

# **RODENTS AS MODELS FOR BIOMEDICAL RESEARCH**

Michael S. Rand, DVM, DACLAM  
Associate Director  
University Animal Care  
University of Arizona  
Tucson, AZ

Last Updated: July 2011

## Table of Contents

MICE.....	3
INTRODUCTION .....	3
USES IN RESEARCH .....	3
Immunodeficient Rodents .....	3
Immunodeficiency Mutations In Mice.....	4
Applications of Immune-Deficient Mice.....	6
Transgenic Technology .....	6
Methods for Genetic Modification.....	7
Models for Human Disease.....	8
Social and Ethical Concerns and Safety .....	9
RATS .....	11
INTRODUCTION .....	11
USES IN RESEARCH.....	11
HAMSTERS and GERBILS.....	12
INTRODUCTION .....	12
USES IN RESEARCH.....	13
HAMSTER.....	13
GERBIL .....	13
GUINEA PIGS .....	14
WILD RODENTS.....	14
GLOSSARY .....	16
REFERENCES.....	20

## MICE

### I. INTRODUCTION

The house mouse, *Mus musculus*, has a name, which originally meant *to steal*, but despite its propensity for petty thievery, it has enjoyed a far better reputation than its fellow rodent, the rat. The mouse, once honored in ancient coins, writings, and paintings, has the dubious distinction of being employed in research studies beginning in the early 1600s. However, development of the laboratory mouse as a research model really began with genetic experiments in the early 1900s.

### II. USES IN RESEARCH

Mice represent the primary species used in research, comprising 67% of all animals used in biomedical research and testing. Their small size, short life span, proclivity for reproduction, known genetic background, and minimal expense for purchase and maintenance has made them a desirable animal model. Today, the laboratory mouse is recognized as the preeminent model for modern genetic research. Mice are also used in a variety of other types of research, including cancer, immunology, toxicology, metabolism, developmental biology, diabetes, obesity, aging, and cardiovascular research.

#### Immunodeficient Rodents

Immunodeficient rodents are indispensable research models for biomedical investigators for studies in oncology, immunology, and infectious diseases. Today, biomedical researchers use a number of naturally occurring and transgenic strains of immunodeficient mice and rats to study the immune system, rejection of tissue transplants, infections, cancer, and tumor growth.

Like other blood cells, lymphocytes differentiate from pluripotent stem cells in bone marrow. Lymphocytes that continue their maturation in bone marrow develop into B cells, while those that migrate to the thymus and complete maturation there become T cells. Mature B cells and T cells are most concentrated in lymph nodes, the spleen, and other lymphatic organs where the lymphocytes are most likely to encounter antigens—foreign substances that evoke the production of antibodies and cytotoxic cellular responses.

Both B and T cells are able to recognize antigens. B cells are responsible for *humoral, or serum immunity* by producing immunoglobulins, or Igs. These Igs are divided into five chief classes—IgG, IgM, IgA, IgD, and IgE—each with special properties. T cells, making up about 70 percent of all lymphocytes, are responsible for *cellular immunity*, meaning they attack and kill antigens directly. T cells do not themselves make antibodies but they help regulate the production of antibodies by the B cells. There are four types of T lymphocytes—helper, cytotoxic, memory, and suppressor.

Differentiation of B and T cells into a vast variety of cloned cell types, each responding to a

specific antigen, involves two phases—the primary or antigen-independent phase and the secondary or antigen-dependent phase. During the primary phase, stem cells proceed through stages of differentiation to generate vast amounts of B or T cell clones, each with unique antigen receptors. The immune system generates an incredibly diverse range of gene sequences, or antigen-binding specificities for antibodies.

The secondary (antigen-dependent) phase involves only B cells, which can recognize an infinite number of antigens (but each individual B cell recognizes only one antigen). When a particular antigen binds to the antigen receptors on the appropriate B cell, that B cell is triggered to proliferate into a large clone of cells, all responsive to the specific antigen. In this *clonal selection* process, some of the cloned B cells become long-lived memory cells and others differentiate into plasma cells secreting antibodies.

Another type of immune cell was discovered in 1975 -- the natural killer (NK) cell, which looks like a lymphocyte but contains granules resembling granulocytes. NK cells apparently recognize some feature of the target cells, either directly or via receptors that attach to the tails of antibodies on the target cell's surface. As a result, NK cells act by releasing the contents of their granules to kill the target cells or by recruiting the help of other immune cells.

Any compromise in host defense may be designated as an immune deficiency. Immune deficiencies found or induced in mice range from very mild, of limited known biological importance, to complete absence of functions or cell types.

Early investigators of immune deficits did not have our current luxury of ordering sets of genetically modified animals from commercial suppliers. Their studies usually required use of animals of restricted age or with deficits induced “in house.” These approaches are still useful today, and include study of very young or very old mice, diet restriction including during the period of fetal development, preconditioning with irradiation or antibody infusion, toxins and immunotoxins, pharmaceuticals, hormones, stress, or surgery such as thymectomy. These types of approaches have numerous limitations. Foremost is variability in the extent of the induced immune deficits. Variability can be between animals, between various organ systems within a single animal, or in duration of the induced immune suppression. These approaches also tend to be costly in labor, materials, and/or equipment. Mice with genetic immune deficiencies have now largely replaced or been combined with the techniques for externally induced immune deficits.

### **Immunodeficiency Mutations in Mice**

More than 4,000 genes of the mouse have now been assigned to specific chromosomal locations. Many genes were first identified following spontaneous mutations that produced distinctive physical characteristics. Single-gene mouse mutants have provided highly useful experimental research models. Two key single-gene, naturally occurring mutations are the nude (*nu*) and the severe combined immunodeficiency (*SCID*); both are important research models for the study of xenografts, transplanted tissues, and tumors from foreign species. Other single-gene mutations commonly used as research models include the beige (*bg*) and the X-linked immune deficiency

(*xid*).

