SOURCEBOOK OF
MODELS FOR
BIOMEDICAL
RESEARCH

EDITED BY

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Preface

The collection of systems represented in *Sourcebook of Models for Biomedical Research* is an effort to reflect the diversity and utility of models that are used in biomedicine. That utility is based on the consideration that observations made in particular organisms will provide insight into the workings of other, more complex, systems. Even the cell cycle in the simple yeast cell has similarities to that in humans and regulation with similar proteins occurs.

Some models have the advantage that the reproductive, mitotic, development or aging cycles are rapid compared with those in humans; others are utilized because individual proteins may be studied in an advantageous way and that have human homologs. Other organisms are facile to grow in laboratory settings or lend themselves to convenient analyses, have defined genomes or present especially good human models of human or animal disease.

We have made an effort not to be seduced into making the entire book homage to the remarkable success of the genomic programs, although this work is certainly well represented and indexed.

Some models have been omitted due to page limitations and we have encouraged the authors to use tables and figures to make comparisons of models so that observations not available in primary publications can become useful to the reader.

We thank Richard Lansing and the staff at Humana for guidance through the publication process.

As this book was entering production, we learned of the loss of Tom Lanigan, Sr. Tom was a leader and innovator in scientific publishing and a good friend and colleague to all in the exploratory enterprise. We dedicate this book to his memory. We will miss him greatly.

*P. Michael Conn*
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Color plates to follow page 240.

Figure 8–1. Example of enrichment items for laboratory mice. The hut is tinted such that the mouse cannot see out, but human caretakers can see in. (Photo by Jill Rawlins.)

Figure 13–4. Imaging in live zebrafish embryos. (A) Whole embryo can be quickly imaged on a fluorescent dissecting microscope in their chorions to sort positive transgenics (left) from wild-type siblings (right). These embryos were not treated with PTU so the melanocytes are visible. (B–E) Confocal microscopy permits much higher resolution imaging. (B) A quick method for labeling is to inject RNA encoding fluorescent proteins, in this case a histone2B-EGFP fusion, and a membrane localized mCherry was used to image all the cells of the inner ear. (C) GFP transgenics can be used to image neuronal projections from the trigeminal ganglion as they extend. (D) GFP transgenics can mark specific populations of cells, in this case rhombomes 3 and 5. (E) GFP fusion proteins can reveal the subcellular localization pattern of proteins, in this case a cytoplasmic protein in the Rohon-Beard and motor neurons of the spinal cord. (Images form S.G. Megason, L.A. Trinh, and S.E. Fraser, unpublished.)

Figure 13–5. Creating genetic mosaics in zebrafish. (A) Donor cells are first lineage labeled with a tracer dye at the one-cell stage. Donor embryos can also be injected with morpholinos, RNA, or DNA. At the 1000-cell stage, totipotent blastula cells are transplanted into regions of the host embryo fated to give rise to specific structures. Donor and host embryos can be of either mutant or wild-type genotypes. Resultant chimeras are grown for subsequent analysis. (B) Fate-map of the pregastrula stage embryos. (Modified from Woo and Fraser, 1995.) (C) Mosaic embryo at 24hpf showing bright-field (left) and fluorescent image (right) of rhodamine-dextran-labeled donor cells targeted to the eye and forebrain. (B.A. Link, unpublished.)

Figure 25–1. Feline chromosome maps (labeled at top) and homologous syteny blocks (HSBs) in the human (H) and dog (D) genomes. HSBs are shown to the right of each cat chromosome map (only the map scale is shown). The dark cross-marks on each cat chromosome correspond to 100-cR5000 intervals. The inferred centromere positions are shown by dark circles. HSBs are color coded by human or dog chromosome, defined by the key in the bottom right corner. (Reprinted from Murphy et al. Copyright 2007, with permission from Elsevier.)

Figure 51–3. Changes in the CBV response to amphetamine (A) before and (B) 4 months after cessation of MPTP treatment in a cynomolgus monkey, showing an almost complete loss of amphetamine-induced CBV signal changes in dopaminergic regions. Parkinsonian primates had a prominent loss of response to amphetamine, with relative sparing of the nucleus accumbens and para-fascicular thalamus. (Modified from Jenkins et al. Copyright 2004 Society for Neurosciences.)

Figure 51–4. (A) Patterns of the fMRI response in the mouse main olfactory bulb (MOB) and accessory olfactory bulb (AOB, pink circle in slice 5) to the pheromone 2-heptanone, one of the urinary chemosignal compounds in mouse. The arrows point to two foci of activation suggestive of a pair of the nearly mirror projections of the receptor neuron subsets to the same MOB. Scale bar = 500µm. (B) A flattened view of the olfactory bulb indicating the orientation of the odor maps shown in (C) and (D): A, anterior; D, dorsal; L, lateral; M, medial; P, posterior, V, ventral. (C, D) The odor maps of 2-heptanone (Hep) in two different mice show that this pheromone not only activates large regions of the MOB but also generates similar patterns across subjects. Interestingly, the odor map for amyl acetate (AA), a common odorant with an odor quality similar to that of 2-heptanone, was also similar to that of 2-heptanoneb. (Adapted from Xu et al. Copyright 2005 Wiley-Liss, Inc.)

Figure 51–5. Signal changes in CBV-weighted fMRI obtained during stimulation of the cat visual cortex according to predetermined stimulus orientations. (A) Raw gray-scale functional map obtained by subtraction of images during 0º stimulation from prestimulus control. The center of each patch is marked with a green + sign. (B) Four different grating orientations (0º, 45º, 90º, and 135º) were presented in the study of one animal, enabling a composite angle map to be generated through pixel-by-pixel vector addition of the four single-condition maps. In the left hemisphere (marked by a white rectangular box), changes between pixels preferentially activated by a particular stimulus orientation were smoothed by a 3 × 3 Gaussian Kernel. (C) A composite angle map was generated with the region indicated by the white ROI in (B) “Pinwheel” structures indicated by small white dots were observed where domains for all orientations converge. (Adapted from Zhao et al. Copyright 2005 Elsevier.)

Figure 51–6. Auditory activation in the songbird telencephalon shows (A) statistical maps illustrating the localization of
significant signal intensity changes during auditory stimulation consisting of white noise (wn), a concerto of Bach (music), and a stimulation with song from a male starling (song). (B) The location of the activated areas (top), together with the average BOLD response amplitude for each stimulus (bottom). (Adapted from Van Meir et al.\textsuperscript{231} Copyright 2005 Elsevier.)

**Figure 61–1.** The effect of single PSS exposure versus unexposed control on rat anxiety-like behavior and acoustic startle response and habituation. The representation of the data from both paradigms (EPM and ASR) shows two obvious and rather distinct features. First, it is clear that PSS exposure alters the response of the majority of individuals to at least some degree. Second, the cluster of individuals that forms in the upper left hand corner of the graph (i.e., had the more extreme responses to exposure) is quite distinct from the majority of individuals.\textsuperscript{36,48}

**Figure 68–2.** Diabetic nephropathy induced by streptozotocin (STZ) in uninephrectomized mice. One week after uninephrectomy, male CD1 mice received an intravenous injection of STZ. (A–D) Representative micrographs demonstrate glomerular enlargement, mesangial expansion, and segmental glomerulosclerosis (periodic acid–Schiff staining; A, B) and glomerular collagen deposition (Masson–Trichrome staining; C, D) (A and C) Normal control; (B and D) diabetic mice. Arrows indicate injured glomeruli. (E) Albuminuria develops in diabetic mice in a time-dependent manner. Data are presented as means ± SEM. *$p < 0.05$. (F) Glomerular collagen deposition score in diabetic and normal mice. *$p < 0.05$. (Adapted and modified from Dai et al.\textsuperscript{46})

**Figure 68–3.** Interstitial fibrosis in the mouse model of obstructive nephropathy. (A, B) Representative micrographs show the cross sections and gross morphology of the obstructed kidneys at day 14 after ureteral ligation. (A) Sham control; (B) UUO. (C) Western blot analyses demonstrate a marked induction of α-smooth muscle actin (α-SMA), a molecular marker for myofibroblasts, in the obstructed kidney at day 14 after UUO. (D) Double immunofluorescence staining shows the α-SMA (red) and proximal tubular epithelial cell marker, fluorescein isothiocyanate (FITC)-conjugated lectin from *Tetragonolobus purpureas* (green). (E) Quantitative determination of total kidney collagen contents in sham and obstructed kidneys. Data are presented as means ± SEM. *$p < 0.01$. (Adapted and modified from Yang et al.\textsuperscript{59})

**Figure 73–4.** A pathogenetic model for the cytokine-mediated stromal reaction observed in MMM.
1 Animal Models for Human Diseases
An Overview

JANN HAU

ABSTRACT

This chapter provides an introduction to the concept of laboratory animal models, focusing on a general classification of animal models for the study of human diseases. Animal models can be grouped into one of the following five categories: (1) induced (experimental) models, (2) spontaneous (genetic, mutant) models, (3) genetically modified models, (4) negative models, and (5) orphan models. This is followed by a discussion of how knowledge concerning human biology and pathobiology can be extrapolation from results obtained from studies of animals. Finally the chapter discusses how the difference in body size and metabolic rate between small laboratory animals and humans has an impact on the calculation of relevant doses for animals used as models for humans in experimental studies.

