Fundamentals of

Laboratory Animal Science

Edited by Enqi Liu • Jianglin Fan

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Edited by
Enqi Liu
Research Institute of Atherosclerotic Disease,
Xi'an Jiaotong University Cardiovascular
Research Center, Xi'an, Shaanxi, China
and
Laboratory Animal Center, Xi'an Jiaotong University
Health Science Center, Xi'an, Shaanxi, China
Jianglin Fan
Department of Molecular Pathology, Faculty of
Medicine, Graduate School of Medical Sciences,
University of Yamanashi, Yamanashi, Japan

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Preface

Laboratory animals have become increasingly important for biomedical research, and it is estimated that approximately 70% of biomedical research is associated with the use of experimental animals. For just over a century the Nobel Prize has been awarded each year in recognition of the world's greatest biomedical advances. Of the 106 Nobel Prizes awarded for physiology or medicine, 95 were directly dependent on animal study. Animal experiments not only expand our knowledge of science, but also greatly improve human and animal health. Since 1900, animal research achievements have helped extend the longevity of Americans by approximately 25 years. However, there are those who disapprove of animal research because they believe such experiments to be expensive and harmful to animals. Therefore, it is incumbent on researchers to find a way to effectively implement animal study based on the 3Rs (reduction, replacement, and refinement) principle and animal welfare.

The curriculum of Laboratory Animal Science is provided in the majority of medical schools and agriculture universities in the world. However, a suitable and practical textbook that contains state-of-the-art techniques is still lacking. In 2004, 2008, and 2014, with the assistance of the Chinese Ministry of Health, we successfully published two books in Chinese titled *Medical Laboratory Animal Science* and *Animal Models of Human Diseases*, which have been used as the standard textbooks for graduate school students in China and have become best sellers in medical schools in China. Recently, after consultation with professionals who perform biomedical studies using laboratory animal models, we feel that it is necessary to publish a textbook of *Laboratory Animal Science* in English for readers worldwide. For this purpose, we decided to compile the current textbook, which will be useful for teaching and training in this field. This book focuses on theoretical instruction, experimental operation, and skills training, and systematically covers both basic information of laboratory animals and techniques for animal experiments.

This book is divided into eight chapters. Chapter 1 provides an overview of laboratory animal science, summarizing some basic concepts and categories of laboratory animals and animal experiments. In addition, it introduces a brief history, applications in biomedicine, and management of laboratory animals. The welfare of laboratory animals has attracted increasing attention in recent years. Chapter 2 describes in detail the definition of animal welfare and the measures taken to ensure protection of experimental animals. Chapter 3 discusses the standardization of laboratory animals and stresses its importance for animal experimentation. This chapter outlines several key elements that are closely related to animal experimentation such as animal genetics, health, facilities, nutrition, and the like. Chapter 4 introduces the most commonly used laboratory animals, such as mice, rats, hamsters, guinea pigs, rabbits, dogs, pigs, rhesus monkeys, chickens, and fish and mainly discusses and compares animal anatomy, biology, husbandry, and applications in biomedical research. Chapter 5 discusses models of human disease and introduces how to select and create an animal model for studying specific human diseases. Information about the newest genetically modified animals is also included in this chapter. Chapter 6 provides a summary of the technologies used in animal experimentation. All of these skills ensure research goes smoothly while greatly reducing animal pain and distress. Chapter 7 discusses how to design a suitable animal experiment. It introduces the content and procedure of animal experimental design. Chapter 8 in which readers will learn the whole process and protocol of an animal experiment, summarizes the organization and management of animal experiments. We believe that this book will become not only a standard textbook for undergraduate and postgraduate students, but also a life tool on the bookshelf for veterinarians, researchers, animal-care staff, and other professionals who are involved in animal experiments.

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Enqi Liu
Jianglin Fan

Editors

Enqi Liu, PhD is a professor and director of the Research Institute of Atherosclerotic Disease, Xi'an Jiaotong University Cardiovascular Research Center and Laboratory Animal Center, Xi'an Jiaotong University Health Science Center. Dr. Liu earned his bachelor's degree in animal genetics and master's degree in veterinary medicine from China Agricultural University, and his PhD from Saga University in Japan.

Currently, Dr. Liu also holds the chair of the professor at Xi'an Jiaotong University Health Science Center, Xi'an. His research interests include laboratory animal science, experimental pathology, atherosclerosis, lipid metabolism, and translational research. He has published more than 70 articles in peer-reviewed journals. He has also published several professional books including *Laboratory Animal Genetics and Breeding* (2002), *Medical Laboratory Animal Science* (2004, 2008), and *Animal Models of Human Diseases* (2014).

Jianglin Fan, MD, PhD, FAHA is a professor and chairman of molecular pathology at University of Yamanashi School of Medicine, Yamanashi, Japan. He also holds the chair of adjunct professor at Xi'an Medical University and Xi'an Jiaotong University. Dr. Fan earned his MD from the Yanbian University, School of Medicine and his PhD from Saga Medical School. He completed a postdoctoral fellowship at Gladstone Institute at the University of California, San Francisco. Dr. Fan's research focuses on atherosclerosis, including elucidation of the molecular mechanisms of atherosclerosis, lipid and lipoprotein metabolism, and inflammation. One of his major achievements was developing novel transgenic and knockout rabbit models for the study of cardiovascular disease. His work has led to more than 150 publications in prestigious peer-reviewed journals.

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Contributors

Changqing Gao
Department of Laboratory Animal
Central South University
Changsha, Hunan, China

Dongmei Tan
Laboratory Animal Center
Chongqing Medical University
Chongqing, China

Yi Tan
Laboratory Animal Center
Chongqing Medical University
Chongqing, China

Qi Yu
Institute of Basic and Translational Medicine
Xi'an Medical University
Xi'an, Shaanxi, China

Sihai Zhao
Laboratory Animal Center
Xi'an Jiaotong University Health Science Center
Xi'an, Shaanxi, China

CHAPTER 1

Laboratory Animals and Biomedical Research

Enqi Liu and Jianglin Fan

CONTENTS

1.1 INTRODUCTION OF LABORATORY ANIMAL SCIENCE

1.1.1 Laboratory Animal Science

High-quality laboratory animals and accurate animal experimental results in biomedical research were initially called for in the 1950s. Thus, an independent specialty on laboratory animals and animal experimentation came into being. The content of laboratory animal science includes animal heredity, breeding, quality control, disease prevention, and animal welfare. The science of animal experimentation refers to animal experiments to obtain novel, scientific experimental data under an animal welfare guarantee.

Some of the major tasks of laboratory animal science are to provide laboratory animals, obtain accurate, reproducible data, and collect scientific information for biomedical research.

Animal experimentation is mainly applied in the fields of medicine, biology, veterinary medicine, and agriculture. In terms of animal quantities used in experimentation, most of the animals are used in the medical field, including teaching and training, medical research, and function detection and security inspection for medicines, biological products, and food. Thus, the science of medical laboratory animals and medical animal experimentation is referred to as medical laboratory animal science.

As a new and independent discipline, laboratory animal science has been rapidly developed since the rediscovering of Mendelism. In 1944, the American Academy of Science put the issue of laboratory animal standardization on the agenda for the first time, which is usually regarded as the starting point of modern laboratory animal science. In 1966, "laboratory animal science" first appeared in scientific literatures, marking the birth of this new discipline.