The *nu* mutation was first reported in 1966 in a closed stock of mice in a laboratory in Glasgow, Scotland. It was not until 1968, however, that it was discovered that the homozygous nude mouse also lacked a functional thymus, i.e., it was *athymic*. The mutation produces a hairless state, generating the name “nude.” The other, unique defect of nude mice is the failure of the thymus to develop normally to maturity. The thymus remains rudimentary and produces reduced numbers of mature T cells. This means nude homozygotes (animals with identical mutant genes at corresponding chromosome loci) do not reject allografts and often do not reject xenografts (tissue from another species). The discovery that human neoplasms (tumors) could be grown in nude mice was immediately recognized as an important research tool. Thus, the spontaneous mutation of *nu* among laboratory mice was a serendipitous development that led to the nude mouse becoming the first animal model of a severe immunodeficiency. In the decades since, the nude mouse has been widely utilized by researchers studying factors regulating transplantable human tumor growth and cancer metastasis.

Nude mice support only a limited number of normal tissue allografts and xenografts. The major application and enduring usefulness of the nude mouse has been in oncology. This is due to growth patterns of transplanted tumors that include metastasis to sites such as brain and the relative resistance of the host nude mice to therapeutic interventions, which permits investigators to accurately evaluate the effects of their new compounds and protocols on tumor growth and metastasis. The nude mouse is not an immunologically inert mouse, and most graft failures in the nude strains are attributed to unusually high NK cell activity.

To promote applied investigations and to obtain growth of a broader range of normal xenogenic tissue in nude mice, a number of laboratories crossbred nude mice to other known, spontaneously mutant mice to generate larger immune defects. Nude-beige and beige/nude/*xid* (BNX) mice are examples. The BNX mice have been extensively used as hosts for normal human hematopoietic stem cells. The beige mutation reduces lytic activity of NK cells, but not numbers of NK cells or their cytokine production. The *Xid* mutation reduces B-cell activity.

Serendipity also played a role in the discovery of another important mutant strain of immunodeficient mice, which lacks both B and T cells, called severe combined immunodeficiency (SCID) mice. During routine lab tests on the immune system in mice, Dr. Melvin J. Bosma of the Fox Chase Cancer Center in Philadelphia discovered the strain in 1980. The first SCID mice were an accident of nature, the product of chance matings of apparently normal mice that carried a recessive mutant gene now called *SCID*. Some of the offspring inherited a complete pair of *SCID* genes and were born with the *SCID* defect.

The SCID mouse was also a superior recipient for transplantation studies because it supported a broader range of tissues, including normal cells of the human immune system. The SCID mouse is not a perfect “tissue culture” vessel for grafted tissue. Important limitations include the presence of NK cells, a “leaky” phenotype in which immunoglobulins are produced,

development of thymomas, and high sensitivity to irradiation. Mice with a similar T- and B-cell deficient phenotype were created by deletion of either of the recombinase activation genes Rag1 or Rag2. These genes initiate rearrangements in the antigen-specific receptors of T and B cells and act slightly earlier in T- and B-cell differentiation than does the SCID gene.

### Applications of Immune-Deficient Mice

In addition to insights into lymphocyte biology, immune-deficient mice have been applied to questions in other fields. In oncology, gene expression in biopsy specimens of human tumors has been found to be maintained after engraftment into immunodeficient mice. Changes in expressions of genes have also been followed after therapeutic interventions in the tumor-bearing mice. This suggests that the xenogenic transplantation models have accurate predictive value for tumor progression and tumor treatment.

In the field of lymphohematopoietic stem cell research, SCID RAG (Recombinase-Activating Gene) nulls and their improved, multiple mutation strains have played key roles in assessing developmental and differentiation potentials of human cells. Reference to human cells with “SCID-repopulating activity” is commonplace. Current work with these models also addresses the feasibility of gene therapy in bone marrow-derived cells and in muscle or endothelium. Use of a severely immunodeficient host assesses the efficacy of the construct and duration of therapy in the absence of antigen specific immunity, which would be directed against products of the therapeutic gene in normal mice.

Other major topics to which immune-deficient mice have made substantial contributions are the pathophysiology and treatment of diabetes mellitus, arthritis, ocular and cardiovascular diseases, dermatology, and human antibody production biotechnology. However, one of the largest bodies of work on applied uses of immune deficient mice is in challenge studies using infective agents. Challenges with bacteria, viruses, parasites, or multiple components are feasible. By comparing responses in mice with differing immune competencies or between different organisms in a single type of immune-deficient mouse, fine dissection of the specific pathways involved in resistance or susceptibility to individual organisms has been achieved.

### Transgenic Technology

The term ‘transgenic’ was coined in 1981 by Gordon and Ruddle to describe an animal in which an exogenous gene was introduced into its genome. In the late 1980s, the term transgenic was extended to gene-targeting experimentation and the production of chimeric or ‘knockout’ mice in which a gene (or genes) has been selectively removed from the host genome. Today, a transgenic animal can be defined as one having any specific, targeted genetic modification. Transgenic animals are most commonly produced through: (i) germline modifications of gametes; (ii) microinjection of DNA or gene constructs into zygotes [unicellular embryos]; or (iii) incorporating modified cells, including embryonic stem (ES) cells, into later stage embryos. After

gamete or embryo modifications, the resultant embryos are matured to term in a recipient female. Several scientific paths converged in the early 1980s to establish transgenic animal technologies. Through the 1990s, a host of new techniques and modeling systems further extended the scope, utility and commercial aspects associated with animal transgenesis.

Transgenic animals are designed to exhibit either a *gain of function* (expression of a novel cell-surface receptor) or a *loss of function* (knockout of a cellular function). All transgenic models, whether targeted or untargeted, still may present unpredictable expression patterns due to incomplete knockout of the targeted gene, redundancy within the genome or unanticipated genetic interactions, such as down-regulation of other genes. The mouse is the only mammalian species in which scientists have the ability to specifically delete one gene at a time from the genome, which makes it possible to discover what these genes do in normal physiological processes and in pathology. By 2012, it is expected to have up to 10,000 new lines of knockout mice, tripling the number currently in existence from all sources.