Key Words: Animal model concept, Induced animal model, Spontaneous animal model, Transgenic animal model, Negative animal model, Orphan animal model, Body size—scaling, Extrapolation, Laboratory animal science—definition.

INTRODUCTION

Throughout history ethical and religious considerations as well as social prohibitions have prevented experimental studies of human biology and pathobiology. Even studies of human anatomy were for long periods of time in history a criminal offense and thus not possible. Although impressive anatomical teaching theaters were established in many old European universities, postmortem dissection was often restricted exclusively to criminals executed for their offenses. In the more quiet corners of Europe the teaching theaters remained unused for decades. Consequently, most of our present basic knowledge of human biology, physiology, endocrinology, and pharmacology has been derived from initial studies of mechanisms in animal models.1 Throughout history scientists have performed experiments on animals to obtain knowledge of animal and human biological structure and function.2,3 Often such studies have not been conducted, and are not possible to conduct, in humans. This may not only be due to ethical or religious considerations. Often practical, economic, and scientific reasons make initial studies in animals the best solution for studies of a biological phenomenon.

Laboratory animal science may be defined as the study of the scientific, ethical, and legal use of animals in biomedical research, i.e., a multidisciplinary field encompassing comparative biological and pathobiological specialties for the optimal scientific use of animals as models for human or other species. Basic laboratory animal science is concerned with the quality of animals as sentient tools in biomedical research. It encompasses the comparative biology of laboratory animals, aspects of breeding, housing, and husbandry, anesthesia, euthanasia, and experimental techniques. For animal welfare reasons as well as for scientific reasons it is vital that scientists using animal models in their research are competent and have a good knowledge of basic laboratory animal science.

This sourcebook provides a thorough introduction to the use of animal models for human diseases. High quality animals combined with first class animal care ensure the highest possible health and welfare status of the animals and are a prerequisite for good science and public acceptance of the use of animals in research.

THE ANIMAL MODEL CONCEPT

A laboratory animal model describes a biological phenomenon that the species has in common with the target species. A key word for understanding the concept of animal models is “analogy.”4 A model should not be considered a claim of identity with what is being modeled, but a convergent set of several kinds of analogies between the “target” phenomenon to be understood and the system that is being studied as a substitute for the target phenomenon. A more comprehensive definition has been given by Held on the basis of Wessler’s original definition5: “a living organism in which normative biology or behavior can be studied, or in which a spontaneous or induced pathological process can be investigated, and in which the phenomenon in one or more respects resembles the same phenomenon in humans or other species of animal.”

What is generally understood by the term animal model is modeling humans. It is not the image of the used animal that is the focus of research but the analogy of the physiological behavior of this animal to our own (or another) species. It would thus perhaps be more correct to refer to animals as “man models” in this context. Laboratory animal science, comparative medicine, and animal experiments are indeed much more about humans than about any other animal species.6

The practice of studying biological phenomena and diseases in laboratory animals and transferring the findings into solutions
and treatments for improving human health and welfare has a long history and is well established in biomedical sciences. The significance and validity with respect to usefulness in terms of "extrapolatability" of results generated in an animal model depend on the selection of a suitable animal model. A good knowledge of comparative anatomy and physiology is an obvious advantage when developing an animal model. Animal models may be found throughout the animal kingdom, and knowledge about human physiology has been obtained from species far removed from humans in terms of taxonomy. A good example is the importance of the fruit fly for the original studies of basic genetics. Animal models are used in most fields of biomedical research as reflected in the respective chapters of this book.

CLASSIFICATION OF ANIMAL MODELS

A plethora of animal models has been used and is being used and developed for studies of biological structure and function in humans. The models may be “exploratory,” aiming to understand a biological mechanism whether this is a mechanism operative in fundamental normal biology or a mechanism associated with an abnormal biological function. Models may also be developed and applied as so-called “explanatory” models, aiming to understand a more or less complex biological problem. Explanatory models need not necessarily be reliant on the use of animals but may also be physical or mathematical model systems developed to unravel complex mechanisms. A third important group of animal models is “predictive” models. These models are used to discover and quantify the impact of a treatment, whether this is to cure a disease or to assess toxicity of a chemical compound. The anatomy or morphology of the model structure of relevance to the studies may be of importance in all three of these model systems. The extent of resemblance of the biological structure in the animal to the corresponding structure in humans has been termed fidelity. A high fidelity model with close resemblance to humans may seem an obvious advantage when developing certain models. What is often more important, however, is the discriminating ability of the models, in particular the predictive models. When using models to assess the carcinogenicity of a substance it is essential that at least one of the model species chosen responds in a manner that is predictive of the human response to this substance. Thus the least one of the model species chosen responds in a manner that is "predictive" models. These models are used to discover and quantify the impact of a treatment, whether this is to cure a disease or to assess toxicity of a chemical compound. The anatomy or morphology of the model structure of relevance to the studies may be of importance in all three of these model systems. The extent of resemblance of the biological structure in the animal to the corresponding structure in humans has been termed fidelity. A high fidelity model with close resemblance to humans may seem an obvious advantage when developing certain models. What is often more important, however, is the discriminating ability of the models, in particular the predictive models. When using models to assess the carcinogenicity of a substance it is essential that at least one of the model species chosen responds in a manner that is predictive of the human response to this substance. Thus the similarity between human and model species with respect to relevant biological mechanisms is often more important than the fidelity of the model. Often the two go hand in hand and high fidelity models offer the best opportunity to study a particular biological function.

An animal model may be considered homologous if the symptoms shown by the animal and the course of the condition are identical to those of humans. Models fulfilling these requirements are relatively few, but an example is well-defined lesion syndromes in, e.g., neuroscience. An animal model is considered isomorphic if the animal symptoms are similar, but the cause of the symptoms differs in humans and the model. However, most models are neither homologous nor isomorphic but may rather be termed partial. These models do not mimic the entire human disease, but may be used to study certain aspects or treatments of the human disease.

CLASSIFICATION OF DISEASE MODELS

The majority of laboratory animal models are developed and used to study the cause, nature, and cure of human disorders. Disease models may conveniently be categorized in one of the following five groups, of which the three first are the numerically most important:

1. Induced (experimental) models
2. Spontaneous (genetic, mutant) models
3. Genetically modified models
4. Negative models
5. Orphan models

INDUCED (EXPERIMENTAL) MODELS As the name implies, induced models involve healthy animals in which the condition to be investigated is experimentally induced, e.g., the induction of diabetes mellitus with encephalomyocarditis virus, allergy against cow’s milk through immunization with minute doses of protein, or partial hepatectomy to study liver regeneration. The induced model group is the only category that theoretically allows a free choice of species. Although it might be presumed that extrapolation from an animal species to the human species is better the closer this species resembles humans (high fidelity), phylogenetic closeness, as fulfilled by primate models, is not a guarantee of validity of extrapolation, as the unsuccessful chimpanzee models in AIDS research have demonstrated. It is just as decisive that the pathology and outcome of an induced disease or disorder in the model species resemble the respective lesions of the target species. Feline immunodeficiency virus (FIV) infection in cats may therefore for many studies be a better model for human AIDS than HIV infection in simians. Although mice and rats have many biological characteristics in common, they do not necessarily serve equally well as models of human disease. For example, schistosomiasis (mansoni) infection may be studied in experimentally infected mice, but not in rats whose immune system is able to fight the infection effectively.

Most induced models are partial or isomorphic because the etiology of a disease experimentally induced in an animal is often different from that of the corresponding disease in humans. Few induced models completely mimic the etiology, course, and pathology of the target disease in humans.

