CHAPTER 31

Handling and Restraint

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Introduction

The use of the mouse in biomedical research can be traced back to the 1600s and since then this species has contributed to a vast number of scientific findings and to progress in basic biological and pharmaceutical research (Grieder and Strandberg, 2003). Nowadays, an enormous number of different inbred and outbred mouse strains, including genetically modified mouse lines are available and used in research laboratories worldwide. Within the scope of experimental work with rodents, mice are not necessarily regarded as a species with a strong drive to cooperate. Despite the long time of selective breeding in captivity, the natural behavioural pattern of the wild mouse – although less prevalent – still persists. Normally laboratory mice have to be 'involved' by means of professional handling/restraint in order to perform all procedures necessary during husbandry and/or experimentation. Nevertheless, mice (with strain differences) are usually not very aggressive and can be handled or restrained without major problems. Correct handling should not only be imperative during experimental work, but should already start at breeding sites and be continued as part of daily husbandry procedures in order to familiarize the animals with people and manipulations.

Although little scientific information on handling and restraint of mice is available, many general text books touch upon the technical approach of this topic. (Cunliffe-Beamer, 1983; Anderson and Edney, 1991; Biological Council, 1992; Harkness and Wagner, 1995; Hrapkiewicz *et al.*, 1998; Wolfensohn and Lloyd, 1998; Baumans, 1999; Suckow *et al.*, 2001; Pekow and Baumans, 2003). This chapter compiles comprehensive information about handling and restraint, including the personal experience of the authors with this species.

Occupational health and risks

Injuries

Work with laboratory mice does not usually bear the risk of severe injuries. Still, minor injuries through mouse bites, mainly into fingers, may occur, especially if the staff is not very experienced and/or adequate protective measures are not properly applied when mice are handled and restrained. Such events can be traced back to the fact that mice are extremely fast in their

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movements and usually tend to escape or defend themselves if they are given the opportunity to do so. In addition to proper handling and restraint, the wearing of single layered synthetic hypo-allergenic gloves, or preferentially a double layer of both, cotton and synthetic gloves have considerable potential to reduce the number of mouse bites that perforate gloves and intact skin at the same time. Initial reluctance by staff to the use of double gloves is overcome eventually because it can increase comfort and well being of people handling and restraining animals.

Human infection and disease

Most purpose-bred laboratory mice from defined sources are specified pathogen free and their hygiene status should undergo repeated microbiological testing during housing and experimentation (*Baker, 1998; FELASA Working Group on Health Monitoring of Rodent and Rabbit Colonies*, 2002). Despite those precautions, the laboratory mouse and its excretions still harbour the potential to transmit opportunistic agents and cause human disease. Infection of skin scratches and bite wounds with mouse or human-borne opportunistic microbes demands attention through a strict occupational medical treatment program (National Research Council, 1997). Immediate cleansing and disinfection of the wound represents the first step in order to prevent infection. Special attention should be given to mice infected with human-pathogenic or zoonotic agents or genetically modified mice that harbour receptors for human pathogens. Both cases require work in higher biosafety level containments, additional screening methods and special guidelines for the handling and restraint of such animals. Tumour cells that are implanted into mice should be microbiologically screened for human and mouse pathogens and excluded if found positive before injection.