Laboratory animal science is a relatively young science. However, it has built an entire theory system and has derived subdisciplines such as laboratory animal breeding, laboratory animal microbiology, laboratory animal environmental ecology, laboratory animal nutrition, laboratory animal medicine, comparative medicine, and laboratory animal husbandry.

Laboratory animal science has great significance. First, it is an important means to study biomedicine and can directly affect the establishment, implementation, and reliability of the biomedical projects. Second, the improvement and development of laboratory animal science has brought various projects into a new field of vision and promoted the development of biomedicine.

1.1.2 Laboratory Animals

1.1.2.1 Species of Laboratory Animals

There are a great variety of animals in nature. Currently, there are more than 1.5 million animal species in the world. Based on natural classification, morphological characters, internal structure, biogenesis, and kinship, animals can be classified into *phylum*, *class, order, family, genus,* and *species*. Additionally, animals can be subdivided into *subphylum, subclass, suborder, subfamily, subgenus,* and *subspecies*.

A species is often defined as a group of living organisms consisting of similar individuals capable of exchanging genes or interbreeding, which is one of the basic units of biological classification and a taxonomic rank. Species is the basic unit of biological classification and its formation is the result of natural selection. For example, C57BL/6 inbred strain mice, which are the most commonly used experimental tool in modern biomedical research, belong to phylum Vertebrata, class Mammalia, subclass Eutheria, order Rodentia, suborder Myomorpha, family Muridae, genus *Mus*, and species *Mus musculus*. At present, the laboratory mice used worldwide in biomedical research are mainly derived from four subspecies: *Mus musculus domesticus, Mus musculus musculus, Mus musculus molossinus*, and *Mus musculus castaneus*.

Only a few animals are used for scientific research and animal experimental study in nature. Except a small number of invertebrates, the majority of laboratory animals are mammalian animals that belong to phylum Vertebrata. Among them, the quantity of rodents accounts for more than 80% of all vertebrates, and mice account for more than 70% of the rodents.

1.1.2.2 Laboratory Animals and Animals for Research

To distinguish animals used in scientific research from other animals, all animals in nature can be classified as laboratory animals, economical animals, or wild animals based on the existing state.

In a narrow sense, laboratory animals are often defined as animals specially bred for biomedical research. To meet the requirements of biomedical research, teaching, therapeutics, assessment, diagnosis, biological product manufacturing, etc., laboratory animals are artificially bred and reproduced into standardized species or strains. According to the definition, laboratory animals should have the following three features:

- From the perspective of genetic control, laboratory animals must come from a clear source, be bred artificially, and have defined genetic background. Therefore, laboratory animals belong to genetically defined animals. In accordance with genetic backgrounds, they can be divided into isogenic animals and heterogeneic animals. Isogenic animals refer to inbred strain and F1 hybrid animals. Heterogeneic animals are mainly outbred strain and F2 hybrid animals. In addition, inbred strain animals include common inbred strain, congenic inbred strain, coisogenic inbred strain, recombinant inbred strain, separate inbred strain animals, and others.

- From the perspective of microbial control, the microorganisms and parasites carried by laboratory animals should be controlled strictly. To guarantee the accuracy, sensitivity, and repeatability of animal experimentation, microbiological and parasitological control not only exclude animal diseases, but also asymptomatic infection and nonpathogenic pathogens, which may interfere with experimental results. Accordingly, laboratory animals in some countries are classified arbitrarily into four levels: conventional (CV), clean (CL), specific-pathogen free (SPF), germ free (GF), and gnotobiotic animals (GA). Microorganisms and parasites carried by SPF and GF animals are artificially monitored and animals themselves must be depolluted by artificial cesarean section or embryo transfer.
- From the perspective of application, the ultimate goal of all laboratory animals is to be used for scientific study. Currently, laboratory animals serve as frontier sentries in the fields of biomedicine, pharmacy, chemical industry, agriculture, husbandry, environmental protection, commodity inspection, foreign trade, military industry, and aerospace. Rather than humans, laboratory animals are used to perform scientific research and verify many scientific truths. On account of the complexity of biological phenomena, no other methods can completely replace laboratory animals so far.

According to the narrow concept of laboratory animals, mice, rats, hamsters, guinea pigs, rabbits, and dogs have completely become qualified laboratory animals after being artificial bred for many years. However, the work of laboratory animalization on other mammals, birds, fish, and nonhuman primates is in progress. Thus, they are not actually laboratory animals in a strict sense.

Economic animals are also known as domestic animals. They are directionally domesticated, bred, and produced for economic traits (meat, milk, eggs, fur, wool, etc.) as indicators of artificial selection to meet the needs of human social life. Many economic animals, such as pigs, horses, cattle, sheep, goats, chickens, ducks, geese, pigeons, and fish, are also applied in biomedical research. Some of these economic animals have become very close to meeting the strict standards of being laboratory animals. However, compared with the "standard" animals like mice and rats, their quality is not perfect.

Wild animals refer to animals living in natural conditions. For the purpose of biomedical research, humans sometimes capture these animals from nature to conduct animal experimental studies rather than to perform artificial breeding or feeding. Except in rare cases, wild animals such as frogs, toads, and salamanders, which are extensively used in biomedicine teaching, wild fish, invertebrates, birds, and nonhuman primates, which are also used in scientific research, are generally not used in artificial breeding.

Compared with the narrow concept of laboratory animals, there is a so-called broad concept, that is, animals for research or laboratory animals. It refers to animals from wild animals, economic animals, or other artificial breeding animals, selected artificially and bred directionally for biomedical research.

The scope of animals for research is much more than that of laboratory animals. Distinguishing animals for research from laboratory animals is important but also has some limitations.

In the aspect of animal experimental reproducibility, there is big difference between animals for research and laboratory animals. The reproducibility of study means that different workers who perform the same experiments can obtain the same results using the same strain animals, albeit at different locations and times. Reproducibility demands that the accuracy of animal experimentation reaches that of a chemical reaction, which requires the laboratory animals to also possess the "purity" of chemical reagents. To achieve good reproducibility and comparability for scientific research, it is necessary to strictly conduct genetic control, microbiological control, nutritional control, and environmental control. As wild animals live in the wild and survive through natural selection in accordance with the principle of "survival of the fittest," it is difficult for wild animals and economic animals to meet these strict requirements, which can only be achieved by laboratory animals. However, economic animals, such as domestic animals, are under artificial selection and their stock selection is based on economic traits and maximum benefit. Although laboratory animals are also under artificial selection, their strain selection is based on the requirements of biomedical research. In the process of strain selection, laboratory animals are bred into a stock or strain to serve as ordinary "reagents" for scientific research. The diseases occurring in laboratory animals, which are similar to human diseases, can also be reserved with genetic methods to build "disease model" strains as special "reagents" for biomedical research or human substitutes.

Obviously, from the narrow concept of laboratory animals, it is difficult to establish popular models for biomedical research. For example, most zebra fish, *Caenorhabditis elegans*, and drosophila, which bring epoch-making discovery to genetics, do not belong to outbred strain, inbred strain, or SPF animals. The narrow concept of laboratory animals has been developed on the basis of rodents such as mice and rats, and achieved great success. Nevertheless, this concept does not necessarily apply to other animals. For example, inbred strain laboratory animals mean animals from continuous sibmating over 20 generations and the inbreeding coefficients are greater than 0.99. In addition to rodents, most animals cannot tolerate such high inbreeding to produce inbred strain animals. Thus, chickens, quail, and other birds can be considered as inbred strain animals as long as the inbreeding coefficient is greater than 0.5.

