Part 5

MSUM IUCAC
Basic Principles of Laboratory Animal Care

Rats

Numerous species of animals serve as subjects for education demonstration and research. However, the laboratory rat seems to be the most used subject. The rat's small size and excellent health make it a superior choice for experimentation. The following presents information about anatomy, general physiology, general care, and breeding of rats as well as euthanasia techniques used on rats.

Anatomy

Many texts of rat anatomy are presently available. These range from early line drawings of gross anatomy and morphology (Wills, 1964; Smith and Calhoun, 1968) to a plate atlas of rat gross anatomy (Olds and Olds, 1979). The latter atlas is a particularly useful atlas of the peripheral anatomy of the rat. In addition, several atlases describe the central nervous system of the rat in stereotaxic coordinates. These include the early line-drawn atlas of deGroot (1959) and the photographically enlarged plates of Konig and Klippel (1963), Pellegrino and Cushman (1967), Skinner (1971) and Thompson (1978).

Physiological Data for the Rat

Table 1 presents average values for several physiological variables of the adult rat. These average values are intended as general information about the rat; most important among these are the values for body weight, food intake, sexual maturity and body temperature.

Table 1. Normative physiological values for the adult rat.

Adult Weight	
Male	300-400 grams
Female	250-300 grams
Life Span	
Usual	2.5-3 years
Maximum Reported	4 years, 8 months
Surface Area	0.03-0.06 sq. meters
Water Consumption	80-110 ml/kg/day
Food Consumption	100 gm/kg/day
Body Temperature	99.5 degree F (37.5 C)
Puberty	50 +/- 10 days
Breeding Season	None
Gestation	21-23 days
Litter Size	8-14 pups
Birth Weight	5-6 gm
Weaning	21 days
Heart Rate	330-480 beats/mn
Blood Pressure	
Systolic	88-184 mm Hg
Diastolic	58-145 mm Hg
Cardiac Output	50 (10-80) ml/min
Respiration Frequency	85.5 (66-114)/min
Urine	
pH	7.3-8.5

Specific Gravity	1.04-1.07

Table adapted from: H.J. Baker, J.R. Llndsey and S.H. Weisbroth (Eds). (1979) The Laboratory Rat, New York: Academic Press.

Housing

Several organizations have developed explicit guidelines to regulate the care and housing of rodents used in the laboratory (Author, 1978; NIH Guidelines, 1984).. According to the National Academy of Sciences (National Research Council), rats should be housed in spacious, ventilated, and dry cages constructed of either plastic or stainless steel. Bedding (wood shavings, sawdust or commercial litter such as Beta-Chip) should be changed frequently (2-3 times weekly) to minimize odor and to reduce the possibility of disease in the colony. Cages should be sterilized frequently with steam or chemical disinfectants. Humidity within the colony room should be maintained between 40 and 60 percent whereas the temperature should be kept between 70 and 74 degrees F. Lighting should be diffuse throughout the colony and of an intensity (75-125 footcandles) sufficient to allow laboratory procedures to be carried out. Light schedules should be diurnal (i.e. 12 hr/12 hr day/night schedule) because continuous lighting schedules may produce partial retinal degeneration in rats. Tap water and nutritionally complete feed such as Purina Rat & Mouse Diet should be freely available from water bottles and feeders suspended outside of the cage except where inconsistent with the experimental procedures. While it is recognized that not all rat facilities can meet these stringent guidelines, you should be aware of their existence and strive to meet this standard of care.

Handling

Rodents that are individually housed over a long period frequently display increased emotionality (urination, defecation and, perhaps, aggression) upon handling. Each rat should be weighted to the nearest gram on a balance (scale) daily to assess its general health and to provide daily handling. You should be aware that rapid weight losses (e.g. 10-20 grams overnight) are frequently the first indicator of disease. To remove a rat from a cage, grasp the rat with your thumb and forefinger around the neck preventing biting movements by the rat. If the rat grasps the cage floor with its paws as you attempt to remove it, do not exert excessive pull to remove the rat as a toenail may be torn. Practice picking up the rat, holding it and then replacing it into the home cage. Body handling is the preferred method of handling rats. If tail handling is done, you should pick up the rat by the base of the tail (that part of the tail closest to the body). This method is not recommended for novices, however, as grasping the tip of the tail will frequently shear off the flesh surrounding the tail tip.

