CHAPTER 4

Guidelines for the Use and Care of Small Laboratory Animals in Transplantation Research

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Introduction

In many cases biomedical research requires the use of experimental animals. Investigators must deal with animal welfare issues which have not only scientific but also ethical, legal, and social implications. Although the availability and development of viable alternatives, for example, mathematical models, computer simulation, and in vitro biological systems, are increasing, in many cases live animal research in biosciences currently has no acceptable alternative. This is the case particularly in transplantation research because of the complex interactions involved in whole animal systems.

Investigators have moral obligations towards their experimental animals [1]. The scientific purpose of the research should be of sufficient potential significance to justify the use of animals. Several models for the ethical evaluation of animal experiments have been developed to help the scientist to consider the ethical justification of his project [2–4]. Experimental procedures that induce pain, suffering, or distress in the animals require greater justification and surveillance.

Animal welfare acts (e.g., directive 86/609/EEC for the protection of vertebrate animals used for experimental and other scientific purposes, 1986; national laws; guidelines, e.g., [5]) and results of laboratory animal science must be considered for the acquisition, care, housing, and use of animals. Scientists are obliged to use only appropriate species for research, reduce the number of animals needed, minimize pain, distress and suffering, and prefer alternatives to the use of live animals. To ensure experimental standard and comparability physical and microbial environments of kept animals must be stable and their stability must be monitored. The description of the local methods of experimental animal housing, care and choice of species is an essential constituent of scientific publications [6].

Investigators must be familiar with the behavioral and biological characteristics of their animal subjects. They must be able to distinguish between normal, species-specific behaviors and unusual behaviors that could be indicative of health problems.

This chapter summarizes guidelines and recommendations for the housing, care, and use of small laboratory animals with regard to standardization, quality control, and animal welfare.

W. Timmermann et al. (eds.), *Organtransplantation in Rats and Mice*

Biological Data of Mice and Rats

As an essential prerequisite for the responsible use of animals in biomedical research the investigator must acquaint himself with the biology and ethology of the intended species. Only then can he judge the suitability of the chosen species or strain, and recognize signs of suffering or illness soon enough.

Mice and rats (order, *Rodentia*) are today the most frequently used laboratory animals. This is not least a result of their extremely high ability to adapt to new and different environments. The laboratory mouse (*Mus musculus*) is descended from the European house mouse and was domesticated only approx. 150 years ago. House mice exist in family groups dominated by a male. The territory and its boundaries are marked by the dominating male with urine. Mice are active during the night and spend the day in community nests. Competing mature males fight with each other until the inferior animal flees from the territory. Because of their competitive and aggressive behavior, mature males should generally not be housed together.

The laboratory rat is descended from the brown rat (*Rattus norvegicus*), which is widespread throughout the world. Rats have been domesticated and bred for about 100 years. They are very social animals and live in groups

Table 1. Reference values for the biology and physiology of mice and rats

	Rat	Mouse
Adult weight (g)		
Male	300–500	20–40
Female	250–300	25–40
Life span (years)	2–3	1–2
Age at sexual maturity (weeks)	12–16	8–10
Weaning (days)	21	21
Heart rate (/min)	300–500	300–800
Blood pressure (mmHg)		
Systolic	116	133–160
Diastolic	90	102–110
Respiration rate (/min)	70–110	100–200
Body temperature (°C)	37.5–38.5	36.5–38
Blood volume (ml/100g)	5–7	6–8
PCV (%)	36–48	39–49
RBC (10^{12}/l)	7.2–9.6	7.7–12.5
Hgb (g/dl)	14–20	10–17
MCV (fl)	48–70	41–49
WBC (×1000)	6–17	5–12
platelets/µl (×1000)	500–1300	160–410
TP (g/dl)	5.6–7.6	3.5–7.2
Alb (g/dl)	3.8–4.8	2.5–3
Glucose (mg/dl)	134–219	124–262
Na^+ (mEq/l)	140–150	140–160
K^+ (mEq/l)	4.3–5.6	5–7.5

with clear hierarchical structure. Although rank fights occur between the animals, the winner accepts the subjection of the loser and allow him to live on in the same group. Rats use community nests for sleeping, as do mice; however, the pups are raised only by the own mother in a separate nest. They are omnivorous and nocturnal.

