others.com, for detailed information on veterinary books, visit our website www.jaypeebrothers.com, for detailed information on veterinary books

Handbook on Laboratory Animals



Second Edition

PV Desai
P Saravanan

Forewords
Suresh S Honnappagol
Ashok S Mallapur
Yasmeen A Maniyar

JAYPEE

Contents

Section 1: Common Laboratory Animals

1. **Laboratory Animals for Research Purpose** **3**
   - *Breeding and Care of Laboratory Animals 4*
2. **Laboratory Mouse** **10**
   - *Taxonomy 10*
   - *Biology 10*
   - *Reproduction and Breeding 11*
   - *Feeding 14*
   - *Husbandry 15*
   - *Disease Management 15*
   - *Research Uses 16*
3. **Laboratory Rat** **17**
   - *Taxonomy 17*
   - *Biology 18*
   - *Reproduction and Breeding 19*
   - *Feeding 20*
   - *Husbandry 20*
   - *Disease Management 20*
   - *Research Uses 21*
   - *Similarities and Differences Between Rat and Mouse 21*
4. **Guinea Pig** **23**
   - *Taxonomy 23*
   - *Biology 24*
   - *Reproduction and Breeding 25*
   - *Feeding 26*
   - *Research Uses 27*
   - *Analgesics, Anesthetics and Euthanasia in Guinea Pigs 31*
5. **Hamster** **33**
   - *Taxonomy 33*
   - *Biology and Behavior 34*
   - *Care and Breeding Management 34*
   - *Feeding 35*

- *Disease Management 36*
- *Research Uses 36*

6. Laboratory Rabbit 37

- *Taxonomy 37*
- *Biology 37*
- *Reproduction and Breeding 38*
- *Feeding 40*
- *Husbandry 40*
- *Disease Management 40*
- *Research Uses 41*

7. Sheep 42

- *Taxonomy 42*
- *Biology 42*
- *Reproduction and Breeding 42*
- *Feeding 43*
- *Husbandry 43*
- *Laboratory Procedures 43*

Section 2: Dogs and Cats

8. Care and Management of Dogs 47

- *Important Points for Care/Management of Dogs 48*
- *Breeds of Dog 48*
- *Nutrition 49*
- *Disinfection and Hygiene 49*
- *Enrichment 50*
- *Animal Transportation 51*
- *Animal Housing 52*
- *Vaccination 53*
- *Common Diseases or Problems in India 56*
- *Puppy Care 62*
- *Novel Dog Identification Systems Using Microchipping 63*
- *How to Bathe Your Dog? 66*
- *How to Trim Your Dog's Toe Nails? 68*
- *How to Brush Your Dog's Teeth? 69*
- *Importance of an Annual Physical Examination in Dogs 70*
- *Nasal Vaccination for Dogs (A Recent Trend) 72*

9. Diseases and Treatment for Dogs **74**

- *Canine Parvovirus 74*
- *Infectious Canine Hepatitis 77*
- *Canine Distemper 78*
- *Rabies in Dogs 81*
- *Canine Parainfluenza 86*
- *Leptospirosis 87*
- *Diabetes Mellitus in Dogs 90*
- *Gastrointestinal Disorders 91*
- *Hematoma of the Ear Flap 92*
- *Bacterial Infections 93*
- *Fungal Infections 94*
- *Protozoal Diseases 94*
- *Parasites in Dogs 95*

10. Cat Management **98**

- *Breeds of Cats 98*
- *Diseases and Treatment of Cats 100*
- *Antibiotics for Cats 122*
- *Cat Vaccination Schedule 123*

Section 3: Essential Feeding Requirements

11. Food and Essential Nutrient Requirements for Various Species of Laboratory Animals **129**

- *Carbohydrates 129*
- *Proteins 129*
- *Fat 130*
- *Minerals 130*
- *Vitamins 131*

12. Feeding Schedule of Different Species of Laboratory Animals **134**

- *Commercially Pelleted Feed 135*
- *Self-compounded Feed 135*
- *Conventional Natural Feed 136*

13. Feeding and Watering Devices for Laboratory Animals **137**

- *Feeding Devices 137*
- *Watering Utensils 139*

Section 4: Procedures

14. Sterilization and Disinfection Methods 143

- *Methods of Sterilization 143*
- *Service Equipment Required in Animal House Facility 146*
- *Duties of Animal House Technician 147*