Animal Identification

Although rats are frequently group housed for reasons related to available space and expense, experimenters must keep track of individual animal data. Some method, therefore, must be used to identify individual rats. The simplest technique is to place a numbered metal tag in the ear (National Band and Tag Co., 721 York Street, Newport, KY 41071). Other investigators prefer to use an ear notching system in which a punch is used to notch the rats ears using the system illustrated in Figure 1. In this system, one ear is used to represent single digits whereas the other ear is used to represent 10's. A particular number is represented by where on the ear a notch or hole is made. Dyes may be used to identify individual rats. These include India ink on the palmar surface or the inner surface of the ear. In addition, either picric acid (yellow) or carbolfuchsin (red) or an indelible felt-tipped pen can be used to stain the fur on the rat's back. The dyes are typically prepared as 1-5% solutions in 70% alcohol and are applied to the fur using dye-soaked cotton tips.

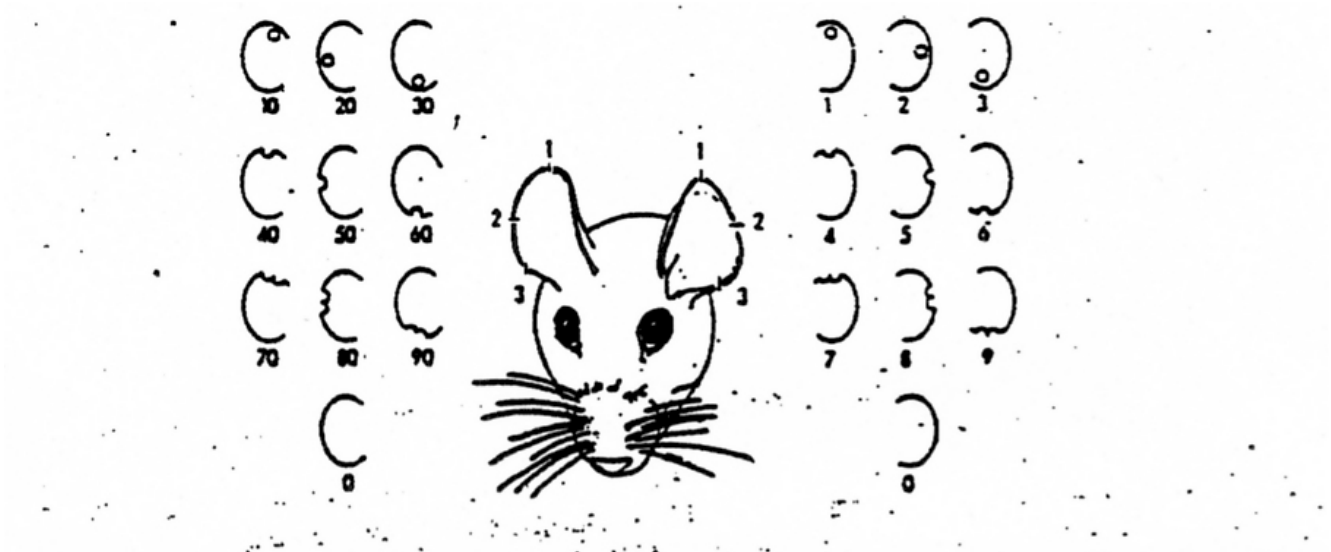

Figure 1. An ear notch system used to identify individual rats. The left ear is notched to represent single digits whereas the right ear is notched to indicate 10's. Adapted with permission from Kraus, A.L. (1980) Research Methodology. In: H. J. Baker et al (Eds.), The Laboratory Rat , Volume 2, New York: Academic Press.

Breeding

The Classic work of Long (1922) described the estrous cycle of the female rat and its clinical characteristics. The rodent estrous cycle is approximately 4 days in length and consists of 4 stages: proestrus, estrus, metestrus and diestrus. Maximal sexual receptivity of the female accompanies estrus, which in the rat occurs 24 hours into the cycle and is indicated by a dry vagina and swollen vulva. Because the estrus cycle is associated with regular changes in the cell type found within the vaginal fluids, cervical smears can be taken daily to estimate the occurrence of estrus. To do so, insert the blunt tip of a disposable pipette containing 1.0 ml of saline into the vagina of the female to be examined. Expel the saline and then a few minutes later, reinsert the pipette and withdraw .25 ml of vaginal fluld. This sample can be smeared on a microscope slide, dipped into 100% alcohol, air-dried and then stained by dipping into a 5% solution of Giemsa stain (Sigma Chemical). Clear the slide by dipping into distilled water, air-dry and then examine the cells on the slide using a light microscope. Using this method, estrus is indicated by the presence of large cornified cells in the vaginal smear (See Figure 2). Other clinical signs of estrus include an ear quiver response induced by stroking the head and back (Farris and Griffith, 1949) or the lordosis response (arched back) induced by manual stimulation of the vulva using a cotton swab.