Table 1 summarized several reference values for the biology and physiology of mice and rats [7–9]. Individual values may vary depending on strain, age, environment, and microbial status. Species- and strain-specific biochemical data are given in an extensive listing in [10].

Physiological and biochemical data vary considerably when housing conditions are modified or a change of location occurs. To achieve a new steady state laboratory animals should be allowed to acclimate to new housing conditions for a least 7–14 days [7, 11, 12]. Furthermore, laboratory animals should as far as possible be obtained from the same breeder. In spite of all efforts at standardization the biotic and abiotic factors of animal housing (the ecology of the animal facility) differ between breeders, and the use of their animals in the same project may therefore distinctly increase the variance of results.

Housing Conditions

The housing conditions of laboratory animals must meet their species-specific needs and contribute to their health and comfort. These ethical demands confront a high degree of standardization, which currently is achieved in the housing of mice and rats and entails the extensive reduction in structure

Table 2. Minimum recommendations for space and climate for laboratory mice and rats

	Mouse	Rat
Minimum cage floor required		
Individual housing (cm^2)	200	350
Each additional animal (cm^2)	60–150	120–350
cm^2/g body weight	3	1
Maximal group size (depending on body size)		
cage type II (360 cm^2)	3–6	Unsuited
cage type III (810 cm^2)	5–10	2–4
cage type IV (1800 cm^2)	10–15[a]	5–10
Cage height (cm)	12	19
Ventilation (changes of room air/h)	15–20	10–15
Relative humidity (%)	55±10	55±10
Temperature (°C)	22±2	22±2
Intensity of light, maximum (lux)	300	300
Usually adjusted light:dark cycle (h)	12:12	12:12
Noise level [dB (A)]	<50	<50

[a] Hierarchical structure in groups of more than ten individuals leads to instability and social stress.

and variability in housing systems. Standardization serves to reduce intraindividual variation in experimental results, and the condition of housing is one of the main reasons for variation. Several sets of regulations and guidelines have been developed to reconcile ethical and standardization demands[5, 13].

Usually mice and rats are housed in transparent macrolon cages with a wire cover made of stainless steel. Coarse-grained sawdust is used as bedding material. Paper tissues should be offered as nesting material. Food is administered in the form of pellets; water and food both are given ad libitum. With the exception of mature male mice, mice and rats should be housed in small groups, unless the experimental conditions require the separation of individuals. Group size depends upon cage size. Minimum recommendations for space and climate for laboratory mice and rats are given in Table 2. The specifications given in Table 2 prescribe housing conditions appropriate to the kept species. Further influences of the environment, in particular through penetrating wild animals (insects, wild strains of mice and rats), and micro-organisms, must be reduced as far as possible by suitable organizational and structural measures. Several animal housing systems have been developed, starting from the germ-free animal keeping in isolators (axenic animals) via intermediate barrier systems to the conventional animal keeping which is open for exchange with the environment [14, 15].

In the most cases biomedical research uses animals certified as specified pathogen free (SPF). These SPF animals are reared within barrier systems where specific micro-organisms or parasites known to be pathogenic to the animals or to influence experimental results [16, 17] are excluded.

Health Monitoring and Quality Assurance

In spite of standardization efforts experimental animals show variations concerning their whole phenotype. It is well known that the microbial environment in the animal facility may greatly influence experimental results. Therefore good scientific methodology seeks to control the microbial environment and to monitor animal health. For animal disease diagnosis and the analysis of animal health currently immunoassay techniques (e.g., enzyme-linked immunosorbent assay) or polymerase chain reactions are used.

