A Volume in The Laboratory Animal Pocket Reference Series

The Laboratory RAT

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CRC Press
Boca Raton Boston London New York Washington, D.C.

Library of Congress Cataloging-in-Publication Data

Sharp, Patrick E.
The laboratory rat/ Patrick E. Sharp and Marie C. La Regina
p. cm. — (The laboratory animal pocket reference series)
Includes bibliographical references and index.
ISBN 0-8493-2565-1
1. Rat—research. 2. Biology—laboratory animals. I. La Regina, Marie C., II. Title. III. Series.
BR849.H79G52 1998
616'.0349—dc20

98-29112
CIP

Visit the CRC Press Web site at www.crcpress.com

International Standard Book Number 0-8493-2565-1
Library of Congress Card Number 98-29112
Printed in the United States of America 3 4 5 6 7 8 9
Printed on acid-free paper

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dedication

P.E.S. To my father, James L. Sharp and Jamie Weaver, a childhood friend. “This one goes out to the one I love...This one goes out to the one I left behind” (REM).

M.L.R. In appreciation to my mentors, colleagues, and researchers who have made my career as a laboratory animal diagnostician challenging, rewarding, and always enjoyable.

preface

The use of laboratory animals, including rats, continues to be an important part of biomedical research. In many instances, individuals performing such research are charged with broad responsibilities, including animal facility management, animal husbandry, regulatory compliance, and performance of technical procedures directly related to research projects. In this regard, this handbook was written to provide a quick reference source for investigators, technicians, and animal caretakers charged with the care and/or use of rats in a research setting. It should be particularly valuable to those at small institutions or facilities lacking a large, well-organized animal resource unit and to those individuals who need to conduct research programs using rats starting from scratch.

This handbook is organized into six chapters: Important Biological Features (Chapter 1), Husbandry (Chapter 2), Management (Chapter 3), Veterinary Care (Chapter 4), Experimental Methodology (Chapter 5), and Resources (Chapter 6). Basic information and common procedures are presented in detail. Other information regarding alternative techniques, or details of procedures and methods which are beyond the scope of this handbook is referenced extensively so the user is directed toward additional information without having to wade through a burdensome volume of detail here. In this sense, this handbook should be viewed as a basic reference source and not as an exhaustive review of the biology and use of the rat.

The final chapter, “Resources,” provides the user with lists of possible sources and suppliers of additional information, rats, feed, sanitation supplies, cages, and research and veterinary

supplies. The lists are not exhaustive and do not imply endorsement of listed suppliers over suppliers not listed. Rather, these lists are meant as a starting point for users to develop their own lists of preferred vendors of such items.

A final point to be considered is that all individuals performing procedures described in this handbook should be properly trained. The humane care and use of rats is improved by initial and continuing education of personnel and will facilitate the overall success of programs using rats in research, teaching, or testing.

the authors

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From 1980 to 1991, Dr. La Regina was on staff at St. Louis University in St. Louis where her duties included diagnostics and clinical rodent medicine. In 1991, she joined the staff of the Washington University.

Dr. La Regina has been an active member in ACLAM since 1980, having served on the Board of Directors and as an Officer in the organization. She has published on a variety of topics related to laboratory animal disease and has been an active co-investigator in the area of pathology.

acknowledgment

The authors wish to acknowledge the generous contributions of Sherilyn Hall, Victoria Culbreth, Holly Kuepfert, Christina Gutting, and George Vogler.

contents

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important biological features

introduction

Taxonomy of the laboratory rat:
Kingdom: Animalia
Phylum: Chordata
Class: Mammalia
Order: Rodentia
Suborder: Myomorpha
Family: Muridae
Genus: *Rattus*
Species: *norvegicus*

Rats are thought to have originated in the area of Asia currently occupied by southern Russia and northern China. *Rattus rattus* (black or ship rat, 2n = 38) was well established in Europe by 1100 A.D. (following the Crusades), with *Rattus norvegicus* (brown rat, 2n = 42) commonly found in Europe in the 1700s. This recent reappearance followed thousands of years absence. Fossilized rat remains dating to the Pliocene and Pleistocene

periods were found in Europe. Until the writings of Giraldus Cambrensis (1147–1223), there was no distinction between the *R. rattus* and mice. The late arrival of *R. norvegicus* to Europe is offset by its ferocious nature, essentially eradicating the black rat from its former strong holds. Today, the black rat is restricted to areas near water, and the brown rat has conquered the planet because of its climatic adaptability and ability to parasitize human refuse.

Today's laboratory rats are the domesticated descendants of *Rattus norvegicus*. Albino animals were held and used for rat shows, and frequent handling is thought to have tamed these animals. By the 1800s these animals were used for breeding and neuroanatomy studies in the United States and Europe. It was in the late 1800s and early 1900s that individual stocks and strains had their beginnings.

The laboratory rat has been, and continues to be a mainstay of biomedical research. Both albino and pigmented animals are available. There are recognized differences between wild and laboratory rodents. For example, laboratory rats have smaller adrenals and preputial glands, earlier sexual maturity, no reproductive cycle seasonability, better fecundity, and a shorter life span than their free-ranging wild counterparts.

nomenclature

Rats fall into two basic groups depending on whether they are inbred or outbred. One generally refers to inbred animals as strains, and outbred animals as stocks. One develops inbred strains through at least 20 generations of brother–sister matings, whereas outbred stocks have less than 1% inbreeding per generation and have been maintained in a closed colony for at least four generations.

Different stocks and strains show variability in many biological parameters including hematology, clinical chemistry, and anesthesia response. This variability is also observed in stocks and strains from different suppliers, so one should exercise caution in changing suppliers during mid-study.

There are specific nomenclature guidelines, with assistance available in using these guidelines. One may wish to consult the following:

ILAR, National Research Council, 2101 Constitution Avenue, Washington, D.C. 20418, U.S.A. Telephone: (202) 334-2590, Fax: (202) 334-1687;

PALM Institute, N29 W4 2-1-215 Sapporo 001, Japan. Telephone: 81-11-746-3988, Fax: 81-11-746-6722;

Registry of Inbred Strains, Dr. Michael F.W. Festing, IRC for Human Toxicology, Leicester University, University Road, Leicester LE2 7RH, UK;

Rat News Letter, 2542 Harlo Dr., Allison Park, Pittsburgh, PA 15101, U.S.A., Telephone: (412) 487-4289;

Transgenic Animal Database, TABD Coordinator, Oak Ridge National Laboratory, PO Box 2008, MS 6050, Oak Ridge, TN 37831-6050, USA, Telephone: (615) 574-7776, Fax: (615) 574-9888;

The Jackson Laboratory, Bar Harbor, ME 04609, U.S.A., Telephone: (207) 288-3371, Fax: (207) 288-8982.

behavior

Rats are nocturnal animals with most activity occurring at night and in the early morning. Changing the light cycle permits rats and investigators to share peak activity periods. This 12-hour shift will require a 2-week accommodation period for the rat. Although there are strain differences, rats are typically non-aggressive, inquisitive, and easily trainable. Frequent handling encourages their non-aggressive nature as they adapt to new surroundings or experimental situations. Improper handling, nutritional deficiencies, and vocalizations from other rats can result in undesired behavior. Males are usually more aggressive than females and when striking, bite once. Rats feel most comfortable in small, dark, confined spaces; a behavior investigators may use as a reward. When designing experiments, it is important to understand the rat's coprophagic behavior and its potential impact on metabolic, drug, and other studies. Male rats, unlike mice, are unlikely to fight when housed together. Rats also differ from mice in their willingness and acceptance of single housing.

anatomic and physiologic features

This section briefly summarizes the anatomic and physiologic characteristics of the laboratory rat. Special emphasis is placed upon those characteristics which are unique to the rat. Table 1 summarizes the basic biological parameters of the rat.

TABLE 1. BASIC BIOLOGIC PARAMETERS

Parameter	Value
Lifespan (years)	2.5–3.5
Mammary glands	6 pr
Male body weight (g)*	450–520
Female body weight (g)*	250–300
Body temperature (rectal)	35.9–37.5°C 96.6–99.5°F
O_2 consumption (ml/m^2/g body weight)†	0.84
Body surface area (cm^2)	10.5 (bw in g)$^{2/3}$
Food intake (g/100 g bw/day)	5–6
Water intake (ml/100 g bw/day)	10–12
Gi transit time (hours)	12–24
Urine volume (ml/100 g bw/day)	5.5
Urine specific gravity	1.04–1.07
Urine pH	7.3–8.5
Total body water (ml)*	167
Intracellular fluid (ml)*	92.8
Extracellular fluid (ml)*	74.2
Plasma volume (ml)*	7.8

* Body weights will vary with stock or strain.

† Based on a 250 g rat.

Oropharynx

- Dental formula: 2 (incisors 1/1 canines 0/0 premolars 0/0 molars 3/3) = 16.
- The incisors grow continuously.
- Lack water taste receptors found in other animals.
- Lack tonsils.

Salivary glands

- Three pair of salivary glands: parotid, submaxillary (submandibular), and sublingual.
    - **Parotid —** The parotid salivary gland secretes a serous product, and consists of 3–4 lobes. The parotid gland extends ventrodorsally from behind the ear to the clavicle. The parotid duct opens opposite the molar teeth. The protein concentration (2%) of the saliva is unique.
    - **Submaxillary —** The submaxillary (submandibular) glands are mixed glands secreting a serous and mucous product. They are found in a ventral region between the mandibles and thoracic inlet. There are two types of secretory granules found in the submaxillary glands, one in the acinar cells and the other in the granulated portion of the secretory ducts. Secretory duct granules of immature animals contain substructures not found in adult animals.
    - **Sublingual —** The sublingual glands are the smallest of the salivary glands and secrete a mucous product. The rounded glands may be found at the rostral aspect of the submaxillary glands, and may be found embedded in them.

Note: Brown fat is found in the ventral cervical region, and one should not confuse this structure with salivary glands or lymph nodes.

Esophagus

- The esophagus enters the lesser curvature of the stomach through a fold in the limiting ridge of the stomach. The fold prevents rats from vomiting.
- The esophageal lining is entirely keratinized epithelium.

Stomach

- The rat's stomach has nonglandular and glandular portions separated by the "limiting ridge." The nonglandular forestomach has a lining similar to the esophagus.

Small Intestine

Small intestine lengths and transit times vary with the age of the rat. The length values listed below are adult averages. The authors wish to direct the reader to the discussion by Varga concerning the interaction among age, intestine length, and transit time.

- **duodenum**: 10 cm in length
- **jejunum**: 100 cm in length
- **ileum**: 3 cm in length

Large Intestine

- **Cecum —** The cecum is a thin-walled, comma-shaped pouch with a prominent lymphoid area found on the lateral aspect of the apex. Although the rat cecum does not possess an inner septa as seen in other rodents it has an inner constriction which divides the structure into apical and basilar sections. The lymphoid tissue is thought to be analogous to the vermiform appendix found in human beings.
- **Colon —** The colon has three divisions: ascending, transverse, and descending. The ascending portion has oblique mucosal ridges, whereas the mucosal folds of the transverse and descending regions have longitudinal mucosal folds.
- **Rectum —** The rectum is that region of the gastrointestinal tract found in the pelvic canal.

Liver

- Liver weight: 10.0 g/250 g rat.
- Liver volume: 19.6 mL/250 g rat.
- Bile flow: 22.5 mL/d/250 g rat.

- Consists of four lobes: median, right lateral, left, and caudate.
- The rat has no gall bladder.
- The bile from each lobe leaves via ducts. These ducts then form the common bile duct, which enters the duodenum approximately 25 mm distal to the pyloric sphincter.

Pancreas

- Consists of a lobulated, diffuse organ, extending from the duodenal loop to the gastrosplenic omentum. The pancreas has a darker color and firmer texture than the surrounding adipose tissue.
- The diffuse nature of the organ results in a network of ducts which coalesce into 2–8 larger ducts emptying into the common bile duct.

Urinary System

- Kidney weight: 2.0 g/250 g rat.
- Kidney volume: 3.7 mL/250 g rat.
- Rats, like other rodents, possess a unipapillate kidney, which consists of one papilla and one calyx and enters the ureter directly.
- Long and short nephrons are present.
- Only animal whose kidneys contain significant amounts of L-amino acid oxidase.
- Female urethral orifice is at the base of the clitoris.

Reproductive System

To distinguish males and females, note that males have a greater anogenital distance than females, and larger genital papillae (Fig. 1).

female

- Six pair of mammary glands — 3 thoracic, 1 abdominal, 2 inguinal.

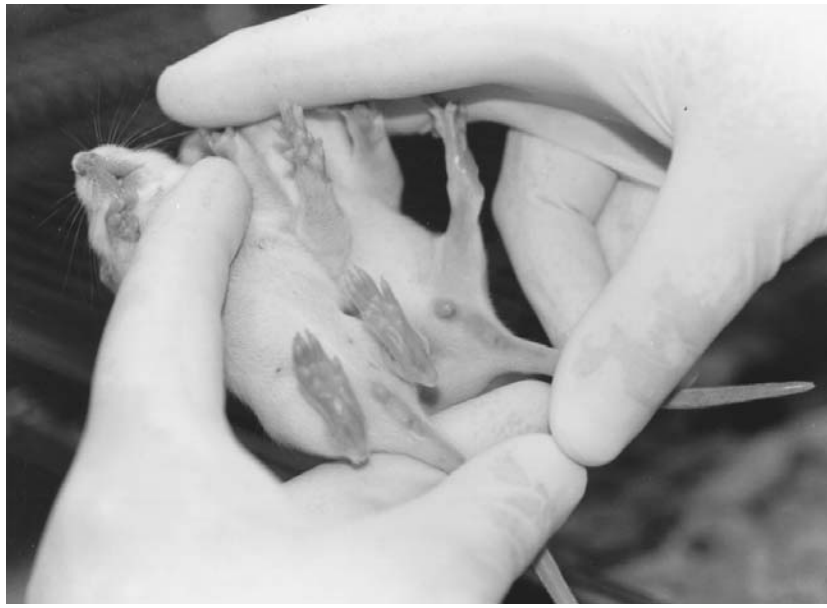


Fig. 1. Note the greater anogenital distance in the preweanling male rat on the right compared to the female rat on the left.

- Uterus is bicornuate and duplex consisting of two uterine horns, two cervices, and one vagina.
- Hemochorial discoid placentation.
- A **copulatory plug** forms from semen coagulation following copulation. Specifically it forms from secretions of the vesicular and coagulating glands, filling the reproductive tract from the vulva to the cervix. It will remain for a few hours following copulation, and then will decrease in size and fall out.

male

- Inguinal canal remains open throughout the animal's life.
- No nipples.
- Has an os penis.

- Accessory sex glands consist of:
  - ampulla
  - seminal vesicle
  - prostate
  - bulbourethral glands
  - coagulating glands
  - preputial glands

Respiratory

- Normal respiratory function values are found in Table 2.
- Lung weight: 1.5 g/250 g rat.
- Lung volume: 2.1 mL/250 g rat.
- Left lung consists of 1 lobe.
- Right lung consists of 4 lobes: cranial, middle, accessory, and caudal.
- Pulmonary veins contain striated heart muscle fibers continuous with those found in the heart.
- Bronchial constriction is under the control of the vagus nerve, not the adrenergic nerve supply.
- The lung is immature at birth and consequently devoid of alveoli, alveolar ducts, and respiratory bronchioles. Air exchange occurs through the smooth walled channels and saccules until 4–7 days following birth when remodeling occurs. Respiratory bronchioles are present 10 days following birth.
- There have been at least ten morphologically distinct cell types identified in the intrapulmonary airways. The epithelial serous cell is thought to be unique to the rat. It secretes a product which has a viscosity less than the mucous cell, and is thought to be responsible for the low-viscosity pericilliary liquid layer found at all levels of the rat's respiratory tract.

- Regulation of the respiratory system occurs through tissue CO_2 exchange in the medullary respiratory center, with the carotid bodies playing a role. The carotid bodies, however, respond to low blood oxygen tension.
- The rat has the thinnest pulmonary artery and the thickest pulmonary vein studied. The thickness of the pulmonary vein is due to the cardiac striated fibers, which are mainly observed in the intrapulmonary branches. Unfortunately, this arrangement permits infectious agents to spread from the heart, through the pulmonary veins, into the lungs.
- As in man, precapillary anastomoses occur in the lungs, and are limited to the hilar region. Pulmonary vasculature will vasoconstrict in response to acetylcholine (0.2–0.5 μg).
- Rats have a high neuronal density, high serotonin activity, and low histamine activity in the lungs.

Note: Papers by Stahl and Leith present methods for comparing respiratory variables between mammals.

TABLE 2. VALUES FOR RESPIRATORY FUNCTION

Parameter	Value
Tidal volume (ml)	0.6–2.0
Respiratory rate (breaths per minute)	70–115
Trachea diameter (mm)	1.6–7.7
Minute ventilation (ml/minute)	75–130
Alveolar diameter (μm) [mean]	57–112 [70]
Total surface area (400 g animal, m^2)	7.5
Thickness air-blood barrier (μm)	1.5
Alveolar length (μm)	288–624
Branches per alveolar duct	2–5
Atria diameter (μm)	15–262
Total lung capacity (ml)*	11.3 ± 1.4
Vital capacity (ml)*	8.4 ± 1.7
Functional residual capacity (ml)*	3.9 ± 0.8
Residual volume (ml)*	2.9 ± 1.0

* 60–84 day old anesthetized rats. The paper by Yokoyama presents values on older and younger rats.

Circulatory

- Normal cadiovascular values are found in Table 3.
- Heart weight: 1.0 g/250 g rat.
- Heart volume: 1.2 mL/250 g rat.
- The cardiac blood supply originates from both the coronary and extracoronary arteries (internal mammary and subclavian arteries). The right coronary arteries supply the right and left atria, with the left cardiac arteries supplying a small portion of the left atria.
- There are two precavae present in the rat vena cava, with the right precava emptying into the right atrium, and the left precava joining with the azygous vein before dumping into the right atrium.

TABLE 3. VALUES FOR CARDIOVASCULAR FUNCTION

Parameter	Value
Heart rate (beats per minute)	250–450
pO_2 (mm Hg)	93.2
pCO_2 (mm Hg)	39.9
Arterial blood pH	7.41
H^+ (nM)	38.6 ± 0.6
Base excess	+1.8 ± 0.4
Arterial systolic pressure (mm Hg) (mean)	88–184 (116)
Arterial diastolic pressure (mm Hg) (mean)	58–145 (90)
Cardiac output (ml/min)	10–80
Blood volume (ml/kg)	57.5–69.9

Musculoskeletal

- Vertebral formula: C7 T13 L6 S4 Cd27-30.
- Bone maturation in the rat is slower than most mammals with ossification incomplete until after the first year of life.

Glands

- **Lacrimal glands —** extraorbital lacrimal glands lie ventral and rostral to the ear and intraorbital lacrimal glands lie in the caudal angle of the orbit.

- **Steno's gland** lies in the maxillary sinus and is homologous to the salt gland of marine birds. The gland regulates mucus viscosity and humidifies inspired air.
- **Harderian glands** are found behind the eye and produce a porphyrin secretion which usually goes unseen, except in cases of illness or stress. The porphyrin component causes crusts or tears to form which will fluoresce red under ultraviolet light (Wood's lamp) — blood does not. This porphyrin secretion is often mistaken for blood.
- **Zymbal's gland** is found at the base of the ear.
- Like other rodents, rats have no sweat glands, cannot pant, and are poor regulators of core body temperature. They do not increase water intake at high ambient temperatures, with heat appearing to inhibit drinking. Relief is sought through behavioral means: increased salivation, burrowing, and shade. Adaption to cold is better than to heat. The rat's tail plays a role in thermoregulation. The vasculature vasodilates to dissipate heat and vasoconstricts when heat conservation is essential. Development of thermoregulating mechanisms in neonatal animals is nonexistent until the end of the first week.

Nervous System

The discussion of the rat nervous system will be a cursory overview in comparison to Paxinos' *The Rat Nervous System*. The reader is directed there for in-depth information.

- Brain weight: 1.8 g/250 g.
- Brain volume: 1.2 mL/250 g.

peripheral nervous system (PNS)

The rat's PNS consists of 34 pairs of spinal nerves arising from the intervertebral foramina of the cervical (8), thoracic (13), lumbar (6), sacral (4), and caudal (3) regions. Plexi form in the cervical, brachial, and lumbrosacral regions. The autonomic nervous system forms the greater and lesser splanchnic nerves along with their plexi and ganglia.

Table 4. Cerebrospinal Fluid Values for the Laboratory Rat

Parameter	Value
Osmolarity (mOsm/kg)	302 ± 4
Na (meq/l)	156 ± 2
K (meq/l)	2.8 ± 0.1
Cl (meq/l)	126 ± 1
Glucose (mg/dl)	65
Formation rate (µl/min)	2.83 ± 0.18
TCO_2 (mmol/l)	27.4 ± 0.4
H^+ (nM)	44.3 ± 0.6
pH	7.35
Lactate (mmol/l)	2.8 ± 0.2
PCO_2 (mm Hg)	48.5
CSF volume*(µl)	250 ± 16
CSF pressure*(mm Hg)	38 ± 4

* 30-day old animals. The rate of CSF formation in these animals was 1.88 ± 0.17 µl/min (Bass and Lundborg, 1973).

central nervous system (cns)

The brain and spinal cord form the CNS. The brain consists of the cerebrum and cerebellum. The CNS has three coverings or meninges — dura mater, arachnoid, and pia mater. The dura mater is a tough fibrous material exterior to the other two meninges. The arachnoid is between the dura and pia mater, with the pia being significantly more delicate and thin than the dura.

The brain and spinal cord are bathed in cerebrospinal fluid (CSF). Normative values for CSF of the laboratory rat may be found in Table 4. The ventricles serve as a conduit for the CSF within the brain. In the rat the two lateral ventricles feed the singular third ventricle via the interventricular foramen. Connection of the third and fourth ventricles is through the cerebral aqueduct. Although there is no median aperture in the rat communicating with the subarachnoid space, there are two lateral apertures.

special senses

The senses of hearing and smell are well developed in rats; however, rats have poor eyesight. The rat's hearing threshold is

in the neighborhood of 15–25 kHz and peaks around 80 kHz. Cones are virtually absent from the retina of the rat, leaving the animal color blind. This inability to see long-wave, red light has some advantages when visual inspection of animals is necessary after hours; however, their ability to perceive dim light is good. Since an ophthalmic exam is a component of systemic toxicology studies, the review article by Kuiper et al. would be of interest to individuals performing ophthalmic examinations. This source provides an overview of the testing procedures, equipment, international guidelines, and ophthalmic terminology.

hematology and clinical chemistry

Hematologic and clinical chemistry values for healthy rats vary with age, strain, sex, and site of collection. Red blood cell count (RBC), packed cell volume (PCV), and hemoglobin levels are lower in juvenile rats than in adult rats. Relative and absolute neutrophil and lymphocyte numbers also vary with age. Certain enzymes such as lactate dehydrogenase (LDH) and alkaline phophatase (ALP) are highly variable in the rat, and dependent on sampling technique, type of restraint, etc. Others do not have the significance found in non-rodent species. For example, alanine aminotransferase (ALT) is liver specific in the rat, but aspartate aminotransferase (AST) is non-specific and of little diagnostic value in this species. It is imperative that laboratories performing these assays in rats establish in-house reference ranges for each strain or stock, and encourage submission of samples from control animals to assure reliable results.

In general, hematologic samples should be submitted in tubes containing the anticoagulant EDTA. Heparin is not a suitable anticoagulant for determining complete blood cell counts (CBC) and interferes with Wright's stain for differential counts. Heparin, sodium citrate, and other anticoagulants should be selected based on the assay being performed. Complete blood cell counts should be run the same day as sampling. Holding samples overnight, even if refrigerated, can cause inaccurate counts.

Most clinical chemistry assays are performed on serum, but for some, plasma is acceptable. Hemolysis invalidates many

clinical chemistry tests. Even minor hemolysis can falsely elevate such enzymes as lactate dehydrogenase (LDH). Storage of samples (serum or plasma) in a refrigerator or freezer may be possible, but some enzymes are unstable for longer periods of storage at any temperature. If storage of samples is unavoidable, care must be taken to place the samples in tight containers to avoid evaporation which occurs even in frozen samples.

