GRANT NO: DAMD17-93-J-3016

TITLE: INSTITUTE OF LABORATORY ANIMAL RESOURCES (ILAR)

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CONTRACTING
ORGANIZATION: Institute of Laboratory Animal Resources
National Research Council
2101 Constitution Avenue, NW
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REPORT DATE: May 12, 1994

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research, Development,
Acquisition and Logistics Command (Provisional),
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	5/12/94	Annual Report, 12/31/92-12/30/93

4. TITLE AND SUBTITLE
Institute of Laboratory Animal Resources (ILAR)

5. FUNDING NUMBERS
Grant No.
DAMD17-93-J-3016

6. AUTHOR(S)
Thomas L. Wolfle

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Institute of Laboratory Animal Resources
National Research Council
2101 Constitution Avenue, NW
Washington, DC 20418

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research, Development,
Acquisition and Logistics Command (Provisional)
Fort Detrick
Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release;
distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

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). 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 is the Guide for the Care and Use of Laboratory Animals (Guide), of which the 6th revision was initiated during this grant year. Oversight for the work of ILAR is provided by ILAR Council, a standing committee of 13 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 those of staff in the publishing of ILAR News, in the activities of the Animal Models and Genetic Stocks Information Program, and International Activities.

14. SUBJECT TERMS	15. NUMBER OF PAGES
laboratory animal care and use, animal research, international activities, collection and importation of biologic materials, biologic databases, ILAR News, animal models and genetic stocks	12
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

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PI - Signature / Date

TABLE OF CONTENTS

1993 Annual Report
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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 the seventh edition was initiated 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 13 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 (roster attached).

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 1993, approximately 50 scientists, veterinarians, and medical ethicists served on ILAR committees.

I. Core Activities

A. **Meetings of ILAR Council.** Council met three times in 1993 to review ongoing work and plan new activitiess–March 3-4 at the Arnold and Mabel Beckman Center, Irvine, California; July 12-13 at the J. Erik Jonsson Woods Hole Center, Woods Hole, Massachusetts; and October 7-8, at the National Academy Sciences Building, Washington, D.C. The Beckman and Woods Hole centers are study sites of the Academy and enable greater participation of west and east coast members, respectively. In addition to the ongoing projects and new initiatives (see II. Special Projects), Council concentrated on the activities of the following three subcommittees of core activities.

B. *ILAR News.* One of the most visible and important of ILAR's activities is its quarterly journal, *ILAR News*. Published as a resource for animal care and use committees, scientists, and veterinarians, it provides timely news and information and includes peer-reviewed scientific articles solicited from experts, articles of interest to members of institutional animal care and use committees, short news items, and announcements of upcoming meetings and new books. *ILAR News* also periodically contains special inserts, usually ILAR committee reports, that can be distributed separately. Of particular interest to DOD investigators are the topical issues contained in each issue. The following issues were published in 1993 and are planned for 1994:

Winter 1993: Models of type I diabetes (first of two-part series).
Spring 1993: Models of type I diabetes (second of two-part series).
Summer/Fall 1993: Issues for institutional animal care and use committees. Includes a discussion of the role of the unaffiliated member, a perspective from an unaffiliated member, and a list of commonly asked questions and answers by the Office of Protection from Research Risks, U.S. Public Health Service.
Winter 1994: Farm animals in biomedical research. The first of a two-part series, this issue includes reviews of swine and transgenic farm animals in biomedical research, as well as an article discussing oversight of farm animals in biomedical research.
Spring 1994: Farm animals in biomedical research. The second of a two-part series, this issue includes reviews of the use of poultry and goats in biomedical research, and a discussion of integrating biomedical and agricultural research policies.
Summer/Fall 1994: Advances in gene therapy.

ILAR News has quietly changed in recent years from an informative newsletter to a reliable source of information on topics affecting biomedical investigators, animal care and use committees, and veterinarians. Concurrent with this change is the peer review of all original articles and oversight by a three-member Editorial Panel, a subcommittee of the ILAR Council, which is chaired by Dr. Margaret Jones of Michigan State University. This

change will be heralded in the Winter 1995 issue with a new masthead carrying the name *ILAR Journal* and announcements of editorial policies consistent with a journal carrying the imprimatur of the Academy. *ILAR Journal* will continue to fulfill the mission and goals currently performed by *ILAR News*. The redesign will permit the addition of new features that will enhance the ability of this periodical to serve the community.

C. **Animal Models and Genetic Stocks Information Program (AMGS).** The AMGS Information Program is ILAR's multifaceted communication and educational program for those with research and managerial responsibilities in biomedical research, as well as for members of Congress and congressional staff, students, and other members of the public. Through this program, which is managed by staff with oversight by a subcommittee of the Council, ILAR distributes information on a wide range of topics relating to the availability, care, and use of animals in research, testing, and teaching. To answer requests, staff draw on many resources: ILAR's two databases (discussed below); ILAR publications, including *ILAR News*; ILAR's resource library; and the expertise of the many scientists worldwide with whom ILAR interacts.

The number of requests that the program receives increased during the past several years. In 1993 there was a 54 percent increase in the number of questions received from 1,752 in 1992 to 2,691 in 1993. Inquiries took the form of requests for ILAR publications (27 percent) or requests for specific information on laboratory animals (73 percent). Of the 1,965 non-publication requests, 721 (37 percent) were for sources of rats or mice. Other animals requested include guinea pigs, hamsters, rabbits, primates, farm animals, amphibians and reptiles, fish, dogs and cats, birds, and invertebrates. Information on standardized nomenclature, appropriate animal models, use of animals in biomedical research, and guidelines and regulations was also requested. In addition, 2,697 ILAR publications were distributed free-of-charge, and 1,086 were sold by the National Academy Press.

At the heart of the program are two databases: the Animals for Research (AFR) database–which comprises commercial and investigator colonies of laboratory, farm, and wild animals available for research–and the Registry of Laboratory Codes–which lists the one- to four-letter symbols used in conjunction with standardized nomenclature as unique identifiers of laboratories or institutions that maintain breeding colonies of rodents and rabbits. ILAR has maintained the registry since 1984 at the request of the International Committee on Standardized Genetic Nomenclature for Mice. The registry has become increasingly important in the past few years as two large databases, GBASE (the Jackson Laboratory's mouse genome database) and TBASE (Oak Ridge National Laboratory's transgenic animal and targeted mutation database, now maintained at and made available on-line by The Johns Hopkins University) have required that investigators acquire a laboratory code before entering information into the databases.

The ILAR databases are currently maintained on a Wang VS 100 mainframe computer; however, the databases must be moved to another system to comply with NRC

administrative directives. Consequently, the AMGS subcommittee recommended to the ILAR Council that ILAR explore making the databases available on-line through a computer network. To seek outside advice, ILAR will convene a workshop supported by NRC Program Initiation Funds on January 7, 1994. More than 50 experts in computer science, scientists who use biological resource databases, users of the AMGS Information Program, and representatives of federal agencies will meet to discuss whether the database should be developed as a computer network resource and, if so, what the format should be and how it should be established. This meeting will follow a meeting on January 6, 1994, at which the participants will deliberate on problems associated with biological resource database programs. Five issues will be discussed: integration, accessibility, quality control, intellectual property rights and custodianship.

D. **International Activities.** ILAR is recognized nationally and internationally as an independent, scientific authority. In 1993, a subcommittee of ILAR Council was formed to help achieve ILAR's two primary goals in international activity, which are (1) endorsement of policies that enhance biomedical science globally and that empower scientific efforts in developing countries, and (2) promotion of the exchange of information and education in partnership with developing countries. In collaboration with the Pan American Health Organization, the Fogarty International Center, and the International Council on Laboratory Animal Science (ICLAS), ILAR's International Subcommittee focused on the following program objectives:

- Form an information resource for new developments both in international biomedical science that depends on research using animals and in guidelines and standards for laboratory animal care and use.
- Catalyze and assist in the recognition of new scientific opportunities in human health, together with animal resources (such as transgenics) required for rapid development.
- Provide high quality information in current topics affecting health research based on the animal sciences.
- Foster training, symposia, and fellowship in collaboration with other agencies, where mutual objectives coincide.
- Make ILAR documents available in the languages represented by our priority interests, with first priority being Spanish.
- Form partnerships wherever possible with science-based industries in developing the above objectives, in technology transfer, and in the availability of reagents and assays.
- Develop specific plans for the support of ILAR international activities in conjunction with other agencies and industry as appropriate.

From these international objectives the following priorities were established:

1. Work with **Canadian** colleagues to foster greater cooperation in the animal sciences between our two countries and to foster the development of scientific and commercial opportunities within the framework of the North American Free Trade Agreement (NAFTA).

2. Commencing with **Mexico**, and then other Caribbean, Central, and South American countries, translate and widely distribute our key publications, such as the *Guide for Care and Use of Laboratory Animals*, the *Laboratory Animal Management* series, and *ILAR News*. Develop a database, working with the Mexican Academy of Science and other National Academies where possible, that will list major laboratories, organizations, and individuals in the field, and invite Canadian and Mexican representatives to meetings of ILAR Council.

3. In cooperation with **Japan** and NIH, continue programs under the U.S.-Japan Non-energy Agreement that in 1993 led to the development and adoption of rat nomenclature and genetic strains agreements, transgenic nomenclature, the Manual of Murine Diseases, and ICLAS Monitoring Centers for genetic and microbiological quality control of laboratory rodents. ILAR will assume a more active role in the Agreement for future projects.

4. In response to recent General Agreement on Tariffs and Trade (GATT) meetings in **Europe** and rapid changes in the former **Soviet Union**, monitor activities that may lead to non-tariff trade barriers, impediments to free exchange of U.S. products, and recognition of scientific property rights. Seek to collaborate with individuals and organizations in these countries who can provide early information about new regulations in the animal sciences, drug and food testing protocols, and the import and export of animals and biologicals. A partnership with U.S. industry will be explored to sustain these activities.

Rapid communication is key to each of these areas of activity. ILAR is already developing a list of biomedical databases that should be internationally accessible within a few years. Much additional work is needed, however, to achieve truly efficient on-line exchange of information. Electronic communication is a goal of the Biological Resource Database workshop and a workshop to be held in March (see below) on U.S. policies and regulations on access to and importation of animals, plants, and biological products.

The *ILAR News* Editorial panel, AMGS Subcommittee, and International Subcommittee met together to discuss mutual areas of activity. Forthcoming will be regular columns on international activity in *ILAR News*, and the coordination of international access to ILAR databases.

II. Special Projects

ILAR's special projects represent the second primary focus of ILAR's activity. These projects are requested and funded by sponsoring agencies and normally result in published NRC reports written by NRC-appointed committees. Although supported independently from the core activities, special projects represent many of the activities for which ILAR is

best known. 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 activities, including a summary of accomplishments during 1993 in each, and plans for the future.

A. **Revision of the *Guide for Care and Use of Laboratory Animals* (*Guide*).** The current edition of ILAR's best known report was last published in 1985. The normal 4-5 year revision cycle was delayed due to the 1985 passage of amendments to the Animal Welfare Act and the Health Extension Act of 1985. It was considered desirable to delay revision until the strengths and weaknesses of the regulations promulgated by the U.S. Department of Agriculture and the Public Health Service were evaluated. Following recommendations of an advisory panel meeting in 1992, proposals were developed and submitted to NIH and other federal agencies for development of the seventh edition. In 1993, a grant was awarded from NIH as lead agency and the committee was appointed.

One of the first tasks of this committee (roster attached) was to hold public meetings to gather information from members of the public, scientists, veterinarians, scientific associations, and animal protection organizations. Two public meetings were held in 1993–at the national meeting of the American Association of Laboratory Animal Science (AALAS) in Nashville, Tennessee and at the National Academy of Sciences in Washington, D.C. Four other public meetings are planned for 1994–in San Francisco, St. Louis, and at the Public Responsibility in Medicine and Research (PRIM&R) and Applied Research Ethics National Association (ARENA) meetings in Boston.

At the October 1993 meeting in Washington, D.C., several presenters asked that ILAR appoint an additional member who could represent the views of the public. In response to this request, ILAR is consulting with scientists, members of humane societies, and the senior management of the NRC and plans to appoint an additional public member.

The seventh edition of the *Guide* is expected to be published in mid-1995.

B. **Laboratory Animal Management Series.** As companions to the *Guide*, ILAR extensively revised two species-specific reports in the *Laboratory Animal Management* series. *Rodents: Laboratory Animal Management* revises and combines three earlier reports, *Laboratory Animal Management: Rodents* (1977); *Laboratory Animal Management: Genetics* (1979); and *Long-Term Holding of Laboratory Rodents* (1976). *Dogs: Laboratory Animal Management* revises a 1973 report entitled *Dogs: Standards and Guidelines for the Breeding, Care, and Management of Laboratory Animals* and includes a new section on care and management of dogs characterized for specific research protocols. Both reports will be published by the National Academy Press by summer 1994.

Plans were initiated in 1993 to revise three other Laboratory Animal Management reports on nonhuman primates, swine, and ruminants. Proposals have been prepared for

these reports. Committees will be appointed and the revisions will begin when funding is obtained.

The selection and prioritization of these and other ILAR committee reports originates with ILAR Council and represents one area in which core supported activities (Council) merge with special projects.

C. **Occupational Health and Safety of Personnel in Research Animal Facilities.** At the request of the federal Interagency Research Animal Committee (IRAC) and with the leadership of the NIH, National Center for Research Resources, a committee with expertise in occupational health and safety will provide detailed recommendations for institutional programs on occupational health and safety, as required by the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy–as prescribed by the Health Research Extension Act of 1985), the *Guide*, and the American Association for Accreditation of Laboratory Animal Care. The committee convened five times in 1993, and the report is being prepared for NRC peer review. The report, cosponsored by multiple federal agencies and pharmaceutical companies, is scheduled for publication in the fall of 1994.

Key sections of the report will address zoonotic diseases transmissible from animals to humans; essential institutional policies, responsibilities, and authorities; allergies to animals; safety assessment; employees' serum banking and routine physical examinations; and recommendations for determining whether, for whom, and how often procedures should be performed.

D. **Psychological Well-being of Nonhuman Primates.** Mandated by the Animal Welfare Regulations, nonhuman primates bred, maintained, or used for research, testing, education, and exhibition must be provided a physical environment suitable to enhance their psychological well-being. At the request of the National Institutes of Health and the U.S. Department of Agriculture, an NRC-appointed committee began work in 1992 to prepare recommendations for compliance with this requirement. During 1993, seven drafts of the report were prepared, which is nearing completion for submission for NRC peer review. The report is scheduled for publication by the National Academy Press in the summer of 1994.

The committee will provide readers with a structure by which to develop a functional psychological well-being program. It will provide strategies for animal care personnel to use in developing enrichment techniques, for animal care and use committees and veterinarians to use in assessing compliance with federal requirements, and for animal welfare inspectors and site visitors to use in assessing the success of the program in achieving the goals of well-being. The recommendations will not provide prescriptive blueprints of a program that can be transported to many institutions. Variations among species; among individuals of a species; and among research institutions, zoos, and research goals are discussed and provide the basis for recommending performance assessment and professional judgement.

Should the appeal of a pending law suit to overturn current Animal Welfare Regulation be rejected, the committee's report will be available for U.S. Department of Agriculture's consideration in rewriting the standards. If the existing regulations stand, the committee's report will provide guidelines by which each institution can develop thoughtful programs for each animal in the facility. Unlike a single engineering standard used for all animals, even those of a single species, the committee's report will provide the background and basis by which each institution can tailor well-being plans to meet its needs while ensuring the well-being of each animal. Of equal importance, the report will enable site visitors, federal inspectors, and members of the public to be able to assess the adequacy of the institutional plan in fulfilling its goal.

Committees of the NRC, such as ILAR's Committee on the Psychological Well-being of Nonhuman Primates, provide unbiased, in-depth reports on issues of national interest. It is unlikely that any other institution or mechanism could provide the balance and credibility needed in times of intense national debate.

E. **Workshop on Biological Resource Databases.** The rapid growth of biological resource databases is providing a major new research resource for life scientists in disciplines ranging from molecular genetics to landscape ecology. At the same time, questions and concerns are growing about such issues as access to databases, custodianship, and integration. A coherent national strategy is needed to address these issues in a timely fashion so that this major new component of our research infrastructure can develop smoothly and effectively.

To discuss whether an NRC study could assist in instituting such a strategy, ILAR will hold a workshop on January 6-7, 1994. The workshop participants will be asked to consider whether the NRC should establish a committee to develop a plan for a **National Biological Information Infrastructure** that will make key databases comparable and easily accessible to the scientific community. The recommendations of the workshop participants are expected to call for the NRC to assist in identifying and providing the kinds of information that are needed; quality control measures to ensure adequate evaluation of components of a database; measures for ensuring adequate support for the infrastructure; criteria for developing new databases, evaluating database management, and deciding when and how best to terminate or archive a database; and procedures for implementing the plan.

ILAR's **AMGS Information Program** (see p. 3), which includes a database of sources of animals for research (AFR), is a small piece of the growing information network and will also be discussed at the January 6-7 Workshop on Biological Resource Databases. The participants will be asked to review the current structure and function of AFR; recommend how this database can be updated and expanded; whether this type of data could effectively be made available on-line, either as a stand-alone system or as a component of an existing database such as GBASE; and the advisability of establishing and maintaining a bulletin board to facilitate communication among users and between users and ILAR staff.

Following NRC Governing Board approval, it is anticipated that NRC-appointed committee(s) will examine the proposal to pursue the recommendations of the National Biological Information Infrastructure and the ILAR AFR database. It is expected that these committees will be appointed before the end of 1994. Expressions of support have been received from the National Science Foundation, the Department of Energy, the National Institutes of Health, and the Department of Agriculture. Examples of questions to be addressed include the following:

- How can available databases be accessed by those who need the information?
- How should potential users be informed about the databases within and across disciplines?
- How should these resources be integrated for more efficient and effective use within and across disciplines?
- To what extent and how should they be standardized to simplify access, data entry, and data retrieval?
- How can these powerful resources be used to their full potential?
- Who owns or should own the information contained in a database?
- What are the best approaches for ongoing quality control, maintenance, and support of these databases?

F. **Workshop on Collection and Importation of Biological Material, Animals, and Plants.** Rules and regulations governing the collection, importation, and movement of biological materials across state and international boundaries have become increasingly complex and time consuming. Achieving compliance has become a serious concern for both investigators in a variety of scientific disciplines and for agency personnel who administer the regulations.

Understanding and complying with these laws, guidelines, and constraints is difficult, time consuming, and costly. Research institutions lack the specific information required to change the regulations or improve their compliance with them. Investigators are only generally informed about the regulations, and the majority of the public is totally unaware of them. Regulating agencies recognize the cost and difficulty of compliance but have limited resources by which to improve the permitting process. To discuss whether an NRC study could assist with these problems, ILAR is planning a workshop during the 1994 grant year, which will be held on March 16-17, 1994.

These issues are of interest to regulatory agencies and to public and private institutions. Regulations administered by the Department of Commerce, National Oceanographic and Atmospheric Administration; Department of Health and Human Services/Public Health Service, Centers for Disease Control and Prevention; Department of Interior, Fish and Wildlife Service; the Department of Agriculture/Animal and Plant Health Inspection Service, National Center for Import/Export; and Department of Treasury, Customs Service

have authority over some aspect of these issues. Regulated entities whose activities may come under the jurisdiction of one or more of these agencies include biomedical and zoological institutions; natural history museums; biology, zoology, and botany departments in academic institutions; pharmaceutical firms; and conservation organizations. Department of Defense medical research laboratories likewise must comply with the policies and regulations of many of these agencies when collecting or transporting biological materials, animals, or plants.

To discuss the impact of these policies and regulations and to make recommendations for further NRC involvement, ILAR is organizing a workshop to be attended by scientists and administrators concerned with *obtaining* permits and scientists and administrators concerned with *granting* permits. There will be presentations from the representatives of the pharmaceutical industry, field biologists, and representatives of agencies concerned with permitting. Customs issues and packaging issues will also be discussed. After the presentations, three smaller working groups will meet to discuss the permitting process, current policies and regulations, and the scientific basis for the regulations. It is expected that the consensus of the workshop will be that the NRC be asked to form a study committee and a forum, both composed of scientists, regulators, scientific societies, and members of the public.

Examples of the types of issues a **committee** might be asked to study, include the following:

- managing resources including personnel training and retention;
- simplifying and harmonizing regulations, redesigning application forms, and exploring the federal regulatory process to enhance interaction among scientists, the public, and agency personnel when writing regulations;
- educating both scientists and personnel who grant permits about permitting requirements; educating scientists on the opportunities presented by notices in the Federal Register;
- electronically distributing educational material and permit forms, such as by a telephone menu driven fax system, an on-line query database, or ultimately creating a central electronic clearinghouse; and
- implementing procedures at local, state, federal, and international levels that would facilitate opportunistic collection.

A **forum**, convened periodically, might be asked to discuss:

- agency policies and permitting practices;
- form design; and
- specific topics for in depth study by separate NRC-appointed committees.

G. A Workshop to Examine the Appropriate Use of Animals and Their Alternatives in Education. A project of high priority for the NRC is one that examines the merits of the use of animals and their alternatives to enhance the precollege educational experience of students. A larger three-report version of this study was approved previously by the NRC, but failed to materialize due to lack of funding. This refocused study is being proposed as a three-day workshop in which an NRC-appointed committee will define the objectives of animal use, examine proper treatment of animals by students and teachers, and develop two reports. The first report will be a technical document that reflects the workshop's discussions. The second will be a summary document for lay audiences. The two reports will be of interest to teachers at the K-12 levels, school administrators, science supervisors, local, state, and federal officials, parents, federal agencies, and professional societies. Funds have been budgeted to cover the costs of production, printing and distribution of 2,000 copies of the workshop's technical summary and 50,000 copies of the summary report. Fundraising for the project is continuing.

CONCLUSIONS AND FUTURE DIRECTIONS

Four reports discussed above will be published in 1994 (1) *Occupational Health and Safety of Personnel in Research Animal Facilities*, (2) *Psychological Well-being of Nonhuman Primates*, (3) *Dogs: Laboratory Animal Management*, and (4) *Rodents: Laboratory Animal Management*. The seventh edition of the *Guide* will be published in 1995.

The **Biological Resource Database** workshop and the workshop on **Collection and Importation of Biological Material, Animals, and Plants** will both be held in early 1994. Recommendations from the workshops will be used to develop future projects.

The application of transgenic technologies to animals is having a major impact on society and has caused many concerns in relation to risks, benefits, and ethical issues. ILAR plans on organizing a conference on *Transgenic Animals: Benefits and Risks* as one of a series of conferences in the Arnold and Mabel Beckman Conference Series (An Irvine, California NAS Study Center). Approximately 12-15 scientists will be invited to make presentations to prepare a white-paper report for dissemination to the media, the Congress, and the public. The report is intended to convey information and perspective to the pubic and to the Congress. Following the publication of *Standardized Nomenclature for Transgenic Animals* (*ILAR News*, 34:4, 1992) this conference proposes to explore the ethical and public policy issues involved in the development and use of biologically modified organisms.

ILAR reports continue to provide valuable information on emerging topics and to reevaluate procedures for the care and use of research animals. As important as the content of ILAR reports is the identification of shortcomings in scientific knowledge around which guidance is developed. This approach substantiates the use of performance-standards used in each report. ILAR's constructive, responsible guidelines identify qualitative (results

oriented) rather than quantitative (process or engineering oriented) approaches. These reports continue to provide valuable leadership for agencies responsible for the oversight of national animal welfare regulations.

ILAR emphasized dissemination of its reports and recommendations in 1993. The principal reason a sponsor requests an NRC study is to obtain the scientific credibility that is part of the imprimatur of the National Academy of Sciences. Increasingly, sponsors have asked that work of the staff and authoring committee extend beyond the publication of a report to include dissemination activities. Cost-effective dissemination strategies are being developed, which in 1993 included participation in ten regional and national scientific meetings, presentations to scientific societies, and institutional sponsored workshops to which ILAR representatives were invited.

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Appendix 1
ILAR Committee Rosters

Institute of Laboratory Animal Resources Council
Committee on Dogs
Committee on Rodents
Committee on Occupational Health and Safety of Personnel in Research Animal Facilities
Committee on the Psychological Well-being of Nonhuman Primates
Committee to Revise the *Guide for the Care and Use of Laboratory Animals*
Committee on Transgenic Nomenclature
Committee on Rat Nomenclature

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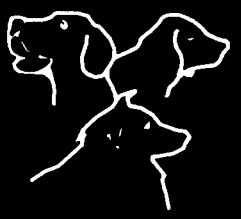
1993 Annual Report
Institute of Laboratory Animal Resources
National Research Council
Grant Number DAMD17-93-J-3016

Appendix 2
ILAR Reports

Dogs: Laboratory Animal Management
ILAR News, Volume 35, Number 1, Winter 1993
ILAR News, Volume 35, Number 2, Spring 1993
ILAR News, Volume 35, Number 3-4, Summer/Fall 1993
Standardized Nomenclature for Transgenic Animals
Definition, Nomenclature and Conservation of Rat Strains

Laboratory Animal Management

DOGS



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DOGS

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NATIONAL ACADEMY PRESS
Washington, D.C. 1994

National Academy Press • 2101 Constitution Avenue, N.W. • Washington, D.C. 20418

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 competencies 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.

This study was supported by the U.S. Department of Health and Human Services (DHHS) through contract number NO1-CM-07316 with the Division of Cancer Treatment, National Cancer Institute; the Animal Welfare Information Center, National Agricultural Library, U.S. Department of Agriculture (USDA), through grant number 59-32U4-8-60; and Regulatory Enforcement and Animal Care, Animal and Plant Health Inspection Service, USDA, through grant number 59-32U4-8-60. Additional support was provided by the following members of the Pharmaceutical Manufacturers Association: Berlex Laboratories, Inc., Cedar Knolls, New Jersey; Bristol-Myers Squibb Co., New York, New York; Bristol-Myers Research, Princeton, New Jersey; Burroughs Wellcome Co., Research Triangle Park, North Carolina; Dupont Merck Research & Development, Wilmington, Delaware; Johnson & Johnson, New Brunswick, New Jersey; Marion Merrell Dow Inc., Kansas City, Missouri; Pfizer Inc., Groton, Connecticut; Schering-Plough Research, Bloomfield, New Jersey; SmithKline Beecham Pharmaceuticals, Swedeland, Pennsylvania; and Syntex Research, Palo Alto, California.

ILAR's core program is supported by grants from the National Center for Research Resources, National Institutes of Health; National Science Foundation; American Cancer Society, Inc.; and U.S. Army Medical Research and Development Command, which is the lead agency for combined Department of Defense funding also received from the Human Systems Division, 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.

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

Library of Congress Cataloging-in-Publication Data

Dogs : laboratory animal management / Committee on Dogs, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council.
p. cm.
Includes bibliographical references and index.
ISBN 0-309-04744-7
1. Dogs as laboratory animals. I. Institute of Laboratory Animal Resources (U.S.). Committee on Dogs.
SF407.D6D64 1994
636.7'0885—dc20 94-960
CIP

Printed in the United States of America

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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 serves as a coordinating agency and a national and international resource for compiling and disseminating information on laboratory animals, promoting education, planning and conducting conferences and symposia, surveying existing and required facilities and resources, upgrading laboratory animal resources, and promoting high-quality, humane care of laboratory animals in the United States.

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Preface

It has been 2 decades since the Institute of Laboratory Animal Resources first published *Dogs: Standards and Guidelines for the Breeding, Care, and Management of Laboratory Animals* (National Academy of Sciences, Washington, D.C., 1973). During that period, great strides have been made in improving care and management techniques, making available specific-pathogen-free and purpose-bred dogs, and identifying dogs with precisely defined genetic disorders. The dog has proved to be "man's best friend," not only because it is considered a companion and family member, but also because its use in research has been associated with many breakthrough discoveries in human medicine (e.g., the discovery of insulin as a treatment for type I diabetes mellitus).

The same period has been characterized by increased public awareness and scrutiny of research funding, occupational health and safety, and animal welfare. New federal and state laws specifically intended to protect research animals have been promulgated and regulations established. In addition to presenting information relevant to the care and use of dogs in research and making recommendations based on an objective evaluation of that information, it was the committee's intent to incorporate in this report those aspects of canine husbandry embodied in federal law. Federal regulations and policies protecting dogs in research are therefore summarized in Chapter 1, which provides information for obtaining copies. Specific details of the regulations and policies are given throughout the text.

The committee firmly believes that good research requires a good animal-care program. The committee is also aware of the tremendous variation in physiologic traits among canine models. Dogs vary greatly in size, age, health status, physical conformation of the breed, behavioral characteristics, and experience. Therefore, no standard of animal care is likely to be optimal for all dogs. The committee recommends that performance standards be used with sound professional judgment in implementing the animal-care program.

Readers who detect errors of omission or commission or who have evidence to support improved procedures are invited to send suggestions to ILAR, National Research Council, 2101 Constitution Avenue, Washington, DC 20418.

The committee wishes to thank the entire staff of ILAR, but especially Dr. Dorothy Greenhouse and Ms. Amanda Hull, for assisting in the production of this manuscript. The committee also acknowledges the many fine contributions made to this report by scientists specializing in the care and use of dogs in research; their names appear on pages *iv* and *v*.

Fred W. Quimby, *Chairman*
Committee on Dogs

Contents

Laboratory

Animal

Management

DOGS

1

Introduction

Dogs make valuable contributions in biomedical research because they share many biochemical and physiologic characteristics with humans and spontaneously develop disorders that are homologous to pathologic conditions in humans. While using them as models for human disease, we have also learned much about normal physiologic processes in dogs themselves. Advances in molecular genetics, reproduction, behavior, immunology, hematology, endocrinology, microbiology, nutrition, pharmacology, and oncology, to name a few, have made dogs more valuable as models and, at the same time, have provided veterinarians with useful information for the diagnosis and treatment of canine diseases.

In the past 2 decades, two amendments to the Animal Welfare Act (in 1976 and 1985) and a section added to the Health Research Extension Act of 1985 have resulted in revised standards for dogs. Institutions that use dogs must comply with the Code of Federal Regulations, Title 9, Subchapter A, Parts 1-3 (9 CFR 1-3), commonly called the Animal Welfare Regulations (AWRs), which were promulgated to administer the Animal Welfare Act. Institutions receiving Public Health Service (PHS) funding must also comply with the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (hereafter called the *PHS Policy*) (PHS, 1986), which in turn requires compliance with the *Guide for the Care and Use of Laboratory Animals* (hereafter called the *Guide*) (NRC, 1985). Some of the AWRs are based on engineering standards (e.g., that on space requirements for dogs), but most rely on performance standards (i.e., the demonstration of

animal well-being). It is expected, therefore, that professional judgment will be used in applying the AWRs. It is also incumbent on all people using dogs to seek improvements in the methods for using them.

This edition of *Dogs: Laboratory Animal Management* incorporates features of housing, management, and care that are related to the expanded use of dogs as models of human diseases and an intrepretative summary of the AWRs and requirements of the *PHS Policy*. The appendix lists subjects within this text by page number with cross references to corresponding sections in the AWRs and the *Guide*. The regulations, policies, and guidelines that are applicable to dogs include the following:

- Code of Federal Regulations, Title 9, Subchapter A, Parts 1-3 (commonly called the Animal Welfare Regulations). Available from Regulatory Enforcement and Animal Care, APHIS, USDA, Federal Building, Room 565, 6505 Belcrest Road, Hyattsville, MD 20782 (telephone, 301-436-7833).
- *Public Health Service Policy on Humane Care and Use of Laboratory Animals*. Available in English or Spanish from the Office for Protection from Research Risks, Building 31, Room 5B59, NIH, Bethesda, MD 20892 (telephone, 301-496-7163).
- *Guide for the Care and Use of Laboratory Animals*. Available in English or Spanish from the Office for Protection from Research Risks, Building 31, Room 5B59, NIH, Bethesda, MD 20892 (telephone: 301-496-7163). Single copies (English only) available from the Institute of Laboratory Animal Resources, National Research Council, 2101 Constitution Avenue NW, Washington, DC 20418 (telephone, 202-334-2590).
- Code of Federal Regulations, Title 21, Part 58; Title 40, Part 160; and Title 40, Part 792 (commonly called the Good Laboratory Practice, or GLP, Standards). Available from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402 (telephone, 202-783-3238).
- IATA Live Animal Regulations. Available in English, French, or Spanish from the International Air Transport Association (IATA), 2000 Peel Street, Montreal, Quebec, Canada H3A 2R4 (telephone, 514-844-6311).

All animals used in research must be treated with the dignity and respect due living beings. Those who use animals in experiments must therefore be properly trained in methods appropriate for the species used. It is the responsibility of each research facility to develop educational programs for animal-care providers and the research staff (9 CFR 2.32). Recommendations for establishing such programs have recently been published (NRC, 1991).

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2

Criteria for Selecting Experimental Animals

Scientists who are planning experiments evaluate both animal and nonanimal approaches. If there are no suitable alternatives to the use of live animals, the appropriate species is selected on the basis of various scientific and practical factors, including the following:

- Which species will yield the most scientifically accurate and interpretable results?
- According to critical review of the scientific literature, which species have provided the best, most applicable historical data?
- On which species will data from the proposed experiments be most relevant and useful to present and future investigators?
- Which species have special biologic or behavioral characteristics that make them most suitable for the planned studies?
- Which species have features that render them inappropriate for the planned studies?
- Which species present the fewest or least severe biologic hazards to the research team?
- Which species require the fewest number of animals?
- Which species that meet the above criteria are most economical to acquire and house?

For many scientific experiments, the answer to those questions will be the domestic dog, *Canis familiaris*. The size, biologic features, and coop-

erative, docile nature of the well-socialized dog make it the model of choice for a variety of scientific inquiries. The contributions of the dog to human health and well-being are numerous (Gay, 1984).

Although research with dogs is often primarily to benefit humans, it has also greatly benefited dogs that are kept as companion animals. Examples of the benefits to dogs are improvements in diagnostic techniques; treatments for diabetes and arthritis; surgical procedures for correcting or treating cardiovascular, orthopedic, and neurologic disorders; and therapies for bacterial, neoplastic, and autoimmune diseases. Moreover, dogs have been necessary for the development of vaccines that protect companion animals against viral diseases (e.g., distemper and parvovirus disease) and drugs that prevent parasitic diseases (e.g., dirofilariasis, or heartworm disease).

GENETIC FACTORS

All domestic dogs, irrespective of breed, are *Canis familiaris*. Canine genotypes and phenotypes vary among breeds as a result of selective breeding, which has created variations in allele frequency between breeds. Although "pure" breeds might have a higher frequency of some genes, much genetic variation remains in most breeds.

The canine karyotype consists of 78 chromosomes (Minouchi, 1928). Most of the autosomes are acrocentric or telocentric, and many pairs do not differ markedly in size. Recently, an improved method for staining canine chromosomes has been developed that makes karyotyping with Giemsa banding feasible (Stone et al., 1991).

A number of loci have been identified that code for the antigens of the canine major histocompatibility complex, which has been designated DLA (Vriesendorp et al., 1977). Initially, several alleles were defined with serologic techniques at three class I loci, and several alleles were defined with cellular techniques at a DLA class II locus (Bull et al., 1987; Deeg et al., 1986). Molecular techniques are being used to refine the definition of the DLA class I loci, and at least eight class I genes have been demonstrated in the dog (Sarmiento and Storb, 1989). Molecular-genetic studies to characterize canine class II loci correlate well with earlier work in which techniques for cell typing for class II antigens were used (Sarmiento and Storb, 1988a,b). The characterization of canine DLA loci is extremely useful for transplantation studies (Ladiges et al., 1985) and for demonstrating an association between the major histocompatibility complex and some inherited canine diseases (Teichner et al., 1990).

Attempts are under way to develop maps that identify the location of canine genes that control particular traits (e.g., inherited diseases and such behavioral tendencies as herding and aggression). Two approaches are used. The first relies on the principle that the relative positions of genes in a

particular region of DNA are comparable in humans, dogs, and other species. Conserved regions can be identified in DNA samples with restriction-fragment length polymorphisms (usually called RFLPs) that have been identified with probes for human and murine genes whose chromosomal locations are known. To enhance the detection of polymorphisms, investigators sometimes produce dog-coyote hybrids, cross-breed two widely divergent dog breeds, or analyze a large, well-defined canine kindred (Joe Templeton, Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, Tex., personal communication, 1993). The second approach uses simple sequence-repeat polymorphisms (microsatellite probes). Specific simple sequence-repeat markers that are highly polymorphic in dogs have been developed to study the canine genome (Ostrander et al., 1992, 1993). These and other techniques, such as chromosomal in situ hybridization and somatic cell hybridization, will likely greatly increase our understanding of canine genetics.

Inherited defects—including lysosomal storage diseases, retinal degenerations, coagulopathies, complement deficiency, and various musculoskeletal, hematopoietic, immunologic, and neurologic diseases—are common in purebred dogs, and many specific disorders are found most commonly in particular breeds (Patterson et al., 1989). This phenomenon might be related, in part, to breeders' inadvertent selection for mutant alleles that are closely linked to loci that determine breed-typical traits or to the chance increase in frequency of particular mutant alleles caused by the founder effect or random genetic drift. The high frequency of inherited canine disorders (compared with murine disorders) was recognized as early as 1969 (Cornelius, 1969). During the 20-year period 1960-1980, 20 percent of more than 1,200 literature citations on naturally occurring animal models of human diseases involved dogs (Hegreberg and Leathers, 1980). A compilation in 1989 noted that 281 inherited disease entities had been reported in dogs (Patterson et al., 1989). Many of those constitute the only animal models for investigating the corresponding human diseases (Patterson et al., 1988). The 19-fascicle *Handbook: Animal Models of Human Disease* (RCP, 1972-1993) lists 83 canine models of human diseases, many of which are hereditary, and the two-volume *Spontaneous Animal Models of Human Disease* (Andrews et al., 1979) describes many canine models.

In scientific studies in which genetic uniformity is desirable or in long-term studies in which the expected differences between experimental and control subjects are likely to be small, purpose-bred dogs (e.g., beagles) might be a more appropriate choice than dogs of unknown provenance. An advantage of using beagles, as opposed to other purpose-bred dogs, is the potential availability of other members of the kindred. But if the studies are to determine the greatest range of a variable that is likely to occur among the experimental subjects or if the experiments are of short duration, ran-

dom-source dogs might be more useful and less expensive (see "Procurement" in Chapter 5).

BIOLOGIC FACTORS

Dogs are monogastric carnivores with a short generation time (i.e., the calculated interval between when a pup is born and when its first offspring could be born) and a maximum life span of approximately 20 years; larger breeds appear to have a shorter maximum life span than smaller breeds. The canine mortality rate doubles every 3 years, compared with every 0.3 year for the rat (maximum life span, 5.5 years), every 15 years for the rhesus monkey (maximum life span, more than 35 years), and every 8 years for humans (maximum life span, more than 110 years) (Finch et al., 1990). Dogs are useful models for studying the lifetime effects of environmental factors, and there is an extensive literature on their use in radiation biology (see Gay, 1984; Shifrine and Wilson, 1980).

Selective breeding has resulted in a spectrum of behaviors and a large range of canine body sizes, from the giant breeds (e.g., Irish wolfhound), which can measure 91 cm (36 in) at the shoulder and weigh more than 56 kg (124 lb), to the toy breeds (e.g., Pomeranian), which can measure less than 31 cm (12 in) in height and weigh less than 4.5 kg (10 lb). Larger dogs, which can include mongrels or dogs of unknown breeding, are particularly well suited to cardiovascular, transplantation, and orthopedic studies, because body weights and blood volumes approximate those of humans (see Gay, 1984; Shifrine and Wilson, 1980; Swindle and Adams, 1988). The dog's size also lends itself to procedures that cannot be carried out in smaller species, e.g., when the instrumentation essential for collecting scientific data is bulky and cannot be miniaturized and when the resolution of imaging equipment requires a larger target field than is available in a small animal.

An individual dog often can be studied in great detail or in many ways, which might reduce the number of subjects needed for a study and generate a more definitive data set. For example, it is possible to take multiple blood samples of several milliliters each from a single dog over some period without compromising the dog's well-being, but taking samples of similar size during the same period from a single mouse or rat would be impossible.

BEHAVIORAL FACTORS

The social unit for dogs is the pack, and most dogs can be socialized to accept humans as the dominant individual in their social hierarchy, especially if the techniques used to socialize them provide rewarding experiences (e.g., food treats, petting, and verbal reinforcements) and minimize

TABLE 2.1 Selected Canine[a] Zoonoses

Disease in Humans	Agent	Mode of Transmission (Intermediate Host or Vector)[b]
Acariasis	*Cheyletiella yasguri*	Direct
Amebiasis	*Entamoeba histolytica*	Direct
American trypanosomiasis (Chagas' disease)	*Trypanosoma cruzi*	Indirect (triatomine insect)
Brucellosis	*Brucella canis*	Direct
Campylobacteriosis	*Campylobacter jejuni*	Direct
Coenurosis	*Taenia multiceps*	Direct
Colibacillosis	Enteropathogenic *Escherichia coli*	Direct
Cutaneous larva migrans	*Ancylostoma braziliense*	
	Ancylostoma caninum	Direct
Dipylidiasis	*Dipylidium caninum*	Indirect (dog flea)
Df2 infections	Dysgonic fermenter-2	Direct
Dirofilariasis	*Dirofilaria immitis*	
	Dirofilaria repens	Indirect (mosquito)
Giardiasis	*Giardia intestinalis* (*canis*)	Direct
Hydatidosis	*Echinococcus granulosus*	Direct
Larva currens	*Strongyloides stercoralis*	Direct
Leishmaniasis (cutaneous)	*Leishmania braziliensis peruviana*	Indirect (phlebotomine flies)
Leishmaniasis (visceral)	*Leishmania donovani*	Indirect (phlebotomine flies)
Leptospirosis	*Leptospira* spp. (usually *L. canicola*)	Direct
Pasteurellosis	*Pasteurella multocida*	Direct
Rabies	Rabies virus	Direct
Ringworm (dermatomycoses)	*Microsporum canis*	Direct
	Trichophyton mentagrophytes	
Rocky Mountain spotted fever	*Rickettsia rickettsii*	Indirect (tick)
Salmonellosis	*Salmonella* spp.	Direct
Scabies	*Sarcoptes scabiei*	Direct
Tularemia	*Francisella tularensis*	Indirect (tick)
Visceral larva migrans	*Toxacara canis*	Direct
	Toxascaris leonina	
Yersiniosis	*Yersinia enterocolitica*	Direct

[a]North, Central, and South American dogs.

[b]Direct = transmission by direct contact with the dog, its excretions, or its secretions; no other vector or intermediate host is required.

aversive experiences. Different breeds and individual dogs differ in the ease and rapidity with which they can be socialized to humans (Scott and Fuller, 1965). However, properly socialized dogs can be docile and can be trained to cooperate in procedures that require repeated contacts with research personnel. For example, most dogs will allow venipuncture with

minimal restraint and will cooperate during detailed physical and neurologic evaluations.

HAZARDS

Unvaccinated dogs might harbor rabies virus, and preexposure immunization should be made available to personnel who are at substantial risk of infection (NRC, 1985). Dogs also have internal and external parasites that can be shared with humans (see "Parasitic Diseases" in Chapter 5). Table 2.1 lists selected zoonoses, zoonotic agents, and modes of transmission. Detailed discussions of zoonoses have been published (Acha and Szyfres, 1987; August, 1988; Elliot et al., 1985; Fishbein and Robinson, 1993; Hubbert et al., 1975). Personnel can develop allergies to canine dander and saliva, can be bitten or scratched, might suffer hearing impairment from prolonged exposure to excessive noise generated by barking dogs or mechanical equipment, or can be injured while lifting or transporting large dogs. To deal with these and other animal-related health problems, institutions must provide occupational health programs for personnel who work in animal facilities or have substantial animal contact (NRC, 1985).

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3

Husbandry

This chapter provides guidelines for the care of laboratory dogs. The first section, on housing, details design and construction considerations for facilities that house dogs, as well as for primary enclosures (here defined as cages and pens). The subsection on facilities contains information on buildings, rooms, and outside areas for containment of dogs, and that on environment and environmental control describes mechanisms for controlling the environment and gives the legislatively mandated ranges for temperature, humidity, and ventilation.

The remaining information in this chapter is supplemented by discussions in other parts of this report. For example, Chapter 4 ("Management of Breeding Colonies") contains sections on food for puppies and gestational or lactating dams and on record-keeping for a breeding colony that amplify the sections on food and identification and records in this chapter. Socialization of puppies is also discussed in Chapter 4. Modified primary enclosures and bedding for dogs with specific disorders are described in Chapter 6 ("Special Considerations").

The 1985 amendment to the Animal Welfare Act required the U.S. Department of Agriculture (USDA) to establish standards for exercise for laboratory dogs, and they were established in 1991. A federal court has now found that the regulations concerning exercise for dogs are inadequate and ordered that new regulations be written. This committee has reviewed the available information relevant to exercise, space, and well-being of dogs,

and it has found that, as was the case in 1985, it is inadequate to formulate objective standards.

Although knowledge of canine behavior is leading to a consensus that opportunities for social interaction with people, other dogs, or both are important for promoting canine well-being, no similar consensus is available concerning fitness and exercise. Another issue is the notion that a single standard can provide optimal care for all dogs. It is generally recognized that such factors as breed, physical conformation, age, health status, past experiences, and general behavioral characteristics influence what constitutes adequate space and exercise. For example, a dog undergoing a surgical procedure might require a restricted space to limit its activity. Once the dog has recovered from the surgical procedure, a different space and exercise regimen can be implemented. Likewise, the space and type and duration of exercise required for Alaskan sled dogs in working condition is quite different from that required for Shih Tzu and other brachycephalic breeds. Finally, medical research benefits from the availability of dogs with inherited disorders similar to those of humans, and the presence of these disorders in dogs imposes the same types of restrictions that human patients must endure. Unsupervised exercise is often contraindicated in dogs with heart and metabolic diseases. Similarly, the construction and layout of primary enclosures for dogs with such conditions as muscular dystrophy, bleeding disorders, blindness, or Ehlers-Danlos syndrome must be carefully considered to avoid compromising their health and well-being.

The most important objective for those responsible for housing dogs should be to achieve an overall high level of care, rather than to conform rigidly to specific standards. Animal well-being must be assessed case by case by those qualified to do so. The regular evaluation of animal well-being is an important aspect of any husbandry and animal-care program and serves as a measure of the appropriateness of animal-care procedures. Procedures that are ultimately linked to the well-being of the individual are defined as performance standards. The committee strongly recommends that performance standards, coupled with sound professional judgment, be used to develop space requirements and exercise programs for dogs. This committee is firmly convinced that performance standards are ultimately better for each dog's physical and behavioral well-being than engineering standards, which might lack the flexiblity necessary to meet the needs of all dogs.

HOUSING

Facilities

Housing facilities for dogs must be designed and constructed so that they are structurally sound, protect animals from injury, contain animals

securely, and prevent entry of other animals (9 CFR 3.1a). Dog facilities vary in size and complexity, depending on their purpose (e.g., holding or breeding), colony size and type (e.g., specific-pathogen-free or conventional), and breed. The design of breeding facilities should address the following:

- The design should facilitate the conduct of research.
- There should be sufficient space for expansion, both for adding animals and for increasing ancillary operations.
- Breeding facilities should have sufficient space to house dams with litters and the progeny.
- The design should promote effective sanitation and husbandry procedures.
- Operation of the facility should be efficient and cost-effective.
- Construction should be economical.

The physical facilities and equipment should be constructed and operated to fulfill the following criteria:

- Contamination from areas adjacent to, but not part of, the facility should be minimized. The locations of equipment washing and sterilizing, food and bedding storage, quarantine, treatment, receiving and shipping, shipping-crate storage, mechanical services, shops, offices, and laboratories should minimize crossovers from soiled or contaminated to clean areas. Clean material and equipment should not come into contact with soiled and contaminated material and equipment.
- There should be sufficient control of temperature, humidity, ventilation, and lighting to provide the animals with appropriate conditions for their comfort and well-being.
- Behavioral well-being should be considered by allowing for visual contact between dogs, social housing, exercise areas, and other appropriate areas.
- The entry of vermin should be prevented.
- Provisions should be made for lunchrooms, locker rooms, and toilets for animal-care personnel.
- Caging equipment and feeding and watering devices should provide a safe environment, make food and water readily available, minimize the opportunity for transmission of diseases and parasites, and make sanitation and sterilization efficient.
- Auxiliary equipment—such as washing machines, cage racks, rolling equipment (e.g., dollies, tables, and carts), and fixed equipment (e.g., cabinets, sinks, and shelving)—should be designed, fabricated, and used to promote maximal sanitation and operating efficiency.

When a dog facility is designed to be part of a larger facility housing other species of animals or part of a multipurpose building with offices and research laboratories, the physical relationships between areas must be carefully planned (NRC, 1985a). Those establishing operating procedures should use the best available information on physiology; nutrition; genetics; behavior; animal breeding, care, and maintenance; colony management (production and research); and disease control.

Dogs can be housed in indoor facilities, outdoor facilities, or a combination of the two (sheltered housing facilities). If the site is exclusively indoors, the only factors that influence site selection are local zoning regulations, the ability to control odors and noise, the availability of appropriate utilities (e.g., sewerage and water) (9 CFR 3.1d), and the proximity to other businesses. Indoor facilities should be constructed and maintained in compliance with CFR, Title 9, Part 3.2 and the *Guide* (NRC, 1985a), as summarized below.

Indoor Facilities

Walls. Exterior walls should be fire-resistant and impervious to vermin. To facilitate cleaning, interior walls should be smooth, hard, and without pits or cracks, and they should be capable of withstanding the impact of water under high pressure and scrubbing with cleaning agents (e.g., detergents) and sanitizing agents (e.g., disinfectants). They should be protected from damage caused by movable equipment.

Ceilings. Ceilings should be smooth, moistureproof, and free of imperfect junctions. Surface materials should be capable of withstanding scrubbing with detergents and disinfectants. Exposed pipes and fixtures are undesirable.

Floors. Floors can be constructed of a variety of materials that are smooth, moistureproof, nonabsorbent, and skidproof; that are resistant to wear and the adverse effects of detergents, disinfectants, acid, and solvents; and that are able to support heavy equipment without being gouged, cracked, or pitted. They should also be easy to clean.

Drainage. Drainage must be adequate to allow rapid removal of water (9 CFR 3.1f). If floor drains are used, they should be constructed and maintained in accordance with the *Guide* (NRC, 1985a). Rim flush drains should be at least 6 in (15.2 cm) in diameter. Porous trap buckets installed in the drains aid in cleaning and screen out solid waste. Floor drains must contain traps that prevent backflow of sewage and gases (9 CFR 3.1f). If

unused floor drains are present, they should be closed with gastight seals that are flush with the floor surface.

Doors. All rooms should have doors. External doors should have adequate latches and locks and should be verminproof when closed. If they are left open during warm weather, adequate screening is essential. All door frames should be sealed to walls and partitions with caulking compound or a similar material.

Ports in animal-room doors allow personnel to observe the dogs without entering the rooms, prevent injury to personnel while they are opening doors, and provide a way to verify that room lights are on at appropriate times. Experience has shown that doors at least 42 in (107 cm) wide and 84 in (213 cm) high allow free passage of cages and equipment. The doors should be equipped with locks and kickplates and should be self-closing.

Outside windows. Outside windows and skylights might not be desirable, because they can contribute to unacceptable variations in temperature and photoperiod. Other problems associated with outside windows and skylights include dust and bacteria buildup on frames, drafts, and increased ventilation costs.

Washrooms and sinks. Washing facilities for personnel (e.g., basins, sinks, or showers) must be provided and must be readily accessible (9 CFR 3.1g).

Sheltered Housing Facilities

A sheltered housing facility, as defined by the Animal Welfare Regulations (AWRs), is a facility that provides shelter, protection from the elements, and protection from temperature extremes at all times (9 CFR 1.1). It can consist of runs or pens in a totally enclosed building or indoor-outdoor runs with the indoor runs in a totally enclosed building. The requirements for the sheltered portion of such facilities are identical with those for indoor facilities, with the additional stipulation that the shelter structure must be large enough to permit each animal to sit, stand, and lie down in a normal manner and to turn around freely (9 CFR 3.3).

The outdoor portion of a sheltered housing facility should be constructed to prevent the introduction of vermin. Outdoor floor areas in contact with animals should be constructed of hard, moisture-resistant material and be properly drained. The use of compacted earth, sand, gravel, or grass is discouraged. The sides of runs can be constructed of chain-link fencing and steel posts or pipe frames or, when necessary to prevent fighting or injury, of solid concrete block coated with sealant. Fencing at the lower ends of

runs and pens should be high enough above the surface to permit adequate drainage but not high enough to allow young puppies to escape. Curbs at least 6 in (15.2 cm) high should be constructed between runs to help prevent the spread of microorganisms during washing. Curbs 24-30 in (61.0-76.2 cm) high might be necessary in runs in which the dog population is constantly changing. Higher curbs might be beneficial in whelping-pen runs to reduce the anxiety of nursing bitches. Run doors or gates should have well-made latches that can be easily opened by animal-care personnel but not by the dogs. Special consideration must be given to removing animal wastes and controlling noise.

Outdoor Facilities

The AWRs, with some restrictions, permit facilities to house dogs solely outdoors, provided that each animal has access to a structure (consisting of a roof, four sides, and a floor) that furnishes adequate protection from cold, heat, the direct rays of the sun, and the direct effects of wind, rain, and snow (9 CFR 3.4). In general, this type of housing is discouraged for dogs being used in an experimental protocol, because environmental factors, infectious agents, and vermin are difficult to control. In other instances (e.g., in protocols requiring acclimation or in breeding colonies maintained in temperate climates), outdoor facilities might be adequate.

Environment and Environmental Control

An important part of maintaining the health and well-being of laboratory animals is control of the environment. In nature, animals respond to environmental changes both behaviorally and physiologically in a manner that will maintain homeostasis. In an animal room, a behavioral response might not be possible, and the animal must deal with an altered environment physiologically. Therefore, it is necessary to control the environment to avoid physiologic changes. Besch (1985) has reviewed environmental factors that can effect the biologic responses of laboratory animals.

Temperature and Humidity

Temperature and humidity are important considerations in a dog facility (Besch, 1985). Dogs can tolerate moderate ranges of temperature and weather, provided that they have appropriate amounts of food and water, have access to shelter, and are allowed sufficient time to acclimate to their environment. The *Guide* recommends that room temperature for dogs be maintained within a range of 18-29°C (64.4-84.2°F) and relative humidity within a range of 30-70 percent. The AWRs require that the ambient temperature in indoor

facilities not fall below 7.2°C (45°F) or rise above 29.4°C (85°F) for more than 4 consecutive hours when dogs are present (9 CFR 3.2a). Except as approved by the attending veterinarian, ambient temperature must not fall below 10°C (50°F) for dogs not acclimated to lower temperatures, breeds that cannot tolerate lower temperatures, and young, old, sick, or infirm dogs (9 CFR 3.2a).

Dogs recovering from general anesthesia are frequently hypothermic. Every attempt should be made to maintain normal body temperature during surgery and recovery. This can be accomplished by using supplemental sources of heat (e.g., heating pads and heat lamps), by avoiding direct contact with heat-conducting surfaces (e.g., metal), and by maintaining the postoperative recovery cage at 27-29°C (80.6-84.2°F) (NRC, 1985a). Newborn pups have poorly developed thermoregulatory mechanisms and might require supplemental sources of heat. Temperatures of 29.4-32.2°C (85-90°F) have been suggested for the first week of life (Poffenbarger et al., 1990).

Each room should be provided with temperature controls and high- and low-temperature alarms. Graphic recorders are useful for monitoring system performance. Ideally, the temperature controls should allow individual adjustments in dry-bulb temperature of ± 1°C (± 2°F) within the range of 18.3-29.4°C (65-85°F).

Relative humidity should be maintained at 30-70 percent throughout the year (NRC, 1985a). It is important to control sources of humidity, such as cage-cleaning equipment, transient loads from cleaning water (Gorton and Besch, 1974), and thermal and mass loads from animals (Besch, 1991). Low humidity can contribute to respiratory distress; and coughs, pneumonitis, and other problems can follow. High humidity impairs efficient body-cooling (Besch, 1991).

Ventilation

Ventilation serves multiple functions. It supplies oxygen; removes heat generated by animals, lights, and equipment; dilutes gaseous contaminants; and helps to control the effects of infiltration and exfiltration (Clough and Gamble, 1976; Edwards et al., 1983). Gorton et al. (1976) have reported a method for estimating laboratory animal heat loads.

Indoor facilities must be sufficiently ventilated when dogs are present to provide for their comfort and well-being and to minimize odors, ammonia concentrations, drafts, and moisture condensation. Auxiliary ventilation must be provided when the ambient temperature is 29.5°C (85°F) or higher (9 CFR 3.2b). It is commonly thought that 10-15 volumetric changes per hour with outside air must be provided to animal rooms and that air must not be recirculated. As a consequence, animal facilities are generally venti-

lated with "one-pass" air, although the *Guide* (NRC, 1985a) includes provisions for alternative methods of providing equal or more effective ventilation. Besch (1992) has reviewed alternative methods of ventilation.

Ventilation system design and construction considerations include the following:

- Diffusers and exhaust openings should be located and controlled to prevent drafts.
- Outside openings and exhaust-ventilation grillework should be screened to prevent entry of vermin. Screening should be cleaned regularly.
- Air pressure in clean areas and animal rooms should be greater than that in public and refuse areas. Where pathogenic organisms are present, a negative-pressure system is necessary.
- Ventilating mechanisms should be equipped with suitable alarm systems that will be activated if the temperature moves outside the desired range or if power fails.
- Supplemental exhaust fans or exhaust systems increase drying and reduce humidity when fixed equipment is being washed. If such systems are used, they should be permanently mounted in external windows or wall openings, their frames should be sealed to the building structure, and the systems should be screened.
- Emergency power sources should be available in case of power failure.

Power and Lighting

Electric systems should be safe, furnish appropriate lighting, and provide a sufficient number of outlets. Lighting systems should allow for either manual or timer-controlled changes in illumination levels or photoperiods, and timer performance should be checked regularly. Lighting fixtures, switches, and outlets should be sealed to prevent entry or harboring of vermin. Moistureproof switches and outlets should be installed where water is used in cleaning. Emergency power should be available.

Illumination must be adequate and uniformly diffuse throughout each animal room to allow proper cleaning and housekeeping, to permit inspection of animals, and to maintain the animals' well-being (9 CFR 3.2c). Light levels of 323 lx (30 ft-candles), measured 1.0 m (3.3 ft) above the floor, appear to provide sufficient illumination for routine animal care (Bellhorn, 1980; NRC, 1985a). A regular diurnal lighting cycle must be provided (9 CFR 3.2c).

Noise Control

Barking dogs can be a nuisance both to personnel working in animal facilities and to the adjacent population. Self-generated noise of 80-110 dB (Peterson, 1980; Sierens, 1976) has been measured in dog rooms. The effects of noise on animals are reviewed in the *Guide* (NRC, 1985a).

Noise-control measures should be implemented in both indoor and outdoor environments. Sound transmission can be reduced by using concrete to build walls, covering concrete walls with sound-attenuating material, and eliminating windows (NRC, 1985a). Pekrul (1991) has discussed other means of decreasing noise in animal facilities. Sound-attenuating materials may be bonded to walls or ceilings only if they can be sanitized and will not harbor vermin. Outdoor runs must be designed and constructed to comply with local noise ordinances.

Chemicals and Toxic Substances

Many of the chemicals used in animal facilities for cleaning, sanitizing, pest control, and other purposes can be toxic to housed animals and personnel. In addition, some materials used in construction for coating surfaces can react with certain cleaning and sanitizing agents to produce toxic gases, including chlorine. Where possible, the use of chemicals should be avoided. For example, adequate ventilation is more effective than chemicals in eliminating most animal-room odors, provided that air inlets are not placed near the building exhaust. Newberne and Fox (1978) and Besch (1990) have reviewed chemicals and other toxicants found in animal facilities.

Where chemical agents must be employed, it is essential to be familiar with their potential toxicity and to develop procedures for using and disposing of them properly. Noxious chemicals should not be used to clean animal facilities. Adequate rinsing is essential to prevent the skin irritation or allergic reactions that can be caused by some cleaning and sanitizing agents (e.g., pine oil).

Primary Enclosures

Primary enclosures should facilitate research while maintaining the health and well-being of the dogs. They must confine dogs securely, enable them to remain clean and dry, protect them from injury, and contain sufficient space to allow them to sit, lie, stand, turn around, and walk normally (9 CFR 3.6a). The design should allow inspection of cage or pen occupants without disturbing them and provide easy access to feeding and watering devices for filling, changing, cleaning, and servicing.

Cages or pens should be fabricated of smooth, moisture-impervious, corrosion-resistant materials that can be easily sanitized and sterilized. Floors must be constructed to preclude entrapping toes, dew claws, or collars. Expanded metal or plastic-covered metal mesh is satisfactory for pens or runs, provided that the dogs' feet cannot pass through the openings (9 CFR 3.6a2x). Pen floors must have adequate drainage.

Each cage and pen should have a hinged or sliding door that covers the opening sufficiently to prevent escape of the occupants. Each door should have a latch that holds the door securely closed.

Space Recommendations

The AWRs require that the floor space for each dog equal at least the "mathematical square of the sum of the length of the dog in inches (measured from the tip of its nose to the base of its tail) plus 6 inches [15.24 cm]," expressed in square feet (9 CFR 3.6c1i). In addition, the interior height of each enclosure must be "at least 6 inches [15.24 cm] higher than the head of the tallest dog in the enclosure when it is in a normal standing position" (9 CFR 3.6c1iii). Each bitch with nursing pups must be given additional floor space based on breed and behavioral characteristics and in accordance with generally accepted husbandry practices, as determined by the attending veterinarian (9 CFR 3.6c1ii). The additional space for each nursing pup must be at least 5 percent of the minimum required for the bitch, unless otherwise approved by the attending veterinarian (9 CFR 3.6c1ii). Minimal space recommendations for dogs are also given in the *Guide* (NRC, 1985a, p. 14). These requirements and recommendations are based primarily on professional judgment and convention.

The few scientific studies on this subject have focused on how enclosure size affects movement, activity patterns, and physical fitness. Clark et al. (1991) found no decreases in physical fitness, as measured by heart rate and muscle enzyme (succinate dehydrogenase) activity, when dogs were housed in cages or runs of various sizes that complied with federal standards and guidelines; however, modest decreases in fitness were found when dogs were housed in cages smaller than mandated by the AWRs. It has been shown that, in general, dogs are more active in pens and runs than in cages; however, dogs housed in the largest enclosures are not always the most active (Hetts et al., 1992; Hite et al., 1977; Hughes and Campbell, 1990; Hughes et al., 1989; Neamand et al., 1975). Enclosure size has not been demonstrated to affect the musculoskeletal system (Newton, 1972), cortisol concentrations (Campbell et al., 1988; Clark et al., 1991), or selected measures of immune function (Campbell et al., 1988). Although they provide interesting and relevant information, the studies do not provide

sufficient objective, scientific data on which to base space requirements for dogs.

To set standards based on scientific data, one must show a correlation between cage size and behavioral well-being. That poses two problems: it is not clear how to define and measure behavioral well-being, and the determination of well-being depends on human interpretations of the data. Movement and activity patterns are unlikely to be sensitive behavioral measures, because a dog's activity can be increased without improving its well-being (e.g., if there is locomotor stereotypy or increased activity caused by social isolation or competition for space). Moreover, the definition of movement varies between studies, so it is difficult to compare and interpret results. It is generally accepted that a variety of perspectives are needed to assess well-being, including measures of physical health, of neuroendocrine and immunologic responses to stress, of the ability to respond effectively to social and nonsocial environments, and of behavior. Scientific data on dogs are inadequate to support any such assessment relative to enclosure size.

EXERCISE AND ENVIRONMENTAL ENRICHMENT

The requirements for providing opportunities for dogs to exercise are specified in the AWRs (9 CFR 3.8). The following paragraph summarizes the AWRs now in effect. It is incumbent on the reader to keep abreast of changes that might occur as the result of further federal court or USDA actions.

Dogs over 12 weeks old, except bitches with litters, must be given the opportunity for regular exercise if they are kept individually in cages, pens, or runs that are less than 2 times the AWR-required floor space. Dogs housed in groups do not require exercise periods, provided that the total floor space of the cages, pens, or runs equals the sum of the AWR-required spaces for the dogs if housed individually. If a dog is housed without sensory contact with other dogs, it must receive positive physical contact with humans at least once a day. Forced-exercise programs (e.g., swimming or walking on treadmills or carousel devices) are not considered to comply with the AWRs. Each institution is responsible for developing a plan for providing exercise. The plan must be approved by the attending veterinarian and must be made available to USDA on request. Exceptions to the requirement for exercise can be made by the attending veterinarian case by case or, if exercise is inappropriate for a scientific protocol, by the institutional animal care and use committee (IACUC). In the former instance, the exemption from exercise must be reviewed every 30 days, unless it was granted because of a permanent condition (9 CFR 3.8d). In the latter instance, exemptions must be reviewed at appropriate intervals, as determined by the IACUC, but not less often than every 6 months (9 CFR 2.31)

Recent studies have provided some information on exercise and well-being. Clark et al. (1991) and Hetts et al. (1992) found that 30 minutes of forced treadmill exercise five times a week did not affect physical fitness or behavior as measured in the study. Campbell et al. (1988) reported that releasing dogs either singly or as a group into a large area for 35-minute exercise periods three times a week did not affect cage activity patterns or weekly measures of selected hematologic or serum biochemical values. However, dogs were more active during the release periods than in their cages, and dogs released individually had different activity patterns from those of dogs released in groups. Studies on enclosure size and exercise are cited in the section above on space recommendations. Although the studies have provided important and relevant information, sufficient data are still not available to support definitive conclusions about the relationship between exercise and well-being. Future studies should be based on larger samples, use a variety of behavioral measures to evaluate well-being (activity patterns are not likely to be sensitive indicators of well-being), and consider the substantial individual variations in physiologic characteristics that have been reported.

It is well known that dogs are highly social animals, and social isolation and solitary housing are considered to be important stressors of social species (Wolfle, 1990). Solitary housing has been shown to be associated with less activity and with nonsocial repetitive behaviors (Hubrecht et al., 1992). Hetts et al. (1992) have found that socially isolated dogs (i.e., dogs having only auditory contact with other dogs and contact with people only during routine husbandry procedures) display bizarre movement patterns and tend to vocalize more than dogs that have more social contact. Several studies have reported that dogs are more active in the presence of humans (Campbell et al., 1988; Hetts et al., 1992; Hughes and Campbell, 1990; Hughes et al., 1989), especially when human presence is relatively rare (Hubrecht et al., 1992). It has also been shown that dogs housed in pairs sleep more than dogs housed singly (Hetts et al., 1992). Although the relationship between sleep patterns and well-being has not been studied in dogs, there is evidence in other species that normal sleep can be disrupted by a variety of environmental stressors and that return to normal sleep patterns can be a sensitive indicator of an animal's adaptation to environmental changes (Ruckenbusch, 1975).

Evidence of the importance of social interactions for dogs is strong enough to support a recommendation that dogs be socially housed in compatible groups, be given opportunities for social interaction during the exercise period, or both. The AWRs address the compatible grouping of dogs in the same primary enclosure (9 CFR 3.7). Age, sex, experience, and genetic differences in social behavior between individuals and breeds influence how dogs accept social housing and respond to social interaction (Fuller, 1970;

King, 1954; Scott and Fuller, 1965). Social interactions should minimize fearful and aggressive behaviors.

Examples of plans that provide social interactions are leash walking and release of dogs in an enclosed area for specified periods. In the latter, several compatible dogs that are housed in the same room can be released together; however, females in proestrus or estrus should not be released with males. Exercise rooms should be cleaned and sanitized between uses by dogs from separate rooms to minimize disease transmission. Only dogs of similar microbiologic status should be combined in groups (see Chapter 5).

If dogs are to be group-released, the composition of the group should remain as stable as possible (i.e., the members of the group should be the same dogs each time), because how readily a group of dogs accepts new members varies a great deal. Some dogs form closed social groups and attack new members (King, 1954). Changes in group composition often cause instability in the social dominance hierarchy, which in turn can result in intraspecific aggression. It is important to remember that two dogs make a pack, and the behavior of a pack is often very different from that of an individual dog. A thorough understanding of pack structure and social behavior is important for those managing research dogs. Any dog that is being attacked or threatened by the group to the extent that it cannot move about freely should be removed and given an alternative method of exercise. Group-released dogs should be observed frequently during the exercise period to ensure their safety.

Positive social interactions with humans can be achieved by having one or more people in the room during the exercise period. There is evidence that passive contact with a person is more reinforcing to dogs that have been socially isolated than is active contact (Stanley, 1965; Stanley and Elliot, 1962). If a dog displays fearful behavior when handled or petted, the handler should sit passively, avoid eye contact, and allow the dog to approach at will. As fearful behavior decreases, contact can gradually become more active.

Information on other types of environmental enrichment for dogs is scarce. The need for complex or varied environments has not been studied. Dogs have been observed to manipulate and direct attention to loose objects they find in their enclosures (Hetts et al., 1992), and dogs provided with toys spent an average of 24 percent of their time using them (Hubrecht, 1993). The toys reduced the dogs' inactive time and decreased destructive behavior aimed at cage apparatuses (Hubrecht, 1993). The relevance of these behavioral changes to well-being is not yet known. Nonetheless, such devices as balls, chew toys, and ropes might be considered for dogs in restricted environments. It is recommended that an ethologist, comparative psychologist, or animal behaviorist knowledgeable about dog behavior be

consulted by those designing exercise and social interaction plans or when other questions arise concerning the behavioral well-being of dogs.

FOOD

Selecting Optimal Rations

Many commercially available dog foods contain all essential nutrients in their required proportions, as outlined in *Nutrient Requirements of Dogs* (NRC, 1985b) and the Association of American Feed Control Officials' *Official Publication 1993* (AAFCO, 1993). These foods are manufactured in dry, semimoist, and canned forms. Dogs should be fed only complete and balanced diets. Specific procedures should be followed to ensure that stored foods do not become deficient in nutrients (NRC, 1985a).

Diet quality can be evaluated by examining the label for a statement of nutritional adequacy, which must be present on all dog-food products sold across state lines. This statement informs the purchaser whether the product has been approved for use as a complete ration for specified life stages (i.e., growth, maintenance, or pregnancy and lactation). Approval is obtained by one of the following means:

- Each of the diet's individual ingredients is analyzed for all essential nutrients; the sum of these nutrients in all ingredients must meet or exceed the nutritional requirements of the animal for specified life stages.
- The product itself is chemically analyzed and shown to meet or exceed the essential-nutrient requirements for specified life stages.
- The product passes a feeding trial as specified by the Association of American Feed Control Officials.

If the product fails to be approved, it must be labeled for use as a dietary supplement only and is not appropriate for use as a dog food. Of the three means of approval, only the feeding trial evaluates the availability of the nutrients in the product. Dog foods approved by that method should be used whenever possible. If such a diet cannot be used, because it would interfere with the experimental design (e.g., nutritional studies with purified diets), the manufacturer of the diet to be used should be consulted about experience with the diet's performance under given conditions.

Many commercially available dog foods, although designed for a specified life stage, are approved and adequate for use during all life stages. Most growth formulations will meet the requirements for gestation, lactation, and maintenance. Similarly, most gestation-lactation products also meet requirements for growth and maintenance. Some foods intended for maintenance will meet the criteria for more than one life stage. However,

no food should be used for growth, gestation, and lactation unless its label states that it meets or exceeds nutrient requirements for these life stages.