Microbial agents may cause overt disease in laboratory rodents, but more often they alter research data through changes in biological responses at the cellular level without changing animal health. In addition, infectious diseases may be transmitted to the employees (zoonosis). Aspects of biosafety will even increase in importance when xenotransplantation becomes introduced as clinical application, and transmission of known or unknown pathogens to human patients may occur. Currently the risk to employees of developing allergy to laboratory animals is even higher than the risk of zoonotic diseases [18, 19]. To ensure both animal quality and personal safety against biological hazards several barrier facility systems and production techniques have been developed to produce rodents free of unwanted micro-organisms and to reduce direct contact between animals and employees.

Table 3. Prevalent infectious agents among populations of laboratory mice and rats (from [23])

	Mouse	Rat
Viruses	Mouse hepatitis virus Sendai virus Pneumonia virus of mice Minute virus of mice	Sendai virus Pneumonia virus of mice Kilham rat virus Rat coronavirus
Bacteria	*Mycoplasma pulmonis* *Pseudomonas* sp. *Pasteurella pneumotropica*	*Mycoplasma pulmonis* *Pseudomonas* sp. *Pasteurella pneumotropica*
Parasites	*Entamoeba muris* *Spironucleus muris* Mites	*Entamoeba muris* *Spironucleus muris* *Tritrichomonas* sp.

The effectiveness of the used barrier system and the quality of the produced animals must be monitored by serological, bacteriological, parasitological, and pathological examinations at regular time intervals. Measures of hygiene [20] for facilities, materials, and personnel must be adjusted to the results of these controls. For animal facilities only certified disinfectants, for example, by the German Veterinary Society (Deutsche Veterinärmedizinische Gesellschaft) [21], should be used.

Currently SPF animals are generally used in biomedical research. SPF standards are confirmed when the above examinations show no evidence of certain micro-organisms which are known to induce overt disease and/or alter biological responses. Since it is difficult to define the term "pathogen free," a specific list of organisms to be excluded from small laboratory animals has been defined to fulfill the status as SPF [22]. Mouse hepatitis virus, Sendai virus, sialodacryoadenitis virus, Kilham rat virus, lymphocytic choriomeningitis virus, *Mycoplasma pulmonis*, *Salmonella* sp., and *Citrobacter freundii* 4280 are examples of agents with known disease-causing potential and known interference with research. Table 3 lists the most prevalent infectious agents among populations of laboratory mice and rats (after [23]).

Opportunistic pathogens have a low potential for causing diseases. Usually they are associated with animals or humans. Organisms such as *Klebsiella pneumoniae, Pasteurella pneumotropica, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus pneumoniae* may induce diseases in animals whose immune response is disturbed. Immune deficiency may be inherited (e.g., nude mice and rats, SCID mice), experimentally induced, concomitant with a performed operation (e.g., transplantation), or accompany a previous infection caused by an obligate pathogen.

The goal of a routine health-monitoring program is to detect the presence of disease-causing agents in a sample of the housed population. The Federation of European Laboratory Animal Science Associations [22] has defined a standard list of pathogenic organisms to be excluded from laboratory animal facilities. If the screening for infectious agents provides evidence of the specific pathogen, measures must be carried out to eliminate this pathogen and to rehabilitate the population.

An important component of this program is the histopathological examination of animals that died spontaneously.

Assessing the frequency of screening and the number of samples at each screening interval depends upon the size of the housed population and the morbidity of an infection. The aim is to detect the presence of a pathogen in at least one individual of the monitored population. However, aspects of practicability and costs must also be considered. Experience shows that the probability of detecting an infectious organism is greater than 95 % when 8–10 animals out of 100 are analyzed [24] at least every 3 months.