SPONTANEOUS ANIMAL MODELS These models of human disease utilize naturally occurring genetic variants (mutants). Many hundreds of strains stocks with inherited disorders modeling similar conditions in humans have been characterized and conserved (see, e.g., www.jax.org). The best known spontaneous models include the athymic nude mouse, the use of which represented a turning point in the study of heterotransplanted tumors and enabled the first description of natural killer cells. Some of these mutants were discovered almost a century ago, like the famous spontaneous model Snell’s Dwarf mouse without a functional pituitary and the curly tail mouse in which the symptoms differs in humans and the model. However, most models are neither homologous nor isomorphic but may rather be termed partial. These models do not mimic the entire human disease, but may be used to study certain aspects or treatments of the human disease.

An extensive literature is available on spontaneous models and the majority of these involve mice and rat models, although a wide range of mutants in many different species has been described. A good example of the amount of information available is the publication of Migaki, referencing more than 200 diseases in animals exclusively caused by inborn errors of metabolism.
The spontaneous models are often isomorphic displaying phenotypic similarity between the disease in the animal and the corresponding disease in man, the so-called face validity, e.g., type I diabetes in humans and insulin requiring diabetes in the BB rat. This phenotypic similarity often extends to similar reactions to treatment in the model animal and the human patient, and spontaneous models have been important in the development of treatment regimens for human diseases.

However, if the object of a project is to study the genetic causes and etiology of a particular disease then comparable genomic segments involved in the etiology of the disorder—construct validity—is normally a requirement. It should be remembered, however, that an impaired gene or sequence of genes very often results in activation of other genes and mobilization of compensating metabolic processes. These compensatory mechanisms may of course differ between humans and the animal model species.

**GENETICALLY MODIFIED MODELS** The rapid developments in genetic engineering and embryo manipulation technology during the past decade have made transgenic disease models the most important category—in terms of numbers—of animal disease models. The technology and ability to genetically modify mice resulted in a substantial increase in the numbers of laboratory animals used worldwide, which is a trend that seems to continue. A multitude of animal models for important diseases have been developed since this technology became available in the 1980s, and the number of models has increased quickly. Mice are by far the most important animals for transgenic research purposes, but farm animals and fish are also receiving considerable interest.

Many physiological functions are polygenic and controlled by more than one gene, and it will require considerable research activities to identify the contribution of multiple genes to normal as well as abnormal biological mechanisms. The insertion of DNA into the genome of animals, or the deletion of specific genes, gives rise to sometimes unpredictable outcomes in terms of scientific results as well as animal well-being in the first generations of animals produced. Thereafter transgenic lines can be selected and bred or cloned to avoid or select for a specific genotype. It is not an accurate science, although the methodology is constantly improving, with the aim of eliminating unwanted effects. The embryo manipulation procedures in themselves do not appear to affect the welfare of offspring in the mouse, and the large offspring syndrome common in farm animals has so far not been reported in the literature for rodents, although it has been observed (Johannes Wilbertz, Karolinska Institute, personal communication).

Mutations induced by the use of mutagens like ethylnitrosourea is another approach to the generation of new mutants, which may serve as models of human disorders. In many aspects these mutants may be similar to spontaneous mutants and to the ones generated by transgenic embryo manipulation. The maintenance of a line raises issues for chemically induced genetic mutants similar to those for animals genetically modified through embryo manipulation.

The recent completion of the maps of the genomes of mouse (and other model animals) and humans will increase the research activities in functional genomics and proteomics, and using high density microarray DNA chip technology in human patients as well as in animals will make it possible to investigate which genes are switched on or off in different diseases.

Having both the human and the mouse genome maps available, this new technology is expected to rapidly increase our knowledge on the genetic background and etiology of important diseases. This paves the way for a range of new homologous animal models with homology between animal and humans (construct validity) for genotype as well as for phenotype. This development may result in a change in animal use from models for the identification of causative genes to models for studying the effects of changes in genetic pathways, gene–gene interactions, and gene–environment interactions. The characterization and application of genetically modified mouse models are slowed down by difficulties in phenotyping the animals. There is a need for accurate and reliable behavioral assessment, biotechnology development for physiological assessment, and analysis of complex data as well as training scientists in whole-organism research.

**NEGATIVE MODELS** Negative model is the term used for species, strains, or breeds in which a certain disease does not develop, e.g., gonococcal infection in rabbits following an experimental treatment that induces the disease in other animal(s). Models of infectious diseases are often restricted to a limited number of susceptible species and the remaining unresponsive species may be regarded as negative models for this particular human pathogenic organism. Negative models thus include animals demonstrating a lack of reactivity to a particular stimulus. Their main application is studies on the mechanism of resistance to gain insight into its physiological basis. Occasionally negative models give rise to the characterization of new spontaneous (mutant) models. Examples are found in studies of infectious diseases and carcinogenicity where individuals exhibiting resistance to a treatment may be developed into new spontaneous models.

**ORPHAN MODELS** An orphan model disease is the term used to describe a functional disorder that occurs naturally in a nonhuman species but has not yet been described in humans, and which is recognized when a similar human disease is later identified. Examples include Marek’s disease, papillomatosis, and bovine spongiform encephalopathy (BSE), Visna virus in sheep, and feline leukemia virus. When discovering that humans may suffer from a disease similar to one that has already been described in animals the literature generated in veterinary medicine may be very useful.

**EXTRAPOLATION FROM ANIMALS TO HUMANS**

When experimental results have been generated in an animal model they have to be validated with respect to their applicability to the target species, which normally is the human. The term extrapolation is often used to describe how data obtained from animal studies reliably can be used to apply to humans. However, extrapolation is generally not performed in its mathematical sense where data fit a certain function that may be described graphically, and the graph extended beyond the highest or lowest sets of data to describe a situation outside the window of observation. Establishing toxicity data in animals and using these to determine safe levels of exposure for people is perhaps what comes closest to mathematical extrapolation in animal studies. However, most studies of animal structure and function are never extrapolated to be applicable to describing the corresponding features in humans; this is not relevant. What laboratory animal experimentation is about is very similar to other types of experiments. The scientists aim to obtain answers to specific questions. Hypotheses are being
tested and the answers obtained, analyzed, and published. As an example of this, we might question the possible health hazards of a new synthetic steroid and ask a number of relevant questions to be answered in animal studies before deciding on its potential usefulness as a human hormonal contraceptive: Does it exist in the same form in humans and animals? How does it affect the estrus cycle in rodents? How does it affect endogenous hormone levels in rodents and other species? How soon after withdrawal do the animals revert to normal cyclicity? Does it interfere with pregnancy in rodents and primates? Does it affect fetal development in rodents and primates? Is the frequency of fetal malformations in mice affected? Are puberty, the ovarian cycle, and pregnancy in rodent and dog offspring of mothers treated with the substance affected? Analyzing the data from experiments of this nature would provide information on the potential of the new synthetic steroid as a hormonal contraceptive in humans.

A large multinational pharmaceutical company surveyed data compiled from 150 compounds for the concordance between adverse findings in human clinical data with data that had been generated in preclinical tests in animals. The concordance rate was found to be 71% for rodent and nonrodent species, with nonrodents alone being predictive for 63% of human toxicity (HT) and rodents alone for 43%. High concordance rates were found, e.g., for cardiovascular HTs (80%), hematological HTs (91%), and gastrointestinal HTs (85%). Lower concordance rates were observed, e.g., for the neurological group, because it is difficult to identify symptoms such as headache and dizziness in the animals studied. The only gastrointestinal HT that did not correlate with animal studies was, not surprisingly, nausea. One of the conclusions reached in this study was that the choice of species used might be subject to more thoughtful consideration. By tradition studies are often carried out using rats and dogs, without an open-minded consideration of whether alternative species might be more appropriate for testing a specific compound.

Although the predictive value of animal studies may seem high if they are conducted thoroughly and have included several species, uncritical reliance on the results of animal tests can be dangerously misleading and has resulted in damage to human health in several cases, including drugs developed by large pharmaceutical companies. What is noxious or ineffective in nonhuman species can be innocuous or effective in humans and vice versa. For example, penicillin is fatal for guinea pigs but generally well tolerated by humans; aspirin is teratogenic in cats, dogs, guinea pigs, rats, mice, and monkeys but obviously not in pregnant women despite frequent consumption. Thalidomide, which crippled 10,000 children, does not cause birth defects in rats but many other species, but does so in primates. Close phylogenetic relationship or anatomical similarity is not a guarantee of identical biochemical mechanisms and parallel physiological response, although this is the case in many instances.

The validity of extrapolation may be further complicated by the question of which humans. As desirable as it often is to obtain results from a genetically defined and uniform animal model, the humans to whom the results are extrapolated are genetically highly variable, with cultural, dietary, and environmental differences. This may be of minor importance for many disease models but can become significant for pharmacological and toxicological models.