Allergies

A more severe problem in people working with mice has been observed for more than 25 years: the development of a human allergy to mice. This phenomenon, also called 'Laboratory Animal Allergy' (LAA), is a form of occupational allergic disease and includes a great number of laboratory animal species to which people may develop allergic reactions. After the phase of sensitization, resulting from complex processes within the immune system, allergy occurs and usually is represented by nasal symptoms (e.g. sneezing, watery discharge etc.) eye reactions or skin rashes. Asthma and, rarely, bite related anaphylaxis, a life threatening allergic reaction may occur. The level of exposure to the laboratory animal allergen is crucial to the nature and intensity of the symptoms (Bush, 2001). In mice, the major allergen is MUP (Mus m 1) the major urinary protein which is a prealbumin and may be found in urine as well as in hair follicles and dander. As the level of production of this protein within the liver is testosterone dependent, it is predominant in adult male mice. The second mouse allergen Mus m 2 is a glycoprotein, found in hair and dander and the third one is albumin, a serum protein. Mouse allergens can be distributed and found throughout an animal facility and even spread into separate buildings adjacent to the facility. Although the wide distribution of particles may also cause problems of sensitization and allergy to people not directly working with mice, the highest exposure to the allergens has been reported in people dealing with cage cleaning and feeding of the animals (Wood, 2001). In order to reduce exposure to mouse allergens and prevent LAA, the following personal protection measures like (a) reducing skin contact with animal products such as urine, dander and serum by using long-necked, non-allergic gloves, laboratory coats and adequate respiratory protective equipment, (b) avoidance of wearing street clothes while working with animals as well as (c) leaving work clothes at the workplace, should be taken. Furthermore, processes and procedures in animal husbandry and handling can be adapted, for example, directing airflow away from workers, performing manipulations within ventilated hoods where possible, installing ventilated animal cage racks or filter-top cages, using absorbent pads for bedding etc. (Harrison, 2001). It has been shown that the combined use of ventilated micro-isolators, ventilated cage change stations, ventilated benches for procedures and robotics for automatic cage emptying and cleaning, together with the use of a centralized vacuum cleaning system, resulted in considerably lower exposure levels to allergens (Thulin *et al.*, 2002).

Definitions

Handling

Handling within this context is defined as dealing with a mouse by hands, in a direct or indirect way – with or without touching the animal. Handling should always be done in a species-specific, calm and firm way in order not to harm the animal and to provide as much safety as possible to the experimenter. In order to reduce the stressful component of any handling procedure to a minimum for both parties, the personnel involved should be dedicated to animals, motivated and well trained. Aims of training are attainment of sovereign handling skills as well as habituation of animals to people and manipulations with as little disturbance of their physical and psychological well being as possible. In the best case, animals can even be motivated to cooperate with their trainers, a fact which does not only facilitate work and enhance safety for people but also helps to reduce stress-induced changes in physiological parameters in animals under experimental conditions.

Restraint

Restraint is described as immobilization of an animal by keeping it or parts of it, in a comfortable but safe hold by hand or by means of a physical device. Physical restraint is performed on conscious animals undergoing manipulations, which do not require sedation or anaesthesia, but necessitate exact positioning of the animal as well as prevention of unexpected movements during the manipulation. Restraining measures therefore are indispensable for the performance of experimental work as they not only facilitate avoidance of injuries in animals but also provide an adequate level of safety to the participating members of staff. In instances where unacceptable stress or pain may occur to the animal, physical restraining measures may be facilitated by sedation or by general anaesthesia and analgesia of an animal, respectively.

Handling of mice

Despite the general non-aggressiveness of the laboratory mouse, only marginal success can be expected regarding cooperation even after weeks of training. Therefore, reinforced individual cooperative training has not become common practice when handling mice. Handling is generally restricted to individual or group transfer from cage to cage during cage change or to transfer of animals from and to the experimental environment. As with other species, hectic and jerky movements should be avoided. Time should be given to animals to investigate the handler's hand and become adapted to the smell of the gloves.

Transfer of groups of mice

By hand

Small groups of mice, often sitting together in a corner of the cage, can be surrounded from two sides with the palms of both hands cupped. Without exerting any pressure, the hands are then slid towards each other beneath the mice and the whole group is lifted up and transferred to, for example, another cage, where they are gently put back on to the bottom (Figures 31.1 and 31.2). This method is very effective when animals are not trained and/or the transfer must be time-efficient.

By means of a device

Another way of transferring groups of mice or individuals, is by using a glass or synthetic bowl. The vessel is brought close to the mice with its open end directed

Figure 31.1 Grasping a group of C57BL/6 mice.

