useful for ensuring that there is adequate space for side-wall or cage-top feeders and adequate clearance for sipper tubes or other watering devices.

In summary, the space required to maintain rodents, either individually or in groups, depends on a number of factors, including age, weight, body size, sexual maturity, experimental intervention, behavioral characteristics, the duration of housing, group size, breeding activities, and availability of enrichment devices or sheltering areas within the cage. The relationships among those factors are complex, and there is not necessarily a direct correlation between body weight or surface area of the animals and the absolute floor area of

the cage required or used by them. Guidelines should be used with professional judgment based on assessment of the animals' well-being. However, alterations that bring floor area or height of cages below recommended levels should be adequately justified and approved by the IACUC.

ENVIRONMENT

Microenvironment

The microenvironment of a rodent is the physical environment that immediately surrounds it and is usually considered to be bounded by the primary enclosure or cage in which it resides. In contrast, the physical conditions in the secondary enclosure or animal room make up the macroenvironment. The components of the macroenvironment are often easier to measure and characterize than those of the microenvironment. The two environments are linked or coupled, but the character of each is often quite different and depends on a variety of factors, such as the numbers and species of rodents housed in the microenvironment, the design and construction of the cages, and the types of bedding materials used (Besch, 1975; Woods, 1975; Woods et al., 1975).

The measurement of constituents of the microenvironment of rodents is often difficult because of the relatively small volume of the primary enclosure. Available data show that temperature, humidity, and concentrations of gases and particulate matter—such as carbon dioxide, ammonia, methane, sulfur dioxide, respirable particles, and bacteria—are often higher in the microenvironment than in the macroenvironment (Besch, 1980; Clough, 1976;

Flynn, 1968; Gamble and Clough, 1976; Murakami, 1971; Serrano, 1971). Although there is little information on the relation between the magnitude of exposure to some of those components and alterations in disease susceptibility or changes in metabolic or physiologic processes, the available data clearly suggest that the characteristics of the microenvironment can have a substantial impact on research results (Broderson et al., 1976; Vessell et al., 1973, 1976).

Temperature

Temperature and relative humidity are important components of the environment of all animals because they directly affect an animal's ability to regulate internal heat. They act synergistically to affect heat loss in rodents, which lose heat by insensible means, rather than by perspiring. Studies in the older literature, which were conducted without the benefit of modern systems for controlling conditions precisely or modern instrumentation, have established that extremes in temperature can cause harmful effects (Lee, 1942; Mills, 1945; Mills and Schmidt, 1942; Ogle, 1934; Sunstroem, 1927). However, those studies were done on only a few laboratory species.

Studies in the past generally focused on prolonged exposure of laboratory animals to temperatures above 85°F (29.4°C) or below 40°F (4.4°C), which are required to achieve clinical effects (Baetjer, 1968; Mills, 1945; Weihe, 1965). When exposed to those extreme temperatures, rodents use behavioral means (e.g., nest-building, curling up, huddling with others in the cage, and adjusting activity level) to attempt to adapt. If the temperature

change is brief or small, behavioral adaptation is sufficient; profound or prolonged temperature changes generally require physiologic or structural adaptation as well. Physiologic adaptation includes alterations in metabolic rate, growth rate, and food or water consumption; hibernation or estivation; and the initiation of nonshivering thermogenesis. Structural adaptation includes alterations in fat stores, density of the hair coat, and structure or perfusion of heat-radiating tissues and organs (e.g., tail, ears, scrotum, and soles of the feet). Initiation of such changes usually requires exposure to an extreme temperature for at least 14 days.

For routine housing of laboratory rodents, a consistent temperature range should be provided. However, there is little scientific evidence from which optimal temperature ranges for laboratory rodents can be determined. For each species, there is a narrow range of environmental temperatures at which oxygen consumption is minimal and virtually independent of change in ambient temperature. The range in which little energy is expended to maintain body temperature is called the thermal neutral zone, and some have suggested that it is a range of comfortable temperatures for rodents (Besch, 1985; Weihe, 1965, 1976a). However, other evidence suggests that animals held within this temperature range do not necessarily achieve optimal growth and reproductive performance, and the optimal temperature range might be age-dependent (Blackmore, 1970; Weihe, 1965). Moreover, measurements of thermal neutral zones are generally made on resting animals and do not take into account periods of increased activity or altered metabolic states, such as pregnancy. Thermal neutrality does not necessarily equate with comfort. In the absence of well-controlled studies that used objective measures for determining optimal ranges, recommended

temperature ranges for laboratory rodents have been independently developed by several groups on the basis of professional judgment and experience (e.g., CCAC, 1980; Council of Europe, 1990; NRC, 1985 et seq.).

Humidity

Relative humidity varies considerably with husbandry and caging practices. In addition, there is usually a difference between the relative humidity in the room and that in the animal cages. Many factors—including cage material and construction, use of filter tops, number of animals per cage, frequency of bedding changes, and bedding type—can affect the relative humidity in the rodents' immediate environment.

Variations in relative humidity appear to be tolerated much better at some temperatures than at others. Studies in humans and limited in vitro work on survival of microorganisms have established a loose association between humidity and susceptibility to disease (Baetjer, 1968; Dunklin and Puck, 1948; Green, 1974; Webb et al., 1963), but there is no good evidence to establish this link in animals. Low relative humidity has been reported to be associated with the development of "ring tail" in rodents (Flynn, 1959; Njaa et al., 1957; Stuhlman and Wagner, 1971); however, this condition has not been adequately studied and does not appear to be reproducible by lowering relative humidity in controlled laboratory experiments.

Guidelines have been established for relative-humidity ranges based on experience and professional judgment (CCAC, 1980; Council of Europe, 1990; NRC, 1985 et seq.). There

is no evidence to support limiting the variation of relative humidity within these ranges: however, the combination of high relative humidity and high environmental temperature can affect the ability of rodents to dissipate heat by insensible means and should be avoided.

Ventilation

Ventilation Rate

Ventilation refers to the process of using conditioned air to affect temperature, humidity, and concentrations of gaseous and particulate contaminants in the environment. Ventilation is often characterized at the animal-room level as air exchanges per hour. However, as for other environmental conditions, there are no definitive data showing that the air-exchange range in existing guidelines (i.e., 10-15 air changes/hour) provides optimal ventilation for laboratory rodents.

Existing guidelines have been criticized as being based mainly on keeping odors below objectionable limits for humans (Besch, 1980; Runkle, 1964) and, in recent years, as being energy-intensive. An often-quoted study by Munkelt (1938) appears to support the first contention: his measure of adequate ventilation was the ability to smell ammonia in the environment. Besch (1980) suggested that ventilation should be based on air-exchange rate per animal or animal cage because room air-exchange rates do not consider such factors as population density, room configuration, and cage placement within a room. Ultimately, however, the ventilation rate in animal rooms is governed by the heat loads produced in the

rooms, which include not only heat produced by animals but also that produced by other heat-radiating devices, such as lighting (Curd, 1976).

Available evidence suggests that little additional control of the concentrations of gaseous and particulate contaminants is gained by using air-exchange rates higher than those recommended in current guidelines (Barkley, 1978; Besch, 1980). The recommendation of providing a room air-exchange rate of 10-15 changes/hour is still useful; however, this ventilation range might not be appropriate in some circumstances, especially if the diffusion of air within the room is inappropriate for the type and placement of cages. Other methods of providing equal or more effective ventilation, including the use of individually ventilated cages or enclosures and the adjustment of ventilation rate to accommodate unusual population densities, are also acceptable.

Calculation of the amount of cooling required to control expected sensible and latent heat loads generated by the species to be housed and the largest expected population (ASHRAE, 1994) can be used to determine minimal ventilation requirements. However, that calculation does not take into account the generation of odors, particles, and gases, which might necessitate greater ventilation.

Air Quality

The quality of air used to ventilate animal rooms is another important consideration. Ventilation systems for rodent rooms incorporate various types of filtration of incoming air. Coarse filtration of the air supply is a minimal requirement for proper operation of

ventilating equipment. Most facilities maintaining rodents of defined microbiologic status also use high-efficiency particulate air (commonly called HEPA) filters to decrease the risk of introducing rodent pathogens into the animal room through the fresh-air supply (Dyment, 1976; Harstad et al., 1967). The required filter efficiency is a matter of professional judgment, and selection should be based on the perceived likelihood of introducing contaminated air into the room. Filtration of exhaust air from rodent rooms when air is not recycled is usually deemed unnecessary unless the exhaust air is likely to contain hazardous or infectious materials. Filters designed to remove chemicals from air are sometimes incorporated into exhaust systems to remove animal odors. Activated-chemical filters (e.g., those with activated charcoal) are often used for this purpose; however, their efficiency in removing odoriferous compounds, including ammonia, varies, and they require substantial maintenance to remain effective.

The use of recycled air to ventilate animal rooms can save considerable amounts of energy. However, many animal pathogens can be airborne or travel on fomites, such as dust, so recycling of exhaust air into heating, ventilating, and air-conditioning systems that serve multiple rooms presents a risk of cross contamination. Exhaust air that is to be recycled should be HEPA-filtered to remove particles. HEPA filters are available in various efficiencies; the extent and efficiency of filtration should be proportional to the risk. Toxic or odor-causing gases produced by decomposition of animal wastes can be removed by the ventilating system with chemical absorption or scrubbing, but those methods might not be completely effective. Frequent bedding changes and cage-cleaning, a reduction in number of animals housed in a room, and a decrease in environmental temperature and

humidity—within limits recommended in the *Guide* (NRC, 1985 et seq.)—can also assist in reducing the concentration of toxic or odor-causing gases. Treatment of recycled air to remove either particulate or gaseous contaminants is expensive and can be ineffective if filtration systems are improperly or insufficiently maintained. Therefore, recycling systems require regular monitoring for effective use.

An energy-recovery system that reclaims heat and thereby makes it energy-efficient to exhaust animal-room air totally to the outside is also acceptable, but these systems often require much maintenance to be effective. The recycling of air from nonanimal areas can be considered as an alternative to the recycling of animal-room air, but this air might require filtering and treatment to remove odors, toxic chemicals, and particles (White, 1982).

Relative Air Pressures

To minimize the potential for airborne cross-contamination between adjacent rodent rooms or between rodent rooms and other areas where contaminants might be generated, it is important to consider controlling relative air pressures. By adjusting the rates of air flow to and from individual areas, one can produce a negative or positive pressure relative to adjoining areas. When the intent is to contain contaminants (e.g., in areas used to quarantine newly arrived animals, isolate animals infected or suspected of being infected with rodent pathogens, house animals or materials inoculated with biohazardous substances, or keep soiled equipment), air pressure in the containment area should be lower than that in surrounding areas. When the intent is to prevent the entry of contaminants, as in areas used

to house specific-pathogen-free rodents or keep clean equipment, air pressure in the controlled area should be greater than that in surrounding areas. It is important to remember, however, that many factors influence disease transmission between adjacent rooms; simply controlling air pressure is not sufficient to prevent transmission.

Cage Ventilation

Ventilation can easily be measured in rodent-holding rooms; however, conditions monitored in a room do not necessarily reflect conditions in the cages in the room. The large sample volumes required by the commonly used instruments that measure ventilation, as well as the size of the intruments themselves, preclude accurate measurement in cages (Johnstone and Scholes, 1976). The degree to which cages are ventilated by the room air supply is affected by cage design; room air-diffuser type and location; number, size, and type of animals in the cages; presence of filter tops; and location of the cages. For example, cages without filter tops provide better air and heat exchange than those with filter tops, in which ventilation is substantially decreased (Keller et al., 1989). Rigidly maintaining room air quality and ventilation will not necessarily provide the same environment for similar groups of animals or even for similar cages in the same room. Individually ventilated cages provide better ventilation for the animals and, possibly, a more consistent environment (Lipman et al., 1992), but those systems are generally expensive.

It has not been established whether rodents are uncomfortable when exposed to air movements (drafts) or that exposure to drafts has biologic consequences. However,

movement of air in a room influences the ventilation of an animal's primary enclosure and so is an important determinant of microenvironment.

Illumination

Animal-room lighting can affect the eyes of laboratory rodents, especially albino rodents. In examining the effects, there is a tendency to think only in terms of light intensity. However, it is the interaction of the three characteristics of light (spectral distribution, photoperiod, and intensity) that produces the effects (Brainard, 1988; Gutman et al., 1989; Wurtman et al., 1985. Also contributing to the effects of light exposure is the amount of time that rodents have their eyes open during the hours when the room is lit. Those factors should be kept in mind in reading the following discussion.

Spectral Distribution

Artificial lighting with white incandescent or fluorescent fixtures is preferred for rodent housing facilities because it provides consistent illumination. The two types of lighting have similar spectra, although incandescent lighting generally has more energy in the red wavelengths and less energy in the blue and ultraviolet (UV) wavelengths than white fluorescent lighting. Although some fluorescent lighting more closely simulates the wavelength distribution of sunlight than incandescent lighting, no artificial lighting truly duplicates sunlight, and there is little reason to believe that the spectral distribution of one

type of artificial lighting is superior to that of any other for rodent rooms. There is some evidence that UV light can increase the incidence of cataract formation in humans (Zigman et al., 1979) and in rodents exposed to very high levels (Zigman and Vaughan, 1974; Zigman et al., 1973). However, there is no evidence that UV-associated cataracts develop in rodents maintained under levels of illumination normally found in animal rooms. UV radiation from fluorescent lights is eliminated when the lights are covered by plastic diffusing screens (Kaufman, 1987; Thorington, 1985).

Photoperiod

Photoperiod (cycles of light and dark during the course of a single day) affects various physiologic and metabolic characteristics, including reproductive cycles, behavioral activity, and the release of hormones into the blood (Brainard, 1989; Reiter, 1991). The rods and cones in the eye are influenced by photoperiod, requiring an interval of darkness for regeneration (LaVail, 1976; Williams et al., 1980, 1989). There is evidence that exposure to even low-intensity light—64.6-193.7 lx (6-18 ft-candles)—continuously for 4 days can cause degenerative retinal changes (Anderson et al., 1972; O'Steen, 1970; Williams, 1989).

Photoperiods in rodent rooms are usually controlled by automatic timers. The cycles usually recommended are either 12 hours of light and 12 hours of dark or 14 hours of light and 10 hours of dark. For some mammals (e.g., hamsters), a longer period of light is important for normal reproduction (Reiter, 1989). In general, lighting in laboratory animal facilities does not reproduce that in nature, in that most light-timing devices do not provide

any interval of reduced lighting intensity (simulating dawn and dusk). Changes or interruptions in light-dark cycles should be avoided because of the importance of photoperiod in normal rodent reproduction and other light-affected physiologic processes (Weihe, 1976b). Similarly, light from exterior windows and uncontrolled hallway lighting are usually undesirable.

Light-timing devices in rodent facilities should be checked regularly for correct operation. Any system that can be overridden manually should be equipped with an indicator, such as a light, to remind personnel to turn off the override device or with a timer to turn it off automatically. Photoperiod can also be checked by photosensors linked to a computer-based monitor.

Intensity

The intensity of illumination is inversely proportional to the square of the distance from the source. Therefore, statements about intensity should indicate where it was measured. In animal facilities, such statements generally specify distance above the floor; that implies that the illumination is uniformly diffused throughout the room. The actual intensity experienced by a rodent in an animal room is influenced not only by the relative locations of its cage and the room lights, but also by cage material and design.

The optimal light intensity required to maintain normal physiology and good health of laboratory rodents is not known. In the past, illumination of 807-1076 lx (75-100 ft-candles) or higher has been recommended to allow adequate observation of the animals and safe

husbandry practices (NRC, 1978). The point of measurement for that recommendation was never clearly stated, but it has been generally assumed that the recommendation referred to the illumination at maximal cage height in the center of the room. The recommended intensities, however, have been shown to cause retinal damage in albino mice (Greenman et al., 1982) and rats (Lai et al., 1978; Stotzer et al., 1970; Williams et al., 1980).

More recently, a light intensity of 323 lx (30 ft-candles) measured about 1.0 m (3.3 ft) above the floor has been recommended as adequate for routine animal care (Bellhorn, 1980; NRC, 1985 et seq.). That intensity has been calculated to provide 32-40 lx (3.0-3.7 ft-candles) to rodents in the front of a cage that is in the upper portion of a cage rack. Exposure for up to 90 days to an intensity of around 300 lx during the light cycle has been reported not to cause retinal lesions in rats (Stotzer et al., 1970); however, it is still questionable whether exposure to light of even this intensity can cause retinal lesions in albino animals if they are exposed for longer periods (Weisse et al., 1974).

Alternatives to providing a single light intensity in a room are to use variable- intensity controls and to divide rooms into zones, each lighted by a separate switching mechanism. Those alternatives conserve energy and provide sufficient illumination for personnel to perform their tasks adequately and safely. However, caution is necessary when instituting those alternatives. Boosting daytime room illumination for maintenance purposes has been shown to change photoreceptor physiology and can alter circadian regulation (Remé et al., 1991; Society for Research on Biological Rhythms, 1993; Terman et al., 1991).

Noise

Many sounds of varied frequencies and intensities are generated in animal facilities during normal operation. Rodents emit ultrasonic vocalizations that are an important part of their social and sexual behavior. Rats can hear frequencies as high as about 60-80 kHz but are relatively insensitive to frequencies less than 500 Hz (Kelly and Masterton, 1977; Peterson, 1980). Sounds are also produced by mechanical equipment (less than 500 Hz): by dog, cat, nonhuman primate, and pig vocalizations (up to 120 dB at 500 Hz); and by exterior conditions (e.g., highway noise).

If acoustic energy is high enough (80-100 dB), both auditory and nonauditory changes can be detected in laboratory animals (Algers and Ekesbo, 1978; Moller, 1978). The type of change produced depends on the pattern of sound presentation. Sound of uniform frequency and unchanging intensity can cause hearing loss in some rodents (Bock and Saunders, 1977; Burdick et al., 1978; Kelly and Masterton, 1977; Kraak and Hofmann, 1977). Hamsters, guinea pigs, rats, and mice pass through developmental stages during which they are very susceptible to injury from sound of this type (Kelly and Masterton, 1977). Sound of irregular frequency and rapidly changing intensity that is presented to animals in an unpredictable fashion can cause stress-induced mechanical and metabolic changes (Anthony and Harclerode, 1959; Geber, 1973; Guha et al., 1976; Kimmel et al., 1976; Peterson et al., 1981). Continuous exposure to acoustic energy above 85 dB can cause eosinopenia (Geber et al., 1966; Nayfield and Besch, 1981), increased adrenal weights (Geber et al., 1966; Nayfield and Besch, 1981), and reduced fertility (Zondek and Tamari, 1964).

Few studies are available on the long-term effects on rodents of sound comparable with that normally encountered in rodent rooms, and there are hardly any data on the sensitivity

of rodents to intensity as a function of frequency (Peterson, 1980). In addition, no comparative damage-risk criteria have been established for rodents; therefore, recommendations for acceptable noise in animal facilities are often based on extrapolations from humans (Peterson, 1980). As a general guideline, an effort should be made to separate rodent-housing areas from human-use areas, especially human-use areas where mechanical equipment is used or where noisy operations are conducted. Common soundproofing materials are not compatible with some of the construction requirements for animal facilities designed to house rodents, but attention can be given to separating rooms housing rodents from those housing noisy species, such as nonhuman primates, dogs, cats, and swine. The location of loud, unpredictable sources of noise—such as intercoms, paging systems, telephones, radios, and alarms—should be carefully considered because the noise from such sources can be stressful to some rodents. Extra care should be taken to control noise in rooms that house rodents that are subject to audiogenic seizures. Every reasonable effort should be made to house rodents in areas away from environmental sources of noise.

FOOD

Nutrition has a major influence on the growth, reproduction, health, and longevity of laboratory rodents, including their ability to resist pathogens and other environmental stresses and their susceptibility to neoplastic and nonneoplastic lesions (BARR, 1978). Providing nutritionally adequate diets is important not only for the rodents' welfare, but also to ensure that experimental results are not biased by unintended or unknown nutritional factors.

Providing nutritionally adequate diets for laboratory rodents involves establishing requirements for about 50 essential dietary nutrients, formulating and manufacturing diets with the required nutrient concentrations, and managing numerous factors related to diet quality. Factors that potentially affect diet quality include bioavailability of nutrients, palatability or acceptance by the animals, preparation and storage procedures, and concentrations of chemical contaminants. The estimated nutrient requirements of laboratory animal species are periodically reviewed and updated by a committee of the National Research Council (NRC, 1995), and information about the formulation, manufacture, and management of laboratory animal diets is available elsewhere (Coates, 1987; Knapka, 1983, 1985; McEllhiney, 1985; Navia, 1977; Rao and Knapka, 1987).

Types of Diets

Adequate nutrition can be provided for laboratory rodents in different types of diets that are classified by the degree of refinement of the ingredients used in their formulation (NRC, 1995).

Natural-ingredient diets are formulated with agricultural products and byproducts, such as whole grains (e.g., ground corn and ground wheat), mill byproducts (e.g., wheat bran, wheat middlings, and corn gluten meal), high-protein meals (e.g., soybean meal and fish meal), processed mineral sources (e.g., bone meal), and other livestock feed ingredients (e.g., dried molasses and alfalfa meal). Commercial diets are the most commonly used natural-ingredient diets, but special diets for specific research programs can also be of this

type if appropriate attention is given to ingredient selection and diet formulation. Natural-ingredient diets are relatively inexpensive to manufacture and are readily consumed by laboratory rodents.

A natural-ingredient diet can be either an open-formula diet (information on the amount of each ingredient in the diet is readily available) or a closed-formula diet (information on the amount of each ingredient is privileged). The advantages of using natural-ingredient, open-formula diets in biomedical research have been discussed (Knapka et al., 1974).

There are two concerns about the use of natural-ingredient diets in biomedical research. First, such factors as varieties of plants, soil compositions, weather conditions, harvesting and storage procedures, and manufacturing and milling methods influence the nutrient composition of ingredients used in this type of diet to the extent that no two production batches of the same diet are identical (Knapka, 1983). This variation in dietary-nutrient concentrations introduces an uncontrolled variable that could affect experimental results. Second, natural ingredients can be exposed to various naturally occurring or human-made contaminants, such as pesticide residues, heavy metals, and estrogen. Diets manufactured from natural ingredients can contain low concentrations of contaminants that might have no influence on animal health but could affect experimental results. For example, a lead concentration of 0.5-1 part per million is inherent in natural-ingredient rodent diets and is not generally detrimental to animal health; but it could substantially influence the results of toxicology studies designed to evaluate lead-containing test compounds.

Purified diets are formulated with ingredients that have been refined so that in effect each ingredient contains a single nutrient or nutrient class. Examples of the ingredients are

casein or soy protein isolate, which provide protein and amino acids; sugar and starch, which provide carbohydrates; vegetable oil and lard, which provide essential fatty acids and fat; a chemically extracted form of cellulose, which provides fiber; and chemically pure inorganic salts and vitamins. The nutrient concentrations in this type of diet are less variable and more readily controlled than those in natural-ingredient diets. However, purified ingredients can contain low and variable concentrations of trace minerals, and batch-to-batch variation in their concentrations is inherent in the manufacture of purified diets. The potential for chemical contamination of purified diets is low; however, they are not always readily consumed by laboratory rodents, and they are more expensive to produce than natural-ingredient diets.

Chemically defined diets are formulated with the most elemental ingredients available, such as individual amino acids, specific sugars, chemically defined triglycerides, essential fatty acids, inorganic salts, and pure vitamins. Use of this type of diet provides the highest degree of control over dietary nutrient concentrations. However, chemically defined diets are not readily consumed by laboratory rodents, and they are usually too expensive for general use.

The dietary nutrient concentrations in chemically defined diets are theoretically fixed at the time of manufacture; however, the bioavailability of nutrients can be altered by oxidation or nutrient interactions during diet storage. Liquid chemically defined diets that can be sterilized by filtration have been developed (Pleasants, 1984; Pleasants et al., 1986).

Criteria for Selecting Optimal Rations

Selection of the most appropriate type of diet for a particular animal colony depends on the reproductive or experimental objectives. One of the most important considerations is the amount of control over dietary-nutrient composition that is necessary to attain the objectives. For example, the use of a purified diet is essential for studies designed to establish quantitative requirements for micronutrients because the batch-to-batch variation in nutrient concentrations inherent in natural-ingredient diets would compromise experimental results. Conversely, the variation in nutrient concentrations in natural-ingredient diets would have no detectable influence on rodent production colonies because the nutrient concentrations are generally greater than those required in a nutritionally adequate diet. The use of chemically defined diets might be required for studies whose objectives involve dietary concentrations of single amino or fatty acids.

The potential for chemical contamination is an important consideration in selecting a diet for rodents that will be used in toxicology studies. Even though the concentrations of chemical contaminants in natural-ingredient diets are so low that they generally do not jeopardize animal health, they might be high enough to compromise results of toxicology studies. The results of some immunology studies might also be influenced by the use of natural-ingredient diets because some ingredients, particularly those of animal origin, contain antigens. Purified diets should be considered for animals used in both kinds of studies, although their cost can increase the cost of conducting the research, especially in life-span studies that use large numbers of rodents.

Any diet selected should be accepted by the animals, otherwise considerable amounts will be wasted. This is expensive and constitutes a major disadvantage in studies that require

quantification of dietary consumption. Diets should be nutritionally balanced and free of toxic or infectious agents. If diet is a factor in a study, the diet selected should be readily reproducible to ensure that research results can be verified by replication.

Quality Assurance

Although reputable laboratory animal feed manufacturers develop elaborate programs to ensure the production of high-quality products, additional procedures are often required to ensure that the diets are nutritionally adequate. The shelf life of any particular feed lot depends on the environmental conditions during storage. Nutrient stability of animal feeds generally increases as temperature and humidity in the storage environment decrease. Natural-ingredient rodent diets stored in air-conditioned areas in which the temperature is maintained below 21°C (70°F) and the humidity below 60 percent should be used within 180 days of manufacture. Vitamin C in diets stored under these conditions has a shelflife of only 90 days. If a vitamin C-containing diet stored for more than 90 days is to be fed to guinea pigs, an appropriate vitamin supplement should be added. To monitor compliance with these guidelines, storage containers should be marked with the date of manufacture of the food stored therein.

Diets stored for longer periods or under conditions other than those recommended above should be assayed for the most labile nutrients (i.e., vitamin A, thiamine, and vitamin C)

before use. Diets formulated without antioxidants or with large amounts of highly perishable ingredients, such as fat, might require special handling or storage procedures.

Given the potential importance of diet quality and consistency to experimental results, a routine program of nutrient testing should be implemented to verify the composition of diets fed to research animals. Accidental omission or inclusion of ingredients in the manufacturing process, although uncommon, can have disastrous consequences on research projects. Discrepancies between expected and actual nutrient concentrations in laboratory animal diets can arise from errors in formulation, which can result in hazardous concentrations of nutrients that are toxic when present in excess of requirements (e.g., vitamins A and D, copper, and selenium); losses of labile nutrients during manufacture or storage; variation in nutrient content of ingredients used in diet formulation; and errors associated with diet sampling or analysis. Although most laboratory animal feed manufacturers will provide data on the complete nutrient composition of rodent diets, it is often difficult to ascertain the source of these data (i.e., whether they are calculated, representative of several diet production batches, or representative of a single production batch). Therefore, it is suggested that feed manufacturers routinely be asked to provide the results of nutrient assays of representative samples of their diets.

Testing samples of natural-ingredient diets used in research colonies is particularly important because the nutrient concentrations measured by analysis can differ from the expected concentrations. Samples for assay should be collected from multiple bags or containers within a single production batch of feed (i.e., in which all containers bear the same manufacture date). The containers sampled should be selected at random; traditionally,

the number sampled equals the square root of the total number of containers in a single shipment or production batch. The objective is to obtain a sample of diet that is representative of the entire lot being assayed. Nutrient analyses should be conducted by a laboratory with an established reputation in assaying feed samples, and all assays should be conducted in accordance with the most recent methods published by the Association of Official Analytical Chemists (Helrich, 1990). Analyses should include at least proximate constituents (i.e., moisture, crude protein, ether extract, ash, and crude fiber) and any nutrients that are under study or that could influence the study. Some vitamins and other nutrients required at trace concentrations might be difficult to assay because of low concentrations, interfering compounds, or both.

The presence of biologic contaminants in diets is a cause for concern in most research and production rodent colonies. Unwanted agents in the diet include pathogenic bacteria and viruses, insects, and mites. Diets for axenic and microbiologically associated rodents should be sterilized before use, as should those for severely immunodeficient rodents (i.e., athymic rodents and mice homozygous for the mutation *scid*) (NRC, 1989). Diets for specific-pathogen-free (SPF) rodents should be subjected to some degree of decontamination, such as pasteurization. It is also prudent to decontaminate diets, at least partially, for conventionally maintained rodents, particularly when they are used in long-term studies. Steam autoclaving is the most widely used method for eliminating biologic contaminants from diets (Coates, 1987; Foster et al., 1964; Williams et al., 1968). However, this process can decrease the concentrations of heat-labile nutrients (Zimmerman and Wostmann, 1963). To ensure that adequate amounts of the most heat-labile vitamins (e.g., vitamins A and C and some of the B

complex) will remain after autoclaving, consideration should be given to purchasing autoclavable diets that have been fortified with those vitamins. The magnitude of fortification in autoclavable diets is not generally high enough to be toxic to rodents; however, the routine use of autoclavable diets without autoclaving is not recommended, because the increased vitamin concentrations could influence experimental results.

The level of sterility required for axenic or microbiologically associated rodents requires that the temperature of the diet be raised above 100°C (212°F). To ensure that all the diet in the autoclave attains this temperature, it is recommended that the diet be exposed to a temperature of 121°C (250°F) for 15-20 minutes. Diets should not be subjected to the maximal autoclaving temperature longer than necessary to achieve sterilization (Coates, 1987).

To ensure proper operation of the autoclave, sterility of the diet, and adequate concentrations of labile nutrients, validation procedures are required, including periodic evaluation of autoclave operation by qualified personnel, use of commercially available heat indicators, culture of autoclaved feed samples for biologic contaminants, and assay of autoclaved feed samples to verify nutritional adequacy. Clarke et al. (1977) have described procedures for sampling and assaying feeds for various pathogenic organisms and provided standards for the number and kinds of organisms that are acceptable in diets.

Autoclaving at 80°C (176°F) for 5-10 min is required for pasteurization of diets. At that temperature, vegetative forms, but not spores, of microorganisms are destroyed (Coates, 1987). Pasteurized diets are generally acceptable for use in both specific-pathogen-free and

conventional rodent colonies. Pasteurization, rather than sterilization, is used because there is less nutrient loss, and the diets are more readily consumed than are sterilized diets.

Laboratory rodent diets also can be decontaminated by ionizing radiation (Coates, 1987; Coates et al., 1969; Ley et al., 1969), and diets sterilized in this way are now commercially available. Ethylene oxide fumigation has also been used to decontaminate diets (Meier and Hoag, 1966).

All animal diets, particularly those produced from natural ingredients, can contain or become contaminated with various manufactured or naturally occurring chemicals, including pesticide residues, bacterial or plant toxins, mycotoxins, nitrates, nitrites, nitrosamines, and heavy metals (Fox et al., 1976; Newberne, 1975; Yang et al., 1976). Procedures, if any, for detecting these chemicals are often difficult and expensive. Testing for contaminant concentrations in natural-ingredient diets should be routine in toxicologic research and might be valuable in some other studies.

On the basis of observed contaminant concentrations and potential toxic effects, Rao and Knapka (1987) developed a list of recommended limits for about 40 chemical contaminants. The authors also proposed a scoring system for diets used in chemical toxicology studies that permits separation of tested diets into those acceptable for long-term use, those acceptable only for short-term or transitory use, and those which should be rejected.

Laboratory animal diets designated as "certified" are commercially available. Although the term is subject to different interpretations, in most cases the certification guarantees that the concentration of each contaminant on a specific list will not exceed the indicated maximum. Because the maximal concentrations usually are established by the diet

manufacturer, the use of certified diets might not be appropriate for studies in which the acceptable concentrations of contaminants could influence experimental data independently or through an additive effect. In addition, a diet might have contaminants that are not included in the certification but are of concern in specific research projects.

Caloric Restriction

Traditionally, the criterion used to evaluate laboratory rodent diets for nutritional adequacy has been maximal growth or reproduction of the animals consuming the diet. Laboratory rodents generally are given ad libitum access to such diets throughout their lives. However, during the past 60 years, many studies have shown beneficial effects of caloric restriction in various species, including laboratory rodents (Bucci, 1992; Snyder, 1989; Weindruch and Walford, 1988; Yu, 1990). It has been reported that caloric restriction increases life expectancy and life span, decreases the incidence and severity of degenerative diseases, and delays the onset of various neoplasias.

The objective of caloric restriction is to reduce calories without malnourishing the animals. That objective is generally accomplished by supplementing a diet with micronutrients and then limiting dietary consumption to 60-80 percent of the dietary consumption of animals that are fed ad libitum; this procedure results in decreased total caloric consumption. Although studies have been conducted in which the total fat (Iwasaki, et al., 1988), protein (Davis et al., 1983; Goodrick, 1978), or carbohydrate (Kubo et al., 1984; Yu et al., 1985) consumption has been limited individually, only reduction in caloric

intake results in the full range of dietary-restriction-related beneficial effects (Turturro et al., 1993). Hypotheses explaining the results of dietary restriction studies have been reviewed and discussed (Keenan et al., 1994).

Numerous questions still need to be addressed to determine by what mechanisms dietary or caloric restriction influences various life processes, and the quantitative nutrient or energy requirements necessary to achieve the effects associated with dietary restriction have not been established. However, the reported data show that ad libitum feeding might not be universally desirable for rodents used in long-term toxicologic or aging studies, and this factor should be a prime consideration when designing such studies.

WATER

Laboratory rodents should have ad libitum access to fresh, potable, uncontaminated drinking water, which can be provided by using water bottles and drinking tubes or an automatic watering system. Occasionally, it is necessary to train animals to use automatic watering devices. If water bottles are used, it is better to replace than to refill them; however, if they are refilled, each bottle should be returned to the cage of origin to minimize potential cross-contamination with microbial agents. If automatic watering devices are used, they should be examined routinely to ensure proper operation. The drinking nozzles on these devices should be sanitized regularly, and the pipe distribution system should be flushed or disinfected routinely.

Water is a potential source of microbial or chemical contaminants. Although a water source might be in compliance with standards that ensure purity of water supplied for human consumption, additional treatment might be required to ensure that water constituents do not compromise animal-colony objectives. Treatments used to limit or eliminate bacteria in water intended for laboratory rodents maintained in axenic or SPF environments include distillation, sterilization by autoclaving, hyperacidification, reverse osmosis, ultraviolet treatment, ultrafiltration, ozonation, halogenation, and irradiation (Bank et al., 1990; Engelbrecht et al., 1980; Fidler, 1977; Green and Stumpf, 19946; Hall et al., 1980; Hann, 1965; Hermann et al., 1982; Kool and Hrubec, 1986; Newell, 1980; Tobin, 1987; Tobin et al., 1981; Wegan, 1982). The advantages, disadvantages, and potential effects of water treatment on an animal's physiologic response to experimental treatments should be evaluated before a method of water decontamination is initiated. In general, any treatment that decreases water consumption is potentially detrimental to the animals' health and welfare.

Drinking water of animals used in toxicology experiments, particularly those of long duration, should be periodically assayed for compounds that might influence experimental results, even when exposures are small. Mineral concentrations in water can have a profound influence on experimental results in studies designed to establish dietary mineral requirements for laboratory rodents. Distilled or deionized drinking water should be provided to rodents used in studies in which the amounts of minerals consumed are critical.

BEDDING

Bedding materials are used to absorb spilled water, minimize urinary and fecal soiling of the animals, and assist in decreasing the generation of odors and gaseous contaminants caused by bacterial decomposition of urine and feces. Bedding material can be used either as contact bedding in solid-bottom cages or as noncontact bedding in waste-collection pans placed beneath wire-bottom cages. Contact bedding provides thermal insulation for the animals and is often used as nesting material in breeding colonies. Abrasive or toxic materials should not be used as contact bedding.

Most products used for bedding in rodent colonies are byproducts of various industries. During the manufacturing process, these byproducts are occasionally subjected to conditions that are conducive to microbial contamination. Many of the commercially available rodent bedding materials are subjected to heat treatment before packaging; however, microbiologic recontamination can occur during shipment from the manufacturing plant to the animal facility. For maximal protection from potential microbiologic contamination, contact and noncontact bedding products should be sterilized before use.

Hardwood and softwood are the most commonly used rodent bedding materials. Wood products should be screened to eliminate splinters or slivers and should be free of foreign materials, such as paint, wood preservatives, chemicals, heavy metals, and pesticides. Some manufacturers will provide an assurance that the bedding is free of specified contaminants. The moisture content of wood products should be high enough to prevent excessive dust but low enough to provide adequate absorbency. Cedar-wood products are often mixed with

other bedding material to mask animal-room odors; however, their use is not recommended because the aromatic hydrocarbons inherent in these products can alter hepatic microsomal enzyme activity and potentially influence experimental results (Cunliffe-Beamer et al., 1981; Ferguson 1966; Porter and Lane-Petter, 1965; Vesell, 1967; Vesell et al., 1976). Furthermore, masking animal-room odors with cedar products is not a substitute for good sanitation practices.

Plant byproducts and other cellulose-containing materials (including ground corncobs) are readily available as bedding for laboratory rodents. Laminated-paper products are available for use in waste-collection pans, and shredded-paper products are marketed for use as contact bedding for rodents. Corncob and paper products treated with germicides or antibiotics to control bacterial growth are also available. However, the routine use of antibiotic-treated bedding materials might cause antibiotic-resistant strains of bacteria to develop or influence experimental results.

Bedding products manufactured specifically for use as rodent nesting materials are available. The use of such products, which might enhance neonatal survival in inbred rodent strains with inherently low reproduction rates, should be considered.

All rodent bedding products should be packaged in sealed, nonporous bags. Bags of bedding material should be stored in vermin-proof areas on pallets that do not touch the walls. When the bedding material is removed from the bags, it should be stored in metal or plastic containers that can be closed securely. The storage containers should be sanitized routinely.

SANITATION

Cleaning

Adequate sanitation is an integral part of maintaining laboratory rodents. Clean, sanitary conditions limit the presence of adventitious and opportunistic microorganisms, thereby decreasing their potential for compromising rodent health or causing adverse interactions with experimental procedures. Complete sterilization of the rodents' environment is seldom practical or necessary unless animals of highly defined microbiologic status or compromised immune status are used.

All components of the animal facility should undergo regular and thorough cleaning, including animal rooms, support areas (e.g., storage areas), cage-washing facilities, corridors, and procedure rooms. They should be cleaned with detergents and, when appropriate, disinfectant solutions to rid them of accumulated dirt and debris. Many such products are available. Selection of a cleaning agent should be based on how much and what kind of material is adhering to surfaces, as well as on the type of microbiologic contamination present (Block, 1991).

Monitoring of sanitation procedures should be appropriate to the process and materials used and might include visual inspection, monitoring of water temperatures, and microbiologic monitoring. It has been suggested that the effectiveness of sanitation procedures can be assessed by the intensity of animal odors, particularly ammonia; however, this should not be the sole means of assessing cleanliness, because too many variables are involved. Agents used to mask animal odors should not be used in rodent housing facilities;

these agents cannot substitute for good sanitation practices, and their use exposes animals to volatile substances that can alter basic physiologic and metabolic processes.

The frequency with which surfaces are cleaned should be determined by how much use an area receives and the nature of potential contamination. Sweeping, mopping, and scrubbing with disinfectant agents should take place in a logical sequence. Cleaning utensils should be constructed of materials that resist corrosion and do not absorb dirt or debris. They should be stored in a neat, organized fashion. Wall-mounted hangers are useful for storing cleaning utensils because they reduce clutter, facilitate drying, and minimize contamination by keeping utensils off the floor. Cleaning utensils should be assigned to specific areas and should not be transported between areas. They should be regularly cleaned and dried, and there should be a regular schedule for replacing worn-out utensils.

Soiled bedding material should be removed and replaced with clean, dry bedding as often as is necessary to keep the animals clean and dry. The frequency is a matter of professional judgment and should be based on various factors, including the number and size of the animals housed in each cage, the anticipated urinary and fecal output, and the presence of debilitating conditions that might limit an animal's ability to access clean areas of the cage.

Bedding should be changed in a manner that reduces exposure of the animals and personnel to aerosolized waste materials. Laminar-flow bedding dump stations or similar devices can be used to control aerosol materials. If animals have been exposed to hazardous materials that are excreted in the urine or feces, additional precautions might be needed to prevent exposure of personnel while they are changing the bedding.

Frequent bedding changes can sometimes be counterproductive, for example, during portions of the postpartum period, changing the bedding removes pheromones, which are essential for successful reproduction (e.g., pheromones are necessary for synchronization of ovulation). Research objectives might also preclude frequent bedding changes. Under such circumstances, an exception to the regular bedding-change and cage-cleaning schedule can be justified.

Cages, cage racks, and accessory equipment, such as feeders and watering devices, should be cleaned and sanitized regularly to minimize the buildup of debris and to keep them free from contamination. Extra caging makes it easier to maintain a systematic schedule. Cleaning frequency will depend on the amount of bedding used, the frequency of bedding changes, the number of animals per cage, and other factors. In general, rodent cages and cage accessories will need to be washed at least once every 2 weeks. Solid-bottom rodent cages, water bottles, and sipper tubes usually require weekly cleaning. Some types of cage racking, large cages with very low animal density and frequent bedding changes, cages housing animals in gnotobiotic conditions, and cages used under other special circumstances might require less frequent cage-cleaning. Filter-top cages without forced-air ventilation and cages containing rodents with increased rates of production of feces or urine might require more frequent cleaning.

Cage-cleaning, debris removal, and disinfection can be accomplished in a single step or in multiple steps. Cage-cleaning and debris removal usually require the application of a detergent or surfactant solution coupled with mechanical action to remove adherent material from cage surfaces. Some laboratory rodents, such as guinea pigs and hamsters, produce

urine with high concentrations of proteins and minerals. Their urine often binds aggressively to cage surfaces, which therefore require treatment with acid solutions before washing. Some detergents are rendered inactive at high temperatures, so, it is important to follow the manufacturer's instructions carefully.

Disinfection of cages is the process of killing vegetative forms of pathogenic bacteria. It can be accomplished by the action of either chemicals or hot water. If chemicals are used as the sole means of disinfection, careful attention should be paid to the concentration of the disinfectant solution's active ingredients, and the solution should be regularly changed in accordance with the manufacturer's instructions. When hot water is used either alone or in combination with disinfectant chemicals, temperatures and exposure times should be appropriate for adequate disinfection. Generally, the water temperature required for adequate disinfection precludes its use in anything but mechanical cage-washing equipment.

Cleaning and disinfection of cages can be done efficiently in mechanical cage washers. Washing times and conditions should be sufficient to kill vegetative forms of common bacteria and other microorganisms that are presumed to be controllable by sanitization. Microorganisms are killed by a combination of heat and the length of exposure to that heat (called the cumulative heat factor). Using high temperatures for short periods will produce the same cumulative heat factor and have the same effect on microorganisms as using lower temperatures for longer periods (Wardrip et al., 1994). To achieve effective disinfection, water temperatures for washing and rinsing can vary from 58°C (143°F) to 82°C (180°F) or more. Recommendations for some types of mechanical cage washers using hot water alone for disinfection have been developed by the National Sanitation Foundation International

(1990). Detergents and chemical disinfectants are known to enhance the effectiveness of hot water but must be thoroughly rinsed from surfaces to avoid harm to personnel and animals.

Cages and equipment can be effectively washed and disinfected by hand if appropriate attention is given to detail. Chemicals should be completely rinsed from surfaces, and personnel should have appropriate equipment to protect them from prolonged exposure.

Large pieces of caging equipment, such as racks, can be washed by hand; if large numbers are to be cleaned, portable cleaning equipment that dispenses detergent and hot water or steam under pressure might be more efficient. Large mechanical washing machines designed to accommodate racks and other pieces of large equipment are also commercially available.

Water bottles, sipper tubes, stoppers, and other small pieces of equipment should be washed with detergents, hot water, and, if appropriate, chemical agents to destroy vegetative forms of microorganisms. This process can be manual, if high-temperature rinse water is not used, or performed with mechanical washing equipment built especially for this purpose or a multiple-purpose cage-washing machine. Water bottles and sipper tubes can also be autoclaved after routine washing to ensure adequate sanitation.

If large numbers of water bottles or other small pieces of equipment are to be washed by hand, powered rotating brushes can be used to ensure adequate cleaning. Small items should be dipped or soaked in detergent and disinfectant solutions to maximize contact time. Therefore, large, two-compartment sinks are generally required if small items are to be hand washed.

If automatic watering systems are used, they should incorporate some mechanism to ensure that bacteria and debris do not build up in the watering devices. These systems are usually periodically flushed with large volumes of water or appropriate chemical agents and then rinsed to remove chemicals and associated debris. Constant-recirculation loops that use filters, ultraviolet light, or other treatment procedures to sterilize recirculated water can also be used.

Common methods of disinfection and sanitization are adequate for most rodent holding facilities. However, if pathogenic microorganisms are present or if rodents with highly defined microbiologic flora or compromised immune systems are maintained, it might be necessary to sterilize caging and other associated equipment after cleaning and disinfection. In such instances, access to an autoclave, gas sterilizer, or device capable of sterilizing with ionizing radiation is required. Whenever such sterilization processes are used, some form of regular monitoring is required to ensure their effectiveness.

Waste Containment and Disposal

Proper sanitation of an animal facility requires adequate containment, as well as regular and frequent removal of waste. Waste containers should be constructed of either metal or plastic materials and should be leakproof. They should be equipped with tight-fitting lids and, where appropriate, provided with disposable plastic liners for ease of waste removal. They should also be adequately labeled to distinguish between containers for hazardous and nonhazardous wastes; a color-coding system often proves useful.

If hazardous biologic waste is generated, an inventory sheet might be necessary for each waste container, so that the type of waste and the approximate quantity of hazardous material can be recorded. Waste containers for animal tissues or carcasses should be lined with leakproof, disposable liners that will withstand being refrigerated or frozen to reduce tissue decomposition. If wastes are collected and stored before removal from the site, the storage area should be physically separated from other facilities used to house animals or store animal-related materials. Waste-storage areas should be cleaned regularly and kept free of insects and other vermin. All waste containers and associated implements should be cleaned and disinfected frequently.

Waste materials from rodent housing facilities can be disposed of in various ways (depending on the type of waste), including incineration, agricultural composting, and landfill disposal. Hazardous waste must be separated from other waste, and its classification and handling are controlled by a variety of local, state, and federal agencies. Some form of pretreatment—such as autoclaving, chemical neutralization, or compaction with absorbents—might be required. The National Safety Council (1979) has recommended procedures for disposal of hazardous waste. It is the institution's responsibility to comply with all federal, state, and municipal statutes and ordinances regarding the control, movement, and disposal of hazardous waste.

Pest Control

All rodent housing facilities should have a program to prevent, control, or eliminate infestation by pests (including insects and wild and escaped rodents). The program should include regular inspection of the premises for signs of pests, a monitoring system that uses rodent traps and insect-collection devices to capture pests, and regular evaluation of the integrity and condition of the animal facilities. The pest-control program should focus on preventing the entry of vermin into the facility (by sealing potential points of entry and eliminating sites outside the facility where vermin can breed or be harbored) and maintaining an environment in which pests cannot sustain themselves and reproduce. Only if those methods are ineffective should the use of pesticides be considered.

If pesticides are required, relatively nontoxic substances (e.g., boric acid, amorphous silica gel, and insect-growth regulating hormones) and mechanical devices (e.g., adhesive traps, air curtains, and insect-electrocution devices) should be used in preference to toxic materials, especially for controlling insect pests. If a toxic compound is to be used in animal areas, it should be used only after consultation with the investigators whose animals are housed in the facility because of potential effects on the animals' health and possible interference with research results. The application of toxic pesticides should be coordinated with those responsible for the management of the animal-care program and carried out by licensed applicators in compliance with local, state, and federal regulations.

The pest-control program should be adequately documented, including records of dates and methods of application of pesticides and possibly records of inspection, results of monitoring and trapping programs, records of sightings and identification of pests, and maintenance schedules.

IDENTIFICATION AND RECORDS

Adequate individual or group identification of rodents and appropriate records of their care and use are essential to the conduct of biomedical research programs. Individual identification of rodents is not always required; when necessary, it can be accomplished in various ways, including ear-punching, use of ear tags, tattooing (usually on the tail), or implanting electromagnetic transponders. If ear tags are used, they should be light enough so that they do not visibly change the animal's head posture, and surrounding tissues should be monitored for inflammation. Dyes are occasionally used on the fur, skin, or tail for temporary identification. In general, amputation of digits (toe-clipping) is no longer an acceptable method of identification, because more humane methods can usually be substituted.

Individual animals or groups of animals can also be identified with cage identification cards. If cards are used, sufficient information is required to identify and characterize the animals in the cage adequately. This information can include such details as the name and location (e.g., office location, telephone number, and division or department name) of the responsible investigator; the species, strain, or stock of the animals; the sex of the animals; the number of animals in the cage; the source of the animals; institutional identification numbers (e.g., IACUC-approved protocol number and purchase-order number); and, when appropriate, other identifying information pertaining to the project (e.g., group designation and age or weight specifications). Bar-code identifiers can also be included on the cage card to aid in identifying the animals and linking their identification with other, more detailed

records. Color-coding the cage cards and labeling cage racks and animal holding rooms are effective management tools for locating and identifying animals.

Some research protocols require that records be kept on individual animals, for example, when animals are used in breeding programs or are exposed to hazardous agents. Detailed surgical records are not commonly maintained on individual rodents but might be helpful in some situations such as when complex surgical procedures are being used or when new procedures are being developed.

RODENTS OTHER THAN RATS AND MICE

Guinea Pigs

One of the most striking ways in which guinea pigs (*Cavia porcellus*) differ from rats and mice is the guinea pigs' absolute requirement for exogenous vitamin C, a requirement that is shared with humans and only a few other species. Because of that requirement, guinea pig diets must be fortified with vitamin C. As an alternative, vitamin C can be added to the drinking water or provided in the form of food supplements, including such vegetables as kale, that are high in vitamin C. The use of food supplements should be approached with some caution because of the possibility of contamination with chemicals or microorganisms that could influence the course of experimentation. Vitamin C is a very labile compound, so

storage conditions of foods containing it and heat treatment of such foods, including autoclaving, are of particular concern.

The guinea pigs' body conformation makes design and placement of feeders important. Feeders should be designed to avoid trauma to the chin and neck area of guinea pigs. Guinea pigs will occasionally rear up on their hind legs, but they will not accept food from feeders suspended overhead. Bowls for food and water can be used instead of more conventional feeding and watering devices; but guinea pigs like to nest in such receptacles, and that causes waste and contamination of food. Feeders that have a J shape are best suited to address these concerns and are used most commonly.

Guinea pigs, like other rodents, tend to eat and drink throughout the day and night. They become habituated to a particular diet and have defined taste preferences. Any changes in the composition of the food—especially changes in size, shape, consistency, or taste—can cause a sharp decline in food consumption. If the animals fail to adapt to the new food, severe weight loss or even starvation and death can occur; therefore, new food should be introduced gradually.

Guinea pigs often grow to weigh more than 1 kg and have relatively small feet. They have a well-developed startle response that causes them to make sudden movements in response to unfamiliar sounds; when they are housed in groups, this might be manifested as a stampede. Those two traits make cage-floor design particularly important. Wire-bottom cages should be designed to provide sufficient support for the animals' feet to prevent pressure sores, and the space between the wires in the floor grid should be small enough to preclude entrapment of animals' feet.

Guinea pigs also differ substantially from rats and mice in having a vaginal closure membrane and a long gestation period. Gestation in guinea pigs can range from 59 to 72 days; 63 to 68 days is the average. Gestation length can be affected by several characteristics, including litter size, which is usually one to three pups (McKeown and Macmahon, 1956). Female and male guinea pigs reach puberty as early as 4-5 weeks old and 8-10 weeks old, respectively, but are best mated when 2.5-3 months old or when they weigh 450-600 g (Ediger, 1976). Because a relatively large fetal mass is expelled at parturition, a female should be bred before she is 6 months old to minimize the likelihood of being excessively fat or having firm fusion of the symphysis pubis. If the symphysis pubis is fused, it cannot separate the approximate 0.5 in. needed for passage of fetuses through the birth canal; the result can be severe reproductive problems and death of both fetus and mother.

Strain 13 guinea pigs, which are highly inbred, should be housed to protect them from or immunized against the common bacterium *Bordetella bronchiseptica* (Ganaway et al., 1965). Treating guinea pigs for bacterial infections should be approached with caution because antibiotics can cause acute effects. Some can be administered safely; others, such as penicillin, can cause toxemia and death (reviewed by Pakes et al., 1984 and Wagner, 1976). The problem appears to be associated with the excretion of the antibiotics into the gastrointestinal tract and the resulting disturbance of the microbiologic flora on which the guinea pig depends for much of its digestive processes.

Guinea pigs produce large volumes of urine that contain substantial quantities of dissolved minerals and protein. Their urine adheres tenaciously to surfaces, and soaking in

dilute solutions of organic acids is often required before cages are cleaned. Urination and dragging the perineum across the floor of the cage are common methods by which guinea pigs mark freshly cleaned cages.

Hamsters

Laboratory hamsters belong to the subfamily Cricetidae. The most common and most readily available commercially is the Syrian hamster, *Mesocricetus auratus* (sometimes called the golden hamster). Syrian hamsters are native to arid regions of the Middle East and have become well adapted to conserving water, which they obtain principally through food. In a laboratory environment, hamsters will drink water from water bottles, bowls, or automatic watering systems. Hamsters secrete highly concentrated urine that contains large quantities of mineral salts; their urine tends to leave deposits on cage surfaces that are often difficult to remove and might require the application of dilute acids.

Hamsters are often aggressive toward each other, and care should be taken when they are housed in groups. Hamsters that fight must be separated to prevent injury. Cannibalization can occur in group-housed animals when an animal becomes sick or debilitated. It is important to separate animals that are observed to be clinically abnormal.

Vitamin E is an important nutritional requirement of hamsters; vitamin E deficiency has been associated with muscular dystrophy (West and Mason, 1958) and fetal central nervous system hemorrhagic necrosis (Keeler and Young, 1979). Most commercial rodent diets are supplemented with vitamin E, but care is required to ensure the adequacy of vitamin E if

special-formula, purified, or semipurified diets are used (Balk and Slater, 1987). The method of food presentation is important. If food is placed in suspended feeders, hamsters will remove it from the feeder and pile it on the floor. Location of the food pile is peculiar to individual hamsters and will vary from one cage environment to the next. Moving food away from a pile will cause the hamsters to retrieve it and move it back. Given that behavioral pattern, feeding hamsters on the floor of the cage is considered acceptable (9 CFR 3.29). Hamsters have cheek pouches in which they hold and transport food; a full cheek pouch should not be mistaken for a pathologic condition.

Hamsters have very loose skin, particularly over the shoulders. Care should be taken when picking them up so that they do not turn around and bite the handler. Hamsters can be tamed by regular, gentle handling. Without such taming, they can be aggressive toward the handler.

Many species of hamsters hibernate if conditions are right. Various environmental influences seem important, including seasonality, photoperiod, ambient temperature, availability of food, and isolation. To avoid hibernation, temperatures should be maintained within ranges specified in the *Guide* (NRC, 1985 et seq.).

Hamsters, like guinea pigs, are susceptible to antibiotic associated toxicity and enterocolitis. Although successful use of antibiotics in hamsters has been reported, the reports usually involve smaller than therapeutic dosages of antibiotics or the use of particular antibiotic preparations that are not excreted into the gastrointestinal tract (reviewed by Pakes et al., 1984; Small, 1987). As a general rule, antibiotics should be avoided in hamsters.

Estrus in hamsters is similar to that in mice, lasting 4-5 days; however, the gestation period is considerably shorter than that in mice—an average of 16 days. Hamsters are commonly pair-mated; the female is taken to the male's cage for breeding on detection of a stringy vaginal discharge that occurs when the female is in estrus. The female can be removed from the male's cage after mating is observed; however, conception is sometimes improved by leaving her with the male for 24 hours. Removing the female after that time minimizes fighting and allows the male to breed with other females. For optimal reproduction, the light cycle should be maintained at 14 hours of light and 10 hours of dark, which is slightly different from that for other rodents. Litter size ranges from 4 to 16 pups; first litters tend to be smaller than subsequent litters. Cannibalism of pups is common, especially in first litters. It is important to furnish enough bedding or nesting material for the neonates to stay well hidden and to provide the dam with enough food to allow her to be undisturbed from about 2-3 days before birth until about 7-10 days after birth (Balk and Slater, 1987; Harkness and Wagner, 1989).

Gerbils

Gerbils (*Meriones unguiculatus*) do well in solid-bottom cages. Gerbils tend to stand and sit upright and often exhibit a digging or scratching behavior in the corners of cages while in an upright posture. Therefore, cages that are tall enough for this behavior are generally preferred.

Gerbils tend to form social relationships early in life, and groups established at puberty tend to exhibit minimal fighting or other aggressive behavior; aggressive behavior is more common when individual animals are put together later in life. New mates are not accepted easily. For those reasons, it is prudent to select a paired-mating scheme for establishment of colonies and not to regroup gerbils often.

Estrus in gerbils lasts 4-6 days; gestation in nonlactating females is about 24-26 days. If females are bred in the postpartum period, implantation is delayed, and gestation can be as long as 48 days. To avoid postpartum mating, the male can be removed from the cage, but he should be returned to his mate within 2 weeks to decrease the possibility of fighting (Harkness and Wagner, 1989). Average litter size is 3-7.

Gerbils are generally very tame and rarely bite unless mishandled. When they are excited, they will jump and dart about to resist being caught. Gerbils should not be suspended by holding their tails, because the skin over the tail is relatively loose and can be pulled off easily.

Commercial rodent diets are usually acceptable for gerbils, provided that they have a low fat content. Because of the gerbils' unique fat metabolism, it is not uncommon for them to develop high blood cholesterol concentrations on diets containing fat at 4 percent or more. When fed a diet high in fat, gerbils tend to store the fat and become obese. In females, the fat accumulation can be associated with reproductive difficulty.

Chinchillas

Chinchillas (*Chinchilla laniger*) have been farmed for pelts since 13 animals were imported from South America to California in 1927. Most domestic stock is believed to be descended from those animals (Anderson and Jones, 1984). Chinchillas can be housed in wire-mesh or solid-bottom cages; the latter are preferred for breeding (Weir, 1976; Clark, 1984). They are fastidious groomers and should be provided with a box containing a mixture of silver sand and Fuller's earth for a short period daily to allow dust bathing (Clark, 1984). Chinchillas tolerate cold but are very sensitive to heat; the suggested temperature is 20°C (68°F) (Weir, 1976). Commercial chinchilla feed is available, but standard guinea pig rations can also be used (Weir, 1976; Clark, 1984). They might require a source of roughage, such as hay (Weir, 1967). Water and food should be made available ad libitum.

The system used most commonly for breeding chinchillas is to put one male with several females in a large cage. However, females are larger than males and are very aggressive toward both males and other females, and it is necessary to provide refuges, such as nesting boxes, for animals that are being attacked. An "Elizabethan collar" can be used to keep an aggressive female from following an animal that she is attacking into its refuge. A light:dark ratio of 14:10 hours is adequate (Weir, 1967). The mean gestation period is 111 days, with a range of 105-118 days (Clark, 1984). Chinchilla litter size ranges from one to six, with a mean of two. The young are born fully furred and with open eyes, and they begin eating solid food within 1 week but are not completely weaned until they are 6-8 weeks old. Females do not build nests.

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6

Veterinary Care

Veterinary care in laboratory animal facilities includes monitoring of animal care and welfare, as well as the prevention, diagnosis, treatment, and control of diseases. It entails providing guidance to investigators on handling animals and preventing or reducing pain and distress. To perform those and related functions, attending veterinarians must be trained or have experience in the care and management of the species under their care. The responsibilities of an attending veterinarian are specified by the Animal Welfare Regulations (AWRs; 9 CFR 2.33 for research facilities and 9 CFR 2.40 for dealers and exhibitors), the *Public Health Service Policy on Humane Care and Use of Laboratory Animals*, or *PHS Policy* (PHS, 1986), and the *Guide for the Care and Use of Laboratory Animals*, known as the *Guide* (NRC, 1985 et seq.).

PREVENTIVE MEDICINE

Procurement

Rodents (excluding mice of the genus *Mus* and rats of the genus *Rattus*) that are acquired from outside a research facility's breeding program must be obtained from dealers licensed by the U.S. Department of Agriculture (USDA) or sources that are exempted from licensing (9 CFR 2.1). Although laboratory mice and rats are excluded from direct USDA oversight, it is recommended that they be acquired from dealers whose facilities and programs conform to the *Guide* (NRC, 1985 et seq.). Documentation of animal health status, site visits by users, history of client satisfaction, USDA licensing for production of other rodent species in the same facilities, and accreditation by the American Association for Accreditation of Laboratory Animal Care can be used to assess dealers.

Sources

Rapid advances in animal-production technology and disease-control methods during the past 20 years have made it easier to obtain laboratory rodents of known health status and genetic definition. Commercial animal producers often maintain colonies of hysterectomy-derived mice, rats, and guinea pigs in barrier facilities designed and operated to prevent the introduction of microbial agents. Those producers regularly monitor their colonies for evidence of infection and infestation and publish the test results in health reports, which they make available to their clients. There is an increasing trend toward maintaining other

rodents (e.g., hamsters and gerbils) under similar conditions, although usually not produced from hysterectomy-derived stock. It is recommended that animals be acquired from such sources whenever it is possible and appropriate for the study. When animals that are not barrier-reared are acquired, precautions should be taken to isolate them until health evaluations are conducted and decisions are made regarding their care and use.

Transportation

The protection of the health status of specific-pathogen-free (SPF) rodents during transportation to the user has improved greatly in recent years. USDA supervision of animal carriers has resulted in important changes, including the requirements that rodents covered by the AWRs not be warehoused for long periods before and after shipment, that adequate space be provided in shipping enclosures, and that acceptable temperatures and ventilation be maintained during all phases of transportation (9 CFR 3.35-3.41) The International Airline Transport Association (IATA) has developed guidelines for shipping all animal species, including recommendations for shipping rodents (IATA, 1993 et seq.). Another major improvement has been in the commercial development of disposable shipping containers with filter-protected ventilation openings. In addition, sterile food and moisture sources have become available for use in such containers.

Despite the many changes for the better, problems remain. For example, the potential still exists for contamination of container surfaces during shipment. It is recommended that the surfaces of shipping containers be decontaminated before the containers are moved into

clean areas of animal facilities. Several types of disinfectants—including quaternary ammonium solutions, iodinated alcohols, sodium hypochlorite solutions, and chlorine dioxide-containing solutions—can be applied with a small hand sprayer. Chlorine-containing solutions are considered to be very effective against stable agents, such as parvoviruses and spore-forming bacteria (Ganaway, 1980; Orcutt and Bhatt, 1986).

The handling of imported rodents on arrival in U.S. airports can also constitute a problem. Laboratory rodents and rodent tissues that are not inoculated with infectious agents do not require a USDA permit; however, U.S. customs inspectors do not always acknowledge this. Unclear lines of authority often cause unnecessary delays in customs clearance, and such delays can have disastrous effects on the health of the animals. To lessen the probability of delays, as much information as possible should be obtained from the involved authorities (USDA, U.S. Customs, and U.S. Department of the Interior) well in advance of ordering rodents from any foreign source. A permit must also be obtained from the Division of Quarantine, Centers for Disease Control and Prevention, before rodents that can carry zoonotic agents are imported (42 CFR 1, 71.54). Sources of information are listed in the appendix. All necessary documentation should also be obtained before one attempts to export rodents. Specific instructions are usually obtained from the embassy of the country of destination and from the person or institution receiving the animals.

Quarantine and Stabilization

Ideally, rodents being introduced into an animal facility are isolated until their health status can be determined. The period of quarantine also provides time for physiologic and behavioral stabilization after shipment. The users, in cooperation with the veterinarian, should make decisions about the method and duration of quarantine for different kinds of facilities, studies, and types of animals. Unless it is inconsistent with the goals of the study, animals should be allowed to stabilize before the experiment begins.

One of the most common methods of quarantine is to place each group of incoming animals in the same room in which they will eventually be studied. No animals other than those being quarantined should be housed in the quarantine area. For this system to work, each room requires a separate air supply and effective sanitization between studies. Daily animal-care and support activities for quarantine rooms should be conducted after all necessary tasks in the nonquarantine rooms have been performed.