It is not possible to provide reliable general rules for the validity of extrapolation from one species to another. This has to be assessed individually for each experiment and can often be verified only after first trials in the target species. An extensive and useful overview on the problem of predictive anthropomorphization, especially in the field of toxicology research, is Principles of Animal Extrapolation by Calabrese. The rationale behind extrapolating results to other species is based on the extensive homology and evolutionary similarity between morphological structures and physiological processes among different animal species and between animals and humans.

### MODEL BODY SIZE AND SCALING

The use of laboratory animals as models for humans is often based on the premise that animals are more or less similar with respect to many biological characteristics and thus can be compared. However, there is one striking difference between mouse and human, and that is body size. In proportion to their body size mammals generally often have very similar organ sizes expressed as percentage of body weight. Take the heart, for instance, which constitutes 5 or 6 g/kg body weight, or blood, which is often approximately 7% of total body weight.

It is well known that the metabolic rate of small animals is much higher than that of large animals. It has also been demonstrated that capillary density in animals smaller than rabbits increases dramatically with decreasing body weight. However, considering that most animals are similar in having heart weights just above 0.5% of their body weight and a blood volume corresponding to 7% of their body weight, it becomes obvious that in order to supply the tissues of small animals with sufficient oxygen for their high metabolic rate it is not sufficient to increase the stroke volume. The stroke volume is limited by the size of the heart and heart frequency is the only parameter to increase, which results in heart rates well over 500 per minute in the smallest mammals. Other physiological variables, like respiration and food intake, are similarly affected by the high metabolic rate of small mammals.

This means that scaling must be an object for some consideration when calculating dosages of drugs and other compounds administered to animals in experiments.

If the object is to achieve equal concentrations of a substance in the body fluids of animals of different body size then the doses should be calculated in simple proportion to their body weights. If the object is to achieve a given concentration in a particular organ over a certain time period the calculation of dosage becomes more complicated and other factors including the physicochemical properties of the drug become important. Drugs and toxins exert their effect on an organism because of the way they are metabolized, the way they and their metabolites are distributed and bound in the body tissues, and how and when they are finally excreted.

However, metabolism or detoxification and excretion of a drug are not directly correlated with body size, but more accurately with the metabolic rate of the animal (see Schmidt-Nielsen for more details). Kleiber in 1932 was the first to demonstrate that in a log–log plot of mammalian body weight to metabolism the graph forms a straight line with a slope of 0.75.

The metabolic rate of an animal as expressed by oxygen consumption per gram body weight per hour is related to body weight in the following manner:

\[ M = 3.8 \times BW^{-0.25} \]
where $M$ is the metabolic rate (oxygen consumption in milliliter per gram body weight per hour) and $BW$ is body weight in grams. This equation may be used to calculate dosages for animals of different body weights if the dose for one animal (or man) is known.33

$$Dose_1/Dose_2 = BW_1^{-0.25}/BW_2^{-0.25}$$

$$Dose_1 = Dose_2 \times BW_1^{-0.25}/BW_2^{-0.25}$$

These equations should be considered as assistance for calculating dosages, but caution should be exerted with respect to too broad a generalization of their use, and the 0.50 power of body weight should be employed when dealing with animals having body weights below 100 g.34 Some species react with particular sensitivity toward certain drugs and marked variations in the reaction of animals within a species occur with respect to strain, pigmentation, nutritional state, time of day, stress level, type of bedding, ambient temperature, etc.35

### CONCLUSIONS

The selection of an animal model depends on a number of factors relating to the hypothesis to be tested, but often more practical aspects associated with the project and with project staff and experimental facilities play a significant role. The usefulness of a laboratory animal model should be judged on how well it answers the specific questions it is being used to answer, rather than how well it mimics the human disease.35

Often a number of different models may advantageously be used in order to scrutinize a biological phenomenon and for major human diseases such as diabetes, a whole range of well-described induced models are available as are spontaneous models in both mouse and rat strains.

Most of the regulating authorities require two species in toxicology screening, one of which has to be nonrodent. This does not imply that excessive numbers of animals will be used because an uncritical use of one-species models may mean that experimental data retrospectively turn out to be invalid for extrapolation, representing a waste of animals. The appropriateness of any laboratory animal model should be judged on how well it mimics the human disease.35

The free choice of species when developing animal models is more or less restricted to the induced models making use of clinically healthy animals, in which a condition deviating from normality is experimentally induced. Although all laboratory animal species are in principle available for model development, it has malsality is experimentally induced. Although all laboratory animal models for human diseases such as diabetes, a whole range of well-described induced models are available as are spontaneous models in both mouse and rat strains.

### REFERENCES


LABORATORY ANIMALS PLAY A CRUCIAL ROLE IN RESEARCH DISCOVERY AND TECHNOLOGICAL ADVANCES, AND THEY WILL CONTINUE TO TAKE PART IN IMPROVING THE LIVES OF PEOPLE AND OTHER ANIMALS. IT IS INCUMBENT UPON THE RESEARCHER TO KNOW HIS SUBJECT WELL IN ORDER TO PROVIDE RELEVANT INFORMATION TO THE SCIENTIFIC WORLD. IN AN EFFORT TO ASSIST THE BIO MEDICAL RESEARCHER IN GAINING THIS KNOWLEDGE, THIS CHAPTER PROVIDES THE FOLLOWING KE Y ELEMENTS: DEFINITION OF TYPES OF ANIMAL MODELS, LEGISLATIVE AND LEGAL REQUIREMENTS, CRITERIA FOR CHOOSING A MODEL, EXTRAPOLATION VALIDITY RECOMMENDATIONS, AND DESCRIPTIVE FEATURES FOR PUBLICATION.

KEY WORDS: ANIMAL MODELS, LABORATORY ANIMAL(S), ANIMAL MODEL TYPES, ANIMAL USE CRITERIA, CHOOSING ANIMAL MODEL, ANIMAL FACTORS, EXTRAPOLATION, ANIMAL DESCRIPTION.

INTRODUCTION

OVER THE LAST ONE AND ONE-HALF CENTURIES, ALMOST ALL MEDICAL KNOWLEDGE, TREATMENT REGIMES, AND MEDICAL DEVICE DEVELOPMENT HAVE INVOLVED RESEARCH USING ANIMALS. THE KEY FACTOR IN USING ANIMALS IN RESEARCH IS IN ITS EXTRAPOLATABILITY OF RESULTS TO HUMANS. ANIMALS IN RESEARCH HAVE BEEN AND STILL ARE ESSENTIAL IN DEVELOPING TREATMENTS FOR ASTHMA, HIV/AIDS, CANCER, BIRTH DEFECTS, BIOTERRORISM, MEDICAL COUNTERMEASURES, VACCINES, ANTIMICROBIALS, HIGH BLOOD PRESSURE, AND MUCH MORE. ADDITIONALLY, THEY HAVE BEEN VITAL IN THE DEVELOPMENT OF ANTIBIOTICS, VACCINES, AND ORGAN TRANSPLANTATION TECHNIQUES. AS THE RISE IN EMERGING INFECTIOUS DISEASES (E.G., WEST NILE VIRUS AND AVIAN INFLUENZA) CONTINUES, ANIMALS WILL BE KEY AND ESSENTIAL IN THE DEVELOPMENT OF PREVENTIVE AND TREATMENT MODALITIES.

THE HISTORY OF ANIMAL USE IN RESEARCH

THE USE OF ANIMALS TO STUDY HUMAN PHYSIOLOGY AND ANATOMY CAN BE TRACED BACK TO THE SECOND CENTURY AD IN WHICH GALEN WAS A GREEK PHYSICIAN AND PHILOSOPHER. HIS RESEARCH WAS BASED ALMOST EXCLUSIVELY ON STUDIES USING APES AND PIGS. UNFORTUNATELY, THIS INITIATED MANY ERRORS BASED ON HIS ACCEPTED AUTHORITY AND THE PROHIBITION BY THE CHURCH OF USING HUMAN CADAVERS FOR RESEARCH PURPOSES. GALEN WAS LATER BLAMED FOR USING INCORRECT METHODS IN RESEARCH WHEN IN TRUTH IT WOULD BE MORE ACCURATE TO SAY THAT HE DREW WRONG CONCLUSIONS BASED ON UNCRI TICAL INTERSPECIES EXTRAPOLATION OF DATA. THAT IS, HE ASSUMED THAT ALL EXTRACTED INFORMATION DERIVED FROM HIS USE OF ANIMALS COULD BE DIRECTLY APPLIED TO HUMANS. IT WAS NOT UNTIL THE LATE SIXTEENTH CENTURY THAT THIS ERROR BEGAN TO BE RECOGNIZED.