If in doubt as to the type of sample to collect, contact the laboratory before taking the sample. This is particularly important if one is shipping samples overnight, as the assay may indicate the use of either a simple cold pack or dry ice in the shipping container. Table 5 lists ranges of hematologic and clinical chemistry measurements in rats.

reproduction

The following is a list of rat reproductive characteristics and reproductive values (Table 6):

The **Bruce effect**: pheromones from a strange male preventing implantation, does not occur in rats.

The **Whitten effect**: synchronization of estrus in females following the introduction of a male, although disputed is not thought to occur in rats.

Induction of anestrous in some members of a group of females housed together is known as the **Lee Boot effect**. This does not occur as strongly in rats as it does in mice.

The estrous cycle is light sensitive, with constant light resulting in persistent estrus and polycystic ovaries.

The estrous cycle is a 4–5 day cycle with the following cytologic characteristics of vaginal smears (Fig. 2):

- **Proestrus** (~12 h duration, Fig. 2A)

  nucleated epithelial cells

  leukocytes

  occasional cornified cells

TABLE 5. CLINICAL CHEMISTRY AND HEMATOLOGIC RANGES FOR RATS

Value	Normal Range[a]
Packed cell volume (PCV)	35–57%
Red blood cell count (RBC)	5–10 × 10^6/µl
White blood cell count (WBC)	3–17 × 10^3/µl
Hemoglobin (Hb)	11–19 g/dl
Mean corpuscular volume (MCV)	46–65 fl
Mean corpuscular Hb concentration (MCHC)	31–40 g/dl
Mean corpuscular Hb (MCH)	18–23 pg
Reticulocytes	0–25%[b]
Platelets	200–1500 × 10^3/µl
Neutrophils	13–26%[c]
Lymphocytes	65–83%[c]
Monocytes	0–4%[c]
Eosinophils	0–4%[c]
Basophils	0–1%[c]
Glucose	80–300 mg/dl[d]
Alanine aminotransferase (ALT)	52–224 IU/l
Calcium	9.1–15.1 mg/dl
Phosphorus, inorganic	4.7–16 mg/dl
Sodium	142–154 mEq/l
Potassium	3.6–9.2 mEq/l
Chloride	84–110 mEq/l
Blood urea nitrogen	11–23 mg/dl
Creatinine	0.4–1.4 mg/dl
Total protein	4.5–8.4 mg/dl
Albumin	2.9–5.9 g/dl
Total bilirubin	0.0–0.64 mg/dl
Activated partial thromboplastin time	19.3 seconds
Prothrombin time	28.8 seconds
Thrombin time	32.6 seconds

[a] Ranges are wide, reflecting the variability due to strain, age, and sex differences.

[b] Value is highly age related, higher values are normal for weanling rats.

[c] Percent of total white blood cells.

[d] Enzyme values are collection method dependent and may be expected to vary among laboratories.

- **Estrus** (~12 h duration, Fig. 2B)

  75% nucleated cells

  25% cornified cells

  Cornified cells will predominate as estrus progresses

TABLE 6. REPRODUCTIVE VALUES FOR THE LABORATORY RAT

Parameter	Value
Puberty (days)	50 ± 10
Gestation (days)	21–23
Estrous cycle (days)	4–5
Maximum fertility (days)	100–300
Birth weight (grams)	5–6
Eyes open (days)	10–14
Ears open (days)	12–14
Haircoat (days)	8–9
Weaning (days)	21
Solid food (days)	11–13
Postpartum estrus	Yes
Vaginal opening (days)	28–60
Litter size (pups)	3–18

- **Metestrus** (~21 h duration, Fig. 2C)

  large numbers of leukocytes and cornified cells

  cellular debris

  large, flat nucleated (pavement) cells

- **Diestrus** (~57 h duration)

  consists mainly of leukocytes

Parturition lasts about 90 minutes

Post-partum estrus occurs, with about 50% fertility, and delayed implantation (4–7 d)

Breeding systems can be either monogamous (1 male, 1 female) or polygamous (1 male, 2–6 females)

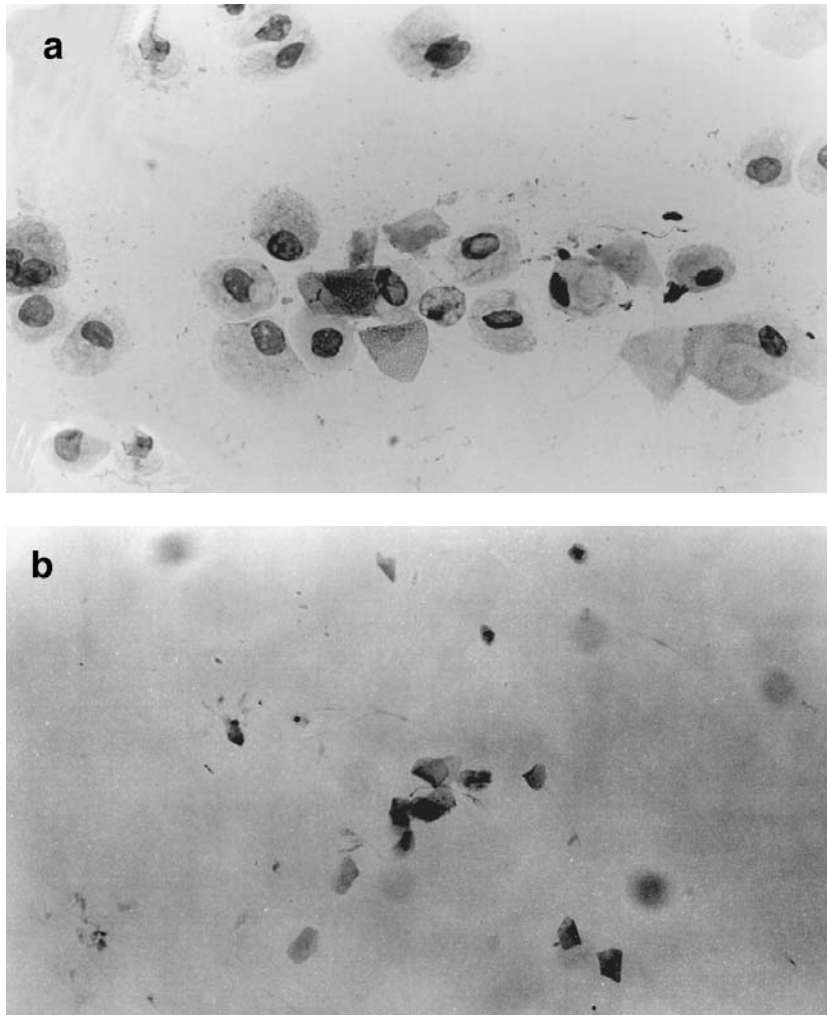


Fig. 2. Cytology of vaginal smears of the various stages of the rat estrous cycle are represented above by: (a) proestrus, (b) late estrus, (c) metestrus.

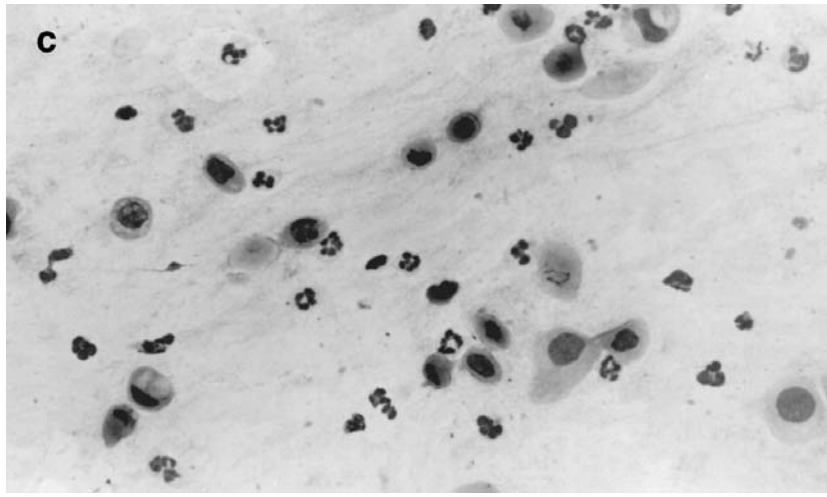


Fig. 2 (continued)

2

husbandry

Good animal husbandry is an important component of laboratory animal care. Proper husbandry includes meeting the housing, enrichment, nutritional, and sanitation needs of rats in a manner which causes minimal stress to the animals, caretakers, and other scientific personnel. Other important aspects involve animal transportation and record keeping.

housing

One may view animal housing as three interrelated, yet successively larger components or environments — microenvironment, macroenvironment, and megaenvironment. The microenvironment consists of items in an animal's cage or primary enclosure. Items in an animal's room constitute the macroenvironment, and the overall facility or building is the megaenvironment.

Microenvironment

The primary enclosure should address the following items for rats:

- Provide for the rat's behavioral and physiological needs.
- Provide for social interaction and hierarchical development.

- Provide a clean, dry, and safe area for the animal with adequate ventilation, food, and water.
- Permit visualization by personnel with minimal disturbance to the animal.

Other important considerations for the primary enclosure include the following:

size

Cage space must be sufficient to allow the rat to turn around and make normal postural movements. *The Guide for the Care and Use of Laboratory Animals* establishes specifications for floor area and height of the primary enclosure (Table 7). Rats larger than 500 g may require additional surface area to make normal postural movements.

TABLE 7. SPACE RECOMMENDATIONS FOR GROUP-HOUSED LABORATORY RATS

Weight	Floor Area in² (cm²)	Height in (cm)
<100 g	17 (109.65)	7 (17.78)
Up to 200 g	23 (148.35)	7 (17.78)
Up to 300 g	29 (187.05)	7 (17.78)
Up to 400 g	40 (258.00)	7 (17.78)
Up to 500 g	60 (387.00)	7 (17.78)
>500 g	Š70 (451.5)	7 (17.78)

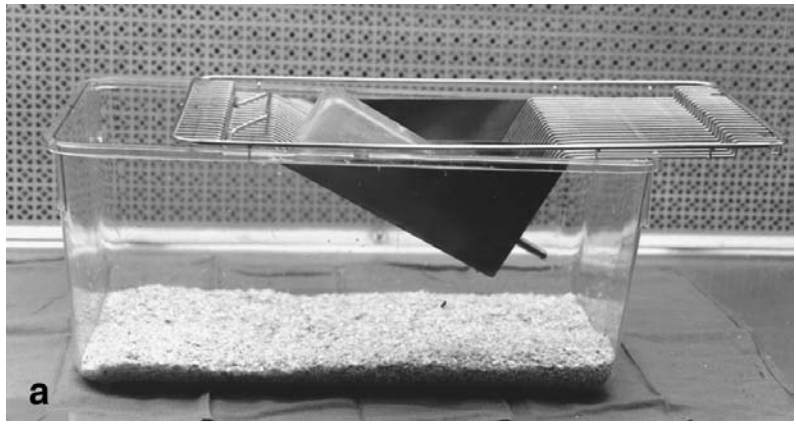
design

The primary enclosure usually consists of a stainless steel wire mesh (Fig. 3) or solid bottom flooring (Fig. 4). Although wire mesh has some advantages, rats prefer solid bottom enclosures with bedding. A further modification of the primary enclosure is the filter or microisolator top (Fig. 5), which reduces the spread of various pathogens. This advantage far outweighs the increase in handling time and elevations in temperature, humidity, gaseous, and particulate levels associated with use of primary enclosures. The increases in temperature and humidity can be kept to a minimum with good husbandry and cleaning practices. Some forms of research require elevation of the animal(s) above the solid bottom cage floor to minimize contact with excreta. Stainless steel wire inserts are available for this purpose.

Fig. 3. The stainless steel primary enclosure (a) has a V-shaped notch to hold the water bottle and a feed bin immediately to the left; (b) the circular hole at the rear may be used for entry of an automatic watering spout; (c) the flanges at the top of the enclosure permit the cage to slide in and out of the rack.

cage materials

The primary enclosure should be constructed of a material which is easy to clean and disinfect, durable, and resists corrosion. In addition, it is important for caging materials to be smooth, have impervious surfaces, and be kept in good repair. Stainless steel and durable plastics meet these criteria with most



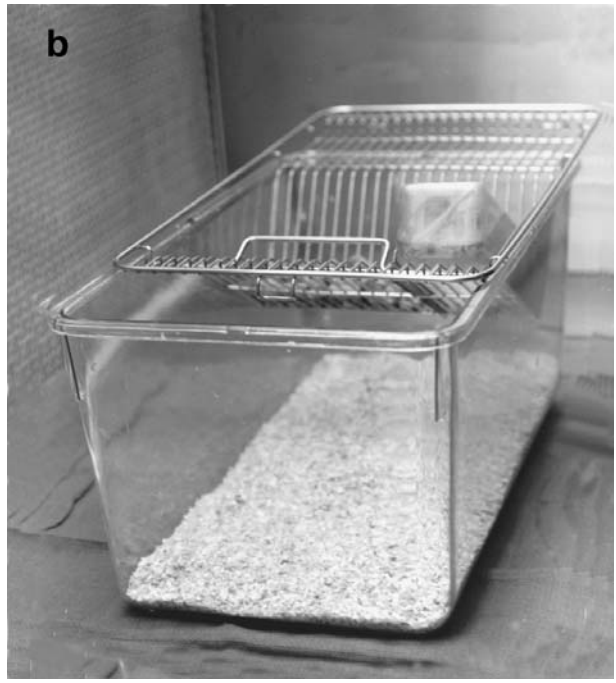


Fig. 4. Plastic primary enclosure. Example of a plastic (polycarbonate) enclosure with a stainless steel top. The stainless steel top supplies an area for the water bottle and an adjacent area for food. (a) side view, (b) front view.

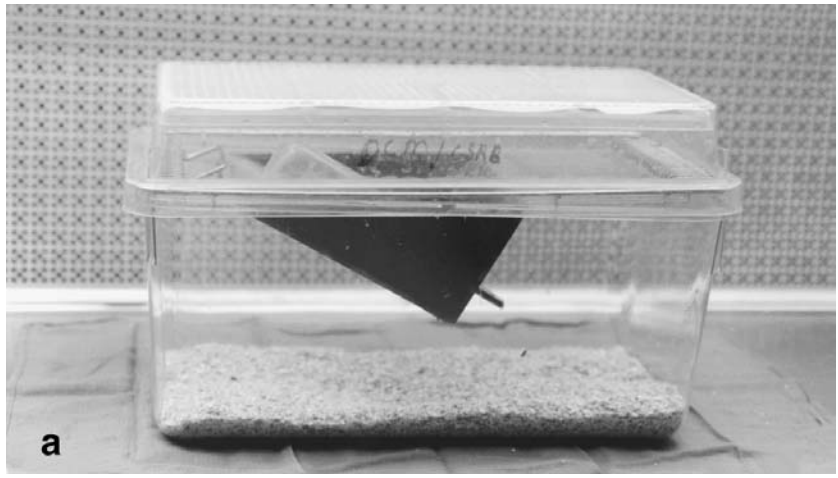


Fig. 5. The addition of the microisolator top prevents contamination from the micro- and macroenvironment. (a) side view, (b) front view.

contemporary caging being the latter. Plastic primary enclosures may be constructed of either polycarbonate or polypropylene construction, with polycarbonate permitting easy visualization of animals because it is clear.

The stainless steel wire top of the plastic primary enclosures acts to contain the rats, and to provide a feeding area. Although adult rats can easily gnaw at the food between the bars, younger or debilitated rats may be unable to feed in this manner, and other accommodations should be made. This may include either placing feed or moistened feed on the cage bottom.

One may use either water bottles or automatic watering systems to provide the water for the animals. Optimally, one should sanitize the water bottles before refilling, and it is necessary to drain and flush automatic systems when sanitizing cage racks. In order to control some bacterial infections, such as *Pseudomonas aeruginosa* and *Pasteurella pneumotropica*, water acidification (pH 2.3–2.5) or chlorination (8–12 ppm) may be necessary. One may acidify water by adding 0.8 mL of 25% hydrochloric acid to each liter of water and checking the resulting pH with a pH meter. Acidification at this level has no impact on body weight gain, hematology, biochemical analysis, or acid-base balance. One should be aware of water acidification's impact on mineral leaching from rubber stoppers, especially zinc and chromium. Acidified deionized water will leach more minerals than deionized water, which may impact some studies. Although automatic watering systems offer labor savings, daily inspections for inadequate or excessive flow are necessary.

Contact bedding material (Fig. 6), bedding in physical contact with the animals, should be absorbent, dust-free, nontoxic, inexpensive, sterilizable, contaminant free, and easily disposable. The type of contact bedding one selects can impact microenvironmental contaminants such as ammonia. Bacterial fermentation is thought to result from the humidity exceeding the moisture threshold of a given bedding. Above this threshold, urease-positive bacteria thrive, resulting in ammonia production. Contact bedding materials would include recycled paper, ground corncob, cellulose, and most commonly wood chips (pine, aspen, or hardwood chips). Studies of various bedding types indicate corncob bedding, unbleached eucalyptus pulp, and virgin cellulose maintain the lowest levels of

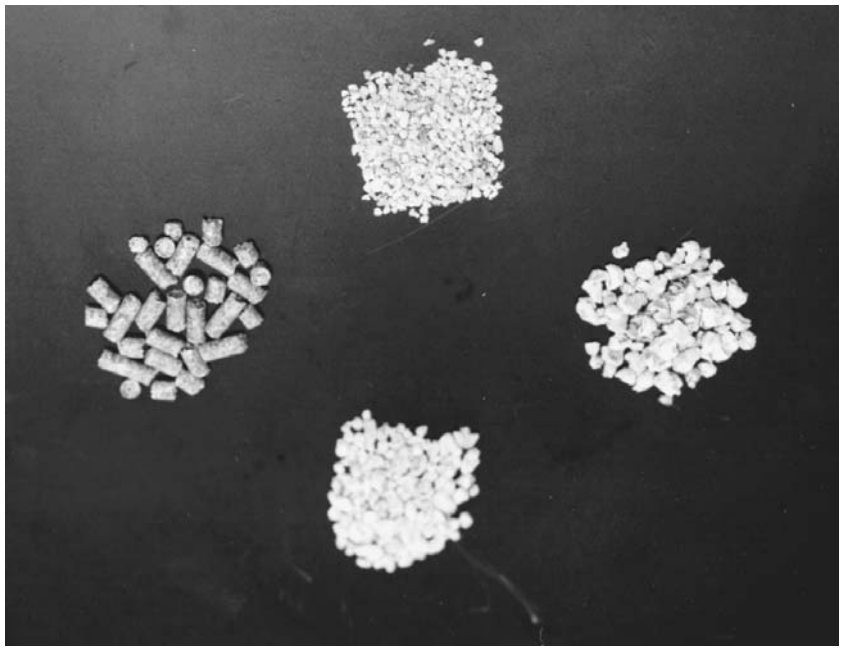


Fig. 6. Examples of bedding materials. Clockwise from the top, 1/8 inch corncob, 1/4 inch corncob, corncob mix, and pelleted noncontact bedding for suspended cages.

ammonia concentration. Previous contamination of the wood with chemical (pesticides) and biologic agents (aflatoxins) can present a potential risk. Some wood products contain natural compounds which may alter research. Specifically, cedar and some other softwoods contain cedrene and cedrol which are known inducers of hepatic microsomal enzymes. Failure to identify similar compounds in hardwoods is responsible for their popularity.

Macroenvironment

The macroenvironment is the room or secondary enclosure (Fig. 7). There are five items of importance with respect to the macroenvironment: temperature, humidity, ventilation, illumination, and noise. Temperature and humidity will be given joint consideration due to their synergistic effects on heat loss and comfort.

Fig. 7. Typical secondary enclosure setup. The primary enclosures in this barrier facility are placed onto racks with cage changes occurring in the hood at the back of the room.

temperature and humidity

The synergy of temperature and humidity will impact an animal's thermal balance. Recommendations for dry-bulb temperature and relative humidity for laboratory rats are 18–20°C (64–79°F) and 30–70%, respectively. Wild rats may require deviation from the laboratory rat recommendations. The deviations for wild rats should be in accord with their previous climate. Temperature and relative humidity in the microenvironment may vary significantly from the macroenvironment, due to factors such as animal density, microisolator top use, bedding type,

and cage changing frequency. Increased animal density and microisolator use can result in increases in the temperature and humidity in the microenvironment. Certain bedding materials, such as corncob and virgin cellulose, and an increased changing frequency result in a lower humidity in the microenvironment.

ventilation

The microenvironment and macroenvironment interact most closely with respect to ventilation. Ventilation impacts the temperature, relative humidity, illumination (removal of thermal loads), and noise within the macroenvironment. The air supply to the room must be sufficient for the number of primary enclosures and other research equipment. Ventilation not only provides an oxygen supply, but must account for the thermal loads and moisture content within a room. Providing pressure differentials to maintain a biohazard room with negative pressure relative to the hallway or to maintain a barrier room positive relative to a hallway, is yet another important role of ventilation. The general recommendation of 10–15 fresh-air changes per hour per room may result in over- or underventilation. One should therefore determine the air handling requirements based on animals and equipment present in a room being careful to accommodate odor and allergen control, metabolic gases, and particulate debris. Ventilation equipment will contribute to noise within the environment through such mechanisms as fans and flow-through conduits. Specialized equipment such as force-filtered caging, Trexler flexible plastic film isolator, or microisolators may further impact ventilation requirements.

illumination

Illumination must permit minimal interruption in the rat's behavior and physiology, yet enable individuals to carry out their charge of routine animal care. The three most important aspects of illumination are spectral quality, photoperiod, and photointensity. The albino rat seems most sensitive to photointensity and therefore most recommendations are based on phototoxic retinopathy. Light levels at the cage level should be between 130 and 325 lux. Levels of 325 lux, 1 m above the floor are satisfactory for routine animal care. Photoperiod can have a significant impact on reproduction, with 13–14 hours of light thought to be optimal for breeding colonies. Timing devices must be

checked regularly for proper function, as a longer (constant) photoperiod may result in the interruption of the normal estrous cycle.

noise

Noise frequency (Hz) and energy (dB) can have an impact on laboratory rats. There are three sources of noise in the animal facility: technical devices, work in the room, and the animals themselves. The rat's hearing ranges from 500 Hz to 60–80 kHz, and plays a significant role in social behaviors. High energy sounds may result in auditory and nonauditory changes in rats. Nonauditory changes include stress, metabolic alterations, and reduced fertility. Specific responses include increases in adrenal weights and total leukocyte counts and decreases in numbers of eosinophils (eosinopenia) and food intake. Individual behavioral differences correlate with corticosteroid elevations. Sound insulating materials may conflict with the desirable construction qualities of laboratory animal facilities as they may not be impervious to moisture, therefore placement of rats near "loud" animals (dogs, primates, etc.) and "loud" areas (cagewash, breakrooms, etc.) should be minimized. Voipio has published on the reaction of rats to specific sounds, including rat screams.

Megaenvironment

The megaenvironment provides quality facilities where research may be performed. There are many sources of in depth information concerning facilities and facility planning. The authors recommend the following:

The Animal Welfare Act — Although rats are not a covered species, it would be the exception to have facilities designed solely for rats, therefore, general concepts should be followed.

Good Laboratory Practice Standards (Sections 58.43, animal care facilities, and 58.90, animal care).

The Guide for the Care and Use of Laboratory Animals.

Public Health Service Policy on Humane Care and Use of Laboratory Animals.

The Handbook of Facilities Planning, Volume 2: Laboratory Animal Facilities.

The Laboratory Rat (ACLAM series).

Electronic Resources — Compmed and Compmed Archives.

