

# The Laboratory Rabbit

## Rabbit Users Wetlab

Administered by  
Laboratory Animals Centre  
National University of Singapore



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## THE LABORATORY RABBIT

### Taxonomy:

Kingdom	Animal
Phylum	Chordata
Class	Mammalia
Order	Lagomorpha
Family	Leporidae
Genus	<i>Oryctolagus</i>
Species	<i>cuniculus</i>

### Introduction:

The domestic and laboratory rabbits with which we are familiar were derived from the European wild rabbit of the same genus and species. True domestication is thought to have begun in the 16<sup>th</sup> century after monks began breeding rabbits in captivity. Following domestication, the coat color and body weight of the rabbit began to vary greatly. Currently there are approximately 50 well-recognized breeds of rabbits. The rabbit has an average life span of 5-6 years, and a possible longevity of 15 years. Males are referred to as *bucks*, females are called *does*, and the neonates are known as *kits* or *bunnies*.

### Behavior:

Rabbits are usually curious in the presence of humans but may be easily frightened when approached. In general most rabbits are not aggressive towards humans, but rabbits that have been repeatedly bled and injected may show signs of aggression (stomping, snorting, leaping forward at handlers) when approached by a human. Rabbits rarely bite but are capable of inflicting deep and painful wounds with their hind claws; hence, good restraint is a necessity to work safely with these animals. Rabbits that are frightened or in pain can produce a very loud and high-pitched scream.

Sexually mature rabbits are territorial and will usually fight if housed together. Immature sibling females 3 months of age or younger housed together may form stable groups; however, trauma, pseudo-pregnancies, and infertility may occur in group-housed rabbits. The benefits of social enrichment and increased exercise must be weighed against the risk of fighting in group-housed females. Rabbits can be trained to use a litter box in a manner similar to cats, since they generally choose one corner of their cage for defecation and urination.

Rabbits housed indoors under controlled environmental conditions tend to have alternating periods of wakefulness and rest throughout the day and night.

Submandibular (or submental) and anal secretions, as well as piles of feces or urine sprays, are used by adult rabbits for individual and territorial marking. Chin-marking of does by bucks may be done after coitus or in new surroundings.

Housing:

Cage space requirements for individually-housed rabbits as listed in the most recent “*Guidelines on the Care and Use of Animals for Scientific Purposes*” (NACLAR, 2004) are shown in Table 1. Cage space requirements for females with litters are listed in Table 2.

Table 1. Cage space requirements for individually-housed rabbits.

Body weight in kg	Floor area/animal, m <sup>2</sup>	Minimum cage height, cm
<2	1.35	35
Up to 4	0.27	35
Up to 5.4	0.36	35
> 5.4*	> 0.45	35

\*According to the guide, rabbits at these sizes might require more space to meet performance standards. “An animal’s space needs are complex, and consideration of only the animal’s body weight or surface area is insufficient. Space allocations should be reviewed and modified as necessary to address individual housing situations and animal needs (for example, for prenatal and postnatal care, obese animals, and group or individual housing). Such animal-performance indices as health, reproduction, growth, behavior, activity, and use of space can be used to assess the adequacy of housing.” Cage floor space does not include that which is taken up by food or water containers.

Table 2. Cage space requirements for females with litters.

Body weight in kg	Minimum floor space, m <sup>2</sup>	Minimum cage height, cm
<2	0.36	35
2-4	0.45	35
4-5.4	0.56	35
>5.4	0.7	35

Under the NACLAR guidelines, it is recommended that all females with litters be provided a nest box containing clean nesting material. Nest boxes should be placed into the cage when the female is nearing the end of her gestation period. The female will pull hair from her body to line the nest in addition to any other nesting materials provided. Nesting materials should be nontoxic and digestible in the event they are eaten. Wood shavings are not recommended.

Rabbits in the laboratory setting are usually housed in front-opening stainless steel cages that have mesh or grid floors. Some of the newer cage designs incorporate a grid plastic floor which is warmer and more comfortable for the rabbit, and decreases the noise that occurs when animals move about in the cage. Most cage designs incorporate a removable tray containing an absorbent bedding which catches the urine and feces. If neonates are present the mesh size on a wire cage floor should not exceed 1 X 2.5 cm on the floor and lower walls. Animals housed in solid-bottom cages with bedding should have the bedding replaced weekly. Cages used to house rabbits must be pretreated to remove the urine scale prior to cleaning.