MODERN RESEARCH PRINCIPLES CAN BE ATTRIBUTED TO THREE PHYSIOLOGISTS FROM THE 1860S. IN 1865, CLAUDE BERNARD, A FRENCH PHYSIOLOGIST, PUBLISHED AN INTRODUCTION TO THE STUDY OF EXPERIMENTAL MEDICINE. THIS BOOK WAS INTENDED TO PROVIDE PHYSICIANS WITH GUIDANCE IN EXPERIMENTAL RESEARCH. IT PROPOSED THE USE OF CHEMICAL AND PHYSICAL INDUCTION OF DISEASE IN ANIMALS, THUS BECOMING THE FIRST PUBLISHED BOOK TO ADVOCATE CREATING “INDUCED ANIMAL MODELS” FOR BIO MEDICAL RESEARCH. HIS PEERS OF THE TIME WERE LOUIS PASTEUR IN FRANCE AND ROBERT KOCH IN GERMANY. LOUIS PASTEUR AND ROBERT KOCH INTRO DUCED THE CONCEPT OF SPECIFICITY INTO MEDICINE AND THE “GERM THEORY OF DISEASE.” THE TURNING OF THE CENTURY SAW THE DEVELOPMENT AND USE OF ANIMAL MODELS FOR INFECTIOUS DISEASES AND SCREENING AND THE EVALUATION OF NEW ANTIMICROBIAL DRUGS BASED UPON THE WORK OF THESE THREE RESEARCHERS.

DURING THE FIRST QUARTER OF THE NINETEENTH CENTURY, ANIMAL STUDIES WERE CRUCIAL FOR LESS THAN ONE-THIRD OF THE MAJOR ADVANCES THAT OCCURRED. WITH THE CONTRIBUTIONS OF CLAUDE BERNARD, LOUIS PASTEUR, AND ROBERT KOCH, ANIMAL STUDIES CONTRIBUTED TO MORE THAN HALF OF THE SIGNIFICANT DISCOVERIES MADE THEREAFTER. SINCE 1901, TWO-THIRDS AND 7 OF THE LAST 10 NOBEL PRIZES IN MEDICINE HAVE RELIED AT LEAST IN PART ON ANIMAL RESEARCH. TODAY, RESEARCHERS RELY ON THE IDENTIFICATION AND DEVELOPMENT OF ANIMAL MODELS TO EXPLORE ALL AVENUES OF MEDICAL SCIENCE TO INCLUDE ASSESSMENT OF PATHOGENIC MECHANISMS, DIAGNOSTIC AND THERAPEUTIC PROCEDURES, NUTRITION AND METABOLIC DISEASES, AND THE EFFICACY OF NOVEL DRUG DEVELOPMENT.

THE CONCEPT OF ANIMAL MODELS

WHAT IS AN ANIMAL? ETYMOLOGICALLY, THE WORD “ANIMAL” DERIVES FROM THE LATIN ANIMAL MEANING SOUL/SPRIT, THUS DESCRIBING LIVING ORGANISMS THAT ARE ANIMATED.

WHAT IS A MODEL? A MODEL IS AN OBJECT OF IMITATION, SOMETHING THAT ACCURATELY RESembLES SOMETHING ELSE, A PERSON OR THING THAT IS THE LIKENESS OR IMAGE OF ANOTHER. THE HOLY BIBLE TELLS US THAT GOD SAID, “LET US MAKE MAN IN OUR IMAGE, IN OUR LIKENESS, SO GOD CREATED MAN IN HIS OWN IMAGE, IN THE IMAGE OF GOD HE CREATED HIM; MALE AND FEMALE HE CREATED THEM.” GOD CREATED MAN OUT OF THE DUST OF THE GROUND AND THEN BREATHED INTO HIS NOSTRILS THE BREATH OF LIFE TO ANIMATE HIM. THUS, HUMANS ARE “ANIMAL MODELS” OF GOD.

CONSEQUENTLY, COMBINING THE TWO DEFINITIONS, AN “ANIMAL MODEL” IS AN ANIMATED OBJECT OF IMITATION, AN “IMAGE OF MAN” (OR OTHER SPECIES), USED TO INVESTIGATE A PHYSIOLOGICAL OR PATHOLOGICAL CIRCUMSTANCE IN QUESTION.
WHAT IS AN ANIMAL MODEL? The U.S. National Research Committee on Animal Models for Research on Aging attempted to define the term “laboratory animal model” as “an animal in which normative biology or behavior can be studied, or in which a spontaneous or induced pathological process can be investigated, and in which the phenomenon in one or more respects resembles the same phenomenon in humans or other species of animal.”

Using the term animal model can be confusing because what is often meant by the term “animal model” is actually studying human conditions. In other words, it is not the image of the preferred animal that is the focus of research but the analogy of the physiological behavior of this animal to our own (or another) species. It would, thus, be more correct to speak of “human models” in this context. Indeed, although using animals in research can benefit other animals, it is much more focused on improving the human condition.

TYPES OF ANIMAL MODELS

When animals are used in research to study biological and functional systems in humans, they are broken down into the following categories:

1. Exploratory. Animals used in this category are used to gain an understanding of fundamental biological mechanisms, whether normal or abnormal. An example would be the use of a novel animal model of aging, particularly for identifying genes and biochemical pathways regulating longevity.

2. Explanatory. Animals used in this category are used to gain an understanding of complex biological problems. An example would be the use of cognitive and psychosocial animal models to provide an etiology for anorexia nervosa.5

3. Predictive. Animals used in this category are used to discover and quantify the impact of investigative treatments whether for diseases or chemical toxicities. Predictive animal testing models are important in improving the success of a drug or medical device in clinical trials and for generating new data in support of the ongoing marketing of existing products.

4. Negative. Negative models fail to react to a disease or chemical stimulus. Thus, their main use in biomedical research is for studies on the mechanism of disease resistance. A classic example is the failure of gonococcal infection to develop in rabbits after an experimental treatment that induces the disease in other animals. Negative animal models have become increasingly important with the advent of transgenic technology. For example, a novel transgenic mouse was created to study the lack of development of autoimmune thyroiditis with the injection of self-thyroglobulin. This strain of mice lacked certain surface epitopes to account for this negative reaction.6

5. Orphan. Orphan models are the opposite of negative models. Orphan models are animals in which a disease occurs but there is not a corresponding disease in humans. Orphan models may become induced models when a similar disease is recognized in humans later on. Historically, scrapie in sheep was such a model, but now is useful as a model for the human spongiform encephalopathies that are of so much concern (e.g., BSE, “mad cow disease,” and CWD, chronic wasting disease in deer).

All categories above may be further subcategorized with the following divisions:

1. Fidelity. The extent a biological structure in an animal resembles that of a human. Thus, a high fidelity animal model gives a highly relevant biological closeness to the human structure. Model fidelity is best conceptualized as a continuous spectrum, ranging from low to high fidelity. Examples of low-fidelity models include bench models made of simple materials that often have little anatomical resemblance to reality. However, these models incorporate some of the key constructs of the simulated tasks. At the other end of the spectrum are high-fidelity models such as human or animal cadavers or the new array of virtual reality simulators. These simulators usually incorporate highly realistic visual and tactile cues in the midst of a highly interactive model. In between these two extremes, almost any kind of intermediate fidelity can exist.
2. **Homologous.** The symptoms shown in the animal are identical to those shown in the human. For instance, the recent discoveries of swine hepatitis E virus (HEV) from pigs and avian HEV from chickens afforded an opportunity to develop small homologous animal models for HEV.9

3. **Isomorphic.** The animal’s symptoms or anatomy are similar to those in the human but the etiology or genetic character is different. For example, there is a set isomorphism between the human and mouse heart at the organ level and also at the organ part level: each species has a heart and a corresponding set of cardiac chambers (right and left atrium, right and left ventricle) and the wall of each chamber has a corresponding set of layers (epicardium, myocardium, endocardium).