Selected Methods for Genetic Modification:

- **Classical pronuclear microinjection:** introduction of foreign DNA into embryonic pronuclei resulting in random integration and expression. Classical pronuclear microinjection techniques have been used for 15 years to create mouse models, which express unique phenotypes. The major flaw in the pronuclear microinjection models has been the random nature of transgene integration locus and copy number. Expression patterns may vary significantly in a series of lines expressing identical transgenes. Modifiers of expression such as age, sex and health status further confound the process, increasing potential for variability.
- **Embryonic stem (ES) cell-mediated gene targeting:** introduction of genetically modified ES cells into recipient embryos resulting in the ablation (knockout) or modification of a specific genetic expression. By using ES cell gene knockout technology, an investigator can produce an animal model in which expression (or the lack of expression) is highly predictable. A clone of cultured ES cells is selected in which a specific DNA sequence in the mouse genome has been modified (usually inactivated).
- **Retrovirus-mediated gene transfer:** four- to 16-cell stage embryos are primarily used for infection with one or more recombinant retroviruses (effectively transducing only mitotically active cells). Immediately following infection, the retrovirus produces a DNA copy of its RNA genome using the viral enzyme reverse transcriptase. The DNA copy of the viral genome, or provirus, integrates randomly into the host cell genome, usually without deletions or rearrangements. High rates of gene transfer, approaching 100% efficiency, are achieved with the use of retroviruses. However, as is the case for gene transfer by microinjection, integration events are random.
- **Nuclear transfer:** nuclear transfer (commonly referred to as ‘cloning’) involves the introduction of donor nuclei obtained from either stem cells or differentiated adult cells into enucleated oocytes, thereby reprogramming future development. Reconstructed oocytes are then transferred to a surrogate dam for the remainder of gestation.
- **Gene knockdown and RNA interference:** the emerging technology of RNA interference (RNAi) has broadened the possibilities for the creation of loss-of-function

models. Short interfering RNA (siRNA) exists in a double-stranded state and inhibits endogenous genes (and/or exogenous sequences, as in viral genes) as the result of complementary sequence homology. The short oligonucleotides that silence gene expression (i.e. protein synthesis) are referred to as siRNAs. siRNA has been shown to be a potent inhibitor of gene function *in vivo*. Mouse and rat models harboring small hairpin RNA transgenes, following shRNA transcription, produced lower levels of the homologous protein when compared with controls. Gene silencing and knockdown technology has potential medical and agricultural applications, including the inhibition of viral gene transcription and inhibition of endogenous genes coding for deleterious gene products. In the mouse, RNAi has several advantages over homologous recombination and ES cell-mediated gene knockout methodologies. RNAi can be synthesized directly, thus avoiding laborious cloning steps. Most notably, this methodology is currently the most significant advance since nuclear transfer in effecting efficient loss-of-function modeling in mammalian species (particularly for nonmurine species where ES cell transgenesis has not been successful).

- **Sperm as vectors:** genetically modified spermatogonia are transferred into testes lacking germ cells. These cells take up residence in the testis, divide and produce fully mature and fertile spermatozoa. Pluripotent spermatogonial stem cells have been isolated and grown under conditions allowing long-term maintenance and proliferation.
- **Mitochondrial gene transfer:** studies revolving around mitochondrial transfer and techniques to produce animals harboring foreign mitochondrial genomes have been initiated. The creation of transmitochondrial animals represents a new model system that will provide a greater understanding of mitochondrial dynamics, leading to the development of genetically engineered production animals, as well as therapeutic strategies for human metabolic diseases affected by mitochondrial mutation or function.

## Models for Human Disease

The biomedical sciences rely heavily on animal models as tools for the discovery and development of therapeutic interventions. Of the many human conditions that medicine seeks to address, few naturally occur in animals. Instead, transgenic modifications, particularly in mice, are commonly used to model the human condition and studies in these animals have provided fertile ground for drug discovery across a continuum of human developmental and pathological conditions including Huntington's, Alzheimer's, cystic fibrosis, emphysema, diabetes, inflammatory arthritis, and cancer. Some examples include:

- (i) Gene therapy.
- (ii) Genetic basis of human and animal disease and the design and testing of strategies for therapy.
- (iii) Disease resistance in humans and animals.
- (iv) Drug and product testing and/or screening.

- (v) Novel product development through molecular pharming. Biologically active proteins have been developed in transgenic animals, which are used as bioreactors instead of traditional bacterial and cell culture-based systems.
  - (vi) Transgenic expression of immunoglobulins.
  - (vii) Xenotransplantation.
- 

### Social and Ethical Concerns and Safety

The advent of biotechnology, more so than any other technological innovation, has made it clear to scientists that they can no longer dismiss ethical concerns in society as irrelevant to the scientific enterprise, on the grounds that, by its very nature, science is and ought to be “value-free” or “value-neutral”. More pragmatically, it is evident that society is extremely exercised about the genetic engineering of animals, and that a failure on the part of scientists to enter into the dialogue concerning the ethical issues raised by transgenics is likely to leave the development of principles governing the field to those who shout the loudest.

In the first place, it is necessary to separate out genuine moral issues from spurious ones, which nonetheless command and galvanize a great deal of public attention. For example, religious groups complain that creation of transgenic animals violates divine law, or fails to show proper respect for life. Other critics argue that humans were not “meant” to create life. Still others see species as inviolable, or assert that humans should not alter “nature.” Such objections have stirred public skepticism and fear regarding biotechnology. When scientists fail to exhaustively discuss - and deal with - the genuine moral issues growing out of genetic engineering of animals, one is inexorably led to a version of Gresham’s Law, wherein, instead of bad money driving good money out of circulation, bad (or spurious) ethical and social concerns drive genuine and legitimate ethical and social concerns out of the social mind. Thus, it is imperative that researchers planning to develop or use transgenic animals are fully cognizant of, and prepared to address, the genuine social and moral questions raised by such activities, lest the sensationalistic, baseless concerns inform and shape the public response to biotechnology.

The first genuine bioethical issue relevant to the creation and use of transgenic animals in biomedical research concerns the possible danger to humans and/or other animals, which might be presented by the animals in question.

