A Handbook of Mouse Models of Cardiovascular Disease

Editor

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Contents

Preface

Cardiovascular disease, the principal cause of heart attack, stroke, and gangrene of the extremities, remains a major contributor to morbidity and mortality in the Western World. Many factors, such as genetic polymorphisms, hypercholesterolemia, modified lipoproteins, hypertension, diabetes mellitus, autoimmune responses, infections and smoking have been implicated in the development of cardiovascular diseases. The etiology and pathogenesis of many cardiovascular diseases have not been fully elucidated. Although clinical investigation of these diseases is an important approach for understanding their etiology and therapy, animal models are essential tools for understanding the mechanisms of the pathogenesis as well as of interventions.

Cardiovascular research with animal models, as known today, is nearly 100 years old. The use of animal models in the study of cardiovascular diseases is essential to answer many questions. For instance, evaluation of a risk factor as a single independent variable, with almost complete exclusion of other factors, can best be performed in animals free of intercurrent diseases or abnormalities and with well known genetic characteristics. Furthermore, experiments using animals are the only way to develop and test new diagnostic, preventive, and therapeutic procedures for both ethical and practical reasons. The investigator can choose the time and method, as well as obtain tissue and serum samples and other material needed for measurements under optimal conditions and selective circumstances that are difficult, if not impossible, in studies with human subjects.

Attracted by the availability of well-defined genetic systems of transgenic and knockout mice, a number of investigators have begun to use the mouse as an experimental system for arteriosclerosis research. Hundreds of inbred lines have been established, the genetic map is relatively well defined, and both congenic strains and recombinant strains are available to facilitate genetic experimentation. In just a few years, murine lipoproteins have been characterised, genetic variants of apolipoproteins identified, and genetic variation in susceptibility to atherosclerosis among inbred mouse strains demonstrated. Because inbred strains have low variability, fewer are needed for each experiment, which is also of economic benefit. The mouse is becoming a widely used model for studying all aspects of cardiovascular diseases.

Although the mouse model is widely used by many laboratories, some problems often appear due to insufficient knowledge of the specific animal models, especially technical issues. Many mouse models involve techniques of microsurgery, which cannot be performed without training following specific guidelines. Investigators who are not experienced in using mouse models need to consider several issues before starting their experiments. They need some essential information about experimental

procedures, specific instruments and technical know-how. The present handbook provides a brief background on each individual disease, describes detailed methods and materials used for establishing the mouse model, discusses the problems that may appear in the experiments, and gives some examples for applications of the model. Importantly, the movies on the accompanying CD allow researchers to learn the techniques by directly watching the whole operation. This book covers most, if not all, mouse models of cardiovascular diseases authored by experts in the research field. I believe that this book will be useful for all researchers working with mouse models in cardiovascular research.

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Mice – general information

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Abstract

The gentle and careful use of laboratory animals requires specific knowledge on the general biology and the characteristic hallmarks of the used animals and the adequate consideration of legal requirements, ethical aspects, and scientific guidelines. In this chapter, important topics of laboratory animal science regarding the mouse as laboratory animal are addressed. Initially, a brief historical overview of the mouse is given and the step from the house mouse to the worldwide actually mostly used laboratory animal species. Moreover, housing conditions of mice, maintaining, and selected genetic principles are described. Commonly used handling techniques are explained in detail. For practical benefit, adequate methods for the sampling of blood and bone marrow cells and the application of substances, in particular, the intravenous injection technique, are described. Finally, useful techniques for anesthesia, analgesia, and euthanasia are emphasized.

Keywords

mice, laboratory animal, housing, breeding, genetics, blood, injection techniques, anesthesia, analgesia, euthanasia

Historical perspective of house mice as laboratory animals

The step of the wild mouse to the worldwide most-used laboratory animal was principally caused by the usefulness of this species for different interests. Historically, mice have been used in biomedical research since the 16th century, when Robert Hooke used mice in England to study the biological consequences of increasing air pressure. Morse[1] reported in 1981 that William Harvey used mice for his fundamental studies on reproduction and blood circulation. In the 19th century several fanciers in

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Europe, the United States and Asia were breeding and exchanging in particular pet mice and rats (rarely other rodents like hamsters, guinea pigs, etc.) showing a variety of coat colour or behavioural mutations.

