

**DLAR TRAINING SERIES**  
**INVESTIGATING THE RAT AND MOUSE**  
**PRESENTED BY:**  
**THE DIVISION OF LABORATORY ANIMAL RESOURCES**  
**AND**  
**THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE**  
**WAYNE STATE UNIVERSITY**  
**TRAINING SESSION OBJECTIVES**

At the completion of this laboratory, participants should be able to:

- Confidently and safely restrain rats and mice.
- Determine the sex of rats and mice
- Be familiar with the appearance of a normal healthy animal and be able to recognize common signs of distress, injury, or illness.
- Apply methods to identify individual animals.
- Know how to obtain a blood sample.
- Know how to administer SC, IM, IP, IV injections.
- Know how to orally gavage a rat and mouse.
- Know what appearances to expect from a mouse when under pentobarbital anesthesia (we will be using pentobarbital anesthesia in this lab). It is one of several anesthetic agents which can be used in rats and mice and should be adequate for our needs today. Pentobarbital is not always the best choice of agents for routine use. Information on inhalant and other injectable agents which might be better suited for individual research projects is available through the DLAR Hospital.
- Know what appearance to expect from a rat under Ketamine/Rompun anesthesia.
- To humanely euthanize rats and mice and provide an assurance of death. (We will euthanize mice used in this lab today by using the methods of pentobarbital overdose, or cervical dislocation, or exposure to carbon dioxide. Rats will be euthanized by pentobarbital overdose or exposure to carbon dioxide. Death will be assured by performing a bilateral pneumothorax.)

**TYPICAL BEHAVIORAL SIGNS OF ILLNESS, PAIN, OR DISTRESS**

- Guarding (protecting the painful area)
- Vocalizing (especially when the animal moves or the painful area is palpated)
- Licking, biting, scratching, or shaking a particular area
- Restlessness, such as pacing and repeatedly lying down and getting up again
- Lack of mobility (with joint, colic, or gut pain)
- Failure to groom, causing an unkempt appearance
- Abnormal resting postures in which the animal appears to be sleeping or is hunched up
- Failure to show normal patterns of inquisitiveness

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I. Physical Exam

<u>OBSERVATIONS</u>	<u>RATIONALE/AMPLIFICATION</u>
1. Behavior in its own cage.	1. Animal should be active, curious, able to use all four legs.
2. Physical condition.	2. Animals weight should be proportional to age and sex and documented as normal, obese, or emaciated.
3. Hair coat.	3. A ruffled coat may indicate illness.
4. Rate and pattern of breathing	4. Breathing should appear effortless and the rate should be the same as that of other animals in the group. Sneezing or noisy breathing are abnormal signs.
5. Nose and eyes.	5. Discharge from nose and eyes is abnormal.
6. Skin color (foot pads, ears, lips).	6. Pink color of non-pigmented skin indicates good circulation and red blood cell count. Abnormal findings would include blue, yellow, or very pale.
7. Skin condition.	7. Crusts, sores, and swelling are examples of abnormal findings.
8. Cage condition observations	8. Food/water levels, normal fecal pellets present?

## **DIVISION OF LABORATORY ANIMAL RESOURCES**

### **MOUSE HANDOUT**

Use this outline to guide you through the variety of hands-on techniques offered to review and refresh skills and interests. Comments offered after each technique listed are helpful tips or keep in minds for each procedure. If we haven't listed a particular technique of interest to you, don't hesitate to ask for a demonstration or explanation - it's your wet lab!

#### I. [Restraint Techniques](#)

A. Grasp near base of tail to gently lift mouse out of cage. Grasping too near the tip of the tail could result in tearing and dislodging of the overlaying tail skin.

B. To restrain, apply pressure to the dorsal region of the mouse while grasping the skin at the neck and back area at the same time. The tail can be placed between two fingers to provide additional stabilization. This will allow you to restrain the mouse with one hand and inject with the other.

#### II. [Ear Tagging/Notching](#)

A. Tag close to the head, numbers up for greater visibility.

B. Unless you ear tag routinely, you may find it easier to work in pairs; one tagger, and one restrainer.

#### III. Injection Techniques

A. IM- not recommended due to small muscle mass in this specie.

B. [SQ](#)- skin is tented, injectate is delivered into space between tented skin and muscle.

C. [IP](#)- tilt the head toward floor, use caudal abdominal quadrant off midline. Watch syringe hub for aspirate! Discard contaminated solutions.