For convenience, laboratory animals mentioned in this textbook refer to animals for research unless otherwise specified. Animals for research include vertebrates and invertebrates such as *Drosophila* and *C. elegans*. This textbook is mainly about vertebrates for research, which are commonly used in biomedical science. Mammals for research account for the vast majority of vertebrates and they are the main laboratory animal groups that should be provided protection by Animal Welfare Act or animal welfare board/organization.

1.1.2.3 Standardization of Animals for Research

Standardization of animals for research refers to standardization of production conditions (environment, facilities, and nutrition), quality (microbiological, parasitological, and genetic quality control), and animal experimental conditions. In order to reach "standardization," it is necessary to carry out genetic control, microbiological and parasitological control, nutritional control, and environmental and facility control.

Genetic, microbiological and parasitological, nutritional, environmental, and facility control will be introduced in Chapter 3.

1.1.2.4 Genetically Modified Laboratory Animals

Gordon et al. (1980) injected exogenous DNA into the male pronucleus of a mouse zygote by means of microinjection technology and successfully obtained transgenic animals, which carried exogenous genes and exhibited stable transgenic hereditary characteristics. Meanwhile, embryonic stem (ES) cells carrying exogenous genes were injected into normal blastocysts and developed into chimera mice. Soon afterwards, genetically modified (GM) mice whose endogenous genes were knocked out (gene inactivation) and knock-in mice in which either of two genes associated with certain physiological phenomena were replaced by the other gene (gene functional change) were successfully produced. Subsequently, the cloned animals were produced by nuclear transfer technology, mutant model animals were induced by *N*-ethyl-*N*-nitrosourea (ENU), and knock-down animals were obtained using RNA interference (RNAi) technology. In recent years, new knock-out technologies, such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regulatory interspaced short palindromic repeat (CRISPR)/Cas-based RNA-guided DNA endonucleases are being introduced following the increasing maturity of animal genetic engineering. The biggest advantage of these technologies is that they are independent of ES cells and enable a broad range of genetic modifications by inducing DNA double-strand breaks that stimulate error-prone nonhomologous end joining or homology-directed repair at specific genomic locations. Therefore, a large number of GM animals are generated through the application of these advanced technologies, which greatly increases the resource abundance of laboratory animals.

From 1990 through 1999, the number of animals for scientific research in Great Britain decreased from 3,207,094 to 2,656,753, a decrease of 17%. However, the use of GM animals increased from 48,255 to 511,607, a more than 10 times increase. It has increased by 14% in 2000 from 1991 and reached 581,740, accounting for 21.4% of Britain's total use of animals in 2000. Figure 1.1 shows that the use of GM animals increased considerably over the period from 215,300 in 1995 to 2.03 million animals in 2013 (+1.82 million or +845%), with the breeding of GM animals and fundamental biological research being the main primary purposes accounting for the rise. A total of 1.94 million GM animals were used in biomedical study in 2014, and nearly all were mice (91% or 1.76 million), zebrafish (8% or 154,000), or rats (1% or 20,100 procedures). We retrieved five journals related to cardiovascular diseases, including *Circulation*, *Circulation Research, Arteriosclerosis, Thrombosis, Vascular Biology*, *Cardiovascular Research*, and *Atherosclerosis*, and found that 184 articles published were related to animal experimentation for the study of human atherosclerosis in

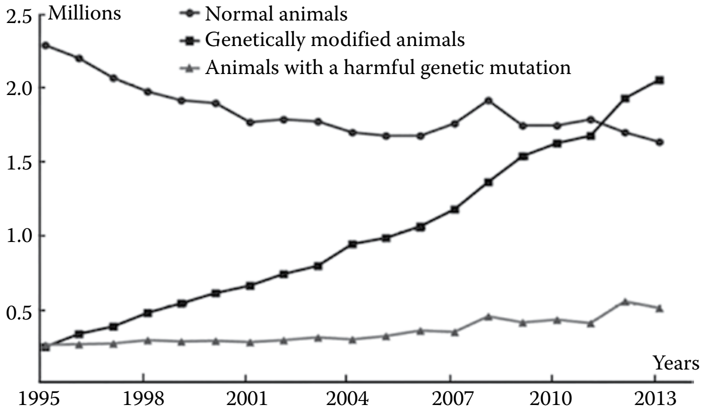

Figure 1.1 Procedures by genetic status of animal in Great Britain, 1995–2013. All EU countries count the total number of procedures for each species rather than the total number of animals, these figures differ by 1%–2% due to reuse and longer studies taking place over several years (with the same animal).

2012. Among them, GM mice were used in 162 articles, while articles that involved using normal mice, rabbits, rats, nonhuman primates, and hamsters were only 9, 6, 5, 1, and 1, respectively. It has been proven that GM animal models play an irreplaceable role in atherosclerosis study, which is the foundation of modern cardiovascular pathology.

Currently, almost all pathogeneses of human disease can be simulated by GM animal models. The utilization of GM animals has greatly shortened the time, reduced the research cost, and improved the results reliability for biomedical research. Therefore, GM animal models are the core model for current biomedical research, and GM mice are predominant in the application of GM animal models.

The generation and application of GM animals are dealt with in Chapter 5.

1.1.3 Animal Experimentation

1.1.3.1 Implications of Animal Experimentation

Large evidence demonstrated that many of the landmark achievements in the field of biomedical research have come from laboratory animals. According to the statistics from the Congress Office of Technology Assessment and National Association for Biomedical Research, laboratory animals have been used for more than 70% of the biomedical research projects funded by the United States government. Since the beginning of the twentieth century, these prizes have charted the world's greatest medical advances. Of the 107 Nobel Prizes awarded for physiology or medicine, 95 were dependent on research using animals. This includes every prize awarded for the past 30 years.

From the viewpoints of science, economy, and ethics, viewpoints, it is necessary to conduct animal experimentation for development of biomedicines. The type of animal experimentation indicated in this textbook refers to animals in biomedical research and the experimental processes that may bring pain or injury to the animals. Experimental study *in vitro*, which involves animal organs, tissues, and cells, is outside the scope of animal experimentation.

The life cycles of most laboratory animals are very short. For example, mice can only survive 2–3 years and their reproductive cycles are only 70 days. Laboratory animals used for experiments are convenient for observing and studying the whole breeding process and life course of an animal in a very short period. The developmental process of human disease is very complicated. Humans cannot be used for experimentation study to explore the pathogenesis and prevention mechanism of diseases. However, animal diseases and biological phenomena can be analogized to humans. Laboratory animals are prone to infection from some diseases, which are similar to human diseases. Study on laboratory animals can mimic human diseases and provide prevention and therapy strategies, both for humans and other animals. Therefore, animal experimentation is beneficial for both humans and animals.