Perhaps the mouse model exemplifies the most dramatic real case of such a concern for AIDS created at the National Institute of Allergy and Infectious Disease of the National Institutes of Health. HIV- 1, the pathogen causing human AIDS, naturally infects only humans and chimpanzees, and chimpanzees remain asymptomatic. In an attempt to create an “animal model” for the disease, researchers introduced the HIV genome into mouse embryos by microinjection and propagated these mice by breeding. Although the purpose of the model was primarily to study viral latency, F<sub>1</sub> progeny resulting from the mating of one of the founder animals, its nontransgenic mates developed symptoms similar in some respects to human AIDS. Furthermore, the tissues of these animals produced infectious HIV particles.

Obviously, the creation of mice capable of harboring infectious HIV virus represents a genuine

and legitimate social concern of both a moral and prudential nature regarding biosafety. The moral question is, of course, whether one ought to produce such a potentially dangerous organism. The prudential question is, given the decision that the benefit outweighs the risk, and therefore that such an animal should be created (leaving aside separate moral questions regarding animal suffering), how does one reduce the risk to an acceptable minimum?

These sorts of concerns are legitimate and understandable and encapsulate questions, which any researcher planning to develop or utilize transgenic organisms should carefully address. Indeed, potential dangers from a transgenic organism may be far subtler than just transmission of the disease it is designed to host. For example, if one were genetically engineering for resistance to a given pathogen in an animal, one could conceivably be selecting new variants among the natural mutations of that microbe to which the modified animal would not be resistant. One possible example of this sort of reaction has recently been discussed above regarding the SCID-hu mouse developed as a model for AIDS. These animals are genetically engineered to possess a human immune system, and are then infected with the AIDS virus. Some researchers suggest that, in such a mouse, the AIDS virus could become more virulent and infectious through new routes of transmission in virtue of interacting with native mouse viruses. This could conceivably wreak havoc both with animals and humans. Genetically engineered animals could conceivably damage extant ecosystems if such animals are not confined. Thus, it would seem morally incumbent both to do careful cost-benefit analysis before creating any transgenic animal, and to build in an additional safety mechanism by demanding **significant** balance of benefit over cost to compensate, at least in some measure, for unknown or unrecognized dangers. Michael Crichton's novel, *Jurassic Park*, provides an ingenious, scientifically based, albeit fanciful account of how small, unanticipated problems in genetic engineering can amplify into major catastrophes along lines describable by the mathematical theory of chaos.

Thus, the first ethical requirement relevant to creating transgenic animals is making a case that benefits clearly outweigh risks, with significant allowance made for unforeseeable risk. If this demand is met, the next ethical requirement is the demand that one control for the remaining risks.

Fortunately, the research community has been extremely sensitive to the theoretical dimensions of laboratory biosafety. Researchers utilizing transgenic animals for disease-related study should familiarize themselves with the principles encoded in the CDC-NIH publications *Guidelines for Research Involving Recombinant DNA Molecules* and *Biosafety in Microbiological and Biomedical Laboratories*. These volumes describe four increasingly stringent levels of biological containment (Biosafety levels 1 to 4).

In the case of the AIDS mice mentioned above, the microinjected embryos were inserted into surrogate female mice, which were transferred to a stainless steel glove box within a BL4 facility.

All transgenic animals were maintained in the glove box closed system for the duration of the experiment. Indeed, one can characterize the containment procedures for this experiment as BL4+, for, in addition to standard BL4 procedures, bleach-filled moats and traps to provide "overkill" assurances that the danger was contained surrounded the containment boxes.

# **RATS**

## ***I. INTRODUCTION***

Rats have been regarded as vile, insidious vermin, damned for their role in the Black Plague of the Middle Ages, and for the injury and destruction they have caused to humans and property throughout the ages. However, one species of rat, *Rattus norvegicus*, has been used in research since the mid 1800s, in what Lindsey described as an ascendancy from the gutter to a place of nobility through its contributions to human health and well-being. In an ever-evolving process since the first crude uses of rats in research, investigators have sought, through trial and error, to develop appropriate husbandry, care, and use techniques to minimize or eliminate the impact of variables such as nutrition and disease on research results. Early ignorance of basic needs translated into inappropriate or substandard care, which affected the health and well being of the animals first, but also affected the quality and reliability of the research data that was generated. Advances were made in understanding the requirements for and provision of adequate nutrition, in recognition and control/elimination of latent infectious diseases, and in providing sanitary environments to minimize contamination of and disease in the rats.

## ***II. USES IN RESEARCH***

The rat is the second most commonly used animal species in biomedical research and testing. Rats comprise 21% of all animals used, a somewhat deceptively low figure, but when coupled with mice, these two species account for 88% of all animals used in research and testing. Rats possess a number of characteristics which make them ideal animal models: they are readily available from many commercial and private sources, they possess genetic uniformity, they are inexpensive to purchase and maintain, they are easy to handle, they are adaptable to novel situations and environments, they have well-defined physiologic parameters, they have known microflora, some have spontaneous diseases useful in modeling, their short life-span affords an opportunity to study long-term effects of experimental treatments on health and well-being, and, lastly, the sequencing of the rat genome was completed in 2004.

The laboratory rat is useful for lifetime studies, whether the questions are related to toxicology, neoplasia, or aging, because of its short lifespan, moderate size, and low maintenance cost. The Fischer 344 rat, the Brown Norway rat, and the F1 hybrid of these two are the three most commonly used inbred rat strains for aging studies in the United States. The Sprague Dawley, Wistar, and Long Evans outbred stocks are common in aging studies in the U.S.

Along with mice, rats are the most common species used in studies to identify the toxic, carcinogenic, and teratogenic potential of chemicals and drugs. In addition, they are frequently used to study the carcinogenic process, both tumor initiation and promotion. Rat models of various cancers also are important in the development of cancer therapy. Experimentally induced tumors are those that arise in various organs in the rat following administration of known cancer causing agents. Spontaneous tumors are those that arise during the natural course of a rat's lifetime. The incidence of spontaneous tumors varies with age, strain, and sex of the rat. The

choice of model will depend on the nature of the oncology study. A model, which produces a tumor in a target tissue with high relevance to human cancer, may be most useful in a chemotherapy trial. On the other hand, a model with spontaneous tumors that are less common in humans may be the better of choice for understanding the basic mechanisms of tumor initiation or promotion.