A booming use of mice have been reported since the 20th century in many areas of biomedical research. Mice had played and continue to play an instrumental role in several scientific fields[2], such as genetics, physiology, immunology, metabolism, pathology, oncology, cardiovascular diseases, etc. Several geneticists had created highly standardised mouse strains, whose genetic characteristics were precisely known. To retain those genetic standards, every breeding generation of mice had to be monitored by specific methods.

With a few exceptions, historical records concerning the genealogy of most laboratory inbred mouse strains are well documented and several reviews on this subject are available.[3–5] A chart[6] concerning the genealogy of these strains has been published recently and regularly updated data on the genetics, genomics, and biology of the laboratory mouse[7] are available. One of the worldwide top ranked research institutions and biggest non-profit breeding organisations of laboratory mice is The Jackson Laboratory, which was founded in 1929 in Bar Harbor (Maine, USA) by C.C. Little. The 'Jax-Lab' has played a pivotal role in the promotion of mice[8] as a very useful laboratory animal and still is a unique source of various mouse strains.[9] Several other institutions, like the Oak Ridge National Laboratory in Tennessee (USA) and the MRC Centre at Harwell in the UK have also played an important role in the development of the mouse as a laboratory model for research projects on genetics, oncology and immunology. Recently the European Union has decided to support the establishment of a network of genetic repositories (the so-called European Mouse Mutant Archive or EMMA[10]), with major breeding institutions in Italy (EMMA Headquarters is in Monterotondo, near Roma), in the UK (MRC-Mammalian Genetics Unit, Harwell), in France (CNRS-Centre de Distribution, de Typage et d'Archivage Animal, Orléans-la-Source), in Portugal (FCG-Instituto Gulbenkian de Ciência, Oeiras), and in Germany (GSF-Institute for Experimental Genetics, Munich). Finally, more recently, Japan has established a Bioresource Center at the RIKEN[11] in Tsukuba.

In addition, well established commercial breeding institutions (e.g. Charles River Laboratories, Harlan-Winkelmann, Taconic, etc.) provide laboratory animals worldwide. Those companies also offer special services to scientists, such as cryoconservation, re-derivations of contaminated and infected animals, respectively, health monitoring programs, isolator- and barrier-housing, immunisation protocols for antibody production, and the service of mouse hotels, if limitations of space, technical equipment, adequate hygienic conditions, and trained staff exist in the basic animal house.

Various genetic backgrounds of laboratory mice are available to achieve the envisaged goal of a scientific analysis,[12] e.g. outbred, inbred, hybrid, congenic, etc. In particular, the use of inbred strains of mice has offered investigations at the genomic level, because they can be achieved with a high level of refinement and can be correlated in a very reliable way to the phenotype of the living animal. It can be affirmed that the new types of mouse strains became of expanding interests for the

biomedical research programmes. Scientists predict that mice will be more useful for scientific research that it has been over the last centuries, especially due to the mouse genome project[13] with its contributions worldwide.

In addition, numerous transgenic mouse models were developed to study human diseases, and it is nearly impossible to depict all of them. Animal models are used for numerous diseases of man, e.g. genetic diseases, infectious diseases (parasites, fungi, bacteria, viruses, prion diseases), apoptosis research, oncology, aging, Alzheimer's disease, immunology and xenografting, reproduction research and endocrine disorders, and cardiovascular diseases. Using hereditary, experimentally induced, and transgenic mouse models, specific (patho-)mechanisms and characteristics of diseases can be better understood and reveal a more detailed and mostly superior insight into complex structures and functions, and even allows to define new therapeutic strategies and drugs capable of protecting humans against diseases. Especially transgenic mice are an essential tool to study human diseases, and this methodology is expected to be even more extensively used in the coming decades.