Auxiliary ventilation by means of exhaust fans or air conditioning must be provided when the ambient temperature is 29.4°C or higher in indoor facilities. Rabbits housed outdoors must be provided with a sprinkling system or another means of cooling when the ambient temperature reaches 32.2 °C. The general temperature recommendation for rabbits as listed in the Guide is a range of 16°C-22°C. Overheated bucks may experience heat stress, which may lead to death or infertility, and overheated does may experience death or embryonic mortality. Heat lowers feed consumption and increases water consumption, whereas cold temperatures increase both.

The number of air changes/hour are the same as for other species (10-15 minimum, or greater if required for to handle odors and ammonia), and the recommended humidity is 30%-70%. Rabbits should be protected from extreme temperature changes and drafts should be minimized. The light cycle is usually controlled to provide 12-14 hours of light per day in non-breeding animals.

#### Diet and Nutrition:

Rabbits are fastidious eaters and may reject foods solely on the basis of odor, texture, form, or method of presentation. They prefer the low-fiber, high-protein, high-carbohydrate portions of plants, which are usually the tender, succulent portions. Rabbits are also fond of sweet foods, such as those containing sucrose or molasses. The optimal level of protein is a minimum of 15-19%, and crude fiber levels should be at least 10%. Dry food is consumed by juveniles beginning at 16-18 days of age.

Table 3. Daily pelleted food consumption of rabbits by stage of life.

Adult rabbits	5 g/100 g body weight
Pregnant does	175-225 g
Lactating does	225-450 g
3-6 weeks	50-75 g
6-8 weeks	125-150 g

Rabbits may be fed *ad libitum* (also referred to as “*full fed*”), but in the laboratory situation they must often be “*limit fed*” to prevent obesity. Reducing the amount of pelleted diet (by 2-3 oz) that is fed and substituting a good quality grass hay may be employed to keep rabbits from gaining excess weight and to prevent the formation of hairballs. Does with more than 5 kits should be full fed, and those with smaller litters should be limit fed. Peaks of food consumption occur in midpregnancy and at 2-3 weeks into lactation.

Because the rabbit’s intestinal microflora are sensitive to the intestinal contents (pH, carbohydrate content, etc.), food changes should be made gradually, especially for 4-12 week old rabbits. The new food should be gradually added and the old food decreased over a 4-5 day period to allow the flora to adjust. Under certain conditions, rapid food changes can result in proliferation of Clostridial and coliform bacteria, which may result in the death of the animal.

Rabbits are *coprophagous*, and ingest large quantities of the protein- and vitamin-rich feces directly from the anus. The fecal pellets that are ingested are referred to as *cecotrophs*, and represent food that has undergone fermentation in the cecum. Cecotrophs provide B-complex and K vitamins, fatty acids, and 10% of the rabbit’s intake of amino acids. Vitamin supplementation is normally not necessary for rabbits on an alfalfa-based diet.

Rabbits have a high water turnover compared to other species of animals, and consume twice as much water as food on a per weight basis. The average water consumption is 120 ml/kg body weight per day. The amount of water consumed increases with higher ambient temperatures. Rabbits on high-fiber or high-protein diets also drink more water to moisten and move food, and to excrete the urea load, respectively.

#### Reproduction:

The onset of puberty varies with the breed of rabbit, with smaller breeds maturing earlier than the larger breeds. Does are generally bred at 4-6 months of age (corresponding weight of 3-4 kg) and bucks are used at about 6 months of age. Does do not have an estrous cycle like other laboratory rodents; the cycle

is characterized by 7-10 days of receptivity to the male followed by a period of 1-2 days of non-receptivity. Receptivity of the female is characterized by a swollen, reddened vulva, restlessness, chin rubbing, and by the doe's willingness to stand and be mounted. Rabbits are *induced ovulators*, which means that ovulation is stimulated by coitus. Ova are released 9-13 hours after copulation. Approximately 25% of matings are anovulatory and are referred to as *sterile matings*. *Pseudopregnancy* is a common occurrence in rabbits following sterile matings, mounting by other does, or stimulation by a nearby male, and lasts approximately 15-17 days. Shortened light cycles, such as those occurring in the winter, diminish ovarian activity in the doe. To maintain breeding efficiency, does should be maintained on a 16:8 or 14:10 light:dark cycle, should receive adequate caloric intake, and should be housed under slightly higher ambient temperature conditions.