D. [IV](#)- lateral tail veins- heat aids visualization

#### IV. Gavage Techniques

A. Measure balled gavage needle length for placement- mouth to the most caudal rib.

B. Gently advance needle, monitor mucous membrane color for respiratory distress.

#### V. Parental Anesthesia

A. Sodium Pentobarbital dose range in the mouse is 40-70 mg/kg IP. The commercially available concentration is 65 mg/ml, or a 6.5% solution. However, to produce a more manageable volume of fluid and decrease the risk of peritoneal irritation, you will be provided with a 1:10 dilution of the commercial product. This equates to a concentration of 6.5 mg/ml. Use a dose of 60 mg/kg IP.

B. To calculate your dose, use the formula: weight times dose, divided by concentration equals volume in ml=s. Example: 0.035 kg (weight) X 60 mg/kg (dose) = 2.1 mg 6.5 mg/ml (concentration) = 0.32 ml

#### VI. Blood Collection

A. [Lateral tail vein](#)- located laterally on each side of the tail, immerse the tail in warm water to dilate the vessel. Practice with 25 gg or 27 gg needle and micro capillary tube and/or 24 gg indwelling catheter. Insert the tip of the needle bevel up at a shallow angle, if blood appears, collect in capillary tube. If injecting, inject a small amount of solution to ensure venous injection. If small bleb appears, needle is not into vein. First attempt should start as close to tail tip as possible; work cranially on successive attempts.

B. [Infraorbital sinus](#) (sinus is located behind orbit) gentle, patient rotation & pressure at the medial canthus with capillary tube, relax pressure intermittently and as blood begins to flow into tube. Hold lids shut approximately 30 seconds post collection to facilitate hemostasis. Always use protective eye ointment after collection.

C. Cardiac puncture- approximately 45 degree needle entry at sternum. Immediately begin and maintain gentle aspiration pressure until blood flows into syringe. Balance and stabilize needle placement once puncture has been achieved. This is a terminal procedure which must be accomplished under general anesthesia.

## MOUSE HANDOUT

### Mus musculus

#### Physiological Data:

- body temperature 99 degrees F.
- respiratory rate 94-163/minute
- heart rate 325-780/minute
- life span 1.2 - 2.2 years (some inbred strains may develop tumor or conditions such as leukemia and die by 1 year of age).
- breeding onset 50-60 days

- female comes into estrus every 4 days (spontaneous ovulator); if mated by fertile mate= pregnant; if mated by non-fertile mate= pseudo pregnant, up to 3 weeks.
- post-partum estrus (other than this, estrus does not occur during lactations).
- gestation 3 weeks; dystocia rare, normal parturition completed within one hour
- breeding life: males- retire after 6-9 months of age; females- retire after 6-9 months of age, litters decrease in frequency and size.
- selection of breeders: larger individuals- be careful in selection, may have come from a small litter; avoid closely related breedings (non-inbred); cull females with small litters, poor lactation, abnormal young.
- male can successfully mate with 2-3 females per night; any additional can cause him to be infertile and result in pseudo pregnancy.
- vaginal smears can be used to identify stage of estrus cycle
- detection of pregnancy: vaginal plug within 24 hours; weight gain of 5-10 gms. after 13 days gestation; mammary development 14 days plus.
- birth: cannibalism is uncommon; should remain undisturbed 2 days post partum; pups are hairless, eyes and ears closed; young are very active from birth; by three days hair begins to grow over body; 2 weeks of age, eyes and ears open; wean at 3 weeks of age.
- pheromones: Whitten effect- synchronize estrus by proper male exposure; Bruce effect- mated female may fail to become pregnant if exposed to second strange male within 24 hours.

## **DIVISION OF LABORATORY ANIMAL RESOURCES**

### **RAT HANDOUT**

Use this outline to guide you through the variety of hands-on techniques offered to review and refresh skills and interests. Comments offered after each technique listed are helpful tips or keep in minds for each procedure. If we haven't listed a particular technique of interest to you, don't hesitate to ask for a demonstration or explanation- it's your wet lab!