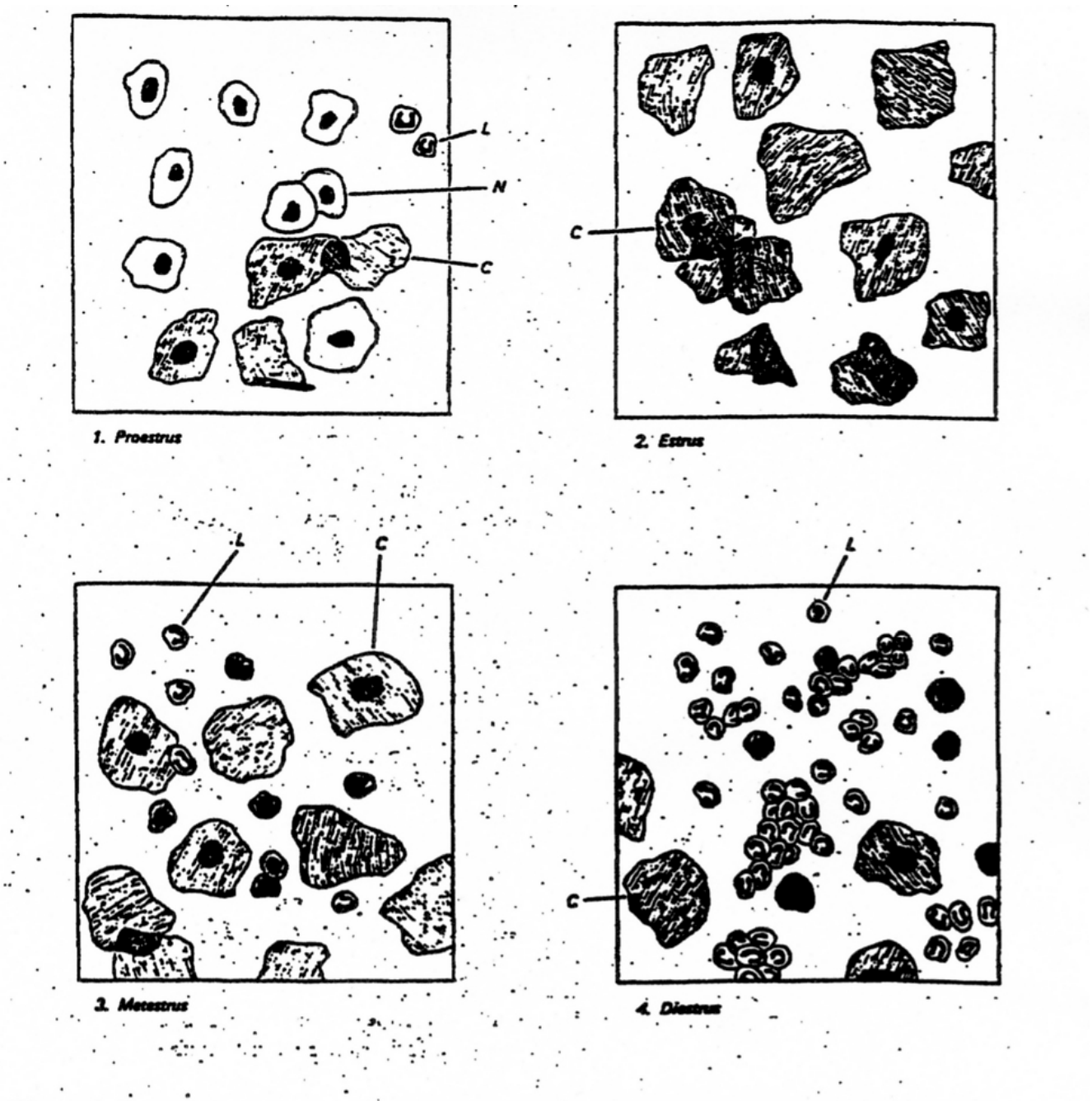

Figure 2 Cell types (C, cornified; L, Leutenized) associated with various stages of the estrus cycle. Reprinted with permission from: Adler, N.T. (1976) Induction of pregnancy in female rats by external stimulation. In: Hart, B.J. (Ed) Experimental Psychobiology, San Francisco: W.H. Freeman, Co.