For the understanding of complex correlations in the pathogenesis of cardiovascular diseases, in particular to elucidate pathomechanisms of atherosclerosis, the use of adequate mouse models increased during the past two decades.[14–21] However, some authors had estimated the small size of rodents[22] as impedimentary to be used in cardiovascular research.

Maintaining and breeding of mice

Housing conditions of mice includes several parameters, which can influence growth and development as well as well-being and social behaviour of mice. A well-organised colony management and exactly followed animal care regulations[23] are essential prerequisites for animal experiments of high quality and reproducibility. In general, national and international laws and guidelines for the care and use of laboratory animals exist in line with animal welfare regulations. For details, see relevant textbooks,[24–25] national laws and international conventions. Several recommendations on proper housing conditions of mice[24,26–28] have been published. These guidelines refer to requirements on ventilation, temperature, humidity, light, noise levels, health status, feeding, water supply, animal enclosures, handling and experimentation procedures, including anesthesia, analgesia and euthanasia.

The environmental requirements of mice are summarized in Table 1.1. The standard procedures have to be strictly performed by a specifically educated and well-trained staff.[29] Such standard procedures are (i) daily monitoring of animals for adequate environmental conditions and general health, (ii) food and water control, (iii) regular changes of cages and bedding, (iv) cleaning and sterilisation programs of cages, racks, cage covers, filter hoods, water bottles, and other equipment, and (v) sanitation programmes.

Genetic monitoring of mice is important to analyse mutations and differential fixations of alleles in inbred strains. Phenotypic differences detected among substrains have been shown to be caused by genetic factors. The techniques for

Table 1.1 Environmental requirements of mice

Temperature	20–24°C
Relative humidity	$50 \pm 10\%$
Ventilation (air exchange per h)	8–20 (in IVC cages 30–80)
Photoperiod (light-dark-rhythm)	12/12 or 14/10 hrs
Light intensity	60–400 lx
Noise	$\approx$50 to $\leq$85 dB
Water intake	5–8 ml per day
Food intake	4–8 g per day

See also Fox et al.,[55] GV-SOLAS[26]
From: Hedrich HJ, Mossmann H, Nicklas W. Housing and Maintenance. In: Hedrich HJ, Bullock G, eds. The Handbook of Experimental Animals – The Laboratory Mouse, Amsterdam: Elsevier, 2004:395–408. With permission of Elsevier publishers.

genotyping have to be adjusted to the specific needs of an institution or a group, and may depend on laboratory equipment, maintenance conditions, and the scientific purpose of such analyses. The determination of a genetic profile allows to distinguish among different strains maintained in one animal unit. In general, this genetic profile is composed of monogenetic polymorphic markers which may be further differentiated by biochemical, immunological, morphological, cytogenetic, and DNA markers. Those classic markers have been almost fully replaced in routine applications by the microsatellite marker technique. A huge number of primer pairs is available and can be purchased from companies worldwide.

Laboratory animals have to be housed under specific environmental conditions, which prevent animals from infections by microbial agents, and established the status of 'specific pathogen-free' (SPF) animals. Locks and showers were commonly used for personnel, autoclaving for food, water, materials, and equipment, and mice were maintained in technically adapted (positive pressure, filtered air, etc.) and regularly disinfected animal rooms. During the last decade, filter cabinets, microisolator cages and individually ventilated cage (IVC)-systems have been increasingly used in laboratory animal facilities. Those new cage types offer the advantage of separating small populations from each other and are also frequently used for housing immunocompromised or infected mice. The transmission of infectious agents can be efficiently prevented by such cage systems, which must be considered as self-contained microbiological entities. In IVC systems, every single cage has its individual route for air supply and air exhaust. The rate of ventilation is much more higher in IVCs than in conventional open cages and allows some extension of the cage-changing interval. The major effect of filter cabinets, microisolator cages, and IVCs is to erect an effective hygienic barrier between the animals and their environment, which can provide a better microbiological status of animals used. However, a well-trained staff[29] is needed to run those cage systems effectively. Because of the higher expenditure of time for concomitant work, e.g. using clean benches with laminar flow conditions, manipulation of animal cages under sterile environment, marking of animals, and care and management of animals in breeding procedures, the number of mice per animal technician is

definitely reduced to about 50–70% compared to the hitherto existing open housing systems.