Figure 31.2 Carrying a group of C57BL/6 mice in the cupped hand.

towards the cage wall. Mice can then be encouraged to climb into the beaker (Figures 31.3 and 31.4). A reel may also serve as means of transfer, as mice like to crawl into the dark tube or climb onto the device. They can be placed back by allowing them to climb freely from the device into the cage (Figure 31.5).

Transfer of single mice

By hand

For a short transfer of less than 2–3 s, mice are gripped by the base of the tail, lifted up and carried to the new destination. This does not apply to very heavy, obese or pregnant mice, which have to be supported by the other hand. The tail remains held by one hand in order to prevent the animal from escaping (Figure 31.6). In case of transferring mice over a longer distance, they should be placed on the hand and must not be carried by the tail. Otherwise the overlying skin of the tail may become detached from the body due to the force exerted on it. Again mice are put back into the cage gently. After weighing for example, they can be released directly from the scales pan into the cage.

By means of a device

In case of special hygienic precaution requirements (e.g. specific pathogen free (SPF) or immuno-compromised animals) where exposure of the animals to potential pathogens and opportunistic microbes should be kept to a minimum, mice can also be transferred by means of a pair of forceps (25–30 cm long, with rubber protected tips). The loose skin at the rear of the neck (neck fold) is grasped with the forceps. In order not to harm the animal, it is approached from behind with the forceps and carefully lifted (Figure 31.7). The animal is released gently by opening the forceps after putting it on to the bottom of the new cage. This method mimics the behaviour of a mouse pup carried by its mother by

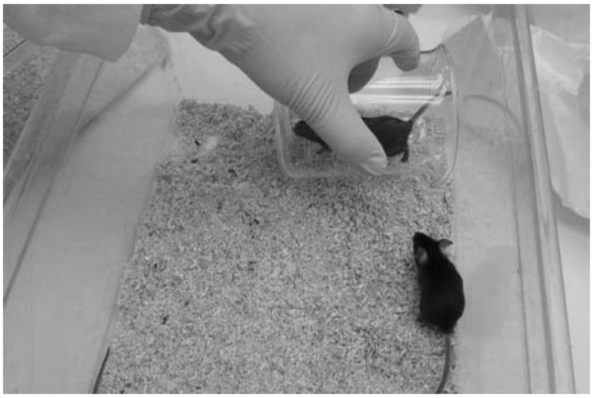


Figure 31.3 Climbing of a C57BL/6 mouse into a glass beaker.

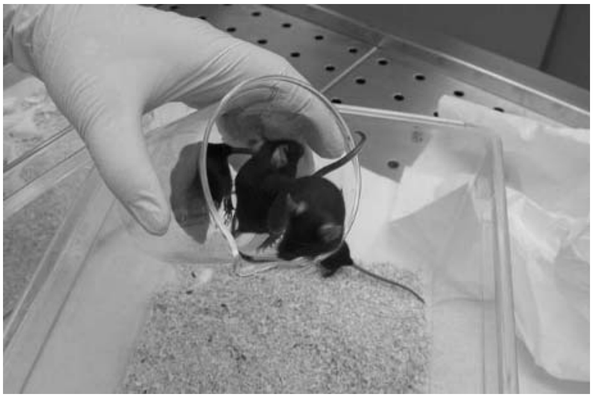


Figure 31.4 Transferring a group of C57BL/6 mice within a glass beaker.

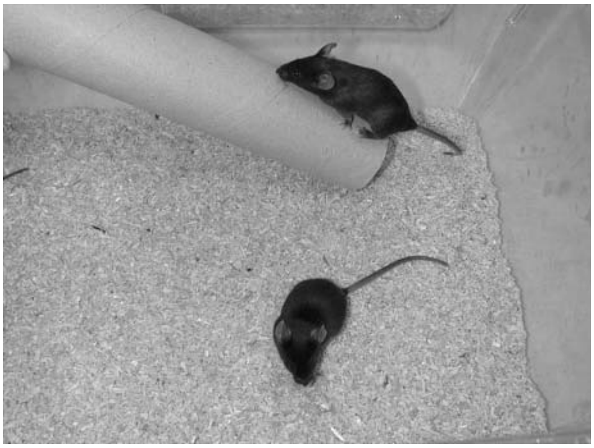


Figure 31.5 C57BL/6 mouse climbing onto a reel for further transfer.