#### **I. [Restraint Techniques](#)**

A. Grasp near base of tail to gently lift rat out of cage. Grasping too near the tip of the tail could result in tearing and dislodging of the overlaying tail skin.

B. The rat can be restrained by placing your thumb and index finger around the thorax of the rat, just under it's forelegs, gently applying pressure to the legs so as to push them under the rat's chin, keeping the head stabilized. Another method involves the placement of the index and adjacent finger over and behind either side of the rat's head, then gently encircling the thorax with the thumb and ring and little fingers.

#### **II. [Ear Tagging/Notching](#)**

A. Tag close to the head, numbers up for greater visibility.

B. Unless you ear tag routinely, you may find it easier to work in pairs; one tagger, one restrainer.

#### **III. Injection Techniques**

- A. [IM](#)- small volumes, split sites if necessary, avoid sciatic nerve path.
- B. [SQ](#)- larger volumes
- C. [IP](#)- tilt head toward floor, use caudal abdominal quadrant off midline. Watch syringe hub for aspirate! Discard contaminated solutions.
- D. [IV](#)- lateral tail veins- heat aids visualization.

#### IV. Gavage Techniques

- A. Measure balled gavage needle length for placement- mouth to most caudal rib.
- B. Gently advance gavage needle, monitor mucous membrane color for respiratory distress.

#### V. Parental Anesthesia

A. The anesthetic combination of Ketamine and Rompun is commonly used in the rat as it provides good depth of anesthesia with muscle relaxation and accompanying analgesia which extends into the recovery period. Accepted ranges are 40-80 mg/kg for Ketamine and 5-13 mg/kg for Rompun. Use a dose of 67 mg/kg for Ketamine and 10 mg/kg Rompun IP. The concentration of Ketamine is 100 mg/ml, Rompun 20 mg/ml. IP is the preferred route for this combination as IM administration has been implicated in muscle necrosis and histological changes. Example:  $0.35\text{kg (weight)} \times 67\text{ mg/kg (dose)} = 23.4\text{ mg}$   $100\text{mg/ml (concentration)} = 0.23\text{ ml Ketamine}$ .

Remember to utilize a bland, sterile ophthalmic base ointment in survival procedures as Ketamine temporarily suppresses the blink reflex which can lead to corneal drying and scarification.

#### VI. Blood Collection

- A. [Lateral tail vein](#)- located laterally on each side of the tail, immerse the tail in warm water to dilate the vessel. Practice with 22 gg or 25 gg needle and micro capillary tube and/or 22 gg indwelling catheter. Insert the tip of the needle bevel up at a shallow angle, if blood appears, collect in capillary tube. If injecting, inject a small amount of solution to ensure venous injection. If small bleb appears, needle is not into vein. First attempt should start as close to tail tip as possible; work cranially on successive attempts.
- B. [Infraorbital sinus](#) (sinus is located behind orbit) - starting with gentle patient rotation and pressure at the medial canthus with capillary tube, rotate the tube slightly dorsal as you tip it at a 45% angle to the back of the globe. Intermittently relax pressure to allow blood to flow into tube. Hold lids shut approximately 30 seconds post collection to facilitate hemostasis. Always use protective eye ointment after collection attempts.
- C. Cardiac puncture- approximately 45 degree needle entry at sternum. Immediately begin and maintain gentle aspiration pressure. Balance and stabilize needle placement once puncture has been achieved. This is a terminal procedure which must be accomplished under general anesthesia.

