

XVI. RABBITS*

A. INTRODUCTION

1. Breeds

The Order *Lagomorpha* contains the rabbits, hares, cottontails and pikas. Present day breeds of domestic rabbits, including those used for laboratory studies, are all derived from the European rabbit (*Oryctolagus cuniculus*) (Sheail, 1971; Fox, 1974). The breeds most commonly used for research include the New Zealand White (albino), Dutch, Polish, Californian and New Zealand Red (Fox, 1974). Approximately 30 breeds and some 80 varieties of rabbits are recognized in North America by rabbit breeding associations, and numerous strains and specific mutations are available commercially for research purposes (Fox, 1974; Lindsey and Fox, 1974; The Jackson Laboratory, 1975; NRC U.S., 1979). Rabbit breeds vary greatly in size and are often classed on the basis of body weight as being: small—less than 2 kg (Dutch, Polish); medium—2 to 5 kg (New Zealand and Californian); large—greater than 5 kg (Flemish).

2. Research Uses

The New Zealand White (albino) is the most widely used breed in Canadian laboratories. Their medium size and very docile nature make these rabbits particularly easy to maintain, handle and restrain, while their large, unpigmented ears facilitate repeated venous injections and bleeding. Unfortunately, though classed as a medium sized breed, these rabbits, with age, usually become very heavy. This results in their tending to be awkward to handle and too large for the standard cage; they also tend to consume excessive amounts of food and to develop sore feet.

Rabbits of various varieties have provided very useful models in many different areas of biomedical research (embryology, toxicology, virology, etc.), and are also widely used in safety testing (pyrogen, teratogenicity, etc.). They are universally and routinely used in serology because of the readiness with which they produce serum antibodies in response to a wide variety of antigenic stimuli. Two major disadvantages of rabbits as research animals are the difficulties encountered with anesthesia, and the fact that they are plagued by a large number of spontaneous diseases (Russell and Schilling, 1973).

B. BIOLOGY

1. General Characteristics

The rabbit's life expectancy in the laboratory or breeding colony will rarely exceed four to five years, although under natural conditions they may, particularly in the case of males, live at least twice that long (Adams, 1976).

Rabbits are alert, docile, timid animals that adapt readily to cage housing. In the wild state they are nocturnal.

The dental formula of the adult rabbit is: Incisors 2/1, canines 0/0, premolars 3/2, molars 3/3. These teeth are peculiar in that they all exhibit continuous growth. Growth of the incisors is particularly rapid, up to 12 cm/yr. The incisors are worn down by normal chewing provided that occlusion is normal (Harkness and Wagner, 1983).

Average body temperature is 39.5°C (range 38.5-40°C) which usually fluctuates considerably with excitement caused by handling, even when other signs of disturbance are not obvious.

Rabbit urine is normally thick and cloudy, and contains crystalline material. Urinary sediment tends to accumulate on the excreta trays.

Summaries on physiological, biochemical and hematological data for the rabbit may be found in the Appendices to Volume I of this Guide.

2. Nutrition

Rabbits are herbivorous and may, under normal conditions, be very adequately maintained on any one of the numerous complete pelleted rations widely available commercially. Fresh water should be provided daily and *ad libitum*. Hay supplements may be provided.

Reingestion of feces (coprophagy) is a normal practice amongst rabbits and is important, indeed essential, to maintain adequate nutrition and normal intestinal physiology (Thacker and Brandt, 1955). Nutrient requirements of rabbits are well established and have been published; they should be consulted when semi-purified or purified diets are to be prepared (NRC U.S., 1977; Hunt and Harrington, 1974).

3. Reproduction

Spontaneous ovulation does not occur in the female rabbit (doe). Coitus is normally required to induce ovulation. Because of this, there is no defined estrus cycle in this species. Under optimal conditions the mature doe will remain ready to accept the male (buck) indefinitely, and will have a constant but fluctuating number of ripe (graafian) follicles ready to rupture if coitus occurs. Whether or not the doe is actually ready to mate can be determined by the state of her vulva, which in heat should be enlarged and somewhat red, and by her immediate behavior when placed with a buck.

Generally, the doe is taken to the buck's cage for breeding. Semen collection and artificial insemination techniques have been described (Bevin and Timmons, 1974; Adams, 1972). The pregnant doe should be housed individually in a quiet area, and provided with an enclosed nesting box in which she will make a hairlined nest for her naked born litter. Summaries of reproductive data are available in the Appendices to Volume I of this Guide.