15. Blood Collection in Laboratory Animals 148

- *Blood Examination 148*
- *Blood Collection 149*

16. Handling and Sexing of Laboratory Animals 154

- *Mice 154*
- *Rat 155*
- *Guinea Pig 156*
- *Rabbit 157*

17. Anesthesia in Laboratory Animals 158

- *General Anesthesia 158*
- *Inhalation Anesthetics 160*
- *Injectable Anesthetics 160*

18. Euthanasia in Experimental Animals 169

- *Choice of Euthanasia Method 170*
- *Methods for Euthanasia 170*
- *Scientific Considerations 174*
- *Location of Euthanasia and Disposal of Carcasses 175*
- *General Considerations on Techniques of Euthanasia Being Employed for Laboratory Animals 175*
- *Standard Euthanasia Methods for Commonly Used Species 182*
- *Methods Not Acceptable for Any Species of Animals 184*

Section 5: Disease Management

19. Bacterial and Viral Diseases of Laboratory Animals and their Management 189

- *Bacterial Diseases 189*
- *Viral Diseases 194*

20. Parasites and their Effects on Laboratory Animals 198

- *General Classification 199*
- *Life Cycle of Parasites 199*
- *Harmful Effects of Parasites 200*
- *Diagnosis of Parasitism 200*
- *Endoparasites of Laboratory Animals and their Control 201*

21. Understanding Mechanism of Tumor Immunology Using Nude Mouse 204

- *History and Significance 204*
- *Genetics 205*
- *Lifespan 205*
- *Research Applications 205*

Section 6: Ethical/Legal Factors

22. Ethical and Legal Provisions in Use of Laboratory Animals 209

- *Ethical Provisions 209*
- *Legal Provisions 211*

23. Role of CPCSEA and IAEC in Conducting Experiments on Animals 213

- *Controlling Agencies for Experiments on Animals 213*
- *Guidelines for Teaching Institutions to Follow Ethical Norms in Animal Use 220*

Section 7: Animal House

24. Animal House Hazards and Safety Precautions 225

- *General Hazards 225*
- *Environmental Hazards 225*
- *Infectious Hazards 226*
- *Chemical Hazards 226*

25. Budget Requirement for Setting up Laboratory Animal Facility 227

- *Animal Housing Facility and Staff (No Breeding Facility and Animals Need to be Purchased) 227*

- *Animal House for Medical Colleges (Breeding as well as Teaching and Research are Being Undertaken) 228*

Section 8: Miscellaneous

26. Hematological and Biochemical Data 233

- *Hematological and Biochemical Data of Commonly Used Laboratory Animals 233*

Index 237

Chapter

2

Laboratory Mouse

Laboratory mouse is the most widely used and convenient animal available to biomedical research scientists (Fig. 2.1). Though initially used in experimental biology (embryology, genetics and nutrition), its use spread with the advent of inbred strains. With emerging capabilities in molecular biology (particularly models of gene transfer methods) development of new transgenic mice has come to stay. Mouse is the extensively used mammalian model for several diseases causing pathogens, development of vaccines, therapeutics and gene therapy besides they are routinely used for toxicological and microbiological research.

TAXONOMY

The taxonomic position of laboratory mouse is described as belong:

- Class: Mammalian
- Subclass: Theria
- Order: Rodentia
- Genus: *Mus*
- Subgenus: *Mus*
- Species: *Mus musculus.*

BIOLOGY

Mouse is a cosmopolitan species, commensally and highly adaptable to a wide range of climatic conditions. However, they prefer drier than wetter places. The species owes



FIGURE 2.1: Laboratory mice colony with their young puppies

JAYPEE BROTHERS

its success to its frugal diet, high fecundity and short interbirth interval. They have a relatively large surface area per gram of body weight. Fluctuation in ambient temperature (22–25°C) may result in dramatic physiological changes. Also, it has greater sensitivity than most mammals to water loss. Mice have acute hearing and sense of smell. To accommodate for the high metabolic rate, mouse has a rapid respiratory rate, a short air passage, high red blood cell (RBC), hemoglobin and high blood oxygen capacity. Mice excrete only a drop or two of urine at a time, which is highly concentrated. The gastrointestinal flora (Fig. 2.2) consists of many species of bacteria that begin to colonize the alimentary canal after birth. They form an ecosystem that provides beneficial effects (production of essential vitamins and homeostasis of important physiological functions). The normal vertebral formula for the mouse is 'C7 T13 L6 S4 C28' with some variations between strains in the thoracic and lumbar regions.