Mouse genetics

The management of breeding colonies of mice requires specific knowledge on the goals of inbred and outbred populations. The objectives for 'outbred' animals are to ensure that the population remains constant in all characteristics for as many generations as possible to maintain the highest level of heterozygosity and to preserve the population's original allelic forms and frequencies as stable as possible over generations. In contrast, for 'inbred' strains efforts are focused on the preservation of isogeneity and a maximum inbreeding coefficient.[4] To achieve the goal of homogenous populations, the main three causes of divergence have to be avoided: genetic contamination, mutations, and residual heterozygosity. Several factors can affect the genetic characteristics of inbred and outbred populations,[30] e.g. the population size, the type of mating scheme, spontaneous mutations, and (positive and negative) selection procedures.

Outbred stocks are characterized by maximum genetic variability (polymorphism) in the population, avoidance of the introduction of new allelic forms, and minimisation of increasing inbreeding from generation to generation by using breeding partners, which are preferably not related to each another. Usually, outbred populations have large numbers of permanent breeding pairs (sometimes more than 200 pairs), no selection criteria (i.e. loss of allelic forms), high interval between generations (to slow down genetic drift), and closed mating populations (no introduction of new breeders).

A different mating scheme is used for inbred strains. Inbred strains are defined as those derived from 20 or more consecutive sister $\times$ brother matings. The main goal of inbreeding is a maximum level of homozygosity at virtually all of the loci, which entails a genetic and phenotypic uniformity. This uniformity reduces the number of mice, which have to be used for experimental approaches, because experimental variability in phenotype is limited to variations in epigenetic, extragenetic, and/or varying uncontrolled environmental factors. However, inbred animals are usually characterised by a lower degree of robustness for infectious agents and environmental factors, a decreased fecundity (fertility rate, litter size, number of weaned pups, etc.), and relatively higher costs than outbred animals. In addition, specific phenotypes can be expressed or even inability to live because of homozygosity for a certain recessive trait[31] and/or lethal gene(s).

The crossing of two inbred strains generates hybrid mice, which are genetically and phenotypically uniform like inbred mice. F_1 hybrids display an overall hybrid vigor, i.e. increased resistance to diseases, better survival under stress situations, greater natural longevity, higher numbers of litter, and are therefore useful as recipients of tissue transplants from mice of either parental strain. Crossings of $F_1 \times F_1$ result in the F_2 progeny, in which alleles of the two parental strains segregate following Mendelian principles of inheritance.

Special breed of inbred lines are recombinant inbred (RI) and congenic strains. RI strains are derived by systematic inbreeding from a cross of two different inbred strains, recombinant congenic (RC) strains are established by backcrossing of F_1 hybrids to mice of one of the parental lines[32,33] (for details see also Chapter 2). Congenic strains are inbred strains carrying a mutant gene or 'foreign' polymorphic allele from a different strain or stock and are expected to be identical at virtually all loci except for the transferred locus ('locus of interest') and a linked segment of the chromosome. A strain is considered congenic after 10 generations of backcrossing to a recipient inbred strain (N_{10}). Using marker-assisted technologies, congenic strains can be established in <10 generations, creating so-called 'speed congenics', from which DNA microsatellite markers are extensively mapped.[7,34]

With regards to cardiovascular research, mice of the C57BL/6J strain are most susceptible for hyperlipidemia-induced atherosclerosis. However, it is known that inbred mice of another inbred strains, for example C3H/HeJ, BALB/cJ and A/J are not sensitive to a cholesterol diet and atherosclerotic lesions cannot be induced by feeding of cholesterol-enriched diets. Concerning the injury models, data indicate that neointimal lesions vary between different strains, but differ from diet-induced atherosclerosis, suggesting that injury-induced neointimal hyperplasia and diet-induced atherosclerosis are controlled by distinct sets of genes; the former appeared to be determined by recessive genes at minimally two loci.[35] Neointimal lesions in vein isografts between C57BL/6J and BALB/c strains revealed no significant difference in either inflammatory responses or the thickness of lesions, suggesting there is less effect of genetics on vein graft models. For transplant arteriosclerosis, different major histocompatibility complex class II antigens between donors and recipients are needed, e.g. between C57BL/6J and BALB/c mice. Thus, careful selection of the model with different genetic background for such experiments is essential for the successful performance of the study.[21]