Special therapeutic diets are available for dogs with specific nutrient requirements caused by the presence of disease (Kirk and Bonagura, 1992; Lewis et al., 1987). Such diets should be fed only under the supervision of a veterinarian.

Feeding

Most commercial rations are formulated to meet all nutrient requirements if a dog eats enough to fulfill its caloric requirements. Estimates of daily caloric requirements can be obtained from several sources, including the manufacturer of the specific food being used. These estimates can be used to initiate feeding programs, but they might need substantial modification because of variations in metabolic rates of individual dogs.

Under most kennel conditions, meal feeding is preferable to free-choice feeding, and individual feeding is preferable to group feeding for the following reasons:

- Restricted feeding has been shown to decrease the incidence of metabolic bone disease in growing dogs that mature at greater than 30 lb (Kealy et al., 1992).
- Restricted feeding has been shown to decrease the incidence of obesity in young beagles and Labrador retrievers (Kendall and Burger, 1980).
- The continual ingestion of small amounts of food observed in free-choice feeding programs stimulates oral bacterial growth and might promote dental disease and gingivitis (Dr. John Saidla, Department of Clinical Sciences, New York State College of Veterinary Medicine, Cornell University, Ithaca, N.Y., unpublished).
- When dogs are fed in groups, dominant dogs might overeat and might prevent subordinate dogs from eating enough to fulfill their daily needs.
- When dogs are fed individually, their food intake can be monitored.

Some kennels have successfully used free-choice feeding to maintain dogs. This practice is most successful when the diet used is a food of relatively low energy density and palatability.

Dogs must be fed at least once a day, except as required for adequate veterinary care (9 CFR 3.9a). Each healthy adult dog should be fed enough to maintain its optimal body weight; this amount will vary with the environment and with the dog's age, sex, breed, temperament, and activity. Within an individual breed, there is often a wide variety of *normal* sizes. It is better to evaluate a dog's size according to how it looks and how it feels than according to body weight alone. With the hands-on approach, a dog's

rib cage, spinous processes, and ileal wings should be easily palpable. They should not protrude from under the skin, nor should they be buried under a layer of adipose tissue. Once an adult dog is being maintained at its ideal body size, its weight can be used as a reference for future evaluation of food requirement. However, the loss of muscle mass and gain of adipose tissue, such as are observed in several endocrine disorders, and shifts in fluid balance might make body weight an inaccurate means of assessing nutritional status; therefore, body weight should not completely replace appearance and feel as assessment methods.

Contaminants

Animal-colony managers should be judicious in purchasing, transporting, storing, and handling food to ensure that it does not introduce diseases, potential disease vectors, or parasites. Food must be stored in a manner that prevents spoilage, contamination, and vermin infestation. Open bags must be stored in leakproof containers with tightly fitting lids (9 CFR 3.1e; NRC, 1985a).

Contaminants in food can have dramatic effects on biochemical and physiologic processes. In general, food for dogs should not be manufactured or stored in facilities used for farm foods or any products containing additives, such as rodenticides, insecticides, hormones, antibiotics, fumigants, or other potential toxicants.

WATER

Ordinarily, all dogs should receive fresh, clean, potable water ad libitum. If water is not continuously available, the AWRs require that it be made available at least twice a day for at least 1 hour each time, unless it is restricted by the attending veterinarian (9 CFR 3.10).

Watering devices can be either portable or self-watering. Self-watering devices are convenient and reduce labor, but they require scheduled observations to ensure proper function. Portable watering devices should be easily removable for daily rinsing and periodic sanitizing.

BEDDING AND RESTING APPARATUSES

Bedding can be used in some husbandry situations. For example, if drains are not available, it can be used as an absorbent to help to keep dogs clean and dry. Kinds of bedding typically used for dogs are wood shavings and shredded paper. Bedding must be stored in a manner that protects it from contamination and vermin infestation (9 CFR 3.1e).

Resting apparatuses, especially those made of high-density polyethyl-

ene (Britz, 1990), are useful for minimizing loss of body heat from dogs in postoperative recovery, dogs in ill health, and young pups with poorly developed heat-control mechanisms.

SANITATION

The schedule for cleaning and disinfecting dog facilities will vary according to the physical makeup of pens, cages, or runs and other factors. Generally, primary enclosures should be cleaned as needed and sanitized at least once every 2 weeks. Excrement pans and runs should be cleaned daily. If pens and runs composed of materials that cannot be sanitized (e.g., gravel, sand, or pea stone) are used, the contaminated materials should be replaced as often as necessary to prevent odors, diseases, and vermin infestation. Procedures outlined in the AWRs (9 CFR 3.11) should be followed. Dogs must be removed before the floors of primary enclosures are thoroughly cleaned. Primary enclosures containing bitches near parturition, dams with litters, or dogs in quarantine require a cleaning schedule that disturbs them as little as possible.

Equipment and peripheral areas should be cleaned according to the recommendations of the *Guide* (NRC, 1985a). Waste should be removed regularly and frequently, and safe, sanitary procedures should be used to collect and discard it (NRC, 1985a).

IDENTIFICATION AND RECORDS

Identification

Each dog held in a research facility must be marked either with the official USDA tag or tattoo that was on the dog at the time it was acquired or with a tag, tattoo, or collar applied by the facility that individually identifies the dog by number (9 CFR 2.38g1).

Unweaned puppies need not be individually numbered as long as they are maintained in the same primary enclosure as their dam (9 CFR 2.38g3). However, they can be marked for identification with a variety of methods. Colored yarns or spots made with such marking substances as nail polish or paint provide a quick visual reference. Subcutaneous dots can be made by injecting a small amount of tattoo ink beneath the abdominal skin with a tuberculin syringe and 25-gauge needle. Ink dots should be placed in a different location for each pup (e.g., left axilla and right side of abdomen). The location or pattern of the dots and the sex and markings of each pup provide individual identification until permanent tattoos can be applied.

Tattooing of the inner surface of a dog's ear is common. Before the tattoo is applied, the ear should be cleaned thoroughly. Tattoos can be

applied with special pliers or an electrovibrator. A tattoo might have to be reapplied after several years. An ancillary method for individually identifying dogs uses a subcutaneously implanted, permanently encoded microchip (transponder) that, when activated by an electronic scanner, broadcasts the encoded number; the scanner transfers the broadcast to a processor that produces either a digital readout or a printed copy. This identification system can be useful during daily examination of dogs being used in studies, but it has not been approved by USDA as the sole source of identification because there is no standard implantation site, no standardized scanner, and no definitive information on whether the microchip migrates from the implantation site. USDA has approved the trial use of the microchips for a few commercial organizations (Richard L. Crawford, Assistant Deputy Administrator for Animal Care, Regulatory Enforcement and Animal Care, APHIS, USDA, Beltsville, Md., personal communication, 1993).

Record-Keeping

Record-Keeping for Scientists and Animal-Care Staff

A life-long, day-to-day log of individual events and experimental procedures experienced by each dog—especially surgery, postsurgical analgesia, and other veterinary interventions—should be carefully maintained. The log will assist animal-care personnel in providing appropriate care, investigators in interpreting research results, and the institution in preparing its annual report to USDA (9 CFR 2.36). Computer programs for maintaining such logs are commercially available (Riley and Blackford, 1991). For small colonies, hand-kept records on each dog might be more appropriate. McKelvie and Shultz (1964) described a record system for long-term studies that is still relevant; it covers clinical examination and includes a coded daily log entry of all events that the animal has experienced.

Records Required by Federal Regulations

Research facilities are obliged to maintain records on procurement, transport, and disposal of all dogs and an inventory of dogs in the facility. When dogs are procured, facilities are required to obtain detailed information on the seller—including name, address, USDA license or registration number or vehicle license number and state—and a description of each dog (9 CFR 2.35b). Likewise, when a dog is transferred to another owner, records must include the name and address of the purchaser, the date and method of transport, and a certificate of health (9 CFR 2.35c). Additional information is available in the section of this chapter entitled "Transportation."

A variety of forms are available to assist institutions in keeping records.

Among them are USDA Interstate and International Certificate of Health Examination for Small Animals (VS Form 18-1), Record of Dogs and Cats on Hand (VS Form 18-5), and Record of Disposition of Dogs and Cats (VS Form 18-6). These forms can be obtained from Regulatory Enforcement and Animal Care, APHIS, USDA, Federal Building, Room 565, 6505 Belcrest Road, Hyattsville, MD 20782 (telephone: 301-436-7833). All records should be maintained for at least 3 years (9 CFR 2.35f).

Records must also be maintained on all offspring born to dogs in the colony (9 CFR 2.35b) and on exceptions to the requirements for exercise (9 CFR 3.8d). Facilities conducting research on any vertebrate animal, including dogs, are obliged to maintain additional records that include the following:

- minutes of meetings of the IACUC;
- semiannual IACUC reports;
- protocols involving animal use;
- scientifically justified deviations from the AWRs; and
- studies involving pain in which analgesics cannot be used.

Some of the information must be reported annually to USDA (9 CFR 2.36); other information, such as approved protocols, must be maintained for 3 years after the study ends (9 CFR 2.35f).

EMERGENCY, WEEKEND, AND HOLIDAY CARE

Dogs should be observed and cared for by qualified personnel every day, including weekends and holidays, as outlined in the *Guide* (NRC, 1985a). Emergency veterinary care should be available after working hours and on weekends and holidays. For dogs undergoing particular experimental procedures and dogs with conditions that might require emergency care, investigators should develop written protocols and provide appropriate additional coverage.

TRANSPORTATION

Transportation over long distances is known to be a stressor for animals. Proper attention to environmental conditions, cage design, and care in transit will minimize the stress. The AWRs specify the requirements for transporting dogs (9 CFR 3.13-3.19). Before a dog is transported, special arrangements must be made between the shipper (consignor), the carrier(s) or intermediate handlers, and the recipient (consignee). The shipper must certify that the dog was offered food and water during the 4 hours before delivery to the carrier and must prepare a written certification, which must

be securely attached to the cage and must contain the shipper's name and address, the animal identification number, the time and date when the dog was last offered food and water, specific instructions for feeding and watering the dog for a 24-hour period, and the signature of the shipper with the date and time when the certification was signed.

Primary Enclosures

Carriers must not accept dogs for shipment if their primary enclosures do not meet the requirements of the AWRs (9 CFR 3.14). The primary enclosure must be large enough to allow a dog to turn around while standing, to stand and sit erect, and to lie in a natural position. Primary enclosures must be structurally sound, free of internal protrusions that could cause injury, constructed of nontoxic materials, and able to withstand the normal rigors of transportation. The container must secure the animal and all parts of its body inside the enclosure. Devices, such as handles, must be attached to the outside to allow the container to be lifted without tilting. The container must have a leakproof, solid floor or have a raised floor and a leakproof collection tray. If animals are housed directly on the floor, absorbent bedding material must be provided. Primary enclosures must be cleaned and any litter replaced if dogs are in transit for more than 24 hours. Primary enclosures should be well ventilated to minimize the potential for a thermal gradient during shipment. Additional specifications for transport cages are in the AWRs (9 CFR 3.14) and the *IATA Live Animal Regulations* (IATA, 1993 et seq.).

Puppies 4 months old or younger must not be transported in the same primary enclosure with adult dogs other than their dams. For puppies shipped during sensitive periods of behavioral development (i.e., 8-14 weeks of age; see Scott and Fuller, 1965), shipping stress should be minimized. Dogs likely to display aggressive behavior must be shipped individually, and females in heat must not be transported in the same primary enclosures as males. No more than two live puppies 8 weeks to 6 months old, of comparable size, and weighing 9 kg (20 lb) or less each may be transported by air in the same primary enclosure. Older dogs and puppies weighing more than 9 kg (20 lb) should be individually housed. Weaned littermates that are less than 8 weeks old and are accompanied by their dam may be transported in the same enclosure to research facilities, either by air or surface transport. During transport by surface vehicle, no more than four dogs 8 weeks old or older and of comparable size may be transported in the same primary enclosure.

When viral-antibody-free (unvaccinated) dogs are transported between facilities, precautions must be taken to avoid contact with infectious agents. Some commercial suppliers have developed filtered shipping containers to

transport those dogs. IATA rules require that special measures be taken to ensure that ventilation rates are maintained within the container, that the container be appropriately labeled, that sufficient water be provided for the entire journey, and that food, if required, be provided at the point of origin (IATA, 1993).

Environmental Conditions

At all times, containers holding dogs should be placed in climate-controlled areas that provide protection from the elements (9 CFR 3.13, 3.15, 3.18-3.19). Trucks and planes must be ventilated and provide air that has adequate oxygen and is free of harmful gases and particulate contaminants. Airlines should always place dogs in pressurized compartments. Dogs may be shipped if temperatures will fall below 7.2°C (45°F) during any portion of their journey only if a veterinarian certifies in writing that they have been acclimated to lower temperatures and states the lowest temperature to which they have been acclimated. During transit, dogs must not be exposed to ambient temperatures exceeding 29.4°C (85°F) for a period of more than 4 hours.

Food and Water

All dogs must be offered food and water within 4 hours of delivery to the carrier (9 CFR 3.13c). Carriers must offer water to each dog at 12-hour intervals beginning 12 hours after the shipper last offered water. Adult dogs must be fed at least once every 24 hours, and puppies less than 16 weeks old must be fed every 12 hours throughout the trip. Feeding and watering utensils must be firmly secured to the inside of the container and placed so that they can be filled from outside the container. Written instructions for feeding and watering in transit must be attached to the primary enclosure in such a way that they are easily seen and read (9 CFR 3.16).

Other Requirements

There are special requirements for animal holding areas of terminal facilities, including rules for sanitization, pest control, ventilation, temperature control, and shelter from direct sunlight, rain, snow, and extreme heat (9 CFR 3.18).

Each dog must be accompanied by a health certificate, issued by a licensed veterinarian not more than 10 days before shipping, that states that the dog is free of any infectious disease or physical abnormality that would endanger it or other animals or pose a threat to public health. An exemp-

tion can be made by the secretary of USDA for individual animals shipped to research facilities if the facilities require animals that are not elegible for certification (9 CFR 2.78b). Instructions for the administration of drugs or provision of other special care must be firmly attached to the outside of the container (9 CFR 3.14h). A pregnant bitch should be accompanied by a certificate, signed by a veterinarian, that states that there is no risk of birth during transit (IATA, 1993).

Carriers and intermediate handlers must not accept dogs more than 4 hours before the scheduled departure (6 hours by special arrangement). An attempt must be made to notify the recipient on arrival at the destination and at least once every 6 hours thereafter (9 CFR 3.13f). During shipment by surface transportation, the operator of the conveyance or someone accompanying the operator must observe the dogs at least once every 4 hours to ascertain that they have sufficient air for normal breathing and are not in distress and that the rules for ambient temperature and all other AWR requirements are met. The same rules apply in air carriers if the animal cargo area is accessible during flight. If it is not accessible, the carrier must observe the dogs at loading and unloading. Dogs in physical distress must receive veterinary care as soon as possible (9 CFR 3.17).

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4

Management of Breeding Colonies

REPRODUCTION

To maintain the breeding efficiency of a colony or to breed an important individual dog successfully, staff must understand the unique reproductive characteristics of dogs. The biology of canine reproduction has been extensively reviewed (Burke, 1986; Christiansen, 1984; Concannon, 1991; Concannon and Lein, 1989; Concannon et al., 1989). Information on heritability of physical and other characteristics of dogs, Mendelian genetics of breeding, the incidence and characteristics of diseases that have a genetic basis, and methods for demonstrating heritability is also available (Patterson, 1975; Patterson et al., 1989; Shultz, 1970; Willis, 1989).

Reproductive Cycle of the Bitch

Most bitches can become pregnant once or twice a year. Each ovarian cycle consists of the following phases:

- A follicular phase, or proestrus, during which there is progressive vulval swelling and a serosanguineous (bloody) vaginal discharge. During this period, which can last from 3 days to 3 weeks, the bitch's blood has high concentrations of estrogen. The male will show interest, but he either does not or is not allowed to mount.
- A periovulatory period, or estrus, during which estrogen declines

and progesterone increases as the ovarian corpora lutea form. This period is also the early luteal phase of the cycle. During estrus, which can last from 3 days to 3 weeks, the bitch assumes a characteristic posture in the presence of a male in which the rump is raised and there is a curvature of the back (lordosis) and the tail is held to one side (flagging). The male is allowed to mount, and copulation occurs.

- A midluteal and late luteal phase or metestrus (either pregnant or nonpregnant metestrus), which lasts about 2 months and during which serum progesterone remains elevated above 1 ng/ml.
- A period of weak ovarian activity, or anestrus, lasting 2-10 months, in which progesterone concentration is low, and there is no evidence of estrogen stimulation of the uterus or vulva.

In constant photoperiods of 12 hours of light and 12 hours of darkness or 14 hours of light and 10 hours of darkness, estrous periods should occur with equal incidence throughout the year. Possible effects of constant light have not been studied. With natural circannual changes in photoperiod, bitches come into estrus more frequently in winter and spring months than in summer and autumn months. In most breeds, the interval between estrous periods averages 7-8 months. After the age of 8 years, however, the interval between cycles begins to lengthen, reaching 12 months or longer by the age of 12 years (Andersen and Simpson, 1973).

Successful breeding requires that observation of reproductive conditions be given high priority, and staff must be able to recognize the start of proestrus. A swollen vulva might not be obvious on a dark or long-haired dog, and bitches often lick away the bloody discharge; therefore, the vulva of each breeding bitch must be examined closely two or three times a week, beginning 4 months after estrus.

Vaginal cytology can be useful for estimating the best time for breeding (Concannon and DiGregorio, 1986; Holst, 1986; Olson et al., 1984) and predicting the time of whelping, which will be 55-60 days after a change in the smear indicates late estrus. Vaginal smears in anestrus are nondescript, with a few leukocytes and small epithelial cells. In early proestrus, smears include a high proportion of rounded epithelial cells, erythrocytes, and sometimes a few leukocytes. During midproestrus, there is an increasing percentage of cornified (flakelike) epithelial cells but no leukocytes. All or nearly all epithelial cells in the smear are cornified from 2-8 days before ovulation until 4-9 days after ovulation, when these cells predictably and abruptly decline. In early metestrus, cornified cells are replaced by rounded, smaller superficial cells, and there is usually an influx of leukocytes. The metestrus smear slowly regresses to the nondescript anestrus smear. Smears should be taken from the anterior vagina. They should be obtained and prepared carefully with saline-moistened swabs and should not be contaminated with

vulval material. In the case of bitches that have had reproductive problems, when a successful breeding is important, or both, more accurate predictions can be made by monitoring the progesterone concentration in the serum or plasma with an enzyme-linked immunosorbent assay (ELISA) kit (Bouchard et al., 1991a; Hegstad and Johnston, 1992; Johnston and Romagnoli, 1991). In this test, ovulation occurs a mean of 1-2 days after the initial rise in progesterone, peak fertility a mean of 0-4 days after the initial rise, loss of fertility 6-11 days after, implantation 18-20 days after, and parturition 63-65 days after (Concannon, 1991).

Mating

Theoretically, it is sufficient to maintain one male for every 10-20 females; however, in practice this ratio might not be adequate, for several reasons. First, a bitch in proestrus produces pheromones that will start proestrus in other bitches in the colony, making it likely that several bitches will be in estrus simultaneously. Because mating an individual male more often than once each day can reduce its sperm output after 1 week (Amann, 1986), a greater ratio of males to females might be required to maintain breeding efficiency. Second, except under special circumstances, such as reproducing a disease model, breeding programs should conscientiously avoid inbreeding, and it has been estimated that a ratio greater than two males for each 10 females is needed to prevent an increase in the coefficient of inbreeding (Shultz, 1970).

Natural Mating

Mating can be done naturally or by artificial insemination with fresh or frozen and thawed semen. Provided that the male is healthy, it is not necessary to take special precautions or to use medications to treat the genitalia because the vagina is not a sterile environment. However, it is important to ascertain that neither the dog nor the bitch has canine brucellosis, a disease that seriously affects reproduction and is a zoonosis (see "Control of Infectious Diseases" in Chapter 5). The bitch is usually taken to the stud dog's pen or cage, because a dog will often ignore the bitch or spend an inordinate amount of time scent-marking if he is moved to new surroundings. The bitch should be mated on 2 or 3 days over a 3- to 5-day period. Unless the staff is experienced in distinguishing early proestrus from estrus, the bitch should be presented to the male for 10-15 minutes every day or every other day from the time she is found to be in proestrus until she is mated. Breeding pairs should not be left unattended, because some bitches are highly selective in choosing mates and it is not uncommon for a bitch to attack a dog that is not of her choosing. In addition, the dog might need

assistance until he attains a copulatory lock. Mating should be recorded only on the basis of observations of copulatory locks that last several minutes or more. If a bitch refuses a particular dog, even when signs of estrus (lordosis and flagging) are present, placing her with a different dog might solve the problem. If it is important to the breeding program that a bitch be bred to a dog that she is refusing, a caretaker should restrain her in a manner that will prevent her biting either the caretaker or the stud dog during breeding, or artificial insemination (AI) should be used.

To ensure accuracy of parentage, the same stud must be used for every breeding within a single estrus to avoid multiple-sire litters. Bitches allow dogs to mate from several days before ovulation until several days after ovulation. Because the events of pregnancy are related to the time of ovulation—not necessarily to the time of mating—parturition can occur 56-68 days after a single mating and up to 70 days after the first of multiple matings. Sperm can survive 6 days or more in the bitch, and ovulated eggs can remain fertile for 3-7 days. Parturition should occur 62, 63, or 64 days after ovulation in nearly every bitch (Concannon et al., 1983). Bitches that whelp 56-60 days after the first mating often have small litters, probably because they were bred at the end of the fertile period (P. Concannon, New York State College of Veterinary Medicine, Cornell University, Ithaca, N. Y., unpublished).

Artificial Insemination

AI can be helpful when males cannot be moved easily within or between facilities, when breeding females with weak or selective estrus behavior, when using males that cannot provide natural service, and for preserving valuable animal models. Semen collection, handling of semen, and insemination are described in detail elsewhere (Christiansen, 1984; Concannon and Battista, 1989).

Insemination with fresh semen. Semen can be collected in a clean paper cup or in a latex cone (artificial vagina) attached to a 15-ml conical polypropylene centrifuge tube. An advantage of the former method is that debris from the penis is less likely to become mixed in the ejaculate. Ejaculate should be maintained at room or skin temperature and should be checked microscopically for sperm viability and malformations. Any variation from the expected chalky white color or 1- to 5-cc volume should be recorded. The full ejaculate should be deposited into the anterior vagina with a clean plastic pipet attached to a syringe with nonrubber (e.g., polypropylene) tubing. The hindquarters of the bitch should be raised for 10 minutes while the vagina is manipulated digitally by an attendant wearing a clean glove. The bitch should not be allowed to sit for 20 minutes, and pressure on her

abdomen should be avoided. AI should be performed every other day until two or three inseminations have been accomplished. The precise timing for performing AI can be predicted by checking for softening of the vulva, which often occurs around the time of ovulation; by demonstrating the appropriate vaginal cytologic characteristics of advanced estrus; or by measuring the initial rise in serum or plasma progesterone. Ideally, two inseminations should occur before vaginal smears show reduced cornification.

Insemination with fresh chilled semen. Fresh semen can be diluted or extended in one of several laboratory buffers or commercial extenders and shipped refrigerated by overnight express for use in insemination in another location (Concannon and Battista, 1989). At 4°C (39.2°F), sperm motility remains nearly normal for 3-4 days if the semen is diluted in an appropriate diluent and for 1 day if undiluted (see Morton and Bruce, 1989).

Insemination with frozen semen. Frozen semen should be thawed and handled according to the instructions provided by the laboratory that processed it, because each freezing technique has stringent requirements for rate of thawing, dilution, and site of deposition. Although sperm live for several days in fresh semen, they normally die within a few hours after thawing; therefore, precise timing of insemination is important for successful impregnation. The best time to inseminate is usually shortly after oocyte maturation, which occurs 5-6 days after the initial rise in progesterone, around the time of a surge in leutinizing hormone. In most bitches, the inseminations should also take place 2-4 days before the decrease in vaginal cornification. Reported success rates for vaginal insemination range from 0 to 70 percent (Concannon and Battista, 1989); success probably depends heavily on the freezing method and the number of viable sperm inseminated. Success rates of 50-90 percent have been reported for uterine insemination, which is accomplished surgically or with special instrumentation to deposit sperm through the cervix (Concannon and Battista, 1989).

Pregnancy and Parturition

Pregnancy can be determined at 25 days after ovulation by ultrasonography, at 20-35 days after ovulation with palpation, and at 45 days after ovulation with radiography (Johnson, 1986; Yeager and Concannon, 1990). There are no well-documented biochemical or immunologic canine pregnancy tests available. Concannon (1991) has reviewed changes in body weight during pregnancy and pregnancy-specific changes in hematocrit, serum chemistry, and metabolism.

Whelping facilities should provide seclusion from excessive noise and other disturbances. The whelping box should be large enough to accommo-

date the bitch and pups and have sides high enough to prevent neonates from wandering out of the box. The bottom of a large, fiberglass shipping crate works well for beagle-size dogs. The whelping box should be provided about a week before expected parturition.

Johnson (1986) has reviewed the management of the pregnant bitch. A nonpurulent green discharge, anorexia, and restlessness are normal just before parturition. Birth of a litter can be either rapid or protracted over much of a day. Intervals between pups normally range from 20 minutes to 3 hours. Intervals greater than 3 hours can indicate a problem with fetal position or uterine function and warrant veterinary attention. Persistent, unproductive labor of more than 1 hour also requires veterinary attention (Johnston and Romagnoli, 1991; Jones and Joshua, 1988).

NEONATAL CARE

Newborn pups, like all neonatal mammals, have poorly developed temperature-control mechanisms; therefore, it is necessary to keep the temperature in the whelping box higher than room temperature. Temperatures of 29.4-32.2°C (85-90°F) have been suggested for the first 7 days of life, 26.7°C (80°F) for days 8-28, 21.1-23.9°C (70-75°F) for days 29-35, and 23.9°C (70°F) thereafter (Poffenbarger et al., 1990). That can be done by raising the temperature of the room and placing insulation between the whelping box and the cage or floor or by using heating devices, such as heat lamps or built-in heating elements. However, caution is necessary in using such heating devices; because pups younger than 7 days old have very slow withdrawal reflexes (Breazile, 1978), they can be overheated or severely burned by these devices. Circulating-water heating pads or commercial pig warmers are useful, because they maintain heat at a safe level.

Whelping boxes should be examined two or more times a day for evidence of maternal neglect or cannibalism and for problems with the pups. A normal pup is plump and round, its head is mobile, and it exhibits a rooting reflex. Breathing is regular and unlabored, and the coat is shiny and free of debris. Abdominal enlargement after nursing is normal, but abdominal enlargement accompanied by restlessness, weakness, and either excessive vocalization or complete silence can indicate illness or aerophagia. Failure to gain weight is often the first sign of illness in a newborn animal (Greco and Watters, 1990). Andersen (1970) reported expected weight gains for beagle pups.

Dead pups should be removed from the box. Andersen (1970) and Lawler (1989) have reviewed causes of neonatal deaths and have reported an average rate of death of about 20 percent. Necropsy examination is suggested for all pups that die or are euthanatized with severe illness. Such examinations are necessary to distinguish between congenital defects, which affect only the pups in which they occur; infectious diseases, whose spread

might be prevented; and problems with the dam (e.g., insufficient milk) or the environment (e.g., room temperature too low), which can be corrected.

REPRODUCTIVE PROBLEMS

False Estrus and Anestrus

Recurrent frequent false estrus (estrus without ovulation) has been reported (Shille et al., 1984). In false estrus, estrus appears normal, and bitches will mate but fail to conceive. False estrus can be confirmed by demonstrating with a progesterone ELISA kit that the serum or plasma progesterone concentration has not risen above 1 ng/ml, as would be expected for 50 days or more after ovulation if estrus were normal. Bitches that often have false estrus or have false estrus followed in a few weeks by normal estrus cause problems in maintaining breeding colonies. Except in special circumstances, such as reproducing a disease model, it is preferable to cull these animals. Culling based on small litter size, problems with whelping or maternal behavior, chronic infertility, or persistent anestrus is also appropriate. Methods for assessment and treatment for potential causes of infertility in females have been extensively reviewed (Feldman and Nelson, 1987; Johnston and Romagnoli, 1991; Shille, 1986). Persistent anestrus can be distinguished from unobserved cycles only through extremely careful examinations for signs of proestrus or progesterone assays every 6 weeks. Estradiol assays are not particularly informative, and assays of canine gonadotropin to diagnose primary gonadal failure are not readily available. Attempts to induce estrus in anestrus bitches have had variable success (Bouchard et al., 1991b; Concannon, 1992; Concannon et al., 1989).

Delayed Parturition

Whelping should not be considered overdue until 67 days after the last mating or possibly 70 or more days after the first of several matings. Cesarean section should not be contemplated earlier unless there are obvious signs of distress in the bitch. Johnson (1986) and Jones and Joshua (1988) have reviewed veterinary management of dystocia.

Pseudopregnancy

Bitches that are not bred or that are bred but fail to become pregnant frequently exhibit pseudopregnancy because of the progesterone secretion that always follows ovulation. Signs of pseudopregnancy include extensive mammary development, lactation, and maternal behavior. Pseudopregnancy is rare in beagles but more common in other breeds. It is self-limiting and usually does not require intervention (Feldman and Nelson, 1987).

SPECIAL NUTRITIONAL REQUIREMENTS

Bitches

During pregnancy and lactation, bitches should be fed a diet approved by the Association of American Feed Control Officials for all life stages or a diet specially formulated for gestation and lactation (see "Selecting Optimal Rations" in Chapter 3). When a quality diet is fed, supplementation with vitamins and minerals is neither necessary nor desirable.

During the first two-thirds of pregnancy, the amount fed should be the same as that fed before pregnancy. During the last trimester, food intake should be gradually increased so that at parturition it is 150 percent of the daily maintenance requirement. Bitches should not be permitted to become obese during gestation, because this condition can increase the risk of dystocia and postparturient metabolic disorders (Johnston, 1986). Bitches that are underfed during gestation tend to have a higher incidence of stillbirths than bitches that are fed appropriate amounts, and their pups often weigh less at birth (Holme, 1982).

Lactation represents the greatest nutrient challenge that bitches experience during their lifetimes. For the first 3 weeks after parturition, nutrient requirements increase rapidly, leveling off at 200-250 percent of daily maintenance requirements, or even more, depending on the number of nursing pups (NRC, 1985). The nutritional demands of lactation are met best through free access to both food and water. At the time of weaning, food is generally withheld for 24 hours to decrease milk production. Food intake for the first day after weaning should be one-fourth of the amount required for maintenance and then gradually increased to the maintenance requirement by day 4. Ideally, lactating bitches should be within 15 percent of their prebreeding body weight at the time of weaning (AAFCO, 1993).

Pups

Pups should be maintained exclusively on their dams' milk until they are 3 weeks old. They can then begin to eat small amounts of a moistened gestation-lactation diet or a growth diet. Most pups can be weaned completely onto this type of diet by the age of 6-8 weeks. For the development of normal social behavior, it is desirable that they not be completely weaned before they are 6 weeks old. Pups that cannot be nursed by their dam or a foster dam before they are 5 weeks old should be fed one of the commercially available, complete milk replacers. Pups can be fed with bottles and nipples or stomach tubes. Bottles and nipples should be thoroughly cleaned after each use. If a stomach tube is used, its proper placement can be

ensured by inserting it to a distance equal to the premeasured distance from the mouth to the last rib. A small amount of sterile saline solution should be introduced through the tube before milk replacer is injected. After each meal, orphaned pups should be massaged in the anal-genital region with a warm, wet cotton ball to stimulate urination and defecation. Most orphans can be completely weaned onto solid food by 5 weeks of age.

Young pups most readily eat canned or moistened dry food; older pups can be fed dry, semi-moist, or canned food. Pups can be fed on either a free-choice or meal-feeding program. If a meal-feeding program is used, they should be fed at least four times a day until they are 3 months old, three times a day until they reach two-thirds of their adult weight, and two times a day thereafter. After the age of 3 months, free-choice programs can lead to obesity in small breeds and faster than optimal growth in large breeds. Excessively rapid growth in breeds whose weight at maturity is more than 30 lb has been associated with an increase in the incidence of several metabolic bone diseases (Hedhammer, 1981; Hedhammer et al., 1974; Kealy et al., 1992). Pups should be fed so that they grow at near optimal rates; growth-curve data are often available from pet-food manufacturers. When an appropriate growth ration is fed, no supplementation is necessary. If a product is not capable of supporting an optimal growth rate, it is generally safer, less expensive, and more convenient to switch to a better-quality growth diet. As a general rule, pups gain approximately 1-2 g/day per pound of anticipated adult body weight (Lewis et al., 1987). An inappropriate growth rate usually reflects a problem with the ration being fed or with the pups' access to it.

VACCINATION AND DEWORMING

Annual vaccinations and deworming of brood bitches should be scheduled for anestrus or weaning periods, not when bitches are in proestrus or are pregnant.

Pups that have nursed on colostrum during the first 12 hours after birth have received passive immunity to viruses against which the dam was immunized. If pups cannot nurse on colostrum, 16 ml of pooled serum administered subcutaneously has been shown to be a successful alternative (Bouchard et al., 1992). Maternally acquired immunity declines over time, and the rate of decline, although variable, depends on the level of the dam's immunity at parturition and the amount of colostrum ingested by each pup. About 30-50 percent of pups will be susceptible to disease and capable of being effectively vaccinated by the age of 6-7 weeks. Most pups (more than 95 percent) can be effectively vaccinated by the age of 16 weeks. General principles of immunity in newborn animals and of immunoprophylaxis are reviewed elsewhere (Carmichael, 1983; Tizard, 1977a,b). Diseases to which pups are

susceptible and vaccination schedules are discussed in Chapter 5, Veterinary Care.

Roundworms (*Toxocara canis*) and hookworms (*Ancylostoma caninum* and *A. braziliense*) are endoparasites that commonly infect young pups. Roundworms are typically transmitted from bitches to pups in utero, and pups begin to shed eggs in their feces 3 weeks after birth. Pups infected with hookworm larvae in their dams' milk typically begin to pass eggs in their feces 2 weeks after birth. It is important that pups receive treatment early in life if infection with roundworms or hookworms is suspected. To prevent peracute hookworm disease in unweaned pups of bitches harboring large numbers of somatic larvae, it might be necessary to treat the pups before hookworm eggs are detectable in fecal examinations. Canine endoparasites are reviewed in Chapter 5 and discussed fully elsewhere (Georgi and Georgi, 1992).

SOCIALIZATION OF PUPS

There is ample evidence of the importance of adequate socialization for the normal behavioral development of dogs (Clarke et al., 1951; Fox, 1968; Freedman et al., 1961; Houpt, 1991; Scott and Fuller, 1965). The term *socialization* is somewhat confusing because it has been used to describe events, processes, and procedures. In the narrowest sense, socialization is the development of the primary social attachments that form between a pup, its dam, and its littermates during a critical or sensitive period in its behavioral development (Scott, 1968). The process is not peculiar to dogs but occurs in many species of social mammals (see, for example, Cairns, 1966; Harlow and Harlow, 1969). In a broader sense, socialization is the process by which pups form attachments to other dogs, people, and environments. Attachment formation might require nothing more than sufficient exposure to or experience with other dogs, people, and elements of the environment, which results in familiarity with a variety of stimuli (Cairns, 1966; Scott, 1963). Breeds and individual pups differ in ease of socialization (Scott, 1970). In any case, adequate socialization allows a pup to develop normal social relationships with other dogs and to adapt to pair or group housing, to adjust more easily to unfamiliar stimuli and environmental changes, and to accept handling with little or no fear and distress (Scott, 1980).

Sensitive Period for Socialization

There is a sensitive period for socialization during which attachments form most readily and rapidly (Scott and Fuller, 1965). The beginning of the period is marked by the startle response to sound at the age of approximately 3 weeks. Also at 3 weeks, a pup begins to display distress vocaliza-

tions when separated from its dam. Distress vocalizations are distinct from those made in response to fear (Davis et al., 1977), hunger (Compton and Scott, 1971; Scott and Bronson, 1964), or physical discomfort (Gurski et al., 1980). Separation distress is greater in an unfamiliar pen (Elliot and Scott, 1961). To minimize separation distress, pups should remain with their dams for at least their first 6 weeks.

Ease of attachment formation varies between breeds and individuals but generally peaks between the age of 6-8 weeks (Scott and Bronson, 1964). Although socialization probably occurs at a low rate throughout life, the end of the sensitive period is marked by the pup's increasing fear of the unfamiliar at the age of 12-14 weeks (Scott, 1962).

Consequences of Inadequate Socialization

Pups that are inadequately socialized during the sensitive period exhibit abnormal behaviors, called kennel-dog or isolation syndromes, that are characterized by one or more of the following behaviors: generalized fearfulness, fear-motivated aggression, timidity, immobility, or hyperactivity (Scott et al., 1967). Dogs that, as a result of inadequate socialization, become highly distressed when subjected to common laboratory procedures (e.g., handling, walking on a leash, restraint, venipuncture, moves to different enclosures, and contact with other dogs) probably do not make good research subjects and might be in a compromised state of well-being. It has been reported that physiologic measurements on such dogs can fall outside normal limits (Vanderlip et al., 1985b).

Socialization Programs

Providing contact and handling only during routine husbandry procedures might not be sufficient to produce behaviorally normal, cooperative research animals (Vanderlip et al., 1985a,b). Specific programs that address each aspect of socialization—to dogs, to people, and to the environment—should be implemented. Programs that can be used as examples for providing adequate socialization have been reported (Vanderlip et al., 1985a,b; Wolfle, 1990).

The following are examples of elements that might be included in socialization programs: positive contacts with more than one person, opportunities to follow handlers, introduction to some type of restraint (e.g., a collar and leash), contacts with conspecifics other than littermates, and opportunities to explore outside the kennel. Exploration might include exposure to floors of different textures, to a room with different lighting, to stairs, and to such equipment as exam tables, clippers, and scales. Exposures to those elements should be gradual and paired with positive reinforc-

ers, such as food, petting, or verbal praise. Negative reinforcement and physical punishment can elicit aggressive or fearful behaviors and will make pups more difficult to handle. It is not necessary, or practical, to introduce pups to every type of environment, person, or animal to which they will later be exposed in order to provide adequate socialization. Evidence suggests that experience in coping successfully with change facilitates later success (Scott, 1980). Thus, the adequacy of any socialization program can be determined by the ability of pups to adapt successfully to environmental changes with minimal behavioral and physiologic disruption.

RECORD KEEPING

Records on colony reproduction are essential. Individual records should contain the following minimal information on each bitch:

- start date of each proestrus;
- dates of mating and stud dog's identification number;
- date on which bitch's diet should be increased (day 42 of gestation), date to move bitch to whelping facility (day 50), and range of expected whelping dates;
- actual whelping date, whelping complications, number and sex of live pups, number of stillbirths, and any obvious abnormalities in the pups;
- date to start weaning, bitch's distemper antibody titer (if known), and dates to deworm and vaccinate litter; and
- date(s) and details of disposition of litter.

In addition, missed cycles, abortions, or any abnormal maternal behavior should be recorded.

To facilitate review of the reproduction records of an entire colony, it is helpful to have a separate computerized or manual-entry spreadsheet that displays every reproductive cycle of each bitch in the colony. The spreadsheet is most useful if it lists the following information, organized chronologically by date of proestrus:

- identification number of each bitch whose proestrus was first observed on that date;
- for each bitch bred, identification number of stud dog, first and last dates of mating, total number of matings, and calculated or expected dates for medical examinations, moving to whelping facility, and whelping; and
- expected date of next cycle.

The spreadsheet should be updated periodically to include for each bitch the actual whelping date; the length of gestation; litter information, as described

above; the actual date of the next cycle; and the calculated interestrus interval. A computerized list can be sorted to review the breeding records of individual bitches and males over several years. Such a list also allows examination for trends in low fertility, long or short gestation lengths as indicators of poorly timed inseminations, number of matings per cycle, projected periods during which several bitches will be in heat at the same time or no bitches will be in heat, and other matters that could reflect husbandry, management, or staff problems that need correction.

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5

Veterinary Care

Veterinary care in laboratory animal facilities goes beyond the prevention, diagnosis, treatment, and control of disease. It also includes monitoring animal care and welfare and providing guidance to investigators on handling and immobilizing animals and preventing or reducing their pain and distress (NRC, 1985, 1992). Responsibilities of the attending veterinarian are specified by the Animal Welfare Regulations (9 CFR 2.33, research facilities; 9 CFR 2.40, dealers and exhibitors).

The first sections of this chapter deal with the procurement and conditioning of research dogs and the control of infectious and parasitic diseases. Aspects of veterinary care dealing with the use of anesthetics and analgesics, surgery and postsurgical care, and euthanasia are taken up in the last three sections. The medical aspects of reproductive disorders are discussed in Chapter 4; special care for pups is also reviewed in Chapter 4 and addressed in detail elsewhere (Hoskins, 1990). Reference values for blood analytes can be found in textbooks by Kaneko (1989) and Loeb and Quimby (1989).

Dogs can be afflicted with many uncommonly occurring but scientifically interesting diseases and disorders, many of which also afflict humans. Some breeds have predispositions to particular diseases and disorders (e.g., dalmatians are prone to urate bladder stones); a comprehensive review of this subject is available (Willis, 1989). Chapter 6 of this book addresses the maintenance of dogs with selected genetic disorders.

PROCUREMENT

General Considerations

Dogs acquired from outside a research facility's breeding program must be obtained lawfully from dealers licensed by the U.S. Department of Agriculture (USDA) or sources that the USDA has exempted from licensing (7 USC 2137). A *List of Licensed Dealers* can be obtained from Regulatory Enforcement and Animal Care, Animal and Plant Health Inspection Service, USDA, Federal Building, Room 268, 6505 Belcrest Road, Hyattsville, MD 20782. Examples of exempt sources are municipal pounds and people who provide dogs without compensation.

Procurement of dogs for research requires planning by a knowledgeable person to ensure that the dogs receive good care and that the needs of the investigator are met. The person should be familiar with federal regulations applicable to the acquisition of dogs (9 CFR, parts 2 and 3) and with state and local ordinances applicable to the aquisition of dogs from pounds and shelters. *It is strongly recommended that institutions inspect vendors' premises for compliance with procurement specifications agreed on by contract before the first dogs are purchased and periodically thereafter.*

Sources

Both random-source and purpose-bred dogs can be purchased for research purposes. Random-source dogs are those raised under unknown conditions of breeding and health. Sometimes they are stabilized and conditioned (see below) by the dealer before sale. Purpose-bred dogs are those from known matings that have limited exposure to infectious diseases.

Random-source dogs that have not been stabilized and conditioned by the vendor (often called nonconditioned random-source dogs) are usually acquired from USDA-licensed dealers or, less commonly, from pounds. If a number of dogs of similar weight or body conformation are needed, the purchaser must allow sufficient time for the group to be assembled by the vendor. Random-source dogs that have been stabilized and conditioned (often called conditioned random-source dogs) should be purchased only from vendors that have written standard procedures for their conditioning programs. Purpose-bred dogs are acquired from USDA-licensed dealers that breed dogs specifically for research or from an institutional breeding program. Dogs with diseases of research interest are often acquired from exempt sources, such as pet owners referred by clinical veterinarians.

Conditioning

Conditioning is defined as physiologic and behavioral adjustment to a new environment. The period required for that adjustment to occur is called the conditioning period. Conditioning consists of adjustment to a new regimen, including new people, diet, climate, and exercise. The adjustment can be hastened if the using institution provides the same type of food as the dealer or vendor and uses the same type of automatic watering devices. Physiologic status, as well as the presence of diseases, can be determined by assessing red-cell counts, packed-cell volumes, and white-cell differential counts and by using blood urea nitrogen tests and other examinations of blood and urine. Those tests are most valuable when samples are taken several days after arrival, by which time initial adjustments to the new environment have been made. Abnormal findings on any of the tests might warrant followup examinations.

Evidence of behavioral adjustment includes decreases in fearful behaviors, increases in friendly behaviors, increases in playfulness, and normal grooming behaviors. Some dogs might not adapt to human handling or the environment and are therefore inappropriate for use in long-term studies. The dealer should be questioned about the sources and histories of such dogs to determine whether additional dogs purchased from that dealer will be similarly distressed. Information on maladaptive canine behavior has been published elsewhere (Scott, 1970).

Many procedures—such as trimming of nails, removal of matted fur, bathing, and teeth-cleaning—can be performed during the conditioning period.

There is no definitive rule about the optimal period for conditioning. The intended use of the dogs, the season, prevalence of canine diseases in the area, and other factors influence the length of the conditioning period. If the dogs are well selected, adequately socialized, immunized, and treated for parasites before delivery, the conditioning period can be reduced. Random-source dogs that have been held for 10 days or more by the dealer usually require at least 21 days of conditioning at the institution before one can be confident that they have adapted fully. Some prefer a minimal conditioning period of 45 days. The importance of humane treatment and proper care during conditioning must be emphasized.

CONTROL OF INFECTIOUS DISEASES

General Considerations

There are three important strategies for controlling canine infectious diseases: examining dogs on arrival and refusing to accept dogs that exhibit

signs of disease, placing all newly acquired dogs in quarantine, and isolating dogs that become sick. Some infectious pathogens to which dogs are susceptible could be introduced into an established colony by new arrivals, especially by random-source dogs, which are commonly unvaccinated. The most common of these pathogens are canine distemper virus (CDV); canine parvovirus (CPV-2); canine herpesvirus (CHV); the respiratory agents canine parainfluenza (PI-2), *Bordetella bronchiseptica*, and *Mycoplasma* spp.; canine adenovirus type 1 (CAV-1, infectious canine hepatitis) and type 2 (CAV-2, tracheobronchitis virus); and canine coronavirus (CCV). An additional problem that warrants careful consideration is the possibility that unvaccinated random-source dogs can harbor rabies virus, which can have a long incubation period (Acha and Szyfres, 1987). Dermatophytosis (ringworm, principally *Microsporum canis* and *M. gypseum*) and canine papillomatosis (warts) can also present problems. Protection against these pathogens is discussed briefly below. Detailed information on canine infectious diseases is available in a number of general references (e.g., Appel, 1987; Barlough, 1988; Greene, 1990).

Quarantine

Quarantine (in this context, the isolation of newly acquired animals until their health status has been evaluated) minimizes the risk of spreading diseases from newly arrived dogs to those already in the colony. In most facilities, the quarantine and conditioning periods overlap. During the quarantine period, most attention is directed to the control of infectious diseases and parasites. Procurement of dogs that are free of infectious diseases and parasites (i.e., conditioned random-source dogs or dogs bred specifically for research) reduces the time necessary for both quarantine and conditioning and might result in more reliable research results. Nonconditioned random-source dogs should be quarantined as a group, and no additional nonconditioned dogs should be introduced into the group.

Quarantine facilities should be designed to provide physical barriers to the spread of infectious diseases (e.g., unidirectional airflow). That is especially important when the research and quarantine facilities are parts of a single building. It is preferable for a quarantine facility to have its own animal-care technicians; however, if this is not possible, quarantined dogs should be cared for last.

Newly arrived dogs should be housed singly to enable veterinarians and technicians to determine which dogs are not eating well, exhibit signs of disease, or are abnormal in other ways. Ideally, dogs are vaccinated by the dealer. If not, they should be vaccinated as soon as possible after arrival against CDV, CPV-2, and CAV-2 (such vaccination also protects against

CAV-1). Vaccination for PI-2 and *B. bronchiseptica* should be considered in institutions where respiratory disease is common. If the dogs are to be vaccinated against leptospirosis and rabies, that is usually done at the same time. If a person is bitten or scratched, the injured area should be cleansed, the person should be referred to appropriate medical personnel, and the dog should be isolated for at least 10 days, as recommended by the National Association of State Public Health Veterinarians (1993).

Research and Breeding Colonies

The major threat to an established colony is that newly introduced dogs might harbor an infectious-disease agent or that personnel might carry such an agent into the colony on their hands or clothing. A regular immunization program, quarantine of nonconditioned random-source dogs, and rigorous sanitation practices will help to protect against infectious agents inadvertently introduced into an established colony. Annual vaccination with a multivalent vaccine is generally recommended, although immunity to CDV and CPV-2 generally persists for at least 3 years. In areas in which respiratory disease is common, frequent vaccination (every 3 months) might be indicated. Frequent vaccination (every 6 months) with leptospira bacterins is recommended in areas in which leptospirosis is endemic or is a proven problem.

Breeding Stock

Some infectious diseases are of special concern in breeding colonies. CHV can remain undetected in a breeding kennel for years. When susceptible, pregnant (usually young) bitches are introduced into the colony, latent CHV manifests itself by causing abortions or fetal or neonatal deaths. A detailed discussion of CHV is available elsewhere (Carmichael and Greene, 1990a).

Canine brucellosis can severely affect reproduction in a breeding kennel. It is also a zoonosis. All dogs purchased for breeding stock should be tested for *Brucella canis* antibodies on arrival and placed in quarantine for at least 1 month, at which time a second brucellosis test should be run. New dogs should not be introduced into a breeding colony unless both tests are negative. An infected dog should not be used for breeding or for long-term studies. Beagles have an unusually high prevalence of brucellosis, although it is occasionally diagnosed in random-source dogs (Carmichael, 1979). For detailed discussions of canine brucellosis see Carmichael (1990) and Carmichael and Greene (1990b).

Pups

CDV and CPV-2 infections are the principal viral diseases that threaten pups during the first 4 months of life, and prevention of these diseases should be the principal objective of an immunization program. Maternal antibody to CDV interferes with the development of an immune response to CDV vaccine; measles vaccine protects against disease but not infection in pups in which maternal antibody is still present (pups about 6-10 weeks old) (Baker, 1970). CDV vaccine should be given to dogs by 14 weeks of age. No vaccines can prevent parvovirus infection in pups during one critical period—that during which they still have maternal antibodies that inhibit the response to vaccination but do not protect against virulent CPV-2 (Carmichael, 1983). Proper management practices are critical in preventing this infection. If a pup does contract the disease, it should be isolated immediately, and rigorous disinfection procedures should be implemented. Diseases caused by adenoviruses and CCV can occur in pups, but they are less common.

It is generally recommended that modified live-virus vaccines be used for immunization, if available. A killed-virus vaccine is used for rabies. A multivalent vaccine that protects against distemper, hepatitis, leptospirosis, and parvovirus and parainfluenza infections can be used. An intranasal vaccine against *Bordetella bronchiseptica*, which causes kennel cough, is generally recommended. Several vaccination regimens have been proposed (Baker et al., 1961; Carmichael, 1983; Swango, 1983); one of them is given in Table 5.1 as a guide, but others are acceptable. The vaccination schedule should be adapted to address the perceived risk of infection.

Pups can be vaccinated with intranasal vaccine against *B. bronchiseptica* at 3-4 weeks of age. Other than that, vaccinating pups less than 6 weeks old is not recommended, because vaccine safety has not been studied in very young pups. Isolation is more important than vaccination in preventing disease in such pups.

TABLE 5.1 A Vaccination Schedule for Pups

Age	Vaccine
6 weeks	CDV or CDV combined with measles and CPV-2
8-10 weeks	CPV-2
12-14 weeks	Multivalent vaccine
16 weeks	CPV-2 (or multivalent vaccine) and rabies

Specific-Pathogen-Free Colonies

Dogs from known matings that have never been exposed to specific infectious agents are called specific-pathogen-free (SPF) for those agents. These dogs are used in infectious-disease and vaccine-development research in which animals are required not only to be free from pathogenic agents, but never to have been exposed, either naturally or through vaccination, to pathogenic agents. It might also be preferable to use SPF dogs in some transplantation studies, because in profound immunosuppression, native and vaccinal viruses (e.g., CDV and CAV-1) might be activated and cause disease (Thomas and Ferrebee, 1961).

The objective in preventing the outbreak of disease in SPF colonies is to isolate, rather than immunize, the dogs. Disease prevention depends on the establishment of physical barriers to preclude the introduction of disease agents, rigorous management practices, and control of personnel movement into and within the facility (Sheffy et al., 1961). Rodents and other pests that can transmit disease mechanically must be excluded. Purpose-bred SPF dogs are available commercially. If bred by the institution, initial breeding stock should be procured from dogs free of latent infectious agents, and all offspring taken by hysterectomy or cesarean section. Embryo-transfer technology offers additional possibilities for SPF colonies. Population immune status should be assessed periodically (at least once a year) by monitoring for antibodies to the common infectious diseases. In the event of an inadvertent infection that would compromise the use of the animals, the colony should be depopulated and re-established.

CONTROL OF PARASITIC DISEASES

Parasites are common in dogs, particularly random-source dogs. They can be found on the skin and hair and in the ears (ectoparasites) and in many internal organs, including the digestive tract, heart, lung, and blood vessels (endoparasites). Specific canine parasites are discussed briefly below; details on life cycles of, treatment for, and prevention or control of these parasites are found elsewhere (Georgi and Georgi, 1992).

Ectoparasites

Ectoparasites include ticks, mites, lice, and fleas. Most can be easily eradicated with insecticides. Three ectoparasites commonly carried by random-source dogs can pose problems if they are not eliminated during quarantine.

- The most damaging is probably the *Rhipicephalus sanguineus* tick. This tick can feed on dogs during all life-cycle stages, and once it enters a facility, it can be expensive to remove.
- Mange caused by *Sarcoptes scabei* is sometimes inadvertently introduced into a facility on a dog that shows no overt signs of dermatosis. This parasite can be a particular problem in dogs that are group-housed or housed in cages or runs that allow the touching of body parts among animals (e.g., through wire-mesh walls). Sarcoptic mange is treated by dipping the affected dogs and all dogs in contact with them in insecticide. It is probably also worthwhile to steam-clean enclosures and floors.
- Fleas are commonly brought into facilities by random-source dogs. The flea life cycle can be disrupted by cleaning enclosures daily to remove developing eggs and larvae. Another strategy is to house dogs in enclosures raised more than 33 cm above the floor. Fleas cannot jump higher than 33 cm, so fleas that fall to or develop on the floor cannot reach the dog to feed.

Additional ectoparasitic infestations that might persist in kennel settings include infestation with ear mites (*Otodectes cynotis*), "walking dandruff" (*Cheyletiella yasguri*), and lice (*Linognathus setosus*, *Trichodectes canis*, and *Heterodoxus spiniger*). The canine nasal mite (*Pneumonyssoides caninum*) can also persist, but it is not known how often infestations with this mite occur in random-source dogs.

To prevent the introduction of skin-dwelling ectoparasites, random-source dogs should be bathed or dipped before they are moved to the housing facility. Their ears should be examined and, if appropriate, treated for ear mites. Mites should be considered as the cause of persistant skin lesions, and appropriate action should be taken to make a correct diagnosis.

All dogs, including random-source and breeding-colony dogs, are probably host to the hair-follicle mite *Demodex canis*. Dogs probably become infected as puppies while nursing. Typically, the infestation is nonpathogenic; in rare instances, the mite causes severe mange. The development of demodectic mange in large numbers of kennel dogs is rare but has occurred. Treatment with topical applications or dips is possible as long as the lesions remain focal, but generalized demodectic mange often indicates some underlying problem (e.g., an inherited susceptibility to demodectic mange or a compromised immune system), and its treatment is difficult or impossible.

Endoparasites

SPF dogs and purpose-bred dogs often host both protozoan and helminthic endoparasites. The protozoa include *Isospora* spp., *Giardia* spp., trichomonads, *Cryptosporidium* spp., *Balantidium* spp., and amebas. The helminths include ascarids (e.g., *Toxocara canis* and *Toxascaris leonina*), *Filaroides*

spp., *Strongyloides stercoralis*, and occasionally hookworms and whipworms. If dogs are housed in a manner that allows mosquitoes access to them, they are also susceptible to infection with heartworm, *Dirofilaria immitis*.

Isospora spp. have direct life cycles (i.e., no intermediate host is required). Oocysts of these coccidia are commonly present in the feces of young dogs raised in colonies, and more than one species can be present in one dog. The oocysts of *I. canis*, *I. ohioensis*, *I. neorivoltos*, and *I. burrowsi* are morphologically similar; however, those of *I. canis* are larger than those of the other three. Clinical signs include increased temperature and diarrhea that is occasionally bloody. Infections usually subside after several days to weeks. Oocyst shedding decreases to low numbers 4 weeks after it begins. Chemoprophylaxis and basic sanitation are necessary to control the infection if it causes problems.

Cryptosporidium is occasionally present in dogs in closed colonies, although it typically does not cause disease. In immunocompetent dogs, the small oocysts of *Cryptosporidium* are shed in low numbers, if at all, for a limited period; however, in immunosuppressed or immunocompromised dogs, *Cryptosporidium* can cause fatal disease. There is no proven method of chemoprophylaxis or treatment, but routine sanitation procedures, accompanied by regular steam cleaning of areas that might be contaminated, will assist in reducing exposure to oocysts.

Giardia canis is commonly present in both purpose-bred and SPF dogs. The prevalence is high in pups and decreases with age. The organism is spread between dogs by the fecal-oral transmission of resistant cysts. Typically, pups are infected with *Giardia* and one or more species of *Isospora*; however, the infection usually causes little or no disease. As dogs mature, the number of organisms decreases. As with *Isospora*, chemoprophylaxis and basic sanitation are the most effective means of controlling *Giardia*.

Trichomonas canistomae is a commensal organism present in the mouths of many dogs. It has no cyst stage and is transmitted between dogs by direct oral contact. There is usually no need for treatment. Species of *Trichomonas* and *Pentatrichomonas* are present in the large intestines of many laboratory-reared dogs. None of these species has a cyst stage; transmission is by the fecal-oral route. These organisms are sometimes observed in diarrheic feces in very large numbers, but they are usually not the cause of the diarrhea. Treatment is available but usually not necessary.

Balantidium coli, a large ciliated parasite that is rarely found in dogs, and *Entamoeba coli* and *E. histolytica*, smaller ameboid parasites, are transmitted by cysts passed in the feces. These parasites are present in the large bowel. Their life cycles are similar to that of *Giardia*, and once they are established in a colony, they are easily perpetuated.

The ascaridoid nematode (roundworm), *Toxocara canis*, is a common parasite of the small intestines of dogs, even in closed breeding colonies.

The parasite is transmitted from bitches to pups in utero, and pups begin to shed eggs in their feces a few weeks after birth. Once the eggs enter the environment, they require about 2 weeks to become infectious; they are very resistant to environmental extremes of heat, cold, and humidity. Pups should be treated soon after birth and several times during early life to prevent the development of adult roundworms from the stages obtained prenatally. Control measures should include steam cleaning of floors and disinfection of floors with a 1:4 (20 percent) solution of chlorine bleach. Adult dogs can have larvae in their tissues whether or not they are shedding eggs in their feces. It is possible to determine whether a dog has ever been infected by measuring antibody concentrations, and dogs that are *Toxocara canis*-naive are available commercially.

The other canine ascaridoid, *Toxascaris leonina*, has a direct life cycle and does not infect pups transplacentally. The eggs of this parasite develop more rapidly than those of *Toxocara canis* but are just as resistant to extremes of heat, cold, and humidity. *Toxascaris leonina* is commonly present in the small intestines of older purpose-bred and SPF dogs, but it is not known how the cycle is maintained in these colonies. Control and treatment are the same as those used for *Toxocara canis*.

Filaroides hirthi is present in the lung parenchyma of many purpose-bred and SPF dogs. The lung lesions caused by the parasite can confuse histopathologic evaluations in toxicologic experiments. The life cycle is direct, and infective larvae are transmitted between dogs by oral or fecal-oral contact. Immunosuppressed dogs can become seriously ill as a result of auto-reinfection that leads to heavy parasite burdens. Infections can be treated, but control is difficult because fecal assays are insensitive. Therefore, all dogs in a contaminated room must be treated, not just those with positive fecal tests. Proper sanitation is helpful, but the larvae do not persist for long periods in the environment.

Strongyloides stercoralis lives as a parthenogenetic female in the mucosa of the canine small intestine. Larvae develop to the infective stage 4-5 days after they are passed in feces. Transmission is by penetration of the skin by infective-stage larvae and by passage of tissue-dwelling larvae in the milk of lactating bitches. Immunosuppressed or immunocompromised dogs can develop severe disease as a result of auto-reinfection. *S. stercoralis* is also transmissible to humans. Although treatment is available, elimination of the parasite from a breeding colony is difficult because it is not certain that transmammary transmission can be interrupted by chemotherapeutic measures. Routine removal of feces and cleaning of cage or pen floors reduce transmission.

Adult hookworms live in the small intestine, where they cause blood loss and anemia. The hookworms *Ancylostoma caninum* and *A. braziliense*, like *S. stercoralis*, are transmitted through the milk or by larval penetration

of the skin. However, infective-stage larvae are more likely to develop in soil than on a moist cage bottom fouled with feces, and transmission is more likely when dogs are housed outside on such surfaces as gravel or sand. Unlike dogs infected with *S. stercoralis*, dogs infected with hookworms often show signs of overt disease, characterized by bloody diarrhea. In addition, hookworm eggs are much easier to detect in feces than are *S. stercoralis* larvae. Those differences and the dissimilarity of conditions required for larval development make it much less likely that hookworms will persist undetected in a colony. The hookworm *Uncinaria stenocephala*, which is present in more temperate climates, is transmitted mainly by larval ingestion; skin penetration and transmission in milk are uncommon. Thus, *U. stenocephala* is less likely to be perpetuated in a closed colony.

Whipworms, *Trichuris vulpis*, live in the cecums and colons of dogs and cause large bowel disease that can produce bloody stools. The life cycle of this parasite is direct. Eggs are passed in feces and take several weeks to become infectious. They are highly resistant to environmental extremes, so contamination is very peristent if eggs get into the soil of earthen-floored runs. Dogs become infected by ingesting the infective eggs on soil-contaminated items. In the dog, the worms take about 3 months to develop to the adult stage, and reinfection is common. Treatment is available but often has to be repeated.

The filarioid nematode *Dirofilaria immitis* causes heartworm disease. It is transmitted between dogs by the bite of a mosquito. The prepatent period (the time between the inoculation of maturing forms by the mosquito and the first appearance of microfilariae in the host's blood) is slightly more than 6 months. The infection is often manifested as cardiopulmonary disease accompanied by respiratory distress and right-sided heart enlargement. In dogs with patent disease, infections can be diagnosed by demonstrating microfilariae in the blood; however, some infected dogs do not have circulating microfilariae (Glickman et al., 1984.). When it is important to ascertain that dogs are heartworm-free, serum or plasma can be examined with antigen-detection tests. Treatment for heartworm infection is generally precluded by its high cost, the stress it causes the dog, the length of time necessary for recovery, and the possibility of residual pathologic changes in the cardiovascular system.

Where *D. immitis* is enzootic, dogs given access to outside runs should be protected by chemical prophylaxis. If dogs cannot be placed on chemical prophylaxis, because of a study design or for other reasons, they can be protected by enclosing the outside kennels with screening.

In addition to infection with the same parasites found in purpose-bred and SPF dogs, random-source dogs are likely to be infected with parasites that are relatively rare or that require intermediate hosts as part of their life cycles. If the intermediate hosts are uncommon (e.g., snails, then crayfish

for the lung fluke *Paragonimus kellicotti*), there is little chance that the infection will be maintained in a kennel. However, if the intermediate host is commonly present around dogs (e.g., fleas for the tapeworm *Dipylidium caninum*), the parasite will probably persist in the facility as long as the intermediate host is present. Additional parasites that can be found in random-source dogs include the tapeworm *Taenia* spp. (intermediate hosts, mammals), the intestinal fluke *Alaria canis* (snails, then frogs), the esophageal nematode *Spirocerca lupi* (beetles), and the stomach nematode *Physaloptera* spp. (beetles).

Two parasites that are found rarely in random-source dogs, *Echinococcus* spp. and *Trypanosoma cruzi*, are important because they cause zoonoses. Larval stages of the canine tapeworms *Echinococcus granulosus* and *E. multilocularis* can be transmitted to humans in contaminated feces and cause unilocular and multilocular hydatid disease, respectively. Eggs of *Echinococcus* spp. are infectious when passed in feces and cannot be distinguished morphologically from eggs of taeniid tapeworms. *E. granulosus* is present in focal areas of the United States; *E. multilocularis* is present in the far northern continental United States, Alaska, and Canada. *Trypanosoma cruzi*, which is present in the southern United States, is a hemoflagellated protozoan that can infect the blood and tissues of opossums, armadillos, dogs, humans, and other mammals. Humans are infected by accidental self-inoculation with blood products from an infected animal. People handling dogs from areas where *Echinococcus* spp. and *T. cruzi* are enzootic should be made aware that such infections, although rare, are possible and can be associated with life-threatening conditions in humans.

Three other uncommon canine pathogens, all requiring arthropod vectors, have occasionally been diagnosed in dog facilities: *Leishmania* spp., *Babesia* spp., and *Ehrlichia canis*. The clinical signs caused by these pathogens are often poorly deliniated, so they can be harder to diagnose than common helminth infections.

Cutaneous and visceral leishmaniasis, caused by infections with various species of *Leishmania*, have been reported in both kennels and research colonies. The organism is typically transmitted between dogs by the bite of a phlebotomine sandfly, although the mode of transmission in the reported cases is not certain. Diagnosis is typically made by identifying the organisms histopathologically or serologically. Treatment is difficult but possible.

Babesiosis, caused by *Babesia canis* or *Babesia gibsoni*, can be introduced into colonies or kennels through an infected dog, an infected tick, or a blood transfusion. Once it is in an establishment, horizontal transmission typically occurs through exposure to infected blood that is not handled properly or through ticks, particularly *Rhipicephalus sanguineus*. Dogs with babesiosis display regenerative anemia, i.e., the bone marrow remains

functional, and increased numbers of immature erythrocytes appear in the blood. The disease can be diagnosed by demonstrating the organisms in erythrocytes on stained blood films. Treatment is difficult, and drugs routinely used in parts of the world where babesiosis is common are not easily obtained in the United States.

Transmission of ehrlichiosis, a rickettsial infection caused by *Ehrlichia canis*, is similar to transmission of babesiosis. Signs of ehrlichiosis in dogs include fever, anorexia, epistaxis (nosebleeds), and reduced kidney function. Diagnosis is made serologically or by demonstrating the presence of the organism in blood smears. Treatment can alter the course of the disease but does not prevent an affected dog from becoming a carrier of the infection.

Good sanitation is probably the major means for controlling endoparasites in a dog facility. In facilities that house purpose-bred or SPF dogs, feces from healthy animals of different ages should be examined periodically for subclinical helminth or protozoan infections. Fecal and blood examinations can be used to screen random-source dogs for parasites on arrival at the facility. To prevent the introduction of helminth parasites into a facility, random-source dogs might be treated for some infections with an anthelminthic. A practical choice would be a broad-spectrum anthelminthic that is active against both nematodes and tapeworms.

RECOGNITION AND ALLEVIATION OF PAIN AND DISTRESS

Recognition of Distress Induced by Pain

Distress can be defined as "an aversive state in which an animal is unable to adapt completely to stressors and the resulting stress . . ." (NRC, 1992, p. 4). Scientists have legal, ethical, and humane obligations to minimize distress in experimental animals. Moreover, there is a pragmatic reason to minimize distress. Unless a stressor (such as pain) is the subject of the experiment, distressed animals might provide erroneous data (Amyx, 1987). Pain is an important cause of distress and is usually produced by disease, injury, or surgery.

Table 5.2 lists some of the signs of pain in dogs. Dogs usually respond to acute pain by vocalizing and by protecting or guarding the area of perceived pain. Signs include withdrawing, attempting to bite if touched, and adopting unusual postures (e.g., the laterally flexed position commonly adopted after lateral thoracotomy). Low-grade pain can produce restlessness. Severe pain, especially if chronic, usually makes dogs appear depressed and lethargic. The decrease in activity can be accompanied by one or more of the following: shivering, inappetence, panting, howling, or whining.

The *U.S. Government Principles for Utilization and Care of Vertebrate*

TABLE 5.2 Signs of Pain in Dogs[a]

Sign	Comment
Guarding	Attempting to protect or move painful part away (e.g., hunched position after celiotomy or laterally flexed position after lateral thoracotomy), attempting to bite
Vocalization	Whining or whimpering when touched or forced to use affected part
Mutilation	Licking, biting, scratching, shaking, or rubbing affected part
Restlessness	Pacing, lying down and getting up, or shifting weight
Recumbency	For unusual length of time
Depression	Inappetence, reluctance to move, or difficulty in rising
Pallor	Pale mucous membranes, probably a result of vasoconstriction caused by an increase in sympathetic tone

[a]Adapted from Soma, 1987; printed with permission of the author, the American Association for Laboratory Animal Science, and the Scientists Center for Animal Welfare.

Animals Used in Testing, Research, and Training (published in NRC, 1985) states that "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." This statement makes it clear that most surgical interventions must be accompanied by adequate anesthesia and suitable postoperative analgesia. Table 5.3 lists the degree and duration of pain that can be expected after surgery on various parts of a dog's body. Although pain thresholds are similar between individuals and even between species, pain tolerance varies widely. Therefore, each dog should be observed and treated as an individual in determining the need to administer analgesics.

Alleviation of Pain

Anesthetics

General anesthesia is the most important way of alleviating pain associated with surgery, and several textbooks contain detailed descriptions of acceptable techniques for inducing general anesthesia in dogs (Booth, 1988a; Hall and Clarke, 1991; Lumb and Jones, 1984; Muir and Hubbell, 1989; Short, 1987). Inhaltant agents (e.g., isoflurane, methoxyflurane, and halothane) are often best for this purpose because they allow close regulation of the duration and depth of anesthesia and rapid and controlled reversibility. However, special equipment is required for administering them. Nitrous oxide is not a general anesthetic in dogs and should be used only as an adjunct to other, more potent anesthetics.

General anesthesia can also be provided with injectable drugs, such as barbiturates (e.g., thiamylal, thiopental, and pentobarbital), propofol, or Telazol

TABLE 5.3 Signs, Degree, and Length of Surgically Produced Pain[a]

Surgical Site	Signs of Pain	Degree of Pain	Length of Pain
Head, eye, ear, mouth	Attempts to rub or scratch; self-mutilation; shaking; reluctance to eat, drink, or swallow; reluctance to move	Moderate to high	Intermittent to continual
Rectal area	Rubbing, licking, biting, abnormal bowel movement or excretory behavior	Moderate to high	Intermittent to continual
Bones	Reluctance to move, lameness, abnormal posture, guarding, licking, self-mutilation	Moderate to high: upper part of axial skeleton (humerus, *femur*) especially painful	Intermittent
Abdomen	Abnormal posture (hunched), anorexia, guarding	Not obvious to moderate	Short
Thorax	Reluctance to move, respiratory changes (rapid, shallow), depression	Sternal approach, high; lateral approach, slight to moderate	Continual
Spine, cervical	Abnormal posture of head and neck, reluctance to move, abnormal gait-"walking on eggs"	Moderate to severe	Continual
Spine, thoracic or lumbar	Few signs, often moving immediately	Slight	Short

[a]Based on observations of dogs. Reprinted from NRC, 1992.