4. **Partial.** These models do not mimic the entire human disease but enough similarities exist to allow their use in studying aspects of the disease or treatments. For instance, animal models of Alzheimer’s disease can be created based on the accumulation of many amyloid plaque deposits; however, they have only subtle behavioral and electrophysiological deficits, thus providing only a partial model of the human condition.10

5. **Face validity.** The degree to which there is a similar phenotypic display between the disease in the animal and the corresponding disease in the human. For example, it could be argued that the demonstration of drug effects in an animal model for depression after a period of chronic administration is important for establishing its face validity, but is not relevant to the model’s predictiveness and therefore to its ability to serve as a screening test for treatments for the modeled disease.10

6. **Construct validity.** The degree to which there is a similar genetic display between the disease in the animal and the corresponding disease in the human. As an example of high construct validity, research was performed on three candidate dopaminergic genes (DRD2, DRD4, and DAT-1) that were sequenced in spontaneous hypertensive (SHR) and Wistar Kyoto (WKY) rats. No differences were found in DRD2 or DRD4 genes, but several variations were found in the DAT-1 gene that are of significance because several ADHD families show linkage to DAT-1. It also strengthened the validity of using WKY as a control for SHR, because their behavioral characteristics are similar to those of other rat strains.12

**LEGISLATIVE AND LEGAL REQUIREMENTS FOR USING ANIMALS IN RESEARCH**

Biomedical research is among the most regulated industries in the world. A comprehensive overview of global requirements can be found in the *Handbook of Laboratory Animal Science*, 2nd edition, Chapter 3.13 Failure to comply with regulatory requirements can result in fines levied against the institution, suspension of authority to operate, permanent revocation of the facility’s license, and withdrawal of public funding.

One newly regulated aspect of biomedical research not covered in this chapter occurred after the terrorist attack on September 11, 2001. The attack increased concerns in the United States for the possibility of bioterrorism using agents that would destroy human, animal, and plant life. This concern escalated the need for research that involved the development of therapeutic and preventive measures against such agents. In response, congress passed and President Bush signed into law the “Public Health Security and Bioterrorism Preparedness and Response Act of 2002” (Public Law 107–188) on June 12. The purpose of the act was to improve the capacity of the United States to prevent, prepare for, and respond to bioterrorism and other public health emergencies and to enhance the control of dangerous biological agents and toxins. The Centers for Disease Control and Prevention (CDC) is the agency with the primary responsibility for implementing the provisions of the Act with regard to human pathogens and toxins and the United States Department of Agriculture (USDA) with regard to animal and plant pathogens and toxins. The regulation provides for expanded regulatory oversight of select agents and toxins, and a process for limiting access to persons who have a legitimate need to possess, use, or store these agents. The regulation also establishes a requirement for a security risk assessment performed by the Federal Bureau of Investigation for those persons needing access to select agents and toxins. It also establishes and enforces safety and security procedures, including measures to ensure proper training and appropriate skills to handle agents and toxins; a requirement to designate an institutional Responsible Official to ensure compliance with the regulations; and a requirement to obtain a certificate of registration when there is a need to possess, use, or transfer select agents and toxins. Infectious agents labeled as “select” as determined by the CDC and USDA, registration forms, and other information concerning the Select Agents Program may be found at http://www.selectagents.gov.14

**CHOOSING THE RIGHT MODEL**

To quote the philosopher, Bernard Rollin, “The most brilliant design, the most elegant procedures, the purest reagents, along with investigator talent, public money, and animal life are all wasted if the choice of animal is incorrect.” Once it has been determined that the use of laboratory animals is necessary, the most appropriate species, breed, and strain with the closest homology to humans must be chosen in order to give the research face and/or construct validity. Because new animal models are continually being identified and characterized and the field of biomedical research has become global in nature, the search for the appropriate animal model should start with a thorough literature search and a check of appropriate web sites (see Chapter 7). The Institute for Laboratory Animal Research maintains a very practical and useful search engine for this purpose.15

Selection of a species should not be based solely on availability, familiarity, or cost. Animals that meet these criteria may not provide the genetic, physiological, or psychological facets needed or wanted for the proposed project. It is almost impossible to give specific rules for the choice of the best animal model, because the many considerations that have to be made before an experiment can take place differ with each research project and its objectives. Nevertheless, some general rules can be given.

**RESEARCH FACTORS**

- Appropriateness as an analogue. Ensuring that the part or organ being studied has a function similar to the target species is vital in applying research-derived data from the chosen model.
- Transferability of information. The usual goal of research using an animal model is to define a process in a system with the hope of transferring the data gained to a more complex system. Traditionally, one-to-one modeling is
sought: modeling in one group of organisms that can be transferred to another group that has several analogous features of interest. This is especially helpful in modeling disease states. However, in modern research, many-to-many models are mainly used. This technique begins by analyzing the component parts of a process or disease, and then finding for each component analogous models in many taxa of living species. This is especially helpful when a plurispecies approach is needed to gain approval for new medications or medical devices.

- Generalizability of the results. The ability to generalize results to the target species is important. Federal regulations prohibiting the unnecessary duplication of previous research highlights the importance of choosing an animal model in which testing results can be easily repeatable and verifiable on which to build new research. In May, 2006, the world was shocked when famed South Korean cloning scientist Dr. Hwang Woo-suk was charged with fraud and embezzlement when scientists could not verify his published data. In addition, if the ultimate target species is human, it is well known that this species is genetically highly variable, with cultural, dietary, and environmental differences. This may be of lesser importance in disease modeling since most diseases do not choose its victim based on genetic variability. However, this is now well known to be of importance in pharmacological and toxicological modeling and has opened up the new field of research in pharmacogenetics.

- Ethical implications. Certainly research must start with justification for using an animal at all. Federal regulations require the use of alternate methods if feasible. Alternate methods could consist of using cell lines, bacteria, computer models, or even human volunteers. The three Rs of Russell and Burch (replacement of existing experiments with animal-free alternatives, or reduction in the number of animals used, or refined methods to reduce animal suffering) help to meet the ethical concerns.

- Numbers needed. Certainly consultation with a biostatistical analyst prior to submitting a proposal is highly recommended. Numbers needed to provide scientific validity, especially for publication, will impact many other factors such as cost and housing availability.

- Customary practice within a particular discipline. Caution must be displayed when using this criterion. Customary practices may not always mean that the most appropriate animal model has been used. The “customary” animal may not represent the most accurate genetic, microbiological, physiological, or psychological facets needed for the study. Historical evidence has revealed that using animal models just because others have is led to substandard results. However, customary practices when justified and supported by the other criteria listed can be a satisfactory and faster route of choosing the animal model needed.

- Existing body of knowledge of the problem under consideration. This criterion again emphasizes the need for a thorough literature search before forming the basis for the research project. The literature search will emphasize what is already known to prevent accidental duplication, but will also reveal what is not known. It will also make known published authorities in the discipline that may serve as a consultation source to prevent unnecessary and competitive research projects.

- Natural versus experimentally produced models. Unavailability of natural models will require the use of experimentally produced models. Depending on the objectives of the study, both may be needed.

**ANIMAL CARE FACTORS**

- Cost and availability. Certainly cost and availability are important factors when choosing an animal model, but they can be disastrous if the decision is based solely on cost and not the other listed factors. Cost also includes ongoing care not only for husbandry but also from experimental manipulations. Certainly, the best animal model can be in short supply as illustrated by the CNN news report on August 9, 2003. This report emphasized the increased demands in research due to public health crises such as AIDS and the threat of bioterrorism. The increased demands have led to a national shortage of rhesus macaques. In addition, the shortage has skyrocketed the cost per monkey.

- Housing availability. Another practical consideration in choosing the animal model is the accessibility of housing. Research animal housing requirements are stringent and may lessen the availability according to the species chosen. For instance, choosing a nonhuman primate may require the purchase of new caging and the hiring of additional personnel to provide specialized husbandry care, as opposed to choosing mice, which can be placed several to a cage and hundreds in a room.

- Husbandry expertise. Some models require not only special housing, but also special care.

- Stress factors. Stress sources from many different causes can affect the animal’s physiology, biochemistry, and behavior. Sources of stress can be transportation, handling and manipulations, overcrowding, lack of environmental enrichment, and the research project itself.

**PHYSICAL AND ENVIRONMENTAL FACTORS**

- Ecological consequences. While the best animal model may be available only by capturing in the wild, ecological consequences must be considered in its removal. In addition, safety measures must be in place to prevent accidental escape from the research facility. A prime example is the *Xenopus* spp. frog. If it escapes, it can overrun local ponds and rivers endangering natural amphibian populations. Furthermore, care must be taken not to violate the Endangered Species Act (http://www.fws.gov/endangered/wildlife.html) or the Convention on International Trade in Endangered Species of Wild Fauna and Flora (http://www.cites.org).

- Hazardous components. Many research projects entail the use of chemicals, infectious agents, and radioisotopes. The uses of these components are highly regulated and the appropriate proper authority (Institutional Biosafety Committee, Radiation Safety Committee, Occupational Health and Safety Committee, Environmental Health and Safety Committee, and Institutional Animal Care and Use Committee) within each institution must give approval for
its use. In addition, all those who will be exposed, whether from the research side or the animal care side, must be notified.