C. ACQUISITION

1. Procurement

Rabbits that are to be introduced into a laboratory colony, either for research use or as replacement breeding stock, should always be ones of known and assured genetic quality. This requirement necessitates dealing with reputable breeders who maintain proper records on such matters as the origin of breeding stock, mating system(s) used, animal identification, and other information pertinent to maintaining a genetically defined population. The supplier's facilities, caging, husbandry and management should be consistent with the principles laid down by the CCAC. The rabbits should be healthy, and free from overt signs of disease or parasitism. It is always advisable, insofar as it may be possible, to use a single, reliable supplier for all replacement stock.

2. Transportation

Newly acquired rabbits should have been transported to the research facility in disposable containers with sufficient space to allow the animals to stand, lie down and turn around. A source of water and food, sufficient for the duration of transportation, should have been provided; carrots are often used for this. Generally speaking, rabbits tend not to travel well over long distances and periods of time; thus it is often advantageous to use local suppliers.

3. Quarantine and Conditioning

Newly acquired rabbits should be held in quarantine for at least three weeks, and examined regularly for disease, for which they should be treated as necessary. The quarantine period may also serve as a conditioning period to acclimatize the animals to the institution's facilities, husbandry and feeding (dietary) practices. All animals dying during this period should be subjected to complete postmortem examinations.

D. HUSBANDRY

1. Housing and Environment

Adult rabbits are housed individually in metal, preferably stainless steel, cages with wire mesh floors, with excreta collection trays below. The latter should periodically be treated with acidic compounds to remove the sedimentary accumulation that is peculiar to rabbit urine.

Space and basic environmental requirements are available in Appendix I, Volume I, of this Guide. It must be emphasized that adequate, free ventilation is vital to maintaining rabbits free of respiratory disease.

Routinely, a 12-14 hour light schedule will prove satisfactory for the research colony. Breeding colony females should be provided with 14-16 hours light, with 8-10 hours recommended for the males (Harkness and Wagner, 1983). Sudden switching on of lights without prior warning should be avoided during

the dark period as it may cause consternation and even spontaneous ovulation and occasional self injury. Similarly, sudden, loud noises should be guarded against, particularly in the breeding colony where they may interfere with estrus and maternal behavior.

2. Management

The caging should be washed at least once a week. Regular cleaning and sanitizing of racks, equipment and room surfaces should also be regularly undertaken. Excreta trays should be cleaned as often as necessary to minimize ammonia buildup in the rooms. Disposal of dirty bedding and cleaning of excreta trays should not be done inside the animal room.

Fresh food and water should be provided daily. All animals should be observed at least once daily, with food and water consumption and nature of excrements being noted. Sick or dead animals should be removed immediately, with the latter being handled in accordance with the provisions of the experimental protocol.

E. HANDLING AND RESTRAINT

1. Handling

When removing a rabbit from a cage, or picking it up, the loose skin over the shoulders can be grasped with one hand, the other hand also grasping the loose skin along the back (in large rabbits) or supporting the abdomen. Rabbits should never be picked up by the ears as these are easily hurt and injured; the ears are sensitive organs that play a role in body temperature regulation, as well as hearing, in animals of this family. Rabbits can safely be carried in the crook of the arm with the rabbit's head tucked in behind the elbow, while the forearm supports the body and the hand is placed around the rabbit's rump.

Improperly handled rabbits may struggle furiously, and in doing so may injure themselves or the handler. It should be remembered that the bones of the rabbit are extremely light and brittle and consequently fracture easily. Traumatic injury to the spine of the rabbit may occur during violent struggling, resulting in a broken back (usually lumbar vertebrae) with posterior limb paralysis and urinary and bowel dysfunction. Such rabbits should be humanely killed as soon as possible (Flatt, Weisbroth and Kraus, 1974). The strongly muscled hind limbs and sharp claws can inflict deep scratches on the hands and arms of the handler, and it is recommended that long sleeved laboratory coats be worn while handling rabbits.