(a mixture of tiletamine and zolazepam). Each injectable drug has properties that determine its duration of action and the route by which it is best administered. Ketamine is used as an anesthetic but its effectiveness as an analgesic for visceral pain is disputed (Booth, 1988b; Hughes and Lang, 1983). It should be used in combination with another analgesic agent when visceral pain is expected. It can also induce seizure-like activity in dogs unless it is used in conjunction with another drug, such as diazepam, acepromazine, or xylazine. Chloralose and urethane are injectable anesthetics that have been used in some experiments; however, chloralose alone is a poor anesthetic that produces little analgesia unless it is combined with an opiate such as morphine (Rubal and Buchanan, 1986), or a short-acting anesthetic (Flecknell, 1987). Urethane is mutagenic and carcinogenic (Auerbach, 1967; Mirvish, 1968); it should be used with caution and only for nonsurvival surgery.

Neuromuscular blocking agents (e.g., succinylcholine, atracurium, curare,

gallamine, pancuronium, and vecuronium) have no anesthetic or analgesic properties. They must not be used alone for surgical restraint, although they may be used in conjunction with anesthetic doses of general anesthetic drugs (NRC, 1985).

Local anesthetics (e.g., lidocaine, mepivacaine, and bupivacaine) act to disrupt nerve conduction temporarily. When applied around a nerve, they produce analgesia in the region served by that nerve. However, these drugs have no depressant effect on the brain; dogs undergoing procedures under local anesthesia usually must be restrained physically or chemically (e.g., with tranquilizers or sedatives). Specific techniques for regional anesthesia are described in several texts (Hall and Clarke, 1991; Lumb and Jones, 1984; Muir and Hubbell, 1989; Skarda, 1987; Soma, 1971). Local anesthetics alone are ordinarily used for only the most minor of surgical interventions; but they can be given either intrathecally or epidurally (usually via the lumbosacral space) to provide segmental anesthesia of caudal body parts sufficient for major surgery (e.g., celiotomy) (Skarda, 1987).

Analgesics

Opioid analgesics are compounds that act at specific opioid receptor sites in the central nervous system to produce analgesia. Table 5.4 lists some of these compounds. They are not general anesthetics, but can be used for surgery when combined with other appropriate drugs (NRC, 1992). Opioid analgesics (e.g., oxymorphone) can be injected epidurally to control postsurgical pain for extended periods with minimal systemic effects (Popilskis et al., 1991).

Opioid agonists have been combined with tranquilizers to produce so-called neuroleptanalgesic combinations (e.g., a mixture of fentanyl and droperidol known by the trade name Innovar-Vet and produced by Pitman Moore, Mundelein, Ill.). Such combinations are capable of producing a state that

TABLE 5.4 Opioid Analgesics Used in Dogs[a]

Drug	Dose (mg/kg)	Route[b]
Buprenorphine	0.01-0.2	IV, IM
Butorphanol	0.2-0.5	IV, IM
Fentanyl	0.04	IV, IM
Meperidine	2.0-6.0	IM
Morphine	0.5-1.0	SC
Oxymorphone	0.2-0.4	IV, IM

[a]Data from Harvey and Walberg, 1987.
[b]IV = intravenous; IM = intramuscular; SC = subcutaneous

sufficiently resembles general anesthesia to permit some surgical procedures (Muir and Hubbell, 1989; Soma and Shields, 1964). Xylazine, which is classified as a sedative, has analgesic properties because of its action on central alpha-2 receptor sites.

The nonsteroidal anti-inflammatory analgesics include acetaminophen, aspirin, flunixin, and ibuprofen. These drugs inhibit prostaglandin synthesis. They are ordinarily used to relieve the acute or chronic pain associated with inflammation and have little place in the management of severe or acute pain that is not associated with inflammation (NRC, 1992).

Recognition of Distress Not Induced by Pain

Signs of distress caused by stressors other than pain include changes in behavior (e.g., unexpected aggression), maladaptive behaviors (e.g., stereotypies), and physical changes (e.g., weight loss). Experienced and attentive animal caretakers are of the utmost importance in early recognition of signs of distress. Changes in biochemical measurements (e.g., plasma cortisol concentration) can also help in recognition of distress.

Alleviation of Distress Not Induced by Pain

Distress caused by stressors other than pain is often related to husbandry practices. Understanding and meeting dogs' social and physical needs will minimize or prevent such distress (NRC, 1992).

Phenothiazine tranquilizers, such as acepromazine (0.03-0.05 mg/kg intravenously or intramuscularly, 1.0-3.0 mg/kg by mouth), are useful as preanesthetic drugs because they make unruly animals more tractable, reduce the doses of anesthetic drugs necessary to maintain anesthesia, and make recovery from anesthesia smoother. However, they can have unpredictable effects and cause some animals to become excited rather than tranquil (Voith, 1984). The phenothiazines have minimal antianxiety effects, and they are not the drugs of choice for decreasing fearful reactions (Marder, 1991).

Alpha-2 agonists, such as xylazine (0.3-1.0 mg/kg intravenously, 0.5-2.0 mg/kg intramuscularly), have many of the advantages of the phenothiazines and are also good analgesics (Gleed, 1987). However, they can cause serious cardiovascular depression, hyperglycemia, and depressed thermoregulation, which can be reversed with yohimbine if necessary (Denhart, 1992).

Benzodiazepines, such as diazepam (0.1-0.5 mg/kg intravenously, 0.3-0.5 mg/kg intramuscularly) are often used as adjuncts to injectable anesthetic drugs, such as the barbiturates and ketamine, because they reduce the dose necessary to produce anesthesia and provide muscle relaxation (Gleed, 1987). Diazepam (Valium) is also used alone to treat seizures. Like the

phenothiazines, the benzodiazepines have an excitatory effect on some animals. Because they are the drugs of choice for the treatment of fearful behaviors (Marder, 1991), especially fear of people (Hart, 1985), they can be useful in reducing distress in unsocialized dogs. However, the benzodiazepines must be used with care in dogs that display fear-motivated aggression. Decreasing the fear might make such dogs more likely to attack (Marder, 1991).

SURGERY AND POSTSURGICAL CARE

Surgery in dogs should be performed in accordance with the tenets in the *Guide* (NRC, 1985). The requirements for minor and nonsurvival surgical procedures are less stringent than those for major survival surgical procedures.

Personnel performing surgical procedures must be adequately trained. Facilities for performing surgical procedures should be available as outlined in the *Guide* (NRC, 1985). The successful practice of survival surgery requires strict adherence to aseptic surgical technique, as well as provision of adequate postoperative care and analgesia for the experimental subject. Aseptic techniques also have some value in major nonsurvival surgical procedures (Slattum et al., 1991). Generally, only healthy conditioned or purpose-bred dogs should be used for survival surgery. Familiarizing the dog with the laboratory environment can assist investigators in identifying intractable subjects and can be beneficial in decreasing postoperative stress.

Presurgical Preparation

Dogs should be surgically prepared by careful shaving to remove all hair from the surgical field. Shaving reduces contamination of the wound and avoids delays in healing that can occur if hair becomes matted in the incision. If a thermal cautery is to be used, an area should also be shaved for placement of a ground lead. Adherent grounding pads are available. The surgical field should be thoroughly cleaned with Betadine (povidone-iodine) or another appropriate surgical scrubbing material. Betadine sterile solution or other appropriate preparation should be applied to the entire field and allowed to dry. Underpadding used to absorb such solutions can be flammable and should be removed before surgery.

All surgical instruments and chronic instrumentation must be sterilized with steam (autoclaving) or gas (ethylene oxide with proper poststerilization aeration time). Cold chemical sterilization is appropriate for minor surgical procedures, but exposure time must be adequate, and the instruments must be thoroughly rinsed in sterile saline before they come into contact with body tissues. All items should be packaged for sterilization in such a way

that they can be opened and positioned for use without compromising sterility. Investigators should follow standard surgical practices: donning surgical caps and masks, scrubbing, and donning surgical gowns and gloves. Sterile drapes should be positioned on the dog to define the surgical field. During the course of surgery, procedures for preserving sterility should be strictly followed.

Generally, dogs should be treated with the appropriate preanesthetic medications (e.g., tranquilizers and atropine) to provide a degree of sedation and facilitate handling. General anesthesia is reviewed in the section "Alleviation of Pain" (see pages 64-67); the type used depends on the type and duration of the surgical procedure. The adequacy of anesthesia can be assessed by the absence of the eyelid reflex and by the lack of withdrawal in response to painful stimuli (e.g , toe pinch). Insertion of a cuffed endotracheal tube will ensure patency of the respiratory tract.

The physiologic status of dogs under general anesthesia should be assessed by monitoring such parameters as pulse rate, systemic blood pressure, and respiratory rate. Electrocardiography can be used to monitor the status of the heart. A heating pad is useful for maintaining body temperature. If inhalant anesthetics are used, the anesthetized dog should be ventilated (tidal volume, 15-20 ml/kg; respiratory rate, 13-20 breaths/minute), and carbon dioxide should be monitored. Respiratory rate, tidal volume, and inspiratory-expiratory ratio can be adjusted to achieve acceptable end-tidal carbon dioxide (38-40 torr) and blood oxygen saturation greater than 90 percent.

An intravenous catheter should be placed in the cephalic vein to provide a continuous intravenous drip (e.g., of lactated Ringer's solution) for volume replacement and to ensure rapid access to the circulatory system. Depending on the situation, antibiotics can be administered through the catheter or intramuscularly. There is evidence that giving antibiotics during the 2 hours before surgery is more beneficial than giving them either during or after surgery (Classen, 1992).

Postsurgical Care

Appropriate analgesics should be administered for postoperative pain, as needed (see pp. 66-67 and NRC, 1992). Surgical wounds and sites of instrument entry into the body should be cleaned and treated daily (e.g., with 0.3 percent hydrogen peroxide or dilute Betadine solution). Topical antibiotics (e.g., bacitracin ointment) can be applied. Surgical dressings should be changed every day.

Basic biologic functions—including urination, defecation, and appetite—are good indicators of a dog's overall physical well-being. These are easy to observe and should be monitored regularly and often. Followup clinical

examinations and laboratory tests can be used to identify specific problems. Appropriate supportive care should be provided as needed.

A commonly used experimental protocol involving major survival surgery in the dog is the implantation of instruments that allow physiologic measurements over a long period while the dog is conscious. The dog is particularly suitable for this type of protocol because of its size, its equable temperament, and the close parallelism of its physiologic functions with those of humans. Strict adherence to the recommendations above will minimize confounding effects.

EUTHANASIA

Euthanasia is a method of killing an animal rapidly and painlessly (NRC, 1985). It should be carried out by trained personnel following current guidelines established by the American Veterinary Medical Association (AVMA) Panel on Euthanasia (AVMA, 1993 et seq.; NRC, 1985) The method used must produce rapid unconsciousness and subsequent death without evidence of pain or distress, or the animal must be anesthetized before being killed (9 CFR 1.1). The method used should also be safe for attending personnel, be easy to perform, and cause death without producing changes in tissues that might interfere with necropsy evaluation. Methods of euthanasia recommended by the AVMA Panel on Euthanasia (AVMA, 1993) are discussed below.

Injection of Lethal Substances

Injection of a lethal substance is probably the most suitable method for euthanatizing laboratory dogs. It usually involves the intravenous injection of a large dose of a barbiturate anesthetic, such as pentobarbital (more than 100 mg/kg). The advantage of this method is that the animal is anesthetized within seconds and does not undergo the pain or distress that might be associated with later respiratory and cardiac arrest. In fact, cardiac arrest can be delayed for many minutes after the onset of anesthesia; therefore, cardiotoxins (e.g., large doses of dibucaine) are sometimes used to hasten death (Wallach et al., 1981). Unruly or aggressive dogs should be sedated or tranquilized to facilitate the restraint necessary for smooth intravenous injection. Intravenous injection is the preferred route of administration because venipuncture is easily performed on most dogs by trained, experienced personnel. Injection outside the circulatory system is less reliable, is potentially painful, and almost invariably produces a slow onset of action.

Injectable drugs—such as magnesium sulfate, potassium chloride, and neuromuscular blocking agents (e.g., atracurium, curare, gallamine, pancuronium, succinylcholine, and vecuronium)—may be used (Bowen et al., 1970;

Hicks and Bailey, 1978); however, the dogs must be in a deep plane of anesthesia before drug administration (AVMA, 1993). Strychnine and nicotine are not suitable for euthanasia, because their stimulant properties might cause distress even in anesthetized animals.

Inhalation Methods

Overdose of a potent inhalant anesthetic (e.g., halothane and isoflurane) is satisfactory for performing euthanasia on dogs and is particularly appropriate for young dogs, in which venipuncture can be difficult. Anesthetic vapors tend to be irritating; therefore, the animals should be tranquilized first. If anesthetic vapors are used, a system for scavenging excess vapor is necessary to comply with federal guidelines on anesthetic-vapor pollution (CDC, 1977). Ether, unlike most contemporary inhalant anesthetics, is flammable and explosive; therefore, its use is not recommended.

Carbon monoxide and carbon dioxide both cause death by hypoxia. Carbon monoxide is impractical in most instances because of the risk to operators and the complexity of the equipment to administer it. Carbon dioxide has anesthetic properties and can be used for euthanasia (Carding, 1968; Leake and Waters, 1929); however, unless the chamber is well designed and used properly, dogs can become distressed before becoming unconscious. Hypoxia is not satisfactory for euthanatizing pups because young animals tolerate hypoxia better than older dogs and can survive for more than 30 minutes (Glass et al., 1944).

Physical Methods

Exsanguination is acceptable for euthanasia; however, the dog must be anesthetized because the decreasing blood flow causes anxiety and autonomic stimulation (Gregory and Wotton, 1984). Electrocution is considered a humane method of euthanasia, provided that sufficient current passes through the animal's brain to produce unconsciousness before or coincidentally with the onset of cardiac arrest. However, this method of euthanasia is not practical in most laboratories because of the danger to personnel (AVMA, 1993; Roberts, 1954; Warrington, 1974). Decapitation of pups is not recommended by the AVMA Panel on Euthanasia (1993).

Human Considerations

Euthanasia of dogs or any other animals can be stressful for the personnel performing the procedure. The degree of distress experienced by people observing or performing euthanasia depends on their backgrounds, personal philosophies, and ethical views on the use of animals in research (Arluke,

1988). People often transfer to the death of animals their unpleasant reactions to human death, and their responses to euthanasia can be magnified when strong bonds exist between them and the dogs being killed (e.g., strong bonds often develop between animal-care personnel and seriously ill canine models that require a great deal of care and rely totally on their human guardians). The stress experienced can be manifested as absenteeism, belligerence, careless and callous handling of animals, and high turnover rate. To be responsive to those concerns, institutional officials and supervisors should be aware of and sensitive to the issues and should provide opportunities for individual and group discussion and support and for educational programs that furnish factual information about euthanasia and teach stress-management and coping skills (NRC, 1991).

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6

Special Considerations

PROTOCOL REVIEW

One of the many important responsibilities of an institutional animal care and use committee (IACUC) is to review the protocols of research projects in which dogs will be used (9 CFR 2.31; PHS, 1986). The protocol-review mechanism is designed to ensure that investigators consider the care and use of their animals and that protocols 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 matters involving the use of animals. Although the discussion below is directed to the use of dogs in research, the review requirements apply to all vertebrate species.

Each research protocol must completely (but concisely) delineate the proposed study, including a description of each of the following:

- the purpose of the study;
- the rationale for selecting dogs as the research subjects;
- the breed, age, and sex of the dogs to be used;
- the numbers of dogs in various groups of the protocol and the total number to be used;
- experimental methods and manipulations;
- experimental manipulations that will be performed repeatedly on an individual dog;

- preprocedural and postprocedural care and medications;
- procedures that will be used to minimize discomfort, pain, and distress, including, where appropriate, the use of anesthetics, analgesics, tranquilizers, and comfortable restraining devices;
- the euthanasia method, including the reasons why it was selected and whether it is consistent with the recommendations of the American Veterinary Medical Association Panel on Euthanasia (AVMA, 1993, et seq.);
- the process undertaken to ensure that there are no appropriate in vitro alternatives, that there are no alternative methods that would decrease the number of animals to be used, and that the protocol does not unnecessarily duplicate previous work; and
- the qualifications of the investigators who will perform the procedures outlined.

One approach used by IACUCs is to have a scientifically knowledgeable member thoroughly review the protocol. The reviewer contacts the investigator directly to clarify issues in question. Later, at an IACUC meeting, the reviewer presents and discusses the protocol and relates discussions with the investigator. Changes or clarifications in the protocol that have resulted from the reviewer's discussions with the investigator are submitted to the IACUC in writing. After presentation of the protocol, the reviewer recommends a course of action, which is then voted on by the IACUC. Another kind of protocol review (which is especially effective in small institutions with few grants) is initial review by the entire IACUC; results are generally available to the investigator within a short period.

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 accorded due process and be given the opportunity to rebut the IACUC's critique in writing, to appear in person at an IACUC meeting to present his or her viewpoint, or both. It is also important that provision be made for expedited review, in which a decision is reached within 24-48 hours. Expedited reviews should be used only for emergency or extenuating circumstances. When a protocol is submitted for expedited review, each member of the IACUC must have an opportunity to review it and may call for a full committee review before approval is given and before animal work begins (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. In

the case of 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 the signer 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 investigators 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.

RESTRAINT

Some form of restraint is generally necessary to control a dog during a procedure (see guidelines in NRC, 1985, p. 9). The method used should provide the least restraint required to allow the specific procedure to be performed properly, should protect both the dogs and personnel from harm, and should avoid causing distress, physical harm, or unnecessary discomfort. In handling and restraining dogs, it is helpful to understand species-typical behavior patterns and communication systems.

A small or medium-size dog can be picked up by placing one hand under the chest and abdomen while restraining the head with a leash. Lifting a large dog might require two people. It is important to remember that males are sensitive to touch near their genitalia. Minor procedures, such as taking a rectal temperature or administering a subcutaneous injection, can usually be accomplished by one person using minimal restraint. During venipuncture, sufficient restraint should be used to avoid repeated needle insertions and to prevent the development of painful hematomas. Kesel and Neil (1990) detail methods for handling and restraining animals.

If dogs are to be restrained frequently or for long periods or if the restraint method used is especially rigorous, it might be necessary to train them to tolerate the restraint. Training sessions should use positive-reinforcement techniques; negative-reinforcement techniques are not desirable. Physical abuse (9 CFR 2.38f2i) and food or water deprivation (9 CFR 2.38f2ii) must not be used to train, work, or handle dogs, although food and water may be withheld for short periods when specified in an IACUC-approved protocol (9 CFR 2.38f2ii).

SPECIAL CARE FOR ANIMAL MODELS

The remainder of this chapter deals with some common uses of laboratory dogs in which aspects of care vary from the general guidelines provided in previous chapters. It is not intended to present an exhaustive list

of canine models that require special housing and husbandry, but rather to provide the reader with different types of canine models that can serve as examples of how housing and husbandry can be modified to achieve animal well-being. The suggestions offered here are not to be construed as the only ones possible. The committee recognizes that not every research procedure and circumstance can be anticipated, and it assumes that sound professional judgment, good veterinary practices, and adherence to the spirit of this guide will prevail in unusual situations.

The final subsection of this chapter introduces the reader to the technique of somatic cell gene therapy. Many disorders of dogs, like those of humans, are caused by single-gene mutations. Scientists are working to develop techniques to cure these disorders permanently by replacing mutant genes with normal ones. For many reasons (see Chapter 2), the dog is an ideal model for evaluating the safety and efficacy of gene therapy.

Aging

Clinical Features

Life expectancy and disease incidences vary among breeds of dogs; therefore, it is not possible to state a specific age at which dogs become old. Common laboratory dogs, such as beagles, begin some aging changes when they are 8-10 years old. Such physical features as graying of the haircoat, especially around the face, are often apparent as aging begins.

As dogs age, they tend to become less active and to exhibit such signs of mental deterioration as poor recognition of caretakers, excessive sleeping, and changes in personality. Senile plaques, similar to those found in humans with senile dementias, have been reported in the brains of old dogs (Wiśniewski et al., 1970). Various forms of arthritis, spondylosis, and degenerative joint disease are common and contribute to problems in mobility and to the apparent diminution of mental alertness. Older dogs might decrease their daily food intake, become slow eaters, or become irregular in their eating habits. Dental problems—including periodontal disease, tooth abscesses, and oral-nasal fistulas—increase; the importance of these problems is probably underestimated (Tholen and Hoyt, 1983). Dogs more than 6 years old develop lenticular sclerosis, which results in a bluish appearance within the pupil. Visual acuity decreases with age and is often associated with cataracts, secondary glaucoma, and other diseases (Fischer, 1989). There is also apparent hearing loss.

Atrophy of the thyroid gland and an increased number of thyroid tumors have been reported, and signs of hypothyroidism are common (Haley et al., 1989; Milne and Hayes, 1981). Thyroid atrophy and the propensity of older dogs to develop hypothermia might be related (B. A. Muggenburg,

Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, Albuquerque, N.M., unpublished). A decreased response to antigens and changes in lymphocyte function might indicate that the older dog is less able to resist infectious diseases (Bice and Muggenburg, 1985). Some changes in common blood-cell measures and serum chemistry become important when these are used for diagnosis (Lowseth et al., 1990a). The incidence of neoplasia increases strikingly (MacVean et al., 1978); for example, lung tumors, nearly unknown in young dogs, can reach an incidence as high as 10 percent in dogs over 10 years old (Ogilive et al., 1989). Pulmonary function decreases with age because of reduced lung volumes and decreased elasticity (Mauderly and Hahn, 1982). Chronic renal diseases often occur and require frequent monitoring. Chronic heart disease is also fairly common, and clinical signs can appear suddenly in old dogs.

Husbandry and Veterinary Care

Housing and environment. Accommodation should be made for dogs that have problems moving comfortably on floor grates or through guillotine-like doors in kennel buildings. Because of their decreased mobility and impaired thermoregulatory function, aging dogs with access to outdoor areas should be checked frequently to be certain that they are able to get inside to escape the cold or heat. Automatic watering devices might become difficult to use; for some old dogs, it might be necessary to switch to water pans placed on the floor.

Nutrition. Differentiation between age-related and disease-caused changes in eating habits might be difficult. It is important that animal-care personnel become familiar with and closely monitor daily eating habits of older dogs. Frequent checking and recording of body weights can help in assessing whether food intake is adequate. Changes in diet are sometimes dictated by the clinical diagnosis of disease (e.g., a low-protein diet for chronic, progressive renal disease and a low-sodium diet for chronic heart failure).

Physical characteristics of food can affect dental hygiene. Soft and wet food fed over many years can contribute to dental disease. Feeding dry dog food and providing hard objects for chewing can be helpful in the long-term management of dental problems. Routine dental care, including the removal of calculus and polishing, is essential.

Veterinary care. The extent of chronic disease problems in older dogs requires more intensive veterinary care, extensive diagnostic investigations, and good nursing. Dosages of some medications might have to be reduced, because drugs are commonly metabolized more slowly in old than in young adult dogs. Such drugs as digoxin should be monitored by measuring blood

concentrations to decrease the risk of overdosing (De Rick et al., 1978). A useful reference on geriatric veterinary medicine is *Geriatrics and Gerontology* (Goldston, 1989).

Reproduction

Bitches. Andersen and Simpson (1973) have described reproductive senescence in beagle bitches. Intact bitches exhibit irregular estrous cycles, accompanied by decreased fertility, and prolonged periods of anestrus. The mortality rate is higher among puppies born to older bitches than among puppies born to bitches less than 3 years old.

The most common pathologic condition of the uterus of aged bitches is pyometra (Andersen and Simpson, 1973; Järvinen, 1981; Whitney, 1967). Vaginal fibromuscular polyps are also common (Andersen and Simpson, 1973). The age-specific incidence of mammary gland neoplasms in intact beagle bitches continues to increase throughout life (Taylor et al., 1976).

Dogs. Aging dogs have testicular atrophy and often develop prostatic hypertrophy and hyperplasia and have episodes of prostatitis (Lowseth et al., 1990b). There are also metaplastic changes in the bladder (Lage et al., 1989).

Cardiovascular Diseases

Congenital Heart Defects

Clinical Features

Dogs with hereditary cardiovascular malformations have been used to investigate the role of genetic and embryologic factors in the cause and pathogenesis of congenital heart defects, including hereditary patent ductus arteriosus, conotruncal defects (e.g., ventricular septal defect, tetralogy of Fallot, and persistent truncus arteriosus), discrete subaortic stenosis, and pulmonary valve dysplasia. Congenital heart defects in dogs have been summarized by Buchanan (1992) and Eyster (1992). Table 6.1 describes and lists the clinical signs of selected heart defects. Each of those defects is transmitted as a lesion-specific genetic defect in one or more breeds. A model for each defect has been developed at the University of Pennsylvania School of Veterinary Medicine by selective breeding of affected dogs (Patterson, 1968), as follows: patent ductus arteriosus, toy and miniature poodles (Ackerman et al., 1978; De Reeder et al., 1988; Gittenberger-de Groot et al., 1985; Knight et al., 1973; Patterson et al., 1971); conotruncal defects, keeshonden (Patterson et al., 1974, 1993; Van Mierop et al., 1977); discrete subaortic

TABLE 6.1 Selected Congenital Cardiac Defects in Dogs

Defect	Description	Clinical Signs
Patent ductus arteriosus	Failure of ductus arteriosus to close after birth. If pulmonary vascular resistance is low, blood flows through ductus from left to right. Pulmonary hypertension and left ventricular hypertrophy result unless ductus opening is small. If ductus is large and pulmonary vascular resistance is high, pulmonary arterial pressure can exceed aortic pressure, and blood will flow from right to left, sending venous blood into ascending aorta.	Vary with size of duct and pulmonary vascular resistance from subclinical to heart failure. Early signs include poor growth, coughing, and dyspnea. Aneurysm can occur at site of ductus arteriosus. Polycythemia occurs in cyanotic dogs with a large patent ductus arteriosus (PDA), pulmonary hypertension, and right to left blood flow through the PDA.
Conotruncal defects		
Ventricular septal defect	Failure to complete formation of the conotruncal septum results in ventricular septal defects (VSDs) of varied size, involving the lower and middle portions of the crista supraventricularis (Type I, subarterial VSD). Pups with large VSDs usually die from pulmonary edema in the neonatal period. Smaller VSDs are compatible with long life unless complicated by pulmonary hypertension and congestive heart failure.	Vary with size of defect from subclinical to signs of respiratory and right-side heart failure, including cyanosis, dyspnea, weakness, and anorexia.
Tetralogy of Fallot	Consists of pulmonic stenosis (valvular, infundibular, or both), conal ventricular septal defects, dextroposition of aorta with overriding of ventricular septum, and right ventricular hypertrophy. Some dogs have pulmonary valve atresia (pseudo-truncus arteriosus).	Depend on severity of pulmonic stenosis and ventricular septal defect. Can include decreased body size, fatigue, cyanosis, and secondary polycythemia.
Persistent truncus arteriosus	Severe but rare anomaly. Complete failure of septation of conus and truncus regions, producing large conal ventricular septal defect and single arterial outlet vessel.	Cyanosis and dyspnea. Dogs rarely survive neonatal period.

TABLE 6.1 *Continued*

Defect	Description	Clinical Signs
Discrete subaortic stenosis	Narrowing of left ventricular outflow tract, most commonly by fibrous ring just below aortic semilunar valves, with concomitant obstruction of blood flow, left ventricular hypertrophy, and increased left ventricular pressure.	Vary with degree of stenosis from asymptomatic to poor growth, exercise intolerance, syncope, ventricular arrhythmias, pulmonary edema, and sudden death.
Pulmonary valve dysplasia	Varies from mild thickening of leaflets surrounding narrowed pulmonary orifice to complete fusion of leaflets and doming of valve. Interferes with emptying of right ventricle.	Vary from asymptomatic to dyspnea, fatigability, and right-side heart failure.

stenosis, Newfoundlands (Patterson, 1984; Pyle et al., 1976); and pulmonary valve dysplasia, beagles (Patterson, 1984; Patterson et al., 1981). Conotruncal defects in the keeshond breed are determined by the effect of a single major gene defect (Patterson et al., 1993). Subaortic stenosis in Newfoundlands also appears to be monogenic with variable expression (Patterson, 1984). Patent ductus arteriosus and pulmonary valve dysplasia are inherited in a non-Mendelian pattern.

Husbandry and Veterinary Care

Animals with cardiac defects often require exercise restriction to avoid cyanosis and congestive heart failure. The need for restriction must be decided for each dog on the basis of cardiac status. If the clinical manifestations of severe defects (e.g., respiratory distress, severe cyanosis, and congestive heart failure) cannot be relieved with appropriate surgical methods or cardiovascular drugs (e.g., cardiac glycosides and diuretics), the dog should be humanely killed (see Chapter 5).

Reproduction

Only dogs with mild to moderate cardiac defects or those in which the defects have been surgically corrected should be selected for breeding. Severely affected dogs do not survive to breeding age, or they develop clinical manifestations that preclude their use for reproduction (e.g., marked cyanosis

and congestive heart failure). Methods of modern clinical cardiology—including auscultation, radiography, echocardiography, cardiac catheterization, and angiocardiography—are necessary for accurate diagnosis and evaluation of the severity of defects in candidates for breeding. Therefore, appropriate facilities and equipment and personnel qualified to use such equipment must be available before a breeding colony is established. Once it is established, the health status of breeding stock and their offspring must be carefully monitored.

Induced Heart Defects

Clinical Features

Many animal models of cardiac disease are surgically induced in physiologically normal animals. Aims of the research protocol and humane considerations must often be carefully balanced to ensure that the maximal amount of information is derived from each animal.

Surgically induced models can be broadly divided into models of volume or pressure overload produced by creating valvular or interchamber defects, models of ischemic injury, and models of arrhythmia (Gardner and Johnson, 1988). Long-term management of these models can be difficult because they are frequently on the verge of physiologic decompensation and at risk of sudden death. Table 6.2 lists the signs of cardiac failure.

TABLE 6.2 Clinical Signs of Heart Failure in Dogs

Type of Heart Failure	Clinical Signs
Left-side	Exercise tolerance decreases. Inappropriate dyspnea follows exercise. Pulmonary venous pressure increases, initially causing pulmonary and bronchial congestion and reflexogenic bronchoconstriction. Repetitive coughing follows exercise. Orthopnea, with a reluctance to lie down; restlessness at night; and paroxysmal dyspnea are common. In severe failure, pulmonary edema, severe dyspnea at rest, and rales on auscultation become evident.
Right-side	Systemic venous congestion occurs with engorgement of jugular veins. Liver and spleen are enlarged and often palpable. Fluid retention is usually first manifested as ascites; subcutantous edema, hydrothorax, or hydropericardium can follow. Disturbances of gastrointestinal function, with diarrhea, can occur.
Generalized	Signs of both left- and right-side failure occur.

Husbandry and Veterinary Care

The management of chronic dog models of induced heart failure is most successful if the approach used is interdisciplinary, involving cardiologists, surgeons, and veterinary-care staff. Goals of long-term management include identifying potential complications, selecting therapeutic regimens, and developing long-term monitoring protocols. The following general guidelines should be tailored to the type of disorder induced, the dogs' well-being, and the goals of the research protocol.

Postoperative care. Postoperative care depends on the type of heart disease induced. Medical management should continue after successful recovery from surgery because a specific surgical protocol does not always produce a physiologically consistent model. Some dogs achieve a stable, compensated postoperative condition; others undergoing the same procedure develop signs of acute heart failure immediately after surgery.

Careful monitoring on the days after surgery is critical. Meticulous physical examinations should be performed on physiologically stable dogs at least once a day until they have recovered from surgery. Physiologically unstable dogs should be examined more often. Vital signs should be monitored, and particular attention should be given to physical findings related to the cardiovascular system. Mucous membrane color, capillary-refill time, and temperature of extremities can be abnormal if peripheral perfusion is seriously impaired. The pulse quality of the femoral artery can be used to assess systemic perfusion. Auscultation should be used to detect abnormal cardiac sounds, and electrocardiography should be performed to diagnose arrhythmias. Assessment of respiratory rate and depth should be combined with careful auscultation of all lung fields to detect early signs of pulmonary complications. Echocardiography, if available, can be used to evaluate cardiac function and contractility.

Good nursing care is important. Special diets, such as canned dog food or dry food mixed with chicken broth, can be offered to encourage food intake. Ideally, dogs should be housed in a dedicated recovery room and returned to the regular housing area only when they are physiologically stable and have recovered fully from surgery. Decreased exercise tolerance secondary to diminished cardiac reserve might affect the extent of activity that a dog can withstand.

Complications. Potential complications associated with surgical and catheterization procedures should be anticipated, including infection of the operative site, bacteremia, and endocarditis. Dogs at high risk for complications are the ones that undergo serial catheterization procedures and those with bioimplants, such as prosthetic valves and pacemakers (Dougherty,

1986). Baseline monitoring should include scheduled physical examinations and complete blood counts (CBCs). A blood culture should be submitted to the laboratory for any animal with a persistent fever or an intermittently increased temperature. If infection is suspected, a broad-spectrum antibiotic, such as one of the cephalosporins, should be administered pending receipt of culture and sensitivity results.

Banding of the great vessels with various materials is a standard procedure for producing volume- and pressure-overload models of ventricular hypertrophy, coarctation of the aorta, and obstruction of right ventricular outflow. Vessel erosion caused by the material used (Gardner and Johnson, 1988) and hemorrhage secondary to banding procedures are common complications that should be included in the differential diagnosis of any banded animal that suffers an acute onset of lethargy, paleness of the mucous membranes, or dyspnea. Those are also clinical signs of heart failure, so it is important to perform auscultation of the chest and suitable diagnostic tests, such as radiography or thoracentesis, to make an accurate diagnosis. A dog that is hemorrhaging should be euthanatized.

Surgical procedures used to induce cardiac disease invariably cause disruption of the endothelium and put the dogs at risk for thrombosis and embolism. Dogs undergoing cardiac catheterization or surgery of the cardiac valves are at greatest risk. Clinical signs reflect the organs involved.

Long-term monitoring. In a study of extended duration, assessment of each dog's general health and cardiovascular system should be continuous. The type and frequency of examinations will depend on whether the model is physiologically stable or unstable. For example, a dog with induced mitral regurgitation, which is defined as a 50 percent reduction in forward stroke volume and a pulmonary capillary wedge pressure of 20 mm Hg, can develop life-threatening pulmonary edema (Nakano et al., 1991; Swindle et al., 1991). Frequent monitoring and auscultation are required to detect early signs of respiratory compromise so that the dog will not die before therapy can be initiated or the dog can be studied. Similarly, a dog with induced right ventricular pressure overload requires frequent monitoring because decreased coronary blood flow can lead to acute right-side heart failure (Fixler et al., 1973; Vlahakes et al., 1981). Conversely, a stable model of left ventricular hypertrophy can be produced in 8-week-old pups by aortic banding, which causes a systolic pressure gradient of 15-20 mm Hg (O'Kane et al., 1973). Dogs with induced tricuspid valve insufficiency can tolerate increased venous pressure and a slight reduction in cardiac output for years, although some develop ascites and reduced serum albumin (Arbulu et al., 1975). These models require less frequent monitoring.

Equipment. Follow-up care and monitoring require appropriate equip-

ment and laboratory support for obtaining CBCs, blood cultures, serum chemistry profiles, and blood-gas analyses. Electrocardiography and echocardiography should be available for assessing cardiac rhythm and function, respectively. Echocardiography is also a useful noninvasive method for monitoring changes in cardiac wall thickness, cardiac motion, and chamber size as cardiac disease progresses. A cardiac catheterization laboratory should be available for performing hemodynamic and angiographic studies.

Pharmacologic therapy. Pharmacologic management of dogs that develop complications or clinical signs of heart failure must be coordinated between the veterinary unit and the investigator to prevent the administration of medications that could compromise the scientific aims of the study. Diuretics can be used to treat pulmonary edema and reduce plasma volume, but their effects on serum electrolytes and the reduction of venous return and cardiac output should be considered. Vasodilators, calcium antagonists, β-blocking drugs, and positive inotropic agents should be available for managing acute clinical events; however, long-term use of these drugs is usually contraindicated because of their effects on the disease process being studied (Bonagura, 1986; Swindle et al., 1991).

Hypertension

Clinical Features

To provide proper care for hypertensive dogs and to avoid inappropriate treatment that can be detrimental to the dogs and compromise the study, it is necessary to have a full understanding of the pathophysiology of hypertension and of the specific method that is used to induce it. Generally, hypertension in dogs is induced by constricting the renal artery. The resulting reduction in renal perfusion causes systemic arterial pressure—and renal arterial pressure distal to the constriction—to rise enough to maintain renal function. A discussion of the relationship between renal function and the long-term control of blood pressure can be found in any standard physiology textbook (e.g., Guyton, 1991).

Two methods are most commonly used to induce renal vascular hypertension: partial constriction of one renal artery (the 2-kidney, 1-clip method) and unilateral nephrectomy and partial constriction of the remaining renal artery (the 1-kidney, 1-clip method). Both those methods produce what is called Goldblatt hypertension, but the mechanisms responsible for the hypertension are different. The 2-kidney, 1-clip model depends more heavily on the renin-angiotensin system than the 1-kidney, 1-clip model and responds to acute treatment with angiotensin-converting enzyme (ACE) inhibitors, which block the conversion of angiotensin I to angiotensin II. The

1-kidney, 1-clip model requires chronic treatment with ACE inhibitors to lower blood pressure. The reason for that difference is described in detail by Guyton (1991).

The greatest success in producing hypertension while reducing the incidence of malignant hypertension and renal failure is achieved by reducing renal arterial flow by exactly 50 percent. Renal blood flow is usually measured when the arterial clamp (Goldblatt clamp) is adjusted during surgery; this obviates later surgery to readjust the degree of constriction. Methods have been developed for measuring renal blood flow chronically and adjusting the renal artery clamp (Ferrario et al., 1971), and more recently a technique has been described for producing hypertension reliably by gradually constricting the renal artery with constrictors fabricated of ameroid, a hydroscopic material made of compressed casein cured in formalin (Ben et al., 1984; Brooks and Fredrickson, 1992).

Other methods that have been used for inducing hypertension include a 2-kidney, 2-clip model in which Goldblatt clamps or ameroid constrictors are applied to both renal arteries; wrapping of one or both kidneys with silk or cellophane; a combination of unilateral nephrectomy and wrapping of one kidney; and placing sutures in a figure 8 configuration on the surface of one or both kidneys (the Grollman model). The creation of hypertension with deoxycorticosterone acetate (DOCA) and common salt has not been as successful in dogs as it has in rats, because dogs are reluctant to eat a high-salt diet or drink a saline solution. However, moderate hypertension in dogs can be achieved with DOCA administration alone. A colony of spontaneously hypertensive dogs has been described (Bovée et al., 1986).

Husbandry and Veterinary Care

Proper care of hypertensive dogs involves the following:

- careful design and establishment of the hypertensive model to produce stable hypertension;
- routine evaluation of renal function;
- regular and frequent monitoring of blood pressure;
- regular monitoring of the retinas;
- appropriate treatment with antihypertensives when required; and
- careful husbandry.

Evaluation of renal function. Routine evaluation of renal function is essential because renal failure is a common complication in dogs with experimental hypertension. Renal failure can be caused by too much constriction of the renal artery, a rapid increase in both systolic and diastolic pressures (malignant hypertension), or the inappropriate use of antihypertensives.

Evaluation of renal function is especially important with use of the 1-kidney, 1-clip and 2-kidney, 2-clip models (which cause the most severe hypertension) and during antihypertensive therapy. In hypertensive dogs, renal function is compromised to such an extent that blood pressure must be raised to maintain sodium balance. If antihypertensive therapy lowers blood pressure too much, acute renal failure will ensue.

The most reliable and easily measured indicators of renal function are serum creatinine concentration and blood urea nitrogen (BUN). Although they depend somewhat on the type of assay, normal serum creatinine for the dog ranges between 0.4 and 1.3 mg/dL and BUN between 10 and 25 mg/dL. Serum creatinine and BUN should be determined in each dog before hypertension is induced to avoid using dogs with already-compromised renal function. In Goldblatt hypertensive models, serum creatinine should be determined daily for the first 5 days after surgery and twice a week thereafter. If ameroid constrictors are used, daily evaluations should continue through the second week after surgery because it takes 4-5 days for ameroid constrictors to reach maximal constriction. In models in which hypertension is not as severe, such as the 2-kidney, 1-clip and 1-kidney, 1-wrapped hypertensive models, renal function is less likely to be impaired, and serum creatinine concentration and BUN might not be increased, but they should be evaluated at least once during the 10-day postoperative period.

If renal-function tests show signs of renal failure, corrective action should be taken. Too-severe constriction of the renal artery can be corrected surgically, or the study can be terminated by euthanatizing the animal. Renal failure caused by lowering blood pressure to below the renal autoregulatory range should be corrected by reducing the dose of the antihypertensive drug to a point that allows blood pressure to remain high enough to maintain renal function. Malignant hypertension can be treated with antihypertensives and reduced salt intake (Ross, 1989; see below).

Measurement of arterial blood pressure. Blood pressure should be determined routinely after surgery. It can be done with indirect methods, such as placing a pressure cuff at the base of the tail (Petersen et al., 1988) or above the hock, or with direct methods, such as chronic implantation of arterial catheters or acute femoral arterial catheterization. To avoid complications associated with exteriorized catheters, some investigators now use methods that do not require exteriorized components, such as a Vascular Access Port (Access Technologies, Skokie, Ill.) (Mann et al., 1987), or chronic instrumentation, such as constriction of the carotid loop (Brooks et al., 1991). In addition, improved telemetric monitoring (Lange et al., 1991) has the potential to allow continuous monitoring of blood pressure over a number of days or weeks.

It is important to establish a baseline blood pressure before inducing

hypertension. Measuring blood pressure several times permits the dog to become accustomed to the monitoring technique and thereby avoids increases in blood pressure caused by stress. Some investigators measure blood pressure indirectly (e.g., with the tailcuff method) before surgery and use more direct methods later. That is done in recognition that indirect methods can lead to a deviation of up to 10 mm Hg from true arterial pressure. Normal systolic blood pressure ranges from 112 to 142 mm Hg; normal diastolic pressure from 56 to 110 mg Hg. Measurements greater than 160/95 indicate hypertension.

Treatment for hypertension. When induced correctly, surgically created hypertension is sustained and has few complications. If necessary, hypertensive dogs can be maintained on special diets (see below) and given diuretics or other antihypertensive drugs when needed. Some drugs readily available for treatment of hypertensive dogs are listed in Table 6.3.

Malignant hypertension must be diagnosed quickly and treated aggressively. The most striking clinical sign of malignant hypertension can be blindness caused by retinal detachment, which is usually preceded by retinal hemorrhage, dilation of retinal vessels, and subretinal edema. The dogs do not appear to be in pain but often bump into walls and might become disoriented or sit quietly in their pens or cages. Diagnosis can easily be confirmed with an ophthalmologic examination. If blood pressure can be controlled and retinal disinsertion (detachment from the ora ciliaris retinae)

TABLE 6.3 Drugs Available for the Oral Treatment of Hypertension in Dogs[a]

Generic Name	Dosage, mg/kg	Frequency of Administration	Class
Chlorothiazide	20-40	Every 12 hr or daily	Diuretic
Hydrochlorothiazide	2-4	Every 12 hr or daily	Diuretic
Furosemide[b]	2-4	Every 8-12 hr	Diuretic
Propranolol	0.25-0.5	Every 8 hr	β-Adrenergic antagonist
Hydralazine	1-3	Every 12 hr	Vasodilator
Prazosin	0.25-2	Every 8 hr	Vasodilator
Verapamil[c]	1-2	Every 8 hr	Vasodilator; calcium-channel blocker
Captopril	0.5-1	Every 8-12 hr	Angiotensin-converting enzyme inhibitor

[a]Adapted from Ross, 1989; printed with permission of the author and W. B. Saunders, Philadelphia, Pennsylvania.

[b]Can also be given intramuscularly or intravenously at 2-4 mg/kg.

[c]Can also be given intravenously at 0.05-0.15 mg/kg.

does not occur, some vision might be restored in 2-3 weeks. Malignant hypertension often responds well to treatment with ACE inhibitors. Diuretics can also be administered if care is taken to avoid a precipitous drop in renal blood flow. Vasodilators can be used with caution. If the cause of the malignant hypertension is overconstriction of the renal artery, ACE inhibitors can be used to stabilize the dog while the stricture is surgically corrected.

Husbandry. Routine care of hypertensive dogs must include a consideration of diet because both salt intake and protein intake will affect blood pressure and renal function. A high salt intake will exacerbate hypertension, and a high protein intake might accelerate the loss of renal function. To avoid unintended changes in diet that could compromise their dogs and studies, investigators, veterinarians, and others caring for hypertensive dogs should establish dietary requirements before beginning studies.

For dogs with hypertension and renal failure, the diet should contain 0.1-0.3 percent sodium on a dry-weight basis or 10-40 mg/kg per day (5-20 mg/lb per day) (Ross, 1989). Low-protein diets (less than 15 percent) that are also low in sodium (e.g., K/D, Hill's Pet Products, Inc., Topeka, Kansas) are available and should be fed in adequate amounts—generally 1 can or 2 cups of dry food for each 10 kg (20 lb) of presurgical body weight. The protein content of some commercially available diets might be too low to maintain ideal body weight, but diets that combine a higher protein content with a lower sodium content are available (e.g., R/D, Hill's Pet Products, Inc., Topeka, Kansas). As in any dog model, following the body weight of an animal regularly is a good way to monitor the animal's overall health.

There is usually no reason to restrict primary enclosure size for hypertensive dogs. Whether they should be exempted from an exercise program depends on their postoperative course. If the hypertensive condition stabilizes and there are no complications, exemption from exercise should not be necessary. Blood pressure is known to increase in stressful conditions; therefore, it is important that such conditions be avoided (e.g., dogs that are housed or exercised in pairs or groups should be monitored to ensure that they are compatible).

Ehlers-Danlos Syndrome

Clinical Features

Ehlers-Danlos syndrome type I is an autosomal dominant condition of humans for which there are analogues in dogs and other mammals (Hegreberg et al., 1969, 1970). The disease is caused by a defect in metabolism of

dermal collagen that results in a skin tensile strength less than 10 percent of normal. Fibrous tissue and bone are subclinically affected in some cases (Minor et al., 1987). Multiple lacerations are often observed. The hyperextensible skin can cause superior entropion, inferior ectropion, or both.

Husbandry and Veterinary Care

The extreme fragility of the skin must be considered in managing dogs with this syndrome. Affected dogs should be housed singly in smooth-surfaced pens of glass, concrete, or sheet steel. Automatic watering valves and other projections should be avoided. The dogs' nails should be kept trimmed. Some dogs might have to wear Elizabethan collars for extended periods to prevent self-inflicted wounds. Dogs should be given opportunities for exercise, either singly or in small groups, by being released under supervision into an exercise pen or room that is free of sharp projections. Leash-walking should be avoided.

Wound management is relatively simple. Wounds tend to heal well, possibly because hyperextensible skin places little tension on wounds. Cutting suture needles and single-stranded nylon suture material can tear through the skin, but tapered needles and braided sutures, such as those of polygalactin 910 (Vicryl), are well tolerated. It is important to avoid placing too much tension along a single suture line. Hygromas and hematomas, which can become large under loose skin, can be encountered, either as sequelae to lacerations or as primary events. Adhesive tape should never be applied directly to the skin or fur during bandaging because it can tear the skin when the bandage is removed. Entropion and ectropion can be corrected surgically; however, repeated correction might be necessary.

Reproduction

All affected dogs appear to be heterozygotes; affected homozygotes probably die in utero. To increase fertility, to avoid injury of affected animals, and to prevent conception of homozygotes, it is preferable to select normal bitches and affected males for breeding and to use artificial insemination. Heterozygous affected pups can be identified at birth by the fragility and hyperextensibility of their skin, as can heterozygous fetuses in late gestation.

Endocrinologic Diseases

Clinical Features

Endocrinopathies in the dog pose diagnostic and therapeutic challenges because they are complicated physiologic derangements that often involve multiple organ systems. An endocrinopathy might be a desired element of an experimental design or simply a spontaneous random occurrence that would be expected in any canine population. Table 6.4 lists the major endocrinopathies that have been documented in dogs. Discussions in this section are limited to endocrinopathies that either are induced in experimental animals or are undesired results of management procedures or investigational protocols. Hypothyroidism and hyperadrenocorticism (Cushing's disease), two major endocrinopathies often seen in clinical veterinary practice, are not discussed here but are well described in the veterinary medical literature (e.g., Capen and Martin, 1989; Chester, 1987, Drazner, 1987a; Feldman, 1989; Hsu and Crump, 1989; Peterson and Ferguson, 1989). A brief review of disorders of calcium metabolism is included because hypocalcemia caused by iatrogenic hypoparathyroidism occasionally occurs in a research setting, and hypercalcemia is often mistakenly attributed to parathyroid dysfunction.

TABLE 6.4 Selected Endocrine Disorders in Dogs

Affected Organ	Diseases
Adrenal cortex	Hyperadrenocorticism Hypoadrenocorticism
Adrenal medulla	Pheochromocytoma
Pancreas	Diabetes mellitus Gastrinoma
Parathyroid	Hyperparathyroidism Hypoparathyroidism
Pituitary	Acromegaly Diabetes insipidus Hypopituitarism
Thyroid	Hyperthyroidism Hypothyroidism
Multiple glands	Hyperlipidemia Hypoglycemia

TABLE 6.5 Common Clinical Signs of Selected Canine Endocrinopathies

Endocrinopathy	Common Clinical Signs
Diabetes mellitus	Hyperglycemia, polydipsia, polyuria, glycosuria, increased food consumption but loss of weight, bilateral cataract development, weakness
Hypoadrenocorticism	Weakness, vomiting, diarrhea, bradycardia, acute collapse
Acromegaly	Respiratory stridor, increased interdental spaces, prominent skin folds, abdominal enlargement, fatigue
Hypercalcemia	Mental dullness; muscular weakness; tachycardia; upper gastrointestinal signs, including anorexia, nausea, and vomiting; signs of renal disease, including nephrocalcinosis, renal calculi, and secondary renal failure
Hypocalcemia	Muscle tremors, tetany, seizures

Common clinical signs of the endocrinopathies to be discussed are listed in Table 6.5. They range from very subtle changes to acute crises. Most are nonspecific and can also be seen in various nonendocrine disorders. Detailed discussions of endocrinopathies can be found in the veterinary medical literature (e.g., Drazner, 1987b; Ettinger, 1989; Feldman and Nelson, 1987; McDonald and Pineda, 1989; Morgan, 1992).

Husbandry and Veterinary Care

Procedures for managing dogs with endocrinopathies are dictated by both the experimental design and the animals' welfare.

Diabetes mellitus. Diabetes mellitus in the dog is a recognized spontaneously occurring model (Kramer, 1981), and the disease is readily induced either by chemical ablation of the pancreatic β-cells or by total pancreatectomy (Mordes and Rossini, 1985). Frequent monitoring is mandatory for the successful management of dogs with diabetes mellitus. Daily measurements, before the first meal of the day and 6-12 hours later, are required to stabilize and control blood glucose in diabetic dogs. The second glucose measurement can be eliminated only when the afternoon blood glucose of an individual dog is consistent from day to day and the insulin requirement for that dog is well established. Blood glucose monitoring should begin after initial administration of diabetogenic chemicals or during the first 24 hours after pancreatectomy. Fasting blood glucose, as measured by the plasma or serum glucose oxidase method, ranges from 65-118 mg/dL (3.6-6.5 mmol/L) in normal adult dogs (Kaneko, 1989).

A number of insulin preparations can be used either singly or in combination in dogs: regular, NPH, lente, and ultralente. Unit doses and prepara-

tion types must be determined for and adjusted to the response of each dog. Insulin should be started at a dose of 1 U/kg per day injected subcutaneously at the time of feeding the first meal of the day. Daily proportions of each preparation included in a therapeutic regimen are determined by trial and error as guided by the results of serial blood glucose measurements. Detailed information on dosage and characteristics of various insulin preparations is available (Nelson, 1989; Schaer, 1992).

In addition to insulin administration, stresses from environmental and experimental manipulation, exercise, concurrent disease, estrus, and changes in food and water intake can cause profound fluctuations in blood glucose concentrations. Blood glucose can be manipulated by adjusting insulin types and dosages. As a general rule, it is preferable to have a slightly hyperglycemic dog rather than a hypoglycemic one because of the potentially disastrous results of a hypoglycemic crisis. If such a crisis occurs, it should be treated with intravenous dextrose and supportive care (Kirk and Bistner, 1985). Supplemental glucose can be given orally if the dog is able to swallow. Obviously, a necessary follow-up includes reviewing and adjusting the insulin dosage and the ratio of short- to long-acting insulin preparations given.

The amount of food fed to each diabetic dog should be standardized at what is necessary to maintain its optimal body weight. The same amount should be fed each day. Once an eating pattern (amount of food eaten and time required for meal consumption) is established for a given dog, its appetite can be used as an indicator of general well-being.

In pancreatectomized dogs, it is necessary to compensate for lost pancreatic exocrine function. That can be accomplished by adding a commercially available digestive enzyme to the food. Some dogs find the product unpalatable, but it is generally accepted if it is mixed with canned food.

Diabetic dogs can be maintained for long periods, but sequelae of diabetes mellitus—including neuropathy, immune system compromise, and delayed healing—do occur, and a shorter than normal life span should be expected.

Hypoadrenocorticism. The canine model of hypoadrenocorticism (Addison's disease) is a classic model in biomedical research (Brown-Sequard, 1856). Hypoadrenocorticism can be induced in dogs by administering the drug mitotane,[1] which chemically ablates the adrenal cortex (Nelson and Woodard, 1949). During induction, a presumptive diagnosis can be made by monitoring changes in serum electrolytes, specifically sodium and potassium. The normal ranges of sodium and potassium concentrations in dog serum are

[1] Chemical name, 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethane; trivial name, *o,p'*-DDD; brand name, Lysodren.

140-155 mEq/L and 3.7-5.8 mEq/L, respectively (Carlson, 1989). In dogs with hypoadrenocorticism, the sodium-to-potassium ratio is decreased to less than 27:1 (Schrader, 1988), although this hyperkalemia is not pathognomonic. The adrenal corticotropic hormone stimulation test is required for definitive diagnosis (Nichols and Peterson, 1992). In a crisis, resuscitation requires recognizing the problem, intravenously administering 0.9 percent saline solution, replacing glucocorticoids and mineralocorticoids, and possibly providing therapy for hyperkalemia. Long-term maintenance entails glucocorticoid (cortisone) administration, mineralocorticoid supplementation with 9-fluorohydrocortisone acetate,[2] and the addition of sodium chloride to the diet. Electrolytes should be monitored at least weekly once stabilization is achieved. Environmental and experimental stresses and alterations in water and food availability can have substantial effects on electrolyte balance and homeostasis. Additional glucocorticoid (increased by a factor of 2-10) should be administered during periods of stress.

Acromegaly. Acromegaly can be iatrogenically induced in bitches when progesterone is given to prevent estrous cycling (Eigenmann, 1985, 1989). It can also be secondary to increased production of progesterone during diestrus. Progesterone induces acromegaly by increasing the production of growth hormone in the anterior pituitary gland. The excessive release of growth hormone can also induce a "pituitary diabetes" that can be difficult to control with insulin. Cessation of progesterone administration or spaying will reverse acromegalic changes.

Calcium derangements. Although disorders of the parathyroid glands are usually suspected when hypercalcemia or hypocalcemia is present, the calcium abnormality is more often associated with other conditions, including pseudohyperparathyroidism, the most common cause of hypercalcemia (Feldman and Nelson, 1987); hypoadrenocorticism; renal failure; bone lesions; and hypervitaminosis D. Primary hyperparathyroidism in the dog is rare. Pseudohyperparathyroidism (hypercalcemia of malignancy) is a paraneoplastic syndrome that has been recognized in dogs with lymphosarcoma, adenocarcinoma of the anal apocrine glands, multiple myeloma, osteosarcoma, and other neoplasms (Meuten et al., 1982, 1986). Signs of hypercalcemia are not always overt, and treatment should be directed toward the underlying cause.

Causes of hypocalcemia include calcium imbalance during lactation, renal disease, acute pancreatitis, intestinal malabsorption, hypoalbuminemia, and primary hypoparathyroidism (idiopathic or iatrogenic). Iatrogenic

[2] Brand name, Florinef.

hypoparathyroidism is associated with inadvertent damage or removal of the parathyroid glands and is an important consideration in research settings. Surgery involving the ventral neck area or the laryngeal-tracheal area or removal of the thyroid glands carries an increased risk of complications related to parathyroid function. Treatment includes calcium replacement and appropriate management of the precipitating disorder.

Hematologic Disorders

Clinical Features

Canine models of human hematologic disorders have been reviewed (Dodds, 1988, 1989, 1992; Hall and Giger, 1992; Harvey, 1989; Kaneko, 1987; Knoll, 1992). Clinical signs of some of these disorders are listed in Table 6.6.

TABLE 6.6 Inheritance and Signs of Selected Hematologic Disorders in Dogs

Disorder	Inheritance	Clinical signs
Hemophilia A	X-linked	Low factor VIII coagulant activity but normal or increased von Willebrand factor antigen concentrations; spontaneous bleeding diathesis of varied severity, depending on factor VIII activity; severely affected dogs often exhibit spontaneous hemarthroses and large joints. The most common severe inherited bleeding disease. Recognized in most purebreds and in mongrels.
Hemophilia B (Christmas disease)	X-linked	Deficiency of factor IX activity; signs similar to those of hemophilia A. Recognized in 17 breeds.
von Willebrand's disease type I	Autosomal incompletely dominant	Variable deficiency of von Willebrand factor; factor VIII activity might be reduced; and prolonged bleeding time; moderately severe bleeding diathesis of mucosal surfaces. Signs are exacerbated by stress, hypothyroidism, intercurrent disease, trauma, and surgery. Recognized in more than 50 breeds.

continued on next page

TABLE 6.6 Continued

Disorder	Inheritance	Clinical signs
von Willebrand's disease type III	Autosomal recessive	Severe deficiency of von Willebrand factor; factor VIII activity is usually low; indefinitely prolonged bleeding time; mucosal surface bleeding diathesis, which can be severe and is exacerbated by stress, hypothyroidism, trauma, surgery, and intercurrent disease. Recognized in Chesapeake Bay retrievers, Scottish terriers, and Shetland sheepdogs.
Factor X deficiency	Autosomal incompletely dominant	Homozygotes are stillborn or die shortly after birth; affected pups might live for up to 2 weeks and then die of massive internal bleeding; young adults can also exhibit life-threatening hemorrhage, but signs in mature adults are usually mild and confined to mucosal surfaces. Found only in one large family of cocker spaniels.
Thrombopathia	Autosomal	Affected dogs can have no clinical signs or show increased bleeding tendency that can be exacerbated by trauma or surgery. Found in basset hounds and otterhounds.
Cyclic hematopoiesis	Autosomal recessive	Regularly occurring interruptions of bone marrow hematopoiesis with loss of neutrophils from peripheral blood; during these periods, dogs exhibit fever, enteritis, keratitis, pneumonia, and skin infections; infections can become life-threatening if not treated. Found in gray collies.
Pyruvate kinase deficiency	Autosomal recessive	Affected dogs exhibit severe anemia with reticulocytosis, macrocytosis, and polychromasia; hyperbilirubinemia; splenomegaly with extramedullary hematopoiesis; and decreased red cell survival. Found in basenjis, beagles, and cairn terriers.
Erythrocyte phosphofructokinase deficiency	Autosomal recessive	Persistent compensated hemolytic anemia with episodes of intravascular hemolysis, hemoglobinuria, and fever associated with stress or exercise; hemolytic crises follow hyperventilation-induced alkalemia; red cells of affected dogs are extremely alkaline and fragile in vitro. Found in English springer spaniels.

Husbandry and Veterinary Care

Bleeding disorders. Dogs with congenital and acquired bleeding disorders require special housing to minimize the risk of spontaneous or injury-induced bleeding. This is important not only for the animals' welfare, but also for experimental reasons. The basal state of animals that experience repetitive bleeding can be altered by the physiologic stress that such bleeding causes and, if bleeding is severe enough to require transfusions, by repeated exposure to homologous plasma proteins and blood cells. That is of particular concern for dogs with severe disorders, such as hemophilia.

Dogs with bleeding disorders should be housed in enclosures that have smooth sides and fronts with smooth vertical or cross-hatched bars. It is not advisable to use materials that can be climbed (e.g., chain-link fencing) because dogs with bleeding disorders can suffer foot injuries caused by weight-bearing pressure between the toes. Enclosure size is also important. To prevent injury, affected animals should have sufficient space to move about freely but not enough to permit vigorous exercise if they become excited. Enclosures should be square or oblong; injury is more likely to occur in a long, narrow run, especially in dogs with long tails, which during wagging can be traumatized by hitting against the sides. Experience has shown that for dogs weighing from 13.6-36.3 kg (30-80 lb), primary housing measuring about 4 × 6 ft (1.22 × 1.83 m) or 5 × 5 ft (1.52 × 1.52 m) minimizes the risk of injury.

Severely affected dogs should be housed individually because the risk of injury in playing with other dogs is substantial. To provide socialization, it is advisable to construct pens that allow visual contact between dogs; this can be achieved by building pens across an aisle from or perpendicular to each other. Partitions between the runs should be solid for the first 4 ft (1.22 m) in height to prevent injury caused by dogs in adjacent pens playing or fighting through the partition, and the seam with the floor should be smooth.

To avoid foot-pad abrasions, nonslip flooring should not be too rough. A poured rubberized flooring with a small amount of sand added to the last coat should create enough friction to prevent sliding. Nontoxic bedding (e.g., shredded newspaper or shavings) can be used to minimize injuries if sliding does occur. English rubber coits or tennis balls can be used to provide environmental enrichment.

Special arrangements are required for feeding and watering. Automatic watering devices are generally not recommended because the spigots can cause mouth injuries, and bleeding from such injuries is usually difficult to control. It is better to use large water buckets anchored to the sides or fronts of the pens. Dry food should be softened before feeding and supple-

mented with good-quality canned or cooked meat. A hematinic can be added to the food for conditioning. Hard biscuits should not be fed.

Bleeding from small surface injuries to the gums or nose or from toenails that are cut too short can be stopped by using sealant materials, such as Nexaband glue (Tri-Point Medical, LP, Raleigh, N.C.). Bleeding toenails can also be packed with styptic powder, and the soft rubber end cap from an intravenous set or catheter can be wedged tightly over the nail. If necessary, the foot can be bandaged; this supplies enough local pressure to control the bleeding. For animals that experience severe bleeding episodes, transfusion is the treatment of choice. Fresh-frozen plasma, plasma concentrates, platelet concentrates, or packed red cells should be given as required for the specific disorder. Details of management and treatment are summarized elsewhere (Dodds, 1989, 1992).

Another management procedure to keep animals healthy and reduce bleeding risk is prophylactic dentistry, which must be performed very carefully to avoid injury to the gums. Booster vaccinations should not be given during bleeding episodes because they create a transient platelet deficit (Dodds, 1992). In addition, dogs are at increased risk for bleeding episodes for 10-14 days after vaccinations. Affected females sometimes bleed excessively both during estrus and during the 30-40 days beforehand when estrogen concentrations are elevated.

Cyclic hematopoiesis. Colonies of grey collies with cyclic hematopoiesis (formerly called cyclic neutropenia) have special requirements because they are susceptible to recurring infections and anemia (Knoll, 1992). They have a cyclic, profound drop in all their blood-cell classes, although the numbers of each cell type rise and fall at different times. Affected animals rarely live beyond the age of 3 years and experience frequent bleeding episodes from cyclic thrombocytopenia. Respiratory tract and enteric infections are the most debilitating.

Affected animals can often be housed together, but they need scrupulously clean facilities to minimize infection, close clinical monitoring, and supportive therapy. They should be monitored for neutropenia, and prophylactic antibiotics should be administered as neutrophil counts begin to decline.

Other hematologic disorders. Dogs with various other inherited and acquired hematologic diseases also require special care. For example, basenjis with pyruvate kinase deficiency and recurring anemia must be closely monitored because of their increased susceptibility to infection or stress (Hall and Giger, 1992; Harvey, 1989); beagles with hereditary nonspherocytic hemolytic anemia must be closely monitored for episodes of hemolytic crisis (Maggio-Price et al., 1988); and English springer spaniels with erythrocyte phospho-

fructokinase deficiency require special care during episodes of hemoglobinuria or myoglobinuria (Hall and Giger, 1992; Harvey, 1989).

Reproduction

For dogs with severe inherited bleeding disorders—such as hemophilia, von Willebrand's disease, factor X deficiency, and platelet dysfunction (thrombopathia)—special care is needed for breeding, whelping, and rearing of the offspring. Immediately after birth, each pup should be carefully examined for signs of bleeding, its umbilical cord should be ligated, and the potential for trauma from the dam should be minimized. It might be necessary to tranquilize first-time dams slightly to protect the young. When the pups are weaned and start to become more active, blood samples should be taken to determine which pups are affected. In hemophilia, the affected pups from a carrier (heterozygous) dam will be males, unless the sire is a hemophiliac (hemizygote), in which case both affected hemizygote males and homozygote females can be produced. Generally, male pups should be watched more closely, and the affected ones should be removed and housed separately if the litter is too rambunctious. Cages should be relatively small; a floor area of about 30 × 36 in (76 × 91 cm) is recommended for the average hemophilic pup.

Affected pups should be watched carefully after vaccinations. Modified live-virus vaccines might induce a relative thrombocytopenia and platelet dysfunction during the period of viremia (i.e., 3-10 days after vaccination) (Dodds, 1992). The pups are at substantial risk for spontaneous or traumatic bleeding at this time because the vaccine effect on platelet function superimposes another hemostatic burden. All vaccinations should be given subcutaneously with a small-gauge needle, preferably 23 or 25 gauge, in the loose skin folds of the neck. Intramuscular injections in affected animals should be avoided.

Affected pups should be housed initially in cages and eventually in small pens. At teething, affected puppies often bleed excessively from the gums; this necessitates use of a topically applied sealant and, on occasion, transfusion therapy.

Immunologic Diseases

Primary Immunodeficiency and Autoimmune Diseases

Clinical Features

Immunodeficiency is characterized by failure to manifest a normal immune response when challenged by infectious agents or other substances

that are foreign to the body. The subnormal response can result from a defect in the afferent, central, or efferent limb of the immune system (see review in NRC, 1989). Immunodeficiency disorders can be primary (i.e., inherited) or secondary (i.e., acquired). Primary immune deficiency can result from an inherited defect in immunocompetent cells or effector mechanisms (e.g., complement or phagocytes) or can be associated with autoimmune disease or a deficiency in growth factors necessary for the optimal function of immunocompetent cells (WHO Scientific Group, 1986). Secondary immune deficiency can be caused by various environmental factors, including x rays, viral agents, toxic chemicals, and dietary deficiencies.

Several primary immunodeficiency diseases have been described in dogs, including selective IgA deficiency (Campbell, 1991; Felsburg et al., 1985; Moroff et al., 1986), IgM deficiency (Mill and Campbell, 1992; Plechner, 1979), common variable immunodeficiency (A. Rivas, New York State College of Veterinary Medicine, Cornell University, Ithaca, N.Y., unpublished), and severe combined immunodeficiency disease (Jezyk et al., 1989; Patterson et al., 1982). Dogs with particular autoimmune diseases also suffer from immunodeficiency. A high incidence of septicemia has been observed in dogs that were bred to develop systemic lupus erythematosus (SLE) (Quimby et al., 1979). Autoimmune hemolytic anemia (AHA) (Bull et al., 1971; Dodds, 1983; Klag et al., 1993), immune thrombocytopenic purpura (ITP) (Dodds, 1983, 1992; Waye, 1960), SLE (Grindem and Johnson, 1983; Monier et al., 1988; Quimby, 1981), rheumatoid arthritis (RA) (Bell et al., 1991; Carter et al., 1989; Quimby et al., 1978), Sjögren's syndrome (Kaswan et al., 1985; Quimby et al., 1979), autoimmune thyroiditis (Gosselin et al., 1982; Quimby et al., 1979; Rajatanavin et al., 1989; Thacker et al., 1992), and thyrogastric disease (Quimby et al., 1978) have been found in research dogs. Primary immunodeficiencies in dogs have also been associated with the absence of the third component of complement (Winkelstein et al., 1981); deficits in neutrophil function, including cyclic hematopoiesis (see page 100) (Knoll, 1992; Lund et al., 1967) and granulocytopathy (Knoll, 1992; Renshaw and Davis, 1979); dysregulation of interleukin-6 (DiBartola et al., 1990; Rivas et al., 1992); and deficiency of growth hormone (Roth et al., 1980). Clinical signs of these diseases are presented in Table 6.7.

All dogs with primary immunodeficiencies are predisposed to infection. Dogs with disorders associated primarily with hypogammmaglobulinemia, complement, or phagocytic function are predisposed to bacterial infection (Blum et al., 1985; Lund et al., 1967; Moroff et al., 1986; Renshaw and Davis, 1979). Those with disorders of cell-mediated immunity have increased susceptibility to fungi and viruses (Jezyk et al., 1989).

TABLE 6.7 Clinical Signs of Selected Primary Immunodeficiency and Autoimmune Diseases in Dogs

Immunologic Disease	Clinical Signs
Common variable immunodeficiency	Increased susceptibility to infectious diseases; clinical presentation after the age of 6 months
IgM deficiency	Increased susceptibility to bacterial diseases
Selective IgA deficiency	Increased susceptibility of some dogs to infectious diseases of mucosal surfaces, such as those of gastrointestinal, respiratory, and urogenital tracts
Severe combined immunodeficiency	Extreme susceptibility to bacterial, viral, and fungal infections; clinical presentation in first few weeks of life; death before reaching maturity
Autoimmune hemolytic anemia	Pallor, slight jaundice, splenomegaly, lymphadenopathy, weakness, and shortness of breath; profound anemia and recurrent episodes of hemolytic disease in approximately 50% of affected dogs
Immune thrombocytopenic purpura	Bruise easily, prolonged bleeding after trauma
Systemic lupus erythematosus	Rash, hemolytic anemia, immunothrombocytopenic purpura, polyarthritis, and proteinuria; females affected more frequently than males
Rheumatoid arthritis	Swollen painful joints—generally multiple small articular joints
Sjögren's syndrome	Keratoconjunctivitis sicca (dry eyes); corneal ulcers associated with dry eyes; excessive dental caries; inflamed gums; signs associated with hypothyroidism, including tendency to obesity, tendency to seek warm places, bilaterally symmetrical hair loss, and changes in skin thickness
Autoimmune (lymphocytic) thyroiditis	Signs associated with hypothyroidism, including tendency to obesity, tendency to seek warm places, bilaterally symmetrical hair loss, and changes in skin thickness
Thyrogastric disease	Signs associated with hypothyroidism, inappetence, megaloblastic anemia, and atrophic gastritis
Granulocytopathy	Increased susceptibility to bacterial infections
Dysregulation of interleukin-6	Familial Mediterranean fever, characterized by fever, synovitis, and renal failure
Deficiency of growth hormone	Small body stature; generalized increase in susceptibility to infectious diseases

Husbandry and Veterinary Care

Immunodeficient dogs pose special management problems. Immune diseases must be diagnosed, their prognosis determined, and their therapy monitored. A number of tests have been developed for those purposes, including tests that assay T- and B-cell function (Ladiges et al., 1988, 1989), identify serologic markers of autoimmune diseases (Kaplan and Quimby, 1983; Quimby et al., 1980), identify circulating immune complexes in rheumatic and neoplastic diseases (Carter et al., 1989; Terman et al., 1979), and assay phagocyte function (Smith and Lumsden, 1983).