• Environmental influences. Environmental aspects may be important to a particular animal species. Environmental factors that fit into the broad categories of physical, chemical, biological, and social may impact the physiological and behavioral responses of animals. These factors include humidity, ventilation, light cycle and quality, noise, cage size and bedding materials, diet and water, and room temperature. As an example, high temperature and humidity have been proven to impair memory in mice.

ANIMAL-RELATED FACTORS

• Genetic aspects. Uniformity of organisms may be necessary where applicable. “This insidious evolution of the inbred genotype is known as genetic drift. It is capable of subverting the conclusions reached about comparable research results coming from different laboratories when each uses its own strain of the same inbred strain (Bailey DW, 1977).” The importance and methods of preventing genetic drift in biomedical research can be found at http://jaxmice.jax.org/geneticquality/drift.html. In addition, it is important to remember that to be in compliance with the National Institutes of Health’s Guidelines, work with transgenic animals requires the approval of the Institutional Biosafety Committee as well as the Institutional Animal Care and Use Committee.

• Background knowledge of biological properties. Knowledge of biological properties such as generalized and specialized function of body components is needed in order to validate interspecies transfer of information. Certainly, a rat would not be the best choice in biliary studies due to the absence of a gallbladder. Knowing the biological properties also aids in the decision of whether the animal is a spontaneous model or must be experimentally induced.

• Ease of and adaptability to experimental manipulation. This is unquestionably a practical matter. Guinea pigs have highly inaccessible blood vessels and would be impractical in studies requiring repeated blood sampling. Prairie dogs and woodchucks can be vicious to handle; therefore, knowing the response to experimental manipulation may also influence the choice.

• Size of the animal. This item is important from several different aspects. The size of the animal impacts housing and husbandry availability. However, size is also important to consider when tissue sampling or blood collection is necessary. For instance, many proposals are rejected because the researcher failed to abide by published guidelines for removal of blood. In addition, it is also important to incorporate the size of the animal into the decision-making apparatus when physiological or morphological properties such as joint strain or organ size must be identical to that of a human, especially when designing medical devices.

• Life span and age. Studies requiring components at different stages of life can certainly impact the species chosen. The average lifespan of a rat is 2.5–3.5 years, whereas it can be over 30 years for a rhesus monkey.

• Sex. The alternating cycle of hormonal production in the female gender and its influence on the data outcome must be considered when planning for the research project.

• Progeny needed. Female mice and rats can produce 5–10 progeny per month, whereas the rhesus monkeys only one or two per year. Xenopus sp. frogs produce thousands of ova during their lifetime, whereas mammals produce only dozens.

• Diseases or conditions that might complicate results. An excellent historical review on the struggle against pathogens in laboratory rodents can be found in Weisbroth. Both publications emphasize the need for disease-free animals in research to prevent adverse effects on resultant data. Just as in human AIDS, the realm of disease-causing organisms changed with the advent of immune deficient models. Special caging and care procedures are fundamental in minimizing such infections.

• Special features of the animal such as unique responses or microflora. It is important to be familiar with unique anatomical or physiological features of the species you will be working with. The results could be quite unexpected otherwise. For example, in rabbits, the terminal portion of the ileum empties into an enlarged rounded viscus called the sacculus rotundus and not the colon as in humans. This unique feature of the rabbit is important to know when designing gastrointestinal studies.

Forming the above standards into a checklist will help to fulfill the criteria needed to choose the best model for the proposed research project. Model selection is the privilege of individual researchers, but they must be very cautious in their selection because in the end, it is up to them to convince the rest of the scientific community that they made the right choice.

Before choosing, consultations should occur with scientists who have already used the animal model. Just as with equipment purchase, communicating with previous users can be very helpful in learning unique features of the selected species, breed, and strain. Not all attributes (especially negative ones) are published, making it even more important to contact those who have experience with the animal model you choose.

Preparatory consultation should also occur with those who will be responsible for housing and maintaining the animals, as they will be the most familiar with the care of the animal and its physical and environmental needs. Preparatory consultation with the laboratory animal veterinary practitioner should also occur to discuss the animal-related factors.

EXTRAPOLATION FROM ANIMALS TO HUMANS

Extrapolation from animals to humans does not necessarily mean that biomedical research data obtained from using animals are then used to find a corollary in a human. Rather, a hypothesis is formed first based on human relevancy, and then tested on an animal. Answers are obtained, analyzed, and published based on the hypothesis. Although true in many cases, caution must be exerted in assuming that a close phylogenetic relationship or anatomical similarity guarantees an identical biochemical or physiological response in the animal. In addition, it must be realized that humans to whom the results are being extrapolated are genetically highly variable due to cultural, dietary, and environ-
ment differences. This is of minor importance when developing
disease models but is highly important for pharmacological and
toxicological models.

So, how can the validity of extrapolation be verified? Complete
reliability cannot be guaranteed; however, following the follow-
ing vital requirements will help to avoid several of the mistakes
of the past and overcome problems of the future:

- **Taking a plurispecies approach.** Most of the regulating
  authorities require two species in toxicology screening,
  one of which has to be nonrodent. This does not necessar-
  ily imply that excessive numbers of animals will be used.
The uncritical use of one-species models can mean that
experimental data retrospectively turn out to be invalid for
extrapolation, representing real and complete waste of
animals. Using more than one species is, of course, no
 guarantee for successful extrapolation either.
- **Metabolic patterns and speed and body size must match
  between species.** The use of laboratory animals as models
for humans is often based on the premise that animals
are more or less similar with respect to many biological
characteristics and thus can be compared with humans.
However, there is one striking difference between mouse
and human, and that is body size. In proportion to their
body size, mammals generally have very similar organ
sizes expressed as percentage of body weight. Take the
heart for instance, which often constitutes 5 or 6 g per
kilogram of body weight, or blood, which is often approxi-
 mately 7% of total body weight. It is well known that the
metabolic rate of small animals is much higher than that
of large animals and, thus, provisions must be made to
adjust the study accordingly. Drugs and toxins exert their
effect on an organism not per se but because of the way
that they are metabolized, the way that they and their
metabolites are distributed and bound in the body tissues,
and how and when they are finally excreted. Adjusted
doses should include the following provisions:
  - If the object is to achieve equal concentrations of a
    substance in the body fluids of animals of different body
    size, then the doses should be calculated in simple pro-
    portion to the animals’ body weights.
  - If the object is to achieve a given concentration in a
    particular organ over a certain time period, the calcula-
    tion of dosage becomes more complicated, and other
    factors, including the physicochemical properties of the
    drug, become important.
  - Metabolism or detoxification and excretion of a drug are
    not directly correlated with body size but, more accu-
    rately, to the metabolic rate of the animal.
  - Some species react with particular sensitivity toward
certain drugs, and marked variations in the reaction of
animals within a species occur with respect to strain,
pigmentation, nutritional state, stress level, type of
bedding, ambient temperature, age, sex, route or time of
administration and sampling, diurnal variation, and
season of the year. As much as possible, these items
must be controlled.4
- **Experimental design and the life situation of the target
  species must correspond.** A model cannot be separated
  from the experimental design itself. If the design inade-
  quately represents the “normal” life conditions of the
target species, inaccurate conclusions may be drawn,
regardless of the value of the model itself.

**DESCRIPTION OF ANIMAL MODELS16**

Unlike the old days when the researcher could write in the
materials and methods section “black mice were used in the
study,” modern obligations require an exact description of
the model. The description should include the following.

- Genetic strain and substrain using correct international
  nomenclature.26,27
- Special genetic features.
- Microbial status of the animal.
- Age.
- Housing standards.
- Maintenance procedures.
- Diet.
- If used in infectious disease studies, the description should
  also include
  - Strain of the organism.
  - Route of inoculation.

**CONCLUSIONS**

Laboratory animals play a crucial role in research discovery
and technological advances, and they will continue to take part
in improving the lives of people and other animals. It is incumbent
upon the researcher to know the subject well in order to provide
relevant information to the scientific world. The final judgment
in the choice of the animal model will always be in its ability
to elucidate and predict the observed effects in the target
species.

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ABSTRACT

Experimental animal models are critical to understand gene function and human disease. Many rodent models are presently available providing avenues to elucidate gene function and/or to recapitulate specific pathological conditions. To a large extent, successful translation of clinical evidence or analytical data into appropriate mouse models is possible through progress in transgenic or gene deletion technology. Despite these significant improvements, major limitations still exist in manipulating the mouse genome. For this reason and to maximize success, the design and planning of mouse models need good knowledge concerning the requirements and limitations of commonly used strategies and emerging technologies. The purpose of this chapter is to provide a current overview of strategies for manipulating the mouse genome.