2. Physical Restraint

A variety of restraint devices (rabbit boxes, rabbit boards) are available commercially for rabbit restraint while injecting, bleeding or performing other non-painful manipulations. These restraint devices must be used with care on rabbits not accustomed to them; however, the acclimatization period is usually short, particularly for the more docile breeds (N.Z. White, etc.). For short holding and simple procedures, rabbits can often be restrained by

wrapping a towel snugly around the animal's body, ensuring that the legs are contained.

A state of "hypnosis" or tonic immobility may be induced in rabbits placed in dorsal recumbency and held in that position. Animals so immobilized exhibit reduced responses to stimuli. Because it is unclear whether there is a reduced response to pain during "hypnosis", it should not be used as an alternative to adequate anesthesia (Bevin and Timmons, 1974; Carli, 1977).

F. SAMPLING AND MANIPULATIONS

1. Marginal Ear Vein Puncture

Marginal ear vein puncture can readily be used to obtain venous blood samples. The hair directly over the vein is plucked or shaved and 70% alcohol is applied to clean the area and wet the surrounding hair, making visualization of the vein easy. Petroleum jelly is then applied to the site. To distend the vein, the venous return is occluded by digital pressure on the marginal ear vein at the base of the ear or by applying a paper clip across it. A small nick may be made with a scalpel through the vein, from which blood may be collected directly into a pipette or into a tube held below the cut. Care must be taken not to cut through the entire ear edge while making the nick in the vein.

Where large amounts of blood are required, or in breeds in which there may be difficulty visualizing the marginal vein, a small amount of xylene (xylol) swabbed on the tip of the ear will result in a markedly increased blood flow, caused by local irritation. The xylene should be washed off with 70% alcohol at the end of the procedure in order to minimize skin irritation. A procedure for placing a vacuum bottle over the entire ear to enhance blood collection has been described (Hoppe, Laird and Fox, 1971).

2. Cardiac Puncture

Cardiac puncture on anesthetized rabbits allows for the collection of large volumes of blood. The rabbit, once anesthetized, is placed in dorsal recumbency, and held in that position or placed on a board for that purpose. The needle (18 gauge 1 1/2 in) is directed horizontally through the rib cage at a point where the heartbeat can be palpated most strongly. An alternate approach can be made medially from the xyphoid cartilage, with the needle being directed anteriorly, at an angle of 30 degrees, into the chest (Bevin and Timmons, 1974).

3. Puncture of the Central Artery of the Ear

Puncture of the central artery of the ear can be used as a technique for obtaining arterial blood samples. Care should be taken to prevent formation of an ear hematoma when sampling is completed, by applying adequate manual pressure over the puncture site until bleeding has ceased.

4. Oral Intubation

Oral Intubation for force-feeding procedures can be accomplished by using a mouth gag and a small diameter (#8 French) pediatric feeding tube (Hunt and Harrington, 1974).

5. Palpation to Determine Sex

Palpation of the genital area with eversion of the penis of the male to determine the sex of the animal may be undertaken at any age. However, if this is undertaken on the newborn, the doe should be removed until after the young settle down from the disturbance. In older animals, restraint for this procedure may be accomplished by grasping the loose skin over the shoulders in one hand, and the hind legs in the other hand. The rabbit is then turned over in the lap of the handler, with the hind legs pressed against its chest. In this way the genital area is exposed for examination. The small, slit-like vulva of the doe and the rounded, papilla-like penis of the buck, with its urethral opening positioned immediately adjacent to the anus, can be readily visualized when the skin of the perianal region is stretched.

Palpation for pregnancy should be done carefully and gently with the female sitting in a normal position on a table.

G. ANESTHESIA AND SURGERY

1. Precautions and Premedication

Anesthesia of rabbits presents some particular problems because: a) the response to the commonly used anesthetic agents is extremely variable and the margin of safety is small (Green, 1979), and, b) there is a high incidence of chronic upper respiratory disease (snuffles) which may complicate anesthesia (Bevin and Timmons, 1974; Green, 1979; Cramlet and Jones, 1976). Risk from these problems can be minimized by careful clinical examination of the animal prior to anesthesia, and by close monitoring of anesthesia.

The depth of anesthesia is monitored primarily by:

- a. the respiratory rate, and b) the pedal or toe pinch reflex. Surgical anesthesia is generally achieved when the respiratory rate is reduced to 15-20 respirations per minute (normal respiratory rate is 35-56 per minute), and the reflex limb withdrawal to a toe pinch is diminished or absent (Bevin and Timmons, 1974; Green, 1979).