Another approach is to have a single quarantine room for all incoming shipments of animals. This approach has regained favor since the development of isolation-type caging systems, which permit true isolation of many small groups of animals in a single room. Filter-top cages, for example, can be used as miniature rooms within a room. This system works well if animals are moved from dirty to clean cages, one cage at a time in a laminar-flow hood; soiled cages are then closed and autoclaved before they are emptied outside the hood; and appropriate protocols for handling the cages and animals are followed strictly. An advantage of this system is that investigators trained to use it can enter a room and complete short-term studies while the animals are in quarantine. Other variations of quarantine systems have been described elsewhere (NRC, 1991a).

The extent of testing (e.g., serology and parasitology) that is needed during quarantine depends on professional judgment; however, any rodent that dies or becomes ill during quarantine should be subjected to careful diagnostic evaluation. SPF rodents purchased from an established commercial supplier and received in clean, disposable transport cages with filter-protected ventilation openings might not require testing. If the animals are to be used in short-term studies where other short-term studies are performed and relatively few animals are at risk, clinical observations and reliance on the supplier's health program might be adequate. Periodic confirmation of an animal supplier's health report by an independent laboratory provides added safety. If the animals are to be used in a facility where long-term studies might be jeopardized or large numbers of animals are at risk, testing for selected agents of concern is advisable. Maximal protection against the entry of pathogens into a facility is provided by introducing only animals that are delivered by hysterectomy and reared in protective isolation until they are old enough to be tested for the presence of undesirable agents (including agents that can inhabit the female reproductive tract), such as *Mycoplasma pulmonis*, *Corynebacterium kutscheri*, and *Pasteurella pneumotropica*. This course of action is usually followed only in long-standing, ordinarily "closed" breeding colonies.

Animals of undocumented microbiologic status received from any outside source should be serologically tested for a comprehensive list of infectious agents. Animals from such sources might harbor clinically inapparent infectious diseases of major concern. For example, mousepox can be difficult to detect clinically in resistant strains of mice or in mice from colonies with long-standing infections. When introduced into a disease-free colony,

mousepox usually becomes evident as an epizootic that can substantially interfere with research (New, 1981). Laboratory rodents and some wild rodents can be subclinically infected with zoonotic agents—e.g., hantaviruses, lymphocytic choriomeningitis (LCM) virus, Lassa fever virus, Machupo virus, and Junin virus—that pose a serious or even deadly health threat to personnel (CDC, 1993; LeDuc et al., 1986; Oldstone, 1987; Skinner and Knight, 1979; Smith et al., 1984). The time of quarantine should be long enough for reasonable expectation that incubating infections will become evident, either clinically or by appropriate testing procedures. As many as 30 percent of the animals should be tested if the microbiologic status of the source colony is completely unknown. In this situation, it is preferable to obtain extra animals for testing so that not only serology, but bacterial cultures, examinations for parasites, and histopathologic evaluations can be performed if needed.

Some pathogens pose special problems for quarantine programs. For example, the chronic form of LCM viral infection in mice, which is contracted in utero or immediately after birth, might not be detectable with antibody tests commonly used in commercial testing laboratories. Mice infected at that time develop persistently high titers of virus that is complexed with humoral antibody, rendering the antibody undetectable by complement-fixation or neutralization tests (Bishop, 1990; Oldstone and Dixon, 1967, 1969). The more-sensitive immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA) give weak reactions and cannot be depended on to detect circulating antibody in persistently infected mice (Parker, 1986; Shek, 1994). That is an important problem because the primary route of transmission in the mouse is vertical, and the infected offspring become lifelong, relatively asymptomatic shedders of virus (Rawls et al., 1981). An alternative method for

detecting LCM virus in asymptomatic virus shedders is to use virus-free sentinels over the age of weaning (Smith et al., 1984). Once beyond neonatal age, exposed mice develop a short-lived infection and have readily detectable antibodies to LCM virus (Rawls, 1981). Intracranial inoculation of blood or tissue homogenates into the sentinels is a faster screening method. If virus is present, neurologic disease and death will ensue in 6-9 days (Parker, 1986). Additional laboratory procedures would have to be performed to confirm the presence of LCM virus in the dead mice. In testing laboratories that maintain cell lines, such as Vero or BHK-21, the quickest method is to inoculate cell-line cultures with blood from the suspect mice and use the IFA 4-5 days later to test for LCM-virus antigen in the cells. The mouse antibody-production (MAP) test can also be used to detect LCM virus. Antibody to LCM virus in rodents other than persistently infected mice is readily detected with the ELISA or IFA procedures.

Viable rodent tissues—including blood, ascitic fluid, tissue cultures, transplantable tumors, and hybridomas—can harbor undesirable agents, and tissues of undocumented microbiologic status should not be introduced into rodent colonies until they are shown to be free of undesirable agents by diagnostic testing (e.g., MAP testing).

Separation by Species, Source, and Health Status

Pressures to maintain different rodent species in separate rooms have lessened with advances in knowledge of rodent infections. For example, the AWRs do not require species separation, and the *Guide* (NRC, 1985 et seq.) allows considerable latitude on this issue. It

has become recognized that more infectious agents are transmissible among animals of the same species than among those of different species. A more important concern is the microbiologic status of rodents from different sources (or from different locations at the same source), regardless of species. Common sense dictates that if it is necessary to place rodents from different sources in the same room because of space constraints or for other practical reasons, it should be done only with animals of comparable microbiologic status. Such decisions should be made with input from people knowledgeable in rodent-disease pathogenesis and with adequate health-status information about the source colonies.

Interspecies anxiety does not appear to be a problem if different rodent species or rodents and rabbits are housed in the same room, although systematic studies are needed to support the validity of this premise. However, it is unacceptable to house rodents with species that are their natural predators, that produce intimidating noises and odors, or that can harbor infectious agents of known or unknown consequences in rodents (e.g., cats, dogs, and monkeys).

SURVEILLANCE, DIAGNOSIS, TREATMENT, AND CONTROL OF DISEASE

Daily Observations of Animals

One important way to track the health status of rodent colonies is to observe the appearance and behavior of the animals daily. A wide range of abnormal signs can be

detected in this manner, including weight loss, ruffled hair coat, dry skin, lacerations, abnormal gait or posture, head tilt, lethargy, swellings, diarrhea, seizures, discharge from orifices, and dyspnea. Underlying causes for those signs include such things as malfunctioning watering systems, fighting, infectious diseases, and experimentally induced changes. Observations are usually made by animal-care staff and technicians, who should be trained to look for spontaneous and experimentally induced abnormalities and report them to the supervisory staff, the attending veterinarian, and study directors. Veterinary oversight of this process and training given by the attending veterinarian are important. Veterinary programs for overseeing the health of laboratory rodents should have readily available, up-to-date references on the biology and diseases of rodents.

Control of Infectious Diseases

First and foremost, control of infectious diseases in rodent colonies means preventing their introduction. That is accomplished by using good management practices, such as purchasing pathogen-free animals; using well-planned quarantine systems for incoming animals and animal-derived specimens; training animal-care staff to make accurate clinical observations; using protective clothing; vermin-proofing the facility; using filter-protected cages, filtered-air ventilation systems, or both; and controlling the movement of personnel and visitors within the facility. In addition, animal-care staff should be encouraged not to maintain pet rodents, because of the possibility of transferring infectious agents into the animal quarters.

Even with good management, infections occasionally gain entrance into colonies. Routine monitoring systems should be in place to detect them as quickly as possible, thereby permitting the start of specific measures to eliminate them or prevent their spread. The key elements of an effective monitoring program are daily observation of the animals to detect clinical diseases and regular microbiologic monitoring to detect subclinical infections. Daily observations are extremely important because they quickly reveal signs of spontaneous disease. To achieve full effectiveness, monitoring activities require diagnostic capability to investigate disease outbreaks.

Microbiologic monitoring can include many kinds of tests, depending on the needs of the facility. Animal suppliers often test for all infectious agents of rodents for which there are commercially available tests so that fully characterized animals can be offered for research use. In research facilities, the staff might choose to test initially or annually for all known pathogenic agents and test more frequently for a smaller number of "core" agents of special concern. Table 6.1 lists typical "core" agents. The research requirements or special interests of the staff will dictate what other agents should be added to the list.

Several newly recognized viruses that are not listed as core agents deserve mention because of their apparent high prevalence. These are the so-called orphan parvoviruses of mice and rats that appear to be widespread in laboratory colonies but are of unknown character and pathogenicity. Although field strains of the viruses are yet to be isolated, the mouse orphan parvovirus (MOPV) has been demonstrated in tissues by in situ hybridization (Smith et al., 1993), and a closely related laboratory strain has been isolated (McKisic et al., 1993). In routine testing, the viruses of both mice and rats have been detected indirectly by

IFA demonstration of antibody against nonstructural proteins of the rodent parvovirus group followed by negative results with hemagglutination inhibition (HAI) tests that are specific for recognized parvoviruses (i.e., MVM, KRV, and Toolan H-1 virus). An HAI test specific for MOPV has been developed by using the laboratory strain (Fitch isolate) but is not yet in general use.

It is debatable whether Sendai virus and simian virus 5 (SV5) should continue to be listed as core agents for guinea pigs and hamsters. Although serologic positivity is often found, it is is believed by some to be caused by infection with antigenically related parainfluenza viruses, possibly from human sources. Isolation of Sendai virus from guinea pigs has been attempted rarely and described only anecdotally (Parker, reported by Van Hoosier and Robinette, 1976). Failure of transmission of Sendai virus from serologically positive guinea pigs to mice also has been found (W. White, Charles River Laboratories, Wilmington, Massachusetts, unpublished). Isolation of Sendai virus from hamsters has been reported rarely (Parker et al., 1987). Serologic positivity for Sendai and SV5 viruses might be caused by cross reactions with human parainfluenza viruses, but isolation of the human agents from these animals has not been documented.

Monitoring can be performed for many combinations of agents and with various frequencies. Emphasis is often on serologic testing because many of the agents of concern cause subclinical infections and are detectable quickly and inexpensively with this method. Table 6.2 lists infectious agents of commonly used laboratory rodents for which serologic (antibody) tests are available.

Bacteriologic testing usually entails culturing for primary and opportunistic pathogens from the upper respiratory tract and intestines. Table 6.3 lists the primary pathogens culturable from these sites.

Monitoring for ectoparasites is done usually by examining the skin and pelage over the head and back with a dissection microscope. For parasites that invade the skin, skin scrapings in immersion oil or 5 percent potassium hydroxide are examined microscopically. Monitoring for endoparasites is performed by using fecal flotation and sedimentation procedures to search for eggs and oocysts, using the Cellophane-tape method to look for *Syphacia* eggs, examining the cecocolic contents for helminths, and examining the bladder mucosa for *Trichosomoides crassicauda* (in rats) and fecal wet smears for protozoa. Descriptions of ectoparasites and endoparasites and their effects on rodents have been published (Farrar et al., 1986; Flynn, 1973; Hsu, 1979, 1982; Ronald and Wagner, 1976; Vetterling, 1976; Wagner, 1987; Wagner et al., 1986; Weisbroth, 1982; Wescott, 1976, 1982). Pathologic monitoring can be used to detect diseases that produce characteristic lesions that are observable at necropsy or detectable by histopathologic evaluation. Infectious diseases for which this approach is useful include Tyzzer's disease (*Clostridium piliforme* [formerly called *Bacillis piliformis*] infection), pneumocystosis (*Pneumocystis carinii* infection) in some immunodeficient animals, and CAR bacillus infections. Special stains are required to demonstrate those causative agents (e.g., methenamine silver for *P. carinii* and Warthin Starry silver for *C. piliforme* and CAR bacillus). Pathologic monitoring can also be used to detect noninfectious conditions, such as nutritional deficiencies, heritable metabolic diseases, and neoplasms. The necropsy is usually the first step in the diagnostic workup of

clinical diseases, often providing the impetus for using other measures, such as virus isolation, bacterial cultures, or histopathology. Complete descriptions of these procedures and the manifestation of infections in rodents are beyond the scope of this report, but such information is available in a number of books, manuals, and review articles (ACLAD, 1991; Baker et al., 1979; Bhatt et al., 1986; Flynn, 1973; Foster et al., 1982; Hamm, 1986; NRC, 1991a; Van Hoosier and McPherson, 1987; Waggie et al., 1994; Wagner and Manning, 1976).

Sample Size for Monitoring

All animals should be monitored for clinical disease by daily observations. This type of monitoring, combined with a diagnostic workup of animals with unexplained abnormalities, is particularly important for early detection of clinical disease outbreaks. It is complementary to microbiologic monitoring in that diseases that spread slowly and smolder for a considerable time in a few cages in a room (Bhatt and Jacoby, 1987; Wallace et al., 1981) might be missed in the statistical sampling used in microbiologic monitoring. Daily observations should quickly reveal these kinds of diseases.

Microbiologic monitoring for evidence of subclinical infections is accomplished by testing regularly a randomly selected sample of the population of animals at risk. How to determine the appropriate sample size is a much debated subject. A formula has been used to predict the number of randomly selected animals in a population of 100 or more that must

be tested to detect a single case of disease within 95 percent confidence limits, assuming a known prevalence rate (NRC, 1976):

$$\text{No. to be sampled} = \frac{\log 0.05}{\log N} .$$

In that formula, N is the percentage of animals expected to be normal. The percentage is derived by subtracting the expected prevalence rate of the disease from 100 percent. The formula is useful for helping to understand the considerations involved in sampling to detect a single disease. In practice, however, its use is limited by several factors. One factor is that sampling of a rodent population is usually aimed at detecting more than one disease, each with a different expected prevalence. Another problem is that infectious-disease prevalences are affected by population density, caging methods, ventilation systems, and a host of other variables that affect the rate of spread of infections; a disease prevalence expected to be 30 percent in open cages might be only 1 percent in filter-top cages. Still another consideration is that much of the monitoring is done by testing for antibody. If an infection with an expected prevalence of 30 percent has been in a colony for several months, the number of surviving animals with antibody can approach 100 percent. Because of those variables, the formula serves only as a rough estimate. If it is used, one prevalence is selected for all diseases and conditions, even though screening is usually for multiple organisms. For example, a prevalence of 30 percent might be assumed for more contagious infections, and a sample size of 8-10 would be used. This sample size would, of course, be unlikely to detect infections that are less contagious (NRC, 1991a).

Similar calculations can be made for populations of fewer than 100 with other formulas. More complex calculations can be used once the monitoring program is in place and sufficient data have been accrued on the incidence of positive findings and frequency of disease outbreaks. Those calculations can be used to adjust the sample size and frequency of sampling to achieve the desired confidence levels for disease detection (Selwyn and Shek, 1994).

In summary, there is no easy way to determine sample sizes and frequencies for monitoring. Although a mathematical approach can be taken, the inability to conform to the assumptions on which the formulas are based or the lack of precise knowledge of prevalence rates or disease outbreaks makes such an approach difficult to apply. For that reason, it is still common to choose sample size and frequency of monitoring in an arbitrary manner, which is often influenced by economic constraints.

An alternative method of monitoring uses *known* pathogen-free sentinel animals to detect infections. Typically, they are randomly dispersed in multiple locations in the facility, and various means are used to promote contagion of any infections that might be present from the animals being monitored by the sentinels. The most effective method is to place the sentinels in the cages with the study animals and move them to cages of different study animals every 1-2 weeks. If such a procedure is not practical, the sentinels should at least be caged on the same rack with the study animals, preferably on a lower shelf, and soiled bedding from the cages of the study animals should be transferred regularly to the cages of the sentinel animals (Thigpen et al., 1989). Because natural transmission of some pathogens might not occur quickly, the time allowed for seroconversion or production of disease should be about 6-8

weeks. Those pathogens include *Mycoplasma pulmonis* (Cassell et al., 1986; Ganaway et al., 1973), ectromelia virus (Wallace et al., 1981), and cilia-associated respiratory (CAR) bacillus (Matsushita et al., 1989); a preferable alternative is to test the animals being introduced into the colony rather than the sentinels.

Treatment and Control

Health-monitoring data should be reviewed regularly, and a plan of action should be in place for dealing with positive test results. Such plans usually include the names and telephone numbers of research and veterinary staff to be notified, a system for confirming the test results, and appropriate measures for controlling or eliminating infection. Decisions about ways to prevent spread to contiguous areas should be made quickly. They usually involve placing the room under strict quarantine and developing strategies for controlling access and for handling potentially contaminated items, such as cages and bedding, that will be removed from the room periodically. Investigations are usually initiated immediately to identify the sources of causative agents. Approaches to control depend on the characteristics of the agents, the value of the infected animals, and the type and design of the facility.

Bacterial diseases of rodents can be treated with antibiotics. However, when large numbers of animals are involved, this is often considered practical only for temporary control. Failure to eliminate the agent from every animal, as well as from contaminated surfaces, might result in re-emergence of the disease when antibiotics are discontinued. In some instances, antibiotics can adversely affect rodents, especially guinea pigs and hamsters,

by causing an imbalance of the intestinal microflora and overgrowth of deleterious bacteria (Fekety et al., 1979; Small, 1968; Wagner, 1976). Other problems include the lack of information on proper dosages, the difficulty of accurately administering antibiotics in food and water, and confounding influences of drug residues and interactions on research results.

Parasitic diseases can also be treated; however, even with highly effective antiparasitic drugs, it is very difficult to eliminate from large colonies such parasites as pinworms and mites. It might be possible in small colonies if the treatment schedule is adjusted to overlap the time of the parasite life cycle and if sanitation procedures are stringently performed simultaneously (e.g., frequent washing of floors, walls, and cages) (Findon and Miller, 1987; Flynn et al., 1989; Silverman et al., 1983; Taylor 1992; West et al., 1992).

Viral, bacterial, and parasitic infections are usually eliminated by euthanatizing and repopulating the colony with disease-free animals after the room, cages, and other equipment have been decontaminated or, in the case of particular viruses, by allowing the infection to run its course in a closed population to produce noninfected, immune survivors. The latter procedure has been used successfully with such viruses as Sendai virus and mouse hepatitis virus, which are highly contagious, usually remain in the animals for a short time, and are relatively unstable in the animal-room environment (Barthold, 1986; Fujiwara and Wagner, 1986). For it to be successful, ample opportunity for contagion is required, and new animals, even newborns, must not be introduced for a period long enough for all animals to become infected, recover, and stop shedding the virus. Contagion can be promoted by transferring infected bedding to numerous cages, placing cage racks near each other, and removing filter tops. Sentinels can be introduced and tested 6-8 weeks later to determine the

success of the procedure. No sentinels should be introduced into the room, and no naive animals of any type should be allowed to be introduced or maintained in the room until 6-8 weeks after breeding has been stopped.

Necropsies

When an animal is unexpectedly found dead or moribund, it is good practice to determine the cause by necropsy. Necropsy, coupled with daily observations by the animal technicians, usually provides the first indication of important clinical infectious and noninfectious diseases. Lesions will often be characteristic enough to permit presumptive diagnoses or point to appropriate additional diagnostic procedures. Routine histopathologic tests are performed in some facilities.

EMERGENCY, WEEKEND, AND HOLIDAY CARE

The need for adequate animal care does not diminish during holidays and weekends. As stated in the *Guide*, laboratory animals should be cared for daily (NRC, 1985 et seq.) Security personnel should be able to contact responsible people in the event of emergencies. Therefore, a list of names and phone numbers should be posted prominently in the facility and maintained in the security office. Provisions for emergency veterinary care should be made as well (9 CFR 2.33b2; NRC, 1985 et seq.).

MINIMIZATION OF PAIN AND DISTRESS

Many internal and external environmental factors can induce physiologic or behavioral changes in laboratory animals. These factors are called stressors, and their effect is called stress (NRC, 1992). The intensity of the stress experienced by an animal is influenced by other factors, including age, sex, genetics, previous exposure, health status, nutrition, and medication (Blass and Fitzgerald, 1988; NRC, 1992). If an animal is unable to adapt to stressors, it will develop abnormal physiologic or behavioral responses; when this occurs, the animal is in distress (NRC, 1992). Sometimes, the effect induced by the stressor is pain. Pain can be described as a physical discomfort perceived by an organism as the result of injury, surgery, or disease. Once pain is perceived by an animal, it can itself become a secondary stressor and elicit other responses, such as fear, anxiety, and avoidance.

To prevent or alleviate pain and distress in laboratory rodents, the research team should anticipate procedures or situations that will elicit these conditions. According to the *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training*, "unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain and distress in other animals" (published in NRC, 1985, p. 82). Classifications of the magnitude of pain or distress estimated to be associated with different types of experimental procedures are available in the literature (OTA, 1986; NRC, 1992). It is the responsibility of the institutional animal care and use committee (IACUC) to evaluate each animal procedure for the potential to cause pain or distress and to ensure that anesthetics, analgesics, and

tranquilizers are used, when appropriate, to prevent or alleviate pain and distress in the animals. Anesthetics or analgesics should be given before the painful insult, because it is easier to prevent pain, by blocking nociceptive neurons, than to alleviate it. The exposure of nociceptive neurons to painful stimuli produces chemical changes that cause the neurons to be hypersensitive to additional pain stimuli for a long period (Hardie, 1991; Kehlet, 1989). In addition, a cascade of physiologic changes occur that can have substantial effect on the recovery of an animal from surgery or on the information that is obtained in the procedure in which the animal is used. Depending on whether the pain is acute or chronic, responses might include protein catabolism, sodium retention, immunosuppression, decreases in pulmonary and cardiovascular function, and increases in plasma concentrations of catecholamines and corticosteroids (Engquist et al., 1977; Flecknell, 1987; S. A. Green, 1991; Yeager, 1989).

Recognition of Pain and Distress

Every person involved in the procurement, care, and use of laboratory rodents plays a major role in contributing to the total well-being of these animals. It is important to understand and consider species-specific behavior and husbandry needs when standard operating procedures and research protocols are developed to minimize exposure of the animals to situations that have a high probability of inducing pain and distress (Amyx, 1987; Montgomery, 1987).

Clinical signs and abnormal behavior displayed by rodents in response to pain and distress can include decreases in food and water consumption, accumulation of reddish-brown exudate around the eyes and nostrils (chromodacryorrhea), weight loss, decrease in activity, hunched posture, piloerection, poor grooming habits, labored respiration, vocalization, increase or decrease in aggressiveness, and self-mutilation (Flecknell, 1987; Flecknell and Liles, 1992; Harvey and Walberg, 1987; Heavner, 1992; NRC, 1992; Sanford, 1992). The degree to which clinical signs are displayed varies within a species and between species. For behavior to be a useful indication of pain or distress, members of the research team, from animal caretakers to principal investigators, should be knowledgeable about the normal behavior of the animals with which they are working. Regular communication among all members of the research team, including the veterinary staff, is critical to ensuring timely evaluation and treatment of animals in pain or distress.

Alleviation of Pain

The *Guide* recommends the use of appropriate anesthetics, analgesics, and tranquilizers for the prevention and control of pain and distress. However, if for justifiable scientific reasons these agents cannot be administered when a painful procedure is to be conducted, the *Guide* states that "the procedure must be approved by the committee [IACUC] and supervised directly by the responsible investigator" (NRC, 1985, p. 37).

The drugs routinely used to prevent or control pain in laboratory rodents are generally classified as either opioids or nonsteroidal anti-inflammatory agents. Drugs reported to be

effective analgesics in rodents are published elsewhere (Blum, 1988; CCAC, 1980; Clifford, 1984; Flecknell, 1984, 1987; C. J. Green, 1982; Hughes, 1981; Hughes et al., 1975; Jenkins, 1987; Kruckenburg, 1979; Lumb and Jones, 1984; Soma, 1983; Vanderlip and Gilroy, 1981; White and Field, 1987). In some cases, the doses quoted are extrapolations from doses for other species, with little or no scientific evidence to support the recommended use.. Because some of these drugs might have systemic side effects that could interfere with a research protocol, it is important to select and use them carefully. Additional factors that should be considered in selecting an analgesic include species, strain, age, sex, health status, nutritional status, period for which pain prevention or control will be required, recommended route of administration, volume of drug required for effect, compatibility with other pharmacologic agents that the animal will be receiving, cost, and availability (C. J. Green, 1982; Kanarek et al., 1991; Pick et al., 1991). Principal investigators should get assistance from the attending veterinarian in selecting the most appropriate agent.

Alleviation of Stress and Distress

The use of tranquilizers can be considered when a laboratory rodent is restrained for long periods or used in a procedure that might cause fear, anxiety, or severe distress. Dosages of tranquilizing agents for rodents have been reported elsewhere (Blum, 1988; CCAC, 1980; Flecknell, 1987; C. J. Green, 1982; Harkness and Wagner, 1989; NRC, 1992; Vanderlip and Gilroy, 1981; White and Field, 1987). It should be noted, however, that tranquilizers have not been well studied in rodents. The drugs might interfere with

experimental results, and suggested dosages might not produce the desired effects. Gradual conditioning to restraint before initiation of a study should also be considered as a means of decreasing associated anxiety or distress.

SURVIVAL SURGERY AND POSTSURGICAL CARE

Surgical procedures on rodents must be performed only by appropriately trained personnel or under the direct supervision of trained personnel (9 CFR 2.32; NRC, 1985 et seq., 1991b). It is essential that personnel given the responsibility to perform surgery be knowledgeable about the principles of aseptic technique and the correct methods for handling tissues and using surgical instruments (McCurin and Jones, 1985). It is the responsibility of the IACUC to ensure that people approved to perform surgery on rodents have the required training or experience (9 CFR 2.32).

Standards and guidelines for conducting survival surgery have been established by the *Guide* (NRC, 1985 et seq.) and for rodents other than mice and rats by the AWRs (9 CFR 2.31). Aseptic technique is required whenever a *major* survival surgical procedure is performed. Aseptic technique is used to reduce microbial contamination to the lowest practical level (Cunliffe-Beamer, 1993) and includes preparation of the animal, preparation of the surgeon, sterilization of instruments and supplies, and the use of operative procedures that reduce the likelihood of infection. A major surgical procedure has been defined as any surgical intervention that penetrates a body cavity or produces permanent impairment of physical or physiologic function (9 CFR 1.1; NRC, 1985 et seq.). Other surgical

procedures, classified as *minor*, include catheterization of peripheral vessels and wound suturing. Less stringent conditions are permitted for minor surgical procedures (NRC, 1985, p. 38), but sterile instruments should be used and precautions should be taken to reduce the likelihood of infection. Deviations from those guidelines and standards should not be undertaken unless reviewed and approved by the IACUC.

The susceptibility of rodents to surgical infection has been debated; however, available data suggest that subclinical infections can cause adverse physiologic and behavioral responses (Beamer, 1972-1973; Bradfield et al., 1992; Cunliffe-Beamer, 1990; Waynforth, 1980, 1987), which can affect both surgical success and research results. Characteristics of surgery on rodents that can justify modifications in standard aseptic technique include smaller incision sites, multiple operations at one time, shorter procedures, and complications caused by the use of antibiotics (Brown, 1994; Cunliffe-Beamer, 1993; Small, 1987; Wagner, 1976). Strategies have been published that provide useful suggestions for dealing with some of the unique challenges of rodent surgery (Cunliffe-Beamer, 1983, 1993). The area used for surgery, whether or not it is dedicated for that use, must be easily sanitized, must not be used for any other purpose during the time of surgery, and should be large enough to enable the surgeon to conduct the procedure without breaking aseptic technique.

It might be necessary to perform experimental surgery on animals whose health has been compromised by naturally occurring or experimentally induced disease, but generally only healthy rodents should be used in experimental surgical procedures. Before being used in experimental surgery, rodents should be allowed sufficient time to acclimate to a new environment and overcome the stress of transportation. Results of several studies have

shown that mice experience increased corticosterone concentrations and depressed immune function after transport; these functions return to baseline values within 4-8 hours. The length of time might vary with the species and the mode and duration of transportation (Aguila et al., 1988; Dymsza et al., 1963; Landi et al., 1982; Selye, 1946). During the acclimation period, the animals should be examined to ensure that they are not exhibiting clinical signs of disease.

To reduce or prevent stress preoperatively, researchers should be trained to handle and restrain animals and give them injections properly (NRC, 1991b). The animals should be conditioned to being picked up and handled by the people that will be doing the preoperative procedures. Fasting for periods of 12 hours or more is neither recommended nor generally required. However, it is often desirable to remove food at least 4 hours before anesthesia to promote consistent absorption of intraperitoneal anesthetics (White and Field, 1987). Access to water should be allowed up to the time that preoperative procedures are to begin (C. J. Green, 1982).

Anesthetics and Tranquilizers

Administration of tranquilizers, sedatives, or anesthetics might prevent or alleviate stress in the animals, as well as making it easier for surgical personnel to prepare them for surgery. Dosages of tranquilizers and anesthetics that can be used in rodents have been reported elsewhere (Blum, 1988; Flecknell, 1987; C. J. Green, 1982; Harkness and Wagner, 1989; Hughes, 1981; Kruckenburg, 1979; Soma, 1983; Stickrod, 1979; White and Field, 1987). In

addition to injectable and inhalational anesthetics, hypothermia has been recommended as a means of anesthesia in neonatal animals (C. J. Green, 1982; NRC, 1992; Phifer and Terry, 1986). Criteria for selecting tranquilizers and anesthetics and their dosages should include species, strain, age, sex, health status, temperament, environmental conditions of the animal holding rooms, drug availability, drug side effects, recommended route of administration, equipment required, length of time that drug effect is desired, and skills and experience of the anesthetist. Doses quoted are often extrapolations from doses for other species with little or no scientific evidence to support them. It is important to select and use these drugs carefully to avoid interference with research protocols.

Preparation for Survival Surgery

Once the animal is tranquilized, sedated, or anesthetized, the operative site should be prepared. The extent of this preparation will depend on the species and maturity of the animal and on the complexity of the surgical procedure to be performed. The preparation might include removing body hair along the surgical site and surrounding areas with clippers, razors, or depilatory agents or by manual plucking. Care should be taken to avoid physical or chemical damage to the skin. Loose hairs should be thoroughly cleared from the surgical site. Various commercially available agents are appropriate for disinfecting the skin, including povidone iodine, alcohol, and chlorohexidine. Because the blink reflex is often lost under general anesthesia, consideration should be given to applying a sterile ophthalmic lubricant before surgery to prevent drying of the corneas (Powers, 1985).

Heat loss can affect the course and success of anesthesia in rodents. Rodents lose body heat rapidly to surfaces such as operating tables, bench tops, and instruments. To preserve body heat, a circulating hot-water blanket, hot-water bottles, or an incandescent lamp placed 12-14 inches from the animal can be used to supply supplemental heat during the surgical procedure and recovery. Positioning the animal on an insulating surface, such as cloth or paper, will also help to decrease heat loss.

The animal should be positioned to provide adequate fixation and exposure of the operative site. Tape, positional ties, or similar mechanical means should be used to ensure that the animal's position will not be changed by pressure exerted by the surgeon. Care should be taken so that the selected method of restraint does not impede circulation or cause injury to the animal.

Depending on the complexity of the surgical procedure, it might be necessary to place a sterile drape over the animal to prevent contamination of the operative site. Various commercially available cloth, paper, and plastic materials are suitable for use as surgical drapes.

In preparation for the procedure, the surgeon should scrub his or her hands and forearms with a povidone iodine scrub, alcohol foam product, or other equally effective disinfectant-detergent. At a minimum, surgical personnel must wear sterile gloves while performing surgery (9 CFR 2.31; NRC, 1985 et seq.). For rodents other than mice of the genus *Mus* and rats of the genus *Rattus*, masks are also required by the AWRs (9 CFR 2.31). Although caps and gowns are not required for rodent surgery, their use can decrease the risk of contaminating the surgical site and sterile supplies.

Sterilization of Instruments

The AWRs (9 CFR 2.31) and the *Guide* (NRC, 1985 et seq.) require that all instruments used in survival surgery be sterilized. As many sets of sterilized instruments as possible should be available when a surgical procedure will be performed on multiple animals during the same operative period. If it is necessary to use the same instruments on several animals, instruments that were sterile at the beginning of the procedure should, at a minimum, be disinfected by chemical or other means (e.g., heated glass beads) before they are used on another animal.

Various methods and materials are available for sterilization of instruments and surgical supplies, including heat, steam under pressure, ethylene oxide gas, gamma irradiation, electron-beam sterilization, and such chemical agents as phenols and glutaraldehyde. The method selected should be periodically monitored (e.g., with spore strips in autoclaves) to ensure that sterilization is achieved. When ethylene oxide gas or a liquid chemical agent is used, care should be taken to ensure that all toxic residues are eliminated before the instruments and supplies are used for surgical procedures.

Instruments and supplies that are to be sterilized with methods other than contact with liquid agents should be wrapped in paper, cloth, plastic, or similar materials in such a way as to prevent contamination after sterilization. The choice of material should be appropriate for the method of sterilization. Each package should bear some indication that it has undergone sterilization. The package should also be marked with the date of sterilization. The shelf-life of sterilized items will depend on the type of material used to wrap them and on how

they are stored (Berg and Blass, 1985; Gurevich, 1991; Knecht et al., 1981). Items that are sterilized with liquid agents are generally prepared near the operating room or area and used immediately after they are removed from the liquid and rinsed with sterile water or sterile irrigation solution.

Monitoring During Surgery

Surgical procedures should not be initiated until the animal has reached a surgical plane of anesthesia. In most rodents, loss of toe-pinch and pedal reflexes indicates that the plane of anesthesia is adequate for surgery. Guinea pigs, however, can maintain a pedal reflex under anesthesia; for them, the pinna reflex is more appropriate for assessing the plane of anesthesia (C. J. Green, 1982). The animals should be closely monitored throughout the procedure. An animal's status can be determined by monitoring respiration, eyes, and mucous membranes. Slow, labored respiration, loss of reflected eye color in albino animals, and pale or cyanotic mucous membranes are all indicators of compromised cardiovascular and respiratory functions. If resuscitation is necessary, a modified bulb syringe can be fitted over the animal's muzzle and gently pumped to force air into its lungs. A gentle, rhythmic pressure can be applied over the apical area of the thorax to induce cardiac contractions. Doxapram can be used to stimulate respiration (Flecknell, 1987). The attending veterinarian can instruct investigators about those and other resuscitative techniques most appropriate for the species and procedures used.

Postoperative Care

A rodent recovering from surgery should be observed regularly until it is conscious and has regained its righting reflex. It should be housed singly in a cage on absorbent material that minimizes heat loss until it is conscious. Recovery is facilitated by providing supplemental heat as previously described. Care should be taken to prevent thermal injuries if water bottles, electric heating pads, or heating lamps are used.

If necessary, body fluid lost during the surgical procedure should be replaced with subcutaneously or intraperitoneally administered fluids. A decision to administer fluids should be based on the nature and length of the surgical procedure and an estimation of fluid loss. Sterile saline, lactated Ringer's and 5 percent glucose solutions are often used. Guidelines on fluid-replacement therapy are available (Cunliffe-Beamer and Les, 1987; Lumb and Jones, 1984).

If recovery takes longer than 30 minutes, the animal's position should be rotated to prevent congestion in dependent organs. If there is concern that its toes will become entangled in sutures or that it will harm the incision or damage the bandage or other protective devices, its toenails should be clipped during the postoperative recovery period.

Analgesics should be administered as needed during the postoperative recovery period. Possible side effects and drug interactions should be taken into consideration when specific agents are selected for use (Harkness and Wagner, 1989).

Surgical wounds should be examined daily for dehiscence, drainage, and signs of infection. Appropriate nursing care should be given to prevent drainage from the incision

from irritating the surrounding skin. If nonabsorbable sutures or medical staples are used to close the skin, they should be removed when the incision is adequately healed.

EUTHANASIA

Euthanasia is the act of producing a painless death. It entails disrupting the transmission of signals from peripheral pain receptors to the central nervous system (CNS) and rendering the cerebral cortex, thalamus, and subcortical structures of the CNS nonfunctional. The "endpoint" (the point at which euthanasia will be performed) should be specified in any protocol for a terminal study or for a study in which the animals are likely to experience pain and distress that cannot be adequately controlled or prevented with pharmacologic agents, including studies associated with infectious diseases or tumor growth. Each investigator should consult with the attending veterinarian to decide on a humane endpoint that will allow collection of the required data without causing undue pain and distress (Amyx, 1987; Montgomery, 1987).

The technique selected for performing euthanasia on laboratory rodents should be based on a number of factors, including the following:

- species;
- animal age and condition;
- objectives of the study;

- histologic artifacts and biochemical changes induced by the agent or method selected;
- number of animals to be euthanatized;
- available personnel;
- cost and availability of supplies and equipment;
- controlled-substance use; and
- skills of assigned personnel.

To avoid causing stress in the animals that will be euthanatized, the following principles should be adhered to:

- Animals should not be euthanatized in the same room in which other animals are being held. The visual, acoustic, and olfactory stimulants that can be present at euthanasia can cause distress in other animals.
- Animals should be handled gently and humanely during transport from the holding room and during the actual euthanasia process.
- If a euthanasia chamber is used, overcrowding should be avoided.
- Euthanasia should be performed only by people trained in the method selected. It is important that the training received include basic information on how the technique works to produce a quick and painless death and on the advantages of using a specific method in a specific protocol.

- Counseling should be available for those performing euthanasia to help them understand feelings and reactions that might develop as a result of performing this task.
- Death should be verified at the end of the procedure. Possible methods might include exsanguination, decapitation, creation of a pneumothorax by performing a bilateral thoracotomy or incising the diaphragm, and a physical examination to verify the absence of vital signs.

PHS Policy (PHS, 1986) requires that methods of euthanasia be consistent with the recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia (AVMA, 1993 et seq.). AVMA-recommended methods cause death by direct or indirect hypoxia, direct depression of CNS neurons, or physical damage to brain tissues. The approved pharmacologic agents and physical methods include barbiturates, inhalant anesthetics, carbon dioxide, carbon monoxide, nitrogen, argon, and microwave irradiation. Two additional techniques, cervical dislocation and decapitation, can be used if scientifically justified and approved by the IACUC (AVMA, 1993). Of these agents and methods, four are commonly used for rodents: carbon dioxide, sodium pentobarbital, cervical dislocation, and decapitation.

Carbon dioxide is a very safe and inexpensive agent for euthanatizing laboratory rodents. In all but neonates, it causes rapid, painless death by a combination of CNS depression, which is produced by a fall in the pH of the cerebrospinal fluid, and hypoxia. Other methods of euthanasia can be used in newborn animals, which are more resistant to acute respiratory acidosis and hypoxia than older animals. Commercially available cylinders

of compressed carbon dioxide or blocks of dry ice can used as the source of carbon dioxide. Compressed gas is preferable because inflow to the chamber can be regulated precisely (AVMA, 1993). If dry ice is used, it should be placed in the bottom of the chamber and separated from the rodent by a barrier to prevent direct contact that could cause chilling or freezing and associated stress.

Sodium pentobarbital is the barbiturate drug most commonly used for euthanatizing animals and can be administered to rodents either intraperitoneally or intravenously. When administered intravenously to rodents at a dose of 150-200 mg/kg of body weight (NRC, 1992), it causes rapid death by CNS depression and hypoxia. Intracardiac and intrapulmonary routes of administration can cause pain and distress because of the required methods of restraint and other procedural difficulties. Therefore, those routes of administration should not be used unless the animal is anesthetized.

Cervical dislocation is an acceptable method for euthanatizing rodents, provided that it is performed by appropriately trained personnel. Death is instantaneous and is caused by physical damage that occurs as the brain and spinal cord are manually separated by anteriorly directed pressure applied to the base of the skull. This technique might be more difficult to perform in hamsters, rats, and guinea pigs than in other rodents because of the strong muscles and loose skin of the neck region. If the method is selected, it should be remembered that it can produce pulmonary artifacts—blood in the alveoli and vascular congestion (Feldman and Gupta, 1976).

For decapitation, only a sharp, clean guillotine or large shears should be used to ensure a clean cut on the first attempt. It is also essential that the cut be made between the

atlanto-occipital joint to ensure that all afferent nerves are severed (NRC, 1992). Decapitation is more difficult in hamsters, rats, and guinea pigs than in other rodents because of the strong muscles and loose skin of the neck region. There has been considerable controversy about how rapidly unconsciousness occurs when this method is used and whether animals should be anesthetized before they are decapitated. There is evidence that unconsciousness occurs very rapidly (in less than 2.7 seconds) after decapitation (Allred and Bernston, 1986; Derr, 1991). Recent studies have shown that anesthesia can cause substantial alterations in arachidonic acid metabolism; lymphocyte assays; and plasma concentrations of glucose, triglycerides, and insulin (Bhathena, 1992; Butler et al., 1990; Howard et al., 1990). It can be concluded that in some cases anesthesia can interfere with the interpretation of data obtained from postmortem tissue samples and that appropriately trained personnel can perform decapitation humanely in rodents without anesthesia.

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Table 6.1 Typical "Core" Agents Monitored in Research Facilities[a]

Agent	Mice	Rats	Guinea Pigs	Hamsters
Kilham rat virus		+		
Minute virus of mice	+			
Mouse hepatitis virus	+			
Mycoplasma pulmonis		+		
Pneumonia virus of mice	+	+	+	+
Rotavirus	+			
Sendai virus	+	+	+[b]	+[b]
Sialodacryoadenitis virus (rat coronavirus)		+		
Simian virus 5			+[b]	+[b]
Theiler's murine encephalomeylitis virus	+			

[a]"Core" agents for each species are indicated by plus signs.
[b]Infection with related parainfluenza viruses can cause false-positive results of tests for Sendai virus and simian virus 5 (Parker et al., 1987).

Table 6.2 Infectious Agents of Rodents for Which Serologic Tests Are Available

Agent	Serologic Test Available[a]			
	Mice	Rats	Guinea Pigs	Hamsters
Clostridium piliforme (formerly called *Bacillus piliformis*)	+	+		
Cilia-associated respiratory (CAR) bacillus	+	+		
Ectromelia virus	+			
Encephalitozoon cuniculi	+	+	+	
Hantavirus	+	+		
K virus	+			
Kilham rat virus		+		
Lymphocytic choriomeningitis virus	+		+	+
Minute virus of mice	+			
Mouse adenovirus (MAd-FL, MAd-K87)	+	+		
Mouse cytomegalovirus	+			
Mouse hepatitis virus	+			
Mouse "orphan" parvovirus	+			
Mouse rotavirus	+			
Mouse thymic virus	+			
Mycoplasma arthritidis	+	+		
Mycoplasma pulmonis	+	+		
Pneumonia virus of mice	+	+	+	+
Polyoma virus	+			
Rat coronavirus and sialodacryoadenitis virus		+		
Rat cytomegalovirus		+		
Rat "orphan" parvovirus		+		
Reovirus 3	+	+	+	+
Sendai virus	+	+	+	+
Simian virus 5			+	+
Theiler's murine encephalomyelitis virus	+	+		
Toolen's H-1 virus		+		

[a]Agents for which serologic tests are available are indicated by plus signs.

Table 6.3 Important Rodent Bacterial Pathogens Culturable from Upper Respiratory Tract and Intestines[a]

Agent	Mice	Rats	Guinea Pigs	Hamsters	Gerbils
Bordetella bronchiseptica			+		
Campylobacter jejuni				+	
Citrobacter freundii (biotype 4280)	+				
Corynebacterium kutscheri	+	+		+	
Helicobacter spp.	+				
Mycoplasma pulmonis	+	+			
Salmonella spp.	+	+	+	+	+
Streptobacillus moniliformis		+			
Streptococcus equis (*zooepidemicus*)			+		
Yersinia pseudotuberculosis			+		

[a]Culturable pathogens are indicated by plus signs. Many commonly occurring bacteria can be present as pathogenic strains (e.g., *Escherichia coli* and *Streptococcus pneumoniae*) or as opportunistic pathogens (e.g., *Klebsiella* spp., *Pasteurella pneumotropica*, and *Pseudomonas aeruginosa*) in stressed or immunocompromised animals, or as agents of importance when transmitted from a carrier to a susceptible animal host (e.g., *Bordetella bronchiseptica*).

7

Facilities

Productive research programs that yield reproducible results depend on laboratory animal-care programs that combine good management and appropriate facilities. Such factors as facility location, design, construction, and maintenance influence the quality of animal care and the efficiency of operation. The general guidelines for planning and operating animal facilities described below provide a framework in which specific designs and procedures can be implemented on the basis of professional judgment. Minimal standards applying to the housing of guinea pigs and hamsters are published in *Animal Welfare Standards* (9 CFR 3.25-3.41). The *Good Laboratory Practice Standards* apply to the housing of animals used for studying substances regulated by the Food and Drug Administration (21 CFR 58) and the Environmental Protection Agency (40 CFR 160, and 40 CFR 792). Reports prepared by the Institute of Laboratory Animal Resources for the

National Research Council, such as this one, supplement the more general information contained in the *Guide* (NRC, 1985 et seq.). A series of texts on laboratory animals, sponsored by the American College of Laboratory Animal Medicine, provides specific information about the housing needs of mice, rats, hamsters, and guinea pigs (Ediger, 1976; Wagner and Foster, 1976; Baker et al., 1979; Lang, 1983; Otis and Foster, 1983; Small, 1983; Hessler and Moreland, 1984; Balk and Slater, 1987). The *Handbook of Facilities Planning, Volume 2: Laboratory Animal Facilities* (Ruys, 1991) addresses such topics as facility planning and basic design principles. Finally, articles having to do with facility design, construction, and management can be found in various journals and trade magazines.

LOCATION AND DESIGN

The location and design of an animal facility depend on the scope of institutional research activities, animals to be housed, need for facility flexibility, physical relationship to other functional areas, space availability, and financial constraints. The site and design might further depend on whether the facility is located in space initially constructed for housing animals or in remodeled space.

Careful consideration should be given to the location of an animal facility. Initial construction and subsequent operating costs can be influenced by the following:

- local geologic features;
- accessibility of the site;

- prevailing winds and other climatic conditions;
- availability and adequacy of utility and waste-disposal services;
- adjacent properties and buildings;
- suitability of the site for future expansion or building modification;
- state and local regulations and codes; and
- security needs.

Initial construction and subsequent operating costs of a facility can usually be minimized by placing support, care, and treatment areas adjacent to animal-housing space and on a single floor. If the facility extends into adjacent buildings, consideration should be given to placing the animal space on the same level and connecting it by a covered, climate-controlled passage to facilitate movement of animals and equipment.

Centralization Versus Decentralization

In a *centralized* animal facility, support, care, and treatment areas are adjacent to animal-housing space. The facility usually occupies a single floor or building; if it extends into adjacent buildings, the spaces are contiguous. Research personnel come to the animals. In a *decentralized* facility, areas where animals are housed and used are scattered among rooms, floors, or buildings separated by space that is not dedicated to animal care or support. Animal-housing areas are often adjacent to the laboratories in which the animals are used. In this situation, animal-care personnel come to the animals.

Centralization reduces operating costs of a facility because there is a more efficient flow of animal-care supplies. equipment, and personnel; more efficient use of environmental controls; and less duplication of support services. Centralization reduces the need to transport animals between housing and study sites, thereby minimizing the risk of disease exposure. It might also offer greater security by providing more control over access to the facilities and increasing the ease of monitoring staff and animals. A decentralized facility potentially costs more for initial construction because of requirements for environmental systems and controls for separate sites. Multiple cage washers might also be required. Although duplication increases costs, it does provide backups that can be used if a system or equipment fails at one site. Decentralization can reduce traffic at a single site, thereby facilitating disease- or hazard-control or containment programs. Decentralized facilities are generally more accessible to investigators and might offer a more efficent flow of research supplies, equipment, and personnel.

Functional Areas

In addition to the areas used for actual housing of animals, the *Guide* (NRC, 1985 et seq.) recommends making provisions for the following:

- specialized laboratories or individual areas for such activities as surgery, intensive care, necropsy, radiography, preparation of special diets, experimental manipulation, treatment, and diagnostic laboratory procedures;

- containment facilities or equipment if hazardous biologic, physical, or chemical agents are to be used;
- receiving and storage areas for food, bedding, pharmaceuticals and biologics, and supplies;
- space for the administration, supervision, and direction of the facility;
- showers, sinks, lockers, and toilets for personnel;
- an area separate from animal rooms for eating, drinking, smoking, and applying cosmetics;
- an area for washing and sterilizing equipment and supplies and, depending on the volume of work, machines for washing cages, bottles, glassware, racks, and waste cans; a utility sink; an autoclave for equipment, food, and bedding; and separate areas for holding soiled and clean equipment;
- an area for repairing cages and equipment; and
- an area to store wastes before incineration or removal.

Space Requirements

The total space occupied by an animal facility includes program (net) and nonprogram (gross minus net) space. Program space consists of the space allocated to animal housing and various functional areas. Nonprogram space consists of wall thicknesses, dead space, mechanical chases, corridors, stairwells, and elevators. The ratio of program to nonprogram space for facilities designed to house rodents and rabbits has been estimated to be 1:1, and

the ratio of housing to support space about 2:3 (Ruys, 1991). Many design factors influence those ratios, and they serve only as gross estimates of space allocation during planning of a facility. The animal-facility program space required in research institutions can be estimated more accurately by considering the number of faculty or staff using animals, anticipated animal populations, how the animals will be used, the health status of the animals, whether animals of differing health status will be used, and the dimensions of caging and support equipment.

The size of individual animal-holding rooms should be adequate to accommodate standard equipment, especially caging, and to allow adequate space to service both animals and equipment. Room dimensions also should provide flexibility of use. Rooms of 12 $\times$ 20 ft (3.7 $\times$ 6.1 m) have been suggested as the most efficient for housing mice, rats, hamsters, guinea pigs, and rabbits (Lang, 1980). However, room size should be based on the needs of the program. For example, preference might be given to smaller rooms or cubicles because they offer more opportunity to isolate animals by health status or use. Every effort should be made to provide the greatest amount of space for caging. Aisle space should be kept at a minimum but should be sufficient to allow cage changing, rack sanitation, and other husbandry manipulations.

Relative Relationships of Space

The relative relationship of animal rooms, support rooms, and administrative space should be such that traffic from contaminated to clean areas is eliminated and the efficiency

of movement of personnel, equipment, supplies, and animals is maximized. The location of animal-holding space will be determined to a great extent by the location of cage-sanitation facilities.

Corridors, Vestibules, and Anterooms

Rooms in an animal facility can be arranged along single or multiple corridors. The single-corridor arrangement provides more efficient use of space and can be as much as 20 percent less expensive to construct and also less expensive to operate than a comparable facility with multiple corridors (Graves, 1990). A multiple-corridor arrangement allows unidirectional movement, is less congested, and minimizes the potential for cross contamination of the animals.

Corridors should be wide enough to facilitate the movement of personnel and equipment. Although the *Guide* (NRC, 1985 et seq.) recommends a corridor width of 7 ft (2.1 m), single-corridor facilities might require wider corridors to reduce congestion.

Entry and exit airlocks and anterooms provide transitional areas between corridors and animal space. They can serve as sound barriers and should reduce the spread of contaminants and allergens. Although airlocks and anterooms slow movement of personnel, animals, supplies, and equipment by doubling the number of doors that must be passed, this slowing provides additional security. Storage of supplies and equipment in airlocks and anterooms should be limited to that essential to support activities in the adjoining animal rooms.

Interstitial Space

Service crews need access to the HVAC system, water lines, drainpipes, and electric connections. The *Guide* recommends making these utilities accessible through service panels or shafts in corridors outside the animal rooms (NRC, 1985 et seq.). Another option is to use an interstitial floor on which equipment can be checked or repaired without requiring entry into the animal facility.

CONSTRUCTION AND ARCHITECTURAL FINISHES

The *Guide* (NRC, 1985 et seq.) describes construction details and architectural finishes suitable for facilities that house rodents. In general, room surfaces should be moistureproof and free of cracks, unsealed utility penetrations, or imperfect junctions that could harbor vermin or impede cleaning. If rooms will be gas sterilized, they should be sealable. The finishes should be able to withstand scrubbing with detergents and disinfectants. All surfaces should be smooth enough to allow rapid removal of water, but floors should have enough traction to be skid-resistant. Surfaces that might be subjected to movement of equipment should be constructed of material that can withstand such movement. Curbs, guardrails, bumpers, door kickplates, and steel reinforcement of exposed corners help to minimize damage. Exterior windows and skylights are not recommended in animal rooms, because they can contribute to unacceptable variations in temperature and photoperiod.

MONITORING

Within an animal facility, the equipment and systems should be monitored to determine whether they are functioning or conforming to predetermined limits or guidelines necessary for successful operation. Temperature, humidity, airflow, air-pressure gradients, and illumination (intensity and photoperiod) in individual animal rooms should be checked. To be effective, a monitoring program should provide accurate, dependable, and timely results. The data collected should be reviewed by personnel who are trained to interpret the results, and the results should be provided to those who are authorized to take corrective action.

SPECIAL REQUIREMENTS

An animal's health status, genotype, or research use might require that it receive special housing. In addition to conventional animal rooms, various levels of barrier or containment housing or other specialized housing might be required to minimize variations that can modify an animal's response to an experimental regimen.

Barrier housing isolates animals from contamination. The degree of isolation depends on the equipment and procedures used and the design and construction of the barrier facility. Rodents usually housed in barrier facilities include microbiologically associated (defined-flora) and specific-pathogen-free rodents, severely immunosuppressed rodents, and transgenic rodents.

In a complete barrier system, isolator-maintained animals are introduced through entry

ports. Equipment and supplies enter through an autoclave or other sterilization or disinfection system. Personnel enter through a series of locks in which they remove their clothes and shower before donning barrier-room attire. Cage-washing and quarantine space might be included within such a barrier. Partial barriers differ from complete barriers in construction features, equipment, or operating procedures.

Facilities for animals used in projects that involve hazardous biologic, chemical, or physical agents should be designed so that exposure of personnel and other animals is minimized or prevented. *Biosafety in the Laboratory* (NRC, 1989b) describes four combinations of practices, safety equipment, and facilities (animal biosafety levels 1-4) recommended for infectious-disease activities in which laboratory animals are used. Conventional facilities that are consistent in design and operation with the standards described in the *Guide* (NRC, 1985 et seq.) also meet the standards for biosafety levels 1 and 2. Levels 3 and 4 require increasing degrees of containment.

Rodents are sensitive to noise and should be housed away from noise sources (see Chapter 5). The *Guide* describes design and construction features that control noise transmission, including double-door airlocks, concrete (rather than metal or plaster) walls, the elimination of windows, and the application of sound-attenuating materials to walls or ceilings (NRC, 1985 et seq.).

SECURITY

Each facility should consider developing a plan for preventing or minimizing the damage or work disruption that can result from a break-in or malicious damage. Procedures adopted should protect animals and personnel from injury and should protect equipment from theft or damage without creating limitations that adversely affect the quality of care or impede legitimate access to the facility. Administrative responsibility for security should be assigned, with the lines of authority clearly delineated. The plan should be reviewed regularly and modified as needed.

The number, design, and location of windows and doors influences the ability of a facility manager to control access. At the most basic level, physical security consists of key locks on doors. Computer-controlled card-access systems offer the ability to control and record entrance and egress; however, the computer network should be properly maintained and should be tamperproof. Closed-circuit television and motion monitors complement the efforts of security guards.

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8

Rodents that Require Special Consideration

Rodents with a wide variety of valuable genetic characteristics are available for use in many kinds of research (Altman and Katz, 1979; Festing, 1993; Festing and Greenhouse, 1992; Hansen et al., 1981; Hedrich, 1990; Lyon and Searle, 1989). Most are easily maintained with the husbandry techniques discussed in Chapter 5. However, some important research models, especially those with deleterious mutations, require special care. Some—such as mice that carry the homozygous mutation *scid* (severe combined immune deficiency), some strains of mice that carry the homozygous mutation *nu* (nude), and rodents exposed to sublethal irradiation—are so severely immunodeficient that contact with infectious agents of even low pathogenicity can cause severe illness and death, and they require isolation for survival (NRC, 1989). Others have specific requirements for the presentation of food and water; for example, food pellets must be placed on the cage floors and longer than

normal sipper tubes are necessary for rodents with mutations that cause dwarfing, and soft diets are essential for mice and rats with mutations in which the incisors fail to erupt (Marks, 1987). Many mutants are subfertile or sterile and require special breeding techniques to maintain the mutation.

A detailed description of the unique husbandry and breeding requirements for each model is beyond the scope of this book. Mating strategies for propagating lethal, sterile, or deleterious mutations have been described (Green, 1981). Those wishing to use mutant rodents should discuss with the investigator or company providing the animals whether there are special requirements for the animals' care and breeding. This chapter will address selected research models: immunodeficient rodents, wild rodents, rodents used for studying aging, mouse and rat models for type I (insulin-dependent) diabetes mellitus, and transgenic mice. Those models are relatively commonly used in research, and information on their husbandry is often difficult to find.

IMMUNODEFICIENT RODENTS

Rodents whose immune systems have been altered through spontaneous mutation, transgenic manipulation, or the application of immunosuppressive drugs or other treatments have long been useful models in biomedical research. However, the immunologic deficiencies that make these animals useful as models often render them susceptible to a host of opportunistic and adventitious infectious agents that would produce few or no effects in immunologically competent animals (Powles et al., 1992; Soulez et al., 1991). The

recommendations in this report that cover various rodent species generally apply to immunologically compromised rodents, but much more stringent housing conditions are often required to ensure the health of immunodeficient rodents.

Husbandry

In general, the cages or other implements used to house immunodeficient rodents should be capable of being adequately disinfected or sterilized on a regular basis. The housing systems should be capable of eliminating airborne contamination of the animals and should be capable of being manipulated without exposing the animals to microbiologic contamination during experimentation and routine husbandry procedures. In determining housing and husbandry requirements for maintaining immunodeficient rodents, it is important to consider the effects of various opportunistic and adventitious microorganisms on the type of research being conducted. The length of the study and the research goals will influence the attention to detail needed to prevent infection with such organisms. Maintaining animals in an axenic or microbiologically associated (defined-flora) state might involve a level of effort that is too great and techniques that are too complex for most experimental studies.

Plastic Cages with Filter Tops

This housing system consists of a shoebox cage usually constructed of transparent autoclavable plastic and a separate filter top—a plastic cap with a removable filtration surface in the top. The cap and cage fit together snugly but do not necessarily form a perfect seal. A stainless-steel wire-bar top keeps animals from gaining access to the filter top and provides a food hopper and a holder for a water bottle. An opaque cage can be used, but a transparent cage facilitates routine animal observation without the need to open the cage except for feeding and watering, sanitation, and experimentation. Cages and filter tops and all food, water, and bedding used in those cages should be sterilized.

All changing and manipulation of animals should be done in a laminar-flow work station using aseptic technique. Sterile gloves or disinfected forceps should be used to manipulate animals in any individual cage, and all experimental manipulations should be done so as to minimize or eliminate contamination of the animals. The successful maintenance of animals with this housing system depends directly on rigid adherence to aseptic technique in all aspects of animal and cage manipulation. Although the initial purchase cost of this housing system might seem relatively low compared with that of other systems for housing immunodeficient rodents, the requirement for laminar-flow change stations, sterile supplies, and other operating expenses leads to a substantial continuing cost. Moreover, only minimal mechanical safeguards are built into this system, and success depends absolutely on technique.

A major drawback to using plastic cages with filter tops is that there is a low rate of air

exchange between the cage and the room. As a result, bedding might have to be changed more frequently to minimize the buildup of toxic wastes and gases and keep relative humidity appropriately low.

Individually Ventilated Plastic Cages with Filter Tops

This housing system uses plastic cages with filter tops that are constructed and maintained like those previously described. However, an air supply has been introduced into each cage with a special coupling device similar in appearance to the fittings used for automatic watering. Air is supplied to a cage under positive pressure and is exhausted through the filter top. Other ventilation options with respect to positive and negative pressure, as well as a separate exhaust, are also available. Usually, the air supplied to these cages is filtered with a high-efficiency particulate air (HEPA) filter. This system has advantages over the nonventilated plastic cages, but its principal disadvantage is the potential for contamination of the fittings that are used to introduce air into the cages. Rigorous attention must be paid to disinfection of these fittings. The efficiency of this system in protecting immunodeficient animals from infectious agents has not been extensively evaluated.

Isolators

Large isolators capable of housing many rodent cages are commercially available. As discussed elsewhere in this report, isolators are ideal for excluding microorganisms in that they rely very little on individual technique for many husbandry procedures or experimental manipulations. Traditionally, they have been used for housing axenic or microbiologically associated animals. Many varieties of isolators are available; the most common are those made of a flexible bag of vinyl or other plastic material, such as polyurethane. Modern isolators are relatively easy to use and provide investigators and animal-care technicians with easy access to the animals. Special precautions are not needed, because all manipulation is done through built-in glove sleeves with attached gloves. All supplies provided to the isolator are sterilized and are introduced through a port; a chemical sterilization and disinfection procedure is used to decontaminate the outside of the items that have been previously sterilized and wrapped with plastic or other materials that can withstand chemical sterilization or disinfection. Air into and out of the isolator is usually highly filtered. As opposed to plastic cages with filter tops, the isolator offers an advantage in health assessment, in that a large number of animals are maintained as a single biologic unit. Isolators made of rigid plastic with a flexible front offer additional advantages, such as integrated racking, individual lighting, lower operating air pressures, and conservation of space.

Recent advances in construction coupled with the availability of vacuum-packed and irradiated supplies has made isolators for housing immunologically compromised animals a cost-competitive alternative to cages with plastic filter tops.

HEPA-Filtered Airflow Systems

These systems have a variety of forms, including modular chambers, hoods, and racks that are designed to hold cages under a positive flow of HEPA-filtered air. In some instances, plastic cages with filter tops have been used in laminar airflow racks that supply a steady stream of HEPA-filtered air across the cage tops to facilitate air diffusion through the filters. The design of such racks usually involves a blower that pushes air across a HEPA filter and then into a large space (or plenum) that contains thousands of small holes. The holes are designed to permit air to be blown across shelves on which cages are placed. Because many cages must fit on the shelves, there is considerable eddying or turbulence of air across the tops of the cages. Once the cages are pulled forward 10-20 cm beyond the lip of a shelf, the air no longer flows laminarly and mixes with room air. Another system consists of a flexible-film enclosure in which HEPA-filtered air is supplied under positive pressure to a standard rack or group of racks containing filter-topped cages. For both systems, all manipulations must be made in a laminar-flow work station using aseptic technique.

Environmental Considerations

Immunodeficient rodents have been successfully maintained at recommended room temperatures for rodents (NRC, 1985 et seq.). Several theoretical considerations suggest that some immunodeficient rodents, specifically those lacking hair or thyroid glands, might

require a higher ambient temperature because of hypothyroidism and poorly developed brown adipose tissue, which reduce the capability for nonshivering thermogenesis (Bripois et al., 1980; Pierpaoli and Besedovsky, 1975; Weihe, 1984). In practice, such temperatures are not necessary and in fact can be detrimental because they tend to create husbandry problems, including increased decomposition of feed and bedding, increased rate of growth of environmental bacteria, and an uncomfortable working environment for animal-care personnel. In addition, because housing of immunocompromised animals generally requires systems that restrict airflow and heat transfer, temperatures in the animal cages tend to be higher than ambient temperature; therefore, increasing the room temperatures is generally not necessary.

Humidity and ventilation should be consistent with recommendations in the *Guide* (NRC, 1985 et seq.). It is important to remember that many of the containment systems result in increased relative humidity and restrict ventilation. Therefore, animal density, bedding-change frequency, and the relative humidity of incoming air should be adjusted to compensate for some of these differences.

Food and Bedding

Food and bedding for immunocompromised animals should be sterilized or pasteurized to eliminate vegetative organisms. Depending on the method of sterilization selected, fortification of feed with vitamins might be required. Steam sterilization can drastically reduce concentrations of some vitamins and can accelerate the decomposition of some

vitamins during storage. Other treatments, such as irradiation, result in much less destruction of these nutrients and so might not require the same degree of fortification of feed before or after sterilization. Adequate validation of the sterilization process is essential to ensure that food or bedding does not serve as a source of contamination.

Water

The water supplied to immunodeficient animals must be free of microbiologic contamination. Sterilization of water is the only sure method of eliminating such contamination. Sterilization can be accomplished by heat treatment, zonation, or filtration. All those processes must be adequately controlled and validated. Other water treatments have been advocated for use with immunocompromised animals, including acidification, chlorination, chloramination, and the use of antibiotics and vitamins. The principal purpose of adding treatment materials to water is to reduce bacterial growth and hence the likelihood of cross contamination in case bacteria are introduced into the water supply. The treatments are not without effects, which can include alteration of bacterial flora, alterations in macrophage and lymphocyte function, reduction in water consumption, and exposure to chlorinated hydrocarbons (Fidler, 1977; Hall et al., 1980; Herman, 1982; McPherson, 1963; Reed and Jutila, 1972). In general, the use of the treatments is not an adequate substitute for sterilization of water and should be used only as an adjunct.