Tephromyelitis (commonly known as poliomyelitis) is an ancient and terrible disease. After the World War II, there was an epidemic in Europe and America. From 1948 to 1952, 11,000 patients died of poliomyelitis, and paralysis and limb atrophy caused by poliomyelitis occurred in 200,000 people in the United States. Currently, it is believed that children will no longer be infected by poliomyelitis after injection of the poliomyelitis vaccine. In addition to poliomyelitis, children can also be protected from typhoid, tetanus, diphtheria, whooping cough, and smallpox infection after injection of the corresponding vaccines. Most healthy adults also benefit from vaccines. However, few people know that the vaccines have come from numerous animal experimentations.

In addition, animal experimental study is in favor of improving animal welfare. For example, canine distemper is an infectious dog disease that is caused by viruses and spreads easily. In the past, 80% of puppies died of canine distemper infection in Great Britain. From the 1920s to 1930s, scientists conducted animal experimentation to study a canine distemper vaccine, but they were met with fierce opposition from organizations that opposed animal use for research. These organizations even proposed dog protection bills to the British House of Commons and tried to make using dogs in biomedical research illegal. Fortunately, biomedical research organizations persuaded the government to accept animal experimentation. Scientists finally developed a canine distemper vaccine and 200,000 puppies survive in Great Britain every year now. In addition, feline immunodeficiency virus (FIV) and leukemia virus (FeLV) are the main causes of cat death, and about 15% of cats were infected by FIV or FeLV. Since the successful development of vaccines by animal experimentation, cats have been protected from FIV and FeLV.

1.1.3.2 Principles of Animal Experimentation

Currently, although most laboratory animals are far removed from humans in evolutionary history, they have been of great value in animal experimentation. As tissues, organs, physiological, and metabolic characteristics of laboratory animals are similar to those of humans and other animals, the basic principles of physiology, anatomy, medicine, and surgery can be applied to all animals, including humans.

The discoveries of the genome and post-genome era have proven that any animal (even invertebrates) could become a valuable model for studying human diseases. For example, *C. elegans* is less than 1 mm in length, lives in the soil and is not similar with humans. However, it contains a 40% genome sequence homology with humans and shares 49% of the protein amino acids with humans. Through the experimental study of *C. elegans*, the key genes governing organ development and programmed cell death were discovered, and it was proven that these genes also existed in higher animals (including humans). The discoveries from *C. elegans* have opened the door to explore human cell differentiation and evolution, and have profound influences on studying the pathogenesis of many human diseases.

In the history of biological evolution, mice parted ways with humans 75 million years ago. Since the genome sequence was completed in December 2002, it has been estimated that there were 27,000–30,500 protein-coding genes in mice, and 99% genes have "matched" sequences in the human genome sequence. Studying every mouse gene exerts the most profound effect on understanding human self and human diseases.

The reactions to drugs or some chemicals are quite different between laboratory animals and humans. Animal genomes can be engineered by modern biological technology. "Humanized" animals reduce the difference with humans in certain aspects and become more appropriate models. For example, GM technology can change the drug metabolism of rodent animals and narrow the difference with humans.

In the field of biomedical research, animal experimentation has been one of the most effective methods. Routine animal experimentation being replaced by other methods is known as alternative methods.

Laboratory animals are not available for all biomedical research. Due to the restriction of ethical and moral laws and regulations, not every animal can be used in biomedical research.

1.1.3.3 Laboratory Animals and Animal Models of Human Disease

In the field of biomedical research, laboratory animals are both carriers of experimental study and models of human diseases. They can also be used to produce biological products and perform biological detection. Laboratory animals are "heroes" in military, aerospace, and many other fields. In the future, laboratory animals may not only turn into donors for human organ transplants, but may also become the "core models" of functional genomics and disease genomics.

Most laboratory animal models are created for studying the occurrence, development, and treatment of human diseases. Although this textbook focuses on the generation and application of animal models of human diseases, not all laboratory animals used in biomedical research can be considered as human disease models. However, it is difficult to distinguish animal models of human diseases from laboratory animals for biomedical research.

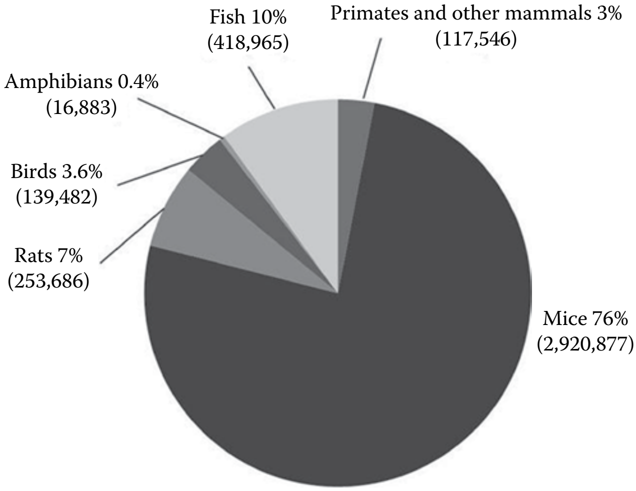

Figure 1.2 Distribution of laboratory animal species used in Great Britain, 2014.

In 2014, a total of 3.87 million animals were used in Great Britain (Figure 1.2). Of those, 1.94 million (50%) were GM animals that were not used in further procedures. The remaining 1.93 million animals (50%) were used for experimental purposes, and of these, 1.04 million (27% of the total 3.87 million animals) were used for basic research; 508,000 (13% of the total 3.87 million) were for regulatory use; 358,000 (9% of the total 3.87 million) were used for translational/applied research; and 20,000 (0.5% of the total 3.87 million) for other purposes. Of those 1.04 million animals used for basic research purposes, 89% (924,000 animals) were for the study of oncology or specified organ systems, 7% (78,000) were for the study of animal biology (including ethology/animal behavior), and 4% (38,000) were for other purposes. Of all the 1.04 million animals used for basic research purposes, the three most common purposes were targeted at the nervous system (23% or 235,000), the immune system (22% or 224,000), or for oncology (13% or 133,000).

Based on the above analysis and the British statistical results in 2014, if most GM animals and harmful genetic defect animals can be used to study human disease models, it can be simply presumed that about half of the laboratory animals for biomedical research served as animal models to study human diseases.

In China, 16 million animals have been used every year, and about 7 million animals were used for teaching, drugs, and chemical tests. Unlike other countries, there is a great usage of hamsters, accounting for about 30% (nearly 5 million) of the laboratory animal use. Hamsters are mainly used as biological materials to produce vaccines. Less than one-third of the laboratory animals are actually used as animal models of human disease for biomedical research.

The abovementioned statistic results simply reflect the relationship between laboratory animals for animal models of human disease and for biomedical research.

The concept, creation principles, and selection of animal models of human diseases for biomedical research will be introduced in Chapter 5.

1.1.3.4 Results of Animal Experimentation

In animal experimentation, animal reactions (animal experimental results) can be summarized with the following formula:

$$R = (A + B + C) \times (D + E) + F$$

where R represents the reaction of animals, that is, experimental processing results; A, the animal (not plant) response; and B, the different species causing different effects on experimental results. By means of gene manipulation, animal species can be transformed to influence animal experimental results; C represents the individual reaction, genetic quality control can reduce the difference between individuals; D, the influence on experimental results caused by the interaction among environmental factors, species, and individual animals; E, the stress reaction. More accurate results can be obtained through improving animal welfare and relieving animal pain or distress; F represents environmental error.