The five general areas of concern when building or renovating a facility are (1) location and design, (2) construction and architectural finishes, (3) facility monitoring, (4) special housing and research needs, and (5) security.

location and design

Consideration should be given to many different issues when deciding where to put facilities. Among these are:

- special concerns — earthquake, hurricane, tornado, and other potential disasters
- geologic features of the proposed site(s)
- climate — prevailing winds, severity of winter and summer, etc.
- utilities and waste management
- state and local regulations and codes
- potential for future expansion and remodeling
- adjacent property utilization
- accessibility and security
- centralized vs. decentralized facilities
- functional areas
- space requirements

construction and architectural finishes

Surfaces in rooms should permit ease of cleaning and be moisture and skid proof. Furthermore, the surfaces must withstand the detergents and disinfectants used for cleaning and disinfection and permit rapid water removal. One may minimize wear and tear to the facilities with proper placement of guardrails, curbs, and kickplates. It is important to seal any cracks, as they will prevent adequate cleaning and provide an area for vermin to hide.

facility monitoring

By monitoring and maintaining records on various facility parameters (temperature, humidity, photoperiod, etc.) one can determine if the facility is meeting the desired performance standards. One may monitor electronically (computer records of data parameters), manually, or via a combination. Monitoring is also a security issue as it may indicate after-hours access, or alert personnel to dangerous parameters such as excessive temperature in a room.

special housing and research needs

The animals in an investigator's study may have special needs. This may include specialized housing (reverse light cycle, elevated room temperature, etc.) or research (cryopreservation, biohazard, irradiator, etc.) needs. Housing needs may include a barrier system to maintain the microbial status of a group of animals or prevent the introduction of undesirable agents. Research needs may involve the use of biohazard, physical, and/or chemical agents. Preventing or minimizing unwanted animal and personnel exposure to these agents must be a priority. Biohazard agents are assigned a biosafety level which indicates the ease of contracting these agents and the severity of the disease associated with the agent. Identification of animal biosafety level is through a numbering system (1, 2, 3, or 4). Studies involving agents in categories 1 and 2 can occur in most barrier facilities, but those studies of category 3 and 4 agents will need a higher level of containment. The assignment of biosafety levels depends on the particular agent and quantity of agent under study. Further description of the biosafety levels and the containment needs of an agent may be found in *Biosafety in the Laboratory*.

housing systems

Rats are maintained under one of the following schemes: conventional, barrier, or isolation.

Conventional animal maintenance uses no special containment procedures to reduce the risk of pathogen introduction or spread. Although their use is quite uncommon in conventional facilities, one may use filter top cages to minimize pathogen transmission between animals of varying microbiologic or pathogen status.

Barrier facilities require more restriction to reduce the potential for pathogen introduction. The preparation of barrier facilities for occupation will require measures effective against bacteria, viruses, coccidia, and arthropods. Sebesteny provides an excellent discussion of a barrier facility treatment protocol. Barriers will use either filter top micro-isolators (static system) or ventilated racks (Fig. 8) to reduce the potential for contamination.

Fig. 8. Ventilated cage rack system. (Photo courtesy Ron Orta, Allentown Caging Equipment Company.)

Ventilated systems offer many advantages over static systems with regards to reducing the cage changing frequency and the buildup of ammonia, water vapor, carbon dioxide, and other gasses and particulates within the microenvironment. Disadvantages include infrequent animal handling, and a potential reduction in the frequency and quality of animal health observations. Personnel follow a given procedure to enter a facility which may require some or all of the following to prevent pathogen entry:

- disrobing
- showering
- use of clothing dedicated to a facility or part of a facility
- gowning
- gloving
- cap
- mask
- booties

Note: Rats should be handled and transferred to clean cages in HEPA-filter hood.

Isolation facilities may use either rigid or plastic isolators (Figs. 9a and b) to contain the animals and equipment. All materials and supplies must be sterilized prior to entry into the isolator, including air. This setup is the most restrictive with manipulation of animals and equipment within the isolator occurring through the attached gloves.

security

Security should alert appropriate individuals in case of a break-in or mechanical breakdown. Either event could result in damage to the facility, disruption of research, and loss of valuable research animals. A security tree should be established

with definite points of contact in case of such an emergency. This plan should be subject to periodic review.

Besides locks and keys, one may wish to consider some type of computer card key access. Although this will help indicate who is accessing the facility and at what time, it is far from perfect. Card keys can still be passed from one individual to another or become lost. Mobile security guards and closed circuit monitoring will also enhance security.

environmental enrichment

Rats, like other laboratory animals, prefer objects to manipulate within their microenvironment. The objects which provide environmental enrichment are usually an extension of the rat's normal behavior — gnawing and burrowing. Although some of the following devices may provide environmental enrichment, there may be limitations on their use due to an object's ability to undergo sterilization, or a study's objectives (nutrition or toxicology studies):

- wood blocks
- nest boxes or nesting material
- shredded paper
- social housing
- stainless steel nuts
- colored marbles
- plastic piping
- plastic bones

nutrition

The nutrient requirements of the rat varies with this omnivore's life cycle, research requirements, environment, microbiological status, and genetics. A nutritionally complete, uncontaminated, and palatable diet should be available to meet these requirements. The National Research Council (NRC, United States) has established the nutrient requirements for various animal species, including rats. The basis of the nutrient requirements are the lowest nutrient amount which fails to show a deficiency, equilibrium between intake and excretion, or maintenance of normal metabolite levels in the blood and urine.

Fig. 9. (a) The front view of a flexible film isolator. There is no need for microisolator tops for the cages as animal manipulations are performed by individuals using the attached gloves. (b) The back view of a flexible film isolator. The unit on the bottom shelf is responsible for moving HEPA (High Efficiency Particulate Arrestance) filtered air in and out of the unit. The two large circular ports in the top two shelves are used to move materials in and out of the isolator. (Photo courtesy Karen Holland, Class Biologically Clean, Ltd., 2901 Latham, Madison, WI 53173.)

Fig. 9 (continued)

Dietary Requirements

The rat's dietary requirements may be classified into five categories: energy, protein, minerals, vitamins, and other potentially beneficial dietary constituents. The specific nutrient requirements for maintenance, growth, and reproduction may be found in Table 8. Specific areas of concern to investigators include interactions among nutrients, which may affect availability and husbandry issues such as animal housing. Housing

TABLE 8. ESTIMATED NUTRIENT REQUIREMENTS FOR MAINTENANCE, GROWTH, AND REPRODUCTION IN THE RAT

		Amount per kg of Diet		
Nutrient	**Unit**	**Maintenance**	**Growth**	**Reproduction (Female)**
Fat	g	50.0	50.0	50.0
Linoleic acid (n-6)	g	2.0	6.0	3.0
Linolenic acid (n-3)	g	R	R	R
Protein	g	50.0	150.0	150.0
Amino Acids				
Arginine	g	ND	4.3	4.3
Aromatic AA	g	1.9	10.2	10.2
Histidine	g	0.8	2.8	2.8
Isoleucine	g	3.1	6.2	6.2
Leucine	g	1.8	10.7	10.7
Lysine	g	1.1	9.2	9.2
Methionine + cystine	g	2.3	9.8	9.8
Threonine	g	1.8	6.2	6.2
Tryptophan	g	0.5	2.0	2.0
Valine	g	2.3	7.4	7.4
Other AA (including nonessential)	g	[a]	66.0	66.0
Minerals				
Calcium	g	[b]	5.0	6.3
Chloride	g	[b]	0.5	0.5
Magnesium	g	[b]	0.5	0.6
Phosphorus	g	[b]	3.0	3.7
Potassium	g	[b]	3.6	3.6
Sodium	g	[b]	0.5	0.5
Copper	mg	[b]	5.0	8.0
Iron	mg	[b]	35.0	75.0
Manganese	mg	[b]	10.0	10.0
Zinc	mg	[b]	12.0	25.0
Iodine	µg	[b]	150.0	150.0
Molybdenum	µg	[b]	150.0	150.0
Selenium	µg	[b]	150.0	400.0
Vitamins				
A (retinol)	mg	[b]	0.7	0.7
D (cholecalciferol)	mg	[b]	0.025	0.025
E (RRR-α-tocopherol)	mg	[b]	18.0	18.0
K (phylloquinone)	mg	[b]	1.0	1.0
Biotin (*d*-biotin)	mg	[b]	0.2	0.2
Choline (free base)	mg	[b]	750.0	750.0
Folic Acid	mg	[b]	1.0	1.0
Niacin (nicotinic acid)	mg	[b]	15.0	15.0

TABLE 8. ESTIMATED NUTRIENT REQUIREMENTS FOR MAINTENANCE, GROWTH, AND REPRODUCTION IN THE RAT

Nutrient	Unit	Amount per kg of Diet		
		Maintenance	Growth	Reproduction (Female)
Pantothenate (Ca-*d*-pantothenate)	mg	[b]	10.0	10.0
Riboflavin	mg	[b]	3.0	4.0
Thiamin (thiamin-HCl)	mg	[b]	4.0	4.0
B_6 (pyridoxine)	mg	[b]	6.0	6.0
B_{12}	µg	[b]	50.0	50.0

Nutrient requirements are expressed on an as-fed basis for diets containing 10% moisture and 3.8–4.1 kcal ME/g (16–17 kJ ME/g) and should be adjusted for diets of differing moisture and energy concentrations. Unless otherwise specified, the listed nutrient concentrations represent minimal requirements and do not include a margin of safety. Higher concentrations for many nutrients may be warranted in natural-ingredient diets. R, required but no concentration determined; other long-chain n-3 polyunsaturated fatty acids may substitute for linolenic acid. ND, not determined.

[a] 41.3 g/kg diet as a mixture of glycine, L-alanine, and L-serine.

[b] Separate requirements for maintenance have not been determined for minerals and vitamins. Requirements presented for growth will meet maintenance requirements.

From *Nutrient Requirements of Laboratory Animals*, 4th rev.ed., Copyright 1995 by the National Academy of Sciences. Courtesy of the National Academy Press, Washington, D.C.

which prevents coprophagy and/or has galvanized (zinc) caging may significantly alter an animal's nutritional status. Coprophagy serves as a source of nutrients for the rat, and restricting access to feces may impact nutritional status. The use of galvanized caging results in a lower zinc requirement since the animal may meet part of its requirement from gnawing on the caging. The NRC nutrient requirements discuss deficiency and toxicity signs which may be found in rats.

energy

Rats are usually fed on an ad libitum basis. The animal's energy requirements may be met by feeding carbohydrates, lipids, or protein; however, protein is an expensive way to meet the requirement. Rats consume a diet to meet their own energy requirement.

The energy requirement may vary depending on temperature, age, and activity level. The basal metabolic rate (BMR) in mature rats can be estimated using the following formula:

$$H_{kcal} = 72\ BW_{kg}^{0.75}$$

where

H_{kcal} is the heat production in kcal per day

BW is the body weight in kg

72 is the average heat production (kcal) per $kg^{0.75}$

To determine the BMR in Joules the equation is:

$$H_J = 301\ BW_{kg}^{0.75}.$$

The usual means to meet the energy requirement is through the addition of carbohydrates and lipids to the diet. Although there is no specific carbohydrate requirement, glucose, fructose, sucrose, starch, dextrins, and maltose are the most common. It should be kept in mind that xylose is toxic, and other carbohydrates may yield less than adequate performance. Lipids provide a source of energy and the essential fatty acids (EFAs), assist fat-soluble vitamin absorption, and increase diet palatability. The two EFAs of nutritional concern are the n-6 and n-3 fatty acids. Linoleic acid will supply the n-6 fatty acids, and linolenic acid the n-3 fatty acids. Although the n-3 fatty acid requirement remains undefined, their inclusion in the diet is advisable.

caloric restriction

Results from numerous studies indicate that restrictions in caloric intake (30–40% restriction from ad libitum feeding) tend to increase lifespan and life expectancy, decrease the incidence and severity of degenerative diseases, and delays neoplasia onset in the rat. The concept is to reduce caloric intake without causing malnourishment. There are several theories to explain these results. In any event, employing ad libitum feeding in some studies (toxicology, aging) may warrant reconsideration.

proteins and amino acids

The latest NRC estimates (Table 8) recommend a 5% protein concentration for maintenance, and a 15% protein concentration for growth and reproduction (females). Specific amino acid (AA) requirements are, on average, 23% higher than previous NRC recommendations.

minerals

Minerals are divided into two categories — macrominerals and microminerals — based on their dietary levels. There are six macrominerals of concern: calcium, phosphorus, chloride, magnesium, potassium, and sodium. The seven microminerals or trace minerals include copper, iodine, iron, manganese, molybendum, selenium, and zinc.

vitamins

One may classify vitamins as either fat-soluble or water-soluble. Fat-soluble vitamins include A, D, E, K. The water-soluble vitamins are B6, B12, biotin, choline, folate, niacin, pantothenic acid, riboflavin, and thiamin.

potentially beneficial dietary constituents

The latest NRC dietary recommendations do not have requirements for potentially beneficial dietary constituents; however, animals on natural ingredient diets appear to respond better to stressors than those animals on more purified diets. These potentially beneficial dietary constituents include fiber, chromium, lithium, nickel, silicon, sulfur, vanadium, ascorbic acid, and myo-*inositol*.

Diet Types

There are three types of diets for laboratory animals, varying in their level of refinement. They are natural ingredient, purified, and chemically defined diets.

natural ingredient diets

Natural ingredient diets are the most widely used diets due to their low cost and palatability. Diet formulations may vary in nutrient concentrations and are subject to contamination. These two concerns over natural ingredient diets may influence their

use in some studies. Open- and closed-formula diets are the two subclassifications of commercially available natural ingredient diets. Open-formula diets specify the exact quantity of each ingredient in the diet, whereas, closed-formula diets do not. "Certified diets" undergo analysis for nutrients and contaminants following preparation. These results are then supplied with the diet.

purified diets

The dietary ingredients in a purified diet are from a single nutrient or nutrient class. This level of refinement results in less nutrient variability and contamination than natural ingredient diets. These diets are less palatable and more expensive than natural ingredient diets.

chemically defined diets

The dietary ingredients in chemically defined diets are individual AAs, specific sugars, chemically defined triglycerides, EFAs, inorganic salts, and pure vitamins. This level of refinement results in nutrient consistency and little contamination; however, the chemically defined diet offers poor palatability and high diet expense as significant concerns.

Dietary Forms

Many dietary forms are available to the investigator; however, the most common is the pelleted diet. Other forms include extruded diets, meal, gel, crumbled, and liquid diets. Pelleted diets offer many advantages over the other forms with regard to handling, storage, use, and minimizing waste and dust. Meal and extruded diets are not commonly used because of wastage and the generation of dust with the meal form. Gel and liquid diets need refrigeration to reduce bacterial growth; however, due to their form, they permit addition of toxic test materials without concern over dust formation. Müller describes the use of a commercial elementary diet for long-term enteral nutrition in rats.

Diet Sterilization

Sterilization of the diet is a requirement for defined flora animals (specific pathogen-free and germ-free) and can be desirable for

conventional animals. One may sterilize a diet by autoclaving, ionizing radiation, and even filtration (some chemically defined diets). Autoclaving typically results in greater nutritional deterioration over ionizing radiation, under ideal conditions (low moisture and packaging under vacuum or nitrogen). Since vitamins are especially vulnerable to damage during autoclaving, autoclavable diets contain, two to four times the required levels, to compensate for potential loss.

Diet Contamination

Dietary contamination may have a detrimental effect on a given research project, therefore prevention is key. It should be kept in mind that contamination may occur well before manufacture of the diet. Contamination may be biological, chemical, or accidental; falling into one of the following eight categories:

1. pesticides
2. pests
3. bacteria, bacterial toxins, and mycotoxins
4. natural plant toxins
5. breakdown products of nutrients
6. nitrates, nitrites, and nitrosamines
7. heavy metals
8. formulation or manufacture errors

Several sources have recommendations for acceptable levels of contamination (EPA, 1979; FDA, 1978; ICLAS, 1987; and Rao and Knapka, 1987).

Diet Storage

Storage of commercially available diets should be in a clean area, which prevents vermin contamination, and has a controllable environment to avoid environmental extremes. Recommendations are to store commercially available diets at 21°C, and purified or chemically defined diets at 4°C. In general natural ingredient diets may be used for up to 6 months after milling, whereas more refined diets may have significantly shorter lifetimes. One should store diets in containers with tight-fitting lids.

Water

Pelleted diets usually have a 7–12% moisture content, with either water bottles or automatic watering systems providing the remainder of the ad libitum water requirement. Ambient temperature, relative humidity, and the diet's moisture content determine the rat's water requirement. Although each watering system has its own advantages and disadvantages, each is capable of supplying fresh water if adequate precautions are taken. Ultimately, it is important for the water reaching the animal to be drinkable and free of contamination. The availability of free choice water is very important. It is possible for rats to consume 1/4 to 1/3 of their body weight in water daily. For instance, at 22°C a rat's water consumption will outpace its food consumption by about 20%, and at 30°C water consumption will be about double food consumption on a per weight basis.

Although tap water may meet or exceed the standards and regulations for human consumption, the water may need further treatment before offering it to a research animal. Such treatments (i.e., acidification with hydrochloric acid to pH 2.3–2.5) may be necessary to reduce or eliminate bacteria (for gnotobiotic or some SPF animals) or to remove compounds from the water (e.g., the use of distilled water to reduce minerals in a nutrition study). It is important to periodically determine if the water meets the research study's quality criteria, especially if there are any specific water treatments. Commercial laboratories are available which will perform water analysis. It is also important to ensure that water treatment does not interfere with the research (e.g., erosion of the enamel and dentine when using acidified water for rats used in dental research).

sanitation

Proper sanitation should include the micro-, macro-, and megaenvironment. Good sanitation acts to restrict the growth of microorganisms, reduce the risk of animal disease, and reduce the potential for experimental variables. Sanitation should impact all areas of an animal facility, and the detergents and disinfectants used should be carefully selected and used according to manufacturer's recommendations.

Note: It is important NOT to mask odors. Odor masking compounds do not replace good sanitation, and can result in physiologic and metabolic alterations.

Sanitation of any item which comes into contact with an animal is a logical, step-by-step process involving:

- cleaning
- washing/disinfection
- rinsing
- sterilization (if needed)

In addition, one should monitor the effectiveness of sanitation with regard to (1) visual inspection, (2) water temperature, (3) microbiologic monitoring. Visual inspection ensures there has been adequate physical cleaning prior to washing and disinfection of an item. Physical cleaning acts to reduce organic matter which may inactivate disinfecting agents. One should monitor water temperatures (58–82°C) to ensure they are adequate for killing the vegatative forms of pathogenic bacteria for a given duration of exposure. One may monitor the temperature through either contact indicators that change color or through organic indicators which contain organisms that are killed when an adequate temperature is acheived (Fig. 10). Microbiologic monitoring ensures adequate sanitation through culturing a surface by using either a sterile swab or RODAC (Replicate Organism Direct Agar Contact) plates.

Microenvironment

Bedding changes should be frequent enough to keep rats clean and dry. This frequency is a function of physiologic status, caging density, and experimental design. For instance, one would expect cages holding rats with diabetes mellitus to have more frequent cages than animals in a breeding colony, where frequent changes may alter pheromone concentrations. During bedding changes, it is wise to limit personnel exposure to aerosols by using laminar flow hoods, respirators, and/or protective clothing.

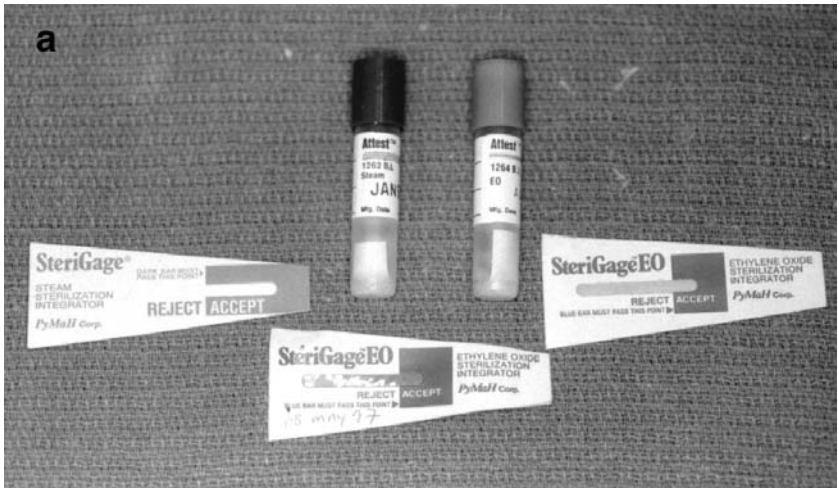


Fig. 10. (a) The two tubes at the top are examples of organic indicators which will require incubation following exposure to steam (left) and ethylene oxide (right). The two strips are examples of contact indicators. Although they do not require incubation, they do contain a color bar which must darken into the "Accept" region. The strip on the left is for steam sterilization and the strip on the right is for ethylene oxide. The strip at the bottom is an example of an acceptable sterilization with the ethylene oxide indicator.

- Sanitation of solid bottom cages and their accessories should occur at least weekly, and other primary enclosures (suspended stainless steel cages) every two weeks.
- One should disinfect cages following cleaning, which destroys vegetative forms of pathogens using chemical agents (disinfectants) and hot water.
- Detergents and disinfectants will enhance the effectiveness of hot water, but necessitate thorough rinsing to avoid problems when animals and human beings come into contact with these agents.
- The concept of cumulative heat factor involves hot water temperatures, 58–82°C (143–180°F), and time. As water temperature decreases, the minimum contact time increases, and vice versa.

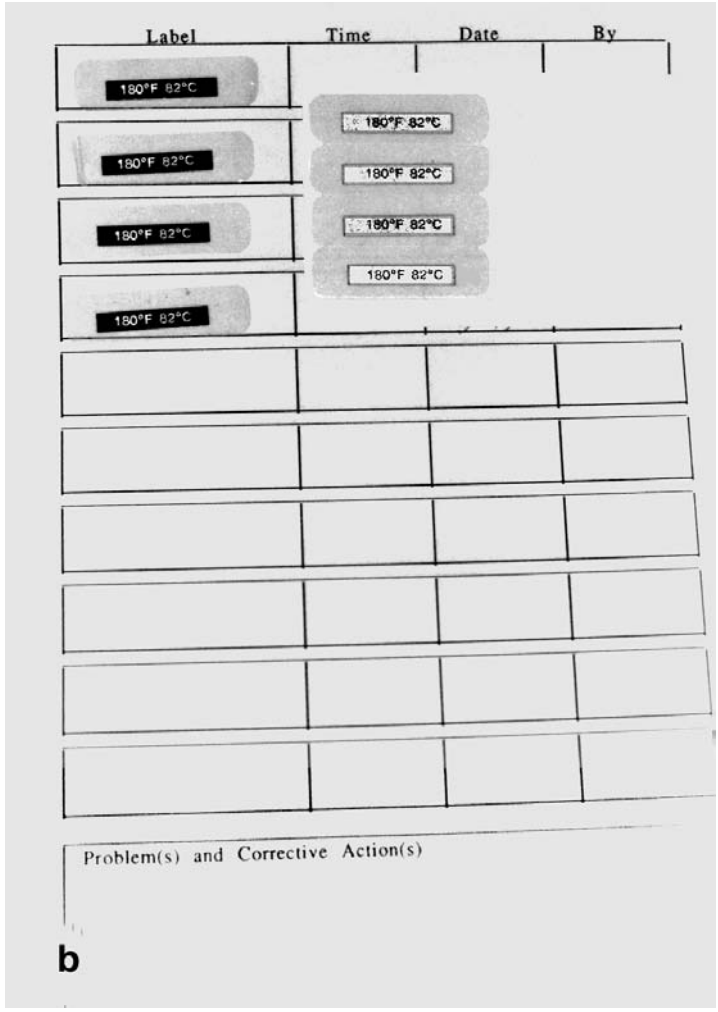


Fig. 10 (continued). (b) This photograph demonstrates a page from a logbook which shows the darkened reacted sanitation labels (180°F 82°C) in the "Label" column. To the right are four of the unreacted self-adhesive labels. Logbooks are good for monitoring the device(s) being sanitized and the sanitizing equipment (e.g., cagewashers). It is best to place the labels over more than one site on the equipment being sanitized.

- It is acceptable to hand wash cages and cage accessories, but it is labor intensive and requires attention to detail. In addition, there is a greater risk of exposure of personnel to hot water and harmful chemicals. An automatic cagewasher is preferable to manual cleaning.

Automatic watering systems will need periodic flushing with copious amounts of water or chemical agents followed by complete rinsing. Some institutions choose to utilize filters or UV light to sterilize water in automatic watering systems.

Some research requires the sterilization of cages and cage accessories after cleaning and disinfection. One may sterilize cages and cage accessories by autoclave, gas sterilization, or ionizing radiation; however, most institutions use a large autoclave. Sterilization will require a method of regular monitoring to ensure sterility.

Macro- and Megaenvironment

The room and building will need a regular schedule of cleaning and disinfection. The cleaning equipment should be dedicated to a given area and NOT transported between rooms, suites, floors, or facilities. The equipment should be of sound, durable, non-corrosive construction, kept in good repair, and stored appropriately to avoid clutter, permit drying, and avoid contamination.

Waste containers should meet the following criteria:

- Easily available.
- Constructed of plastic or metal.
- Leakproof.
- Have tight-fitting lids.
- Have plastic liners available.
- Easily distinguishable between food, hazardous, and nonhazardous waste.
- Those containers used for animal carcasses should have leakproof, disposable liners able to withstand refrigeration and freezing.