Health Monitoring

Many immunodeficient rodents are susceptible to a greater range and incidence of diseases caused by microorganisms than are immunocompetent animals. The lack of a completely functioning immune system often results in more dramatic clinical signs and pathologic changes than would be seen in immunocompetent animals. Because some immunodeficient animals often lack the ability to produce antibodies in the presence of microorganisms, serology is often not useful for diagnosis. Screening for such agents might require the use of immunocompetent sentinel animals of the appropriate microbiologic status. Most commonly, soiled bedding is used as a means of exposing sentinel animals to the immunocompromised animals, and a period of 4-6 weeks of exposure is often required before samples can be taken. Sentinels must be housed under the same environmental conditions and microbiologic barriers as the immunocompromised animals. Health monitoring of animals maintained in individual plastic cages with filter tops is complicated by the potential for contamination of individual cages, as opposed to large groups of cages, with a particular microorganism. Because frequent screening of every cage is not economically feasible, statistical schemes for sampling or batching soiled bedding for exposure of sentinel animals is often required. That is less of a problem with the use of isolators in which large numbers of cages are kept in the same microbiologic space.

Purchase of animals from commercial sources or transfer of animals from other institutions entails some risk with respect to immunocompromised animals. Health status can be compromised during packing, transport, unpacking, and housing of animals. It is important to provide adequate quarantine and stabilization time to allow assessment of the health status of these animals before they are used in experimental procedures. Appropriate

precautions should be taken to disinfect the outside of transport containers and to examine them for integrity. Specialized containers have been developed for transport of immunocompromised rodents and should be used whenever possible.

WILD RODENTS

A large number of rodent species have been maintained and bred in a laboratory environment. Wild rodents are used in many fields of research, including genetics, reproduction, immunology, aging, and comparative physiology and behavior. Hibernating rodents, such as woodchucks (*Marmota monax*) and 13-lined ground squirrels (*Spermophilus tridecemlineatus*), are used to study control of appetite and food consumption, control of endocrine function, and other physiologic changes associated with hibernation. Woodchucks are also used as models to study viral hepatitis and virus-induced carcinoma of the liver.

Wild rodents can be obtained by trapping or, in a few instances, from investigators who are maintaining them in the laboratory. Trapping is the simplest way to acquire wild rodents. However, a collector's permit is required in most states, and it is also important to confirm that the species to be trapped, as well as other species in the trapping area, are not threatened or endangered. It is best to begin trapping with an experienced mammalogist.

A search of the literature will locate investigators who maintain feral rodents in a laboratory environment; however, these scientists usually do not maintain enough animals to permit distribution of more than a few. Colonies of wild rodents are listed in the *International Index of Laboratory Animals* (Festing, 1993), in *Annotated Bibliography on*

Uncommonly Used Laboratory Animals: Mammals (Fine et al., 1986), and in the Institute of Laboratory Resources (ILAR) Animal Models and Genetics Stocks Data Base (contact: ILAR, 2101 Constitution Avenue, Washington, DC 20418; telephone, 1-202-334-2590; fax, 1-202-334-1687). Several species of the genera *Mus* and *Peromyscus* are more widely used and are available from laboratory-bred sources.

Hazards

Wild-trapped rodents commonly carry pathogens and parasites that are usually not found in or have been eliminated from animal facilities; therefore, appropriate precautions must be taken to prevent disease transmission between feral and laboratory stocks (see Chapter 6). The primary hazard to personnel is getting bitten. Personnel should always wear protective gloves when handling wild rodents. Mice can be handled with cotton gloves (Dewsbury, 1984) or can be moved from place to place in a tall, thin bottle (Sage, 1981). Metal meat-cutter's gloves can be worn under leather gloves for handling larger, more powerful species, such as black rats (*Rattus rattus*) (Dewsbury, 1984). Elbow-length protection, such as leather gloves and gauntlets, should be worn for handling woodchucks because the animals can turn rapidly and bite the inside of the handler's forearm.

Wild rodents can carry zoonotic diseases, such as leptospirosis and lymphocytic choriomeningitis, that are not usually encountered in laboratory-bred rodents (Redfern and Rowe, 1976). Personnel should be offered immunization for tetanus, and anyone that is bitten should receive prompt medical attention. Wild-caught mastomys [*Praomys* (*Mastomys*)

natalensis] cannot be imported into the United States, because it is a host for the arenavirus that causes the highly fatal Lassa fever.

Care and Breeding

Many small species can be housed in standard mouse and rat cages (Boice, 1971; Dewsbury, 1974a); solid-bottom cages with wood shavings or other bedding are preferred (Dewsbury, 1984). Most small wild rodents are much quicker than domesticated rodents and can easily escape if the handler is not careful. It is advisable to open cages inside a larger container, such as a tub or deep box, to avoid escapes (Sage, 1981; Dewsbury, 1984). Most species do well if given ad libitum access to water and standard rodent diets; however, voles do better on rabbit diets (Dewsbury, 1984; Fine et al., 1986). General guidelines for caring for wild rodents have been published (Redfern and Rowe, 1976; CCAC, 1984). Fine et al. (1986) have summarized and provided references for laboratory care and breeding of kangaroo rats (*Dipodomys* spp.); grasshopper mice (*Onychomys* spp.); dwarf, Siberian, or Djungarian hamsters (*Phodopus sungorus*); Chinese hamsters (*Cricetulus barabensis*, also called *C. griseus* or *C. barabensis griseus*); common, black-bellied, or European hamsters (*Cricetus cricetus*); white-tailed rats (*Mystromys albicaudatus*), fat sand rats (*Psammomys obesus*), voles (*Microtus* spp.), four-striped grass mice (*Rhabdomys pumilio*), and degus (*Octodon degus*). Guidelines on laboratory maintenance of hystricomorph (Weir, 1967, 1976; Rowlands and Weir, 1974) and heteromyid (Eisenberg, 1976) rodents have been published. Mammalogists and other investigators experienced in working with specific

species are also excellent sources of information.

Breeding of many wild species is similar to that of domesticated rodents. Some (e.g., voles and deer mice) breed almost as well in captivity as do domesticated species (Dewsbury, 1984). Others (e.g., four-striped grass mice) require special conditions (Dewsbury and Dawson, 1979; Dewsbury, 1974b). A few investigators have reported that breeding of wild *Mus* species is difficult unless running wheels are provided; exercise (up to 10-15 miles/day) apparently causes females to come into estrus and begin a normal breeding cycle (Schneider, 1946; Andervont and Dunn, 1962). Others have not had this problem (Sage, 1981). Pheromones are extremely important in the reproduction of some wild rodents; too frequent bedding changes preclude successful reproduction. A nesting enclosure might be appropriate and should be constructed of a durable material that is easily sanitized, such as plastic or corrosion-resistant metal. Nesting material might improve neonatal survival.

Peromyscus

Peromyscus maniculatus (the deer mouse) and *P. leucopus* (the white-footed mouse) can be maintained with the same husbandry procedures as laboratory mice. A maximum of seven can be housed in 7 x 10 inch plastic cages. Standard rodent feed and water should be give ad libitum. Rabbit or guinea pig feed should not be used, nor should such supplements as fresh vegetables, raisins, and sunflower seeds. Except for breeding, sexes should be housed separately. *Peromyscus* are reasonably cold-tolerant; the suggested temperature is 22-25°C (71.6-77.0°F), and the ambient temperature should not exceed 33°C (91.4°F).

For breeding, single male-female pairs are formed at the age of about 90 days and remain together throughout life. The estrous cycle is 5 days (Clark, 1936). Females caged alone or with other females will not come into estrus. The average reproductive life of *Peromyscus* is 18-36 months. Females should be checked regularly for pregnancies. Copulatory plugs are not a reliable indication of mating, because they are inconspicuous. Lighting is very important in breeding. A 16:8-hour light:dark ratio is generally satisfactory. Continuous light will produce anestrus, and breeding difficulties can sometimes be overcome by reducing the light cycle to a light:dark ratio of 12:12 hours and gradually increasing it to 16:8 over a 3-week period (W. D. Dawson, Peromyscus Stock Center, unpublished). Introduction of a strange male into a cage with a pregnant female can block the pregnancy (Bronson and Eleftheriou, 1963). Gestation is 22 days, except in lactating females, in which it is delayed by 4-5 days. Females enter postpartum estrus about 12 hours after delivery and then remate; therefore, serial litters are produced at 26- to 27-day intervals. Litter size is usually three to six and rarely exceeds eight. Males provide some of the care for the young. Additional information on the care and breeding of *Peromyscus* can be obtained from the Peromyscus Stock Center, Department of Biology, University of South Carolina, Columbia, SC 29208 (telephone, 803-777-3107; fax, 803-777-4002).

Woodchucks

Woodchucks (*Marmota monax*) have been successfully housed indoors in standard cat, dog, or rabbit cages (Young and Sims, 1979; Snyder, 1985) and outdoors in pens or runs

(Albert et al., 1976). Enclosures must be carefully secured because a woodchuck can squeeze through any hole large enough to admit its head (Young and Sims, 1979). Each animal should be provided with a nesting box and nesting material, especially if it is housed under conditions that will induce hibernation, for example, in a cold room or, in a cold climate, outdoors in an unheated enclosure. Very thin woodchucks will not survive hibernation (Young and Sims, 1979). Usually, adult females are housed in small groups, and males are housed individually except during breeding season. However, young males and females can be kept together through their first year (Young and Sims, 1979). Food and water should be made available ad libitum. Water should be provided in heavy porcelain bowls. Standard bottles and sipper tubes are not satisfactory, because the animals grip the tubes in their teeth and shake them until they are dislodged from the bottles (Young and Sims, 1979; Snyder, 1985). Woodchucks do well on commercial rabbit diet (Young and Sims, 1979).

AGING COHORTS

Mice and rats have been favored by mammalian gerontologists as experimental models because of their relatively short and well-defined life spans, small size, comparatively low cost, and the large and growing store of information on their genetics, reproductive biology, physiology, biochemistry, endocrinology, neurobiology, pathology, microbiology, and behavior. However, the term *comparatively low cost* is used advisedly. The true cost in 1994 of producing one 24-month-old rat was approximately $200 and a similarly aged mouse

$95; the cost for producing one 36-month-old rat was approximately $350 and a similarly aged mouse $175 (DeWitt Hazzard, National Institute on Aging, National Institutes of Health. Bethesda, Maryland, unpublished). The cost to investigators is slightly more than half that amount because production is subsidized by the National Institute on Aging. A problem faced by investigators who use aged animals is periodic shortages in older cohorts of some strains.

General Considerations

Strictly speaking, *aging* can refer to all changes in structure and function of an organism from birth to death; however, mammalian gerontologists generally confine their experiments to alterations that occur after the onset of sexual maturity and the transition from the juvenile to the young adult phenotype. In sampling for some measure of aging or accruing pathologic conditions, 6-month-old animals will usually provide a normal baseline, and sampling should be carried out at 6-month intervals. Many investigators consider a 24-month-old rodent to be "old"; however, age-related changes in a number of characteristics are often more pronounced in still older animals.

The mean life span (MnLS) of ad libitum-fed (AL-fed), hybrid strains of specific-pathogen-free (SPF) mice or rats is often around 30 months, whereas that for calorically restricted (CR) animals, depending on the regimen used, can be 30 percent longer

Because caloric restriction retards or eliminates common forms of chronic renal disease and a variety of neoplasms, some gerontologists believe that such nutritional management should be the norm. Comparative changes in AL-fed versus CR rodents are increasingly used to test the validity of putative biologic markers of aging rates.

Survivorship in any colony used for gerontologic research should be determined repeatedly. Survival curves for SPF mice and rats should exhibit a classic "rectangularization pattern," that is, a survival curve should nearly parallel the X axis close to the 100-percent survival level for a prolonged period and then decline sharply as the population nears the species' maximum life span (MxLS), which is defined as the age at which only 10 percent of the animals are surviving. A linear survival curve indicates a problem in the population (e.g., exposure to infectious disease). Patterns of age-related pathology within a colony should be repeatedly evaluated through systematic sampling and necropsy of cohorts of various ages (including histologic examination of the major organs). Any animal euthanatized during the course of a study on aging should be necropsied to determine whether the cause of death, such as a specific lesion or neoplasm, could seriously affect the interpretation of the experimental data. For example, the occurrence of lymphoma involving primarily the spleen of old mice of some strains not only decreases survival, but might cause death before other expected findings can occur; this limits the value of these strains in some studies of age-related immunology. A good deal of information is now available on the pathology of aging cohorts of commonly used laboratory mice and rats (Burek, 1978; Myers, 1978; Altman, 1985; Wolf et al., 1988; Bronson, 1990).

Laboratory Mice

There are obvious advantages to using genetically defined strains for research on aging. Inbred or F1 hybrid strains provide a reproducible gene pool, and so permit a more rigorous evaluation of environmental variables, such as caloric restriction. However, in some circumstances, such as longitudinal studies with markers of aging or searches for longevity-assurance genes, the widest possible allelic variability might be desired. For those purposes, systematically outbred animals might suffice, although in the development of such lines, including so-called Swiss mice, the tendency to select breeding pairs for docility and breeding efficiency has resulted in a loss of genetic heterogeneity. An alternative approach is to develop an 8- or 16-way cross between established inbred lines (van Abeelen et al., 1989).

Recombinant inbred mice can also be useful for aging research because they provide a reassortment of linked parental genes (see Chapter 3). Recombinant congenic strains are of special interest for the analysis of polygenic traits (Démant and Hart, 1986; van Zutphen et al., 1991) because they contain a small fraction of the genome of a genetically defined donor line against a genetic background derived from another genetically well-defined strain. For a discussion of the specific uses and relative values of inbred, congenic, recombinant inbred, and nongenetically defined populations, see Gill (1980).

Eight SPF mouse strains, commonly used for gerontologic studies are available from the NIA: inbred strains A/HeNNia, BALB/cNNia, CBA/CaHNNia, C57BL/6NNia, and DBA/2NNia and hybrid strains BALB/cNNia x C57BL/6NNia F1 (CB6F1), C57BL/6NNia x C3H/NNia F1 (B6C3F1), and C57BL/6NNia x DBA/2NNia F1 (B6D2F1). Crl:SW outbred

stock is available commercially. Nude mice have also been suggested for gerontologic research (Masoro, 1990), but they are not available from NIA. By using mouse stocks obtained from NIA for research on aging, an investigator avoids changes in genetic characteristics and phenotypes caused by genetic drift in animals from disparate sources (see Chapter 3). An advantage to using well-studied strains is that historical baseline measures are available for comparison, including characteristic age-related pathologic conditions that might complicate the research (see Hazzard and Soban, 1989, 1991, for bibliographies). Life tables for most mouse strains have been published and are summarized by Abbey (1979), and Masoro (1990) presents accumulated data from several sources (see also Green and Witham, 1991). MnLS and MxLS are required in most cases as background data when choosing a strain. More extensive survival data can be obtained from survival curves like those compiled for the SPF colonies of aging NIA mice maintained at the Division of Veterinary Services, National Center for Toxicological Research (NCTR) in Jefferson, Arkansas.

A group of related sublines derived from AKR mice and known as SAM (senescence-accelerated mice) have also been developed. SAM mice display multiple pathologic conditions, have an MnLS of as little as 200 days, and have an MxLS of as little as 290 days. They respond to caloric restriction in the same manner as do other strains of mice (Takeda et al., 1981; Umezawa et al., 1990).

Rats (*Rattus norvegicus*)

Four strains are available from NIA: inbred strains BN/RijNia (Brown Norway) and F344/NNia (Fischer 344) and hybrid strains BN/RijNia x F344/NNia F1 (BNFF1) and F344/NNia x BN/RijNia F1 (FBNF1). Inbred strains BUF/N (Buffalo) and LEW (Lewis) and outbred stocks LE (Long Evans), SD (Sprague Dawley), and WI (Wistar) have also been used in research on aging. These are available commercially as young animals but seldom as old animals. Life tables are available for each of those stocks and strains (Hoffman, 1979; Masoro, 1990).

Although rats were previously believed to have longer life spans than mice, recent studies indicate that, the life spans of rats and mice are similar (Table 8.1). Rats' larger size might make them more useful than mice for some studies of aging, such as those involving surgery, and rats are widely used in studies on the neurobiology of aging. As do mice, aging cohorts of rats exhibit an increased prevalence of various neoplasms. The prevalence of specific kinds of neoplasms varies among strains. Infectious diseases, including a chronic respiratory complex associated with *Mycoplasma pulmonis*, can also affect life span. The incidence of *M. pulmonis* in rats has been found to be 38 percent in conventionally housed colonies and 0 percent in SPF colonies (NRC, 1991). Thus, cesarean derivation and barrier maintenance can eliminate *M. pulmonis* associated with chronic respiratory disease of rats.

Husbandry

There is evidence of an age-related decline in immune response (Miller, 1991), therefore, maintenance of an SPF microbiologic status, under clearly defined and regularly monitored conditions, is a requirement for an aging colony. Mice and rats in an aging colony can be housed in groups (usually four to five animals per cage) or individually. The latter is necessary for both test (CR) and control (AL-fed) animals in caloric-restriction studies. In some colonies, an exercise device, such as a wheel, is provided. The results of studies on whether group housing or exercise facilitation extend MnLS or MxLS vary (Menich and Baron, 1984; Skalicky et al., 1984; Clough, 1991; Holloszy and Schechtman, 1991; Masoro, 1991). A complication of group housing occurs as the old animals begin to die. When that occurs, cages no longer have identical conditions; some contain several animals and others contain only one or two animals. Another complication of group housing, especially among males, is the fighting and threat stress that occurs between animals when dominance is being asserted. The effect of such stress can substantially affect the results of studies on survival, metabolism, and behavior. If males are to be group-housed, they should be grouped immediately after weaning. In some strains, however, this will not prevent fighting. In some instances, the death of one animal in a cage will be followed by the deaths of the rest of the animals in that cage; whether this is caused by an opportunistic pathogen or by the stress of the first animal's death is not clear. Conversely, individual housing is probably stressful initially and might promote inactivity. Thus, the choice of a housing plan depends on the sex and strain of the experimental animals and on the experimental protocol.

Room lighting is especially important in gerontologic research in which performance is measured. Because of the retinal damage that can be caused in albino rodents by exposure to

moderately bright light (see Chapter 5), placement of individual cages in relation to the lighting source could influence performance over time. An additional consideration is the light:dark cycle. When CR animals are being compared with AL-fed controls, it is desirable to regulate the light cycle so that both groups will begin eating simultaneously, and activity, cell division, hormone concentrations, and other characteristics will be measured in both groups at similar times on the blood-glucose and -insulin curves. Mice and rats are essentially nocturnal, and AL-fed animals naturally begin feeding shortly after the dark cycle begins. CR animals, in contrast, begin to eat immediately after they are fed, which is usually during the light cycle, and consume most of their food quickly. Both sets of animals can be induced to eat at the same time by reversing the light:dark cycle so that the animal room is dark during the workday. If the light:dark cycle is reversed, the illumination used in the room during the workday should not be visible to the animals.

The temperature of the room and heat-retaining characteristics of the cages are important in studying old or CR animals, which have difficulty in adjusting to cold. Masoro (1991) discusses environmental conditions for aging rats, including the desirability of providing a room temperature somewhat higher than normal. Given the limited knowledge in this regard, a room temperature of 25-27°C (77.0-80.6°F) is suggested for individually housed aging mice and rats, and a somewhat lower temperature for group-housed animals. Variables that will affect this recommendation are the characteristics of the caging (e.g., dispersion of heat through plastic versus through metal and the number of surfaces open to the air) and the airflow and air currents in the room (see Chapter 5).

As discussed previously, diet is a major consideration for aging animals. It affects

longevity, perhaps by influencing metabolism and certainly by influencing pathology. Not only caloric restriction, but also the effect of quantity and quality of the protein fed is important (Iwasaki et al., 1988), particularly for strains susceptible to kidney disease. One good high-quality diet is NIH-31, which is used by NCTR for the NIA colonies and by institutions that use animals from the NIA colonies.

Record-Keeping

Record-keeping is discussed in Chapters 4 and 5. Some special considerations apply in aging rodent colonies. In long-term breeding colonies, records of paired-mated sublines should be kept so that selection for life-table characteristics can be either enhanced or limited. Careful records are obviously required for four- or eight-way matings and for the development of recombinant inbred strains. A few animals should be euthanatized and necropsied at regular intervals throughout the study. In the case of mice and rats, this process should begin no later than the age of 18 months.

Transportation and Stabilization

Aged mice and rats are especially susceptible to physical stresses, and this should be a consideration in shipping, as well as in housing the animals. If animals are shipped in very hot or very cold weather, especially if there will be an intermediate holding period in an airport building, they can become debilitated or die. CR mice, in particular, have reduced

resistance to cold because of their limited metabolic reserves. It is also difficult to maintain a diet regimen if shipping requires more than 24 hours. The best course of action is to pick up the animals at the airport as soon as they arrive. Transport cartons designed to protect against temperature changes and to maintain SPF status should be used. Arriving shipments of aged SPF rodents should be placed in a barrier facility immediately, even if they will be euthanatized soon after arrival. Failure to do so might lead to bacterial or viral infections that will affect physical performance, immune function, enzyme concentrations, standard blood values, or other characteristics that will be measured. A 2-week quarantine period should be imposed on all arriving shipments of aged animals before they are used in experiments to allow time for incipient infections, if present, to be expressed. Small (1986) has reviewed quarantine periods, particularly with regard to the introduction of communicable diseases (see also Chapter 6). The value of a period to stabilize physiologic and behavioral responses probably varies with the study and should be established by each investigator.

Veterinary Care and Surveillance

Because there is an age-related decline in immune response (Miller, 1991), old mice and rats are especially susceptible to infectious diseases. Therefore, regular microbiologic monitoring (see Chapter 6) is essential for maintaining their SPF status. Sentinel animals should be used for monitoring because aged animals are usually too valuable to euthanatize or to subject to multiple blood-collection procedures. Infectious agents of particular concern

to gerontologists are mouse hepatitus virus, Sendai virus, rotavirus, and *Mycoplasma pulmonis* in mice and Sendai virus, Kilham rat virus, rat corona/sialodacryoadenitis virus, and *Mycoplasma pulmonis* in rats (Lindsey, 1986; NRC, 1991). Those agents are of concern because they affect either immune function or general health.

Care of the animals and maintenance of their microbiologic status are usually overseen by the veterinary staff. However, to provide an early warning of incipient health problems, the research staff should observe each animal daily, including weekends and holidays. Moribund or dead animals should be picked up daily before postmortem changes make useful necropsy impossible. A full discussion of barrier facilities and surveillance programs and a summary of infectious disease agents and the systems that they affect have been published (NRC, 1991).

Important considerations to investigators who use aging animals are the timing and method of euthanasia of moribund animals. It is generally considered inhumane to allow old and sick animals to die naturally; however, gerontologic research often requires an accurate record of the time of death. Even if a recorded time of death accurate only to within 24-48 hours would satisfy the experimental protocol, it is difficult to obtain because fragile old mice or rats can appear moribund for days or weeks before they die. Signs of imminent death that can be used to decide when to perform euthanasia are cessation of eating for 48 hours, reduction of body temperature (determined by touching the animals with alcohol-washed fingers or measuring with an electronic thermometer), or maintenance of an immobile posture even if given a gentle stimulus. Each investigator should develop his or her own system with the guidance of the attending veterinarian and, having chosen it, should

adhere to it rigorously. An advantage for the investigator of euthanatizing the animal is the ability to obtain usable tissue specimens and necropsy findings. Methods of euthanasia are discussed in Chapter 6.

Other Rodent Species Used for Gerontological Research

Other Species of *Mus*

A number of interesting species of wild *Mus* and wild subspecies of *Mus musculus* are being adapted for laboratory use (Potter et al., 1986; Bonhomme and Guénet, 1989), but little is known about their life-table characteristics. *Mus caroli* (a rice-field mouse of Southeast Asia) is the single exception. Data on survival, reproductive life span, and age-related pathology have recently been published (Zitnik et al., 1992). The MxLS observed from among cohorts of 249 males and 231 females were 1,560 and 1,568 days, respectively. Gompertz analysis indicated an aging rate only slightly less than that published for wild *Mus musculus*. The shape of the survival curve (especially for females), however, suggests that many animals have died from causes not related to aging, such as fighting and acute stress.

Peromyscus spp.

The best studied member of the genus *Peromyscus* is *Peromyscus leucopus*, the white-footed mouse (Sacher and Hart, 1978), which has a life span about twice that of the laboratory mouse (Sacher, 1977). *Peromyscus*, however, is only "mouse-like"; it has been

separated from *Mus musculus* for 15-37 million years (Brownwell, 1983). Given that caveat, *Peromyscus* will continue to be useful in broader comparative gerontologic studies because it has adapted well to laboratory conditions. As with all such "domesticated" wild strains, however, a substantial degree of genetic diversity is lost because of the small numbers of animals used to initiate laboratory populations.

Guinea Pigs

The guinea pig (*Cavia porcellus*) has been somewhat neglected by gerontologists because of its comparatively large size, relatively long life span, and relatively high cost of maintenance. Although published survival curves have indicated an MxLS of around 80 months (Rust et al., 1966), some have recorded an MxLS of close to 10 years (Kunst'yr and Naumann, 1984). As with all iteroparous species (species that reproduce more than once in a lifetime) that have not been extensively used for research on aging, the MxLS is likely to be underestimated because record longevities are a function of population size. At least three aspects of guinea pig biology make them of special interest to gerontologists: Like humans, guinea pigs are unable to synthesize ascorbic acid and so are candidates for studies of the free-radical theory of aging (Harman, 1986); their cells appear to be resistant to transformation in vitro (like those of humans and unlike those of mice and rats) (T. H. Norwood and E. M. Bryant, Department of Pathology, University of Washington, Seattle, Washington, unpublished); and the considerable body of research that has been carried out on their auditory system (McCormack and Nutall, 1976) might provide useful background in

studies on the pathogenesis of presbycusis.

Guinea pigs are highly susceptible to a variety of infectious diseases; therefore, it is important to maintain them under SPF conditions for gerontologic research. Several such colonies have been established. Husbandry and dietary requirements of guinea pigs have been discussed in Chapter 5.

Hamsters

Primary cultures of Syrian hamster (*Mesocricetus auratus*) somatic cells are often used to study the cellular basis of aging. Cellular function, particularly replicative capacity, can be analyzed in culture with a degree of experimental control that cannot be achieved in living organisms. Normal diploid somatic cells of all studied mammalian species initially divide rapidly in culture, but the replicative capacity or life span of cells is limited, that is it eventually declines. Some of the cells from some species, however, are spontaneously "transformed" and exhibit indefinite replicative potential (Rubin et al., 1990; Wright and Shay, 1992). Transformation in primary cultures of mouse somatic cells is very rapid and difficult to study, whereas primary cultures of guinea pig somatic cells are resistant to transformation. Syrian hamsters exhibit transformation properties intermediate between those of mice and those of guinea pigs. Investigators interested in a manageable system for studying both the limited replicative life span of cells and their ability escape from such a limitation have found this species to be useful (e.g., Sugawara et al., 1990; Bols et al., 1991; Deamond and Bruce, 1991).

Recent data on survival and pathology are available for a colony of outbred male Syrian hamsters (Deamond et al., 1990). On the basis of 150 spontaneous deaths, the MnLS was 19.5 months, and the MxLS was 36 months. More than 35 inbred strains of Syrian hamsters have been described; most of these have not been carefully investigated in gerontologic research, and many are extinct.

The Turkish hamster (*Mesocricetus brandti*), like other hamsters, offers an opportunity to investigate how hibernation might modify rates of aging and life span (Lyman et al., 1981). The direct correlation found between life span and the amount of time spent in hibernation is consistent with the hypothesis that one or more processes of aging are slowed during hibernation (Lyman et al., 1981).

Chinese hamsters (*Cricetulus griseus*) are of interest to cytogeneticists because their chromosomes are rather easy to study (Brooks et al., 1973). Several outbred, inbred, and mutant stocks have been developed, but they are not as readily available as some other rodents. The life span characteristics of this species have not been rigorously investigated; however, although typical survival curves have been demonstrated for females, the curves for males, which usually live longer, are atypical. An MxLS of about 45-50 months has been reported for males (Benjamin and Brooks, 1977). Information on pathology is available for the colony maintained at the Lovelace Foundation Inhalation Toxicology Research Institute, Albuquerque, New Mexico (Benjamin and Brooks, 1977). Husbandry and dietary requirements have been discussed in Chapter 5.

Gerbils

Cheal (1986) has provided a comprehensive review of the Mongolian gerbil (*Meriones unguicultatus*) as a model for research on aging and has concluded that its ease of handling, ready availability, and particular physiologic and behavioral attributes establish it as a valuable model system. However, the gerbil exhibits an atypical survival curve and much more must be learned about the causes for this, including susceptibility to various infectious diseases and nutritional requirements. All gerbils in the United States are descended from only nine animals (Cheal, 1986), and there is some concern that deleterious recessive or dominant mutations might have become fixed in the population (M. Cheal, University of Dayton Research Institute, Higley, Arizona, unpublished). The husbandry of gerbils is discussed in Chapter 5.

RODENT MODELS OF INSULIN-DEPENDENT DIABETES MELLITUS

With rare exceptions, the rat and mouse models of human autoimmune diabetes mellitus have appeared spontaneously, presumably as a result of mutation, rather than deliberate genetic manipulation. The discussion below focuses on two models of insulin-dependent diabetes mellitus: the BB rat and the NOD mouse. The management principles suggested are easily superimposed on standard rodent-management techniques.

Diabetes-Prone and Diabetes-Resistant Rats

In 1974, some animals were found in a closed colony of outbred WI rats (Bio-Breeding Labs, Ottawa, Ontario) that spontaneously developed autoimmune diabetes mellitus (Chappel and Chappel, 1983). Several inbred diabetes-prone and diabetes-resistant strains were developed from this outbred stock at the Department of Pathology, University of Massachusetts Medical School. The diabetes-prone strains are designated BBBA/Wor, BBDP/Wor, BBBE/Wor, BBNB/Wor, and BBPA/Wor; the diabetes-resistant strains are designated BBDR/Wor and BBVB/Wor.[1] The genetics and pathophysiology of the diabetes-prone strains have been reviewed (Guberski, 1993; NRC, 1989).

Breeding Techniques and Genetic Records

Foundation colonies of diabetes-prone and -resistant strains are maintained strictly by full-sib matings. However, the selection of litters from which future generations of breeders will be derived is influenced by the presence of desired phenotypic traits (e.g., incidence of diabetes, age at onset of diabetes, fertility, litter size, and survival of pups to weaning). Although it is recognized that the imposition of selection criteria can delay achieving inbred status, the goals of any breeding strategy must include preservation of the desired phenotypic characteristics (e.g., the development of diabetes mellitus).

Essential data on each litter produced in the foundation colonies must be recorded to permit genetic tracing of breeding stock from one generation to another. To achieve this, a system of identification of each member of the primary and secondary breeding branches

[1]The designation BB/Wor was originally used as a group name for all seven inbred strains.

must be established. The records should include the occurrence of phenotypic characteristics, such as diabetes, thyroiditis, and lymphopenia.

Husbandry and Care

It is desirable that diabetes-prone and -resistant rats be maintained free of rodent pathogens in appropriate barrier facilities (see Chapter 5) because of the effect of these pathogens on phenotypic expression of diabetes (reviewed by Guberski, 1993). Microbiologic status should be monitored and recorded; records should include the tests performed and the frequency of testing. Experience has shown that these animals do well on a conventional light:dark ratio of 12:12 hours.

Detection and treatment of diabetes mellitus. The most cost-effective method of screening for diabetes is to test for glycosuria. Urine is expressed from the bladder manually by gently compressing the bladder against the pubic symphysis. Urinary glucose concentration is measured with a glucose test strip. Positive urine tests are confirmed with blood glucose measurements. Blood samples should be obtained from the tail within 2 hours of the urine test and tested with an appropriate technique. Animals testing 4+ for glycosuria and having blood glucose concentrations greater than 250 mg/dL are considered diabetic.

The age at which to begin testing and the frequency of testing for diabetes depend on the unique characteristics of the particular model and the environmental conditions under which it is kept. Testing for glycosuria should be started before the expected onset of diabetes and

performed at least three times per week at the start of the light period in the light-dark cycles. The frequency of glycosuria testing can be reduced after about 120 days because new occurrences are less likely.

Daily treatment of diabetic rats with insulin is mandatory and should begin on the day that glycosuria is found and diabetes is confirmed. The daily dose of insulin will be a function of age, body weight, the presence of ketoacidosis and dehydration, and the presence of pregnancy or lactation. Table 8.2 provides guidelines for the initial doses of insulin for animals that become diabetic after the age of 65 days. Animals that become diabetic *on or before the age of 65 days* should receive 0.2 U of insulin per 100 g of body weight in addition to the dose indicated. As animals increase in weight, the dose of insulin is increased by 0.2 U/10 g of body weight if the animals became diabetic on or before the age of 65 days, and by 0.2 U/16 g of body weight if the animals became diabetic after the age of 65 days. The maximal daily dose should not exceed 1.4 U/100 g of body weight for animals that became diabetic on or before 65 days of age, and 1.25 U/100 g of body weight for animals that became diabetic after the age of 65 days.

If ketonuria (as detected with a test strip) develops, the dose of insulin should be increased, and lactated Ringer's solution with sodium bicarbonate should be administered in the amounts shown in Table 8.3. Injections of fluids are well tolerated when given under the loose skin on the back (distal to the nape of the neck).

Treatment of hypoglycemia. Hypoglycemia is defined as severe if blood glucose is less than 40 mg/dL, moderate if blood glucose is 40-60 mg/dL, and mild if blood glucose is 60-80

mg/dL. The successful treatment of hypoglycemia requires a decrease in insulin dose combined with subcutaneous injections of fluid. Suggested regimens are outlined in Table 8.3.

Care of pregnant females. If pregnant animals become aglycosuric, the course of action depends on the ratio of insulin to "ideal" body weight (IBW). The IBW of a pregnant female at the age of 90 days is considered to be 270 g. If the animal is more than 90 days old, the body weight of a nonpregnant female sibling should be used as the IBW. The following procedures are recommended:

- If the ratio of insulin to IBW is greater than 1.0 U/100 g, the dose of insulin should be reduced by 15 percent.
- If the ratio of insulin to IBW is 0.9-1.0 U/100 g, the dose of insulin should be reduced by 10 percent and 10 cm^3 of lactated Ringer's solution should be administered.
- If the ratio of insulin to IBW ratio is less than 0.9 U/100 g, the dose of insulin should be reduced by 0.2 U/100 g and 10 cm^3 lactated Ringers solution should be administered.

If pregnant animals are severely hypoglycemic, follow the instructions for treating hypoglycemia in Table 8.4.

If a female becomes ketotic at parturition, the insulin dose should not be changed. Instead, lactated Ringer's solution and sodium bicarbonate should be injected subcutaneously

in the amounts indicated in Table 8.3.

Care of lactating females. Beginning 12-14 days after delivery, insulin should be decreased by 10-15 percent each day until a dose of 0.8-1.0 U/100 g of IBW is achieved. To prevent hypoglycemia in lactating females, food should be made readily accessible by placing it on the cage floors. If hypoglycemia occurs, it should be treated as indicated in Table 8.4.

Use of Spleen Cells to Reduce Frequency of Diabetes and Improve Breeding Efficiency

Diabetes-prone rat strains are profoundly T-cell lymphopenic. Injections of neonatal bone marrow, fresh spleen cells, or concanavalin-A-stimulated spleen cells correct the T-cell lymphopenia and substantially reduce the frequency of spontaneous diabetes (Naji et al., 1981; Rossini et al., 1984). Fresh spleen cells are obtained from diabetes-resistant rats, which are histocompatible with diabetes-prone rats but are not lymphopenic. Spleens are prepared with standard techniques (Burstein et al., 1989). Diabetes prone rats between 21 and 40 days old receive one spleen equivalent of fresh donor cells in 1 cm^3 of RPMI medium 1640, administered intraperitoneally. This procedure reduces the incidence of diabetes from greater than 85 percent to about 15 percent. Nondiabetic females do not require daily insulin injections (this reduces the workload of the staff) and are more productive breeders, as shown in Table 8.5.

Shipping Pathogen-Free Rats

Diabetes-prone rats have severely compromised immune systems and should be shipped in crates designed to keep them free of rodent pathogens (see Chapter 6). Drinking water or a water-rich material must be provided, especially for diabetic rats showing signs of polydipsia and polyuria, because these animals are prone to dehydration. Commercial carriers should be instructed to use climate-controlled trucks and holding rooms because diabetic rats are more susceptible than normal rats to fluctuations in temperature. In addition, commercial carriers must guarantee delivery within 24 hours because shipping delays are hazardous for animals that require daily insulin injections.

NOD Mice

NOD (nonobese diabetic) is an inbred strain derived from Jcl:ICR mice with selection for the spontaneous development of insulin-dependent diabetes (Makino et al., 1980). The expression of diabetes in this strain is under polygenic control (Leiter, 1993). Clinical features of diabetes in NOD mice are similar to those in humans. Females develop diabetes at a higher incidence and at an earlier age than males. The genetics and pathophysiology of this model have been reviewed (NRC, 1989; Leiter, 1993).

Insulin treatment is required to maintain diabetic NOD mice; without insulin, they survive only 1-2 months after diagnosis. Diabetes is diagnosed by determining that the blood (nonfasting) or plasma glucose concentration is increased. This determination can be made

by measuring blood glucose directly or by measuring urinary glucose with a glucose test strip. Glycosuria, as read on the test strip, usually denotes a plasma glucose of 300 mg/dL. Large numbers of mice can be easily screened by this method.

It is difficult to keep serum glucose within a normal range with insulin treatment, but body weight can be maintained and life prolonged (Ohneda et al., 1984). Morning and evening intraperitoneal injections of a 1:1 mixture of regular and NPH insulin are satisfactory. The dose will be 1-3 U, depending on the extent of glycosuria.

Environmental factors are extremely important in the expression of diabetes in NOD mice. Keeping them in SPF environment increases the occurrence of diabetes; exposure to a variety of murine viruses, including mouse hepatitis virus (Wilberz et al., 1991) and lymphocytic choriomeningitis virus (Oldstone, 1988), prevents diabetes development. That various types of exogenous immunomodulators prevent the development of diabetes (Leiter, 1990) suggests that infectious agents prevent diabetes by general immunostimulation. Diet also has an important effect on diabetes development: natural-ingredient diets, including standard, commercially available mouse feed, promote a high incidence of diabetes (Coleman et al., 1990).

NOD is an inbred strain and should be maintained by brother × sister mating. NOD mice have an excitable disposition but breed well. Siblings bred before the development of overt diabetes can usually produce two large litters (9-14 pups each) of which nearly all the pups survive to weaning. Breeders can be protected from developing diabetes by a single injection of complete Freund's adjuvant (Sadelain et al., 1990).

TRANSGENIC MICE

Since the late 1970s, advances in molecular biology and embryology have enabled scientists to introduce new genetic material experimentally into the germ lines of mice and other animals. The term *transgenic mice*, as used here, means that foreign DNA has been introduced into mice and is transmitted through the germ line. The gene transfer can be performed to introduce new genetic traits or to negate or "knock out" host-gene function by targeted mutagenesis.

Foreign genetic sequences can be introduced into mouse cells, especially in early embryos, by several different methods. The most commonly used method is pronuclear microinjection, in which a solution of purified DNA is injected into either of the two pronuclei visible in a newly fertilized egg (Gordon et al., 1980). Other, less reliable methods include the carrying of the proviral DNA into the cell with a retroviral vector (Jaenisch, 1976) or by electroporation (Toneguzzo et al., 1986) and transformation of totipotent embryonic stem (ES) cells, which are derived from cultured blastocyst-stage embryos (Doetschman et al., 1987). In contrast with microinjection or retroviral insertion, integration of foreign DNA into ES-cell chromosomes can be targeted to specific loci. The specifically modified, undifferentiated ES cells can then be introduced into a recipient embryo in which (it is hoped) they will incorporate into the developing germ line. This approach is used not only for modifying gene expression, but often for introducing targeted mutations by replacement of genes with nonfunctional counterparts, that is, for producing "knockouts" (Mansour et al., 1988).

Colony Management

Although a transgene causes only a small change in a genome, it can produce dramatic and unpredictable changes that make colony maintenance a challenge. Husbandry and production of transgenic mice have been reviewed (Gordon, 1993) and will be described briefly here.

Colony management can be complicated by several characteristics of transgenic mice, including unpredictable phenotypic effects of transgene expression, pathologic effects of the transgene that compromise viability, unpredictable interactions between the transgene and other host genes (e.g., insertional mutagenesis), altered responses to microorganisms or other environmental variables, compromised fertility, and possible instability of transgene expression through generations. Depending on the presence and severity of those characteristics, barrier maintenance might be advisable. Filter-top caging systems are usually sufficient if proper precautions are taken. Flexible-film or rigid isolator systems, however, permit the most complete control of the physical and microbiologic environment. Microbiologic status should be monitored regularly and should include testing for standard murine infectious agents. Both transgenic and sentinel mice should be evaluated if the integration or expression of a foreign gene alters immune competence.

Transgenic mice should be observed daily, and all visible clinical events should be recorded. Animal-care technicians should be trained to recognize clinical events and to report their occurrences with appropriate descriptive terminology. Unexpected deaths should be discussed with an animal-health professional, such as an animal pathologist, to determine

whether necropsy and histologic examination are warranted. It is imperative that deceased animals be collected and preserved properly as soon as they are discovered. Corpses can be placed in fixative, refrigerated, or frozen, depending on the specific postmortem procedures that are planned.

Management of a transgenic-mouse facility includes special requirements for embryo donors, embryo recipients, and offspring. In many transgenic facilities, embryo collection and culture, DNA introduction, and embryo transfer are performed outside the barrier; therefore, the embryos and embryo-transfer recipients might no longer be SPF and should not be returned to the barrier.

Embryo Donors

Embryos into which DNA will be introduced to generate founder mice are obtained by administering exogenous gonadotropic hormones intraperitoneally to virgin females. The hormones elicit synchronized ovulation of a relatively large cohort of mature oocytes (i.e., superovulation); therefore, fertilization and later preimplantation development will also be synchronized. Very young females—28-40 days old, depending on the stock or strain—usually respond best to superovulatory hormones. Outbred mice were originally used as embryo donors; more recently, inbred FVB mice have also been used. FVB mice are highly inbred, they respond well to superovulatory hormones, and their embryos have large pronuclei (Taketo et al., 1991).

Males should be individually housed; females can be group-housed before mating.

Breeding is most effective if a 3- to 8-month-old male that is a proven breeder is paired and bred with one or two females every 2 or 3 days. Mating should always occur in the cage of the male.

An uninterrupted dark phase of the lighting cycle is critical for efficient superovulatory breeding; a light:dark ratio of 14 to 10 hours is effective. Two gonadotropic hormones, pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (HCG), are each administered 7-9 hours before the beginning of the dark cycle, but PMSG is administered 2 days before HCG. Pronuclear embryos are generally collected 14-17 hours after the beginning of the dark cycle. For example, if the dark cycle begins at 10 p.m., PMSG would be administered between 1 and 3 p.m. 2 days before the day of mating, HCG would be administered between 1 and 3 p.m. on the day of mating, and pronuclear embryos would be collected between noon and 3 p.m. the next day.

Embryo Recipients

Group-housed females are used; outbred or hybrid mice generally make the best dams. Good choices of stocks to carry transferred embryos include outbred ICR mice (if a white coat is desired) and C57BL/6 $\times$ DBA/2 F1 (B6D2F1) hybrid mice (if a colored coat is desired). Housing strategies that avoid synchronization of estrus in group-housed females have been described (Gordon, 1993).

A colony of vasectomized males is required. It is preferable for the males to be test mated to ensure sterility; however, if 5- to 6-week-old males are vasectomized, there is no

sperm yet in the vas deferens, and test mating is not necessary. Even if test mated, males used to produce pseudopregnant females should be a different color from the embryo donor so that "accidental" offspring of males that have recovered their fertility can be distinguished from transgenic offspring.

Embryo-donor females should be 0-1 day more advanced in the reproductive cycle than pseudopregnant females. Early (one or two cells) embryos are transferred into the oviduct of the embryo recipient; morula and blastocyst embryos are transferred directly into the uterus. Recipient females should be used only once.

Offspring

Individual litters should be separated by sex at weaning and housed in cages that clearly indicate the litter number, date of birth, lineage, and parental identities. In general, fewer than 25 percent of live-born pups that receive transgene DNA as embryos will have integrated transgenes; 10 percent is considered average if microinjection is used. Most transgenic mice are identified by Southern blotting or polymerase chain reaction (PCR) analysis of DNA extracted from tissue taken from the tip of the tail; approximately 1 cm of tissue is sufficient. Rarely, it is possible to identify transgenic mice by detecting gene products from the introduced DNA.

Breeding Transgenic Mice

Once a mouse is identified as transgenic, it should be bred to verify that the transgene has been integrated into its germ cells. The development of a colony of mice homozygous for the transgene is achieved by standard breeding and test-mating procedures. Homozygous transgenic mice will produce 100 percent transgenic progeny on mating with a nontransgenic mate, whereas hemizygotes will produce both transgenic and nontransgenic offspring. It is recommended that multiple test litters be analyzed before the homozygosity of a breeder is considered established. Transgenic inheritance patterns do not always conform to classical Mendelian patterns, because the integration and expression of a transgene can affect implantation, in utero development, and postnatal survival. When mice are not homozygous for the transgene, all offspring must be screened for the transgene.

Reproductive performance of transgenic mice can differ substantially from that of the nontransgenic parental or background strains. Insertional phenomena can compromise fertility and affect embryo survival. Although breeding mice to homozygosity for the transgene is often desirable, homozygotes might be inviable, infertile, or subfertile. If fertility problems are encountered in homozygotes, whether caused by transgene expression or insertional mutagenesis, the problem can often be effectively managed by maintaining the transgene in the hemizygous state. Even in hemizygous mice, however, the effects of transgene integration, transgene expression, or both can be detrimental to survival and reproduction, and investigators and animal-care personnel should be alert to the necessity for establishing aggressive breeding programs. In extreme cases, assisted-reproduction technologies (e.g., superovulation and in vitro fertilization) might be helpful.

Identification, Records, and Genetic Monitoring

Identity, breeding, and pedigree records must be fastidiously kept because breeding errors in transgenic colonies are difficult to detect. For example, classic genetic monitoring will not necessarily distinguish between different transgenic lines on the same background strain. Even direct examination of the transgenic DNA sequence (e.g., with Southern blotting or PCR analysis) might not definitively identify a specific mouse. It is recommended that a combination of methods for identification and genetic monitoring be used in a colony of transgenic mice. Purified DNA samples from important animals can be frozen and stored at -70°C; these might be useful for future analyses, especially if DNA rearrangement is suspected.

Individual animals can be marked rapidly and inexpensively by tattooing, clipping ears, or using ear tags. The most reliable, albeit most expensive, system for identifying an individual animal is subcutaneous implantation of a transponder encoded with data on the animal. Transponder identification chips are durable for the life of the animal and suitable for computerized data-handling. Whatever method is chosen should be used in conjunction with a well-maintained cage-card system. One issue that arises in colonies of genetically engineered animals that does not arise in other colonies is confidentiality specifically related to patentability of the animals; information displayed on cage cards should be reviewed with the principal investigator.

The identity of each transgene-bearing breeder should be verified before mating. Important information on the transgenic parent includes transponder code or other

identification code, lineage, date of birth, date of pairing, administration of exogenous hormones (if any), and date of separation of breeding pair. If mice escape, all unidentifiable animals should be euthanatized, and recaptured identifiable females should be isolated for at least 3 weeks to determine whether they are pregnant. Litters derived from questionable or unverified matings should be euthanatized.

Embryo Cryopreservation

Because each transgenic line is unique, embryo cryopreservation might be considered. In general, cryopreservation issues relevant to transgenic lines are the same as those relevant to for other rodents (see Chapter 4). However, some lines cannot be made homozygous, are reproductively compromised, or both, so it might be prudent to freeze more embryos than would be necessary for preservation of an inbred strain.

Data Management

A large amount of data accumulates in a transgenic colony and must be managed efficiently. Daily or weekly records include data on breeding, birth, weaning, death, and laboratory analyses; they also include documentation of observations on such things as characteristics that are possibly related to gene manipulation, pathologic conditions, and unusual behaviors.

Shipment and Receipt of Transgenic Rodents

In general, it is not necessary to use extraordinary containment procedures for shipping transgenic mice. To reduce the risk of loss, shipments can be split so that accidents or errors during transit do not compromise the entire shipment. The following information should accompany transgenic mice shipped from a facility and be requested for transgenic mice brought into a facility:

- genetic identity, including the species and strains from which the transgene originated, the designations of all transgene components, the ancestry of the transgenic founder, and the exact lineage designation and generation number of each mouse;
- standardized transgene symbol (see NRC, 1992);
- individual identification numbers accompanied by an explicit description of the identification method (e.g., subcutaneous transponders, 16-digit codes, or an ear-marking scheme with a drawn key);
- description of the predicted phenotype and relationship of transgene expression to such factors as age, sex, pregnancy, and lactation;
- identification of potential human health hazards related to transgene expression (e.g., active expression of intact virus particles or potentially immunogenic viral structural proteins);
- general health status of the mice and probable morbidity or mortality associated with transgene expression, including available data on serologic, bacteriologic, and

parasitologic screening; and

- information important to maintenance and breeding, such as breeding strategies, pregnancy rates, gestation times, litter sizes, and sex distribution within litters.

Human Health Hazards

Consideration must be given to possible zoonotic hazards posed by transgenic mice. For example, viral replication has been demonstrated in mice carrying the entire hepatitis B virus genome (Araki et al., 1989). Preliminary banking of employees' sera should be considered (see Chapter 2).

Administrative Issues

In maintaining colonies of transgenic animals, all relevant legal requirements must be addressed. Examples include laws governing patent applications or awards, international regulations governing the importation or exportation of genetically engineered animals, and quarantine laws.

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Table 8.1 Mortality for Selected Strains of Mice and Rats Fed Ad Libitum

		Age, weeks			
		Females		Males	
	Strain	50% Mortality	90% Mortality	50% Mortality	90% Mortality
Mice	C57BL/6NNia	117	143	120	141
	DBA/2NNia	77	123	88	126
	C57BL/6NNia × DBA/2NNia F1 (B6D2F1)	128	152	138	171
	C57BL/6NNia × C3H/NNia F1 (B6C3F1)	132	158	140	177
Rats	F344/NNia	116	144	103	121
	BN/RijNia	133	157	129	155
	F344/NNia × BN/RijNia F1	137	166	146	171

Source: Data on National Institute on Aging colonies from the Division of Veterinary Services, National Center for Toxicological Research, Jefferson, Arkansas

Table 8.2 Starting Doses of Insulin for BB/Wor Rats That Become Diabetic After the Age of 65 Days.

	Initial Blood Glucose Concentration, mg/dL					
	250	300	350	400	450	500+
Body weight, g[a]	Starting Dose of Insulin,[b] U					
100	0.4	0.6	0.6	0.6	0.8	0.8
125	0.4	0.6	0.6	0.8	0.8	0.8
150	0.6	0.8	0.8	1.0	1.0	1.2
175	0.8	1.0	1.0	1.2	1.2	1.4
200	1.0	1.2	1.2	1.4	1.6	1.6
225	1.2	1.4	1.4	1.6	1.6	1.8
250	1.4	1.6	1.6	1.8	1.8	2.0
275	1.4	1.6	1.8	1.8	2.0	2.0
300	1.4	1.6	1.8	2.0	2.0	2.2
325	1.6	1.8	2.0	2.0	2.2	2.2
350	1.6	1.8	2.0	2.2	2.2	2.4
375	1.8	2.0	2.2	2.2	2.4	2.4
400	2.0	2.2	2.4	2.4	2.6	2.6
425	2.2	2.4	2.6	2.6	2.8	3.0
450	2.2	2.4	2.6	2.8	3.0	3.2

[a]Assumes that rat is well hydrated and that ketosis, if present, is being corrected.
[b]PZI U40 (Eli Lilly) insulin and a U/100 Lo-dose syringe (B-D) are used. U40 insulin + U/100 syringe = 0.4 units per gradation mark. Add 0.2 U/100 g of body weight to the dose for animals that develop diabetes on or before the age 65 days. Maximal daily dose equals 1.4 U/100 g of body weight for animals that become diabetic on or before the age of 65 days and 1.25U/100 g of body weight for animals that become diabetic after the age of 65 days.

Table 8.3 Treatment for Ketonuria in BB/Wor Rats

Ketones	Increased Insulin,[a] U/100 g body wt	Lactated Ringer's Solution, cm^3	Sodium Bicarbonate, mEq[b]
2+	0.2	10.0	0.0
3+	0.2	9.0	1.0
4+	0.2	18.0	2.0

[a]Insulin dose of lactating females should not exceed 1.0 U/100 g of "ideal" body weight (see Care of pregnant females). Dose should not be increased during mild episodes of ketonuria.
[b]1 cm^3 of 8.4% sodium bicarbonate equals 1 mEq.

Source: Guberski, 1993

Table 8.4 Treatment for Hypoglycemia in Diabetic BB/Wor Rats

Classification (blood glucose concentration)	Subcutaneous Fluid Therapy	Change in Insulin Dose	Change in Time of Insulin Administration
Severe (<40 mg/dL)	Give 1 cm^3 50% dextrose; 2 hrs later give lactated Ringers olution with 5% dextrose	Reduce by 30-50%	Delay by 2-3 hrs
Moderate (40-60 mg/dL)	Give 10 cm^3 lactated Ringers solution with 5% dextrose	Reduce by 20-30%	Delay by 2-3 hrs
Mild (60-80 mg/dL)	Give 10 cm^3 lactated Ringers solution	Reduce by 10-15%	No delay

Source: Guberski, 1993

Table 8.5 Reproduction in Diabetes-Prone BB/Wor Rats Before and After Receiving Splenocytes from Diabetes-Resistant BB/Wor Rats

	Diabetes-Prone Females Not Treated with Splenocytes (N = 1,238)	Diabetes-Prone Females Treated with Splenocytes (N = 1,022)
Incidence of diabetes	86%	16%
No. pups born	7,160	12,434
No. pups weaned	5,766	10,918
Pup survival through weaning	80.5%	87.8%
No. pups weaned per female mated	4.7	10.7

Source: Guberski, 1993

Appendix

Sources of Information on Importing Rodents

Information on All Categories of Rodents

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services, Import/Export Products
Federal Building 22, Room 756
Hyattsville, MD 20782
Telephone: 301-436-7885

Information on Wild Rodents

U.S. Department of the Interior
Fish and Wildlife Service

Contact at one of the following addresses

New York, New York

700 Rockaway Turnpike
Lawrence, NY 11559
718-553-1767

Baltimore, Maryland

40 South Gay Street, Room 405
Baltimore, MD 21202
410-962-7980

Los Angeles, California

370 Amapola Avenue, Room 114
Torrance, CA 90501
310-297-0063

San Francisco, California

1633 Bayshore Highway, Suite 248
Burlingame, CA 94010
415-876-9078

Miami, Florida

10426 NW 31st Terrace
Miami, FL 33172
305-526-2789

Honolulu, Hawaii

PO Box 50223
Honolulu, HI
808-541-2681

Chicago, Illinois

10600 Higgens Road, Suite 200
Rosemont, IL 60018
708-298-3250

New Orleans, Louisiana

2424 Edenborn Road, Room 100
Metairie, LA 70001
504-589-4956

Seattle, Washington

121 107th NE, Suite 127
Bellevue, WA 98004
206-553-5543

Dallas/Fort Worth, Texas

PO Box 610069
D/FW Airport, TX 75261-0069
214-574-3254

Portland, Oregon

9025 SW Hillman Court, Suite 3134
Wilsonville, OR 97070
503-682-6131

For Customs Regulations

U.S. Department of the Treasury
U.S. Customs Service

(For local office, check lisings in telephone directory.)

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ILAR JOURNAL

National Research Council
INSTITUTE OF LABORATORY ANIMAL RESOURCES

Perspectives on Xenotransplantation

Ethical Aspects of Animal to Human Xenografts

Xenotransplantation: A Historical Perspective

The Application of Xenotransplantation In Humans—Reasons to Delay

The Immunologic Response to Xenografts

Xenotransplantation: The Need, The Immunologic Hurdles, and The Prospects For Success

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A quarterly publication for biomedical investigators, laboratory animal scientists, instituional officials for research, and members of animal care and use committees.



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Introduction

Ralph B. Dell

Many physicians, patients, and their families hope that animal to human transplantation could become an option for individuals with critical organ failure for whom no human organs are available. Xenotransplantation has been tried several times in the past 10 years without long term success. For example, baby Fae and, more recently, baboon-to-human liver transplants at the University of Pittsburgh have attracted considerable attention. These experiments and the shortage of human organs have combined to spur research on xenotransplantation. At the same time, the prospect of using animal organs in people has stimulated much interest in the public and among thoughtful observers, raising a number of ethical, legal, social, and scientific issues. There is considerable hope that with further research and experimentation the technique can become a viable option for desperately ill people.

Nonetheless, confusion, doubt, controversy and opposition have led to fear that xenotransplantation will result in chimeric monsters. There is some theological and philosophical opposition. Some of these fears are groundless and should be calmed with better public education while other concerns should be and are being discussed and debated in many different forums. Ethicists and philosophers have debated the proper conduct of human experimentation and the use of animals in research which has lead to careful review procedures for both humans and animals.

There is concern that xenotransplantation hasn't worked and won't work and that it is too expensive or is a misallocation of scarce resources. Economic arguments for and against xenotransplantation involve questions of proper resource allocation and the cost of human life, questions which are not easily answered. Another aspect of the cost issue is quality of life, an important consideration for all considering receiving grafts.

Interest in xenotransplantation has sparked considerable immunological research which has significantly improved the probability of success. Rejection of transplanted organs is now recognized to occur in several stages with each stage representing numerous and complex biochemical reactions. While there are many similarities among the immune systems of mammals, the details of the human immune system will require study of humans. Many of the proteins involved in immune reactions are species-specific. Therefore detection and quantification of these proteins requires species-specific reagents. There is also hope that transgenic animals will express human proteins and modify the response to the transplanted organ by the human immune system.

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The possibility that organ transplantation from animals can transmit infectious disease to humans is of concern. This possibility warrants careful consideration and merits taking precautions to protect the public health. The precise nature of these precautions will have to be decided by experts in infectious and emerging diseases. It should also be recognized that no monitoring scheme will be able to guard against the unknown.

The use of animal organs in the treatment of human disease has raised significant issues in a number of spheres. Thoughtful discussion and communication with the public and its representatives are needed with all of these concerns.

In this first issue of *ILAR Journal,* seven authors, chosen to represent a diversity of views, consider many of the concerns raised by the prospect of xenotransplantation research in humans. These concerns include ethical, legal, and social issues; the current status of immunological research; and the possible infectious disease consequences of using either nonhuman primates or other species as donor animals.

All of these issues are of interest and concern to both the institutional animal care and use committee (IACUC), which reviews the use of animals, and the institutional review board (IRB), which reviews the use of human subjects in research, at institutions that are contemplating research on xenotransplantation, either direct organ transplant, tissue implantation, or various extracorporeal devices containing animal tissue. Consideration must be given to the issues discussed in this report in order to adequately review the proposed experiment. Furthermore, these experiments have facility and husbandry implications, raise regulatory issues, and cause the concerned people to grapple with understanding the ethical, legal, social, and scientific issues of xeno-transplantation. This issue of *ILAR Journal* provides an overview that is a beginning to understanding this complex and developing area of research.

In the opening article, Charles McCarthy reviews the ethical, legal, and social issues surrounding the use of xenografts in humans. While he supports the use of animals in research, he points out that the complexities go far beyond the use of animals. He outlines conditions that should be met for approval from the IACUCs and IRBs, and from the institution itself. All of these conditions require careful thought and discussion by all concerned: committee members, investigative staff, and institutional officials.

Keith Reemtsma reviews the history of xenotransplantation from ancient Greece to the present, including his own considerable contributions to the field through studies in both humans and nonhuman primates. He concludes that the most difficult question is when to transfer work from the laboratory to the clinical setting. The decision to proceed must be made on an individual basis (including, it might be added, discussion with local committees and accounting for public concerns), but, as Reemtsma goes on to state, "clinical success is probable, although not assured, in the near future." In contrast, David Steele and Hugh Auchincloss Jr. believe that animals used in laboratory trials of xenotransplantation have not yet reached the length of survival that justifies moving to human experimentation. The authors assert that more work is needed to understand rejection of xenogeneic tissue and, at a minimum, they want to see long-term survival in animal studies before proceeding to human cases. Thus, the current state of knowledge concerning the immunologic response to xenografts is raised as a potential barrier to proceeding with human studies.

The immunology of transplantation is reviewed in two complementary articles by David Sachs and Jeffrey Platt. Sachs focuses on cellular and serologic mechanisms in both concordant (between similar species) and discordant (between species phylogenetically disparate) xenotransplants. The author notes that because there are many immunosuppressive drugs available, long-term results may depend on increasing the recipient's tolerance to the graft. Platt provides the reader with a thorough review of antibody- and complement-mediated xenograft rejection. Because there are immunological responses to organs transplanted from nonhuman primates, there is a hyperacute response to organs from animals such as swine, which are phylogenetically far from human. This hyperacute rejection of swine organs involves complement reaction to certain cell surface proteins. Using transgenic techniques, these proteins can be made to be more like human proteins. Swine have a number of desirable features as organ donors if hyperacute rejection can be overcome.

The final section explores the likelihood that a virulent organism may be transferred from the animal to the xenograft recipient. For some, this is a theoretical issue that can be safely ignored. For others, the potential consequences are so great that xenotransplantation should not be performed unless there are compelling reasons for doing so. Many agree that cautiously proceeding with xenotransplantation research in humans is appropriate given the shortage of human donors and the need to do immunologic research in human xenotransplantation. Seymour Kalter and R.L. Heberling provide an overview that explains why there is a concern but state that thus far infectious disease has been a minor factor in transplantation. Jonathan Allan takes a more cautious view of transmission of organisms and the likelihood of the occurrence of disease. In his view, transfer of organisms is bound to occur, but the question really is—will it be harmful? He ends with a call for the formation of a panel of experts to address these issues.

Just such a workshop is being planned by the Institute of Medicine (IOM) of the National Academy of Sciences. Currently in the planning stages (see summary p. 50 by Dr. Constance Pechura, associate director the Division of Behavioral Sciences and Mental Disorders of the IOM), the 3-day workshop will cover many of the issues considered in this edition of *ILAR Journal*, including a full day

devoted to the infectious-disease problem and ways of minimizing the potential threat to the public health. In addition, a number of federal agencies, including the Food and Drug Administration, the Centers for Disease Control and Prevention, National Institutes of Health, and Health Resources Services Administration, and a number of professional societies are interested in a study of the issues raised by xenotransplantation. The IOM workshop will be held on June 25 to 27, 1995.

The success of transplantation in treating life-threatening organ failure has exceeded the supply of human organs, despite concerted efforts by many organizations to recruit donors. The need to turn to nonhuman animals for organs raises a host of complex ethical, social, and scientific issues that must be considered by all those contemplating working in this difficult area. Many of these issues are dealt with in this first edition of *ILAR Journal* and will be further addressed by the IOM workshop. Clearly, with issues as complicated as these, there will be ongoing discussion for a number of years.

Ethical Aspects of Animal-to-Human Xenografts

Charles R. McCarthy

INTRODUCTION

The demand for organs suitable for transplantation into human beings is increasing. It has been estimated that, in the United States, as many as 15,000 human patients per year could benefit from heart transplantation. The demand for livers, kidneys, pancreases, lungs, corneas, and other organs is also on the rise. Even if efforts to persuade persons to donate organs are increased, and even if consent to donate should become a legal presumption, there is little prospect of developing a sufficient supply of transplantable human organs to meet the growing demand (Evans and others 1986). The United Network for Organ Sharing reported that 560 patients on the UNOS waiting list died while waiting for a liver transplant during calendar year 1993 (Annual Report 1994).

New drugs and new combinations of existing drugs have improved the chances of successful medical outcomes for patients. It is now recognized that serial application of immunosuppressive drugs can reduce the likelihood of both short- and long-term organ rejection (Makowka 1994). Improved understanding of both human and animal immune systems; insights into histocompatibility; new agents to control graft-versus-host disease; animal breeding programs for the production of transgenic animals; and perfection of surgical techniques have opened, as never before, the possibility of successful transfer of organs from animals to humans. Some rate the chances of good patient outcomes as high, others make more conservative predictions, but virtually all experts believe that the chances for successful patient outcomes resulting from xenotransplantation are improved.

The demand for organs to treat human beings coupled with the new scientific understanding of the immune systems of both humans and animals make it probable that animal-to-human xenografts will soon be attempted at a frequency rate unknown in the past. Optimism for animal to human organ transplantation is at an all time high. As technical and biological barriers to successful xenotransplantation are lowered, the prospect of raising dedicated animal colonies to provide a ready supply of organs for human transplantation is now seriously discussed. Nelson (1993) calls for careful consideration of the ethics of whether we morally wrong animals in taking their organs and their lives. He cautions against rushing to create colonies of primates dedicated to xenografts.