Behavior during breeding is characterized by chasing, squealing, tail flagging, enurination (releasing of a small amount of urine), and combat. The doe should be brought to the buck's cage for breeding, and if the female is receptive copulation will take place rapidly (within 30 seconds) after their introduction. At least 2 intromissions should occur. Females may attempt to castrate males, so rabbits should be paired for only short periods of time (not greater than 30 minutes) and should be watched closely while together. Young bucks (6 months old) should be placed with gentler does, because aggressive does may attack and ruin the buck as a breeder.

The gestation period of the rabbit lasts from 29-35 days, with 31 days being the average. Litters retained longer than 34 days are often stillborn or contain large or abnormal fetuses. Fetuses can be palpated beginning on days 14-16. Females begin to build a nest at 3-4 days before parturition, and a nest box should be in place at that time. *Kindling*, or parturition usually occurs at night and is generally rapid, but may last 1-2 days. *Dystocia* (difficulty giving birth) is uncommon in the rabbit. Does may be rebred at 2-7 days postpartum. Cannibalism of the kits is rare. Litters generally consist of 6-7 bunnies, but may range from 4-10.

The *kits*, or *bunnies*, are *altricial* at birth; that is, they are born blind, deaf, hairless, and helpless. Birth weight is generally 40-80 grams depending on breed and litter size. The young are normally nursed only once per day, either early in the morning or late in the evening. Newborn rabbits do not require colostrum because all passive immunity is received through the placenta. For the first 2 weeks of life, regulation of body temperature depends on the metabolism of *brown fat* stores. The ears open at 8 days, the eyes open at 10 days, and they begin to eat some solid food at about 16-17 days. Kits also begin to eat maternal feces at 8 days of age.

Rabbits are weaned at 4-6 weeks of age. Sibling does may be left together, but the bucks should be separated and housed individually at weaning. Weaning is a stressful time, and may be best accomplished by removing the doe and leaving the young in the cage with which they are familiar. Decreased food consumption is generally seen for about 3 days after weaning, and may be associated with having to learn to use feeders and water systems.

Litter survival is dependent on having nests and nest boxes with adequate nesting material. Kits that are removed from the nest box are not retrieved by the doe, will be ignored, will not be nursed, and will eventually become hypothermic and die. Kits that are cold may be rewarmed and placed back into the nest box; human scent will not prevent the doe from nursing this kit. Nesting material that has become wet should be replaced immediately.

An ideal set-up for a nesting box is one in which the level of the box is lower than the cage floor, thus simulating a natural burrow. Does probably do not retrieve young that have fallen out of the nest box because this would not occur in the wild with the young in a burrow, and there is no need for this

behavior to be present. The nest box is generally removed from the cage when the kits are 15-21 days old.

### Anatomic and Physiologic Peculiarities:

Normal body temperature for the rabbit is 38.5°-40°C. Heart rate is from 130-325 beats per minute, depending on the excitement level of the animal. Normal respiratory rate is from 30-60 breaths per minute. Average adult body weight is up to 5kg for males and 6kg for females.

Rabbit teeth are *open-rooted*, and grow continuously throughout the life of the animal. The dental formula is I(2/1) C(0/0) P(3/2) M(3/3). *Lagomorphs* are characterized by having a second pair of incisors in the upper jaw located directly behind the first pair of incisors. This second set of incisors may also be referred to as the *peg teeth*. Malocclusion is most commonly a problem with the incisors which grow 10-12 cm/year; however, the second set of incisors and cheek teeth may also overgrow. Because malocclusion can be hereditary, affected animals should not be bred.

The rabbit has a heavily muscled back and rear legs, but the lightweight skeleton only comprises 7%-8% of its total body weight. This disparity makes the rabbit prone to fracturing its lumbar vertebrae.

Rabbits, like rats and horses, cannot vomit. The stomach is thin-walled, and may be ruptured during necropsy.

The rabbit has 2 unique lymphoid organs associated with the gastrointestinal system. The *sacculus rotundus* is located at the ileocecal junction, and the *appendix or vermiform process* is at the tip of the cecum. These 2 lymphoid organs account for greater than 50% of the total lymphoid tissue in the rabbit. The spleen is correspondingly much smaller in the rabbit.

The cecum is about 10 times the size of the stomach, and is the site of fermentation of the plant diet. Rabbits are referred to as *hind-gut fermenters* because of the location of the cecum. Two types of feces are produced by rabbits: firm, dry daytime feces, and soft, moist nighttime feces which are also called *cecotrophs*. Cecotrophs may be distinguished by their covering of mucus, which helps prevent breakdown in the acidic environment of the stomach. Healthy rabbits never have an empty stomach due to the practice of coprophagy.

Rabbits, like rodents, have Harderian lacrimal glands behind the eyes.