Normal animal experimental technology will be introduced in Chapter 6. Animal experimental design will be introduced in Chapter 7. The basic knowledge and skills of animal experimentation can be mastered through learning, which can lay the foundation to work in biomedical research.

1.2 HISTORY AND APPLICATION OF LABORATORY ANIMALS

1.2.1 History of Laboratory Animals

The most commonly used laboratory mice originated in Africa, while the laboratory rats originated in Central Asia. According to historical records, the history of mice bred by humans is at least 3000 years. In 1100 BC, breeding of spot mice was recorded in ancient Chinese books. In the eighteenth century, fancy mice in China and Japan spread to Europe and mated with local species. Their offspring is the main source of modern laboratory mice.

The use of mouse in scientific research has a long history. As early as 1664, Robert Hooke used mice to study the characteristics of the air. Books recording mouse color inheritance were published in Europe in the eighteenth century. In general, animal use for scientific research was far less systematic and continuous before the twentieth century.

In 1902, William Castle from Harvard University had started to breed and use mice for biomedical research. At that time, mice specially bred for research use mainly came from a farm created by Abbie Lathrop in Massachusetts. Lathrop not only sold mice to scientific research institutions, but also performed production and breeding research. She also successfully generated spontaneous tumor mouse strains.

Many of the most common inbred strains used in modern biomedical research belong to strains of mice bred by William Castle and Abbie Lathrop.

William Castle and his student Clarence Little realized the value of mice in studying Mendelism and began producing mice strains using a breeding system. Little utilized consecutive inbreeding (sibmating) and created the world's first inbred strain mice—DBA in 1909. DBA represents the coat color gene, that is, the abbreviation of dilute, brown, and non-agouti. Over the next 10 years, Clarence Little and other scientists created several famous inbred strains of mice such as A, C57BL, C3H, CBA, BALB/c, 101, 129, and AKR. In fact, the most widely used inbred mouse strains were basically created from 1920 to 1930. C57BL/6 mice generated by Clarence Little in 1921 were considered as the world recognized "standard" of laboratory inbred animals. The most commonly used inbreeding rats were also bred in this period. Most of them, such as F344, M520, Z61, A732, were bred by Maynie R. Curtis and Wilhelmina F. Dunning in the Columbia University Institute for Cancer Research. By 1930, there were 12 inbred strains of rats. Since 1906, George Rommel of the United States Department of Agriculture had started making use of guinea pigs in inbreeding studies; some of those inbred strains are still widely used.

According to the latest statistics of the International Committee on Standardized Genetic Nomenclature for Mice, there are 478 inbred mouse strains and 234 inbred rat strains (Festing: University of Leicester, UK, 1998) in the world. The inbred mouse strains TA1, 615, LIBP/1, NJS, and T739 were created in China. At present, some new inbreed strain mice and rats have been produced in the world and some strains have been gradually eliminated.

Although there are many mouse and rat strains, the commonly used strains in biomedical research only include several mouse strains such as A, AKR, BALB/c, CBA, C3H, C57BL/6, DBA/2, 129, and SJL. C57BL/6 mice have been utilized for genomic sequence analysis. Commonly used rat strains include five or six strains such as F344, LEW, LOW, SHR, and SD. Inbred strain animals that lack in special biological characteristics are rarely used in scientific research. Information about the inbred strain mice and rats in the world is available at http://www.informatics.jax.org/external/festing.

In addition to mice and rats, inbred strain hamsters, guinea pigs, rabbits, and chickens have also been successfully produced and used for biomedical research.

In 1918, China began to breed mice for research and also imported mice, rats, rabbits, and golden hamsters from abroad. Kunming mice, which were introduced from India albino mice to Kunming in 1947, have been distributed across China and have become the most widely used outbred strain mice. Up to now, to provide more choices for biomedical research, scientific and technical workers have done much work on developing Chinese characteristic laboratory animals such as the Mongolian gerbil, Chinese hamster, tree shrews, Himalayan marmot, pika, reed vole (*Microtus fortis*), green swordtail (*Xiphophorus helleri*), and miniature pigs.

1.2.2 Animal Experimentation and Biomedical Research

Laboratory animals, as human substitutes, have been developed for biomedicine. In the field of modern biomedical research, the conditions to conduct experimentation can be summarized as involving four basic elements, AEIR: A represents animals; E, equipment; I, information; and R, reagents. Animals are always the most important element.

Western medicine has mainly originated in ancient Greece. In 400 BC, *Corpus Hippocraticum*, the first medical handbook, recorded using animals to conduct experimentation. Since Claudius Galen (130–201 AD), physicist and physician of ancient Rome, used pigs, dogs, and monkeys in experimentation of medical physiology, making use of animals in medical research prevailed for several centuries. Since Christianity developed and became dominant in Rome, experimental research almost completely disappeared. Not until the Renaissance was medical research carried out, although most research was about anatomy at that time. During this period, French philosopher René Descartes (1596–1650) explained life by pure mechanical principles. He believed that having a soul or not was the difference between animals and humans. He thought animals had no soul, so they did not have consciousness. He also thought humans could think and feel pain, but animals behaved like machines without feelings. Rene theoretically supported making use of animals in research. By the eighteenth century, people gradually realized that medical experimentation was very important to improve human lifestyle and health. Thus, studies on experimental medicine began. Laboratory medicine has developed since only 300 years to modern medicine.

From the following examples, we can clearly find the contributions of experimental medicine to modern medicine. In 1878, German scientist Robert Koch (1843–1910) observed *Mycobacterium tuberculosis* and then found the relationship between bacteria and disease by studying diseases in cattle and sheep. During the end of the nineteenth century and the beginning of the twentieth century, dogs were used by Ivan Petrovich Pavlov (1849–1936), a Russian physiologist, to study digestive physiology and higher neural activity, and he put forward the concept of conditioned reflex and started the physiological research on higher neural activity. In the end of the nineteenth century, German bacteriologist Friedrich Loeffler (1852–1915) used guinea pigs and other animals to study *Corynebacterium diphtheria*. He found that bacterial toxin rather than the bacteria itself was the real cause of animal death. This helped the discovery of immune therapy, which could prevent diphtheria, and opened a new era for antibiotic therapy. In 1914, Katsusaburō Yamagiwa and Kōichi Ichikawa coated asphalt on the ears of rabbits and induced skin cancer. Further research found that 3,4-benzopyrene within asphalt was a chemical carcinogen and proved the carcinogenic effects of chemicals. French physiologist Charles Ricet (1850–1935) found from animal experimentation that the essence of allergies was antigen–antibody reactions and made large contributions to the study of allergic diseases. In 1975, Georges Köhler (1946–1995) and César Milstein (1927–2002), scientists at the University of Cambridge, successfully produced monoclonal antibodies by the hybridoma technique and brought revolutionary change to antigen identification, infectious disease diagnosis, and cancer research and treatment. This was a major breakthrough for modern biomedical research. The main materials were BALB/c mice and their passaged myeloma cells. The understanding and utilization of inbred strain mice has led to the technique of fusing myeloma cells with immunized spleen cells of BALB/c mice and monoclonal antibody technique.