The potential resource of an ample supply of animal organs, genetically altered to reduce the probability that human hosts will reject them, now appears to be technically feasible. Some believe that investment in colonies of purpose-bred animals to serve as a source of transplantable organs is close at hand (Leventhal 1994).

HISTORY SUGGESTS CAUTION

Nevertheless, the history of previous failed attempts to carry out xenografts should send a caution signal to the research community. Some will perceive the history of xenografts as flashing an amber light, while others are likely to interpret that historical data as a red stoplight. Past efforts to prolong human life by implanting animal organs into human recipients have all met with failure—usually relatively quickly. Past failures, summarized below, should serve to dampen enthusiasm for efforts to use animals as a source of spare organs for human beings.

Xenotransplantation, resulting in early death for all recipients, was attempted early in the twentieth century (Neuhof 1923). More sophisticated efforts occurred in the late 1960s and early 1970s during the period when anti-rejection drugs began to make allografts more feasible. Although one recipient of a xenograft survived for 9 months, most patients died within a matter of minutes, hours, or days after engraftment of organs from chimpanzees or baboons (Starzl and others 1964; see also Millard and others 1985).

A well-known xenograft involved the transfer in 1984 of

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a baboon's heart into 14-day-old "Baby Fae" by Dr. Leonard Bailey at Loma Linda Hospital. The "Baby Fae" xenograft was occasioned by the introduction of cyclosporin A into the armamentarium of drugs used to prevent organ rejection. Because human organs suitable for transplantation into infants are extremely rare, a search for an alternative organ source to fill this need led to a decision to transplant a baboon heart into a human baby whose own heart was unable to sustain life for more than a few days. Coming as it did at the zenith of organized protest against the use of laboratory animals, the "Baby Fae" case generated enormous controversy and unprecedented coverage by news media. In general, the public press first praised and subsequently condemned the Loma Linda xenograft. "Baby Fae" died of progressive graft necrosis 20 days after receiving the baboon heart (For a sampling of public and professional comment on the "Baby Fae" case, see Ammas 1985).

With the advent of the new drug FK506 and other immunosuppressive agents, at least two new xenotransplant efforts have been attempted in the 1990s (Starzl and others 1993). Liver transplants from animals to humans are under consideration in several centers, either as permanent organ transplants or as a "bridging" procedure to sustain patients awaiting human liver transplants. One technique involves a donor baboon liver used as a bridge that can be removed with relative ease because, although connected to the subject's liver, it remains extracorporeal (Prentice and others 1994). Similar experimental techniques are planned involving baboon hearts to be used as bridges for pediatric cardiac patients. Plans to conduct animal-to-human xenotransplants for bridging are under consideration in at least five major U.S. transplant centers.

Before major new animal-to-human xenograft research programs are initiated, both the scientific and the ethical aspects of such programs should be carefully considered and debated. Many of the scientific issues are addressed in other articles in this issue of *ILAR Journal*. This article will address ethical aspects of xenotransplantation from animals to humans.

The position taken in this article is intended to be a part of the debate over xenotransplantation and should not be considered as a final word on the ethics of research in this area.

MORAL OPPOSITION TO XENOGRAFT RESEARCH

Animal-to-human xenograft research will encounter strong opposition from persons who regard such research as unethical or immoral. Xenotransplantation requires the sacrifice of healthy animals whose organs, engrafted into human hosts, will constitute one of the most intimate associations possible. It is not surprising that many regard it as morally offensive. Those who oppose xenografts on ethical or moral grounds are likely to fall into one or more of the following groups:

- Those who regard animal-to-human transplantation as immoral for theological reasons may include: (a) some Jewish groups that consider the engrafting of animal parts into human beings as defiling to the recipient, particularly if the animal parts are derived from a species regarded as unclean; (b) some Christian groups that object to xenotransplantation because they regard the action of engrafting animal parts into human beings as "playing God," that is, attempting to usurp or interfere with God's role as Creator (Scientific creation of chimeras composed of both human and animal parts is regarded as blasphemous by such persons); and (c) because successful xenotransplants will almost always require blood transfusion, Jehovah's witnesses, who are morally opposed to transfusion of blood ipso facto and opposed to xenotransplantation.

- Those who object to xenotransplantation on philosophical grounds. These include: (a) those who regard any sacrifice of higher vertebrate animals for purposes that can only benefit human recipients as morally objectionable; (b) anyone who regards animals as the subjects of rights, particularly the right not to be interfered with; and (c) those who consider that the interest of each animal in maintaining life and bodily integrity is at least equal to any human interest in receipt of an animal organ. (In this view, the deliberate taking of the healthy animal's life—a life for a life—can never be justified by resultant human benefits); (d) those who oppose the use of highly intelligent, and perhaps self-conscious, nonhuman primates as the source of organs for xenotransplants, but are not inexorably opposed to the use of other less advanced animals for xenograft purposes (pigs, for example); (e) those who argue that prior to obtaining organs derived from robust, healthy animals, organs should be taken from humans whose quality of life is poor (such as anencephalics or people in a permanent vegetative state; and (e) those who object to the transplantation of animal organs into humans on the grounds that such transplants are aesthetically repugnant.[1]

- Those who may be opposed to initiating animal-to-human xenograft research on the grounds that the benefits

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[1] For references to a range of literature on the morality of using animals for research purposes, and a summary of moral positions opposing or questioning the use of animals in research see: Donnelley, S., and K. Nolan, eds. 1990. A Special Supplement to The Hastings Center Report entitled Ethical Theory and the Moral Status of Animals by Lilly Marlene Russow, in collaboration with K. Danner Clouser, David DeGrazia, and James Stephens, Sec. I, pp. 4-8. A more complete summary and critique of leading philosophical positions is presented by David DeGrazia, March, 1991, in the Kennedy Institute of Ethics Journal, 48-69. DeGrazia seeks to find some degree of convergence in the thought of leading opponents to the use of (some or all) animals in research, Singer, Frey, Regan, Midgley, and Sapontzis. Nevertheless, he is not entirely satisfied with the thought of any of them. He dismisses the arguments of Cohen and H.J. McCloskey (proponents of the use of animals in research) because, in his view, they do not represent significant contributions. By finding more consensus than may actually exist, DeGrazia seems to suggest more unanimity in positions opposed to the use of animals than this author believes is justified.

do not justify the economic costs, and that xenotransplantation will introduce additional inequities into a health care delivery system that is already inherently unfair. They argue that even if the research results in long-term survival of some who would otherwise almost certainly die, xenotransplantation will introduce an extremely costly form of high technology into a health care system that already overburdens the economy and that is tilted in favor of the affluent. They contend that it is unethical to conduct costly research that, if successful, can only lead to additional high cost, high technology medical service. They argue that xenotransplants will be available only or primarily to the privileged few who are already relatively wealthy or who are able to afford expensive comprehensive health insurance, while lack of funds prevents the provision of even minimal, routine health care for a significant segment of the society.

IN DEFENSE OF THE MORAL USE OF ANIMALS

This article cannot deal in depth with the arguments summarized above. However, I believe that each of the moral objections can be met with a counterargument that is at least as compelling. I want to advance the argument that it is morally acceptable to use animals in research such as xenotransplantation designed solely or primarily to benefit human beings. The argument contends that both theology and philosophy support the position that humans have an obligation to exercise wise stewardship over the entire ecosphere, including animals. The degree of animal care and the kind of use that humans permit will vary according to circumstances. Obligations to provide for humane care and use of laboratory animals will differ from obligations to animals used as pets, industrial animals, and animals in the wild. Although all animals have a claim on human stewardship, higher obligations may justify the use of animals for ends not consistent with the best interests of particular animals.

The argument has three parts:

1. A well-established traditional Judeo-Christian view of the proper relationship of humans to animals in creation rests on the belief that, although animals and other creatures manifest God's creative power, only humans are made in God's image. This position holds that humans occupy a higher niche in creation, and have a transcendent moral relationship to God, which surpasses the relationship of other animals to their Creator. The position assigns to humans responsibility to be stewards for all creatures, including animals (A similar case is presented in Loeb and others 1989 and Bulger 1987).

2. Humankind enjoys relative moral superiority over nonhuman animals. Humans are characterized as possessing capabilities for: conceptualization, making judgments, exercising wisdom, exercising creativity, freedom of choice, moral decision making, social adaptability, appreciation of beauty, seeking justice, exercising compassion, learning from the past, planning for the future, altruism, and exercise of responsibility for valued and valuable things (ranging from one's own self and family to the entire ecosphere). Although many human traits are possessed in limited ways by animals, the level and complexity of these traits as they exist in humans, establishes humankind, taken as a whole, as morally superior to nonhuman animals. Of course not all humans exercise all of these capabilities, and some humans possess few or none of them. This is not the place to defend the relative moral superiority of humans who are incapable of exercising full human potential (such as infants, the senile, and those in permanent vegetative state). This problem has vexed ethicists for a very long time. My own view is that a defense of the moral superiority of humans who do not manifest the full range of human characteristics is best made on theological rather than philosophical grounds.

3. Since the dawn of history, humans have domesticated animals for their use. All domestic and many wild animals depend on humans for survival and well being. Conversely, major elements of human civilization, including the understanding and preservation of the environment, nutrition (for animals and plants as well as for humans), clothing, tools, and the preservation and promotion of health for animals and humans are dependent on and inextricably intertwined with human use of both domestic and wild animals. To argue that the use of animals for human ends is immoral, is tantamount to saying that humans cannot and never have been able to live morally upright lives. In effect, such a position condemns the conduct of all living human beings (since all participate in societies that require the use of animals for human ends), and the conduct of previous generations of human beings as well. To say the least, this would be an eccentric conception of morality.

However, if the fact that humans and animals depend on each other in an unequal web of relationships argues against condemnation of all use of animals for human ends, it does not justify the moral permissibility of every use of animals. Rather it makes it incumbent on human society to strive for consensus concerning how to use animals in appropriate ways that reflect both the relative moral superiority and the responsibility of humans for animals. Creation of colonies of animals ideally suited to provide replacement organs for humans appears to be consistent with good stewardship. Surely the sacrifice of animals to preserve human life is at least as defensible as creating and sacrificing colonies of animals for use in the food chain. Both xenotransplantation and use of animals for food appear to be reasonable if one accepts the moral superiority of humans; neither can be justified if the moral superiority of humans is denied. These summary arguments are presented, not as a complete case for the use of animals in research, but to suggest to the reader that powerful philosophical and theological arguments can be made to support the responsible use of animals in research designed primarily for human ends.

SPECIFIC CRITERIA FOR XENOGRAFTS

If one accepts the argument that xenotransplantation is not immoral a priori, then one must ask: What are the conditions that must be met in order to carry out xenografts in a reasonable, defensible manner? The following conditions are suggested as necessary for reasonable xenotransplantation:

- Prior to animal-to-human xenotransplantation, successful organ transfer between species other than human must have provided credible evidence of a significant chance of successful transplantation of organs from animals to humans, with the prospect of long-term human survival.
- The subsequent animal-to-human xenograft research must be carefully designed to achieve a non-trivial outcome for human subjects, and to provide more good than harm to society.
- A sufficient, but not excessive, number of animals has been used to accomplish the research. (In the case of xenotransplantation, research investigators may tend to use an insufficient number of animals by failing to carry out enough carefully controlled xenografts between animal species to provide a solid scientific basis for animal-to-human xenotransplantation and to evaluate both short- and long-term consequences of the procedure.)
- Pain and distress to the animals must be minimized.
- Until xenotransplantation is well established each animal-to-human xenograft must be thoroughly evaluated before additional animal-to-human xenograft research efforts are undertaken. If any xenograft research produces serious harmful consequences, then further research should be suspended until the harms can be understood and prevented or overcome in the future.

RESPECT FOR CONSCIENTIOUS OBJECTION

It must be recognized, nevertheless, that many competent, thoughtful, conscientious persons do not agree with either the philosophical or theological positions regarding the moral permissibility and the responsible use of animals outlined above. Even among those that consider some use of animals as morally acceptable there are some who consider xenotransplantation morally wrong. Most people who object to the use of animals for human ends or who object specifically to xenotransplantation appear to be principled and conscientious in their objection to the use of some or all animals in research, or to the use of animals in xenograft research that benefits humans but offers few, if any, benefits to animals. Therefore, any research program that proposes to conduct animal-to-human xenografts must make allowance for conscientious objection.

Those who object to animal-to-human xenotransplantation research on moral grounds must not be coerced or pressured to cooperate with xenograft research in any way. Without prejudice, animal vendors, caretakers, veterinarians, technicians, co-investigators, surgeons, transplant recipients, and nursing staff must be given the opportunity to separate themselves from cooperation with xenograft research. The employment, wages, opportunity for advancement, working conditions, and right to actively oppose xenotransplantation must not be adversely affected by a person's conscience-based refusal to cooperate with animal-to-human xenograft activities.

REACHING A DECISION CONCERNING PROPOSED XENOTRANSPLANTATION RESEARCH

Actual decision-making should occur in a manner similar to other research decisions. Xenotransplantation may go forward if:

- The proposed research complies fully with federal, state, and local laws, policies, and regulations governing both the care and use of laboratory animals and the protection of the rights and welfare of human research subjects. Compliance, at the very least, will require careful review and approval of the research activity by the relevant Institutional Animal Care and Use Committee (IACUC) and by the relevant Institutional Review Board (IRB).[2] In the case of animal-to-human xenograft research proposals it is recommended—but not required—that the IACUC and the IRB meet together to review and evaluate the research design. Joint meetings are likely to enhance the quality of the review by both committees. Of course each committee must take action on each research proposal (each IRB/IACUC may approve, approve with modification, or disapprove) independently of the other. Disapproval by either committee will be sufficient to prevent the research from going forward.
- The proposed research has the approval of the institution under whose auspices the research is conducted.

CONDITIONS OF IACUC APPROVAL[3]

Prior to giving approval to the proposed research activity, the IACUC should find and document that the following conditions have been met:

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[2] Careful attention must be given to assure compliance with the *Public Health Service Policy on Humane Care and Use of Laboratory Animals*, revised, Sept. 1986 (U.S. Govt. Printing Office: 1991-294-776). See also The Improved Standards for Laboratory Animals Act, December 23, 1985 (Subtitle F of the Food Security Act of 1985 (P.L. 99-198, #1751-1759). This Act amended the Animal Welfare Act (AWA) of 1966 (P.L. 89-544), amended in 1970 (P.L.91-579), and 1976 (P.L. 94-279). Regulations implementing the 1985 law and amending parts 1 and 2 of the Animal Welfare Act Regulations were promulgated by the USDA March 15, 1989 Federal Register 54:10822-10954. Final regulations amending part 3 of the AWA regulations were promulgated Feb. 15, 1991, F.R. 56:6426-6505.

- The animals must not be members of a rare or endangered species (Donnelley and Gaylin 1989). Although most past xenograft attempts have used nonhuman primates, the committee should give careful consideration to other species, such as pigs, whose organs appear to be well suited for xenotransplantation, and whose genetic suitability and pathogen-free status is more easily ascertained than is the case with primates.
- The animals will be obtained from an approved vendor, dealer, or breeding program. The species must be chosen on grounds that it offers the best chance of successful xenograft to human recipients. To the extent possible within the state of the art, principal investigators must demonstrate that candidate animals are free of zoonotic and pathogenic agents, even if these agents are usually considered harmless. Primates that are considered acceptable as xenotransplant donors should be screened for *Toxoplasma gondii*, *Mycobacterium tuberculosis*, Marburg virus, herpes virus, Simian cytomegalovirus, herpes simplex 1 and 2, and any other pathogens known to reside in the species. Blood from candidate animals must be screened for HIV-1, HIV-2, HTLV and hepatitis (Prentice and others 1994). Animals found to harbor pathogens or zoonotic agents must not be used as xenograft donors. Animals found to be free, or as free as possible, from zoonotic or pathogenic organisms should be selected on the basis of histocompatibility and suitable organ size and morphology for successful xenotransplantation.
- Donor animals are to be maintained in as healthy a condition as possible prior to their being sacrificed for xenograft purposes. To this end, the IACUC should consider how long, and under what conditions the animals will be quarantined prior to use as xenograft donors. Careful attention to the animals' environment as well as to food and fluid intake must be given to assure that the animal is healthy.
- Donor animals must be sacrificed in a humane and painless manner in accord with American Veterinary Medical Association standards.
- Those removing the animal organ (or organs) must possess the requisite skills, staff, and equipment needed for prompt removal and preservation of animal parts with a minimum of insult to those parts prior to transplantation into the human recipient.

CONDITIONS OF IRB APPROVAL

The IRB should find and document that the following conditions have been met prior to giving approval to the research activity (Federal Policy 1991):

- Human candidates for receipt of animal organs have a genuine need for an organ that cannot be met by other morally acceptable, less risky means.
- Human candidates meet the selection criteria factors established by the United Network for Organ Sharing, for receiving organ transplants. Human candidates who elect not to participate in xenograft research must not lose their standing in the list of those seeking human organs.
- The surgeon or surgical team introducing the animal organ into the human recipient must be well-qualified to carry out the transplant.
- Nursing and other support staff must be well trained in monitoring and caring for transplant patients.
- Potential human recipients must be well informed concerning all aspects of the study, especially risks that they face if they consent to xenotransplantation. Informed consent must include the following:

(a) A clear statement that animal-to-human xenografts are in a very early stage of development. For that reason xenografts are classified as research. Xenotransplantation must not be equated with standard treatment.

(b) Data showing how many persons have been recipients of xenotransplants; how long they survived (mortality); and what the quality of life of survivors has been (morbidity).

(c) Alternative options for subjects, including the option of no treatment.

(d) A fair estimate of risks (including both probability and magnitude) associated with: (i) major surgery (and, in the case of bridging, double major surgery); (ii) transferring zoonotic agents or pathogens to the recipient via xenotransplantation, and an explanation of what steps will be taken to identify such agents or pathogens, and to treat the recipient if he or she becomes infected; (iii) quarantine of the subjects after transplantation for a period of time to make sure that the subjects will not spread new and dangerous pathogens; (iv) the probability of infection of subjects whose immune system has been compromised by opportunistic pathogens; (v) the probability of rejection of the transplanted organ; (vi) the probability of graft-versus-host disease, and the probability and magnitude of minor, moderate, or serious graft-versus-host disease; (vii) for those who receive an animal organ as a bridging technique—a fair estimate of the time that will elapse between surgical implantation of the bridge and obtaining and transferring a human organ, and the extent to which the bridge organ may increase the probability of rejection of the subsequent transplant of a human organ; (viii) financial costs (if any) to subjects or their family or heirs; (ix) the probability of short- and long-term survival, including the expected quality of life of subjects; and (x) a determination by the IRB that risks, taken as an aggregate, are reasonable in the light of expected benefits.

- A statement of the care and compensation that will be provided to subjects during the course of and subsequent to the research.
- Frank disclosure of the fact that participation must be entirely voluntary on the part of subjects; disclosure of how

[3] See U.S. Department of Agriculture Regulations implementing the Animal Welfare Act as amended in December, 1985. The Regulations are found at Title 9 CFR Part 3, Subparts A through D. See Also the *Public Health Service Policy on Humane Care and Use of Laboratory Animals*, May, 1985.

subjects will be cared for if they elect not to participate as research subjects; disclosure of a source to provide additional information or to answer additional questions.

- Disclosure to subjects of the degree of confidentiality that can be provided. This disclosure must include frank discussion of expected media coverage of the project, and the probability that subjects, family, friends, and staff may be subjected to unwanted attention by the media. Disclosure should also deal with the fact that media may try to "buy" stories from subjects, family, friends, or staff. Agreement should be reached prior to initiation of the research concerning who will make statements to the press, and who, if anyone, will be permitted to sell stories or interviews.
- If the research involves children, then the IRB must meet all relevant additional legal and regulatory requirements for children.

CONDITIONS OF INSTITUTIONAL APPROVAL

The research institution may allow the research to go forward if and only if, the following conditions are met:

- The institution will provide the necessary staff, personnel, and logistical and financial support necessary to conduct both preliminary research in animal models and the actual animal-to-human xenotransplantation.
- The institution will provide fiduciary support of the investigative team including handling public relations, legal council, and a willingness to defend investigators against criticism.
- The institution is willing to continue to provide xenotransplant services if the technique proves to be useful and effective. This willingness includes facing the fact that xenografts used as a bridging device involve not only high cost in dollars and personnel for human-to-human transplant, but additional high cost in dollars and personnel for the bridging technique (If bridging is contemplated, the institution must be aware that bridging will not help to solve the problem of greater demand for transplantable human organs than can be met by the existing supply of human organs).

The research should not be initiated unless the institution is prepared to support xenotransplantation, assuming that the research proves it to be successful, until it becomes a program for standard health care delivery. In other words, the institution should not start xenotransplantation research unless it is prepared to develop the research to the point where it may be adopted as a standard therapy for future patients.

Institutions must also be aware that IRBs are responsible for the rights and welfare of the subjects. IRBs are not required to assess public health risks. Nevertheless, there remains a real but unspecified possibility that a pathogen that was harmless when it was in the animal host, could be transformed in its new, immunosuppressed human host into a serious or deadly disease that could attack the organ recipient or others who come in contact with the transformed pathogen (Allan 1994).[4]

CONCLUSION

Institutions must decide whether they are willing to assume responsibility for the low probability but high magnitude risk to the public health of introducing a new disease into society (Science 1995).[5]

Institutions must also consider carefully whether they are willing to undertake the heavy costs and responsibilities associated with xenotransplantation. Meeting all of the conditions cited above will be no easy task.

No doubt a few institutions will express a willingness to shoulder the costs and responsibilities that accompany xenotransplantation and will make the judgement that they have met the required conditions. Citing the desperate need of subjects whose best hope for survival lies in the receipt of transplanted organs from any reasonable source, a few institutions are likely to proceed with xenotransplantation. Many institutions, however, will choose to wait until such time as (1) xenograft research demonstrates greater, more efficient, long-lasting success of xenografts between species of nonhuman animals; (2) colonies of pathogen-free, purpose-bred animals ideally suited for xenotransplants are readily available; and (3) additional evidence is developed to show that xenotransplantation researchers will not be risking the public health by inadvertently creating or releasing new pathogens.

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[4]Allan's letter stated that, "T*he identification of a previously unknown virus in nonhuman primates illustrates the possibility of doing more harm than good through xenograft transplantation: any pathogen carried by a baboon donor would be introduced to the human recipient along with the baboon organ. Most new pandemics arise through inadvertent transmission of viruses from another species (which functions as a natural reservoir) to humans.*"

[5]In Science's section entitled Sciencescape, the following statement appeared: "*Renewed interest in transplanting animal organs into people is causing consternation at the Food and Drug Administration (FDA), where officials plan to issue a warning that could slow clinical trials set to begin this year. The FDA's concern: "Xenografts" might allow dangerous pathogens lurking in animals to jump to humans....But screening for known viruses does little to apprehend novel pathogens. So FDA officials want stricter safeguards that could include improved tests for pathogens, protocols to quarantine patients, and the creation of colonies of "clean" animals. FDA has the muscle to demand such provisions...but for now... the agency only plans to alert surgeons, health officials and review boards to xenograft risks.*"

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Xenotransplantation: A Historical Perspective

Keith Reemtsma

INTRODUCTION

The increasing success of organ transplantation over the past several decades has had the paradoxical effect of highlighting the scarcity of human donors. One response to the need for organs has been an increased effort in research into cross-species transplantation, usually referred to as xenotransplantation.

While the donor-organ shortage is the most frequently cited reason for renewed interest in xenotransplantation, there are other compelling reasons for pursuing this approach, such as the logistic advantages and the ability to prepare the donor, the recipient, or both should preoperative immunologic modification prove feasible.

The overriding question often asked about xenotransplantation is: Will it work? The answer is that xenotransplantation has worked, and the appropriate questions, therefore are: Under what circumstances can we predict success? And which species, which organs, and which form of immune suppression or immune modification should be used?

The history of xenotransplantation is both interesting and informative. Although the modern history can be dated to 1963, the earlier work provides some background for more recent efforts.

THE MYTHOLOGY OF TRANSPLANTATION

The idea of transplanting organs from animals to humans has intrigued humanity for as long as he recorded his myths and his history. Daedelus, who grafted bird feathers to his arms, was perhaps the first to transplant across the species barrier successfully. He escaped from his island prison in Crete and flew to the mainland of Greece. A similar experiment by his son, Icarus, ended in acute graft rejection, attributed to a thermolabile adhesive. After flying too close to the sun he plunged into the water which is now called, in his honor, the Icaran Sea (Hamilton 1940).

EARLY ATTEMPTS AT RENAL XENOGRAFTING

Early in the twentieth century reports on cross-species grafting (then called heterotransplantation) appeared in the scientific literature. In 1905 in France, Princeteau inserted slices of rabbit kidney into a nephrotomy in a child with renal

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insufficiency. "The immediate results were excellent," he wrote. "The volume of the urine increased; vomiting stopped . . . On the 16th the child died of pulmonary congestion . . ." (Princeteau 1905).

In the following year, Jaboulay, also in France, attempted renal heterotransplantation into humans on two occasions, using vascular anastomoses (Jaboulay 1906). The xenografts, one from a pig and another from a goat, were inserted into the antecubital space. Neither graft functioned, and failure was attributed to vascular thromboses.

In 1910, Unger, in Germany, described his attempt at transplantation of kidneys from a nonhuman primate into man. The patient died 32 hours after transplantation, and autopsy showed venous thromboses (Unger 1910).

In New York in 1923, Neuhof attempted treatment of a patient with mercury bichloride poisoning by renal heterotransplantation. When he was unable to obtain a human kidney, he transplanted the kidney of a lamb into the patient. The patient died 9 days later, but Neuhof was not totally discouraged. He wrote, "[This case] proves, however, that a heterografted kidney in a human being does not necessarily become gangrenous and the procedure is, therefore, not necessarily a dangerous one, as had been supposed. It also demonstrates that thrombosis or hemorrhage at the anastomosis is not inevitable. I believe that this case report should turn attention anew . . ." (Neuhof 1923).

However, scientific interest in transplantation declined when the immunological basis of the rejection process was established. With the demonstration of effectiveness of immunosuppressive drugs, there was renewed interest in transplantation. An accelerated effort in renal allotransplantation was accompanied by problems in procuring organs. Ethical considerations posed difficult problems, particularly in the use of volunteer human donors. The use of organs harvested from human cadavers depended on rapid transfer or preservation and imposed restrictions of supply, selection, and scheduling.

THE TULANE UNIVERSITY CHIMPANZEE-TO-MAN RENAL XENOGRAFT EXPERIENCE

In our renal allografting at Tulane University in New Orleans, we had increasing difficulty obtaining donor organs. Attempts to use cadaveric kidneys were inadequate. We were reluctant to press the use of volunteer humans for ethical, scientific, and legal reasons. Chronic dialysis was not available.

As this impasse was developing, we decided to explore the use of nonhuman sources for clinical renal transplantation. This decision was prompted, in part, by clinical urgency. Additionally, a regional primate center in the vicinity brought scientists experienced in primatology. Furthermore, an active program in transplantation immunology had been developed to give an added base to the study.

Our basic conjuncture was that kidneys from nonhuman sources closely related to humans would respond similarly to human kidneys following transplantation into man. The problem of presumably more strenuous immune suppression was balanced against the advantages in the use of nonhuman donors.

In practice, all patients were terminal uremics, maintained on dialysis, who were presented with the following alternatives: (1) supportive treatment only, (2) an allograft from a relative, (3) a cadaveric allograft if available, or (4) a heterograft (xenograft).

The risks, the uncertainties, and the experimental nature of the work were discussed with the patients and their families. If they chose to proceed with transplantation and had no volunteer donor, a search was made for a cadaveric kidney. If no suitable cadaver kidney became available, a xenograft was used, with the patient's understanding and consent.

The Chimpanzee as Donor

The chimpanzee was selected as the donor for several reasons. They included (1) the chimpanzee's close taxonomic relationship to man; (2) its range of size, which approximates that of man, a factor that might have significance in the transplantation of other organs in addition to kidneys; (3) its renal function corresponds closely to that of man; and (4) chimpanzees have been found to be of blood types A and O, thereby offering the possibility of the universal donor from the standpoint of blood groups.

Between November 5, 1963, and February 10, 1964, six patients received renal heterotransplants from chimpanzees. All patients were in terminal uremia necessitating dialysis and all patients received pretransplantation treatment with azathioprine, actinomycin C, and steroids. The donor was selected based on body size and blood typing of both donor and recipient. Creatinine clearance was determined in each donor.

In each instance the donor received general endotracheal anesthesia with monitoring of blood pressure, electrocardiogram, and body temperature. A moderate hypothermia (about 30°C) of the entire renal complex, including both kidneys and ureters, aorta, and vena cava, was removed en bloc after anticoagulation and was irrigated. Patients were prepared simultaneously by extraperitoneal exposure of the external iliac artery and vein. In each instance the aorta and vena cava of the graft were anastomosed to the recipient's external iliac artery and vein, respectively, in an end-to-side fashion. The periods of ischemia, from the time of vessel clamping in the donor until blood flow was restored through the graft in the recipient, varied from 36 to 43 minutes.

All patients received postoperative azathioprine, actinomycin C, steroids, and X-irradiation to the graft. Two cases are summarized below.

Case 1

A 43-year-old former dock worker with a history of hypertension since 1957 was admitted to the Veterans Administration Hospital, New Orleans, in 1959. Renal biopsies showed nephrosclerosis and chronic glomerulonephritis. He was treated with dietary management, including salt restriction. He was readmitted in June 1963 because of progressive uremia, hypertension, and congestive heart failure. Laboratory studies included the following: blood urea nitrogen (BUN) 240 mg%, creatinine 14 mg%, and creatinine clearance 8 ml/min. There was no improvement with dietary management, and peritoneal dialysis was required.

On November 5, 1963, he received a renal xenograft. During the first 14 hours after transplantation, the urinary output was 5700 ml. The BUN, which was 112 mg% on the day of operation, decreased to 39 mg% by the 4th day of operation, and fell to 1.5 mg% 48 hours after transplantation. Four days after transplantation, rejection occurred, but was reversed following local irradiation to the graft and increased doses of immunosuppressive drugs. His early course has been reported previously in detail. Function of the graft was confirmed by renograms, scans, and intravenous urogram.

Serial renograms demonstrated a progressive delay in the appearance of the peak uptake. Changes in the renogram, however, were not correlated with biochemical changes in renal function.

Hemagglutination studies demonstrated a precipitous rise in titer beginning on the 4th day following transplantation. The titer fell to pretransplantation levels at the end of 1 month and remained at this level throughout the 2d month. Data on cytotoxicity studies are shown.

On December 18, he was allowed to leave the hospital because he was asymptomatic and had normal renal function. He was readmitted on December 20 with a temperature of 39.4° C, and radiographic evidence of an infiltrate in the right middle lobe with pleural effusion. Culture of the sputum revealed *Aerobacter aerogenes*. The dosage of azathioprine was lowered because of leukopenia, but renal function continued satisfactorily. The patient's condition later deteriorated rapidly, and he died 63 days after transplantation following a period of shock, apparently due to sepsis.

Autopsy showed acute bronchopneumonia (right lower lobe) and acute tracheobronchitis with resolving abscess (right middle lobe). The transplanted kidneys showed acute tubular necrosis, consistent with shock. There were no cellular infiltrates or changes in the blood vessels (Reemtsma and others 1964).

Case 2

A 23-year-old schoolteacher was admitted in November 1963 with chronic glomerulonephritis and progressive uremia. She had experienced an episode of acute glomerulonephritis at age 14, and demonstrated persisting proteinuria. She had remained asymptomatic until approximately 5 months before admission, when she noted weakness and dizziness.

On admission her blood pressure was 190/120 mmHg, and laboratory studies included BUN of 184 mg%, creatinine of 40 mg%, and creatinine clearance of 4 ml/min. Rapid deterioration of her condition necessitated peritoneal dialysis.

On January 13, 1964, she received a renal heterotransplant. Diuresis occurred with a urinary output on the day of operation of 7 liters. By the 3d day following transplantation the BUN had fallen from a preoperative level of 116 mg% to 12 mg%, and the serum creatinine from a preoperative level of 21 mg% to 0.9 mg%. Creatinine clearance was 50 ml/min. Her blood pressure fell to normotensive levels (110/70 mmHg). Her subsequent course demonstrated satisfactory renal function until the 23d day following operation when threatened rejection was expected.

Urinary output decreased to 1,000 ml/24 h, BUN creatinine rose to 28 and 1.9 mg%, respectively. Creatinine clearance fell to 23 ml/min, and urinary sodium content to 11.6 mEq for a 24-hour period. Gradual reversal of rejection occurred during the following 2 weeks, although unexplained fever persisted for 3 months. She became asymptomatic and had normal renal function 8 months after transplantation.

Serial renograms in this patient demonstrated a delay in peak activity, which coincided with clinical and biochemical evidence of threatened rejection. Following reversal of rejection, the renogram resumed a more normal pattern. An intravenous urogram 12 weeks after transplantation showed function of both transplanted kidneys.

Agglutination studies demonstrated a slight rise in titer at approximately 3 weeks after transplantation. The agglutation titer subsequently returned to previous levels.

This patient died 9 months after transplantation. The cause of death was thought to be acute electrolyte imbalance. Autopsy showed no other cause of death. Histology of the transplanted kidneys showed no other cellular infiltration, but subintimal hyperplasia of the arterioles (Reemtsma and others 1964).

SUBSEQUENT CLINICAL STUDIES

Following the initial experience in New Orleans, there was a flurry of activity in the field of primate-to-man transplantation. In December, 1964, three distinguished transplant surgeons, Drs. J.D. Hardy, D.M. Hume, and T.E. Starzl, were attending a surgical meeting in New Orleans. At the end of the meeting I showed these three surgeons the first patient, who was doing well with normal renal function 7 weeks after transplantation. Each of these three surgeons began working in clinical xenografting.

Hardy and others (1964) reported a few months later the first case of heart transplantation in man. He used the heart of a chimpanzee, but was unsuccessful in this attempt. Hume (1964) did a chimpanzee-to-man renal transplant, and the patient died the following day of excessive diuresis.

Starzl (1964) began a series of baboon-to-man renal transplants which were studied extensively and have been reported in detail. The histopathological studies in our chimpanzee-to-man work and in Starzl's baboon-to-man cases were all carried out by Dr. Ken Porter of London.

By 1965 we had dialysis facilities available at Tulane University, and we had developed a successful cadaver organ procurement program. For these reasons we discontinued our clinical renal xenotransplantation.

I have subsequently, however, maintained experimental programs in xenotransplantation, including transplantation of islet cells in several animal models, and xenotransplantation of the heart between different species of primate.

ETHICAL CONSIDERATIONS

In considering the use of nonhuman species as donors for transplants into humans there are ethical issues concerning both the recipient and the donor, and, in addition, the process of experimental procedures in general.

For this discussion, I shall confine my remarks to the use of nonhuman donors. The questions that arise are these:

1. Is it *ever* acceptable, from an ethical point of view, to use nonhuman animals to treat human illness?
2. What species should we use?
3. Which organs and tissues should be used?
4. Under what circumstances should xenotransplantation be moved from the laboratory to the clinical setting?

The general use of animals for treating human illnesses now is widely accepted. Insulin from animal sources has been used to treat diabetes in humans for most of this century. Heart valves from animals now are routinely used in cardiac surgery worldwide.

The selection of the species poses several problems. From an immunologic standpoint, we would prefer species most closely related to man. The chimpanzee, however, is an endangered species and cannot be used in terminal experiments. The baboon is more distant from humans and does not reach the size of the chimpanzee or adult human. Although the baboon is not an endangered species, it is, nevertheless, a primate, and as such this work raises ethical concerns.

The use of nonprimate donors, such as pigs, reduces ethical concerns, but the use of organs and tissues from pigs into humans involves a higher immunologic barrier than with primates. Extensive studies now are underway to modify pig donors, such as with transgenic techniques to reduce problems involved in transplantation.

The final, and most difficult question, involves the transfer of work from the laboratory to the clinical setting. There is no single criterion that can be applied to this decision. The variables include success of laboratory work, the applicability of animal studies to clinical experiments, the degree of urgency, and the availability of alternate solutions.

Xenotransplantation in the future may involve a broad spectrum of tissues, from cells and subcellular components to organ grafts. Some problems, such as transmission of microorganisms, may be similar across this spectrum, but other aspects of xenotransplantation may vary with the organ or tissue used. Furthermore, with the immunologic approaches to xenotransplantation continuing to undergo rapid evolution, it would be premature to prescribe guidelines or regulations governing the translation of work from the laboratory to the clinic.

The current increase in interest in xenotransplantation is based both on clinical needs and on promising leads being pursued in different laboratories. When and how these advances are translated into clinical programs are decisions best left to the groups of investigators involved in this work. The trend is unmistakable, and clinical success is probable, although not assured, in the near future.

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The Application of Xenotransplantation In Humans—Reasons to Delay

David J. R. Steele and Hugh Auchincloss, Jr

INTRODUCTION

There is consensus, among those involved in the field of clinical transplantation, that a need exists for a larger pool of organ donors. Efforts to enlarge the current pool of cadaver organ donors have not alleviated what has now become a critical shortage of donors. As a result, substantial numbers of patients with end stage disease of vital organs, 2,359 in 1991—an increase of 20 percent over the previous year (according to the most recently available UNOS figures)—are dying while awaiting transplantation (DHHS 1993). Those on the waiting list are waiting longer for their organs, and even if efforts currently in place were able to maximize the pool of cadaver donors, there would still be a shortfall in meeting potential demand. This situation is particularly frustrating to clinicians given the high rate of successful outcome in those transplants that do occur.

For these reasons interest is focused on xenogeneic tissue as an alternative for those requiring transplantation. The progress made in the field of experimental xenotransplantation over the past few years has further encouraged clinicians, particularly the measurable success that has been achieved in the survival of transplanted xenogeneic organs under certain circumstances, most notably in concordant rodent species (Hassan and others 1992). However, the barriers to successful clinical xenotransplantation are much greater than those to allogeneic transplantation, and clinically successful xenotransplantation has yet to be achieved.

CLINICAL EXPERIENCE

Previous clinical experience in xenotransplantation is summarized in Table 1. In all more than 20 patients have received xenografts. Although all these experiments have been failures in the sense that long-term organ survival was not achieved, they were able to show that xenogeneic tissue is able to support human life for a period of time. Rejection of xenogeneic tissue was both humoral and cellular and was more difficult to control than allograft rejection, although in the short term, it could be controlled with conventional immunosuppressants, albeit in large doses.

TABLE 1 Experience in clinical xenotransplantation

Donor	Organ	Outcome	No. Cases	Year
Chimpanzee	Kidney	<9 months	12	1964
Monkey	Kidney	10d	1	1964
Baboon	Kidney	4.5d	1	1964
Baboon	Kidney	<2 months	6	1964
Chimpanzee	Heart	<1d	1	1964
Chimpanzee	Liver	<14d	3	1969-74
Baboon	Heart	<1d	1	1977
Chimpanzee	Heart	4d	1	1977
Baboon	Heart	4 weeks	1	1985
Baboon	Liver	70d, 26d	2	1993

Over and above the immunological and physiological barriers to xenotransplantation, these early cases raised a number of other issues. They included: the rights of humans to use animals to suit their best interests, particularly in the case of primate donors (Singer 1992); concerns about the possibility of transmission of xenotransplant associated zoonoses to recipients under immunosuppression (Michaels 1994); ethical questions regarding the rights of patients, and the performance of extreme medical interventions, in those with reduced life expectancy (Caplan 1992). While it is important that these concerns be addressed when considering clinical xenotransplantation, we believe that none of them represent a barrier to it as such, and that it is reasonable to consider xenotransplantation because too many patients are dying while waiting for an organ. There are, however, other fundamental reasons for not yet performing this procedure. These include a lack of data in support of long-term engraftment, the considerable immunosuppression required to prevent rejection of xenogeneic tissue, and the inability to select appropriate recipients from those currently awaiting allotransplantation. Thus we tend to see the issue as primarily scientific and logistical, rather than ethical.

STATUS OF CURRENT EXPERIMENTATION

Much of the current enthusiasm for clinical xenotransplantation is based on the potential for success indicated by

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research advances achieved in the field over the past few years. However, many of these advances have been made in rodents and do not provide an adequate basis for human experimentation. At the same time, investigators are reporting increased survival times for solid organ transplantation in certain concordant and some discordant species involving nonhuman primate recipients. Monkey to baboon heart transplants have survived for months, and in the case of one group of investigators for more than a year (Michler 1987; Sadeghi and others 1987). Recipients also survived after the heart xenograft was removed and replaced by an allograft in an experiment aimed at proving that a xenograft may be used as a bridge to allotransplantation (Alonso de Begona and others 1992). However, the data for orthotopic heart transplantation in primates are not as encouraging as for heterotopic heart transplantation, and the immunosuppression used was quite toxic in those models documenting long-term successful outcomes (Sadeghi and others 1987).

The animal research data reported to date highlights several important issues relevant to xenotransplantation. For instance, sudden rejection of the Pittsburgh groups most recently performed baboon liver transplant was thought to be due to activation of complement in the recipient, possibly provoked by the transfusion of blood products. Uncontrolled activation of complement will occur in a setting where complement, and the regulators of complement activation, originate from different species (Starzl and others 1994).

In addition, a role for induced antibodies in the rejection of concordant xenograft tissue appears likely. Although treatment strategies have been developed against induced antibody responses, these strategies have involved the use of large doses of cyclophosphamide and other drugs, with resultant severe immunosuppression, to the detriment of the recipient in at least one of the most recently performed baboon-to-human liver transplants (Starzl and others 1994). Alternative, less toxic, therapies are needed to deal with this problem.

Finally, rapid humoral rejection of both concordant and discordant xenografts has in the past limited the ability of investigators to study the mechanisms of cellular rejection in vivo. However, essentially every direct comparison of cell-mediated xenograft compared with allograft rejection has indicated that larger doses of nonspecific immunosuppressive drugs are needed to control cell-mediated xenograft rejection. Recent in vitro evidence showing intact direct recognition of discordant xenogeneic tissue, coupled with in vivo work in those concordant transplants with prolonged survival times, will allow further clarification of the role of cellular xenogeneic rejection, and the interventions necessary for its control (Murray and others 1994; Auchincloss 1994).

These considerations have led us to conclude that the immunological barriers to xenotransplantation are greater than for allotransplantation and, therefore, that higher levels of immunosuppression will be needed to accomplish long-term xenograft survival.

Patient selection for xenotransplantation

In considering all the available data, including previous clinical experiences, it is now apparent that if the large doses of immune suppression are tolerated, some xenogeneic transplants will probably survive in human candidates for a period of time. Assuming that concordant xenotransplantation will work in a given number of cases, and a small percentage of transplants may even achieve prolonged engraftment, the question then is how to identify appropriate recipients for these second best organ transplants.

In the past, selection of patients for xenotransplantation has been based essentially on two criteria (1) the unsuitability of the recipient to receive an allotransplant (e.g. Hepatitis B infected patients for liver transplants because of the high recurrence rate in the allotransplanted organ and the resistance of baboon livers to Hepatitis B virus), or (2) the unavailability of an organ for a dying patient. The exclusion of Hepatitis B infected patients from liver allotransplantation is not absolute and a number of centers will transplant these patients. In fact, the survival rates reported for allotransplantation in these patients with Hepatitis B is superior to that which we could expect from xenotransplantation at this time. Another possible group might be in patients who run out of dialysis options, who have failed previous attempts at allotransplantation, and who are highly sensitized. We do not know, however, whether such patients are also likely to be sensitized to concordant donor antigens.

For organs such as the heart and the liver where there are limited options for chronic replacement therapy other than transplantation, failure to obtain a human organ in time often leads to the patient's demise. However, current policies favoring allograft allocation to the sickest patients, means that even as the patients approach imminent death, they still have a better chance of long-term survival by waiting for a last-minute human organ than by opting for a xenotransplant. Under our current system of organ allocation, some patients waiting for a heart or a liver transplant will die of organ failure while waiting for human organ. The ideal circumstance would be to offer a xenotransplant to those who would die without achieving allotransplantation. However, to achieve this will require changes in our organ allocation policy (Auchincloss 1993).

XENOTRANSPLANTATION AS A BRIDGE TO ALLOTRANSPLANTATION

One intermediate proposal is to use xenotransplantation as a bridge to allotransplantation in patients who are approaching death while waiting for a graft. This proposal is superficially compelling, particularly in the pediatric group, for whom the size of the baboon heart is well suited, and for whom there is not only a waiting list mortality comparable to that of adults, but also some waste of organs due to availability, timing, and size of donors and recipients (Michler and Chen 1994). Once

again though, it is hard to select patients to receive the less favorable option of xenotransplantation, instead of possibly waiting longer for an allotransplant. Furthermore, the performance of two major surgical procedures instead of one (allotransplant following xenotransplant) will diminish survival following the allograft, and the xenogeneic tissue may sensitize the recipient against a future allograft (Sachs and others 1971).

Most importantly, the use of xenografts as a bridge to allografting does not address the fundamental issue of the shortage of human donor organs (Gundry 1994). Indeed, the use of xenografting as a bridge will probably diminish the overall survival of those patients who receive our limited number of human organ transplants. Thus, the use of xenotransplants as a bridge to allotransplants will probably benefit some individual patients and provide valuable information to our society about xenotransplantation in human beings, but at a short-term cost of less good overall survival for patients with organ failure.

AN APPROACH TO CLINICAL XENOTRANSPLANTATION

In order that there be a high likelihood of a successful outcome for the early patients entering a trial of clinical xenotransplantation we would recommend further experimental documentation of successful long-term xenotransplantation using tolerable doses of immunosuppression, in nonhuman primate models. It is accepted that there exists a need for an alternative therapy in a large number of patients who will wait unsuccessfully for a transplant. Even though xenotransplantation offers the potential for an expanded pool of donor organs, which could be obtained electively, it is competing with an established successful therapy, namely allotransplantation. A large part of the problem with xenotransplantation as it currently stands, is that the reduced chances of long-term graft survival compared to allotransplants make it an unacceptable therapeutic option in the clinical setting, most of the time.

For situations where xenografting might be considered, when no other alternative is available for instance, a system needs to be developed so that potential recipients of xenografts can be clearly identified. A solution would be to change allograft allocation policy, such that healthier candidates are more readily able to have access to an allograft, and sicker patients after a defined wait at highest priority, would lose that advantage (as their chances of a successful outcome are reduced). Selected patients from this group might then become candidates for xenotransplantation.

Ultimately, if xenografting can be shown to offer predictable long-term successful outcomes, patient selection for the procedure will be simplified.

CONCLUSION

Clinical xenotransplantation cannot yet be offered as an acceptable form of organ replacement therapy. Fundamental questions remain about the rejection of xenogeneic tissue and how to deliver the least amount of immune suppression safely to prevent rejection. Referral of appropriate candidates for xenotransplantation will remain problematic in this setting. Good animal models exist in which these issues can be investigated, and hopefully solved, so that xenotransplantation could be offered to selected patients with at least the equivalent hope of success of allotransplantation. It would be under these circumstances that this form of treatment should be applied to a patient population to their best advantage.

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The Immunologic Response to Xenografts

David H. Sachs

INTRODUCTION

The rejection of xenografts is clearly an immunologic phenomenon, since in the absence of an immune response, animals accept transplants even across widely disparate xenogeneic barriers. For example, nude mice, in which the absence of the thymus leads to defective T-cell immunity, have been shown to accept xenogeneic skin grafts (Manning and others 1973), even to the point of growing chicken feathers. Similarly, some of the lymphohematopoietic compartments in severe combined immune-deficient (*scid*) mice can be repopulated by highly disparate xenogeneic hematopoietic cells (Mosier and others 1988; McCune and others 1988). In theory, eliminating the immune response to a xenograft should be sufficient to assure its success. This premise is of more than just theoretical importance to the field of xenotransplantation, since there certainly might have been other, non-immunologic barriers that could have prevented xenotransplantation even in the absence of an immune response. For example, the cell surfaces of xenogeneic tissues might have been physiologically incompatible, or the red cells of the recipient might have been physiologically incapable of delivering oxygen to xenogeneic tissues or even unable to negotiate xenogeneic capillaries. Nevertheless, at least between mammalian species, it now seems likely that the immune response is the barrier of greatest importance.

As is the case for other immune responses, the reaction to xenografts involves both humoral and cellular immunity. Xenografts have been further categorized as concordant or discordant on the basis of phylogenetic distance and vigor of the immune response (Calne 1970). The most notable immunologic difference between concordant and discordant xenografts involves the presence in the latter of natural antibodies capable of causing hyperacute rejection of vascularized organs. As will be described in more detail below, although natural antibodies have posed a formidable barrier to discordant xenografting in the past, there are now numerous methods for eliminating these antibodies or controlling their effects, which have shown promising results in avoiding hyperacute rejection. However, both humoral and cellular immune responses to xenografts will undoubtedly be as strong as, or stronger than, responses to allografts, and will have to be overcome if xenotransplantation is to become a reality. I will review here our present understanding of these two arms of the immune response for both concordant and discordant xenografts and will try to identify strategies that may overcome the resultant barriers that each of these responses poses to successful xenotransplantation.

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CONCORDANT XENOGRAFTS

Humoral Responses

The most widely studied concordant xenograft systems involve closely related rodent species such as the mouse and rat. In many respects, the humoral response to transplants between such species is similar to that observed for MHC-mismatched allotransplants, and as implied by the definition of concordant species, hyperacute rejection is not observed when primarily vascularized transplants are performed. However, the absence of natural antibodies between concordant species is relative rather than absolute, and even in the rat-mouse system, natural antibodies have been detected when carefully sought (Aksentijevich and others 1991a). Thus, using flow cytometry and cytotoxicity assays, it has been determined that normal mouse serum contains natural antibodies with specific binding to, and cytotoxicity against, *scid* rat bone-marrow cells (Aksentijevich and others 1991a). These natural antibodies were predominantly of the IgM and IgG3 classes, and activity toward bone marrow cells was much greater than that toward spleen cells. Such antibodies probably explain the observation that much greater numbers of rat than of murine bone-marrow cells are required to achieve engraftment in mice (Ildstad and Sachs 1984). To more directly evaluate the effect of these natural antibodies on engraftment of rat bone-marrow cells in mice, adoptive transfer studies were performed using T- and B-cell-deficient *scid* mice as recipients (Aksentijevich and others 1991b). Because of their immunodeficiency, *scid* mice accepted rat bone-marrow cells readily, with only a low dose of whole body irradiation being necessary for conditioning. Passive transfer studies showed that normal mouse serum could markedly inhibit the engraftment of rat bone-marrow cells even in this phylogenetically close species combination, consistent with the hypothesis that natural antibodies provide a barrier to engraftment of xenogeneic bone marrow.

Natural antibodies are probably present in other concor-

dant species combinations, including primates. This should hardly be surprising, given the existence of ABO antibodies even within the human species. For the most part, these antibodies are probably of sufficiently low titer that they do not lead to hyperacute rejection. Nevertheless, pretransplant cross matching will certainly be required in order to avoid those situations in which relatively high titers of such antibodies might lead to catastrophic loss of a transplanted organ. As has been demonstrated for ABO antibodies, it should be possible to eliminate hyperacute rejection due to such natural antibodies by plasmapheresis or absorption techniques (Alexandre and others 1987).

In contrast to natural antibodies, the induced humoral response across concordant species barriers is likely to pose a major barrier to xenotransplantation. In the case of rat anti-mouse responses, immunization similar to that used to produce alloantisera was effective in raising high titers of xenoantibodies very rapidly (Sachs and others 1971). Within such xenoantisera considerable titers of antibodies reactive with mouse MHC alloantigens were detected, along with other antibodies of equal or greater titer reacting with species-specific antigens (Sachs and others 1971). The initial humoral response following immunization consisted predominantly of IgM, and this shifted to IgG after 2-3 weeks, suggesting a typical T-cell-dependent response (Davie and Paul 1974).

Similarly, humoral antibodies have been a major feature of the response to concordant xenografts in several other systems. For example, the rejection of heterotopically transplanted hearts from cynomolgus monkeys to baboons was correlated with the development of cytotoxic antibodies in the recipients' sera (Sadeghi and others 1987). For this reason, splenectomy and cyclophosphamide treatment or both have been used to prolong xenograft survival in several concordant species combinations by diminishing antibody responses, presumably by removing or inhibiting antibody-producing B-cell populations (Edwards and Rose 1989; Thomas and others 1992). Antibody was likewise a prominent feature of the immune response of human-to-baboon liver transplants, which was apparently controlled by high-dose immunosuppressive medications (Starzl and others 1993b). It therefore seems clear that the measures that will have to be taken to avoid the induced antibody response to concordant xenografts will have to be as least as vigorous as they are for MHC-mismatched allografts. One fortunate note is that because the predominant problem following sensitization appears to be the T-cell-dependent IgG response, it seems possible that induction of tolerance at the T-cell level (see below) will carry with it tolerance at the B-cell level with respect to this induced humoral response.

Cellular responses

The cellular response to concordant xenografts is, in general, similar to that of allografts (Auchincloss 1988). In vitro cellular assays such as the mixed lymphocyte reaction (MLR) and the cell-mediated lympholysis (CML) assays have been shown to be qualitatively and quantitatively similar for concordant combinations of primate (Martinis and Bach 1977), canine (Hammer 1991) and rodent (Ildstad and others 1984) species to the corresponding assays within each species. Since the acute rejection of untreated vascularized allografts is predominantly a cell-mediated phenomenon, as one might expect, survival times for vascularized concordant xenografts are similar to those for MHC-mismatched allografts (Auchincloss 1988). Likewise, immunosuppressive agents such as anti-thymocyte globulin and Cyclosporin A, which are successful in controlling allogeneic cellular rejection, have also been effective in suppressing the cellular response to concordant xenografts (Russell and Monaco 1967; Sadeghi and others 1987).

Indeed, as early as 1964, Reemtsma and colleagues reported survival of a chimpanzee kidney in a patient for 9 months using azathioprine, Actinomycin C, steroids, and irradiation of the kidney transplant (all of these being the recommended treatment for allotransplants in that era) as the only immunosuppression (Reemtsma and others 1964). The long-term survival obtained suggests that for the chimpanzee-to-human combination, current drug therapy for preventing allotransplant rejection would probably suffice. However, since chimpanzees are now considered an endangered species, it is highly unlikely that these animals will see further use as clinical concordant xenograft donors. The two clinical baboon-to-human liver xenografts reported recently from Pittsburgh were likewise treated with a four-drug immunosuppressive regimen including three agents previously used in varying combinations for allotransplantation (FK506, prednisone, and prostaglandin E). Cyclophosphamide was added to the treatment for its potential effect on the humoral response. Although neither patient survived long-term (70 and 26 days, respectively) it was noteworthy that both showed little or no evidence of cellular rejection in biopsy or autopsy specimens (Starzl and others 1993a, 1994). Infectious complications were the immediate cause of death in both patients, and the investigators attributed this complication at least in part to over-immunosuppression. It therefore remains unclear whether or not levels of immunosuppression identical to those that are effective for allotransplantation would have sufficed to avoid cellular xenograft rejection for this concordant baboon-to-human xenograft combination.

DISCORDANT XENOGRAFTS

Humoral responses

As noted above, one of the most important differences between concordant and discordant xenografts is that discordant xenografts contain preformed or natural antibodies capable of causing hyperacute rejection of vascularized organs. In general, the further the phylogenetic distance between two species, the greater the detectable levels of such preformed antibodies (Hammer and others 1973), which increases the

likelihood of hyperacute rejection. Although natural antibodies are defined as those that are present in the absence of immunization, it is likely that these antibodies actually represent cross-reactions between antibodies directed against bacterial cell-wall antigens and antigens on the surface of xenogeneic cells. Consistent with this hypothesis is the fact that germ-free animals have been shown to have very low levels of natural antibodies (Hammer 1987).

The majority of natural antibodies are of the IgM subclass (Hammer 1987; Latime and others 1994). It has been clear for some time that these antibodies are cross-reactive and bind to carbohydrate determinants on glycoproteins and glycolipids (Galili and others 1984), and there is increasing evidence that the majority of natural antibodies in the human anti-pig combination are directed to the α1-3-galactose linkage (Oriol and others 1993; Sandrin and others 1993). IgM antibodies are known to be excellent activators of complement, and the effects of these antibodies on xenografts often involve activation of the complement system (Dalmasso and others 1991). For this reason, several approaches have been taken to avoid hyperacute rejection by eliminating the complement-mediated cytotoxicity pathway. These include use of complement inhibitors (Pruitt and others 1991; Miyagawa and others 1993) and potentially the production of transgenic pigs bearing species-specific complement inhibitory molecules such as decay accelerating factor (Langford and others 1994). However, natural antibodies also appear to be effective in activating endothelial cells, which may play a major role in the hyperacute rejection process (Platt and others 1991), and which may not be avoided by complement inhibition.

The fact that natural antibodies are predominantly IgM may be advantageous from the point of view of xenotransplantation, since IgM responses are generally primary and do not involve long-term immunologic memory. Memory for antibody formation is generally thought to occur at the stage of the IgM to IgG switch and to involve T-cell help (Davie and Paul 1974). Therefore, one might hope that if natural antibodies can be removed prior to xenotransplantation, and if T-cell tolerance to xenografts can then be induced, natural antibodies may not recur.

As indicated in Table 1, there are several procedures which have been suggested as potential ways to eliminate xenoreactive antibodies. Alexandre and colleagues have pioneered the use of extensive plasmapheresis as a means of removing natural antibodies (Alexandre and others 1989). In our own laboratory we have chosen an absorption technique using a pig liver to absorb antibodies in vivo prior to the xenotransplant (Latinne and others 1993; Tanaka and others in press). A one-hour perfusion was found sufficient to remove the vast majority of natural antibodies and to eliminate hyperacute rejection. More recently, we have begun to use columns in which an insoluble matrix bearing α1-3-gal epitopes is substituted for the liver in our perfusion step, and the results are very encouraging (Sablinski and others unpublished data).

The induced antibody response to discordant xenografts may pose a more difficult problem for xenotransplantation than do natural antibodies. The induced antibodies that occur in response to such transplants are predominantly IgG and result from a T-cell-dependent response. As such, they involve long-term memory, so that absorption procedures are unlikely to provide a lasting solution to their elimination. It is not yet clear how much of the induced response is directed to the same determinants that are detected by natural antibodies and how much is directed to additional antigens. Clearly, immunization across disparate species barriers leads to antibodies to many surface molecules, with antigenic determinants carried both by proteins and carbohydrates. Methods aimed at avoiding T-cell responses to xenografts should also mitigate against the generation of induced antibodies. Perhaps the most effective way to eliminate this problem will be to avoid it, by inducing tolerance at the T-cell level, as discussed below.

TABLE 1 Strategies for eliminating natural antibodies

1. Plasmapheresis
2. Absorption
   - A. Organ perfusion
   - B. Insolubilized antigen
     - 1) Natural
     - 2) Synthetic
3. Anti-Ig
   - A. Class-specific (e.g. anti-IgM)
   - B. Anti-Idiotype
4. Anti B kcell/Plasma-cell Rx
5. Induction of B-cell tolerance

Cellular response

The primary cellular response to all transplants takes time, since both an afferent response (that is, sensitization and proliferation) and an efferent arm (mobilization of effector T cells) are required. Since natural antibodies cause hyperacute rejection within hours, most in vivo studies of discordant xenografts have concentrated on humoral and complement-mediated mechanisms rather than on the cellular immune response.

However, in vitro cellular responses to xenogeneic cells have been studied extensively. Early studies produced the surprising result that cellular immunity to xenogeneic antigens appeared to be weaker than the corresponding responses to allogeneic antigens (Wilson and Fox 1971; Engelhard and others 1988; Widmer and Bach 1972; Simonsen 1967). Such analyses suggested lower precursor frequencies both for proliferative and for cellular cytotoxic responses. On the basis

of these lower frequencies, it was hypothesized by Jerne (1971) that the primary reactivity of T cells in the immune response was directed toward alloantigens (that is, minor variants of self-MHC antigens). However, there are numerous other reasons why cellular reactivity across discordant barriers could appear weaker than alloreactivity besides a difference in the T-cell receptor repertoire. One other potentially significant factor is the species-specificity of some accessory molecule interactions. For example, it is now clear that CD4 molecules on T-helper cells add to the affinity of the interaction of these cells with stimulator cells expressing class II antigens by interacting directly with the constant portion of class II molecules (Gay and others 1988). CD4 molecules are highly conserved within species, but divergent between species (Pames 1989), and this is also true of the constant portions of class II molecules (Klein 1986). Therefore, depending on the species combination studied, the interaction between CD4 and class II antigens could lead to diminished apparent frequencies of responder cells, because only T cells bearing receptors with high enough affinity for xenogeneic class II to react effectively even without a CD4 class II interaction would be counted as positive. Similarly, one might expect second-signal interactions (such as CD28-B7), which are needed for activation (Azuma and others 1992), to depend on the effectiveness of the receptor-ligand interaction across the species difference under study. Still another possible reason for lower apparent reactivities for xenogeneic interactions may be the species-specificity of certain cytokine interactions (Benfield and others 1991).

Extensive studies by Auchincloss and colleagues using the mouse as the responding species and human, monkey and pig skin grafts as the discordant donor transplants, have demonstrated that many of these potential defects may explain the apparent decreased rejection response (Moses and others 1992). These authors have demonstrated that the failure of direct CD4 class II and CD8 class I interactions in these species combinations leads to exclusive use of the indirect pathway for sensitization to xenografts (Moses and others 1990), that is, presentation of xenogeneic class I peptides on self class 11 molecules (Sayegh and others 1994). If this were true for all xenogeneic cell-mediated responses, one might actually expect the xenograft reaction to be easier to control than an allograft reaction, which includes both direct and indirect pathways of sensitization. On the other hand, with the exception of skin grafts on mice, the cellular responses that have been encountered for discordant xenografts in vivo have been faster and stronger than those for allografts (Auchincloss 1991). In addition, more recent studies in several laboratories, including our own, have indicated that the human anti-pig cellular response is mediated both by direct and indirect pathways of recognition (Murray and others 1994; Kumagai-Braesch and others 1993; Yamada and others in press). Therefore, at least in this highly relevant discordant species combination, it is likely that regimens at least as potent as those required to suppress allograft rejection will undoubtedly be needed.

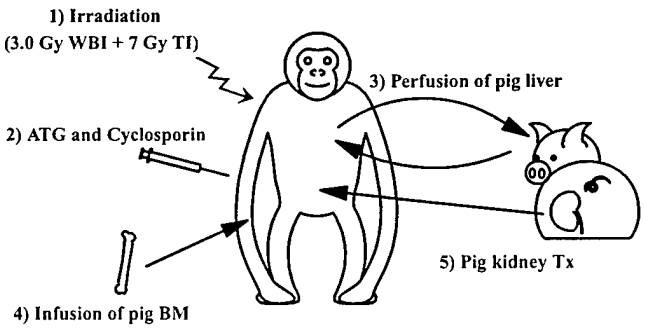


Figure 1. Pig-to-cynomolgus monkey protocol.

MIXED CHIMERISM AND TOLERANCE

Considering the nature of the discordant xenograft response, and the fact that even for allografts the titration of immunosuppressive drugs places the transplant patient on the border between rejection and infection, the amount of nonspecific immunosuppression that will be required to avoid xenograft rejection may be so great that too many patients would succumb to infectious complications. For this reason, it seems likely that the success of clinical xenografting will depend, at least in part, on finding ways of inducing tolerance across xenogeneic barriers rather than relying entirely on nonspecific immunosuppressive agents. In our laboratory, we are pursuing the use of mixed chimerism as a means of inducing tolerance across xenogeneic barriers.

The methodology we have developed is based on previous work in allogeneic and concordant xenogeneic systems in rodents (Ildstad and Sachs 1984; Sharabi and Sachs 1989; Sharabi and others 1990). The approach has also recently been extended successfully to an allogeneic system in primates (Kawai and others in press). In essence, mature T cells are depleted from the recipient animals, and sufficient ablation is administered to make room for donor bone-marrow cells to engraft. In contrast to the use of bone marrow transplantation as a treatment of leukemia, in which case complete ablation of host bone-marrow elements is required, such ablation is neither necessary nor desirable when bone marrow transplantation is used as a tolerance-inducing regimen. Instead, it is advantageous to achieve a state of mixed chimerism, in which the presence of certain donor-derived elements induce specific tolerance, while host-type antigen presenting cells maintain normal immunocompetence. Such mixed chimeras show long-term specific tolerance to transplants from the donor strain.