REPRODUCTION AND BREEDING

The female mouse normally has five pairs of mammary glands. External influences such as noise, diet, light and population density play important role in reproduction, which in turn

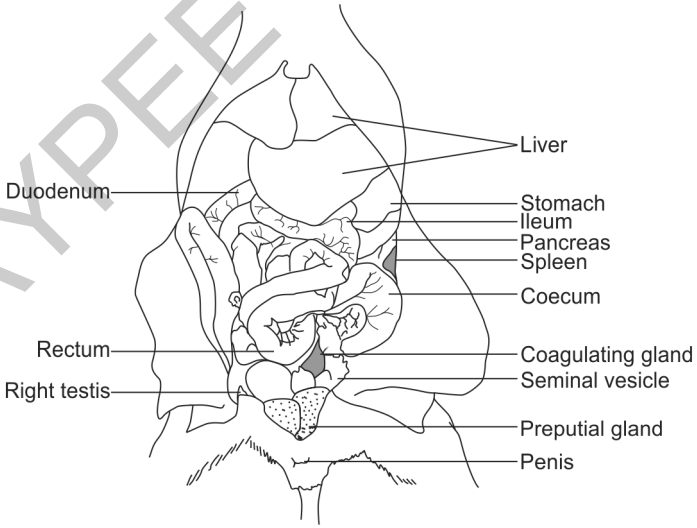


FIGURE 2.2: Abdominal visceral organs of a mouse

JAYPEE BROTHERS

directly or indirectly influence the hypothalamic pituitary axis for hormonal control of ovarian and testicular function. Genotype also influences the reproductive performance of the mouse. Sexual maturation varies among the stains and is subject to seasonal and environmental influences. The mouse is polyestrous and cycles every 4–5 days. Estrus is routinely observed in mice at about 12–24 hours after parturition (postpartum estrus). The cycles of estrus and ovulation are controlled by the diurnal rhythm of the photoperiod. Mating, estrus and ovulation most often occur during the dark phase of the photoperiod. Pheromones and social environment also affect the estrus cycle. For example, estrus is suppressed in mice housed in large group due to pseudopregnancy (Whitten effect). By contrast, pheromones from a strange male mouse, particularly of a different strain may prevent implantation or pseudopregnancy in recently breed females (Bruce effect). Estrus can be synchronized by group housing females prior to pairing with males. Group housing suppresses estrus, but exposure to male pheromones restarts the cycle and leads to estrus in most females 3 days after pairing. During successful mating vaginal plug is formed (mixture of secretions of the vesicular and coagulating glands of the male). Gestation is usually 19–21 days and because of postpartum estrus, lactation and gestation can occur simultaneously. Though, females have effective reproductive life of 1–2 years, litter size decreases as the female ages. Therefore, females are usually retired by 1 year of age. Milk production increases up to 12 days postpartum and then declines until weaning at 21 days. Some important biological, physiological and reproductive parameters of laboratory mice are described in Table 2.1.

Breeding System

Laboratory mice are identified by strain or system of breeding (Table 2.2). The breeding systems followed in mice are detailed below.

Inbred Strains

Inbred strains are produced by brother-sister mating for at least 20 generations. For all purposes they are genetically identical to other mice of the same strain and sex. The primary colony should be maintained by brother-sister mating in monogamous permanently

JAYPEE BROTHERS

Table 2.1: Biological data of laboratory mouse

Parameters	Values
Male (adult weight)	20–40 g
Female (adult weight)	18–35 g
Life span	2–3 year
Surface area	0.03–0.06 cm
Chromosome number (2n)	40
Rectal temperature	37.5°C
Respiratory rate	138 (90–180) breaths per minute
Heart rate	470 (300–650) beats per minute
Water required	3–7 mL/day
Urine excreted	1–3 mL/day
Food (pellet, concentrated)	5–8 g/day
Space per mouse, floor area	70–100 cm^2
Minimum height	13–15 cm
Room temperature	22–25°C
Relative humidity	50–55%
Ventilation changes/hour	8–12 hour
Age at pairing	6 week
Weight at pairing	18–25 g
Type of estrus cycle	Polyestrus
Length of estrus cycle	4–5 day
Duration estrus	10–20 hour
Mechanism of ovulation	Spontaneous
Time of ovulation	2–3 hour after onset of estrus
Breeding season	Round the year
Time of implantation	Early day 5
Length of gestation	19–21 day
Litter size	8 (6–12)
Weight at birth	1–3 g
Weaning age	21 day
Weaning weight	10–12 g