#### VII. Necropsy (ask if interested)

## RAT HANDOUT

## Rattus norvegicus

### Physiological Data

- body temperature is 99.5 degrees F
- heart rate 260-600 (338 average per minute)
- respiratory rate 75-115 per minute (94/minute average)
- reproduction:
  1. estrus cycle is 5 days; vaginal smears can be taken to determine stage of estrous cycle; pH of vaginal secretion decreases in estrus
  2. gestation period 21 days; dystocia rare, normal parturition completed within one hour
  3. litter size 8-12 pups
    - a. cross fostering possible at 1-3 days
    - b. female can feed 14 young, over that is stressing to mother
    - c. don't disturb litter for 2-3 days, cannibalism
    - d. young born hairless and ear and eye canals closed; scarlet in color
    - e. eyes open at 10-12 days of age
    - f. wean litter at 21 days; during first 7-10 days post weaning should not separate litter- heat loss and death may result
  4. mating:
    - a. monogamous mating- one female and one male; record keeping easier
    - b. polygamous mating- one male to 4 females (remove pregnant female, litter in isolation; female will tolerate another male, but not other females when she has a litter)
    - c. breeding life 6-10 litters, 14 months of age
    - d. post partum estrus ( however, generally not used in rats )
    - e. regarded as spontaneous ovulators
    - f. receptive to males only in estrus; receptive for 12 hours
    - g. vaginal plug within 24 hours post breeding
    - h. mammary development is evident at 14 days gestation
    - i. select breeders- good mothers, good lactaters, good size litters
- sexually mature at 7 weeks of age (males can retract testicles throughout life)
- life span 3 years

## ANIMAL GAVAGING

**RECOMMENDED SIZES OF BALLADE GAVAGE NEEDLES**

<b>SPECIES</b>	<b>WT. IN GRAMS</b>	<b>GAUGE</b>	<b>LENGTH</b>	<b>BALL DIAMETER</b>	<b>SHAPE</b>
mice	to 14 gms	24	1"	1 1/4 mm	straight
mice	15-20 gms	22	1", 1 1/2"	1 1/4 mm	straight
mice	20-25 gms	20	1", 1 1/2", 3"	2 1/4 mm	str. / curved
mice	25-30 gms	18	1", 1 1/2", 2"	2 1/4 mm	str. / curved
mice	30-35 gms	18	2", 3"	2 1/4 mm	str. / curved
rats	50-75 gms	20	1", 1 1/2"	2 1/4 mm	str.
rats	75-120 gms	18	1", 1 1/2"	2 1/4 mm	str. / curved
rats	100-200 gms	18	2", 3"	2 1/4 mm	curved
		16	2"	3mm	str./curved
rats	150-300 gms	16	3", 4"	3 mm	curved
rats	200-350 gms	14	3"	4 mm	curved
		13	3"	4mm	straight

## GAVAGE METHOD (STOMACH TUBING)

Stomach tubes, also known as gavage tubes, are used to instill liquids directly into the stomach. Using a rigid metal feeding needle with a protective ball on the tip is safer than using a flexible tube. The properly sized metal ball is too large to enter the opening of the larynx, yet small enough to pass easily into the esophagus. Metal gavage tubes of the appropriate size are available in a variety of lengths and diameters.

1. Restrain the rat and carefully measure the distance from the tip of the rat's nose to the last rib. Excessively long or improperly handled stomach tubes may penetrate and rupture the pharyngeal mucosa or the stomach wall.
2. To achieve correct positioning, use the stomach tube as a lever to move the rat's head slightly upward and back, approximating a straight line. Positioning the tube to the right or left in the mouth will facilitate passage into the esophagus. You should attempt to time the passage of the tube from the pharynx through the esophagus and into the stomach, to coincide with the animal's next swallowing reflex.
3. Resistance to insertion of the tube to the premeasured length, or a struggling rat, usually indicates a problem. Stop, remove the tube, and carefully start over.
4. Observe the rat after the procedure for any signs of distress, such as gasping and frothing at the mouth or nose. These signs may indicate injury or inhalation of the foreign materials. Euthanasia should be administered to animals that do not respond to therapeutic intervention.

*Practical Methodology: Humane Handling and Laboratory Techniques for the Rat*; School of Veterinary Medicine, University of California, Davis, 1987

\* These techniques are also applicable to other rodent models.