In our initial studies using mixed chimerism to induce allograft and concordant xenograft tolerance, we used lethal irradiation and reconstitution with mixtures of T-cell-depleted host and donor bone-marrow cells (Ildstad and Sachs 1984). These studies demonstrated that stable mixed chimerism established specific tolerance to other donor-derived

tissue transplants. However, lethal irradiation is too toxic a preparative regimen to be considered for clinical transplantation. We have therefore turned more recently to a nonmyeloablative regimen that produces mixed chimerism and tolerance without the use of lethal irradiation (Sharabi and Sachs 1989; Sharabi and others 1990). We have demonstrated that treatment of recipient mice with monoclonal antibodies to the two mature T-cell subsets, CD4 and CD8, followed by sublethal irradiation (300R) and a dose of irradiation to the thymus (700R thymic irradiation) permits engraftment of a subsequent injection of allogeneic bone marrow, and the production of mixed allogeneic chimeras (Sharabi and Sachs 1989). Such animals develop long-term mixed chimerism in all lymphohematopoietic compartments and are indistinguishable by two-color FACS analysis from mixed chimeras prepared by lethal irradiation and reconstitution with mixtures of T-cell depleted syngeneic plus allogeneic bone marrow. They have been shown to be stable mixed chimeras and to be specifically tolerant, as demonstrated by long-term acceptance of donor strain skin grafts and prompt rejection of third-party grafts. Using a very similar regimen, we have now demonstrated multilineage mixed chimerism and long-term tolerance to kidney allografts in a cynomolgus monkey model (Kawai and others in press).

We are now attempting to use a similar methodology to induce tolerance across the discordant xenogeneic barrier of pig-to-cynomolgus monkey. As a donor, we are using partially inbred miniature swine, which were developed in this laboratory over a 20-year period as both a large animal model for studies of transplantation biology (Sachs 1992) and a potential xenograft donor (Sachs 1994). The protocol being attempted is illustrated in Figure 1. We have recently reported the preliminary results of our first studies using this model (Latinne and others 1993; Tanaka and others in press). Recipient cynomolgus monkeys were treated with anti-thymocyte globulin to remove mature T-cell subsets and NK cells. They were treated with sublethal irradiation, similar to that used in our previous rat-mouse studies, and received bone marrow from the pig donor. At the time of operation, the recipient's blood was perfused from the aorta through a freshly isolated pig liver and back to the recipient vena cava for one hour, using Silastic catheters. Following this procedure, a pig renal xenograft was transplanted as a test organ for the induction of transplant tolerance. Our results to date have shown no sign of hyperacute rejection by the absorption , and kidney grafts have survived as long as 15 days. However, we did not achieve persistence of mixed xenogeneic chimerism in this model, nor was long-term tolerance established. Subsequent studies have administered recombinant pig cytokines (IL-3 and SCF) postoperatively in order to favor engraftment of pig bone-marrow elements. These studies remain preliminary at the time of this writing, but are encouraging, especially since the recipients have maintained normal renal function for more than 2 weeks with only a functioning pig xenograft kidney.

SUMMARY

Because xenografts are readily accepted by mutant mice that lack cellular immune function, it seems likely that successful xenografting, even across discordant species barriers, will depend predominantly on effective manipulation of immune responses. However, species-specificity of cytokine interactions and accessory molecule interactions may also have to be considered for long-term success. While immune responses to xenografts show many similarities to immune responses that have been studied extensively for allografts, they also show some differences especially for discordant species barriers. Most work to date for discordant xenografts has concentrated on the problems of humoral immunity. However, cellular responses in vivo are at least as strong or stronger than their allogeneic counterparts. For this reason, the amount of nonspecific immunosuppression required to avoid xenograft rejection is likely to lead to an unacceptably high incidence of infectious complications. It therefore seems likely that the success of clinical xenografting will depend, at least in part, on finding ways of inducing tolerance across xenogeneic barriers rather than relying entirely on nonspecific immunosuppressive agents. One such method that is being pursued in this laboratory involves the use of mixed lymphohematopoietic chimerism to establish specific transplantation tolerance, and the data so far are early but encouraging.

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Xenotransplantation: The Need, The Immunologic Hurdles, and The Prospects For Success

Jeffrey L. Platt

INTRODUCTION

The most urgent problem in clinical transplantation is the shortage of donor organs. Only 15% of patients awaiting a heart transplant in the United States can undergo the procedure in a given year; if the current criteria for cardiac transplantation were extended to all potential recipients, the donor pool would provide less than 5% of the organs needed (Evans 1991). The shortage of other organs for transplantation procedures is nearly as severe (Figure 1). The dimensions of this problem, as well as recent advances in the biomedical sciences including the ability to "genetically engineer" large animals, have provoked interest in the potential use of animals in lieu of humans as organ donors, that is, interspecies or xenotransplantation.

CLINICAL EXPERIENCE IN XENOTRANSPLANTATION

In the early 1900s, the development of surgical techniques that would allow one end of a severed blood vessel to be connected to another (vascular anastomosis) opened the door to organ transplantation. Experimental surgeons such as Alexis Carrel, Emrich Ullman, and Charles Guthrie realized that this surgical technique might be exploited to transplant healthy organs into individuals with chronic organ failure (Guthrie 1912). Because it was believed that human organs in a suitable condition for transplantation would rarely become available, initial efforts in organ replacement focused on xenotransplantation. A few procedures in which sheep or pig kidneys were connected to the circulation of patients with renal failure were performed. However, at best, the xenotransplants functioned only briefly (Ullman 1914; Neuhof 1923). Experimental allografts did function, but inevitably failed within days to weeks (Carrel 1908). Thus, further attempts at clinical transplantation were rarely undertaken until the 1950s when the immunological basis for graft failure became apparent and therapeutic approaches for dealing with immune responses began to emerge.

The availability of immunosuppressive agents in the early 1960s finally made transplantation a rational approach to the treatment of organ failure. Because human organs remained scarce, researchers turned again to the possibility of xenotransplantation. Reemtsma and others (1964) transplanted a series of chimpanzee kidneys into humans. The clinical course of the grafts was characterized by episodes of decreased renal function which, consistent with acute cellular rejection, responded to immunosuppressive therapy. In two chimpanzee-to-human renal transplants, graft loss was associated with infection; in six recipients it was associated with acute cellular rejection. However, some of the grafts lasted

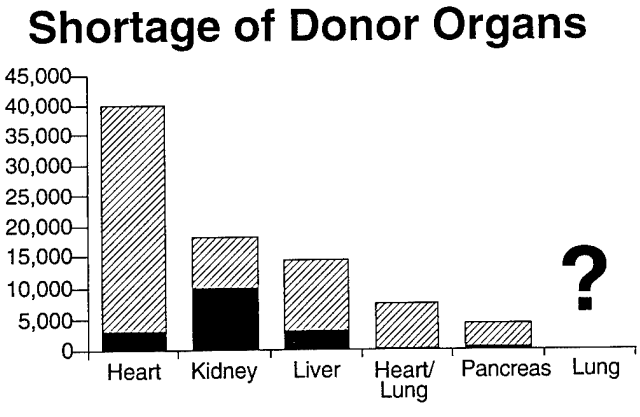


Figure 1. The shortage of donor organs for transplantation. The number of transplants carried out yearly in the United States is indicated in the solid bars. The number of transplants that might be carried out if an unlimited supply of organs were available (Evans 1991) is shown in the open bars.

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for months, the longest of which survived for 9 months. It would not be unreasonable to think that in recipients treated with modern immunosuppressive agents and with the availability of new antibiotics, these grafts might have functioned and the recipients might have survived longer.

At the same time that Reemtsma was reporting the first clinical xenografts, Starzl and others (1964) and Hitchcock and others (1964) performed baboon-to-human renal grafts. The clinical courses of the grafts were characterized by rejection episodes that responded, in part, to immunosuppression and graft irradiation. Graft survival was 10–49 days. The histologic picture of the rejected kidneys suggested that the failure of these grafts was due to acute vascular and cellular rejection. More recently, baboon livers were transplanted into two human subjects with hepatic failure (Starzl and others 1993). Although the recipients of the baboon livers ultimately died, the transplanted organs appeared to be free of rejection at the time of the patients' deaths (Nalesnik and others 1994).

While the results described above might be viewed as promising, there are some serious limitations to the use of nonhuman primates as donors for clinical transplants. First, many nonhuman primates are too small to be used as organ donors in adult patients, and large nonhuman primates are not available in sufficient numbers to address the current need for hearts or kidneys. Second, nonhuman primates may harbor viruses that would be lethal if transmitted to humans. Third, social opposition to the extensive use of nonhuman primates would pose a hurdle. Fourth, current technology does not allow genetic manipulation of primates. Even if suitable techniques were to be developed and societal concerns allayed, the long period between birth and maturity would discourage this approach.

Given the urgent need for donor organs and the problems associated with the use of nonhuman primates, many laboratories are focusing on strategies for overcoming the significant immunological barriers to using nonprimates as organ donors for clinical transplantation. The species generally viewed as being the most suitable for this purpose is the pig. Pigs are of the appropriate size and, are as far as it is known, physiologically compatible with humans. The pig harbors relatively few infectious agents that could be communicated to humans (Michaels and Simmons 1994; Ye and others 1994). Those infections that do pose a risk to humans can be detected by screening procedures. Pigs are born in litters, after a comparatively brief gestation, facilitating the breeding of animals with desired traits. Finally, current technology is available to genetically manipulate pigs, thus allowing suitable donor animals to be genetically engineered.

THE IMMUNOLOGICAL BARRIER TO XENOTRANSPLANTATION

There are some formidable immunologic barriers to transplanting organs from nonprimates into humans. In addition to the immunologic processes that cause injury to organs transplanted between individuals of the same or closely related species, there are some immunologic problems that are especially characteristic of organ transplants between species that are widely disparate. The clinical and pathologic outcomes of such xenografts, reflecting immune-mediated changes, are summarized in Figure 2.

Hyperacute xenograft rejection

Organ xenografts carried out between species that are phylogenetically distant, such as pig-to-dog or pig-to-primate, exhibit a course that is dramatically different from that of allografts, free tissue grafts, and concordant xenografts. These xenografts are subject to a rapid and violent rejection reaction, called hyperacute xenograft rejection, which abolishes organ function in minutes or a few hours (Auchincloss 1988; Perper and Najarian 1966; Platt 1995; Platt and others 1990a). Species combinations that are subject to the hyperacute xenograft rejection of vascularized xenografts are called "discordant" whereas species combinations not subject to this type of rejection are called "concordant" (Calne 1970). For a comprehensive review of hyperacute xenograft rejection, the reader is referred to a recent monograph (Platt 1995).

Hyperacute rejection of xenografts is clinically and pathologically similar to hyperacute rejection of allografts seen in recipients exposed to donor antigens (Platt 1994b). After being connected to the recipient's circulation, the xenograft

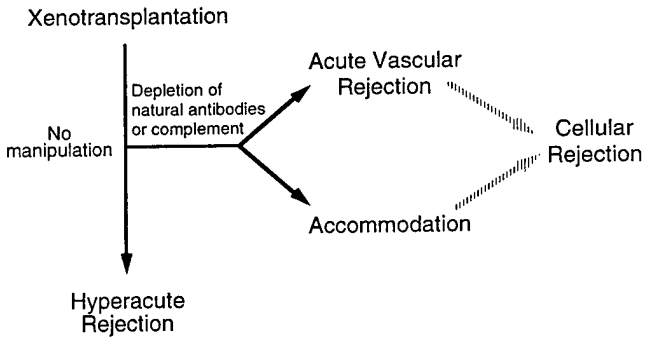


Figure 2. The immunological barrier to xenotransplantation. Transplantation of an organ from one species into a phylogenetically-disparate recipient leads inevitably to hyperacute rejection. Hyperacute rejection can be averted by depletion of anti-donor antibodies from the recipient or by inhibition of the complement system. When hyperacute rejection is averted in this way a delayed form of rejection—acute vascular xenograft rejection—may supervene one to four days later. Acute vascular xenograft rejection is not prevented by inhibition of complement but may be prevented or delayed by further depletion of anti-donor antibodies. In some cases the removal of anti-donor antibodies from the circulation of the recipient allows the development of accommodation—a condition in which the vascularized graft functions without apparent injury caused by the return of anti-donor antibodies. A xenograft may also be subject to cellular rejection more or less similar to cellular rejection of allografts.

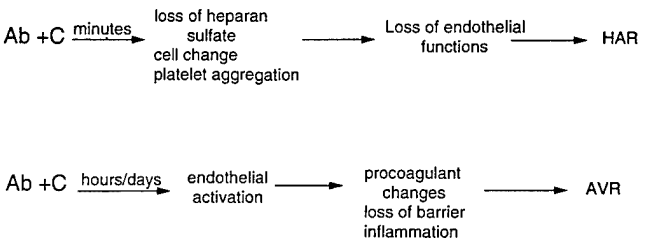


Figure 3. The pathogenesis of xenograft rejection.

may regain color and begin to function; however, the graft rapidly takes on a mottled appearance and the function declines. The histologic features of hyperacute rejection are characterized by interstitial hemorrhage, edema, and thrombosis (Platt 1994a). The immunopathology invariably reveals the presence of complement proteins and usually antibodies of recipient origin in the blood vessel walls (Platt and others 1991b). The events which lead so quickly to hyperacute xenograft rejection involve complement-mediated injury to graft blood vessels (Platt and others 1990b). Complement activation on endothelial cells causes loss of heparan sulfate from the cells and the formation of intercellular gaps (Saadi and Platt 1994). These, and perhaps other changes lead to a loss of the normal barrier and antithrombotic functions of endothelium (Platt and others 1995).

Hyperacute rejection of allografts and of some xenografts, particularly those of porcine organs transplanted into primates, is initiated when recipient antibodies are bound to blood vessels in the donor organ (Platt 1995). Xenoreactive natural antibodies which might initiate hyperacute rejection have been found in the circulation of all mammalian species (Boyden 1964); their presence in the circulation is not linked to prior sensitization with xenogeneic cells. The binding of xenoreactive antibodies activates the recipient's complement system which in turn mediates severe injury to the endothelial lining of blood vessels in the graft (Figure 3).

The importance of natural antibodies in triggering hyperacute xenograft rejection is widely accepted (Hardy and others 1984; Auchincloss 1988), and there is much evidence to support the concept that when a porcine organ is transplanted into a nonhuman primate or into a human, complement activation and thus organ injury is initiated predominantly by this mechanism (Platt and others 1991b; Dalmasso and others 1992; Platt 1995). For example, depletion of xenoreactive antibodies from a primate allows the prolonged survival of a vascularized xenograft (Cooper and others 1988) even if the complement system of the recipient remains intact (Dalmasso and others 1992). Moreover, porcine cardiac xenografts in newborn baboons, which have very low levels of natural antibodies but intact complement activity, are not subject to hyperacute rejection (Kaplon and others 1994).

In some experimental models, however, the complement system of the recipient is activated directly on the surface of donor cells, without the involvement of antibodies (Miyagawa and others 1988). For example, guinea pig hearts transplanted into rats that have been depleted of natural antibodies and rabbit hearts transplanted into newborn pigs which possess no natural antibodies are rejected immediately (Leventhal and others 1993b; Johnston and others 1992). Activation of complement in these models leading to hyperacute rejection of the organ grafts is thought to involve the alternative pathway of complement. The mechanism underlying activation of complement through the alternative pathway probably involves the failure of factor H in recipient plasma to control the spontaneous generation of the C3 cleaving enzyme (C3bBb) on a xenogeneic cell surface. This restricted functioning of factor H has obvious advantages in promoting host defense as it allows activation of complement on the surface of invasive organisms while protecting autologous cells from inadvertent injury. Fortunately, human factor H appears to effectively control alternative pathway activation on porcine and bovine cell surfaces (Edwards 1981).

Another mechanism that renders pig-to-primate xenografts especially susceptible to complement-mediated injury involves the impaired functioning of cell-associated complement regulatory proteins such as decay accelerating factor and CD59. These proteins, which are present in the cell membranes of all mammalian species, protect cells against inadvertent injury during the activation of complement. Thus, when complement is activated on the surface of a microorganism, soluble reaction products, which attach to adjacent endothelium, are prevented from catalyzing further reactions on endothelium. The points in the complement cascade at which complement regulatory proteins exert control are shown in Figure 4. Decay accelerating factor and membrane cofactor protein inhibit the formation and integrity of C3 convertase, the pivotal enzyme complex for the activation of complement. CD59 and homologous restriction factor inhibit the lytic properties of C8 and C9. Some complement regulatory proteins control the activation of homologous complement more effectively than heterologous complement. Thus, the limited ability of complement regulatory proteins in a xenograft to control activation of the complement system of the recipient may contribute to the susceptibility of a xenograft-to-complement-mediated injury.

Regardless of whether natural antibodies or the alternative complement pathway initiate hyperacute rejection, activation of the complement system is an essential event in the

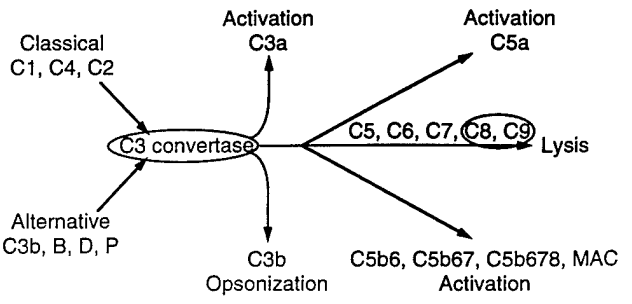


Figure 4. Activation and control of the complement system.

pathogenesis of hyperacute rejection. How does complement cause within minutes the devastating vascular injury characteristic of hyperacute rejection? Figure 4 summarizes the effector mechanisms that may contribute to graft injury. One mechanism of tissue injury could involve the killing of endothelial cells caused by the formation of C5b6789n complexes, called the membrane attack complex, on the cells. Lysis of endothelial cells, mediated by the membrane attack complex, may be important in some cases of hyperacute rejection. However, in the pig-to-primate models that the author has studied, endothelial cell death is not a major lesion (Platt and others 1991b). Rather, complement-mediated changes appear to involve non-cytotoxic processes. In addition to causing cell death, the membrane attack complex of complement may trigger changes in cellular behavior. For example, the membrane attack complex activates endothelial cells leading potentially to procoagulant changes (Hamilton and others 1990).

Complement components other than the membrane attack complex may contribute to graft injury. C5a together with binding of natural antibodies heparan sulfate, an acidic polysaccharide that participates in many of the normal functions of endothelium, to be released from endothelial cells (Platt and others 1991a). The author has postulated that the loss of heparan sulfate from graft endothelium could be responsible in part for the rapid loss of endothelial integrity seen in hyperacute rejection. C5b67 complexes mediate changes in endothelial morphology leading to the formation of intercellular gaps (Saadi and others 1995). The formation of gaps in endothelium may explain the rapid onset and progression of hyperacute rejection. Formation of iC3b a proteolytic product of C3 on endothelial surfaces provides a mechanism for adhesion of neutrophils (Vercellotti and others 1991). To the extent that neutrophils are involved in xenograft rejection, iC3b generation may thus be a critical pathogenic event.

Acute Vascular Xenograft Rejection

If a xenograft recipient is depleted of natural antibodies or complement, hyperacute rejection does not occur. Instead, the vascularized xenograft is subject to a delayed type of rejection which we have called acute vascular xenograft rejection (Leventhal and others 1993b). Acute vascular rejection, which may also be seen in allografts and concordant xenografts, is characterized by the swelling of endothelial cells, prominent fibrin deposition, focal edema, and hemorrhage, changes similar to those seen in hyperacute rejection. Although the histologic picture of acute vascular rejection bears some resemblance to that of hyperacute rejection, its pathogenesis is probably quite different. We have postulated that acute vascular rejection arises as a consequence of the activation of endothelial cells in the graft. This activation leads to the endothelial cells acquiring new functions, including the elaboration of inflammatory cytokines, expression of cell adhesion molecules, and conversion of the endothelial cell surface from anticoagulant to procoagulant (Platt and others 1995). These changes cause the formation of blood clots and the infiltration of leukocytes, which are so characteristic of this type of rejection. The mechanisms contributing to the development of acute vascular xenograft rejection are an issue of current interest because this type of rejection is now viewed as the major barrier to clinical application of xenotransplantation.

Accommodation

Fortunately, acute vascular xenograft rejection is not the only outcome seen in xenografts if hyperacute rejection is prevented. In some cases, the depletion of recipient xenoreactive antibodies and the manipulation of complement allows the long-term survival of the graft even after the antibodies return to the circulation and the complement system is restored. The author has called this condition "accommodation" (Platt and others 1990a) to denote what appears to be an acquired resistance of the graft to humoral reactions, which under other circumstances would cause hyperacute or acute vascular rejection. The "accommodated" xenograft appears histologically normal. It may contain recipient immunoglobulin, but evidence of complement activation and thrombosis is not seen (Platt and others 1991b).

Accommodation was first observed in the clinical transplantation of blood group A or B kidneys into recipients who had antibodies against these blood groups (Chopek and others 1987; Alexandre and others 1987). The temporary depletion of anti-A or anti-B antibodies from the recipient allowed the engraftment of kidneys bearing A or B blood group antigens. In many instances, vascular rejection did not occur after those antibodies returned to the circulation.

Accommodation has also been observed in a few experimental xenografts. Alexandre and others (1989) used plasmapheresis and immunosuppressive therapy to prolong the survival of swine-to-baboon renal xenografts. In three cases, graft function in excess of 20 days was achieved. Fischel and others (1992) reported one case of extended survival of a pig cardiac xenograft in a rhesus monkey that had been treated with plasma exchange and organ perfusion in combination with immunosuppression.

The mechanism or mechanisms that allow accommodation to develop have not been elucidated. Potential causes of accommodation include a change in the levels or repertoire of xenoreactive antibodies or a change in the expression of the antigen or antigens they recognize. We have shown that in the accommodation of ABO-incompatible renal allografts, recipients have antibodies specific for donor blood groups, and the organ transplant continues to express that blood group antigen based on immunopathologic analysis (Chopek and others 1987; Bannett and others 1989). Since in most cases there is no evidence of recipient antibody deposited in the ABO-incompatible graft, it is likely that the mechanism of accommodation involves a qualitative alteration in the antigen leading to decreased interaction of host antibody with

the graft. Less information is available about the potential mechanisms that could bring about accommodation in a xenograft. In addition to the possible importance of a change in antigen expression, we have speculated that the slow return of natural antibodies to the circulation of the recipient and interaction of those antibodies with the graft, perhaps with the activation of small amounts of complement, may cause the endothelium of the graft to develop resistance to humoral injury. This idea is supported by in vitro studies demonstrating that endothelial cells exposed for prolonged periods to noncytotoxic stimuli such as endotoxin or cytokines develop resistance to those stimuli (Busso and others 1991). Yet another mechanism that may underlie accommodation involves recovery of the graft from injury incurred in the peritransplant period. Thus, temporary depletion of natural antibodies from a recipient may allow the graft to recover from ischemia-reperfusion injury prior to exposure to active complement proteins (Magee and Platt 1994). Since ischemia-reperfusion injury depends in part on the activation of complement (Maroko and others 1978; Weisman and others 1990), such recovery may have the net effect of reducing the amount of complement-mediated injury at the time of transplantation. Regardless of the mechanism, the development of accommodation has an obvious impact on the ability to apply xenotransplantation clinically, without the need for continuing manipulation of the recipient.

Cellular mechanisms of xenograft injury

Although hyperacutely rejected organ grafts are often found to contain host leukocytes (Colman and others 1976), there is scarce evidence suggesting that leukocytes contribute to the pathogenesis of that process. Indeed, Forbes and others (1976) showed that depletion of neutrophils has no impact on the course of hyperacute rejection. Zehr and others (1994) showed that administration of an agent that inhibits neutrophil-endothelial cell interaction fails to prolong xenograft survival in otherwise untreated recipients. Furthermore, a number of cases of hyperacute xenograft rejection have been studied in which the infiltration of neutrophils is very focal and sometimes absent (Platt and others 1991b). Some studies have suggested that natural killer cells are able to accumulate in xenogeneic organs and mediate endothelial cell injury (Inverardi and others 1992); however, histologic and immunopathologic analysis fails to reveal significant numbers of these cells in hyperacutely rejecting grafts.

Given these findings it would seem reasonable to conclude that neutrophils and other inflammatory cells are not essential for the development of hyperacute rejection. On the other hand, it would seem not unlikely that the activation of neutrophils, their adherence to blood-vessel walls, and their influx into tissue adds to the injury caused by antibodies and complement.

Cellular infiltrates are commonly seen in acute vascular xenograft rejection. The infiltrating cells include neutrophils, macrophages, and lymphocytes, any combination of which may contribute to the pathogenesis of graft injury. Studies by Zehr and others (1994) demonstrate that administration of an agent that inhibits neutrophil expression of CD11b/CD18 lessens the microvascular injury in acute vascular rejection supports the importance of cellular mechanisms. Although immunopathologic studies have failed to reveal evidence that such cells are present in hyperacute rejection (Platt and others 1991b; Leventhal and others 1993b); natural killer cells may constitute an important component of the cellular infiltrate seen in acute vascular rejection (Blakely and others 1994). In fact, the extent to which these cells actually mediate tissue injury in xenografts is a subject of current inquiry.

Alexandre and others (1989) support the importance of lymphocytes in mediating xenograft injury by studying the transplantation of porcine kidneys into baboons. These studies showed that decreases in graft function can be reversed by administering immunosuppressive agents. The mechanisms by which T cells might cause the rejection of xenografts has been considered in recent years (Auchincloss 1988; Geller and others 1993).

Cellular immune responses to a xenograft might differ in certain respects from the cellular immune responses to allografts. One important difference concerns the mechanisms by which T cells become activated in response to xenogeneic cells. Owing to the events leading to development of the repertoire of mature T cells, the number of different T cells able to recognize xenogeneic cells may be fewer than the number able to recognize allogeneic cells. Furthermore, the cytokines and cell adhesion molecules synthesized by donor cells may function ineffectively on the recipient's T cells. Thus, some have postulated that the cellular response to a xenograft might actually be less intense than the cellular response to an allograft (Auchincloss Jr., 1988; Alter and Bach, 1990). The relative intensities of cellular responses to xenogeneic and allogeneic grafts have not been compared critically; however, to the extent that cellular immune responses to xenogeneic tissues have been evaluated, they appear to be nearly as strong and sometimes stronger than the response to allogeneic tissues (Murray and others 1994). Thus, there is every reason to believe that an intense cellular response would contribute to the immunologic barrier to xenotransplantation. The most important question from a practical perspective is whether there are unique aspects of this cellular response, which might require therapeutic agents or strategies distinct from those used for allotransplantation.

THERAPEUTIC STRATEGIES FOR XENOTRANSPLANTATION

The last 5 years have brought much progress in understanding the immunological barriers to xenotransplantation and in developing strategies to overcome those barriers.

This progress has been summarized in several recent reviews (Platt 1994a, 1994c, 1995). The sections that follow will summarize work in the author's laboratory which may help advance xenotransplantation toward the clinical arena.

Natural antibodies-antigens

The repertoire of natural antibodies in a serum might be expected to recognize a vast array of xenogeneic antigens, which might differ from species to species and even from individual to individual. In fact, recent studies demonstrate that human xenoreactive antibodies predominately recognize one carbohydrate antigen, Galα(1-3)Gal, which was extensively studied in the 1980s by Galili (Galili and others 1984; Galili 1993). Galα(1-3)Gal is expressed on the cells of New World monkeys and lower mammals but is not expressed by humans, apes, or baboons (Collins and others 1994b). At the same time, humans, apes, and baboons have natural antibodies specific for that structure while lower mammals do not (Galili and others 1987). Recent studies by Good and others (1992) and Neethling and others (1994) demonstrated that purified Galα(1-3)Gal and similar sugars block the binding of human xenoreactive antibodies to porcine cells and prevent complement mediated cytotoxicity. These authors also demonstrated that antibodies directed against Galα(1-3)Gal can be eluted from porcine organs perfused by human plasma. Sandrin and others (1993) demonstrated that transfection of COS cells with the murine α1,3-galactosyl transferase gene, which is responsible for adding terminal αGal residues to oligosaccharides, induces binding of human natural antibodies to the transfected cells. Collins and others (1994a) demonstrated that expression of Galα(1-3)Gal in the heart of a New World monkey provides a sufficient basis for the development of hyperacute xenograft rejection when that heart is transplanted into a baboon which has antibodies specific for αGal. Collins and others (1994b) also demonstrated that enzymatic removal of α-galactose decreases the binding of xenoreactive antibodies to porcine endothelial cells (Collins and others 1994b).

Although the importance of Galα(1-3)Gal as a target antigen is now widely accepted, the conditions that allow xenoreactive antibodies to bind to that structure are complex. Parker and others (1994), Platt and Holzknecht (1994), and Cotterell and others (1995) have shown that the affinity of natural antibodies for Galα(1-3)Gal is very low and therefore expression of that structure on a cell surface may not by itself be sufficient to result in significant binding of complement fixing in xenoreactive antibodies. Rather, it appears that the sugar must be expressed as a posttranslational modification of certain glycoproteins (Holzknecht and Platt, 1995). The apparent avidity of natural IgM antibodies for the Galα(1-3)Gal on the glycoproteins is seven orders of magnitude higher than the avidity for the simple sugar. Further evidence that the manner in which αGal is expressed dictates the extent of antibody binding to a xenogeneic cell derives from the work of Cotterell and others (1995) and Alvarado and others (1995) demonstrating that although binding of IgM to porcine cells depends on the presence of αGal, IgM binding varies over a nearly tenfold range and is independent of total expression of αGal.

Based on the finding that xenoreactive antibodies bind predominantly to Galα(1-3)Gal, it is possible to devise specific strategies for immunodepletion of those antibodies from the circulation of a xenograft recipient. A number of groups are actively pursuing approaches to antibody depletion. Affinity columns have been used previously for depleting isohemagglutinins from human patients allowing transplantation of organs across ABO barriers (Bannett and others 1987) and it is reasonable to think this approach could also be used for depletion of xenoreactive antibodies. One hope is that temporary depletion of xenoreactive antibodies would allow the development of accommodation for xenografts as it does for allografts.

Another way to prevent humoral injury by xenoreactive antibodies is to inhibit their binding using soluble ligands. This approach has also been used to prevent the hyperacute rejection of ABO-incompatible grafts (Chopek and others 1987; Cooper others 1993). Unfortunately, because the binding of xenoreactive antibodies to cell surfaces is very avid (Parker and others 1994), high concentrations of a monomeric inhibitor would be needed.

Yet another approach to preventing the interaction of xenoreactive antibodies with a xenograft is to seek out or develop pigs that have low levels of xenoantigen expression. With the identification and cloning of the gene for the glycosyltransferase responsible for the synthesis of Galα(1-3)Gal (Sandrin and others 1994; Strahan and others 1995), the possibility of genetically engineering donor animals with decreased expression has been advanced. Unfortunately, the most direct strategy which involves "knocking out" the gene can be achieved only in mice because it requires embryonic stem cell technology, which is not yet proven in larger animals. An alternative strategy could involve introducing another glycosyl transferase that would compete with α1,3-galactosyl transferase for the growing oligosaccharide chain. This approach has been pursued by Sandrin and others (1994).

The development of animals with low levels of expression of antigens recognized by xenoreactive natural antibodies does not necessarily require genetic engineering. Geller and others (1994) and Cotterell and others (1995) with the author found that the level of antigen expression varies over a tenfold range among pigs. Variation in antigen expression appears to have a genetic basis. Indeed, perfusion of baboon blood through organs from pigs that express low levels of antigen leads to the deposition of very little IgM and C4 in contrast with similar experiments in which organs from normal animals are perfused. These results suggest that preferred donor animals might be selected or bred.

Complement activation

Over the past 30 years a number of studies have demonstrated that if complement is depleted or inhibited, hyperacute rejection does not occur (Gewurz and others 1967; Leventhal and others 1993a; Pruitt and others 1994; Platt 1995). Most of these studies used cobra venom factor, which depletes complement components by activating the alternative pathway complement in the blood. A more recently developed agent, soluble CR1, functions by a different mechanism to inhibit complement. Soluble CR1 causes decay of active complement convertases and serves as a cofactor for proteolytic cleavage of those convertases (Weisman and others 1990). Administration of cobra venom factor or soluble CR1 at optimal doses prevents hyperacute rejection and yields graft survival of 3-4 days. The grafts ultimately succumb to acute vascular rejection (Leventhal and others 1993b). Combining the use of cobra venom factor with antibody depletion results in more prolonged graft survival (Leventhal and others 1994). However, the recipient may be subject to a heightened risk of infection as complement and natural antibodies play important roles in host defense. One way to overcome this problem might be to use agents that would selectively inhibit the classical complement pathway (Dalmasso and Platt 1993), which is used in the activation of complement in pig-to-primate xenografts (Platt and others 1991b; Dalmasso and others 1992), sparing the alternative complement pathway for host defense. Another approach that may function by a similar mechanism was recently tried by Magee and others (1995) based on the work of Frank and others (1992). This approach involved the administration of purified human IgG, which may function as an alternative acceptor for activated complement proteins by directing enzymatically-active complexes away from xenograft endothelium.

Complement regulatory proteins

The concept that a xenograft might be uniquely susceptible to complement-mediated injury because of the restricted ability of xenogeneic cells to control activation of heterologous complement was first proposed by Dalmasso (Dalmasso and others 1991; Platt and others 1990a). This concept has spurred efforts in a number of laboratories to introduce human complement regulatory proteins into potential donor animals in order to ameliorate the effects of complement activation. Although human complement regulatory proteins might be introduced extrinsically into porcine endothelium by various techniques (Dalmasso and others 1991; McClellan and others 1994), most attention has focused on the introduction of genes encoding human complement regulatory genes into potential donor animals. Cary and others (1993) developed transgenic mice and more recently transgenic pigs expressing human decay accelerating factor under control of the decay accelerating factor promoter. Subsequent studies demonstrated that cells from the transgenic mice have increased resistance to complement-mediated lysis. Transgenic mice and transgenic pigs have also been developed that express combinations of CD59, decay accelerating factor, and membrane cofactor protein under the control of various promoters (Kooyman and others 1994; Diamond and others 1994; Kagan and others 1994). Hearts from transgenic mice were shown to resist the activation of complement during perfusion with human plasma or baboon blood (McCurry and others 1995). Recently McCurry, with the author, carried out a series of transplants from transgenic pigs expressing human CD59 and decay accelerating factor into baboons. Although expression of the human proteins was lower than optimum and the experiments were preliminary, the grafts did not undergo hyperacute rejection and functioned for prolonged periods of time. Histologic analysis of the grafts revealed remarkably little tissue injury.

FUTURE PROSPECTS FOR XENOTRANSPLANTATION

What are the prospects for clinical application of xenotransplantation? The path that will lead to safe, reliable, and effective xenotransplantation is not yet clear. However, certain major steps along that path clearly have been taken and from the vantage point of 1995 it is possible to see some of the elements that will likely contribute to a successful strategy. The identification of the major antibody-antigen system involved in hyperacute rejection, and the development of effective strategies for the inhibition of complement are significant steps. The ability to genetically engineer, breed, and select donor animals will lower the immunological barriers to xenotransplantation and thus play an important role in launching it into the clinical arena. It would thus seem if one can identify a "molecular" hurdle to xenotransplantation, a strategy for overcoming that hurdle can be devised.

While hyperacute rejection was once viewed as the major hurdle to successful xenotransplantation, that view is no longer a correct one. Hyperacute rejection can be prevented reliably, reproducibly, and effectively. The next and perhaps most daunting barrier to xenotransplantation is acute vascular rejection. Although some progress has been made in elucidating the pathogenesis of acute vascular rejection, there is as yet no certain way to prevent or overcome it. Besides acute vascular rejection, humoral and cellular responses to the myriad of donor antigens remains a significant concern. Such responses as hurdles to allogeneic transplantation and in some humorally-mediated diseases have been overcome. Whether special approaches will be needed to prevent elicited responses to the xenograft is yet unknown.

If the immediate prospects for clinical xenotransplantation remain uncertain there can be little doubt that biomedical science and patient care will benefit from those efforts that are made. Studies in xenotransplantation have provided a context for testing anti-inflammatory agents (Pruitt and others 1992; Miyagawa and others 1993; Zehr

and others 1994; Magee and others 1994) and at least one patient's life has been saved by temporary perfusion of xenogeneic livers to correct metabolic abnormalities associated with fulminant hepatic failure (Chari and others 1994).

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Xenotransplantation and Infectious Diseases

S.S. Kalter and R.L. Heberling

For want of a nail the *shoe was lost...* Herbert, G. 1651.

INTRODUCTION

Organ transplantation is now an accepted, and in many instances necessary, mechanism for the preservation of human life when disease has resulted in the loss of organ function. The exact number of organs required for transplants is probably unknown, but there is unquestionably an insufficient supply of human organs, and another source or method is required. Hilts (1993) estimated that approximately 60,000-80,000 lives could be saved if organs were available. According to the United Network for Organ Sharing, in 1994 there were over 37,000 registrants for organ transplants; approximately half will die for lack of an available organ. It has been suggested that improving the logistics of delivery and matching of supply and need would be helpful in supplying human organs. Mechanical replacement is currently an inadequate option, and it is not a permanent solution. With limited human organ sources, what are the alternatives?

Leaving social and ethical aspects aside, animals could create a potential supply of temporary or perhaps permanent organs for transplantation. Assuming that xenotransplantation is viable, numerous queries enter into such a decision. For example, are infectious diseases acquired from transplanted animal organs and are body fluids the major conceivable danger? Humans are susceptible to animal-associated diseases (zoonoses). As it would appear that there is little choice between human and animal organs for transplants, and that there is an insufficient supply of human organs, problems with animal organs must be resolved.

Of the animals under consideration as organ donors, nonhuman primates and swine are most frequently suggested. Currently, nonhuman primates, because of their phylogenetic relationship to humans, are preeminent as suitable organ donors. When contemplating the nonhuman primate, anxieties that continue to counter their usage are fears engendered by the possible presence of infectious agents. It would appear, however, that "the jury is still out" with regard to this concept. Brack (1987) provides a background of infectious agents present in nonhuman primates. A potential for humans to become infected as a result of heterotransplantation exists and has been discussed in numerous reports (see references). Conceivable transfer of infectious agents as a result of xenotransplantation using nonhuman primates or pig sources is reviewed in Cooper and others (1991), Kalter (1991), and more recently by Michaels and Simmons (1994). Hardy (1989) does not consider infectious diseases.

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If animals are to be a source of organs, it has been suggested that the spread of infectious diseases may be minimized by employing germfree (gnotobiotic) or specific pathogen-free (SPF) animals for xenotransplants. Because of the numbers needed, the use of germfree or SPF animals would be impractical. Further, such animals still harbor endogenous agents. Limited experience suggests that well-characterized animals born in captivity and allowed no contact with animals introduced from the wild may be a more practical source of organs for xenotransplants.

When considering the choice of an animal, disease potential is a moot point. Infectious agents are unfortunately universal. A precise understanding of the specificity of infectious agents is often lacking and in many instances is not clearly defined. An alien species infected by certain agents may change the virulence of that agent, such as B virus infection of humans (*Herpesvirus simiae* from *Macaca sp.*). Many agents are host-specific and not infectious when crossing species barriers. Two recently described highly fatal simian viruses, Ebola-Reston and Simian Hemorrhagic fever do not cause human disease. The simian immunodeficiency viruses (SIVs) are not known to cause human disease although infection is suspected (Khabbaz and others 1994). Other agents, such as measles and tuberculosis are not influenced by species barriers and can cause infection or disease in diverse host species. Although the exact epidemiology of the Marburg virus is unknown, it was fatal for both human and nonhuman primates (Kalter 1986). The human immunodeficiency viruses are considered to be derived from simian sources, and others may be forthcoming. It is not known if natural immunodepression is a cause of altered virulence of infection or disease in a new host.

Many mammals are also infected by agents of nonmammalian origin (such as arboviruses, bacteria, parasites, and fungi). Arboviruses require a vector for transmission from one host to another and are not a transplant problem. Infection and disease are complicated by a number of extraneous considerations: immunological status, age, nutrition, socioeconomic factors, and geographic locale. If history foretells the future, "new" strains of infectious organisms most certainly will be forthcoming and could be a source of human infection and disease. Such occurrences have been observed in the past.

Infectious agents that can cause zoonotic disease are frequently of little hazard in the host of origin; infection usually results in subclinical disease with antibody production. Zoonoses are generally initiated in immunologically naive individuals and are devastating as such to any population. A transplant patient, because of immunosuppression, is immunologically naive. Because overtly sick animals would not be considered for organ donation, it is unlikely an agent would be transferred from them. However, latent infections of donor or recipient, which are not discernible, may be activated as a result of the transplant. Endogenous agents are also an unknown factor, and their presence may need consideration. In pursuing possible sources of transplant organs for humans from nonhuman primates and swine, human contact with these animals over the years has not been overshadowed by human infection and disease. However, these contacts have been by immunologically "normal", not immunosuppressed, individuals.

Immunosuppression increases the susceptibility of an organ recipient to infection and frequently permits nonpathogenic organisms to become pathogenic. As a consequence, activation of dormant or latent infectious agents in the recipient should be anticipated. Similarly, transferring a nonpathogenic or latent infection from an organ donor to an immunosuppressed individual may also result in enhanced pathogenesis. Past infections of both organ donor and recipient may be detected by antibody surveys. The presence of antibody may serve as a protective mechanism in transplants (Peterson and others 1980). However, antibody surveys are not infallible as titers may fall below perceptible levels or inappropriate methodologies may be employed.

While there is reason to be concerned about the transfer of infectious agents from an animal source, experience has indicated thus far that infection has not been a major problem in heterotransplants. Infections from animal donors do occur, but as in human-to-human transplants where infections are recognized (Ho 1977; Michaels and Simmons 1994), careful selection of a donor may limit the spread of infectious agents. Human infection from swine has not been as extensively studied as from nonhuman primates.

Of all the nonhuman primate viruses, B virus has received the most attention because of its pathogenicity for humans. This virus, which is closely related antigenically to human herpes simplex, is common to *Macaca sp.* B virus infection in the macaque simulates human infection with herpes simplex, while infection in humans is fatal. Macaques, however, are now not considered for xenotransplants largely because they are hosts for B virus, so the question of B virus transfer is irrelevant. SA8, a herpesvirus found in baboons that is related to B virus but not considered a human pathogen, frequently causes misinterpretation of serologic results because of its antigenic cross reactivity with B virus.

Among nonhuman-primate organ donors, apes and principally chimpanzees are most desirable. While apes other than chimpanzees (such as gorillas, orangutans, gibbons) have been considered, they are not available. Immunologically these apes are also more remote from humans than the chimpanzee and would be less enticing as donors. The availability of chimpanzees is extremely limited and for all practical purposes this animal cannot be considered as an organ donor because they are an endangered species. Development of chimpanzee breeding colonies, although intriguing, would be inappropriate because of cost as well as for ethical reasons. The baboon (*Papio sp.*), a monkey that is readily available and raised in captivity, is currently the most frequently used nonhuman primate considered for xenotransplantation. Of the nonprimates, considerable attention has been given to the pig (*Sus scrofa*). Both animals, the baboon and the pig, have a microflora.

FLORA AND FAUNA

The baboon and pig meet the various criteria established for donor animals: size, ease of breeding in captivity, a source of food (pig), phylogenetic relationship (baboon), and many other physiological, anatomical, and other similarities to humans. The major negative feature shared by both animals, but also a problem in human-to-human transplants, is the continuing need for immunosuppression and the conceivable danger of infection.

Nonhuman Primates

Baboons have been extensively studied, and preliminary data are available on their acceptance as organ donors for humans (Starzl and others 1964, 1993; Hitchcock and others 1964; Reemtsma and others 1964, 1969; Murphy and others 1970; Brede and Murphy 1972; Van der Riet and others 1987; Bailey and others 1985; Human and others 1987). The baboon is currently the nonhuman primate of choice for xenotransplants in the United States and South Africa, the major areas of xenotransplantation activity. Other nonhuman primates, particularly the chimpanzee, have been used in limited instances over the years. In South Africa, the Chacma (*Papio ursinus*) baboon is locally available. In the United States the much smaller yellow baboon (*P. cynocephalus*), derived from Kenya, Tanzania, and Ethiopia, is used. Breeding of *P. cynocephalus* in the United States is well established (Goodwin and Coelho 1982). For practical purposes there does not appear to be any major differences in the normal flora of both species (Human and others 1987; Michaels and others 1994). Baboons breed well in captivity, do not appear to be endangered, and are readily available. In areas of origin, baboons are considered agricultural pests.

An extensive bibliography is available on the baboon, and its overall microbiology has been reported (Kalter 1967; Brede and Murphy 1968, 1972; Kalter and Heberling 1971; Kalter 1973, 1986). Noteworthy are the studies done on the baboon immediately following capture in Kenya providing information on natural infections (Kalter 1973). As suggested above, the phylogenetic relationship of nonhuman-to-human primates emphasizes their common flora and fauna, including bacteria, fungi, parasites, and viruses and their enhanced susceptibility to many of these agents. In addition, most nonhuman primates have an extensive specific pattern of infectious agents that are related, but distinct from their human counterparts (Kalter and others 1980). Widespread geographic and species differences in flora and fauna among nonhuman primate species is recognized. When contemplating nonhuman-primates as a source of organs, it would appear that the choice is limited. Apes, as indicated, cannot be considered. South American monkeys are generally too small, many species are restricted in availability, and all species have a definite viral flora that includes several oncogenic viruses. Of the Asian and African nonhuman primates, macaques are not desirable because of the presence of Herpes B virus. Of the remaining African species, none offer any advantages over the baboon.

Despite the large number of viruses and other organisms recovered from the nonhuman primate, as well as the recognition of disease among these animals (Benirschke 1986), there is little evidence to indicate that *extensive* human infection, other than B virus, has been recognized. B virus infection of humans following contact with macaques is well described (Keeble and others 1958; Hunt and Melendez 1969; Hull 1968). Information on human infection with nonhuman primate agents following xenotranplants is very limited and is unquestionably due to the restricted number of simian-to-human xenotransplants completed. If the practice of using nonhuman primates as organ donors on immunosuppressed recipients continues, infection and disease must increase.

In nontransplant situations, hepatitis A contracted from chimpanzees is well substantiated (Hillis 1961; Smetana and others 1970). In 1967 an outbreak of a highly fatal disease occurred among humans as a result of contact with African green monkeys (*Cercopithecus aethiops*) blood and tissues. Monkeys carrying the virus (Marburg virus) rapidly succumbed to the disease and would not have been considered as organ donors (Kalter 1986). Monkeypox virus, a close relative of smallpox and vaccinia viruses, has infected humans following contact with monkeys (Kalter 1986). There are other examples of human infections resulting from nonhuman primate contact. Human vaccines (such as polio and adenovirus) made in monkey tissues have resulted in the transfer of SV40 virus, a monkey papovavirus, to human recipients. This virus is oncogenic in experimental animals, but human disease has not been observed (Shah and Nathanson 1976). Unquestionably, other viruses have also been transferred by this mechanism. Human-to-human organ transplants are known to result in infection (Fulginiti and others 1968; Ho 1977), principally with herpesviruses (cytomegalovirus). As a consequence, it must be assumed that similar infections may occur as a result of xenotransplants (Michaels and Simmons 1994).

Several antibody studies on donor baboons have been conducted and antibody to a number of viruses has been demonstrated (Van der Riet and others 1987; Human and others 1987; Michaels and others 1994). In the limited number of instances where xenotransplants were conducted, few adverse effects from infectious agents were noted (Hitchcock and others 1964; Starzl and others 1964; Reemtsma 1964; Reemtsma 1969; Bailey and others 1985; Starzl and others 1993). Infections, possibly leading to death, have been observed as a result of agents other than viruses (Starzl and others 1964; Reemtsma and others 1964; Brede and Murphy 1968; Michaels and Simmons 1994). It is quite clear that until more xenotransplants have been performed, infection and disease resulting from the donor organs will not be known.

Table 1 provides a listing of virus antigens and the results of screening baboon candidates for xenotransplants from the

TABLE 1 Serologic findings on baboons; accumulated data

Herpesviruses		
Herpes simplex	+ (16/20)**	DIAdot®
Cytomegalovirus	+ (52/65)	DIAdot
Epstein-Barr	+ (32/65)	IFA
Varicella-zoster	+ (7/24)	DIAdot
SA 6 (Simian CMV)	+ (24/34)	DIAdot
SA 8	+ (7/24)	DIAdot
Lymphotropic herpes	+ (?/30)	IFA
Retroviruses		
SIV	+ (5/51)	DIAdot
STLV 1	+ (5/31)	DIAdot
SRV	+ (1/85)	DIAdot
Foamy virus	+ (30/31)	DIAdot
Type C	+ (?/30)	EM
Miscellaneous		
Hepatitis A	+ (3/31)	ELISA
Hepatitis B	+ (0/31)	ELISA
Influenza A	+ (0/34)	DIAdot
Influenza B	- (0/34)	DIAdot
Measles	+ (23/55)	DIAdot
Rubella	- (0/34)	DIAdot
Mumps	- (0/34)	DIAdot
Simian hemorrhagic fever	- (0/31)	DIAdot
Marburg	- (0/34)	DIAdot
Ebola-Reston	- (0/30)	DIAdot
Lymphocytic choriomeningitis	- (0/65)	DIAdot
Reovirus (SA 11)	+ (31/39)	DIAdot
Monkey pox	- (0/31)	DIAdot
Encephalomyocarditis (EMC)	- (0/30)	DIAdot

*Serologic studies on both groups of animals performed at the Virus Reference Laboratory, Inc® The presence (+) or absence (-) of antibody at a 1:5 or 1:10 serum dilution, ? - no accurate count.

**Numerator (number positive)/Denominator (number tested). DIAdot® DIA-dot immunobinding assay, IFA-immunofluoresence, EM-electron microscopy, ELISA-enzyme linked immunosorbent assay.

Note: Other groups of baboon have been found with antibody to many of these viruses (Kalter and Heberling 1971; Kalter 1986). The above data were obtained only from the two specifically referenced groups of animals. Antigens employed were derived from nonhuman primate sources and were highly specific. Cross reactions among the herpesviruses were observed, but distinguishable.

South African and Pittsburgh transplant groups. An important consideration when selecting animals based on antibody detection is the source of antigen. In a recent comparative study in which two laboratories tested the same serum sample for antibody, it became apparent that there was a need to develop specific test antigens against the animal species used in the xenotransplant (Michaels and others 1994). It is evident that extensive additional information, not only about the donor but about test methodologies employed, is required before making any judgement on the development of infection and disease following xenotransplants.

A major virus family that must be regarded with suspicion and that is a possible cause for concern in xenotransplants, is the Retroviridae. Retroviruses comprise a large family of viruses divided into a number of subfamilies all with varied biologic and clinical characteristics. Certain retroviruses (HTLV-BLV group and the genus lentivirus), are considered to be associated with B-or T-cell leukemia/lymphoma as well as human AIDS. Simian-related viruses are known to produce a disease in nonhuman primates similar to human AIDS. Both endogenous and exogenous retroviruses are recognized, and they may be transmitted horizontally, vertically, or both. Oncoviruses, which have long been recognized as tumor-producing and are associated with both human and simian AIDS, have furthered concern about the disease potential of retroviruses.

The status of human infection with SIV is not clear. The precise relationship between human and simian immunodeficiency viruses, particularly HIV-2, has also not been clarified. Antibody development in "two human infections" along with other infection markers, but no disease, is of interest in this continuous concern over infectivity by simian retroviruses (Khabbaz and others 1994). Waning antibody obviously indicates lack of persistent infection. Existence of other SIV strains with differences in pathogenicity need consideration and one may speculate that human pathogens exist. However, until such pathogenicity is demonstrated or a sufficient time has passed in the case of the two seropositive individuals, SIV strains should not be considered as the cause of human disease. Antibody development, in response to a foreign antigen, is to be expected.

Foamy viruses (Spumavirus), another group of retro-viruses, are prevalent in nonhuman primates and other animal species. Thus far, foamy viruses have shown little indication that they are other than nuisances with no recognized disease production. Their continued presence in host animals, however, strongly suggests that their status continue to be monitored.

Little studied and generally ignored are the endogenous retroviruses. Both types C and D endogenous viruses have been recognized, usually in placental or embryonic tissue, by electron microscopic examination, but rarely seen in adult tissue. Genomic material of these viruses probably resides within all living animals, but expression varies from cell to cell. While such genes are difficult to detect, molecular probes may be of value in determining their presence. Although a number of type C and D viruses have been isolated, little is known of their pathogenicity with the exception of the baboon (type C) isolate. This virus, as a pseudotype containing the Kirsten murine sarcoma genome, produced metastatic disease in dogs and nonhuman primates (Heberling and others 1976). The potential for the baboon

endogenous type C virus, which can replicate in human cell cultures, to acquire a sarcoma gene present in the transplant host, is unknown.

In addition to viruses, other organisms have been recognized in nonhuman primates: bacteria (Brede and Murphy 1968; McClure and others 1986), parasites (Toft 1986), and fungi (Migaki 1986). These agents may have a detrimental effect on nonhuman primates especially in the colony habitat. Proper colony management is an integral aspect of maintaining healthy animals and preventing transmission to humans. Breakdowns in disease control do occur, and require rapid and precise management to minimize the devastating effects of disease expansion. Many of these organisms are recognized as infectious for the human and a cause for concern in xenotransplantation (Michaels and others 1992). Usually, unless donor tissue is in direct contact with human blood, agent transmission is minimal. Blood dyscrasias require careful examination. Overtly ill animals would be rejected as donor candidates. Routine laboratory monitoring should detect most infections and disease including those due to agents other than viruses. Unfortunately current monitoring is dependent on available methodologies, but even more so on accessible reagents including recognized antigens. While some cross-reactions may indicate infection by agents other than those included in the test menu, dependence upon such a reaction is extremely limited and open to criticism. Previously unrecognized disease incitants cannot be included in monitoring programs until the etiological agent is isolated. Accordingly, in addition to continuous colony antibody surveys, the need for laboratory support in identifying disease outbreaks is essential.

The effects of immunosuppression on infection were realized in early heterotransplants even though the primary cause of failure was organ rejection. "The continued need for high-dose immunosuppressive therapy precipitates lethal infections in the majority of cases"(Starzl and others 1964). Contributing infectious agents detected were: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus fumigatus, Candida albicans*, Herpesvirus varicella, and Klebsiella-Aerobacter. The epidemiology of these infections remains unknown. Was it caused by the baboon or activation of recipients organisms? Reemtsma and others (1964) did observe that their patient receiving chimpanzee kidneys, who had been immunosuppressed, died of infection without evidence of organ rejection. Whether infection will be a significant problem in xenotransplantation remains to be determined.

Nonprimates

Several small animals have been suggested as organ donors and a number have been used. Swine have received considerable attention and are involved in a number of studies (Cooper and others 1991). The pig shares a number of characteristics with humans including size, structure, physiology, and dietary habits. As would be expected, the pig also has a microflora, which includes bacteria, parasites, fungi, and viruses. Many of these are known to be associated with human infection and disease (Cooper and others 1991). Endogenous viruses are suspected in swine but have not been reported. As with nonhuman primate tissues, use of the pig is contingent upon development of a successful method for overcoming rejection. Breeding pigs is more efficient and offers a greater opportunity than breeding nonhuman primates, and their additional usage as a source of food is another possible advantage. The availability of large numbers of pigs permits further exploration into the development of transgenic swine that could be immunologically acceptable to humans (Sachs 1992). Would the same mechanism be applicable to the nonhuman primate? Fewer ethical considerations is another major factor when contemplating the pig as an organ donor.

SUMMARY AND QUESTIONS FOR CONSIDERATION

The need for a source of organs as support systems for humans in the immediate future is unquestioned. The supply of needed organs from acceptable human donors is extremely limited, and mechanical organ replacements are currently impractical. The venture into using animals as organ donors, with full recognition of their deficiencies, is based on the realization that there is an insufficient supply of human organs and no adequate mechanical replacement.

In this brief overview, the nonhuman primate (baboon) and the pig are examined as potential organ donors. For practical purposes, either animal would be acceptable, if, or when, the problem of rejection is solved. Is the phylogenetic position of the baboon (concordant) versus that of the pig (discordant) sufficient to recommend the baboon over the pig as the donor of choice? Pigs offer a number of positive considerations: they are easy to breed, are a source of food, share many characteristics with humans, and their use raises fewer ethical issues than the use of baboons. Careful analysis suggests that infection and disease, while they occur, thus far have been a minor factor in the few xenotransplants attempted. However, the influence of immunosuppression must be weighed. Immunosuppression, used to control rejection, is the major underlying cause for infection either as a result of reactivation or enhanced susceptibility. In human allotransplants, immunosuppression and infection are closely linked. Rubin (1981) states that clinically significant infection occurs in 75% of all transplant recipients and is the leading cause of death.

Both baboons and pigs are known to harbor agents of human infection and disease, and careful colony husbandry may solve this problem. Conceivably, unrecognized endogenous retroviruses in the nonhuman primate and perhaps in the pig could be a source of disease. These agents, passed from mother to offspring, are maintained as genomic material and are neither observed nor recovered from adult tis-

sues. Endogenous viruses would not be eliminated by use of either gnotobiotic or SPF animals. Some limited experimental data suggest an oncogenic capability for the baboon type C endogenous viruses, but more information is needed. What are the potential dangers to organ transplant recipients with regard to lymphoproliferative diseases? Such diseases have been observed in nonhuman primates including baboons (Kalter 1991). The existence of a herpesvirus closely related to human herpes simplex and the macaque B virus, that is SA8, may alarm some baboon advocates. However, human disease with SA8 has not been recognized.

While the question of alternatives has been asked, mechanical substitutes are so far unacceptable. Even given the ability to eliminate rejection, using animals raises some social and ethical opposition, particularly if choosing a nonhuman primate. Without question apes, and particularly chimpanzees, would be inappropriate. Their supply is limited in the wild and we are unable to raise sufficient numbers in captivity. Any use of present populations, regardless of the purpose, would not only be unethical, but would constitute an irreplaceable loss. Would the same protest result from the choice of the baboon or pig?

Is prevaccination with a battery of antigens of value in protecting an individual? There is evidence to indicate that seronegative immunosuppressed individuals receiving organs from seropositive donors have a higher risk of disease (Peterson and others 1980). Effective vaccines require availability of immunizing antigens. Until desired antigens are provided, "protection" would be limited only to those antigens in the vaccine. Of concern would be the failure to provide protection against the unknown. It has been suggested that the use of immunosuppressive drugs have enhanced the survival of HIV-infected liver recipients (Jacobson and others 1991).

The major difficulty in xenotransplants still seems to reside in the inability of the human body to accept a foreign tissue. Progress in developing mechanisms to modify rejection by means of immunosuppressive drugs has been somewhat successful and continues to improve (Starzl and others 1993). Are molecular and transgenic modifications of the donor animal (or even the recipient) worthy of consideration? Transgenic animals could be developed providing organs that would be immunologically acceptable to humans (Sachs 1992). However, will development of transgenic donors result in the appearance of new "uncontrollable" diseases, from which human fatalities may result? Current infections apparently are under control.

Can ethical and social thinking about the use of animal organs for xenotransplants be revised by solving the problem of organ rejection, controlling infection, increasing survival rates, choosing an animal host other than a primate and that also serves as a source of food? Because of the limited supply of human organs, more than half of the needy individuals die. Providing organs from animal sources for the approximately 50,000 patients waiting for transplants, even if not a permanent solution, should subdue any opposition to the use of an animal source of organs.

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Xenograft Transplantation and the Infectious Disease Conundrum

Jonathan S. Allan

INTRODUCTION

Transplant surgeons and AIDS clinicians are on the verge of implementing a host of new procedures that will revolutionize transplantation and adoptive cell transfer techniques in a quest for cures to such diverse medical conditions as heart disease and AIDS. Just as gene therapy is expected to directly impact inherited diseases, new advances in immunosuppressive drug combinations and new discoveries in our understanding of the fundamental processes of immune tolerance have led to the real possibility of engineering tissue and cell transplants from other living species such as baboons and pigs into humans (xenogeneic transplantation) (Starzl and others 1994). At the same time, interest in emerging viruses as a discipline for virologists and infectious disease specialists has surfaced, and it appears that these two fields may be on a collision course at the present moment (Michaels and Simmons 1994; Allan 1994).

Several recent reviews have focused attention on the notion that new human diseases continue to present danger with the appearance of new viruses, a consequence of cross-species transmission from animal reservoirs to humans (zoonoses) (Morse 1993; Morse and Schluederberg 1990; Murphy and Nathanson 1994; Murphy 1994). A zoonosis is generally defined as an infection of one or relatively few humans with an animal virus without necessarily establish-

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ing itself in that population. The real threat of an emerging virus is when, or if, it is transmitted from one human to another, and this is where the danger lies with xenotransplantation. Numerous examples of the emergence of human viral diseases are available but the most studied culprit and one that reaches deep into the consciousness of most Americans is AIDS. In this review, I will try to put into perspective, in some cases through example, the reasons why the risk to the human population from xenogeneic transplantation is unacceptable.

We have had ample historical warnings of the dangers associated with animal-to-human zoonoses yet we continually ignore these signals. Isn't it ironic that the most notorious infectious disease known to humankind appears to have arisen through inadvertent transmission from an African nonhuman primate. Yet there are those who now want to use tissues from African monkeys in an attempt to cure AIDS, in my opinion, without sufficient forethought as to the consequences and risk to the human population of such procedures. It is also prophetic that we are having to revisit the plague with recent headlines decrying the exodus of 400,000 people from a city in India as panic sets in over the mounting death toll from pneumonic plague. Also known as the Black Plague, Yersinias pestis was responsible for the death of one-third of the total population of Europe in the late thirteenth and early fourteenth century (Morse 1993). If we think we have eradicated most forms of pestilence from our civilization, then it is time to think again, for we will continue to be bombarded either by reemerging diseases, as is the case with the plague, or by newly emerging diseases such as AIDS.

HISTORICAL PERSPECTIVES

Recently, there has been an explosion of interest surrounding emerging viral diseases. To put this into perspective, public health agencies are devoting considerable, though sometimes inadequate, resources toward epidemiological surveillance in preparing to identify, characterize, and contain new viral epidemics in humans. Instances where humans are in greater contact with animal species, as is seen in the destruction of the rain forests, allow for far greater risks in transmitting new viruses from rodents and monkeys to humans. This has been observed with the emergence of human monkeypox, a virus originating in African monkeys, which led to 10% case fatality rate in humans (Fenner 1993). In West Africa, the reemergence of yellow fever generally coincides with high viral burdens in monkey reservoirs even though the virus is transmitted by insect vectors (Monath 1994). Past successes in controlling new emerging diseases are proudly recounted, such as tracking down the Hantavirus outbreak in the Southwest United States (Ion-Nedelcu and others 1994). Fortunately, this newly identified strain of Hantavirus is a zoonotic disease resulting from the inhalation of soil and other material contaminated with rodent urine and is apparently not easily transmitted from person to person (Hjelle and others 1994). The infection is usually associated with an acute febrile illness and rapid recovery or death from pneumonia, which also severely reduces the chances of transmission to another person.

Another example of the public health agencies ability to successfully intercede in cases of zoonotic transmission of disease was the identification and containment of a recent Ebola-like virus (Reston Virus) outbreak in a macaque colony in Virginia for which the virus received its name (Peters and others 1994; Jahrling and others 1990). A second virus, simian hemorraghic fever virus (SHF), was also found in the cynomolgus macaques, confusing the initial efforts to identify the etiologic agent (Dalgard and others 1992). The SHF virus is generally associated with cross-species transmission from an African monkey to macaques, however, seroepidemiologic studies revealed that many monkey species had antibodies reactive with Ebola viruses so it is uncertain what events are necessary for Ebola-associated disease. Nevertheless, the disease spread quickly resulting in the deaths of many animals. Curiously, serological studies of four of the staff members at the facility indicated that humans were infected with the Reston virus. Luckily, no disease was observed and there were apparently no human-to-human transmissions.

In both of the above cases, the major reason that a new emerging disease did not materialize had less to do with human intervention than with the characteristics of the virus. In these cases, virus infection was apparently self-limiting and for the Reston virus not clinically significant to humans. However, the most serious zoonotic infections are ones that establish themselves in humans but leave no animal signature, and thus the false impression of a newly evolved human disease. Many animal viruses that pose a risk for initiating new diseases in humans generally cause no overt damage to their natural host reservoir so that identification of potential pathogens from those animals is not possible. What seems to be missing in all of these surveillance efforts is the fact that we are not devoting our efforts towards preventing the initial introduction of these new viruses into humans. Once the virus has silently found its way into one human sentinel case, there can be little chance of reversing the process. As Stephen Morse put it, "We might consider the emergence of new viruses as a two-step process, the first step being the introduction of the virus into a human population, and the second step dissemination within the population" (Morse 1994). Xenotransplantation may well accomplish the first step in the process.

Unfortunately, there are also many instances in which the outcome has not been a success story. Herpes B virus is a common oral and genital infection of macaques with symptoms similar to herpes simplex virus infection in humans (Weigler 1992). In a limited number of cases, humans infected with this virus develop a rapidly fatal neurologic disease. Since the advent of acyclovir therapy, a few individuals have survived their infections, and in one case, an infected person appears to have infected his spouse. However, further spread of herpes B virus has not been observed (Holmes and others 1990). Again, contact with primates can lead to

fatal consequences, yet the difficulty in transmission from person to person naturally limits this disease to a few individuals; generally those whose occupation involves frequent contact with macaques. It should be noted that since we now have developed therapies to control B virus infection in humans, in one sense we may have increased the risk to the overall population by promoting the survival of the virus in its human host.