Figure 31.6 Carrying a C57BL/6 mouse within the hand while fixing the tail.

Figure 31.7 Gripping a C57BL/6 mouse by rubber-tipped forceps.

gripping of the pup's neck fold with its mouth. This relaxation can still be seen in adult animals when being handled (Figure 31.8a and b).

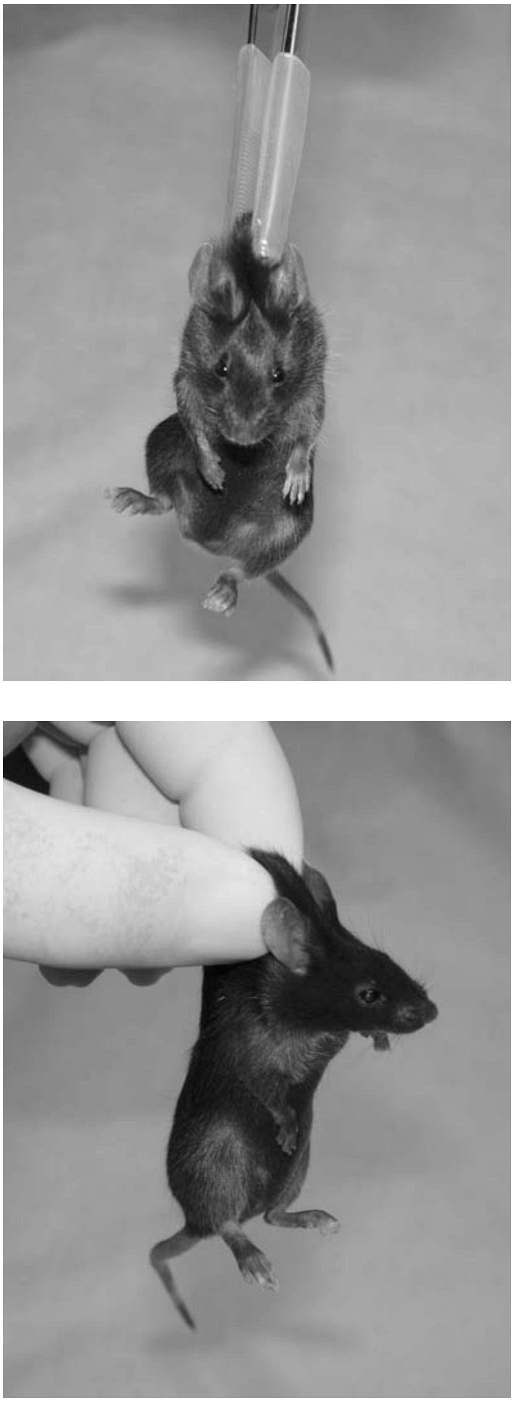
Transfer of litters and mother

In case of transferring a mother with her litter, the mother is removed first, in order not to provoke defensive reactions by her when the nest is taken out of the cage. The female is transferred according to the procedure described above for single mice and placed into the new cage. The litter, i.e. nesting material and pups together, is grasped with both hands forming a cup and sliding beneath the nest. The whole nest with its contents is then lifted up, carried to the new cage and gently placed back, preferably not touching the pups by unprotected hands. By transferring litters this way, the female usually immediately approaches the nest and accepts her pups without any problems (Figures 31.9 and 31.10).

Restraining of mice

Limited cooperation of the mouse, its unpredictable behaviour and continuous readiness to bite when being restrained demands careful action and proper restraining of each individual animal. This includes secure immobilizing that minimizes movements of the animal but still allows it to breath normally. Such action avoids casualties even in very sensitive strains and reduces animal accidents that may be caused by mouse bites and unexpected reflexes of the bitten person. Gentle release into the researcher's hands before return of the animal into the cage can contribute to adaption of the animals to restraining procedures.