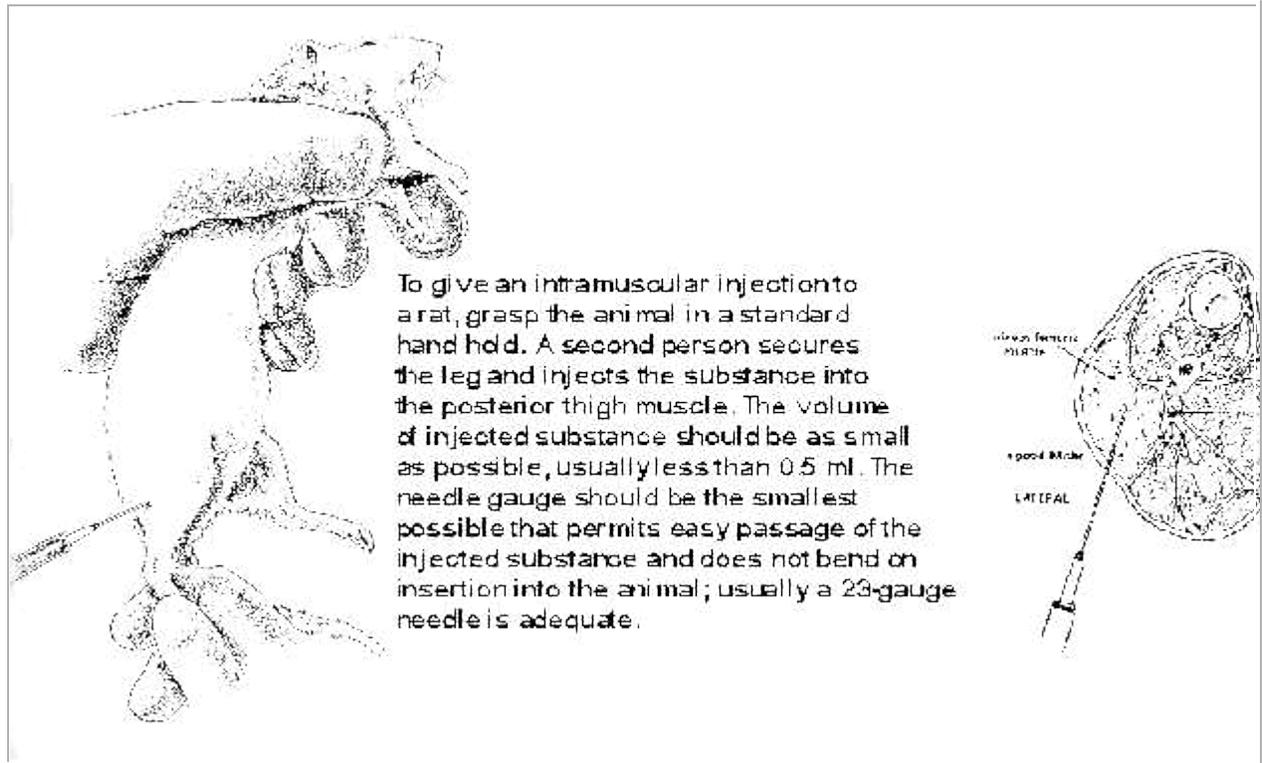
## DLAR SMALL ANIMAL BLOOD WITHDRAWAL GUIDELINES FOR SURVIVAL PROCEDURES

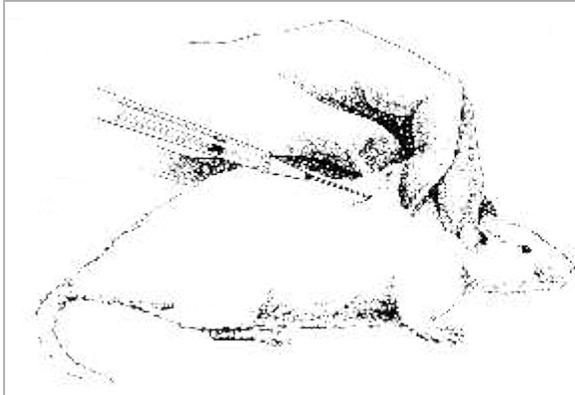
Total body blood volume equals 6% of the animal's body weight (example: a 500 gm guinea pig has about 30cc total blood volume.  $500 \text{ gm} \times 0.06 = 30 \text{ gm} = 30\text{cc}$ ). Blood withdrawal (with like volume fluid replacement) should not exceed 10% ( or 2.5mg/lb or 5ml/kg) of the total body blood volume in any two week period. Up to 20% of the total body blood volume may be withdrawn over any two week period if it is accompanied with double the volume fluid replacement (preferably lactated ringers) given SC, IP, or IV at the time of withdrawal.

It is recommended that the hematocrit (packed cell volume) is checked weekly to confirm when it is safe to draw blood again. Be sure to obtain correct hematocrit range for the appropriate species. Restraint is necessary. Sedation is recommended.