Blood flow through the relatively large ears is a primary means of thermoregulation. This also means that the ears are an excellent site for performing i.v. injections or for collecting blood.

The uterus of the doe is *bicornuate* (having 2 horns), and each of the 2 horns enters the vagina via a separate cervical opening. The uterine horns are curled and lie in the caudal abdomen dorsal to the urinary bladder. The female has 4-5 pairs of mammary glands. Males do not have nipples. The inguinal canals of the male remain open for life, and the testes generally descend at about 12 weeks of age. Paired, hairless *inguinal glands* are located laterally to the external genitalia, and often contain a white-to-brown secretion. These glands may be mistaken for the anus. Mature female rabbits often have a more

prominent *dewlap* (skin flap) under their chin than do males. Mature male rabbits have a bigger head than do the females.

Rabbit neutrophils are referred to as *pseudoeosinophils* or *heterophils* because the cells have eosinophilic (reddish) granules causing them to resemble eosinophils. Lymphocytes are the predominant white blood cell in peripheral circulation. Basophils are more commonly found in rabbits than in other species of animals, and may comprise 2-7% of the white cell population.

The urine of rabbits has an alkaline pH of about 8.2, and has a cloudy appearance due to the high concentration of triple phosphate and calcium carbonate crystals. Rabbits commonly have a high urine output, and the 24 hour urine volume is about 130 ml/kg body weight per day.

### Common Health Problems:

*Pasteurellosis* is the most common and problematic disease of the laboratory rabbit, and is caused by the bacterium *Pasturella multocida*. Many rabbits are inapparent carriers of the organism in their upper respiratory tract, and do not show clinical signs of disease. When stressed, rabbits may present with a multitude of syndromes, including rhinitis, conjunctivitis, bronchopneumonia, otitis media, abscesses, pyometra, or septicemia. Different isolates of *Pasturella* vary in virulence. Rhinitis, or “*snuffles*” as it is commonly called, is characterized by sneezing and production of a mucopurulent nasal discharge. This discharge is commonly seen on the inside of the forelegs as well, as rabbits use their forelimbs to wipe their noses. Some animals may exhibit chronic signs of snuffles, or may progress to more severe forms of the disease. A suppurative conjunctivitis may also be observed, and often the fur may be rubbed off around the eye. Otitis media (middle ear infection) may progress to inner ear infection (otitis interna), with the clinical signs of *torticollis* (head tilt) and balance problems. This syndrome is also referred to as “*wry neck*”. Although antibiotics may improve clinical signs, treatment does not completely eliminate the organism. The best prevention lies in the purchase of animals from *Pasturella*-free rabbitries.

Enteric (intestinal) diseases resulting in diarrhea are the second most common problem in rabbits, but often the etiology of the disease is not understood. Symptomatic treatment may be required to maintain hydration and body temperature.

*Enterotoxemia* is one cause of diarrheal disease in rabbits, and is caused by the overgrowth of one or more species of bacteria of the genus *Clostridium*. Overgrowth of Clostridial organisms may follow stress, dietary changes, weaning, or treatment with antibiotics. Affected rabbits are depressed and anorexic. Enterotoxemia results in the death of the animal, and at necropsy the cecum is found to be hemorrhagic and edematous.

*Coccidiosis* is a common cause of diarrhea in rabbits and is caused by infection with one of several species of the protozoan *Eimeria*. Adult rabbits may show no clinical signs, but young rabbits may be pot-bellied and may have weight loss and diarrhea. Diarrhea may be intermittent to profuse, and may sometimes contain flecks of blood. Affected animals may be treated by coccidiostats, and prevention and control is by strict attention to cleaning and sanitation procedures.

*Muroid enteropathy* is a disease problem most frequently seen in rabbits that are 7-10 weeks old. Clinical signs include abdominal distention with sloshing sounds from the intestine, hunched posture, depression, polydipsia, anorexia, hypothermia, and constipation followed by profuse muroid diarrhea. Treatment is usually ineffective.

The rabbit *ear mite* is a common problem in conventionally-raised rabbits. This is a non-burrowing mite that chews on the epidermal surface inside the ear and causes an intense inflammatory response. Affected

rabbits may be seen to have a dark brown, crusty material inside the ear, and often shake their heads and scratch at their ears. Occasionally lesions caused by scratching may be seen on the skin on the back of the head as well. Treatment with ivermectin is effective in killing the mites, and the ear canal should be gently cleaned to remove accumulated debris.