Return to estrus (postpartum) estrus regular cycle at end of lactation

JAYPEE BROTHERS

mated pairs, offspring's from this should be used firstly to perpetuate the primary colony and secondly to perpetuate the breeding stock for production colony. In the production colony, animals may be mated, at random (as pairs or trios) provided the breeders are derived only from inbred primary colony.

Table 2.2: Some popular inbred mice strains and their characteristics

Strains	Characteristics
AKR	High leukemia incidence, source of thymus cell antigen
BALB/c (Bagg albino)	Model for leishmaniasis, host for many transplantable tumors, also for hybridoma production
C57 BL/6	Genetically modified mice used as models of human diseases

Outbred Strains

Outbred animals are those whose genotype at any given locus is usually unknown. The objective of maintaining an outbred stock is to minimize the rate of change over a period of time. This is maintained by random mating and whenever possible, deliberate selection should be avoided.

F1 Hybrids

F1 hybrids are cross between two inbred strains. They are similar to inbred strains in that they are genetically and phenotypically uniform. Unlike the parental strains they do not breed true. Thus, they cannot be perpetuated. Apart from the above classes, other strains like congenic strains, congenic and recombinant inbred strain are available. Their breeding and maintenance are somewhat elaborate, and can be found in any good textbook dealing with laboratory animal genetics.

FEEDING

The most commonly used diets for laboratory mice are natural ingredients. These are based on one or more grains or grain byproducts to which fats, protein, vitamins and minerals are added. The source of fat is usually vegetable oil and protein sources include fish meal, and groundnut or soybean meal. Minimum protein levels necessary for maintenance are 12–14% and for reproduction 17–19%. Diet for barrier maintained animals must be decontaminated by sterilization

JAYPEE BROTHERS

or irradiation. The feed consumption would depend upon age, sex and strain, but average pellet consumption of a nutritionally balanced feed for adult mice would range from 5 to 8 g/day and clean water is provided ad libitum. Many facilities practice acidification of water to pH 2.5 for the control of microflora.

HUSBANDRY

In large facilities, work assignments are usually divided. Some people transfer mice from soiled to clean cages, other wean litter and prepare new mating. Another person may be responsible for removing cleaning of soiled cages. In small colonies one or two people may perform all the jobs necessary to maintain the mouse colony. Cages are available in a variety of sizes, materials and designs. At the SN Medical College, polypropylene cages of the size 14 cm wide 30 cm long and 12 cm deep work quite well for a pair of breeding mice and their litter. Stock animals are generally kept in larger cages. Regardless of type of materials used to construct the cage, hopper or basket for feed should be about 5 cm above the cage floor. A hopper that is integral part of cage cover is more convenient. Water can be provided from bottle attached to cage. Bottles should be of glass or polycarbonate because these can be sterilized. Paddy husk is most commonly used bedding material in Indian conditions because it is readily available, is not harmful to make, absorbs moisture and has no smell for washing. Sanitation and environmental control are available. Temperature should be in the range of 22–25°C and relative humidity is 50–55%.

DISEASE MANAGEMENT

The safeguard of health within a mouse colony is very important. The golden rule is that prevention is better than cure. Health care is considered from three aspects:

1. Prevention of conditions conducive to ill health.
2. Detection of latent disease by regular health monitoring.
3. Management of disease in the event of outbreak.

The nucleus colony should only be procured from a facility, which is known to keep animals under strict hygienic conditions, strict attention to sanitation, avoidance of overcrowding; proper husbandry and nutrition and a vigilant lookout for signs of ill health are extremely important steps. Samples of feed, bedding and dead animals should be regularly monitored for parasitological,

JAYPEE BROTHERS

pathological and microbiological examination. If the animal facility is small and monitoring laboratories absent, these samples should be sent to laboratories elsewhere. Similarly, in case of outbreaks a qualified veterinarian experienced with small animals should be consulted immediately.