Premedication with tranquilizers may be useful in calming the animal in order to facilitate induction with a general anesthetic. Tranquilization also reduces the total amount of general anesthetic required to achieve and maintain surgical anesthesia levels. Use of tranquilizers is particularly recommended prior to induction with the inhalant anesthetic methoxyflurane (McCormick and Ashworth, 1971). A significant proportion of the rabbit population

contains high levels of serum and hepatic atropinesterase, and in these rabbits premedication with atropine will prove of little value (Sawin and Blick, 1943). A list of pre-anesthetic agents with recommended dosages is presented in Table I:

TABLE I - PREANESTHETIC MEDICATION OF RABBITS^a

Drug	Dosage	Route	Effect or Response	References
Atropine sulfate ^b	0.04-0.1 mg/kg	IM IV		18
Acetylpromazine	1.0 mg/kg	IM	tranquilization for 30-60 minutes	18, 25
Chlorpromazine ^c	25 mg/kg	IM	tranquilization	12, 18
Diazepam	5-10 mg/kg	IM	sedation 60-100 minutes	12, 25
Propiopromazine	5-10 mg/kg	IM	tranquilization	18
Ketamine HCl	20-44 mg/kg	IM	immobilization ^d	26
Xylazine	3-5 mg/kg	IM	sedation	25

^aWhere these drug dosages differ from Appendix IX, X of Volume 1 of this Guide, the dosage in this table should be used.

^bSee note regarding atropinesterase levels in text.

^cIntramuscular injections produce myositis, swelling and lameness at injection site.

^dAnalgesia not sufficient for surgery.

2. **Injectable Anesthetics**

Intravenously administered anesthetic agents should be introduced slowly, in dilute form, with the total amount given being titrated to the response of the animal.

The barbiturates are not recommended for use in rabbits because their respiratory depressant action dangerously reduces the margin of safety (Bevin and Timmons, 1974). Manual pressure on the chest wall may stimulate breathing reflexes in severely depressed patients (Coates, Hogg and Thurlbeck, 1974).

Dosages and routes of injection for a number of drugs and drug combinations have been reported for use in rabbits (Table II). It should be noted that all

these drug agents affect physiological responses, and thus may adversely affect physiological, biochemical or hematological parameters under study. Investigators should familiarize themselves with the pharmacology of each drug prior to its use in an experiment, in order to avoid potential complications.

**TABLE II - INJECTABLE ANESTHETICS (HYPNOTICS/SEDATIVES)
IN RABBITS^a**

Drug	Dosage	Route	Effect or Response	References
Ketamine HCl + Xylazine ^b	35 mg/kg 5 mg/kg	IM	Sedation for 20-70 minutes	26, 27
Fentanyl/Droperidol (Innovar Vet)	0.17 ml/kg	IM	Sedation	12
Fentanyl/Fluanisone (Hypnorm)	0.3-0.5 ml	IM	Sedation- Anesthesia	27
Pentobarbital	20-40 mg/kg	IV	Anesthesia	12
Thiopental	50 mg/kg	IV	Anesthesia for 5-10 minutes	17
Thiamylal	22-54 mg/kg	IV	Anesthesia for 5-10 minutes	17
Urethan ^b	1.5 gm/kg	IP	Anesthesia onset 45-60 minutes duration 5-6 hr	17

^aWhere these drug dosages differ from Appendix IX, X of Volume 1 of this Guide, the dosage in this table should be used.

^bSuitable only for non-survival (acute) studies.

3. Inhalation Anesthetics

Halothane and methoxyflurane can safely be administered to rabbits using a face mask with a semi-open anesthetic system and an Ayers T-piece breathing system. Halothane is a potent anesthetic, and should only be used with a precision vaporizer anesthetic machine (Lumb and Jones, 1973). Induction time is prolonged with methoxyflurane, and the excitement stage

may be difficult to manage unless premedication with a tranquilizer is used (McCormick and Ashworth, 1971).

Endotracheal intubation is difficult in rabbits because the narrow pharynx makes visualization of the larynx difficult. A variety of techniques for endotracheal intubation of rabbits have been described (Star, 1979; Hoge, Hodesson, Snow *et al.* 1969).