During the last two decades genetically engineered strains of mice were created by 'transgenesis' and 'targeted mutagenesis'. Transgenic mice have genetic material randomly added to their genome, whereas knockout and knockin mice are produced by the gene targeting technique using mouse embryonic stem (ES) cells. Gene targeting replaces the gene sequence resident in the mouse genome by means of homologous recombination of a related sequence that has been modified in the laboratory to contain a mutation.

Thousands of transgenic mouse strains have been used to study gene function and expression and have resulted[7] in many important disease models. The phenotype of a knockout mouse provides important clues about the gene's normal role. One major application of this technology is the modelling of human diseases caused by loss of gene function. Such knockout mouse models are useful tools to investigate the biochemical and physiological aspects of diseases. Interesting examples of knockout mice for transplant arteriosclerosis research are animals, which lack gene function for interferon-gamma,[36] nitric oxide synthase (NOS),[37] P-selectin,[38] intercellular adhesion molecule-1 (ICAM-1),[17,38] and Apo E.[39]

Blood and bone marrow collection methods

For different analyses in hematology, immunology and similar disciplines, the collection of blood samples[40] is necessary. In this chapter, the scientifically accepted blood collection methods from mice are described. Blood collection techniques should be performed with a minimum of tissue trauma and thus a minimum of pain and suffer to the animals. Methods that enable blood sampling directly from a vessel or plexus are preferred to those that may cause more tissue trauma. The following sites are commonly used for blood collection in mice: orbital sinus, tail vein, heart, aorta, and vena cava.[41] Depending on the site that is used for blood collection, either a terminal procedure (heart, aorta, vena cava, jugular vein) or a survival procedure (orbital sinus, tail vein) is applied. Terminal blood collection procedures must be performed in anesthetised mice only.[12]

Proper handling techniques are necessary for blood sampling from orbital sinus or from the tail vein. After removal from the cage by grasping the animal's tail and, if required, identification of mice, the restraining can be performed either by an immobilisation device or by fixing a skin fold at the rear of the neck with thumb and forefinger. For the collection of blood samples from the orbital sinus an adequate sedation or a brief anesthesia is recommended. For routine hematology, the collected blood should be immediately placed in a tube to let the blood clot for serum extraction. In the case that anticoagulation should be achieved to separate plasma from cell compartments, EDTA (ethylenediamine tetra-acetic acid), heparin, sodium citrate (3.8 per cent) and others are valuable anticoagulants.[41]

For qualitative and quantitative analyses, bone marrow cells are used for smears and counting to evaluate for relative proportions, maturity of precursor cells, storage pools, and other changes. Among the feasible methods used to prepare bone marrow cells are cytospin preparations, paint brush smears and squash preparations.[41] Most commonly, the femur (or other appropriate long bone) is cracked and bone marrow material is either rinsed from the marrow cavity using sterile medium and a 22-gauge $(0.7 \times 30\ \text{mm})$ or 23-gauge needle $(0.6 \times 25\ \text{mm})$, or a slightly moistened paint-brush is introduced into the marrow cavity to pick up a small amount of bone marrow, or bone marrow cells are harvested by centrifugation of the cracked bone in a 0.6 ml centrifugation tube and adequate medium.