In the case of viruses and several bacteria evidence for their presence is acquired by serological screening for their antibodies. Immune response of an animal and spreading of the infectious agent throughout the population are time-consuming processes. Therefore up to 6 weeks may elapse until a new pathogen can be detected in only a small number of sampled animals. Immunologically deficient or very young animals which cannot develop antibodies are obviously unsuited for health monitoring. In these cases and in order to spare valuable animals sentinels can be used. Sentinels are conspecific animals housed together with the population of animals to be monitored. Sentinels usually are exbreeders, the conventional origin of a transgenic strain, or simply individuals that are not used for experimental purposes.

Sampling for bacteriological and parasitological analyses should be carried out in the same manner as described above. Detailed methods are described in [24].

Recognition of Pain and Distress

Experimentation on animals may cause pain, injury, suffering, and distress in the animals. It is the humanitarian duty of the responsible investigator to minimize pain and distress. While pain and injuries are easily recognizable, suffering and distress also represent types of prepathological states. These may result from pain and are difficult to recognize. Nevertheless, suffering and distress may considerably influence experimental results. Assessments of pain and suffering in animals by an investigator are very often inaccurate because they are based on subjective and anthropomorphic assumptions [25]. To detect the species-specific signs of pain and suffering one must be familiar with the normal behavior, appearance, physiology, and anatomy of the animal. The absence of components of the normal species-specific behavior indicates the existence of pain or suffering. In mice and rats typical behavioral signs of pain or suffering include [2, 26–28]:

Behavior

- Protecting the painful area
- Licking, biting, scratching, or shaking the painful area
- Squealing when taken into the hand
- Automutilation
- Unusual aggressiveness, biting of group members or investigator
- Restlessness, lack of mobility

- Lack of normal interest in surroundings, reduced vigilance, apathy
- Failure to groom, withdrawal from the group

Appearance
- Abnormal postures
- Bulged back, contracted or flabby abdominal wall
- Distended stomach
- Stilted walking as a sign of stomach
- Staring coat, piloerection, ungroomed appearance
- Sunken eyes
- Pale or blue skin at ears, nose or feet (anemia, icterus)

Physiology
- Dehydration
- Urine or feces altered in volume, color, or consistency
- Increase of body weight (ascites!), weight loss, hollow flanks
- Respiratory sounds, panting
- Animal feels cold
- Signs of paralysis
- Tremor, convulsions
- Traces of blood at orifices of the body
- Red and swollen wounds, edema

It is an essential constituent of the entire planning of a project to define criteria for termination of experiments that could cause severe or chronic pain and suffering in laboratory animals. Such experiments must have a human end point, that is, to take measures to prevent the unnecessary prolongation of pain or suffering. Experience has shown that animal models for transplantation research can be associated with greatly varying strains on the animals. For example, if cells or organs are to be implanted subcutaneously or to the heterologous position without having a physiological function for the recipient, the animal's welfare will only moderately be affected (e.g., abdominal heart, islet cell transplants beneath the renal capsule). The replacement of an animal's organs by those of a donor must be assumed to evoke severe distress and suffering depending on whether and when the physiological function is achieved again, and whether the immune response will lead to a rejection of the transplanted organ (e.g., transplantations of heart, liver, small intestine, induction of a graft-versus-host reaction). In these cases precautions must be taken to ensure frequent controls and medical care for the animals, and mandatory criteria must be defined for the timely termination of the experiment to shorten severe suffering of an animal.

If any of the above features are observed simultaneously, or severe signs of pain and distress develop, the animal should be excluded from the experiment. For example, the human end point of an experiment should be set when:

- Body weight decreases more than 20 % of the animal's initial weight.
- Ascites is visible (abdominal distension).
- Diameter of tumors increases more than 2cm.
- The animals feel cold, and legs or abdominal skin are darkly discolored because of circulation disturbance.

- Animals show persistent tremors or convulsions.
- Paralysis or convulsions prevent the animals from uptake of water and food.
- Animals show bleeding wounds from automutilation.
- Prolonged shallow and labored breathing is observed.