*Buphthalmia* is a form of glaucoma in rabbits and represents a common inherited disease. The eyes of affected rabbits gradually bulge outward, and the cornea eventually becomes opaque. The condition develops slowly and does not require treatment. Affected animals should not be used as breeding stock.

*Gastric trichobezoars* (hairballs) are common in rabbits, and are a result of excess grooming. Affected rabbits may suddenly stop eating and drinking and may pass no feces, but otherwise appear bright and alert. A high fiber diet is beneficial in preventing the formation of hairballs, and brushing rabbits may help remove excess hair.

*Ulcerative pododermatitis or sore hock* is often seen in heavy rabbits maintained on wire-mesh bottom cages (not steel bar cages). Lesions are characterized by an ulcerated area of skin on the ventral metatarsal region that becomes infected by bacteria such as *Staphylococcus*; abscesses may also form in the area. Treatment consists of cleaning and bandaging the feet and housing the animal in a solid-bottom cage with deep, soft bedding.

### Handling and Restraint:

When rabbits are picked up they are prone to kick with their hind limbs, which can result in wounds to the handler as well as in a broken back for the rabbit. Rabbits have extremely well-developed and strong musculature in comparison to their weaker bone structure, which makes the lumbar portion of the vertebral column prone to fracture. An animal with a broken back must be euthanized; thus, the handler must use care to ensure that the rabbit is restrained and held properly.

Rabbits should be handled firmly but gently. To pick up a rabbit, firmly grip the loose skin over the scruff of the neck with one hand while using the other hand to support the rabbit's hindquarters and to control the movement of the hind limbs (Figure 1). Do not approach the rabbit from on top, always come in from the side to pick it up so it will know that you are there. Rabbits are easily frightened if approached when they cannot see you. *The hind limbs must always be supported or the rabbit may break its back if it struggles.* The rabbit should not be picked up using only the loin area as this often results in struggling and the animal may break its back. *Never use the ears to pick up a rabbit!* An extremely aggressive rabbit may attack or posture aggressively when attempts are made to remove it from the cage. A towel can be put over the head of such an animal in order to allow the handler to safely restrain the rabbit with less resistance. The aggression may be related to the rabbit being in its home territory (the cage) or because the rabbit has had prior experience with painful procedures.

To carry the rabbit, place it snugly against your body with its head tucked into the bend of the elbow (Figure 2). Keeping the eyes covered helps the rabbit to relax. Rabbits feel insecure on stainless steel tables or any type of slick surface; therefore, if an animal must be examined on such a surface it is helpful to place the animal on a towel. Commercial restrainers are available which restrain the body of the rabbit while allowing access to the head and ears. These restrainers may be made of metal, plastic or plexiglass. Restraint bags designed for cats may also be used for rabbits. If you need to transport your rabbits from one room to another, please obtain a plastic rabbit transport cage from any of the Laboratory officers at AHU.

To put the rabbit back into the cage, place its rump first into the cage while placing the back feet on the floor of the cage. Once the rabbit is inside the cage, slowly release the scruff of the neck and release your hand.

Rabbits held in the arms or placed in a V trough can be immobilized or hypnotized by gently stroking the abdomen while the rabbit's eyes are covered. Use one hand to cover the eyes of the rabbit with its ears, and grasp the head firmly placing the chin against the neck. The hind legs should be held with the other hand and the body stretched out. The rabbit should be stroked beginning at the thorax and continuing over the hind legs, and after a few strokes the rabbit should be in a trance. Not all rabbits will respond alike, but if the rabbit relaxes the respiration rate will drop from 50 to about 20 breaths per minute. This type of immobilization is suitable for minor procedures such as toenail or incisor clipping but not for invasive procedures such as injections or blood collection. Animals may be conditioned by repeatedly performing this procedure.

#### Recognition of Pain and Distress in Rabbits:

Because animals cannot volunteer to participate in medical research, we are ethically constrained to provide humane care, and to alleviate as much pain and distress as is possible in such animals. We must *always* work with the assumption that if a procedure causes pain in human beings it will also cause pain in an animal. This concept is mandated by the NACLAR Guidelines: "Pain and distress cannot always be adequately evaluated in animals and investigators must therefore assume that animals experience pain in a manner similar to humans. Decisions regarding their welfare in experiments must be based on this assumption unless there is evidence to the contrary. The investigator should anticipate any potentially adverse effects of a manipulation and take all possible steps to avoid or minimize pain and distress." The proper use of anesthetic and analgesic drugs helps to alleviate pain and distress during procedures and it is imperative that we as researchers learn to recognize the signs of pain and distress in rabbits. If you are unsure what drugs to use for your research procedures, please do not hesitate to discuss this with one of our veterinarians.