Anesthesia and Analgesia

Anesthesia and analgesia protocols require specific knowledge of the mouse physiology and is a real challenge to the mouse anesthetist. The three major 'pillars' of an appropriate anesthesia are adequate analgesia, sedation/hypnosis, and relaxation of skeletal muscles. Hence, an expertly performed anesthesia needs special knowledge and experience. The choice for the used anesthetic regimen is particularly affected by the aim of the scheduled study, the age, sex, and size of the animal species, and the

advantages and disadvantages of various anesthetic drugs. Anesthetics can be administered by injection or by inhalation (and via the route of tank water, if animals are aquatic residents). For major surgery and other long-term procedures a combination of injectable anesthetics with an inhalant anesthetic may be considered.[42]

Several problems can occur in association with the small body size of mice, e.g. the higher surface area relative to body mass, increased sensitivity to blood loss entailing cardiovascular failure, and the lack of intra-anesthetic monitoring. In addition, strain, sex-, and age-dependent variability in the effective dose of some injectable anesthetics may result in inadequate depth of anesthesia or even in a lethal overdose.[42]

For larger animals a fasting period of 12–24 hours is usually emphasized in the preanesthetic phase. In contrast, it is undesirable to withhold food and water before anesthetising mice.[43] For anticholinergic premedication mice should be treated with atropine (0.04 mg/kg, subcutaneously)[44] about 30 minutes prior the induction of anesthesia.

However, anesthetics are usually injected into mice because of easy administration by the subcutaneous (Figure 1.1), intraperitoneal (Figure 1.2), or intramuscular (Figure 1.3) routes. Drug absorption is slow via those routes, and it needs a couple of minutes until the anesthetic drug(s) provide(s) an adequate anesthesia. Rapid

Figure 1.1 Subcutaneous injection. Several locations can be used for subcutaneous application of substances. One example is shown here: After an adequately restraint, the mouse is injected into the skin fold between the knee joint and abdomen. Recommended injection volume is 0.1 to 0.5 ml. *Note*: do not damage one of the dugs (mamma), which can be clearly recognized in the picture. In male mice and sometimes in females the dugs cannot be clearly seen. Another location for subcutaneous injection is the neck region (near or between the fingers of the experimenter, who restraint the mouse) and in the hip region of the mouse. In principle, each location can be used, which is capable for administering substances under the skin. For a color version of this figure, please see the images supplied on the accompanying CD

Figure 1.2 Intraperitoneal injection. Administration of substances into the peritoneal cavity of mice is a frequently used technique. The mouse is properly restraint by grasping the animal's skin in the neck region, and turned the abdominal site upwards. The right injection location can be found as follows: an imaginary line runs between the *processus xyphoideus* (end of the sternum) to the cranial edge of the pubic bone (*os pubis*). This distance is cut in half, and this particular site is used for the i.p. injection. To avoid injuries of the intestine, liver, and of other abdominal organs by the needle, it is important to keep the head of the mouse deeper that the abdominal/pelvic region. The needle is carefully placed at the above described site and inserted in an angle of about 50–60 degree to keep the way through the various skin layers as short as possible (a minimum number of sensitive nerves should be irritated by the needlepoint). The recommended injection volume is 0.1 to 0.5 ml. For a color version of this figure, please see the images supplied on the accompanying CD

absorption, which entails a nearly simultaneous anesthetic effect can be achieved by intravenous application (Figure 1.4).

For the performance of intravenous injections mice are first placed under a heating lamp for about 10 minutes to warm the animals' body. Overheating of mice must be avoided by permanent careful observation. Unless the mice show increasing activity for self-grooming, expressed by distinct wiping movements of the forepaws over face and snout, mice are placed in a restriction device made from plastic or glass. The free movement of the body is restricted, the experimenter has free access to the tail, where the veins can be well seen due to their heat-induced dilatation. On the dorsal site as well as at the left and right lateral site the three tail veins can be observed, if the warming procedure of the mouse has been properly performed (Figure 1.5). At the ventral site of the tail the artery runs along the tail to the tip and should not be used for intravenous applications. One of the three tail veins can be punctured using a 30-gauge needle $(0.3 \times 13\,\text{mm})$. It is emphasized to insert the needle into the vein lumen in an angle of $5–10^{\circ}$, meaning nearly parallel to the surface of the tail skin. The