RESEARCH USES

The mouse is a popular laboratory animal because of its relatively small body size, ease of handling and low cost of maintenance. Also, the large number of inbred strains and mutants that are available make this little rodent a favorite animal model of geneticists, and biomedical researchers. The short life span makes it a useful model for aging or chronic toxicological studies. It is also used in the field of immunology, transplantation studies, physiology, biochemistry, behavior, microbiology and teratology. However, the majority of mice are probably used to evaluate the safety and effectiveness of pharmaceuticals, cosmetics, and drugs.

Mouse as an Experimental Animal

Mice are employed widely in acute toxicity studies. They are also used for the assay of insulin and analgesics, the latter especially against chemically induced pain. General screening of chemotherapeutic agents is undertaken in this species. Specially bred mice are used for study of problem in genetics and in cancer research. Mice are most frequently used for the testing of drugs for teratogenicity. The development of nude mouse, a hairless genetic mutant that lacks a thymus and thus deficient in T lymphocytes, has been of fundamental interest in the field of tissue immunity, cell-mediated immunity, and transplantation research. They are less susceptible to cancer, the reason being that they possess normal or perhaps elevated number of natural killer cells that kill both tumor cells and those infected by viruses (Marx, 1980). Beige mice on the other hand, which lack killer cells, are usually susceptible to cancer. Mice are important animal models in various techniques of oncology and molecular biology.

JAYPEE BROTHERS

r detailed information on veterinary books, visit our website www.jaypeebrothers.com, for detailed information on veterinary books, visit our web

Handbook on Laboratory Animals

Salient Features

- First of its kind
- One of the rare books dealing with basics and applied information required for researchers involved in laboratory animal experiments
- Serves as guide for physicians, veterinarians and persons involved in maintaining laboratory animals in research/academic institutions and medical colleges
- Reference book for doctors, scientists, researchers and postgraduate students, while taking up research projects involving animals
- Ready reckoner for pet lovers as it covers recent and useful topics on diseases, treatment and vaccination of dogs and cats
- Ethical/legal factors in animal experimentation are explained
- Written by authors who have vast experience in handling, breeding and management of laboratory animals
- Written in a simple and lucid manner.



PV Desai BVSc is a renowned veterinarian who has vast experience in working with laboratory animals for more than 40 years. He is a highly experienced veterinary doctor who served in the Department of Animal Husbandry, Government of Karnataka, India, in various capacities from Field Veterinary Officer to Deputy Director. Later, he moved to S Nijalingappa Medical College and HSK Hospital and Research Centre, Bagalkot, Karnataka, and brought animal house facility to an excellent facility there. He has vast practical experience in laboratory animal management and breeding. Besides, he is actively involved in social activities as President and Zonal Chairman in Lions Club, Vidyagiri Development Sangha, etc. and continues to provide help and support for the needy public and animal lovers. He is a dedicated social worker with special emphasis on animal welfare and upliftment of downtrodden people.



P Saravanan BVSc MSc PhD was graduated from Madras Veterinary College, Chennai, Tamil Nadu, India, and completed Master's degree from National Dairy Research Institute, Karnal, Haryana, India, and Doctoral degree from Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India. He is working as Senior Scientist at Indian Veterinary Research Institute Campus, Bengaluru, Karnataka, with tremendous experience in scientific research involving development of novel vaccines and diagnostics for animal diseases. He has working experience of more than 15 years in large and small animal management, feeding, breeding and treatment. He serves as an expert consultant for breeding, housing, nutrition, health care and management of laboratory animals and pet animals. He serves as CPCSEA expert for inspection and nominee to approve experiments on animals. He provides scientific advice and suggestions for research scientists, veterinarians and students to design and conduct experiments on animals and creates social awareness among public on current issues concerning animal welfare for the benefit of mankind.

Available at all medical bookstores or buy online at www.jaypeebrothers.com

JAYPEE BROTHERS
Medical Publishers (P) Ltd.
www.jaypeebrothers.com

Join us on facebook.com/JaypeeMedicalPublishers

Shelving Recommendation
VETERINARY SCIENCE

ISBN 978-93-5152-952-1