Behavioral signs of pain and distress specific to rabbits include an "anxious" look, facing the back of the cage (hiding posture), hypersensitivity to environmental stimuli, vocalization by means of a piercing squeal, kicking and scratching, and in the case of females with pups, cannibalization of the litter. Rabbits experiencing pain may also grind their teeth. Some individuals may become overtly aggressive. It is important to be familiar with the normal behavior for a species and strain as well as for an individual animal to be able to assess changes.

Nonspecific signs of pain and distress in rabbits listed in order of increasing severity, include: (1) ruffled or "spikey" fur (rabbit looks unkempt); (2) weight loss (mild to severe), anorexia, or dehydration; (3) ocular discharge; (4) lethargy, depression, or reluctance to move; (5) sitting with the back in a hunched position; (6) ataxia (uncoordinated muscle movements), regional or generalized weakness; (7) tremors which may be intermittent to persistent depending on the condition of the animal; (8) hypothermia characterized by cool extremities; (9) labored respiration; and (10) cyanosis, or a blue tinge to the mucous membranes. Any animals exhibiting combinations of 2 to 3 minor signs, or a single major sign should be euthanized immediately. Rabbits are also apt to break their backs if handled incorrectly; any animal which becomes paralyzed following such an accident should be euthanized immediately.

#### Blood Collection:

The marginal ear, cephalic, and lateral saphenous veins can be used to obtain small amounts of blood. Blood must be withdrawn slowly as too much negative pressure will cause these vessels to collapse. If larger blood samples are required it is best to use the auricular artery which runs medially in the ear

(Figure 3). After obtaining blood from the auricular artery, pressure should be applied to the site with a piece of gauze until bleeding has stopped to avoid the formation of hematomas.

Rabbits are most commonly bled from the ear as restraint is easily accomplished leaving the ear free for blood collection. Several methods may be employed to increase blood flow to the ear. Mechanical methods of stimulating blood flow include warming the ear with a heat lamp or warm (not hot) water or by gently stroking the base of the ear. A topical irritant such as wintergreen oil may be used to stimulate vasodilation of the vessels, but recently (+)-limonene at a concentration of 25-40% in 95% ethanol has been recommended since it is much less irritating. Two ml of the limonene solution can be applied to the ear and the vessels should dilate in 2-5 minutes. *Xylene may not be used as a topical irritant.* Vasodilation will last for at least 10 minutes. If irritants are used the ear should be washed well with 95% ethanol after the blood has been collected to avoid further irritation of the skin. Acepromazine (1-2 mg/kg) may also be given subcutaneously to the rabbit to induce vasodilation and sedation. 2% xylocaine, a local anesthetic, can also be injected subcutaneously close to the base of the targeted vessel; however, xylocaine is slightly acidic and pain is experienced on administration.

The lateral saphenous vein may be used to collect blood quickly and easily in large rabbits. This vein runs medial to lateral in a diagonal fashion across the lateral aspect of the tibia. The skin over the lateral tibia should be shaved to make the vessel visible, and an assistant should restrain the rabbit on its side while holding off the vein just above the hock. Not all rabbits will be amenable to blood collection in this manner without sedation.

Cardiac punctures can be used to collect larger volumes of blood terminally, but the animal must be anesthetized for this procedure. The animal should be positioned dorsally on a restraining board, and an 18 gauge, 1.5 inch needle is inserted in a craniodorsal direction at a approximately 30° angle from the plane of the sternum and immediately caudal to the xiphoid cartilage. It is a good idea to examine the thoracic cavity of the carcass of a rabbit beforehand to become more familiar with the location and size of the heart.

#### Guidelines for Blood Collection:

The total blood volume of a rabbit ranges from 160-480 ml depending on the size of the animal, and represents 6-8% of the lean body weight. Approximately 5% of the blood volume is within the capillary bed at any given time. For any animal, the total blood volume is approximately 67 ml/kg.

Up to 15% of the blood volume may be removed at one bleeding, and this volume may be collected weekly. If rabbits are being used to produce antibodies, blood should be collected no more frequently than every 2-4 weeks to ensure adequate antibody titers. If more than 15% of the blood volume is removed the hematocrit and serum proteins must be monitored. *Acute loss of 25-30% of the blood volume is fatal in 50% of rabbits!*

#### Injection Sites:

*Subcutaneous (s.c.) injections:* Subcutaneous injections are generally administered under the loose skin between the shoulder blades. Thirty to 100 ml may be given in this manner depending on the size of the rabbit. This is a common route for injecting adjuvants for antibody production.