Figure 1.3 Intramuscular injection. Because of the small muscle mass of mice, the intramuscular application of substances is not a very common technique used in mice. In principle, parts of the *M. glutaeus* are used to be injected. The mouse has to be restraint as described. The appropriate muscles are found between the ischium and the popliteal region. The needle is inserted into the *M. glutaeus* and the injection is performed. The recommended injection volumes should not exceed 0.05 ml for an adult mouse.
Note: take care not to injure the ischiatic and femoralis nerve or the femoralis blood vessels with the needletip. For a color version of this figure, please see the images supplied on the accompanying CD

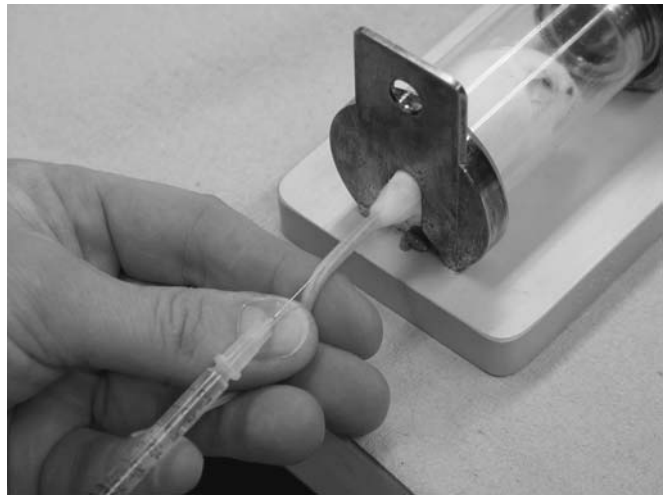

Figure 1.4 Intravenous injection. Before a intravenous injection mice are warmed by a heating lamp for about 10 minutes to warm the animal's body. Tail veins became distinctly visible and can be punctured using a 30-gauge needle. The needle must be inserted into the vein lumen in an angle of 5–10°, nearly parallel to the surface of the tail skin. Because of the application the blood in the vein lumen is displaced and thus indicate the proper placement of the needle in the vessel lumen. Recommended volume for intravenous application into adult mice is 0.05 to 0.15 ml. The intravenous injection technique must be properly educated and trained to ensure successful intravenous injections in mice. For a color version of this figure, please see the images supplied on the accompanying CD

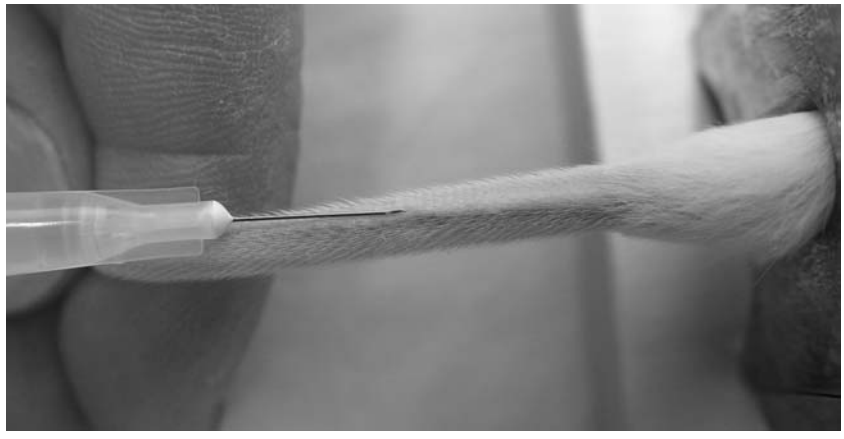

Figure 1.5 'Mouse tail vein'. This picture clearly shows the course of the dorsal tail vein, which can be used for the intravenous injection technique. For a color version of this figure, please see the images supplied on the accompanying CD

application of drugs must be performed slowly and displacement of blood by the injected fluid assure that the injection is properly placed in the vessel lumen. Adequate education and training is required to conduct intravenous injections in mice successfully.

Recommended injection volumes[44–45] range from 0.1 to 0.5 ml for intraperitoneal and subcutaneous applications, from 0.05 to 0.15 ml for intravenous application and should not exceed 0.05 ml for intramuscular injection to an adult mouse. Young mice and animals with a lower body weight should receive reduced injection volumes accordingly.