(a)



(b)

Figure 31.8 (a) Carrying a C57BL/6 mouse by rubber-tipped forceps in a relaxed position; (b) C57BL/6 mouse carried by hand at neck skin fold; mimics carriage by mother. (*Note*: Natural relaxation.)

Restraining by hand

The tail of the mouse is gripped at its base and the mouse is lifted onto the grid cage top. By gently pulling the tail backward, the animal tends to move forward and to hold on to the grid with its forelegs. At this

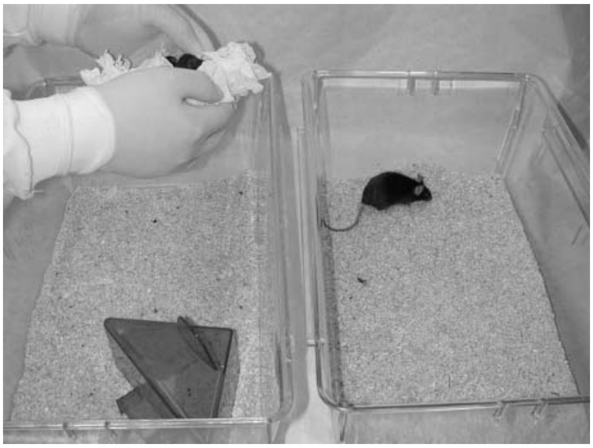


Figure 31.9 Grasping a nest with litter from the bottom of a cage. (Note: C57BL/6 mother has been transferred first.)

Figure 31.10 Placing a complete nest with litter and nesting material back into a cage.

moment, the other hand approaches the rear of the neck and a skin fold, quite close to the ears, is grasped with the thumb and the forefinger, while the loose skin extending over the back is gripped with the other fingers. It is important to grip the loose skin in the rear of the neck properly, in order to prevent the animals from turning its head and biting into the handler's fingers. At the same time, care must be taken not to impair the animal's breathing and venous blood backflow from the head to the chest. By turning the hand upwards, the mouse is positioned with its ventral side uppermost. The tail is then gripped between the third finger and the ball of the thumb. The head and body of the animal are brought into a straight and comfortable position with its back being supported by the palm of the hand. In this position the mouse is held safely for any further manipulations (Figures 31.11–31.15).

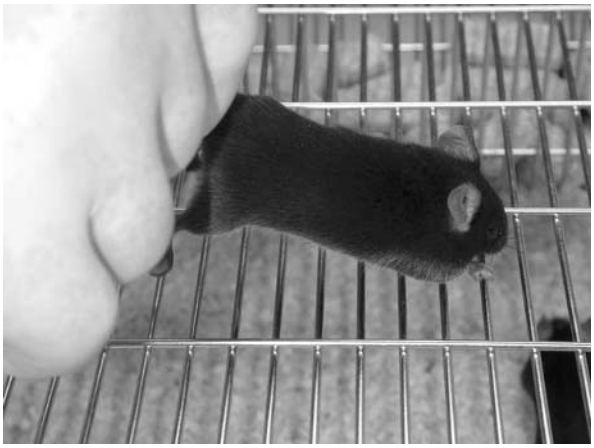


Figure 31.11 Fixation of a C57BL/6 mouse by the base of its tail.

Figure 31.12 Grasping a skin fold at the rear of the neck with thumb and forefinger.

Figure 31.13 Fixing loose skin along the back and tail of the C57BL/6 mouse.