Another example that illustrates the potential for monkey-to-human transmission through human intervention involves the pioneering work of the poliovirus vaccine researchers. A recent exposé in the Rolling Stone accused Dr. Koprowski, whose clinical vaccine trials in Africa and Poland were instrumental in the development of the live attenuated poliovirus vaccine, of being responsible for creating the AIDS epidemic (Curtis 1992). The crux of the story centered upon the idea that the poliovirus used in the vaccine had been grown on monkey cells and that these cells may have harbored immunodeficiency viruses that then became the progenitor to the human AIDS virus. Common sense however, tells us that this could not have been the case (Koprowski 1992). First, the monkey kidneys used in making the primary cell cultures were from Asian macaques, which do not carry an SIV-like virus in the wild. Second, the virus does not replicate in kidney cells. Third, the human AIDS virus, HIV-1 virus, is most closely related to the chimpanzee virus SIVcpz, which is therefore most likely responsible for the original infections in humans (Peeters and others 1992). Nevertheless, these types of stories continue to surface in regard to the origins of AIDS. One cautionary note, although the early vaccine preparation were generally prepared on macaque cell lines, until very recently, most poliovirus seed stocks were propagated in primary kidney cultures from African green monkeys. Seed stocks refer to those virus stocks that are generated every few years and are used as a source to expand and propagate virus for large scale vaccine production. In most cases, these African monkeys were not tested for SIVagm infection, yet 40-50% of African green monkeys carry SIV in the wild (Kanki and others 1985a). Again, no evidence of an SIVagm-like virus has been observed in humans, and SIVagm does not replicate in kidney cells.

The poliovirus vaccines did in fact lead to inadvertent exposure of millions of people to another monkey virus, SV40, a DNA virus that is known to transform human cells in culture and has the capacity to induce cancer in experimental animals (Shah and Nathanson 1976). SV40 was recovered from the macaque kidney cells along with poliovirus. In one 20-year follow-up study, no evidence of any link to cancer caused by SV40 in these vaccines was uncovered (Mortimer and others 1981). Recently, however controversy resurfaced in regard to SV40 contaminated poliovirus vaccine lots. In a new study, a significant number of mesotheliomas contained viral DNA (29 of 48 cases), which, of any of the known papovaviruses, most closely resembled SV40 (Carbone and others 1994). While preliminary in scope, these findings do suggest that monkey viruses may play a role in human cancer and will require further analysis to resolve this issue.

The poliovirus vaccine efforts led to yet another serious infectious disease outbreak. In 1967, 31 cases of an acute hemorraghic disease afflicted laboratory personnel in Germany and Yugoslavia, which included seven deaths (Kissling and others 1968). The illness was a direct result of exposure of laboratory workers to African green monkey kidneys being used to propagate poliovirus. It seems that the African green monkeys harbored a new virus which was later identified as Marburg virus, a member of the filovirus group. A similar outbreak caused by an antigenically related filovirus, Ebola, was seen in 1976 in Africa, which led to the deaths of over 400 people living in Central Africa with a 90% mortality rate (WHO 1978). The Ebola virus was originally thought to be linked to African monkeys, yet it is still uncertain how the African monkeys contracted the infection since serologic studies have not indicated a primate reservoir. The acute and lethal nature of these viral infections was an important factor in containing the infection because infected individuals could be quarantined thus limiting contact and further spread of infection. It is still uncertain if baboons harbor filoviruses, although one report suggests that Chacma baboons may carry serologically related viruses (Lecatsas and others 1992).

What these stories do tell us is that we have been remarkably lucky that our efforts to cure one disease have not led to more deadly consequences in spite of our repeated encounters with unexpected microbial agents. These types of cases also serve to illustrate that one cannot know what the consequences will be of introducing a new virus into an unnatural host (humans). A virus that seems ubiquitous in nature and induces little or no pathology in its natural host may under the proper conditions wreak havoc in a foreign host. Of course many of these infections may become inapparent, or abortive in humans. Monkeys are genetically closely related to humans and thus the receptors for various virus types may be highly conserved. Indeed, most monkey viruses are isolated and characterized by their growth on human cell cultures. However, it is impossible to predict what effect introduction of a "non-pathogenic" animal virus into humans will have.

While monkey-to-human infections may result in new epidemics, human-to-human transplantations and blood transfusions have routinely led to morbidity and mortality. From herpesviruses to hepatitis and AIDS, our attempts to save lives have had isolated but disastrous consequences. At least six cases of rabies have been reported in humans receiving corneal transplants from cadavers, where the individuals were only later determined to have died from rabies (Houff and others 1979). In 1974, Creutzfeldt-Jakob disease, a slowly progressive and transmissible dementia was also acquired following corneal transplantation (Duffy and othesr 1974). The most notorious cases of human error are those related to AIDS in the early 1980s. Hemophiliacs and blood transfusion recipients became inadvertently infected with HIV when receiving blood and blood products. Upwards of 90 percent of all hemophiliacs and transfusion recipients in

this country and elsewhere became infected within just a few short years and many have since died (Peterman and others 1987). The controversy surrounding blood transfusions and acquired HIV infection continues in the press with administrators from blood centers in France imprisoned for not moving quickly in testing blood preparations because a French test kit was not yet on the market, while an American commercial kit was already available. It has been estimated that over 12,000 transfusion recipients became infected with HIV before HIV antibody testing of the U.S. blood supply was instituted. In addition, many human transplant recipients were inadvertently infected with HIV-1 before testing was instituted in the early 1980s. One can well imagine a similar scenario emanating from just a few successful transplant recipients, with donors and recipients participating in the grand experiment of life, the donor providing not only a viable organ but also all of its resident microbes.

It should be mentioned that an important reason for using baboon tissue is not because of the shortage of human organs but that the baboon is resistant to some known human viral infections such as the hepatitis B virus. This virus continues to be a major problem in liver transplant recipients (Hollinger 1990). Some of these patients are in need of a new liver precisely because their own has been destroyed by hepatitis viruses. Implanting a new human liver generally leads to the same consequence in chronic carriers where the donated liver also becomes infected and is destroyed. Baboon livers are seen as a way around this infectious disease problem due to species restriction in hepatitis virus replication. Similarly, baboon bone-marrow cells are being considered as a last resort in AIDS patients who have lost their CD4 T-cell subset population and are in the late stages of AIDS. Studies in my laboratory indicate that baboon CD4 T cells resist infection with HIV-1 (Allan unpublished data). What this means is that if one can generate chimerized bone marrow where the baboon immune cells can function normally in a human host, then one can essentially reconstitute a functional chimeric baboon-human immune system with natural resistance to HIV conferred by baboon T cells. Success in rodent models has laid the foundation for these studies (Ildstad and others 1992), although a single attempt at baboon-to-human bone marrow transplantation has failed (Ricordi and others 1994), and graft-versus-host disease is still a concern for those studies.

Obviously, some baboon viruses will not be transmitted to humans due to constraints either at the level of viral entry or during their replication. However most, if not all, of the known baboon viruses have been shown to grow in human cell lines, so there is no great leap of faith in surmising that viruses will set up shop in a new human host. Whether the transplanted material is liver or bone marrow, the sheer numbers and variety of cell types that will be introduced is phenomenal and will likely include several microbes as well. There may be less enthusiasm for using organs from phylogenetically more distantly related species such as swine for human transplantation, but the distance also belies the fact that viruses carried by pigs are theoretically less likely to be infectious to humans due to a higher degree of variation in cellular receptors used by these viruses, whereas the close genetic relationship between baboons and humans enhances the possibility of transmitting animal viruses to humans.

The lessons being learned from the AIDS epidemic are also relevant to this discussion. The human viruses, HIV-1 and HIV-2, share a great amount of homology with chimp and mangabey simian immunodeficiency virus (SIV) isolates, respectively (Myers and others 1992). It is generally accepted that cross-species transmission of SIV viruses to humans were the sources for the emergence of human AIDS. No one will ever know who was first infected and when, yet most epidemiological studies point to the late 1950s for the onset of HIV-1 and the early 1970s for HIV-2. Interestingly, an animal model was discovered by coincidence soon after HIV was identified. At the New England Regional Primate Research Center in Southborough, Massachusetts, a severe immunodeficiency-like disease was observed in a few macaques that were involved in studies to understand the pathogenesis of STLV, a retrovirus that had been identified with lymphoid cancers in macaques (Daniel and others 1985; Kanki and others 1985b). Careful analysis led to the discovery of a related but distinct virus from HIV called SIVmac. This virus was demonstrated to induce classic AIDS like disease including a loss in CD4+ T cells in Asian macaques (Letvin and others 1985). Soon after, seroepidemiologic studies provided a link to African green monkeys and a related virus was isolated and designated SIVagm (Kanki and others 1985a). Unlike SIV in macaques, African green monkeys showed no signs of illness when naturally infected or by experimental infection. A direct link between SIVmac and a natural African monkey reservoir was determined at both the Yerkes Regional Primate Research Center in Atlanta, Georgia and the Tulane Regional Primate Research Center in Covington, Louisiana (Murphey-Corb and others 1986; Fultz and others 1986). Another African monkey, the sooty mangabey, had high prevalence rates to SIVsm, and genetic analysis revealed a close relationship to the macaque virus, which strongly implicated the mangabey virus as the cause of the macaque outbreak (Hirsch and others 1989). A total lack of SIV infection in the wild for macaques supports the possibility of cross-species transmission and is consistent with the theory that unnatural host species are likely to be more susceptible to disease from infection with a new virus.

Coincidentally, a second human virus (HIV-2) was found in West Africans and was also highly related to the mangabey virus (Barin and others 1985; Clavel and others 1986). It is interesting that the sooty mangabey's geographic distribution in nature is limited to West Africa which is precisely where HIV-2 is found. This story also points out another important aspect to new viral diseases in terms of their unpredictable nature for causing disease. Whereas HIV-1 is considered an important health problem due to its high morbidity and mortality, it appears that HIV-2 is much less pathogenic, and may not be transmitted as easily as HIV-1 (Marlink and others 1994). One can speculate that the relative replication rates for these viruses differ substantially in

their respective human hosts, which might account for this variation in pathogenesis. There are several ways that this dispersion may have arisen. First, HIV-1 has been in the human population considerably longer than HIV-2 and has had a greater adaptive advantage. Alternatively, HIV-1 may simply be intrinsically more pathogenic while HIV-2 is not likely to reach the same levels of replication in humans.

The significance of these studies in relation to this discussion is that it is apparent that African monkeys have been infected with their own virus types for perhaps thousands of years. This can be inferred because the relative phylogeny for the SIVs parallels the phylogeny of the monkeys. For example, there are four distinct SIVagm viruses that are related to each other to the same extent that the monkeys are related (Allan and others 1991). This suggests that the viruses have co-evolved and then diverged along with their respective hosts.

While these viruses have probably reached a delicate balance with the natural host, the existence of these viruses was not discovered until after many thousands of humans began developing a new clinical entity called AIDS. In some ways, the medical community was lucky, the virus grew in CD4 T cells and T-cell growth factor (IL-2) had only recently been discovered allowing for the cultivation in vitro of the HIV in primary lymphocyte cultures. Also, the tropism of the virus was quickly determined rather easily because commercial antibodies used to type T-cell subsets also blocked HIV infection in culture (Dalgleish and others 1985). Imagine the difficulties that would have been encountered had the receptor not been previously identified. Seroepidemiologic studies are still incomplete but it is likely that there are about 30 distinct SIV types harbored in African monkeys, and some of these viruses may have the potential of becoming a new human AIDS variant (Myers and others 1992; Allan 1992). It is important to keep this in mind in discussing monkey-to-human transplants for AIDS.

Direct evidence for the transmissibility of monkey SIVs to humans comes from a recent report on the accidental infection of two laboratory workers with the SIVmac virus (Khabbaz and others 1994). While not certain, it appears that these people were exposed while handling large quantities of infectious tissue culture fluids. Seroconversion was evident, although initial attempts to isolate the virus have failed. It is probable that accidental infection with SIVmac might also require sufficient replication and adaptation before host-specific disease or transmission among close contacts is manifested (Essex 1994).

BABOON VIRUSES TRANSMISSIBLE TO HUMANS

One must also keep in mind that the greatest risk of xenotransplantation to humans comes from viruses that are waiting to be discovered. In general, viruses from newly emerging diseases are only identified once the target population has been significantly affected. That is, until the human disease is evident there is no reasonable means for evaluating potential pathogens currently harbored by nonhuman primates. While numerous baboon viruses have been identified, it is not unreasonable to suggest that there may be just as many viruses that have yet to be discovered and are as much of a threat as the established ones. It should be noted that detailed analysis of the types of baboon viruses in nature is still only a poor estimation. Most of these viruses were discovered in the late 1960s or early 1970s, and their detailed relationship to other mammalian viruses is still slowly evolving (Kalter and Heberling 1990; Barahona and others 1974). Very little basic research has focused on the characterization of baboon viruses within the last twenty years. In fact, the assays available for detecting some of these viruses and for their isolation have not changed substantially over the years. Furthermore, some of the commercial assays to detect antibodies to viral antigens may not be optimal in detecting baboon viruses. For example commercial ELISA (enzyme-linked immunosorbent assay) kits are sometimes used to screen baboons for antibodies to STLV, a retrovirus closely related to the human counterpart HTLV. However, detailed studies to determine the sensitivity and specificity of the baboon response for detection with this kit have not been fully elucidated. For other viruses, even less is known about the serological utility of baboon antibodies for viral analysis. Until more detailed studies are conducted to assess the validity of these assays it is uncertain whether most animals are truly virus-negative for some of the pathogens for which they are being screened. In addition, there are circumstances where an animal may be viremic yet lack detectable antibodies to that virus either because of a newly acquired infection or a host-specific block in generating anti-viral antibodies. One must then contend with these possibilities before embarking on these transplantation procedures.

It should be mentioned that in 1985 an attempt at successful heart xenotransplantation from a baboon to a human (Baby Fae) failed (Bailey and others 1985). Efforts directed at prescreening the baboon for potential pathogens were deficient. Furthermore, six baboon kidneys were transplanted to patients over 30 years ago and although the kidneys were vigorously rejected, success in any form might have changed human evolution in regard to infectious agents (Starzl and others 1964). Although AIDS was not present in this country 30 years ago, African primates harbored these viruses or ones like the human form, and the potential was there to begin the first human infection. Viruses that are commonly referred to as human infections, ranging from influenza to measles, may have had a monkey virus ancestor. HTLV and SIV are two such viruses where a strong case can be made for cross-species transmission to humans. Perhaps chronic fatigue or one of the new herpesviruses discovered in humans are actually a recent accidental introduction from monkeys rather than a distant ancestral link.

A list of viruses and their potential for causing disease are given in Table 1. Selected viruses will be described be-

low and readers are therefore referred elsewhere for more information regarding other virus types.

Retroviruses

AIDS viruses. Fortunately, the human AIDS viruses do not have a baboon homologue. However, in one study, antibodies to an African green monkey virus (SIVagm) were found in two baboons from a large seroepidemiologic survey in Tanzania (Kodama and others 1989). Recent highly sensitive polymerase chain reaction (PCR) methods were used to amplify viral DNA from peripheral lymphocytes from one of the animals, and the nucleic acid sequence matched that of a vervet SIVagm (Jin and others 1994). Other studies including our own serologic studies have failed to detect SIV infected baboons. It should be pointed out, however, that baboons might harbor a distantly related SIV that may not be detected using standard virologic and serologic assays. High background reactivity to both HIV and SIV proteins is routinely observed from African nonhuman-primate sera, including baboon sera, which does not preclude the possibility of a distantly related virus in baboons. Our studies and those of others have shown that baboon lymphocytes are resistant to infection with HIV-1 viruses (Allan unpublished data; Morrow and others 1989). On the other hand, recombinant viruses composed of the envelope from HIV-1 chimerized with SIVmac provirus easily infect baboons. Other studies have shown that baboons are susceptible to infection with HIV-2 and SIVmac and may develop AIDS-like disease (Castro and others 1991; Benveniste and others 1988). What these studies indicate is that there is no innate reason why baboons do not harbor their own SIV.

TABLE 1 Viruses of significance to baboon—human transplantation

Virus	Examples of Pathogenicity
Retroviruses	AIDS in humans and Asian macaques
Human and Simian Immunodeficiency Viruses	
HIV-1	
SIVagm	
HIV-2	
SIVmac	
SHIV	
SIVbab?	
STLV/HTLV	Leukemia/Lymphoma
BaEV—baboon endogenous virus	Unknown—cancer
Spumaviruses (Foamy)	Unknown—cancer, Graves diseases,other
Other—Type D, other endogenous	Unknown—cancer
Herpesviruses	
SA8	Genital lesions, abortions
H.Papio	Cancer?
CMV	Immunosuppression, cancer?
Papoviruses	
SA12	Unknown—cancer?
Picornaviruses	
EMCV	Acutely fatal myocarditis
Spongiform encephalopathies	Kuru, Creutzfeldt Jakob disease, scrapie agent
Uncharacterized	
reovirus	Encephalitis
Other	
Rabies	Rapidly fatal neurologic
monkeypox	Like smallpox, fatal in 10% of human cases

HTLV/STLV family. The first human retrovirus (HTLV) associated with disease was discovered in 1980 and in part emanated from past achievements in developing methods for growing T cells in culture. Virus could be propagated in culture and thus isolated and characterized. A disease entity recognized in Japanese populations called Adult T-cell leukemia/lymphoma was recognized in 1977 and later linked to HTLV (Cann and Chen 1990). Further studies led to the identification of a highly related monkey virus called STLV which is found in most Old World primates and its association with lymphoma has been described (Homma and others 1984; Fultz 1994; Mone and others 1992; Hubbard and others 1993). Our own studies in baboons have demonstrated that 40 percent of our colony of approximately 3,000 baboons carry STLV (Mone and others 1992). Only about 4 percent of infected animals develop lymphoma during their lifetimes, an incidence that is remarkably similar to rates seen in HTLV infected humans. Molecular analysis of baboon lymphomas showed monoclonal integration of STLV provirus indicating a role for STLV in the induction of the lymphomas (Mone and others 1992). As yet we have not identified animals with neurologic manifestations of tropical spastic paraparesis (TSP) seen in some HTLV-infected humans. STLV and HTLV-1 are genetically almost indistinguishable having over 90 percent nucleic acid sequence similarity in the env gene. HTLV can be viewed as a significant public health problem and screening of the nation's blood supply is mandatory.

Spumaviruses. Foamy viruses were first described in humans in the early 1970s and were originally linked to nasopharyngeal carcinoma, which later proved to be an erroneous association (Achong and others 1971). Seroepidemiologic surveys have found significant rates of infection in

Africans while no foamy virus infection was observed in North America (Achong and Epstein 1983). On the other hand, most other animal species, including baboons, harbor species-specific foamy viruses (Neumann-Haefelin and others 1993). Unlike oncoviruses, the foamy viruses act much like immunodeficiency viruses in that they generally remain latent in many cell types including lymphocytes and when expressed, induce large multinucleated giant cells, or syncytia, and cell death, which is almost pathognomonic in SIV negative animal cultures (Flugel 1991; Allan unpublished data). Curiously, no definitive evidence as to pathogenicity of this virus has been found. The natural history of infection of foamy viruses is not well described but it would appear that these animals are infected early in life, perhaps even in utero. Obviously, generating animals that are free of foamy virus will be no small task. It must be remembered that like other retroviruses, foamy viruses must be considered capable of inducing cancers in susceptible animals. From Grave's disease to thyroiditis (Neumann-Haefelin and others 1993; Wick and others 1993), the search continues for a direct association with disease (Weiss 1988). Bone marrow induced tolerance to foamy virus infection could potentially have disastrous consequences due to the inherent pathogenicity of the virus for human cells in vitro.

Baboon endogenous viruses (BaEV). A Type C virus, recovered from placental tissue from baboons, represented the first nonhuman primate retrovirus (Benveniste and others 1974). Efforts to produce disease in experimental animals have failed, however, this virus could still represent a potential hazard in humans (Huang and others 1989). In general, retroviruses represent a serious hazard in that they integrate randomly into the host genome and under the right circumstances may induce cancers by insertional mutagenesis. A second problem that might arise is the possibility of recombinational events leading to a "new" virus. It has been estimated that as much as 0.6-1.0 % of the human genome consists of retroviral-like elements including human endogenous viruses or HERVs (Leib-Mosch and others 1992). Integration or recombination could ultimately result in the generation of mutant forms with varying degrees of pathogenicity. Just as the more lethal influenza epidemics arise by reassortment/recombination between avian and human viruses, recombinational events between baboon and human retroviruses could result in new virus types (Smith 1993; Zhang and Temin 1993). In spite of this cautionary note, there is currently no direct evidence to substantiate this possibility.

The notion that a retrovirus might induce cancer was recently realized in nonhuman primates that had been experimentally infected with murine retroviruses used in gene therapy (Vanin and others 1994). Three of ten monkeys which had received autologous bone-marrow stem cells transduced with a replication-competent MuLV, developed T-cell lymphoma. In fact, it appears that the animals had become tolerant to the viral antigens. Even though high titers of virus were recovered from the peripheral blood, no antibodies to MuLV were detected in the lymphomatous animals. In addition, a clonal pattern of MuLV integration was observed in one animal. There are several conclusions that one can draw from this study. First, retroviruses from other animal species including mice may be pathogenic in humans under the right circumstances. Second, AIDS patients treated with baboon bone-marrow stem cells may become tolerant to not only the baboon cells but also to the very pathogens contained within the baboons. While this scenario is more remote it still deserves serious consideration. Replication of baboon viruses in an immunocompromised host may additionally lead to a more rapid rate of variation and more rapid adaptation. Furthermore, retrovirus infections by themselves are generally associated with a certain incidence of cancers. Recently, studies with HIV-1 induced lymphomas have demonstrated that in addition to immunodepletion, HIV is also capable of inducing cancer through site-directed mutagenesis (Herndier and others 1992). One can also imagine that infecting an immunocompromised host with oncogenic herpesviruses, such as Herpes papio, might also accelerate leukemogenesis in that host.

Herpesviruses. There are a number of known baboon herpesviruses that are potentially hazardous in humans (Barahona and others 1974; Hilliard and others 1989). SA8 is an alphaherpesvirus that shares many properties with human herpes simplex viruses and typically causes genital and oral lesions in baboons (Borchers and Ludwig 1991). By sexual maturity, almost all baboons are infected with this agent. It is presently unknown what effect transmission of this virus would have in an immunocompromised human host. The macaque equivalent to SA8, herpes B virus, is acutely lethal in humans as mentioned previously (Kalter and Heberling 1990). The pathogenicity in humans must be considered for the baboon herpesviruses as well. Herpes papio is another herpesvirus whose human homologue is Epstein Barr virus. EBV is well-known as the primary cause of mononucleosis, or "kissing" disease, and has been linked to a variety of human cancers (Stevens 1994). Like EBV, H. papio is capable of transforming to B cells of both baboons and humans. Baboons also carry cytomegaloviruses (CMV), which are related to the human CMV strains (Hilliard and others 1989). One must consider all of these viruses as potentially harmful to humans. Efforts to eliminate these viruses from the baboon donors should be mandatory, however, there are likely to be other related herpesviruses that have not yet been identified just as new human herpesviruses are continually being discovered. The unknown consequences of infected humans with baboon herpesviruses makes this endeavor a risky proposition.

Reoviruses. Recently, an outbreak of encephalitis was observed in the baboon colony at the Southwest Foundation for Biomedical Research (SFBR). Although preliminary, it appears that the agent responsible for this disease is a previously unrecognized reovirus (Michelle Leland, personal communication, SFBR, San Antonio, Texas). It is unknown whether this virus represents a baboon reovirus or resulted

from infection with a rodent or avian virus. Again, this outbreak does point out that baboons might harbor viruses that are presently uncharacterized but may represent a significant public health hazard to humans.

Picornaviruses. Although baboons carry several picornaviruses, including coxsackie viruses (Kalter and Heberling 1990), a recent epidemic in baboons of the SFBR baboon colony was associated with high mortality. More than 80 animals died of acute myocarditis while many more animals became infected and survived. An encephalomyocarditis virus (EMCV) was identified and was apparently contracted from resident rodent populations (Hubbard and others 1992). The severity of the outbreak has not been fully investigated but several hundred animals became clinically ill during one 9-month period. The fact that baboons were infected with a rodent virus that was acutely lethal points out that it is imperative that baboons used for organ donation are raised from infancy to adulthood in an environment free from the possibility of contracting a virus from either rodents or birds. Most of the baboons at the SFBR are reared in conditions that expose them to the outside environment, which is also a source of enrichment. However, contact with other species can occur leading to avian-primate or rodent-primate transmissions.

Spongiform encephalopathies. Although not strictly members of the virus family, a group of maladies with a common thread are the slowly progressive encephalopathies, each manifested by an abnormal accumulation of amyloid deposits in the brain (Chesebro 1990). While a baboon scrapie-like agent has yet to be described, it is not unreasonable to imagine a similar virus-like entity found naturally in baboons. Since scrapie was first described in sheep (Sigurdsson 1954), a number of similar syndromes have been elucidated with the more famous study related to Kuru, a disease linked to cannibalism in New Guinea. Gajdusek found that humans that had consumed brains and other tissues from deceased relatives developed a debilitating neurodegenerative disease with pathologic findings remarkably similar to scrapie in sheep (Gajdusek and Zigas 1957). Indeed, the infection and disease could be transmitted experimentally to chimps (Gajdusek and others 1967). Other scrapie-like disease entities have also been reported in humans and include Creutzfeldt-Jakob Disease (CJD) and Gerstmann-Straussler Syndrome (Duffy and others 1974; Masters and others 1981). Most recently, "Mad Cow" disease or bovine spongiform encephalopathy arose by feeding cows bone meal from sheep (Wells and others 1987). A similar disease has not been observed in baboons, but a detailed study has not been undertaken. In the event that a scrapie-like agent was present in baboon tissue and transmitted to humans, it is unlikely to be transmitted to others since its route of transmission is primarily through cannibalism or transplantation.

ANDROMEDA STRAIN REVISITED

While Michael Crichton's best-selling science fiction novel is firmly implanted into the public's awareness, the relative risk of an acutely lethal and highly contagious viral disease is mostly improbable. Consider that humans have been in contact with most other nonhuman primates whether in zoos or in the wild for hundreds if not thousands of years, and any catastrophic viral disease emanating from easily transmitted viruses would have materialized by now. Viruses transmitted by fecal-oral or respiratory routes have probably been transmitted from monkey to human over the years and it is possible that some of the viral strains circulating in human populations today might have had their origins in monkeys. The more serious known zoonotic infections from monkeys to humans have already been mentioned. Many bacterial pathogens are readily transmitted among primates such as Shigella, and tuberculosis is a serious concern at primate centers, since macaques are highly susceptible to infection accompanied by advanced disease (Michaels and Simmons 1994). A new emerging viral disease resulting from xenogeneic transplantation could take many forms with as many outcomes as the mind can imagine. The most insidious threats are those viral infections that are largely silent ones which might only become fulminant at some later time and perhaps only in a small percentage of the infected population.

By understanding the intricacies of how AIDS is spread, and the pathogenesis of other more slowly progressive diseases such as the spongiform encephalopathies, multiple sclerosis, or Alzheimer's disease, one can easily weave together possible candidate diseases. It should be remembered that our public health agencies are most successful at investigating infections that have acute morbidity and mortality. On the other hand, our success at stemming the tide of persistent slow virus infections is abysmal. From AIDS to hepatitis, we are still struggling to find cures and vaccines to safeguard the uninfected population. Despite our best efforts, the percentage of people infected with HIV continues to rise due to our inability to moderate human behavior and the persistency of the infection. The fact that the virus tags along with human sexual activity really hinders our efforts at control. It is therefore easy to imagine that the more difficult viruses to detect and eliminate will be sexually transmitted or blood borne, similar to AIDS.

Another level of complexity is apparent when one considers the risk associated with bone marrow transplants from baboons. Given that the baboon bone-marrow cells harbor several virus infections with proven pathogenicity for human cells in vitro, a healthy baboon immune system would maintain a full repertoire of anti-viral responses that would limit virus replication in the human recipient. Should the baboon bone marrow also function in limiting progression to AIDS and should the recipient recover, that individual might become a cauldron for silent epidemics, a modern day Typhoid Mary. Even though his or her chimerized immune system may keep those monkey viruses in check, transmission through intimate contact to sexual partners could then lead to demonstrable

illness since the partner's immune system has likely never seen the baboon viruses. Efforts to pinpoint the origins of these new diseases would also be more difficult since the cases are once removed from the transplant recipients.

FUTURE CONSIDERATIONS AND RECOMMENDATIONS

Transplant specialists are determined to proceed with studies directed toward xenogeneic transplantation. Over 30 years have gone into its development and these baboon xenogeneic studies are a culmination of those efforts. I cannot separate the good of saving a human life through transplantation from the risk of introducing a pathogen that will likely change human evolution in some profound way. I view xenograft tissues as essentially very complex vectors for shuttling new viruses into humans. All major natural barriers to viral infections that have evolved during the millennia will have been circumvented by a single surgical procedure. It is time to stop and weigh the evidence against and in favor of transplantation. Once the pendulum is set in motion, baboon viruses will certainly become established in the human population. The only real questions will then be, how serious will be the consequences of such actions? How serious will the inevitable disease (or diseases) be? How will they manifest themselves? Obviously we can't predict the outcome.

There are a few recommendations that might aid in reducing the overall risk to humans from these types of procedures, if it is decided that xenogeneic transplantations are to continue. Selected recommendations are as follows:

- Form an independent panel of scientists and surgeons to address the issues discussed above. This panel may be chartered either through the Institute of Medicine or through the auspices of the National Institutes of Health and the Centers for Disease Control and Prevention. Their charge should be to thoroughly weigh the facts and relative risk that these operations are likely to create, which should include ethical considerations.
- Initiate studies that address the virologic repertoire in baboons. Is this the best nonhuman primate species to use for such studies based on its viral flora? Thorough examination of virus types through state-of-the-art viral detection and isolation methodologies should be instituted. Most of the viruses harbored by baboons have been only marginally characterized. For example, is there a significant population of baboons that carries STLV without detectable antibody responses? How are foamy viruses transmitted and at what age?
- Scrutinize currently available tests for the known viral pathogens. Are diagnostic tests adequate for screening animals for a multitude of viral etiologies? A negative test result should not be misconstrued as anything other than negative as far as that test is concerned. This finding in no way determines actual virus status, and is only predictive of virus burden. Most of the assays used to screen for viral infections in baboons were developed for use in humans. For example, most African green monkeys would not be considered SIVagm positive by immunoblotting methods if the criteria used for humans infected with HIV-1 were used in monkeys.
- Develop tests for viruses that we know are carried by baboons but for which we have no reliable assays. One important example of this problem is the lack of reliable testing for foamy viruses. Serologic tests may discriminate among the various foamy virus types but the sensitivity of these types of assays are suspect. Most of the assays specific for nonhuman primates have not been put under the same microscope as those used for human testing. It is time to develop more stringent criteria and testing methods to screen potential animal donors. In some cases, direct virus isolation techniques should follow an antibody based assays so as to decrease the possibility of a virus positive, and antibody negative animal.
- Provide specific pathogen-free (SPF) colony-bred animals. It's not enough to limit the use of baboons to colony raised animals in place of wild caught animals. SPF baboon colonies can be developed but would be expensive and would create a long lag time until the first baboon could be furnished as a donor. SPF colonies have been established for rhesus monkeys for use in AIDS vaccine testing; these monkeys are free of SIV, STLV, Herpes B virus and Type D retroviruses (Lerche and others 1994; Ward and Hilliard 1994). Very stringent screening methods followed by housing constraints require at least three years before the first group of animals become available. It should be emphasized that SPF only denotes that the animal is free of specific pathogens and not free of all pathogens. One can easily be lulled into thinking that somehow these animals are safe for transplantation when in fact they still harbor any number of viruses (those viruses not part of the SPF list of agents). Because some viruses may be transmitted early in life, cesarean section delivery and removal of the newborns to a sterile environment where they can be reared away from viral flora circulating in the baboon colony, might aid in reducing their virus burden. In addition, animals prescreened and selected as donors can be extensively treated with gancyclovir or acyclovir along with zidovidine to reduce the expression of herpesviruses and retroviruses respectively. Yet, even with these measures, baboon viruses will surely take up residence in human recipients.

Despite all of the best efforts to provide a "clean" baboon for donating organs or cells to humans, the best strategy for preventing xenotransmission is still not to do them. In this brief article, I have provided several examples of primate viruses that have escaped into the human population. Are we to surmise that we have found all there is in these monkeys? I think not. It's time to think about what we can learn from these diseases before we jeopardize the human race. Trying to cure a disease (AIDS) that presumably emerged by close human contact with monkeys by implanting monkey tissue into AIDS patients makes very little sense when viewed from a public health perspective.

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ACKNOWLEDGMENTS

I wish to thank Julia Hilliard for helpful discussions and critical review and Michelle Leland for providing unpublished data concerning the epidemiology of baboon reovirus-like viral encephalitis.

In the News

SCAW to Conduct New IACUC Survey

The Scientists Center for Animal Welfare (SCAW) has developed a new survey to study how IACUCs in both academic and industry function at different institutions and how effective they are at completing their tasks. The study will use a questionnaire mailed to IACUC members in hopes of documenting the IACUC's role in research-animal care and use, as well as indicating some best practices for IACUC functions. The mailing is scheduled for September 1995 with a final report submitted to SCAW in January 1996 by Cygnus Corporation, a Washington D.C.-based marketing group that was chosen to orchestrate the distribution and retrieval of the survey. A conference to discuss the findings will be held in Spring 1996. The study results and the comments and analyses made at the conference will be published.

Humane Society Solicits Nominations

The Russell and Burch Award is given annually to a scientist who has made an outstanding contribution toward the advancement of alternative methods in the areas of biomedical research, testing, or higher education. Alternative methods are those that can replace or reduce the use of animals in specific procedures, or refine procedures so animals experience less pain or suffering. The award, which includes a monetary prize, is named in honor of William M. Russell and Rex L. Burch, British scientists who first articulated the 3Rs approach of replace, reduction, and refinement.

Ideal candidates are scientists who: 1) have made an outstanding contribution toward developing or validating alternative methods in biomedical research, testing, or education, 2) were motivated—at least in part—by humaneness, and 3) have a history of laboratory work that is above reproach on humane grounds. Individuals who have questions about their suitability for the award or the suitability of someone they wish to nominate should contact the HSUS.

Nominations should be sent by June 1st to: Philip Mendoza, Laboratory Animal Programs, The HSUS, 2100 L Street, NW, Washington D.C., 20037 Tel: (301) 258-3042, Fax: (301) 258-3082. Persons nominating themselves or others should submit a letter explaining the nominee's suitability and arrange for supporting documentation to be forwarded. Winners are selected with the aid of a scientific advisory panel and are announced in the fall.

Past winners include Alan M. Goldberg, Ph.D., Director of the Center for Alternatives to Animal Testing, Johns Hopkins University (1991), and Charles E. Branch, Ph.D., Professor of Physiology, College of Veterinary Medicine, Auburn University (1992).

In Memoriam

Earl L. Green, Mouse Geneticist. 1913–1995

Earl L. Green, 81, died in Bar Harbor on January 18, 1995. Until his retirement in 1975, Dr. Green was director of The Jackson Laboratory, a biomedical research institution devoted to the use of genetically defined mice for attacking basic problems in biology and medicine.

He was born on August 7, 1913, in Meadville, Pennsylvania, the fourth child of George Graytric Green and Iva Pearl (Lewis) Green. He attended public schools and Allegheny College in Meadville. He received a Ph.D. in biology from Brown University in Providence, Rhode Island, in 1940.

He and Margaret Creighton of New London, Connecticut, were married on July 4, 1940, in Chicago, Illinois, and spent the ensuing year as postdoctoral fellows at the University of Chicago.

Dr. Green held a faculty position at The Ohio State University in Columbus from 1941 to 1956, where he advised graduate students and taught courses in genetics. During a military leave of absence from 1943 to 1946, he served in the U.S. Army Air Force as chief, department of statistics, of the School of Aviation Medicine, at Randolph Field in Texas. During another leave of absence from 1953-1955, he served as geneticist in the division of biology and medicine of the U.S. Atomic Energy Commission in Washington.

Dr. Green was chosen as director of The Jackson Laboratory in 1956, succeeding the founder and first director, Clarence Cook Little. He was the author or coauthor of 66 papers published in scientific journals. He was the editor of the second edition of the *Biology of the Laboratory Mouse*, published in 1966, and author of *Genetics and Probability in Animal Breeding Experiments*, published in 1981. Dr. Green also served in a variety of advisory positions in several Federal agencies, including the U.S. Atomic Energy Commission, the National Bureau of Standards, the National Institutes of Health, and the National Science Foundation.

He was associated as a volunteer with a variety of private institutions, including the Mount Desert Island Biological Laboratory, Harvard College, New England Regional Primate Research Center, the Center for Human Genetics in Bar Harbor, and the Bigelow Laboratory for Ocean Sciences in West Boothbay Harbor.

Dr. Green was a member at various times of the American Association for the Advancement of Science, the American Cancer Society, the American Genetic Association, the American Institute for Biological Sciences, the American Society of Naturalists, the American Statistics Association, the Biometric Society, the Genetics Society of American, and the Radiation Effects Research Foundation of Japan.

After he retired, Dr. Green taught genetics courses and statistical reasoning at College of the Atlantic in Bar Harbor from 1976 to 1981 and in Elements of Genetics at The Jackson Laboratory in 1987-1988. He also served as a member of the Advisory Committee for the Maine Center for the Arts at the University of Maine from 1985 to 1987.

Dr. Green was honored by election to Phi Beta Kappa at Allegheny College and to Sigma Xi at Brown University. He was awarded a Doctor of Science degree, honoris causa, by Allegheny College in 1960 and received a Graduate Citation for Distinguished Achievement at the Brown University Graduate Convocation in 1980. Along with eight others, he was cited for pioneering the development of inbred strains by the National Cancer Institute, the National Institute of Allergy and Infectious Diseases, and the Cancer Research Institute in 1978.

On the occasion of his retirement from The Jackson Laboratory, the Trustees decided to name a new building the "Earl L. Green Mammalian Genetics Laboratory."

Margaret C. Green, Mouse Geneticist, 1914–1995

Margaret C. Green, 81, died at her home in Bar Harbor on January 16, 1995. Until her retirement in 1975, she was a senior staff scientist of The Jackson Laboratory. Both before and after her retirement, she devoted most of her energies to compiling information about the mutated genes and normal genetic variants of the mouse and publishing the information in linkage maps and catalogs.

She was born on January 11, 1914, in Prince Albert, Saskatchewan, Canada, the second of three children of Allison Graham Creighton and Jean (Mackinnon) Creighton. She moved with her parents to New London, Connecticut, in 1920 and became a naturalized citizen in 1928. She attended public schools and Connecticut College in New London. She received a Master of Science degree from Brown University in Providence, Rhode Island, in 1937, and a Ph.D. degree in genetics and cytology from the State University of Iowa, Iowa City, in 1940. She and Earl Green of Meadville, Pennsylvania, were married on July 4, 1940, in Chicago, Illinois, and spent the ensuing year as postdoctoral fellows at the University of Chicago. Dr. Green held a variety of positions in the Department of Zoology of The Ohio State University in Columbus between 1941 and 1956. She taught courses in biology and genetics and served as research associate in genetics.

From 1953 to 1955, on leave from The Ohio State University, she was employed at the National Science Foundation, in Washington, D.C., where in 1954 she served as the first program director for genetic and developmental biology in the division of biological and medical sciences. In 1956, she and her husband moved to Bar Harbor where she became a staff member of The Jackson Laboratory, a position she occupied until her retirement.

Dr. Green's research consisted of analyzing the genetic basis of several new mutations and of determining their intimate effects on the anatomy and development of the mouse. She was the author or coauthor of 70 papers published in the scientific literature. She was the editor of *Genetic Variants and Strains of the Laboratory Mouse*, published in 1981.

Dr. Green was a member of or consultant to a variety of national and international organizations, including the National Science Foundation, the National Academy of Sciences-National Research Council, the National Institutes of Health, the International Committee of Standardized Genetic Nomenclature for Mice.

Dr. Green was a member at various times of the American Association of University Women, the American Society of Naturalists, the American Genetics Association, the Genetics Society of America, and Sigma Delta Upsilon.

She was honored by election to membership in Phi Beta Kappa at Connecticut College in 1935 and in the Society of Sigma Xi at the State University of Iowa in 1939. Along with eight others, she was cited for pioneering the development of inbred strains of mice by the National Cancer Institute, the National Institute of Allergy and Infectious Diseases, and the Cancer Research Institute in 1978.

In the town of Bar Harbor, Dr. Green served as a member of the Warrant Committee and a member of the Board of Appeals.

She is survived by a sister Jane C. Orr of Annapolis, Maryland, and several nieces and nephews. Contribution in her memory may be sent to the "Earl and Margaret Green Endowment Fund" of The Jackson Laboratory, Bar Harbor, Maine 04609.

Coming Meetings

May 1995

8–9 The Well-being of Animal Research Models in Zoos and Aquaria—New Orleans, Louisiana. This two-day international conference will focus on areas of concern regarding animals used for research in U.S. zoos and aquaria. The conference is sponsored by the Scientists Center for Animal Welfare (SCAW) and the American Veterinary Medical Association. General sessions include discussions on how research concerns differ in zoos and aquaria, ethical dilemmas for conservation research, trends in environmental enrichment, and the role of the institutional animal care and use committee at zoos and aquaria. For more information contact SCAW, Golden Triangle Building One, 7833 Walker Drive, Suite 340, Greenbelt, MD 20770. Tel: (301) 345-3500; Fax: (301) 345-3503.

June 1995

11–14 CALAS/ACTAL Annual Conference—Saskatoon, Saskatchewan, Canada. The Thirty-fourth Annual Conference of the Canadian Association for Laboratory Animal Science/L'association canadienne pour la technologie des animaux de laboratoire (CALAS/ACTAL) will include workshops and scientific sessions in laboratory animal science. For more information contact Dr. Don McKay, CALAS/ACTAL National Office, Biosciences Animal Service, CW 401 Biological Sciences Building, Edmonton, Alberta, Canada T6G 2E9. Tel: (403) 492-5193; Fax: (403) 492-7257; Email: dmckay@gpu.srv.ualberta.ca.

21–24 Eighteenth Annual Meeting of the American Society of Primatologists (ASP)—The meeting is hosted by the Primate Foundation of Arizona and Arizona State University in Scottsdale, Arizona. All paper sessions, symposia, posters, exhibits, and business meetings will take place at The Safari Resort in Scottsdale. Registration costs are now $125 for regular members, $88 for student members, and $410 for non-members through May 15. After May 15, registration costs for regular and student members and non-members are $145, $108, and $106, respectively. For registration forms or more information contact Jo Fritz, Primate Foundation of Arizona, P.O. Box 20027, Mesa, AZ 85277-0027. Voice: (602) 832-3780; Fax: (602) 830-7039; Compuserve: 75031,3052 (Internet users send to: 75031.3052@compuserve).

24–29 Ethical Issues of Animal Research—This summer course will be held on the campus of Georgetown University, Washington, D.C. The course is open to college faculty and others who would like to improve their skills in teaching about ethical issues surrounding the use of animals as research subjects. Emphasis will be on how to use the course material in classroom instruction. Topics include the moral status of nonhuman animals, justification for using animals as experimental subjects, ethical concerns about vulnerable subjects, student objections, the use of alternatives, animal harms and pain, legal issues, and the importance of species. For more information contact Moheba Hanif, Georgetown University, Washington, D.C. 20057. Tel: (202) 687-6833; Fax: (202) 687-8089 Email: hanifm@guvax.georgetown.edu.

25–27 Workshop on Xenograft Transplantation: Ethical Issues and Public Policy

The Institute of Medicine (IOM) will convene a workshop to explore some of the problems related to transplantation of xenografts (animal organs in humans). It will be the centerpiece of a planned study by a committee and will address key scientific, social, legal, and ethical issues that attend the use of xenografts. The committee will later produce a report highlighting possible ways of thinking about issues related to future xenograft transplantation. The report will be widely distributed to interested parties such as Institutional Review Boards, Animal Care and Use Committees, clinicians, scientific investigators, and health policy analysts.

On June 28, 1992, a baboon liver was transplanted into a 35-year-old father of two young children at the University of Pittsburgh. Hepatitis B virus had destroyed his own liver. The patient died on September 6, 1992, just about two months later. On October 11, 1992, a pig liver was transplanted into a 26-year-old female at the Cedars-Sinai Medical Center in Los Angeles. This patient also died within months of the transplant. Such xenografts are often, but not exclusively, undertaken to bridge the time between expected patient death and availability of a transplantable human organ, although the patients often die before a suitable transplant is obtained for use. These recent events have rekindled long-standing debates about the technical feasibility and the wisdom of employing xenografts.

When human organs are not available or otherwise not appropriate, xenografts are often performed as experimental procedures. Many scientific, ethical, and social questions relating to these procedures remain unaddressed. For example: Who, if anyone, should regulate the availability of xenograft transplantation? What is adequate informed consent for such an experimental process? What are the ethical issues involved in using animals for these procedures? What are the health policy and financing issues attendant on the expanded use of xenografts?

The advantages of xenograft transplantation include the potentially high availability of donor animals (possibly from the farming of donor animals such as pigs), the fact that surgi-

cal procedures could be done on an elective basis, and the fact that rejection of concordant xenografts qualitatively resembles allograft rejection (although quantitative differences stemming from genetic disparity between discordant donors and recipients remain). Disadvantages include the historically poor survival of patients and grafts, the ethics of using animals in experimental procedures, especially endangered species such as chimpanzees (a species closely related to human beings), the embryonic state of the scientific information on which these procedures are based, and the suffering of patients and families that become involved for little obvious gain.

Newer research on the development of transgenic animals (particularly pigs) offers renewed enthusiasm for continued exploration of xenografting. At the Second International Conference on Xenotransplantation, held in England in October 1993, two groups reported that they had successfully inserted human genes into pigs.

Because at present no standing process or mechanism exists to permit systematic examination of the social, ethical, and legal issues related to advances in biomedicine, the Institute of Medicine, through its Boards on Health Sciences Policy and Health Care Services, plans to assemble a committee to lay out these issues as they relate to xenograft transplantation. This committee will highlight possible ways of thinking about issues related to future xenograft transplantation.

The workshop will provide a time for in-depth presentations of the many viewpoints on aspects of this study and time for meaningful multidisciplinary interactions of participants. Topics that could be considered at the workshop relating to the background and the context will include the following:

- Biomedical sciences: History and current status of xenografts; emerging knowledge on immunologic reactions across species; newer technical developments that may influence the field (transgenic animals and new immunosuppressive drugs); and lessons learned about introduction of animal infectious agents into human systems (SIV infected animal caretakers).
- Human issues: setting ethical standards for xenograft transplantation; assessing competency of medical teams; developing procedures for informed consent; possible national review mechanisms; psychological aspects of receiving animal tissues; social, cultural, and religious viewpoints on xenograft transplantation; and ideas concerning mortality, aging, and acceptance of death in our culture.
- Use of animals: the likelihood and acceptability of breeding or genetically engineering animals for xenografts.

An additional workshop day will focus entirely on the issue of transmission of microorganisms from nonhuman primate tissues and organs, including how to screen for such agents, how to monitor patients, how to protect health care workers, and other issues.

For more information about this upcoming workshop, contact Valerie P. Setlow, Division of Health Sciences Policy, Institute of Medicine, 2101 Constitution Avenue, NW, Washington, DC 20418. Tel: (202)334-2351; or Constance M. Pechura, Board on Biobehavioral Sciences and Mental Disorders, Institute of Medicine, 2101 Constitution Avenue, NW, Washington, D.C. 20418. Tel: (202) 334-3387.

July 1995

2–6 The International Congress of Toxicology VII—Seattle, Washington. The Society of Toxicology is hosting this congress 5-day conference entitled, "Horizons in Toxicology: Preparing for the Twenty-first Century." The scientific program will include lectures, symposia, workshops, and debates. The congress will also offer platform and poster presentations, continuing education courses, a social program, and commercial exhibits. For more information contact International Congress of Toxicology—VII, The Sterling Group, P.O. Box 12227, Overland Park, KS 66282-2227.

2-6 Frontiers in Laboratory Animal Science: XI ICLAS General Assembly and Joint Conference of ICLAS, ScandLAS and FinLAS—Kuopio, Finland. This conference aims to give an overview of latest research results and their applications. Workshop topics deal with immunization, nutrition, pharmacokinetics, euthanasia, and welfare assessment. There are also six seminars, amongst them one by ICLAS and ScandLAS 25 years Jubileum seminar, six platform sessions, three discussions and six plenary lectures. Abstract deadline is March 15, 1995. For more information contact Dr. Tarja Kohila, Lab Animal Center, P.O. Box 17 (Arkadiankatu 7), FIN-00014 University of Helsinki, Finland. Tel: 358-0-1917281; Fax: 358-0-1917284; Email: tarja.kohila@helsinki.fi

September 1995

14–15 Internal Audits of the Animal Care and Use Program—Augusta, Georgia. Sponsored by the National Institutes of Health Office for Protection from Research Risks, the Medical College of Georgia, and Albany State College, this workshop will address processes by which institutional animal care and use committees (IACUCs) can effectively evaluate their institutions' animal care and use program. The *Public Health Service Policy on Humane Care and Use of Research Animals (PHS Policy)* and U.S. Department of Agriculture (USDA) regulations state that at least once every 6 months the institution's program is to be evaluated by the IACUC using the *Guide for the Care and Use of Laboratory Animals (Guide)* and USDA regulations (Title 9, Chapter 1, subchapter A-Animal Welfare) as a basis. Topics include a review of the program as described in the *Guide*; institutional policy issues such as the occupational health and safety program, personnel training, and the activities of the IACUC and how effectively it meets its mandates; veterinary care; the animal environment; and record reviews. Reports of the

IACUC semiannual program and facility reviews will also be discussed. Approaches useful to IACUCs serving both small and large institutions will be included. This workshop is part of an ongoing series sponsored by the National Institutes of Health, Office for Protection from Research Risks on implementing the *PHS Policy*. Workshops are open to institutional administrators, members of IACUCs, laboratory animal veterinarians, investigators, and other institutional staff who have responsibility for high-quality management of sound institutional animal care and use programs. Ample opportunities will be provided to exchange ideas and interests through question and answer sessions and informal discussions. For more information contact Ms. Katrinka Akeson, Department of Continuing Education HM 100, Medical College of Georgia, Augusta, GA 30912. Tel: (706) 721-3967; Fax: (706) 721-4642.

28–29 The Care and Use of Fish, Amphibians and Reptiles in Research—Toronto, Canada. This international conference sponsored by the Scientists Center for Animal Welfare (SCAW) and the Canadian Council on Animal Care (CCAC) will include general sessions on: regulations and guidelines; ACC/IACUC Concerns; the relief of pain in cold-blooded vertebrates (except fish); housing, handling, and nutrition; field research, tagging, capture/recapture monitoring evaluation; aquaculture; stress, disease and euthanasia and other topics. For more information, contact: SCAW, 7833 Walker Drive, Suite 340, Greenbelt, MD 20770, Tel: (301) 345-3500; fax: (301) 315-3505 or CCAC, 315-350 Albert, Ottawa, Ontario KIR 1B1, Canada, Tel: (613) 238-4031; Fax: (613) 238-2837, Email: CCAC@carleton.ca

October 1995

22–25 Swine in Biomedical Research: The International Symposium—College Park, Maryland. This international symposium, sponsored by the University of Minnesota and the University of Illinois at Urbana-Champaign, is accepting abstracts relating to transplantation, pharmacology, nutrition, genetic models, toxicology, behavior, infectious diseases, immunology, physiology, obesity, dermatology, and other subjects. For more information contact Secretariat International Symposium, College of Veterinary Medicine, 295 AS/VM Building, 1988 Fitch Avenue, St. Paul, MN 55108-6009. Email: pigmodel@gold.tc.umn.edu

January 1996

27–31 Fourth National Symposium on Biosafety: Working Safely with Research Animals—Atlanta, Georgia. This national symposium is sponsored by the Centers for Disease Control and Prevention, Office of Health and Safety; National Institutes of Health, Office for Protection from Research Risks; American Biological Safety Association; and Emory University School of Medicine and Yerkes Primate Center. It is intended to provide a forum to stimulate an exchange of ideas and information that promote the identification of hazards, assessment of risks, and implementation of measures to ensure the health and safety of personnel and animals. Biosafety officers, occupational health physicians, veterinarians, principal investigators, members of institutional animal care and use committees, architects, engineers, animal care givers and supervisors, facility managers, administrators, and others are encouraged to attend. For more information contact Centers for Disease Control and Prevention, Office of Health and Safety, Atlanta, GA 30333 (Attention: Jonathan C. Richmond, Ph.D.). Fax: (404) 639-2294.

June 1996

19–26 Sixth FELASA Symposium on International Harmonization of Laboratory Animal Husbandry Requirements—Basel, Switzerland. The aim of this symposium is to exchange useful information among scientists and regulatory agencies in order to increase our knowledge and harmonize the requirements of laboratory animal husbandry. For more information, contact Sixth FELASA Symposium, Kongresszentrum Messe Basel, Messeplatz 21, CH-4021 Basel, Switzerland. Tel: 61-686-2828; Fax: 61-686-2185.

October 1996

20–25 Second World Congress on Alternatives and Animal Use in the Life Sciences—Utrecht, The Netherlands. The aim of this congress is to exchange information on recent developments in the field of alternatives (replacement, reduction, refinement) within the various areas of animal use, such as toxicology, pharmacology, pharmacy, cancer research, bioassays, and safety testing. Alternatives in education and training, ethical aspects of animal use and developments aiming at the improvement of animal welfare will be covered. For more information contact World Congress Alternatives 1996, FBU Congress Agency, P.O. Box 80.125, 3508 TC Utrecht, The Netherlands. Tel: 31-30535044; Fax: 31-30533667.

New Books

Sharing Laboratory Resources. This summary of a workshop held at the National Academy of Sciences explores factors that influence the sharing of valuable laboratory resources in biological research, using the distribution of genetically altered mice as a case study. The report describes factors that influence the action of the investigators who generate the mice, the funding agencies that support the research, the institutions in which the research is performed, the organizations that gain rights to distribute the mice, and the academic and industrial investigators who wish to pursue further work with genetically altered mice. It identifies and discusses ideas for solutions to three key subjects of concern relative to this issue: intellectual property rights, safe and efficient distribution of the mice to researchers, and handling the proliferation of strains. It is available from ILAR at no charge (while supplies last). Tel: (202) 334-2590.

Nutrient Requirements of Laboratory Animals, Fourth Revised Edition, National Research Council. This book integrates new information gained in the latest review of the world literature on nutrient requirements of laboratory animals. The committee sought to make it a valuable reference to investigators whose expertise was other than nutrition. Examples of natural-ingredient and purified diets reported in the literature are provided. Chapters cover the rat, mouse, guinea pig, hamster, gerbil, and vole. The report provides information on the expected growth rates and reproductive performance as well as general information on selection and appropriateness of various types of diets based on research goals. New appendix tables are provided, detailing the amino acid and fatty acid composition of some ingredients commonly used in purified diets as well as molecular weights and international unit standards of various forms of vitamins. It is available from the National Academy Press, 1995. Soft cover, 174 pp, $29.95 ($4 shipping and handling). ISBN 0-309-05126-6. 1-800-624-6242.

Veterinary Drug Handbook, Second Edition, Donald C. Plumb. This single-volume reference contains essential information about hundreds of systemic drugs. It covers "drugs approved for use in veterinary species as well as nonapproved (human) drugs that are routinely used in veterinary practice today."

More than 350 drug monographs cover, respectively: Chemical characteristics. Storage, stability, and physical compatibility. Pharmacology. Pharmacokinetics. Contraindications, precautions, and reproductive safety. Adverse effects and warnings. Overdosage and/or acute toxicity. Drug-drug and drug-laboratory test interactions. Dosages by species and indication (fully referenced). Monitoring parameters. Client information. Dosage forms available. Approval status and withdrawal times. An appendix of additional information and an index containing trade names and generic names enhance the book.

New to the second edition are: more than 100 additional drugs; a new section on topical ophthalmic drugs; monographs by generic name, for rapid location without reference to the index.

Available through ISU Press, 1994. Pocket Edition: 800 pp., 5 x 8 flex cover, $44.95. Desk edition: 732 pp., 7 x 10, flex cover, $44.95. ISBN 0-8138-2443-5. 2121 S. State Ave., Ames, IA, 50014-8300, 1-800-862-6657.

International Directory of Primatology, 2nd edition, Lawrence Jacobsen and Raymond Hamel, eds. This directory enhances communications among organizations and individuals involved in primate research, conservation, and education. It can be used by primatologists as a desktop working tool or by guidance counselors, educators, librarians, students and the general public as a guide to primate programs and information resources. The directory covers more than 300 organizations and 2,000 people active in the field. More emphasis has been placed on educational opportunities for students. Also, an ISIS listing of primates held in zoological gardens worldwide was added. Coverage includes: (1) detailed entries for major primate centers, laboratories, educational programs, foundations, conservation agencies and sanctuaries, (2) a listing of primates held in zoological gardens worldwide, (3) professional primate societies, including the membership roster of the International Primatological Society, and (4) major information resources in the field.

Copies are available from the Wisconsin Regional Primate Research Center, 1994. Spiral bound, 354 pp., $15 or outside the U.S. $23 (prices include postage and handling). ISSN 1064-3826. Orders by telephone: (608) 263-3512, fax (608) 263-4031, or via email: library@primate.wisc.edu. Credit card orders not accepted.

*New Publication

Publications Available

Single copies of the following publications are available without charge from the Institute of Laboratory Animal Resources (ILAR), National Research Council, 2101 Constitution Avenue, NW, Washington, DC 20418. Tel: 1-202-334-2590; Fax: 1-202-334-1687.

Annotated Bibliography on Uncommonly Used Laboratory Animals: Mammals. 1986
Control of Diets in Laboratory Animal Experimentation. 1978
Definition, Nomenclature and Conservation of Rat Strains. 1993
Guide to Infectious Diseases of Guinea Pigs, Gerbils, Hamsters, and Rabbits. 1974
Important Laboratory Animal Resources: Selection Criteria and Funding Mechanisms for their Preservation. 1990
Laboratory Animal Management: Cats. 1978
Laboratory Animal Management: Genetics. 1979
Laboratory Animal Management: Nonhuman Primates. 1980
Laboratory Animal Medicine: Guidelines for Education and Training. 1979
Long-Term Holding of Laboratory Rodents. 1976
Principles and Guidelines for the Use of Animals in Precollege Education. 1989
Recommendations for the Care of Amphibians and Reptiles in Academic Institutions. 1991.
Standardized Nomenclature for Transgenic Animals. 1993
Third International Registry of Animal Models of Thrombosis and Hemorrhagic Diseases. 1988

To obtain single copies of the *Guide for the Care and Use of Laboratory Animals* (1985) write **Office for Protection from Research Risks, Division of Animal Welfare, National Institutes of Health, 6100 Executive Boulevard, MSC 7507, Rockville, MD 20892-7507.**

The following ILAR and Board on Agriculture publications, for which there is a charge, can be ordered from the **National Academy Press, P.O. Box 285, Washington, DC 20055. Tel: 1-202-334-3313 or 1-800-624-6242; Fax: 1-202-334-2451**. All orders must be prepaid by check, money order, or credit card unless accompanied by a bona fide purchase order. Please add $3.50 per item for shipping and handling. Quantity discounts are as follows: 5-24 copies of one title—15%; 25-499 copies of one title—25%. To be eligible for a discount, all copies must be shipped and billed to one address. Please note that the following prices are those for the United States, Canada, Puerto Rico, and Mexico and are subject to change without notice. Ordering information outside these areas can be obtained from the National Academy Press at the address above, or at any of the following locations:

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Japan: Maruzen Co., Ltd., P.O. Box 5050, Tokyo International 100-31, Japan (accept letters only)
Brunei, People's Republic of China, Hong Kong, India, Indonesia, Korea, Malaysia, Philippines, Singapore, Taiwan, and Thailand: World Scientific Publishing Co. Pte. Ltd., Farrer Road, P.O. Box 128, Singapore 9128. Tel: 65-3825663; Fax: 65-3825919.

Dogs. Laboratory Animal Management Series. 1994.
Rodents. Laboratory Animal Management Series. In press.
Recognition and Alleviation of Pain and Distress in Laboratory Animals. 1992. $29.95. ISBN 0-309-04275-5
Education and Training in the Care and Use of Laboratory Animals: A Guide for Developing Institutional Programs. 1991. $11.95 each; $10.50 if purchasing 2-9 copies; $9.95 if purchasing ten or more copies. ISBN 0-309-04382-4
Infectious Diseases of Mice and Rats. 1991. $60.00. ISBN 0-309-03794-8
Companion Guide to Infectious Diseases of Mice and Rats. 1991. $12.00 each (free with purchase of Infectious Diseases of Mice and Rats). ISBN 0-309-04487-1
Immunodeficient Rodents: A Guide to Their Immunobiology, Husbandry, and Use. 1989. $29.95. ISBN 0-309-03796-4
Use of Laboratory Animals in Biomedical and Behavioral Research. 1988. $14.95. ISBN 0-309-03839-1
Nutrient Requirements of Laboratory Animals. 3d rev. ed. 1978. $12.95. ISBN 0-309-02767-5
Amphibians. Guidelines for the Breeding, Care, and Management of Laboratory Animals. 1974. $29.75. (photocopy of original, bound in paper cover). ISBN 0-309-00151-0
Nutrient Requirements of Domestic Animals: A Series - contact the National Academy Press for information on specific reports and prices.

The following ILAR publications are available from the **National Technical Information Service, 5282 Port Royal Road, Springfield, VA 22161.** Add $3 to the total order for the cost of shipping and handling.
Techniques for the Study of Primate Population Ecology. 1981. Paper cover, $31.00, Accession no. PB82 183120
National Survey of Laboratory Animal Facilities and Resources, Fiscal Year 1978. 1980. $17.00 Accession no. PB83 181347

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Emerging Issues

The Impact of International Free Trade Agreements on Animal Research

A quarterly publication for biomedical investigators, laboratory animal scientists, institutional officials for research, and members of animal care and use committees.



The Institute of Laboratory Animal Resources (ILAR) was founded in 1952 under the auspices of the National Research Council, National Academy of Sciences, which serves as an independent adviser to the federal government on scientific and technical questions of national importance. Jointly administered by the National Academy of Sciences and the National Academy of Engineering, the National Research Council brings the resources of the entire scientific and technical community to bear on national problems through its volunteer advisory committees.

ILAR is a component of the Commission on Life Sciences. Among its goals are to develop and make available scientific and technical information on laboratory animals and other biologic research resources to the federal government, the laboratory animal science and biomedical research communities, and the public. Guidelines developed by ILAR form a foundation for institutional and governmental policies on animal care and use.

ILAR Journal is published quarterly by the Institute of Laboratory Animal Resources, National Research Council, 2101 Constitution Avenue, NW, Washington, DC 20418 (Tel: 202-334-2590; Fax: 202-334-1687; Email: ilarj@nas.edu). It is circulated by request to investigators in the field of biomedical and related research.

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Introduction

John P. Hearn

To those doing research that requires the study of animals, the array of legislative and regulatory guidelines, instructions, or orders that have multiplied in the past 20 years can be bewildering. During the same period, the Institute of Laboratory Animal Resources (ILAR) has charted a responsible, balanced course by producing highly respected, science-based guidelines and advice for the biological research community. Many of ILAR's documents have won international acclaim. Yet the multiplication of regulations continues, coming from an alphabet soup of national or international organizations whose committees may sometimes decide to start afresh, without the benefit of already tested knowledge.

Guidelines and regulations must retain the flexibility for improvement as knowledge advances. Therefore it is vital to adopt performance-based standards that are open to improvement, and not engineering standards that can block or inhibit further progress. By the same token guidelines are far more positive in encouraging improvements than are standards or regulations. The international research committee is committed to improved animal care and welfare based on advancing knowledge. The state of the art is not static art. Flexibility is needed because local conditions may vary enormously, for example in climatic, financial, or cultural differences between developed and less developed countries. Often there is no single answer to suit these local variables. With the best of intentions, rigidity in "standards" or "regulations" can be self-defeating.

This issue of *ILAR Journal* examines and compares national laws and guidelines, in the hopes that we can move towards greater synthesis and simplicity. It is important that we do so. Animal science is about new discovery and knowledge, but it is also about the international adoption and transfer of that knowledge to all aspects of human and animal life, including the improvement of the procedures for experiments and for animal care.