A recommendable anesthesia can be achieved by intraperitoneal injection of medetomidine (0.5 mg/kg), midazolam (5 mg/kg) and fentanyl (0.05 mg/kg), because those drugs can be neutralized by specific antagonists (atipamezole (2.5 mg/kg), flumazenil 0.5 mg/kg, and naloxone (1.2 mg/kg), subcutaneous application). Optimised and safe intraperitoneal injection anesthesia in mice was analysed by Arras[46] by using combinations of dissociative anesthetics (ketamine, tiletamine), α_2-agonists (xylazine, medetomidine), and/or sedatives (acepromazine, azaperone, zolazepam).

Alternatively, volatile anesthetic drugs, such as enflurane, isoflurane, sevoflurane, etc. are widely used for inhalation anesthesia for mice because of the better controllability on the anesthetic depth. Those volatile agents are applied by a specific device, which vaporises the liquid substances by higher pressure. For the initiation of anesthesia the volatile agent is conducted into a chamber, in which the animal had been placed. Anesthesia is continued by inhalation of the anesthetic vapor via fitting face masks. As an easily manageable system syringes can be used from which the pistols had been removed. For mice, aged >6 weeks, 5 ml-syringes can serve as face masks, for older mice 10 ml-syringes are commonly used. The major advantages of inhalation anesthesia are rapid onset of analgesia, proper sedation, adequate relaxation, and a precise adjustment of the anesthetic depth and period. In larger animal species a tube is inserted into the trachea[45] allowing artificial respiration in case of apnea. Table 1.2 summarizes the use of anesthetics and tranquilizers in mice.

Table 1.2 Anesthetics and tranquilizers used in mice

Drug	Dosage (mg/kg)	Comments	References
α-Chloralose	114 i.p.	5% solution, only in combination with analgetics and/or other anesthetic agents	Windholz[56]
Alphaxolone/alphadolone (Saffan, Althesin)	10–20 i.v.	Unpredictable anesthetic effect following i.p., volume too large for i.m.	Green,[44] Flecknell[43]
Chloral hydrate	60–90 i.p. 370–400 i.p.	Light surgical anesthesia Considerable strain differences	Green[44] Flecknell[43]
Fentanyl/fluanisone (Hypnorm)	0.4 ml/kg i.m.	Muscle rigidity, pronounced respiratory depression, 1:10 dilution	Flecknell[43]
Hypnorm/midazolam	10 ml/kg i.p.	2 parts *water for injection* + 1 part Hypnorm + 1 part midazolam (5 mg/ml)	Flecknell[43]
Fentany/droperidol (Innovar Vet)	0.5 i.m.	Irritant, tissue necrosis, self-trauma following i.m. application	Flecknell[43]
Ketamine	80–100 i.m. 100–200 i.m.	Sedation, muscle	Green[44] Flecknell[43]
Methohexital (Brevital, Brevimytal)	10 i.v. 44 i.p.	Short-term anesthesia	Flecknell[43] Dörr[57]
Medomitate/fentanyl	60/ 0,06 s.c.		Green,[44] Flecknell[43]
Pentobarbital (Nembutal, Vetanarcol)	45 i.p. 50 i.p. 60 i.p.	1:10 dilution, narrow safety margin, marked strain differences in response, severe respiratory depression	Flecknell[43] Erhardt[58] Zeller,[59] Koizumi[60]
Propofol (Rapinovet, Diprivan)	26 i.v. 30 i.v.	Short-term anesthesia, i.v. injection required	Flecknell[43] Koizumi[60]
Thiopental (Penthotal, Trapanal)	30 i.v.	Short-term anesthesia, i.v. injection required, dose dependent hypothermia and respiratory depression	Flecknell[43]
Tiletamine/zolazepam (Telazol)	40 i.p. 80–100 i.p.		Flecknell[43] Silverman[61]
Tribromethanol (Avertin)	125–300 i.p. 240 i.p.	1.2% solution, possible peritonitis, serositis	Flecknell[43] Zeller[59]

i.p., intraperitoneally; i.m., intramuscularly; i.v., intravenously; s.c., subcutaneously.

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