Placement of an adult female rat into a cage with one or more adult male rats for a 6 day period will result in detection of sperm in the vaginal tract and pregnancy (Baker, 1980). Sperm can be detected in the vaginal smear (using the technique described above without staining the vaginal smear) or one can examine the bedding of the rat cage in search of the so-called vaginal plug (a dried mass of sperm and vaginal secretions) that is dislodged from the vagina after

successful copulation. To prevent cannibalism of the offspring by the male, the pregnant female should be isolated in a large cage provided with adequate amounts of food, water and bedding. Gestation in the rat is approximately 21-23 days. The abdomen of a pregnant female rat is distinctly swollen at 13 days of gestation. This is most easily observed by suspending the rat vertically by the tail. Litter size is approximately 8-14. The number of male and female pups are approximately equal in most litters. If the litter size is large (12 or more pups), the litter size should be reduced or culled to 8-10 pups. The pups that are to be euthanized are those that are smallest for their sex. To determine pup sex, one can use the ano-genital distance as an indicator of sex (Myer, 1971). In general, male pups exhibit a larger distance between the anus and the genitals than do females (see Table 2). Moreover, female pups may display rudimentary nipples at about 9-15 days post-partum. Litters should be weaned (i.e. removed from the mother) between 23 and 28 days post-partum with pups placed into either individual or group cages with chow and water freely available. If facilities are not available for breeding, commercial breeders supply rodents of either sex and a given weight range. Moreover, special surgical procedures (i.e. ovariectomy, hypophysectomy etc) are often available from the breeder for a nominal charge.

Rat Diseases

Great advances in the production of disease-free laboratory rats have been made in the last two decades. Commercial breeders often derive their stock using barrier techniques in which a litter is delivered by caesarean section and raised in a germ free environment. Such rats are remarkably free from diseases of the respiratory and digestive tracts. Upon arrival in the laboratory, however, rats obtained from commercial suppliers should be placed in quarantine for a 7 day period. If skin sores indicating lice or mites are observed, commercially available powders such as Dichlorovose or Equigard (the latter is placed on the cage bedding) will control lice and mites. Viral or mycoplasmal infections may produce upper respiratory difficulties in rats, often indicated by a chattering or wheezing sound. Such infections are highly contagious. Because antibiotic treatments do not readily reverse these conditions, infected animals (or whole colonies) should be euthanized (killed) and the colony housing cages should be disinfected with steam or chemical disinfectant. Another common disease observed in rats is labyrinthitis, a bacterial infection of the middle ear. Infected rats display a marked twisting of the body when suspended by the tail. No therapy is available for this disease; infected animals should be euthanized and the colony disinfected. If you are concerned about the health of animals in your care, you should alert your laboratory instructor.

Table 2. Average ano'genital distance (mm's) in rat pups.

Age	Male	Female
Newborn	2.8	1.2
7 Days	5.2	2.7
14 Days	8.2	4.9
20 Days	12.0	7.0
42-50 Days	21.0	13.0

Table adapted from: Myers, R.D. (Ed), (1971) Methods in Psychobiology, Volume 1, New York: Academic Press.

Animal Euthanasia

Care should be taken during the course of an experiment that animals are not subjected to unnecessary pain or discomfort. Rats that undergo surgical procedures must be rendered incapable of feeling pain via suitable anesthesia. Certainly, the same principle applies to animals that are to be sacrificed or euthanized at the end of a study. The term euthanasia means "good death". Methods of euthanasia should result in a rapid, painless, and humane death for an experimental subject. An accepted technique of euthanasia for rats is lethal injection of pentobarbital (80 mg/ml/kg, intraperitoneal). Such injections rapidly produce unconsciousness and then death. Although this technique may not be suitable for experiments in which biochemical samples are to be collected after death, it is the most humane. Other techniques are described below. They vary in terms of method (physical vs inhalant) and rapidity of death.