The susceptibility of immunodeficient dogs to infectious diseases is handled in various ways. All immunodeficient dogs can benefit from an environment that minimizes contact with canine pathogens; however, for some of these conditions (e.g., severe combined immunodeficiency), cesarean derivation and maintenance in a gnotobiotic chamber are required to ensure survival. Pups with humoral deficiencies born to normal dams profit from receiving maternal antibodies in colostrum, and their dams should be immunized before being bred to ensure that high concentrations of antibodies will be present. Adult dogs with humoral deficiencies can be helped by transfusions of normal or hyperimmune serum or plasma or by administration of purified gamma globulin. Some dogs that are genetically predisposed to autoimmune diseases can be spared clinical illness for years by housing them in gnotobiotic chambers; however, if they are moved to a conventional environment, they quickly develop autoimmune disease (Schwartz et al., 1978).

Dogs with autoimmune diseases should be carefully monitored and appropriately treated. Treatment might involve immunosuppressive therapy (e.g., for SLE, AHA, ITP, and RA), transfusions of red cells and platelets (for AHA and ITP), splenectomy (for AHA and ITP), renal dialysis (for SLE), administration of thyroxine (for autoimmune thyroiditis), administration of thyroxine and vitamin B_{12} (for thyrogastric disease), and administration of artificial tears (see the section on ophthalmologic disorders) and special dental care (for Sjögren's syndrome). Some dogs with growth hormone deficiency benefit from injections of thymosin (Roth et al., 1980). Bone marrow transplantation and systemic antibiotics are effective in treating dogs with neutrophil defects. Dogs with thrombocytopenia (as in SLE, ITP, or Evan's syndrome) are predisposed to bleeding and bruising and should be housed and maintained as described in the section on hematologic disorders. Preliminary studies suggest that oral levamisole therapy is efficacious in treating one type of canine common variable immunodeficiency that is associated with ulcerative colitis and a predisposition to adenocarcinoma of the intestine (A. Rivas, New York State College of Veterinary Medicine, Cornell University, Ithaca, N.Y., unpublished). Trials involving the use of colchicine to delay the onset of amyloidosis in dogs with interleukin-6

dysregulation are in progress (L. Tintle, Wurtsboro Veterinary Hospital, Wurtsboro, N.Y., unpublished). The care of dogs with C3 deficiency and dogs that have been exposed to total body irradiation and immunosuppressive drugs associated with organ transplantation is described below. Dogs should be immunized against known canine pathogens before being exposed to agents that will induce immunodeficiency.

Reproduction

In colonies where the objective is to reproduce dogs with SLE by selecting breeders with serologic evidence of the disorder (i.e., by using antinuclear antibody and LE-cell tests), many progeny develop autoimmune diseases not apparent in the parents (Monier et al., 1988; Quimby et al., 1979). That observation has led to the hypothesis that multiple genes control the susceptibility and specificity of autoimmune diseases (Monier et al., 1988; Quimby and Schwartz, 1978). In some cases, an unanticipated result is compromised fertility (e.g., immune-mediated aspermatogenesis), which necessitates the use of littermates or repeat breeding of the parents to continue the lineage (Quimby et al., 1978). Hypothyroidism caused by lymphocytic thyroiditis (Beierwaltes and Nishiyama, 1968; Gosselin et al., 1982; Mizejewski et al., 1971; Rajatanavin et al., 1989; Thacker et al., 1992) can lead to poor reproductive performance that can be corrected with thyroxine-replacement therapy. Details on monitoring blood thyroxine and oral supplementation have been published (DePaolo and Masoro, 1989; Ferguson, 1986). For some autoimmune diseases, such as immune-mediated aspermatogenesis, no therapy has been found.

Complement Deficiency

Clinical Features

Dogs deficient in the third component of complement (C3) are particularly susceptible to bacterial infections (Blum et al., 1985). They also develop a membranoproliferative glomerulonephritis, which can be detected histologically by the age of 1 year (Cork et al., 1991). Affected dogs are normally active and appear well; the only clinical sign of this renal disease is proteinuria. Renal disease progresses inexorably and culminates in a nephrotic syndrome with azotemia when the dogs are 6-8 years old.

Husbandry and Veterinary Care

Dogs deficient in C3 can be reared and housed in standard laboratory dog facilities. Because the dogs are susceptible to bacterial infections (Chick et al., 1984), animal technicians should be alert to any deviations from

normal behavior that might indicate illness (e.g., inappetence and lethargy). C3-deficient dogs that show these clinical signs must immediately be evaluated for increased body temperature and leukocytosis. Blood samples should be taken and submitted for culturing to identify and determine the antibiotic sensitivity of the microorganisms; however, treatment with intravenous bactericidal antibiotics should not await diagnosis but should begin as soon as clinical signs are detected and a blood sample has been drawn. Although that protocol undoubtedly results in overtreating and might preclude a definitive diagnosis, it will in most cases ensure the recovery and survival of the affected dog. If an invasive procedure (e.g., renal biopsy or placement of an indwelling catheter) is required, antibiotic prophylaxis should begin 24 hours beforehand, and it is essential to follow strict aseptic technique while performing the procedure.

The presence of proteinuria can be detected by testing for total-protein excretion in the urine over a 24-hour period, and renal biopsies can be used to evaluate the progression of renal disease. As dogs age, periodic measurements of total serum protein, albumin, and serum urea nitrogen can be used to identify dogs whose renal disease is becoming severe or those in which a nephrotic syndrome might lead to fluid accumulation in body cavities. Repeated blood transfusions or infusions of canine plasma are contraindicated because they exacerbate renal disease.

Reproduction

C3 deficiency is inherited as an autosomal recessive trait (Johnson et al., 1986; Winkelstein et al., 1982). Affected pups are produced by breeding heterozygous females with homozygous males. Homozygous females are fertile but have rarely produced viable young. Pups should be tested at birth, and the ones that are C3-deficient should be placed on antibiotic therapy for the first 4 days after birth. C3-deficient dogs do not respond normally to immunization; therefore, it is recommended that immunizations against the common canine pathogens be given at 2-week intervals until the pups are 18 weeks old (Krakowka et al., 1987; O'Neil et al., 1988; Winkelstein et al., 1986).

Organ Transplantation

Clinical Features

Dogs that are used in organ-transplantation studies must first be made immunodeficient. Immunosuppressive methods include total-body irradiation and administration of cytotoxic chemicals (Ladiges et al., 1989). Im-

munosuppressed dogs are very susceptible to infectious diseases and might have gastrointestinal tract problems.

Husbandry and Veterinary Care

Dogs that undergo experimental organ transplantation generally require intensive postoperative supportive care, the level of which depends on the transplantation procedure used and the degree of immunosuppression required to overcome graft rejection. Supportive care includes fluid therapy, blood and platelet transfusions, preoperative and postoperative administration of appropriate antibiotics, and intensive husbandry practices. Regular monitoring of white cells is critical for ascertaining health status and determining the necessity for treatment. Blood should be cultured if clinical signs suggest septicemia. Nutritional needs are critical for dogs undergoing bowel transplantation or for those suffering from gastrointestinal tract problems caused by the immunosuppressive procedures. Dogs might need to be housed individually in intensive-care facilities during early convalescence.

Dogs undergoing bone marrow transplantation are profoundly immunodeficient for 200-300 days after lethal total-body irradiation and successful marrow engraftment, and they require intensive supportive care (Ladiges et al., 1990). Recovery of granulocyte count and function is complete by the twenty-fifth day after engraftment; blood lymphocyte count does not return to normal until day 200. Antibody response to bacteriophage and sheep and chicken red cells is lower than normal during the first 200 days, with IgM being the primary isotype. Lymphocyte stimulation by phytohemagglutinin, the mixed-leukocyte reaction, and the response to first- and second-set skin grafts are impaired. Long-term survivors (dogs that survive more than 200 days) generally regain their health and are no longer more susceptible than normal to infectious diseases. The development of graft-versus-host disease and its treatment drastically affect recovery of the immune system and place the dogs at increased risk for contracting infections.

Lysosomal Storage Diseases

Clinical Features

Clinical manifestations of canine lysosomal storage diseases (LSDs) generally fall into three categories: severe neurologic signs, mainly skeletal signs, and a mixture of visceral, skeletal, and neurologic signs. The following discussion addresses techniques for managing dogs in each category, using a single LSD as an example. The techniques can be extended to manage dogs with other LSDs.

Fucosidosis. Fucosidosis is caused by a deficiency of α-L-fucosidase (Healy et al., 1984). Affected dogs exhibit mainly neurologic signs. By the age of 12 months, affected dogs show subtle behavioral changes and might have an overextended posture. From 12 to 18 months, they develop mild ataxia and hypermetria. Signs progress rapidly between the ages of 18 and 24 months to more severe deficits in gait, proprioceptive defects, hyperclonus, nystagmus, kyphosis, and a loss of learned behavior. The dogs become dull and unresponsive. Hearing and vision might be impaired. Signs in severely affected, 24- to 36-month-old dogs include severe incoordination, opisthotonos, muscle spasms, muscle wasting, circling, head tilt, abnormal pupillary light reflexes, dysphagia, and cranial nerve deficits. The dogs become severely obtunded and suffer from self-inflicted injury. If not euthanatized, they usually die by the age of 3 years.

Mucopolysaccharidosis VII. The majority of clinical signs in canine mucopolysaccharidosis VII (MPS VII), a condition caused by a deficiency of β-glucuronidase, are related to skeletal and joint abnormalities (Haskins et al., 1984). Progressive noninflammatory arthrosis develops, and joints become lax and deformed. By the age of 3-6 months, affected dogs are unable to stand, and the muscles of locomotion atrophy. Corneal clouding can lead to decreased vision in dogs with MPS VII, but the impairment is generally less severe than in dogs with MPS I. At the age of 15-22 months, MPS VII-affected dogs often become dull and lethargic and lose interest in their environment and in animal-care personnel. Those signs might be associated with progressive hydrocephalus.

Mucopolysaccharidosis I. Canine mucopolysaccharidosis I (MPS I), a condition caused by a deficiency of α-L-iduronidase, is most similar to the human MPS I phenotype of intermediate severity (Hurler's syndrome and Scheie's syndrome) (Shull et al., 1982). Clinical signs refer to visceral, skeletal, and mild neurologic injury. Dogs with MPS I appear normal at birth, although there is a higher than normal incidence of umbilical hernias. Affected pups remain generally healthy for 4-6 months and then show stunted growth, corneal clouding, and progressive, degenerative, noninflammatory joint disease caused by mucopolysaccharide deposition in synovial and periarticular tissues. Joint laxity caused by abnormalities in ligaments and tendons is also common and, in combination with the arthroses, causes decreased ambulation. Degeneration of intervertebral disks, collapse of disk spaces, vertebral and long-bone osteopenia, and spondylosis also develop. Mucopolysaccharide accumulation in heart valves and coronary arteries can cause rapidly progressing heart failure. Affected dogs remain alert and responsive until their death by natural causes or euthanasia, often between the ages of 2 and 3 years.

Husbandry and Veterinary Care

Dogs with LSD present unique and serious medical and husbandry problems. Proper care of these valuable, critically ill animal models requires compassion, diligence, hard work, and specialized knowledge of the diseases involved. Technicians must be well trained and observant.

Fucosidosis. As the clinical signs progress, affected dogs should be handled carefully to prevent injury. They should be fed, exercised, and housed separately from normal dogs. Severely ill dogs should be moved by carrying. Affected dogs should always be housed on a raised trampoline bed and kept dry during cage cleaning to prevent self-soiling and pressure sores. Particular attention should be given to dogs with long hair; they should be bathed weekly and clipped several times a year. Ears should be checked daily for signs of infection. Dogs with moderate to advanced disease should be fed more frequently, and canned or moistened dry food should be used to aid prehension. Dogs with advanced disease often have a poor appetite, and the addition of highly palatable foods assists in maintaining body weight. Excess dental tartar must be removed regularly. At the age of 2-3 years, motor and mental impairment will have progressed to the point that euthanasia will be indicated.

Mucopolysaccharidosis VII. MPS VII-affected dogs should be housed in cages with floors of coated wire mesh; this aids sanitation and helps to prevent decubital sores. Once the dogs are unable to walk, food and water intake must be carefully managed. Recumbent animals will usually eat and drink if pans of food and water are placed on the cage floor; however, hand feeding might become necessary. Euthanasia should be considered when a dog's response to human attention begins to diminish.

Mucopolysaccharidosis I. Except for surgical correction of umbilical hernias, special care is not usually required for dogs with MPS I that are less than 1 year old. However, as the disease progresses and the vertebral column deteriorates, the dogs become extremely fragile, and especially gentle handling is necessary when working with them or moving them between cages. Acute disk herniation can occur with even very minor trauma or inappropriate handling. Once skeletal disease has developed, exercise must be limited, and affected dogs must be protected from more rambunctious colony members. Decubital sores are a frequent consequence of the increase in time spent lying down. Housing affected dogs on shredded newspaper or elevated wire mesh provides both comfort and better sanitation.

Appetite generally remains normal, although hand feeding or varying the diet might become necessary, especially in dogs with pronounced cor-

neal clouding, impaired hearing, or the rare decrease in cerebral sensorium. Some dogs have enlarged tongues; however, prehension of food is generally not a problem. The teeth of dogs that are fed a diet composed mainly of canned food require periodic scaling of tartar.

MPS I-affected dogs are rarely maintained until they die naturally. By the age of 24-36 months, the symptoms of skeletal disease are generally so marked that euthanasia is indicated before debilitation becomes unacceptable.

Reproduction

Most LSDs can impair fertility in dogs. MPS I- and VII-affected males have sired litters by artificial insemination. Males with fucosidosis show copulatory behavior before they become severely uncoordinated, but they are infertile because of epididymal lesions, which probably impair spermatozoan capacitation. Females with fucosidosis are fertile but are very poor mothers; their pups usually must be fostered or hand-reared. Pups with LSDs are generally produced by breeding heterozygous carriers that are clinically normal.

Muscular Dystrophy

Clinical Features

A genetic disorder homologous to Duchenne's muscular dystrophy of humans—a devastating, fatal disorder predominantly of boys—occurs in various breeds of dogs. The disorder in dogs, which is inherited as a simple sex-linked recessive gene with full penetrance, is known as canine X-linked muscular dystrophy, and dogs with the condition are called *xmd* dogs. The mutation has been found in golden retrievers and rottweilers, and a similar mutation is suspected to have occurred in samoyeds, malamutes, and Irish terriers. The golden retriever is the best studied of the affected breeds, and the following discussion is based on data on this breed.

Both Duchenne's muscular dystrophy and canine X-linked muscular dystrophy are caused by a defect in the production of dystrophin, a skeletal muscle cytoskeletal protein. The mutation in the dystrophin gene results in massive continuing skeletal muscle degeneration that occurs from birth onward. In dogs, progressive cardiac muscle degeneration begins in hemizygous males at the age of about 6 months. Carrier bitches appear clinically normal but have subtle lesions in their cardiac muscles. Because of the homology to Duchenne's muscular dystrophy, the *xmd* dog can serve as an animal model for studies leading to better understanding of the pathogen-

esis of Duchenne's muscular dystrophy, as well as for studies designed to assess therapeutic approaches (Valentine et al., 1992).

Clinical signs of obvious weakness, muscle wasting, and abnormal gait appear in *xmd* dogs at the age of about 8 weeks. After that time, clinical signs progress, and they are most severe at the age of about 6 months, at which time the dogs have a markedly stiff, shuffling gait. There is frequently a severely abnormal posture, with carpal overextension, tarsal overflexion, and splaying of the limbs. The dogs are unable to open their jaws fully, their tongues are thickened and cannot be fully extended, and they frequently drool excessively. After the age of 6 months, the clinical disease appears to stabilize, and many dogs seem to gain strength as they age. However, there is still a progressive degeneration and fibrosis of cardiac muscle that results in the characteristic Duchenne-type cardiomyopathy.

Husbandry and Veterinary Care

Dystrophic dogs do not require special caging. Shavings provide a soft, warm surface, but the shavings must be free of dust so that the dogs do not inhale particles and develop granulomatous pneumonia. Temperature and humidity must be carefully controlled. Older dystrophic dogs should be monitored carefully for signs of cardiac failure. Treatment for heart failure has been described (Fraser et al., 1991). Euthanasia should be considered when treatment fails to alleviate clinical signs (e.g., when the dog has difficulty breathing and when fluid accumulates in the abdomen).

Dystrophic dogs require high-calorie food that is easy to prehend and swallow because of the weakness of their tongue, jaw, and esophageal muscles. Canned food mixed with moistened dry food seems to constitute an adequate diet, but careful monitoring of food intake and weight is necessary. Regurgitation of food is common because of the esophageal skeletal muscle dysfunction. Severely disabled dogs might not be able to use automatic watering devices and might have to be given water in bowls or buckets. Their water might need frequent changing because of a buildup of saliva.

Adequate exercise is crucial during the period of rapid growth. Although dystrophic dogs might prefer to lie down, restricted exercise will result in more severe joint contractures. The presence of a slightly more active dystrophic cagemate is ideal, provided that competition for food does not impair food intake. The kennel must have a nonslippery surface to provide traction, and daily release for exercise is advised. These dogs should not be forced to exercise, however, because it might lead to increased muscle damage.

Dystrophic dogs cannot groom themselves adequately. Regular brushing of their haircoat and clipping of overgrown toenails is required. To

prevent skin irritation, the mouth and jaw should be kept free of the saliva and food that accumulate.

Reproduction

Many dystrophic dogs survive to breeding age, and breeding colonies can be established. Some affected males are able to breed naturally; others are hampered by their physical disability and require artificial insemination techniques. An *xmd* male that breeds naturally might need assistance to remain upright once he has "tied" with the female. Breeding dystrophic bitches, which are produced by mating dystrophic males to carrier bitches, is possible but not advised. Pregnant dystrophic bitches require constant monitoring, are likely to have respiratory and cardiac complications, will require cesarean section, and might not be able to care for their pups adequately.

At whelping, a safe, warm environment and proper maternal care are essential for the survival of dystrophic pups. If dystrophic pups are stressed by cold, separation from the litter, or inability to compete with normal pups in a large litter, some of them will develop massive skeletal necrosis within the first few days of life. Once signs of severe weakness have developed in a pup, it is virtually impossible to save it. Severe diaphragmatic necrosis resulting in respiratory failure appears to be the cause of death. Dystrophic pups can be identified in the first week of life by their markedly increased serum concentrations of creatine kinase released from degenerating muscles. Dystrophic pups that survive the first week grow more slowly than their littermates. Euthanasia should be considered for pups that are too weak to nurse during the first week of life; tube feeding has not been successful in keeping such pups alive (B. A. Valentine, Department of Pathology, New York State College of Veterinary Medicine, Cornell University, Ithaca, N.Y., unpublished).

Neurologic Disorders

Clinical Features

Dogs with hereditary or induced neurologic disorders are often used to study equivalent human disorders. Clinical signs in these dogs include abnormal gait, hyperactivity, nervousness, tremors, convulsions, visual impairment, blindness, deafness, quadriplegia, and tetraplegia. Obviously, these dogs commonly require extra care to ensure that they are as comfortable as possible. Inherited canine neurologic diseases and their clinical signs have been reviewed by Cummings (1979) and Oliver and Lorenz

(1993); the pattern of inheritance of specific diseases has been discussed by Willis (1989).

Husbandry and Veterinary Care

Food and water must be placed where a neurologically impaired dog can find and reach them easily, and, if the dog is blind, placement should be consistent. That might require using water bowls instead of automatic waterers or, for dogs with severe impairment, intravenous or subcutaneous administration of fluids. Food might have to be placed in flat dishes, softened, or made into a gruel so that it can more easily be reached, masticated, and swallowed. Food and water intake should be monitored. Dogs should be weighed regularly to ensure that body weight is maintained. Nasogastric, lavage, pharyngotomy, or intragastric feeding might be required in some circumstances to provide adequate nutrition.

Dogs with sensory deficits can experience dysesthesias and might respond by chewing the affected limb or body part or another, more accessible body part. Several strategies can be used to deal with such behavior. Dogs should be closely monitored to detect the beginning of self-directed behaviors. A dog can sometimes be distracted by housing it where it has more external visual and social stimulation. If a nonaggressive cagemate can be identified, social housing might be sufficiently distracting—provided that the cagemate does not harass the affected dog or prevent it from eating and drinking. Toys, such as rawhide bones, might also be useful. If bandages must be used, they should not be too tight and should be checked regularly. Elizabethan collars or muzzles can be used to limit access to the body. Light tranquilization, if it does not interfere with the experimental protocol, might be helpful.

Dogs with sensory deficits might require extra or different bedding to prevent unintentional self-injury. The dogs' primary housing must be free of rough or sharp edges and projections. Dogs with motor deficits might have difficulty in positioning their bodies for urination and defecation. Sometimes all that is necessary is to provide flooring with better traction (e.g., plastic-coated grids or rubber mats). If necessary, research or animal-care personnel should assist the dog to position itself. Catheterization or manually expressing the bladder might be required to prevent urinary retention. Careful husbandry and nursing will avoid decubitus ulcers.

In dogs with respiratory deficits, the normal ability to thermoregulate by panting has been compromised. For these animals, exertion must be avoided and comfortable temperatures maintained.

Reproduction

Dogs with some neurologic disorders can reproduce, even though they are severely impaired. Such dogs usually need assistance for mating or require artificial insemination. Bitches with marked sensory or motor deficits or ataxia should be closely attended at parturition and while nursing to protect the pups from accidental injury. If the neurologic deficits of the dam interfere with her ability to care for her offspring, hand rearing or foster rearing will be required.

Ophthalmologic Disorders

Clinical Features

Dogs are affected by various ophthalmologic problems, either as inherent aspects of the research in which they are being used, as complications, or as acquired conditions unrelated to the research. Descriptions of canine eye diseases can be found in any standard text on veterinary ophthalmology (e.g., Gelatt, 1991; Helper, 1989). In the research setting, ocular problems that require special management techniques are visual impairment, painful ocular conditions, untoward sequelae of interfering with the eye's external protective mechanisms, and combinations of these conditions.

Blindness. Visual impairment in dogs usually cannot be measured precisely. For purposes of this discussion, *blindness* is used, in a loosely defined manner, to refer to any condition in which visual impairment is sufficient to interfere with a dog's ability to perform visually guided tasks or to exhibit normal visually guided behavior. In general, dogs maintained in a familiar environment adapt well to visual deficits that are congenital, are gradual in onset, or have been present for an extended time (weeks to months). A dog that has adapted to its blindness, that is maintained in a familiar environment, and that is not subjected to stressful experiences will move about actively and engage in all normal canine behavior. Its adaptation, or compensation, might be so successful that a naive observer will not recognize that it is blind.

Ocular pain. Painful ocular conditions fit broadly into three categories. External ocular pain is usually associated with corneal irritation and commonly causes obvious signs, such as blinking, excessive tearing, and redness. Uveal pain is caused by intraocular inflammation, which might not be evident without careful examination of the eye; uveal pain is usually more painful than corneal irritation. Glaucomatous pain is often the most insidious and most severe ocular pain. All these conditions are not only painful, but can threaten a dog's vision and the integrity of the affected eye.

Conditions associated with failure of the eye's external protective mechanisms. Untoward sequelae can arise from any condition that interferes with the eye's external defense mechanisms. These mechanisms depend on such funcitons as corneal sensitivity, lid movement, and tear production. Anything that reduces corneal sensation, interferes with lid movement, or lowers tear production can lead rapidly to painful ocular inflammation, impairment of vision, and loss of the affected eye. Common causes include anesthesia, radiation, surgical procedures, and drugs.

Husbandry and Veterinary Care

It is recommended that all experimental protocols involving dogs with ophthalmologic problems—whether the problems are "natural" (i.e., genetic), acquired, or induced—be reviewed by a veterinarian or a physician with training in ophthalmology (e.g., a veterinarian certified by the American College of Veterinary Ophthalmologists). Such protocols should include an adequate program for monitoring the dogs' ophthalmologic problems and written procedures for dealing with ocular emergencies.

Blindness. In spite of the ease with which dogs can adapt to blindness, they require special protection from a variety of environmental dangers, the more obvious of which are protruding objects, sharp edges, openings through which a dog might fall, and sources of electric or thermal injury. More insidious risks can arise because blind dogs lack the menace reflex, which normally protects the cornea from damage by causing the eyelids to blink in response to seen objects approaching the eye. Personnel responsible for the care and handling of blind dogs must be aware of these risks and keep them to a minimum and must watch for signs of acute or chronic corneal injury.

Dogs that have adapted to their blindness can become decompensated in response to rapid changes in their environment or other stressful experiences, such as anesthesia (e.g., for diagnostic, surgical, or experimental procedures), illness, and alterations in their daily routine. A decompensated chronically blind dog might look as though it has suddenly become blind and might exhibit behaviors compatible with a general stress reaction—from stiff-limbed hesitancy in walking and an apparent fear of its surroundings to anorexia or polydipsia, polyphagia, and polyuria. Similar signs can be observed in some dogs that have recently and rapidly lost their sight. Given time and a restricted, safe, and consistent environment, the blind dog will readapt and once again exhibit compensated normal behavior. Personnel responsible for the care and handling of blind dogs must be aware that these dogs need consistent familiar surroundings and that they might react adversely to stressful experiences. When approaching a blind dog, animal technicians should talk to it so that the dog will be more likely to perceive the approach as friendly.

Ocular pain. Ocular pain can vary from moderate to excruciating. Dogs in ophthalmologic research colonies are often at risk of developing ocular pain, either as a direct result of a study or as an unpredictable occasional side effect. In some cases, particularly if the pain is chronic or develops gradually, it will not be readily apparent without special examination procedures, especially if the observer is inexperienced. Personnel responsible for the care and handling of dogs used in ophthalmologic research should suspect that ocular pain is present when there is periocular soiling or when there are behavioral changes, such as decreased activity, decreased appetite, increased yawning, and changes in vocalization patterns.

Conditions associated with failure of the eye's external protective mechanisms. All protocols should be reviewed for potential adverse effects on external ocular defense mechanisms, and dogs subject to such risks should be monitored carefully for evidence of adverse effects.

Reproduction

Most dogs with ophthalmologic disorders can breed normally.

Orthopedic Disorders

Clinical Features

Dogs serve as models for both canine and human orthopedic diseases. Spontaneous bone and joint diseases of dogs have been reviewed (Lipowitz et al., 1993; Newton and Nunamaker, 1985; Whittick, 1990; Young, 1979). Orthopedic diseases can also be induced in dogs.

Husbandry and Veterinary Care

When inducing an orthopedic disease in dogs, one must first evaluate the dogs to be certain that natural bone and joint diseases are absent. Radiography is used to diagnose hip dysplasia, osteochondrosis, osteoarthritis, elbow dysplasia, and patellar luxation. These are considered heritable disorders because offspring of affected parents often have them and they occur in siblings.

Ideally, the surgical suite, the radiographic diagnostic facility, and an anesthesia recovery box lined with foam-rubber padding should be located near the primary housing facility. The floors of both the orthopedic research facility and the primary housing should be kept dry and have a nonslippery surface to provide good, steady footing.

The amount of food consumed should be monitored because excess body weight will exacerbate orthopedic conditions. Limiting food consumption during the growth period has been shown to reduce signs of orthopedic disease in dogs that mature at greater than 30 lb (Kealy et al., 1992). Dogs that refuse to eat because of pain might require a palatable high-energy food to maintain body weight. Human socialization is desirable to allow caregivers to detect abnormalities more readily and to facilitate handling and, when necessary, treatment.

Mild exercise, such as walking, is beneficial to keep muscles limber, promote bone formation, and increase lubrication and nutrition of joints. However, excessive exercise aggravates pain and causes further bone or joint damage. Anti-inflammatory drugs, given with food, can be used to relieve pain. Glucocorticosteroids, although potent anti-inflammatory agents that relieve pain, can also accelerate disease progression and should be used only in advanced cases of joint disease. Warm packs can ease the pain of chronic osteoarthritis. Dogs affected with skeletal diseases should be kept warm and dry, although pain associated with a recent injury can be eased by applying crushed ice in a plastic bag to the affected region.

Reproduction

Dogs with joint and bone diseases can generally be bred, although it might be necessary to guide and hold a male affected with moderate or severe hip dysplasia. If the orthopedic problem is so severe that mating is not possible, artificial insemination can be used.

Radiation Injury

Clinical Features

Radiation is commonly used in experimental protocols involving dogs. Total-body irradiation (TBI) is generally delivered by a cobalt-60 source or medical x-ray therapy machine. Doses of radiation up to 2 Gy can result in signs of illness related to mild gastrointestinal toxicity and decreased white-cell counts. At doses of 2-4 Gy, signs become progressively more severe. Doses greater than 4 Gy cause destruction of bone marrow, loss of circulating blood cells, immunosuppression, increased tendency to bleed, and moderate to severe gastrointestinal toxicity. Bone-marrow transplantation can prevent severe clinical signs and death in dogs. The high radiation doses are similar to the doses that human transplantation patients receive.

Several side effects occur in dogs that survive for long periods after TBI and bone-marrow rescue (Ladiges et al., 1989): pancreatic fibrosis,

malabsorption and malnutrition, radiation-induced cataracts, and malignancies. A consistent finding is graying of the hair.

Radionuclides that are ingested, inhaled, or injected rarely produce signs of illness. However, knowledge of the chemical form and metabolism of the radionuclide is necessary to determine possible side effects. For example, inhaled particles of oxides of cesium-144 are relatively insoluble in the lungs and potentially remain there for some time. Signs of radiation pneumonitis might then be expected (Mauderly et al., 1980). Conversely, strontium-90 as a chloride is relatively soluble in the lungs. When inhaled, it is translocated to the bones, where it can cause prolonged thrombocytopenia and neutropenia (Gillett et al., 1987).

Types of radiation. Radiation emissions can be alpha particles, beta particles, gamma rays, and x rays. The distinctions between those emissions are important for providing care for laboratory animals.

Alpha emissions from radionuclides, such as plutonium or americium, are generally high-energy emissions, but they travel very short distances in tissue. These radionuclides are rarely used in animals unless the study is specifically intended to assess metabolic or biologic effects of alpha emissions. No special precautions are needed for direct contact with animals contaminated with alpha-particle-emitting radionuclides because the radiation energy is absorbed within the animals' tissues. However, personnel should wear disposable clothing, shoe covers, gloves, eye protection, and respiratory protection to prevent inadvertent ingestion of, inhalation of, or wound contamination with alpha particles from contaminated feces, urine, bedding, cleaning water, or surfaces.

Beta-emitting radionuclides, such as cesium-144 and strontium-90, penetrate farther into animal tissues than alpha particles but still only a short distance. The same precautions should be taken as are taken for alpha particles. Dogs can usually be handled without taking further precautions 10-12 days after administration of radionuclides.

Gamma rays and x rays from internally deposited radionuclides penetrate tissues for considerable distances. These emissions can cause some radiation exposure of personnel, and it is important to know the potential exposure levels. These are generally low-energy kinds of radiation with short half-lives. Procedures for monitoring radiation must be in place to be certain that exposures of personnel are within accepted standards. The facility radiation-protection officer should participate in planning of animal-care procedures.

Disposal of radioactive wastes is regulated by both federal and state governments. It is important to have procedures in place for collecting, packaging, and labeling radioactive wastes before studies are initiated.

Biohazards associated with radioactivity. Dogs exposed to external radiation sources do not pose a hazard to personnel once exposure is complete; the concern is for the effects on the health of the exposed animals. However, dogs that are administered radionuclides by ingestion, injection, or inhalation might present a continuing hazard to personnel because the radionuclide will be excreted in feces, in urine, and in some instances in exhaled air for some period after exposure. Standard operating procedures must be developed and followed for collecting and disposing of all contaminated materials to protect animals and personnel. Animal health is of immediate concern only when large quantities of radionuclides are given.

Husbandry and Veterinary Care

Dogs exposed to external radiation can be housed in the usual manner (see Chapter 3); however, it is critical that immunosuppressed dogs be protected from other dogs that might harbor pathogens. Dogs given internally deposited radionuclides should be housed individually. To facilitate collection of contaminated excreta and cage-cleaning water, the cages should be designed for collection of urine and feces and should be easy to clean. Dog rooms must have adequate ventilation, and ventilated air should not be recirculated. It might also be necessary to filter exhaust air. To prevent cross-contamination and simplify monitoring, it is recommended that dogs exposed to the same radionuclide be housed in the same room.

Clinical observations and frequent peripheral-blood-cell counts are useful for monitoring dogs exposed to large doses of radiation. Treatment for reduced numbers of blood cells is supportive, and euthanasia should be considered if illness becomes too severe. Marrow "rescue" can prevent severe illness. Supportive care should consist of aggressive antibiotic and fluid therapy, and a semiliquid diet is necessary during the immediate post-irradiation period. Euthanasia should be considered in long-term survivors experiencing pancreatic fibrosis, malignancies, or pneumonitis.

Reproduction

Dogs that have received TBI are usually sterile. Lower doses of radiation have variable effects on reproduction.

Gene Therapy

Gene therapy can be used to correct inborn errors of metabolism, hemoglobinopathies, and blood factor A deficiencies; to insert genes into normal cells of the host (e.g., marrow stem cells) to increase their resistance to the toxic effects of chemotherapy; to introduce genes into cancer cells

that will restore suppressor-gene function or neutralize the function of activated oncogenes; and to induce tolerance to transplantation antigens by transferring genes that code for such antigens (Anderson, 1984). The use of the dog as a preclinical, large, random-bred animal model has set the stage for clinical gene therapy. A number of target tissues for gene therapy have been used; this section will cover three of them.

Hematopoietic Stem Cells

In preparation for gene transfer, marrow is aspirated while the dog is under general anesthesia. The hair over the shoulder and hip joints is clipped. The skin is cleaned with povidone iodine, washed with 70 percent ethyl alcohol, and cleansed with sterile Ringer's solution. Under sterile conditions, a needle 20 cm long and 2.5 mm in internal diameter is inserted into the marrow cavity through the proximal intertubercular groove of the humerus or trochanteric fossa of the femur. The needle is then connected with polyvinyl tubing to a suction flask, and marrow is aspirated by placing a suction flask, which contains tissue-culture medium and preservative-free heparin, under negative pressure with a pump. The procedure can be completed on all four limbs in approximately 20 minutes, during which 70-80 ml of a mixture of blood and bone marrow is collected. The marrow suspension is then passed through stainless-steel screens with 0.307- and 0.201-mm mesh diameters. A 1 ml sample is taken for marrow cell counts, and the remainder of the marrow is placed in plastic containers. The aspiration procedure is well tolerated without any sequelae. Dogs are capable of walking unimpaired after recovery from anesthesia.

Nucleated marrow cells are then cocultivated with virus-producing packaging cells at a ratio of 2:1 for 24 hours in 850-ml roller bottles. The gene-containing vector is replication-defective. Retrovirus-producing packaging cells are seeded in roller bottles 48 hours before the addition of marrow and are cultured in vitro with established techniques. After cocultivation, marrow cells are used to boost long-term cultures established 1 week earlier. The cultures are harvested after 6 days of incubation, and marrow cells are carefully removed without dislodging the virus-producing packaging cells, washed, resuspended in serum-free medium, and infused intravenously into the dog from which the marrow was taken.

In preparation for the infusion, the dog is exposed to total-body irradiation to create room for the infused marrow to seed. Total-body irradiation is administered at doses of 4-10 Gy and is usually delivered at a rate of 7 cGy/minute from two opposing cobalt-60 sources. For that purpose, an unanesthetized dog is housed in a polyurethane cage that is midway between the two cobalt-60 sources. The long axis of the cage is perpendicular to a line between the sources. After irradiation, the dog is returned to the

animal-care facility for supportive care. Total-body irradiation can cause nausea, vomiting, and diarrhea. Its destruction of normal marrow leads to a disappearance of red cells, white cells, and platelets. The temporary absence of those blood components produces a risk of anemia, infection, and bleeding that persists unless the dog receives a marrow graft and the graft begins to function. Dogs are monitored daily and receive parenteral fluids and electrolytes as required. Appropriate preoperative and postoperative antibiotics are routinely used to prevent and treat infections. Platelet and red-cell transfusions are given as needed. Marrow-graft function is monitored by evaluating daily blood counts.

The success of gene transfer can be assessed by repeated aspiration of marrow under general anesthesia and examination of the samples for the appropriate marker gene with culture techniques, the polymerase chain reaction, or other appropriate methods (Stead et al., 1988). Peripheral blood cells can be tested in a similar manner, as can lymph node lymphocytes and pulmonary macrophages (Stead et al., 1988).

Skin Keratinocytes

Skin keratinocytes provide another good target for gene insertion. For some gene products, such as adenosine deaminase, gene transfer can take place in any replicating tissue. A 2 × 1.5-cm skin biopsy is obtained from the recipient under general anesthesia. Keratinocytes are derived from the biopsy material and cocultivated in vitro with replication-deficient retroviral vectors that contain the gene of interest. Keratinocytes are then cultured in a liquid-air interface culture, which gives rise to the various layers of skin in an in vitro system. After some time in culture, the skin grown in vitro is transplanted into a prepared bed on the flank of the dog under general anesthesia. The transplant site is treated with topical antibiotic powder, protected by nonadhering dressing, and inspected daily by the investigators. Generally, the skin grows in and is functional in 3-4 weeks. Punch biopsies of 2-3 mm allow assessment of gene transfer (Flowers et al., 1990).

Smooth Muscle Transplantation

Because of their location, genetically modified vascular smooth muscle cells can be particularly useful for the treatment of some diseases (e.g., hemophilia). Studies have demonstrated that vascular smooth muscle cells are easily obtained, cultured, and genetically modified and replaced and provide a good target tissue for gene therapy that involves both secreted and nonsecreted proteins (Lim et al., 1991). A segment of femoral artery or vein is surgically removed from a dog for preparation of smooth muscle cell cultures. The procedure of removing femoral artery and vein segments will

not compromise the dog, because there is extensive collateral circulation in this region. With the dog under general anesthesia, as long a segment of vessel as possible (at least 2 cm) is isolated from the circulation with ligatures. Any side branches in the two ends are permanently ligated before the vessel is removed. The smooth muscle cells are isolated, cultured, and infected with replication-defective amphotropic retroviruses that carry the genes of interest, in accordance with National Institutes of Health recombinant-DNA guidelines. The genetically modified smooth muscle cells are returned to the animal from which they were obtained. With the dog once again under general anesthesia, the transduced cells are seeded into the left and right carotid arteries and into the remaining femoral arteries (Lim et al., 1991).

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Appendix

Cross Reference

Subject	Page No. in This Report	Part No. in AWRs (9 CFR)	Page No. in *Guide*
Bedding storage	26	3.1e	24
Chemicals and toxic substances	19	—	22, 25
Emergency power	18	—	46
Exercise	21-24	3.8	17
Feeding	25-26	3.9	22-23
Food storage	26	3.1e	23, 46
Handling	78	2.131	—
Housing facilities			
General construction	12-14	3.1a-b, 3.4c	42-43
Drains	14-15	3.1f	44
Lockers, washrooms, and toilet areas	13, 15	3.1g	6, 42
Physical relationship of animal facilities to laboratories	14	—	41
Power and Lighting	18	3.1d	46
Surfaces	14-15	3.1c ,3.2d	43-45
Humidity, indoor	16-17	3.2b	18-19, 45
Identification	27-28	2.38g, 2.50	27
Illumination	18	3.2c	20-21
Noise	19	—	21

Subject	Page No. in This Report	Part No. in AWRs (9 CFR)	Page No. in *Guide*
Outside runs	15-16	3.3e	—
Primary enclosures	19-20	3.6a,c,d	11-12
Procurement	52-53	2.60	34
Protocol review	76-78	2.31c,d	—
Record-keeping			27
Annual or semiannual reports	29	2.36	
Dogs on hand	28-29	2.35b-e	
Dog procurement	28-29	2.35b	
Institutional animal care and use committee	29	2.35a	—
Restraint	78	—	9
Sanitation	27	3.11	24-27
Social interaction	22-24	3.6c3, 3.7	12-13
Space	20-21	3.6a,c	13-17
Temperature, indoor	16-17	3.2a	18-19, 45
Training employees	2	2.32, 3.12	4–5
Transportation	29-32	3.13-3.19	—
Ventilation, indoor	17-18	3.2b	19-20, 45-46
Veterinary care			
Analgesia	66-67	2.33b	37
Anesthesia	64-66	2.33b	37
Conditioning	53	—	34-35
Emergency, weekend, and holiday care	29	2.33b	28
Euthanasia	70-72	2.33b	38-39
General	51	2.33, 2.4	33
Postsurgical care	69-70	2.33b	37-38
Quarantine	54-55	—	34-35
Surgery	68-69	2.31d	9-10, 37-38, 47-48
Surveillance, diagnosis, treatment, and control of infectious diseases	53-57	2.33b	36-37
Waste disposal	27	3.1f	26-27
Watering	26	3.10	24

Index

DOGS

Laboratory Animal Management

This newly revised edition–a must for anyone using dogs for research or supervising that use–incorporates regulatory requirements and improved practices for laboratory animal care that have developed over the past two decades. It covers selection of dogs as research models; design, construction, and maintenance of indoor and outdoor facilities; temperature, humidity, food, water, bedding, sanitation, animal identification, record keeping, and transportation; and general veterinary care, as well as special care of breeding animals and random-source animals. *Laboratory Animal Management:* **Dogs** examines controversies over proper cage sizes and interpretation of federal requirements for exercise and offers recommendations for researchers. Guidelines are provided on how to recognize and alleviate pain and distress in research dogs, and the sensitive topic of euthanasia is covered in detail. It discusses how to assemble a proper research protocol and how to handle conflicts; outlines procedures for institutional animal care and use and committee review; and presents guidelines for handling aging dogs, use of radiation in experiments, and a wide range of other special circumstances.

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ISBN 0-309-04275-5;
1991. 160 pages, 6 x 9,
index, hardbound

ISBN 0-309-04744-7
90000
9 780309 047449

INSTITUTE OF LABORATORY ANIMAL RESOURCES

Volume 35, Number 1 Winter 1993 National Research Council

Letters

More on IACUCs and Merit Review

Models of Type I Diabetes—Part One

The NOD Mouse: A Model for Analyzing the Interplay Between Heredity and Environment in Development of Autoimmune Disease

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ILAR News is published quarterly by the Institute of Laboratory Animal Resources, National Research Council, 2101 Constitution Avenue, NW, Washington, DC 20418. It is circulated by request to investigators in the field of biomedical and related research.

Publication of this issue of *ILAR News* was supported by grants from the National Center for Research Resources, National Institutes of Health; National Science Foundation; American Cancer Society, Inc.; and U.S. Army Medical Research and Development Command, which is the lead agency for combined Department of Defense funding also received from the Human Systems Division, 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.

ILAR News ISSN 0018-9960
Volume 35, Number 1 Winter

ILAR NEWS

INSTITUTE OF LABORATORY ANIMAL RESOURCES

VOLUME 35, NUMBER 1 WINTER 1993

CONTENTS

Letters

More on IACUCs and Merit Review

To the Editor:

A recent article in *ILAR News* [Prentice et al. 34 (1–2):15, 1992] examines the rationale for institutional merit review of research involving live animals. In my view, although the citations are probably appropriate, the authors' analysis leading to the main conclusion that there is an institutional responsibility for review of scientific merit, over and above a justified concern for parsimonious and humane use of animals, is incorrect on several grounds.

1. Prentice et al. equate the phrase "relevance to human and animal health" with "acceptable level of scientific merit." This is simply not the case. "Relevance" implies that the outcome of the research, positive or negative, will have some impact on human or animal health, while "merit" is a much more ephemeral issue that may or may not include a test for relevance within its context. Much fundamental research is seen to have relevance only long after it is completed, or it may take on relevance in a manner much different from original intentions. Furthermore, equation of human and of animal health by inclusion in a single phrase accepts as de facto the inappropriate assertions made by PETA [People for the Ethical Treatment of Animals] and other organizations of the equivalence, and in some cases the primacy, of ethical obligations to animals over those to humans. This is a not generally acceptable minority viewpoint.

2. It is well established that merit review depends upon review by a principal investigator's peers. On a national level, peer review by a funding agency or a journal is made possible by reaching out to other investigators across the country and occasionally in other countries. Within any one institution, certainly there are individuals who can judge proposed research at the "fundamental level," but equally there are usually not those who can make peer judgements at the "knowledge-based level" (Prentice et al.'s terms). Imposing the need for external reviewers in internal decisions would increase delay and cost of research considerably, while reducing its timeliness and eventual relevance.

3. There is a profound difference in the consequences of external and internal review of research. The external review (by agency or journal) may result in a lack of funding or refusal to publish results. However, the investigator is free to pursue alternate funding and/or publication sources and ultimately, to perform the research as planned. Internally, especially in the context of review by the IACUC, a negative review effectively prevents the research from being conducted. Furthermore, despite the positive "spin" that Prentice et al. attempt to place on the interaction between the investigator and the internal review committee (IACUC or other), that interaction has a distinctly negative aspect; since the investigator is reacting to the review process by altering the proposed research, his/her autonomy is clearly being limited. While Prentice et al. refer to "academic freedom" in the second paragraph of their discussion, they show a remarkable insensitivity to the real meaning of the phrase. External peer review groups, such as NIH study sections, take great pains not to plan or redirect proposed research when reviewing its merit, admissibility and fundability. Concern for noninterference with individual academic autonomy (freedom) should be viewed as even more important within the principal investigator's own institution, since the investigator and the institutional reviewers are colleagues.

Finally, on a related subject, while I certainly agree with Prentice et al. that an ethical "cost-benefit" calculation must be made in research involving either animals or humans, I must disagree very strongly that, in the latter case, the IRB [Internal Review Board] affects such a calculation by transferring "the decision-making responsibility to human subjects" and, by implication, transferring the ethical responsibility. In either case, the ethical responsibility resides with the principal investigator and remains there, whatever the advice of the IACUC or the IRB, respectively, may be. Consent must be sought from patients out of proper (ethical) respect for their humanity and autonomy. However, ethical responsibility is an *individual* attribute of the "doer" rather than of the "done to" and no consent and/or corporate decision, however well considered or rationalized, can alter the situation. We can enjoin individuals to act responsibly and punish lack of ethical behavior after the fact, but we cannot dictate the metes and bounds of such behavior prospectively. Recognition and assumption of individual moral responsibility is a distinguishing feature of mature, well-socialized human beings. Thus, there is an almost exact parallelism in this aspect of animal and of human research; the ethical "buck" begins and stops with the principal investigator.

I welcome Prentice et al.'s thoughtful discussion but suggest that they re-examine their conclusions in the light of individual academic freedom and ethical responsibility.

Sincerely,

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To The Editor:

We wholeheartedly agree with Dr. Black's statement that "much fundamental research is seen to have relevance only long after it is completed or it may take on relevance in a manner much different from original intentions." As has been shown many times, it is not unusual for significant scientific discoveries to be serendipitous in nature. However, we wish to point out that assessment of a research project's relevance in advance of the completion of the research represents only a judgement that the proposed hypothesis to be tested appears to have *potential* relevance. As we suggest in our paper, the term "relevance" in biomedical research means the research has potential value to human or animal health, the advancement of knowledge, or the good of society. Indeed, a statement of the importance of proposed experiments with respect to "health relevance" is specifically requested in the PHS 398 grant application. Relevance is obviously one of the key characteristics of scientific merit, along with a sound experimental design. If a research proposal has potential relevance or value *and* the experimental design is sound, we would contend that the research has scientific merit. It should, therefore, be conducted providing the ethical cost-benefit is acceptable and funding is available.

Dr. Black takes exception to the inclusion of the terms "human or animal health" in the same phrase and suggests this implies an equivalence "and in some cases the primacy of ethical obligations to animals over those to humans." We dispute Black's interpretation and strongly disagree with his contention that use of the phrase in any way "accepts as de facto the inappropriate assertions made by PETA" and other equivalent organizations. The phrase to which Black refers is part of Principle II of the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training that were developed by the Interagency Research Animal Committee and implemented by the PHS Policy (PHS, 1986). Certainly, the framers of the PHS Policy, in response to the Health Research Extension Act (42 USC 289d) and amended Animal Welfare Act (7 USC 2131–2157) never intended to imply any equivalence between humans and animals. We suggest the phrase in question reflects the fact that legitimate animal research can be designed to benefit animals alone without any direct relevance to human beings. Clearly, veterinary projects that may result in animal health benefits should be an active area of research.

Dr. Black expresses concern that "imposing the need for external reviewers in internal decisions would increase delay and cost of research considerably, while reducing its timeliness and eventual relevance." Whereas we do not understand how an external review can possibly reduce the eventual relevance of research, we do agree that over reliance by IACUCs on external reviews would have a negative impact on research. However, as we indicated in our paper, protocols at our institution have rarely required outside review. Indeed, the few external reviews sought by our IACUC have been delivered in a timely fashion and have not prevented grant applications from meeting funding agency deadlines. In addition, the PHS Policy specifically authorizes such reviews in recognition that IACUCs may not have the prerequisite scientific expertise in all fields of research.

Perhaps the most disturbing aspect of Dr. Black's letter is his concern for preservation of academic freedom or, as he states, "noninterference with individual academic autonomy (freedom)." Black apparently considers the use of animals in research to be an academic right as opposed to a privilege that is granted by society. Freedom, be it academic or other, does not imply license under any guarantee of the constitution. Given the current threat to animal research and the concern expressed by the general public over the need to ensure appropriate animal welfare in this nation's research laboratories, we find Black's attitude to be both alarming and remarkably naive. The public, which overwhelmingly supports valuable animal research that is humanely conducted, demands accountability. Quite simply put, investigators can no longer operate in relative autonomy in an environment devoid of any comprehensive system of checks and balances. Indeed, as we point out in our paper, the local institution now bears the ultimate legal responsibility for the research conducted within its walls. It is not in compliance with the PHS Policy for an IACUC to give approval for a protocol conditional upon successful peer review by the NIH because the committee cannot make a judgement regarding scientific merit. More importantly, Black fails to acknowledge that many research projects are initiated with in-house funds and, therefore, do not receive peer review at any grant agency level. Internally funded projects conducted without appropriate review can place the institution in a precarious position. It takes only one well-publicized research project labeled as unjustified to significantly damage the reputation of the institution and compromise the credibility of biomedical research in general. Therefore, if IACUC review implies interference with individual academic autonomy, it clearly does so with federal and public support. As we have learned, painfully, in the area of scientific misconduct, it is far better for science to police itself than have some external agency perform this function.

Dr. Black characterizes IACUC review that results in alteration of a proposed research project as being "distinctly negative." Apparently, he either does not understand or does not agree with the provisions of the PHS Policy. The Congress has the power to make whatever laws are consistent with the constitution, and it is Congress that has decided that animal research must be regulated and monitored by the PHS and the USDA. And, it is these organizations that have decided that the IACUC must serve that role at the institutional level. According to the PHS Policy, the institution through its IACUC has

a legal obligation to assess the experimental design of a research project in order to ensure its soundness, while at the same time minimizing potential pain, discomfort, and distress the animals may experience and the number of animals to be utilized. Certainly, any IACUC review that takes into consideration the aforementioned criteria may, indeed, alter the proposed research after appropriate consultation with the investigator. However, what Black fails to recognize is that any alterations that may result will improve the research from an ethical standpoint without compromising its scientific validity. As a matter of fact, a properly performed IACUC review can enhance the probability of a research proposal being funded. Impetus for the alteration of research also occurs in the NIH review process. Contrary to Black's assertion that "NIH study sections take great pains not to plan or redirect proposed research," in reality, many investigators have found themselves involved in revision and resubmission of a proposal *until* the study section finds it acceptable.

Finally, we wish to comment on Dr. Black's last point regarding a transference by implication of the ethical responsibility for human subjects research to the human participants. In our paper, we specifically linked the transference of responsibility to the informed consent process. While an IRB must conclude that a given research project has a favorable risk-benefit relationship, the board, nevertheless, has the luxury of knowing that any prospective subject who finds the risk-benefit relationship of research participation unacceptable may simply choose not to participate. Anyone who has served on a medical IRB realizes that in an ethically complex and/or risky therapeutic experiment, respect for the patient's autonomy may become the overriding factor in determining the approvability of the research. We do not think our paper implied either directly or indirectly that investigators are not responsible for the ethical conduct of their research. Most assuredly, as Black points out, the ethical "buck" does begin and stop with the investigator. However, we wish to add that both the IRB and the IACUC serve to help researchers prospectively delineate their moral responsibility to human and animal subjects, respectively. Both the IRB and the IACUC are regulatory bodies empowered by the federal government and appointed by the institution. While Black implies that the advice of these review committees can be effectively disregarded, since "the ethical responsibility resides with the principal investigator," we can most assuredly state that the PHS, USDA, and FDA would strongly disagree with this characterization, implied or otherwise, of IRB/IACUC authority.

We thank Dr. Black for his thoughtful review of our article, and while we disagree with many of his points, we are grateful for the opportunity to exchange ideas and opinions. The IACUC, in its present form, has been in existence only 7 years. As IACUCs and investigators continue to work together, we are confident that consensus will be reached and our privilege to continue to use animals in justifiable research will be better protected.

Sincerely,

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Reference

PHS (Public Health Service). 1986. U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. Pp 27-28 in Public Health Service Policy on Humane Care and Use of Laboratory Animals. Washington, D.C.: U.S. Department of Health and Human Services. (Available from Office for Protection from Research Risks, Building 31, Room 5B59, National Institutes of Health, Bethesda, MD 20892.)

Models of Type I Diabetes—Part One

The NOD Mouse: A Model for Analyzing the Interplay Between Heredity and Environment in Development of Autoimmune Disease

Edward H. Leiter

INTRODUCTION

Inbred colonies of NOD (nonobese diabetic) mice have become widely established around the world since the publication of the initial report (Makino et al., 1980) describing this strain's susceptibility to spontaneous development of autoimmune (type I) insulin-dependent diabetes mellitus (IDDM). The availability of NOD mice has provided important new immunogenetic and pathophysiologic insights into autoimmune disease and its prevention. Because of their genetic predisposition to develop IDDM, NOD mice are in great demand by investigators wishing to test compounds or devices that will either prevent the development of diabetes or provide therapy after the disease has developed. These mice present a special challenge to laboratory animal health specialists and colony managers. One of the most important lessons "taught" by the NOD mouse is that the quality of the breeding and maintenance environment is not a trivial matter. Whereas there is only circumstantial evidence supporting environmental triggers for IDDM in humans (e.g., dietary ingredients and pathogens), studies using the NOD mouse clearly demonstrate that the penetrance of a diabetes-susceptible genotype is strongly modified by both the microbial and the dietary environment. The immunopathogenic features associated with IDDM in this mouse have recently been reviewed (Kikutani and Makino, 1992). Because of the large numbers of publications reporting on research with the NOD mouse, particularly research on immunotherapy, this review cannot be comprehensive. Our purpose will be to provide an overview of the NOD mouse as a model for analysis of autoimmune diabetes, to identify those characteristics most closely associated with development of autoimmune disease, and to provide a basis for understanding why stringent control of the environment is essential for research in which this model is used.

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DEVELOPMENT OF THE NOD STRAIN

The NOD and related inbred strains were developed at the Shionogi Research Laboratories in Aburahi, Japan, by Dr. S. Makino (Makino et al., 1980, 1985). A combination of inbreeding and selective breeding began in 1966 with progeny from an outbred Jcl:ICR female mouse that exhibited cataracts and microphthalmia. This breeding program led to the development of one strain in which all mice develop cataracts (now designated CTS, for cataract Shionogi) and a second, control strain that is cataract-free (designated NCT). Both are now beyond 100 generations (F100) of brother × sister matings.

At F6, mice free of cataracts but exhibiting anomalously high fasting blood glucose, were separated from the cataract-prone line; breeders were selected on the basis of the high fasting blood glucose trait. At F13, mice exhibiting normal fasting blood glucose were separated from the hyperglycemic line as a potential control strain (Kikutani and Makino, 1992). In 1974, at F20, a female spontaneously developed overt IDDM. Paradoxically, this female was found not in the line with fasting hyperglycemia, in which diabetes development was expected, but instead in the line being inbred as a diabetes-free control line. The NOD/Shi strain originated from subsequent selective breeding of glycosuric mice from this control line. Based upon a recent report, the diabetes incidence in NOD/Shi mice beyond F20 in the specific-pathogen-free (SPF) source colony at Aburahi Laboratories has remained relatively constant, with a 70–80 percent incidence in females versus a 20 percent incidence in males (Kikutani and Makino, 1992). This gender dimorphism is controlled, in part, by gonadal sex steroids. Gonadectomy at 5 weeks of age markedly increases diabetes development in NOD/Shi males, while it depresses diabetes incidence in females (Makino et al., 1981).

The inbred strain that was developed from the subline with fasting hyperglycemia has been designated NON (nonobese nondiabetic). Because of its similarity to the

NOD strain, NON mice are extremely useful for genetic analyses; they apparently share with NOD some, but not all, of the diabetogenic loci predisposing them to diabetes (Leiter, 1989). Although NON mice do not develop autoimmune diabetes, NON/Lt males at The Jackson Laboratory develop marked obesity by 20 weeks of age (NRC, 1989, pp. 103–4). Moreover, NON mice of both sexes are intolerant to glucose loading and develop severe glomerulosclerotic kidney lesions (Tochino et al., 1983). As NON/Lt mice age, immunoregulatory anomalies become apparent; for example, by 20 weeks of age, T-lymphocytopenia and functional T-cell anergy develop in NON/Lt splenocytes (Leiter et al., 1986). Thus, NON mice are not necessarily the best controls for establishing certain "normative" physiologic or immunologic baselines. The issue of potential controls to match with the NOD strain will be discussed later.

PATHOPHYSIOLOGY OF DIABETES IN NOD MICE

Prior to 1986, the availability of NOD mice was limited to a group in Japan called the NOD Mouse Study Group. A book reporting the results of these early studies has been published and contains a wealth of pathophysiologic information (Tarui et al., 1986).

Diabetes development in NOD mice is characterized by insulitis, which is a leukocytic infiltrate of the pancreatic islets. In the NOD/Lt colony at the Jackson Laboratory, a pervasive leukocytic infiltrate emanating from the pancreatic vasculature and secretory ducts is first observed at a time when the islets are free of lesions. Pancreatic islets are concentrated in the perivascular/periductular areas; consequently, large numbers of leukocytes aggregate at the periphery of islets (peri-insulitis). The aggregates usually start at one pole but eventually surround the entire islet perimeter. Widespread insulitis, entailing the erosion of β-cell mass as leukocytes penetrate into the islet core, develops between 5 and 7 weeks of age in females and several weeks later in males. Interestingly, a period of islet growth partially compensates for this early insulitis. NOD/Lt islets in mice between 5 and 12 weeks of age are quite large in comparison with those of the closely-related NON/Lt strain, despite the heavy leukocytic aggregations surrounding many of them.

Marked decreases in pancreatic insulin content are demonstrable in NOD/Lt females at around 12 weeks of age (Gaskins et al., 1992a) and several weeks later in males (E. Leiter, unpublished observation). Onset of diabetes is marked by the appearance of moderate glycosuria (1^+ reading on Lilly Tes-Tape™) and by a non-fasting plasma glucose higher than 250 mg/dl. Both glycosuria and hyperglycemia become progressively more severe over a 3–4 week period during which weight loss, polydipsia, and polyuria occur. Diabetic mice are hypoinsulinemic and hyperglucagonemic, a finding that correlates with the histologic profile of selective destruction of β, but not non-β, islet cells. Without insulin treatment, diabetic mice become severely hyperglycemic and ketonemic, but they do not become ketoacidotic (Harano et al., 1986).

In most specific-pathogen-free colonies, untreated diabetic mice will survive for 3–4 weeks after the first detection of glycosuria. Most investigators monitor NOD mice for development of glycosuria at weekly intervals beginning after 10 weeks of age. Weight loss and the appearance of polydipsia and polyuria indicate the onset of hyperglycemia. Documentation of increasing levels of glycosuria over 2 consecutive weeks, coupled with a serum or plasma glucose measurement in excess of 300 mg/dl, are acceptable measures for a diagnosis of IDDM. It is difficult in diabetic NOD mice to maintain serum glucose within a normal range by insulin treatment, although body weight can be maintained and lifespan prolonged (Ohneda et al., 1984). A morning and evening injection of a 1:1 mixture of regular and NPH insulin at a dose between 1 and 3 units per mouse, depending upon the level of glycosuria as measured using Tes-Tape™, has been reported (Doi et al., 1990).

IMMUNOPATHOGENESIS

Although B lymphocytes and macrophages are present in the early insulitic infiltrates (Jarpe et al., 1991; Signore et al., 1989), T lymphocytes predominate (Miyazaki et al., 1985). Diabetogenesis is T-lymphocyte dependent and to a large extent T-cell mediated, with both $CD4^+$ and $CD8^+$ subsets required for the initiation of destructive insulitis (Christianson et al., 1993; Miller et al., 1988; Yagi et al., 1992). Unlike the diabetes-prone BBDP/Wor rat, which is T-lymphopenic and exhibits strong natural killer cell activity, NOD/Lt mice exhibit elevated percentages of T lymphocytes in peripheral lymphoid organs (T-lymphoaccumulation), whereas NK cells are functionally defective (Serreze and Leiter, 1988). Table 1 presents a partial listing of some of the aberrant immunophenotypes described in the NOD mouse. NOD/Lt mice homozygous for the severe combined immune deficiency (*scid*) mutation fail to develop functional T and B lymphocytes and consequently, are insulitis- and diabetes-free (Christianson et al., 1993). Purified splenic $CD4^+$, but not $CD8^+$, T lymphocytes from *diabetic* NOD/Lt donors (already primed in vivo to the β-cell antigens released as a consequence of destructive insulitis) adoptively transfer insulitis and diabetes into unirradiated NOD/Lt-*scid/scid* recipients (Christianson et al., 1993). However, purified splenic $CD4^+$ T lymphocytes from *prediabetic* NOD/Lt mice are incapable of transferring disease in the absence of $CD8^+$ T lymphocytes, confirming that $CD8^+$ T lymphocytes are required for the initiation of β-cell destruction during the natural course of the disease in euthymic NOD mice (Christianson et al., 1993). Macro-

TABLE 1 Aberrant Immunophenotypes in NOD Mice: A Partial Listing

Bone Marrow
- Transfers diabetes to irradiated F1 hybrids nominally diabetes resistant
- Contains defects in tolerance induction traced to marrow-derived APC

Macrophages/APC
- Are defective in the differentiation/maturation from marrow
- Have low Il-1 secretory responses to LPS stimulation
- Display defective activation of regulatory T lymphocytes in a syngeneic mixed lymphocyte reaction (SMLR).
- Respond to gamma interferon with an aberrant MHC class I down-regulation
- Contribute to defective negative selection of islet autoreactive T lymphocytes

T Lymphocytes
- Thymocyte proliferation is not stimulated by Con A
- T-lymphoaccumulation (high percentage of T lymphocytes in lymphoid organs)
- Have low IL-2 and IL-4 secretion
- SMLR blasts fail to suppress a mixed lymphocyte reaction (MLR)

NK Cells
- Are functionally defective against targets in vitro

phage infiltration into the islets is required to recruit or activate diabetogenic T lymphocytes (Hutchings et al., 1990; Ihm and Yoon, 1990). A longitudinal flow cytometric analysis of islet-infiltrating leukocytes in NOD/Uf (detected at 5 weeks of age in females and 7 weeks of age in males) indicates that class II$^+$, Ig$^-$ monocytes and CD8$^+$ T lymphocytes are the first cell types to infiltrate pancreatic islets (Jarpe et al., 1991). The failure to detect CD4$^+$ T lymphocytes in the earliest infiltrates by flow cytometry apparently reflects an activation-associated down-regulation of the CD4 molecule because CD4 transcripts are detected by polymerase chain reaction amplification of reverse transcriptase products (Dr. A. B. Peck, University of Florida, Department of Pathology, Gainesville, personal communication). A second influx of CD8$^+$, as well as a major influx of CD4$^+$ cells has been observed between 10 and 12 weeks of age, quickly followed by the development of overt diabetes (Jarpe et al., 1991). Of further interest is the finding that the cytokine profile of CD4$^+$ T lymphocytes from the islets of 14-week-old females is that of a T-helper 2 cell (A. B. Peck, personal communication). Interestingly, pancreatic β cells from 3-week-old mice of both sexes are coated with autoantibody, suggesting that the initial lesion may entail antibody-dependent cell-mediated cytotoxicity. NOD β cells are distinguished at the ultrastructural level from non-β islet cells by the presence in the former of an aberrant intracisternal type C virus particle (Nakagawa et al., 1992), which is apparently encoded by an endogenous xenotropic (*Xmv*) proviral gene (Gaskins et al., 1992a). Since autoimmune-prone mouse strains commonly develop high titers of antibodies to retroviral gene products, it is conceivable that maternally-transmitted antibodies may "target" β cells in juvenile NOD mice for T-lymphocyte-mediated destruction. Adoptive transfer of autoantibodies from mother to offspring would explain the finding that diabetogenic T-effector cells will adoptively transfer disease into neonates whose endogenous B-lymphocyte functions have been suppressed by treatment with anti-μ chain monoclonal antibody (Bendelac et al., 1988).

The spontaneously occurring autoimmune disease in NOD mice differs significantly from experimentally induced models of organ-specific autoimmunity. Although myelin basic protein-autoreactive T lymphocytes elicited in experimentally induced allergic encephalomyelitis (EAE) in susceptible strains of mice show very restricted T-cell receptor (TCR) Vβ gene utilization, analysis of TCR Vβ gene utilization by islet-infiltrating T lymphocytes has indicated an extensive polyclonal expression of the repertoire (Maeda et al., 1991; Waters et al., 1992). Similarly, the immune system of NOD mice responds to a plethora of different candidate β-cell antigens, including insulin, glutamic acid decarboxylase (formerly a 64 kilodalton [kd] autoantigen) and a 52 kd autoantigen, as well as to endogenous retroviral gene products (Gaskins et al., 1992a).

Although most analyses of diabetogenesis in NOD mice focus on T lymphocytes because of their role as disease effectors, functional defects in bone marrow-derived antigen-presenting cells (APCs), which are sum-

TABLE 2 Spectrum of Aging-associated Pathologies Present in NOD/Lt Mice in Addition to Insulitis

Tumors
- Lymphomas*
- Lymphosarcomas
- Osteosarcoma/osteochondrosarcoma
- Myoepitheliocarcinoma
- Rhabdomyosarcoma
- Mammary carcinoma
- Hepatoma

Infiltrates
- Colitis
- Sialitis
- Harderian/Lacrimal adenitis
- Myositis
- Neuritis/meningitis
- Thyroiditis
- Osteomyelitis
- Nephritis

*80–100% incidence of thymic lymphomas in NOD-*scid/scid* mice.

marized in Table 1, very likely underlie the generation of β-cell-autoreactive T lymphocytes. F1 hybrids between NOD and other inbred strains are diabetes-resistant; however, diabetogenic T lymphocytes develop when those F1 hybrids are lethally-irradiated and reconstituted with NOD bone marrow (Serreze et al., 1988; Wicker et al., 1988). These defects have been attributed to APCs, such as macrophages and dendritic cells, that develop from myelocytic precursors in the marrow. APCs play an important role in shaping the T-lymphocyte repertoire by presenting self-antigens intrathymically, and they stimulate T-lymphocyte responses in the periphery by presenting processed peptides in association with major histocompatibility complex (MHC) molecules to antigen-specific T lymphocytes. Two immunoregulatory defects that are probably central to the diabetes susceptibility of NOD/Lt mice have been ascribed to defective APC functions, including an inability to activate functional immunoregulatory T (suppressor) cells in the periphery, as measured in a syngeneic mixed lymphocyte reaction (SMLR) (Serreze and Leiter, 1988; Serreze et al., 1990), and an inability to block the development of β-cell-autoreactive T lymphocytes in the thymus (Serreze and Leiter, 1991). The genetic basis for the expression of these defects at the APC level entails a complex trans-interaction between diabetogenic MHC and non-MHC-linked genes (Leiter and Serreze, 1992).

It should be noted that aging NOD mice exhibit a spectrum of autoimmune pathologies distributed through a variety of organs (Leiter, 1990a). These multi-organ lesions are a reflection of the T-lymphoaccumulation peculiar to this strain. The thymus gland is very slow to involute in NOD/Lt mice when compared with NON/Lt mice, and NOD thymocytes do not proliferate in response to mitogen (Zipris et al., 1991), potentially reflecting an underlying defect in normal intrathymic apoptosis. Morphologic anomalies in the NOD thymus have also been reported (Savino et al., 1991). As in other inbred strains with autoimmune susceptibilities, aging NOD mice develop a wide spectrum of neoplasms, the most common of which are lymphomas (Leiter, 1990a).

GENETICS

The susceptibility of NOD mice to type I autoimmune IDDM is under complex polygenic control, with environmental factors also exerting strong effects on gene penetrances. However, it is quite clear from genetic analyses (reviewed in Kikutani and Makino, 1992; Leiter, 1990a; and Leiter and Serreze, 1992) that the major component of this susceptibility is the unique MHC haplotype (*H-2*^{g7}, on Chr 17). The MHC-encoded susceptibility entails both a lack of expression of *I-E* (homologous to *DR* in humans) and expression of a unique *I-Aβ* locus (histidine at residue 56, serine at residue 57; homologous to "diabetogenic" HLA-*DQβ* non-aspartic acid^{57}-containing alleles) (Todd et al., 1988). In genetic outcross/backcross analyses, heterozygous expression of the diabetogenic *H-2*^{g7} haplotype is permissive for insulitis development, but insulitis sufficiently widespread to produce the clinical phenotype of diabetes is rarely observed in segregants that are not homozygous for the diabetogenic class II MHC alleles. Thus, if only insulitis induction is considered, the haplotype functions in a codominant fashion with other genetic factors in the NOD strain background. However, when development of overt IDDM is considered, recessive components within this haplotype are clearly recognizable. An example of a recessive gene effect is the inability of the *H-2*^{g7} haplotype to express cell surface I-E molecules on APCs due to mutations in the *Eα* locus. NOD mice expressing a functional *Eα*^{d} transgene have been reported to be both insulitis- and diabetes-resistant when compared with standard (I-E null) NOD mice (Lund et al., 1990; Uehira et al., 1989).