Among the reasons this is important are (1) the quality of the science, (2) the need for efficiency in costs of research, (3) the need for similar standards to govern the care and use of animals involved in international research protocols, (4) facilitation of the movement and exchange of research animals and animal products, (5) conservation of the time of scientists on the bench, (6) the achievement of optimal care of traditional or nontraditional animal or cell stocks, and (7) the need to identify the critical research questions that will lead to further improvements in animal care and use.

As we witness encouraging developments with the North American Free Trade Agreement (NAFTA) and the World Trade Organization, we

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in ILAR would like to see science-based advice included early in the process to influence and help develop rational, efficient guidelines, regulations, and legislation, both nationally and internationally. The results will be vital for human health, pharmaceuticals, many aspects of trade and also for animal welfare and conservation. Time spent now in providing expert knowledge and in liaising with the numerous regulatory agencies will save much more time later, especially if untenable regulations were to be developed by default.

In this issue we have presented a range of national approaches to animal research and some opportunities for the future. We have compared national laws and regulations in a table on page 78. We ask that those driven to write new regulations consider what is already tried and true, in furthering and improving the field rather than in reinventing the wheel. We welcome comments on the enclosed articles and are prepared to include such comments in a future issue. ILAR looks forward to working with all interested parties to benefit science and improve animal care and welfare.

EDITOR'S NOTE:

With this issue, *ILAR Journal* brings together perspectives on laboratory animal care programs in Canada, Japan, New Zealand, the United States, and the United Kingdom. In an effort to focus each piece, contributors were asked to describe animal care policies and regulations in their country and comment on how, in their opinion, these policies and regulations affect biomedical research. Authors responded to a set of questions (below), which broadly cover oversight, funding, and enforcement; applicability; administration and costs; strengths and weaknesses; and the future. The final sections include the authors' personal assessments of each system and their predictions for the future. For purposes of this issue, we have used the words "law or policy" very broadly to refer to whatever system a country has in place to ensure that research animals are cared for humanely.

This issue is the beginning of what we hope will become a continuing forum of perspectives, commentary, and information about laboratory animal care around the world. Expect to see submissions from Mexico and Australia in the near future. As always, we welcome all comments (*ILAR Journal*, Institute of Laboratory Animal Resources, 2101 Constitution Avenue, NW, Washington, DC 20418. Tel: 202-334-2590; Fax: 202-334-1687; Email: ilarj@nas.edu).

QUESTIONS ADDRESSED BY EACH AUTHOR

Oversight, Funding, and Enforcement

1. Is oversight of animal care and use in your country provided by national law, state or local law, institutional policy or guidelines, or another system? Please explain.
2. How is this law or policy funded on a national level?
3. How is this law or policy enforced? Who is disciplined for noncompliance? Can infractions by a single investigator adversely impact on the institution's ability to do animal research? Please explain.

Applicability

4. Does this law or policy apply to the institution, the investigator, or other? Please explain.
5. Does this law or policy apply to the animals, protocols, housing, anesthetics, or other?

Administration and Cost

6. Describe the method by which your animal care and use program is administered, including the role of the institutional official, deans, investigators, veterinarians, oversight committees, or others who may have some responsibility.
7. How is the cost of this administration defrayed? By individual research grants, institutional overhead, or other means?

Strengths and Weaknesses

8. In your opinion, what are the current strengths and weaknesses of the administration and oversight of the care and use of animals for research in your country?

The Future

9. How is the administration and oversight of the care and use of animals for research in your country likely to change in the next 10 years?

Canada

James Wong

OVERSIGHT

In Canada, all scientific use of vertebrates and cephalopods is subject to the requirements of the Canadian Council on Animal Care (CCAC), a national, peer-review organization founded in Ottawa in 1968. While Canadian federal legislation covers the prevention of cruelty to animals, research is exempt if it can be shown to be necessary. The provinces of Ontario, Saskatchewan, and Alberta have legislation dealing with laboratory animal use. Their provincial programs and the nationwide programs operated by the CCAC are mutually complementary.

The province of Ontario has the most comprehensive legislation, which regulates the use of animals in connection with research, teaching, testing, and production under the Animals for Research Act (1971). The Provinces of Alberta and Quebec are considering introducing legislation that will empower the use of CCAC guidelines in regulating the use of animals in research, teaching, and testing.

The CCAC comprises 20 member organizations, whose representatives include scientists, educators, and delegates from industry and the animal welfare movement.

The mandate of the CCAC states

> The purpose of the Canadian Council on Animal Care is to act on behalf of the people of Canada to ensure, through programs of education, assessment and persuasion that the use of animals in Canada, where necessary for research, teaching and testing employs physical and psychological care according to acceptable scientific standards, and to promote an increased level of knowledge, awareness and sensitivity to the relevant ethical principles. (CCAC, 1995)

The CCAC does not act as an advocate for the use of animals in science nor does it act to oppose the responsible and ethical use of animals in Canadian science. Its mandate is to work with institutions, scientists, and animal care personnel to develop programs to optimize laboratory animal care and to make changes as required, based on sound expertise and input. CCAC guidelines are not all-encompassing or "etched in stone." Their application requires good judgement and common sense, based on training and experience. The CCAC programs encourage the development of consensus among those using the guidelines and those required to oversee their application.

James Wong, D.V.M., is director of assessments for the Canadian Council on Animal Care

FUNDING

From its inception in 1968 till the end of 1994, the costs of CCAC programs (which include assessment visits, publications, and development of guidelines), have been entirely funded by annual grants from the Medical Research Council (MRC) and the Natural Sciences and Engineering Research Council (NSERC), Canada's two main research granting agencies. In 1968, and for the first few years after it was established, the CCAC assessment program extended only to academic facilities. As the assessment program gained acceptance both in Canada and worldwide, other facilities began to participate in the program. The government and industry research sector now make up a significant proportion of the 201 facilities covered by the CCAC. Beginning in April of 1995, NSERC and MRC will no longer underwrite the costs associated with assessment of government and private industry sectors, due to their own budgetary constraints. The CCAC will therefore follow a user-pay system for facilities not covered by the MRC/NSERC umbrella.

ENFORCEMENT

The cornerstone of surveillance of the care and use of animals in Canadian science is maintained by CCAC's program of peer review. Essential to this program is the institutional animal care committee (ACC). The ACC, set up according to terms of reference laid down by the CCAC, is responsible for the standards of animal care and use within the institution and for evaluation of the ethical acceptability of the animal-based research conducted at the institution.

The effectiveness of each institutional ACC, and the appropriateness of animal care facilities, practices, and procedures, are subject to regular review as part of the CCAC assessment program. CCAC assessment panels are composed of scientists, veterinarians, and members of the animal welfare movement.

In-depth site visits are conducted at least every 3 years. Follow-up visits, most of which are unannounced, are often carried out by members of the CCAC secretariat. Assessments are based on CCAC's two-volume *Guide to the Care and Use of Experimental Animals* (CCAC, 1984, 1995) which includes the following regularly updated policy statements and guidelines on specific issues: (1) Ethics of Animal Investigation, (2) CCAC Guidelines on Acceptable Immu-

nological Procedures, (3) Categories of Invasiveness in Animal Experiments, and (4) Social and Behavioral Requirements of Experimental Animals. The CCAC guide covers a wide range of topics from veterinary care to the social and behavioral requirements of experimental animals.

Assessment Panel Selection

The assessment visit is a key component of the CCAC program and assessment panel members assure that the program is applied fairly and consistently throughout Canada. Panel members are selected from institutions and animal welfare associations across the country. The spokesperson for each panel will have previously served on a number of assessment panels, but as a general rule, the Director and Associate Director of Assessments select panel members from a wide pool of volunteers. In this way, the program is subjected to a large cross section of researchers, administrators, and public representatives. Panel selection also involves inclusion of members who have a particular expertise in the assessed institution's area of research. Assessment panels can offer pertinent advice while at the same time assuring that the interpretation and application of CCAC's mandate comes under the scrutiny of a competent group of experts.

Visit Preparation

Prior to each assessment visit, the CCAC requests and receives pre-assessment documentation pertaining to the institution's (1) administration of animal care program; (2) animal care personnel; (3) space allocation and location of animal housing and use; (4) animal care procedures; (5) veterinary care; (6) statistics on annual animal use; (7) ongoing research and teaching protocols; and (8) occupational health and safety program.

Most assessments begin with a meeting with the ACC and senior administrative personnel. All areas that house or hold animals are visited, as are all areas in which procedures on animals are performed, such as surgical suites and laboratory testing areas.

An integral part of the assessment visit is the summary meeting, where institutional representatives and ACC members can introduce any topic for discussion. This meeting has also become a forum for an exchange of views on animal care and use during which the panel summarizes its findings and relays items of immediate concern to its ACC and institutional representatives. Panel members often use their experience with other institutions to suggest possible solutions to concerns raised at the summary meeting. Participants are encouraged to bring any animal care related concern to the attention of panel members during this meeting for discussion.

A subsequent in-depth report containing recommendations, prepared by the panel, is aimed at helping the institution to improve its animal care practices and facilities to a standard in keeping with the guidelines laid down by the CCAC. These reports are circulated to the members of the CCAC's Assessment Standing Committee (which reviews all assessment reports to ensure continuity) prior to being forwarded to the senior administrative official of the institution.

In response to the panel's report, the institution is required to submit to the CCAC within 6 months, a report describing how it proposes to implement the report's recommendations. Should this implementation report be considered unsatisfactory, the CCAC may instruct its secretariat to determine the reasons for noncompliance and to take such further actions as deemed necessary. For example, the CCAC notifies the MRC and NSERC of any institution that is in noncompliance with CCAC standards and has not responded satisfactorily within the time given to correct the situation. NSERC and MRC hold powerful enforcement options. On receipt of a statement of noncompliance and after reviewing the full evidence, the granting agencies reserve the right, either separately or together, to bring their concerns to the appropriate authorities in the research institution and, if they deem it necessary, to implement such financial or other sanctions as may be in the power of either research council. Such sanctions may include the freezing or withdrawal of research funds.

APPLICABILITY

When carrying out assessments of the various animal facilities, assessment panels focus on five main areas:

1. *The functioning of the animal care committee.* In particular, assessment panels look for appropriate membership, the quality of the ACC's documentation, the interaction with animal care personnel and investigators, and the ethical review process for scientific protocols. Animal care committees are also required to make sure that standard operating procedures are developed for routine techniques, and to put in place a crisis management program to cope with situations such as fire, electrical failure, and threats to facilities.
2. *The animal holding facilities.* An assessment is made of the state of the infrastructure and its maintenance, and also of the day-to-day operational management of the facilities.
3. *The animal care and management practice.* This includes examining the state, size, and appropriateness of the caging; the cleaning and feeding schedules; and the provision of environmental enrichment.
4. *Veterinary practices.* In particular assessments are made of the use of anesthetics and analgesics; effectiveness of health monitoring programs; and interaction between the animal care staff, the veterinarian, and the investigator.
5. *The provision of continuing education and training for animal care staff and investigators.*

The CCAC assessment program is aimed at the institutional level. Failure to comply with the requirements of the CCAC can result in the institution being placed in a state of "noncompliance" if severe deficiencies are found in any of the above categories. Potentially, an investigator who re-

fuses to comply with CCAC guidelines could jeopardize the status of the institution.

ADMINISTRATIVE METHODS AND THEIR COSTS

Institutional ACCs were introduced in 1968 to administer the CCAC program at the local level, and are now embodied in American legislation and have been introduced in other countries. The ACC must function under terms of reference that describe its membership (at least one person must represent the community's interests and concerns), its authority (the ACC must be able to terminate any procedure if it considers that unnecessary pain is being experienced by the animal), its responsibilities, and its meetings (such as for protocol review and site visits).

At the local level the program cost is defrayed by the institution. In some instances, institutions charge a per diem for housing animals. This money, drawn from the investigators' budgets, covers animal care costs, including space, veterinary care, and other animal care items.

STRENGTHS AND WEAKNESSES

The CCAC program has helped to improve animal care in Canada since its inception 25 years ago. The program unites researchers with animal welfare societies in the common goal of promoting the concept of the 3Rs of Russell and Burch (1959). As the concepts of replacement, refinement, and reduction of the scientific use of animals are difficult to encourage through legislation, the CCAC program succeeds where the legislative approach fails. ACCs are mandated in their terms of reference to question all aspects of animal use, including whether animals should be used, whether protocols can be refined to minimize suffering, whether the number of animals used can be reduced, and whether there are any viable alternatives to the use of animals.

One of the most powerful attributes of the CCAC program, which is not evident in legislative strategies, is the in-depth review of all research protocols as part of the assessment process. Assessment panels not only examine every protocol, but they also evaluate the institutional process that led to protocol approval. Lack of, or the ineffective operation of such a process leads to a critical recommendation in the assessment report.

Another important aspect of the CCAC program is the promotion of the social and behavioral welfare requirements of animals in institutions. Through its newsletter, conferences, workshops, and discussions during assessment visits, the CCAC promotes the improvement of animal care through environmental enrichment. This field is constantly changing, and the CCAC disseminates information among institutions as researchers, technicians, and veterinarians all find new ways to improve the daily lives of animals. These types of improvements in animal care would be very difficult to achieve through legislation.

Through its program of peer review, the CCAC is able to identify the successes or failures of an institution's animal care program. Whether weaknesses are due to the administration's failure to allocate adequate funding, a lack of veterinary care, or the improper functioning of an animal care committee, the CCAC assessment panel is able to target a deficient area in its report.

Although assessment panels are generally considered to be one of the programs strongest assets, they can also be considered to be one of the program's weaknesses. Panel members are volunteers, and as such must be drawn from a large pool of potential candidates. The size of the pool allows for inconsistencies between reports. To overcome this, the CCAC Assessment Standing Committee reviews all reports so that oversights can be corrected, inconsistencies eliminated, and recommendations strengthened. To ensure continuity, one of two CCAC staff laboratory animal veterinarians participates in each assessment.

Another drawback to using volunteers on the assessment panel is that members must fit their CCAC duties into otherwise busy schedules, which can delay the production of final reports. To avoid delay in implementation of important animal care issues, a summary of the most serious recommendations is sent to the institution prior to completion of the assessment report.

THE FUTURE

Through continual contact with the animal welfare community and member institutions, the CCAC system will continue to evolve. Initially, emphasis was placed on animal research facilities and infrastructure deficiencies. As new facilities are built and older ones improved, the emphasis has shifted to ensuring that the ACC is strong and functional. Future directions will include further emphasis on enrichment strategies, alternatives, and education.

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Japan

Tatsuji Nomura

OVERSIGHT

Animal protection in Japan is based more on ethical codes than on laws and regulations. The laws and regulations that do exist are not enforced by strict punitive measures as are the laws in most Western countries.

The Prime Minister's Office is the competent authority for animal protection laws and regulations in Japan. Japanese animal care and use legislation consists of the Law for the Protection and Management of Animals in 1973 (Law No. 105, October 1, 1973; hereinafter referred to as "the animal protection law") (Law for the Protection and Management of Animals, 1982) and the Standards Relating to the Care and Management of Experimental Animals (Notice No. 6 of the Prime Minister's office, March 27, 1980; hereinafter referred to as "the experimental animal standards") (Standards Relating to the Care and Management of Experimental Animals, 1982). Both the animal protection law and the experimental animal standards apply to all universities and other national and private research institutions, even though institutions may be under the jurisdiction of various government agencies. Japan has no other national or local laws or regulations related to animal care and use. Since the enactment of the animal protection law the Prime Minister's Office has made efforts to educate the public about the moral importance of animal protection.

The standards call for humane handling of laboratory animals during rearing, transport, and experimental procedures, as well as at the completion of experiments. Consideration is also given to conservation of the environment. Animals must be disposed of painlessly after the experiment is completed.

In 1987, the Ministry of Education, Science, and Culture issued a notification called "Animal Experimentation in Universities" (Notification No. 141 of the Science and International Affairs Bureau, Ministry of Education, Science, and Culture, May 25, 1987; hereinafter referred to as "the ministry notification") (Science Council of Japan, 1981) to the deans of national, public, and private universities throughout Japan. This ministry notification, although not legally binding, is followed by the majority of Japanese universities. Among other measures, it calls for the establishment of an animal experimentation committee by the dean of the university. As a result, all medical schools in Japan have animal experimentation committees, and research protocols involving animals are reviewed in some manner in more than 80 percent of medical schools.

The Ministry of Education, Science, and Culture prepared the ministry notification after carefully deliberating on a 1980 recommendation submitted to the Prime Minister by the Science Council of Japan, to establish animal experimentation guidelines. In connection with the Science Council of Japan and the Ministry of Education, Science, and Culture, the Japanese Association for Laboratory Animal Science (JALAS) published its own "Guidelines on Animal Experimentation" in 1987 (JALAS, 1987). These guidelines are used by various institutions for reference.

ENFORCEMENT

The animal protection law specifies that "any person who cruelly treats or who abandons a protected animal shall be liable to a fine of not more than 30,000 yen [approximately U.S. $360]." The experimental animal standards have no clear punitive measures. The laws and regulations are not aggressively enforced because animal protection in Japan is not based on legalism and the policy of the government is to promote animal protection through educational activities. However, if researchers do not follow the animal experimentation guidelines of their respective institutions, they are generally issued a severe warning or admonition by the animal experimentation committee. In extreme cases, the director may order research to be suspended. This is part of the "administrative guidance" system in Japan, which, although not legally binding, carries great weight in universities and other institutions with close government connections.

FUNDING

Because the animal protection law has no severe punitive measures or inspection systems, no particular funding is required for its administration.

APPLICABILITY

The experimental animal standards insist that humane animal care is the responsibility of managers of laboratory animal facilities, laboratory animal caretakers, and researchers.

ADMINISTRATIVE METHODS AND THEIR COSTS

As there is no formal requirement in Japanese law for reviewing animal experiments, there is very little administration required. However, the use of animal experimentation committees, as recommended by the ministry notification, has become widespread. The role of the animal experimen-

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tation committee is to provide guidance and advice so that animal experiments are performed in accordance with the respective guidelines. The committee must make important decisions concerning the scientific validity and adequacy of animal care in connection with particular protocols. Therefore, the members include several experts in laboratory animal science such as veterinarians, researchers with a wide range of experience in performing animal experiments, and others who are able to make decisions on conformity with related laws and regulations and who are knowledgeable in matters related to ethics and animal welfare. The members are usually appointed by the dean of the university or director of the institution, who bears final responsibility in all matters. Animal experimentation committees are generally made up of individuals from the institution concerned, and the meetings are not open to the public. Investigators must receive approval from the committee in order to conduct animal research.

There are no provisions in Japanese laws or regulations concerning who should pay for costs incurred from meeting the experimental animal standards. The costs concerned appear to be covered by the operating expenses of research institutions.

STRENGTHS AND WEAKNESSES

When viewed by the West, Japanese animal protection laws and regulations appear to contain many loopholes. Details of compliance are left up to the good judgment of the researchers, there are essentially no punitive measures, there is no formal approval system for animal researchers and animal experimentation facilities, and there is no formal system for reviewing animal experiment protocols. Japanese laws and regulations based on researchers' good will have been criticized by animal rights groups, animal welfare groups, and the media. There have also been demands that the laws and regulations be revised.

Japanese society, however, appears to support the level of protection afforded by the current laws and regulations, and it is a good system for ordinary researchers. However, many people connected with the management and operation of laboratory animal facilities would like to see the responsibilities of the heads of research institutions clarified concerning laboratory animal welfare, as well as the inclusion of stricter punitive measures and budgetary provisions.

Researchers in Japan tend to adopt the concept of the 3 Rs of reduction, refinement, and replacement of laboratory animals (Russell and Burch, 1959). At the same time social movements calling for animal rights and welfare are gradually becoming more active. Researchers, government officials, and executives are realizing that international harmonization of experimental animal welfare is becoming necessary. The animal protection law and the experimental animal standards will begin to be revised in the near future and will likely be similar in content to the ministry notification.

CONCLUSION

Japanese laws and regulations are basically ethical codes that rely on the good sense of the researchers and do not contain any strict punitive measures such as those seen in Western countries. While there are many details in the current system in Japan that should be improved, the basic policy as a whole is supported by the general public and functions well as it stands. Japan has a long tradition of animal protection based on the Buddhist teaching against senseless killing as part of its ethical system. Japanese people act according to this fundamental concept of personal morality with little need for laws or regulations. Although modern Japanese society is not known for its religious conviction, each year the biomedical faculties of universities and employees of research institutes that perform animal experiments hold a memorial service for the spirits of the animals sacrificed for biomedical research. This illustrates the widespread influence of Buddhist ethical concepts in Japanese society.

Because of this way of thinking, Japanese people generally accept the importance of living things but also recognize that in some cases, they must be sacrificed. It is not in the Japanese people's character to loudly proclaim their agreement or disagreement. This is perhaps one reason why the animal welfare movement has become vociferous and even extreme in Western countries but has always remained rather subdued in Japan.

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New Zealand

C.S.W. Reid

INTRODUCTION

All use of live animals in New Zealand for the purposes of research, testing, production of biological agents, or teaching must comply with a code of ethical conduct. The basic contents of the code, which is specific to an institute (such as a university, polytechnic, research organization, or industrial company) are prescribed by the Animals Protection Amendment Act 1983 and its Regulations. Codes must be approved by the Minister of Agriculture, who is advised by the National Animal Ethics Advisory Committee. The code is administered by an Institutional Animal Ethics Committee (IAEC) of 6-8 people including three members who are not affiliated with the institute. The IAEC is appointed by the chief executive of the institute, who is responsible to the Minister. Proposals for research are examined in detail by the IAEC, which has the authority mandated by the chief executive to decide whether to accept the project, request modification, or reject it. The system demands that the researcher give careful consideration to the ethical justification for the work proposed, and ensures that both the researcher and the institute are clearly accountable for the work undertaken. The law does not prescribe how the code will be put into practice, nor does it call for licensing of premises, researchers, or projects. A cumbersome and expensive bureaucracy is thus avoided.

OVERSIGHT

Animals play an important part in the economy and culture of New Zealand. Animal industries (meat, dairy, wool, and fish) employ large numbers of the population and earn approximately half of New Zealand's export income. The people of New Zealand use animals in a variety of ways, including as companion animals (particularly dogs, cats, and cage birds) and as working animals (dogs and horses). They also hunt; fish for trout and salmon; race horses; and participate in show jumping and eventing, agricultural and pastoral shows, aquaria, zoos, and rodeos. A number of introduced vertebrates have become pests; the Australian brush-tailed possum (*Trichosurus vulpecula*) and the rabbit are among the most damaging.

The principal areas of research in New Zealand that involve live animals include animal production, biomedical and veterinary research, basic biology, conservation (especially of native birds), pest control, and testing for natural toxins in food for human consumption.

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In all these interactions between humans and animals, the animals are protected by an umbrella law, the Animals Protection Act 1960 and its amendments.

The main purpose of the 1960 Act is to prevent cruelty to animals. However, in the case of laboratory animals, the original Act was seriously deficient: it exempted "Any research or experimental work carried out on an animal by any bona fide research worker," and it did not bind the Crown, the largest employer of scientists. A watershed amendment—the Animals Protection Amendment Act 1983—closed those gaps and laid down the principle that all manipulations of live animals for the purposes of research; experimental, diagnostic, toxicity, and potency testing; the production of antisera or other biological agents; or teaching, must be carried out in accordance with a code of ethical conduct relating to the welfare and humane treatment of the live animal involved. The definition of "manipulation" and the details of the legal requirements are set out in the Animals Protection (Codes of Ethical Conduct) Regulations 1987 (Table 1). A matter currently under review is the definition of animal. The original Act covered the common land mammals of the day, marine mammals, and birds. The present Act now covers all vertebrates kept in a state of captivity or dependent on humans for their care and sustenance (Animals Protection Amendment Act 1987). Invertebrates are not protected.

The change in 1983 was brought about largely by the advocacy of the animal and medical scientists, who recognized the need for revised legislation. An important coordinating role was played by The Royal Society of New Zealand, which facilitated the development of proposals and communicated these to the Minister of Science (Reid, 1990).

A seminal outcome of the 1983 amendment to the Act was the establishment of the National Animal Ethics Advisory Committee (NAEAC), which has the task of advising the Minister of Agriculture (who has administrative responsibility for the Act) on the acceptability of proposed codes, as well as other matters relating to codes and ethics of animal use. The present composition of NAEAC is given in Table 2.

The 1983 Amendment and the 1987 Regulations legislate only for the use of codes of ethical conduct and their content. They do not prescribe how the codes are to be implemented. Some institutes in 1983 already had codes of ethical conduct administered by an Institutional Animal Ethics Committee (IAEC). NAEAC adopted that model and developed a set of guidelines based on the IAEC, including recommendations as to the composition of the committee, the details of the institutional code, and how the committee should operate (NAEAC, 1988). The members of the IAEC, usually 6-8 in number, are appointed by the chief executive of the institute and include three people not affiliated with

TABLE 1 Extracts from the Animals Protection (Codes of Ethical Conduct) Regulations 1987

Manipulation

"'Manipulation' in relation to any live animal, means interfering with the normal physiological, behavioural, or anatomical integrity of the animal by deliberately

(a) Exposing it to any parasite, micro-organism, drug, chemical, biological product, radiation, electrical stimulation, or environmental condition;
(b) Subjecting it to enforced activity, unusual restraint, or surgical intervention;
(c) Depriving it of usual care;

but does not include any therapy or prophylaxis necessary or desirable for the welfare of the animal"

(Section 2)

Matters to be included in codes of ethical conduct

"Every code of ethical conduct which relates to the welfare and humane treatment of any live animals that are manipulated in any research, experimental, diagnosis, toxicity, or potency testing work, or are used in teaching involving the manipulation of live animals shall.....make specific reference to the following matters:

(a) The measures to be taken to ensure that alternatives to the manipulation of animals are used whenever possible;
(b) The factors to be considered when determining whether (i) the work is likely to result in the extension of the body of knowledge relevant to the health and welfare of humans or animals or the productivity of animals, or (ii) the teaching is a required part of an educational institution's curriculum;
(c) The factors to be considered in choosing an appropriate animal species;
(d) The procedures to be adopted in formulating, approving, and implementing manipulation projects so as to minimise the numbers of animals manipulated in order that no more animals are used than are necessary to ensure unequivocal interpretation of the findings;
(e) The measures to be taken to ensure that the procedures for the obtaining of animals for manipulation are such that they ensure the welfare and humane treatment of the animals;
(f) The responsibilities of the persons undertaking, supervising, and responsible for manipulation and selection of animals, and their care and disposal;
(g) The measures to be taken to ensure the general health and welfare of the animals before, during and after manipulation;
(h) The measures to be taken to minimise any pain or distress caused to live animals manipulated, including the abandonment of manipulation at any stage and the immediate humane destruction of animals where pain and distress cannot be held within reasonable levels;
(i) A requirement that all Acts of Parliament, regulations, and bylaws relating to the obtaining, holding, possession, care, and treatment of animals are complied with;
(j) The measures to be taken within the organisation or body by which the work or teaching is carried out to ensure compliance with the code of ethical conduct."

(Section 4)

TABLE 2 Composition of NAEAC and AWAC, 1994

National Animal Ethics Advisory Committee	Animal Welfare Advisory Committee
Independent chairperson†	Independent chairperson‡
Nominees of: NZ Local Government Association (1*) (Lay person) Chief Veterinary Officer, MAF (1) Royal New Zealand Society for the Prevention of Cruelty to Animals (2) New Zealand Veterinary Association (2) The Royal Society of New Zealand (2) Health Research Council (1) School Trustees' Association (1) (Source : NAEAC, 1994)	Nominees (1 each) of: Chief Veterinary Officer, MAF Australian and New Zealand Federation of Animal Societies Federated Farmers of New Zealand New Zealand Veterinary Association The Royal New Zealand Society for the Prevention of Cruelty to Animals Animal behaviorist NAEAC Chairperson (ex officio) (Source : AWAC, 1995)

*Number of nominees

†Currently a senior Wellington barrister retired from the Crown Law Office. Previous chairpersons were a former Commissioner for the Environment, and a former Dean of the Faculty of Veterinary Science, Massey University.

‡Currently a former Dean of the Faculty of Veterinary Science, Massey University. Previously, a former Professor of Veterinary and Public Health, Massey University.

the institute (the external members): a lay person, currently nominated by the New Zealand Local Government Association; a veterinarian nominated by the New Zealand Veterinary Association; and a nominee of a nationally recognized animal welfare group, usually the Royal New Zealand Society for the Prevention of Cruelty to Animals.

Initially, NAEAC made no attempt to develop a national code. Rather, institutes were—and still are—encouraged to write their own code, incorporating the items required by law, some features imposed by NAEAC (such as the establishment of an IAEC and the inclusion of the external members), and matters specific to the individual institute. The intention is to reinforce the IEAC's mandate to operate the institute's code and, particularly, its responsibility to make its own decisions. All proposed codes are inspected by NAEAC, which may make suggestions for their improvement. NAEAC may discuss with IAECs particular difficulties they encounter in their decisions, but it is not NAEAC's function to be a "higher court." There is now a move towards establishing a New Zealand Code and the possibility of a joint New Zealand-Australian code is being explored (Bayvel, 1993). At present there are 35 IAECs in New Zealand and 63 Codes of Ethical Conduct (some IAECs supervise more than one code), dealing with an annual average of approximately 280,000 live animals (Table 3).

The 1987 Regulations also require a variety of statistics (species of animals, number used, their sources, and how they were disposed of), which are to be supplied to the Director General of the Ministry of Agriculture on request. Statistics are now collected annually and NAEAC interprets them and provides comment. This has led NAEAC to rationalize the set of statistics being requested and to develop a standard return form. A new statistic sought is data relevant to the degree of impact that manipulations or procedures have had on the welfare of animals subjected to them. NAEAC has therefore been evolving a scale of impact (Reid and Mellor, 1993; Mellor and Reid, 1994), which is expected to be in place in 1995. The scale will also help in predicting the impact of a project, which is required when seeking approval for the work.

Unlike the systems used in many other countries, the Act does not call for licensing of institutes, premises, locations, researchers, or their projects. The linchpin of the New Zealand system is the code; the IAEC makes it effectual. While the main task of the IAEC is to deliberate on proposals, it also serves an important educational function. It pro-

TABLE 3 "Experimental" Animal usage 1990-1994 [1,2,3]

Species	1990	1991	1992	1993
Amphibians	2,937	2,531	484	2,606
Birds	9,488	6,874	9,331	12,721
Cats	408	411	274	579
Cattle	51,637	66,821	60,888	66,055
Deer	3,244	2,265	2,782	1,723
Dogs	1,369	772	612	514
Fish, fish eggs	6,229	5,312	5,485	16,252
Goats	4,662	3,606	2,330	2,598
Guinea pigs	5,215	3,577	2,506	2,824
Hamsters	1,924	1,237	1,391	824
Horses/donkeys	4,417	6,276	973	612
Marine mammals	417	1,483	2,031	1,698
Mice	78,380	41,294	62,535	110,445
Mustelids	57	309	213	734
Pigs	260	241	454	445
Possum	1,114	2,524	2,869	2,660
Primates	0	0	12	0
Rabbits	3,479	2,050	2,168	2,576
Rats	20,040	22,763	9,638	18,660
Reptiles	1,748	3,052	3,420	1,980
Sheep	63,378	103,289	109,467	44,954
Miscellaneous species	375	763	240	331
TOTALS	260,778	277,450	280,103	291,801

[1]Sources: 1990-92, NAEAC (1994); 1993, MAF (Bayvel, pers.com.).

[2]Animals used in research, experiment, diagnosis, toxicity testing, potency testing work, the production of antisera or other biological agents, or teaching.

[3]No distinction made in terms of degree of impact of procedures or manipulations on the animals, or purpose of use.

motes consideration for the welfare of animals, disseminates information about the regulations, encourages respect for their spirit, and it advises animal users as to how they may pursue their activities both humanely and effectively. The Act, the letter of the law, is the backstop protecting the animals from ill-treatment or cruelty.

In 1988 a second ministerial advisory committee was established, the Animal Welfare Advisory Committee (AWAC). Its overall task is "to advise the Minister of Agriculture on all matters relating to animal welfare other than those which fall within the jurisdiction of the National Animal Ethics Advisory Committee" (AWAC, 1994). Specific tasks include review of the Animals Protection Act, drawing up or revising codes of recommendations and minimum standards for the welfare of particular classes of animals, and recommending specific areas where research into animal welfare matters is required. A code for the care and use of animals for scientific purposes has now been published (AWAC, 1995); it does not deal with ethical aspects of their use, which is the concern of NAEAC. Codes published by AWAC so far are listed in Table 4. The present composition of the committee is given in Table 2.

TABLE 4 Codes of Recommendation and Minimum Standards Published by the Animal Welfare Advisory Committee[1]

For the Welfare of	Code Number
Circus Animals	01
Sea Transport of Sheep from New Zealand	02
Sheep	03
Dairy Cattle	04
Deer during Removal of Antlers	05
Animals used in Rodeo Events	06
Horses	07
Bobby Calves[2]	08
Animals in Boarding Establishments	09
Slaughter	10
Sale of Companion Animals	11
Farm Animals[3]	12
Pigs	13
Exhibit Animals	14
Animals Transported within New Zealand	15
Welfare of Animals in Saleyards	16
Care and Use of Animals for Scientific Purposes	17

[1]As of August 1993. Source: Ministry of Agriculture and Fisheries, Wellington, NZ from which copies may be obtained.

[2]A "bobby calf" is a calf that is at least 4 days old and is destined for slaughter for human consumption.

[3]Not a code: a general account of the implications of the Animals Protection act for those responsible for farm animals.

FUNDING

The costs associated with NAEAC—fees, travel, publications—are borne by the Ministry of Agriculture and Fisheries (MAF), which also subsidizes national meetings concerning animal welfare matters. The costs associated with the functioning of IAECs are borne by the institute as overheads.

ENFORCEMENT

Compliance with an institute's gazetted code of ethical conduct is required by law. If a researcher does not comply with the code he or she is committing an offense and is liable to prosecution under the Animals Protection Act. Penalties may be fines or, in cases of flagrant cruelty, imprisonment and possibly denial of the right to own animals. The levels of fines have been reviewed recently and increased 5-fold. The researcher may, of course, suffer penalties imposed by the institute.

Although the burden of penalty is borne in the first place by the individual researcher, the institute could be called to task by the Minister who could consider revoking the institute's code, effectively barring it from further work with live animals. This has not happened in the 8 years the Regulations have been in operation. It seems unlikely that the transgression of a single individual would result in curtailing of an institute's activities.

APPLICABILITY

Overall responsibility to administer the Animals Protection Act and its Regulations lies with the Minister of Agriculture. The minister inspects each proposed code of conduct that NAEAC presents on behalf of institutes. If it is accepted, the minister's approval is noted in the government Gazette, from which time the code has standing in law. The minister appoints the members of NAEAC, may declare a particular animal species to be protected under the Act, may call for investigation of any problem related to animal use, and may revoke a code.

Responsibility for the welfare of live animals used for the purposes listed in the Regulations is shared by the researcher and the institute with which the researcher is associated. The system is based on the principle of self-regulation at both levels.

ADMINISTRATIVE METHODS AND THEIR COST

Each institute is responsible to the minister through its chief executive. In the case of universities the Vice-Chancellor is the responsible party. The chief executive appoints the members of the IAEC and normally delegates the authority to administer the institute's code to the chairperson. The ultimate responsibility for the performance of the IAEC and for the activities of the institute that involve live animals, however, remains with the chief executive.

The IAEC has supervisory responsibilities during the

execution of a project. It may stipulate progress reports, including a final report on the actual as compared with the predicted welfare of the animals during the project. All codes of ethical conduct empower the IAEC to inspect the animals at any time and to order a project to be stopped if the welfare of the animals is considered to be compromised beyond what was expected at the time the project was approved. The IAEC has the power to order distressed animals to be euthanized.

The researcher is ethically responsible as an individual for any experiment he or she conducts on a live animal. Discharging that responsibility involves making a careful estimate of the impact the experiment is likely to have on the animal, minimizing the severity of the experiment, and maintaining a continuing concern for all aspects of the welfare of the animal throughout the period it is in the researcher's care. As well, the researcher is responsible for ensuring that others involved in the experiment have the appropriate skills, which may involve training of inexperienced staff.

While supervision is primarily the responsibility of the IAEC, others have the legal power to inspect premises when there is good reason to believe animal suffering may be occurring. These include veterinarians and livestock officers of the Ministry of Agriculture and Fisheries, police officers, and warranted inspectors of the Royal New Zealand Society for the Prevention of Cruelty to Animals.

Submission to IAEC

The proposer of a project involving live animals (which could involve carrying out specified research or teaching, or pursuing a specified commercial purpose) must submit the proposal in writing to the IAEC. It is important that the submission be written in terms understandable to lay people. For a research project, the proposer has to provide the following information:

- the title of the project and a clear statement of its objective;
- the value of the proposed work, that is, the expected benefits;
- explanation of why there is no practical alternative to using live animals;
- details of the proposal—experimental design, dates, methods, species and number of animals to be used and, where appropriate, statistical evidence indicating that there will be enough animals to provide analyzable results;
- the likely impact on the welfare of the animals and a cost-benefit analysis;
- a statement that the protocol is consistent with the 3 Rs (replacement, refinement, reduction) (Russell and Burch, 1959), including the methods chosen to minimize any pain or distress (Marbrook et al., 1994);
- the source of the animals and their history;
- details of the husbandry of the animals—accommodation, diet, and non-experimental treatments such as routine weighing and dosing with anthelminthics;
- how the animals will be disposed of at the conclusion of the protocol; and
- personnel involved, their qualifications, and who has overall responsibility for the project.

The IAEC must satisfy itself that the information given is adequate and correct. It may ask for more information, seek expert help, and inspect facilities as it sees fit. The IAEC must then decide whether to accept the proposal in full, request that it be modified (possibly recommending that a pilot experiment be carried out), or refuse it. That decision will be based primarily on the balance between the ethical cost to the animal and the benefit expected to be obtained from the research. No distinction is drawn between biomedical research and veterinary research or any other usage that meets the cost-benefit test.

STRENGTHS AND WEAKNESSES

The New Zealand system is established and now accepted by all animal users. Improvements continue to be made, which is a process that involves both NAEAC and the IAECs.

The main strengths of the New Zealand system are:

- It is based on trust in the integrity of both the IAEC and the researcher.
- Institutional ownership of its own code reinforces the institute's commitment to the spirit and intent of the legislation.
- The presence on the IAEC of the three non-institutional members allows a range of perspectives to contribute to the committee's decisions and reduces the possibility of the committee becoming a rubber stamp for the institute.
- The researcher is personally responsible for the welfare of the animals used in the project, starting from when they are acquired and continuing until the researcher disposes of them. Including the cost-benefit analysis in the proposal effectively commits the researcher to this responsibility.
- Decision and control are close to the researcher, not in some distant central bureaucracy. New Zealand has avoided the creation of a cumbersome and expensive inspectorial apparatus.
- The code is concerned with performance standards, not engineering standards.
- The IAECs are given support by NAEAC in the form of national guidelines, dissemination of information and ideas, and meetings to discuss problems and solutions.
- NAEAC has direct access to the minister responsible for administering the Animals Protection Act.

As a self-regulating system, it is vulnerable to changes in public opinion based on real or imaginary failure to perform. Vigilance on the part of the IAEC and the experimenter, honest reporting to the public, and willingness to enter into dialogue with critics are all ways that help garner the public trust. The three external members of the IAEC play an important part in judging public reaction to a proposal.

Influence of the System on Animal Research

The total number of animals used annually for research, teaching, and the other purposes listed in the regulations has increased by 12 percent over the past 4 years (Table 3). There are also changes in the numbers of individual species and the relative proportions of different species used in that period. Too little information is available, however, to analyze the causes. There is no national record yet of how the animals were used, nor of the impact the procedures or manipulations had on the animals. It is to rectify these deficiencies that NAEAC seeks to rationalize and improve the collection of statistics.

The system devised over the past 10 years has had some very positive effects. Awareness of ethical and welfare considerations has been heightened. This has increased interest in finding ways to reduce the impact of procedures and manipulations on animals, particularly those that are potentially more severe. Animal housing and animal care have improved. Finally, greater effort is being put into the planning of projects, to optimize the efficiency and effectiveness of animal use.

It is known that the number of live animals used in teaching at all levels has fallen. There is no objective evidence that the system is obstructing worthwhile biomedical, veterinary, or production animal research. Insufficient funding is a much greater constraint to animal research in New Zealand than is the animal welfare legislation.

FUTURE

New developments in animal welfare in New Zealand include:

- *The association with Australia through partnership in the Australian and New Zealand Committee for the Care of Animals in Research and Teaching (ANZCCART).* ANZCCART promotes sharing of animal welfare information between the two countries, identification of common goals, and cooperation to achieve them. It further promotes informed discussion and debate on both ethical and practical aspects of animal welfare among animal scientists, other scientists, the public, and politicians, and it organizes meetings for that purpose. A substantial newsletter, *ANZCCART News*, is published as well as proceedings of meetings and specific information, such as sources of particular strains of experimental animals.
- *The appointment of the first Professor of Animal Welfare Science in New Zealand to the Massey University Faculty of Veterinary Science.* This both consolidates and expands teaching of animal welfare science in the University, and provides a focus for research on the improvement of the welfare of animals. The professor's tasks include educating the public and countering the growing gap between city dwellers and livestock farmers.
- *The formation of the New Zealand Foundation for the Study of the Welfare of Whales.* The aim of the Foundation is to prevent or reduce suffering of whales and dolphins in natural disasters such as mass strandings, or through human activities, such as whaling. No whaling is carried out in New Zealand waters, but as many as 400 animals have been stranded on New Zealand beaches in a single year.

Developments in the near future are expected to include:

- A new Animal Welfare Act based on a duty of care towards animals as well as prevention of cruelty (Bayvel, 1992);
- Better reporting of animal usage;
- Further improvement in the assessment of the impact that projects have on live animals;
- Establishment of a system of reviewing the performance of IAECs; and
- Better linking with international animal welfare information databases.

In the longer term, extending animal welfare science courses to non-veterinarians—companion animal groups, farmers, breeders, stockhandlers, inspectors, and others—will foster an understanding of animal welfare and an increased sensitivity to animals' needs and how they can be met.

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United Kingdom

Paul Townsend and David B. Morton

OVERSIGHT

The United Kingdom enacted a national law in 1986, the Animals (Scientific Procedures) Act (A(SP)A), which put into legislation the European Directive 86/609/EEC. The 1986 Act replaced the earlier Cruelty to Animals Act, which had been in place since 1876. There are no derogations for government or industry except for the very small number of veterinary field trials conducted under an Animal Test Certificate authorized by the Medicines Act, 1968. Anyone carrying out animal research outside the 1986 Act may be prosecuted under other animal protection legislation (such as for causing unnecessary suffering under the Protection of Animals Act 1911 [1912, Scotland]). There is no other state or local legislation pertaining directly to animal research. The Home Office is the ministry responsible for this Act and more generally for law and order issues nationally. It equates broadly to the U.S. Department of Justice or to the Ministry of the Interior in other countries.

The scope of the Act is broad. It protects all living vertebrates, including their free-living immature forms and embryos more than 50 percent of the way through gestation, when used for scientific purposes that may cause the animal pain, distress, suffering, or lasting harm. The Act has recently been extended to cover *Octopus vulgaris*. Perhaps of particular note, it protects genetic mutants that have defects that may potentially compromise their welfare and all transgenic animals until two successive generations have shown the transgene to have no significant detrimental effect.

The Act controls animal research in four main ways (1) by a system of certification and licensing, (2) at the institutional level, (3) by a group of national inspectors, and (4) by a national committee. Each of these is described in more detail below.

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Certification and Licensing

A Certificate of Designation applies to premises (including animal facilities and animal laboratories) deemed to be suitable either for the performance of scientific procedures (such as surgical procedures and the breeding of mutants), or for the breeding and supply of animals for research, or a combination of these. It is granted by the Secretary of State at the Home Office. The certificate is granted in the name of a senior individual at the institution (such as the vice-chancellor, secretary to the university, managing director of a company, or a board member).

A project license is given for a program of research to a named individual, normally a senior researcher, by the Secretary of State. A project license may consist of several "protocols," which are called "procedures" in U.K. jargon. Each procedure forms part of the overall experimental plan (the project) and may consist of a number of different "techniques" applied to the animals, such as anesthesia, blood sampling, and dosing. Different procedures within a project license are usually aimed at investigating different parts of the overall question for which the project license has been granted. Procedures are also classified by the degree of suffering incurred by animals undergoing them. A procedure may be given a severity limit of "mild," "moderate," or substantial." The endpoints for each procedure are dependent on the severity limit; the more severe a procedure the greater the degree of morbidity allowed. Thus within a project there may be procedures of all three severities. Animals must not exceed the severity limit for the procedure in which they are being used and it is the responsibility of the personal and project license holders to prevent this from occurring. In addition the project as a whole receives an overall severity band which is assessed from the severity limits of the procedures and the number of animals likely to reach the limit in each procedure. In the application, it has to be shown that the harms done to the animals are, in the opinion of the Secretary of State, balanced by the potential benefits of the research (that is a harm-benefit analysis is considered) and consequently, the license is limited to that scientific purpose and specifies fairly precisely what can be carried out.

Finally, there is the personal license, which aims to en-

sure adequate education and training of the investigator, and, through supervision, eventual competence in specific techniques. The actual license tightly defines the scientific techniques the investigator is authorized to perform, which are delineated by species and by the use (or not) of anesthesia. The personal licensee is ultimately responsible for the animals he or she is using and can never work without the authority of a project license.

The Institutional Level

The individual who holds the Certificate of Designation (the Certificate Holder) is responsible for ensuring compliance and facilitating implementation of the requirements of the 1986 Act. The Certificate Holder must appoint a Named Veterinary Surgeon (NVS) and a Named Day-to-Day Care Person (NDDCP) to help ensure the well-being of protected animals at the institution (there may be more than one NDDCP according to the size and layout of the institution). The NVS should provide advice on animal health and welfare. The NDDCP (who is normally a senior animal technician or caretaker) is responsible for the husbandry and care during experimental procedures of all protected animals on the premises. In essence, both the NVS and NDDCP act as advocates for the animals.

National Inspectorate

A cadre of Home Office inspectors, who must hold either medical or veterinary qualifications, provide a third level of control. They are appointed by the Secretary of State, and at the end of 1994 there were 20 such inspectors. Their number is determined by the government and is set by the U.K. Treasury in negotiation with the Home Office. Remuneration of the inspectors is based on national pay scales for government employees. There are two women and all inspectors are white apart from one Asian male. The role of the inspector was described in the Report of the Animal Procedures Committee for 1992 (HMSO, 1993). It has three major components and each is reviewed below.

1. *"To consider in detail applications for licenses and advise the Home Secretary how to ensure that only properly justified work is carried out."* In U.S. terms, this means that the inspector is a one-person institutional animal care and use committee (IACUC). The project license application form is similar in many respects to the typical protocol review form in use in many institutions in the United States. The U.K. Act requires the inspector to make the "ethical" harm-benefit analysis on the project and to review (and suggest modifications where appropriate) both the severity limits for individual scientific procedures within the research program specified in the project, and also the severity band of the project as a whole. A project license can be granted for a maximum of 5 years and is not normally subject to further review over that time, unless new cause for concern arises. However when any interim changes are sought, these must be assessed and formally approved by the Home Office. Note that unlike the United States and Canada, protocols do not normally have to be assessed and licensed by the Home Office before applying for funding.

2. *"To carry out visits...to establishments designated under the Act to ensure that its controls and the terms and conditions of licenses issued under it are being observed."* In 1993, inspectors were responsible for 345 designated establishments, at which there were 5,570 project licenses and about 16,800 personal licensees. In that year they made 2,507 visits for the purpose of inspection or assessment of research projects, or an average of just over seven visits per establishment (down from an average of eight in 1992). This is "oversight" in the literal sense, ensuring that license holders are complying with the specifics of both project and personal licenses and meeting their other responsibilities under the 1986 Act, such as keeping records and purchasing animals only from designated suppliers. As the Home Office also issues the Certificate of Designation for the establishment and has produced a "Code of Practice for the Housing and Care of Animals Used in Scientific Procedures" (the equivalent of the *Guide for the Care and Use of Laboratory Animals* (NRC, 1985), the inspectors are also responsible for approving the suitability of the facilities for their required purpose.

3. *"...to give advice and assistance to licensees and other personnel."* As the interpreters of the legislation and associated guidelines and codes, the inspector is required to give his or her opinion and approval in various circumstances regarding the A(SP)A.

National Committee

The fourth level of oversight is the Animal Procedures Committee, a statutory body that passes broad judgements on matters referred to it by the Secretary of State. It can also initiate its own enquiries and pass comments back to the Secretary of State. It has a small fund to support research related to the three Rs (reduction, refinement, and replacement of the use of animals in research) (Russell and Burch, 1959). At least two-thirds of the committee must be scientists, veterinarians, or medical doctors, and at least one member must have legal training. At least half the committee is required to be made up of people who have not been actively involved in scientific procedures using animals in the previous 6 years (that is, they cannot have held a license under the 1876 or 1986 Acts). The Act further states that "the Secretary of State shall have regard to the desirability of ensuring that the interests of animal welfare are adequately represented" but does not spell out further details such as the number of such persons, the overall proportion on the committee, or their background.

FUNDING

All the costs arising from the central administration of the A(SP)A are funded through the central government (through public taxation). However, the charge for certificates and licenses covers some of the administration costs including salaries and the research budget of the Animal Procedures Committee. At present the annual cost of designation for a scientific procedure establishment is £200 ($318), for a breeding and supplying establishment £400 ($636), and for a personal license £110 ($175). There is no charge for a project license.

ENFORCEMENT

Home Office inspectors periodically make unannounced visits to look for breaches of the Act; inspect records (records are required of numbers of animals used, adverse effects incurred during the research, and health of the animal colonies including any screening results and veterinary visits); make suggestions on the husbandry, care, and use of animals; carry out audits of particular procedures (such as those with high severity limits); and discuss the work and give advice to the personal licensees and project license holders.

Depending on their nature, breaches of the terms and conditions attached to a personal or project license can result in criminal penalties (imprisonment for not more than 2 years, a fine, or both) or in a wide range of administrative sanctions, which range from revocation of a license to letters of admonishment. Breaches are not reported in detail (we have no Freedom of Information Act as in the United States) but are summarized annually in the Report of the Animal Procedures Committee. Although 10 to 20 infringements are investigated by the Home Office annually, only one case has been prosecuted since 1987 (of an unauthorized rabbit dealer). It is likely that other "technical breaches" are noted and resolved by the institution and sometimes by individual inspectors without warranting a formal investigation. In such cases, the personal licensee and the project license holder would normally be disciplined; in extreme circumstances (one or two cases each year) licenses are revoked. While Certificate Holders may be indirectly responsible for such offenses on the basis of poor management, the Home Office has only admonished them so far and not yet removed one from office. Unlike personal and project licenses, breaches of the conditions attached to a Certificate of Designation are not criminal offenses.

There are no direct sanctions in regard to research funding for a breach of the Act as in other countries, although funding may be contingent on persons being licensed to carry out the work. If an investigator's license has been revoked then he or she will not be able to do the work, and the granting bodies may decide not to make an award. So far as we are aware this question is not directly addressed by the granting bodies. They are, of course, interested in whether there are already licenses in place to permit the work.

APPLICABILITY

The law applies to individuals within the institution with responsibilities under the Act, such as the Certificate Holder, project license holders, personal licensees, NDDCP, and NVS. The Home Office has refused Certificates of Designation on several occasions to institutions with facilities that did not meet the standards laid out in the Code of Practice (HMSO, 1989). On other occasions, it has prescribed a strict program for upgrading. In all cases Home Office approval is required for the proposed NVS and NDDCP in the application for a certificate.

The Certificate Holder is often a senior executive of the institution and is responsible for many aspects of implementing the Act, including those outlined above, and for compliance with the law (such as keeping species of animals in designated rooms; recording sources of animals; and identifying primates, dogs, and cats). The Certificate Holder must, among other requirements, ensure appropriate staffing, adequate care and accommodation as outlined in the relevant Code of Practice, adequate security (that is, preventing unwanted intrusion and animal escape), competence in killing animals according to a schedule of approved methods, and adequate training for researchers.

A project license is granted for a specific program of research work and is normally explicit in the numbers of animals and scientific procedures to be used. The applicant has to show that the experimental design is sound and the source of animals has to be approved (pound animals cannot be used). Because of the harm-benefit analysis, the license is restricted to its specified purpose; even if the procedures described could be used for another scientific purpose, that purpose would have to be approved in a separate license. Furthermore, the applicant must show that the 3 Rs (replacement, refinement, and reduction) have been addressed, by showing that there are no replacement alternatives, that the number of animals used has been reduced to the minimum, and that the scientific procedures have been refined so as to cause the least amount of suffering. Specific sections of the license deal with the potential benefits of the project as well the adverse effects, their recognition, alleviation, avoidance, and control (including endpoints). Each scientific procedure, which in some circumstances can be interpreted as each animal model, has an upper severity limit and action has to be taken if any animal exceeds that limit. In the U.K. there is a maximum limit to the suffering regardless of whether the scientific objective has been achieved such that any animal in severe pain or severe distress that cannot be alleviated must be killed. Some work may not be authorized on that basis regardless of the predicted benefit.

The Home Office has issued guidance on the conduct of specific procedures and techniques, such as the Draize test and the use of adjuvants, which must be adhered to by project license holders unless they make a scientific case for not doing so. Standard methods of humane killing are listed in Schedule 1 of the Act. If the only "technique" applied to a protected animal is killing it by one of the methods listed in

Schedule 1, then such use is outside the scope of the Act and does not require project license authorization by the Home Office (that is, it does not require protocol review). If animals used in experiments covered by the A(SP)A are to be killed by a method not on Schedule 1, such as by decapitation, then authority to use an alternative method must be sought and approved through a project license.

A second schedule to the 1986 Act lists species of animals that must be specifically bred for research; exceptions can be made but are not common. Animals that must be purpose-bred are rats, mice, hamsters (Golden), rabbits, guinea pigs, dogs, cats, primates, and quail (*Coturnix coturnix*). No restriction is placed on the source of other species.

It is a criminal offense for a project license holder to procure or knowingly permit anybody under his control to carry out a regulated procedure either not authorized by the project license or outside the authority of that individual's personal license. Other examples of more serious offenses with respect to project licenses include carrying out procedures in a non-designated place and using a neuromuscular blocking agent without authority. While it is not a criminal offense for a project license holder to procure or knowingly permit a person under his control to perform experiments carelessly or incompetently or to allow an animal to exceed the severity limit for a particular procedure and take no action, it would be an administrative breach.

For a personal licensee, criminal offenses include performing techniques not authorized by a project license, carrying out a technique not authorized on the personal license, using a species not covered in the license, unauthorized use of neuromuscular blocking agents (for example, in place of an anesthetic), public exhibition of animal research, working in a non-designated place, unauthorized re-use of an animal, or allowing an animal to suffer after the scientific objective has been achieved. Failing to comply with a Home Office requirement to immediately kill an animal that the inspector considers is undergoing excessive suffering would be a breach of the conditions of the personal license but not an offense.

The Named Persons (the NVS and NDDCP), while having broad-ranging responsibilities and a statutory duty to act if an animal gives rise to concern, have no statutory authority in what is really muddy water in terms of the definition of "concern." When a stock animal is suffering and is not the responsibility of any one individual, the Named Persons are free to act in the animal's best interests. But when an experimental animal gives rise to concern because of the techniques being used, the competence of the researcher, or the interpretation of the severity limit, difficulties can arise. Authority can be delegated at a local level by the Certificate Holder to stop an experiment or to kill an animal, but the Certificate Holder has no statutory authority, only that which stems from the management structure within the institution. Only the Home Office inspector has explicit statutory authority to require an animal to be killed.

The NVS has a statutory duty to provide advice on animal health and welfare. The Guidance (HMSO, 1990) delineates further duties, which include making visits to assess health and keep records; maintaining regular contact with the Certificate Holder; having a thorough knowledge of laboratory animal science (in the future it may be that only those veterinarians with appropriate qualifications will be acceptable to the Home Office for approval as an NVS); providing a comprehensive service (such as being on-call 24 hours a day); being familiar with the project licenses and their severity limits including adverse effects and endpoints; and providing advice on anesthesia, analgesia, euthanasia, surgical technique, and the recognition of adverse effects.

The NDDCP is required to help ensure the well-being of the animals along with the NVS. They have a duty to take action when any animal gives rise to concern, even independently of the scientist (for example, if the severity limit has been exceeded and the personal licensee is unavailable). They must also be familiar with and take steps to implement the standards set out in the Code of Practice; keep health records with the NVS as well as records of the environment, of animals coming into the establishment, and of disposal of animals; with their staff, check all animals daily; familiarize themselves with project licenses including severity limits, adverse effects, and humane endpoints; be able to contact personal licensees, the NVS, and the Certificate Holder; and implement the schedule of killing methods to ensure competent destruction of animals.

ADMINISTRATIVE METHODS AND THEIR COSTS

All costs of institutional administration are borne by the institution. How this is paid for depends very much on the establishment concerned, but U.K. universities are working towards devolving real costs down to users. Consequently, there are now moves to recover the costs of licensing and new equipment to meet the code of practice or best experimental standards, from grant-awarding bodies. The national research councils will pay most of the costs apart from premises but research charities are reluctant to do so; commissioned commercial research is usually on a real-cost basis. There are also significant costs to the institution in meeting the mandatory requirements for the training of personnel under the Act.

STRENGTHS AND WEAKNESSES

The U.K. law has created one of the world's strictest environments in which to carry out animal research, in practice as well as in theory. There are many safeguards for animals, and the system contains several checks and balances such as interdependent licensing of research programs and technical implementation; stringent conditions attached to the licenses and certificates; appointment of animals' advocates; assignment of managerial responsibility; requirement of inspec-

tion, monitoring, and record-keeping; restrictions relating to animal supply; and provision of specific guidance and codes of practice.

There is also considerable emphasis on training and competence of those carrying out the research both in manual skills and in implementation of the 3Rs of reduction, refinement, and replacement. For example, there is a mandatory requirement for training for all project and personal license holders.

The Home Office also issues occasional and useful guidance on what constitutes best practice for techniques such as raising antisera, the use of neuromuscular blocking agents, and Draize tests. It has limited funds to support research into refinement. The Home Office issues statistics on various aspects of animal research including the numbers used in research and the reasons for their use, which allows for informed debate.

Assessment of Harm

A further strength of the U.K. system is that it accepts that the use of animals in science does not occur in a moral vacuum, that there is considerable and justified public concern over the performance of procedures that cause animals to suffer in the name of science, and that there are limits to what scientists should be allowed to do to animals. For example, severe pain or severe distress (the upper severity band) is not permitted. This is really an extension of a harm-benefit analysis, which concludes that no justification can be made to cause severe pain or distress.

However, for a harm-benefit analysis to be carried out, assessment of the degree of harm is critical, and it is only after 7 years of working with this system that serious questions are being asked about what is meant by the "mild", "moderate," and "substantial" bands referred to as the severity limits in the A(SP)A. Little work on the assessment of these states has been carried out. It is quite reasonable to state that this concept has been used without any reasonable basis in objective fact. How do we know when a mouse, rat, or *Xenopus laevis* is suffering moderately, and do we have the scientific knowledge to make such a judgement? The degree of suffering is dependent upon many things, including the species, investigator competence, standard of care, and how a person's responsibilities are discharged in practice.

At a practical level a harm-benefit analysis also means that there are some things that we may wish to do to animals for scientific reasons that may not be justified, not because they are not scientifically valid questions but because the suffering caused is not outweighed by the value of the benefits gained (Bateson, 1986; Smith and Boyd, 1991). Thus a Home Office inspector is free to advise refusal of an application on the basis of it being ethically unacceptable, regardless of the degree of refinement that may have been applied to it. The "final" ethical decision does not simply reside in minimizing harms and maximizing benefits but in balancing the two parts. In practice this is very difficult to do.

Decision-making

The question also arises as to who should be involved in making the final ethical decision. The Home Office inspectors all have a scientific background but no formal training in making ethical decisions. In other countries, ethics committees make such decisions, and while their members may also not be trained, their consultation base is broader and may be more likely to represent public concern. Furthermore, such committees can be constituted to include members with training and experience in making ethical decisions. Decisions made by a single person may make the inspector a scapegoat for anything that goes wrong or can be contested, particularly considering the history of antivivisection movements in the U.K. Ethics committees, on the other hand, can provide a forum to debate these issues, raise awareness on both sides, and perhaps defuse tensions and lead to a greater mutual understanding (Boyd, 1995). This in turn may reduce violence of the animal campaigners towards the scientists and veterinarians involved in research.

Ironically, despite high standards employed in animal research in the U.K., the top-down approach of the legislation, because it specifically excludes those who have a serious interest in the debate, leads to discontent. Little opportunity arises for mutual education, unlike in countries where ethics committees have an active role to play. This exclusion makes genuine animal welfarists more skeptical about what goes on under the 1986 Act and gives them little option to make their voices heard other than through acts of protestation, and regrettably, sometimes violence.

The poor representation of community members, professional animal welfarists and Named Persons on the national Animal Procedures Committee contrasts strongly with ethics committees controlling animal research elsewhere in the world. The system of control of the Animals Procedures Committee and the Home Office inspectors gives an overwhelming scientific bias to the implementation of the 1986 Act.

Diffuse Responsibilities

The U.K. system is very dependent on the inspectors for formal oversight. The job of this small group is considerable and in addition to those duties described above they are

> "...increasingly...being called upon to mount detailed retrospective investigations into allegations made by anti-vivisectionist organizations concerning animal handling or facilities at designated places, or the justification for animal work presented in published research papers" (HMSO, 1994).

The U.K. system, in our opinion, places much of the responsibility for overseeing animal research on a small group of civil servants. Because the inspectors are seen to be the ultimate arbiters of the performance of a particular research project and the level of animal suffering it causes, they are the focus of attention when it comes to defending

and justifying such research, even though the U.K. system has multiple individuals with formal responsibility for the use of animals.

Although in theory, the Personal Licensee has ultimate responsibility for an animal, the various responsibilities for animal care and use and ensuring best practice and compliance are spread among the Personal Licensee, Project License holder, NDDCP, NVS, Certificate Holder and Home Office inspector. Perhaps responsibility is spread too widely for tight control. However, only the Project License holder and Personal Licensee can commit criminal offenses through neglecting their responsibilities, the others simply breach conditions attached to their license or do not follow the guidelines set out in the Home Office Guidance on the A(SP)A.

There is also much criticism from the public that the Director of Public Prosecutions, who handles all cases reported by the Home Office, seems remarkably reluctant to prosecute apparent criminal offenses under the Act (similar criticism surrounded the 1876 Act). They may fear a failure will reflect badly on the inspectorate, reveal significant flaws in the wording and application of the Act, and generally encourage further questioning of a system that is largely based on the inspector offering advice and the scientific community accepting and abiding by it.

Within an institution, the responsibility for compliance with the Act rests largely with the Certificate Holder, and the Home Office inspector monitors its effectiveness through regular visits and inspections (sometimes even monthly depending on the type and amount of work being carried out). While this is a strength of the system, the Certificate Holder is normally a very senior administrator responsible for fairly detailed matters and issues, many outside his or her area of expertise, and must delegate responsibility. It is important, therefore, that an effective management system be put in place that includes protecting the important roles and functions of the Named Persons as animal advocates. Apart from any other function, the Named Persons are responsible for ensuring that no animal suffers unnecessarily, from the stock animal not the responsibility of a licensee, to the animal that has exceeded the severity limit when the licensee cannot be contacted.

The Home Office inspector communicates with the individual licensees about their research protocols. While the Certificate Holder (or his deputy) must sign the application before it is submitted to the Home Office, there is no formal requirement for any wider review within the institution. The NDDCP and the NVS are required to be familiar with project licenses, but there is no formal requirement that either be consulted about any aspect of the protocol. It is up to the institution (or the Certificate Holder) to set up a management system that allows this to occur, and many do not appear to have such an infrastructure.

Re-use of Animals

There are also strict controls on the re-use of animals, which in our opinion, are based on the misguided criteria of whether an animal has had an anesthetic during the first protocol, rather than on the amount of suffering an animal might have experienced in total. Thus, an animal that has not had an anesthetic may be re-used in one or more unrelated projects if that is accepted in the project license application. However, an animal that has had an anesthetic cannot be re-used in an unrelated project except under terminal anesthesia. Exceptions are made if the first procedure using anesthesia was essential for the research project, such as surgical preparation of an animal or if the anesthetic was used solely to immobilize an animal. These regulations on re-use are likely to cause an unnecessary increase in the number of animals used (particularly those that cause most public concern such as dogs and primates) without any additional protection for the animals' welfare. In our opinion the harms involved in using a "new" animal are likely greater than the harms involving the re-use of an existing acclimatized animal. It also infers that animals that have had a general anesthetic for what may have been a relatively minor scientific procedure have suffered a significant harm.

Schedule 2 of the Act lists those animals that must be purpose-bred, which provides some reassurance to the public that pets (stray cats and dogs) will not end up in research laboratories. However, no such safeguard is in place for horses.