Decapitation

A physical euthanasia procedure is that of decapitation using one of several commercially available guillotines. The rat's head is carefully introduced between the guillotine blades and then separated from the trunk using a rapid movement of the guillotine arm. This procedure may be used to collect large (approximately 5 ml) blood samples or to collect tissue samples in a way that is not compromised by chemically-induced euthanasia. It should be noted, however, that decapitation is not a routine euthanasia technique. Moreover, recent electroencephalic data suggests that decapitation may not produce death as rapidly as once thought (Mlkeska and Klemm, 1975).

Carbon Dioxide

Placement of rats into a chamber containing highly concentrated carbon dioxide will result in unconsciousness and then death. This technique is often used to euthanize large numbers of rats and is thought to be more humane than decapitation. Inexpensive carbon dioxide euthanasia chambers are described in the literature (Myers, 1971; Kraus, 1980).

Other Inhalant Gases

Ether, chloroform, halothane and metofane have been used in high concentrations to euthanize rats. Ether and chloroform, although inexpensive, are rather caustic to the lungs of the rat and may result in a painful death. In contrast, halothane and metofane are not caustic, are somewhat rapid but can be prohibitively expensive. Moveover, these gases may be harmful to hepatic function in laboratory personnel that are repeatedly exposed to these gases.

Carcass Disposal

Death should be verified (absence of heart rate, cool body and rigidity of the body) in any euthanized animal prior to its disposal. Again, if you are in doubt, consult your instructor. Each carcass should be double-wrapped in plastic bags and tagged as to their source. Disposal of carcasses should be carried out according to IACUC recommended practices.

Mice

Weight: The average mouse weighs less than an ounce (30 grams) and can live up to three years.

Handling: If you are not accustomed to handling mice, it is advisable to wear a snug fitting glove when handling them. To move a mouse, pick it up by its tail with your thumb and first finger grasping it close to the body (if taken by the tip, the whole skin covering may come off). Make sure to leave the mouse in a head down position for only a few seconds, otherwise the mouse may turn, crawl up its own tail, and bite you. To

restrain a mouse, grasp by the base of the tail, place it on a surface that it can grip with its froe feet so that you can stretch the body out, then grasp a fold of the loose skin over the neck and shoulders just tight enough to keep the mouse from turning it's head and biting.

Housing: Mice are normally kept in cages measuring six by twelve by six inches high (15x30x15cm) with a solid bottom. The floor is usually covered with one to two inches of woodchips, ground corncobs, sawdust, hay, or any other material available (do not use newsprint, the ink may make the mice sick). The bedding should be changed at least once weekly.

Feeding: Mice are nocturnal animals and tend to feed more at nights, however, it is advisable for laboratory animals to have food and water ad libitum (except where experimental procedures dictate otherwise). When using pellets, make sure they small fresh and clean. If they smell musty and moldy, the pellets are old and shouldn't be used. Water bottles should be washed and replaced at least once weekly. Diets can be supplemented with snacks such as cracked corn and/or oil sunflower seeds.

Breeding: Female mice are old enough to breed at two months and can have up to seventeen litters a year with an average of five to ten babies per litter. Her reproductive cycle (estrous cycle) makes it possible for her to accept a male every fourth day and so mating occurs often if the male and female are continuously kept in the same cage. More than one female may be kept in one cage with a male (as long as each female has her own nesting box), but it is not recommended to keep two or more males in one cage. When multiple males are housed together they will fight to establish dominance. The Gestation period in mice lasts nineteen to twenty one days. Immediately following birth, babies are tiny, helpless, and hairless weighing approximately one gram. Their eyes are sealed for the first two weeks of life. After they have been weaned, male babies should be placed in separate cages, but female babies can remain together.

Diseases:

Bite wounds: Bite wounds will occur between fighting males or overcrowded females. Treat them with a small amount of antibiotic ointment but be careful not to use too much or the mice will eat it when they groom themselves.

Antibiotic Toxicity: Mice are sensitive to streptomycin which can kill them if injected.

Respiratory Infections and Pneumonia: Mice suffering from respiratory infections and pneumonia sit in a corner with fur ruffled up. Discharge from their nose, and chattering noise as they breathe may also be observed. -Antibiotics such as chloramphenicol or tetracyclene can be given orally (5mg). twice daily for 4 or 5 days. A 250 mg. capsule of antibiotic can be dissolved in a cupful of water (240cc) and placed in their water bottle. A half teaspoon of sugar can be added to sweeten the water. They should receive 4 to 5 mg of antibiotic daily with this procedure and should be kept on the medication for 5 days.