Although immunogenetic analysis has concentrated on the diabetogenic contributions of the MHC class II region of the *H-2*^{g7} haplotype, current evidence suggests that the haplotype as a whole should be considered as contributing to susceptibility. The most compelling evidence comes from the congeneic transfer of the unique MHC haplotype of the related CTS/Shi strain onto the NOD/Shi genetic background. The MHC of CTS mice apparently contains the same class II alleles as NOD but has distinct class I loci, indicating that loci between these markers may differ as well. When this CTS haplotype (*H-2* designation not yet assigned) was transferred onto the NOD inbred background and compared in the homozygous state to segregants homozygous for the *H-2*^{g7} haplotype, a lower incidence of diabetes and insulitis was observed in the mice homozygous for the CTS MHC (Kikutani and Makino, 1992; Makino et al., 1991). The reduced diabetogenic potency of the CTS MHC thus provides strong support for the concept that, while the class II region is clearly important to disease development, other loci within the extended *H-2*^{g7} haplotype are also contributory. Intra-MHC regions both proximal and distal to the *H-2*^{g7} class II region contain rare or unique alleles that may also contribute to diabetes susceptibility. Among these are a unique heat-shock protein 70 (*Hsp70*) allele (Gaskins et al., 1990), as well as rare alleles at *Tap-1* and *Tap-2* (for *t*ransporters associated with *a*ntigen *p*rocessing and formerly designated *Ham-1* and *Ham-2*) (Gaskins et al., 1992b) The products of these loci are members of a superfamily of ATP-dependent transport proteins, and they may function to transport processed antigenic peptide fragments generated in endosomal compartments into the lumen of the endoplasmic reticulum for association with MHC class I molecules (Monaco et al., 1990). Mutant mouse and human cell lines lacking the ability to form stable MHC class I peptide complexes all carry mutations that map to regions encoding *Tap-1* or homologous genes (Monaco et al., 1990). Although defective *Tap* gene transcription in the spleens of NOD

TABLE 3 The Number of Genetic Factors Segregating for Diabetes Susceptibility Varies with the Partner Strain Used in Outcross with NOD

Original outcross	# Diabetic/total (both sexes)	% Diabetic	Minimum estimate of # of chromosomes carrying recessive susceptibility genes
NOD × NON	19/200	9.5	3
NOD × BKs	1/115	0.9	>5
NOD × B6	5/383	1.3	>5
NOD × SWR	15/112 (females)	13.0	3

mice has recently been reported (Faustman et al., 1992), this has not been confirmed in cultured NOD/Lt peritoneal macrophages, in which IFN_γ-induced upregulation of both *Tap-1* and *Tap-2* mRNA is normal, as is upregulation of TAP1 content in NOD β cells (Gaskins et al., 1992b). However, the NOD *Tap* genes have not yet been sequenced, so the possibility remains that TAP functions in NOD macrophages may not be normal. Although the MHC class I *H-2K* and *H-2D* loci in the $H\text{-}2^{g7}$ haplotype of NOD are common alleles (e.g., $H\text{-}2K^d$, $H\text{-}2D^b$), intracellular and cell surface levels of $H\text{-}2K^d$ aberrantly decline in response to IFNγ. This aberrant response is cell-specific since it is observed in NOD macrophages but not in β cells (Serreze et al., 1993). This cell-type-specific defect is apparently not due to defective interaction between *Tap* gene products and MHC class I molecules, because an $H\text{-}2^{g7}$-identical strain called NOR/Lt, which is related to NOD and is discussed below, exhibits normal MHC class I upregulation in response to IFNγ(Serreze et al., 1993).

MHC-unlinked genes also control diabetes development and have been analyzed by outcrossing NOD with other inbred strains; the numbers and locations of these genes is wholly predicated upon the degree of relatedness of the strain chosen for outcross with NOD (Table 3). Analysis of MHC congenic stocks has shown that although the unique $H\text{-}2^{g7}$ haplotype of the NOD strain represents a major component of this strain's genetic susceptibility to IDDM, the diabetogenic $H\text{-}2^{g7}$ haplotype requires interaction with non-MHC linked susceptibility modifiers to mediate diabetogenesis (Leiter and Serreze, 1991; Todd et al., 1991).

Genetic segregation analyses are initiated by an outcross between NOD and a diabetes-resistant strain, followed by either one backcross (BC1) to NOD or, less frequently, by an F1 × F1 intercross to produce an F2 generation. In situations where the diabetes-resistance genes from the second parental strain are incompletely dominant (as usually is the case), the F2 cross will be more informative than a backcross to NOD because the strength of the protective locus can be assessed in the homozygous, as well as in the heterozygous state. The NOD-derived modifiers shown to segregate with disease susceptibility are provisionally designated *Idd* (Insulin-dependent diabetes) loci. The $H\text{-}2^{g7}$ susceptibility (which encompasses at least two loci within the class II region) has been designated *Idd-1*, with $Idd\text{-}1^s$ denoting the susceptibility haplotype and $Idd\text{-}1^r$ denoting the resistance haplotype (Prochazka et al., 1987). Interval mapping strategies have been employed to define chromosomal locations of the non-MHC susceptibility loci segregating in outcrosses with the NON, C57BL/10J (B10), and C57BL/6J strains. These analyses have shown the presence of genetic factors controlling diabetes susceptibility or resistance on at least eight chromosomes (Leiter and Serreze, 1992). These studies require segregation analysis in diabetic versus non-diabetic progeny using mapped DNA or phenotypic polymorphisms distinguishing the two parental strains. The aim is to type as many polymorphic loci as possible within a discrete region of a chromosome that have been identified by linkage analysis as segregating with diabetes susceptibility. Although phenotypic markers (e.g., polymorphic serum or tissue isoenzymes, immunophenotypic cell surface markers, or physiologic responses known to be under single gene control) are employed, the bulk of the genetic screening for diagnostic polymorphisms is at the DNA level (Prochazka et al., 1987; Todd et al., 1991). Identification and mapping of microsatellite repeat sequences scattered throughout the mouse genome, coupled with the development of the polymerase chain reaction (PCR), have revolutionized this type of genetic analysis (Dietrich et al., 1992; Love et al., 1990; Todd et al., 1991). NOD-derived genes showing the strongest statistical linkage association with the diabetic phenotype are assumed to flank an *Idd* locus. A linked marker locus that shows no recombination with the diabetic phenotype may be presumed to be a candidate for the *Idd* locus itself. In practice, only the $H\text{-}2^{g7}$ complex exhibits such tight linkage with diabetes that it can be stated with certainty that this complex contains the *Idd-1* susceptibility. This may reflect the fact that although diabetes in NOD mice is clearly under polygenic control, $H\text{-}2^{g7}$ exerts a stronger diabetogenic influence than many of the non-MHC modifiers. For example, when NOD/Lt is crossed with the related NON/Lt strain, there is no diabetes in the F1 generation. In contrast, in an outcross using an NON/Lt stock congeneic for $H\text{-}2^{g7}$, a low (6.5 percent) incidence of diabetes develops in F1 females, with a 14 percent incidence in F2 females (Leiter and Serreze, 1992). In a cross between NOD and

TABLE 4 Chromosomal Location of *Idd* Susceptibility Modifiers (designations are provisional)

Locus Symbol (provisional)	Chromosome/strongest linkage marker or interval	Outcross in which linkage was established	Comments regarding resistance modifiers from outcross partner strain
Idd-1	17/*Aβ, Eα*	all MHC-disparate outcrosses	whole haplotype may contribute
Idd-2	9/*Thy-1*	NON/Lt(BC1) NON.NOD-*H-2*^{g7} (F2) B10.NOD-*H-2*^{g7} (BC1)	incompletely dominant, potential timing gene
Idd-3	3/*IL-2* in NON *D3Nds1-Tshb* in B10	NON.NOD-*H-2*^{g7} (F2) B10.NOD-*H-2*^{g7} (BC1) C57BL/6J*	stronger than *Idd-2*, incompletely dominant, probably at least 2 distinct resistance loci contributed by B10
Idd-4	11/*Acrb*	B10.NOD-*H-2*^{g7} (BC1) C57BL/6J*	incompletely dominant, potential timing gene
Idd-5	1/*IL1r1-Lsh-Bcl-2*	B10.NOD-*H-2*^{g7} (BC1)	incompletely dominant
Idd-6	6/*Kras-2*	C57BL/6J*	incompletely dominant
Idd-7	7/*Ckmm*	B10.NOD-*H-2*^{g7} (BC1)**	B10 locus contributes to susceptibility
Idd-8	14/*Plau*	B10.NOD-*H-2*^{g7} (BC1)**	B10 locus contributes to susceptibility

*Personal communication, Dr. Edward Wakeland, University of Florida, Gainesville
**Todd et al., 1991; Personal communication, Dr. John Todd, Oxford University

B10 mice congeneic for *H-2*^{g7}, no F1 and less than one percent of F2 progeny develop diabetes, indicating the presence of a larger number of non-MHC genes segregating for susceptibility and resistance when an unrelated inbred strain is employed as the outcross partner (Todd et al., 1991). It should be noted that not all genes derived from a diabetes-resistant strain are disease-protective or disease-neutral. In an outcross between NOD and C57BL/10J mice congeneic for *H-2*^{g7}, B10-derived genes on Chr 7 (*Idd-7*) and Chr 14 (*Idd-8*) have been found to be positively associated with diabetogenesis (Todd et al., 1991). Autoimmune lupus and nephritis are similarly accelerated when NZB mice are outcrossed with lupus-prone NZW mice (Babcock et al., 1989).

ENVIRONMENTAL REQUIREMENTS FOR DIABETOGENESIS

The NOD mouse has provided researchers with the most compelling evidence to date that environmental factors are important modulators of genetic susceptibility to IDDM. By the time they are 30 weeks of age, the incidence of diabetes in NOD females is usually 80 percent or higher, whereas in males, incidence is highly variable among colonies, ranging between 100 and 0 percent at different institutions. The environment accounts for a major component of this variation (Leiter, 1990b). Diabetes incidence in NOD males serves as a useful indicator of the presence of environmental factors affecting the penetrance of this strain's genetic susceptibility to IDDM. Transfer of NOD males from a conventional mouse room in Japan into germfree conditions increased the incidence of diabetes in males from 6 to 70 percent (Suzuki et al., 1987). Exposure of NOD mice to a variety of murine viruses (e.g., encephalomyocarditis virus, lymphocytic choriomeningitis virus, and murine hepatitis virus) prevents diabetes development (Hermite et al., 1990; Oldstone, 1988; Wilberz et al., 1991). These infectious agents apparently protect by providing general immunostimulation, because treatment of prediabetic NOD mice with various types of exogenous immunomodulators, including complete Freund's adjuvant (Sadelain et al., 1990), cytokines (IL-1, TNFα, IL-2, IL-4), and poly I:C, all circumvent the development of diabetes (Leiter, 1990b; Rapoport et al., 1992). Diabetogenic catalysts are also present in natural ingredient diets, which contain lipoidal moieties that are absent or present in low concentration in semi-purified diets (Coleman et al., 1990).

Certain peripheral immunoregulatory functions appear to be defective in NOD mice maintained in specific-pathogen-free (SPF) environments (see Table 1), as exemplified by defective T-suppressor-cell functions measured in vitro, as well as defects in the differentiation and maturation of APCs developing from bone marrow progenitors (Serreze et al., 1993). Defects in the degree of cytokine-elicited differentiation of APCs from bone marrow have been associated with inefficient presentation of

self-antigens (Leiter and Serreze,1992; Serreze et al., 1993), which may explain not only the defective tolerogenic functions of these cells but also the subnormal secretion of monokines by peripheral macrophages in response to lipopolysaccharide stimulation; subnormal secretion of IL-2 and IL-4 by splenic and thymic T lymphocytes, respectively; depressed NK cell activity; depressed thymocyte responses to mitogenic stimulation; and accumulation of T lymphocytes. Presumably, immunomoduiatory effects mediated by environmental components serve to regulate some of these defective APC functions, resulting either in more normal thymic elimination of autoreactive T lymphocytes, more potent activation of immunoregulatory T lymphocytes in the periphery, or both.

Macrophages and neutrophils represent a mammal's first line of defense against infectious agents and irritants. The knowledge that the genetic defects that predispose NOD mice to IDDM are reflected, in part, as maturational or functional defects in macrophages provides a basis for understanding why exposing prediabetic NOD mice to any of a myriad of environmental pathogens and immunoregulatory cytokines circumvents diabetes (Leiter, 1990b). Many of the therapies that do not entail direct immunosuppression of T cells would be expected to activate macrophage function and stimulate antigen processing and presentation. Even when therapeutic intervention is achieved by a semi-synthetic diet rather than by exposure to a virus or treatment with a cytokine, protection is associated with increased rather than decreased immune responsiveness. For example, when the semi-purified diet AIN-76 is substituted for a natural-ingredient diet (such as Old Guilford 96W, the diet fed to NOD/Lt mice at The Jackson Laboratory), the incidence of diabetes in NOD/Lt females is reduced and the age of onset is delayed (Coleman et al., 1990). This protection is associated with development of high titers of anti-BSA antibodies (D. V. Serreze and E. H. Leiter, The Jackson Laboratory, Bar Harbor, Maine, unpublished data), presumably in response to contaminants in the casein used in the diet. Anti-BSA antibodies found in NOD mice have recently been cited to support the speculation that cow's milk protein is a diabetogenic catalyst for development of IDDM in genetically-predisposed Caucasian children (Karjalainen et al., 1992). The finding in NOD mice that high titers of anti-BSA antibodies do not necessarily correlate with early onset diabetes is not consistent with the hypothesis that BSA or another product of milk in the rodent diet is diabetogenic. Interestingly, digestive hydrolysis of β-casein generates immunomodulatory peptides that stimulate macrophage phagocytosis (Migliore-Samour and Jolles, 1988). The protection afforded by Pregestimil™, a hypoallergenic infant formula, is even more potent than that provided by diet AIN-76 (Coleman et al., 1990). Although Pregestimil™ contains casein hydrolysate rather than intact milk protein, small peptides and amino acids are present and may serve as macrophage activators. It is conceivable that either dietary or microbial components are acting as "superantigens" to regulate the T-cell repertoire. However, it now appears that the T-cell response to NOD β-cell antigens is quite polyclonal. Thus, environmental factors probably decrease the penetrance of diabetes susceptibility genes by upregulating APC functions. More normal APC functions, both intrathymically and in the periphery, should block development of autoreactive, polyclonal cytotoxic T lymphocytes.

Since many investigators who study the NOD model want to perform therapeutic intervention studies that often entail the use of monoclonal antibodies and other biologics (Shizuru et al., 1988; Taki et al., 1991), it is prudent that all biologic reagents to be tested in NOD mice be prescreened for the presence of pathogenic agents. The acute sensitivity of NOD mice to environmental modulators places a special burden on colony managers to strive for the maximum possible degree of environmental control. Ideally, the breeding facility should be separate from rooms in which experiments are performed.

APPROPRIATE CONTROLS FOR NOD MICE

The question of the appropriate control to use for experimentation with NOD mice often arises. Table 5 lists some of the inbred and congenic strains without insulitis and diabetes that have been used to establish experimental "baseline" parameters and are potential controls for the NOD strain. In some of the initial immunologic studies of the NOD mouse in Japan, the outbred ICR strain was used as the standard for comparison, and it was erroneously concluded that NOD mice were T-lymphocytopenic (Kataoka et al., 1983). Considerable size differences distinguish ICR from NOD mice. At the Jackson Laboratory, the SWR/J or the SWR/BmJ substrain has provided a suitable, inbred standard for comparison. SWR mice share with ICR (and NOD) mice a common derivation from the "Swiss" mice brought to the United States by Dr. Clara Lynch in the 1920s. SWR/J mice are approximately the same size as NOD/Lt mice, and they exhibit normal immunoregulatory responses for most parameters that are aberrant in NOD/Lt mice. The $H\text{-}2^{q}$ haplotype of SWR, like the $H\text{-}2^{g7}$ haplotype of NOD, does not produce cell surface I-E molecules. The NON/Lt is not considered a normative control strain for immunologic function because, like CTS mice, NON/Lt mice develop an age-related decline in peripheral T-cell numbers, and like NOD/Lt mice, their SMLR is depressed. Thus, as positive controls for cytokine production assays or SMLR analyses, SWR/J mice are a reasonable choice. The recently developed NOR/Lt incipient congenic stock (presently at F20 and available for distribution from The Jackson Laboratory) shares the diabetogenic $H\text{-}2^{g7}$ with NOD, but carries the genome from the C57BL/KsJ strain on portions of at least five chromosomes (Chr 2, 4, 7, 11,

TABLE 5 Potential Diabetes-free Control Strains for NOD Mice

Strain	Advantage	Disadvantage	Best Use	Reference
NON/Lt	• Closely related to NOD • Diabetes resistant MHC	• Develops obesity • Impaired glucose tolerance • Immunodeficiencies • Difficult to breed	• Genetic analysis of *Idd* genes . Potential model for type II diabetes	Leiter et al., 1986
CTS/ShiJos	• Closely related to NOD • Same MHC class II genes • Different class I genes	• Early developing T-lymphocytopenia • Unavailable	• Genetic analysis of the contributory role of MHC class II as well as other *H-2* alleles in the *Idd-1* complex	Ikegami et al., 1988; Kikutani and Makino, 1992
ILI/JicJos	• Closely related to NOD • MHC-identical	• Unavailable	• Genetic analysis of non-MHC-linked *Idd* genes	Hattori et al., 1990
ICR (available as CD-I)	• Progenitor stock for NOD and related strains	• Randomly bred	• Analysis of population frequency of rare genetic polymorphisms present in NOD	Ikegami et al., 1990
SWR/J	• Swiss-derived like ICR and NOD, but inbred and without immunodeficiencies • Available	• Genetically very different from NOD, including MHC	• Control for immune functions that are aberrant in NOD	Serreze and Leiter, 1988
NOR/Lt	• NOD-derived recombinant congenic stock • Same MHC, differs at relatively few non-MHC loci	• Not yet at F20 • Exhibits some but not all of NOD's immune dysfunctions	• Analysis to establish which *Idd* genes control aberrant immunophenotypes essential to pathogenesis	Prochazka et al., 1992a
NOD-Eα^d transgenics	• Closest genetic match to diabetes-developing NOD mice	• Not widely available— stock being developed at The Jackson Laboratory	• Analysis of T-cell repertoire development • Presentation of β-cell antigens	Uehira et al., 1989; Lund et al., 1990
NOD-NON-H-2^{nbl}	• Diabetes-resistant MHC from NON/Lt • Available	• Exhibits some but not all of NOD's immune dysfunctions	• All MHC congenic stocks are extremely useful in dissecting the role of MHC versus non-MHC genes in producing aberrant immunophenotypes	Leiter and Serreze, 1991; Serreze and Leiter, 1991
NON-NOD-H-	• Diabetogenic MHC from NOD/Lt	• Exhibits some but not all of NOD's immune dysfunctions		Leiter and Serreze, 1991; Serreze and Leiter, 1991
NOD-B10-*H-2*^{b}	• Diabetes-resistant MHC from C57BL/10J • Available	• Exhibits some but not all of NOD's immune dysfunctions		Wicker et al., 1992; Todd et al., 1991
NOD-*scid*	• No endogeneous T- or B-lymphocyte functions	• Develops high incidence of thymoma with age	• Delineation of the role of T-cell subsets and autoantibodies	Prochazka et al., 1992b; Christianson et al., 1992

and 12). NOR/Lt mice develop very little insulitis and no diabetes, even after cyclophosphamide treatment (Prochazka, 1992b) In NOD colonies in which the penetrance of diabetogenic genes is suppressed (e.g., in colonies with a very low incidence of spontaneous diabetes in males), cyclophosphamide treatment can be used to rapidly elicit the diabetic phenotype (Harada and Makino, 1984). The NOR/Lt stock exhibits a more robust SMLR than NOD/Lt mice, and as discussed above, should provide an excellent control for comparing the nature of diabetogenic interactions between non-MHC-encoded *trans*-active factors and the diabetogenic *H-2*^{g7} MHC haplotype. NOD mice that are rendered diabetes-free because they express an MHC transgene (Eα^d) or are congeneic for a diabetes-resistant *H-2* haplotype are of obvious value for analyzing the role of the unique *H-2*^{g7} haplotype in selection and peripheral regulation of a normal T-cell repertoire. Diabetes-resistant NOD congenic stocks carrying

chromosomal regions from B10 that are associated with non-MHC-linked resistance are currently under development by Dr. L. Wicker (Merck Research Laboratories, Rahway, New Jersey). These stocks will be essential for associating specific immunodeficiencies in NOD mice with specific *Idd* genes. To identify and isolate specific *Idd* genes, one's choice of an outbred partner strain is limited only by the degree of genetic complexity that the investigator is willing to analyze (see Table 3). Although inbred strains derived from wild mice, such as *Mus spretus*, guarantee widespread genetic polymorphisms at most loci that have been typed, the polygenetics of disease susceptibility are such that very few mice with IDDM will be recovered at the first backcross. In contrast, reasonable numbers of diabetic probands will be obtained when outcrosses with related strains such as NON/Lt or SWR/J are employed, because these related strains share with NOD a certain number of diabetes-permissive genes not shared in unrelated strains, such as B10. However, the disadvantage is that related strains provide reduced numbers of genetic polymorphisms available for segregation analyses.

Congenic stocks of NOD mice rendered genetically T-cell deficient by introduction of the *nu* (Yagi et al., 1992) or the *scid* (Christianson et al., 1993) mutations have recently been described. These mice are obviously useful in establishing an endocrinologic or physiologic baseline in the absence of insulitis and impairment of pancreatic β-cell functions. Further, they are useful for analyzing the pathogenic or diabetes-suppressive competence of various cloned T-cell lines established from the NOD mouse (Bradley et al., 1990; Pankewycz et al., 1991; Reich et al., 1989).

A PROBLEM FOR THE FUTURE: GENETIC DIVERGENCE OF NOD SUBSTRAINS

As more and more institutions maintain their own inbred colonies of NOD mice, substrain divergence can be expected and has already occurred to some extent. During the development of the NOD/Shi inbred strain, and apparently before all segregating loci were fixed to homozygosity, a satellite breeding colony was established in Kyoto, Japan. From this colony, breeders were sent to the Walter and Eliza Hall Institute in Australia in 1984, where a new substrain, NOD/Wehi, was developed. The NOD/Lt mice at the Jackson Laboratory were also derived from a breeding colony held by Dr. M. Hattori, Kyoto, who brought the mice to the United States in 1984.

The incidence of spontaneous diabetes in NOD/Wehi mice is significantly lower in both sexes than in NOD/Lt mice imported from the Jackson Laboratory to Australia in 1987 and maintained in the same environment (Baxter et al., 1989, 1991), indicating that there has been significant genetic divergence between the NOD/Lt and NOD/Wehi substrains. What particularly distinguishes the NOD/Lt substrain from both the NOD/Shi and NOD/Wehi substrains is the higher incidence of diabetes in NOD/Lt males (50-70 percent by 40 weeks of age) when compared with NOD/Shi and NOD/Wehi males (20 percent or less). Because of the gnotobiotic study done in Japan (Suzuki et al., 1987) that showed ar environmental influence on the penetrance of diabetes in NOD/Shi males, it has been assumed that the difference between incidences of diabetes in male NOD/Shi and NOD/Lt mice is probably environmentally-mediated. Surprisingly, however, a direct comparison of diabetes incidence in both substrains maintained in a common environment by Dr. L. Herberg (Diabetes Research Institute, Dusseldorf, Germany) indicates that substrain divergence is occurring. By 28 weeks of age, 78 percent of male NOD/LtHl have diabetes (21 out of 27), compared with 14 percent (8 out of 22) of NOD/ShiHl males obtained from CLEA Japan, Inc. Similarly, 96 percent (25 out of 26) of NOD/LtHl females become diabetic compared with 65 percent (13 out of 20) NOD/ShiHl females obtained from CLEA Japan, Inc. (Dr. L. Herberg, personal communication). Although castration can increase diabetes incidence in NOD/Shi males, which have a low spontaneous incidence of IDDM (Makino et al., 1981), adolescent castration does not further elevate the relatively high spontaneous incidence observed in NOD/Lt mice (E. H. Leiter, unpublished). Thus, substrain as well as environmental differences must be considered when approaching the scientific literature on NOD mice.

Given the likelihood that inbred NOD colonies worldvide are accumulating independent genetic mutations that will eventually lead to substrain divergence, it is essential that inbred colonies be identified by a laboratory code. For example "Hl" is the laboratory code assigned to Dr. L. Herberg. These assignments are available upon request from Dr. Dorothy Greenhouse, Institute of Laboratory Animal Resources, National Research Council, 2101 Constitution Avenue, NW, Washington, DC 20418. Colony managers of inbred NOD mice and related strains are kindly requested to complete a colony registration/data form supplied by the World NOD Registry. Forms are available from Dr. Paolo Pozzilli, Department of Diabetes and Metabolism, St. Bartholomew's Hospital, West Smithfield, London EC1A 7BE, UK. Comparisons of worldwide diabetes incidence in the various NOD colonies will provide important insights into the genetic and environmental factors that, in aggregate, determine the development of diabetes.

ACKNOWLEDGEMENTS

This review was supported by grants from NIH DK 27722 and 36175, The Juvenile Diabetes Foundation International, and the Diabetes Research and Education Foundation. I would like to thank Dr. Lieselotte Herberg for permission to cite her unpublished substrain comparative incidence data.

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The LETL Rat: A Model for IDDM Without Lymphopenia

Takashi Natori and Kazuya Kawano

INTRODUCTION

Diabetes mellitus (DM) is a disease with several different pathogenetic origins. The heterogeneity of the origin of DM is also manifest in experimental animals, in which diabetes is linked in some with sex (NOD mice) and in others with lymphopenia [BBDP (formerly BB) rats]. Although of great interest, these characteristics are restricted to the respective animal models and do not reflect characteristics of human insulin-dependent diabetes mellitus (IDDM). The strain we describe here represents an animal model that more closely reflects the pathology of human IDDM.

FIGURE 1 The LETL rat (photo courtesy of the authors).

ESTABLISHMENT OF LETL STRAIN

The LETL strain originated from a few pairs of outbred Long-Evans rats purchased from Charles River Canada (St. Constant, Quebec) in 1982. During the course of establishing a breeding colony, some rats showed a sudden onset of polyurea and polydipsia and were determined to be diabetic by measuring glucose in the urine and blood glucose levels. Diabetic animals exhibited retarded growth and died within 30 days of the appearance of glycosuria. Unfortunately, diabetic females reproduced poorly, and males could reproduce only at an early stage of diabetes. Consequently, we attempted to establish an inbred strain by selectively breeding brothers in early-stage diabetes with their nondiabetic sisters. Nondiabetic brothers were also used occasionally to avoid increasing the incidence of diabetes to a level that would result in poor overall breeding performance of the colony. Brother × sister matings were continued in this manner for 7 years, maintaining an incidence of diabetes in less than 30 percent of the rats. In 1989, the strain, which was designated LETL (Long-Evans Tokushima Lean), reached the twentieth generation of selective inbreeding (Kawano et al., 1991b) (Figure 1). A nondiabetic line was also established by brother × sister matings of nondiabetic animals from the same parental stock. That line, the LETO (Long Evans Tokushima Otsuka), was used as the normal control for LETL. We have recently established from the same parental stock another diabetic line, called OLETF, that spontaneously develops long-term hyperglycemia and exhibits diabetic complications resembling those of human type II diabetes (Kawano et al., 1991a).

CLINICAL FEATURES

LETL rats display no characteristic clinical signs until the onset of glycosuria. Rats are observed daily for wetness of the bedding. For those rats with bedding wetter than those of the controls, glycosuria is confirmed by Ket-Diastix (Miles-Sankyo, Tokyo). Once glycosuria is confirmed, the rats' blood glucose, urinary glucose, and ketone levels are monitored every 4 weeks with a Glucose B test kit (Wako, Osaka, Japan) throughout their life spans. Onset of diabetic symptoms in LETL rats occurs abruptly between 8 and 20 weeks of age, with equal frequency and severity in both sexes. The mean age of diabetes onset after birth is 15.9 weeks in males and 14.1 weeks in females. Without insulin therapy, most affected rats die within 30 days after diabetes onset. Body weights decrease to 50 percent of prediabetes weight, urinary volumes increase from 10–20 g/day to 50–100 g/day, and water intake increases from 20–25 g/day to 100–150 g/day. A prominent clinical feature in LETL rats is an elevated plasma glucose level. The level is 6–9 mM before diabetes onset but increases to greater than 30mM after onset. Plasma insulin decreases abruptly at onset to

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below the lower limit of detection (8.7 pM). The urinary glucose level correlates well with that of plasma glucose. Urinary ketones are detected a few days after the onset of diabetes.

The average incidence of diabetes during the inbreeding process was approximately 21 percent in males and 15 percent in females. The incidence of diabetes depended on whether diabetic parents were used in mating. Of those animals with two diabetic parents, 64.2 percent (18/28) were diabetic, whereas of those with two nondiabetic parents, 13.7 percent (91/664) were diabetic. When diabetic males were mated with nondiabetic females, the incidence was 41.7 percent (71/170), whereas with the reverse mating (i.e., when diabetic females were mated with nondiabetic males), the incidence was 23.5 percent (4/17). In the LETO control line, no diabetic rats were found during the 20 generations of inbreeding.

HISTOPATHOLOGY

The histological appearance of the pancreas of an LETL rat is shown in Figure 2. A characteristic feature is lymphocyte infiltration into the peri-islet area and the islets. Lymphocyte infiltration appeared approximately 4–5 days before the onset of clinical diabetes in roughly half of the islets examined, then expanded to almost all islets during the clinical onset period. However, the insulitis gradually regressed after the onset of clinical diabetes. Lymphocytes were rarely found in atrophic islets more than a week after diabetes onset, and insulin-containing cells were not immunohistochemically detected in affected animals (Figure 3A). On the other hand, insulitis was still prominent and insulin-positive cells were detected in diabetic rats examined 2 days after onset of clinical diabetes (Figure 3B). The distribution of glucagon and somatostatin were normal. Insulitis was not observed in nondiabetic animals, including LETO control rats.

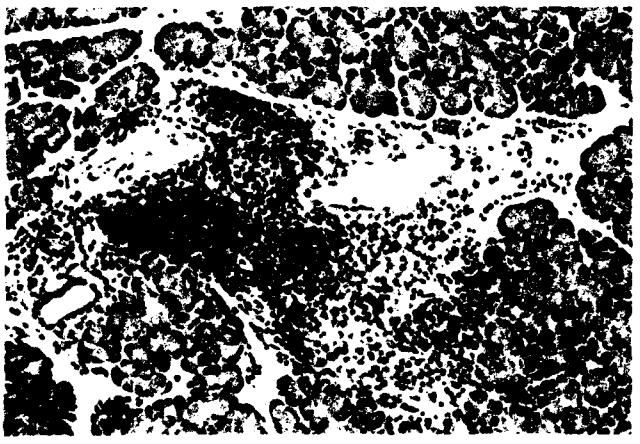

FIGURE 2 Histological appearance of the pancreas of an LETL rat. (Reprinted with permission from Kawano et al., 1991b, p. 1377. Copyright © 1991 by American Diabetes Association, Inc.)

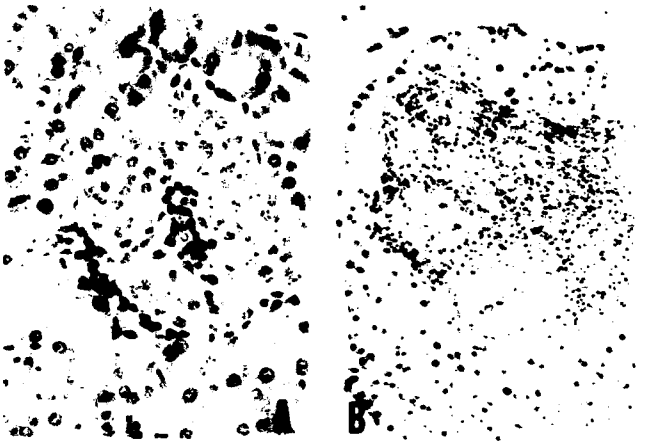

FIGURE 3 Immunohistochemical staining of insulin-containing cells (immunoperoxidase-hematoxylin staining). (a) Pancreatic islet cells of an affected rat (13 weeks old); (b) Pancreatic islets obtained 2 days after onset of clinical diabetes. (Reprinted with permission from Kawano et al., 1991b, p. 1378. Copyright © 1991 by American Diabetes Association, Inc.)

Lymphoid cell infiltration was observed in other organs, such as the salivary gland (21.4 percent) and the lacrimal glands (30 percent)—but not in the thyroid glands, reproductive organs, or thymus—in 559 25-week-old rats, of which 286 were female and 273 were male. At later stages of the disease, glycogen deposition was observed in the kidney tubules, but no glomerular lesions were seen. Lymphocyte subsets in the peripheral blood and the spleen were analyzed in a fluorescence-activated cell sorter (FACS; Spectrum III, Ortho Westwood, Massachusetts) with monoclonal antibodies OX6, OX8, OX33, and OX19 (Table 1). The proportion of pan T lymphocytes was 37 percent in diabetic LETL rats, 45.7 percent in nondiabetic LETL rats, and 42 percent in control LETO rats. The proportion of helper to cytotoxic/suppressor T lymphocytes followed a similar pattern—proportions were lower in diabetic LETL rats than in nondiabetic or control LETO rats—although the differences were not statistically significant. No significant differences in the numbers of splenic lymphocytes were found in the three groups.

GENETICS

The general genetic profile of the LETL strain was reported before, and was compared to the LETO strain (Kawano et al., 1989). The two strains appear to share the same haplotype at the 22 loci that were tested. Both strains carry *RT1*^{u}. The frequencies of insulitis in F1, F2, and backcross progenies are shown in Table 2. No insulitis was found in any F1 hybrids [(F344 × LETL)F1 and (WKAH × LETL)F1]. In [(F344 × LETL)F1 × (F344 × LETL)F1]F2 animals, insulitis was found in 5.4 percent of the offspring, whereas in [(WKAH × LETL)F1 × (WKAH × LETL)F1]F2 animals, the frequency was 3.3 percent. All F2 rats with insulitis (7/130) appeared to carry ho-

TABLE 1 Lymphocyte subsets in LETO, nondiabetic LETL, and diabetic LETL rats

Source/strain	*n*	Ia(OX6[a])	B lymphocytes (OX33[a])	Pan T lymphocytes (OX19[a])	Helper (OX19[a]/OX8[-])	Cytotoxic/suppressor (OX19[a]/OX8[a])
Peripheral blood lymphocytes						
LETO (control)	12	22.6±3.5	23.5±2.7	42.1±6.6	29.1±6.4	12.6±1.7
LETL (nondiabetic)	15	22.5±9.1	21.7±7.5	45.7±9.0	30.8±7.2	15.1±2.5[a]
LETL (diabetic)	13	32.7±6.5[a]	23.5±7.6	37.0±9.9	24.1±8.4	12.1±2.9
Splenic lymphocytes						
LETO (control)	12	40.6±3.2	25.9±3.0	35.3±5.1	25.0±5.4	10.5±2.3
LETL (nondiabetic)	15	49.2±9.6[a]	40.9±5.7[b]	34.9±7.1	23.3±4.1	11.3±4.8
LETL (diabetic)	13	51.4±8.3[b]	39.2±3.8[b]	37.0±5.8	27.6±5.3	9.3±3.8

All values are means ± SD. LETL, Long-Evans Tokushima Lean; LETO, Long-Evans Tokushima Otsuka; OX6, OX33, and OX19 were the monclonal antibodies used. (Reprinted with permission from Kawano et al., 1991b, p. 1379. Copyright © 1991 by American Diabetes Association, Inc.)

[a]P<0.01
[b]P<0.001, vs. LETO

mozygous *RT1[u]*. In the backcross progenies [(F344 × LETL)F1 × LETL], 26.3 percent of the animals developed insulitis, whereas in the [(WKAH × LETL)F1 × LETL] animals, the frequency was 6.6 percent. [(F344 × LETL)F1 × F344] animals appeared to have no insulitis. These results suggest that at least two recessive genes are involved in induction of insulitis, one of which is closely linked with *RT1[u]*. The clinical and pathological features of LETL rats are similar to those of BBDP rats, except that LETL rats do not develop T lymphopenia. The proportions of lymphocyte subsets in peripheral and spleen lymphocytes remained within the normal ranges (Table 1). In addition, the lymphocytes appeared to show normal functional reactivity against allogeneic stimulator cells (Table 3). These features contrast with those of BBDP rat lymphocytes, which are abnormal in quantity (Jackson et al., 1981; Elder and Maclaren, 1983; Woda et al., 1986) and function (Elder and Maclaren, 1983; Bellgrau et al., 1982; Greiner et al., 1986). Like et al. (1986) reported on a strain related to BBDP, called BBDR, that have no lymphopenia, and Guberski et al. (1991) have recently reported that the outbreak of diabetes is associated with Kilham's rat virus infection. It is likely that the lymphopenia itself in BBDR rats is not functionally associated with diabetes, although several reports suggest that it is in BBDP (Guttmann et al., 1983; Jackson et al., 1984; Georgiou et al., 1988). Thus, we have concluded that the LETL strain represents another model of IDDM without lymphopenia and shows no direct association between lymphopenia and diabetes.

Encephalomyocarditis virus (or enterovirus) and Coxsackie B4 virus cause insulitis and subsequently, IDDM in mice (Craighead and McLane, 1968; Burch et al., 1971); however, it is unlikely that infectious agents, such as viruses, are responsible for the IDDM of LETL rats. First, transfer of serum and supernatants of pancreas or spleen homogenates from diabetic LETL rats to naive young adult LETO or LEA/Otk rats does not cause diabetes (unpublished data). Second, the LETO control line has never developed diabetes, even though the animals are kept in the same room or, sometimes, in the same cage as LETL rats. However, we cannot exclude the possibility of involvement of Kilham's rat virus, as reported by Guberski et al. (1991).

TABLE 2 Mode of Inheritance of insulitis in LETL rats

Cross	*n*	Insulitis (*n*)	Insulitis frequency (%)
F_1(WKAH/Hkm × LETL)	30	0	0
F_1(F344/Ducrj × LETL)	43	0	0
F_2(WKAH/Hkm × LETL) × (WKAH/Hkm × LETL)	59	2	3.3
F_2(F344/Ducrj × LETL) × (WKAH/Hkm × LETL)	130	7	5.4
BC_1(WKAH/Hkm × LETL) × LETL	15	1	6.6
BC_1(F344/Ducrj × LETL) × LETL	19	5	26.3
BC_2(F344/Ducrj × LETL) × F344/Ducrj	30	0	0

BC=Backcross
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TABLE 3 Mixed lymphocyte culture responses of LETO, nondiabetic LETL, and diabetic LETL rats

	Stimulator						
Responder	LETO	LETL (non DM)	LETL (DM)	F344 (l)	ACI/N (a)	WM (u)	BN/Sea (n)
LETO (control)	4302±1192 (1.0)	3597±1101 (0.8)	6441±1041 (1.5)	88111±20884 (20.5)	106684±24619 (24.8)	2951±881 (0.7)	148456±25879 (34.5)
LETL (non DM)	6790±1479 (1.0)	6965±1358 (1.0)	5089±1891 (0.7)	56771±9848 (8.2)	47422±8584 (6.8)	6666±1154 (1.0)	52247±10098 (7.5)
LETL (DM)	5525±639 (1.0)	5714±672 (1.0)	5686±1984 (1.0)	60685±8865 (10.7)	73155±1899 (12.9)	4916±803 (0.9)	105017±8883 (18.5)

Values are mean ± SD (n=4) for cpm of [^{3}H]thymidine incorporation in triplicate cultures with stimulation index in parentheses. DM=Diabetic. (Reprinted with permission from Kawano et al., 1991b, p. 1379. Copyright © 1991 by American Diabetes Association, Inc.

It is important to note that LETL rats carry the *RT1^u* haplotype, as do BBDP rats. Results of mixed lymphocyte reaction (MLR) and restriction-fragment length polymorphism (RFLP) (Kawano et al., 1991b) analyses clearly show that LETL rats carry the *u* haplotype for the class II region of *RT1*. This finding is consistent with the results of previous serological typing (unpublished data). In addition, we have reported that the *RT1.A* haplotype of LETL rats is *u* (Kawano et al., 1989). Therefore, the results taken together indicate that LETL rats carry *RT1.A^{u}H^{u}B^{u}D^u*. Results obtained so far indicate no substantial difference in the *u* haplotype of LETL (with or without diabetes) and LETO (control line) rats. However, we cannot exclude the possibility that a few substitutions of base pairs exist in sequences of the class II genes that do not affect the RFLP patterns or serological or MLR reactivity, as demonstrated in NOD mice.

SUMMARY

Spontaneously diabetic rats with polyuria, polyphagia, and polydipsia were discovered in 1983 in an outbred colony of Long-Evans rats purchased from Charles River Canada in 1982. The diabetic LETL strain was bred from these rats. The characteristic features of the disease in LETL rats are:

- sudden onset of polyuria, polyphagia, hyperglycemia, and weight loss;
- no sex differences in the incidence or severity;
- lymphocyte infiltration into the islets, followed by destruction of beta cells and disappearance of lymphocytes at the onset of diabetes;
- no significant T lymphocytopenia;
- lymphocyte infiltration into the salivary glands and lacrimal glands; and
- involvement of at least two recessive genes in the pathogenesis of insulitis, one of which is closely linked with *RT1^u*.

These characteristics closely resemble those of human IDDM.

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Ethical Issues Involved in the Development of Animal Models for Type I Diabetes

Frederick E. Sieber and Richard J. Traystman

Numerous animal models have been developed to mimic human disease states. The underlying assumption in using these animal models in medical research is that they will provide additional knowledge about and insight into disease processes and, hopefully, better methods for treatment or prevention of diseases in humans. Mounting pressure from both the scientific and lay communities makes it necessary for researchers to clearly articulate these assumptions in all animal research. This is most challenging when designing and testing new animal models. All scientific research involving the use of animals should begin with an ethical focus by examining the risk-benefit ratio (i.e., morbidity or mortality to the animal versus the potential importance of the knowledge acquired). Simply stated, if animals are to be used as models of human diseases a clear understanding of the ethical standards that apply should be established. The aim of this paper is to discuss these ethical aspects within the context of research on type I diabetes, using scientific evidence as a framework for the decision-making process.

Wessler defines an animal model as "a living organism with an inherited, naturally acquired, or induced pathological process that in one or more respects closely resembles the same phenomenon occurring in man" (Wessler, 1976). He emphasizes that animal models offer only approximations of human disease and can never actually duplicate the same process. In attempting to approximate type I diabetes in animals, both spontaneous and induced models have been developed. The means by which the disease is induced or how it develops in an animal is important in determining how the model is to be studied.

The first step in the scientific method is a clear statement of the hypothesis, which in medical research originates from the human side of the equation. This hypothesis may involve a clinical question or pathophysiologic mechanism that cannot be tested ethically or appropriately using human subjects or alternative methods. The hypothesis is brought to the laboratory, where an appropriate animal model is chosen. As outlined in the following section, several animal models of type I diabetes are currently available. One must evaluate the appropriate use of these models to answer the hypothesis based on our original contention that clinical relevance and the risk-benefit of expanded knowledge and animal welfare is weighed carefully. It is our contention that these ethical issues must be addressed before the project begins and adhered to throughout the project.

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DIABETIC ANIMAL MODELS AND THEIR USES

Induced Diabetes

Diabetes can be induced by pharmacologic or surgical means. The classic surgical model of type I diabetes is total pancreatectomy, and the animal of choice is the dog, because total pancreatectomy is technically easiest in this species (Sarr, 1988). The advantages of this model include large size, which enables taking multiple tissue samples, frequent blood sampling, and more easily managing insulin regimens; and ease of handling, thereby making long-term care easier (although more expensive). The diabetes obtained is highly reproducible, and in several species, microangiopathy similar to that observed in human type I diabetes develops in both the eyes and kidneys. This model was used by Banting and Best in their studies leading to the discovery of insulin and the mechanism of diabetes. It is also of benefit in the study of diabetic retinopathy (Gelatt et al., 1979) and in chronic studies on tight versus loose control of blood glucose level and the effects of diabetes on end-organ vascular disease. This model has been used to validate various insulin delivery systems, as well as to test various insulin

preparations (Goriya et al., 1978) and combinations thereof. This model has validated the use of the artificial pancreas and led to clinical trials of the artificial pancreas in humans (Grau and Saudek, 1987). In addition, various types of artificial pancreatic implants and the biohybrid artificial implantable pancreas have been tested using this model (Sullivan et al., 1991).

There are, however, some disadvantages to total pancreatectomy, including the following: 1) the procedure requires major surgery, adequate postoperative analgesia, and good postoperative care with administration of antibiotics; 2) following pancreatectomy, the animal has little if any counterregulatory response to hypoglycemia; and 3) chronic pancreatic enzyme supplementation is required. These factors must be taken into account in designing a study using this model.

Type I diabetes may be pharmacologically induced via a number of agents that selectively destroy pancreatic β cells. Streptozotocin and alloxan are the most commonly used drugs. In contrast to total pancreatectomy, the use of these chemical agents leaves the remainder of pancreatic function intact. Pharmacologic induction of diabetes has been performed in many species including nonhuman primates, dogs, cats, rabbits, and rodents. The advantages of this model include the following: 1) the use of these agents allows the investigator to study a reproducible type of diabetes in a variety of species; 2) investigators are able to use smaller animal models for economy of care; 3) many of the end-organ effects of diabetes occur with this model. Disadvantages of pharmacologic induction of diabetes are that the drugs used can be toxic to other organ systems and the response to the drugs can be variable. Models using streptozotocin-induced type I diabetes have been useful in studying the effects of tight versus loose control of blood glucose level on the development of diabetic retinopathy (Engerman and Kern, 1987), examining changes in kidney basement membrane associated with the development of diabetic nephropathy (Bloodworth and Engerman, 1980), the effects of diabetic glucose control on the vasculature and endothelium (Engerman et al., 1977), and validating pancreatic transplant techniques (Barker et al., 1982). Pharmacologically induced models of type I diabetes have been invaluable in advancing knowledge about the pathology of diabetes (e.g., gaining basic information about the impairment of endothelial-dependent dilation of arterioles), and treating this disease (e.g., development of surgical techniques for placement of programmable insulin pumps and the development of therapeutic modalities, such as supplementation of the diet with myo-inositol, for treatment of neuropathy).

Spontaneous Type I Diabetes

There are several spontaneous models of type I diabetes, two of which have been extensively studied: the BBDP rat and the NOD mouse. The diabetic syndrome in BBDP rats is highly reproducible (>50 percent incidence) and closely resembles insulin-dependent, ketosis-prone type I diabetes mellitus in humans. As in human type I diabetes, the syndrome in BBDP rats is characterized by hyperglycemia, lymphocytic insulitis, and the presence of antibodies to islet cell-surface molecules. A major distinguishing feature of diabetic BBDP rats is a genetically transmitted, lifelong lymphopenia characterized by elevated numbers of natural killer cells and depressed numbers of T-helper and T-suppressor cells. The onset of diabetes in BBDP rats is from 60–120 days of age and is associated with lymphocytic insulitis and destruction of pancreatic β cells. It is believed that the development of diabetes in the model is secondary to a cell-mediated autoimmune process and may have implications for the pathophysiology of type I diabetes in humans. In addition to diabetes, some BBDP-related lines develop an autoimmune thyroiditis. This model has been useful in determining several genetic markers of diabetes, studying the immunologic mechanisms of diabetes, and developing cyclosporin therapy for use in early type I diabetes in humans (Stiller et al., 1984). In addition, BBDP rats are prone to several of the long-term complications of diabetes; in particular, the neuropathy and retinopathy that occur in this model are very similar to complications in human diabetics (Marliss et al., 1982).

NOD mice develop an autoimmune lesion involving lymphocytic infiltration and destruction of the pancreatic β cells, which leads to hypoinsulinemia, hyperglycemia, ketoacidosis, and death. These mice are particularly well suited for genetic and immunologic studies, as well as for research on environmental factors that influence the expression of diabetes. Important insights into transplantation techniques (Ikehara et al., 1985; Lum et al., 1991) and the role of lymphotrophic viral infection in preventing the development of diabetes have been achieved using this model (Oldstone, 1988). A more complete discussion of the NOD mouse is found elsewhere in this journal (pp. 15).

ETHICS OF DEVELOPING AND UTILIZING ANIMAL MODELS OF TYPE I DIABETES

Animal models of type I diabetes have clearly provided benefits to humans, including the discovery of insulin, better treatment of ocular and vascular complications, development of pancreatic transplantation techniques, and increased understanding of the immune basis of juvenile onset diabetes (Council on Scientific Affairs, 1989). There are many other areas of investigation, such as those examining diabetic-induced changes in cellular polyol metabolism or $Na\text{-}K^+$ ATPase activity, in which the results cannot be directly applied to therapeutic interventions in humans. However, advances in biomedical sciences of-

en come from combining results of experimentation at ll levels, from the molecular to the clinical. The model elected should depend on the hypothesis and the techni- al requirements. For instance, larger animal models nay be needed for studies requiring numerous proce- lures, including blood collection, tissue biopsy, and sur- gical implantation of catheters and mini-pumps. Smaller nimal models that have high rates of reproduction might e helpful in studies on the effects of genetics and the nvironment on the expression of diabetes.

In the development of type I diabetic animal models it s important to justify the research objectives by care- ully answering the five vital questions posed by Lane- etter (1972), as delineated below. The principles dis- ussed in these five questions have been incorporated nto guidelines for animal protocol review and ethical ssessments of animal experiments (Prentice et al., 1990; orter, 1992) as well as into policy statements of the merican Diabetes Association on the responsible use of nimals (ADA, 1985). Others have discussed these con- epts within the context of the "three R's": replacement, eduction, and refinement (Rowan, 1979). The five questions or the researcher to consider are:

1. Is an animal the best experimental system for the roblem? It is important to carefully evaluate the possi- ilities and ethics of answering the research question, ither in humans or by alternative means. With type I iabetes, we are studying a disease and its end-organ ffects, a situation that virtually eliminates alternative methods of research. The possibilities afforded by clini- al research depend on the questions addressed. On the ther hand, no model of diabetes can accurately and ompletely reproduce the human syndrome. It must be determined if the particular animal model in question will provide new insight into the problem studied. Therefore, one must assess an animal model on the basis of species appropriateness, ability to accurately reproduce the le- sion to be studied, its reproducibility from one animal to another, ability of the animal to survive, and simplicity and versatility of the model (Held, 1983).

2. Must the animal be conscious at any time during the experiment? The study and development of models of type I diabetes are chronic experiments that require con- scious animals.

3. Can pain or discomfort of the procedure be less- ened or alleviated? Both animals and humans share cer- tain capacities for pain and suffering. The investigator who uses animals has both a legal and moral obligation to ensure that the pain and suffering of experimental subjects is minimized and that the experimental methods are continually refined and improved. In chronic experi- ments, such as those involving diabetes, anesthesia is not an option. In developing models of diabetes, one is inducing a serious metabolic disease in an organism. If the animal is allowed to remain diabetic for a protracted period, the end-organ effects of the disease may occur, including retinopathy, microangiopathy, and neuropathy. Investigators must evaluate the experimental needs and endpoint of the study to eliminate as much of the suffer- ing incurred with this disease state as possible. Investi- gators must closely monitor the animals and treat their metabolic disease as is appropriate to the research goals of the protocol. The effects of stress on the animal caused by continual blood glucose measurements or ad- ministration of insulin is undoubtedly species-dependent and must be assessed. In addition, investigators must ask themselves if progression of the disease is absolutely required to meet the research objectives and, if so, how far it is necessary to allow the disease to progress.

4. Could the number of animals used be reduced? Chronic animal studies should be designed for maximum efficiency to obtain the highest possible rate of success and the maximum amount of information from each ex- perimental subject. New investigators to the field should seek advice and counsel from both the institutional ani- mal care and use committee and experienced researchers in the field.

5. Is the problem worth solving? Of all the ethical questions proposed by Lane-Petter, it is our opinion that this is the most important and one that has been asked many times in the lay press and by animal-rights activ- ists. If the hypothesis is clearly articulated as a relevant problem, the benefit of developing such a model is con- firmed. The risk to the animal must then be minimized to obtain the best model to test the hypothesis.

Hoff (1980) asserts that animals should not be used in experiments when substantial benefits, defined as the saving of human lives or the provision of a substantial contribution to human welfare, are not expected to result. This definition, however, is too narrow. Biomedical research in which animals are used does not always pro- vide an immediate benefit to humans. Merely seeking to understand the mechanisms by which biological systems operate is important and may eventually lead to better treatment of human ailments. This is often impossible to predict when developing animal models of human dis- ease. However, if the research hypothesis has met the relevant criterion, the development of an animal model to test the hypothesis is justified. What are the relevant, unanswered questions in diabetes research? Significant morbidity and mortality are the hallmarks of this chronic disease that affects six percent of the American popula- tion. For instance, diabetics have twice the risk of devel- oping heart disease and hypertension as nondiabetics. Diabetes is the second leading cause of blindness and one of the leading causes of renal insufficiency, unques- tionably providing a rationale for using animal models to study the disease. The research questions that arise from this chronic illness encompass such fields as genetics, immunology, nephrology, ophthalmology, cardiovascu- lar disease and stroke. There is no doubt that the devel- opment of animal models of type I diabetes has improved

treatment and provided a better understanding of this disease.

Animal research and the development of animal models of human disease will continue to make important contributions to the treatment of type I diabetes in both animals and humans. Scientists today are compelled to address both their peers and their critics by formally answering the questions posed by Lane-Petter (1972) and strictly adhering to the scientific method in formulating and testing a research hypothesis. Clearly, the development and use of many different animal models to study type I diabetes can stand up to both scientific and lay scrutiny.

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In the News

ILAR Begins Report on Occupational Safety and Health in Research Animal Facilities

The Institute of Laboratory Animal Resources (ILAR) has appointed a committee that will develop guidelines for research animal facilities to develop occupational safety and health programs for their personnel. Institutions using animals in research are required by the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* to have an occupational health and safety program, although no readily accessible recommendations exist for what these programs should include. In response to this requirement and to regulations of the Occupational Safety and Health Administration and other federal agencies, this report will address a wide range of topics, from the ethical and legal aspects of pre-employment discussions to zoonoses. It will provide advice on species-specific strategies and procedures to reduce risk of infection or accident, and it will make recommendations about immunization schedules, serum testing and banking, and prevention of allergies. Special consideration will be given to precautions for people who could potentially be exposed to naturally occurring zoonoses (such as simian B-virus, hepatitis, tuberculosis, and Q fever) and to experimentally induced biohazards (such as transgenic mice carrying the AIDS virus and radioisotopes used for diagnoses or research in animals). The report, which will be published by the National Academy Press, will assemble in one place comprehensive legal requirements and recommended procedures for developing and implementing occupational safety and health programs. Committee members will present a major part of the scientific program at the 1994 American College of Laboratory Animal Medicine forum on occupational safety and health. The committee anticipates that the report should be available in early 1995.

Committee on Occupational Safety and Health of Personnel in Research Animal Facilities: William Emmett Barkley, Ph.D. (chairman), Howard Hughes Medical Institute; Rebecca Bascom, M.D., University of Maryland, School of Medicine; Robert K. Bush, M.D., Allergy Section, William S. Middleton VA Hospital; Diane O. Fleming, Ph.D., Safety Consultant; Peter J. Gerone, Sc.D., Tulane Regional Primate Research Center; Janet C. Gonder, D.V.M., Ph.D., Baxter Healthcare Corporation; A. Wallace Hayes, Ph.D., Bowman Gray School of Medicine; Julia K. Hilliard, Ph.D., Southwest Foundation for Biomedical Research; Christian E. Newcomer, V.M.D., Tufts - New England Med. Center, Inc.; James H. Stewart, Ph.D., Harvard University; and Wayne R. Thomann, D.P.H., Duke University.

Report of the AVMA Panel on Euthanasia

The American Veterinary Medicine Association (AVMA) Panel on Euthanasia has revised its 1986 Panel Report on Euthanasia. The *1993 Report of the AVMA Panel on Euthanasia* [J. Am. Vet. Med. Assoc. 202(2):229-49, January 15, 1993] includes updated information on euthanasia of animals in research and animal care and control facilities, as well as expanded information on poikilothermic, aquatic, and fur-bearing animals. The panel added new information on horses and wildlife and deleted methods or agents considered unacceptable. The report is limited to those methods and agents supported by data from scientific studies. Reprints of the *1993 Report of the AVMA Panel on Euthanasia* are available from AVMA, Division of Scientific Activities, 1931 N. Meacham Road, Suite 100, Schaumburg, IL 60173-4360 (one copy available free to AVMA members; 50 cents each for additional copies and for non-AVMA members).

Primate Bibliographies Available

The Primate Information Center at the University of Washington issued ten new topical bibliographies in 1992. Topics include *Handedness, Cerebral Dominance and Brain Asymmetry in Nonhuman Primates*; *Aged Primate Learning and Behavior*; *Parkinson's Disease: Studies in Nonhuman Primates*; *and Tool Use by Nonhuman Primates*. The center has many more titles available from previous years and also will do custom searches on its 80,000-record data base. For the complete list of topics available, as well as for additional information about the Primate Information Center, contact the Primate Information Center, SJ-50, University of Washington, Seattle, WA 98195; Tel: 1-206-543-4376; Fax: 1-206-685-0305; Email: PLJ@U.WASHINGTON.EDU.

Coming Meetings

June 1993

8–11 FELASA Welfare and Science, Brighton, Sussex, England. This conference, organized by the Laboratory Animal Science Association (LASA) on behalf of the Federation of European Laboratory Animal Science Associations, will cover many aspects of human and animal welfare in laboratory animal science. Parallel sessions are being organized by the Universities Federation for Animal Welfare, the Royal Society for the Prevention of Cruelty to Animals, the British Laboratory Animals Veterinary Association, and the Institute of Animal Technology. For more information contact The Meeting Secretariat, LASA, P.O. Box 803, Sheffield, S10 2RS, United Kingdom. Fax: 44-742-796682.

9–11 Approaches to Design and Development of Cost Effective Laboratory Animal Facilities, Ottawa, Ontario, Canada. This symposium, hosted by the Canadian Council on Animal Care (CCAC), is the first in a series planned on animal facility design that will meet the humane and scientific imperatives of the twenty-first century. It will be valuable to those who are or may be involved in the planning, design, construction, or renovation of laboratory animal facilities. The symposium will cover such topics as programming (defining the need, projecting the need, site selection, and cost projection); process (selecting the team, delegating responsibility, monitoring); architectural design (layout, functional adjacencies, graphic tests, creativity, flexibility, equipment); and mechanical, electrical, and structural engineering. For more information contact CCAC, 1000-151 Slater, Ottawa, Ontario, Canada K1P 5H3. Tel: 1-613-238-4031; Fax: 1-613-238-2837.

14–15 Comparative Pathobiology of Lentiviral Infections, Bethesda, Maryland. The program will address the current understanding of the pathology and pathogenesis of lentiviral diseases in humans and animals. Speakers will discuss the historical importance of lentiviral infections, describe their clinical and pathologic features and host-virus interaction, and make comparisons among lentiviral infections. For more information contact The Registry of Comparative Pathology, Armed Forces Institute of Pathology, Washington, DC 20306-6000. Tel: 1-202-576-2452.

21–22 Ethical Issues of Animal Use in Academe and Industry, Philadelphia, Pennsylvania. The National Institutes of Health, Office for Protection from Research Risks is cosponsoring this animal welfare education workshop with Hahnemann University and Drexel University. The 2-day program will focus on investigator training, animal use in teaching, assessment of morbidity and endpoints, and allegations of noncompliance. The format will include panels on each of the topics and roundtable discussions with each of the panelists. A training session for new IACUC members will run concurrently with the breakout sessions. The workshop is open to institutional administrators, members of institutional animal care and use committees, laboratory animal veterinarians, investigators, and technicians, as well as anyone sharing responsibility for the management of a sound institutional animal care and use program. For more information contact Ms. Eleanore Hersh, Director of Continuing Education, Hahnemann University, Broad and Vine, Mail Stop 623, Philadelphia, PA 19102-1192. Tel: 1-215-762-8263; Fax: 1-215-762-8848.

September 1993

19–22 Eleventh Annual Symposium on Nonhuman Primate Models for AIDS, Madison, Wisconsin. This conference, hosted by the Wisconsin Regional Primate Research Center and the University of Wisconsin Medical School, will include scientific sessions on transmission epizootiology, vaccine development and testing, host factors in pathogenesis, viral factors in pathogenesis, and opportunistic pathogens and intervention strategies. For more information contact Symposium on NHP Models for AIDS, Marie Ellingson, Conference Services, Wisconsin Union, 800 Langdon Street, Madison, WI 53706. Tel: 1-608-262-2755; Fax: 1-608-262-5487.

November 1993

14–19 World Congress on Alternatives and Animal Use in the Life Sciences: Education, Research, Testing, Baltimore, Maryland. This congress will address issues of interest to an international audience of academic researchers, corporate scientists, government regulators, educators, and the interested public. It will cover scientific and ethical issues of alternatives and animal use in education, research, and testing. The congress will offer 5 days of lectures, workshops, and point/counterpoint sessions; scientific and commercial poster sessions; trade exhibitions and educational video screenings; and educational and scientific computer program demonstrations. For more information contact World Congress, 1101 14th Street, NW, Suite 1100, Washington, DC 20005-5601. Tel: 1-202-371-2200; Fax: 1-202-371-1090.

July 1994

10–14 Eighth International Workshop on Immunodeficient Animals, Utrecht, The Netherlands. The International Workshop on Immunodeficient Animals acts as a forum to bring together breeders and users of immunodeficient animals. It combines symposia on general themes in immunodeficient animal research and workshops on special topics. The preliminary list of topics for the symposia and workshops include immunodeficient mouse and rat mutants, spontaneous autoimmunity and allergy, experimentally induced autoimmunity or allergy, transgenesis associated with immunodeficiency, retrovirus infection, T-cell differentiation, autoimmunity, hypersensitivity, infectious diseases, oncogenesis, and molecular genetics. For more information contact P. de Vrey, Central Animal Laboratory, Pb43, National Institute of Public Health and Environmental Protection, P.O. Box 1, 3720 BA Bilthoven, The Netherlands. Tel: 31-30-742335; Fax: 31-30-252178.

New Books

Animal Care Committees: Role and Responsibilities—Canadian Council on Animal Care. This publication contains the proceedings of a workshop of the same title sponsored by the Canadian Council on Animal Care (CCAC) in Ottawa, February 10–11, 1992. As a feature of the workshop, participants were made members of simulated animal care committees (similar to U.S. institutional animal care and use committees) who deliberated and reviewed ten difficult-to-adjudicate protocols. Results were surprisingly consistent. Other subjects include ethical concerns regarding animals in the 1990s, how to deal with the media, the researcher's responsibility, the role of the community representative, personnel training, use of invertebrates as research animals, use of animals in field studies, and many other aspects of experimental animal care and use. CCAC, 1992, 122 pp., paperback, $12.00 U.S. ($15.00 Cdn.) ISBN 0-919087-16-17. (Available from CCAC, 1000-151 Slater Street, Ottawa, Ontario, Canada K1P 5H3. Tel: 1-613-238-4031; Fax: 1-613-238-2837.)

The Care and Use of Amphibians, Reptiles and Fish in Research—Dorcas O. Schaeffer, Kevin M. Kleinow, and Lee Krulisch, eds. The Scientists Center for Animal Welfare (SCAW), aided by a grant from the Animal Welfare Information Center, U.S. Department of Agriculture, has published these proceedings of a conference by the same name. The 2-day conference was held in New Orleans on April 8–9, 1991, where conference faculty and attendees exchanged information about the care and use of fish, amphibians, and reptiles. These species are used as important research models in many biomedical disciplines, yet little has been written about their humane and responsible care in a research situation. Part of the conference also focused on research that is being done for aquaculture and included areas in field research; housing and handling; and anesthesia, analgesia, and euthanasia for different fish species. SCAW, 1991, 196 pp., paperback, $55.00, Library of Congress Card Number 92-61834. (Available from SCAW, 4805 St. Elmo Avenue, Bethesda, MD 20814. Tel: 1-301-654-6390; Fax: 1-301-907-3993.)

Information Resources for Reptiles, Amphibians, Fish, and Cephalopods—Animal Welfare Information Center (AWIC), National Agricultural Library. This volume lists bibliographic references for current information about the care and use of reptiles, amphibians, fish, and cephalopods. The volume is categorized by animal group, including listings of books and articles pertaining to the group's use in biomedical research, as well as information about husbandry, maintenance, regulations, guidelines, and veterinary aspects such as appropriate anesthesia or euthanasia. The last section provides names and addresses of organizations that can provide further information. AWIC, 1992, 87 pp., paperback. (Available free-of-charge from AWIC, National Agricultural Library, 10301 Baltimore Boulevard, Beltsville, MD 20705. Tel: 1-301-504-6212, Fax: 1-301-504-5472.)

Experimental and Surgical Technique in the Rat, Second Edition—H.B. Waynforth and P.A. Flecknell. This manual, which first appeared 12 years ago, is a detailed, up-to-date compilation of illustrated experimental methods for surgical work with the rat. This new edition takes into account the greater appreciation of the well-being of experimental animals and includes references to a more humane and refined approach. Chapters include: administration of substances, methods of obtaining body fluids, anesthesia and post-operative care, surgical technique, specific surgical operations, miscellaneous techniques, and vital statistics and miscellaneous information. Academic Press, 1992, 382 pp., hardback, $69.95, ISBN 0-12-738851-6.

Publications Available

Single copies of the following publications are available without charge from the Institute of Laboratory Animal Resources, 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

Holders of Inbred and Mutant Mice in the United States. Including the Rules for Standardized Nomenclature of Inbred Strains, Gene Loci, and Biochemical Variants. 1984

Important Laboratory Animal Resources: Selection Criteria and Funding Mechanisms for their Preservation. 1990

International Standardized Nomenclature for Outbred Stocks of Laboratory Animals. ICLA bull. no. 30. 1972

Laboratory Animal Management: Cats. 1978

Laboratory Animal Management: Genetics. 1979

Laboratory Animal Management: Nonhuman Primates. 1980

Laboratory Animal Management: Rodents. 1977

Laboratory Animal Management: Wild Birds. 1977

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

Standardized Nomenclature for Transgenic Animals. 1993

Supplement to Animals for Research—A Directory of Sources. 10th ed. 1980

Third International Registry of Animal Models of Thrombosis and Hemorrhagic Diseases. 1988

Your Career in Veterinary Technology. AVMA Brochure. Updated Dec. 1989

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INSTITUTE OF LABORATORY ANIMAL RESOURCES

Volume 35, Number 2 Spring 1993 National Research Council

Models of Type I Diabetes—Part Two

Diabetes-Prone and Diabetes-Resistant BB Rats: Animal Models of Spontaneous and Virally Induced Diabetes Mellitus, Lymphocytic Thyroiditis, and Collagen-Induced Arthritis

Transgenic Models of Insulin-Dependent Diabetes Mellitus

A quarterly publication for biomedical investigators, laboratory animal scientists, institutional officials for research, and members of animal care and use committees.

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ILAR News is published quarterly by the Institute of Laboratory Animal Resources, National Research Council, 2101 Constitution Avenue, NW, Washington, DC 20418. It is circulated by request to investigators in the field of biomedical and related research.

Publication of this issue of *ILAR News* was supported by grants from the National Center for Research Resources, National Institutes of Health; National Science Foundation; American Cancer Society, Inc.; and U.S. Army Medical Research and Development Command, which is the lead agency for combined Department of Defense funding also received from the Human Systems Division, 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.

ILAR News ISSN 0018-9960
Volume 35, Number 2 Spring

ILAR NEWS

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CONTENTS

Models of Type I Diabetes—Part Two

Diabetes-Prone and Diabetes-Resistant BB Rats: Animal Models of Spontaneous and Virally Induced Diabetes Mellitus, Lymphocytic Thyroiditis, and Collagen-Induced Arthritis

Dennis L. Guberski

INTRODUCTION

In 1974, some rats (subsequently called BB) that spontaneously developed autoimmune diabetes mellitus were found in a closed colony of outbred WI (Wistar) rats at the Bio-Breeding Laboratories, Ottawa, Ontario. Since the first report by Nakhooda et al. (1977), colonies of BB rats have been established worldwide. The availability of the model enables investigation into fundamental questions concerning the spontaneous etiopathogenesis (Crisá et al., 1992) and virally induced mechanisms (Guberski et al., 1991) of diabetes. Complications of diabetes, including peripheral neuropathy (Greene et al., 1984; Yagihashi and Sima 1985a,b; Sima et al., 1986; Yagihashi et al., 1986; McEwen and Sima, 1987), impotence (Murray et al., 1985a,b), nephropathy (Chakrabarti et al., 1989; Cohen et al., 1987), and retinal changes (Sima et al., 1985; Chakrabarti et al., 1991), are also widely studied in this model.

This paper will provide an overview of the model, describing the development of the inbred rats, features of the diabetic syndrome, the immunopathology and mechanisms of immune suppression and immune modulation, and the interaction of genetics and the environment, as well as a brief review of recommended husbandry practices.

DEVELOPMENT OF INBRED DIABETIC RATS

In 1977, Butler et al. (1983, 1988, 1990) began inbreeding BB rats at the University of Massachusetts Medical Center (laboratory code Wor) with 300 breeders purchased from the Bio-Breeding Laboratories. In 1983, the National Institutes of Health established the Wor inbred colony as a reference colony.

The first priority was to develop an inbred model with good fecundity and a high incidence of diabetes. Diabetes was defined as positive glycosuria with a blood glucose concentration greater than 250 mg/dl. The incidence of diabetes was determined in breeders held through 150 days of age. Selection of siblings for breeding occurred after the onset of hyperglycemia. In 1978, between the third and fourth generation of inbreeding, an epidemic of *Mycoplasma pumonis* caused a substantial number of animals to die. As a result, a pathogen-free rodent barrier system was constructed for the colony. During caesarian rederivation, animals from litters with the highest incidence of diabetes were introduced into the barrier. Following the successful rederivation, genetic studies were undertaken to determine the mechanism of inheritance of diabetes. From these studies two groups of animals were produced. The first was comprised of the progeny of diabetic × diabetic matings, from which six diabetes prone (DP) strains were developed. The second was comprised of progeny selected to remain diabetes free, including the BBVB/Wor strain, a direct descendant from the original 300 breeders, and the BBDR/Wor strain derived from DP rats in the fifth generation of inbreeding. Characteristics of the DP and DR strains are listed in Table 1.

Genetic studies showed that a single diabetic × diabetic mating fixed the recessive diabetogenic genes. Subsequent progeny all had the same incidence of diabetes whether they were the offspring of a phenotypically diabetic × nondiabetic mating or a nondiabetic × nondiabetic mating (Butler et al., 1983). This revolutionized the breeding at the Wor facility. Instead of waiting until hyperglycemia occurred and then mating chronically diabetic rats, mating was now possible before the onset of diabetes, which substantially improved production. New selection criteria were also developed, including selecting for animals that developed hyperglycemia at an early age and came from large litters with a high incidence of diabetes. Animals with an early onset of hyperglycemia are difficult to breed (Murray et al., 1985b; Butler et al., 1988, 1990) due to the difficulty of controlling blood glucose in females during pregnancy and lactation and impotence in chronically diabetic males (Murray et al., 1985a,b). Selection criteria that reduces fertility can cause residual heterozygosity and delay the development of fully inbred lines (Hedrich, 1993). Because diabetes reduces pro-

Mr. Guberski is the BB/Wor project administrator at the University of Massachusetts Medical Center in Worcester.

TABLE 1 Characteristics of Diabetes Prone and Diabetes Resistant Rats

	Diabetes prone (DP) rat strains						Diabetes Resistant (DR) rat strains	
	BBBA	BBDP	BBBE	BBNA	BBNB	BBPA	BBDR	BBVB
Insulitis	+	+	+	+	+	+	–	–
Incidence of Diabetes (GEN 41-45)	87%	88%	89%	77%	83%	90%	0%	0%
Lymphopenia	+	+	+	+	+	+	–	–
RT6.1$^+$ T cells	–	–	–	–	–	–	+	+
T_{helper}	↓	↓	↓	↓	↓	↓	Normal	Normal
$T_{cytotoxic/suppressor}$	–	–	–	–	–	–	Normal	Normal
Thyroiditis	++	+/–	+	++	++	+	–	–
MHC *RT1^u*	+	+	+	+	+	+	+	+

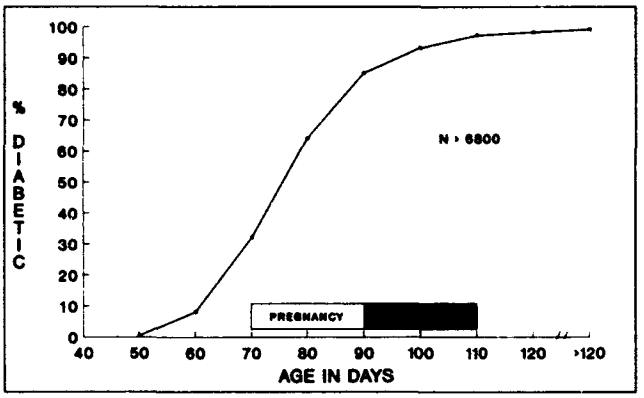

FIGURE 1 Age at onset of diabetes in diabetes-prone BB/Wor rats was determined by glycosuric testing animals 3 times per week. Diabetes was defined as 4+ glycosuria with subsequent sera glucose measurement greater than 250 mg%/(13.8mmol/L). Animals studied represent viral antibody-free breeders from all diabetes-prone lines from generations 37–48.