- Storage of the carcasses and disposed tissues must be physically separate from facilities housing animals or storing any materials which might come into contact with animals.
- The storage areas and containers should undergo frequent cleaning and disinfection. It is also important to keep these storage areas free of insects and other vermin.
- Hazardous waste must be handled differently, and according to local, state, and federal regulations.

transportation

Transportation of animals may be either global or local, both of which exert stress on the rat. The International Airline Transport Association (IATA), Laboratory Animal Breeders Association of Great Britain Limited (LABA), and the Laboratory Animal Science Association (LASA) have recommendations for shipping rats. The goal of transportation is to minimize the stress level of the rat by minimizing transit time and providing sources of food and water and adequate, filtered ventilation. It is also important to avoid overcrowding,physical trauma, and environmental extremes.

Commercial transport enclosures are available which meet the food, water, and ventilation needs, and are disposable to prevent cross contamination. When receiving rats, the outer surface of the container is subject to contamination during shipment and should be decontaminated prior to entering a facility. There are several disinfectants available, including a dilute bleach solution (28 mL/l of water), which may be sprayed onto the container's outer surface prior to entering the facility.

One should permit a stabilization period for the animals following shipment. This may involve quarantine of the animals prior to experimental use. It is good procedure to obtain information concerning the genetic and microbiologic status of animals prior to ordering from vendors, other institutions, and other divisions within the same institution. One should evaluate the information for timeliness (how recent is the information)

Table 9. Rat Adaption Period by Various Research Parameters

Research Parameter	Minimum Adaption Period
Body weight	<24 hours
Body weight gain	2 days
Water intake	17–23 days
Luteinizing hormone (LH)	>7 days
Leukocytes (segmented neutrophils)	12 days
AST, LDH, potassium, cholesterol	12 days

and location (is this information from the same room(s) as the animals received). Table 9 gives adaption periods depending on various research parameters.

When considering international shipments of rats, one should contact the responsible agency or agencies in the importing and exporting countries including the appropriate individuals at the receiving institution. Animals may quickly pass through customs, and end up at a loading dock unbeknownst to those directly responsible for animal care. To minimize delays at customs one should discuss the shipment with the responsible authorities prior to requesting animals. For importation these authorities would include the USDA (United States Department of Agriculture) and U.S. Customs; for exports, the USDA, the importing country's embassy, and individuals at the receiving institution.

quality control

Quality control of rats concerns their microbiologic and genetic background, and is an active process involving the use of sentinel rats and serial serologic analysis for evidence of pathogens. Various microbes can have a negative impact on research projects and result in a loss of time, money, and rats. This is especially important since rats can be in a persistently infected carrier state with some pathogens, even after resolution of clinical signs. For these reasons it is important to receive animals free of known pathogens whenever possible, and maintain them in a manner which prevents infection. The animals received should be SPF (specific pathogen free) or free of viral antibodies to minimize complications. Maintenance may vary from conventional housing to the use of isolators.

The genetic integrity of a rat strain requires sound husbandry, personnel, and management practices. Assessment of a strain's genetic integrity reduces experimental variability. The methods used to assess genetic integrity include DNA-typing, morphometric and biochemical methods. One may obtain this information from vendors.

identification and records

Accurate, concise research records necessitate animal identification. Some areas of research may require meticulous record keeping, such as a GLP (Good Laboratory Practice) study of a drug or medical device. Without adequate identification (individual or group) it is difficult to maintain records. One may identify individual rats by the following methods (listed least to most permanent):

- **Markers** and **dyes** are available for temporary identification on an animal's tail or fur.
- One may use metal **ear tags**, each with a unique number, to identify rats. These tags, even when properly placed, may be removed by the identified animal or its cagemate(s).
- **Ear punches** are an easy and more permanent means to identify animals. Identification depends on the location (left vs. right ear and location on a given ear) and number of notches. This system is highly variable, with individual laboratories developing their own identification systems.
- **Tattoos** are an effective and permanent means to identify rats (Fig. 11). The experience of the individual tattooing the animal has an impact on the quality of the tattoo. Besides placing identification on a rat's tail, tattoos are also an effective means to identify boney prominences in kinesiology studies, tumor placement sites, etc.
- **Electronic transponders** are also available for unique identification of animals. These small devices are implanted subcutaneously and are read with a transponder detector. Some transponder and detector models are programmable and permit temperature measurement

Fig. 11. Tattooing as a method of identification in the rat.

(Fig. 12). Although this method of identification is very efficient, it is also expensive. Another disadvantage is that the technology currently lacks standardization requiring one to purchase the chips and detectors from the same manufacturer, as manufacturer "A"'s chips are typically not read by manufacturer "B"'s detector.

Note: Digit amputation is an unacceptable and inhumane method of identification.

Usually, one will identify groups and individual animals by cage cards. Cage cards should carry the following information:

- Species
- Stock or strain
- Sex
- Source
- Protocol number
- Investigator
- Department
- Other information — contact person; office location, phone number

In addition, individual records may need to be kept. Surgery, breeding programs, and studies involving hazardous materials are instances where record keeping is essential to identify individual animals under study.

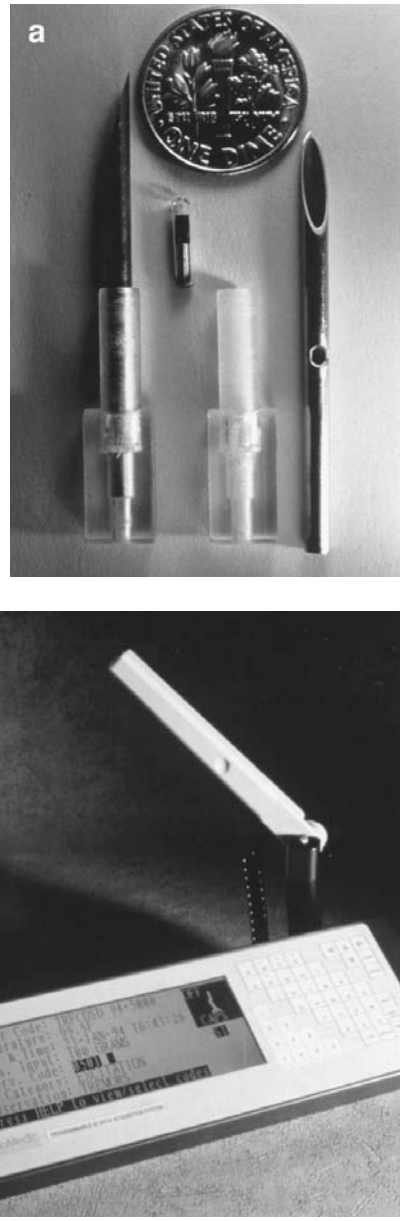


Fig. 12. Electronic transponder. The subcutaneous implant (a) is the small oblong device between the implanting needles and below the dime. The transponder detector (b) permits an investigator to individually identify an animal with the wand and make observation notes with the base unit.

3

management

regulatory agencies and compliance

Australia

The *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* is followed by most Australian states. The Code was written by the National Health and Medical Research Council (NHMRC), Commonwealth Scientific and Industrial Research Organization (CSIRO), and the Australian Agricultural Council (AAC). Although not legislation, researchers receiving funds from the above sources are expected to follow the Code. Some states have additional legislation and have incorporated the Code into their regulations.

- *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*

Canada

The *Canadian Council on Animal Care* sets forth the control of the scientific use of vertebrates and cephalopods in research, teaching, and testing. In addition, some provinces have regulations concerning the use of laboratory animals.

- *Canadian Council on Animal Care*

Europe

Regulation of animal use for experimental and scientific purposes occurs at the national and international level. The two international organizations responsible for regulating animal use are the 26 member Council of Europe (*Council of Europe Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes*) and the 12 member European Communities (*The European Communities Council Directive Regarding the Protection of Animals Used for Experimental and Other Scientific Purposes*). The 12 European Communities (EC) states are also members of the Council of Europe (CE). Enforcement of legislation is the responsibility of the individual country. Neither the CE Convention, nor the EC Directive prevent members from adopting stricter legislation. With a few exceptions, there is a high level of agreement between the two policies. Table 10 describes European legislation by country.

There are six CE members who have not signed the Convention and are without national legislation: Cyprus, Czechoslovakia, Hungary, Malta, Poland, and San Marino. In addition, the following are not CE members and have no national legislation: Albania, Bosnia-Hercegovina, Bulgaria, Croatia, Estonia, Latvia, Lithuania, Macedonia, Romania, Slovenia, Yugoslavia, and the Commonwealth of Independent States (CIS).

Japan

Regulation in Japan consists of an animal protection law, experimental animal standards, and a ministry notification. There is an emphasis on ethical codes rather than legislation in Japan.

- *Law for the Protection and Management of Animals* in 1973.
- *Standards Relating to the Care and Management of Experimental Animals*.
- *Animal Experimentation in Universities* (not legally binding).

New Zealand

Emphasis in New Zealand is placed on a code of conduct for those who use animals in research, teaching, testing, and production of biological agents. This code is specific to an institution.

TABLE 10. SUMMARY OF EUROPEAN LEGISLATION BY COUNTRY

Country	CE	EC	National Legislation
Austria	NS	NM	*Bundesgesetz vom 27 September 1989 über Versuche an lebenedn Tieren*
Belgium	S,R	M	*Loi du 14 Aout 1986 relative á la protection et au bien-être des animaux* (Legislative) *Arrêté royale relatif á protection des animaux d' expérience* (Royal Decree)
Denmark	S,NR	M	*Lov om dyreforsøg*
Finland	S,R	NM	Förordning om försöksdjurverksamhet
France	S,NR	M	*Décret no 87-848 relatif aux expériences pratiquées sur les animaux* and three supplementary *Arrêtés*
Germany	S,R	M	*Tierschutzgesetz*, *Allgemeine Verwaltungsvorschrift zur Durchführung des Tierschutzgesetzes*
Greece	S,R	M	Yes, conforms almost exactly to EC Directive
Iceland	NS	NM	*Reglugeró um notkun dyra í vísindalegum tilgagni*
Ireland	S,NR	M	*Cruelty to Animals Act*, and EC Directive
Italy	NS	M	Yes, conforms to EC Directive on almost every point
Liechtenstein	NS	NM	Total ban on animal use in experimental procedures
Luxembourg	NS	M	Yes
The Netherlands	S,NR	M	*Wet op de Dierproeven*, with a supplemental royal decree
Norway	S,R	NM	*Lov om dyrevern*, *Forskrifter om biologiske forsøk med dyr*
Portugal	NS	M	In preparation
Spain	S,R	M	*Sobre proteccíon de los animales utilizados para experimentacíon y otros fines científicos*
Sweden	S,R	NM	*Djurskyddslag*
Switzerland	S,NR	NM	*Tierschutzgesetz*, *Tierschutzverordnung*
Turkey	S,NR	NM	None
United Kingdom	S,NR	M	*Animals (Scientific Procedures) Act*

CE: Council of Europe Convention
EC: European Communities Directorate
M: Member
NM: Non-member
NR: Not ratified
NS: Not signed
R: Ratified
S: Signed

- *Animals Protection Amendment Act*
- *Animals Protection (Code of Ethical Conduct) Regulations*

United States

Regulatory oversight of animal research in the U.S. is the responsibility of the following agencies:

The United States Department of Agriculture (USDA) is responsible for oversight and enforcement of the **Animal Welfare Act** (AWA) (P.L. 91-579, 94-279, 99-198). This law requires registration of all institutions, except elementary and secondary schools, using animals in research, teaching, and testing. In defining animals, the AWA states "This term excludes: ...rats of the genus Rattus...bred for use in research...." This thereby currently exempts rats from the Act.

Awards from the National Institutes of Health (NIH) and National Science Foundation (NSF) must meet the provisions of the **Health Research Extension Act** (PL 99-158). Standards are those described in the *Guide for the Care and Use of Laboratory Animals*. The NIH Office for Protection from Research Risks (OPRR) is responsible for enforcing the Act.

The **Good Laboratory Practice (GLP) Act** contains regulations for conducting nonclinical laboratory safety studies for products regulated by the **Food and Drug Administration** (FDA). The regulations contain requirements for animal care, animal facilities, and study records.

The **Institutional Animal Care and Use Committee (IACUC)** is the basic unit of an effective animal care and use program. The PHS (when PHS funds are being used) and AAALAC require an IACUC at any institution using rats in research, teaching, and testing. Important points regarding the composition of the IACUC include:

- PHS policy requires a minimum of five members, including:
  - A chairperson.

- A Doctor of Veterinary Medicine who has training or experience in laboratory animal medicine or science, and responsibility for activities involving animals at the research facility.
- An individual who is in no way affiliated with the institution other than as an IACUC member. At some institutions this role has been filled by clergypersons, lawyers, or local humane society or animal shelter officials.
- A practicing scientist with experience in animal research.
- One member whose primary concerns are in a nonscientific area. This individual may be an employee of the institution served by the IACUC.

One individual can fulfill more than one of the above categories.

- IACUC Responsibilities
  - Protocol review for activities involving animals in research, teaching, and testing. Protocol approval by the IACUC must occur before animal use can begin.
  - Inspect and assure the animal research facilities and equipment meet acceptable standards.
  - Assure personnel are adequately trained and qualified to conduct research using animals.
  - Assure adequate handling and care for animals.
  - Assure consideration for alternatives to potentially painful and stressful procedures and determine the research to be nonduplicative.
  - Assure appropriate use of sedatives, analgesics, and anesthetics.
  - Assure that proper surgical preparation and techniques are used.
  - Assure that appropriate euthanasia techniques are used.

voluntary accreditation

Association for Assessment and Accreditation of Laboratory Animal Care International, Inc. (AAALAC International)

- AAALAC International is a nonprofit organization designed to provide peer review-based accreditation to animal research facilities.
- Basis for accreditation is adherence to principles described in *The Guide for the Care and Use of Laboratory Animals*.

occupational health and zoonotic diseases

Zoonoses are infectious diseases that are transmitted from animals to humans. Infectious agents can be transmitted by aerosol, ingestion, through skin wounds, or conjunctiva. Protective clothing, the use of biohazard hoods, and shower facilities may all be required depending on the nature of the biohazard. Specific procedures for dealing with various levels of biohazard agents are discussed elsewhere. Many facilities have environmental safety divisions which can provide the user with information and assistance with biohazard protocols.

At a minimum, gloves, a laboratory coat, and protective eyewear should be worn for any necropsy or when dealing with fresh tissue or body fluids from any animal. Gloves and a laboratory coat should be worn when handling rats. Thorough washing of hands after any procedure using animals is one of the best preventative measures.

Some of the more common zoonotic diseases associated with rats are:

Rat Bite Fever ("Haverhill Fever")

This disease is caused by either of two bacteria, *Streptobacillus moniliformis* and *Spirillum minus*.

- Rats are asymptomatic and the two organisms are often considered commensal bacteria.

- In humans the bacteria cause acute febrile illness. Clinical signs associated with *S. minus* infection include inflammation at the wound site and lymphadenopathy. However, these are inconsistent findings with *S. moniliformis*. Both organisms cause headache, malaise, myalgia, chills, joint pain, and arthritis.
- Transmission is generally from bite wounds or contaminated food.

Hantaan Viruses

Although not a problem in commercially bred laboratory rats in the United States, the virus has been transmitted to humans from laboratory rats or rat tissues in Europe and Asia. Serologic testing for Hanta virus is often required if rats are to be shipped to facilities in other countries.

- The rat develops a persistent subclinical infection. Large amounts of virus can be found in infected rat tissues, yet the rat shows no clinical disease and no pathology. Rats can remain infected for up to two years.
- Clinical signs in humans vary with the strain of virus and can include hemorrhagic fever and adult respiratory distress syndrome (ARDS).
- Transmission is by inhalation of aerosol of infected urine, feces, or saliva. Bites are a minor mode of spread and direct animal contact is not necessary for humans to become infected.

Salmonellosis

Salmonella infection is relatively rare in well managed rodent facilities.

- Clinical signs in the rat include anorexia, decreased activity, rough hair coat, soft or formless feces, and occasionally death loss. Animals can be convalescent carriers, active disease shedders, or act as fomites.
- In humans, clinical signs include abdominal pain, diarrhea, fever, and vomiting.
- Transmission of the bacteria is often through fecal contamination of hands and food.

Hymenolepsis nana

This tapeworm occurs in several rodent species.

- In rats, it may be asymptomatic or cause intestinal obstruction and enteritis.
- In humans, clinical signs include enteritis, anorexia, pruritis, and headache.
- Transmission is fecal-oral via ingestion of feces from infected animals.

Dermatophytosis (Ringworm)

Dermatophytosis is a superficial fungal infection involving the keratinized layers of the skin and its appendages.

- The most common dermatophyte in rodents is *Trichophyton mentagrophytes*. Skin lesions are characterized by alopecia, broken hairs, erythema, or crusts.
- Lesions in the human are papulo-squamous (scaly red elevated area of skin), dry, with a red ring (erythema), and can affect any part of the body.
- Transmission is through physical contact. There are a number of human dermatophytes that can cause ringworm (anthropophilic). These are transmitted from human to human, and are not associated with animals.

Bite or Scratch Wounds

Other than rat bite fever, bites or scratches from rats can become infected with a variety of bacteria if the wound is not thoroughly cleaned. Deep puncture wounds may provide entry for tetanus organisms, and tetanus immunization is highly recommended for anyone working with animals, including rodents.

Allergies

Hypersensitivity reactions in humans to rat allergens in urine and dander are relatively common. Symptoms vary from hay fever-like conditions to asthma, skin wheals, and eczema. Allergic sensitivity usually develops within two years of working with animals. Individuals with a previous history of allergies may develop more serious manifestations to animal allergens, e.g., asthma. Often a variety of laboratory and clinical tests, including

skin tests for animal dander, are needed to establish the diagnosis. Protection against the development of allergic reactions to animal allergens includes the use of masks or fitted respirators, gloves, and other protective clothing; and showering after the work day.

Zoonotic Diseases Associated with Wild Rats

Leptospirosis

Common serovars of *Leptospira* in animals include *canicola*, *hardjo*, *autumnalis*, *icterohemorrhagica*, *grippotyphosa*, and *pomona*.

- Rodents can remain inapparent carriers of the bacteria for life.
- Symptoms in humans include fever, headache, myalgia, nausea, jaundice, stiff neck, chills, rash, and conjunctivitis.
- Transmission is through urine-contaminated water or by direct contact. In the United States the most important sources of infection are from dogs, farm animals, wildlife (especially rodents), and cats. However, human disease has been associated with laboratory and pet rodents.
- Leptospirosis is considered an occupational disease of veterinarians, farmers, military personnel, and abattoir workers.

Wild rats may be host to additional diseases such as bubonic plague (*Yersinia pestis*) and tularemia (*Francisella tularensis*). When these animals are used in the laboratory, extra care should be taken to prevent transmission of infectious agents.

4

veterinary care

physical examination

A good physical exam is the baseline for determining if there are any problems with an animal. Rats are very stoic animals and may be quite ill before they actually show physical signs of disease. To perform the physical exam, one simply needs a stethescope, thermometer, a tongue depressor to aid in visualization of the teeth, and a scale which can measure weight in grams. It is recommended the examiner wear exam gloves. The physical exam of the rat should include the following items:

Temperature: Rats have a normal body temperature of 35.9–37.5°C (96.6–99.5°F). One may determine this parameter either rectally or by the use of an over the counter tympanic membrane thermometer.

Weight: Although weight will vary with an animal's age, weight loss may be an indicator of a disease process. A rat's weight gain will also vary due to stock or strain, with weight gain tables available from most animal vendors.

Face:

- **Eyes —** One should pay particular attention to a rat's face. The development of "red tears" (porphyrin pigment) are an indicator of stress in rats. This

stress may be the result of a disease process. Even though there may be no pigment present around the eyes or face, one should check the forepaws for pigment, as rats will wipe the pigment away from the face during grooming. One may differentiate the pigment from blood by using a Wood's lamp, as the pigment's porphyrin component will fluoresce and blood will not.

- **Ears —** Check ears for any abnormalities, including any swelling near the base of the ear which may indicate a Zymbal's gland tumor.
- **Teeth —** Rats must gnaw to wear off new tooth growth, leaving the incisors susceptible to overgrowth. Overgrowth will occur in instances of tooth breakage of the opposite arcade, or due to malocclusion. Malocclusion is a heritable trait, suggesting that affected animals should not be used as part of a breeding colony. Trimming the teeth is palliative and should be carefully performed to prevent shattering the tooth being trimmed.

Heart and lungs: One may easily auscult the heart and lungs with a pediatric stethoscope. Rats are susceptible to a variety of respiratory pathogens.

General body condition:

- **Lumps and bumps —** Swellings or tumors on any part of the rat's body may indicate abscesses or benign or malignant tumors.
- **Hair coat —** Rats are suseptible to ectoparasites, which may result in a poor haircoat and itching. Poor haircoat may also indicate stress, cold, or poor nutrition.

diagnostic imaging

Diagnostic imaging permits non-invasive viewing of tissues and organs and is available to the investigator in many forms — radiographs, ultrasound, magnetic resonance imaging, computerized

tomography, and positron emission topography. The expense of these imaging techniques may be offset by using fewer animals in a study. One may follow a single animal over a period instead of sacrificing multiple animals over the same period.

Radiographs

Radiographic equipment capable of generating 300 mA, an exposure of 1/120 (0.008) second, and kvp between 50–60 is considered best. Rats should be radiographed using a tabletop technique. Some groups have found the combination of conventional radiographic and mammography techniques useful in imaging tumor-bearing animals.

Ultrasound

Ultrasound is another valuable diagnostic tool which in some cases may yield more information than radiographs (abdominal masses, uterine enlargement, or urinary bladder calculi). For best results use high-frequency transducers (7.5 mHz) with narrow sector angles.

Other Diagnostic Imaging Modalities

Other imaging techniques such as magnetic resonance imaging (MRI), computerized tomography (CT), and positron emission tomography (PET) permit noninvasive analysis. Analysis by these methods will require adequate anesthesia, for immobilization, to provide the best images. The amount of information that these imaging modalities yield is impressive.

diseases of laboratory rats

During the past 20 years animal research has become more sophisticated. The development of molecular techniques now permits investigation at the cellular and subcellular levels. Concurrent infection of rodent colonies used in these studies can produce subtle effects which at best confound and at worst invalidate the data.

This section is divided into a discussion of:

- Specific disease conditions with descriptions of clinical signs and lesions.

- A discussion of treatment.
- Programs designed to prevent the introduction of infectious disease into the colony.

In rodent colonies, an ounce of prevention is truly worth a pound of cure, especially when dealing with infectious agents. Signs are rarely specific to one disease. Any ill rat may have a roughened hair coat, hunched posture, porphyrin (pigment in rat tears) staining around the eyes and nose, and weight loss. None of these signs point to any specific disease. Therefore, definitive diagnosis of a disease in the colony often depends on a complete necropsy of an affected animal. To complicate matters further, rats may not appear sick at all. The only indication of infection may be altered research results. For example, Kilham rat virus, *Mycoplasma pulmonis*, and Sendai virus will contaminate cell cultures and transplantable tumors and cause immune response alterations.

One should always bring sick animals to the attention of the attending laboratory animal veterinarian, as control measures and treatments need to be tailored to the specific colony and research project. Certain treatments may be effective for young outbred rats, but contraindicated in aged inbred strains. Treatments, as well as the disease itself, may adversely affect research protocols. Finally, some treatments — especially antibiotics — may well ameliorate the signs but do not eliminate the disease from the population. The veterinarian will discuss options for the colony or individual animals with the researcher in context of available treatments and control measures, potential adverse effects of the disease and/or treatments on the data and animals, and the impact of the disease on other rats in the facility or animal room.

bacterial diseases of rats

Mycoplasmosis

Mycoplasma pulmonis is an extracellular parasite colonizing the luminal surface of the respiratory epithelium. Virulence depends on the *M. pulmonis* strain as well as the strain of the rat. Lesions may be acute or chronic and consist of rhinitis,

otitis media, laryngitis, tracheitis, bronchitis, bronchiectasis, and pulmonary abscesses.