Diarrhea: Diarrhea may result from improper diet, spoiled food or internal parasites. Replace pellets if they smell musty or smell/look moldy. If there is no improvement in 24 to 48 hours, collect a sample of stool in a plastic vial and have the Vet examine it for internal parasites. If there are parasites the Vet can prescribe the appropriate medication.

Tumor: A tumor is any unusual lump or swelling growing on or in the mouse. Mice are susceptible to tumors and they are common in middle aged and older mice. Some can be removed surgically. Many are malignant and

have probably spread extensively before being observed. These mice need to be euthanized to prevent suffering.

Fur chewing: Fur chewing is an indication that the mice are too crowded. Over crowded mice will chew the fur off each others faces. If this occurs they should be placed in separate cages or in larger cages.

Mites, fleas, lice: Mites, fleas, and lice are not commonly seen in mice. If found, their fur should be treated by applying a rotenone-based flea powder weekly. A Shell pest strip containing Vapona may also be placed on top of the cage one night weekly. The strip should not be left on the cage continuously or placed where the mice can chew on it or lick it.

Rabbits

Weight: Rabbits range from 2 to 20 pounds and can live for up to 15 years. They are born with closed eyes which do not open until about ten days after birth. Bunnies should be weaned at about 4 weeks old.

Handling: To pick up a rabbit, take hold of a handful of loose skin over the shoulders. As you start to lift , slide the other hand under its hind legs and support the body weight by allowing the rabbit to stand on your hand. It is very important that you put your hand under its legs before lifting the rabbit too high. If you fail to do so, it could cause serious damage to the rabbit. The reason for this is if it becomes startled, it will kick its hind legs and possibly break its back. This is particularly true for adult Jack rabbits. Under no circumstances should you grab a rabbit by the ears. It is painful for the rabbit and during the struggle it could damage the fragile blood vessels in the ears. Also, do not grab the rabbit with both hands around its middle. Rabbits do not like this and will try to escape. As they struggle to escape, they may scratch you and can injure their back. In the event that a rabbit is being given sub-cutaneous injections in the back which may produce sores, the animal can be cupped to the chest of the handler using both hands. However, special care has to be taken to prevent the animal from scratching the handler and/or injuring it's back if it struggles to escape.

Housing: A rabbit must have a hutch or a cage for its own security. An adult rabbit weighing 8 to 12 pounds requires at least 1 square yard (1 square meter) of floor space. A rabbit can tolerate cool much better than hot. The hutch should be protected from the sun and from wind. Bedding should be changed 2-3 times per week.

Feeding: Rabbits are herbivorous animals. They eat most types of greens, hay, and vegetables. There are also complete diets available in pellet form. This is the preffered diet in most animal research labs. Fresh, clean water must be available at all times. A rabbit will drink 1-2 ounces of water per pound per day. A salt block or ring can be provided and the rabbit will lick to obtain salt as needed. Rabbits teeth grow continuously so they must chew to keep them down. Providing them with hard wood blocks or dry hay or grass will provide for tooth wearing. Feeding utensils and dishes shoud be kept clean. Wash as needed but they should be washed at least once weekly. Cages should also be cleaned once weekly. All rabbits eat some of their droppings. In the early morning they produce softer pellets covered with mucus, which they swallow as it is passed from their anus. This is a form of recycling called Coprophagy. Disgusting though it may seem to us, the rabbit obtains vitamins produced by intestinal bacteria and additional protein broken down by these bacteria. Thus Coprophagy serves an important function in rabbit nutrition.

Diseases: Singly housed rabbits have few disease problems, however there are a few

commonly seen diseases.

Ear Mites: These are small insects that live and reproduce in the ear canal.

Symptoms: The infection will produce a smelly, dark-colored discharge. The ear will droop, and the rabbit will scratch its ear and shake its head often.

Treatment: Rub mineral oil or olive oil on both canals twice a week for a month.

Diarrhea: Overeating of greens the rabbit is not used to can cause diarrhea. Cut back on the amount of greens offered. Virulent infections can also cause diarrhea in young rabbits. This is usually fatal.