There are 29.1 million diabetes patients in the United States, 415 million people have diabetes worldwide, accounting for 8.3% of the total adult population. Patients with type 1 insulin-dependent diabetes mellitus will quickly die without insulin. At the beginning of the twentieth century, Frederick Banting, Charles Best, and many Canadian scientists repeatedly used dogs and rabbits to conduct experiments. They separated, purified, and identified insulin to determine its function. Currently, more than 30 million diabetes patients in the world have been treated and have a prolonged life span through insulin injections. Patients with high blood pressure (more than 270 million cases in China alone) also benefit from animal experimental study. Scientists have invented many drugs to treat and prevent high blood pressure, stroke, and heart disease caused by high blood pressure through animal experimentation. The success of canine heart surgery and transplantation experiments made it possible to perform similar surgeries for humans. At present, radiation therapy and chemical therapy (drugs), which can both kill cancer cells, have been developed by many experiments using chicken, rats, mice, and rabbits. In the past, nausea and vomiting responses were serious side effects of cancer treatment. However, a new drug was discovered to prevent nausea and vomiting using ferret research. Without animal experimentation, it is difficult to find a good way to treat cancer.

Toxicology testing became important in the twentieth century. In the nineteenth century, laws regulating drugs were more relaxed. For example, in the United States, the government could only ban a drug after a company had been prosecuted for selling products that harmed customers. However, in response to the Elixir Sulfanilamide disaster of 1937 in which the eponymous drug killed more than 100 users, the U.S. congress passed laws that required safety testing of drugs on animals before they could be marketed. Other countries enacted similar legislation. In reaction to the Thalidomide tragedy, further laws requiring safety testing on pregnant animals before a drug could be sold were passed in the 1960s.

People still have a limited understanding of human diseases and a lot of animal experimentation is still in progress. With the increase in human longevity, many people may be subject to geriatric diseases like Parkinson's disease or cancer. The further study of acquired immune deficiency syndrome (AIDS), incurable diseases, and genetic diseases is very important. Insulin injection is not a perfect method to treat diabetes. Scientists have been conducting animal experimentation in order to find ideal ways that may help cure diabetes such as slow releasing insulin technology or tissue transplants.

1.3 USE OF LABORATORY ANIMALS CURRENTLY

According to rough estimates, there were 30 million vertebrates used in scientific research in 1960. By 1970, the number multiplied by several times and reached 100–200 million.

Great Britain is one of the countries with the most accurate statistics on laboratory animals. In 1940, only about 1 million animals were used in scientific research, but the number increased to 3.5 million in 1960. In 1971, it reached 5.6 million and remained at about 5.5 million a few years later. The number has significantly decreased since 1980 and dropped to 2.62 million in 2001, less than half of the use in 1970 (Figure 1.3). In recent years, the number of laboratory animals in Great Britain has increased. It has risen to 4.11 million in 2012, an increase of 57% from 2001. The growing number of laboratory animals brought a lot of pressure to the coalition government of Prime Minister David Cameron (2010–2016).

According to records on animal species for scientific research, more than 70% of laboratory animals are rodents. In 2000, the top five widely used laboratory animals in Great Britain were mice, rats, fish, birds, and guinea pigs, accounting for 59.2%, 19.7%, 9.0%, 4.6%, and 2.6% of the total number, respectively. In 2001, rodents accounted for 82% of animals for research in Great Britain; fish, amphibians, reptiles, and birds, 14%; small mammals (except rodents), 2.3%; large mammals, 1.3%; dogs and cats, 0.4%; and nonhuman primates, 0.1%. In 2014, the most popularly used animals were mice, fish, rats, birds, others, and specially protected species, for 60%, 14%, 12%, 7%, 6%, and 1% of the total number (Figure 1.2), respectively.

In Great Britain, since the beginning of the twentieth century to the early 1970s, the number of laboratory animals for research sharply increased and remained in a stable period until the mid- to late-1970s. The number gradually decreased after 1980 but has plateaued since 1985 (Figure 1.3).

From 1945 to 1971, the number of laboratory animals for scientific research in Great Britain elevated from 1.18 million to 5.61 million, an increase of 4.75 fold, and then declined year by year. It decreased to 3.11 million in 1986, 45% less than in 1971. The number increased slightly after 1987. The reason is that a law (*Animals* [*Scientific Procedures*] *Act*) on laboratory animal statistics was revised by the British

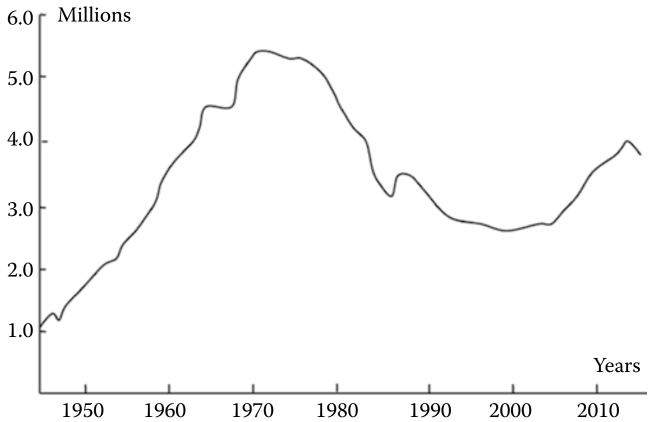

Figure 1.3 The graph of total laboratory animals used in Great Britain, 1945–2014.

government in 1986, and animals for production, treatment, and natural products, which were not counted before, were included. Therefore, the increase in statistical data does not reflect an increase in animals. In 2001, the number decreased to 2.62 million, which was the lowest. Then, it elevated year by year and reached 4.11 million in 2012, an increase of 57% from 2001. When comparing 2014 with 2013, the total of 3.87 million animals represents a decrease of 6% (Figure 1.3).

From a global perspective, the number of animals for biomedical research markedly increased in the early to mid-last century, and then has gradually fell and become relatively stable. However, the general trend is that the number of higher animals has dropped, while the number of lower animals used has increased. Traditional animal numbers have dropped, while GM animal numbers have increased. In addition, animal species have become more abundant.

Over the past 25 years, the total number of animals used in research has fallen by nearly 40% in the United States. Compared with in 1975, the use of animals in research also greatly decreased in Canada in 1992. Mouse and rat use in Japan was 11 million in 1975 and dropped to 7 million in 1989. In 2006, 1530 facilities in China used approximately 16 million laboratory animals, but the number was unknown before 2006. From 1975 to 1992, fish use has increased nearly 4 times in Canada from 1975, and mammals have been replaced by fish in many research fields (e.g., toxicology). In 2000, fish use for animal science research in Britain has increased from 122,438 to 243,019, an increase of 98% from 1999. Currently, fish substitute for rats and have become Great Britain's second most used laboratory species (Figure 1.2). The use of birds (including fertilized eggs) and ferrets has also substantially increased, but the use of primates, livestock, and dogs has been reduced.

The biological characteristics and application of laboratory animals commonly used in biomedical research will be introduced in Chapter 4.

The application areas of animal experimentation in biomedical research include improving the understanding of complex life systems, studying the cause of diseases and bettering human and animal life; looking for new diagnosis and treatment methods; producing biological products that are useful for both humans and animals (e.g., vaccines, insulin); product safety testing such as medicines, vaccines, household goods, cosmetics, food additives, etc.