Analgesics (see below) should be administered immediately when:
- The animal repeatedly licks, bites, or scratches a certain area of its body.
- Automutilation occurs.
- The animal shows bulged back, contracted abdominal wall, or stilted walking
- The animal squeals when taken into the hand.

Analgesia, Anesthesia, and Euthanasia

Analgesia, anesthesia, and euthanasia are measures to reduce or eliminate sensitivity to surgical or otherwise unavoidable painful procedures. The use of these measures must be balanced with regard to the planned interventions or treatments and must be matched among one another.

Preoperative Care

Before an animal is anesthetized, its health status must be checked. In particular, pulmonary and cardiac function must not be impaired. A preoperative withdrawal of food is not necessary in small laboratory rodents. If stomach and intestine must be empty prior to surgical operation, the animals may be fed on liquid food.

To provide a stress-free and anxiety-free animal for the induction of anesthesia, tranquilizers and sedatives are commonly used. However, these substances can in no way replace habituation of the animal to the specific laboratory environment by careful handling by the involved personnel.

Further, preanesthetics are usually given to alleviate some of the undesired side effects (e.g., depression of cardiac function and respiration) and/or to decrease the required dose of the primary anesthetics. Premedication should provide good tranquilization, analgesia, and muscle relaxation. Atropine, acepromazine, xylazine, and diazepam are commonly used in rodents (see Table 4).

General Anesthesia

The aim of anesthesia is to prevent the perception of pain without depression of physiological functions. A number of stages and planes of unconsciousness can be distinguished while carrying out anesthesia [29]:

- Stage I: a state of light analgesia is reached.
- Stage II: excitement, e.g., struggling and uncontrolled movements can still be seen. Therefore, this state must be overcome rapidly.
- Stage III: the level of surgical tolerance is reached. This stage is further divided into planes.
  - Plane 1: loss of the eyelid reflex.

Table 4. Proposed regimens of anesthesia and analgesia [7, 28–31]

	Mouse	Rat
Premedication		
Atropine (mg/kg s.c./i.m.)	0.05	0.05
Diazepam (mg/kg i.p.)	5	2.5–5
Acepromazine (mg/kg s.c.)	2.5	2.5
Xylazine (mg/kg)	5 (i.p.)	10 (s.c.)
Anesthesia (5–10 min)		
Propofol (mg/kg i.v.)	26	10
Thiopentone (mg/kg i.v.)	25–50	20–30
Methohexitone (mg/kg i.v.)	6	10
Anesthesia (20–60 min)		
Ketamine/xylazine (mg/kg)	100/5 (i.p./i.m.)	100/5 (i.m.)
Pentobarbitone (mg/kg i.p.)	40–60	40–55
Anesthesia (inhalation)		
Ether	5 %–15 %	
Halothane	4 %	
Isoflurane	2.5 %–4 %	
Methoxyflurane	3 %–4 %	
Analgesia		
Aspirin (mg/kg p.o., 4 hourly)	120–300	100–120
Buprenorphine (mg/kg s.c., 12 hourly)	2	0.1–0.5
Paracetamol (mg/kg p.o., 4 hourly)	300	100–300

  - Plane 2: eyeball movements cease. Animals respire deep and regular. Usually this is the optimal level for surgical operations.
  - Plane 3: central physiological functions deteriorate. Artificial ventilation becomes essential at this plane.
- Stage IV: this stage is reached if anesthesia is overdosed. It is characterized by total loss of respiratory movements (asphyxia), cyanosis, and finally cardiac arrest. It is therefore called the "toxic stage."

Anesthetics may be given via injection (i.v., s.c., i.p., i.m.) or inhalation. Injectable anesthetic agents are easy to administer, are inexpensive, and do not require elaborate equipment; in most cases spontaneous respiration is preserved. Usually they are combined with preanesthetics and/or analgesics. The major disadvantage is that once the drug is given, the depth and duration of anesthesia are difficult to control or to adjust to changing experimental conditions. Ketamine, pentobarbital (slow but long-acting), thiopental (fast but short-acting) and propofol (for injection or infusion) are examples for most common injectable anesthetic drugs in rodents (see Table 4).