- **Clinical signs —** Animals are often asymptomatic. When present, signs are non-specific. Sniffling, difficult breathing, weight loss, hunched posture, inactivity, head tilt, and porphyrin staining around the eyes and mouth are among the common signs. Signs and lesions become more severe with increasing cage ammonia levels to 19 µg/l of air. Lesions are also more severe with concurrent viral or bacterial infections.
- **Transmission** is primarily through aerosols.
- **Gross lesions —** There is serous to purulent exudate in the nasal passages, trachea, and occasionally the tympanic bullae (middle ear). Gray nodules are present in the lungs, particularly in the apical lobes in a "cobblestone" pattern. The diaphragmatic lobes may also show compensatory emphysema. If the uterus is severely affected, purulent exudate may be present in the lumen. Rats of the LEW strain are highly susceptible to severe genital disease, the primary lesions being purulent endometritis, pyometritis, salpingitis, and perioophoritis. In less susceptible strains, there may be no gross lesions associated with the genitalia, only decreased reproduction.
- **Histopathology —** Microscopically, there are abundant neutrophils in the airways, hyperplasia of the mucosal epithelium, and lymphoid hyperplasia. The hyperplasia of the bronchus-associated lymphoid tissue (BALT) is characteristic of murine respiratory mycoplasmosis, and has been related to the finding that constituents of *Mycoplasma pulmonis* are potent non-specific mitogens for rat lymphocytes. In the rat, there is little inhibitory protection from humoral antibody.
- **Diagnosis —** Diagnosis of *Mycoplasma* infection is based on clinical signs and lesions, bacterial culture, and serologic assays. The most common serologic assay is the enzyme linked immunosorbent assay (ELISA). The length of time for antibody response varies and rats may remain negative up to five months of age. Successful culture of

Mycoplasma organisms requires a multi-site culture in appropriate broths and agars. One may use Hayflicks or similar media to culture *M. pulmonis* from the lung, oropharynx, and trachea.

Corynebacteria kutcheri

The disease caused by this organism is also known as pseudotuberculosis. It is caused by a Gram-positive diptheroid bacillus. Stress will result in disease expression. Infected animals may be carriers, but show no clinical signs. The bacteria colonize the oropharynx, submaxillary lymph nodes, and large intestine.

- **Clinical signs —** The respiratory tract, middle ears, and preputial glands are common sites of infection. The signs of active disease are difficult breathing (dyspnea), ocular and nasal discharge, weight loss, hunched posture, and anorexia.
- **Transmission** is by the fecal–oral route.
- **Gross lesions —** Lesions are primarily pulmonary abscesses (Fig. 13a) of varying diameter from 0.25 mm to 1 cm. Abscessess may occur in the liver, kidney (Fig. 13b), spleen, subcutaneous tissue, and peritoneal cavity as well.
- **Histopathology —** In affected organs, there is multifocal necrosis with neutrophilic infiltrates. Mononuclear inflammatory cells are found in older lesions. There may be pulmonary edema and perivascular cuffing.
- **Diagnosis —** Samples of affected organs or tracheal washings should be taken for bacterial culture. A preliminary diagnosis can be made on finding typical Gram-positive rods on impression smears taken at necropsy. The bacteria grow well on blood agar, although culture may not be effective in subclinical cases.

Pasteurella pneumotropica

The organism is a Gram-negative bipolar staining rod. It is a pathogen of low virulence, and most infections are clinically inapparent. There are few reports of *Pasteurella pneumotropica* as a primary pathogen, causing pneumonia, otitis media, and

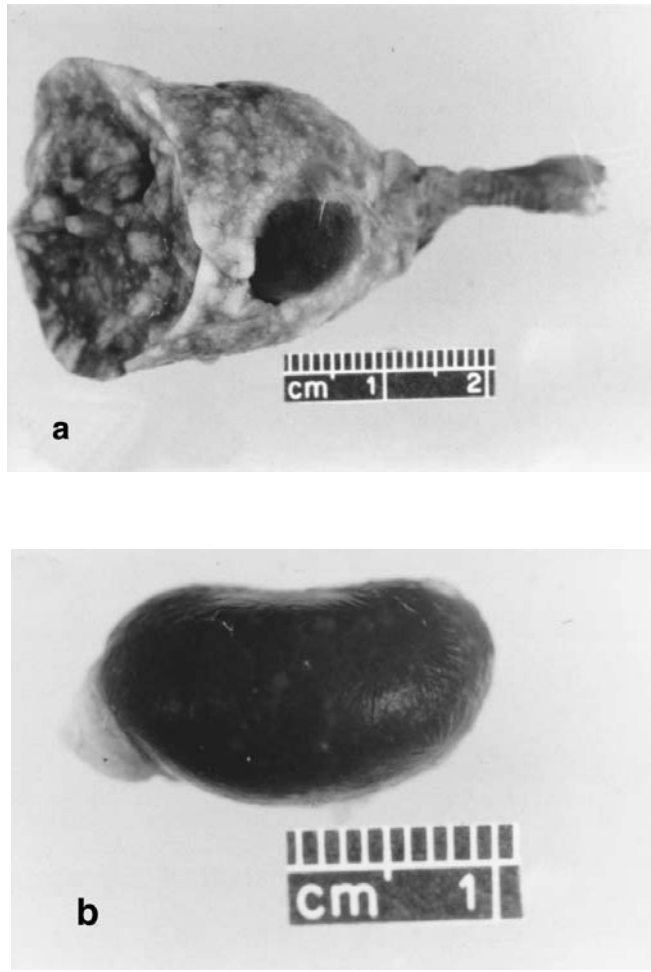


Fig. 13. Typical *Corynebacteria kutcheri* lesions found in the lung (a) and kidney (b) of a rat. The lungs and kidney contain multifocal pale areas of varying diameter.

conjunctivitis. It is a co-pathogen with *Mycoplasma* and Sendai virus, resulting in pneumonia and otitis media.

- **Pathology/Clinical signs —** The pathology is not distinctive. There may be ocular or nasal discharge, conjunctivitis, head tilt, dyspnea, skin abscesses and mastitis, purulent exudate within the tympanic bullae and nasal passages, and bronchopneumonia.

- **Transmission** is by the fecal–oral route; vertical transmission can occur.
- **Diagnosis —** The bacteria can be isolated from the oral cavity, respiratory tract, intestinal tract, and uterus, and grow well on basic media.

Streptococcus pneumoniae

This is a Gram-positive coccus ubiquitous to humans and animals. Rats are usually asymptomatic carriers, and several serotypes can affect rats. Both humans and rats carry some of the same serotypes, but it is not considered a true zoonotic disease. The most common serotypes in rats are 2, 3, and 19. The bacteria are carried in the nasal turbinates and the tympanic bullae. The infection often remains localized. With stress, such as infection with other pathogens, shipping, or experimental manipulation, overt disease may develop.

- **Clinical signs —** Serous to mucopurulent nasal discharge is a common sign, as is porphyrin staining, head tilt, dyspnea, and rales. There may be some death loss. The very young and very old are most susceptible.
- **Gross lesions —** In severe cases lungs are firm and red, with a thick white exudate in the thoracic cavity. The pleura and pericardium are often thickened with fibrin and purulent exudate. This same fibrinopurulent exudate may occur in the abdomen, genital system, and meninges of the brain.
- **Histopathology —** Lesions include fibrinopurulent bronchopneumonia, pleuritis, pericarditis, peritonitis, orchitis, or meningitis.
- **Diagnosis —** The organism is grown on blood agar in a 10% CO_2 atmosphere. Alpha hemolysis (seen as a green tint) is produced around the bacterial colony on blood agar plates. There are many non-pathogenic alpha hemolytic streptococci, and differentiation is by use of optochin disks (hydrocuprein hydrochloride) on sample cultures. Optochin inhibits the growth of *S. pneumoniae*, but allows growth of the non-pathogenic streptococci.

Bordetella bronchiseptica

Bordetella bronchiseptica is a Gram-negative, small rod-shaped bacterium which is a primary pathogen in the rabbit and the guinea pig. In rats, it is an opportunistic pathogen when associated with other primary pathogens such as *Mycoplasma* or viruses. The respiratory infection is characterized by suppurative rhinitis and bronchopneumonia.

Tyzzer's Disease

Tyzzer's disease is caused by *Clostridium piliformis*, a Gram-negative spore-forming rod. It is an intracellular pathogen which cannot be cultivated on artificial media. It can be grown in embryonated eggs or in cell culture. It is widely distributed in many species and clinical signs are not specific. Young adults are most often affected. The spores are relatively stable in the environment remaining viable in contaminated bedding for up to one year. This organism is susceptible to disinfectants such as sodium hypochlorite, and spores are inactivated at 80°C for 30 minutes.

- **Clinical signs —** Clinical signs include lethargy, weight loss, and distended abdomen, but clinically inapparent infections do occur. In rats, clinical signs usually are associated with stress or immunosuppression.
- **Gross lesions —** The major gross lesions involve the liver, ileum, and myocardium. The primary infective sites are the jejunum, ileum, and cecum. The intestinal lesion is flaccid segmental intestinal dilatation, with an edematous atonic ileum. Ileal lesions may extend to adjacent cecum and jejunum. In the liver, there are few to many scattered pale foci up to several millimeters in diameter. Circumscribed grayish foci may be seen in the myocardium in some cases. The mesenteric lymph nodes are usually swollen.
- **Histopathology —** Microscopically, in the liver there are foci of coagulative necrosis with a distinct boundary between the necrotic and adjacent normal tissue. The organism is found in viable hepatocytes on the periphery of the necrotic foci. Myocardial lesions vary in size, ranging from few fibers to complete transmural involvement. Inflammatory infiltrates are variable.

- **Diagnosis —** Definitive diagnosis of the organism is based on finding the organisms in the hepatocytes, intestinal epithelial cells, or myocardium. Special stains such as Gram stain, Giemsa, and Methylene blue are used for impression smears. For tissue stains, the Warthin-Starry, Giemsa, or PAS are valuable. An ELISA for antibody to the bacteria has been developed. One must differentiate the ileal distention of Tyzzer's disease from adynamic ileus, which is associated with intraperitoneal injection of the anesthetic chloral hydrate.

Streptococcal (Enterococcal) Enteropathy of Infant Rats

This is a disease of suckling rats with high morbidity and mortality. *Enterococcus faeceum durans-2* is the causative agent.

- **Clinical signs —** The primary clinical signs are diarrhea in suckling rats and stunted growth.
- **Gross lesions —** Grossly there is abdominal distention and fecal soiling. Stomachs are distended with milk, with concurrent dilation of the small intestine.
- **Histopathology —** Microscopically there is little change in the intestinal villi and minimal or no inflammatory response. However, large numbers of Gram-positive cocci are present over the villar surface.
- **Diagnosis —** Bacterial culture, isolation, and identification of the organism is necessary to confirm the diagnosis.

Staphylococcus aureus

Coagulase positive *S. aureus* has been associated with ulcerative dermatitis in rats. The lesions were most likely due to self-trauma and inoculation of the wound with bacteria.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is an opportunistic bacterial pathogen, and frequently is a nosocomial infection. It is a Gram-negative rod, and often colonizes the oropharynx of rats. Human carriers are frequent. These bacteria have been a major problem in burn research, and research involving immunosuppression. In the immunocompromised animal, there may be facial edema,

conjunctivitis, and nasal discharge. Contaminated water bottles and automatic waterers are a common source of the bacteria. The organism grows well on blood agar, and often produces a blue-green pigment (pyocyanin or fluorescein).

Cilia-Associated Respiratory Bacillus (CAR Bacillus)

This has been reported as an etiologic agent of chronic respiratory disease (CRD) of rats in the Netherlands, United States, and Japan. Signs and lesions are similar to infection with *Mycoplasma pulmonis*, the predominate microscopic lesion being peribronchiolar cuffing with lymphocytes and plasma cells. Infection with CAR bacillus can easily spread from infected rats to noninfected cagemates within two months. Co-infections with CAR bacillus and *Mycoplasma* are common.

- **Clinical signs —** Rats may be asymptomatic or show varying degrees of respiratory distress.
- **Gross lesions —** Lesions in the lung vary from a mild, patchy mottling to scattered gray raised foci resembling that seen in murine mycoplasmosis.
- **Histopathology —** The bronchial epithelium is normal to slightly hyperplastic and hypertrophic. Bronchiectasis and bronchial abscessation are evident in severe cases.
- **Diagnosis —** Attempts to grow the bacteria on a variety of artificial media and under different atmospheric conditions have generally not been successful. The bacteria are successfully cultured only on embryonated chicken eggs or in cell culture. Diagnostic tests include special histopathologic stains, particularly the Warthin-Starry, but also immunoperoxidase stains. Enzyme linked immunosorbent assay and the direct immunofluorescent assay are common serologic tests.

viral diseases of rats

Kilham Rat Virus (KRV)

There are few reports of natural outbreaks with clinical signs involving Kilham rat virus. It is a single-stranded, DNA virus of the family parvoviridae, genus parvovirus. Synonyms include

rat virus or parvovirus R-1. Laboratory and wild rats are the natural hosts. The virus is reported to be shed in the urine, feces, milk, and nasal secretions. A persistent infection occurs in rats for up to 14 weeks, if they are infected as infants, and may persist even in the presence of high antibody titer. Large doses and experimental infections have been used as models for viral teratological effects, cerebellar hypoplasia, hepatitis, and hemorrhagic encephalopathy.

Note: Contaminated cell lines and tumors are important sources of KRV infection.

- **Clinical signs —** The signs are nearly always subclinical. Natural disease in pregnant rats may cause an increase in the number of resorption sites, runting, and ataxia, in the pups. Young adult rats exposed to KRV may develop scrotal cyanosis, abdominal swelling, and dehydration.
- **Transmission** is primarily through the horizontal route, direct contact, or fomites.
- **Gross lesions —** Gross findings vary with the age of the individual and include runting, cerebellar hypoplasia, and jaundice in young rats. In adults, jaundice is a variable finding. There may be hemorrhage within the scrotum, poor body condition, and congestion of lymph nodes.
- **Histopathology —** Microscopic lesions include hemorrhagic infarction with thrombosis in multiple organs, including the brain, spinal cord, testes, and epididymis; and multifocal hepatic necrosis.
- **Diagnosis —** Diagnosis can be confirmed by demonstration of the presence of antigen in the tissue, using immunocytochemistry techniques. Serologic testing, including ELISA and IFA (Indirect Fluorescent Antibody), detects antibody in the sera of infected rats.
- Toolan's H-1, another parvovirus, causes no natural disease. It is a single-stranded DNA parvovirus with epizoologic aspects similar to KRV.

Orphan Parvovirus

Also called rat parvovirus (RPV), this virus is distinct from both KRV or Toolan's H-1. Adult animals show no clinical signs, and although parvovirus requires dividing cells to replicate, no pathology has been associated with infection of RPV in infant rats. However, altered immune responses have been documented in mice infected with mouse parvovirus (MPV). Diagnosis is by serologic assay.

Sialodacryoadenitis Virus/Rat Coronavirus

The two most common coronaviruses in rats are rat coronavirus (RCV), which is also known as Parker's Rat Coronavirus, and sialodacryoadenitis virus (SDAV). The properties of RCV and SDAV are very similar. Both viruses produce infections which have high morbidity and low mortality and are rapidly spread. They share antigens with each other and cross-react with some strains of mouse hepatitis virus. Both SDAV and RCV replicate in the respiratory tract, but SDAV affects the salivary glands and lacrimal glands more severely.

- **Clinical signs —** SDAV is highly infectious but in the uncomplicated infection rarely results in death. For an individual rat, clinical signs usually regress within one week and lesions regress in two to four weeks. Clinical signs are sneezing, photophobia, swelling of the neck (due to inflammation of the submandibular salivary glands), and nasal and ocular discharge that is reddish-brown. Some rats develop chronic keratoconjunctivitis, with corneal opacity, ulcers, pannus, synechia, hypopyon and hyphema, and cataracts.
- **Transmission** is by aerosol or direct contact.
- **Gross lesions —** Lesions include rhinitis, inflamed lacrimal and salivary glands and enlarged cervical lymph nodes. Eye lesions are thought to be caused by corneal drying secondary to a loss of tear production. The virus has not been detected in the cornea or other parts of the globe.
- **Histopathology —** Necrosis and inflammation due to the virus is self-limiting and repairable. The nasal turbinates are most severely affected, and the olfactory epithelium

is usually spared. There are mild similar lesions in the trachea. In the lung, changes include mild hyperplasia of the peribronchiolar lymphoid nodules. In the salivary glands, lesions are characterized by necrosis and inflammation followed by squamous metaplasia during the repair phase. Serous and mixed salivary glands in the oropharynx are affected, but mucous producing salivary glands are resistant. Repair begins during the second week and is usually complete by the third week. In athymic (nude) rats, infections and lesions may persist up to six months, characterized by chronic suppurative rhinitis and bronchopneumonia, with chronic inflammatory lesions of the salivary glands and Harderian gland.

- **Diagnosis —** Infection with SDAV should be differentiated from *Mycoplasma*, Sendai virus, pneumonia virus of mice, and from sequelae to periorbital bleeding, or irritation due to high ammonia levels or stress associated chromodacryorrhea. ELISA and IFA are the most common serologic diagnostic tests. Immunocytochemistry with demonstration of the antigen in tissue can be performed.

Rat coronavirus (RCV) primarily affects the lung, and usually there are no gross lesions. The microscopic lesions are characterized by patchy interstitial pneumonia which is mild and short-lived. There may be transient rhinotracheitis. Lesions in the salivary gland are uncommon and when present, mild and similar to SDAV. The lesions apparently do not occur in the lacrimal gland.

Pneumonia Virus of Mice (PVM)

Pneumonia virus of mice is typically asymptomatic in the rat, with some animals developing a transient, mild interstitial pneumonia. There are usually no gross lesions, but there may be focal to multifocal plum colored to gray foci, less than 2 mm diameter in any lobe of the lung. Microscopically, there is focal interstitial pneumonia with perivascular and peribronchiolar cuffing. Differential diagnosis would include Sendai and rat coronavirus. Diagnosis is by histology and serologic assay, primarily ELISA and IFA.

Sendai Virus

This is a parainfluenza virus of the paramyxovirus family, which is highly contagious. It is an acute respiratory infection, with no carrier state. The virus replicates in Type I and Type II pneumocytes and in alveolar macrophages. It can be superimposed on and contribute to respiratory lesions caused by *Mycoplasma*, and presumably *Pasteurella pneumotropica*. It can mimic changes seen with exposure to halogenated aromatic hydrocarbons, oxidant gases, and other toxicants.

- **Clinical signs —** The disease is usually subclinical but signs include rough coat, dyspnea, and anorexia.
- **Transmission** occurs through aerosols or direct contact.
- **Gross lesions —** There are usually few gross lesions, although there may be patchy consolidation of the lungs.
- **Histopathology —** Histologically, there is focal non-suppurative interstitial pneumonia, mild to severe peribronchiolar and perivascular cuffing, which may persist for several weeks. Rhinitis with focal diffuse necrosis of the respiratory epithelium also occurs. Necrotizing bronchitis has been reported in germ-free rats with experimental infections.
- **Diagnosis —** Diagnosis is confirmed by histologic lesions and serologic titers (ELISA and IFA), although antibody levels may drop below detectable range after nine months.

Other Viral Infections

Rats seroconvert to REO3, but natural and experimental disease does not occur. Rats also seroconvert to mouse encephalomyelitis virus (MEV), and again there is no natural disease, but experimental disease is produced by an MEV (Type NHG) resulting in neurologic signs.

parasitic diseases

Ectoparasites

Lice and mites are the more common ectoparasites of the laboratory rat. However, even these parasites are rare in the well managed colony. Rat fleas (e.g., *Xenopsylla*) are rare in the laboratory rat.

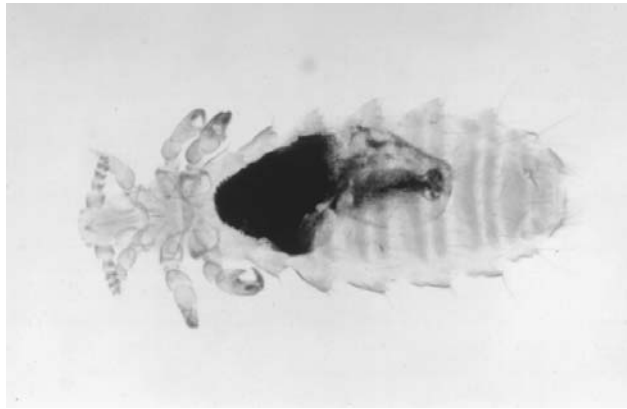


Fig. 14. *Polyplax spinulosa*, the spined rat louse, can cause anemia and generalized skin lesions.

- ***Ornithonyssus bacoti***, the tropical rat mite, and ***Laelaps echidninus***, the spiny rat mite are rarely encountered. Both mites remain on the host only long enough to obtain a blood meal. They are not host-specific and will bite humans. The can act as vectors for several agents pathogenic to man.
- ***Radfordia ensifera***, the rat fur mite, has a limited range and feeds on skin debris. Clinical signs may not be observed, but in heavy infestations alopecia, or self-induced trauma due to scratching are common signs. Transmission is by direct contact.
- ***Polyplax spinulosa***, the spined rat louse (Fig. 14), is a blood sucking louse which completes its life cycle on the host. Clinical signs include anemia, unthrifty appearance, scratching, and small skin wounds. It can transmit a number of infectious agents. Transmission is by direct contact.
- **Diagnosis —** *Ornithonyssus bacoti* and *Laelaps echidninus* require microscopic examination of the pelt and bedding for the mites. *Radfordia ensifera* and *Polyplax spinulosa* can be diagnosed by skin scraping or plucking hairs and examining the specimen microscopically for the mites. If the animal is examined postmortem, the

pelt can be removed and placed in a petri dish or on black paper. As the pelt cools, the parasites will become visible through a dissecting microscope as they migrate to the ends of the hair.

Endoparasites

nematodes

Syphacia muris, the rat pinworm, is a common infection in laboratory rats. The worms inhabit the cecum, and the eggs are deposited in the perianal region.

- **Clinical signs —** Usually, the infection is asymptomatic, but soft stool, enteritis, and perianal irritation have been reported.
- **Microscopically** there is little pathology although a multifocal granulomatous reaction in the lamina propia may be observed in the large intestine. Cross sections of adult worms may be identified in histologic sections of the cecum or colon.
- **Diagnosis —** A cellophane tape impression, made by touching a piece of clear tape to the perianal region and placing the tape on a glass slide, is examined microscopically for the presence of eggs (Fig. 15). Other diagnostic methods include fecal floatation or direct examination of cecal contents for adult worms.

cestodes

Hymenolepis nana has been mentioned under zoonotic diseases. Rats serve as intermediate hosts of ***Taenia taeniaformis***, the cat tapeworm. The cysts are found in the liver of infected rats. Contamination of food or bedding by cat feces is a common mode of transmission.

protozoans

- ***Encephalitozoon cuniculi*** is found in the brain and kidney of apparently normal rats, mice, and rabbits. It usually causes no clinical signs, but induces focal granulomatous encephalitis and nephritis. Clusters form in the brain, kidney, heart, muscle, pancreas, liver, spleen, and other

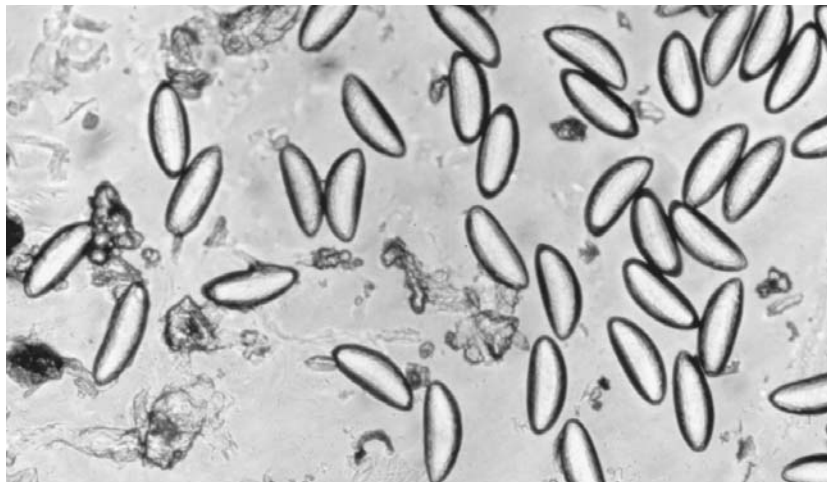


Fig. 15. Eggs of *Syphacia muris*, the rat pinworm, are banana-shaped and easily seen on fecal floatation or cellophane tape test.

organs. The spores have a capsule that stains well with Gram stain, Giemsa, or Goodpasture's carbol fuchsin stains, but stains poorly with hematoxylin and eosin. The life cycle is direct. The mode of transmission is through ingestion of spores.