Paralysis of the rear legs:

Symptoms: The rabbit is unable to use its hind legs and will drag itself along the floor. This is usually the sequel to improper handling, which resulted in the rabbit tearing muscles in the back or fracturing the back. Place food and water close by where the rabbit can reach them without much movement.

Treatment: If the rabbit does not get better in three weeks from keeping still, euthanasia is the humane course to follow.

Heatstroke:

Symptoms: When it is hot and humid, the rabbit may begin to pant and drool. If it is not cooled down, it will go into a coma.

Treatment: If this happens, set up a hose to spray a fine mist or set a tray of ice in the cage when it is hot and humid to prevent it from happening.

Buck Tooth or Malocclusion:

Symptoms: If the rabbit does not have anything to chew on, its teeth will become long and twisted and the rabbit will not be able to eat. There is also an inherited condition in which the rabbits lower incisors can grow out in front of the uppers. If there is no wear the teeth will grow overly long.

Treatment: Trimming the teeth with a cutting pliers if they get long, or Just providing chewing materials should give relief.

Pregnancy Toxemia:

Symptoms: This is a condition that sometimes appears in overweight does at or shortly after kindling. It can result in death even with veterinary treatment. The doe is found lying comatose in the hutch.

Treatment: You should first take it to the Veterinarian. There it will be treated with 10ml/50 percent glucose solution intravenously. If the vet. is not available, and you are forced to treat the rabbit yourself, give the rabbit a simple sugar solution orally. Honey can usually be used as a simple sugar (5cc honey In 30 ml water). Insert a plastic tube (**1/8 or 1/4** inch [3-6mm] by 12 Inches [36cm]) down the rabbits throat in to its stomach. Use a large syringe to inject the liquid in to the tube. If you do not have access to a syringe you can use a plastic food dispenser (ketchup or mustard bottle) or a turkey baster. Lay the

doe on her left side with her head pointed to her right. Measure from the rabbits last rib to its nose and mark the distance on the tube. Insert the tube into the rabbits stomach until the mark on the tube reaches the rabbits nose. The tube can be lubricated with margarine or butter to facilitate its insertion. Make sure to push it gently into the back part of the mouth and if it hits an obstruction, withdraw a little way and start down again until it slides down the throat and the mark on the tube reaches the rabbits nose. If the rabbit is still alive but not up and around in about an hour, repeat the procedure. If it recovers, a solution of one teaspoon of sugar per pint of water should be placed in its waterer for five or six consecutive days.

Upper Respiratory Infection: This is usually a bacterial infection, is frequently caused by a Pasteurella organism and is called snuffles. If left untreated, it can lead to middle ear infection, pneumonia and death.

Symptoms: The symptoms of upper respitory infections are: sneezing, coughing, and mucus discharge from eyes and nose.

Treatment: This can be effectively treated with 80 mg/kg of an antibiotic/day (Panmycin), which should be divided into several doses over the day.

Internal Parasites:

Symptoms: The rabbit can get roundworms, tapeworms, and coccidia.

Treatment: If you see what looks like parasites being passed by a rabbit, collect several samples of the stool in a plastic bottle and take them to the Vet. He or she will identify the parasites and prescribe the appropriate medication.

Guide for the ethical treatment of fish, amphibians and reptiles for research purposes

We generally follow the guidelines developed by consensus among conscientious members of the Animal Behavior Society (published *Animal Behavior*, 1998), the Fisheries Society of the British Isles (published in the *Journal of Fish Biology* 2006) and the American Society of Ichtyologist and Herpetologist (www.asih.org, 2004).

1. Choice of species and non-animal alternatives
   a. Investigators should avoid species that are rare, endangered or listed as species of concern unless the study purports to contribute to species conservation.
   b. Preference should be given to species that are well suited for captivity to minimize stress induced by captivity.
   c. The species should be well-suited for the study question at hand.

2. The number of individuals.
   a. The number of animals used should be the minimum that are sufficient to accomplish the stated research goals.
   b. Animals collected from the wild should not deplete or detrimentally impact natural populations.