1.4 MANAGEMENT OF LABORATORY ANIMALS

1.4.1 Controversy of Animal Experimentation

Animal experimentation is always an attractive topic for politicians and is also the focus of the animal welfare arguments. Currently, the real battle in developed countries exists between scientists who try to reduce the suffering and pain of animals in scientific research and extreme animal advocates such as "animal rights organizations" and "animal liberation fronts" who attempt to clamp down on animal experimentation, animal sourced foods, and even pets. In recent years, these organizations have threatened scientists who conduct animal experimentation, demolished experimental equipment through violence, destroyed experimental data, and released or stolen laboratory animals. They ignore the revolutionary progress brought by animal experimentation to both humans and animals and stubbornly believe that experimental results obtained by “mad scientists” are not reliable because animal experimentation lacks justice and impartiality.

There are also moderate organizations and individuals that oppose animal experimentation worldwide. They believe animal experimentation should be terminated because it brings extra suffering and pain to animals. People who oppose animal experimentation ignore that many people and animals are suffering from diseases that may shorten their life span. Animal experimentation may reduce suffering and pain from diseases for humans and animals, and prolong their life span. Only few research projects referred to as animal experimentation may cause obvious suffering and pain, but animals are given pain medication or anesthesia in advance. Animal pain always leads to stress, which severely interferes with the experimental results. Pain or discomfort may be caused by experimental factors, but it can be overcome by reasonable experimental design. Modern science and technology, and the latest achievements in laboratory animal medicine, may provide a new approach to further reduce and avoid pain or discomfort for animals.

Some people think that scientists have enough medical knowledge, and that there is no need to perform animal experimentation. They believe animal experimentation can be replaced by knowledge or technology such as cell culture or computer models. The fact is that we know little about diseases such as cancer, heart disease, and sudden infant death syndrome. More than 50 million people die from these diseases each year worldwide. Animal models can describe how diseases affect the human body because both human and animal bodies are very complex. Other nonanimal models only partly reflect the characteristics of diseases.

Therefore, the use of laboratory animals is helpful for the study of biomedical research, development of safe and effective drugs, and guarantees the health of human and animals. Meanwhile, it is important to emphasize animal welfare in animal experimental study.

1.4.2 Animal Welfare and Alternative Methods of Animal Experimentation

1.4.2.1 Laboratory Animal Welfare

The status of animals in philosophy, religion, and culture must be considered following the development of life ethics. Animals are no longer “automatons” without feelings. As a life form, animals also have basic living requirements and high-level psychological requirements. Animals have feelings, and are rich in emotions and abilities such as affection and love, memory, concentration and curiosity, imitation and reasoning. While coexisting with humans in an interdependent ecosystem, animals should be cared for, respected, and have their interests considered for equally, which is known as animal welfare.

Animal utilization and animal welfare are the two aspects of the unity of opposites. In terms of biomedical research, excessive requirements of animal welfare may bring a heavy economic burden to scientific research. Moreover, many animal studies have to be terminated due to different restrictions. On the contrary, ignoring unnecessary pain or discomfort of animals would go against human ethics. Therefore, real animal welfare is not only just protecting animals, but paying attention to animal welfare, merging animal welfare and animal utilization, and opposing those who perform animal experimentation by extreme methods or ways.

In animal experimental study, animal welfare has put more emphasis on animal health and comfortable living conditions. Animal welfare will worsen when the basic physiological requirements of the animal cannot be satisfied.

1.4.2.2 3Rs Theory

In the early 1950s, the number of laboratory animals for biological research markedly increased, which has drawn social public attention to animal protection and laboratory animals. The comprehensive and systematic 3Rs theory was first put forward by Zoologists William Russell and microbiologists Rex Burch in 1959. The principles of the 3Rs have subsequently become embedded in national and international legislation regulating the use of animals in scientific procedures.

3Rs is the abbreviation for Reduction, Replacement, and Refinement. More specifically, 3Rs refers to using a smaller number of animals in scientific research to acquire the same amount of experimental data, or using a certain number of animals to gain more experimental data; using other ways rather than conducting animal experimentation to achieve the same results; reducing or avoiding pain or discomfort by improving and perfecting experimental procedures to improve animal welfare.

Biomedical researchers should regard the 3Rs as a branch of biomedicine and further study 3Rs technology. The study and application of 3Rs is an opportunity rather than a threat. 3Rs is an effective approach in biomedicine and other fields. It is helpful to conduct scientific research and obtain more accurate and reliable results.

Laboratory animal welfare and the principles of the 3Rs will be specifically introduced in Chapter 2.

1.4.3 Management of Laboratory Animals

The purpose of laboratory animal management is to humanely treat animals during teaching, biomedical research, testing and other activities, guarantee animal welfare, and improve the level of biomedical research.

Great Britain is the first country that made laws to protect animals in scientific research and promulgated the world's first law of animal experimentation in 1822. The *Cruel Treatment of Cattle Act*, was one of the first pieces of animal welfare legislation. It was the first time to consider animal abuse as a crime. Although this Act was applied only to large domestic animals such as cattle, sheep, pigs, and horses, while dogs, cats, rodents, and birds were excluded, it was still a milestone in the history of animal protection and welfare.

The *Animal Welfare Act* was signed into law in 1966 by the United States, and it is the only Federal law in the United States that regulates the treatment of animals in research, exhibition, transport, and by dealers. The Act was amended 8 times. Animals covered under this Act include any live or dead cat, dog, hamster, rabbit, nonhuman primate, guinea pig, and any other warm-blooded animal determined by the Secretary of Agriculture for research, pet use, or exhibition. Excluded from the Act are birds, rats, mice, farm animals, and all cold-blooded animals.

Other laws, policies, and guidelines may include additional species coverage or specifications for animal care and use, but all refer to the *Animal Welfare Act* as the minimum acceptable standard. The *Health Research Extension Act of 1985* provides the legislative mandate for the Public Health Service (PHS) *Policy on Humane Care and Use of Laboratory Animals*. It directs the Secretary of Health and Human Services to establish guidelines for the proper care and treatment of animals used in research, and for the organization and operation of animal care committees. The Office of Laboratory Animal Welfare (OLAW) implements the PHS Policy. OLAW's responsibility for laboratory animal welfare extends beyond National Institutes of Health (NIH) to all PHS-supported activities involving animals. In Great Britain, the use of animals in scientific procedures is regulated by the *Animals (Scientific Procedures) Act 1986* (it has recently been revised to transpose *European Directive 2010/6 3/EU* on the protection of animals used for scientific purposes), an animal protection measure that requires licensing and oversight of all places, projects, and personnel involved in such work. The *Laboratory Animal Regulations* was issued by the government of China in 1988. Laboratory animal regulations or acts formulated by state/province or city governments were also included. Those laws or regulations are to be obeyed unconditionally by relevant animal producers, institutions, and researchers who perform laboratory animal production or animal experimentation. The *Guide for the Care and Use of Laboratory Animals* was edited and published by the Institute of Laboratory Animal Resources (ILAR) of National Research Council. It is imperative to abide by the *Guide for the Care and Use of Laboratory Animals* for facilities or individuals who plan to apply for relevant grants from the NIH to conduct biomedical research involving vertebrates. Many countries also reference the *Guide for the Care and Use of Laboratory Animals* to manage the work of laboratory animals.