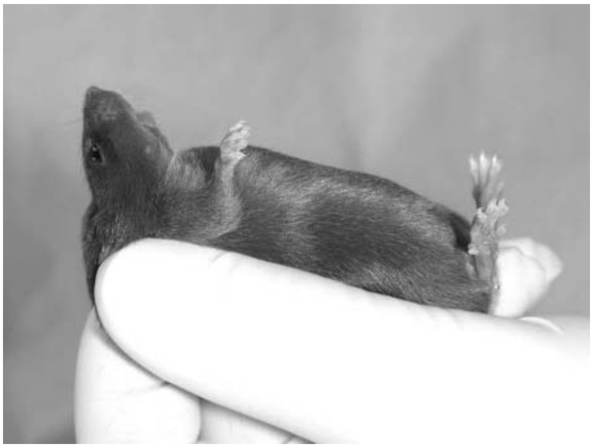


Figure 31.14 Final fixation for further manipulations.

Figure 31.16 Fixation of a C57BL/6 mouse during temperature recording (note: adult animal).

Figure 31.15 Fixation of a C57BL/6 mouse and ip injection into the left, caudal abdomen.

Figure 31.17 Restraining a mouse pup during oral dosing.

Mouse pups can be restrained in two ways: (A) Without any prior handling, the thumb and the first two fingers are placed around the shoulder and thorax region and the animal is picked up from the cage. It is then held in this way and can be positioned for physical examination or rectal temperature recording for example (Figure 31.16). (B) A skin fold in the dorsal neck/shoulder region is first grasped between the thumb and the index finger. Special care has to be taken not to restrict their breathing due to the small size of pups. After positioning the pup in the same way as described for the adult, oral administration of drugs by means of a ball-ended metal tube, for example, can be performed (Figure 31.17).

Two further indications for which manual restraint is performed frequently are tail marking and sexing. *Tail marking* is best performed in the following way: After lifting the mouse by the base of its tail and putting it onto the grid cage top, the tail is gently pulled backwards and different marks can be applied by means of a waterproof text marker. For *sexing*, the mouse is put onto the grid cage top and the tail is carefully pulled backward in the same way as described for tail marking. When the animal reaches an extended position due to its drive to move forward, its back is gently depressed with the third and fourth finger while the tail base and rear legs are lifted up in order to expose the genitalia. The sex can then be determined by checking the ano-genital distance, which is longer in male animals (Figures 31.18 and 31.19).

Restraining by means of a device

New mouse restraining devices are continuously being developed. They are self-made for special purposes or can be supplied from commercial sources. Materials

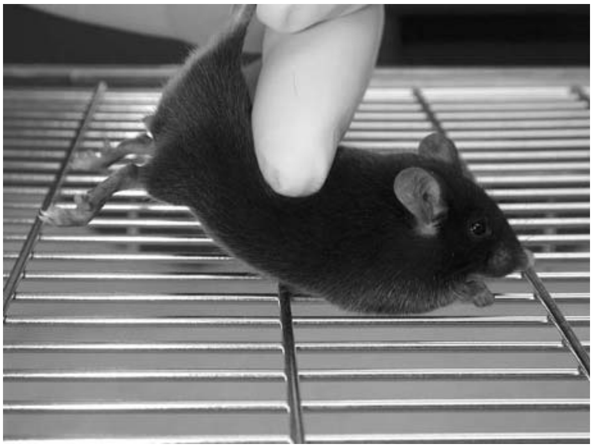


Figure 31.18 Animal positioning for sexing.

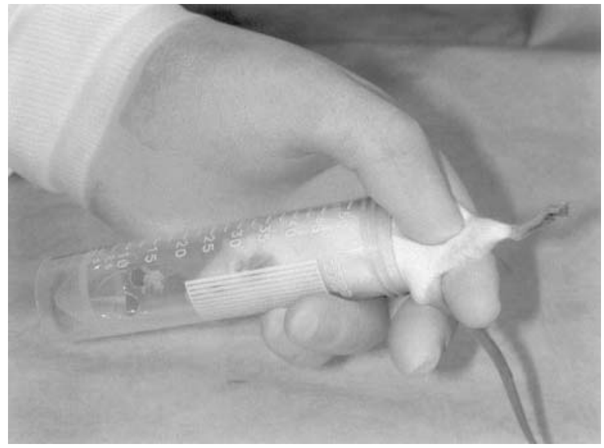


Figure 31.20 Tube restraining of a BALB/c mouse for blood sampling at the vena saphena.