3. Procedures

a. Ethical treatment of fish, amphibians and reptiles assumes that these animals are sentient and sufficiently complex to be cognizant of pain and suffering. Therefore, researchers should take the same general measures followed to minimize pain and suffering in warm-blooded vertebrates.
b. Field studies:
    i. Investigators must always consider and minimize the effect of their study on the natural resident population and the habitat on which it depends. Researchers bear the responsibility of acquiring knowledge of the local habitat and population in order to minimize their effect on the natural population.
    ii. Trapping: live traps should be checked frequently ("frequently" depends on the biology of the species)
    iii. Marking and telemetry: ensure that removed tissue does not affect animal's survival and general killing:
c. Aggression, predation and intraspecific killing:
    i. Reasonable measures should be taken to minimize harm to study animals, including the use of models in lieu of staged encounters with predators, and pre-determined end points.
d. Aversive stimuli, deprivation and motivation:
    i. Care should be exercised to ensure that levels of manipulation are no greater than necessary to produce the desired effect.
e. Social isolation or crowding:
    i. An understanding of the natural behavior of an animal is necessary to minimize these sources of stress. Some species are stressed by crowding while others are stressed by social isolation.
f. Deleterious conditions:
    i. Inducement of disease, increase in parasite load, exposure of animals to pesticides and homeostatic stressors should address possible treatment or alleviation of the condition induced.

4. Housing needs vary for each species. The investigator bears the responsibility to ensure adequate space, shelter, food and water, photoperiod, temperature and hygienic living conditions for their study animals. There is not usually any reason to house each species in separate rooms.
5. Safety: all facilities housing aquatic animals should be equipped with ground fault interrupter circuits to protect against electrical shock.
6. Water quality assurance: the single most important parameter to the health and welfare of fish and amphibians is proper care of water quality.
    a. Dechlorination: city tap water contains chorine and chloramines. These are both highly toxic to fish and must be removed using either a dechlorinating agent or a filter (e.g. charcoal tower.)
    b. Filtration: water filtration can be achieved through a variety of means. There are two main types of filtration: mechanical and biological. Coarse media such as sponges of polyester cartridges mechanically remove suspended particles from the water. Filtration media become clogged with debris and must be inspected regularly and cleaned and/or replaced as necessary. Undergravel filters, sponge filters and trickle filters create large surface area for *Nitrosomonas* and *NItrobacter* bacteria to colonize. These bacteria biologically oxidize ammonia (highly toxic) to nitrite (highly

toxic) to nitrate (low toxicity). Regular water changes remove nitrate and prevent it from accumulating.

c. Water replacement: The general rule of thumb for water replacement is 10% replacement per week. A gravel vacuum should be used to remove feces and uneaten food from the tank floor when siphoning out water during a water change. Turtles and *Xenopus* may require more frequent water changes depending on stocking density and tank volume.
d. Temperature: Depending on the temperature range of the species, heaters or chillers may be required.

7. Feeding: regular feeding

    a. Adequate amount: poikilotherms eat small amounts of food. Excess food fouls the water and clogs the filtration system.
    b. Adequate frequency: for many species, one feeding per day is adequate. More than three feedings per day runs the risk of over-feeding and should usually be limited to larvae.
    c. Appropriate food type: most fish eat generic flake food, but wild fishes in particular (e.g. stickleback, darters, mudminnows, etc) refuse flakes and must be fed specialize food (e.g. brine shrimp)
8. Holding conditions
    a. Appropriate density: crowding increases stress, suppresses the immune system and increases the rate of transmission of parasites and pathogens.
    b. Appropriate availability of refuge: if aggressive individuals are housed together then refuge is required to protect the subordinate individuals from injury or death.
    c. Lighting: fish should not be exposed to constant light or constant dark. A 24-hour L:D cycle of 12:12 to 6:18 should be maintained by use of automatic timers.
    d. Algae: alga is a natural and beneficial part of the natural environment. Algae should be scraped clean of the front viewing pane so that people can easily view the fish and monitor their health and welfare.
9. Disease Control
    a. Hygiene: regular water changes (10 % per week for fish) keeps animals stress low and keeps pathogens at low density
    b. Use of quarantine tank for newly arrived organisms
    c. An antiseptic net dip to prevent transfer of disease organisms among tank
    d. When any of these signs develop: inactivity, loss of appetite, drooping fins, labored swimming, labored ventilation, bloat, red-lined rays in the fins, exopthalmia, fungal hyphae on mouth or body surface:
    e. Remove and euthanize the affected individual by cervical dislocation, or overdoes by methane tricaine sulphonate (MS222)
    f. Increase the frequency and proportion of water changes to 50% of tank volume per day until health of the tank's inhabitants stabilizes

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