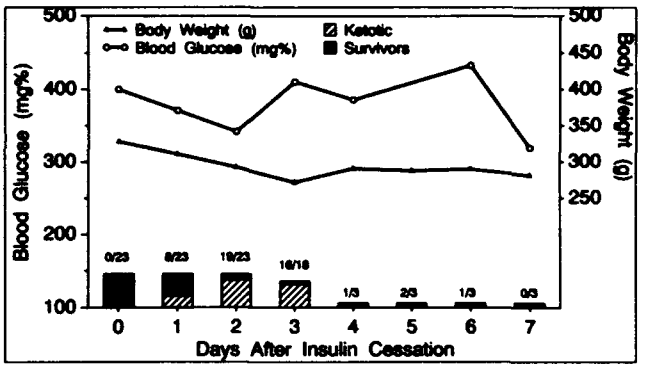

FIGURE 2 Clinical course of diabetes in 23 lean diabetic male rats after cessation of insulin therapy. Twenty out of twenty-three rats died in diabetic ketoacidosis within 4 days after cessation of insulin therapy.

ductivity in DP rats and the onset of the disease typically occurs during pregnancy or lactation (Figure 1), residual heterozygosity may still be present in the Wor reference colony. Sound management practice requires continuation of sibling matings.

DP strains are designated BBBA/Wor, BBDP/Wor, BBBE/Wor, BBNA/Wor, BBNB/Wor, and BBPA/Wor, and DR strains are designated BBDR/Wor and BBVB/Wor (Table 1). All strains have been inbred for more than 48 generations.

DIABETES IN DP RATS

DP rats develop a cell-mediated autoimmune destruction of the pancreatic β cells, resulting in an abrupt onset of insulin-dependent, ketosis-prone diabetes mellitus. The development of diabetes in DP strains occurs between 60 and 120 days of age in both males and females. The abrupt onset of disease is characterized by weight loss, polyuria, polydipsia, glycosuria, hyperglycemia, hypoinsulinemia, and ketosis. In the absence of exogenous insulin therapy, animals usually die in 4–7 days (Figure 2). The overall incidence of diabetes in DP strains is 86 percent, and the average age of onset is 80 days (Like et al., 1991). This peripubertal onset of diabetes is comparable to the peak onset observed in 12- to 14-year-old humans.

Insulitis

Insulitis is a term coined by Gepts (1965), who described mononuclear infiltration of the islets of Langerhans in the pancreas of a child who died with acute type I diabetes mellitus. DP rats develop an intense mononuclear insulitis that precedes the onset of disease by 2–3 weeks. Insulitis destroys only the pancreatic β cells (Figures 3–4), resulting in an islet devoid of insulin-positive cells (Figure 5); pancreatic alpha, delta, and polypeptide cells are not destroyed. Studies to determine which cells make up the insulitis lesion suggest that different lymphoid populations are present, depending on the stage of insulitis. The initial infiltrate is comprised of ED1$^+$ cells (macrophages/dendritic cells) (Kiesel et

al., 1986; Lee et al., 1988). Later, $CD4^+$, $CD8^+$, and natural killer (NK) cells are found. Recently Ellerman et al. (1993) and Shachner et al. (1992) demonstrated that NK cells are not the final effector cells for β-cell destruction. In situ studies have demonstrated the presence of TNF, IL-1, and IL-6 mRNA in islets of acutely diabetic DP rats (Jiang and Woda, 1991). Pancreatic lymphocytic infiltration (PLI) has also been reported to precede the insulitis lesion (Guttmann et al., 1983). However, PLI can also be found in animals that never become diabetic or demonstrate insulitis (Dr. Arthur A. Like, Personal Communication, University of Massachusetts Medical School, Worcester, Massachusetts).

IMMUNOPATHOLOGY

Lymphopenia

DP strains are severely lymphopenic (Elder and Maclaren, 1983), as manifested by a reduction in T lymphocytes in peripheral blood, spleen, and lymph nodes. Peripheral blood samples obtained from venous sinus punctures, after being sequentially incubated with anti-CD8 (OX8) and anti-CD5 (OX19), show a significantly lower than normal percentage of T-helper cells ($CD5^+/CD8^-$) and virtually no cytotoxic and suppressor ($CD5^+/CD8^+$) T cells. DP strains also have a slightly greater than normal percentage of NK cells ($CD8^+/CD5^-$). *DR strains are not lymphopenic* and have a normal number of T cells (Like et al., 1991).

RT6 System

RT6 is a T-cell differentiation alloantigen controlled by two alleles *$RT6^a$* and *$RT6^b$*. Those alleles make two products, RT6.1 and RT6.2, respectively, which are co-expressed in F1 progeny derived from mating a strain that is $RT6.1^+$ with a strain that is $RT6.2^+$ (Crisá et al., 1992). Although normally absent on thymocytes and bone marrow cells, these cell-surface markers can be demonstrated on approximately 50 percent of $CD4^+$ T-helper cells and 70 percent of $CD8^+$ T-cytotoxic/suppressor cells. Overall, 60 percent of peripheral T cells express an RT6 marker. Intraepithelial lymphocytes (IELs) also express RT6 cell markers (Fangman et al., 1991).

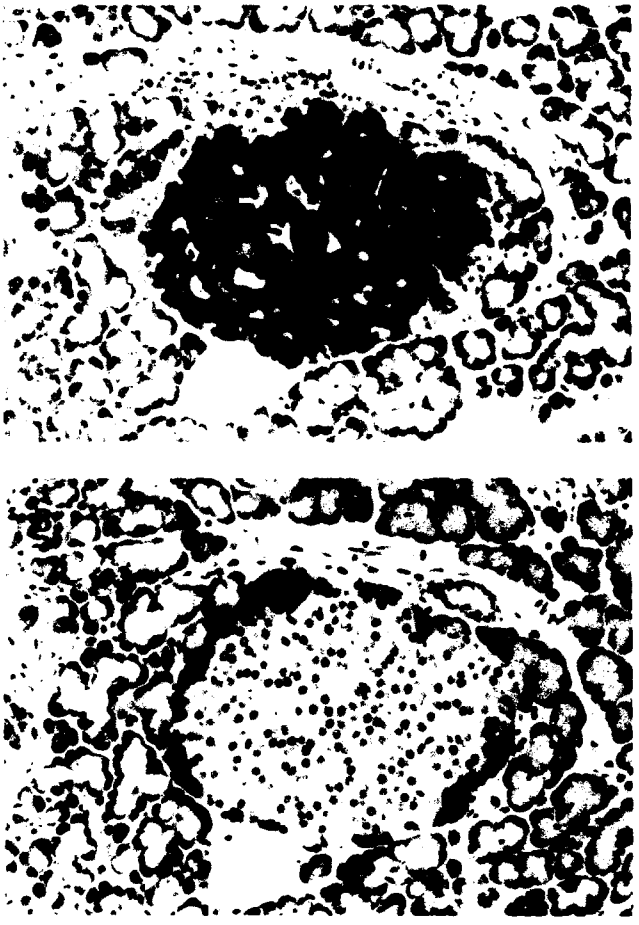

FIGURE 3 Normal islet from a BBDR/Wor rat. The centrally located β cells stain intensely for insulin (3A). Alpha cells located at the islets periphery stain positively for glucagon (3B).

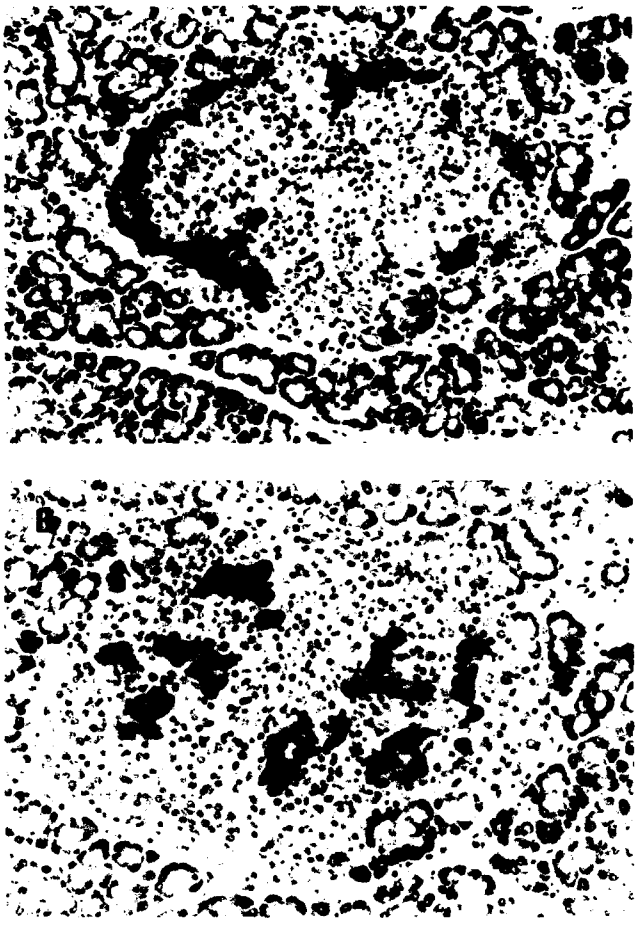

FIGURE 4 Islet from an acutely diabetic BB rat. Insulitis is widespread and is responsible for the destruction of the insulin producing β cells (4A). Glucagon positive cells (4B) are unaffected.

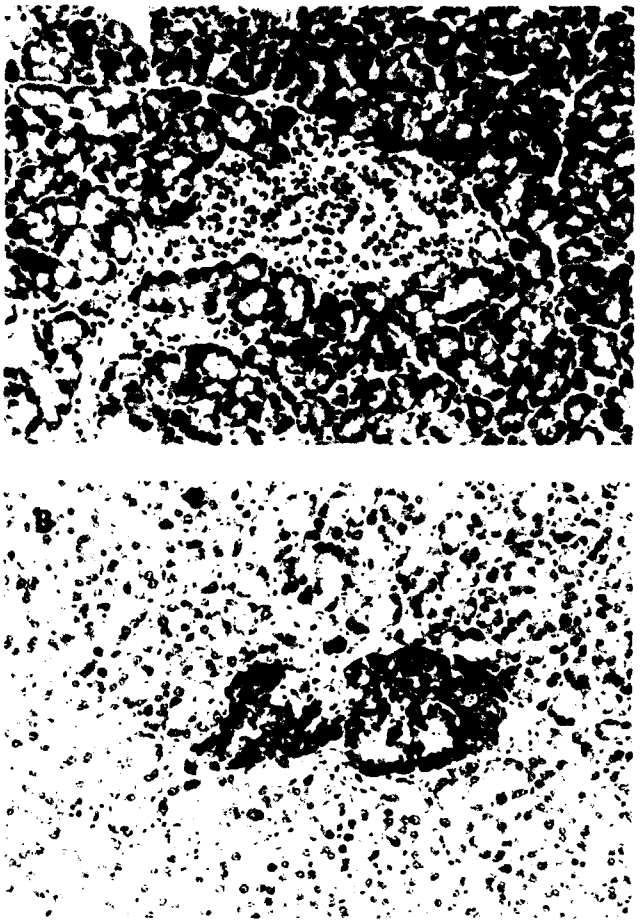

FIGURE 5 End stage islet from a chronically diabetic BB rat. Insulin positive β cells are absent (5A) due to autoimmune destruction of pancreatic β cells. Peripherally located β cells (5B) have clustered following the collapse of the centrally located β cell core.

T cells that express RT6 markers are believed to be immunoregulatory (Greiner et al., 1988). Although DP strains have a functional RT6^a allele (Crisá et al., 1993), they do not express RT6.1 antigen on peripheral T cells, on splenocytes, or in lymph nodes. These rats do, however, express RT6.1 markers on their IELs (Fangman et al., 1991). DR strains express RT6.1 antigen in peripheral T cells, splenocytes, and in lymph nodes (Like et al., 1991).

Other immune abnormalities

DP rats develop autoantibodies to smooth muscle, thyroid colloid, gastric parietal cells, and islet cell-surface markers (Elder et al., 1982; Like et al., 1982a; Pipeleers et al., 1987; Dean et al., 1986). The significance of the production of autoantibodies and its pathogenic consequences to diabetes remains unclear. However, humans with organ-specific autoimmune disease typically produce similar autoantibodies.

More than 90 percent of BBBA/Wor, BBNA/Wor, and BBNB/Wor rats develop lymphocytic thyroiditis (LT) by 150 days of age (Sternthal et al., 1981; Rajatanavin et al., 1991), and the severity of the lymphocytic infiltration is more pronounced in these strains. In addition, the administration of iodine to the drinking water induces LT at an increased frequency by 90 days of age (Allen et al., 1986).

The peripheral T-cell receptor β-chain variable region (V_β) repertoire fails to develop in DP rats (Gold and Bellgrau, 1991). That limited V_β repertoire could be due to a differential T-cell expansion or a selective deletion of T-cell receptors. The importance of the defect and its contribution to the pathogenesis of diabetes requires further study.

DR rats do not spontaneously develop autoantibodies, LT, or insulitis. DR rats are not lymphopenic, but they do harbor an effector-cell population that can be induced to become autoreactive T cells, resulting in diabetes or thyroiditis (Like, 1990).

The DR rat is also an excellent animal model for studying collagen-induced arthritis. When immunized with human type II collagen suspended in incomplete Freunds adjuvant, virtually all DR rats develop arthritis within 10 days (Watson et al., 1990). It is a good model for collagen-induced arthritis because the third hypervariable region of the RT1.D$^\beta$ gene in DR rats encodes a nucleotide sequence identical to the human disease response genes (called HLA DR) associated with rheumatoid arthritis (Watson et al., 1990).

GENETICS

In DP rats, susceptibility to diabetes is complex and requires the interaction of several genes. First, there is a close association of genes present in the major histocompatibility complex (MHC) encoding for the *RT1^u* haplotype on chromosome 20 (Jacob et al., 1992). The development of diabetes is thought to be independent of class I expression but requires the presence of at least one class II *RT1^u* allele (Fuks et al., 1990). Additional support for that hypothesis is derived from in vivo studies, which show that treatment of DP rats with a monoclonal antibody directed against class II RT1.D but not anti-RT1.B prevented diabetes (Boitard et al., 1985). Second, a recessive gene for lymphopenia, *lyp*, is permissive for diabetes and is tightly linked to the neuropeptide Y *Nyp* gene on chromosome 4 (Jacob et al., 1992). Lymphopenia segregates independently of the *RT1^u* haplotype, and studies show that *lyp* is an autosomal recessive gene (Guberski et al., 1989; Herold et al., 1989).

The relationship between lymphopenia and diabetes is also complex. While lymphopenia is permissive for diabetes, it is not an absolute requirement, because nonlymphopenic DR rats (BBDR/Wor and BBVB/Wor) can be induced to develop hyperglycemia by various means (Like et al., 1986a; Like, 1990). Other factors contributing to the diabetic phenotype include a dominant gene for PLI (called *Pli*) (Guttmann et al., 1983) and a proposed genetic factor that controls age at onset (Guberski et al., 1989). To date the use of biochemical and molecular markers have not identified a specific diabetogenic genotype (Kastern et al., 1984; Kryspin-Sorensen et al., 1986; Bjorck et al., 1986).

DR rats share the same MHC haplotype as DP rats but are not lymphopenic (Like et al., 1986a). If maintained in a pathogen-free environment, DR rats do not become diabetic. Since the rederivation of the strains in July 1989, more than ten thousand DR rats have been studied, and no diabetes has been detected. DR rats harbor effector cells capable of causing diabetes, however, and hyperglycemia can be induced by injection of polyinosinicpolycytidylic acid (usually called POLY-IC) (Sobel et al., 1992), x irradiation (Handler et al., 1989; Rossini et al., 1984a), and exposure to or injection of Kilham rat virus (KRV) (Guberski et al., 1991). RT6 depletion of conventionally housed DR rats can also induce diabetes (Greiner et al., 1987).

ENVIRONMENT

The diabetic phenotype is a combination of an animal's genetic predisposition and environmental factors that determine the penetrance of the diabetes genes. In inbred colonies of DP rats the genetic predisposition towards development of diabetes is uniform, but not all of the rats develop diabetes. Further, the incidence of diabetes varies among research institutions. One must therefore consider environmental factors, including viruses, bacteria, and diets.

Viruses

When the BB/Wor colony was rederived in 1989 by caesarian derivation, environmental pathogens were eliminated, causing significant changes in how many animals developed diabetes and at what age Prior to rederivation, Sendai virus and sialodacryoadenitis virus were ubiquitous in the DP and DR colonies. The animals were seronegative for *Mycoplasma pumonis* and intermittently seropositive for KRV. The incidence of diabetes in DP rats was 70 percent, and the average age at onset was 91 days. Seven percent developed diabetes before 72 days of age, 74 percent between 72 and 102 days, and 19 percent after 103 days. After the viruses were eliminated, the incidence of diabetes increased from 70 percent to 86 percent, and the average age at onset was now 80 days. In addition, the distribution of ages at onset of diabetes was significantly earlier: 32 percent developed diabetes before 72 days of age, 62 percent between 72 and 102 days of age, and only 6 percent developed diabetes after 103 days. These studies (Like et al., 1991) suggest that viruses may modulate the immune system of DP rats by interfering with the genetically programmed process of β-cell destruction. Additional support for the hypothesis of viral immune modulation is the observation that injecting lymphocytic choriomeningitis virus into young DP rats prevents diabetes (Dyrberg et al., 1988). Finally, Sadelain et al. (1990) has demonstrated that complete Freund's adjuvent (CFA) administered to young DP rats prevents diabetes, which suggests that CFA-induced cytokine release regulates immune responses. A review on cytokine regulation has recently been published (Rabinovitch, 1993).

Conversely, the incidence of spontaneous diabetes in DR rats was less than three percent before the elimination of environmental pathogens. Two spontaneous outbreaks of diabetes in DR rats were recorded, the first from 1984–1986 (Like et al., 1986a) and the second from 1988–1989 (Thomas et al., 1991; Sadelain et al., 1990). Following the first observations of diabetes in the DR population, breeding studies ruled out a genetic basis for nonlymphopenic diabetes. First, mating nonlymphopenic diabetic rats with normal DR rats did not increase the incidence of nonlymphopenic diabetes in the progeny. Second, matings in which both parents were nonlymphopenic and diabetic failed to produce diabetic offspring (Guberski et al., 1989). Retrospectively, the spontaneous outbreak was linked to the presence of KRV, a rodent parvovirus (Guberski et al., 1991). During the second outbreak of diabetes in the DR population, a virus, later determined to be KRV by sequencing of nonstructural protein (Brown et al., 1993), was isolated from the tissues of a diabetic DR rat. That virus, when injected into 21- to 25-day-old DR rats, induced diabetes and insulitis. Unlike other viruses that induce diabetes, KRV cannot be demonstrated in the pancreatic islets by immunohistochemistry or in situ hybridization techniques (Guberski et al., 1991; Brown et al., 1993). When KRV is present, it is also permissive for induction of diabetes in DR rats following depletion of RT6.1$^+$ T cells (Like, 1990). Injecting KRV into young DP rats fails to induce diabetes unless their immune systems have been reconstituted with splenocyte injections from DR rats. Similarly, immune reconstitution of DP rats with WF (Wistar Furth) splenocytes followed by KRV injection fails to induce diabetes (Guberski et al., 1991). These studies suggest that the immune system is being modified by the virus, possibly by viral molecular mimicry. Viral molecular mimicry could induce the pancreatic β cells to express a viral protein on the surface, thereby attracting immunocytes to destroy the "non-self" tissue. Another possibility is that KRV has a direct effect on the immune system, destroying the delicate balance between autoreactive effector and regulatory T cells. This mechanism could occur by either a virally induced stimulation of effector cells or a virally induced suppression of regulatory T cells. The hypothesis that the DR immune system is affected by KRV is derived from the following observations: 1) KRV injected into 21- to 25-day-old DP rats fails to induce diabetes; 2) DP rats whose immune systems are reconstituted by injections of splenocytes from either DR or WF rats do not become diabetic; 3) DP rats whose immune systems are reconstituted with splenocytes from DR rats and are subsequently injected with KRV become diabetic; and 4) DP rats whose immune systems are reconstituted with splenocytes from WF rats and subsequently injected with KRV do not become diabetic. These data clearly illustrate that KRV-induced diabetes requires a DR immune system since the target DP β cells were constant during these experiments (Guberski et al., 1991; Ellerman et al., 1992).

Bacteria

The concept that environmental pathogens or their extracellular products may initiate autoimmunity was recently tested by Ellerman and Like (1992). In these experiments staphylococcal entertoxins were used to stimulate RT6.1-depleted splenocytes in DR rats. Those stimulated spleen cells were effective in adoptively transferring diabetes, which clearly demonstrates that microbes are capable of activating autoreactive T cells, at least in vitro. Since approximately one-third of clinical isolates of *Staphylococcus aureus* produce enterotoxins, this ubiquitous pathogen may initiate an autoimmune process in genetically susceptible individuals.

Diets

The impacts of diet on development of diabetes have been studied extensively (Scott et al., 1985a,b; Behrens et al., 1986; Scott et al., 1988a,b; Scott and Marliss, 1991). Laboratory diets that utilize plant products, such as wheat gluten flour and soybean meal, are diabetes permissive. Semisynthetic diets rich in L-amino acids and hydrolyzed casein diets reduce the incidence and delay the onset of diabetes (Scott et al., 1988a), which may be caused directly by diet or indirectly by chemicals or microbiologic agents associated with the source of protein (Scott and Marliss, 1991).

TYPE I DIABETES, AN AUTOIMMUNE DISEASE

An immunopathogenesis for diabetes is suggested by the presence of insulitis in the pancreatic islets at the onset of hyperglycemia and by the ability to adoptively transfer disease by injecting spleen cells from diabetic rats into normal rats (Koevary et al., 1983). Cloned T-cell lines from diabetic rats are also capable of initiating β-cell cytotoxicity (Reich et al., 1993). Diabetes can be prevented by neonatal thymectomy (Like et al., 1982b), injection of antilymphocyte sera (Like et al., 1979), and a variety of other immune modulatory techniques (Like et al., 1986b; Like et al., 1984; Rossini et al., 1984b). Finally, recent studies have shown that diabetes and insulitis can be prevented by transplanting islet cells from DP or DR rats into the thymus of neonatal DP rats, which suggests that establishing a state of tolerance prevents autoimmune destruction of pancreatic β cells (Koevary and Blomberg, 1992; Posselt et al., 1992).

HUSBANDRY

Housing

DP and DR rats should be maintained under pathogen-free conditions, such as in laminar-flow hoods, isolator cages, or barrier rooms, to avoid the impact of environmental pathogens (Like et al., 1991).

Detection and Clinical Care of Diabetes

Testing for glycosuria is the most efficient, cost-effective method to screen rats for diabetes. Urine is expressed manually from the bladder by pelvic compression and tested with Testape (Eli Lilly, Indianapolis, Indiana). A blood sample, which can be obtained by nicking the tip of the tail with a razor blade, should be taken within 2 hours of a positive test for glucosuria to ascertain blood sugar. Animals whose blood glucose concentrations exceed 250 mg/dl are considered diabetic and require insulin therapy. Testing for glycosuria should begin before the expected onset of diabetes and be performed at least three times each week at the start of the light cycle.

Insulin therapy. Daily treatment of diabetic rats with insulin is essential for their survival 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, presence of ketoacidosis and dehydration, and whether the animal is pregnant or lactating. The starting dose of insulin should be 0.67 U/100g body weight. Complete direction for dealing with the various clinical emergencies are published in detail in *Rodents: Laboratory Animal Management Series* (NRC, in press). Briefly, the rat should be well hydrated, free of ketosis, gaining body weight, and maintained in a moderate state of glucosuria to avoid hypoglycemia. 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.

Treatment of hypoglycemia. The successful treatment of hypoglycemia requires a decrease in insulin dosage combined with subcutaneous fluid injections. Suggested regimens are reviewed in *Rodents: Laboratory Animal Management Series* (NRC, in press).

TABLE 2 Treatment for ketonuria in diabetes-prone rats

Ketones	Increased insulin[a] (U/100g body wt)	Lactated ringers (cc)	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 IBW. Do not increase insulin during mild episodes of ketonuria.

[b]1 cc of 8.4% sodium bicarbonate equals 1 meq.

TABLE 3 Treatment for hypoglycemia in diabetes-prone (DP) rats

Classification (blood glucose concentration)	Fluid therapy (subcutaneous)	Dose of insulin	Time of insulin administration
Severe <40 mg/dl	Give 1 cc 50% dextrose; 2 hrs later give lactated Ringers with 5% dextrose	Reduce 30–50%	Delay 2–3 hrs
Moderate 40–60 mg/dl	Give 10 cc lactated Ringers with 5% dextrose	Reduce 20–30%	Delay 2–3 hrs
Mild 60–80 mg/dl	Give 10 cc lactated Ringers	Reduce 10–15%	No delay

TABLE 4 Reproduction in diabetes-prone (DP) rats before and after receiving splenocytes from diabetes-resistant (DR) rats

	DP females **not treated** with splenocytes	DP females **treated** with splenocytes
Incidence of diabetes	86% (N = 1,238)	16% (N = 1,022)
Number of pups born	7,160	12,434
Number weaned	5,766	10,918
Percent surviving	80%	88%
Pups weaned/ female mated	4.7	10.7

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Transgenic Models of Insulin-Dependent Diabetes Mellitus

Jun-ichi Miyazaki and Fumi Tashiro

INTRODUCTION

Transgenic mice carry a foreign gene that usually is introduced by direct microinjection into fertilized mouse eggs (Hogan et al., 1986). This technique allows us to study the effects of a single gene during the development and growth of an animal, which cannot be done in cell-culture systems. It has been used to study complex biological processes, such as developmental gene regulation, the role of oncogenes in tumorigenesis, and the function of the immune system (Hanahan, 1989). Thus, transgenic technology provides a powerful tool to explore the mechanisms of human genetic disorders and autoimmune diseases using animal models. Recently, transgenic mouse technology has been used to study type I or insulin-dependent diabetes mellitus (IDDM).

The literature on the pathogenesis of IDDM in mice and rats suggests that there is an autoimmune response directed against insulin-secreting pancreatic β cells (see reviews by Eisenbarth, 1986; Guberski, 1993; Leiter, 1993). Overt diabetes develops during the final stages of the disease. Anti-islet T cells appear to have a dominant role in the progression of the disease (see Guberski, 1993; Leiter, 1993). This disease is strongly correlated with some types of HLA molecules (Todd et al., 1987, 1989). Other susceptibility genes may also be present in humans and in mice (Todd and Bain, 1992). Some environmental factors are believed to trigger the disease, including β-cell toxic agents and β-cell tropic viruses (see Guberski, 1993; Leiter, 1993).

A number of transgenic mice have been produced to investigate the exact sequence of events in IDDM. One line was designed to examine the effects of introducing candidate disease-susceptibility genes into IDDM mouse models, and another to establish mouse models for IDDM that reproduce at least part of the autoimmune process (Table 1). These transgenic mice have provided valuable information on molecular mechanisms of autoimmunity against β cells, roles of major histocompatibility complex (MHC) antigen expression on β cells, and disease susceptibility genes. This review will discuss the current studies.

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TRANSGENIC MOUSE MODELS FOR IDDM

The first transgenic mouse model of IDDM was produced by introducing into one-cell mouse embryos a hybrid gene combining regulatory sequences of the rat insulin II gene with simian virus 40 (SV40) large-T antigen protein coding information (Adams et al., 1987). In the transgenic lineages, large-T antigen was expressed exclusively in the β cells of the islets of Langerhans. In some lineages, transgene expression began before birth, and the progeny were naturally self-tolerant to the T antigen. However, in other lineages, transgene expression was delayed until the age of approximately 2–3 months. Progeny of those lineages failed to become self-tolerant to T antigen; they gradually developed an autoimmune response against β cells and insulitis. This β-cell response was shown to correlate with the MHC haplotype of the mice, although diabetes did not develop in any of the strain backgrounds they tested (Skowronski et al., 1990). This study suggests that β-cell dysfunction damages β-cells and leads to exposure of β-cell-specific antigens to the immune system, which triggers anti-β-cell autoimmunity.

To investigate the potential association between virus and IDDM, Roman et al. (1990) generated three lines of transgenic mice (called RIP-HA) that express the hemagglutinin (HA) of a strain of influenza virus specifically on their β cells. All three expressed HA in the islets by day 12 of embryonic life; therefore, these mice should have been im-

TABLE 1 IDDM-related transgenic mice

Transgene product[a]	Insulitis	Diabetes	Age at onset	References
SV40[b] large T antigen	+	–		Adams et al., 1987
Influenza virus hemagglutinin	+	+	4~9 weeks	Roman et al., 1990
LCMV[c] glycoprotein	+	+	Inducible	Ohashi et al., 1991; Oldstone et al., 1991
Mouse IFN-γ	+	+	6~10 weeks	Sarvetnick et al., 1988, 1990
MHC class I H-2K^b	–	+	2~3 weeks	Allison et al., 1988
MHC class II I-E^b	–	+	3~5 weeks	Lo et al., 1988
MHC class II I-A^d	–	+	~8 weeks	Sarvetnick et al., 1988
MHC class II I-A^k	–	–		Böhme et al., 1989
β2-microglobulin	–	+	~25 days	Allison et al., 1991
Chicken calmodulin	–	+	~3 days	Epstein et al., 1989
Human Ha-*ras*	–	+	~20 weeks	Efrat et al., 1990

[a]Promoters used to drive the transgenes were either that of the rat insulin II gene or that of the human insulin gene.
[b]simian virus 40
[c]lymphocytic choriomeningitis virus

munologically tolerant to HA. However, they developed insulitis, accompanied by a humoral response against β-cell-surface antigens, including HA. Furthermore, they exhibited hyperglycemia, although at a low frequency (approximately 13–27 percent). Interestingly, the incidence of hyperglycemia is affected by the haplotype of the MHC locus, reminiscent of the heritable susceptibility of humans to IDDM (see Analysis of IDDM Susceptibility Genes). RIP-HA mice may provide a useful system in which to study cellular interactions involved with the induction of self-tolerance and autoimmunity.

Ohashi et al. (1991) have produced transgenic mice (called GP mice) that express the glycoprotein (GP) of lymphocytic choriomeningitis virus (LCMV) and have bred them with transgenic mice whose peripheral T cells predominantly express the T-cell receptor (TCR) derived from an anti-GP cytotoxic T-cell line. In the resulting double transgenic mice (called TCR-GP mice), most T cells express GP-specific TCR transgenes in the periphery, although their β cells express GP on the cell surface. Therefore, these T cells seem to be positively selected in the thymus. They do not exhibit an immune attack on their GP-expressing β cells but on macrophages infected with LCMV in vitro. GP transgenic mice develop diabetes approximately 9–11 days after infection with LCMV and die in 15–20 days. The onset of diabetes is accelerated in TCR-GP mice (3–4 days after LCMV infection). The islets of Langerhans in GP mice were infiltrated with cytotoxic T lymphocytes (CTLs) and with CD4$^+$ T cells. Ohashi et al. (1991) suggest that self-reactive CTLs may remain functionally unresponsive due to a lack of appropriate T-cell activation, which may require CD4$^+$ T cells. Oldstone et al. (1991) have reported similar observations.

TRANSGENIC MICE EXPRESSING CYTOKINE

The central role of CD4$^+$ T cells in the activation of anti-islet autoimmunity has also been suggested from studies of NOD mice and diabetes-prone and diabetes-resistant rats (Guberski, 1993; Leiter, 1993). However, it is not clear whether β cells are damaged by CD4$^+$ T cells, CD4$^+$-activated CD8$^+$ T cells, or cytokines (such as interferon, tumor necrosis factor, interleukin-1, and interleukin-2) secreted by CD4$^+$ or other inflammatory cells. To address this question, transgenic mice were produced by microinjection with a hybrid gene comprised of the human insulin gene promoter and the mouse interferon-γ (IFN_γ) gene (Sarvetnick et al., 1988). Some of those transgenic mice exhibited inflammatory destruction of the islets and overt diabetes. However, it is still unclear whether islet destruction was caused by infiltrating lymphocytes or by deleterious effects of IFN_γ. Further analysis showed that engrafted MHC-compatible islets are destroyed in these transgenic mice and that lymphocytes from the transgenic mice are cytotoxic to normal islets in vitro, which indicates that the pancreatic expression of IFN_γ can result in a loss of tolerance to normal islets (Sarvetnick et al., 1990).

TRANSGENIC MICE EXPRESSING MHC ANTIGENS

Enhanced expression of MHC class I molecules and ectopic expression of class II molecules have been observed on β cells with insulitis in humans (Bottazzo et al., 1985) and in animal models (see Guberski, 1993; Leiter, 1993). Such aberrant expression of MHC molecules might be induced by local cytokine production caused by viral infection and might enable the β-cell to function as an antigen-presenting cell, leading to autoimmunity. To examine this possibility, several lines of transgenic mice were produced in which class I H-2K^b (Allison et al., 1988), class II I-E^b (Lo et al., 1988), and class II I-A^d (Sarvetnick et al., 1988) proteins were expressed at high levels exclusively on β cells of MHC-compatible mice. All of these transgenic mice developed early-onset diabetes without evidence of lymphocytic infiltration into the islets. Clonal anergy could be shown in these mice expressing extrathymic antigens (Markmann et al.,

1988). It was also reported that overexpression of β_2-microglobulin in transgenic mouse β cells causes hyperglycemia (Allison et al., 1991). Similarly, transgenic mice with an elevated β-cell expression of calmodulin, which is implicated as a regulator of insulin secretion, (Epstein et al., 1989) and those expressing an activated form of Ha-*ras* (Efrat et al., 1990) exhibit β-cell destruction and diabetes. These results suggest that various transgene molecules that are overexpressed in β cells can cause islet dysfunction, which leads to diabetes. Consistent with this, Böhme et al. (1989) showed that expression of I-A^k cDNA on islet cells at levels comparable with those on resting B lymphocytes does not perturb the function of β cells. It is interesting to note that these transgenic mice were not tolerant to the I-A^k antigen but did not develop diabetes. Therefore, over-expression of MHC class I molecules or aberrant expression of class II molecules does not necessarily result in an autoimmune reaction.

ANALYSIS OF IDDM SUSCEPTIBILITY GENES

The NOD (non-obese diabetic) mouse, which was established from ICR mice (Makino et al., 1980), is a good model of IDDM. Typically, NOD mice develop insulitis at approximately 4 weeks, and by 20 weeks more than 80 percent of the mice have developed insulitis (Figure 1) (Makino et al., 1980). The development of insulitis is followed by the complete destruction of islets and leads to overt diabetes. However, the incidence of diabetes is at most 80 percent in females and 20 percent in males (Makino et al., 1985). Many immunological studies have demonstrated that an autoimmune mechanism is involved in the generation of insulitis (Bendelac et al., 1987; Miller et al., 1988). In addition, breeding studies between NOD and other strains indicate that two or three genetic loci contribute to disease susceptibility (Hattori et al., 1986; Makino et al., 1985; Prochazka et al., 1987; Todd et al., 1991), one of which is linked to the MHC on chromosome 17 (Hattori et al., 1986; Prochazka et al., 1987). There are two characteristic features of the MHC of NOD mice. First, it does not express I-E molecules because of a deletion in the promoter region and the first exon of the E_α gene (Hattori et al., 1986). This defect is not unique to NOD mice, but is shared by other mice of *H-2*^{b} and *H-2*^{s} haplotypes. Second, the I-A molecule of the NOD mouse is unique. Acha-Orbea and McDevitt (1987) demonstrated that the 3' half of the $I\text{-}A_\beta$ chain, including the second external domain, the transmembrane domain, and the intracellular domain, is identical to that of the $I\text{-}A_\beta$ chain in mice of the *H-2*^{d} haplotype. The first external domain carries several amino acid changes and deletions, but most of these differences are shared by at least one other haplotype (Acha-Orbea and McDevitt, 1987). However, one region has five consecutive nucleotide changes that are unique to the NOD mouse and cause substitutions in the amino acids at position 56 (histidine replaces proline) and position 57 (serine replaces aspartic acid) (Acha-Orbea and McDevitt, 1987). In humans, Todd et al. (1987) have demonstrated that HLA-DQ_β alleles in which alanine, valine, or serine are substituted for aspartic acid at position 57 are positively associated with IDDM in Caucasians. These and other studies suggest that the expression of abnormal class II molecules might be involved in the development of autoimmune insulitis (Table 2) (Lund et al., 1990; Miyazaki et al., 1990a, Slattery et al., 1990; Uno et al. 1991).

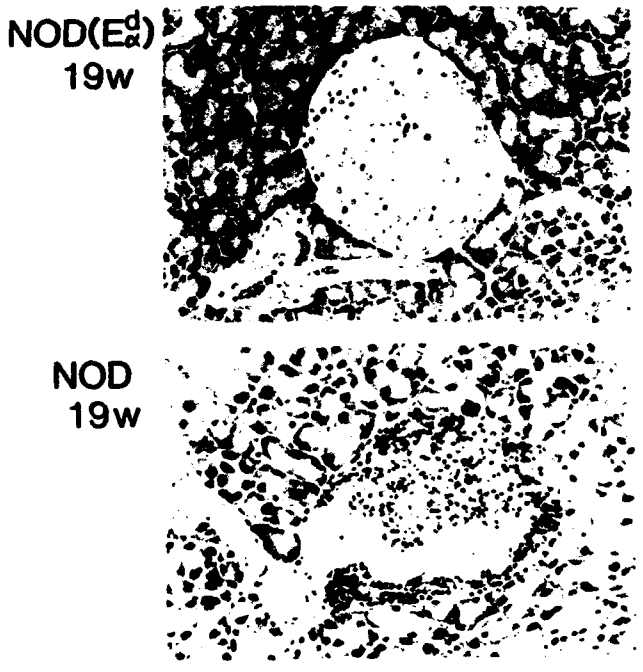

FIGURE 1 *Insulitis was observed in the pancreas of the NOD mouse. Pancreas from an NOD mouse (bottom) and an I-E expressing transgenic NOD mouse (NOD(Eα^d) (top) at 19 weeks of age was sectioned and stained with hematoxylin and eosin. Massive lymphocytic infiltration into the islet (insulitis) was observed in the NOD mouse but not in the transgenic mouse.*

I-E TRANSGENIC NOD MOUSE

Nishimoto et al. (1987) have demonstrated by backcrossing C57BL/6 transgenic mice (B6-Eα^d), which express I-E (Yamamura et al., 1985), with NOD mice, which do not, that the expression of I-E molecules in NOD mice can prevent the development of autoimmune insulitis. Although this is an extremely intriguing finding, it is difficult to exclude the possible involvement of other genes adjacent to the Eα^d transgene from B6-Eα^d mice. Therefore, Uehira et al. (1989) generated NOD mice that carry the Eα^d transgene and confirmed that I-E expression completely prevents the development of insulitis (Figure 1) and cyclophosphamide-induced diabetes (Uno et al., 1991). Lund et al. (1990) obtained similar results. The mechanism by which I-E molecules protect NOD mice from diabetes is not known, although some speculate that this protection is mediated through the deletion of specific V_β families of T-cell receptors (Lund et al., 1990).

I-A TRANSGENIC NOD MICE

Breeding studies between NOD and C57BL/6 mice, both of which lack I-E expression, suggest the presence of an MHC-linked IDDM susceptibility locus other than I-E. To investigate whether the unique I-A molecule of NOD mice ($I\text{-}A^{g7}$)is related to IDDM susceptibility, several lines of transgenic NOD mice were produced with the $A\alpha^k$ gene and the $A\beta^k$ gene (Miyazaki et al., 1990a; Slattery et al., 1990). As summarized in Table 2, when either $A\alpha^k$ or $A\beta^k$ were expressed, there was no effect on insulitis, but when $A\alpha^k A\beta^k$ was expressed (i.e., $I\text{-}A^k$), the incidence of insulitis was decreased to one-third that in normal NOD mice. Further analysis showed that $I\text{-}A^k$ expression almost completely prevents diabetes (F. Tashiro, Department of Disease-related Gene Regulation Research (Sandoz), University of Tokyo, Faculty of Medicine, Tokyo, Japan, unpublished).

Using site-directed mutagenesis, Miyazaki et al. (1990a) have shown that the protective effect of transgenic $I\text{-}A^k$ molecules in which aspartic acid was replaced by serine at position 57 is enhanced (Table 2). Lund et al. (1990) took a complementary approach, making transgenic NOD mice that harbored a mutated $A\beta^{g7}$ gene in which codon 56 is changed from histidine to proline. Interestingly, this transgene also conferred protection against diabetes.

These experiments show that the amino acid sequences in the vicinity of position 57 are related to the diabetogenic propensity of an I-A molecule. A recent study of diabetes in human populations suggests that disease susceptibility is correlated with the presence of DQ molecules formed by particular combinations of α-chains with β-chains (Todd et al., 1989). This conclusion may be supported by Miyazaki et al. (1990a), who showed that the $I\text{-}A^k$ β chain protects NOD mice against insulitis only when it associates with the $I\text{-}A^k$ α chain, but not when it associates with the endogenous $I\text{-}A^d$ α chain, as shown in the $A\beta^k$ single transgenic NOD mouse (Table 2). These transgenic studies revealed that the roles of MHC class II molecules in the pathogenesis of autoimmune insulitis in NOD mice and IDDM patients closely resemble each other. Recently, it has been shown that transgenic expression of class $I\text{-}L^d$ molecules also decrease the incidence of insulitis in NOD mice, suggesting that MHC class I molecules are also involved in the development of insulitis in NOD mice (Miyazaki et al., 1992).

TABLE 2 Effects of MHC transgene expression on the incidence of insulitis in NOD mice[a]

Transgene	Insulitis[b]	(%)	Diabetes
–	+	(80~90)	+
$E_\alpha{}^d$	–	(0)	–
$A_\alpha{}^k$	→	(80~90)	+
$A_\beta{}^k$	→	(80~90)	+
$A_\alpha{}^k A_\beta{}^k$	↓	(20~30)	–
$A_\alpha{}^k A_\beta{}^k$ ($Asp^{57}\rightarrow Ser$)	↓	(10~20)	–
$A_\beta{}^{g7}$ ($His^{56}\rightarrow Pro$)	↓		–
L^d	↓		

→ unaffected
↓ decreased incidence

Based on Miyazaki et al., 1990; Slattery et al., 1990; Lund et al., 1990; Uno et al., 1991; Miyazaki et al., 1992.

FUTURE PERSPECTIVE

Various transgenic mouse models for IDDM have been produced. Although none of these mice seem to be an exact model of the autoimmune process in humans or in NOD mice, each has provided important information about how the mechanisms of β-cell tolerance are maintained or lost. Studies using transgenic NOD mice clearly demonstrate that MHC class II genes participate in conferring susceptibility or resistance to IDDM. The fine structure or amino acid residues of the class II $A\beta^{g7}$ chain, which is directly involved in the development of insulitis, will further be identified by introducing mutated Aβ genes into NOD mice. The transgenic technique has also been applied to establish pancreatic β-cell lines (Efrat et al., 1988; Hamaguchi et al., 1991; Miyazaki et al., 1990b). These cell lines will make it easier to study and identify autoantigen(s) that trigger IDDM. Techniques developed to target genes via homologous recombination in the embryonic stem cells enable us to directly assess the role of a gene in the disease process (Capecchi, 1989). This technique, together with traditional transgenic techniques, will further be applied to address problems in the pathogenesis of IDDM, including identification of the genes conferring disease susceptibility and the authentic autoantigen(s).

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In the News

John VandeBerg Appointed ILAR Council Chairman

John L. VandeBerg, Ph.D., replaced Steven P. Pakes, D.V.M., Ph.D. as chairman of the ILAR Council on July 1, 1993. Dr. Pakes, director of the Animal Resources Center, Southwestern Medical Center in Dallas, served as ILAR Council Chairman since 1987. Dr. Pakes will continue his longstanding relationship with ILAR, serving as liaison to ILAR for the International Council on Laboratory Animal Science (ICLAS), for which he is the U.S. representative.

Dr. VandeBerg, who was recently appointed scientific director of the Southwest Foundation for Biomedical Research (SFBR), has been associated with ILAR since 1982, when he was a member, and later chairman, of the Committee on Animal Models and Genetic Stocks (AMGS). Dr. VandeBerg also played a key role as a member of the committee that wrote the ILAR report *Important Laboratory Animal Resources: Selection Criteria and Funding Mechanisms for their Preservation*, which was published in 1990.

An internationally renowned expert on genetic research with animal models, Dr. VandeBerg assumed the position of scientific director of SFBR on October 1, 1993, after chairing SFBR's Department of Genetics since its inception in 1982. SFBR is a private, nonprofit organization that has pioneered the use of the baboon as an animal model for human heart disease and is an established leader in research on AIDS, genetic susceptibility to common diseases, and atherosclerosis.

Among his scientific accomplishments, Dr. VandeBerg co-discovered paternal X-chromosome inactivation in marsupials and developed this novel system of chromosomal behavior as a model for research on gene regulation; discovered an enzyme, PGK-B, produced only during spermatogenesis and also important as a model for gene regulation; and developed the marsupial *Monodelphis domestica*, the gray short-tailed opossum native to Brazil, as a mammalian model for research on early developmental processes and on genetic susceptibility to skin and eye cancers induced by ultraviolet radiation.

A native of Appleton, Wisconsin, he received his Ph.D. in genetics from Macquarie University, Sydney, Australia, in 1975 and bachelor's degrees in genetics from the University of Wisconsin in 1969 and from La Trobe University in Melbourne, Australia, in 1970. He is the author of more than 150 scientific papers on biochemical and developmental aspects of genetics.

Dr. VandeBerg is a member of the National Research Council's Commission on Life Sciences; a member of the government affairs committee of the Association of Independent Research Institutes (AIRI); president of the Texas Genetics Society; trustee of the South Texas Regional Blood Bank and member of its medical advisory committee; and trustee of the Mind Science Foundation. He is also a professor in the Department of Cellular and Structural Biology and in the Department of Pathology at the University of Texas Health Sciences Center at San Antonio.

Former ILAR Committee Member Wins Charles River Prize

Howard C. Hughes, D.V.M., a former ILAR committee member, was recently awarded the Charles River Prize in recognition of his distinguished contributions to the field of laboratory animal science. Dr. Hughes is vice president of laboratory animal science (worldwide) for SmithKline Beecham Pharmaceuticals and is also a clinical professor of comparative medicine at the College of Medicine, Pennsylvania State University in Hershey, Pennsylvania and an adjunct professor of Laboratory Animal Medicine at the School of Veterinary Medicine, University of Pennsylvania. From 1988 to 1992, Dr. Hughes was an active member of the Institute of Laboratory Animal Resources (ILAR) committee that wrote *Recognition and Alleviation of Pain and Distress in Laboratory Animals*. This handbook, published in 1992, provides clear guidelines on the proper care and use of laboratory animals and a comprehensive overview of current knowledge about pain and distress in laboratory animals.

Revision of the Guide is Underway

The Institute of Laboratory Animal Resources (ILAR), has been asked by the National Institutes of Health and other federal sponsors to appoint a committee that will revise the *Guide for the Care and Use of Laboratory Animals* (*Guide*). The *Guide* is ILAR's most widely distributed work and is accepted by the scientific community as the main resource on animal care and use. It was last revised in 1985.

One of the first priorities of the committee will be to meet with those who wish to make recommendations regarding the content of the seventh edition of the *Guide*. These public meetings were held in Washington, D.C. on December 1, in San Francisco on February 2, and in St. Louis on February 4. In addition to the three public forums, the committee conducted a seminar at the annual meeting of the American Association of Laboratory Animal Science (AALAS) in Nashville on November 15, 1993.

The committee plans to publish the seventh edition of the *Guide* in early 1995. For additional information contact Thomas L. Wolfle, D.V.M., Ph.D., ILAR, National Research Council 2101 Constitution Avenue, NW, Washington, DC 20418. Tel: 1-202-334-2590; Fax: 1-202-334-1687; Bitnet: twolfle@nas Internet: twolfle@nas.edu.

Coming Meetings

February 1994

24–25 Current Issues in IACUC Protocol Review, Raleigh, North Carolina. The National Institutes of Health (NIH), Office for Protection from Research Risks (OPRR), is cosponsoring this Animal Welfare Education Workshop with North Carolina State University. The format will include plenary presentations and panel discussions on topics such as death as an endpoint, exposure routes in toxicology studies, and use of alternatives. The workshop is open to institutional administrators, members of institutional animal care and use committees, laboratory animal veterinarians, investigators, technicians, and others responsible for managing a sound institutional animal care and use program. For more information contact Dr. Thomas Hamm, Jr., Director, Laboratory Animal Resources, North Carolina State University. Tel: 1-919-829-4280; Fax: 1-919-829-4283.

March 1994

23 Applied Research Ethics National Association (ARENA) Annual Conference, ARENA's annual conference, which will focus on the issues surrounding the use of animals in research, will immediately precede Public Responsibility in Medicine and Research (PRIM&R) annual conference (see below). For more information contact PRIM&R, 132 Boylston Street, Boston, MA 02116. Tel: 1-617-423-4112.

23–24 Animal Care and Research at the Crossroads: Expanding Challenges, Boston, Massachusetts. This program, hosted by Public Responsibility in Medicine and Research (PRIM&R) and the Tufts University School of Veterinary Medicine, will include presentations by a diverse faculty of experienced animal researchers, representatives from the National Institutes of Health, U.S. Department of Agriculture, and the American Association for the Accreditation of Laboratory Animal Care; institutional administrators; and representatives of pharmaceutical companies and animal welfare organizations. The program will also include more than 24 workshops, which will cover such topics as regulatory updates, financial and operational effects of animal rights protests, policies on genetic engineering and xenografts, death as an endpoint, and biohazards and occupational safety issues. For more information contact PRIM&R, 132 Boylston Street, Boston, MA 02116. Tel: 1-617-423-4112.

May 1994

5–6 The Ethical Implications of the New Genetics—Boston, Massachusetts. This program is hosted by Public Responsibility in Medicine and Research and the Tufts University School of Medicine and will include presentations by a diverse faculty of genetic researchers, ethicists, policy analysts, representatives of the National Institutes of Health and the Office of Technology Assessment, genetic counselors, research administrators, and representatives from advocacy groups, pharmaceutical and biotechnology companies, and other genetic testing organizations. For more information contact PRIM&R, 132 Boylston Street, Boston, MA 02116. Tel: 1-617-423-4112.

12–13 Research Animal Anesthesia, Analgesia, and Surgery, Atlanta, Georgia. This 2-day conference is sponsored by the Scientists Center for Animal Welfare (SCAW) and will include the following topics: U.S. Department of Agriculture, National Institutes of Health, and American Association for the Accreditation of Laboratory Animal Care requirements for surgical programs; surgical training and personnel qualifications; ethics and science of xenotransplantation and xenoperfusion; recognizing pain and distress in research animals; physiologic effects of anesthetics and analgesics; and post-surgical care. Researchers, regulatory personnel, members of institutional animal care and use committees, administrators, and others interested in these issues are encouraged to attend. For more information contact SCAW, 4805 St. Elmo Ave., Bethesda, MD. Tel: 1-301-654-6390; Fax: 1-301-907-3993.

July 1994

10–14 Eighth International Workshop on Immunodeficient Animals, Utrecht, The Netherlands. The International Workshop on Immunodeficient Animals acts as a forum to bring together breeders and users of immunodeficient animals. It combines symposia on general themes in immunodeficient animal research and workshops on special topics. The preliminary list of topics for the symposia and workshops include immunodeficient mouse and rat mutants, spontaneous autoimmunity and allergy, experimentally induced autoimmunity or allergy, transgenesis associated with immunodeficiency, retrovirus infection, T-cell differentiation, autoimmunity, hypersensitivity, infectious disease, oncogenesis, and molecular genetics. For more information contact P. de Vrey, Central Animal Laboratory, Pb43, National Institute of Public Health and Environmental Protection, P.O. Box 1, 3720 BA Bilthoven, The Netherlands. Tel: 31-30-742335; Fax: 31-30-252178.

9–13 A Summer Course on Ethical Issues of Animal Experimentation, Washington, D.C. This course is open to college faculty who would like to improve their skills in

teaching about ethical issues surrounding animal experimentation to graduate and undergraduate students. Emphasis will be on how to use course materials in classroom instruction. Topics include the moral status of nonhuman animals, the justifications for using animals in research and education, student objections, the use of alternatives, animal pain, legal issues, and the importance of relevant species. For more information contact Marc Favreau, Kennedy Institute of Ethics, Georgetown University, Washington, D.C. 20057. Tel: 1-202-687-6771; Fax: 1-202-687-6770.

27–31 Animal Behavior Society Meeting, Seattle, Washington. The goal of the Animal Behavior Society is "to promote and encourage the biological study of animal behavior in the broadest sense, including studies at all levels of organization using both descriptive and experimental methods under natural and controlled conditions." Presentations at the meeting will be multidisciplinary, ranging across zoology, psychology, and anthropology. Special sessions on applied animal behavior and psychoneuroimmunology will be scheduled. There will be a joint session with the American Society of Primatologists on Thursday, July 28, with an emphasis on the behavior of primates. For more information contact James C. Ha or Carolyn Crockett, Primate Center SJ-50, University of Washington, Seattle, WA 98195. Tel: 1-206-543-1440; Email: jcha@u.washington.edu. or crockett@u.washington.edu.

31 July–4 August International Congress of Vertebrate Morphology, Chicago, Illinois. This will be the first International Congress of Vertebrate Morphology held in North America. It will include contributed oral and poster papers, plenary speakers, workshops, and symposia on such topics as the functional design of musculoskeletal systems, segmentation in vertebrates, genetics and morphology, adaptations of marine amniotes, and amphibian-amniote transition. For more information contact Sue Herring, Chair, ICVM Organizing Committee, Department of Orthodontics, SM-46, University of Washington, Seattle, WA 98195. Tel: 1-206-543-3203; Fax: 1-206-685-8163; email: herring@u.washington.edu.

New Books

International Index of Laboratory Animals, Sixth Edition—Michael F. W. Festing. This index gives the locations of more than 7,000 stocks of laboratory vertebrates throughout the world. It also includes abbreviated rules for nomenclature of genetically defined stocks of laboratory animals, a brief listing of known inbred strains of mice, an abbreviated listing of inbred strains of rats, a list of mouse mutants sorted by name, a list of rat mutants sorted by name, and a common name to Latin name index. Michael F. W. Festing, 1992, 238 pp., paperback, $33.75 (£22.50), ISBN 0-9520975-0-8. (Available from Michael F. W. Festing, MRC Toxicology Unit, Woodmansterne Road, Carshalton, Surrey SM5 4EF, UK.)

Research Protocol and Technician's Manual: A Guide to the Care, Feeding, and Evaluation of Infant Monkeys, Second Edition—Gerald C. Ruppenthal and Gene P. Sackett. The University of Washington Infant Primate Research Laboratory (IPRL) is making this manual available to the scientific community for the cost of production and mailing. Its purpose is to provide researchers and colony managers with a model of a research-oriented nursery for nonhuman primates. The model illustrates the scope of the husbandry activities and types of normative developmental data collection pursued in the IPRL. The manual is divided into three parts: research protocols, which includes maternal prepartum exams, infant blood draws, and playroom testing; a technician's guide, including nursery shift protocols, protocols for data collection and record keeping, and special procedures in the nursery; and an appendix, which includes sample data sheets, records, and logs. IPRL, 1992, 131 pp., paperback, $15.00. (Available from the Primate Information Center, Regional Primate Research Center SJ-50, University of Washington, Seattle, WA 98195. Tel: 1-206-543-4376; Fax: 1-206-685-0305.)

Understanding Chimpanzees: Diversity and Survival—Chicago Academy of Sciences. These symposium abstracts examine the behavioral diversity and survival of chimpanzees and bonobos, both in the wild and in captivity. The abstracts take a comparative approach to a wide range of topics, including social behavior and ecology, the rich variety of cultural traditions between populations, and the significant cognitive abilities of chimpanzees. Abstracts addressing the survival and well-being of chimpanzees and bonobos examine population status in the wild, threats to habitat and survival, development of sanctuaries, and conservation and care in captivity. The abstracts include observations from field sites across Africa and from zoos and captive colonies. The 1991 conference advisory committee included Jane Goodall, Robert Fry, Toshisada Nishida, Randall Susman, Geza Teleki, Frans de Waal, and Richard Wrangham. Chicago Academy of Sciences, 1992, 56 pp., paperback, $4.50. (Available from the Chicago Academy of Sciences, 2001 N. Clark Street, Chicago, IL 60614. Tel: 1-312-549-0606.)

The Venomous Sea Snakes: A Comprehensive Bibliography—Wendy A. Culotta and George V. Pickwell. A collection of approximately 2800 references, this book contains a comprehensive listing of the world's literature on sea snakes from the time of the early Greeks through 1986, with additional references through 1990. It is organized into 24 subject chapters, which include venom, folklore, food, natural history and behavior, and uses by man. Each chapter is followed by a genus and species index and a comprehensive author index. This book should be of interest to anyone in the fields of herpetology, zoology, biology, chemistry, biochemistry, medicine, natural history, ecology, and genetics. Krieger Publishing Company, 1993, 526 pp., hardback, $79.50, ISBN 0-89464-469-6.

Publications Available

The following publications are available free from ILAR:

Animal Technician Certification Program. AALAS pub. 83-1. Rev. 1986

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

Holders of Inbred and Mutant Mice in the United States. Including the Rules for Standardized Nomenclature of Inbred Strains, Gene Loci, and Biochemical Variants. 1984

Important Laboratory Animal Resources: Selection Criteria and Funding Mechanisms for their Preservation. 1990

International Standardized Nomenclature for Outbred Stocks of Laboratory Animals. ICLA bull. no. 30. 1972

Laboratory Animal Management: Cats. 1978

Laboratory Animal Management: Genetics. 1979

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INSTITUTE OF LABORATORY ANIMAL RESOURCES

Volume 35, Numbers 3–4 Summer/Fall 1993 National Research Council

Issues for IACUCs

Frequently Asked Questions About the Public Health Service Policy on Care and Use of Laboratory Animals

Personal Reflections: The Role and Value of the Unaffiliated Member of the Institutional Animal Care and Use Committee

Connecting Two Worlds: A High School Teacher's Work on an IACUC Fosters Communication Between Scientists and Students

Guidelines

IRAC Recommendation on LD_{50} Testing

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, 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, the National Academy of Engineering, and the Institute of Medicine, 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 News is published quarterly by the Institute of Laboratory Animal Resources, National Research Council, 2101 Constitution Avenue, NW, Washington, DC 20418. It is circulated by request to investigators in the field of biomedical and related research.

Publication of this issue of *ILAR News* was supported by grants from the National Center for Research Resources, National Institutes of Health; National Science Foundation; American Cancer Society, Inc.; and U.S. Army Medical Research and Development Command, which is the lead agency for combined Department of Defense funding also received from the Human Systems Division, 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.

ILAR News ISSN 0018-9960
Volume 35, Numbers 3–4 Summer/Fall

ILAR NEWS

INSTITUTE OF LABORATORY ANIMAL RESOURCES

VOLUME 35, NUMBERS 3–4 SUMMER/FALL 1993

CONTENTS

Issues for Institutional Animal Care and Use Committees (IACUCs)

Frequently Asked Questions About the Public Health Service Policy on Care and Use of Laboratory Animals

Prepared by the staff of the Division of Animal Welfare, Office for Protection from Research Risks, National Institutes of Health, Bethesda, Maryland

The Office for Protection from Research Risks (OPRR) of the National Institutes of Health (NIH) develops, implements, and oversees compliance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy) (PHS, 1986). The PHS Policy and the U.S. Department of Agriculture's (USDA's) Animal Welfare Regulations, are the two principal federal documents setting forth requirements for animal care and use by institutions using animals in research, testing, and education. One of OPRR's primary functions is to assist institutions in implementing PHS Policy by responding to policy-related questions.

The following represent several frequently asked questions from institutions and the OPRR responses:

1. What standards does the PHS Policy require our institution to follow in conducting survival rodent surgery, and how do they differ from those applicable to other species?

The PHS Policy requires that the recommendations of the *Guide for the Care and Use of Laboratory Animals* (*Guide*) (NRC, 1985) be adhered to regarding survival surgical procedures and the environments in which they are conducted. The *Guide* presents slightly different standards for rodent and nonrodent species. Briefly, the current requirements are as follows. Survival surgery may be conducted on rodents in an area that is used solely for this purpose while the surgery is being performed (such as a room or a portion of a room). The surgery must be performed using sterile instruments, surgical gloves, and aseptic procedures designed to prevent contamination of the operative site. In addition to the above requirements, survival surgery involving higher vertebrate species must use aseptic surgical techniques such as wearing sterile surgical gloves, gowns, caps, and face masks; using sterile instruments; and preparing an aseptic surgical field. Furthermore a separate, dedicated surgical area, subdivided into a surgical support area, preparation area, and operating room is mandated. Safeguards against hazards surrounding the use of explosive gases are required, and anesthesia scavenging devices or exhaust hoods must be used to eliminate waste anesthetic gases regardless of the species on which surgery is being performed.

2. In the experience of OPRR, what form of administrative organization works best for directing the animal programs and ensuring compliance with the PHS Policy?

It has been OPRR's observation that organizations having simple, clear, direct lines of responsibility and corresponding authority function well and are better able to respond quickly and effectively to the requirements of the PHS Policy. The key components in such organizations are the Institutional Official (IO), the IACUC, and the participating veterinarian. The IO should have the authority to allocate organizational resources needed to maintain a smoothly functioning animal care and use program based on recommendations and advice received from the IACUC and the veterinarian. The IO should also clearly define and assign responsibilities and reporting channels for other essential program elements such as training, occupational health, and maintenance. The IACUC, appointed by the organization's chief executive officer, usually reports directly to the IO and is empowered to perform its duties without undue interference. OPRR'S experience suggests that it is usually best for the veterinarian also to report directly to the IO in connection with his or her responsibilities for implementing those parts of the animal care and use program that are set forth in the PHS Policy, the Animal Welfare Act (Animal Welfare Act of 1966), and the *Guide*.

OPRR recognizes that the size and complexity of Assured institutions vary, and that no single organizational or administrative structure will be compatible with the needs of all institutions. While the Policy allows for such institutional flexibility, OPRR strongly recommends that its organizational channels for implementation be as direct and straightforward as possible. In OPRR's experience, unclear or inappropriate lines of authority and responsibility have been the underlying cause for serious cases of programmatic failure.

3. What is the difference between IACUC animal study proposal review in a convened meeting and "expedited" review, and when is it appropriate to use the latter?

Paragraph IV. C. 2. of the PHS Policy and Part 2, Section 2.31 (d) (2) of the USDA's Animal Welfare Regulations require that, as a minimum, all IACUC members be given for their review a list of proposed research protocols involving

the care and use of animals and that written descriptions of the projects be available to them. Any member of the IACUC may then request full review of any protocol by the full committee. In the absence of such a request, the chairperson may appropriately designate at least one qualified person to review, approve, require modifications, or request full committee review.

This process, protocol review by less than the full committee in a convened meeting, is often referred to as an "expedited" review. This does not correspond, however, to the expedited review process of the Institutional Review Board applicable to Human Subjects Protection. In order to comply with the PHS Policy, *no* animal work may begin before the full committee has either been given the opportunity to review the protocol and call for a full committee review or before the protocol has been approved by (1) the majority of a quorum of the members or (2) the designated reviewer in the absence of a call for full committee review. In this regard, it should be kept in mind that neither the PHS Policy nor the Animal Welfare Regulations recognize "provisional" or "interim" approval of any animal study proposal.

4. Does the PHS Policy place any proscriptions on filling the positions of IO, attending veterinarian, and IACUC chairperson with the same individual?

While there are no specific prohibitions, OPRR strongly recommends against having more than one of these positions filled by the same individual. OPRR considers that the responsibilities and authorities vested in each of the aforementioned positions are distinct, often requiring different skills. Also, the assignment of more than one of these roles to the same individual circumvents the intended checks and balances designed by the framers of the PHS Policy. Circumstances arising from having the same person serving as the IACUC chair, the institutional veterinarian, and the IO have, in the past, been an underlying factor in some of our most serious cases of noncompliance with the PHS Policy. In addition, the mere perception of conflict of interest may lead to allegations of improprieties from various sources. However, the intent of the PHS Policy is to provide levels of responsibility and authority within institutions which would provide an optimal environment for its implementation. Hence, the attending veterinarian, as the only member appointed by virtue of position, serves on the IACUC under the IACUC chairperson, with the latter reporting directly to the IO. This arrangement, however, should not preclude the veterinarian from performing the appropriate management and administrative functions as the institutional veterinarian with direct access to (and preferably reporting channels to) the IO.

5. Under the conditions of the Animal Welfare Assurance, is it necessary for our IACUC to report to OPRR any suspensions of animal-related activities or other sanctions imposed by the IACUC if the subject activities are not PHS supported?

While a few institutional laboratory animal care and use programs can be subdivided into physically and operationally distinct entities, program oversight is almost always exercised *institution-wide* under a single institutional standard for animal care and use. OPRR has found that deviations from IACUC policies and procedures, whether sufficiently serious to impose protocol suspensions or minor in nature, generally include issues that have bearing on compliance with the PHS Policy. Unless OPRR has approved an Institutional Assurance that exempts specific facilities or programs, it is expected that protocol suspensions or other sanctions imposed by the IACUC on non-PHS supported work will be reported. This will allow an evaluation of the potential impact of the infraction on studies that are conducted with PHS support. An additional reason for such reporting is to provide OPRR with advanced knowledge of an incident *prior* to having it appear in the form of an official complaint or congressional inquiry. This knowledge allows OPRR to respond positively to outside inquiries that the system of animal welfare oversight is working as intended.

6. Our institution's animal care and use program is constantly undergoing modification, much of which we consider to be minor. In its annual report to OPRR, how extensively must institutional facility and program changes be described?

The approved Animal Welfare Assurance is the key document in defining the relationship between the institution and the PHS. Institutions should therefore consider any program or facility modification that results in a change to any item described in its Assurance to OPRR as reportable. Facility modifications generally involve changes in gross square footage resulting from the addition (either newly-constructed or otherwise acquired) or elimination of animal space.

Normally, it is not necessary to report preventive maintenance items or remodeling that does not result in changes to the gross square footage or carrying capacity of the facility. Annual reports should identify the affected areas, the number of square feet involved, and any resultant changes in the average daily animal populations. Concerning program matters, OPRR needs to be kept informed of any modifications in institutional lines of authority and responsibility and of any changes in key personnel such as the institutional official, the IACUC chairperson, and the veterinarian. Another consequential programmatic consideration includes changes in the composition and procedures of the IACUC that represent departures from those described in the Assurance. Similarly, any significant changes made in the occupational health and training or instruction programs should be brought to the attention of OPRR in the annual report. Finally, the report should include significant changes in the numbers and species of animals maintained by the institution. These elements are especially important in OPRR's evaluation of the adequacy of veterinary resources, credentials, and support facilities.

The extent to which program or facility modifications or changes need to be described in an annual report will depend on the degree to which they differ from the corresponding

items described in the Assurance. OPRR recommends that the descriptions be comprehensive and in sufficient detail to allow replacement of the affected items as they were described in the original Assurance.

7. Implementing regulations of the Animal Welfare Act require that animal study protocols be reviewed and acted upon by the IACUC annually. The PHS Policy requires that such reviews be conducted every three years. For the purpose of complying with OPRR's oversight policy, how frequently must our IACUC perform such reviews?

The PHS Policy requires that de novo IACUC reviews of all PHS-supported protocols be conducted on a triennial basis. The Policy also states that ". . . institutions are required to comply . . . with the Animal Welfare Act, and other Federal statutes and regulations." To be compliant with the USDA's Animal Welfare Regulations, the IACUC must review those protocols involving dogs, cats, nonhuman primates, rabbits, guinea pigs, and hamsters each year to assure active status and to identify significant changes. Although annual reviews of protocols involving other species are not required under the PHS Policy, many institutions will choose to establish a uniform method covering all vertebrate species that satisfies the USDA's requirement for annual review and the PHS requirement to review and approve proposals for significant changes to ongoing protocols. A relatively simple monitoring mechanism, which meets USDA requirements and serves to monitor animal activities covered by the PHS Policy, can be implemented by the use of a standard form containing basic protocol information (including title, approval number, date, and species). This form is then sent to the PI to (1) verify active status, (2) verify that completed activities were conducted in accordance with the approved protocol, (3) describe any proposed departures from the approved protocols, and (4) solicit information about activities projected for the upcoming year. Information pertaining to the future would indicate either that no changes were proposed or describe changes that the PI would like to implement. Any proposed changes deemed to be significant would then be brought before the IACUC for its consideration and documented as an official IACUC action. Such a monitoring system, however, does not preclude the requirement for triennial review or for PIs to seek IACUC approval when they want to make significant changes in approved protocols at other than the regularly scheduled monitoring periods. Such approval must be obtained *prior* to implementing the changes. Both the USDA and PHS requirements, of course, may also be satisfied by conducting complete de novo reviews of all animal study proposals on an annual basis.

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Personal Reflections

The Role and Value of the Unaffiliated Member and the Nonscientist Member of the Institutional Animal Care and Use Committee

J. Wesley Robb

The Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy) (PHS, 1986) and the Animal Welfare Regulations (AWRs) mandate that one member of the Institutional Animal Care and Use Committee (IACUC) be an "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 Policy IV A 3 b 4 and 9 CFR 2.31 b 3 ii).

PHS Policy gives no criteria that should be applied in the selection of this person or no indication of what the role of this person should be, while the AWRs state that it is intended that the "person will provide representation for general community interests in the proper care and treatment of animals" (9 CFR 2.31 b 3 ii). The PHS Policy further requires that one member of the committee be someone "whose primary concerns are in a nonscientific area" (PHS Policy IV A 3 b 3). It is possible for the unaffiliated member and the nonscientist member to be the same person.

Beyond this, administrative officers of institutions operating under either PHS Policy or the AWRs are left to their own judgments. There is a paucity of material that addresses this issue and considerable variation in practice exists among institutions (see Orlans (1993) pp. 99-117, which deals with many of the issues addressed in this article). The purpose of this essay is to explore some of the questions that are pertinent to the selection and role of the unaffiliated member that is required by both PHS Policy and AWRs and the nonscientist or lay member required by PHS Policy.

WHY AN UNAFFILIATED MEMBER?