Key Words: Transgenic mice, Knockout mice, Conditional mouse models, Cre and Flp recombinase, Tetracycline system, RNAi, Knockdown mice, Functional genomics.

INTRODUCTION

One of the central issues facing biomedical research is the need to transform in vitro data into knowledge about gene function in mice or humans. In this respect genetically engineered laboratory mice are an excellent tool for modeling genetic disorders, assigning function to genes, evaluating the action of drugs and toxins, and answering fundamental questions in basic science. Animal models account for factors such as age, stress, cell-to-cell communication, pathogen–host interaction, physiology, immune response, brain function, and other key issues, providing an advantage over in vitro assays or computer models. For example, tumor initiation, progression, and spreading cannot be recapitulated in vitro but can be addressed with animal models. Nevertheless, animal models represent only an experimental surrogate and results obtained from mouse experiments do not necessarily recapitulate the human situation.

Furthermore, introducing genetic changes to the germ line of the mouse may indeed identify gene function, but also might result in severe developmental consequences, complicating or preventing analysis. Embryonic lethal phenotypes, frequently associated with null alleles or compensatory pleiotropic gene expression, are examples that can prevent the generation of a useful animal model. To overcome these limitations and more precisely control gene expression or gene deletion in a tissue- and time-specific fashion, conditional mouse models are used and are becoming increasingly popular. Therefore, these second-generation models may significantly improve our ability to examine gene function in vivo.

In this chapter, we will discuss different conditional transgenic and gene-targeting techniques, as well as provide a brief overview regarding conventional mouse transgenesis and germ line gene targeting. In addition, we will examine the emerging technology to knock down gene expression in vivo through small interfering RNA (siRNA) methods. While this chapter is not intended to be comprehensive or to provide specific technical details, we encourage the interested reader to explore more focused reviews on this topic.

STANDARD MOUSE TRANSGENESIS

Since the pioneering work from Gordon and colleagues reporting the successful generation of transgenic mice by microinjection of DNA into the pronucleus of one-cell embryos, the genetic manipulation of the mouse embryo has been extremely useful for creating thousands of murine models for biomedical research. Today, two different methods are routinely used for generating genetically modified mice.

TRANSGENIC MICE

First there is microinjection of recombinant DNA into the pronuclei of fertilized mouse eggs or infection of germ cells/early embryos with viral vectors carrying the foreign gene. With this technique the introduced DNA is more or less randomly inserted in one or multiple copies into the mouse genome. Using this approach, it has been possible to characterize the function of well-defined transgenic gene products, dominant negative or constitutively active gene mutations, or specifically designed proteins. Furthermore, in vivo suppression of a particular endogenous gene can be achieved by transgenic expression of antisense mRNA, ribozymes, and small hairpin (shRNAs) or micro-RNAs (miRNAs). Besides gene products, transcriptional control elements as enhancers,
silencers, promoters, or complete locus control regions can be studied using transgenic mice. 16–18

One recurrent problem observed in transgenic mouse models is variegational position effects often disturbing or masking the specific function of the used transcriptional control elements. Theoretically, it might be assumed that after stable integration into the genome the recombinant DNA should readily manifest its predicted mission. However, many experiments reveal that the genetic surrounding of the inserted transgenic construct is modulating the expression pattern of the transgene itself. 19

Obvious reasons for positional variegation are that the injected DNA (1) integrates into or near an endogenous gene locus with strong transcriptional control activity, affecting in cis the promoter of the transgenic construct or (2) integrates in a chromatin structure prone to be inactivated during development, which would lead to silencing of the transgenic construct. To avoid this recurring issue, single-copy integration of transgenes into a selected target locus or the use of so-called insulator elements has been reported. 20,21

Alternatively, large recombinant constructs like bacterial artificial chromosomes (BACs) or yeast artificial chromosomes (YACs) can be used for generating position-independent transgenic mouse lines. 22,23 As one of the consequences of the genome sequencing projects, a collection of well-characterized BAC and YAC clones that cover nearly the entire human and mouse genomes is now available (for example, http://www.rzpd.de/products/clones; http://bapac.chori.org; http://www.sanger.ac.uk). In addition to that, convenient methods for site-specific modification of BACs and YACs have been established. 24–27 However, it has to be emphasized that by the random chromosomal insertion of BAC or YAC constructs endogenous gene loci may be destroyed and also the expression of neighboring genes might be modified. 28 In case these endogenous genes will be indispensable to life, this will become obvious when the transgenic mouse variant can be maintained only as a heterozygous line.

For the above reasons, a correlation between phenotype and transgene function is obvious only when at least two independent transgenic mouse lines with identical BAC, YAC, or transgenic constructs show the same phenotype. If only one transgenic line is analyzed, it can never be completely excluded that the observed phenotype is not linked to the transgene itself but reflects the compromised expression of neighboring endogenous genes.

CONVENTIONAL GENE TARGETING

The second widely used method for the generation of gene manipulated mice makes use of pluripotent mouse embryonic stem (ES) cells. Targeted gene modification is built on the finding that mammalian cells have the enzymatic machinery for exact homologous recombination between identical (homologous) DNA sequences. 29–31 Thus precise predefined modifications of an endogenous gene are possible in cultivated mammalian cells including mouse ES cells. Genetically modified ES cells can then be used to generate gene “knockout” or “knockin” mice that carry the planned DNA modification. 32 Normally, pluripotent ES cells are injected in early mouse embryos at the blastula stage or aggregated with morula stage embryos. During embryonic development the ES cells participate in the formation of different tissues including the germ cells and as a result the embryo is composed of two different genetic backgrounds, derived either from the wild-type cells or the genetically modified ES cells. However, in case ES cells have contributed to the germ cells of the animal, offspring from these chimeric mice will harbor the desired genetic modification in the germline (germline transmission). To generate mice with a defined background, inbreeding with animals of the same genetic background as the original ES cell will produce genetically identical homozygous offspring. However, mice crossed to commonly used ES cell mouse backgrounds often poorly reproduce and are therefore difficult to expand. For this reason, for most experiments chimeric mice are crossed to mouse strains with good breeding efficiencies.

Typically, the targeting construct for homologous recombination is composed of a central core region flanked by two regions—the so-called homology arms—that are identical in sequence to the nucleotide sequence of the target region in the genome. The homology arms are required for correct site-specific integration or replacement of the endogenous gene segment by the DNA of the targeting vector. The core region of the targeting construct incorporates the planned genetic modification together with a positive selection cassette, conferring resistance to ES cells containing the targeting construct. Most of the genomic insertions of the targeting vector are random, somewhere in the genome. In a few cases a replacement of the gene segment by the targeting vector takes place. Therefore, enrichment strategies for correctly recombined ES cell clones have been developed. These enrichment strategies make use of negative selection cassettes such as the herpes simplex type 1 thymidine kinase gene or the gene for the diphtheria toxin α-chain. The negative selection cassettes are placed outside the homology arms and are lost during homologous recombination, whereas during random integration the entire targeting vector including the negative selection marker is inserted into the genome of the ES cell. This permits a counterselection against randomly integrated clones and leads to an enrichment of ES cell clones with correct targeting. 33–35 For complex multistep genome manipulations including large chromosomal deletions or translocations convenient combinations of different selection markers have been described. 34

Most of the published gene targeted mice are summarized in several electronically searchable databases in the internet (see http://www.bioscience.org/knockout/knochome.htm, http://www.nih.gov/science/models/mouse/index.html, and http://www.informatics.jax.org/imst/index.jsp). In addition to the already existing gene targeted mice, academic institutes and commercial companies have generated gene trapped ES cell libraries that can be used as a source for generating knockout mice. Each individual ES cell clone of such a library harbors a single integration of a viral construct, which in turn serves as a signpost for identification of the trapped gene. Recently, the major gene trapping groups have centralized the access to all publicly available gene trap ES cell lines. 36 In this portal (www.genetrap.org) a collection of at the time 45,000 well-characterized ES cell clones is available on a noncollaborative basis. In addition, gene-trap-derived gene-specific knockout ES cell clones are commercially obtainable (http://www.lexicon-genetics.com/discovery/omnibank_ebiology.htm).

Importantly, studies involving gene targeted mice have to consider the genetic background. Depending on the mouse strain that was used the in vivo function or loss of function of a particular gene can be very different. 37,38 For example, γ-protein kinase C knockout mice showed a considerable difference in sensitivity to ethanol that was completely dependent on the genetic background. 39 This example illustrates that the effect of a single genetic