The rat has long been used as a model for toxicity testing of various agents. It is widely used for evaluating the safety and efficacy of new drugs. The rat is useful in teratologic studies because of its short reproductive cycle, large litter size, and relatively few spontaneous congenital anomalies. Common stocks and strains of rats used in toxicological, carcinogenic, and teratologic studies are Sprague Dawley, F344, Wistar, and Long Evans.

Rheumatoid arthritis is a chronic immune-mediated disease of unknown etiology and pathogenesis. Nevertheless, it is generally considered a disease of autoimmune origin. Most of the experiments leading to the discovery of neurogenic inflammation and its possible involvement in rheumatoid arthritis have been carried out in rodents with experimentally induced arthritis. One of the most valuable and widely used animal models is adjuvant-induced arthritis in rats.

The BB-rat syndrome was recognized initially in 1974 by Chappel at the BioBreeding Laboratories, Ottawa, Canada . Overt Type 1 Diabetes Mellitus, associated with hypoinsulinemia occurred sporadically in this commercial breeding colony of non-inbred Wistar-derived laboratory rats kept under strict gnotobiotic conditions. A breeding program was established, and it was decided that the syndrome would be named "BB" after the initials of the breeding laboratory. All BB rats are descendants of the original Ottawa litters, but rats in different colonies now vary in frequency of diabetes. To identify the specific strain, all animals are named after the city or institution in which they are bred, for example, BB/W for Worcester. Diabetes in the BB rat typically has an abrupt onset, with glucosuria, hyperglycemia, hyperketonemia, ketonuria, and hypoinsulinemia.

## **HAMSTERS and GERBILS**

### ***I. INTRODUCTION***

There are more than 15 species of hamsters, but the one used most frequently in biomedical research is the Syrian (golden) hamster, *Mesocricetus auratus*. Commercially available golden hamsters were derived from only three or four littermates collected in Syria in 1930. The Mongolian gerbil, *Meriones unguiculatus*, has been used in research in Europe since the mid to late 1800s. Dr. Victor Schwentker is credited for introducing the Mongolian gerbil to the U.S. in 1954.

Both hamsters and gerbils are hardy creatures, and do not exhibit the wide spectrum of

spontaneous overt and latent diseases common to rats and mice. Their good general health, their susceptibility to induced disease conditions, the low cost of production and maintenance, and literature available on the biology and physiology of these species make them useful animal models.

## **II. USES IN RESEARCH**

### **A. HAMSTER**

Hamsters account for 0.6% (approximately 500,000 used per year) of the total number of animals used in research annually. The American College of Laboratory Animal Medicine published a review of and references for several of the research uses of the hamster in studies involving antibiotic-associated colitis, behavioral and neuroscience research, dental research (caries and periodontal disease), endocrine research, genetics, hibernation and cold adaptation, immunology, infectious disease research, oncology (cheek pouch immunoprivileged transplant site, natural and induced tumors, viral oncogenesis), radiobiology (radioresistance), reproductive physiology, gerontology, tissue culture preparation, teratology, and toxicology.

Male Syrian golden hamsters, or gonadectomized males or females, are susceptible to developing estrogen-dependent renal neoplasia when given stilbestrol. These tumors originate in the cortical tubular epithelium. Tumors can be transplanted after pretreating the recipient with diethylstilbestrol. Recipients develop bilateral, multiple cortical tumors that rupture and locally metastasize; implant metastases are found on the viscera and abdominal wall. Distant metastases by hematogenous route are not found. With continued passage, these tumors lose their dependence on exogenous estrogen and depend more on endogenous estrogen produced in the adrenal cortex and testis.

### **B. GERBIL**

Approximately 70,000 to 80,000 gerbils are used annually in research. Gerbils are used in studies involving aerospace medicine, aging, anatomy (gross, microscopic, ultrastructural), auditory research, behavior, cancer research (immunogenetics, transplantation, oncogenesis), dental research (caries, periodontal disease), endocrinology, genetics, hematology, infectious disease research, metabolism, neurology (spontaneous and induced seizures), nutrition, pharmacology/toxicology, radiobiology (radioresistance), reproduction, and stroke research. Additional areas of research involving gerbils have included investigations of experimental atherosclerosis and temperature regulation. Gerbils are readily susceptible to infection with *Giardia duodenalis*, making them a good bioassay model for evaluating or studying the infection in humans or in environmental monitoring. Gerbils of all ages are susceptible to the human infection, whereas in mice only suckling and weanling age animals are minimally susceptible.

## **GUINEA PIGS**

Guinea pigs are significantly less utilized for research than the murine rodents, mice and rats, and so it is somewhat of a mystery why the expression “guinea pig” came to mean “research animal subject.” Historically, guinea pigs were first widely used in coat color genetics. Of course, being dependent on vitamin C in the diet, they are ideal subjects for investigations of this nutrient. Guinea pigs are not useful for studies that require any but terminal blood samples nor intravenous administration of substances, as they do not have easily accessible peripheral veins. Guinea pigs are a very good source of serum complement, and have been maintained by testing laboratories for this substance.

Guinea pigs are subject to anaphylactic shock and death from bronchospasm due to histamine release. However, they are widely used for skin-testing procedures; a test substance can be applied to the back and the animal wrapped to avoid scratching or biting of the test area. Of course, these animals must be individually caged to prevent cagemates from tearing bandages. Guinea pigs are used in otological research, as the hearing range and structures (except for size, of course) of the ear are similar to those of humans. Hormonal effects during pregnancy are similar to those of humans, and they may therefore model some aspects of human pregnancy.

Generally, in the study of experimental dermatophytes, guinea pigs are the animal of choice over mouse, rat, hamster, rabbit, and dog because these animals make their toilette by licking or scratching, and they bite itching or irritated lesions intensively. In the study of skin lesions caused by dermatophytosis, hairless strains of guinea pigs are preferred because the lesions produced are more like those seen in humans. Moreover, hairless animals are more suitable for the application of topical antifungal agents.