After many years of discussion, *Directive 2010/63/EU* revising *Directive 86/609/EEC* on the protection of animals used for scientific purposes was adopted in 2010. The Directive is firmly based on the principle of the 3Rs, to replace, reduce, and refine the use of animals for scientific purposes. The scope is now wider and includes fetuses of mammalian species in their last trimester of development and cephalopods, as well as animals used for the purposes of basic research, higher education, and training.

From a legislative perspective, there is no specialized, complete law for animal protection or animal welfare in biomedical research in China. The current separate rules such as the *Law of the Protection of Wildlife* and *Animal Epidemic Prevention Law* do not refer to laboratory animals. Thus, the legal system of laboratory animal management in China should be perfected.

1.4.4 Protocol of Animal Experimentation

In accordance with the *Animal Welfare Act* and PHS policy, the Institutional Animal Care and Use Committee (IACUC) is required to oversee the responsible use of animals in university research and instructional activities as described in the PHS *Policy on Humane Care and Use of Laboratory Animals* and the *Guide for the Care and Use of Laboratory Animals*. The IACUC reviews protocols, reviews the animal care and use program, and monitors university animal facilities to ensure compliance with standards and regulatory requirements.

As mandated by the PHS policy, membership of the IACUC will comprise at least five members including the following: one veterinarian with training or experience in laboratory animal science and medicine; one practicing scientist experienced in research with animals; one member whose primary concerns are in a nonscientific area (e.g., ethicist, lawyer); one member who is not affiliated with the institution other than as a member of the IACUC. In addition, IACUC should submit annual summary and evaluation reports to the OLAW.

All animal experimentation should be reviewed and approved by IACUC before study performance. Studies submitted to the IACUC for review must comprise a completed application form, including appropriate signatures. In general, projects of conventional animal experimentation, which use common laboratory animals, can be easily approved by IACUC. However, if the project requires wild animals, higher animals, special GM animals, or nonhuman primates, IACUC will discuss carefully and may submit it to the higher IACUC, or even to national authorities for approval. In Great Britain, all living vertebrates and octopi used in scientific research are protected by the *Animals (Scientific Procedures) Act 1986*, but invertebrates (e.g., *Drosophila*, nematodes, etc.) are not covered. The utilization of wild animals for biomedical research is forbidden in Britain unless the project is approved by the Home Office. Therefore, the government bans animal experimentation using Pongidae.

The study on animal experimentation reflects the complexity of life activities. From the perspective of scientific research, accurate research conclusions are based on the similarities between human model animals and humans. However, these kinds of animals are usually in the public eye and some people vehemently oppose using these animals for experimental research.

The organization and management of animal experimentation will be reviewed and summarized in Chapter 8.

1.4.5 Laboratory Animal Science Organization

Many countries have established laboratory animal science associations, societies, or other scientific organizations, and some of them have become outstanding and influential international organizations. The American Association for Laboratory Animal Science (AALAS) (http://www.aalas.org) is an association of professionals that advances responsible laboratory animal care and uses it to benefit people and animals. AALAS provides educational materials to all laboratory animal care professionals and researchers, and administers certification programs for laboratory animal technicians (LATs) and managers. The Federation of European Laboratory Animal Science Associations (FELASA) (http://www.felasa.org) represents common interests in the furtherance of all aspects of laboratory animal science in Europe and beyond. FELASA focuses on exchanging Laboratory Animal Science information, optimizing experimental conditions, ensuring animals to be treated humanely and properly and promoting the development of European animal experimentation. The Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) (http://aaalac.org/) is a private, nonprofit organization that mainly focuses on promoting the humane care of animals by willingness to accept certification. Currently, over 650 companies, universities, hospitals, and research institutes, including famous institutes and organizations such as NIH and the American Red Cross, have passed the AAALAC certification. International Council for Laboratory Animal Science (ICLAS) (http://www.iclas.org/), which was established by the United Nations Educational, Scientific and Cultural Organization (UNESCO) in 1956, puts emphasis on promoting the health of humans and animals by utilizing and managing laboratory animals humanly in scientific research. The Fund for The Replacement of Animals in Medical Experiments (FRAME) (http://www.frame.org.uk) is committed to solve the problems of animal experimentation using the 3Rs. Chinese Association for Laboratory Animal Science (CALAS) was established in April 1987. CALAS, an academic corporate social group comprising laboratory animal science workers, has made great contributions to the prosperity and development of Chinese laboratory animal science.

The Jackson Laboratory (JAX) (http://www.jax.org/) is an independent, nonprofit biomedical research institution dedicated to contributing to a future of better health care based on the unique genetic makeup of each individual. The Laboratory's mission is to discover precise genomic solutions for disease and empower the global biomedical community in the shared quest to improve human health. JAX is a National Cancer Institute-designated Cancer Center and has NIH centers of excellence in aging and systems genetics. JAX is also the world's source for more than 7000 strains of GM mice, is home of the Mouse Genome Informatics database, and is an international hub for scientific courses, conferences, training, and education. JAX provided 3 million JAX mice annually for more than 20,000 investigators in at least 50 countries for research and drug discovery.

1.4.6 Laboratory Animal Technical Training

Laboratory animal technical training and qualification recognition are divided into animal breeding and animal experimentation technology in many countries.

It is required by the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and *Directive 2010/63/EU* that scientists who conduct animal experimentation be formally trained and educated to gain the professional knowledge of laboratory animal science.

According to the requirements of the Council of Europe and European Union, FELASA has designed detailed training schemes of laboratory animal science.

Based on research field, training and qualifications can be classified into four classes: A, B, C, and D. Class A is applied to persons who take care of animals. In line with master's degree and working time, class A can be subdivided into four grades: grade one, grade two, grade three, and grade four. Class B is applied to persons who carry out animal experiments; class C, to persons who are responsible for directing animal experiments; class D, to laboratory animal science specialists who design and conduct animal experimentation, have mastered enough knowledge to improve the level of animal experimentation, and use animals humanely and scientifically. Class D requires more than 80 class hours of specialized training in laboratory animal science.

AALAS has classified qualifications of LATs into three categories: assistant laboratory animal technician (ALAT), laboratory animal technician (LAT), and laboratory animal technologist (LATG). The qualification certificates can be obtained after full-time theoretical study, technical training, and passing of the examination.

Based on the *Laboratory Animal Regulations* and *Regulation on the Quality Management of Laboratory Animal*, the Chinese government has also demanded that professionals who work in laboratory animal production and animal experimental research must be trained by the provincial or municipal Laboratory Animal Management Committee, pass the exams, and obtain qualification certificates before engaging in the corresponding work. The education and training of laboratory animal science has two purposes. First, the relevant personnel accept the following views: animal experimentation is necessary for biomedical research and cannot be completely replaced by other methods; compared to the pain suffered by animals, the benefits brought by animal experimentation are very valuable; designs and implementation of animal experimentation must protect animal welfare as much as possible. Second, study of the basic theories and knowledge of laboratory animal science and mastering the basic skills of animal breeding and animal experimentation is a must.

Contents of this textbook were selected and compiled according to the above two basic beliefs. Students can master the basic theoretical knowledge and basic skills of animal experimentation after systematic learning and training. Moreover, students can achieve B and C level of FELASA and LATG level of AALAS, and obtain qualification certificates with animal experimental study.

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