Animal Source and Disposal

Interestingly, killing animals for tissue using an approved humane method listed in Schedule 1 of the Act, is not classed as a procedure presumably because this type of use is of no ethical concern. Thus no statistics are kept on such use, which would give an idea of the use of animals in "replacement ex vivo" alternative methods. Animals lives could be wasted in this regard. Furthermore, there are fewer checks and balances on the competence with which killing is carried out compared with the same technical process for a scientific purpose (such as intravenous injection for an overdose of anesthetic, saline, or drug).

Conclusion

The implementation of the Act relies at the crucial level of protocol review on a small, overworked group of inspectors who have a scientific background and so may be more benevolent towards science when asking questions and making decisions about projects. However, the inspectors as a group have amassed a good deal of skill at encouraging best practice, refining animal experiments, and reducing animal waste. The legislation quite correctly emphasizes numerous personal responsibilities in the performance of animal research. However, it may not, in our opinion, have produced an effective practical system, readily applicable across a wide range of institutions, which allows responsibility to be taken by the entire biomedical research community within that institu-

tion, and which integrates public involvement and accountability. In fact it may, inadvertently, have had the opposite effect.

THE FUTURE

In the foreseeable future, the use of animals in science is likely to come under even closer scrutiny from the general public and anti-vivisectionist movement. Because of the U.K.'s centrally administered system of oversight and, as noted by the Animal Procedures Committee in its 1993 report, the increasing resort by anti-vivisection groups to require retrospective analysis of projects, the work load on the inspectorate will also increase. However, it is likely that the resources allocated to the inspectors by the government will fall in real terms (it is projected that the number of field inspectors will be decreased in the near future). The obvious criticism that can then be voiced is that the inspectors are even less likely to perform their functions effectively.

In order to deal with this problem, there is pressure from some quarters for the U.K. to introduce local ethics committees (similar to IACUCs) that would review protocols and help make the harm-benefit analysis currently required by the A(SP)A (Boyd, 1995). The Home Office is reluctant to see itself at the whim of institutional committees and lose some of its influence. Currently the only person who can authorize a program of animal research is the Secretary of State, advised by a Home Office inspector. A change to the present system would raise questions about authorization of projects, of consistency between IACUCs (although this criticism exists now with the national inspector), training of IACUC personnel, committee structure, and other questions similar to those raised in the United States. However, the Home Office inspector would be placed in a much stronger position to truly "oversee" the activities of IACUCs (much like the Canadian and the projected Australian and New Zealand systems) and "audit" the work of investigators. If the IACUC was also to include members of the local community and an animal welfare organization representative, then it would be less likely to be criticized for being an instrument of the scientific community. An argument for the establishment of IACUCs in the United Kingdom has been given by Jennings (1994) and a discussion of their possible role is addressed in LASA (1994).

While the U.K.'s legislation was internally driven, it was also a response to a European Community Directive 86/609/EEC, which outlined a basis for animal research legislation for European Community countries. This Directive has now been ratified by some countries, but there is still pressure on the others to implement it. Therefore it is unlikely that further legislation arising from the European Community will have a major impact on the oversight of animal use in science in the United Kingdom during the next 10 years.

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United States

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OVERSIGHT

Oversight of animal care and use in the United States is provided mainly by two overlapping national laws: the Animal Welfare Act (7 USC 2131-2157) and the Health Research Extension Act (42 USC 289d), which was amended November 20, 1985 by Public Law 99-158 to cover, in addition to many other matters pertaining to animals, the care and use of animals in research.

The regulations that implement the Animal Welfare Act are published in the Code of Federal Regulations (9 CFR 1-3). These regulations are administered by the U.S. Department of Agriculture (USDA) and apply to both the care and any use of laboratory animals covered by the regulations regardless of funding source. Animals are 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, teaching, testing, experimentation, or exhibition purposes, or as a pet" (9 CFR 1.1). Animals currently exempted from these regulations include, "...birds, rats of the genus *Rattus* and mice of the genus *Mus* bred for use in research, and horses not used for research purposes 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 or fiber" (9 CFR 1.1).

The Health Research Extension Act is implemented by the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy) (PHS, 1986). This policy is applicable to all activities conducted and supported by the Public Health Service (PHS) involving any live vertebrate animal used or intended for use in research, research training, experimentation, biological testing, or related purposes. The PHS Policy requires compliance with the Animal Welfare Act and requires institutions to use the *Guide for the Care and Use of Laboratory Animals* (*Guide*) (NRC, 1985) as a basis for developing and implementing an institutional program for activities involving animals.

The *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (Consortium for Developing a Guide, 1988) has been developed for institutions that use agricultural animals in their programs. All states have their own laws governing the humane treatment of animals within their borders (NABR, 1991), but usage by research institutions is usually exempted. Twenty states have simple facility licensure requirements and a few have only very general regulations governing research usage of animals. In reality, nearly all states defer to federal law providing protection for research animals. There also may be local laws, and most institutions have in-house policies governing the use of animals. These are so varied that they cannot be adequately summarized here.

FUNDING

The offices that administer the national laws are currently funded by the national government. The AWA is under the auspices of USDA, while the PHS Policy is handled by the National Institutes of Health. The funding for the implementation of the national, state, and local laws usually comes from the regulated institution and grant funds awarded to individual investigators who use animals in their research.

ENFORCEMENT

The Animal Welfare Act is administered by the USDA's Animal and Plant Health Inspection Service (APHIS), Regulatory Enforcement and Animal Care Branch (REAC). REAC employs approximately 80 veterinary medical officers who conduct unannounced inspections at least once a year at institutions that use animals in research, education, and testing. These inspections involve only the species covered under the Animal Welfare Act. In addition, APHIS inspects dealers and vendors of regulated species. If deficiencies are found by the veterinary medical officer, they are noted on an inspection form, and the institution is given a time interval to correct the deficiencies. If the deficiencies are not corrected within the allotted time period, a warning may be issued. If severe deficiencies are found, or if deficiencies are not corrected, administrative legal proceedings may be initiated, which can result in fines and loss of registration to operate as a research facility.

The PHS Policy is administered by the National Institutes of Health, Office for Protection from Research Risks (OPRR). No activity involving animals may be conducted or supported by the PHS until the institution conducting the activity has provided a written Assurance acceptable to the PHS, which complies with PHS Policy. If an institution restricts its Assurance to those portions of its animal care program supported by PHS funds, then PHS authority ex-

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tends only to those areas. Most institutions assure the PHS that their total animal care program is being conducted in accordance with PHS Policy. In that case, all of the institution's animal care program is open to PHS scrutiny and policy requirements. OPRR investigates complaints against an institution, usually starting with a letter of inquiry. If deemed necessary OPRR will assemble a team, including outside consultants who are experts in laboratory animal medicine, and conduct an on-site review of the institutional Assurance. The purpose of the visit is to determine whether the institution is following the policies outlined in its Assurance and whether all aspects of the animal care and use program are in conformance with the *Guide*. If noncompliance is found, the Institutional Official is appraised of these deficiencies and given a date by which the deficiencies must be fixed. If the deficiencies are severe enough, OPRR can withdraw approval of the Assurance, thereby leading to the suspension of the expenditure of PHS funds for all research at that institution until such time as the deficiencies are corrected.

The American Association for Accreditation of Laboratory Animal Care (AAALAC) is a non-regulatory, not-for-profit organization founded in 1965 whose mission is to promote high standards of animal care, use, and well-being and enhance life sciences research and education through the accreditation process. Participation in the accreditation program is voluntary and at the initiative of individual animal programs. The Council on Accreditation evaluates animal programs by conducting site visits and reviewing annual reports. AAALAC relies on the *Guide* as its primary standard for evaluating laboratory animal care and use programs. In addition, AAALAC uses published references for supplemental information about procedures or techniques related to the care and use of laboratory animals. AAALAC accreditation demonstrates that a program has achieved a standard of excellence beyond the minimums required by law and conforms with the scientific community's accepted ethical practices. AAALAC is currently the only accrediting body recognized by the PHS for activities involving animals.

APPLICABILITY

The responsible party under each law is the institutional official, and it is this person to whom all correspondence is addressed. The institution makes the principle investigator the responsible individual with oversight by the attending veterinarian and the institutional animal care and use committee (IACUC). It is possible that infractions by a single investigator, if not corrected by the institution, could result in sanctions against the institution because it shows a failure to properly administer its animal care and use program.

The Animal Welfare Act and the PHS Policy provide standards for such items as construction of animal facilities, review and approval of proposed animal use, veterinary care, standards for occupational health, handling of hazardous agents, and training of animal care personnel and research staff.

ADMINISTRATIVE METHODS AND THEIR COSTS

PHS Policy and the Animal Welfare Act are administered in broadly similar ways and will be discussed together. Both make an institutional official responsible for the animal care and use program. An IACUC is required by both. The Animal Welfare Act requires a Chairman and at least two additional members.

> "Of the members of the committee:
>
> (i) At least one shall be a Doctor of Veterinary Medicine, with training or experience in laboratory animal science and medicine, who has direct or delegated program responsibility for activities involving animals at the research facility; (ii) At least one shall not be affiliated in any way with the facility other than as a member of the Committee, and shall not be a member of the immediate family of a person who is affiliated with the facility. The Secretary intends that such person will provide representation for general community interests in the proper care and treatment of animals;" (9 CFR 2.31).

The PHS Policy requires a minimum of five members including at least

> "(1) one Doctor of Veterinary Medicine, with training or experience in laboratory animal science and medicine, who has direct or delegated program responsibility for activities involving animals at the institution; (2) one practicing scientist experienced in research involving animals; (3) one member whose primary concerns are in a nonscientific area (for example, ethicist, lawyer, member of the clergy); and (4) one individual who is not affiliated with the institution in any way other than as a member of the IACUC, and is not a member of the immediate family of a person who is affiliated with the institution (PHS, 1986, p. 5).

Many institutions have 10 or more members on the IACUC. The IACUC is charged with performing a semiannual review of the institution's animal care and use program and of the facilities where the animals are housed, using the *Guide* (NRC, 1985) and the standards of the Animal Welfare Act as a basis for evaluation. Reports of these two reviews are sent to the institutional official with recommendations for programmatic improvements if necessary and, if identified, deficiencies requiring timely corrective action. The IACUC is also charged with the task of reviewing all concerns expressed by anyone about the care and use of animals at the facility. The most time-consuming task for most IACUCs is the prior review of all protocols designed to use animals in research, education, or testing. Finally, the IACUC is authorized to suspend an activity that is not being conducted in accordance with the approved protocol. Suspension of an activity or disapproval of an animal use protocol by the IACUC cannot be overturned by the institution.

In reviewing protocols, the committee is to ensure that research projects will be conducted in accordance with USDA regulations and the PHS Policy unless acceptable justification for a departure is approved. Further, the IACUC

must determine that the project conforms with the institution's Assurance and meets the following requirements as set forth in PHS Policy:

> "a. Procedures will avoid or minimize discomfort, distress, and pain to the animals, consistent with sound research design. b. 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. c. Animals that would otherwise experience severe or chromic pain or distress that cannot be relieved will be painlessly sacrificed at the end of the procedure or, if appropriate, during the procedure. d. The living conditions will be appropriate for their species and contribute to their health and comfort. The housing, feeding, and nonmedical care of the animals will be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. e. Medical care for animals will be available and provided as necessary by a qualified veterinarian. f. Personnel conducting procedures on the species being maintained or studied will be appropriately qualified and trained in those procedures. g. Methods of euthanasia used will be consistent with the recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia [AVMA, 1993] unless a deviation is justified for scientific reasons" (PHS, 1986, pp. 7-8).

Annual reports are sent to the USDA and the PHS although the information requested by both is different.

The cost of the administration of the committee (usually one and sometimes two or more staff) is usually borne by the administration using institutional funds, although in some organizations these administrative costs are collected through charges levied by the animal care facility. IACUC members usually serve without any additional compensation. Their salary is a cost that is provided by the individual's department.

STRENGTHS AND WEAKNESSES

One of the greatest strengths of the present system is that many of its rules and regulations are based on performance, as opposed to rigidly delineated "engineering" standards. Performance standards rely on the professional judgement of the institution's veterinary staff and IACUC to devise a suitable means for achieving specified goals, while engineering standards mandate detailed specifications that must be met. A weakness of the present system is that the two major sets of national regulations are not entirely uniform. For example, they differ in the species of animals covered. The Animal Welfare Act covers some species only when they are used in certain types of experiments and exempts the same species when they are used for other types of experiments. The requirements for cage sizes are also not consistent between the regulations. These and other differences have resulted in confusion when non-experts attempt to evaluate the extent of institutional compliance with regulations. An additional weakness is that the law requiring regulations was enacted without adequate provision for its fiscal impact on the research and instructional activities being regulated.

THE FUTURE

One possibility in the future is that new attempts will be made to mandate detailed engineering standards that are expensive to implement and will thwart efforts to employ local professional judgement. It would also erode a strength of the current regulations, which is that the regulations are goal-oriented (leaving much of the details of implementation up to the good judgement of professionals), rather than process-oriented regulations. Such efforts would divert funds from research and teaching with no substantial benefit and possibly causing great harm to animal welfare. Another likely possibility is that the current trend toward increased cooperation between regulating agencies will continue, with the result that a more uniform set of regulations will emerge over time.

The USDA has never had adequate funding to perform the inspections required by the regulations. That is a principal reason why they focus their inspections on species that were of concern when the regulations were enacted. Further decreases in the USDA budget, which at this time seem certain, will greatly decrease its ability to perform the required inspections. USDA will probably consider implementing user fees to pay for inspections in the future. Since funding for research is also decreasing from almost all sources, such fees would be difficult for most institutions to pay. Currently most facilities are inspected twice yearly by their IACUC, at least once a year by the USDA, and approximately one-third are accredited by AAALAC, which conducts on-site inspections at 3-year intervals. This is a very redundant inspection system that could be coordinated to reduce the number of inspections. Perhaps USDA could review IACUC and AAALAC reports and discontinue inspecting those facilities that are in compliance.

Currently some investigators are discontinuing animal-based research because it has become too expensive and, in some cases, too controversial. Undoubtedly in the future some institutions will begin to stop the use of animals for the same reasons. This will result in a diminished ability to make advances in biomedicine in this country.

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Laboratory Animal Care Policies and Regulations: An Overview

	Oversight	Funding Source	Enforcement	Applicability	Administration and Cost
Canada	National peer-review organization (CCAC)	From 1968-1995 annual grants; from 1995 on user-pay system for government facilities and private enterprise	Compliance reviewed by Animal Care Committee; granting agencies may freeze or withdraw research funds if non-compliance is found	Institutions	Institutional Animal Care Committees administer CCAC program; costs defrayed by institution for government facilities and private enterprise and by government grants for academic institutions
Japan	National law	No funding required	Not strictly enforced (small fine); individual institutions may issue warnings or suspend projects	Individuals (managers of lab animal facilities, animal caretakers, researchers, etc.)	No central administration; institutions may have animal experimentation committees, which review all protocols
New Zealand	Code of ethical conduct (unique to each institution)	National government	Liable to prosecution under Animals Protection Act	Shared between institution and researcher	Institutional codes of ethics are approved by Ministry of Agriculture; individual institutions bear costs of institutional animal ethics committees (IAECs)
United Kingdom	National law	Public taxation and user fees	Criminal penalties or administrative sanctions	Individuals (certificate holder, project license holders, personal licensees, etc.)	Certificates and licenses required for premise, project, and individual; national inspectors oversee all licensees and establishments; national committee passes broad judgements on matters of animal welfare
United States	National laws	Public taxation	Fines and loss of registration to operate a research facility	Institutional Official	Institutional animal care and use committee oversees protocols; funded by institutional overhead

The Impact of International Free Trade Agreements on Animal Research

James W. Glosser

The creation of a global common market will require major changes in public policies and initiatives concerning international trade on the part of all countries. Such a market will also require more scientific information to optimally identify and manage risks. A common market will allow the free movement of goods, persons, services, and capital among countries with diverse socioeconomic situations, cultural backgrounds, and regulatory systems. It represents a marked shift away from the traditional policy of refusing animal and plant trade from regions with any degree of health risk (such as pests and diseases) towards assessing the level of risk that would permit safe trade. The U.S. Congress ratified the North American Free Trade Agreement (NAFTA) in November 1993, and the Uruguay Round of the General Agreement for Tariffs and Trade (GATT) in December 1994. Both agreements aim to reduce and eliminate barriers to trade, investment, and services, and they clearly signal the emergence of a "one world" global market.

These agreements introduce a host of questions for the scientific community: What are their implications for biomedical research? Will new trade policies and initiatives affect research priorities in the future? How will research and development of new techniques and assay methods be impacted? Will additional restraints in the form of new guidelines and regulations be forthcoming?

A new world market will, without question, present challenges as well as new opportunities for the scientific community. Therefore, it is important for the researcher to become familiar with the general provisions of NAFTA and GATT in order to identify concerns, participate in discussions, and assume an appropriate role in developing new policy.

At present, Canada, Mexico, and the United States constitute NAFTA. With 360 million people and a gross national product totaling $6 trillion, this agreement creates the largest free-trade area in the world. In the future, it is quite likely that more countries will be added. For example, Chile is preparing to meet the NAFTA provisions, and preliminary discussions are ongoing with some Central American countries. A bill to include the Caribbean countries in NAFTA was recently introduced in Congress. The GATT comprises 115 signatory countries and has provisions similar to NAFTA.

In addition to eliminating traditional barriers such as quotas and tariffs, both agreements have sanitary and phytosanitary (S&P) provisions intended to control the use of nontariff trade barriers such as unjustified technical animal and plant health standards. Key provisions of both agreements include (1) the use of science-based measures (such as risk assessment); (2) recognition of pest-free, disease-free, and low-prevalence areas, thus allowing trade from those areas; (3) participation in the international standard-setting organizations, and wherever possible, basing import requirements on international standards; (4) recognition of equivalent treatments and quarantine practices to facilitate trade (known as "equivalence"); (5) provision by member countries of advance notification of any new or modified regulation or policy that may affect trade (known as "transparency"); and (6) establishment of a dispute settlement process that begins with a consultation of technical representatives from both parties and proceeds, if necessary, to the use of a formal dispute settlement system (NAFTA, Volume 1, Chapter 7, Section B, Articles 709-724; GATT, Uruguay Round of Agreements, Articles XXII, XX(b), XXIII).

The requirement of science-based measures in determining import policy is significant because it means that import decisions must be based on a risk assessment. In other words, scientific data and methodologies must be used. Both agreements require that key factors be considered in determining science-based measures for import policy, which include (1) risk assessment methodologies and techniques developed by the Office of International Epizootics (OIE) for animals and the International Plant Protection Convention (IPPC) for plants; (2) relevant scientific evidence; (3) relevant processes and production methods; (4) relevant inspection, sampling, and testing methods; (5) prevalence of disease or pests; (6) relevant ecological and environmental conditions; (7) relevant treatments, including quarantines; and (8) relevant economic factors such as production or sales losses and the control of costs if a particular disease or pest were introduced (NAFTA, Volume 1, Chapter 7, Section B, Articles 709-724; GATT, Uruguay Round of Agreements, Articles XXII, XX(b), XXIII).

A major challenge in both agreements is to develop an acceptable approach to resolving disputes that involve diverse regulatory systems or trade measures. The solution lies in harmonization with the broadest possible use of international standards and the creation of a workable dispute settlement system.

Both agreements stress that the first step in handling disputes is a technical consultation. If there is no resolution of

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the problem at that level, the plaintiff country can elevate its complaint by evoking the formal dispute settlement process. Panels will be formed to review complaints and make recommendations. The panels may seek recommendations and advice from an international standard-setting organization (such as OIE and IPPC), as well as from a board of experts to evaluate the issue. Advisory panels will determine whether the disputed measure is based on a scientific risk assessment and whether the data and the process used in the assessment was (1) collected in a scientific manner, (2) based on international standards if such standards exist, (3) not discriminatory, and (4) transparent. Prior to NAFTA and GATT, countries entered bilateral negotiations to solve disputes, such as the latest Halifax summit meeting when the president of the United States and prime minister of Japan discussed the threat of 100 percent tariff penalties on a half dozen Japanese car imports.

Although some international animal standards are in place, they are far from being complete. Hopefully, future standards will be adopted from high quality past and existing management practices. Standards for animal welfare and biotechnology are currently nonexistent within the framework of NAFTA and GATT, and considerable discussion and negotiation will be required to harmonize approaches. The current S&P codes do not address the use of animal welfare or other socioeconomic measures as barriers to trade, such as the uncertain status of bovine somatotropin and the total ban of anabolic growth promoters in the European market.

Standardized animal welfare regulations may become even more important in international trade, because one of the major aspects to be considered in a risk assessment is the relevant process and production methods used in a product. Presently, products are assessed on the end product and not on the process by which it is produced. If agreement is reached on principles for regulating commodities according to how they are processed, restrictions potentially could be imposed on veal, pork, and poultry because of the breeding and husbandry practices used to raise those animals.

Animal welfare is an increasingly important issue in the field of biotechnology, particularly regarding recombinant DNA products and transgenic animals. Beyond achieving scientific consensus on safety, efficacy, and quality, genetically engineered products and animals will in all probability be subjected to intense public scrutiny, also known as the "fourth hurdle." Public debates about transgenic animals are similar to those involving the hormone ban and the current moratorium on the licensure and use of bovine somatotropin. Without question, this issue will continue to be a daunting challenge to the research community.

Because of the considerable variation among animal welfare laws and regulations of different countries and regions, it is possible that a country could take an animal welfare issue and turn it into a health issue. European countries have already developed and implemented formal policies concerning the protection and care of animals used for scientific and farming purposes. For example, the Treaty of the European Union (EU) includes a declaration that requires the European Parliament and its subunits (the Council, Commission, as well as member states) to comply with the welfare requirements of animals when drafting and implementing legislation. In addition, there are currently three overriding legal documents at the EU level, which deal specifically with animal biotechnology. These are (1) Council Directive 86/609/EEC, November 24, 1986 dealing with the laws, regulations, and administrative provision of the member states regarding the protection of animals used for experimental and scientific study; (2) Council Directive 90/220/EEC, April 23, 1990 dealing with the deliberate release of genetically modified organisms in the environment; and (3) the European Convention for the Protection of Animals kept for farming purposes, to which the EU is also a contracting party. In contrast, U.S. animal welfare laws regulate the care and use of animals used in biomedical research, but exempt farm animals.

Clearly there is a need to develop international standards for animal welfare to prevent the issue from becoming a nontariff trade barrier as the movement of laboratory animals will only increase between countries with different levels of sophistication and infrastructure regarding the care and welfare of animals. An excellent resource and starting point for developing such international standards for animal welfare is the *Guide for the Care and Use of Laboratory Animals* (NRC, 1985).

Developing international standards is of prime importance to make trade as free as possible by providing acceptable levels of risk. Scientific advice will be vital in providing negotiators with information on which to forge those standards. The results will be critical for human health, pharmaceuticals, trade of animals, and animal welfare. The trade agreements provide an excellent opportunity for the research community, especially individual scientists, to assist in developing technically sound and operationally feasible standard approaches for conducting risk assessment.

Scientists have other opportunities for input. These include (1) participating as key players in discussions to clarify and define levels of risk, (2) standing ready to serve on expert panels to review disputes and provide findings and recommendations, and (3) focusing on research that provides new technologies and assay methods to reduce animal health risks to levels that permit safe trade.

In summary, researchers should seize the opportunity to assist in developing new policies and initiatives during the implementation phase of the dramatic changes in policy brought about by free trade in a global market. The participation of the research community is needed to optimize international scientific exchange of views on how to facilitate the movement of animals, animal products, cell lines, cell products, pharmaceuticals, and agricultural products.

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Call for Comments

Permitting Issues and the Costs of Animal Research

The costs of doing research have long been of concern to the scientific community. They have also recently become the focus of Congressional inquiry. In a June 6, 1995 letter to the Government Accounting Office (GAO), Congressmen John Porter (R-Il) and Dan Miller (R-Fl) expressed their concern for the amount of money being spent on "funding excess paperwork and additional staff due to excessive regulations" governing research.

ILAR is currently focusing on two aspects of the cost issue—the collection and importation of biologics for research and education, and how various costs associated with animal research affect scientific progress. To assist in our work on these two issues, we are seeking comments and examples from the scientific, regulatory, and funding communities regarding problems encountered in these two areas.

PERMITS FOR THE COLLECTION AND IMPORTATION OF BIOLOGICS FOR RESEARCH AND EDUCATION

Biological research often requires the collection and movement of sera, cells, and other specimens as well as whole plants, animals, and fossils from one country to another or from the field to a laboratory. Collection, transport and disposition of such material are closely regulated and often require permits. Four major permitting agencies regulate these activities: the Animal and Plant Health Inspection Service of the U.S. Department of Agriculture, the National Marine Fisheries Service of the National Oceanographic and Atmospheric Administration, the Fish and Wildlife Service of the Department of Interior, and the Centers for Disease Control and Prevention of the Department of Health and Human Services. Components of other agencies also have a role, such as the Department of Transportation, U.S. Post Office, U.S. Customs, Food and Drug Administration, Bureau of Land Management, and the National Park Service. Scientists from both the biomedical and biodiversity sciences, in academe and industry, are affected by the permitting process.

Two goals frequently clash during the permitting process. On the one hand, science requires the use and exchange of biological material and live organisms. On the other, the natural resources of the country and the health of the public must both be protected. The rules and regulations on permitting are complex and can lead to problems for both scientists and regulators. Complying with the laws is time-consuming and can cause delays in the movement of materials. An investigator might have to obtain permits from as many as four or five agencies before collecting or moving biological specimens. Several institutions have full-time staff whose job is to deal with permits. Nonetheless, many investigators are unaware of the regulations or choose to ignore them. Many of the problems might be solved by simplifying the permitting process, streamlining the administration of the granting of permits, and by better communication between scientists and regulators.

ILAR held two meetings in 1994, attended by both regulators and scientists, to plan a series of workshops on permitting issues. Some of the topics identified were

- effects of the permitting process on biomedical and toxicological research requiring the use of live and dead plants and animals; biological materials such as sera, cell lines, DNA, and microorganisms,
- impact of the permitting process on biodiversity research including planned, opportunistic, and existing collections and field research,
- international laws and regulations (such as NAFTA and GATT), and packaging and shipping requirements,
- cataloguing and harmonizing import/export documents used by various agencies to administer the regulations, with a goal of producing a resource handbook and electronic database, and
- examining whether permitting policies could be improved by basing them more explicitly on scientific risk-assessment.

To assist in developing the proposed workshop series and to help foster communication between regulators and scientists, we seek examples and comments from the scientific and regulatory communities regarding problems encountered in obtaining or issuing permits necessary for collection, transport, export, or import of biologic materials, including animals, plants, biologic specimens, cell cultures and reagents, and museum or archaeologic specimens.

THE COSTS OF ANIMAL RESEARCH: IMPACTS ON SCIENTIFIC PROGRESS

In recent years, ILAR has heard increasing concerns from many researchers that high and rising costs of conducting research with animals are impeding scientific progress. Various causes for cost increases have been cited, including the purchase and maintenance of specialized stocks and strains; the regulatory oversight imposed on research institutions by federal, state, and local regulations and policies; requirements

for facility safety and security; and various internal policies of research institutions. Differences in the ways that institutions handle cost components can lead to substantial differences in the costs of performing animal research at different institutions.

Federal requirements have a major impact on costs. Chief among them are the Animal Welfare Act and the Public Health Service Policy on Humane Care and Use of Laboratory Animals. They impose wide-ranging controls and oversight over animal-based research, including review of protocols by institution-based animal care and use committees (IACUCs); cage-size requirements; occupational health and safety programs; facility designs; and heating, ventilation, and air-conditioning requirements. Office of Management and Budget (OMB) directive A-21 prohibits recovery of indirect costs for animal housing since it is a cost center whose use can be assigned to specific investigators. That requirement has caused some institutions to charge very high direct costs to cover heating, ventilation, and cooling; housekeeping; maintenance; and other such indirect costs. Others are finding ways to redefine animal facilities and research space, and still others are decentralizing the animal facilities.

ILAR is exploring the above and related issues to determine what, if any, action the institute should take to help address those issues and how to prioritize those that need to be addressed. The ILAR Council is considering questions such as the following:

- What are the major sources of cost for research involving animals? What factors affect those costs?
- To what extent is it possible to evaluate what impacts those costs have on scientific progress?
- Is there any evidence that the funding success of research proposals in which animals are used is related to the costs associated with their use? To what extent is it known whether interinstitutional variability of direct and indirect costs for animal research affects funding success?
- How might the costs identified be reduced, or at least contained, without sacrificing animal welfare, safety, and other goals?

To help ILAR identify and address concerns about the costs of animal research, we seek comments and examples relating to the above and related questions.

HOW COMMENTS WILL BE USED

The examples and comments we obtain on permitting issues and the cost of animal research will be used internally by ILAR in planning activities that provide advice to the government and the scientific community and foster communication between them. A summary of the information received may be published in a future issue of *ILAR Journal*, but neither contributing individuals nor their institutions would be identified without permission.

Please send comments and examples by letter, fax, or email to: Editor, *ILAR Journal*, Institute of Laboratory Animal Resources, 2101 Constitution Avenue, NW, Washington, DC 20418, Fax: 202-334-1687, E-mail: ilarj@nas.edu.

Future Challenges and Opportunities for Microbial Culture Collections

INTRODUCTION

The U.S. National Committee for the International Union of Microbiological Societies (USNC/IUMS) held a one-day fact-finding meeting on March 27, 1995 to gather information about the current and potential uses of microbial collections and the challenges and opportunities they face in the future. The meeting was organized to answer questions posed by the National Research Council (NRC) Commission on Life Sciences in response to the Committee's proposal for a full-scale NRC study on microbial collections.

The Committee proposed the study after hearing reports of continuing financial difficulties of several collections in the United States and abroad. Although long-term funding has always been a chronic issue for collections, the USNC/IUMS is concerned that substantial resources and expertise are falling into jeopardy at a time when biomedical, basic research, agricultural, biotechnological, and environmental uses of microbes are expanding.

In requesting additional information about the dimensions and magnitude of the collections situation, the Commission on Life Sciences (CLS) asked the following questions: Are funding levels for collections declining relative to past support levels? What are the indicators that underinvestment may lead to negative long-term consequences? Are there new demands being placed on collections that have increased costs or services? Is the maintenance of collections changing in fundamental ways? Have the needs of academic scientists for access to collections changed over the last decade? What is the significance of "commercial" collections to both academic and industrial scientists?

To answer these questions, the meeting brought the USNC/IUMS together with representatives from funding agencies, academic and commercial microbiologists, and curators and database developers from several different collections including the ATCC, the Drosophila collection at Indiana University, the former anaerobe collection at Virginia Polytechnic Institute, the DOE subsurface microbe col-

lection at Florida State University, the U.S. Department of Agriculture (USDA) entomopathologic fungi collection at Cornell, and the Merck Company collection.

The one-day workshop could not completely answer all of the questions asked by the CLS in part because there are no recent systematic data on collections. Nevertheless, the meeting did elucidate major trends influencing the use of collections and the changes underway that have, on the one hand increased funding, space, and personnel problems, and on the other hand, led to the expanded substantive involvement of collections in answering research and commercial questions.

This report is a summary of the major issues presented and discussed at the meeting. It will be used as a basis for the USNC/IUMS response to the Commission on Life Sciences.

CHANGING ROLE OF CULTURE COLLECTIONS

Traditionally viewed as part of the scientific infrastructure, culture collections have long been considered a useful but peripheral tool in support of research. Over the last decade, however, collections have begun to play an increasingly diverse role within companies, research institutions, and in the scientific community at large. In their evolving roles, the expertise and information housed in collections are more closely tied to research, development, and production activities.

What is responsible for encouraging these developing roles? Throughout the workshop, several factors were reiterated as major forces changing the environment in which culture collections operate. These include the economic importance of microorganisms, the emergence of new areas of research, the effect of regulations on activities related to microorganisms, and the expansion of electronic information capabilities.

Economic Importance of Microorganisms

Microorganisms are an expanding part of the world economy and of increasing interest to the pharmaceutical industry. Antibiotic production alone provides annual worldwide revenues of over 16 billion dollars. In the last 10 years, advances in molecular biology have allowed the machinery of microorganisms to be manipulated and exploited, fostering the advent of the biotechnology industry. This 10 billion dollar industry has relied on microbes and their metabolites as the source of many of its products.

Natural products screening is a flourishing enterprise. Microbial collections, the contents of which have been carefully characterized and identified, are a source of value-added material for bio-prospectors—those seeking an organism or metabolic product for the burgeoning new industries of biosensors, bioremediation, energy conservation, environmentally friendly products, and biocontrol.

Polymerase chain reaction (PCR), a method used to amplify small quantities of nucleic acids, was developed using the DNA polymerase of *Thermus aquaticus*, a thermophilic organism deposited in the American Type Culture Collection in the 1960s. PCR is now widely used in basic and applied research, from diagnostics to forensics. The utilization of this unique characteristic of *Thermus aquaticus* has generated interest in exploring other "extremophiles" maintained at the ATCC.

The catalogue of information built by collections can provide a roadmap to researchers trying to find organisms with special properties. For example, Fujisawa was granted the patent on the compound Immunosuppressant FK506, sending its competitors scrambling to find comparable substances. Within months it was discovered that Immunosuppressant FK506 was similar to an antifungal material isolated 20 years ago from an organism that was freely available. Dr. Keith Bostian, CEO of Microcide Pharmaceuticals, estimated that at least 3 years of effort was saved by the information in collections' databases.

New Areas of Research

In addition to research related to medical and industrial biotechnology, microorganisms are also being reexamined in the context of their diversity and ecology. Investigations into the function and relationships of microorganisms to one another and to all other biota in their ecosystems will be aided by the information already recorded in collection databases. Indeed, the taxonomic expertise of curators will be challenged by the expected inundation of biodiversity samples for identification. Given the projection of enormous numbers of undiscovered organisms in the environment, collections may have difficulty meeting the needs of storing and maintaining samples. For example, special handling is needed for microorganisms found in the depths of ocean vents.

Culture collections will also play an important role in research on antibiotic resistance, the emergence of new and old diseases, and accordingly, the causes of virulence. Archival collections, sometimes regarded as microbial "museums" are stockpiles of information waiting to be unlocked with the right scientific tools and questions. An example is the mycobacterium collection, which was rescued by the American Type Culture Collection. As tuberculosis reemerges as a problem in the United States, these cultures are essential resources.

Regulations

Microorganisms used in industry and academia are subject to a number of national and international regulations on their use and handling. Collections are often asked to take responsibility in meeting these requirements. For example, the Food and Drug Administration (FDA) requires biotechnology firms to be able to verify the DNA sequence of any organism producing a product in a fermentation system. The

collections department will often develop a standard assay to comply with this regulation.

The number of patents granted on microorganisms, from wild-type to mutants to genetically modified is growing. Any patented organism must be maintained by the patentee for 30 years (in addition to deposit in an International Depository Authority such as the ATCC or Northern Regional Research Laboratory). Thus, viability and plasmid retention checks are increasingly important work for culture collections.

Collections that ship microorganisms nationally and internationally are affected by overlapping and sometimes conflicting shipping regulations of various U.S. agencies. Collections are also sometimes required to have export licenses for international shipments.

In the case of biocontrol, both Animal and Plant Health Inspection Service of the USDA, and the Environmental Protection Agency regulate the movement and transfer of pathogens and nonindigenous organisms. Although the concerns are primarily related to industrial use and release of microorganisms, collections are caught up in the regulations even though the cultures are contained and simply sent from one qualified lab to another.

Accessibility of Information

Advances in information technology bring the possibility of greater accessibility to information as well as the ability to compare information held in different locations. Many collections have put information about their holdings on electronic databases, but going online is a more recent development that opens up the collection to a broad audience.

Examples of networks being established are the Microbial Strain Data Network, for which fees are associated; the Microbial Germplasm Database in the United States, which focuses mainly on plant pathogens; the World Data Center at RIKEN, funded by the Japanese government; the United Nations Environment Programme and the World Federation for Culture Collections; and the Brazilian Tropical Database, funded by the Brazilian government. Microbial Information Network Europe, funded initially by the CEC, now must stand on its own through user fees. The Microbial Information Network of China is a newly established entity.

Networks provide users with the means to find out about an organism and its properties, and to make comparisons with other organisms. Computer databases are most effective if the information is presented in a consistent way; there will be many challenges to accomplishing this goal in a manner that ensures the integrity of the data. Questions of who owns the data and how electronic access should be paid for must also be addressed in the future.

NEW ROLES, NEW FUNCTIONS, NEW CHALLENGES

As research on microorganisms intensifies, it is not surprising that collections and their managers have had to adapt to new developments in the field. As a result, the specific functions of collections have diversified.

Dr. Jenny Hunter Cevera of the Lawrence Berkeley Lab, and the former curator of the Cetus collection, described how the obvious function of a collection to maintain the growth of organisms overlays an expanding list of other activities, including, for example, the identification of isolates and contaminants, providing taxonomic descriptions of organisms, characterizing plasmids and hosts, comparing 16S and 23S rRNA sequences, conducting photomicroscopy, providing quality assurance and quality control, validating organisms in production and their products, determining the DNA sequence of organisms used in fermentation, fine-tuning fermentation, conducting patent deposits, shipping organisms, and constructing and maintaining computer databases, and conducting research on the organisms themselves.

Compared to the traditional notion of a microbial "warehouse," a successful modern culture collection requires an expanded and diverse source of expertise and adequate resources. In fact, according to Dr. Kathleen Matthews, curator of Indiana University's Drosophila Center, a 1993 NSF-Genetics Society Workshop found that the most successful collections shared three aspects:

1. expert and committed management, including constant evaluation and quality control, and a knowledge of users of the collection and their research;
2. integration with users in the community; that is, act as a center for active dialogue on microbe related issues, such as maintenance of a genetic map, nomenclature issues, databases, or newsletter; and
3. adequate and stable funding.

OPERATIONS OF REPRESENTATIVE CULTURE COLLECTIONS

In order to hear first-hand how different kinds of collections are attempting to successfully meet the needs of the research community, the USNC/IUMS listened to five curators discuss the specific challenges and opportunities facing their collections, and the ways those issues are being addressed.

A Federal Collection

Dr. Richard Humber is the Director of the Agricultural Research Service collection of entomopathogenic fungi (ARSEF). Originally set up as a source of germplasm for biocontrol of invertebrate pests such as insects, mites, and nematodes, it holds approximately 5,000 strains. It is a potentially valuable source of compounds for pharmaceutical and biorational agricultural use, and in fact, through the Boyce Thompson Institute, ARSEF has organized cooperative research and development agreements (CRADAs) with private firms to conduct screening of the collection.

Challenges facing the ARSEF collection include fund-

ing, information resources, regulations, staffing, and decisions about accessions and deaccessions. In contrast to the plant germplasm collections of the USDA, ARSEF is maintained through research funding which does not take into consideration the burden of servicing the collection and user requests. ARSEF has a blanket importation permit and is used widely by the USDA as a quarantine facility for the importation of microbes. Meeting the screening requests of industry can also stretch the staffing and other resources of the collection.

ARSEF publishes a catalog and maintains an elaborate database, which is not yet on the Internet. The collection needs programming expertise to put the database online. Dr. Humber also noted that getting accurate geographical and host (insect) data for these organisms is a major challenge for the collection. Completion of these missing data can increase the utility of the collection.

Taxonomic skills in the area of entomopathogenic fungi is specialized and scarce. Few funds have been available to train the next generation of systematists, leaving the future care of the collection in jeopardy.

Like all collections, space at ARSEF is limited, and a critical concern is how to absorb endangered collections such as the Australian fungi collection, while trying to make more room for new isolates resulting from biodiversity research.

The American Type Culture Collection

The ATCC is often thought of as a national service collection. It is a patent repository, and one of the largest collections in the United States. It is respected and trusted by the academic and private research communities. It is the primary source of "standards" or "reference strains" for quality control, sterility testing, susceptibility testing, evaluation of drug candidates, toxicity testing, and diagnostic reagents. It is a major source of microbial, plant, and tissue cultures. The ATCC distributed 139,000 cultures in 1994.

According to Dr. Robert Gherna, head of bacteriology for the ATCC, 80 percent of the collection is not distributed. Nevertheless, in addition to maintaining these archival cultures, the ATCC continues to absorb parts of endangered or dismantled collections, such as the mycobacterium collection. How to save endangered collections is a primary concern of the ATCC, professional societies, and others.

As a non-profit organization, the ATCC survives through federal and state contracts and grants, through revenues on charges for cultures and patent deposits, and teaching courses. In 1994, the ATCC received 17 percent of its revenues from federal sources, a decline from 24 percent in 1991. The level of federal support can be contrasted to the Deutsche Sammlung von Mikroorganismen (DSM) in Germany, which gets 82 percent of funding from government, the RIKEN in Japan which is fully funded by the government, and the decentralized national collections of the United Kingdom, which receive 35 percent of their funding from government sources.

ATCC's funding concerns are based on the increased cost of personnel (due to competition from the biotechnology industry and others), its need for expansion and renovated space, new requests for storage of DNA material, the preparation of biological materials under strict quality control procedures, and the recent need to purchase cultures in demand (when once it received them gratis).

In addition to funding concerns and accession concerns, the ATCC faces new issues in its role as a patent repository. For example, the ATCC is receiving cultures for storage with restrictions on their distribution. It anticipates being involved in litigation regarding the misappropriation of a patented strain by a third party who received it from the ATCC. The ATCC is even being sued by Gulf War veterans for its distribution of cultures to Iraq, even though the Department of Commerce had issued an export license for the material.

Academic Research Collection

Dr. Edward Moore, former curator of the Virginia Technical Institute anaerobe collection which was recently dismantled, explained its history and background. The collection consisted of 60,000 cultures from clinical infections, periodontal disease, and colon cancer including 6,000 strains not available elsewhere and some isolated before World War I when antibiotics were not in wide use. Dr. Moore estimated that it took 300 person-years of work to build the collection, which was used to develop positive identification techniques for anaerobes.

Dr. Moore was able to distribute most of the collection to the Centers for Disease Control, the ATCC, a dental lab in Boston, and a company called Microbial I.D. Inc. Some of the strains, however, were discarded.

The situation of the Virginia Tech anaerobe collection is representative of many academic collections. Dr. Moore was about to retire when it became clear that the University did not have plans to continue support for the collection. Yet the collection itself was built on publicly funded grants.

Biodiversity Collection

The Department of Energy's subsurface microbial collection housed at Florida State University (FSU) contains microbes isolated from deep aquifer coastal plain sediments. At the time of its inception, it was thought that the unusual locations might reveal novel organisms with interesting properties.

According to Dr. David Balkwill, the collection's curator, the collection has received substantial inquiries from private industry anxious to look for new products. The biggest challenge facing the collection is that it is too young, that is, most of its cultures are uncharacterized microorganisms. Lack of data diminishes the value of the strains to industry, which would have to pay $25-40 per culture not knowing what it was getting or even if many of the cultures

are duplicates. Therefore, a critical issue for the collection is developing fast identification techniques. Much of the work being done at FSU is the design of molecular probes to help determine the phylogeny of the cultures.

A second important issue is that most of the funding for the collection is from the Department of Energy, a situation that is very unstable. Like the VPI collection, there is no guarantee that the University will continue to "host" the collection. The need to diversify funding is therefore critical.

An Industrial Collection

The Merck Co. culture collection for antibiotics was started in 1954 to conduct natural products screening and to have a taxonomic capability for patent purposes. It is one of two major collections at Merck, the other being clinical microbiology.

The antibiotics screening collection holds 17,665 prokaryotic and eukaryotic strains, mostly in lyophilized and frozen forms. It serves as an archival collection, as a depository for Merck's patented strains, as a disaster protection backup for Merck's production line, and as a research center. The collection maintains and characterizes cultures, checks purity and viability, and is the hub for distribution of microorganism inside and outside the company. The collection maintains historical information, growth requirements, utility and other strain data on an internal database. Dr. George Garrity, curator of the collection, estimates that the value of the services it provides to the company is directly around 3.15 million dollars (based on a comparison with ATCC prices). The collection is the basis for many company products.

Nearly one-third of the cultures have come from outside the company, for example, 2000 strains from the ATCC, 600 from the NRRL, others from the U.S. Army, and from universities.

Dr. Garrity noted that the most important challenges to the Merck collection in the future will be finding trained individuals, meeting user needs, keeping up with fixed costs, and keeping management aware of what the collection does and its importance in maintaining the rigor of science, product development, and quality control.

IS A NATIONAL RESEARCH COUNCIL STUDY WARRANTED?

In a final roundtable session of the meeting, participants were asked what would be accomplished by an NRC study. Aside from the specific challenges faced by individual collections, the participants focused on the contribution of an NRC study to the examination of broader policy issues such as the effects of underinvestment in collections, funding and priority issues, and on steps to capitalize on what collections have to offer.

Underinvestment

Although there was disagreement about which parties should carry the burden of collections expenses, there was a strong sentiment among many of the workshop participants that the United States, as a whole, was undervaluing its microbial germplasm collections.

Loss of germplasm. Indicators of this underinvestment included the loss of important germplasm such as that in the Virginia Tech collection, a scenario being repeated frequently across the country and the world. Meeting participants were unable to quantify the loss, however, as a national registry of collections is lacking. It was noted that past efforts to obtain modest funding to establish such a registry were unsuccessful.

Another area of concern is the purchase of entire academic collections by private industry, both domestic and foreign, often inexpensively. That industry might benefit from collections is not the issue. Rather, the fear is that industry might not have the expertise to handle the materials, that access to the cultures will be curtailed, and that resources built with public funds have been transferred to the private sector with too little return.

The latter point leads to the question of who owns a collection (university, individual researchers, federal government) and to the undervalued status of microbial collections at most universities. For example, in one anecdote related at the meeting, Rutgers University was unaware that one of its collections had been dismantled and distributed several years prior by its retiring curator when it established a Center of Biodiversity Research and began frantically looking for the materials.

Lack of trained microbiologists. Another indicator of underinvestment is the lack of adequately trained next generation curators. As one participant noted, taxonomists are a dying breed. The fact that industry must often "contract out" to consultants for microbiological advice signifies a lack of professionals trained not only in classical molecular biology techniques, but with a system of characterization that includes biochemistry, taxonomy, and molecular biology. It was noted at the meeting that a "Microbiology 101" course was no longer offered in many academic institutions. Students may never be taught even the elementary technique of how to transfer a culture. The United States has fallen behind other countries in bringing its students into the microbiology field. A related issue is that manuals on how to preserve germplasm are virtually nonexistent.

Nonstandard materials in circulation. An additional indication of underinvestment in collections is the exchange of nonstandard culture materials by researchers. This practice, which can affect the integrity of data, is possibly due to the increased cost of obtaining standard cultures. The ATCC's decision to increase the prices of cultures for both academic and industrial purchasers 10% over the last 4 years

and 5% this year is a result of increasing costs and declining revenues. The ATCC's prices must also compete with commercial germplasm distributors who buy the most popular strains and grow and resell them, without the burden of maintaining large numbers of infrequently distributed cultures.

Poor public image. The level of public knowledge about microorganisms and the significance of their storage in collections continues to be a concern. Although the public hears about the emergence of new diseases such as the Hanta viruses and HIV, the reemergence of disease such as the plague and tuberculosis, of the growing problem of antibiotic resistance, and of deaths from contaminated food products, workshop participants felt that it was unlikely that the public understands and appreciates the role of microbiology and of collections in addressing these problems.

Some participants argued that this importance was not understood even by the federal agencies that support collections. Collections remain labeled as infrastructure, and as infrastructure, are easy to underfund.

Awareness of the potential. In some cases, opportunities for collections to make a vital contribution are simply missed. For example, Dr. Milton Friend of the National Biological Service explained that the crane population in the United States has been affected by an epivirus believed to be an exotic organism responsible for crane deaths in Austria, Russia, and Japan. Because these organisms have never been cultured and maintained, there is no way to make a comparison.

Similarly, another major disease of water birds is avian botulinum. The Fish and Wildlife Service, in isolating *Clostridium botulinum*, also finds many botulism-inhibiting microbes. In addition to their potential role in finding the solution to the waterfowl condition, the organisms also have economic potential for the canning industry. Again, however, there is no systematic maintenance of these isolates.

Funding Issues

The rational basis for the sponsorship of collections by the federal government must be examined in relationship to benefits received and contributions made by other parties, including industry, universities, and researchers themselves.

Some determination of an appropriate level of investment in collections should be developed, whether that should be a national level in relation to other nations' spending, the contribution of the resource to the economy, or relative to spending on research grants. The National Science Foundation, for example, supports 18 major collections at a cost of $3.5 million annually, or 3% of what is spent on research grants. Is this appropriate?

If there is indeed a role for federal sponsorship, the needs of those agencies in making coordinated, strategically sound funding decisions should also be addressed, including questions on the basis for initiating support for a collection and what a "phase-out" policy should be. Collections supported by public funds should be evaluated upon loss of funding or personnel.

The establishment of alternative sources of funding for collections should also be examined seriously, including the concept of an endowment, or a "royalty fee" fund from revenue on products developed using microorganisms. The potential for interaction with industry screening programs should also be examined.

In academe, the initiation of a collection implies a long-term commitment of which the curator and universities must be aware. An NRC study might help to formulate guidelines for planning a collection's "life-cycle." Although collections may be in existence an indeterminate number of years, plans on how the collection should be dismantled is an issue that should be examined from the start. Collections, and the universities that house them, must be accountable to their sponsors. How the collection will attempt to maximize its utilization by the broader community, and at what cost, should also be examined.

For example, neither users nor sponsors can expect that collections can house all the microbial diversity in the world. Although obviously an important part of understanding the working of ecosystems, the actual potential for holding all strains must be put into rational perspective. As one meeting participant put it, how can we seek funds for preserving biodiversity, when we can't afford to keep the biodiversity we already have preserved?

Scientists also need to develop consensus on the sharing of cultures mentioned in journal publications. The deposit of organisms with restrictions on their use (outside of patent rights) has serious ethical implications for research practice.

Steps to Maximize Use and Benefits of Collections

Throughout the workshop, participants identified many ways in which the contribution of collections could be enhanced to fully realize their potential. Many of these suggestions have applicability beyond collections, because they are tied to research and commercial interests as well. The are briefly listed here.

Need for centralized information. The absence of a comprehensive national or international registry of collections makes information gathering about the numbers of collections and their contents difficult. A registry would be a valuable tool, not only for the evaluation of microbial resources, but for the academic and industrial research communities, for sponsors of collections, and for collections themselves.

Collections related research. If collections are to be exploited for what they can provide, collections-related research should be expanded. The benefit of these kinds of

research questions is that they have a much broader application than for collections. A study might consider the contributions of research on:

- Novel methods for isolation from the environment;
- Storage and use of microbial consortia (mixed cultures);
- Rapid assays for identification;
- Stability of genetic material;
- Artificial extension of generation time;
- Cryopreservation including diapause, cryoprotectants;
- Examination of metabolic pathways under different storage or environmental conditions;
- Mechanisms of virulence and antibiotic resistance; and
- Reconstructing an organism from the gene sequence.

International/Regulatory Issues. Some attention should be given to issues that impede or have an impact on culture collections, such as:

- Transportation standards and regulations,
- Sharing of information and cultures internationally,
- Standardization of organism names (e.g., the International Committee on Taxonomy of Viruses has been funded by NSF to determine common terms),
- Standardizing practices for protecting patented organisms (e.g., eliminating loopholes that allow distribution of microbes to 3rd parties),
- Ownership issues (e.g., the rights to indigenous germplasm; deposits with restrictions), and
- Standardization of identification protocols.

CONCLUSION

The information presented at the meeting on the future challenges and opportunities for culture collections provided a glimpse of the potential impact of these institutions on academic research and commercial prospects. In an era of budgeting restraint, this potential may only be actualized if a national (and perhaps international) perspective on their effective use can be developed. For this reason, the U.S. National Committee for IUMS believes that an NRC study focusing on the issues related to collections may enhance the productive and dynamic capabilities of these important resources.

For more information contact Robin Schoen, Commission on Life Sciences, National Research Council, 2101 Constitution Avenue, N.W., Washington, DC 20418. Tel: 202-334-2233; Fax: 202-334-1687; E-mail: rschoen@nas.edu.

Coming Meetings

September 1995

14–15 Internal Audits of the Animal Care and Use Program—Augusta, Georgia. Sponsored by the National Institutes of Health, Office for Protection from Research Risks, the Medical College of Georgia, and Albany State College, this workshop will address processes by which institutional animal care and use committees (IACUCs) can effectively evaluate their institutions' animal care and use program. The *Public Health Service Policy on Humane Care and Use of Research Animals* (*PHS Policy*) and U.S. Department of Agriculture (USDA) regulations state that at least once every 6 months the institution's program is to be evaluated by the IACUC using the *Guide for the Care and Use of Laboratory Animals* (*Guide*) and USDA regulations (Title 9, Chapter 1, subchapter A-Animal Welfare) as a basis. Topics include a review of the program as described in the *Guide*; institutional policy issues such as the occupational health and safety program, personnel training, and the activities of the IACUC and how effectively it meets its mandates; veterinary care; the animal environment; and record reviews. Reports of the IACUC semiannual program and facility reviews will also be discussed. Approaches useful to IACUCs serving both small and large institutions will be included. This workshop is part of an ongoing series sponsored by the National Institutes of Health, Office for Protection from Research Risks on implementing the *PHS Policy*. Workshops are open to institutional administrators, members of IACUCs, laboratory animal veterinarians, investigators, and other institutional staff who have responsibility for high-quality management of sound institutional animal care and use programs. Ample opportunities will be provided to exchange ideas and interests through question and answer sessions and informal discussions. For more information contact Ms. Katrinka Akeson, Department of Continuing Education HM 100, Medical College of Georgia, Augusta, GA 30912. Tel: 1-706-721-3967; Fax: 1-706-721-4642.

28–29 The Care and Use of Fish, Amphibians and Reptiles in Research—Toronto, Canada. This international conference sponsored by the Scientists Center for Animal Welfare (SCAW) and the Canadian Council on Animal Care (CCAC) will include general sessions on regulations and guidelines; concerns of animal care committees; the relief of pain in cold-blooded vertebrates (except fish); housing, handling, and nutrition; field research; aquaculture; and stress, disease, and euthanasia. For more information contact SCAW, 7833 Walker Drive, Suite 340, Greenbelt, MD 20770, Tel: 1-301-345-3500; Fax: 1-301-315-3503 or CCAC, 315-350 Albert, Ottawa, Ontario KIR 1B1, Canada, Tel: 1-613-238-4031; Fax: 1-613-238-2837; E-mail: ccac@carleton.ca

October 1995

19 Animal Behavior and Laboratory Animal Welfare— Baltimore, Maryland. This half-day seminar, sponsored by the Scientists Center for Animal Welfare and *Lab Animal*, will be held at the national American Association for Laboratory Animal Science (AALAS) annual meeting. The seminar will focus on areas of animal behavior and laboratory animal welfare, and topics will cover why understanding behavior is important for good laboratory animal care; normal and abnormal behaviors of laboratory animals; preference testing to determine the needs of animals; behavior of rodents; and behavior of rabbits. For more information contact SCAW, 7833 Walker Drive, Suite 340, Greenbelt, MD 20770. Tel: 1-301-345-3500; Fax: 1-301-345-3503 or AALAS, Tel: 1-901-754-8620.

22–25 Swine in Biomedical Research: The International Symposium—College Park, Maryland. This international symposium, sponsored by the University of Minnesota and the University of Illinois at Urbana-Champaign, is accepting abstracts relating to transplantation, pharmacology, nutrition, genetic models, toxicology, behavior, infectious diseases, immunology, physiology, obesity, dermatology, and other subjects. For more information contact Secretariat International Symposium, College of Veterinary Medicine, 295 AS/VM Building, 1988 Fitch Avenue, St. Paul, MN 55108-6009. E-mail: pigmodel@gold.tc.umn.edu

January 1996

27–31 Fourth National Symposium on Biosafety: Working Safely with Research Animals—Atlanta, Georgia. This national symposium is sponsored by the Centers for Disease Control and Prevention, Office of Health and Safety; National Institutes of Health, Office for Protection from Research Risks; American Biological Safety Association; and Emory University School of Medicine and Yerkes Primate Center. The meeting will correspond with the release of the report of the Institute of Laboratory Animal Resources entitled *Occupational Health and Safety in the Care and Use of Research Animals*. Many of the speakers are members of the committee that wrote the report. It is intended to provide a forum to stimulate an exchange of ideas and information that promote the identification of hazards, assessment of risks, and implementation of measures to ensure the health and safety of personnel and animals. Biosafety officers, occupational health physicians, veterinarians, principal investigators, members of institutional animal care and use committees, architects, engineers, animal caregivers and supervisors, facility managers, administrators, and others are encouraged to attend. For more information contact Centers for Disease Control and Prevention, Office of Health and Safety, Atlanta, GA 30333 (Attention: Jonathan C. Richmond, Ph.D.). Fax: 1-404-639-2294.

June 1996

19–26 Sixth FELASA Symposium on International Harmonization of Laboratory Animal Husbandry Requirements—Basel, Switzerland. The aim of this symposium is to exchange useful information among scientists and regulatory agencies in order to increase our knowledge and harmonize the requirements of laboratory animal husbandry. For more information, contact Sixth FELASA Symposium, Kongresszentrum Messe Basel, Messeplatz 21, CH-4021 Basel, Switzerland. Tel: 61-686-2828; Fax: 61-686-2185.

October 1996

20–25 Second World Congress on Alternatives and Animal Use in the Life Sciences—Utrecht, The Netherlands. The aim of this congress is to exchange information on recent developments in the field of alternatives (replacement, reduction, refinement) within the various areas of animal use, such as toxicology, pharmacology, pharmacy, cancer research, bioassays, and safety testing. Alternatives in education and training, ethical aspects of animal use and developments aiming at the improvement of animal welfare will be covered. For more information contact World Congress Alternatives 1996, FBU Congress Agency, P.O. Box 80.125, 3508 TC Utrecht, The Netherlands. Tel: 31-30535044; Fax: 31-30533667.

New Books

The Care and Management of Decapod Crustaceans in Captivity, R. W. Ingle. This publication is intended to help all those keeping decapod crustaceans to maintain them under the best conditions currently known to science and to increase awareness of their needs in captivity. It includes sections on decapod biology; management of aquatic environments; management of semi-terrestrial environments; rearing in captivity; food and feeding; special requirements of captive species; collection, handling, and transporting; restraint, anesthesia, and euthanasia; and diseases. This monograph is an extension of the sixth edition of the *UFAW Handbook on the Care and Management of Laboratory Animals*, from which invertebrates were deliberately omitted. A separate volume on cephalopods was published in 1991, and future monographs on invertebrates are planned. Universities Federation for Animal Welfare (UFAW), 1995, 119 pp.,

soft cover, $30.00, ISBN 0 900767 86 3. (Available from UFAW, 8 Hamilton Close, South Mimms, Potters Bar, Hertfordshire EN6 3QD, England. Tel: (44) 1707 685202; Fax: (44) 1707 649279.

Wildlife Mammals as Research Models: In the Laboratory and Field, Kathryn A. L. Bayne and Michael D. Kreger, eds. This volume contains the proceedings of a half-day seminar held at the annual American Veterinary Medical Association (AVMA) conference in San Francisco, California on July 12, 1994. The chapters include, "Wildlife Management in the Laboratory: Non-human Primates" by Kathryn A. L. Bayne; "Wildlife Management in the Laboratory: Other Species" by Michael Kreger; "An Overview of Contraceptive Research and Non-capture Methods for Studying Reproduction in Wildlife" by Jay F. Kirkpatrick; "Marking, Trapping, and Manipulating Animals: Some Methodological and Ethical Considerations" by Marc Bekoff; "Ethics of Keeping Marine Mammals in Captivity" by Michael T. Walsh; and "Use of Positive Reinforcement Techniques to Enhance Animal Care, Research, and Well-being" by Gail E. Laulie. Scientists Center for Animal Welfare (SCAW), 1995, soft cover, 60 pp., $20.00. (Available from SCAW, Golden Triangle Building One, 7833 Walker Drive, Suite 340, Greenbelt, MD 20770. Tel: 1-301-345-3500; Fax: 1-301-345-3503.

Publications Available

Single copies of the following publications are available without charge from the Institute of Laboratory Animal Resources (ILAR), National Research Council, 2101 Constitution Avenue, NW, Washington, DC 20418. Tel: 1-202-334-2590; Fax: 1-202-334-1687; E-mail: ilarj@nas.edu

Annotated Bibliography on Uncommonly Used Laboratory Animals: Mammals. 1986
Control of Diets in Laboratory Animal Experimentation. 1978
***Definition, Nomenclature and Conservation of Rat Strains.** 1993
Guide to Infectious Diseases of Guinea Pigs, Gerbils, Hamsters, and Rabbits. 1974
Important Laboratory Animal Resources: Selection Criteria and Funding Mechanisms for their Preservation. 1990
Laboratory Animal Management: Cats. 1978
Laboratory Animal Management: Genetics. 1979
Laboratory Animal Management: Nonhuman Primates. 1980
Laboratory Animal Medicine: Guidelines for Education and Training. 1979
Long-Term Holding of Laboratory Rodents. 1976
Principles and Guidelines for the Use of Animals in Precollege Education. 1989
Recommendations for the Care of Amphibians and Reptiles in Academic Institutions. 1991
***Standardized Nomenclature for Transgenic Animals.** 1993
Third International Registry of Animal Models of Thrombosis and Hemorrhagic Diseases. 1988

*New Publication

The following ILAR and Board on Agriculture publications, for which there is a charge, can be ordered from the **National Academy Press, P.O. Box 285, Washington, DC 20055. Tel: 1-202-334-3313 or 1-800-624-6242; Fax: 1-202-334-2451.** All orders must be prepaid by check, money order, or credit card unless accompanied by a bona fide purchase order. Please add $3.50 per item for shipping and handling. Quantity discounts are as follows: 5-24 copies of one title—15%; 25-499 copies of one title—25%. To be eligible for a discount, all copies must be shipped and billed to one address. Please note that the following prices are those for the United States, Canada, Puerto Rico, and Mexico and are subject to change without notice. Ordering information outside these areas can be obtained from the National Academy Press at the address above, or at any of the following locations:

United Kingdom and Western Europe: Plymbridge Distributors Limited, Estover, Plymouth PL6 7PZ, United Kingdom. Tel: 44(0752) 695745; Fax: 44(0752) 695699
Japan: Maruzen Co., Ltd., P.O. Box 5050, Tokyo International 100-31, Japan (accept letters only)
Brunei, People's Republic of China, Hong Kong, India, Indonesia, Korea, Malaysia, Philippines, Singapore, Taiwan, and Thailand: World Scientific Publishing Co. Pte. Ltd., Farrer Road, P.O. Box 128, Singapore 9128. Tel: 65-3825663; Fax: 65-3825919.

To obtain single copies of the *Guide for the Care and Use of Laboratory Animals* (1985) write **Office for Protection from Research Risks, Division of Animal Welfare, National Institutes of Health, 6100 Executive Boulevard, MSC 7507, Rockville, MD 20892-7507.**

Dogs. Laboratory Animal Management Series. 1994.

Rodents. Laboratory Animal Management Series. In press.

Recognition and Alleviation of Pain and Distress in Laboratory Animals. 1992. $29.95. ISBN 0-309-04275-5

Education and Training in the Care and Use of Laboratory Animals: A Guide for Developing Institutional Programs. 1991. $11.95 each; $10.50 if purchasing 2-9 copies; $9.95 if purchasing ten or more copies. ISBN 0-309-04382-4

Infectious Diseases of Mice and Rats. 1991. $60.00. ISBN 0-309-03794-8

Companion Guide to Infectious Diseases of Mice and Rats. 1991. $12.00 each (free with purchase of Infectious Diseases of Mice and Rats). ISBN 0-309-04487-1

Immunodeficient Rodents: A Guide to Their Immunobiology, Husbandry, and Use. 1989. $29.95. ISBN 0-309-03796-4

Use of Laboratory Animals in Biomedical and Behavioral Research. 1988. $14.95. ISBN 0-309-03839-1

Nutrient Requirements of Laboratory Animals. 3d rev. ed. 1978. $12.95. ISBN 0-309-02767-5

Amphibians. Guidelines for the Breeding, Care, and Management of Laboratory Animals. 1974. $29.75. (photocopy of original, bound in paper cover). ISBN 0-309-00151-0

Nutrient Requirements of Domestic Animals: A Series— contact the National Academy Press for information on specific reports and prices.

The following ILAR publications are available from the **National Technical Information Service, 5282 Port Royal Road, Springfield, VA 22161.** Add $3 to the total order for the cost of shipping and handling.

Techniques for the Study of Primate Population Ecology. 1981. Paper cover, $31.00, Accession no. PB82 183120

National Survey of Laboratory Animal Facilities and Resources, Fiscal Year 1978. 1980. $17.00 Accession no. PB83 181347

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