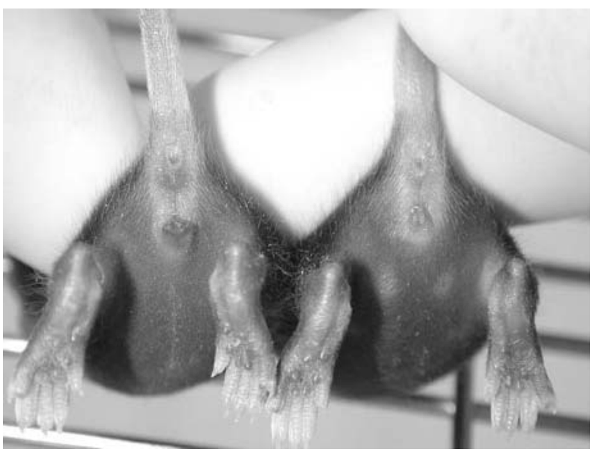


Figure 31.19 Exposing genitalia for determining gender of the animals by checking the ano-genital distance; left: male, right: female.

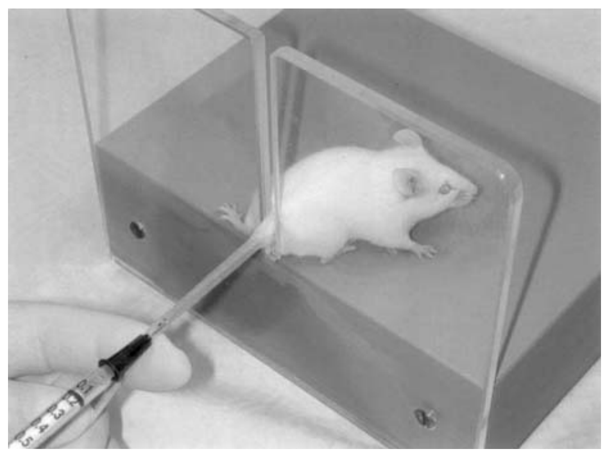


Figure 31.21 Using of a restraining wall for e.g. blood sampling on the lateral tail vein of a BALB/c mouse.

used include soft leather or plastic, hard plexy-glass or macrolon, metal among others. Unlimited design possibilities are restricted by demands on hygienic properties, harm- and stresslessness for animals and man, and optimally, the restraining device allows the experimenter to have both hands free for the execution of procedures on the animal. Long-term restraining devices should allow the animal to fulfil its basic physiological needs. Commercial catalogues are available from various suppliers or devices can be searched for on the internet (AALAS 2002, *Laboratory Animals Buyers' Guide*, 2002).

Some examples of commonly used restraining devices are shown on Figures 31.20–31.22. One example (Figure 31.20) shows a commercially available tube usually used for cell culture technique. The animal is

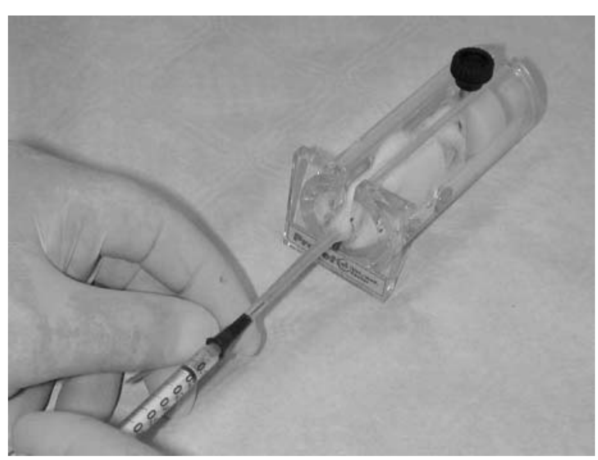


Figure 31.22 Restraining tube for manipulations on the tail of a BALB/c mouse.