Anesthesia by inhalation techniques can be rapidly induced, its depth can be easily adjusted, and recovery is also rapid. In mice and rats face masks are generally used to administer the inhalation agent. Intubation and/or artificial respiration in these small rodents is required only if long-lasting operations are planned, or a very deep anesthesia with a risk of respiratory depression is

needed. For intubation the animals are initially injected with, for example, an ultrashort-acting barbiturate. When using gaseous anesthetic agents such as ether or halothane, safety of the exposed personnel must be ensured by eliminating waste gases. In small rodents commonly used gaseous anesthetic agents include ether, halothane, isoflurane and methoxyflurane (see Table 4).

Muscle relaxants (pancuronium) are seldom necessary in small rodents. Administering muscle relaxants without general anesthesia during surgery is unacceptable since the animals stay fully conscious and sensitive to pain, while most of the reflexes used to control the depth of anesthesia are inactivated.

More detailed information of analgesics and anesthetics are given in [27, 28, 30, 31].

Intraoperative Care, Monitoring of Anesthesia

During anesthesia small laboratory animals are subjected to hypothermia, fluid loss, and metabolic depression. Anesthetized small animals should lie on a heating pad which should be controlled to 37 °C. Losses of fluid must be adequately replaced by warmed physiological saline solution. In cases requiring long-lasting anesthesia of mice or rats the i. v. infusion of glucose is very helpful to preserve physiological functions.

The effort required to control efficacy and depth of anesthesia in animals is less demanding than in humans, mostly from financial and practicability reasons. As a contribution to the protection of animals, improving the assessment of depth of anesthesia in laboratory animals could significantly reduce intraoperative mortality, which because of a lack of control is much higher in animals than in humans [32]. Since anesthetic measures are a strain for the organism, the aim of good anesthesia must be to balance surgical tolerance at the lightest level of anesthesia. To judge the depth of anesthesia one must combine supervising the animal's responses to stimulation of different reflexes with the monitoring of heart rate, blood pressure, etc. (see below):

- Righting reflex. This is usually the first reflex lost. It may be checked by turning the animal over on its back. If an animal can right itself obviously it is not at a surgical level of anesthesia.
- Loss of laryngeal reflex. The loss of this reflex is a prerequisite for placement of an endotracheal tube for facilitated or artificial respiration.
- Eyelid reflex. A slight touch of the eyelashes cause flutter of the eyelid if the reflex is present.
- Pedal or paw pinch reflex. Pinching of the animals toe between a forceps provokes a withdrawal response if this reflex is present. Its absence indicates that surgical depth of anesthesia is reached.
- Pinching the ear can be used in addition to or instead of the paw pinch reflex, especially in rats.
- Pupil width is a rather uncertain criterion since it can indicate either very light anesthesia and the perception of pain or dangerously deep anesthesia.

Methods for the technical monitoring of anesthesia and vital functions in the animal are:

- Palpation or measurement of frequency of heart beat
- Electrocardiogram
- Noninvasive or invasive blood flow or blood pressure monitor
- Oxygenation of blood, peripheral perfusion (color of skin; color of mucous membranes; oxygen analyzer)
- Acid-base status
- Observation of breathing frequency, blood gas monitoring
- Control of body temperature

Anesthetic Emergencies

Human errors are the main cause of anesthetic emergencies. Mistakes occur with inappropriate selection of anesthetic agents or their doses, too late recognition of respiratory or circulatory failure, or with the use of unhealthy animals.

Respiratory failure may be caused by barbiturate overdose or airway obstruction, for example, by secretion or aspiration of fluids. Gasping, heavy movements of the chest, and cyanosis of ears, tongue, and feet are symptoms of respiratory failure in mice and rats. When respiratory failure occurs, the administration of anesthetics must be stopped and artificial ventilation applied, with the addition of oxygen if required.