## **WILD RODENTS**

Traditionally, animals used in laboratory research are mice, rats, guinea pigs, hamsters, gerbils, rabbits, dogs, cats, pigs, and certain primate species; however, there are over 41,700 species of vertebrates. The potential for any of these species to be used as research subjects increases as our knowledge of physiological and ecological processes expands.

Examples of the use of nontraditional laboratory animals in biomedical research are common. Woodchucks (*Marmota monax*) are used as models to study obesity, energy balance, hepatitis, and hepatocellular carcinomas and the opossum (*Didelphis virginiana*) as a model for endocarditis.

The fetal or newborn opossum provides an attractive model for the study of fetal urinary obstruction. Because the young are born shortly after conception and complete their development while attached to the teat, these marsupials are readily accessible to surgical manipulation. This model has been used to identify the roles of insulin-like growth factor and platelet-derived growth factor on obstructed developing kidneys. Histologic evaluation of kidneys with early obstruction as compared with those with late obstruction showed ductal hyperplasia and

medullary and cortical aplasia; obstruction late in development produced less severe medullary dysplasia. These studies may suggest potential therapeutic interventions or markers for early detection of obstruction.

One species used increasingly in toxicological and epidemiological research, as well as in ecological, behavioral, and genetic studies, is the deer mouse (*Peromyscus maniculatus*).

The deer mouse has distinct advantages for certain kinds of studies. Many of the advantages stem from the fact that this native species can be used as a laboratory standard for contrasting wild counterparts. For example, deer mice can be utilized to monitor environmental pollution using exposed wild animals compared with laboratory-bred controls. A representative study is that of Schaubert and others (1997), in which effects of insecticide ingestion were assayed in deer mice. Because deer mice are native, laboratory observations can be extrapolated to natural populations for ecological investigations. Because many genetic variants in deer mice occur at loci homologous with those in the laboratory house mouse and rat, they are useful in parallel studies with more conventional laboratory species. Additionally, they are slightly smaller than most laboratory mice, and odor is negligible. Another major advantage is the ample genetic polymorphism in natural *Peromyscus* populations and various laboratory stocks that can be screened for specific variants of interest. For example, an outbred stock of deer mice was the source of the alcohol dehydrogenase "null" variant extensively employed in ethanol metabolism research.

The most active research areas utilizing deer mice and related species are ecology and epidemiology. *Peromyscus* have been implicated in two human diseases of current interest: (1) Deer mice (*P. maniculatus*) are carriers of the pathogen producing the recent hantaviral pulmonary syndrome (Sin Nombre Virus) outbreak in the southwestern United States. (2) The deer mouse and the congeneric white-footed mouse are known hosts for the larval stage of the tick (*Ixodes*), which transmits the Lyme disease spirochete (*Borrelia*). These species are extensively employed in laboratory studies of the conditions described.

*Peromyscus* are also used in aging research, since they are often long lived compared with house mice (*Mus*). However, less than 25% of research activity with deer mice and their allies is for strictly "biomedical" research, in which more traditional laboratory animals are usually preferred. Deer mice and other *Peromyscus* have long been considered ideal for evolutionary research at the morphological, biochemical, cytogenetic, and molecular levels. Deer mice are a frequent species of choice for studies of biological rhythms, and neurochemistry.

Field and laboratory research on a wide variety of species is conducted to learn more about the species and its biology and ecology. Many studies are conducted in the natural environment where they are found, and others bring animals into the laboratory for study. Scientific societies in North America have developed guidelines for conducting research on the species of their concern. The American Society of Mammalogists has published field methods for mammals. The Wildlife Society includes field research guidelines in its *Research and Management*

*Techniques for Wildlife and Habitats*. The Canadian Council on Animal Care and the Universities Federation for Animal Welfare have published manuals that include guidelines for a wide range of species; and, the U.S. government has adopted the “U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training.”

## **GLOSSARY**

**Adjuvant.** A substance which enhances the body's immune response to an antigen.

**Amplify.** To make multiple identical copies, as in “amplify a segment of DNA.”

**Anaphylaxis.** Hypersensitivity especially in animals to a substance, such as foreign protein or a drug, which is caused by exposure to a foreign substance after a preliminary exposure.

**Aplasia.** Defective development resulting in the absence of all or part of an organ or tissue.

**Atherosclerosis.** A form of arteriosclerosis characterized by irregular fatty deposits on the inner surface of large and medium-sized arteries; the deposits are associated with fibrosis and calcification of the inner layer of the arteries. Similar conditions may be found in swine and fowl. The deposits may become large enough to impede the blood circulation and in some cases may restrict the blood supply to the heart.

**Auditory.** Relating to the sense of hearing.

**Banking.** Storage of cells or embryos by cryopreservation for subsequent use.

**Biopharming.** Production of therapeutic proteins using plant or animal production systems.

**Bronchospasm.** Spasmodic contraction of the muscular walls of the bronchial air passages to the lungs, as in asthma, which makes breathing difficult.

**Caries.** Decay of a bone or tooth, especially dental caries.

**Centromere.** Specialized region of the chromosome to which spindle fibers attach during cell division.

**Chimeric.** An organism, organ, or part consisting of two or more tissues of different genetic composition, produced as a result of organ transplant, grafting, or genetic engineering.

**Colitis.** Inflammation of the lining of the colon.

**Complement.** A group of proteins present in blood plasma and tissue fluid which combine with an antigen-antibody complex to bring about the lysis of foreign cells.

**Complementary.** Of or relating to a group of genes that act in concert to produce a specific phenotype.

**Conditional expression.** Expression of the protein is limited to certain situations; for example, the protein is expressed only in a lactating mammary gland.

**Cortical.** An outer layer of another organ or body part such as a kidney (the renal cortex), the cerebellum, or a hair.