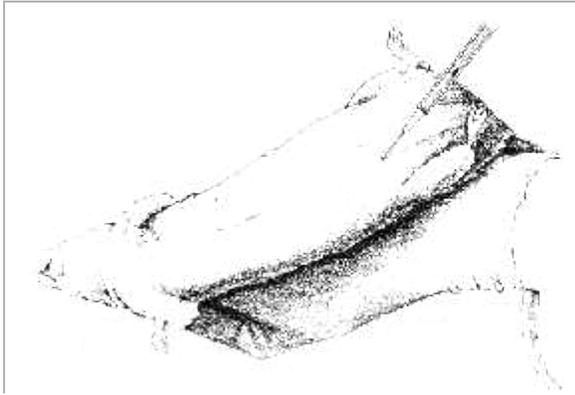
Safe amount of blood (in ml) to draw =  
Animal's wt. (in GRAMS)  $\times 0.06 \times 0.20 =$   
Animal's wt. (in KG)  $\times 0.06 \times 0.20 \times 1000 =$   
Animal's wt. (in LBS)  $\times 0.06 \times 450 \times 0.02 =$

SPECIES	AMOUNT THAT CAN BE SAFELY DRAWN (over a two week period with 2x fluid replacement )
Dog (44lbs.=22kg)	264 ml
Rabbit (10lbs.=4.5kg) (Hematocrit 36-48%)	54 ml
Rabbit (6lbs.=2.7kg) (Hematocrit 36-48%)	32 ml
Ferret (2lbs.=1kg)	12 ml
Guinea pig (500 grams)	6 ml
Rat (400 g)	4.8 ml
Rat (250 g)	3 ml
Hamster (100 g)	1.2 ml
Gerbil (90 g)	1 ml
Mouse (30 g)	0.35 ml

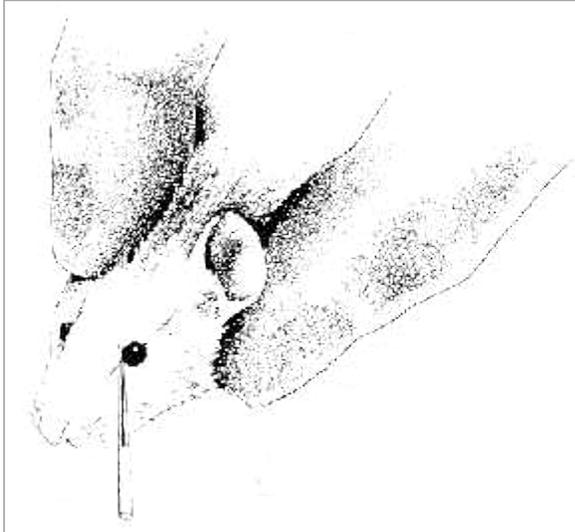




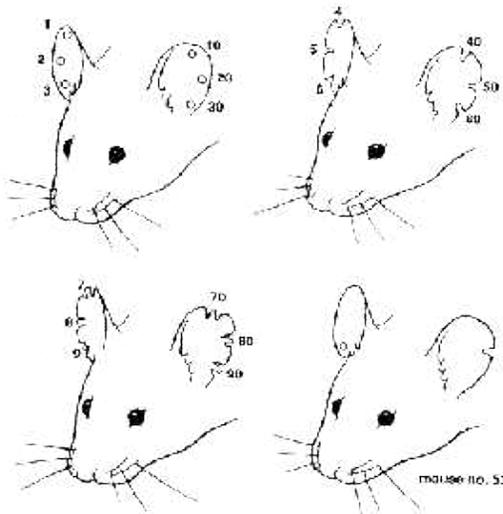
When giving a subcutaneous injection, secure the animal by the nape of the neck, elevate the skin to produce a tent, and insert the needle through the loose skin. Caution must be observed to avoid puncturing the opposite side of the tent before the substance is injected.