As with respiratory failure, cardiac failure is indicated by white or cyanotic mucous membranes and extremities. Blood in arteries is no longer pulsative, and heart beat is hardly or not palpable. Steps to stabilize circulation include discontinuation of anesthetic administration, adequate i.v. fluid replacement, administration of oxygen, and chest massage.

Postoperative Care

Postoperative care including the use of analgesics and antibiotics should be provided to minimize discomfort and to prevent infection and other detrimental consequences of anesthesia and the experimental procedure.

During recovery from anesthesia the animals must be observed. They need to be kept warm. Bedding material must be taken away to avoid inhalation of bedding particles and dust, especially when a tracheal tube is still installed.

Analgesics may be given to minimize pain from surgical wounds (see Table 4). Normally small animals recover (absence of pain) from even severe surgical operation within 72h.

Euthanasia

Euthanasia, the act of killing animals, must be carried out in consideration of animal welfare. Only approved methods and techniques that cause rapid unconsciousness and death without pain or distress are to be applied.

Euthanasia is indicated and justified if it is a constituent of the experimental planning, or if the animal is in a state of severe distress or severe pain that is not essential to the intention of the research and cannot be eased by analgesics, sedatives, or other treatments. Experimental planning and protocols should include criteria for initiating euthanasia (see "Recognition of Pain and Distress") to ensure that a human end point of the experiment is not neglected.

Applied methods of euthanasia must be fast, reliable, without pain or distress for the animals, and with regards to the personnel they must be safe and emotionally acceptable.

If an animal is to be killed, other conspecifics should not be present, because vocalization and release of pheromones by the animal may alarm them.

Euthanasia must be performed by experienced personnel with practical knowledge of the supposed method. Prior to disposal the death of the animal must always be confirmed by the cessation of the species specific vital signs.

Recommended methods for the euthanasia of mice and rats are [28]:

- Overdose of inhalation anesthesia: halothane, isoflurane, or methoxyflurane
- Intraperitoneal injection of overdosed pentobarbital.
- Inhalation of CO_2
- Cervical dislocation (elongation of cervical vertebrae); only mice and small rats (150g)
- Decapitation
- Intracardial or intravenous injection of T61 in anesthetized individuals

Blood Collection

Removal of blood from laboratory animals is a very common practice, for example, for collecting experimental data and for monitoring the animals' health. Nevertheless, the site and the method of blood collection and the physiological consequences of blood withdrawal may adversely affect the animals.

Common sites for blood collection in mice and rats are tail vein, amputation of tail tip, saphenous and subclavian veins (only rats), and orbital sinus [9]. Cardiac puncture is allowed for terminal blood withdrawal without recovery of the animal. Blood collection via orbital sinus and cardiac puncture require anesthesia.

The circulating blood volume in rats and mice amounts to about 7 %/kg body weight (see Table 1). During a single blood draw a maximum of 1 % of the animal's body weight – 10 %–15 % of total blood volume – can be removed. If experimental conditions require more frequent samplings, a maximum of 1 % the circulating blood volume can be removed daily [9].

Animals replace the loss of blood constituents at an average rate of 1 ml/kg per day. Therefore an animal needs a period of 10–14 days to recover from this

blood loss. Blood withdrawals at shorter intervals cause anemia. Generally blood should not be taken from animals showing a hematocrit of less than 35 % or a hemoglobin concentration of less than 10 g/dl.

At the end of blood withdrawal the animal must have achieved complete hemostasis before it is returned to its cage. Hemostasis can be accelerated by pressing sterile gauze onto the collection site.

Terminal bleeding is allowed only on animals under full general anesthesia. As a general rule, half of an animal's blood volume (10 % of its body weight) can be obtained when the animal is bled out (bled out volume=5 % of the animal's body weight). At the end of terminal blood withdrawal and before disposing the animal the investigator must verify its death.

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