**Cryopreservation.** Storage of cells at subfreezing temperatures.

**Dermatophytes.** Any fungus parasitic on the skin and causing a skin disease, as ringworm.

**DNA: d(eoxyribo) n(ucleic) a(cid).** A nucleic acid that carries the genetic information in the cell and is capable of self-replication and synthesis of RNA. DNA consists of two long chains of nucleotides twisted into a double helix and joined by hydrogen bonds between the complementary bases adenine and thymine or cytosine and guanine. The sequence of nucleotides determines individual hereditary characteristics.

**Ductal.** A bodily tube or vessel especially when carrying the secretion of a gland.

**Dysplasia.** Abnormal development or growth of tissues, organs, or cells.

**Electrofusion chamber.** Small chamber used to hold cells during electroporation process.

**Electroporation.** Process using an electrical shock to make cell membranes permeable to allow introduction of new DNA; commonly used in recombinant DNA technology. Also used to fuse two cells together or to fuse a donor cell and oocyte during somatic cell nuclear transfer.

**Embryonic stem cells.** Embryonic cells that can replicate indefinitely, transform into other types of cells, and serve as a continuous source of new cells.

**Endocrinology.** The study of the glands and hormones of the body and their related disorders.

**Endogenous.** Originating or produced within an organism, tissue, or cell.

**Endogenous sequence.** The DNA sequence found within a particular animal as opposed to the exogenous transgene obtained from another DNA source.

**Enucleation.** Removal of the nucleus (nuclear DNA) from an oocyte.

**Epithelium.** A thin layer of tightly packed cells lining internal cavities, ducts, and organs of animals and covering exposed bodily surfaces, especially in wounds that are healing.

**Exogenous.** Derived or developed from outside the body; originating externally.

**Exogenous sequence.** A DNA sequence originating from a source outside of a particular animal as opposed to the animal's own DNA (endogenous sequences).

**Fibroblasts.** Type of cell found in connective tissues, e.g., skin. Fibroblasts can be cultured relatively easily.

**Gamete.** A mature male or female germ cell usually possessing a haploid chromosome set and capable of initiating formation of a new diploid individual by fusion with a gamete of the opposite sex.

**Gene expression.** Process by which a gene's coded information is transcribed into either messenger RNA (mRNA) and then translated into protein, or into RNA but not translated into protein (e.g., transfer and ribosomal RNA's).

**Gene targeting.** Insertion (or removal) of DNA at a specific site (gene) within the genome to alter expression of that gene. *See also* **Homologous recombination.**

**Gerontology.** The scientific study of the biological, psychological, and sociological phenomena associated with old age and aging.

**Giardia.** A single-celled protozoan, some forms of which live as parasites in the gut of humans and other vertebrates, causing an infection **giardiasis.**

**Gonadectomy.** Surgical removal of an ovary or testis.

**Hematogenous.** Originating in or spread by the blood.

**Homologous recombination.** Swapping of DNA fragments between paired chromosomes or between a piece of DNA that can pair with a specific DNA site and the chromosome containing that specific site because of alignment of complementary (matching) DNA sequences. *See also* **Gene targeting; Knock-in; Knock-out.**

**Glucosuria.** The presence of sugar in the urine, usually a sign of diabetes.

**Homology.** Two anatomical structures or behavioral traits within different organisms which originated from a structure or trait of their common ancestral organism.

**Hyperglycemia.** The presence of an abnormally high concentration of glucose in the blood.

**Hyperimmunized.** Strong immunological response resulting in the production of a high level of immunoglobulins (antibodies) after exposure to an antigen.

**Hyperketonemia.** The presence of elevated concentrations of ketone bodies in the blood.

**Hyperplasia.** An abnormal increase in the number of cells in an organ or a tissue with consequent enlargement.

**Hypoinsulinemia.** An abnormally low concentration of insulin in the blood.

**Immunogenetics.** The study of the interrelation between immunity to disease and genetic makeup.

**Ketone.** A chemical produced when there is a shortage of *insulin* in the blood and the body breaks down body *fat* for energy. High levels of ketones can lead to *diabetic ketoacidosis* and *coma*. Sometimes referred to as ketone bodies.

**Ketonuria.** The presence of ketones in the urine, a warning sign of severe and uncontrolled diabetes.

**Knock-in.** Incorporation of an exogenous sequence into a specific site that results in altered gene function. *See also Homologous recombination*

**Knock-out.** Incorporation of an exogenous sequence into a specific site that results in disruption of normal gene function. *See also Homologous recombination.*

**Marsupial.** A mammal of an order whose members are born incompletely developed and are typically carried and suckled in a pouch on the mother's belly. Marsupials are found chiefly in Australia and New Guinea, and also in America.

**Medulla.** The inner region of an organ or tissue, especially when it is distinct from the outer region or cortex (as in a kidney, an adrenal gland, or hair).

**Metastasis.** The development of secondary malignant growths at a distance from a primary site of cancer.

**Microchromosome.** Small, artificially produced chromosome.

**Micromanipulator.** Instruments (attached to a microscope) used to manipulate oocytes and embryos.

**Mitochondria.** In a cell, a long or round piece found in the cytoplasm (= substance surrounding the nucleus) that produces energy for the cell by breaking down food.

**Mitotically.** The usual method of cell division, characterized typically by the resolving of the chromatin of the nucleus into a threadlike form, which condenses into chromosomes, each of which separates longitudinally into two parts, one part of each chromosome being retained in each of two new cells resulting from the original cell.

**Morphology.** The form and structure of anything, usually applied to the shapes, parts, and arrangement of features in living and fossil organisms.

**Mutated rhodopsin gene.** Mutations (alterations) in the genetic code for rhodopsin, a component of the photoreceptor cells in the eye. Mutations can lead to the disease *retinitis pigmentosa*, which can result in degeneration of the photoreceptor cells and blindness.

**Null.** Amounting to nothing; absent or nonexistent.

**Oocyte.** Female germ cell, which after maturation into an ovum (egg) can be fertilized by the sperm (male germ cell).

**Otological.** The study of the ear, including the diagnosis and treatment of its diseases and disorders.

**Periodontal.** Relating to or affecting tissue and structures surrounding and supporting the teeth.

**Peripheral.** An outward structure or surface; the portion of a system outside the central region.

**Pharming.** The production of pharmaceuticals from genetically altered plants or animals.

**Phenotype.** The observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influences.

**Plasmapheresis.** Process of separating cells and other components from plasma in the blood by a machine. This process can be used to remove antibodies from the blood.