- ***Pneumocystis carinii*** is a ubiquitous opportunistic organism, in many species, including rats and mice. It is normally not pathogenic. It can be activated by immunosuppression and fills the lungs with trophozoites, precysts, cysts, and intercystic bodies. There is interstitial alveolitis with lymphocytic infiltration. Infection can occur in immunosuppressed animals, including SCID mice, nude mice, and nude rats. Infection is diagnosed by histologic examination of the lung stained with methenamine silver, which identifies the cyst form.

neoplastic diseases of rats

Mammary Tumors

Mammary tumors are one of the most common neoplasms of rats with some becoming quite large (Fig. 16). Although the tumors occur mostly in females, they can occur in males as well.

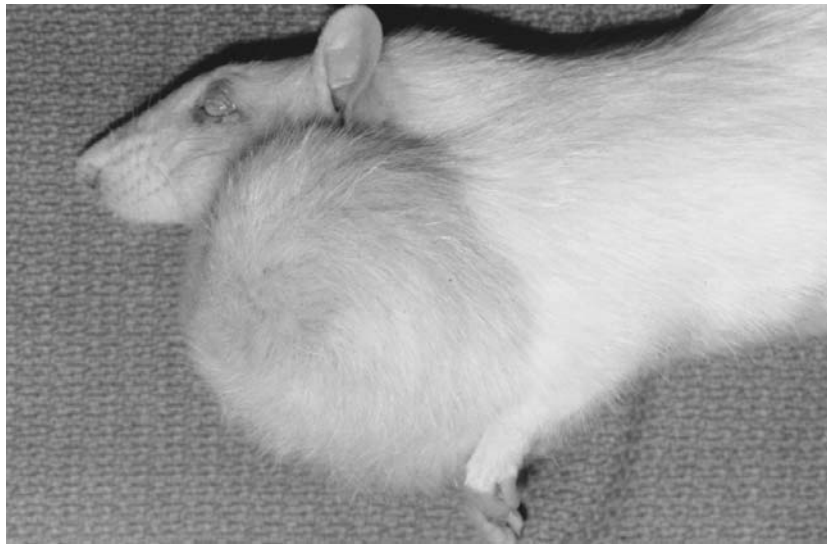


Fig. 16. Rat mammary tumors are usually fibroadenomas and can grow quite large. They may be removed surgically but can recur.

- Incidence increases with age, especially after 18 months.
- The vast majority of mammary tumors are benign fibroadenomas. Malignant adenocarcinomas do occur, but are uncommon.
- These tumors grow slowly, but can become very large. They may ulcerate or, depending on their location, interfere with normal movement or the ability to reach food and water.
- Tumors may be found wherever there is mammary tissue. Mammary tissue in the rat extends from the axillary area to the inguinal region on either side of the ventral midline and back.

Testicular Tumors

The incidence varies greatly between strains and colonies of rats.

- These tumors are discreet, soft, yellow to brown, with areas of hemorrhage, and may be multiple and frequently

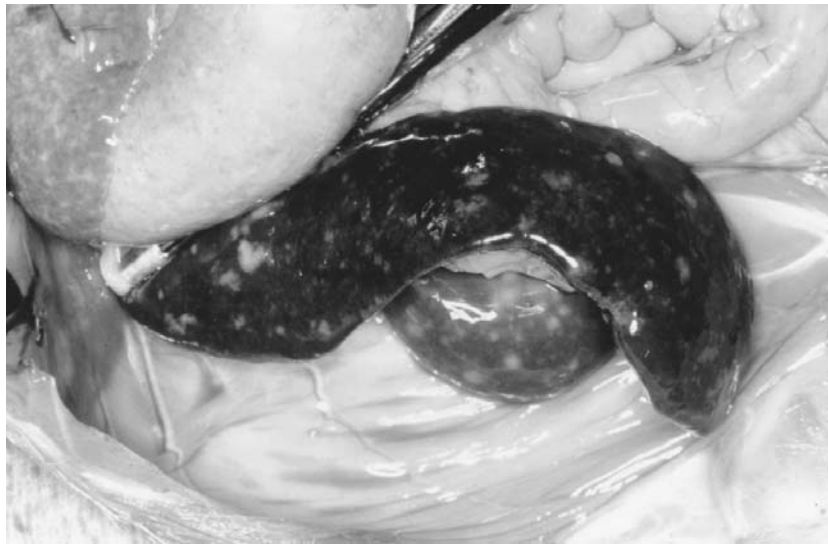


Fig. 17. Splenomegaly with focal raised nodules is common in F344 rats with large granular cell (LGL) leukemia.

are bilateral. The vast majority are interstitial cell tumors (Leydig cell tumors).

- There is a high incidence of these tumors in Fisher 344 (F344) and ACI/N rats.
- These tumors are generally benign.

Mononuclear Cell Leukemia (Large Granular Cell Leukemia)

This is frequent in F344 and Wistar-Firth rats. It occurs primarily in older rats. Splenomegaly is a constant sign (Fig. 17), with hepatomegaly and lymphadenopathy being variable. There is jaundice, anemia, weight loss, and lethargy. This neoplasm eventually causes death of affected rats.

Pituitary Tumors

Neoplasia of the pituitary occurs frequently, with a higher incidence in females.

- **Clinical signs —** Signs vary, but include head tilt, behavioral changes, and sudden death.

- Most common is the chromophobe adenoma of the pars distalis.
- **Gross lesions —** Pituitary tumor size varies from single to multiple foci to large masses that replace the whole gland. The gland can enlarge to 2 cm in diameter and weigh as much as 350 mg or more. It is well-defined, spherical, and soft.
- Diet can affect the incidence. Ad lib feeding of high caloric diet produces a high incidence of spontaneous tumors. The lowest incidence is seen when both caloric and protein intake are decreased.
- It is not unusual for pituitary and mammary gland tumors to occur in the same animal.
- **Prognosis —** The tumor is eventually fatal.

Zymbal's Gland Tumors

These occur in the holocrine gland located at the base of the external ear. Grossly, the tumors are usually a circumscribed mass, frequently with ulceration of the overlying skin. The tumors may be either adenomas or adenocarcinomas (benign or malignant). They are relatively rare as a spontaneous tumor.

Keratoacanthoma

This tumor is a benign neoplasm of the skin. The most common sites in rats are the skin of the chest, back, or tail. The center of the tumor is usually filled with a keratin plug.

age-related lesions and miscellaneous conditions

Chronic Progressive Nephropathy

Chronic progressive nephropathy (CPN) is the major old age disease of rats. The actual pathogenesis remains undetermined; however, age, diet, microflora, and hormonal treatment, especially testosterone, will influence the rate of development.

- **Clinical signs —** The most common clinical signs include renal failure, wasting, and general lethargy.

- **Gross lesions —** Kidneys are pale, irregular, and swollen with a pitted surface. Changes can begin in rats as early as three months of age.
- **Microscopic lesions —** There is multifocal to diffuse dilatation of tubules which are lined by flattened epithelium. There is interstitial fibrosis and frequently accumulations of mononuclear inflammatory cells. Glomerular lesions vary from minimal to marked sclerosis.
- There is earlier onset of the disease and a more rapid progression in male rats. Albino strains and stocks are particularly predisposed. The disease is also related to the quality and quantity of dietary proteins.
- Axenic rats do not seem to develop significant chronic progressive nephropathy.
- **Prognosis —** The condition is eventually fatal, and it is the leading cause of death in old, otherwise healthy, rats.

Myocardial Degeneration

This is common to most stocks and strains, with an onset of 12 to 18 months of age. It occurs more frequently in males.

- **Clinical signs —** Usually absent.
- **Gross lesions —** Although usually microscopic, small grayish foci may be noted grossly. The papillary muscles and their attachment sites in the wall of the left ventricle are the most frequent sites of degeneration.
- **Microscopic lesions —** Degeneration and atrophy with fibrosis and a mononuclear cell infiltrate.
- Atrial thrombosis is also seen as an age-related lesion and may or may not accompany myocardial degeneration.

Urolithiasis

Urinary calculi are occasional findings in older rats (Fig. 18). Clinical signs include hematuria, cystitis, and if obstruction occurs, anuria. Stones may be composed of oxalates, phosphate, carbonate, or a mixture. One may attempt surgical removal, but this is seldom warranted in the laboratory rat.

Fig. 18. Uroliths found in the urinary bladder of a rat.

Cholangiofibrosis

The major age-related change in the liver is cholangiofibrosis. This is common in the F344 and to a lesser extent, Sprague-Dawley rats. It is uncommon in the WAG and BN rat strains.

- **Clinical signs —** Usually none.
- **Microscopic lesions —** There is a proliferation of portal bile ducts with associated mild fibrosis. A few mononuclear inflammatory cells may be noted. Large inflammatory infiltrates are not generally present. The cause is unknown.
- Cystic and telangiectatic lesions also occur in the aged liver.

Pulmonary Foam Cells

Aggregations of pulmonary foam cells, also known as foamy macrophages or alveolar histiocytes, can be an age-related change. The change is characterized by subpleural accumulations of cells containing free fatty acids, cholesterol, and phospholipids. It occurs in rats of various ages. There are no associated clinical signs, and the significance is not understood. Lipid metabolism may play a role or it may be a degenerative age change.

Radiculoneuropathy

This is degeneration of the spinal nerve roots. It generally is a late onset disease in rats older than 24 months of age. The cauda equina and ventral spinal nerve roots are most commonly involved, but the site can vary among strains. The Sprague-Dawley, Wistar, BN/BI/RIJ, and WAG/RIJ have been reported with lesions. There is no treatment.

- **Clinical signs —** Posterior paresis and paralysis have been associated with these lesions, but some suggest this is due to a separate degenerative process in the skeletal muscle and the two conditions are not related.
- **Microscopic lesions —** There is myelin sheath swelling and segmental demyelination. In advanced lesions there is axonal degeneration and loss.

Polyarteritis Nodosa

This is a disease of unknown etiology.

- **Clinical signs —** Not usually apparent, and when present they are they are nonspecific.
- **Gross lesions —** It affects the muscular arteries, most commonly the mesenteric, pancreatic, and spermatic. Lesions may be acute or chronic.
- **Microscopic lesions —** In the **acute disease**, intimal and medial fibrinoid necrosis is noted, with focal thrombosis, destruction of the elastic laminae, and infiltration of polymorphonuclear cells and mononuclear cells. In the **chronic disease**, the arteries are nodular, tortuous, and thick-walled. Affected vessels frequently have aneurysms and thrombi. Both acute and chronic lesions may be present in the same animal.

other disease conditions

Ringtail

This is a lesion of young animals, characterized by annular constrictions of the tail, sometimes with sloughing of the tissue distal to the constrictions. The young rats remain clinically normal other than the tail lesions. It is associated with high

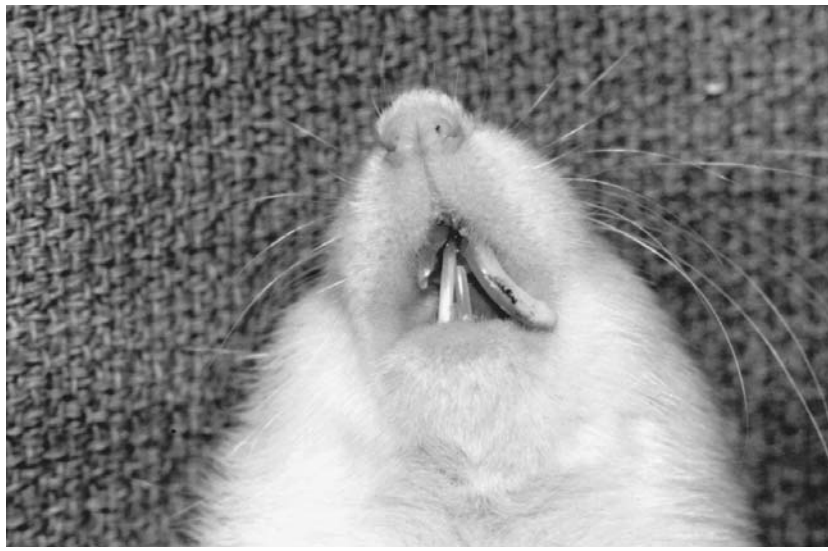


Fig. 19. Malocclussion of the incisors occurs with broken teeth or misalignment of the jaw. Animals which are unthrifty, or anorectic should be checked for malocclusion.

temperature and low humidity (below 40% relative humidity) and is seen especially in young rats raised in wire-bottom caging. If segments of the tail slough, healing usually occurs without further complication.

Malocclusion

In rats, malocclusion most commonly refers to overgrown incisors (Fig. 19). The condition is seen both in younger and older rats.

- **Clinical signs** include an unthrifty appearance, weight loss, and dehydration.
- It is related to broken teeth or congenital misalignment of the jaw.
- The malocclusion can progress to such an extent that the teeth penetrate the opposing gum causing inflammation and abscessation.

Heat Prostration

This condition most often occurs in the summer months. Rats shipped when the ambient temperature is above 85°F are prone to heat exhaustion. Clinical signs include increased respiration

Fig. 20. Heat prostration is not uncommon in rats shipped during very warm weather, especially if shipment is delayed. Signs include increased salivation and wet muzzles and paws as the rats attempt to cool themselves.

and excessive salivation resulting in wet mouths, muzzles, and paws (Fig. 20). There may be congestion of the nail beds and digits. There is often a high death loss.

Retinal Degeneration

Retinal degeneration has been documented in albino rats exposed to light intensities of 130 lux or higher at the cage level. The most severe lesions are seen in rats housed on the top shelf near the light fixtures. Lesions vary from decreased photoreceptor cell nuclei to loss of nearly all retinal layers.

Adynamic Ileus Associated with Chloral Hydrate

Severe dilatation of the intestinal tract and ileus has been associated with intraperitoneal injection of the anesthetic chloral hydrate. The abdomen is distended with dilated loops of atonic bowel. Lesions may not develop for several weeks following injection and do not uniformly develop in all rats injected. The lesions resemble Tyzzer's disease, and infection with *Clostridium piliformis* should be ruled out.

disease treatment and control

There are only a few instances when treatment is warranted or effective in a colony of rats. Table 11 is a partial listing of antiinfectives commonly used in the rat. The listing is by no means complete, with the formulary by Hawk and Leary and drugs listed in the Harkness and Wagner text serving as fine references for the many different classes of compounds one may need to employ for treatment of clinical disease in research rats. Before any treatment is initiated, the laboratory animal veterinarian should be consulted as to the appropriate drug to use. Ivermectin which is a popular treatment for certain parasitic diseases may be lethal in certain breeds or strains of animals with an incomplete blood-brain barrier. Not all antibiotics are effective against all bacteria, and some, such as gentamicin, can be ototoxic and nephrotoxic making it a poor choice for animals used in hearing or kidney research. A review article by Morris discusses the following topics concerning antibiotic use in laboratory animals, including rats:

- Interference by antibiotics with experimental studies.
- Antibiotic toxicity.
- Routes of administration.
- Effects of formulation on bioavailability.
- Antibiotic prophylaxis.
- Use of antibiotic combinations.
- Misuse of antibiotics.
- Regulatory approval for antibiotic use in animals.
- Sources of information on antibiotic indications and dose.
- Extrapolation of dose information from other species.

Bacterial Diseases

- Antibiotics may ameliorate the signs of bacterial infections in rats, but often do not eliminate the disease. This

TABLE 11. COMMONLY USED ANTIINFECTIVES IN RATS

Drug	Dose
Amikacin	2–5 mg/kg q 8–12 h SQ, IM
Ampicillin	6 mg/kg q 8 h SQ
Cephalexin	15 mg/kg SQ q12 h
Chloramphenicol sodium succinate	20–50 mg/kg q 6–12 h SQ
Enrofloxacin	10 mg/kg SQ q12 h
Gentamicin	2–4 mg/kg q 8–24 h SQ, IM
Griseofulvin	25–50 mg/kg q 12 h PO for 14–60 d, or 1.5% (15 mg/ml) DMSO solution applied topically for 5–7 d
Ivermectin	200 µg/kg sid for 5 d gastric gavage; 3 mg/kg PO once; or 2 mg/kg PO for 3 treatments at 7–9 d intervals
Ketoconazole	10–40 mg/kg/d PO for 14 d
Metronidazole	20–60 mg/kg q 8–12 h PO
Neomycin	2 mg/ml drinking water
Penicillin G	40,000–60,000 IU/kg SQ, IM
Piperazine	2 mg/ml drinking water
Trimethoprim-sulfa	30–50 mg/kg PO, SQ q12 h
Tylosin	10 mg/kg SQ q 24 h

is especially true for diseases caused by *Mycoplasma* species and *Streptococcus pneumoniae*.

- Infected bite or fight wounds can be treated locally by cleaning the site and applying a topical antibiotic.
- Ulcerative dermatitis caused by *Staphylococcus aureus* may be treated with local or systemic antibiotics if the initiating factors are removed.
- Infections with *Pseudomonas* can be difficult to control. Many isolates are resistant to a variety of antibiotics. Phenolic disinfectants usually are effective, but the organism can thrive in quaternary ammonium compounds and also thrives in iodine solutions. Hyperchlorinating the drinking water to 12 ppm or acidification to a pH of 2.3 to 2.5 has reduced the incidence of clinical disease.

- Control measures for many bacterial diseases rely on rederivation or cesarean section of new stock and maintenance under barrier conditions. For those bacteria which can be passed vertically, cesarean section is effective only if uteri are culturally negative. Since culture is not 100% sensitive, it can be difficult to recover clean young from an infected colony. In this event, depopulation and restocking with clean animals may be the only solution.

Note: Clipping the rear toenails of rats with staphylococcal dermatitis will help prevent self trauma and reinoculation of the bacteria.

Viral Diseases

- There are no treatments for the viral diseases of rats.
- Where transplacental transmission is not significant, cesarean rederivation is a recommended control measure.
- If rederivation is not possible, isolation of breeders and repopulation with seronegative young may control the disease. The room should be quarantined for four to six weeks, during which time all breeding is stopped, suckling and weanling animals killed, no new animals introduced; and steps taken to prevent other types of cross contamination (e.g., shared lab equipment). Following the quarantine period, sentinels are placed in the room and tested three to four weeks later to ensure that no active disease is present.
- Prevention of viral infection through transplantable cell lines and tumors is best accomplished through the rat antibody production test (RAP test) described below.

Ectoparasites

- Lice or mites can be treated with a variety of drugs including pyrethrins, carbaryl, and ivermectin.
- Ectoparasites may not be completely eliminated by any of these treatments and long-term success often relies on continued routine use over several months.

- The potential effects of long term use of these treatments on research data should be thoroughly explored.
- Cesarean section and maintenance of young in barrier housing may be the only reliable long term solution.

Endoparasites

- Piperazine in the drinking water or fenbendazole medicated feed have been effective in eliminating pinworm infections. Ivermectin has been less successful in rats than mice in the authors' experience due to differences in grooming behavior. Control involves rigid sanitation and the use of filtered cages. The eggs are extremely hardy, very light, and may aerosolize.
- Clinical signs and lesions caused by *Pneumocystis carinii* may be minimized by using antibiotics or acidified drinking water.

Neoplasia

- The only neoplastic disease of rats which can be treated is mammary fibroadenoma. These tumors are relatively easy to to remove surgically; however, new tumors may develop anywhere along the line of mammary tissue.
- The incidence of specific types of neoplasia varies with the strain or stock, and this should be taken into consideration when choosing which rat strain to use in long term studies.

Age-Related Lesions

- Diet can influence the development of age related lesions.
- Food restriction and protein content both affect the occurrence of age-related lesions, especially chronic glomerulonephropathy.

Other Diseases

- Malocclusion of the incisors is best treated using a dental drill or small grinder to evenly grind the teeth to their

appropriate size. Clipping the teeth can cause splitting and the development of tooth root abcesses. Malocclusion can be due to a congenital misalignment of the jaw, and treatment may need to be repeated on a routine basis.

- Heat stress may be treated with careful spraying of the rat with a gentle stream of cool water. Residual effects of heat stress may make recovered animals unsuitable as research subjects.

prevention of infectious disease

Rodent colonies are generally treated on a "herd-health" basis, i.e., prevention, treatment, and control measures are at the colony level rather than the individual level. Preventative measures take the form of three major health status programs:

1. **Colony health surveillance** using sentinel animals or random sampling of the colony on a routine basis throughout the year.
2. The **rat antibody production (RAP) assay** in which transplanted tumors or cell lines are tested for the resence of infectious agents.
3. **Quarantine** of incoming animals or vendor surveillance in which animals arriving from various sources are tested prior to being placed in the colony.

Colony Health Surveillance

Diagnostic testing at the vendor and facility level are important to ensure a quality research rodent. Ideally, rodents are purchased disease free and maintained this way after arrival in the facility. Many facilities house rats under "barrier conditions," designed to prevent the introduction or spread of disease to the colony. This usually means restricted access to the housing rooms, the use of special filtered cages, mask, gloves, and gowns worn by personnel when working with the rats. The type of barrier may vary depending on the dynamics of the colony. The health of the colony can be monitored through a sentinel program.

A sentinel program for rodent populations is a scheduled screening of all colonies for infectious agents. Such programs have been a generally accepted standard since 1976, when the Institute for Laboratory Animal Resources (ILAR) published a formula for the number of animals necessary to screen for infectious agents, based on incidence of disease and the number of animals in the population. Since that time, the concept of a sentinel program has evolved to the use of a high risk population to increase the yield of the screening program. Sentinel rats from a known pathogen-free source are placed in each room or colony and their risk of exposure is increased in several ways, the most common being exposure to soiled bedding from other rats in the room, housing the sentinels in unfiltered cages and rotating the sentinel cages to different positions on the rack. How often animals are tested and for what pathogens depends in large part on facility, strain, and colony dynamics (e.g., breeding colony, aging study, etc.). Most facilities screen the majority of their rooms at least on a quarterly basis. Screening usually occurs after four weeks in a room to assure adequate exposure and development of a detectable antibody titer. The actual number of animals used as sentinels depends on the population of the room, disease incidence, type of housing, and extent of exposure time. No single number or range of numbers will work in all instances.

Depending on the facility and colony dynamics a surveillance program may contain all or a selection of the following examinations and tests:

1. **Gross necropsy** and visual examination of organs for abnormalities.
2. **Bacteriologic culture** for both respiratory and enteric pathogens. This can include the use of specialized media for the recovery of *Salmonella, Mycoplasma,* and other bacteria requiring enrichment or selective growth conditions. Fungal cultures for dermatophytes are not routine unless skin lesions are present.
3. **Serologic assays** are available for sialodacryoadenitis virus/rat coronavirus, pneumonia virus of mice, Sendai virus, *Mycoplasma*, Kilham rat virus, Toolan's H1 virus,

Cilia Associated Respiratory Bacillus, and Orphan Parvovirus (rat parvovirus). Serologic testing for Hantavirus is not done routinely. However, for shipments of animals to Europe, Hantavirus testing of rats is frequently requested. Tumors and cell lines from Europe or Asia especially should be tested via the rat antibody production test described below.

4. **Fecal examination** as described earlier for endoparasites such as pinworms and tapeworms.
5. **Pelt examination** as described earlier for ectoparasites such as lice and mites.
6. **Histopathology** of representative tissues.

Rat Antibody Production Test (RAP Test)

Cell lines and tumors may carry murine viruses which could become active in inoculated mice or rats. This would pose a serious health/research threat to the inoculated animals and the entire colony as well. If a sample of a pathogen-contaminated cell line is inoculated into pathogen-free test rats, they should develop an antibody titer within four weeks following inoculation. Serum is taken and tested for those antibodies which indicate the presence of murine pathogens. Uninoculated sentinel rats may be tested concurrently to rule out the possibility of a pre-existing colony infection confounding the test results. More detailed information on the procedure for the antibody production test is given by Small in the text *Laboratory Animal Medicine*.

Quarantine of Incoming Rats/Vendor Surveillance

Depending on the facility and/or supplier, incoming shipments of rats may be held in quarantine until their health status is confirmed either through testing of a sample from the shipment itself (vendor surveillance), random sampling of the rats in quarantine, or placing sentinels in the same area as the new arrivals and testing them in four to six weeks. Testing would be similar to that of colony sentinels and may be more extensive depending on the source of the animals.