When PHS Policy and AWRs require appointment of an unaffiliated member, it is intended that the person should represent the interests of the public in the humane use of animals in research, testing, and education. Whether or not this stipulation was prompted by the increasing concern of the public for the welfare of research animals over the last decade is an open question. Nonetheless the question remains: What should be the role that an unaffiliated member plays in the deliberations of the committee? In a pluralistic society "general community interests" are increasingly difficult to identify. Since a majority of the American public approves research with animals that will contribute to human health and well-being (from a 1990 poll by the National Association for Biomedical Research), "general community interests" could be interpreted as supporting this position. However, to what extent should the unaffiliated member represent the concerns of animal protection groups (this term is used to denote groups and people who are, to varying degrees, critical of the use of animals in scientific research), particularly those concerns directly related to animal welfare?

Supposedly, legislative bodies that are elected by a constituency are responsible to their public. But in the case of lay members appointed to IACUCs by the administrative official of an institution, there is no provision that they represent any special group other than that of the general public. The unaffiliated member is usually chosen from people who are likely to be cooperative with the review process and not by a public vote nor a comprehensive survey for members of the public willing to serve. (This type of selection process is often followed for the appointment of Institutional Review Board (IRB) and hospital ethics committee unaffiliated members as well.) As the result, far too often, some benign individual is selected who may accept the appointment out of a sense of public service or because of a friendship within the institution. The letter of the obligation to appoint a person not affiliated with the institution is fulfilled, but the spirit of the rule is neglected. The committee then proceeds with its work unchallenged. Neither committee members nor people in animal protection groups are entirely happy with the "outside member." The term "outside" often indicates how the committee feels about the interloper and how the unaffiliated member feels about his or her role. Thus, committee decisions may not be rigorously subjected to debate nor viewpoints of the mythical average layperson represented.

The informed unaffiliated member can be of inestimable value to the committee by (1) acting as a liaison between the community or several communities and the institution and (2) bringing a fresh and different perspective to bear upon the research enterprise.

The Unaffiliated Member as Liaison

People are becoming aware of the fact that large amounts of money are being spent by both public and private sectors on research that uses animals. The press has reported both accusations by animal protection groups of the inhumane use

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of animals and reports by the scientific community that indicate the benefits for humankind resulting from the carefully monitored use of animals in research. However, what sticks in the mind of the public are reports that raise questions whether the use of animals is humane. As a result, the public receives a negative message regarding research and often fails to hear responsible spokespersons who are either intimately involved with research or are lay members of an IACUC. Animal protection groups (that is, that part of the animal protection movement whose aim is to abolish all use of animals in research) are so committed to their cause that dialogue with them is very difficult, if not impossible. Others in the animal protection movement are frequently well-informed and raise plausible concerns about the moral appropriateness of specific research protocols.

On the whole, investigators within the research community, as ardent defenders of their work, find it counterproductive to engage in discussions with these groups. One of the productive roles of the unaffiliated member, who has no vested interest in any particular research project involving animals, is to engage in dialogue with those groups that are critical of, or oppose specific research projects involving animals and who are willing to enter into a rational discussion of the issues.

A far more productive dialogue, however, can be initiated with people in the animal protection movement who recognize the necessity for using animals in research, but who want humane care and use of animals in that research. For the most part, the central issues for these people are: minimization of any pain that animals might suffer, reduction of the number of animals used, and exploration of the possible use of non-animals methods. These principles were first clearly enunciated by Russell and Burch (1959) as the three Rs—Refinement, Reduction, and Replacement. They called for a Refinement of experimental techniques to reduce animal pain and distress, a Reduction in the number of animals used and, where possible, a Replacement of animals by in vitro methods. These principles are widely adhered to by research scientists including those at the National Institutes of Health (Whitney, 1989). The unaffiliated member can suggest bibliographical sources to people in animal protection groups and can cite standards that are being practiced within the local research community. As a result, both animal protection groups and the general public can be reassured that the institution is following high standards in caring for animals. It has been my experience that prejudicial and negative attitudes of many people of good will in the scientific community are based upon misinformation regarding the standards that are mandated and practiced. The unaffiliated member can serve as a resource person in a liaison role with the community.

A Fresh Perspective

The unaffiliated member can bring to the IACUC a fresh, different, and questioning perspective to the animal research enterprise. Some people within the research community believe that almost any type of research that uses animals is justified. Other scientists think that animal welfare can be "objectively" determined. Justification of research and assessment of animal welfare are value judgments and strongly held opinions on this issue often compromise the researcher's ability to be open and receptive to countervailing judgments. Tannenbaum (1991) contends that investigators who have a "pure science" conception of animal welfare investigation make value judgements without realizing it and thus such a "model fundamentally misconstrues the nature of animal welfare." A significant contribution can be made by the unaffiliated member who has less of a vested interest in the conduct of any given research protocol than a scientist and can approach the evaluation of each protocol in a more dispassionate way. The problem of special interest is particularly applicable when the research is to be reviewed and approved for funding by a peer group. Peer review does not include, by definition, non-expert participation, and the only point in the review process where unaffiliated persons are involved is at the IACUC level. As an unaffiliated member of an IACUC, who holds a Ph.D. in comparative literature, states

> There are, however, some very real advantages to being a layperson on the Committee. Because I am not involved in biomedical research, I believe I can be more objective about the outcome of particular research proposals. I have no vested interest...I have no personal stake in the outcome of the individual projects; nor do I personally know most of the faculty members submitting proposals for our review. ...[Laypersons play an essential role] in keeping the Committee 'honest' and ensuring that it conducts its business in a manner and language understandable to all (Baker, 1987).

Another unaffiliated member, who had a background in Greek philosophy and biochemistry, underscored the importance of the liaison role and stated that his task "is to build trust between the community and the research community" (Farr, 1989).

We will now turn to an examination of the role of the lay member, mandated by PHS Policy, on the IACUC. As noted above, the lay member may or may not be the same individual as the unaffiliated member. It should also be noted that the AWRs do not call for a lay member although it is stated in the regulations that it is intended that the unaffiliated member represent community interest in IACUC discussions. This does not say that a scientist could not represent community interests but it is usually interpreted to mean just that.

Review by a lay member is often troubling to investigators because the individual is not a researcher and not a scientist. Conversely, the technical language in the protocol, at least for the lay member, may obscure both the ethical issues and the science involved in the research proposal. Over the years, I have frequently had to inquire, "What are you **really** doing with these animals? How invasive is the procedure?"

In other words, I want to be informed, in layperson's language, exactly what the protocol entails. I have often asked the investigator or the chairperson of the review committee to summarize in ordinary words the nature of the protocol and the scope and methods involved in the proposal before it is discussed by the committee. Lay members of the committee invariably applaud this suggestion. This is a simple suggestion, but it makes the lay member feel that there is a desire on the part of the committee to communicate on a non-technical level. Lay members are often intimidated and frustrated because they are not in the scientists' circle and are often hesitant to ask questions that might facilitate communication.

We come now to the very delicate issue of recognizing pain and distress in animals and the validity of anthropomorphic projection. It is generally recognized that there is a subjective element in addressing the question of pain while at the same time a trained person can recognize patterns of behavior manifested when the animal is experiencing discomfort and pain (NRC, 1992, P. 32). However, it is possible for the investigator to focus on the goals of the research effort and become inured to the stress or pain the animal is experiencing. One of the functions of the committee is to review situations where this might occur. But the question remains: Does the lay member have a unique or special role in protocol review by bringing an intuitive dimension to the process that might be based upon an unsophisticated experience with animals? I raise this as a question only. I recognize full well the dangers of anthropomorphic projection based upon intuition but comments by the lay person that express such feelings might serve to enhance the review process. The committee has the responsibility to consider such views respectfully. As one commentator puts it, "The community member should be made to feel that he will receive prompt, honest answers to his questions about animal care and use" (Gerrity, 1989).

While formerly an affiliated and lay chairperson of an IACUC, I now serve as an unaffiliated and lay committee member. I must confess that, in my present role, I have felt at times that my comments and concerns were considered by some members of the committee as a form of harassment having little or no substantive value. Unless this type of feeling is openly addressed by the "outside member" with the committee, mutual respect and dialogue will be threatened. Lay members are not scientists, that is why they are on the committee. Lay members should not be treated in a patronizing way, nor should lay members have a chip on their shoulders; they should be equally respectful of the scientist's interests and professional expertise. Mutual understanding and respect is fundamental to fulfilling successfully the intent of the mandate that a community representative serve on the committee. When an adversarial relationship develops among committee members, it defeats the purpose of the interdisciplinary nature of the group. This is why selection of the unaffiliated member and the lay member should be made with great care and deliberation.

THE SELECTION OF THE UNAFFILIATED AND LAY MEMBERS

First, who should be appointed? A general profile of the qualifications of both unaffiliated and lay members can be inferred from the preceding discussion of the productive roles these members might play on the committee. The frequent comment is made: Where do we find such people or a person if the unaffiliated member is also to be the lay member? The task is difficult, but not impossible. A few of the desirable characteristics that these members might possess are as follows:

- Someone who owns animals and cares deeply about their welfare but, at the same time, recognizes the value of animal research.
- A member of a local (city, county, or state) animal regulation body who is involved in formulating laws pertaining to animal use and care, who hears claims about the abuse of animals or is responsible for enforcing animal care regulations.
- A member of a local humane society or animal welfare group who understands the legal and ethical standards that apply to the appropriate use of animals.
- A person who loves animals, who has been a patient and who has personally benefitted from research with animals. A helpful resource is the Patient Coalition, a national voluntary health organization. (For more information contact incurably ill For Animal Research (iiFAR) National Headquarters, Box 27954, Lansing, MI 48909. Tel: 1-517-887-1141).
- A science or biology teacher who uses animals in the classroom for instructional purposes.
- A professor of ethics in a neighboring institution.
- A lay volunteer in a medical institution who is concerned about patient care and new developments in medical research that will enhance the well-being of patients (such as an IRB member in another institution).
- A public-spirited citizen who is active in community organizations that serve the needs of the community, e.g. ministers, lawyers, and non-professionals.
- Geologists, engineers, physicists, or other non-biological scientists

You will note that I have not mentioned a veterinarian or a biomedical scientist. These professionals are already amply represented on the committee. I believe that the unaffiliated member should be truly a lay person, i.e. a non **biomedical** scientist, who can faithfully represent the interests of the public. There are others, however, who feel that the unaffiliated member can be a scientist, even a veterinarian in private practice or a biological scientist. This is based on the belief that possessing a scientific background means that they can understand the science in the protocols and what is actually happening to the animals and, thus, are able to better represent community interests during protocol review.

The second issue relates to the question of the selection

process of the unaffiliated member. The following suggestions may be helpful:

- Canvas the groups mentioned above and solicit their recommendations.
- The chairperson of the committee and the administrative liaison officer should interview the candidate.
- Before selection, take the candidate on a tour of the facility, meet investigators and vivaria staff. Respect the staff's reactions to potential members.
- Inquire about what experience the candidate has had with other community and public service organizations. What does the candidate believe to be his or her constituency?
- What does the individual understand by the term, "humane" care and use of animals in research? Is the candidate in sympathy with the principle that research with animals is necessary for the progress of medical science and the advancement of knowledge? Discuss the possibility that the candidate may feel that a particular research protocol is ethically or legally inappropriate and indicate the procedures that are available, in the pursuit of such a grievance, within the administrative structure of the institution other than a negative vote within the committee. I feel strongly about this matter. Occasionally the unaffiliated member will give the only dissenting vote. The vote is properly recorded and that is the end of the matter. The established methods of appeal (which may vary from institution to institution) should be clearly explained to each member. Otherwise, unaffiliated members may find their experience on the committee very frustrating and their voice unheeded.

CONCLUSION

These are some personal observations about the role and selection of unaffiliated and lay members after having served as both as affiliated chairperson of an IACUC and as an unaffiliated member over the past ten years. Hopefully these reflections will be provocative in order to elicit careful consideration of the role of the unaffiliated member. It is my belief that the appointment of unaffiliated members is frequently taken too lightly and is often considered an unwelcome burden that has been necessitated by legal mandate. There is no question that institutions doing research involving the use of animals are often challenged. Therefore, it is very important that they retain or regain the confidence of the public and that their research follows the highest ethical and legal standards. A credible unaffiliated member can be of immeasurable assistance in this regard.

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Connecting Two Worlds

A High School Teacher's Work on an IACUC Fosters Communication Between Scientists and Students

Susan Riddell Sprouse

Seven years ago, I had never even heard of an Institutional Animal Care and Use Committee (IACUC)—despite the fact that I had been teaching biology, life science, and health at Beverly Hills High School for 15 years and was the sponsor of an animal welfare club at my school. Therefore, when Vice-Chancellor Albert Barber from the University of California, Los Angeles (UCLA), asked me to be the federally mandated unaffiliated member of the UCLA Animal Research Committee, I was intrigued yet also apprehensive. I was intrigued by the opportunity to be actively involved in assuring the humane treatment of animals used in research and to gain firsthand knowledge of current research, yet I was apprehensive because I lack a research background and because of the increasing negative public perception of animal research orchestrated by animal rights groups.

During my initial interview with Dr. Barber, I was pleased to learn that the research community has addressed many of the concerns expressed in recent years in regards to the ethical treatment of animals. It was enlightening to learn that under current regulations some of the experiments I did during my college physiology classes are no longer considered appropriate, and in many cases fewer animals are used to achieve the same teaching objective. Much of this change can be attributed to prodding by animal rights groups and to federal policies governing animal research issued in the mid-1980s (Animal Welfare Regulations, 1985; Animal Welfare Act; PHS, 1986). My students often discuss their concerns with me regarding animal research, and participating on this committee seemed like a good opportunity to learn firsthand just what was being done to protect animals in research institutions. After discussing with Dr. Barber the purpose of the committee, the time commitment involved, and the potential risk of harassment from extremist groups, I agreed to give it a try.

I have since learned that the unaffiliated member is often a source of concern for other members of the committee, but I have found that my background as a biology teacher has helped alleviate some of these concerns. Although I do not have a research background, my training in the sciences has definitely helped me in dealing with the technical terminology needed for reviewing research protocols, and it has also given me an understanding of the research process, which allows me to understand problems that may arise in the course of a research project. I also find that the other committee members are more than willing to answer any questions I raise about actual procedures. Because I interact daily with my students, who loudly voice their concerns about animal research, I bring this perspective of the community at large into the research and teaching activities of the university world.

At a recent national conference of biology teachers from various parts of the United States, I concluded that most of the participants were totally unaware of IACUCs and their function. Therefore, when I talk with colleagues about my work with UCLA's committee, I can help bridge the gap between the committee and the public by clarifying the IACUC's work as well as by developing an understanding of the importance of the IACUC process in protecting animals while not restricting ethically sound research.

My work on the committee has been a mutually beneficial arrangement. I have been able to contribute to the committee, and the committee provides me with valuable experience that enriches my teaching and therefore benefits my students. I am somewhat embarrassed to admit the two initial reflections I had after I attended my first monthly meeting. First, I found it a real pleasure to work with adults who did their homework! (I spent 8 to 10 hours reading the protocols for that first meeting, but my preparation time gradually decreased to 2 to 3 hours as I became more familiar with the material). Second, I was struck by how pleasant it was to be in a situation where my opinion and experience were respected and appreciated and where my views were solicited. I have never been made to feel that any question I ask or concern I raise is unworthy of discussion, and I have been made to feel I am an integral part of the process.

In 1987, UCLA sent me to a conference in Boston sponsored by Public Responsibility in Medicine and Research (PRIM&R) and Tufts University School of Veterinary Medicine. This conference proved to be memorable to me for two reasons. First, it was exciting and enlightening for me to meet with so many people actively committed to the welfare of animals and working hard to put their concerns into action, giving me a glimpse of the national commitment and overall process. Secondly, it gave me an opportunity to reunite with a student from my first biology class, Dr. Michael Grodin, an Associate Professor of medical ethics at Boston University and a member of the Board of Directors at PRIM&R—a real treat!

During the past 6 years, because of my IACUC membership, I have had the opportunity to attend other conferences

Ms. Sprouse is a biology teacher at Beverly Hills High School and has been the unaffiliated member of the University of California, Los Angeles IACUC since 1986.

at the University of Southern California (USC) and in San Francisco. Most recently I attended a National Association for Biomedical Research Conference in Washington, D.C., and my reaction was the same as always: renewed excitement and energy toward the task of the IACUC in general and the UCLA committee in particular. Whenever I am tired or feel as if the work is unimportant or not worth the effort, attending the conferences invigorates me and sustains my sense of purpose and dedication to the ultimate goal of humane treatment of laboratory animals used in research.

My work on this committee has been helpful to me in the classroom. When students come to me with concerns about animals used in research, I can tell them based on personal observations about the safeguards that are in place, about the facilities in which the animals are maintained, and how the numbers of animals used have been reduced. Students often come to class with outdated photos and stories of animal torture under the guise of science, and I am able to say with certainty that I know such abuses no longer occur, at least not at UCLA or at any other institution that receives public funds for research and consequently must have an IACUC. Not only have the numbers of animals used in research been reduced, but the committee consistently requests modifications in the protocols in answer to concerns about animal welfare. Reducing stress and promoting the animal's well-being lead to more accurate experimental outcomes for the researcher. My students' interest has encouraged me to continue my involvement with the committee.

I have also been able to talk directly with students who are not in my classes through the animal welfare club I sponsor at school. The club focuses primarily on preservation of endangered species, but has also dealt seriously with alternatives for animal research. I often relate a comment I heard at a USC conference from a strident animal rights activist. She does not wear leather or eat meat, she says, because there are satisfactory alternatives to those uses of animals by humans, but she does support the use of animals in some types of research because to date, there are often no satisfactory alternatives.

Learning about the "cutting edge" of biomedical research has also added a new dimension to my classroom teaching Without compromising confidentiality, I have been able to relay to my students some of the latest ideas and innovative methodologies that are being pursued in research. The university professors on the IACUC have also been helpful in working with some outstanding students from my school, and on occasion research scientists will speak at our high school.

One of the major drawbacks of being involved in an IACUC is the considerable time commitment involved. I read a minimum of 2 to 3 hours each month in preparation for the monthly 2 to 3 hour meeting at UCLA, and this takes place after a full day of teaching. Also, the regulations require biannual "walk-through" inspections of all animal facilities, which take another 4 to 5 hours each. For these reasons I appreciate the fact that the UCLA IACUC has two unaffiliated members, so that in an emergency or when schoolwork is too overwhelming, I can miss a meeting without being concerned that the federal guidelines are being compromised.

I would highly recommend that other IACUCs consider using local high school biology teachers as unaffiliated members. By involving teachers, the committee gains people who are interested in the scientific process, knowledgeable enough to understand most of the protocols, and in touch with community and student concerns about animal welfare. Teachers benefit by participating in a program that helps to ensure the welfare of animals used in research, keeps them informed of current research in their field, and establishes university contacts that can benefit both the teacher and students.

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IRAC Recommendation on LD_{50} Testing

Interagency Research Animal Committee (IRAC)

INTRODUCTION

The Interagency Research Animal Committee (IRAC) recommendations on LD_{50} testing presented below was adopted by IRAC on June 23, 1993. IRAC is a focal point for federal agencies to discuss issues involving all animal species needed for biomedical research and testing, especially their care, use, and conservation. Responsibilities include promoting information exchange, contributing to developing unified federal policies, coordinating animal programs among agencies, and representing the U.S. government on international issues. Agencies represented on the committee are the Veterans Administration, Department of Energy, National Aeronautics and Space Administration, Environmental Protection Agency, Department of the Interior, Department of State, Department of Defense, National Science Foundation, U.S. Department of Agriculture, Consumer Product Safety Commission, and Department of Health and Human Services, including the National Institutes of Health (NIH), Fogarty International Center, Centers for Disease Control and Prevention, Office of International Health, the Health Research Services Administration, and the Food and Drug Administration. The NIH serves as the lead agency and Dr. James F. Taylor, Office of Animal Care and Use, NIH, is the chairman. These recommendations were prepared by a subcommittee as a revision of recommendations adopted in October 1989. The subcommittee included Drs. Kailash Gupta, Consumer Product Safety Commission; Helene Guttman, U.S. Department of Agriculture; Louis Sibal, NIH; William Stokes, National Institute of Environmental Health Sciences; and James Vickers, Food and Drug Administration.

RECOMMENDATION

The LD_{50} test evaluates acute lethality from exposure to a substance or product. An LD_{50} value is the dose at which 50 percent of the test animals can be expected to die. The test is used to (1) classify substances or products for regulatory purposes, including safe transportation and labeling; (2) provide information for treatment of acute intoxications; (3) standardize certain biological products; (4) set dose levels for subsequent toxicity studies; (5) provide comparative information on the chemical's dose response curve; and (6) provide data for evaluation and validation of alternative test methods. The LD_{50} tests have become controversial among toxicologists, animal welfare organizations, legislators, and the public primarily due to the ethics of using a large number of animals and evaluating only mortality. The Interagency Research Animal Committee (IRAC) studied this issue in depth and recommends the following:

1. The **Classical LD_{50}** test should only be conducted when specifically justified for reasons of scientific necessity and approved by the institutional animal care and use committee (IACUC).
2. Toxicity testing procedures based on the principles of reduction and refinement (such as the Limit test) should be used until alternative test methods become validated.

BACKGROUND

IRAC has reviewed the policies and recommendations of federal agencies and international organizations relevant to the LD_{50} test. These organizations include the Environmental Protection Agency, the Food and Drug Administration, the Consumer Product Safety Commission, the Department of Transportation, the National Toxicology Program[1], the Organization for Economic Cooperation and Development, the British Toxicology Society, and the European Chemical Industry Ecology and Toxicology Center. There is consistency among the policies and recommendations on the following points:

1. The use of the **Classical LD_{50}** test, which may require the use of 100 or more animals to establish the desired statistical confidence limits and evaluates only mortality, is unnecessary for the determination of acute oral toxicity.
2. Tests involving animals are currently essential in the determination of acute oral toxicity, which is a step in the assessment of the potential hazard of a chemical or product.
3. There are recommended alternatives to the **Classical LD_{50}** test using the principles of **reduction** and **refinement** (see Summary of Current Policies).
4. A validated in vitro test or battery of tests is presently not available that can be used as a replacement for tests on animals in the determination of the LD_{50} dose. There are tests in various stages of development and validation. Instead of

[1] The National Toxicology Program includes the National Institute of Environmental Health Sciences of the National Institutes of Health, the National Center for Toxicological Research of the Food and Drug Administration, and the National Institute for Occupational Health and Safety of the Center for Disease Control.

waiting for a validated test (or tests) for the universe of chemicals or products, the process of development and validation may be accelerated using a group of related chemicals or products. For that group, a validated test (or tests) could be accepted as a screen or as a replacement of the LD_{50} test on animals.

DEFINITIONS

Classical LD_{50}

The Classical LD_{50} test is used to determine the lethal dose (LD) of a substance that will kill 50 percent of test animals. Typically, this method can use 100 or more animals. The test material is administered in increasing doses, usually five or more, to groups of 10 male and 10 female animals. Mortalities are recorded within a given period, and the LD_{50} is determined with the aid of statistical calculations.

Limit Test

The Limit test is used to determine if the toxicity of a test substance is above or below a specified dose. Five to 10 animals of each sex or 10 animals of the susceptible sex are administered a dose specified by regulations. Toxic responses occurring within a given period are recorded. Based on the results, a regulatory action or additional testing may be required.

SUMMARY OF CURRENT POLICIES

U.S. Organizations

Consumer Product Safety Commission:

- Strongly discourages Classical LD_{50} test.
- Recommends other alternatives—existing animal data, prior human experience, expert opinion.
- Recommends the Limit test.

Department of Transportation

- Discourages the use of the Classical LD_{50} test.
- Recommends the use of existing animal data and human experience.
- Recommends the Limit test.

Environmental Protection Agency

- Discourages the use of the Classical LD_{50} test.
- Uses Structure Activity Relationships (SAR) to obviate the need for testing on animals.
- Recommends the Limit test.
- When testing beyond the Limit test is conducted, recommends use of abbreviated test methods such as the approximate lethal dose, the moving average, and the up-and-down methods. Stresses the need for multiple endpoint evaluations, including onset, nature, reversibility of effects, and gross necropsy.

Food and Drug Administration

- Does not require the use of the Classical LD_{50} test.
- Accepts alternatives
- Refers to the Limit test.

National Toxicology Program

- Does not use Classical LD_{50} test.
- Uses in-depth toxicology with many endpoints.

International Organizations

Organization for Economic Cooperation and Development

- Discourages the use of the Classical LD_{50} test.
- Recommends the Limit test (2 g/kg dose).
- When compound related mortality occurs in the Limit test, recommends five animals per dose and recommends using at least three dose levels to produce a range of toxic effects and mortality rates. Clinical observations and pathological investigations should be conducted.
- Also recommends a fixed dose procedure, which uses morbidity instead of mortality as the endpoint.

British Toxicology Society

- Recommends that the LD_{50} test only be determined with any accuracy where scientifically and ethically justified. Such cases are relatively rare.
- Recommends examination of few animals in detail rather than many for statistical purposes.
- Allows that Limit tests could be used, provided animals in distress are killed humanely unless this would interfere with the study objectives.
- For classification of substances and preparations, suggests using a fixed-dose procedure targeted to acute signs to replace the current practice of LD_{50} determination.

European Chemical Industry Ecology and Toxicology Center

- Recognizes importance of acute toxicity profiles.
- Maintains that the Classical LD_{50} test is seldom necessary.
- Maintains that protocols exist for estimating lethal dose.
- Believes that the LD_{50} above 2 g/kg is irrelevant.
- Recommends that regulations not specify a minimum number of animals.
- Encourages procedures for the selection of a toxicity class.
- States that acute toxicity data from some products may be unnecessary for the protection of human health.
- Encourages predictions based on alternatives as an aid to dose selection.

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Adopted by IRAC—September 22, 1993.

In the News

ILAR Guide Committee Adds Unaffiliated Member

A new member from outside the biomedical research community has been added to the Institute of Laboratory Animal Resources (ILAR) committee to revise the *Guide for the Care and Use of Laboratory Animals* (*Guide*). The *Guide* is considered the standard reference on laboratory animal care by private organizations, federal agencies, and the scientific community.

Jo Ann D. Steggerda, a community representative on the University of Illinois Laboratory Animal Care Advisory Committee, was appointed in response to recommendations made by participants at public meetings held by the Committee to Revise the Guide earlier this year. She has begun seeking issues to present to the committee and requests comments from individuals and organizations interested in animal protection (see address below).

"Mrs. Steggerda adds an important perspective to the committee," said committee chair J. Derrell Clark, professor at the College of Veterinary Medicine, University of Georgia, Athens. "Her commitment to animal welfare and to representing the broad interests of the public will constitute a valuable contribution to our work."

Mrs. Steggerda also is a member of the Humane Society of Champaign County, American Society for the Prevention of Cruelty to Animals, World Wildlife Fund, Incurably Ill for Animal Research, and the Nature Conservancy.

Other committee members include experts in various scientific fields including laboratory animal medicine, behavior, husbandry, philosophy, medical ethics, and the study of common and uncommon animal species used in research, education, and testing.

The *Guide* is recognized as the standard reference on laboratory animal care by private organizations and federal agencies, such as the American Association for the Accreditation of Laboratory Animal Care and the Public Health Service. More than 400,000 copies have been distributed since it first was published in 1963. The committee's revision, expected to be completed by late 1995, will be published as the seventh edition.

For more information contact Dr. Thomas L. Wolfle, ILAR, 2101 Constitution Avenue, NW, Washington, DC 20418. Tel: 1-202-334-2590; Fax: 1-202-334-1687; Email: twolfle@nas.edu; or Dr. J. Derrell Clark, College of Veterinary Medicine, University of Georgia, Athens, GA 30602-7381. Tel: 1-706-542-4173; Fax: 1-706-542-3897; Email: clark.d@calc.vet.uga.edu. Comments for Mrs. Steggerda may be sent to P.O. Box 7314, Champaign, IL 61826-7314.

ILAR Workshop Recommends Study of Permitting Process

Thirty-two scientists, agency representatives and administrators, and representatives of professional societies recently met to discuss how policies and regulations governing the collection and importation of biological materials, animals, and plants affect scientific research. The participants of the workshop, which was sponsored by the Institute of Laboratory Animal Resources (ILAR), National Research Council (NRC), agreed that the NRC should recommend ways to improve the current permitting procedures. They identified the following principal issues: scientists' lack of information about requirements and application procedures; poor communication among regulating agencies and between scientists and regulating agencies; a need for better resource management and for streamlining the permitting process particularly given shrinking resources; and a need for better collaborative arrangements with foreign countries. There was also a call for an NRC-appointed forum of agency personnel, representatives of professional societies, and scientists to meet several times a year to address specific problems and identify issues requiring in-depth study. For more information contact Dr. Ralph Dell, Visiting Scientist, ILAR, National Research Council, 2101 Constitution Avenue, N.W., Washington, D.C. 20418. Tel: 1-202-334-2590; Fax: 1-202-334-1687; Email: rdell@nas.edu.

Tufts University Reports on the Animal Research Controversy

The Center for Animals and Public Policy recently released a report entitled *The Animal Research Controversy: Protest, Process & Public Policy.* The report, funded by the Pew

Charitable Trust, analyzes key issues and discusses arguments and tactics used both by proponents and opponents of animal research. Dr. Franklin M. Loew, co-author of the report and the dean of the Tufts Veterinary School of Medicine, held a press conference in Washington, D.C. on March 2, 1994 to discuss the release of the report. The news conference was attended by scientists, members of scientific societies, members of animal protection groups, and members of the press. The report, Loew said, discusses current public attitudes concerning the use of animals in research, ethical and philosophical concerns, the number of animals used in research, and the fact that the degree of pain and distress animals experience during research is not known. Finally, he stressed that the principal conclusion of the report was that the scientific community and animal protectionists should strive for a middle ground. For more information contact the Center for Animals and Public Policy, Tufts University School of Medicine, 200 Westboro Road, N. Grafton, MA 01536. Tel: 1-508-839-7991; Fax: 1-508-839-2953.

SCAW to Conduct IACUC Survey

The Scientists Center for Animal Welfare (SCAW) has initiated a project to evaluate institutional animal care and use committees (IACUCs). Although certain elements of IACUC functions are defined in the legislation, there are a variety of ways in which IACUCs can be organized, select their members, and carry out their responsibilities. To date, there have been no comprehensive efforts to gather data about IACUC operations

SCAW will sponsor both a survey of regulated research institutions and IACUC chairpersons and members in order to develop an aggregate picture of IACUC operations nationally and to identify "best practices" in IACUC operations. The results of the survey will be published and used to stimulate dialogue about best practices among regulated institutions, and may also become the basis for a conference on the present standing of IACUCs. For more information contact SCAW, 4805 St. Elmo Avenue, Bethesda, MD 20814. Tel: 1-301-654-6390; Fax: 1-301-907-3993.

Animal Care Matters

A 24-minute video program, designed to aid institutions in providing effective education to animal research personnel as required by government regulations and guidelines, is now available. Produced by the Committee on Animal Care at the Massachusetts Institute of Technology (MIT) with major funding provided by the Edna H. Tompkins Trust, the program includes discussions of the ethical and moral issues concerning animal research, the responsibility of research personnel for ensuring humane treatment of research animals, applicable legislative and regulatory guidelines, the roles and functions of institutional animal care and use committees, and the issue of alternatives to animals in research. Copies (VHS format) cost $20.00, including shipping and handling, and can be ordered from Animal Care Matters, MIT, 37 Vassar Street 45-105, Cambridge, MA 02139. Tel: 1-617-253-1758.

Coming Meetings

May 1994

5–6 Ethical Implications of the New Genetics, Boston, Massachusetts. This conference will be hosted by Public Responsibility in Medicine and Research (PRIM&R) and the Tufts University School of Medicine and will include presentations by a diverse faculty of genetic researchers; ethicists; policy analysts; representatives from the National Institutes of Health and the Office of Technology Assessment; genetic counselors; research administrators; and representatives from advocacy groups, pharmaceutical and biotech companies, and other genetic testing organizations. Topics to be covered at the conference include an update on the human genome initiative; issues of race, class, and gender in the new genetics; the ethical and policy issues surrounding pedigree studies; the clinical and psychosocial implications of assisted reproduction; the Institutional Review Board's role in reviewing the new genetics; and the development of training programs for researchers and health care providers who grapple with the ethical implications of the new genetics. For more information contact PRIM&R, 132 Boylston Street, Boston, MA 02116. Tel: 1-617-423-4112.

5–6 Training and Education: Institutional Improvement—Crisis Prevention, Lafayette, Indiana. The National Institutes of Health (NIH), Office for Protection from Research Risks is cosponsoring this Animal Welfare Education Workshop with Purdue University. The workshop will focus on continuing education and training as mandated by the NIH and the U.S. Department of Agriculture. The format will include plenary presentations and panel discussions on the various topics. Concurrent breakout sessions will be offered. The workshop is open to institutional administrators, members of institutional animal care and use committees, laboratory animal veterinarians, investigators, technicians, as well as any person sharing responsibility for the management of an institutional animal care and use program. For more information please contact Ms. Lisa D. Snider, Admin-

istrative Assistant, Laboratory Animal Program, Purdue University, 1071 South Campus Courts-D, West Lafayette, IN 47901-1071. Tel: 1-317-494-7206; Fax: 1-317-494-0793.

12–13 Research Animal Anesthesia, Analgesia, and Surgery, Atlanta, Georgia. The Scientists Center for Animal Welfare (SCAW) will be conducting its 1994 conference on research animal anesthesia, analgesia, and surgery. Topics to be covered include U.S. Department of Agriculture, National Institutes of Health, and American Association for the Accreditation of Laboratory Animal Care requirements for surgical programs, surgical training and personnel qualifications, recognizing pain and distress in research animals, physiological effects of anesthetics and analgesics, cardiopulmonary complications and emergencies in surgery, and post-surgical care. Researchers, regulatory personnel, members of institutional animal care and use committees, administrators, and others interested in these issues are encouraged to attend. For more information contact SCAW, 4805 St. Elmo Avenue, Bethesda, MD 20814. Tel: 1-301-654-6390; Fax: 1-301-907-3993.

May 31–June 3 Descriptive Veterinary Pathology, Bethesda, Maryland. This course is designed to teach attendees how to describe both gross and microscopic lesions in a variety of major organs. The course will also include lectures on interpretation and description of electron micrographs. Practice tests will be given and graded to provide feedback. The objective is to increase skill at describing gross and microscopic lesions in animal tissues, which is necessary for success on the American College of Veterinary Pathologist's certifying examination.

July 1994

10–14 Eighth International Workshop on Immunodeficient Animals, Utrecht, The Netherlands. The International Workshop on Immunodeficient Animals acts as a forum to bring together breeders and users of immunodeficient animals. It combines symposia on general themes in immunodeficient animal research and workshops on special topics. The preliminary list of topics for the symposia and workshops include immunodeficient mouse and rat mutants, spontaneous autoimmunity and allergy, experimentally induced autoimmunity or allergy, transgenesis associated with immunodeficiency, retrovirus infection, T-cell differentiation, autoimmunity, hypersensitivity, infectious disease, oncogenesis, and molecular genetics. For more information contact P. de Vrey, Central Animal Laboratory, Pb43, National Institute of Public Health and Environmental Protection, P.O. Box 1, 3720 BA Bilthoven, The Netherlands. Tel: 31-30-742335; Fax: 31-30-252178.

12 Wildlife Mammals as Research Models: In the Laboratory Animal Field, San Francisco, California. The Scientists Center for Animal Welfare (SCAW) will sponsor this half-day seminar at the 1994 American Veterinary Medicine Association conference. Topics to be covered include wildlife management in the laboratory, contraceptive research and non-capture methods for studying reproduction in wildlife, marking and tracking, aquatic research, and positive reinforcement training. Veterinarians, researchers, regulatory personnel, members of institutional animal care and use committees, administrators, and others interested in these issues are encouraged to attend. For more information contact SCAW, 4805 St. Elmo Avenue, Bethesda, MD 20814. Tel: 1-301-654-6390; Fax: 1-301-907-3993.

10–15 A Summer Course on Issues of Animal Experimentation, Washington, D.C. This course is open to college faculty who would like to improve their skills in teaching about ethical issues surrounding animal experimentation to graduate and undergraduate students. Emphasis will be on how to use course materials in classroom instruction. Topics include the moral status of nonhuman animals, the justifications for using animals in research and education, student objections, the use of alternatives, animal pain, legal issues, and the importance of species. For more information contact Marc Favreau, Kennedy Institute of Ethics, Georgetown University, Washington, D.C. 20057. Tel: 1-202-687-6771; Fax: 1-202-687-6770.

27–31 Animal Behavior Society Meeting, Seattle, Washington. The goal of the Animal Behavior Society is "to promote and encourage the biological study of animal behavior in the broadest sense, including studies at all levels of organization using both descriptive and experimental methods under natural and controlled conditions." Presentations at the meeting will be multidisciplinary, ranging across zoology, psychology, and anthropology. Special sessions on applied animal behavior and psychoneuroimmunology will be scheduled. There will be a joint session with the American Society of Primatologists on Thursday, July 28, with an emphasis on the behavior of primates. For more information contact James C. Ha or Carolyn Crockett, Primate Center SJ-50, University of Washington, Seattle, WA 98195. Tel: 1-206-543-1440; Email: jcha@u.washington.edu. or crockett@u.washington.edu.

31 July–4 August International Congress of Vertebrate Morphology, Chicago, Illinois. This will be the first International Congress of Vertebrate Morphology held in North America. It will include contributed oral and poster papers, plenary speakers, workshops, and symposia on such topics as the functional design of musculoskeletal systems, segmentation in vertebrates, genetics and morphology, adaptations of marine amniotes, and amphibian-amniote transition. For more information contact Sue Herring, Chair, ICVM Organizing Committee, Department of Orthodontics, SM-46, University of Washington, Seattle, WA 98195. Tel: 1-206-543-3203; Fax: 1-206-685-8163; Email: herring@u.washington.edu.

August 1994

4–5 Sharing Animal Welfare Responsibilities Between Affiliated Institutions, Portland, Oregon. This workshop, sponsored by the National Institutes of Health, Office for Protection from Research Risks and by the Department of Veterans Affairs in Portland, Oregon will explore the relationships among academic, government, and industry as they pertain to the care and use of laboratory animals and animal research facilities and programs. The focus of the workshop will address such issues as (1) sorting out collaborations and assuming responsibility, (2) the Veterans Administration vs. academia, (3) costs and benefits of industrial contracts and agreements, (4) building a shared institutional animal care and use committee, and (5) the regulatory agencies' perspective and oversight. This workshop is open to institutional administrators, members of institutional animal care and use committees, laboratory animal veterinarians, investigators, and technicians, as well as anyone sharing responsibility for management of a sound institutional animal care and use program. For more information contact Ms. Margaret Doherty, Veterans Affairs Medical Center, P.O. Box 1034, Portland, OR 92707-1034. Tel: 1-503-220-8262 ex. 7610; Fax: 1-503-273-5351.

8–12 Pathology of Laboratory Animals, Bethesda, Maryland. This 5-day course, offered at the Uniformed Services University of the Health Sciences, will provide a comprehensive overview of the gross and histopathological manifestations of disease in laboratory species, including rodents, rabbits, nonhuman primates, aquatic animals, and birds. Selected topics in clinical and ultrastructural pathology are also included, and an emphasis is placed on the recognition and interpretation of spontaneous lesions in laboratory animals. This course is designed for veterinarians who are either training or certified in veterinary pathology and laboratory animal medicine. For more information contact Uniformed Services University of the Health Sciences, 14th and Alaska Avenues, N.W., Washington, DC 20306-6000. Tel: 1-301-427-5231; Fax: 1-301-427-5001.

September 1994

29–30 Use of Animals in Research and Alternatives, New Orleans, Louisiana. This workshop is cosponsored by the National Institutes of Health, Office for Protection from Research Risks, the Louisiana State University Medical School, and Xavier University. It will address various aspects of the use of animals in research and education, including the adequacy of computer searches; National Institutes of Health, U.S. Department of Agriculture, and Food and Drug Administration initiatives in alternatives; occupational health; and the role of animals and alternatives in education. For more information contact Ms. Lois Herbez, Administrative Secretary, Louisiana State University Medical Center, 1542 Tulane Avenue, New Orleans, LA 70112. Tel: 1-504-568-4198; Fax: 1-504-568-4843.

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.

November 1996

3–7 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

Animals and Alternatives in Testing: History, Science, and Ethics—Joanne Zurlo, Deborah Rudacille, and Alan M. Goldberg. This book addresses the use of animals in toxicity testing and the scientific status of alternatives. It presents a balanced view that neither advocates a unilateral ban on cosmetic testing nor rejects the use of animals in biomedical research. It is a useful reference for researchers as well as legislators, members of the health and biomedical communities, and concerned citizens. It is written with the lay reader in mind and includes a glossary of terms in the back. Mary Ann Liebert, Inc., 1994, 86 pp., paperback, $35.00.

Language and Intelligence in Monkeys and Apes: Comparative Developmental Perspectives—Sue Taylor Parker and Kathleen Rita Gibson, eds. This is the first collection of articles completely and explicitly devoted to the new field of "comparative developmental evolutionary psychology," that is, to studies of primate abilities based on frameworks drawn from developmental psychology and evolutionary biology. These frameworks include Piagetian and neo-Piagetian models as well as psycholinguistic ones. The articles in this collection, originating from Japan, Spain, Italy, France, Canada, and the United States, represent a variety of backgrounds in human and nonhuman primate research, including psycholinguists, developmental psychology, cultural and physical anthropology, ethology, and comparative psychology. The book focuses on such areas as the nature of culture, intelligence, language, and imitation; the differences among species in mental abilities and developmental patterns; and the evolution of life histories and of mental abilities and their neurological bases. The species studied include the African gray parrot, cebus and macaque monkeys, gorillas, orangutans, and both common and pygmy chimpanzees. Cambridge University Press, 1990, 590 pp., hardback.

Viral Diarrheas of Man and Animals—Linda J. Saif and Kenneth W. Theil. This comprehensive book is a source of information for readers interested in the current state of knowledge surrounding enteric viral infections of medical and veterinary importance. It is organized into five sections: Historical Perspective, Comparative Properties and Pathogenicity of Enteric Viruses, Nonenveloped Enteropathogenic Viruses, Enveloped Enteropathogenic Viruses, Mixed Enteric Infections, and Vaccine Strategies and Immunity to Enteropathogenic Viral Infections. CRC Press, 1990, 343 pp., hardback.

Publications Available

The following publications are available free from ILAR:

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 Management: Rodents. 1977
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
***Standardized Nomenclature for Transgenic Animals.** 1993
Supplement to Animals for Research—A Directory of Sources. 10th ed. 1980
Third International Registry of Animal Models of Thrombosis and Hemorrhagic Diseases. 1988
Your Career in Veterinary Technology. AVMA Brochure. Updated Dec. 1989

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, Building 31, Room 5B59, 9000 Rockville Pike, Bethesda, MD 20892.**

* New Publications

PUBLICATIONS AVAILABLE FROM THE NATIONAL ACADEMY PRESS (NAP)

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.

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.

PUBLICATIONS AVAILABLE FROM THE NATIONAL TECHNICAL INFORMATION SERVICE (NTIS)

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

2101 Constitution Avenue, NW
Washington, DC 20418

Standardized Nomenclature for Transgenic Animals

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

Reprinted from *ILAR News*, Volume 34, Number 4. 1992

NATIONAL ACADEMY PRESS
Washington, D.C. 1993

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Preface

As part of its mission to promote efficient, cost-effective, ethical research with animals, and to those ends to identify valuable laboratory animal resources, the Institute of Laboratory Animal Resources (ILAR) of the National Research Council's Commission on Life Sciences was charged with addressing a new and rapidly developing application of molecular biology to animal experimentation termed *transgenic technology*. The development of a large number of new strains of animals with this technology has been accompanied by some specific deficiencies, as follows:

- There is no standardized nomenclature by which to identify transgenic animals.
- There are no guidelines for managing animals that might be difficult to maintain and breed, have the potential for transmitting diseases to humans, or might have an adverse impact on the environment if inadvertently released.
- There are no effective mechanisms for ensuring the preservation of valuable transgenic models.

To address those deficiencies, the ILAR Council organized an advisory panel,[a] which recommended a series of initiatives to assist scientists who work with or care for transgenic animals.

In recognition that the lack of a widely accepted standardized nomenclature for transgenic animals greatly complicates efforts to catalog existing resources, the recommended first initiative was that ILAR develop such a nomenclature. Accordingly, ILAR established the Committee on Transgenic Nomenclature to undertake this task and to delineate a means by which the nomenclature could be used to create a catalog of existing transgenic resources. The committee was constituted to include experts in the production of transgenic animals by various means, scientists with experience in issues related to animal nomenclature, and a member and an invited participant who are involved in the development of a data base on transgenic animals.

The committee felt that it would be sensible to establish a nomenclature that is compatible with the storage and efficient retrieval of information from the data base on transgenic animals. The nomenclature that it developed, therefore, includes the relevant differences in the various approaches to making transgenic animals, encompasses the diversity and complexity of the DNA elements that are introduced into the germline, and provides the conciseness required for the convenient use and efficient storage of information.

The committee acknowledges the assistance of the staff of ILAR which has made this report possible.

Jon W. Gordon, *Chairman*
Committee on Transgenic Nomenclature

Committee on Transgenic Nomenclature

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Contents

This study has been supported by the Howard Hughes Medical Institute (HHMI) through grant number 70209-500104 and by the U.S. Department of Health and Human Services (DHHS) through contract number NO1-CM-07316 with the National Cancer Institute. Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the committee and do not necessarily reflect the views of HHMI or DHHS, nor does the mention of trade names, commercial products, or organizations imply endorsement by the HHMI or the U.S. government.

ILAR's core program is supported by grants from the National Center for Research Resources, National Institutes of Health; National Science Foundation; American Cancer Society, Inc.; and U.S. Army Medical Research and Development Command, which is the lead agency for combined Department of Defense funding also received from the Human Systems Division, 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.

INTRODUCTION

The ability to achieve controlled genetic modifications of multicellular eukaryotic species by the insertion of genetic material, termed *transgenic technology*, has led to an explosion of research in developmental genetics, attracting the interest of many investigators who previously were not engaged in animal research. However, although many new animal models have been produced, there has been no organized effort to identify and catalog them. The lack of a catalog might keep research with the new technology from being conducted with maximal efficiency and effectiveness for several reasons:

- *Duplication of effort.* Many different transgenic animals already exist (perhaps as many as 10,000 strains). Without a catalog, investigators might needlessly reproduce existing models.
- *Loss of valuable transgenic models.* Many transgenic models are not relevant to the research programs of the laboratories in which they are made. When such models are made by insertional mutagenesis, they are often unique and irreplaceable. However, with today's funding constraints, laboratories cannot afford to maintain models that they are not using. In the absence of an effective method to make their existence known, such models would probably be discarded, even though they might be extremely valuable to investigators in other laboratories.
- *Loss of information.* Many of the subtleties of gene regulation are discernible only by comparing the expression of closely related transgenes in different strains. For example, only when different constructs of the human β-globin gene in transgenic mice were compared did it become apparent that DNA sequences derived from cloning vectors, such as bacteriophage or plasmids, could interfere with gene expression and that such interference could be overcome if multiple enhancer elements in the globin gene region were included in the transferred DNA construct. In the absence of a catalog of transgenic animals for comparative study, similar complexities of gene regulation might escape notice.

It is obvious that some registry of transgenic strains and their characteristics is essential. To that end, the Oak Ridge National Laboratory, Oak Ridge, Tennessee, under contract Y01-[illegible]567 from the National Institute of Environmental Health Sciences of the National Institutes of Health has undertaken the development of a data base for cataloging transgenic animals: the Transgenic Animal Data Base (TADB). For the TADB to be effective and to promote effective communication between scientists in different laboratories, a widely accepted standardized nomenclature for transgenic animals is necessary.

ISSUES IN DEVELOPING A TRANSGENIC NOMENCLATURE

For developing a transgenic nomenclature, the committee defined a transgenic organism as an organism in which new genetic material has been experimentally introduced into the germ cells. The new DNA can be derived from the homologous species and need not be genetically active; however, it should be heritable. Thus, animals with new gene insertions resulting from ecotropic retroviral infection are not transgenic, nor are those receiving DNA only in somatic cells. In contrast, mice with an additional mouse β-globin gene that has been inserted into a site other than that of the endogenous gene and transmitted to progeny as a Mendelian trait are transgenic. Animals with genetic modifications introduced by homologous recombination in embryonic stem cells are considered transgenic if the purpose of the homologous recombination approach is to target functional genetic elements into specific sites in the genome.

A transgenic nomenclature must incorporate information related to both the methods used to produce transgenic animals and the diversity of genetic elements that can be inserted into animals. It must also be concise enough to make it easily recognized and remembered. Thus, there is a potential conflict between the need for conciseness and the need for completeness of information. The same conflict was encountered by the International Committee on Standardized Genetic Nomenclature for Mice, which began work on nomenclature for transgenes in 1984. That committee proposed a general form that followed the format used for chromosomal anomalies: an abbreviation of the word *transgenic*, followed by parenthetic information on the inserted segment, followed by a unique laboratory code and a numerical designation. Although this nomenclature was widely circulated for review and the comments of experts in transgenic mice were incorporated, neither it nor the revised nomenclature published a few years later (Lyon, 1989a) has been widely used. A major problem has been deciding how much information on the inserted segment to include. Opinions vary from a three-character symbol to all the information known (the latter would sometimes mean a symbol that requires several lines of text).

Given that history and the present committee's own perception of the problems presented in creating a usable transgenic nomenclature, the committee decided that brief, unique identifiers of transgenic insertions would be most effective for transgene symbols. The full history and description of each transgenic insertion should be recorded in the TADB (described in detail in Appendix II).

In determining the elements of the transgene symbol, the committee concentrated its deliberations on the following issues:

- *The need to incorporate within the nomenclature a symbol that identifies important characteristics of the method used to create the new transgenic strain.* The committee concluded that it is important to distinguish between three approaches to making transgenic animals: methods that use modified retroviral vectors; gene-transfer methods, other than viral infection, that result in

nontargeted (nonspecific) gene insertion; and targeted insertions (homologous recombination).

• *The need to identify the salient characteristics of the new genetic material and the consequences of its insertion into the genome.* The committee recognized that it would not be possible to incorporate within a short symbol all the important features of a given genetic modification. Genes from different species, orders, or even kingdoms can be readily transferred into the germlines of mammals through transgenic technology; recombinant-DNA constructs can contain elements from several sources combined in one molecule; and new genetic material could produce important phenotypic changes even in the absence of its expression. Accordingly, the committee chose a nomenclature that would convey the following major categories of genetic modification: (1) the creation of an insertional mutation, (2) the insertion of coding, as opposed to noncoding, elements, and (3) the most important features of the construct as determined by the investigator. For example, in some cases, the coding region of a transgene might be the most relevant factor in the resulting genetic modification; in others, such as insertion of reporter gene constructs, the regulatory apparatus might be the most relevant characteristic.

• *The need to provide a unique identifier for each transgenic strain.* To facilitate identification of each transgenic strain and its source, it is essential that a unique designation conveying such information be part of the strain symbol.

• *The need to develop a nomenclature that can be adapted to species other than mice.* Although almost all existing transgenic animals are mice, transgenic technology can readily be extended to several other species, and transgenic nonmurine mammals, birds, and fish have already been developed. The committee chose to develop a general nomenclature that can be used in all species.

IMPLEMENTATION OF THE NEW NOMENCLATURE

For the nomenclature to contribute to better transgenic-resource management and preservation of transgenic animals, it must first be accepted for use by the major investigators in the field and then be made available to all those developing or using transgenic animals. To achieve the first objective, the committee sent the proposed nomenclature to several major laboratories involved in transgenic research including two that use species other than the mouse. Comments from those laboratories have been considered in developing the nomenclature.

To achieve the second objective, the committee solicited and has received endorsement of the nomenclature rules from the International Committee on Standardized Genetic Nomenclature for Mice and, in the absence of a functional international nomenclature committee for rats, from the ILAR Committee on Rat Nomenclature. In addition, membership on the present committee of one of the developers of the TADB has allowed us to develop a nomenclature that is compatible with the data base. Promulgation of the nomenclature will simultaneously increase awareness of the existence of the data base.

The committee also identified several other strategies for implementation, including sending the nomenclature for publication in journals that commonly contain papers involving research with transgenic animals and recommending that the editors of the journals make the nomenclature a requirement for acceptance of papers in this field. Moreover, this report has been sent to the categorical institutes of NIH that have an interest in transgenic technology with the request that the rules be provided to the appropriate grantees and contractors and to the study sections that evaluate grant and contract proposals involving transgenic animals.

In the next section, the committee formally proposes its transgene nomenclature. Information on obtaining assistance with naming transgenes or recording transgenes in the data base is contained in Appendix I.

RULES FOR NAMING TRANSGENES

Transgenes are named according to the following conventions.

Symbol

A transgene symbol consists of three parts, all in Roman type, as follows:

TgX(YYYYYY)#####Zzz,

where TgX = mode,
(YYYYYY) = insert designation,
= laboratory-assigned number, and
Zzz = laboratory code.

Mode. The first part of the symbol always consists of the letters Tg (for "transgene") and a letter designating the mode of insertion of the DNA: N for nonhomologous insertion, 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 among three fundamentally different organizations of the introduced sequence relative to the host genome not simply to indicate the method of insertion or nature of the vector. For example, mice derived by infection of embryos with MuLV vectors will be designated TgR, and mice derived by microinjection or electroporation of MuLV DNA into zygotes will be designated TgN; mice derived from ES cells by introduction of DNA followed by recombination with the homologous genomic sequence will be designated TgH, while mice derived by insertions of the same sequence by nonhomologous crossing-over events will be designated TgN.

When a targeted mutation introduced by homologous

recombination does not involve the insertion of a novel functional sequence, the new mutant allele (often called a "knockout" mutation) will be designated in accordance with the guidelines for gene nomenclature for each species. The gene nomenclature will also be 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 Lyon, 1989b). The mode symbol TgH is reserved for a time in the future when homologous recombination might be employed to transfer genes to specific sites in the genome using cloned DNA from the target cite to produce a homologous recombination vector. Such target loci might be anonymous, but might exhibit important regulator features that render them desirable for targeting transgenes. A hypothetical example is given on page 50.

Insert designation. The second part of the symbol indicates 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. The choice of the insert designation is up to the investigator, but the following guidelines should 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 insert designation should identify the inserted sequence and indicate important features. If the insertion uses sequences from a named gene, it is preferable that the insert designation contain the standard symbol for that gene. If the gene symbol would exceed the spaces available, its beginning letters should be used. Hyphens should be omitted when normally hyphenated gene symbols are used. For example, Ins1 should be used in the symbols of transgenes that contain either coding or regulatory sequences from the mouse insulin gene (*Ins-1*) as an important part of the insert designation. Resources are available to identify standard gene symbols (see Appendix I).
- Symbols that are identical with other named genes in the same species should be avoided. For example, the use of Ins to designate "insertion" would be incorrect.
- For consistency, a series of transgenic animals produced with the same construct might be given the same insert designation. However, that is not required; some lines might manifest unique and important characteristics (e.g., insertional mutations) that would warrant a unique insert designation. If two different symbols are used for the same construct in different transgenic lines, the published descriptions should clearly identify the construct as being the same in both lines. Two different gene constructs used for transgenic animal production, either within a laboratory or in separate laboratories, should not be identified by identical insert designations. Designations can be checked through the available resources (see Appendix I).
- A standard abbreviation can be used as part of the insert designation (see below for an example). If a standard abbreviation is used, it should be placed at the end of the insert. These now include

  An (anonymous sequence),
  Ge (genomic clone),
  Im (insertional mutation),
  Nc (noncoding sequence),
  Rp (reporter sequence),
  Sn (synthetic sequence),
  Et (enhancer trap construct), and
  Pt (promoter trap construct).

  This list will be expanded as needed and maintained by appropriate international nomenclature committees.
- The insert designation should identify the inserted sequence, not its location or phenotype.

Laboratory-assigned number and laboratory code. The third part of the symbol consists 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 laboratory code is uniquely assigned to each laboratory that produces transgenic animals. A laboratory that has already been assigned such a code for other genetically defined mice and rats or for DNA loci should use that code. The registry of these codes is maintained by the Institute of Laboratory Animal Resources (ILAR) (see Appendix I).

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. Each insertion retains the same symbol even

if it is placed on a different genetic background. Specific lines of animals carrying the insertion should be additionally distinguished by a stock designator preceding the transgene symbol. In general, this designator will follow the established conventions for the naming of strains or stocks of the particular animal used. If the background is a mixture of several strains, stocks, or both, the transgene symbol should be used without a strain or stock name.

Examples

- C57BL/6J-TgN(CD8Ge)23Jwg. The human CD8 genomic clone (Ge) inserted into C57BL/6 mice from the Jackson Laboratory (J); the 23rd mouse screened in a series of microinjections in the laboratory of Jon W. Gordon (Jwg).
- Crl:ICR-TgN(SVDhfr)432Jwg. The SV40 early promoter driving a mouse dihydrofolate reductase (*Dhfr*) gene; 4 kilobase plasmid; the 32nd animal screened in the laboratory of Jon W. Gordon (Jwg). The ICR outbred mice were obtained from Charles River Laboratories (Crl).
- TgN(GPDHIm)1Bir. The human glycerol phosphate dehydrogenase (GPDH) gene inserted into zygotes retrieved from (C57BL/6J × SJL/J)F1 females; the insertion caused an insertional mutation (Im) and was the 1st transgenic mouse named by Edward H. Birkenmeier (Bir). No strain designation is provided because each zygote derived from such an F1 hybrid mouse has a different complement of alleles derived from the original inbred parental strains.
- 129/J-TgH(SV40Tk)65Rpw (hypothetical). An SV40-thymidine kinase (Tk) transgene targeted by homologous recombination to a specific but anonymous locus using embryonic stem cells derived from mouse strain 129/J. This was the 65th mouse of this series produced by Richard P. Woychik (Rpw).

Abbreviations

Transgene symbols can be abbreviated by omitting the insert. For example, the full symbol TgN(GPDHIm)1Bir would be abbreviated TgN1Bir. The full symbol should be used the first time the transgene is mentioned in a publication; thereafter, the abbreviation may be used.

Insertional Mutations and Phenotypes

The symbol should not be used to identify the specific insertional mutation or phenotype caused directly or indirectly by the transgene. If an insertional mutation that produces an observable phenotype is caused by the insertion, the locus so identified must be named according to standard procedures for the species involved (see Appendix I). The allele of the locus identified by the insertion can then be identified by the abbreviated transgene symbol (see above) according to the conventions adopted for the species.

Examples

- $ho^{TgN447Jwg}$. The insertion of a transgene into the hotfoot locus (*ho*).
- $xxx^{TgN21Jwg}$. The insertion of a transgene that leads to a recessive mutation in a previously unidentified gene. A gene symbol for *xxx* must be obtained from a species-genome data base or member of a nomenclature committee (see Appendix I).

REFERENCES

Lyon, M. F. 1989a. Nomenclature for transgenic mice. Section 1.2.5 of Rules and guidelines for gene nomenclature. Pp. 10–11 in Genetic Variants and Strains of the Laboratory Mouse, 2d ed., M. F. Lyon, and A. G. Searle, eds. London: Oxford University Press.

Lyon, M. F. 1989b. Alleles. Section 1.1.5.6 of Rules and guidelines for gene nomenclature. P. 2 in Genetic Variants and Strains of the Laboratory Mouse, 2d ed., M. F. Lyon, and A. G. Searle, eds. London: Oxford University Press.

APPENDIX I

Resources Available for Assistance with Transgenic Nomenclature

Before naming a transgene, an investigator should obtain a laboratory code from ILAR at the address given in the list below. An investigator who has already been assigned such a code for other genetically defined mice and rats or for DNA loci should use the same code. The transgene should be named as stated in the rules. Assistance in selecting transgene symbols is available from several organizations (see below). Lists of named genes for mice and rats are published periodically in *Mouse Genome* (Oxford University Press, Journal Subscriptions Department, Pinkhill House, Southfield Road, Eynsham, Oxford OX8 1JJ, UK) and *Rat News Letter* (Dr. Viktor Stolc, editor, *Rat News Letter*, 2542 Harlo Drive, Allison Park, Pittsburgh, PA 15101). The list of mouse genes is also maintained in GBASE, a genomic data base for the mouse maintained by Dr. Don P. Doolittle, Dr. Alan L. Hillyard, Ms. Lois J. Maltais, Dr. Muriel T. Davisson, Dr. Thomas H. Roderick, and Mr. John N. Guidi at The Jackson Laboratory (see below). Human gene symbols are recorded in the Genome Data Base (GDB), which is maintained at The Johns Hopkins University (see below).

Institute of Laboratory Animal Resources (ILAR). Assigns laboratory codes; assists in naming transgenes; provides rules for naming transgenes. Contact: Dr. Dorothy D. Greenhouse, ILAR, National Research Council, 2101 Constitution Avenue, NW, Washington, DC 20418. Tel: 1-202-334-2590; Fax: 1-202-334-1687; Bitnet: DGREENHO@NAS).

The Jackson Laboratory. Assists in naming transgenes; provides rules for standardized nomenclature for mice; provides lists of named mouse genes. Contact: Dr. Muriel T. Davisson, The Jackson Laboratory, Bar Harbor, ME 04609. Tel: 1-207-288-3371; Fax: 1-207-288-8982).

Medical Research Council Radiobiology Unit. Assists in naming transgenes; provides lists of named mouse genes. Contact: Dr. Josephine Peters, MRC Radiobiology Unit, Chilton, Didcot, Oxford OX11 0RD, UK. Tel: 44-235-834-393; Fax: 44-235-834-918.

Transgenic Animal Data Base (TADB). Records, stores, and provides information on transgenic animals, including standardized nomenclature and a complete description of each transgenic animal; maintains rules for transgenic nomenclature on electronic bulletin board. Contact: Ms. Karen Schneider, TADB Coordinator, Oak Ridge National Laboratory, PO Box 2008, MS 6050, Oak Ridge, TN 37831-6050. Tel: 1-615-574-7776; Fax: 1-615-574-9888; Bitnet: TUG@ORNLSTC; Internet: OWENSET@IRAVAX.HSR.ORNL.GOV.

Genome Data Base (GDB). Records, stores, and provides information on mapped human genes and clones. Contact: GDB, Welch Medical Library, The Johns Hopkins University, 1830 East Monument Street, Baltimore, MD 21205. Tel: 1-301-955-9705; Fax: 301-955-0054. For assistance in naming human genes, the contact is Dr. Phyllis J. McAlpine, GDB Nomenclature Editor, University of Manitoba, Department of Human Genetics, 250 Old Basic Sciences Building, 770 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E 0W3. Tel: 1-204-788-6393; Fax: 1-204-786-8712; Bitnet: GENMAP@UOFMCC).

APPENDIX II

The Transgenic Animal Data Base

The Transgenic Animal Data Base (TADB) is intended to be a comprehensive, online, computerized record of all lines of transgenic animals and animals with targeted mutations that have been generated worldwide. Although the data base now includes only mice, it will be expanded to include other animal species. Transgenic animals of little interest to one researcher might be of enormous interest to others. This situation is addressed through the data base by making available to the scientific community extensive information about transgenic constructs, including methods, expressions, and phenotypes. Standardized nomenclature should be used to enter information into the data base.

Scientists provide data on their own lines of transgenic animals. The TADB office faxes a set of specific questions to each scientist, who answers the questions on a floppy disk with a word-processing program. The floppy disk is returned to the TADB office, where the data are formatted and transferred to the online computer. The result is that pertinent information, published or unpublished, on each line of animals is organized in the data base by the categories listed below. The data base has an easy-to-use, menu-driven interface that enables new users to search for and retrieve relevant records rapidly.

The data base also furnishes an opportunity for users to enter messages to the system administrator. This fea-

ture enables the system administrator to assist users who report difficulties and to make necessary adjustments in data base operations to maximize its usefulness. Information on various topics related to use of the data base can be accessed online.

The TADB is stored on an IBM RS6000/320 workstation server using BRS, a full-text searching software, for retrieval. It is accessible internationally via a toll-free number through the Tymnet telecommunications network. Access through Telnet is also available. Users need a personal computer, a terminal emulation program (such as White Knight, Versaterm, Procom, or Kermit), and a modem to access the data base. Users can get information at no cost from the TADB office. Contact: Ms. Karin Schneider, TADB Coordinator, Oak Ridge National Laboratory, PO Box 2008, MS 6050, Oak Ridge, TN 37831-6050. Tel: 1-615-574-7776; Fax: 1-615-574-9888; Bitnet: TUG@ORNLSTC; Internet: OWENSET@IRAVAX.HSR.ORNL.GOV.

Development of the TADB is sponsored by the National Institute of Environmental Health Sciences, National Institutes of Health, under contract Y01-ES10067.

Categories in the Transgenic Animal Data Base

DOCN	BRS-Assigned Accession Number
DESG	Designation (Standardized Nomenclature) of Transgenic Line
METH	Method Used
DNA	DNA Fragment Introduced
COPY	Copy Number
BACK	Genetic Background of Host Embryo or ES Cells
EXPR	Expression of Transgene or Targeted Gene
PHEN	Phenotype Due to Expression of Transgene or Targeted Gene
INTG	Description of Integration Site
CROS	Crosses with Other Transgenic Lines
IGEN	Genetics of Host Insertional Mutation
ICOM	Comments on Genetics of Host Insertional Mutation
ICLN	Is Flanking Sequence Cloned?
ICHR	Is Mutant Locus Characterized?
IALL	Is Mutation Allelic with Existing Mutations?
IPHN	Phenotype of Insertional Mutation
FCOM	Further Comments Relating to Transgenic Line
MNT	Is Line Being Maintained?
HNDL	Special Handling Instructions for the Animals
QDTE	Is This Line Presently Available to Other Investigators?
CADD	Name and Address of Contact Person
GADD	Name and Address of Author(s) Entering This Record
PUBL	References for Publications Relating to Line
DATE	Date Document Was Entered or Updated

Definition, Nomenclature, and Conservation of Rat Strains

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

Reprinted from *ILAR News*, Volume 34, Number 4, 1992

NATIONAL ACADEMY PRESS
Washington, D.C. 1993

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 competencies 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.

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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 serves as a coordinating agency and a national and international resource for compiling and disseminating information on laboratory animals, promoting education, planning and conducting conferences and symposia, surveying existing and required facilities and resources, upgrading laboratory animal resources, and promoting high-quality, humane care of laboratory animals in the United States.

This study was supported through grant number 1R13RR06884-01-R by the Comparative Medicine Program, National Center for Research Resources; Biology of Aging Program, National Institute on Aging; and Division of Cancer Biology, Diagnosis, and Centers, National Cancer Institute. Support was also provided by the Division of Cancer Treatment, National Cancer Institute through contract number NO1-CM-07316; the National Toxicology Program, National Institute of Environmental Health Sciences, through purchase order PR242640; the Japanese Ministry of Education, Science, and Culture; Chugai Pharmaceutical Co., Ltd.; Mitsubishi Kasei Co., Ltd.; and Otsuka Pharmaceutical Co., Ltd. Additional support was provided by the B & K Universal Group Ltd., Charles River Laboratories, Inc.; CLEA Japan, Inc.; Harlan Sprague Dawley, Inc.; and Taconic Farms, Inc. Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the committee and do not necessarily reflect the views of the National Institutes of Health; Japanese Ministry of Education, Science, and Culture; or other sponsors, nor does the mention of trade names, commercial products, or organizations imply endorsement by the U.S. or Japanese governments.

ILAR's core program is supported by grants from the National Center for Research Resources, National Institutes of Health; National Science Foundation; American Cancer Society, Inc.; and U.S. Army Medical Research and Development Command, which is the lead agency for combined Department of Defense funding also received from the Human Systems Division, 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.

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Preface

Inbred and other genetically defined rat strains are being increasingly used as research models. With increasing use, however, has come the realization that selecting and obtaining rat strains entail problems that can significantly affect research. The Laboratory Animal Science Group of the U.S.-Japan Non-Energy Research and Development Cooperative Agreement began to look for solutions to those problems in 1987. As a first step, eight inbred rat strains were selected as international reference strains, and institutions where these strains would be maintained were identified (see T. Nomura and S. Potkay, Establishment and Preservation of Reference Strains of Rats for General Purpose Use, *ILAR News*, 33[3]:42-44, 1991). In addition, the group proposed that a committee be established to address the issues further. As a result, the Institute of Laboratory Animal Resources (ILAR) of the National Research Council's Commission on Life Sciences, as part of its continuing mission to encourage high-quality, cost-effective, ethical research with animals, undertook the formation of the Committee on Rat Nomenclature, whose specific goals were as follows:

- To encourage the use of standardized nomenclature for rats by revising and updating the rules for standardized nomenclature.
- To resolve problems that have arisen because of the inappropriate use of nomenclature in naming rat strains.
- To ensure high genetic quality of rats by developing criteria for determining appropriate techniques for genetic monitoring.
- To encourage sharing of unique genetically defined strains by developing criteria for investigators to use in distributing animals to other investigators and to commercial companies.
- To ensure continued availability of unique genetically defined rat strains by developing criteria for determining what strains are of most value to the scientific community and by what mechanism they might be preserved.
- To develop a strategy for establishing communication between rat geneticists and investigators who use rats in fields other than genetics.

Because rats and the data obtained through studying them are shared worldwide, the committee recognized the necessity of addressing those issues in an international forum. Accordingly, it organized an international workshop, which was held January 14-16, 1992, at the Arnold and Mabel Beckman Center of the National Academy of Sciences and National Academy of Engineering, Irvine, California. The committee expresses its thanks to the workshop participants for their information and insights, which form the basis of this report.

The committee also acknowledges the assistance of the staffs of ILAR, Washington, D.C., and the Central Institute for Experimental Animals, Kawasaki, Japan, in organizing the workshop and preparing this report.

Thomas J. Gill III, *Cochairman*
Committee on Rat Nomenclature

Tatsuji Nomura, *Cochairman*
Committee on Rat Nomenclature

Contents

Summary

The increase in the use of genetically defined laboratory rats (*Rattus norvegicus*) in research has been accompanied by several problems. Many investigators have little understanding of the importance of genetic definition and genetic monitoring and are unfamiliar with the rules for standardized nomenclature. In addition, criteria for conserving unique genetic stocks are lacking and communication between geneticists and the general scientific community is inadequate. To address those problems, the Institute of Laboratory Animal Resources (ILAR) established the Committee on Rat Nomenclature. The committee met January 14-16, 1992, with an international group of scientists to discuss the issues and later met alone to recommend methods for dealing with the problems. The committee revised rules for standardized nomenclature for rats (presented in Appendix I) and endorsed the ILAR report *Important Laboratory Animal Resources: Selection Criteria and Funding Mechanisms for Their Preservation* (summarized in Appendix II), which lists criteria for evaluating animal resources and suggests mechanisms for preserving those of scientific value. It also recommended that an international committee be established under the auspices of an appropriate organization such as the International Council for Laboratory Animal Science and have the following functions:

- To encourage genetic characterization and monitoring of stocks and strains of rats used in biomedical research by developing a set of recommendations, including recommendations for practical techniques for genetic characterization and monitoring.
- To implement suitable genetic nomenclature for the rat, taking into account existing nomenclature used for humans, mice, and other relevant species.
- To encourage the conservation of rat strains and stocks by recommending a set of criteria for determining which strains and stocks should be conserved and by what means and by promoting systematic, national efforts to conserve valuable strains and stocks.
- To disseminate information on rat genetics to all appropriate scientific disciplines by publishing new genetic information in journals appropriate to various fields, encouraging journal editors to require the use of standardized nomenclature in submitted manuscripts, and establishing registries with widely available electronic data bases.