**Polyclonal antibodies.** Mixture of immunoglobulin molecules secreted against a specific antigen, each recognizing a different site on the molecule.

**Polymerase chain reaction.** Method for amplifying (copying) a DNA base sequence using a heat-stable enzyme that copies DNA and two primer sequences, complementary to the strands of the DNA. The newly synthesized DNA strands subsequently can serve as additional templates for the same primer sequences. Successive rounds of DNA copying produce rapid and highly specific amplification of the desired sequence. PCR also can be used to detect the existence of the defined sequence in a DNA sample.

**Polymorphism.** The occurrence of different forms of individuals in a single species.

**Prion receptor.** Membrane receptor that allows prions (abnormal proteins responsible for brain disease such as bovine spongiform encephalopathy [mad cow disease]) to enter a cell.

**Promoter.** In molecular biology, the term promoter refers to the binding site on DNA to which the enzyme that transcribes DNA into RNA can attach and initiate transcription.

**Pronuclear microinjection.** Process in which transgenes are injected directly into one or both pronuclei. A pronucleus is the nuclear structure formed by the male- or female-derived chromosomes in a recently fertilized oocyte. Each fertilized oocyte should have two pronuclei, one female derived and one male derived.

**Recombinant DNA technologies.** Procedures used to join together DNA segments (sequences). Under appropriate conditions, a recombinant DNA molecule can enter a cell and replicate there, either autonomously or after it has become integrated into the chromosome(s).

**Recombinant protein.** Protein produced using recombinant DNA technologies.

**Retinitis pigmentosa.** *See Mutated rhodopsin gene.*

**RNA: r(ibo) n(ucleic) a(cid).** A polymeric constituent of all living cells and many viruses, consisting of a long, usually single-stranded chain of alternating phosphate and ribose units with the bases adenine, guanine, cytosine, and uracil bonded to the ribose. The structure and base sequence of RNA are determinants of protein synthesis and the transmission of genetic information.

**Selection.** Process of selecting those cells that have incorporated the transgene into one or more of its chromosomes.

**Selection cassette.** The DNA sequence that contains the coded information for several genes, one being the sequence for the protein of interest and another being a protein that can be used to mark or select those cells that contain the cassette. In some instances, the gene used for selection will code for resistance to a toxin such that only those cells containing the transgene can survive exposure to the toxin.

**Somatic cell nuclear transfer (SCNT).** Process by which an oocyte's DNA is replaced with the DNA from a somatic cell (donor). In a process that is inefficient and poorly understood, the

oocyte is able to reprogram (reset) the somatic cell DNA so that it can direct normal embryonic development.

**Sperm-induced oocyte activation.** An unfertilized oocyte is maintained in a state of arrested development until activated either by the sperm during fertilization or artificially using chemical or electrical stimulation. If the oocyte is not activated, it will degenerate.

**Spider silk proteins.** Proteins produced by a spider, which comprise the silk filaments found in spider webs.

**Stem cell.** An unspecialized cell that gives rise to a specific specialized cell, such as a blood cell.

**Telomere.** Specialized structure located on the end of a chromosome, which is involved in the replication and stability of the chromosome.

**Teratogenic.** An agent or factor which causes malformation of an embryo.

**Therapeutic proteins.** Proteins used in medical therapies to treat disease.

**Transchromosomal technology.** Method of producing a transgenic organism using small artificial chromosomes rather than incorporation of smaller DNA sequences into the organism's own chromosome(s).

**Transfection.** Introduction of foreign DNA into a host cell.

**Transgenic animals.** Experimentally produced animal in which exogenous DNA has been artificially introduced and incorporated into the animal's cells.

**Ultrastructure.** The detailed structure of a biological specimen, such as a cell, tissue, or organ, which can be observed only by electron microscopy.

**Viscera.** The internal organs in the main cavities of the body, especially those in the abdomen, e.g. the intestines.

**Xenotransplantation.** Tissue or organs from an individual of one species transplanted into or grafted onto an organism of another species (e.g., the use of pig heart valves in humans).

## REFERENCES

Croy, B.A., Kinder, K.E., and Yager, J.A. Primer for non-immunologists on immune-deficient mice and their applications in research. *Comparative Medicine* 2001; 51(4): 300-313.

Dunn, D.A., Kooyman, D.L., and Pinkert, C.A. Transgenic animals and their impact on the drug discovery industry. *Drug Discovery Today* 2005; 10(11): 757-767.

Hau, J. and VanHoosier, Jr., G.L.: *Handbook of Laboratory Animal Science, Third Edition.* CRC Press, Boca Raton, FL, 2010.

Immunodeficient rodents opening new doors for investigators. *Research Animal Review* 1996; 1(2): 1-8.

Joyner, C.P., Myrick, L.C., Crossland, J.P., et al: Deer mice as laboratory animals. *ILAR Journal* 1998; 39(4): 322-330.

Keefer, C.L., Pommer, J., and Robl, J.M.: The role of transgenic livestock in the treatment of human disease. *CAST Issue Paper* 2007; 35(6): 1-12.

Patoine, B.: For understanding human disease, the mouse is a knockout. *NCCR Reporter* 2007;

XXXI(2):12.

Pinkert, C.A.: Transgenic animal technology: alternatives in genotyping and phenotyping. *Comparative Medicine*; 2003; 53(2): 126-139.

Rollin, B.E. and Kesel, M.L.: *The Experimental Animal in Biomedical Research*, volume I (1990) and volume II (1995). CRC Press, Boca Raton, FL.

Sharp, P.E. and La Regina, M.C.: *The Laboratory Rat*. 1998. CRC Press, Boca Raton, FL.

Suckow, M.A, Danneman, P, and Brayton, C.: *The Laboratory Mouse*. 2001. CRC Press, Boca Raton, FL.

Sutherland, S.: Embracing the rat. *Drug Discovery Today* 2004; 9(11): 468.