Cesarean section/rederivation

To eradicate pathogens of various etiologies, it may be wise to use cesarean section (CS) rederivation as a means to end pestilence. This requires a definite commitment with regards to preventing the reintroduction of the various disease agents discussed in this text. This method requires less equipment and resources than embryo transfer. The procedure should be performed as quickly and as safely as possible. The delivery of rats via CS and experiencing a period of less than five minutes of anoxia do not appear to cause changes such as spatial learning deficits compared to vaginal birth controls. In contrast, some alterations from vaginally delivered rats were seen with respect to plasma corticosterone, body weights, and body organ weights during the first 35 days of life.

➤ *Procedure for performing a CS*

1. One must determine the gestation period of the stock or strain of rats undergoing CS, since the normal gestation period of the rat ranges from 21 to 23 days. The gestation period is typically measured from the day the vaginal plug is seen or the vaginal smear contains sperm. A few test animals should go through full gestation to determine the specific length of gestation.
2. The CS should occur as close to the time of birth as possible. By observing the animals frequently, one can determine whether the CS should occur in the morning or afternoon. A good schedule is to check the test animals at 7 AM, 11 AM, 1 PM, and 6 PM. If the animals typically deliver by 6 PM, one should perform the CS by late morning or early afternoon.
3. At least an equal number of foster dams should be available. Plan for these foster mothers to deliver the day before the CS is to occur.
4. The room setup should include:
   - A separate area where the contaminated or "dirty" mothers are euthanized (decapitation) and the gravid uterine horns are removed. This is the "dirty" or contaminated area.

- A warm (80–85°F) room
- Warm betadine
- Warm saline
- Heating pads, with chemical heating pouches being very effective for maintaining the neonates body temperature during transport
- Sterile cotton swabs
- Instruments
  - scissors
  - mosquito forceps (3)
  - tissue forceps
- Cotton sponges or Telfa pads
- Doxapram hydrochloride
- Petri dishes
- Towels
- At least two people: one working with the clean animals and one working with the contaminated animals.

One should perform the following procedure as quickly as possible:

➤ *Technique*

1. Administer doxapram hydrochloride 5–10 mg/kg SQ to the contaminated dam and wait 5 minutes.
2. Euthanize the contaminated dam (physical methods of euthanasia, such as decapitation, are preferred to pharmacologic methods such as pentobarbital. The pharmacologic methods can result in cardio-pulmonary depression in the pups.
3. Prepare the area by wetting the fur with alcohol.
4. Remove the gravid uterus through a midline incision, and clamping off the two distal horns of the uterus and the cervix. Cut the necessary attachments being careful not to enter the bowel.

5. While holding onto the three forceps, dunk the gravid uterus into the warm betadine solution.
6. Move to the clean area.
7. Place the betadine-soaked gravid uterus onto the clean area by removing the forceps. The forceps will be returned to the contaminated area.
8. Quickly remove the feti from the uterus along with their fetal membranes. Place the feti onto the sterile petri dishes lined with Telfa pads or cotton sponges. The petri dishes should be atop a heating pad. If using a chemical heat pouch, there should be one layer of the towel between the heat pouch and the petri dish.
9. Stimulate the neonates by rolling them around with the cotton tipped swabs. To prevent them from sticking to the cotton sponges the sponges may be moistened with warm saline. The animals should not stick to the Telfa pads. Moistening the cotton sponges acts to remove amniotic fluid and blood which, if remaining, could result in cannibalization of the young.
10. Exclude any young which do not appear to be responding, to minimize the risk of cannibalization.
11. Transport the animals to the foster or "clean" mother. Care should be taken to keep them warm using the chemical heat pouches and towels for transport.
12. One should ensure the total number of pups (foster + natural born pups) is identical to the original number of pups found in the foster mothers cage. To ensure fostering one may wish to have the foster mother urinate on the foster pups. The foster mother's pups are typically one to two days of age at the time of fostering. The removed foster mother's pups are then euthanized.
13. The foster mother should remain undisturbed for at least 48 hours.

In addition to cesarian rederivation, embryo transfer (ET) may be a viable option for some facilities. The technique for ET in rats is similar to the procedure described in mice. Good resources for this procedure in the rat include articles by Rouleau et al. and Vanderhyden et al.

anesthesia and analgesia

There is much terminology concerning the use of various agents to provide anesthesia, analgesia, and sedation. Below is a partial list of commonly used terms.

Akinesia: The loss of motor responses.

Analgesic: An agent which temporarily reduces or eliminates the sensation of pain.

Anesthesia: A temporary and reversible state characterized by a marked reduction or elimination of sensory and motor responses.

Balanced anesthesia: Producing anesthesia via combinations of two or more drugs or anesthetic regimens.

Light anesthesia: Animals under light anesthesia are immobilized and have lost the ability to right themselves; however, they will react to some painful stimuli.

Local anesthesia: Anesthesia to a region of the body.

Neuroleptanalgesia: A trance-like state accompanied by analgesia seen with the use of sedation and analgesic agents.

Pain: A sensory response evoked by an unpleasant stimuli.

Sedation/tranquilization: Mild CNS depression where the patient is awake and calm.

Surgical anesthesia: The loss of consciousness, analgesia, and muscle relaxation permitting the performance of a surgical procedure without pain or movement by the patient.

Prior to selecting an anesthetic, analgesic, or sedative agent one should evaluate the points listed below with the assistance of a veterinarian or veterinary technician to determine if light anesthesia, surgical anesthesia, or sedation would be appropriate for a given procedure.

- **Purpose**: Physical examination, surgery, body fluid collection.

- **Age, sex, species, temperament, strain, and/or physical condition of the patient.**
- **Study or procedure to be performed,** which may vary from purpose. For instance, one should choose an anesthetic that does not require intraperitoneal administration when performing abdominal surgery.
- **Necessity of postprocedural analgesia**.
- **Equipment and agent availability.**
- **Capability of the anesthetist.**
- **Disease and physiologic status.**
- **Cost.**

Preoperative Medications

Preoperative medications usually fall into two categories: antibiotics and preanesthetic agents. Administration of antibiotics should occur prior to a surgical procedure to ensure adequate blood levels as a way to minimize the risk of postoperative infection. Only a single bolus is usually needed unless there is great likelihood of postoperative infection (gastrointestinal tract procedures, contaminated wound, etc.).

The administration of atropine or glycopyrrolate may be warranted to counter decreased heart rate caused by increased vagal tone, and to decrease salivation. Atropine has a faster onset and shorter duration than glycopyrrolate and is recommended for cardiac emergencies (bradycardia).

Sedation, analgesia, and immobilization are important aspects of preanesthesia. Table 12 lists some preanesthesia agents, dosages, and their effects. Prior to administering anesthetic agents it is recommended to allow at least three days of acclimatization for the animal following arrival and before experimental use, and to limit fasting time to two hours or less due to the rat's high basic metabolic rate (BMR), and their inability to vomit.

Note: DO NOT water restrict animals.

TABLE 12. PREANESTHETIC AGENTS

Agent	Dosage	Effects
Acepromazine	2.5 mg/kg IM, IP	Sedation
Atropine	0.05 mg/kg IP, SQ	Parasympatholytic
Diazepam	2.5–5.0 mg/kg IM, IP	Sedation
Fentanyl/Dropiderol (INNOVAR-VET®)	0.5 mL/kg IM	Immobilization and analgesia
Fentanyl/Fluanisone (HYPNORM®)	0.2–0.5 mL/kg IM 0.3–0.6 mL/kg IP	Sedation with some analgesia
Glycopyrrolate	0.5 mg/kg IM	Parasympatholytic
Ketamine	50–100 mg/kg IM, IP	Sedation, Immobilization
Medetomidine	0.03–0.1 mg/kg IP, SQ	Sedation, some analgesia
Midazolam	5 mg/kg IP	Sedation
Xylazine	1–5 mg/kg IM, IP	Sedation, some analgesia

Anesthesia

One may deliver an anesthetic agent either through injection or inhalation. When using inhalation agents, it is important to determine if the animal has a respiratory tract illness which may interfere with delivery of the agent. One may determine depth of anesthesia by using the toe pinch (pedal withdrawl reflex), eye blink (palpebral reflex), skin pinch, breathing rate and depth, or a combination of all these signs. Foot withdrawl when pinched, blinking when the eyelashes are touched, a movement in response to a skin pinch may all indicate inadequate depth for surgery. Breathing should be deep and regular.

injectable anesthesia

When administering an injectable agent in rats, consider the administration site, method, volume, and discomfort level. Dilution will reduce the discomfort of an irritating compound, and assure adequate dosage of otherwise small volumes. Administration of injectable compounds is most commonly intraperitoneal in rats, although intramuscular, subcutaneous, and intravenous administration are at times useful. If equipment for scavenging of waste anesthetic gases is unavailable, it may be necessary to rely solely upon injectable agents to provide surgical and light anesthesia. Balanced anesthesia is common

when working with injectable agents. Tables 13 and 14 provide information about agents, dosages, anesthesia time, and sleep time of some agents used to provide surgical and light anesthesia. Anesthesia time is that time which one expects the rat to have a marked reduction or elimination of sensory and motor responses. Sleep time is that time which one expects the rat to be unconscious. During sleep time, the anesthetic's effect can gradually diminish, resulting in responces to a painful stimuli and thereby necessitating an additional dose of anesthetic.

One advantage of some injectable agents is the availability of injectable antagonist or reversal agents. One may choose to use an antagonist if the animal appears to be having an adverse reaction to the anesthetic, has received too much anesthetic, or if the procedure has been completed. These agents are valuable tools for the anesthetist. Table 15 lists the antagonists for some injectable anesthetic agents.

notes on injectable agents

Note: Consult with a veterinarian when planning any anesthetic regimen. The surgical anesthesic agents provide better analgesia than the light anesthesic agents. Rat strains vary in response to various anesthetic agents.

- HYPNORM® and IMMOBILON® are currently unavailable in the United States.
- Alphaxolone/alphadolone (steroid anesthetic agents) and propofol (an alkyl phenol) can provide total intravenous anesthesia:
    - Alphaxolone/alphadolone: 10–12 mg/kg IV, then 0.2–0.7 mg/kg/min IV
    - Propofol: 0.5–1.0 mL/kg HYPNORM® premedication, then 4–6 ml/kg/h propofol IV
    - Propofol: 10 mg/kg IV, then 0.5–1.0 mg/kg/min IV.

  Propofol at high infusion rates may produce apnea and extended recovery times, therefore it may be a better decision to use propofol with HYPNORM®, as this combination requires lower infusion rates, will provide adequate

TABLE 13. INJECTABLE AGENTS SUITABLE FOR SURGICAL ANESTHESIA

Anesthesia Time (minutes)	Agent(s)	Dosage	Sleep Time (minutes)
ð5 minutes	Alphazalone/Alphadolone	10–12 mg/kg IV	10
	Methohexital	10–15 mg/kg IV	10
	Propofol[a]	10 mg/kg IV	10
ð10 minutes	Thiopental	30 mg/kg IV	15
at least 20 minutes	Fentanyl/Fluanisone (HYPNORM®) + Diazepam	0.6 mL/kg IP 2.5 mg/kg IP	120–240
	Fentanly/Fluanisone (HYPNORM®) + Midazolam[b]	2.7 mL/kg IP	120–240
	Ketamine + Medetomidine[c]	75 mg/kg IP 0.5 mg/kg IP	120–240
	Ketamine + Xylazine[d]	75–100 mg/kg IP 10 mg/kg IP	120–240
	Tribromoethanol	300 mg/kg IP	—
at least 60 minutes	Etorphine/Methotrimeprazine (IMMOBILON®) + Midazolam[e]	0.5 mL/kg SQ	120–240
	Fentanyl + Medetomidine	0.3 mg/kg IP 0.2–0.3 mg/kg IP	240–360
	Inactin	80 mg/kg IP	60–240
at least 6 hours	Urethane — **NONSURVIVAL PROCEDURES ONLY**	1000–1200 mg/kg IP	360–480

[a] HYPNORM® (Janssen Animal Health Ltd., Grove, Wantage, Oxon OX12 0DQ, UK) premedication (0.5–1.0 mL/kg) and propofol continuous infusion (4–6 mL/kg/h) will provide stress-free induction, well-controlled anesthesia, good analgesia, and muscle relaxation.

[b] Prepare the cocktail by combining 1 part HYPNORM®, 2 parts sterile water for injection, and 1 part midazolam (5 mg/ml).

[c] Prepare a cocktail by combining 0.75 mL (75 mg) of ketamine, 0.5 ml (0.5 mg) of medetomidine, and 0.75 mL sterile water for injection. Administer 0.2 mL per 100 g of body weight.

[d] Although pentobarbital is used by some investigators for anesthesia, there are more reliable agents available which have a wider safety margin, and are less affected by factors such as strain and time since the last meal. For this reason pentobarbital is only recommended for light anesthesia.

[e] One may prepare this cocktail by combining 1 part IMMOBILON® (Grampian Pharmaceuticals Ltd., Marathon Place, Moss Side Industrial Estate, Leyland, Lancs PR5 3QN, UK), 1 part midazolam, and 2 parts sterile water for injection.

analgesia, and the HYPNORM® (neuroleptanalgesic combination) is reversible. The availability of ventilatory support is strongly recommended. Bolus administration of propofol given rapidly, over less than 5 seconds, appears to result in an excitatory response. This response includes forelimb extensor rigidity and vibrissae twitching. The response was not seen with bolus administration given over 5–10 seconds.

- The use of HYPNORM® in fed rats may result in hyperglycemia, possibly due to opioid involvement in glucose metabolism.
- The IMMOBILON®/midazolam (neuroleptanalgesic combination) cocktail may result in severe respiratory depression, therefore oxygen should be available if using this combination.
- The medetomidine in the fentanyl + medetomidine (neuroleptanalgesic combination) cocktail acts to lengthen anesthetic duration.
- Urethane and α-chloralose should only be used in non-survival procedures. Urethane is a carcinogen and one should handle it with caution.
- Reports exist of xylazine administration resulting in pulmonary edema at higher dosages (greater than 20 mg/kg). Care must be taken to use the proper concentration since xylazine is available in two different concentrations (20 mg/ml and 100 mg/ml). Xylazine also appears to affect antidiuretic hormone (ADH) secretion and glucose homeostasis.
- Significant prolongation of thrombin and activated partial thromboplastin times during ketamine/xylazine and urethane anesthesia, repectively, lasting at least 60 minutes was found to occur.
- Although pentobarbital is used by some investigators for anesthesia, there are more reliable agents available which have a wider safety margin, and are less affected by factors such as strain and time since the last meal. For this reason pentobarbital is only recommended for light anesthesia.

TABLE 14. INJECTABLE AGENTS SUITABLE FOR LIGHT ANESTHESIA

Anesthesia Time (minutes)	Agent(s)	Dosage	Sleep Time (minutes)
15 minutes	Pentobarbital	40–50 mg/kg IV	120–240
	Tiletamine/Zolezepam (TELAZOL®)	40 mg/kg IP	60–120
20–30 minutes	Ketamine + Acepromazine[a]	75 mg/kg IP 2.5 mg/kg IP	120
	Ketamine + Diazepam	75 mg/kg IP 5 mg/kg IP	120
	Ketamine + Midazolam[b]	75 mg/kg IP 5 mg/kg IP	120
1 hour	Chloral Hydrate[c]	300–400 mg/kg IP	120–180
at least 8 hours	α-Chloralose — **NONSURVIVAL PROCEDURES ONLY**	55–65 mg/kg IP	480–600

[a] Prepare a cocktail with 0.75 mL (75 mg) ketamine, 0.25 mL (2.5 mg) acepromazine, and 1 mL sterile water for injection. Administer 0.2 mL per 100 g body weight.

[b] One may prepare a cocktail by combining 0.75 mL (75 mg) ketamine, 1 mL (5 mg) midazolam, and 0.25 mL sterile water for injection. Administer 0.2 mL per 100 g body weight.

[c] The use of chloral hydrate may result in a dynamic ileus in rats.

gas anesthesia

The use of inhalation anesthesia is very effective in rats. Concerns include adequate scavenging of waste gases, skill of the anesthetist, and equipment availability. Inhalation agents permit the researcher to exert greater control over the anesthetic event, which greatly impacts survivability. Several inhalation agents are available depending on the research project's goals. The equipment may range from a bell jar to open or closed anesthetic delivery systems. An important concept in inhalation anesthesia is minimum alveolar concentration (MAC). MAC is an indication of potency, and is the alveolar concentration necessary to block painful stimuli in 50% of the animals in a group. The lower the MAC, the more potent the anesthetic agent. It should be clearly understood that 1 MAC does not assure adequate anesthesia. Levels of 1.2–1.3 MAC are commonly used if no preanesthetic analgesics or tranquilizers have been given.

Table 15. Antagonists Available for Injectable Anesthetics

Antagonist for:	Agent	Dosage
Fentanyl	Butorphanol	0.4 mg/kg SQ
	Nalbuphine[a]	2 mg/kg SQ
Medetomidine or Xylazine	Yohimbine[b]	2.1 mg/kg SQ, IP
Medetomidine or Xylazine	Atipamizole[c]	1 mg/kg SQ
Fentanyl + Medetomidine	Butorphanol	0.4 mg/kg
	Atipamizole	1 mg/kg

[a] One may dilute 0.2 mL (2 mg) nalbuphine in 0.8 mL of sterile water for injection and administer 0.1 mL per 100 g body weight to reverse fentanyl.

[b] Yohimbine is not as specific as atipamizole. Atipamizole is preferred by the authors.

[c] Dilute 0.2 mL (1 mg) atipamizole in 0.8 mL of sterile water for injection and administer 0.1 mL per 100 g of body weight.

Table 16. Characteristics of Various Gas Anesthetic Agents

Agent	MAC	Vapor Pressure	% Saturation @ 22°C	Stability in Soda Lime	% Recovered as a Metabolite (Human)
Enfluorane	2.21	172	23	Stable	24
Ether	3.2	443	58	Stable (trace of aldehydes)	—
Halothane	0.95	242	32	Slight decomposition	20–25
Isofluorane	1.38	240	32	Stable	0.17
Methoxyfluorane	0.22	23	3	Slight decomposition	50
Nitrous Oxide	250	39,500	100	Stable	0.004

The bell jar technique requires minimal skill and permits only minimal control over anesthetic administration. It involves placing the animal in an environment where the volatile anesthetic

agent evaporates. There must be some separation between the anesthetic and the rat, as contact with agents such as methoxyfluorane, halothane, and isofluorane are irritating. Besides the lack of anesthetic control, there is potential for the anesthetist to gain high-level exposure to these anesthetic agents. Methoxyfluorane is the preferred agent when using the bell jar technique because room temperature and pressures permit a 3% concentration to easily develop. This concentration of methoxyfluorane is safe, whereas, lethal concentrations of halothane and isofluorane develop under identical conditions. One should discourage use of this technique, unless adequate scavenging (e.g., chemical fume hood) is available to prevent unintentional exposure of humans and animals to waste anesthetic gases. This technique only provides very short term anesthesia for quick procedures such as orbital bleeding or anesthetic induction. A complete anesthetic induction chamber (controlled delivery, minimal contamination of the immediate environment, and scavenging) description is given by Gwynne and Wallace. The system provides for rapid anesthetic induction (<45 seconds) using low halothane levels (2.5%) and minimal contamination of <1 ppm in the immediate environment.

A bell jar can be assembled using a large covered-glass container, the volatile anesthetic, cotton balls, and some means to prevent animal contact with the liquid phase anesthetic. One may place anesthetic-soaked cotton balls on the bottom of the jar and cover them with an elevated wire mesh or place them in a separate container with ventilation holes. The rat is placed in the jar where it becomes anesthetized as it inhales the volatized anesthetic. After removal from the bell jar, one may administer additional anesthetic during a procedure by placing the shaft of a 35- or 60-cc syringe containing anesthetic-soaked cotton or gauze over the rat's face. The rat will inhale supplemental anesthetic as it breathes. Unless adequate scavenging is available this method of prolonging inhalant anesthesia is not recommended.

Endotracheal intubation provides an effective means to deliver adequate inhalant anesthesia for a longer duration procedure (Fig. 21). It is easily accomplished via 14–20 gauge intravenous catheters, an otoscope, a flexible (yet firm) guide wire.

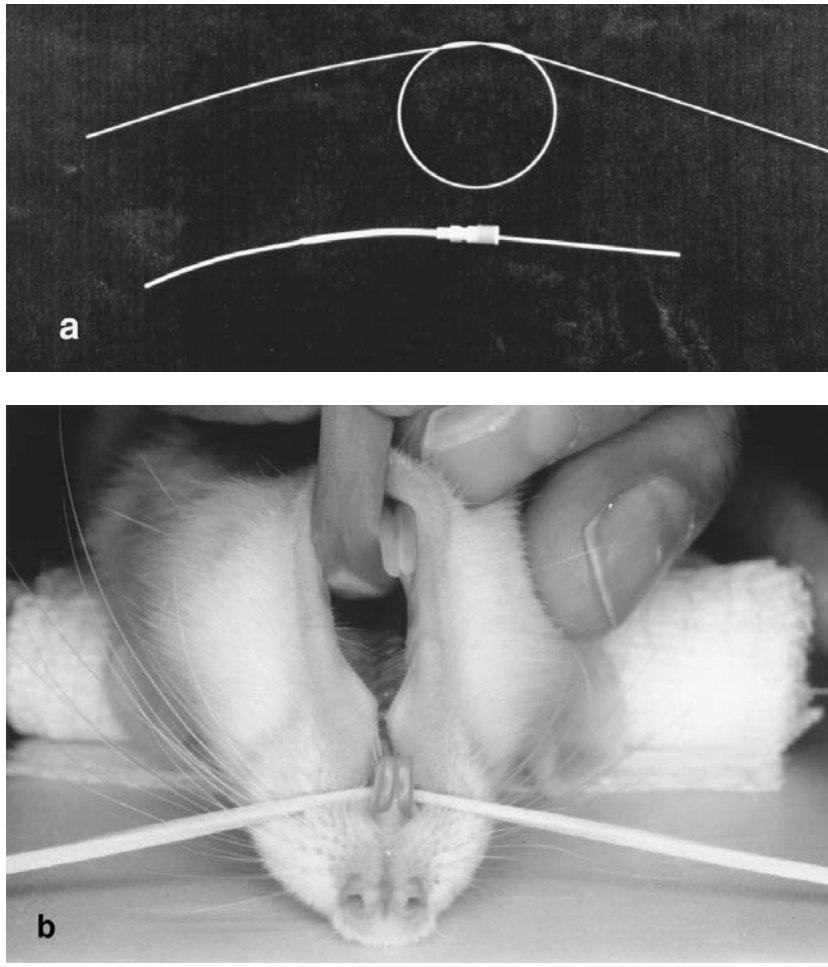


Fig. 21. Endotracheal intubation of the rat. (a) A flexible guidewire (above) and 14-gauge over-the-needle-catheter (below) used for intubation of the rat. Otoscope not shown. (b) Rat in dorsal recumbancy with a rubber band behind the maxillary incisors, gentle traction on the tongue, and a roll of gauze under the dorsal neck. (c) Front view of the rat ready to intubate. Photographs courtesy Dr. George Vogler.

With the rat in dorsal recumbancy, one may use the otoscope to visualize the larynx and place the guide wire in the trachea. The guide wire is kept in place while carefully removing the otoscope. Finally, thread the intravenous catheter over the guide

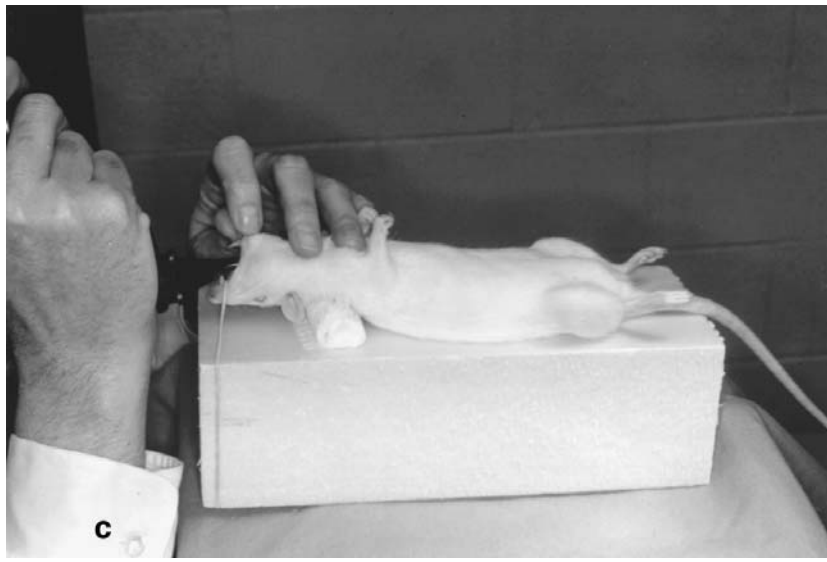


Fig. 21. (continued)

wire. While holding the catheter in place, remove the guide wire and attach the catheter to the anesthetic circuit. It is very important to minimize equipment dead-space in the circuit by placing the connector close to the mouth, since deadspace is the compartment of gas which does not undergo physiologic exchange, and if great enough, can result in the suffocation of an animal.