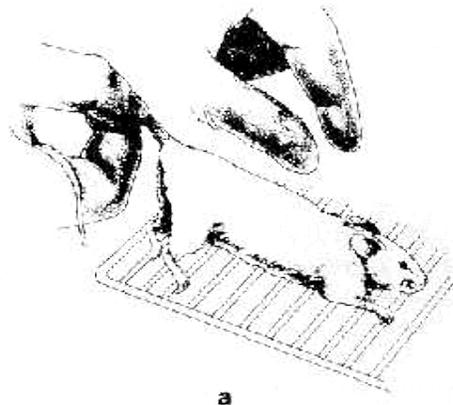
To give an intraperitoneal injection, grasp the animal in a standard hand hold. Tilt the animal head down so that the intestines fall forward, then inject into the lower right quadrant of the abdomen, slightly off the midline, being careful to avoid the bladder. A short needle having a length of approximately 1.5 cm helps prevent organ puncture.



To bleed retro-orbitally, secure its head between the thumb and forefinger, insert a capillary tube at the medial edge of the eyeball, and direct it toward the back of the eye socket. Carefully rotate the tube to puncture the blood sinus. Blood fills the tube by capillary action.



Rodents can be identified with the numbers 1 through 9 by putting a hole, a notch, a double notch or any combination of these three marks in one or both ears. The marks on an animal's right ear denote units or ones: 1, 2, 3, 4, 5, 6, 7, 8, 9. The marks on the left ear denote tens: 10, 20, 30, 40, 50, 60, 70, 80, 90. The sample animal is number 53.



a

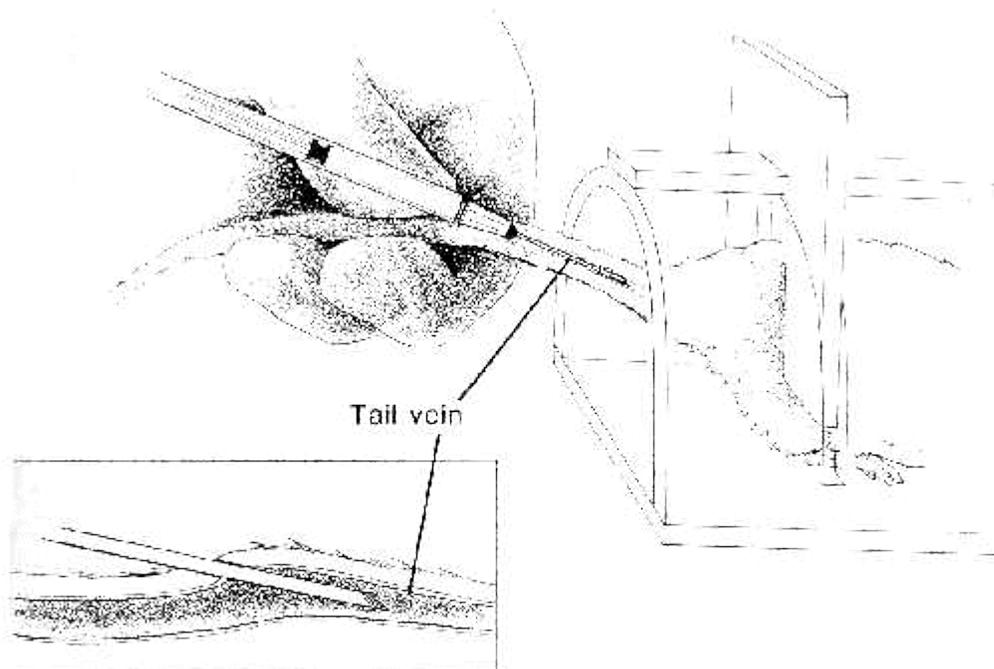


b

An acceptable mouse restraint technique such as this, causes no discomfort for the animal and protects the handler. Initially, the animal is placed on a surface it can grasp, such as a cage top, while it is held near the base of the tail with the dominant hand (a). The neck area is then restrained with the nondominant hand, while the tail is grasped between the fourth and fifth fingers of that hand (b). This manipulation results in restraint that frees the technician's dominant hand for other functions while securely restricting the animal's movement.



To pick up and restrain a rat, first grasp the base of the tail. Then firmly, but without excessive pressure, hold the rat around the thorax and just below the forelegs and restrict movement of the head. It is rarely necessary for a trained technician to wear heavy gloves.



For intravenous injections, secure rodents in restrainers unless they are anesthetized. Locate the lateral tail vein and insert the needle at a slight angle. Make sure the beveled edge of the needle faces outward (inset). A 26 to 28 gauge needle, a low volume and repeated practice are essential for achieving success with this technique.