gripped at the base of the tail and lifted up. After introducing the mouse into the tube, the device is turned into a horizontal position and the animal is gently pushed forward into the tube or the tail slightly drawn backward to motivate the animal to escape ahead into the tube. The tip and bottom of the tube are equipped with self-made holes, which the mouse tends to reach into. When the mouse is completely in the tube, a rear leg can be exposed e.g. for blood sampling at the vena saphena. Different restraining devices for blood sampling from or injections into the lateral tail vein are widely distributed and many of them are commercially available. Another example (Figure 31.21) of a 'restraining wall' has been used and modified. In order to restrain the mouse, the animal is grasped at the base of the tail and lifted up. It is then positioned in front of the wall with its tail being placed in the slit and the mouse is lowered to the underlying platform. When the mouse has reached the bottom, the tail can be gently pulled backwards and blood can be taken from the tail vein with the animal not being squeezed into a narrow tube but allowed to move freely. Figure 31.22 shows a more sophisticated version of a restraining device (Provet AG, Lyssach, CH) for exposing the tail of the animal. The mouse is lifted up by the base of the tail and then placed in front of the open end of the tubular device. It is introduced backwards with its tail being gently pulled along the open longitudinal slit. A head button is slid into the tube up to the animals head in order to prevent the mouse from moving forward. The animal is now ready for further manipulations.

Effect of handling and restraint on well being of mice

Little scientific background information on stress related to handling or short term restraint in mice is currently available. Stress is considered to be influenced by the combination of restraint and procedure and be dependent on the duration and frequency in which the animals are exposed to manipulations. The outcome of continuous restraint stress can be manifold and range from temporary weight loss to restraint induced pathology (Paré and Glavin, 1986). More recent restraint studies have shown that stress response can be more subtle. It has been shown that mice that were restrained for 12–24 h in restraint cages and tubes showed reduction of lymphocyte cell numbers in lymphoid organs and suppression of *in vivo* antibody production (Fukui *et al.,* 1997), elevation of endogenous glucocorticoid and suppression of migration of granulocytes and macrophages to an inflammatory focus (Mizobe *et al.*, 1997), delay of cutaneous wound healing (Padgett *et al.,* 1998) and also impairment of bacterial clearance during wound healing (Rojas *et al.,* 2002). These findings suggest that handling and restraint should be carried out in a firm, confident and gentle manner and permanent care should be taken, not to crush or squeeze the animals (Rodent Refinement Working Party, 1998). There is still some controversy about the question as to whether frequent handling and restraint will reduce or increase stress in the mouse. Although Li *et al.* (1997) have shown that repeated restraint caused significant impairment of anti-tumour T cell responses, further studies are required to clarify the effect of repeated handling and restraint in the mouse. Different temperament, adaptability and stress sensitivity of strains must be taken into account before any final conclusion regarding stress response to handling and restraint can be made.

Summary and recommendation

Despite its limited friendliness and cooperative behaviour but for the many other benefits as e.g. its high reproduction rate, small size and vitality, the laboratory mouse has been most prevalent in the *in vivo* research laboratory. Unlike the rat, the mouse shows generally a less positive response to good handling. The risk of deep bite injuries, however is low. Nevertheless, the animal should be approached, handled and restrained with care and deep respect. All measures shall be taken to ensure competent and least stressful manipulation. This can be achieved by professional training of the experimenter and animal care staff. Proper handling and restraint contribute to refinement of animal research and validity of research data.

Although physical restraining alone can serve to achieve safe and efficient manipulations in the animal as, for example, subcutaneous, intra-peritoneal and intra-muscular injections or gavage applications, procedure-related stress and pain of an animal shall be evaluated carefully.

Safe and efficient anaesthetic agents providing fast onset of anaesthesia together with a short recovery phase may be used for chemical restraint of mice in situations, where physical restraint of conscious animals may not be appropriate for certain procedures from the animal welfare point of view. Such instances may not only be surgical events but also injection of transponders, tattooing of tails, ear punching and injections of compounds (for chemical restraint see Chapter 34 in this book).

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