4. **Surgery**

All experimental surgery in rabbits should be done in strict accordance with acceptable (aseptic) veterinary surgical practices and the principles as detailed under "Standards for Experimental Animal Surgery" in Volume 1 of this Guide.

H. **DISEASES AND HEALTH CARE**

1. **General Comments**

Rabbits are plagued with a wide variety of diseases that may interfere with their use in research. Diseases of the respiratory tract and intestinal diseases with diarrhea, are particularly common.

A number of the common diseases may exist subclinically in a colony, and appear as outbreaks of overt diseases following stress from transport or the initiation of an experiment. In all instances where disease is a problem, expert veterinary advice should be sought.

This section is not intended as a treatise on rabbit diseases, but rather to serve as an introduction to a few conditions that are easily recognized and dealt with by trained laboratory animal care personnel and the non-veterinary investigator. For greater detail on the subject of rabbit health care, the reader is referred to the several detailed texts available on this subject (Harkness and Wagner, 1983; Flatt, Weisbroth and Kraus, 1974).

2. **Infectious Conditions**

- a. **Respiratory Infections:** A number of disease syndromes are collectively designated as Pasteurellosis and involve infection with *Pasteurella multocida*. The most common of these is a chronic upper respiratory infection with rhinitis and conjunctivitis which is termed "snuffles". Affected rabbits exhibit a purulent discharge from the nares and/or eyes, with sneezing. Rabbits affected with snuffles generally remain in otherwise good condition; however, they should not be subjected to anesthesia. Treatment with antibiotics is usually of little value.

Pasteurellosis may give rise to middle ear infection (*otitis media*) with the eventual development of a wry neck (*torticollis*); when this develops, treatment will be of no value and affected rabbits should be humanely killed.

Rabbits should be obtained from breeding colonies that are free from *P. multocida* infection.

- b. **Intestinal Infections:** Diarrhea, as a result of intestinal infections, is common in young rabbits. Intestinal coccidiosis and Tyzzer's Disease may be involved; however, in many cases the etiology is unknown. Often the occurrence of a stressful situation, such as the initiation of an experimental protocol, will precede the onset of intestinal dysfunction. In such cases veterinary expertise should be called upon and an attempt made to determine and eradicate the cause.
- c. **Ear Mange:** Infection of the external ear canal with mites (*Psoroptes* sp., *Chorioptes* sp.) is a common condition amongst rabbits. The presence of typical brownish-grey, scaly debris in the external ear canal is indicative of ear mange. Severely affected ears may be quite painful, and should be handled carefully. Routine examination and treatment of affected animals will minimize the infection even in those colonies where the mites are endemic. Treatment consists of application of mineral oil or a mineral oil/acaricide mixture to the external ear canal of affected rabbits.

3. Physical Diseases

- a. **Dental Malocclusion:** Malocclusion and overgrowth of the incisor teeth has a hereditary basis in rabbits. Animals exhibiting this defect should not be used for breeding. Routine examination of rabbits should include examination of the teeth. The overgrown incisors should be clipped periodically to permit proper feeding. Untreated overgrowth of maloccluded incisors will lead eventually to death from starvation.
- b. **Sore Hocks:** Longterm caging on wire mesh floors may result in the development of pressure sores on the plantar surfaces of the feet, especially in large, obese and older rabbits. Temporary relief may be provided by placing a solid platform in the cage; however, these animals should be culled as soon as possible.

4. Health Care

- a. **Preventive Measures:** The introduction and maintenance of a sound sanitation program and the regular examination of all rabbits will be of great help in minimizing diseases in a rabbit colony. A policy of purchasing healthy rabbits from a reputable, and preferably from a single source is particularly helpful.

The treatment of infectious diseases amongst rabbits tends to be frustrating. The immediate removal of sick animals to an isolation area and dead animals from the colony room will aid in limiting the spread of infections.

As mentioned, where infectious disease appears to be a problem, veterinary expertise should be called upon without delay.

- b. **Antibiotic Sensitivity:** Rabbits appear to be sensitive to the penicillin antibiotics (Boyd, 1960). Administration of penicillins may result in a disturbance of the normal intestinal microflora and produce diarrhea. Ampicillin appears to be particularly toxic to the rabbit (Milhaud, Renault, Vaissaire *et al.* 1976).

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