Introduction

The use of the laboratory rat (*Rattus norvegicus*) in research has grown steadily during the past decade. It is used extensively in research in physiology, toxicology, pharmacology, transplantation, reproductive immunology, immunogenetics, and cancer (see review by Gill et al., 1989). Like the mouse, the rat is genetically well-characterized, but its larger size allows scientists to perform many procedures that can be accomplished in the mouse only with great difficulty. The rat also has both scientific and economic advantages over larger models. However, problems have arisen in using genetically defined rats that have impeded research, sometimes severely. These problems generally are in five areas as follows:

1. *Genetic definition.* Although great strides have been made in the development of techniques for characterizing rat strains, many scientists do not understand the importance of knowing the genetics of the strains and substrains that they are using. As a result, both funds and time have been spent ineffectively in trying to replicate experiments among laboratories.
2. *Standardized nomenclature.* Many scientists who develop rat models are unaware that there are rules for standardized nomenclature of rats or do not follow those rules.
3. *Conservation criteria and strategies.* Resources for maintaining colonies of genetically unique animals are shrinking at an alarming rate in many parts of the world. Criteria are urgently needed for determining which strains should be conserved and in what form (e.g., as living animals or as frozen embryos).
4. *Responsibilities for maintaining colonies and distributing resources.* Questions continually arise about the responsibilities of scientists who develop rat models and then share animals with other investigators or provide breeding stock to commercial companies.
5. *Communication of information.* The communication of information on rat genetics is a long-standing problem. Geneticists obtain information from a journal entitled *Rat News Letter*, but a mechanism for transmitting this knowledge to the general scientific community is lacking.

The ILAR Committee on Rat Nomenclature discussed these issues with an international group comprised of geneticists and other biomedical scientists, representatives of commercial breeders, and funding-agency program officers at a workshop held on January 14–16, 1992, at the Arnold and Mabel Beckman Center of the National Academy of Sciences and National Academy of Engineering, Irvine, California. Although the report presented below generally expresses the consensus of the international group, the opinions and recommendations contained herein are solely the responsibility of the committee.

Genetic Definition of Strains

As scientists strive to understand the molecular biological bases of physiological and pathological processes by using animal models, the precise genetic definition of those models becomes essential. It is important to know not only the strain being used, but also its origin and its relationship to other strains, including those with the same major histocompatibility complex (MHC). Accurate genetic definition (characterization) is necessary to give a strain a proper designation and to select proper control strains for studying pathological processes and clarifying their etiology. The problems caused by using inaccurately defined rat strains include the following:

- Rejection times of organs in transplantation research depend on the source of the rats used and their proximate environment.
- Biological characteristics (e.g., the expression of diabetes, reproductive performance) frequently differ in supposedly identical strains held in different laboratories
- There is often considerable confusion in interpreting experimental results from different laboratories that are ostensibly using the same strain.

Characterization of a strain involves a detailed investigation of its genotype (the genetic endowment of the animal), its phenotype (manifestations of the genotype as influenced by the environment during gestation and the postnatal period), and its dramatype (manifestations of the phenotype as influenced by the proximate environment in which experiments are performed). The following are useful for genetically defining inbred strains:

- *Cytogenetic techniques* (karyotyping) detect important polymorphic morphological markers of chromosomes, especially the C-banding pattern and the position of the nuclear organizer region. Most of the linkage groups have been assigned to specific chromosomes, and the rest should be identified shortly. The synteny groups of the mouse, rat, and human chromosomes will play an important role in gene mapping and disease associations in the three species.
- *Biochemical polymorphisms* are important genetic markers. The panel of markers (generally 15–30) selected should represent a broad sampling of the genome and be reproducible over a long period. Useful protein and enzyme markers can be obtained from blood, tissues, and urine.
- *Immunological markers* include the antigens encoded by the MHC (*RT1*) and by the blood-group loci *RT2* and *RT3*. Skin grafting is an all-encompassing way in which to test histocompatibility, and it can be done whenever strains are compared or when a strain is tested for its degree of inbreeding.
- *Molecular genetic markers* are powerful tools for characterizing the rat genome. The methods to be used

and their interpretation are the subject of intensive research in a number of species. At the moment, the microsatellite DNA profile appears to be the most useful for strain characterization. So-called DNA fingerprinting and mitochondrial DNA restriction-fragment length polymorphism (RFLP) patterns are also useful.

• *Phenotypic traits* remain important components of strain characterization. Useful phenotypic traits are coat color, eye color, reproductive performance, and behavioral patterns. Other unique traits are useful for characterizing strains that are models of human diseases (e.g., hypertension and diabetes).

Each genetically defined strain should have a well-documented pedigree, either in graphic or log book format, and a clear description of the mating scheme used and the reason that it was chosen. Each inbred strain should normally be maintained by a small foundation colony that feeds an expansion colony, both of which are perpetuated by brother × sister mating. A given expansion colony should not be maintained for more than four to seven generations before being reconstituted from the foundation colony. It is especially important that the foundation colony have a detailed pedigree. It should be remembered that there is an implicit selection for breeding performance in developing an inbred strain and that a bias towards reproductive fitness can affect studies in reproduction.

Standardized Nomenclature

To ensure that the scientific community can communicate in precise terms, it is necessary to standardize the nomenclature of the strains used in research. That need was recognized early by those studying mouse genetics, and the International Committee on Standardized Genetic Nomenclature for Mice was established to revise the nomenclature rules as necessary and to encourage their use. The International Committee on Genetic Nomenclature of Rats, established in 1978 under the aegis of the International Council for Laboratory Animal Science (ICLAS), recognized the genetic nomenclature systems that had already been developed for the mouse and endorsed new nomenclature developed by ad hoc working parties: the new RT system for naming rat alloantigens and the revised esterase nomenclature. However, the international committee has not met for some years, and serious problems have arisen, as follows:

1. Inbred strains

• Multiple sets of inbred strains independently derived from the same outbred stock have been given the same designation, although they are genetically different (e.g., there are several different BB, SHR, and WKY strains).

• Some inbred strains (e.g., LOU/Iap and BDII/Cr) have become genetically contaminated but have retained the original strain name.

• Substrains of several commonly used strains differ at several loci but have not officially been given substrain symbols (e.g., BN strains, which differ at the *Pep3* locus, and MNR strains, which differ at the *RT1* locus).

• Obsolete synonyms of inbred strains are still widely used (e.g., Brown Norway for BN and Fischer or Fischer 344 for F344).

2. Gene symbols and mutant loci

• Several lists of gene symbols exist but are not in complete agreement.

• Rules for loci identified by molecular genetic methods have not been established.

3. Outbred stocks. Outbred stocks have generally not been genetically characterized, and lists of these stocks have not been compiled.

4. Exclusivity. To maintain the exclusivity of certain animal models, there has been a recent trend toward trademarking the animal's name or patenting the animal.

• A trademark is usually applied to a brand name, that differs from the standardized nomenclature. This leads to confusion about which of the two names is the proper one. If a trademark is applied to the standardized nomenclature, companies other than the trademark holder that supply the animal tend to change the name to avoid using the trademark, causing further confusion.

• A patented animal model is generally called by some nickname that is thought to be descriptive of the model's main characteristic. That has resulted in several different models with the same nickname and makes it difficult to communicate information.

To address the first three problems, the Committee on Rat Nomenclature has revised the rules for nomenclature of genetically defined and outbred rats and adopted the rules recommended by the ILAR Committee on Transgenic Nomenclature for naming transgenes (see Appendix I). The committee also recommends the re-establishment of an international committee to address future problems and revise the rules as necessary. This recommendation is discussed in detail later.

The trademarking of standardized strain or stock nomenclature is not permitted. A trademark designating a commercial supplier or institution and used in conjunction with a brand name is permissible but not recommended. Each investigator or institution breeding genetically defined rats should request a laboratory code from ILAR, National Research Council, 2101 Constitution Avenue, Washington, DC 20418 (telephone, 1-202-334-2590; fax, 1-202-334-1687). The code, preceded by a slash and appended to the strain name (see the rules for standardized nomenclature in Appendix I), will uniquely identify the model.

The issue of patenting an animal is complicated and involves extensive legal constraints. A detailed discussion of the issue is beyond the scope of this report. Nonetheless, the committee strongly disapproves of any procedures that restrict the free exchange of scientific

resources and information. Furthermore, patented animals should be identified by standardized nomenclature when referred to in the scientific literature.

Conservation Criteria and Strategies

There are no clear-cut guidelines for the conservation of genetic stocks. As a result, valuable mutants and strains can be lost, either by accident or when an investigator loses research funding, retires, or dies. Only in Japan is there a well-organized national commitment to conserving such resources. The problems in the United States have been discussed in detail with suggested solutions in the ILAR report entitled *Important Laboratory Animal Resources: Selection Criteria and Funding Mechanisms for Their Preservation* (NRC, 1990), which is summarized in Appendix II. The present committee endorses the recommendations made in that report.

Embryo cryopreservation has been used to conserve mouse strains for many years and has been adapted for use with rats in several laboratories worldwide (e.g., the Central Institute for Laboratory Animal Breeding, Hannover, Germany, and the MRC Experimental Embryology and Teratology Unit, London, U.K.). However, only the Central Institute for Experimental Animals (CIEA), Kawasaki, Japan, has a well-defined commitment to the cryopreservation of rat strains. Cryopreservation is a valuable technique for preventing loss of strains caused by infection, accident, or fire; for preventing genetic drift; for humanely and economically storing stocks not in use, but of potential future value; and for transporting breeding nuclei economically and without the risk of introducing infection. It is likely to be of particular value in the future for the conservation of transgenic strains, which are being produced in ever-increasing numbers. Techniques for cryopreserving rat embryos have been reviewed (Hedrich and Reetz, 1990). Further research and development will improve efficiency and recovery rate. Because the methods are technically complex, cryopreservation is most suitably performed in centralized facilities.

The committee encourages international collaborative efforts to establish cryopreservation centers worldwide to serve as repositories for valuable rat strains and models and to provide training for individual investigators. The establishment of such centers would provide an incentive for investigators to develop new strains and models, knowing that a cost-effective, reliable method for their preservation is available. In addition, investigators could design their experiments with the knowledge that the needed rat strains can be made available.

Responsibilities For Maintaining Colonies and Distributing Resources

An investigator who develops and then distributes an animal model bears the responsibility for ensuring that the model is genetically pure and has an acceptable health (microbiological) status. Those factors are often critical to an animal's response in an experimental protocol. If investigators are not aware of, or ignore, those responsibilities, the loss in time and money can be considerable.

The purposes of genetic monitoring are to ensure that strains maintain their genetic integrity and to make it possible to detect genetic changes that occur as a result of contamination or mutation. Genetically characterized strains must be monitored routinely and systematically, using a well-defined protocol. The genetic markers selected should be easy to detect, so that they can be used by individual institutions or investigators, testing laboratories, or centralized facilities. Table 1 lists markers useful for monitoring purposes (i.e., those at which there are several polymorphisms). Various techniques for genetic monitoring are described in *Genetic Monitoring of Inbred Strains of Rats* (Hedrich, 1990). A standard protocol for monitoring the nucleus of inbred rat strains has been established by the ICLAS Monitoring Center at the CIEA, Kawasaki, Japan. One male and one female from each strain are tested, and biochemical markers chosen from among those listed in Table 1 are differentiated by isoelectric focusing, polyacrylamide electrophoresis, and cellulose acetate membrane electrophoresis. Immunogenetic markers are differentiated by erythrocyte agglutination. Additional information on the CIEA protocol can be obtained from Dr. Hideki Katoh, CIEA, 1430 Nogawa, Miyamae, Kawasaki 216, Japan (telephone, 81-44-754-4450; fax, 81-44-754-4454).

The valid use of a disease model requires that the characteristic for which it is being used is maintained (e.g., high blood pressure in strains used for studying hypertension or increased blood glucose in strains used for studying diabetes). This type of monitoring requires the use of appropriate techniques.

Microbiological monitoring should be performed routinely and systematically, and microbiological records should be available on all animal colonies. A detailed discussion of microbiological monitoring can be found in *Manual of Microbiologic Monitoring of Laboratory Animals* (Allen and Nomura, 1986) and in the report *Infectious Diseases of Mice and Rats* (NRC, 1991).

Communication of Information

The problem of communication of information between scientific disciplines is a continuing one. Although information on rat genetics is important for scientists in a variety of disciplines, such information rarely extends beyond the genetics community.

The committee discussed the following three ways in which information about rat genetics and nomenclature can be disseminated to scientists outside the field of genetics:

- Rules of nomenclature and revisions thereof can be submitted for publication in various journals, especially

TABLE 1 Selected Markers Useful for Genetic Monitoring of Rats

Locus	Chromosome	Gene Name	Notes
Biochemical markers:			
Acon1	5	Aconitase-1	*a*
Ahdc	13	Aldehyde dehydrogenase-c	*a*
Ahd2	5	Aldehyde dehydrogenase-2	*a*
Akp1	—	Alkaline phosphatase-1	*b*
Alp1	—	Serum alkaline phosphatase-1	*a*
Amy1	2	Amylase-1	*b*
Es1	19	Esterase-1	*b*
Es2	19	Esterase-2	*b*
Es3	19	Esterase-3	*b*
Es4	19	Esterase-3	*b*
Es6	8	Esterase-6	*b*
Es7	19	Esterase-7	*b*
Es9	19	Esterase-9	*b*
Es10	19	Esterase-10	*b*
Es14	19	Sex-influenced esterase	*b*
Fh1	13	Fumarate hydratase-1	*a*
Gc	14	Group-specific component	*b*
Hbb	1	Hemoglobin β-chain	*b*
Mup1	5	Major urinary protein-1	*b*
Pep3	13	Peptidase-3	*b*
Pgd	5	Phosphogluconate dehydrogenase	*c*
Svp1	3	Seminal vesicle protein-1	*a*
Immunogenetic markers:			
RT1	20	MHC	*b*
RT2	19	Red cell antigen-2	*b*
RT3	13	Red cell antigen-3	*a*
RT8	—	Red cell antigen-8	*d*

[a]Useful for characterization.
[b]Useful for routine monitoring.
[c]Useful for differentiating substrains of strains LE, SHRSP, and WKY.
[d]Useful for differentiating SHR substrains.

journals that commonly publish articles on rat genetics and immunology, and the editors of those journals can be urged to require the use of standardized nomenclature in submitted manuscripts.

- Commercial breeders can play an important part in educating those using rats by giving more prominence to the standardized nomenclature in their catalogs and less prominence to obsolete names and brand names. They can also be requested to distribute the rules for nomenclature of rats to their customers.
- Data bases containing information on genetically defined rats now housed in individual institutions can be made widely available by linking to a single, publicly accessible data base such as GBASE, The Genomic Data Base of the Mouse, which is maintained at the Jackson Laboratory, Bar Harbor, Maine.

The committee recommends that responsibility for the appropriate dissemination of information be assigned to the international committee discussed below.

Recommendation

The Committee on Rat Nomenclature recommends that scientists who use rats as research models establish a committee on rat nomenclature under the auspices of an appropriate international organization such as ICLAS. The international committee should consist of 10–15 members from a broad range of scientific disciplines and should demonstrate a suitable geographic distribution. It should meet about every 2 years, preferably in conjunction with an international meeting in a related field.

The functions of the committee should be as follows:

1. *Genetic definition and monitoring of strains.* The international committee should encourage the genetic characterization and monitoring of stocks and strains of rats used in biomedical research by developing a set of recommendations, including practical techniques for genetic, phenotypic, and dramatypic characterization and for monitoring.

2. *Nomenclature.* The international committee should

implement suitable genetic nomenclature systems for the rat, taking into account existing nomenclature used in the human, the mouse, and other relevant species. Registries should be established in appropriate laboratories to maintain laboratory codes; inbred strain, transgenic strain, outbred stock, and gene names and characteristics; genetic maps; DNA probes and primers useful for the rat; chromosomal polymorphisms and anomalies; and rat-human and rat-mouse homologies. The goal should be for each developer of a rat model or genetic resource to communicate with the relevant registry to ensure that the model, gene, or probe will be named correctly and to assist in keeping the registries up to date by contributing appropriate new findings.

3. *Conservation criteria and strategies.* The international committee should encourage the conservation of rat strains and stocks by recommending a set of criteria for determining which strains and stocks should be conserved and by what means (e.g., cryopreservation) and by promoting systematic national efforts to conserve valuable genetic strains and stocks. It should support continued research and development on the cryopreservation of rat embryos, gametes, and ovaries and should explore other methods of conserving genetic resources.

4. *Communication of information.* The international committee should assume the responsibility for disseminating information on rat genetics to all appropriate scientific disciplines by publishing new genetic information in journals appropriate to the field of interest, by encouraging journal editors to require the use of standardized nomenclature in submitted manuscripts, and by establishing registries with electronic data bases whose information is widely available to scientists. It should determine the most appropriate means for communication of information, including the journal *Rat News Letter.*

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APPENDIX I

Rules for Nomenclature of Rats

The following rules have been adopted by the Committee on Rat Nomenclature of the National Research Council's Institute of Laboratory Animal Resources (ILAR). They are based on the rules adopted by the International Committee on Standardized Genetic Nomenclature for Mice (Lyon, 1989a,b). Although, some types of genes (e.g., recessive lethals and homeobox-containing genes) have not yet been described in rats, the rules for their nomenclature are presented because these genes are likely to be discovered in the foreseeable future. In these instances, examples for mice are used to illustrate the rules.

1. INBRED STRAINS

1.1 Definition

A strain is regarded as inbred when it has been mated brother × sister (hereafter called b × s) for 20 or more consecutive generations. To ensure isogenicity, as well as homozygosity, a single b × s pair must be selected in the twentieth or a subsequent generation to perpetuate the strain. Parent × offspring matings may be substituted for b × s matings, provided that in the case of consecutive parent × offspring matings, each mating is to the younger of the two parents; this will prevent repeated backcrossing to a single individual. Exceptionally, other breeding systems may be used, provided that the inbreeding coefficient achieved is at least equal to that at the twentieth generation, theoretically 0.99.

1.2 Symbols

Inbred strains should be designated by a capital letter or letters in Roman type. Brief symbols (four letters or fewer) are preferred (e.g., ACI, DA). An exception is allowed in the case of stocks already widely used and known by a designation that does not conform (e.g., F344, DONRYU).

Strains with a common origin (i.e., from the same outbred base population or arising from the same cross but separated before the twentieth generation) should be regarded as related inbred strains and should be given symbols that indicate the relationship and that bring the strains together in alphabetical lists (e.g., the strain SR, which is resistant to sodium chloride-induced hyperten-

sion, and the strain SS, which is sensitive to sodium chloride-induced hypertension).

To avoid duplication in strain designations, anyone naming a new strain should consult the Registry of Inbred Strains (see Sec. 6, Resources). If two inbred strains are assigned the same symbol, the strain to retain the symbol will be determined by priority in publication. For this purpose, listing in *Rat News Letter* will be regarded as publication. A list of inbred strains is published periodically in *Rat News Letter* (see Sec. 6, Resources).

1.3 Indication of Inbreeding

When it is desired to indicate the number of generations of b × s inbreeding, this should be done by appending in parentheses an F followed by the number of inbred generations (e.g., F87). If only part of the total inbreeding is known, this should be indicated with a question mark and a plus sign (e.g., F? + 10).

1.4 Substrains

An established inbred strain is considered to have divided into substrains when known or probable genetic differences become established in separate branches. Such differences could arise by residual heterozygosity at the time of branching or by new mutation. Hence, substrains should be considered to be formed as follows:

- When branches are separated before F40 (i.e, after 20 and before 40 generations of b × s matings). In such cases, residual heterozygosity might be present.
- When genetic differences from other branches are discovered. Such differences could arise either by residual heterozygosity or mutation. Contamination is likely to lead to numerous genetic differences and might thus be distinguishable from mutation. If contamination is thought likely, the strain should be renamed.
- When a branch is known to have been maintained separately from other branches for 100 generations, even if neither of the above applies. In accordance with the rules of standardized nomenclature for inbred mice, the separate branch is considered a new substrain because the existence of differences arising by mutation is highly probable.

A substrain should be known by the name of the parent strain followed by a slanted line (slash) and, in the case of identifiable genetic differences, a number (e.g., BN/1, BN/2). The founding strain is considered the first substrain; the use of /1 for it is optional (e.g., KGH or KGH/1). In the case of established strains, the first substrain will be the one maintained in the greatest number of laboratories or so determined by the registrar of inbred strains (see Sec. 6, Resources).

When genetic differences are probable but not demonstrated, a laboratory code (e.g., Pit for the University of Pittsburgh Department of Pathology, and N for the NIH Genetic Resource—see Sec. 1.5) is used to designate a substrain (e.g., BN/1PitN becomes BN/1N after 100 generations at the NIH Genetic Resource).

1.5 Laboratory Codes

Each laboratory or institution that breeds rats should obtain a laboratory code from ILAR (see Sec. 6, Resources). This code, which can be used for all species, consists of either a single Roman capital letter or an inital Roman capital letter and one to three lower-case letters. Normally, a strain is designated by the strain name followed by a slanted line (slash), the substrain designation (if any), and the laboratory code (e.g., BN/1Pit). When the strain or substrain is established in another laboratory, the new laboratory code is appended (e.g., BN/1PitN). The first laboratory code should be retained until a genetic difference is demonstrated or a branch has been maintained separately from other branches for 100 generations (e.g., BN/1PitN becomes BN/1N after 100 generations at the NIH Genetic Resource). Intermediate laboratory codes should be dropped to avoid excessively long designations. It is the responsibility of the holder to maintain a history of the strain.

1.6 Recombinant Inbred Strains

Strains formed by crossing two inbred strains, followed by 20 or more generations of b × s mating are called recombinant inbred (RI) strains. The symbol of an RI strain should consist of an abbreviation of both parental strain names separated by a capital X with no intervening spaces (e.g., LXB for an RI strain developed from a cross of LEW and BN). Different RI strains in a series should be distinguished by numbers (e.g., LXB1, LXB2).

1.7 Coisogenic, Congenic, and Segregating Inbred Strains

Two strains that are genetically identical except for a difference at a single locus are called coisogenic. True coisogeneity can probably be achieved only by mutation within an existing inbred strain, whereas lines obtained by inbreeding with forced heterozygosis (segregating inbred strains) or by crossing onto an inbred strain (congenic strains) usually differ in a short chromosomal segment, rather than in a single gene.

Coisogenic and congenic strains (except for alloantigenic systems—see Sec. 3.15) should be designated by the strain symbol, a slash, the substrain symbol (if any), and the laboratory code, followed by a hyphen and the gene symbol in italics (e.g., LEW/Han-*ci*, a coisogenic strain; LEW/N-*rnu*, a congenic strain). When the mutant or introduced gene is maintained in the heterozygous condition, this may be indicated by including a slanted line and a plus sign in the gene symbol (e.g., LEW/N-*rnu*/+).

A strain developed by repeated backcrossing should

be regarded as congenic when a minimum of 10 backcross generations to the background strain have been made, counting the first hybrid or F1 generation as generation 1. The number of backcross generations should be indicated by N followed by a number. If it is necessary to use more complex mating systems, the generations should be expressed as N equivalents (NE) and the strain regarded as congenic at a minimum of NE10.

For segregating inbred strains developed by inbreeding with forced heterozygosis, indication of the segregating locus is optional. The number of generations of such breeding should be indicated by FH followed by a number.

2. HYBRID STRAINS

The first filial generation of a cross between two inbred strains is called an F1 hybrid. It is designated by the full strain designation of the female parent, followed by a multiplication sign and the full strain designation of the male parent, followed by F1 (e.g., F344/NNia × BN/RijNia F1). If there is any chance of confusion, parentheses should be used to enclose the parental strain names [e.g., (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., F344/NNia × BN/RijNia F1 (hereafter called FBNF1)].

Hybrids from backcrosses and three- or four-way crosses are designated on the same basis, that is, by giving in parentheses the designation of the female parent first, followed by a multiplication sign and the designation of the male parent, followed by the generation number, for example, [(F344/NNia × BN/Rij/NNia)F1 × LEW/NHsd]F1.

3. GENES

3.1 Names of Loci

Names of loci should be brief and should be chosen to convey as accurately as possible the characteristic by which the gene is usually recognized, including coat color, a morphological effect, a change in an enzyme or other protein, disease susceptibility or resistance, resemblance to a human syndrome, or a DNA sequence identified by a DNA probe for the gene or by sequence analysis.

3.2 Symbols for Loci

Symbols for loci should typically be two-, three-, or four-letter abbreviations of the name in italics. For ease in finding loci in alphabetical listings, the initial letters of names and symbols should, where possible, be the same. A number may be included for a protein in which a number is part of the recognized name or abbreviation, but the symbol should always begin with a letter (e.g., *C4* and *C6* for the fourth and sixth components of complement). Roman numbers, Greek letters, names of people, and names of places should not be used for gene names or symbols. Except in the case of loci first discovered because of a recessive mutation (see Sec. 3.5), the initial letter of the locus symbol should be a capital, and all others should be in lower case (e.g., *di* for diabetes insipidus; *Hbb* for hemoglobin β chain).

The discovery of a morphological, biochemical, or antigenic variant does not necessarily indicate the discovery of a new locus. Appropriate genetic tests should be conducted to show Mendelian segregation, and identity or lack of identity with known loci should be established as far as possible by mapping or by testing for allelism. Loci can also be identified by somatic cell genetics or studies of DNA.

A proposed new symbol must not duplicate one already used for another locus, even if the gene effect is very different. Listing of a gene symbol in *Rat News Letter* establishes priority (see Sec. 6, Resources).

3.3 Loci That Are Members of a Series

Loci that are members of a series specifying similar proteins or other characteristics (e.g., isoenzymes and alloantigenic loci) should be designated by the same letter symbol and a distinguishing number without a hyphen (e.g., *Es1*, *Es2*, and *Es3* for esterase loci; *RT1*, *RT2*, and *RT3* for alloantigenic loci).

For morphological or "visible" loci with similar effects (e.g., genes that cause hairlessness), distinctive names should be given because the gene actions and gene products might prove to be very different (e.g., *fz* for fuzzy and *rnu* for Rowett nude).

3.4 Homology with Other Organisms

It is highly desirable that terminology for homologous genes be standardized among species. Therefore, for a rat gene that is homologous with a gene in another species, the symbol selected should be that already adopted for the other species, provided that it does not duplicate a symbol already in use for a different locus in the rat. To avoid such duplication, the symbol should be modified to one that resembles that used in the other species but does not duplicate one already in use for a different locus either in the rat or in the other species.

Where possible, the numbering of homologous loci in a series should be made concordant in various species, with locus *1* in the rat corresponding to the locus *A* in other species, locus *2* with locus *B*, and so on.

3.5 Alleles

Alleles should be designated by the locus symbol and a superscript. In computerized symbols the superscript may be denoted by prefixing an asterisk (e.g., Hbb^h or *Hbb*h*). Allele superscripts should typically be one or two lower-case letters and, if possible, should convey additional information about the allele (e.g., c^h for Hima-

layan allele of *c* or albino). If information is too complex to be conveyed conveniently in the symbol (e.g., biochemical properties or antigenic specificities), the alleles are still given superscripts (e.g., $Pgm1^a$, $Pgm1^b$), but the information concerning the allelic properties is shown in catalogs or tables.

For the first discovered allele in cases in which there is clearly a wild type, no superscript is used (e.g., *fa* for fatty). When further alleles are discovered, the first mutant allele may still be written without a superscript (e.g., *fa* for fatty, fa^{cp} for corpulent).

Recessive alleles of a mutant gene should be indicated by a lower-case initial letter (e.g., *a* for nonagouti; *rnu* for Rowett nude). All other alleles—whether dominant, codominant, or having dominance relationships that vary with the method of assessment—should be indicated, as for the locus symbol, by a capital initial letter followed by lower-case letters (e.g., *Ca* for hereditary cataract).

Wild-type alleles should be designated by a plus sign with the locus symbol as a superscript (e.g., $+^d$, $+^c$). Reversions from a mutant allele to the wild type should be distinguished from the original wild-type allele by the locus symbol with a plus sign as a superscript (e.g., d^+, c^+). A plus sign may be used alone when the context leaves no doubt as to the locus represented (e.g., in genetic formulas).

Indistinguishable alleles of independent origin (e.g., recurrences and reversions to wild type) should be designated by the existing gene symbol with a series symbol appended as a superscript. The series symbol should consist of a number corresponding to the serial number of the recurring allele in the laboratory of origin plus the laboratory code. To avoid confusing the number 1 and the letter l, the first-discovered recurring allele may be left unnumbered and the second recurring allele numbered 2 (e.g., in mice, *bg* for beige; bg^J for a recurrence of the mutation *bg* at the Jackson Laboratory; bg^{2J} for a second recurrence of the mutation *bg* at the Jackson Laboratory).

Mutations or other variations that occur in known alleles (except for alloantigenic systems—see Sec. 3.15.2) are 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., $Mup1^{a\text{-}m1Pit}$ for the first mutant allele of $Mup1^a$ found by the University of Pittsburgh Department of Pathology). For known deletions 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 (see also Sec. 3.20, Transgenes).

3.6 Phenotype Symbols

Phenotype symbols, if they are necessary (e.g., antigen loci, enzyme loci), should be the same as genotype symbols but in capital letters, not italicized, and with superscript characters lowered to the line. The phenotypes of heterozygotes should be written as in the following examples: ES1A, ES1C, and ES1AC for phenotypes associated with the *Es1* locus and RT6A and RT6B for phenotypes associated with the *RT6* locus.

3.7 Gene Complexes

Gene complexes are considered to exist when a number of apparently functionally related loci are closely linked. Alternative states of complexes are referred to as haplotypes, rather than as alleles. Known complexes are of two main types: less extensive complexes that involve duplicated loci or in which operators or *cis*-acting regulators of structural genes for protein show little or no recombination with the loci on which they act, and very extensive complexes that might involve hundreds of related loci and for which special rules might be necessary.

The existence of a gene complex, as opposed to the presence of multiple types of variation in a structural gene, should not be postulated without good evidence. Different mutations in a structural gene can affect not only electrophoretic mobility but also activity and stability, and changes in 5′ or 3′ regulatory sequences can cause apparent changes in tissue specificity or inducibility. Thus, such changes should be attributed to mutations in the structural gene unless there is good evidence otherwise.

To distinguish different loci of a complex, the basic symbol should have appended a single lower-case letter in italics designating the presumed function or means of identification of the locus, such as *s* (structural), *e* (electrophoretic), *r* (regulatory), *t* (temporal), or *m* (mitochondrial). This letter should be set off by a hyphen (e.g., *Bgl-e*, β-galactosidase electrophoretic), except for numbered unlinked loci in a series, in which case the letter should follow the number without a hyphen (e.g., *Adh1t* for alcohol dehydrogenase-2 temporal). When it is discovered that a previously described locus is part of a complex, a letter indicative of its function or means of identification should be added to the basic symbol to form the new symbol for the already known locus, and a different letter should be added to form the symbol for the newly discovered locus. For example, hypothetically, the electrophoretically detected *Adh2* locus, after discovery of a temporal regulator, becomes *Adh2e* (i.e., *Adh2* electrophoretic), and the regulator is called *Adh2t* (i.e, *Adh2* temporal). The basic symbol (e.g., *Adh2*) then represents the entire complex. If necessary for clarity, the complex may be additionally indicated by enclosing the basic symbol in parentheses or in brackets.

Haplotypes are designated by the symbol for the complex with a superscript lower-case letter. The components of the haplotype can be briefly indicated as in the following hypothetical example: $Adh2^a$ or $(Adh2)^a$ = $Adh2e^a\ Adh2t^a = Adh2e^at^a$. If two or more closely linked

and functionally related structural loci have been given serial numbers, the complex, loci, and haplotypes should be indicated as in the following hypothetical example: complex, *Amy* or (*Amy*); loci, *Amy1* and *Amy2*; haplotypes, Amy^a or $(Amy)^a = Amy1^a\ Amy2^a = Amy1^a2^a$.

Distantly acting regulators should be given locus symbols different from but related to the locus they regulate and preferably with the same initial letter (e.g., hypothetically, *Gdr1* for a regulator of the glucose-6-phosphate dehydrogenase locus *Gpd*).

The list of extensive complexes with special rules continually increases. Special rules are needed because the various complexes differ widely in their structure, and no suitable single nomenclature system has yet been found that is adequate for all the complexes. Complexes with special rules are the following:

- the major histocompatibility complex and other alloantigenic systems (see Sec. 3.15)
- immunoglobulin complexes (see Sec. 3.16)
- globin gene complexes (see Sec. 3.17)
- homeobox-containing gene complexes (see Sec. 3.18)
- cytochrome P450 (see Section 3.19)

In some cases, the rules for these gene complexes might be formulated by a separate committee that covers more than one species.

3.8 Pseudogenes

The symbol for a pseudogene located away from the main gene complex should consist of the locus symbol followed by a hyphen, the suffix *ps*, and an appropriate serial number (e.g., *cytc-ps1* for the first of approximately 30 known pseudogenes of cytochrome C located away from the structural gene locus).

3.9 Lethals

The symbol for a recessive lethal with no known heterozygous effect and an unidentified function consists of a lower-case letter *l* followed by the chromosome number of location in parentheses and a series symbol that indicates the serial number of the lethal in the laboratory of origin. No examples have yet been described in the rat; in the mouse, *l(17)2Pas* is the second lethal on chromosome 17 found at the Pasteur Institute.

Such symbols should be considered as provisional. The lethal should be renamed if it is found to be allelic with a known gene or if the underlying defect becomes understood.

3.10 Viruses

Nomenclature for genes related to the expression of viral antigens or to sensitivity or resistance to viruses should follow the standard rules for gene nomenclature, i.e., symbols should be italicized with the initial letter a capital and all others in lower case. Where possible and appropriate, the letters of the symbol should be those by which the virus is usually known. Successive loci concerned with the same virus should be distinguished by appending a number. Locus symbols ending in *v* should be reserved for viral loci. Little is known about viral loci in the rat; however, in the mouse, *Mtv-1* is a locus concerned with induction of mammary tumor virus, MTV, and *Fv-1* and *Fv-2* are loci concerned with resistance to Friend virus.

3.11 Oncogenes

Nomenclature for cellular oncogene sequences should follow the standard nomenclature for oncogenes. However, in lists of symbols and maps, the prefix *c-* denoting cellular sequence should be omitted and the initial letter of the symbol should be capitalized if it is not already (e.g., *c-myc* becomes *Myc* for the myelocytoma oncogene, and *c-Hras1* becomes *Hras1* for the Harvey rat sarcoma-1 oncogene).

The names and symbols of oncogenes should be regarded as provisional until the true functions of the genes become known, when they should be renamed (e.g., *Erbb* becomes *Egfr* for the epidermal growth factor receptor, and *Sis* becomes *Pdgfb* for the platelet-derived growth factor, β polypeptide).

3.12 Mitochondrial Genome

The symbol for a locus in the mitochondrial genome should consist of the prefix *mt* followed by a hyphen and the main symbol.

3.13 Restriction-Fragment Length Polymorphisms

Restriction-fragment length polymorphism (commonly known as RFLP) can occur as

- Variation in DNA sequence within exons of a known gene.
- Variation in DNA sequence within introns or within flanking sequences of a known gene.
- Variation in DNA sequence outside exons or introns but detected by a probe for the known gene (e.g., the *Hpa* site variant 5 kb from the 3′ end of the human β-globin structural gene).
- Variation in DNA sequence detected using an arbitrary DNA sequence as a probe.

The first two types of variation should be described according to current rules for nomenclature of gene loci and alleles so that these variants can be listed both in a compilation of restriction-fragment length variants and in lists of gene loci.

For the third type, symbols for the restriction fragments should consist of the capital letter *D* (for DNA), the gene symbol, and a number (e.g., the *Hpa* site variant cited above would be symbolized *DHbb1*, the 1 indicating that this was the first probe found that detected a polymorphism). The variation in possession of the *Hpa* site can be described in terms of alleles. Thus, the presence of the site would be designated $DHbb1^a$ and the absence $DHbb1^b$. If the allele in which the variation occurs is known, it should be indicated in the symbol (e.g., $DHbb^{d1a}$).

For the fourth type, it is not possible to ascertain whether the variation fits into any of the first three categories. The nomenclature should follow that in human gene mapping for provisional nomenclature (Skolnick and Francke, 1981).

An arbitrary probe is given a name composed of four parts: *D* for DNA, the chromosome number or 0 for unassigned segments, the laboratory code, and a number to give uniqueness to the probe (e.g., in mice, *D1Pas5* is the fifth chromosome 1 probe developed at the Pasteur Institute and *D17Leh48* is a chromosome 17 probe designated number 48 by Lehrach). Again, $D1Pas5^a$ could indicate possession of a restriction site for a particular enzyme and $D1Pas5^b$ its absence. If the arbitrary sequence is later shown to be at a known locus, the nomenclature should be altered to take this into account.

Anonymous DNA segments from the human genome that hybridize with rat DNA and are mapped to a rat chromosome should retain their human symbol, and this should be followed by a lower case *h* to denote the human origin (e.g., *D21S56h* for a DNA segment from human chromosome 21).

3.14 Biochemical Variants

Biochemical nomenclature should be in accord with the rules of the International Union of Biochemistry's Commission on Biochemical Nomenclature. The nomenclature recommended by the commission is published periodically in major international biochemical journals, such as the *Journal of Biological Chemistry* and the *Biochemical Journal*. Enzymes and other biochemicals have both formal and trivial names. The correct formal name should be given the first time a substance is mentioned in a publication [e.g., D-glucose-6-phosphate:$NADP^+$ 1-oxidoreductase (E.C. 1.1.1.49)]; trivial names (e.g., glucose-6-phosphate dehydrogenase) or abbreviations (e.g., G6PD or GPD) can be used subsequently. The commission's nomenclature is used in periodicals, reference works, and textbooks of biochemistry.

3.14.1 *Symbols for structural loci.* Symbols for structural loci should typically be two-, three-, or four-letter abbreviations of the official commission name of the enzyme, protein, or other entity. The initial letter of the symbol should be capitalized [e.g., *Gpd1* for the first identified structural locus of GPD). In the case of biochemical variants, beginning the locus symbol with a lower case letter to indicate a recessive mutant gene or a capital letter to indicate a dominant mutant gene should generally be avoided. Such nomenclature is not suited to polymorphic systems of alleles, and the dominant-recessive relationship usually varies and depends on the method used to assess it.

A Greek letter preceding the name of an enzyme or other protein should be changed to an appropriate English letter and placed at the end of the locus symbol (e.g., in mice, *Fuca* for α-fucosidase). That permits a rational alphabetic ordering of locus symbols. Similarly, an adjective describing tissue specificity or another property of an enzyme or protein should be placed after the noun to allow appropriate alphabetic ordering (e.g., in mice, *Actc* for actin, cardiac, and *Acts* for actin, skeletal).

3.14.2 *Symbols for loci specifying isoenzyme structure or polypeptide chains.* A series of loci specifying structurally different isoenzymes that catalyze the same or similar reactions or different polypeptide chains of a protein should be designated by the same letter symbol for the structural locus with the addition of a distinguishing number [e.g., *Acp1* and *Acp2* for loci of structurally different isoenzymes of orthophosphoric-monoester phosphohydrolase, acid optimum (E.C. 3.1.3.2, acid phosphatase-1)].

3.14.3 *Homology with other organisms.* It is highly desirable that terminology for homologous genes be standardized among species. Therefore, as in the standard rules, in choosing a gene symbol an attempt should first be made to discover and use any symbols already adopted for the same locus in other species. However, care should be taken not to duplicate symbols already in use in the rat for other loci. If duplication would occur, the symbol should be modified to resemble that used in the other species without duplicating the symbol used for a different gene in that or another species (e.g., CA is the symbol for carbonic anhydrase, in humans, but it is used in the rat for hereditary cataract, so the symbol used for carbonic anhydrase in the rat should be that used in the mouse, *Car*). Where possible, the numbering of homologous loci in a series should be made concordant in various species, with locus *1* in the mouse and rat corresponding to the locus *A* in other species, locus *2* with locus *B*, and so on (e.g., *Car1*, *Car2*).

It is not appropriate to insert the letter r or R (for rat) as the first letter of the symbol for a locus with homologues in other species because all rat locus symbols would then begin with the same letter.

3.14.4 *Alleles.* An allele should be designated by the locus symbol with an added superscript, as in the standard rules. In describing alleles, whether found in inbred strains or in the wild, it is desirable to report the pheno-

type of a number of widely used inbred strains. One strain should arbitrarily be designated the prototype strain for each allele, because variation that has not been detected by the methods used might be present in each allelic class. If an apparently identical allele in another strain is found by new methods to differ from that in the prototype strain, it should be assigned a new alphabetical symbol as a superscript and a prototype strain for the new allele should be designated. This system permits the orderly assignment of symbols to newly identified alleles and allows ready comparisons of new variants with previously reported variants.

Locus and allele symbols are necessarily brief and cannot contain more than a small fraction of the known information. Additional information can be contained in gene descriptions, which in some cases, can be collected in catalogs or tables. For example, haplotypes or alleles of the mouse hemoglobin α-chain locus *Hba* specify at least five polypeptides. In general, each strain produces a single polypeptide, but in some strains, two polypeptides are produced. The loci encoding the polypeptides of an allele can be assigned letter designations corresponding to the allele, and information about the amino acid composition of the chains produced by the alleles can be shown in tables.

3.14.5 *Proteins detected as spots on 2D-gels but not identified.* Locus symbols for proteins detected as spots on 2D-gels should be given only if genetic variation or gene location is established, if the gene behaves in a Mendelian fashion, and if the protein is known (or strongly believed) to be distinct from those already named. Such a symbol should consist of four parts: the capital letter *P* for protein, a number indicating the chromosome that holds the coding gene (using the number 0 to indicate unknown location), a laboratory code, and a number distinguishing the protein from others found in the same laboratory (e.g., *P0Pas1* for the first 2D protein in a Pasteur Institute series). The number of digits in the distinguishing number should be kept as low as possible for convenience in listing. When the protein is identified, the locus should be given a new and appropriate symbol.

3.14.6 *Phenotype symbols.* Phenotype symbols, if they are necessary, should be the same as genotype symbols but capital letters, not italicized, and with superscript characters lowered to the line (e.g., GST1A and GST1B for phenotypes associated with the *Gst1* locus).

When information concerning subunit structure is available, phenotype symbols should reflect the subunit composition, according to the rules of the International Union of Biochemistry, by use of capital letters (Green, 1979). Details are given in rules for gene nomenclature (see Sec. 3.6).

Identification of loci should not be assumed from the discovery of phenotypic structural variation; crosses should be made to show Mendelian segregation of the alleles. Official gene symbols should not be assigned to variants found in wild rats unless appropriate genetic tests for allelism with known similar variants are carried out. In the absence of genetic tests, phenotypic symbols (as in the standard rules) should be used with a description of the criteria for establishing identity with phenotypes of inbred strains.

3.14.7 *Genetic variants affecting enzyme activity.* Genetic variants that affect enzymes can do so for reasons other than a direct change in the catalytic activity per molecule of the enzyme under study. Presumptive mutations in this group include those affecting enzymatic activity with no discernible alteration in physical or chemical properties of the enzyme and those producing tissue-specific differences in activity. Mutations producing this type of quantitative variation might or might not prove to be allelic or to form a gene complex with the structural locus of the enzyme in question. When allelic with the structural locus, they should be designated according to the standard rules. Even when not allelic, or when the structural locus has not been identified, the new locus should be named on the basis of its discernible phenotype, following the above rules (e.g., *Ak1* for adenylate kinase-1, a locus in rats that controls the level of the enzyme).

3.15 The Major Histocompatibility Complex (MHC) and Other Alloantigenic Systems

3.15.1 *Symbols.* The locus of an alloantigenic system should be designated by *RT* followed by a number (e.g., *RT1*, *RT2*, *RT3*). The numbers should be assigned in the order of discovery of the loci. The MHC is designated *RT1*.

3.15.2 *Haplotypes.* Haplotypes should be given superscript letters as follows:

- Haplotypes of inbred strains of rats should be designated by lower-case letters from *a* to *u* omitting *r* (e.g., $RT1^a$). Used alone, *m* indicates the haplotype of the MNR strain ($RT1^m$). When used with another haplotype symbol, *m* indicates a mutant form of that haplotype (e.g., $RT1^{lm1}$—see below).
- A haplotype of a laboratory recombinant should be designated by the superscript haplotype symbol *r* followed by a series number (e.g., $RT1^{r1}$, $RT1^{r2}$).
- A variant haplotype should be designated by adding the letter *v* and a series number to the haplotype superscript symbol (e.g., LEW = $RT1^l$, F344 = $RT1^{lv1}$).
- A mutant haplotype should be designated by adding the letter *m* and a series number to the haplotype superscript symbol (e.g., $RT1^{lm1}$).

- A haplotype of a wild rat should be designated by a superscript *w* followed by a series number (e.g., *RT1*^{w1}).
- The letters *x*, *y*, and *z* are reserved as generic designations of unknown haplotypes.

3.15.3 *Congenic strains.* A congenic strain involving an alloantigenic system should be designated by the name of the inbred background strain, either a hyphen and the differential locus or a period and an abbreviation of the differential locus, the name of the donor strain enclosed in parentheses, a slash, and the laboratory code of the strain's developer (e.g., BN-*RT1*^{c}(AUG)/Pit or BN.1C(AUG)/Pit). The name may be abbreviated after the first time it is used in a publication by leaving out the name of the donor strain [e.g., BN-*RT1*^{c}(AUG)/Pit is abbreviated BN-*RT1*^{c}/Pit, and BN.1C(AUG)/Pit is abbreviated BN.1C/Pit].

A congenic strain involving an alloantigenic system with a recombinant haplotype should be designated by the name of the inbred background strain, either a hyphen and the differential locus or a period and an abbreviation of the differential locus, a slash, and the laboratory code of the strain's developer (e.g., PVG-*RT1*^{r1}/Ola or PVG.1R1/Ola).

3.15.4 *Loci.* Each locus should be designated by a capital letter. An allele is designated by a superscript denoting the haplotype from which the locus originated. The letters should be assigned in the order of discovery starting with A. The order in which the letters are written should indicate the sequence of loci on the chromosome, as determined by mapping studies (e.g., *RT1.A*^{a}*B*^{a}*D*^{a}*E*^{a}*C*^{a}). Although loci within the MHC should be designated on the basis of laboratory-derived recombinants, uncompromising adherence to this precept greatly reduces the utility of the nomenclature as a shorthand description of the information that is available on a given strain. A reasonable compromise is to restrict genetic diagrams showing the relative positions of the various loci to cases in which each locus is defined by a recombination and to allow the use of locus designations on an inferential basis in other cases. For example, a recombination in a given strain may define two loci, A and B, which encode antigens defined by the appropriate serological test. Then it should be permissible to ascribe these functions to the same loci in other strains, even though they have not yet been defined by recombination in these strains, provided that the inferential nature of the assignment is clearly stated.

3.15.5 *Reporting new systems.* The report of a new antigenic system should include the following data: demonstration that it segregates independently of known systems; strain distribution pattern; tissue distribution pattern; and nomenclature assignment, using first the provisional (local laboratory) name and, after a period of usage and confirmation, the formal name.

3.16 Immunoglobulin Complexes

The following rules were developed for mice by Green (1979) and were adopted for rats at the Fourth International Workshop on Alloantigenic Systems in the Rat (reported by Gutman et al., 1983). The heavy-, kappa-, and lambda-chain regions are designated *Igh*, *Igk*, and *Igl*, respectively. The constant subregions are designated *Igh-C*, *Igk-C*, and *Igl-C*. Individual loci in these subregions are designated by numbers, which are assigned chronologically; however, the hyphen that originally appeared in the designation of an individual locus has been dropped. As a result, the kappa-chain locus in the rat is called *Igk1*, the alpha-chain locus is *Igh1*, the gamma-2b locus is *Igh2*, and the gamma-2c locus is *Igh3*. Although results of DNA cloning studies (Sheppard and Gutman, 1981) make it unlikely that new loci will be discovered for rat kappa chains, the number 1 in the *Igk1* designation is kept for clarity. An allele of an individual locus is designated by the symbol for the locus and a superscript lower-case letter (e.g., *Igh1*^{a}, *Igh1*^{b}).

The variable subregions are designated *Igh-V*, *Igk-V*, and *Igl-V*. An individual locus in one of these subregions that encodes a specific immunoglobulin chain is designated by a hyphen and two or three letters or by a hyphen, two letters, and a number (e.g., in mice, *Igh-Dex*, *Igh-Pc*, *Igk-Efl*). The symbol after the hyphen for a variable-region locus should be related to the antigen for which the immunoglobulin is specific or to the method used for recognizing the variant. Allelic symbols are superscript lower-case letters such as *a* and *b* when allelic markers are well established (e.g., in mice, *Igk-Efl*^{a}, *Igk-Efl*^{b}) or *a* and *o* when the allelic nature of markers is in doubt and the alleles are postulated to determine the presence or absence of a marker (e.g., in mice, *Igh-Dex*^{a}, *Igh-Dex*^{o}).

3.17 Globin Gene Complexes

3.17.1 *Symbols.* The α- and β-globin genes should be considered as constituting gene complexes and should be given the names and symbols hemoglobin-alpha, *Hba*, and hemoglobin-beta, *Hbb*.

3.17.1 *Haplotypes.* The different forms of the complexes should be considered as haplotypes and designated by superscript lower-case letters (e.g., *Hbb*^{a}, *Hbb*^{b}). The letters *m* and *o* should be omitted as they might be confused with 'mutant' or 'null', and the letter *w* should be reserved for wild-derived haplotypes. If the alphabet becomes exhausted, a series of two-letter symbols should be used (e.g., *Hbb*^{aa}, *Hbb*^{bb}).

3.17.3 *Loci within complexes.* The individual loci in the *Hba* and *Hbb* complexes should be denoted by lower-case letters, in some cases followed by numbers and set

off from the main symbol by a hyphen (e.g., in mice, *Hba-x*, *Hbb-y*). The numbers should run from the 5′ end.

3.17.4 *Alleles.* The alleles of genes in the complexes should be denoted by superscript lower-case letters indicating the haplotype of origin (e.g., in mice, *Hbb-y*^{a} and *Hbb-y*^{b} for the alleles of *Hbb-y* occurring in haplotypes *Hbb*^{a} and *Hbb*^{b}).

3.17.5 *Variant haplotypes and alleles.* A new haplotype or allele that arises in a known haplotype by mutation or another change should be denoted by appending a serial number to the haplotype superscript. If the change is known to be caused by mutation or deletion within a particular allele, this should be indicated by adding a hyphen and an appropriate letter symbol and serial number to the allele superscript (e.g., in mice, *Hbb*^{a2} and *Hbb*^{a3} for the first two variants of the haplotype *Hbb*^{a}, *Hbb-b1*$^{d\text{-}m1}$ for the first mutant allele of the gene *Hbb-b1*^{d}, and *Hbb-b1*$^{d\text{-}d11}$ for the first deletion found in the allele *Hbb-b1*^{d}).

3.17.6 *Pseudogenes.* A pseudogene located at a distance from a main complex should be given a locus symbol consisting of the main haplotype symbol, a hyphen, the lower-case letters *ps*, and a serial number (e.g., in mice, *Hba-ps3* and *Hba-ps4* for α-globin pseudogenes located away from the main *Hba* complex).

3.18 Homeobox-containing Genes

The following is based on modifications made by the International Committee for Standardized Nomenclature for Mice to recommended nomenclature drawn up at a meeting on homeobox-containing genes and published by Martin (1987).

Any homeobox-containing gene or genomic fragment may be given the designation *Hox* provided that a substantial fraction of the amino acids that it encodes are identical with those of the homeobox in the *Drosophila* Antennapedia gene.

The criterion for designating a new *Hox* locus is that it occupies a different map position (i.e., it is physically distinct) from all other known *Hox* loci. Until this criterion is met, a new homeobox-containing gene or genomic sequence should be designated by a laboratory name.

The designation of any new *Hox* locus or group of loci (complex), will be determined as follows:

- If the new locus is not apparently closely linked to any previously described *Hox* locus, a number should be be appended. The numbers should be assigned sequentially (e.g., *Hox1*, *Hox2*, *Hox3*). If two or more homeobox-containing loci are present, they will be designated by decimal numbers (e.g., *Hox-2.1*, *Hox-2.2*). Decimal subdesignations should, where possible, reflect the linear order of the *Hox* loci along the chromosome.

- If the new locus is known to be closely linked to a previously designated *Hox* locus, it should be given the numerical designation of that locus (or complex) and the next available decimal subdesignation in the series. Decimal subdesignations should, where possible, reflect the linear order of the *Hox* loci along the chromosome.

3.19 Cytochrome P450

The symbol *Cyp* is used to designate cytochrome P450 loci. The root symbol is followed by a number to indicate the P450 family, a lower-case letter to indicate the subfamily, and another number to indicate the individual gene within the family and subfamily (e.g., *Cyp2a1*, *Cyp2b1*). Numbers are assigned in the order in which genes are identified (e.g., *Cyp2a1*, *Cyp2a2*, *Cyp2a3*). Pseudogenes are designated by appending the lower-case letters *ps* (e.g., *Cyp2c6ps*). Additional details are given by Nebert et al. (1989).

3.20 Transgenes

Transgenes are named according to the following conventions. Examples given are for the mouse.

3.20.1 *Symbols.* A transgene symbol consists of three parts, all in Roman type, as follows:

TgX(YYYYYY)#####Zzz,

where TgX = mode,
(YYYYYY) = insert designation,
= laboratory-assigned number, and
Zzz = laboratory code.

3.20.1.1 *Mode.* The first part of the symbol always consists of the letters Tg (for "transgene") and a letter designating the mode of insertion of the DNA: N for nonhomologous insertion, 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 among three fundamentally different organizations of the introduced sequence relative to the host genome, not simply to indicate the method of insertion or nature of the vector. For example, mice derived by infection of embryos with MuLV vectors will be designated TgR, and mice derived by microinjection or electroporation of MuLV DNA into zygotes will be designated TgN; mice derived from ES cells by introduction of DNA followed by recombination with the homologous genomic sequence will be designated TgH, while mice derived by insertions of the same sequence by nonhomologous crossing-over events will be designated TgN.

When a targeted mutation introduced by homologous recombination does not involve the insertion of a novel functional sequence, the new mutant allele (often called

a "knockout" mutation) will be designated in accordance with the guidelines for gene nomenclature for each species (see Sec. 3.5). The gene nomenclature will also be 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 Lyon, 1989a). The mode symbol TgH is reserved for a time in the future when homologous recombination might be employed to transfer genes to specific sites in the genome using cloned DNA from the target cite to produce a homologous recombination vector. Such target loci might be anonymous, but might exhibit important regulator features that render them desirable for targeting transgenes. A hypothetical example is given in Section 3.20.1.4.

3.20.1.2 *Insert designation.* The second part of the symbol indicates 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. The choice of the insert designation is up to the investigator, but the following guidelines should 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 insert designation should identify the inserted sequence and indicate important features. If the insertion uses sequences from a named gene, it is preferable that the insert designation contain the standard symbol for that gene. If the gene symbol would exceed the spaces available, its beginning letters should be used. Hyphens should be omitted when normally hyphenated gene symbols are used. For example, Ins1 should be used in the symbols of transgenes that contain either coding or regulatory sequences from the mouse insulin gene (*Ins-1*) as an important part of the insert designation. Resources are available to identify standard gene symbols (see Sec. 6).
- Symbols that are identical with other named genes in the same species should be avoided. For example, the use of Ins to designate "insertion" would be incorrect.
- For consistency, a series of transgenic animals produced with the same construct might be given the same insert designation. However, that is not required; some lines might manifest unique and important characteristics (e.g., insertional mutations) that would warrant a unique insert designation. If two different symbols are used for the same construct in different transgenic lines, the published descriptions should clearly identify the construct as being the same in both lines. Two different gene constructs used for transgenic animal production, either within a laboratory or in separate laboratories, should not be identified by identical insert designations. Designations can be checked through the available resources (see Sec. 6).
- A standard abbreviation can be used as part of the insert designation (see Sec. 3.20.1.4 for an example). If a standard abbreviation is used, it should be placed at the end of the insert. These now include

  An (anonymous sequence),
  Ge (genomic clone),
  Im (insertional mutation),
  Nc (noncoding sequence),
  Rp (reporter sequence),
  Sn (synthetic sequence),
  Et (enhancer trap constuct), and
  Pt (promoter trap construct).

This list will be expanded as needed and maintained by appropriate international nomenclature committees.

- The insert designation should identify the inserted sequence, not its location or phenotype.

3.20.1.3 *Laboratory-assigned number and laboratory code.* The third part of the symbol consists 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 laboratory code is uniquely assigned to each laboratory that produces transgenic animals. A laboratory that has already been assigned such a code for other genetically defined mice and rats or for DNA loci should use that code. The registry of these codes is maintained by ILAR (see Secs. 1.5 and 6).

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. Each insertion retains the same symbol even if it is placed on a different genetic background. Specific lines of animals carrying the insertion should be additionally distinguished by a stock designator preceding the transgene symbol. In general, this designator will

follow the established conventions for the naming of strains or stocks of the particular animal used. If the background is a mixture of several strains, stocks, or both, the transgene symbol should be used without a strain or stock name.

3.20.1.4 *Examples.*

- C57BL/6J-TgN(CD8Ge)23Jwg. The human **CD8** genomic clone (**Ge**) inserted into C57BL/6 mice from the Jackson Laboratory (J); the **23**rd mouse screened in a series of microinjections in the laboratory of Jon W. Gordon (**Jwg**).
- Crl:ICR-TgN(SVDhfr)432Jwg. The **SV40** early promoter driving a mouse dihydrofolate reductase (**Dhfr**) gene; **4** kilobase plasmid; the **32**nd animal screened in the laboratory of Jon W. Gordon (**Jwg**). The ICR outbred mice were obtained from Charles River Laboratories (Crl).
- TgN(GPDHIm)1Bir. The human glycerol phosphate dehydrogenase (**GPDH**) gene inserted into zygotes retrieved from (C57BL/6J x SJL/J)F1 females; the insertion caused an insertional mutation (**Im**) and was the **1**st transgenic mouse named by Edward H. Birkenmeier (**Bir**). No strain designation is provided because each zygote derived from such an F1 hybrid mouse has a different complement of alleles derived from the original inbred parental strains.
- 129/J-TgH(SV40Tk)65Rpw (hypothetical). An **SV40**-thymidine kinase (**Tk**) transgene targeted by homologous recombination to a specific but anonymous locus using embryonic stem cells derived from mouse strain 129/J. This was the **65**th mouse of this series produced by Richard P. Woychik (**Rpw**).

3.20.2 *Abbreviations.* Transgene symbols can be abbreviated by omitting the insert. For example, the full symbol TgN(GPDHIm)1Bir would be abbreviated TgN1Bir. The full symbol should be used the first time the transgene is mentioned in a publication; thereafter, the abbreviation may be used.

3.20.3 *Insertional mutations and phenotypes.* The symbol should not be used to identify the specific insertional mutation or phenotype caused directly or indirectly by the transgene. If an insertional mutation that produces an observable phenotype is caused by the insertion, the locus so identified must be named according to standard procedures for the species involved. The allele of the locus identified by the insertion can then be identified by the abbreviated transgene symbol (see Sec. 3.20.2) according to the conventions adopted for the species. Two examples follow.

- $ho^{TgN447Jwg}$. The insertion of a transgene into the hotfoot locus (*ho*).
- $xxx^{TgN21Jwg}$. The insertion of a transgene that leads to a recessive mutation in a previously unidentified gene. A gene symbol for *xxx* must be obtained from a species-genome data base or member of a nomenclature committee (see Sec. 6, Resources).

4. CHROMOSOMES

The rules for nomenclature of rat chromosomes follow the human system for cytogenetic nomenclature, which has been described in detail (Harnden and Klinger, 1985). A standardized system for the numbering of rat chromosomes has been published by the Committee for a Standardized Karyotype of *Rattus norvegicus* (1973). Levan (1974) described the chromosome banding pattern of the rat and assigned numbers to each band in accordance with the human nomenclature system. A high-resolution banded idiogram has been produced by Satoh et al. (1989). The most recent tabulation of rat chromosomes is given in Levan et al. (1992).

5. OUTBRED STOCKS

5.1 Definition

A stock is regarded as outbred when it has been maintained as a closed colony for at least four generations. To minimize changes caused by inbreeding and genetic drift, the population should be maintained in such numbers as to give less than 1 percent inbreeding per generation. Under these conditions, a heterozygous breeding population is expected to reach equilibrium and to produce a stock of stable genetic composition. Formerly inbred strains may be included after four generations of closed outbreeding, provided that continued outbreeding is intended. Outbred stocks are not necessarily highly variable genetically. The degree of genetic variability of any individual stock can only be determined by studying the appropriate genetic markers.

5.2 Symbols

The stock designation consists of a laboratory code, a colon, and two to four capital letters (e.g., Hsd:LE, Crl:WI). The transfer of an outbred stock between breeders is indicated by listing the laboratory codes in chronological order from left to right (e.g., BluHsd:LE for rats obtained by Harlan Sprague Dawley from Blue Spruce Farms). To avoid excessively long designations, only two laboratory codes should be used: that of the current holder preceded by that of the holder from whom the stock was obtained.

An outbred stock that contains a specified mutation is designated by the stock symbol, a hyphen, and the gene symbol (e.g., Crl:ZUC-*fa*).

An outbred stock designation 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. New stock symbols should be registered with Dr. M. F. W. Festing (see Sec. 6, Resources).

5.3 Widely Accepted Outbred-Stock Symbols

The following symbols for outbred rats have been widely accepted for more than 20 years (ILAR, 1970):

Osborne-Mendel	OM
Long-Evans	LE
Sherman	SH
Wistar	WI

6. RESOURCES

Assistance in naming rat strains and stocks can be obtained from the following organizations:

- **Institute of Laboratory Animal Resources (ILAR).** Assigns laboratory codes; assists in naming rat strains and stocks; provides rules for naming rat strains and stocks. Contact: Dr. Dorothy D. Greenhouse, ILAR, National Research Council, 2101 Constitution Avenue, Washington, DC 20418, USA (telephone, 1-202-334-2590; fax, 1-202-334-1687; Bitnet, DGREENHO@NAS).
- **PALM Institute.** Assists in naming rat strains and stocks; provides rules for naming rat strains and stocks. Contact: Dr. Takashi Natori, Director, PALM Institute, N29 W4 2-1-215 Sapporo 001, Japan (telephone, 81-11-746-3988; fax, 81-11-746-6722).
- ***Registry of Inbred Strains.*** Maintains lists of inbred strains and outbred stocks of rats; assists in naming strains and stocks of rats; provides rules for naming rat strains and stocks. Contact: Dr. Michael F. W. Festing, Medical Research Council Toxicology Unit, Woodmansterne Road, Carshalton, Surrey SM5 4EF, UK (telephone, 44-81-643-8000; fax, 44-81-642-6583). As of June 1993, Dr. Festing's address will be IRC for Human Toxicity, Leicester University, University Road, Leicester LE2 7RH, UK.
- ***Rat News Letter.*** Publishes new inbred strain, gene, and other symbols for rats. Periodically publishes lists of strain and gene symbols, chromosome maps, and rules for rat nomenclature. Contact: Dr. Viktor Stolc, Editor, *Rat News Letter*, 2542 Harlo Drive, Allison Park, Pittsburgh, PA 15101 (telephone and fax, 1-412-487-4289).
- **Transgenic Animal Data Base (TADB).** Records, stores, and provides information on transgenic animals, including standardized nomenclature and a complete description of each transgenic animal; maintains rules for transgenic nomenclature on electronic bulletin board. Contact: Ms. Karen Schneider, TADB Coordinator, Oak Ridge National Laboratory, PO Box 2008, MS 6050, Oak Ridge, TN 37831-6050 (telephone, 1-615-574-7776; fax, 1-615-574-9888; Bitnet, TUG@ORNLSTC; Internet, OWENSET@IRAVAX.HSR.ORNL.GOV).
- **The Jackson Laboratory.** Assists in naming transgenes; provides lists of named mouse genes. Contact: Dr. Muriel T. Davisson, The Jackson Laboratory, Bar Harbor, ME 04609 (telephone, 1-207-288-3371; fax, 1-207-288-8982).
- **Genome Data Base (GDB).** Records, stores, and provides information on mapped human genes and clones. Contact: GDB, Welch Medical Library, The Johns Hopkins University, 1830 East Monument Street, Baltimore, MD 21205 (telephone, 1-301-955-9705; fax, 301-955-0054).

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APPENDIX II

Summary: Important Laboratory Animal Resources: Selection Criteria and Funding Mechanisms for Their Preservation[a]

Prepared by the Committee on Preservation of Laboratory Animal Resources,[b] Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council

In response to the perception of U.S. scientists that some genetically unique animal models have been lost or are at risk, partly because of financial problems but also for such other reasons as the death or retirement of scientists responsible for specific stocks, the Committee on Preservation of Laboratory Animal Resources was formed by the Institute of Laboratory Animal Resources. The committee was charged with documenting losses of animal models and resources resulting from funding inadequacies or other reasons, evaluating the long-term effects of such losses on biomedical research, assessing existing animal resources and the current mechanisms for maintaining them, and recommending cost-effective procedures for preserving genetic stocks. The committee identified the following important problems associated with preserving laboratory animal resources: lack of centralized planning, lack of standardized criteria for assessing the value of an animal model or resource, instability of funding, changes in government regulations and funding priorities, and complex maintenance requirements of many animal models.

Criteria for Preservation

To reduce the risk of losing valuable animal models, the committee recommended establishment of a long-term, stable, integrated program for safeguarding the nation's animal resources. The program should include mechanisms for identifying valuable animal resources, maintaining and preserving them, and providing for their financial support. The following criteria were recommended for rigorously evaluating animal models considered for preservation:

- *Importance of disease process or physiologic function.* Animal models of severe or common human pathologic conditions or models used to study normal physiologic function are extremely valuable. Even models representing diseases not yet observed in humans are important. In all cases, the value of such models depends on the ability to maintain and to transmit reliably the relevant traits through breeding.
- *Validity.* The validity of many models depends on proper genetic management to preserve their unique traits and to ensure that the phenotype is predictable.
- *Replaceability.* The difficulty of replacing a model is a measure of its value. Models that are require years of selective breeding to develop or that arise as a consequence of spontaneous mutations (especially in animals with long generation times) must be considered relatively important.
- *Versatility.* The variety of problems that can be studied with a given animal model is a measure of its value.
- *Use.* If an animal model is used by a large number of laboratories, its value is high. The number of investigators using the model in research is a more important measure of use than is the number of animals used.

Dual Review of Requests for Support of Research Involving Investigator-Managed Animal Resources

Funds should be allocated specifically for developing and preserving important laboratory animal resources that are maintained in investigator-managed facilities. Such funds should be administered through a competitive grants program reviewed by an appropriately constituted group. In the case of the National Institutes of Health (NIH), these funds should be administered conjointly by all the institutes to provide a single focus of responsibility.

A Review Group for Laboratory Animal Preservation should be established to evaluate grant proposals that request funds to support investigator-managed resources. The group should be composed primarily of scientists who use animals in their own research. These scientists should represent a broad range of disciplines, including the study of pathogenesis of disease, basic physiologic processes, and fundamental genetics. In addition, there should be at least one geneticist who is capable of evaluating the genetic quality of the animals in a resource and

[a]This study was supported by the National Research Council Fund, a pool of private, discretionary, nonfederal funds that is used to support a program of Academy-initiated studies of national issues in which science and technology figure significantly.

[b]Members of the committee: Dorothea Bennett (*Chairman*), Department of Zoology, University of Texas, Austin, Texas (deceased); Linda C. Cork, Division of Comparative Medicine, Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, Maryland; Thomas J. Gill III, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; Jon W. Gordon, Department of Obstetrics and Gynecology, Mt. Sinai School of Medicine, New York, New York; Andrew G. Hendrickx, California Primate Research Center, University of California, Davis, California; Larry E. Mobraaten, The Jackson Laboratory, Bar Harbor, Maine; and John L. VandeBerg, Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, Texas.

at least one laboratory animal scientist who is capable of evaluating the health of the animals and the husbandry procedures for maintaining them. The responsibility of this group should be the uniform application of the criteria for evaluating animal resources described previously. In addition to reviewing the merits of a resource application, the review group could, if it were deemed preferable, recommend that the proposed resource be maintained at some other established facility.

Applications for support of investigator-managed animal resources, whether or not they are submitted in conjunction with applications for associated research, should be reviewed by the proposed group. Applications seeking support for both research and resource components would also be reviewed by the appropriate study section. Applicants preparing proposals with a resource component should describe and document the resource according to the criteria outlined above. To provide stability to resource colonies, grants should generally be made for a period of 5 years between competing renewals, irrespective of the duration of funding for the research component.

National Center for Laboratory Animal Resources

Many animal models are not used consistently in large numbers, so commercial breeders do not maintain them. They also might not be used continuously enough by any one investigator to warrant maintaining them as an investigator-managed resource. It is in such situations that a national center would meet a major need. A National Center for Laboratory Animal Resources would provide a source of genetically defined and appropriately monitored animals to ensure quality control and cost-effective maintenance. It could also hold duplicates of valuable animal resources, so that if individual colonies housing such animals were lost, the resource would survive. The center could distribute these animals for experimental purposes or as breeding nuclei.

In addition to distributing animals, the center would be a source of information about the various strains and stocks and would work actively to develop new and useful animal resources and unique methods of preserving them (e.g., cryopreservation). The center could form the core of a network of resource colonies, both commercial and investigator-based, to provide extensive national coordination of laboratory animal resources.

A critical part of the structure of the national center should be an advisory committee to set policy and make decisions about which species and which strains and stocks within a species should be maintained and what new animal resources should be developed. The advisory committee should be distinct from the Review Group for Laboratory Animal Preservation, although it should be composed of scientists with a similar scope of expertise. A committee with such a composition is critical to the success of the National Center for Laboratory Animal Resources. The advisory committee would represent the scientific community and ensure appropriate oversight of the hard decisions that are essential in allocating limited resources.

Conclusions

The system recommended in this report should not greatly increase the overall amount spent for animal resources, in as much as the present system is inefficient and has large hidden costs that result in duplication of support for maintaining animal models and animal colonies. An important cost-effective aspect of the system proposed by the committee is that only animal resources that merit support, as determined by an appropriate group using objective criteria, will receive such support.

It was the consensus of the committee members that these recommendations are realistic and cost-effective and can provide the basis for many research initiatives. It is believed that the necessary investment, including the costs of operating the committees suggested in this report, will be offset in part by savings of funds currently committed. For example:

- Central facilities can maintain the very highest standards of animal husbandry and genetic management and provide the healthiest possible animals for research purposes. At the same time, the cost of producing animals will be considerably less than the combined cost of producing them at multiple independent facilities.
- The proposed program should eliminate duplication of stocks by several investigators, who might or might not be actively using the stocks.
- The proposed program will eliminate the necessity of having breeding colonies maintained by investigators who require animals of only one sex, animals of only one age class, only pregnant females, or animals with special requirements. Thus, a single breeding colony of the same size that would be required by each investigator can efficiently satisfy the needs of several investigators.
- The scientists and staff who maintain central facilities can provide critical advice and expertise on the maintenance and experimental manipulation of specialized animal models; such advice and expertise in many instances will lead to more efficient and more humane use of animals in research.

Copies of the full report *Important Laboratory Animal Resources: Selection Criteria and Mechanisms for Their Preservation* [*ILAR News* 32(4):A1-A32, 1990] are available from the Institute of Laboratory Animal Resources, National Research Council, 2101 Constitution Avenue, Washington, DC 20418.

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