*Intramuscular (i.m.) injections:* Intramuscular injections may be administered in the large lumbar (epaxial) muscles that run along the spinal column or in the large quadriceps and thigh muscles of the hindlimbs (Figure 4). When administering an i.m. injection to a rabbit it is helpful to have one person hold the rabbit while the other extends the leg and gives the injection. The muscles should be held firmly

with one hand while the other is used to make the injection. Care must be taken not to hit the sciatic nerve which courses behind the femur. If the sciatic nerve is damaged the rabbit may experience tingling or numbness and may self-mutilate the affected foot. Aspirate lightly after the needle is inserted to ensure that the needle is not placed within a blood vessel. If blood appears in the syringe, reposition the needle slightly. The maximum volume that can be given per site i.m. is 1-1.5 ml.

*Intravenous (i.v.) injections:* Intravenous injections are most commonly given in the marginal ear vein. The ear should be cleaned and prepared for an aseptic injection. Use the fingers to apply pressure at the base of the ear to distend the vein. The needle should be inserted into the skin just beside and parallel to the vein to avoid flattening the vessel. The needle is then inserted into the distended vein and threaded up a short distance to insure that the injection will be intravenous and not subcutaneous. *Use care to avoid the injection of air into the vein or the rabbit may die as a result of air emboli.*

*Intradermal (i.d.) injections:* Intradermal injections should be administered in the loin and flank areas in a position that makes it difficult for the rabbit to reach with its hind feet. Before giving an i.d. injection shave the area of the skin where the injection is to be given. If necessary the remaining hair can be removed with a depilatory, and the area should be prepared aseptically. A 25 gauge needle and tuberculin syringe are most commonly used. The skin should be held tautly and the needle inserted with the bevel facing up until the bevel disappears. The point of the needle should enter just under the epidermis and into the dermis. Small volumes of 0.1ml or less can be injected, and if the needle is seated properly a small bleb should appear at the site of the injection. Examine the injection site after removal of the needle. If material is observed seeping from the site the needle should be inserted further into the dermis for injection.

*Intraperitoneal (i.p.) injections:* Intraperitoneal injections should be made when the rabbit's stomach and urinary bladder are empty. To give the injection in this site, the rabbit should be held with the hindquarters elevated and the needle inserted just lateral to the midline and just posterior to the area of the umbilicus. A one inch needle should be used and inserted toward the spine. Before injecting, aspirate the syringe to make sure the needle is not within an organ or a blood vessel.

#### Guidelines for the Use of Adjuvants in the Rabbit:

Rabbits are commonly used to raise polyclonal antibodies against various antigens, and this is generally done by combining the antigen with an *adjuvant* to increase antigenicity as well as to hold the antigen in depot form for slow and constant presentation to the immune system. The use of adjuvants results in an inflammatory lesion at the injection site which may progress to form a sterile abscess. These lesions may also cause pain and distress in animals, which may result in scratching and self-mutilation at the injection site. Abscesses should be kept clean and dry and monitored for any signs of bacterial infection until the area heals. Animals with numerous injection sites may also require analgesics for control of pain. Consult the veterinary staff if animals appear painful or abscesses develop.

*Freund's complete adjuvant (FCA)*, which contains a killed *Mycobacterium* in mineral oil, generally produces the highest incidence of inflammatory and necrotic lesions, and its use should be avoided whenever possible. The guidelines for the use of FCA, which will be summarized here. FCA can be used for the first antigen injection only, and subsequent injections should be done in *incomplete Freund's adjuvant (IFA)* which consists of mineral oil alone. Subsequent injections of FCA result in severe inflammatory and necrotic lesions; therefore, the use of two or more doses of FCA must have IACUC approval based on scientific justification. FCA should be injected subcutaneously only, as intradermal injections uniformly result in skin necrosis and sloughing, and intramuscular injections may lead to

temporary or permanent lameness. *No more than 0.1 ml should be injected subcutaneously at any one site on the rabbit. The number of injected sites on one animal may not exceed ten (10).*

Other adjuvants are available commercially and their side effects are reviewed in Leenaars et al. These include TiterMax (CytRx, Norcross, GA) and RIBI (RIBI ImmunoChem Research, Inc., Hamilton, MT). These adjuvants generally give good titers with a decreased incidence of side effects compared to FCA.

### Anesthetic and Surgical Considerations:

The general health of the rabbit should always be assessed prior to any anesthetic or surgical event. Any animal showing signs of respiratory disease (sneezing, lacrimal or nasal discharge, matted hair on the medial side of the front paws) or diarrhea/muroid feces should not be considered a suitable surgical candidate.

Always approach the rabbit quietly, and avoid exciting the rabbit as much as possible when removing the animal from its cage. Excitement can cause the release of catecholamines, which increases the possibility of untoward anesthetic complications such as cardiac arrhythmias.

*Anesthesia in rabbits is challenging.* Many anesthetics have a relatively narrow margin of safety, and/or induce profound respiratory depression in this species. In addition, rabbits are difficult to intubate due to the anatomical structure of the oral cavity and larynx. Please consult a veterinarian if you do not have prior experience with these techniques in rabbits.

Adequate monitoring of the rabbit during anesthesia is required for a successful outcome. Parameters which can be used to monitor the physiological status of anesthetized rabbits include anesthetic depth, respiratory rate and pattern, arterial blood gases, blood pH and oxygen saturation, pulse, arterial blood pressure, electrocardiographic (ECG) changes, core body temperature and end tidal CO<sub>2</sub> content. The selection of equipment for monitoring the anesthetized rabbit is beyond the scope of this handout and should be done with the aid of a veterinarian.

Parameters that can be used to assess the depth of anesthesia in rabbits include recumbency and loss of purposeful movements, loss of reflexes, muscle relaxation, changes in jaw tone, chewing motion, vocalization, response to aversive stimuli, alterations in respiration, cardiovascular function, and electroencephalographic (EEG) changes. The best reflex response to use for monitoring the surgical plane of anesthesia in the rabbit is the ear pinch reaction or loss of pinnae reflex. In contrast to other species, the rabbit does not lose the corneal reflex until the plane of anesthesia is dangerously deep. The noxious stimuli used most frequently to monitor anesthetic depth in rabbits include the response to pinching of the abdominal skin, toes or tail.

### Euthanasia:

At LAC, we recommend euthanasia via chemical means (i.e. sodium pentobarbital). If an overdose of a barbiturate is used for euthanasia it should be given i.v. and not by other routes, as the i.v. route is the most reliable method. Exsanguination can be used to ensure death in an unconscious animal but cannot be used as the sole means of euthanasia. Animals that are deeply anesthetized may appear to a casual observer to be dead but may later recover; therefore, it is important to be sure that the animal is actually dead before disposal of the carcass occurs. Cessation of the heartbeat should be used to determine that the animal is no longer alive.

Because neonatal animals are extremely resistant to hypoxia, the use of inhalant anesthetics or carbon dioxide alone requires a much longer exposure time to ensure death. It is best to combine the use of such agents with another method to ensure humane euthanasia of very young animals.

### Record Keeping:

An individual record must be maintained for each rabbit. This record contains medical information and a record of any experimental procedures, blood collection, etc. performed by the investigator. Records are required to be kept by the AVA and may be requested for review during their facility inspections.

### Occupational Health Concerns:

Development of allergies to species of animals used in research, especially rodents and rabbits, is one of the most common problems encountered by both animal care workers and investigators. While the most common manifestations of this sensitivity are nasal symptoms, itchy eyes, and rashes, it is estimated that up to 10% of chronically exposed individuals will develop asthma which can be life-threatening. The majority of allergies induced by rabbits are due to proteins found in the dander. Individuals that are extremely sensitive can be adversely affected by simply walking into a room where rabbits are housed. The use of gloves, laboratory coats, and other protective clothing helps to minimize exposure and prevent the development of allergies; wash well with soap after working with the rabbits. Anaphylaxis may occur in extremely allergic individuals if they are bitten or scratched by a rabbit or receive a puncture wound from a used needle that has rabbit proteins on it. Development of allergies should be reported to your supervisor.

Anyone being bitten by a rabbit should report the injury immediately to any of the Lab Officers at AHU and also to the University Health and Wellness Centre, which is located at Yusof Ishak Hall, Level 4. The phone numbers are 6776-1631 (Nurses Station) and 6516-2880/6516-2390 (Admin Office). For on-campus emergencies and after office hours, please proceed to NUH Accident and Emergency Unit. A bite from a mouse may result in a puncture wound, and any bite wound should be cleaned thoroughly to prevent bacterial infections. A current tetanus immunization is recommended for anyone working with mice, as such injuries may provide entry for the tetanus bacterium.

In general, work with laboratory-reared rabbits does not present any special concerns in terms of zoonotic disease transmission.

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