Analgesia

There are three broad categories of analgesic drugs available for use in the rat and other laboratory animals:

1. Opioids — centrally acting compounds that work in conjunction with the central nervous system's endogenous opioid system.
2. Peripherally acting compounds which block nociceptor impulses (antihistamines, local anesthetics, and α-2 adrenoceptor agonists).
3. Nonsterioidal Anti-inflammatory Drugs (NSAIDs) — compounds which act by inhibiting production of chemical mediators and in turn activate peripheral nociceptors (aspirin, acetaminophen, ketoprofen).

TABLE 17. OPIOID ANALGESICS

Agent	Dosage Recommendation
Buprenorphine*	0.1–0.5 mg/kg IV, SQ q8–12 h
Butorphanol	2 mg/kg SQ q4 h
Morphine	2.5 mg/kg SQ q2–4 h
Nalbuphine	1–2 mg/kg IM q3 h
Pentazocine	10 mg/kg SQ q3–4 h
Meperidine	10–20 mg/kg IM, SQ q2–3 h

* Buprenorphine may have a slight immunostimulatory effect and may result in gastric distention.

TABLE 18. NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAID) ANALGESICS

Agent	Dosage Recommendation
Acetaminophen	200 mg/kg PO
Aspirin	100 mg/kg PO
Carprofen	2–5 mg/kg SQ
Diclofenac	10 mg/kg PO
Flunixin	2.5 mg/kg IM, SQ q12 h
Ibuprofen	15 mg/kg PO
Indomethacin	2 mg/kg PO
Ketoprofen	33 mg/kg PO
Piroxicam	3 mg/kg PO

Animals receiving any analgesic medication should have an individual assessment of their analgesic needs. One should consider administering an analgesic prior to a potentially painful procedure to disrupt the pain cascade. Studies indicate water consumption in rats as the most reliable indicator of pain assessment and analgesic efficacy. The administration of acetaminophen in drinking water does not appear to provide analgesia to the same degree as parenteral administration in rats, whereas oral buprenorphine may provide analgesia. Rats appear to be more susceptible to gastrointestinal ulcers resulting from the use of certain NSAIDs than other rodents.

Local Anesthesia

Local anesthesia involves desensitization of specific, defined sites, often involving the skin and subcutaneous tissues. Injectable lidocaine and bupivicaine can be placed directly into or around the site of injury. Bupivicaine has a longer duration of action than lidocaine. Only a small volume is necessary to infiltrate the area around the wound. A lignocaine-prilocaine cream (EMLA cream, Astra Pharmaceutical Products, Inc. Westborough, MA) is available for skin desensitization. The cream is applied to the skin and covered with an occlusive bandage for about 1 hour before desensitization occurs.

aseptic surgery

General

There is a general misconception first espoused in 1942 that rats are less susceptible to postoperative infections than other animals. This belief in the rat's surgical invulnerability is contrary to the teachings in veterinary and medical schools. Although definitive evidence is lacking, circumstantial evidence strongly indicates, as does common sense, that rats benefit when aseptic technique is used for surgical procedures. It would not seem good scientific judgment to employ lower standards for survival rodent surgical procedures.

The surgical procedure can be divided into three equally important parts: preoperative, intraoperative, and postoperative. One should consider each part individually when attempting any surgical procedure, identifying potential issues which may jeopardize the study, and more importantly the animal's life. It is better to identify such issues in the planning stages of an experiment rather than after an undesirable or questionable experimental outcome. The issues could include items such as anesthetic choice, equipment and staffing requirements, animal health status, animal monitoring requirements, and investigator education. Consideration of these issues may prevent unnecessarily duplicating an experiment and result in a savings of time, money, and animal life. With many potential issues, it is perhaps a wise decision to include a pilot study to identify specific issues and their solutions. In this regard, institutional veterinarians

and veterinary technicians may be a valuable resource in experimental planning to identify issues specific to the rat.

One may classify surgical procedures according to the following:

- **Major —** A procedure in which one penetrates or exposes a body cavity, or induces substantial physical or physiologic impairment.
- **Minor —** A procedure where exposure of a body cavity does not occur or there is little to no physical or physiologic impairment.
- **Survival —** A procedure in which one expects the animal to regain consciousness after anesthesia.
- **Nonsurvival —** Termination of the animal occurs immediately following the procedure and the animal does not regain consciousness.

The preoperative, operative, and postoperative areas will be further divided into issues concerning the following:

- anesthetic and supportive equipment/drugs
- personnel
- animals

Aseptic Technique

Aseptic technique plays an important role in a positive surgical outcome. Several texts are available which discuss in depth this and other elements of surgical procedures (animal, surgical team, and instrument preparation; suture selection, knot tying, etc.). One should view aseptic technique as a state-of-mind in order to minimize contamination and its associated physiologic disruptions. A brief discussion on the components of aseptic technique will include equipment sterilization, animal preparation, location of various components of the procedure, and surgeon preparation.

One may sterilize items by autoclaving (pressurized steam), ethylene oxide (ETO) exposure, cold sterilization (chemical agents), or irradiation. Autoclaving and ETO exposure are most common, with cold sterilization of value when large numbers of rats undergo a procedure. Follow the manufacturer's recommendations for

contact time of cold sterilants and rinse in sterile saline. Most bulk disposable items and other surgical supplies purchased pre-sterilized will have undergone irradiation sterilization.

Animal preparation up to and including anesthesia induction should occur outside of the area designated for operative procedures to minimize contamination with bedding, fur clippings, dander, urine, and feces. One should clip a generous area of fur over the surgical site in the surgical preparation area.

Once this portion of the preoperative preparation is complete, the animal can now be transported into the operative area, positioned, and can receive the final preparation for surgery. The surgical site should receive three alternating preparations with an appropriate surgical scrub (hexachlorophene, chlorhexidine, iodophors, etc.) each followed with an alcohol or sterile water rinse. Following the last rinse, an iodine or chlorhexidine containing preparation is applied to the skin three to five minutes prior to starting the procedure.

The surgeon and/or the assistant can now isolate the surgical site with sterile drapes and start the procedure using sterile instruments. Optimally, the surgeon and assistant should wear shoe covers, cap, mask, and sterile gloves and gown; other individuals in the room not performing or assisting in the actual procedure should wear scrubs (not exposed street clothes), shoe covers, cap, and mask. This optimal level may not be necessary with less complex procedures. At the minimum level, a cap, gown, and shoe covers may be unnecessary.

If one is performing procedures on groups of animals, it is best to have two or three surgical packs per surgeon, to assure an adequate supply of sterile instruments. At the minimum, surgical gloves and drapes will also need changing between animals. If the drapes are dry it is possible for them to follow the rat to the recovery area to aid in maintaining body temperature.

Suture Material

Suture material has two broad classification schemes — absorbable and non-absorbable. Absorbable materials are those which lose their tensile strength within 60 days, whereas non-absorbable materials lose their tensile strength greater than 60 days following placement within the body. Further classifications include natural vs. synthetic and monofilament vs. multifilament.

Natural materials include catgut, steel, or silk; whereas synthetic materials include polyglycolic acid, polydioxanone, and polyester. Preference is given to monofilament materials because multifilaments tend to draw (or wick) body fluids and bacteria into the surgical wound. In addition, multifilaments can harbor organisms between their strands.

suture recommendations

- Consult a veterinarian or veterinary technician.
- Catgut is not recommended for internal wound closure due to the potential for rapid suture breakdown following bacterial contamination.
- Synthetic monofilaments are highly recommended for internal and external wound closure.
- Tissue adhesives, surgical staples, and ligating clips are available to assist in wound closure and hemostasis.
- One usually closes the skin with 3–0 or 4–0 suture material.

suture needles

Needles are available in a variety of sizes, shapes, and diameters. Typically needles are either tapered or cutting, with the suture material attatched to the needle. Suture material without an attached needle is available. Tapered needles are usually for internal closures, and cutting needles are for skin closure and other more dense tissues such as tendon.

suture patterns and knots

Various suture patterns are available with desciptions available in previously described texts; however, the three most commonly used patterns are the simple interupted, continuous, and horizontal mattress. When finishing a given pattern, the suture material is tied into knot(s), depending on a given material's knot security. One may tie these knots by hand or with instruments (needle holders) using either a square knot or a surgeon's knot, a variation of the square knot.

preoperative issues

Anesthetic and Supportive Equipment/Drugs

Assure that the equipment is available and in good working order. One should become familiar with the equipment's operation. The equipment should include heating blankets or other means for providing supplemental heat, monitoring, and anesthetic equipment.

1. **Equipment for providing supplemental heat —** Avoid electric heating blankets and heat lamps to reduce the incidence of burns. Preference is given to warm-water recirculating blankets. Since it is much easier to keep a patient warm than rewarm it, heating blankets should be available while the animal is being anesthetized. Room temperature can also be elevated (80–85°F), and when using inhalation anesthesia, tanked oxygen kept in the warm room overnight to assist in maintaining normothermia. Drapes and bubble wrap will provide additional insulation to reduce heat loss. Holes can be cut in the bubble wrap to permit adequate exposure for the procedure.

2. **Monitoring equipment —** Adequate patient monitoring can indicate problems and enable corrective actions to be taken to prevent the death or serious impairment of a study participant. Monitoring could include measurement of body temperature, respiratory rate, heart rate, arterial blood pressure, and pulse oximetry. Although the level of monitoring will depend on the procedure, body temperature is perhaps the most important parameter to measure, because of the rat's large surface area to body weight ratio predisposes it to hypothermia during surgery.

3. **Anesthetic Equipment —** One should become familiar with the anesthetic equipment no matter how simple or complex. Troubleshooting is very important to prevent needless, and costly, losses.

Drugs

- Check the expiration dates of all compounds which could be used during any component of the procedure.
- Check the concentrations of the compounds to ensure they are appropriate.
- Ensure there are adequate amounts of drugs available.
- Make sure emergency drugs are available:
    - Epinephrine: 0.1 mL/kg (1:10,000)
    - Doxapram hydrochloride: 5–10 mg/kg
    - Atropine: 0.05 mg/kg
    - If using an injectable anesthetic, the appropriate antagonist should be available.
- Administer antibiotics prior to the procedure to ensure adequate tissue concentration at the time of the procedure. Antibiotics are not a substitute for aseptic technique.
- Administer analgesics prior to a procedure. This acts to interrupt the pain cascade and reduce postoperative discomfort.

Personnel

- Make sure the personnel have adequate information and training regarding the following:
    - Experimental protocol
    - Anesthetic and support equipment/drugs
    - Animals — anatomy, physiology, etc.
    - Surgical and other techniques — The personnel should be familiar with aseptic technique, the surgical and other procedures related to the experimental protocol.

Animals

- Use of healthy animals free of clinical and subclinical disease (e.g., SPF animals)
    - Will **reduce morbidity and mortality.**
    - Will **reduce research variability.**
    - Overall will **reduce wasted resources and save money.**
    - Perform a brief **physical exam** to assure health status.
    - **Permit the animals to acclimate** to their new surroundings.
    - One should **fast rats only if performing gastrointestinal surgery (up to 12 hours)**, for other procedures never fast any longer than 2 hours.

Note: Do not water restrict rats prior to a procedure. Rats do not vomit because the esophagus enters the lesser curvature of the stomach through a fold in the limiting ridge of the stomach, which prevents vomiting.

operative issues

Anesthetic and Supportive Equipment/Drugs

Aseptic techniques should always be used for survival surgery.

Ventilator — Several commercial ventilators suitable for rats are available. Respiratory rates of 60–100 breaths per minute and tidal volumes of 10–15 mL/kg should provide adequate ventilation under pressures of 5–15 mm Hg.

Intraoperative monitoring/records — Intraoperative monitoring in rats should include at the minimum the

measurement of temperature, heart rate, and repiratory rate. By measuring and following these and other parameters (direct or indirect blood pressure, pulse oximetry, etc.) one can get an indication of problems before they develop and take corrective action or stop the procedure. When using neuromuscular blocking agents, it is valuable to follow heart rate as increases in rate could indicate inadequate anesthetic depth.

1. **Drugs**

- **Adequate anesthetic agents** should be made available in the event the procedure takes longer than anticipated.
- **Fluid therapy —** One should administer fluids during a long procedure or at the end of a short procedure. One may administer *warm* lactated ringers solution (LRS) or 0.9% (normal) saline. Administering warm fluids at the end of a procedure helps ensure normothermia or near normothermia at the time the animal is placed into a warm recovery area. One should not rely exclusively on warm fluids at the end of a procedure to warm an animal. Other techniques such as heating blankets, bubble wrap, etc. are just as valuable, as it is easier to maintain an animal's temperature rather than rewarm them. One should administer fluids at the rate of 40–80 mL/kg/h and take into consideration any pre-existing, or developing condition related to the procedure (e.g., kidney failure, diabetes mellitus, etc.). One may administer the fluids either SQ, IP, or IV.
- **Emergency drugs —** see previous section.
- **Oxygen** may be useful during the procedure in case of emergency.
- **Ophthalmic ointment —** One should instill ophthalmic ointment to prevent corneal dessication (Fig. 22).
- Consider administering **local anesthetics** (lidocaine, bupivicaine) as a line block along the incision to further reduce postoperative pain.

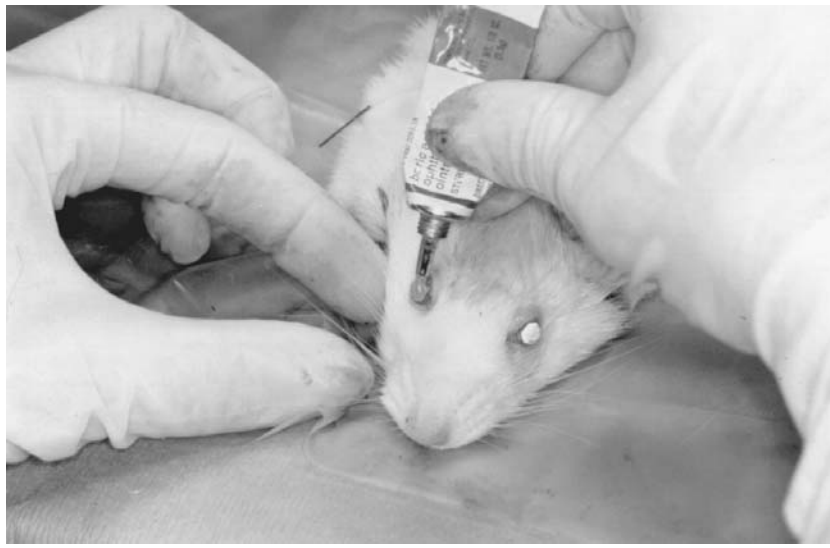


Fig. 22. Application of ophthalmic ointment to prevent corneal dessication.

- **Neuromuscular Blocking (NMB) Agents —** NMB agents fall into two categories: depolarizing and nondepolarizing. Depolarizing agents act similar to acetylcholine and cause muscle contraction. Animals will develop generalized muscular fasiculations prior to general muscle paralysis. Nondepolarizing agents compete with acetylcholine at the neuromuscular junction (NMJ) for receptor sites. An increase in acetylcholine will reverse the blockade. Neostygmine will increase acetylcholine concentration by altering acetylcholinesterase activity. Two concerns with NMB agent use are first to ensure good anesthesia, as the animals are still able to perceive pain, and second to provide ventilatory support, since paralysis of respiratory muscles may occur. Table 19 lists NMB agents commonly used in rats.

TABLE 19. NMB AGENTS COMMONLY USED IN RATS

Agent (All Nondepolarizing)	Dosage
Gallamine	1 mg/kg IV
Pancuronium	2 mg/kg IV
Turbocurarine	0.4 mg/kg IV

2. **Personnel**

- **Adequate personnel —** Enough personnel should be available to perform the procedure in both aseptic (e.g., surgeon and assistant) and nonaseptic (e.g., anesthetist, circulating technician) capacities without compromising the sterility of the procedure and integrity of the study.
- **Adequate knowledge** of anatomy, procedure(s), etc. with particular attention to the following:
    - **Aseptic technique**
    - **Gentle tissue handling**
    - **Hemostasis**
    - **Minimizing desiccation** by keeping tissues moist with normal sterile saline.
    - Use of **appropriate suture material and suture patterns** for wound closure.

3. **Animals**

- **Maintain homeostasis**
    - **Respiratory —** One may provide O_2 throughout a procedure through a face mask.
    - **Cardiovascular —** A 400 g rat has about 32 mL of blood volume, therefore, a 6–8 mL blood loss could result in shock. With the use of inbred strains, there is no need to crossmatch animals of the same strain prior to administering blood. One may store blood in acid citrate dextrose (ACD) at 4°C for several days prior to administration, the proper ratio of ACD:blood is 1:3. The successful storage of whole blood in citrate, citrate-dextrose, and citrate-phosphate-dextrose-adenine (CPDA-1) for 8, 22, or 35 days, respectively, has been reported by Wiersma.
    - **Temperature —** Hypothermia tends to increase anesthetic depth and sleep time.

postoperative issues

Anesthetic and Supportive Equipment/Drugs

Monitor parameters regularly for return to normal levels: respiratory, cardiovascular, and temperature.

1. **Drugs**

- **Anesthetic agent antagonists (injectable agents) —** Post-operative administration of the injectable antagonist will minimize the anesthetic and sleep time for the procedure.
- **Antibiotics —** There may be a need for post-operative antibiotics, especially if sterility has been broken or if the gastrointestinal tract has been entered. One should consult with the veterinarian for further assistance.
- **Analgesics —** Administering post-operative analgesics will be necessary for some procedures. Animals should be individually assessed for pain and medicated accordingly. Some analgesics will have a longer duration than others and this should be taken into consideration when selecting an analgesic.

2. **Personnel**

- **Assessment of surgical outcome** with appropriate alteration of technique in the future.
- **Monitoring of the animal**.

3. **Animals**

- **Support physiologic needs —** body temperature, fluid balance
- **Assess surgical outcome** with respect to the procedure itself and its impact upon the animal. Determine if there are any changes that could be made to yield a better outcome?

- Monitor for **analgesic needs**. Clinical signs and animal behavior are important tools to assess a rat's analgesic needs. Below is a list of clinical signs and behaviors which may indicate pain in rats:
    - Weight loss
    - Reduced food and water intake
    - Self-mutilation
    - Altered stance or gait
    - Unkempt haircoat due to piloerection or inadequate grooming
    - Red tears or dark crusts around the eyes, nostrils, and forearms
    - Increased rate and/or effort of respiration
    - Vocalization
    - Reduced activity
    - Altered behavior — irritable, docile, etc.
- Monitor for **wound healing and surgical complications**
    - Dehiscence of the surgical site
    - Wound infection, indicated by an unusual discharge, redness, and/or swelling at the surgical site
    - Analgesic response
    - Other potential complications (anemia, poor antibiotic response, slow or poor postoperative recovery)

euthanasia

The 1993 *Report of the AVMA Panel on Euthanasia* and the 1995 *Euthanasia of Experimental Animals* (European Union) describe several methods of euthanasia as acceptable for rodents. In the laboratory, the most common methods are overdose with inhalation anesthetic or barbiturates and carbon dioxide

inhalation. Conditionally acceptable methods most used in the laboratory are cervical dislocation and decapitation. Whatever method is chosen, euthanasia should be quick, with a minimum of animal distress, fear, or anxiety. Rats should be handled gently by an experienced individual. Table 20 summarizes the euthanasia techniques.

Inhalant Anesthetics

Ether is generally unacceptable for euthanasia of rodents. The process of placing even moderate numbers of rats euthanized by ether into closed containers for disposal greatly increases the risk of explosion. Other anesthetic agents, such as methoxyflurane, halothane, and isofluorane are more acceptable, but personnel safety requires scavenging of these gases either through an appropriately equipped anesthetic machine or under a chemical fume hood. If anesthetic equipment is not available, a rat may be placed in a closed glass container containing anesthetic soaked gauze or cotton. This should be arranged in such a way to prevent direct contact between the animal and the wet anesthetic agent. If the top of the container has a dome, the wet cotton can be taped to the inside of the top. If not, soaked material can be placed on the floor of the container and a wire mesh placed over it and elevated such that the rat's paws do not contact the material. Methoxyfluorane has a low vapor pressure, and induction may be slow. To overcome this, the container loaded with anesthetic should be covered and allowed to sit for 20 minutes before placing the rat inside. This allows time for the anesthetic to fill the chamber.

Barbiturates

If rats are acclimated to restraint for tail vein injection and the personnel are experienced, euthanasia by intravenous barbiturates is preferred. However, if this technique is likely to cause more distress to the animal, intraperitoneal injection is acceptable. There are several barbiturate products formulated specifically for euthanasia. In the U.S., all barbiturates are regulated drugs, and their use must comply with the regulations of the Drug Enforcement Agency (DEA).

Carbon Dioxide

Carbon dioxide (CO_2) is routinely used for euthanasia of rats and mice. It is inexpensive and poses minimal hazard to personnel. Compressed CO_2 gas in cylinders is recommended. Dry ice may be used, but animal contact with the dry ice must be avoided. A second problem with dry ice is lack of uniformity of chamber fill. Cylinders must be equipped with a regulator and secured to the wall as per safety requirements. The chamber should be primed with CO_2 before placing the rat inside. However, since CO_2 is heavier than air, large amounts may settle to the bottom of the chamber causing distress when the animal goes from air to pure CO_2. The animal is placed in the chamber and the flow rate should be such as to displace at least 20% of the chamber volume per minute. Flow is continued until the rat loses consciousness and stops breathing. It is recommended to wait a brief period following respiratory arrest to assure the animal is dead. Rats with respiratory disease and neonatal rats are resistant to the effects of CO_2. For these animals an injectable anesthetic is a better choice.

Cervical Dislocation

This manual method of euthanasia is confined to immature rats (<200 g) when scientifically justified and approved by the Institutional Animal Care and Use Committee (IACUC). The rat is grasped behind the skull or a rod is placed at the base of the skull and the base of the tail or hind legs are pulled. This technique must be performed by skilled personnel. Mechanical dislocators can be used on larger rats by experienced personnel and if approved by the IACUC.

Decapitation

The correct use of guillotines, which are commercially available, requires well-trained personnel. The rat must be restrained in such a manner that there is little distress to the rat and little risk to the handler from either a rat bite or a guillontined finger. The blade must be dropped quickly and firmly so that the result is a quick cut, not a slow crush. This also means that the blade must be kept clean and sharp. Rats are sensitive to the smell of blood and one must clean the guillotine well before proceeding

TABLE 20. SUMMARY OF EUTHANASIA METHODS

Method	AVMA	EU	Notes
Inhalation Anesthetics	Acceptable	Acceptable	Provide adequate scavenging
Barbiturates	Acceptable	Acceptable	
Carbon Dioxide	Acceptable	Acceptable	
Cervical Dislocation	Conditionally Acceptable	Acceptable	Acceptable for rats <200 g (AVMA) and <150 g (EU)
Decapitation	Conditionally Acceptable	Prefer other methods	

to the next animal. If not, the technician may find the rats becoming more aggressive and more difficult to restrain. Rats also remain calmer if brought into the room individually rather than as a group.

Note: Decapitation must be scientifically justified to the IACUC.

5

experimental methodology

restraint

Manual Restraint

Frequent handling of rats in a firm, yet gentle, manner will accustom them to manipulation. Rats may be removed from their cage or primary enclosure by grasping the tail 1–2 cm from the base. One may restrain the rat by any of the following means:

- Grasp the loose skin over the back and neck with the nondominant hand.
- Firmly grasp the rat around the thorax with one's thumb or index finger under the mandible to prevent being bitten (Fig. 23a). Grasping with the nondominant hand will give the restrainer use of the dominant hand for animal manipulations or to support the animal's hindquarters. With single hand restraint, the tail may be held between the fourth and fifth fingers.
- The rat may be palmed over its back with the index and middle finger placed between its mandible to minimize the chance of being bitten (Fig. 23b).

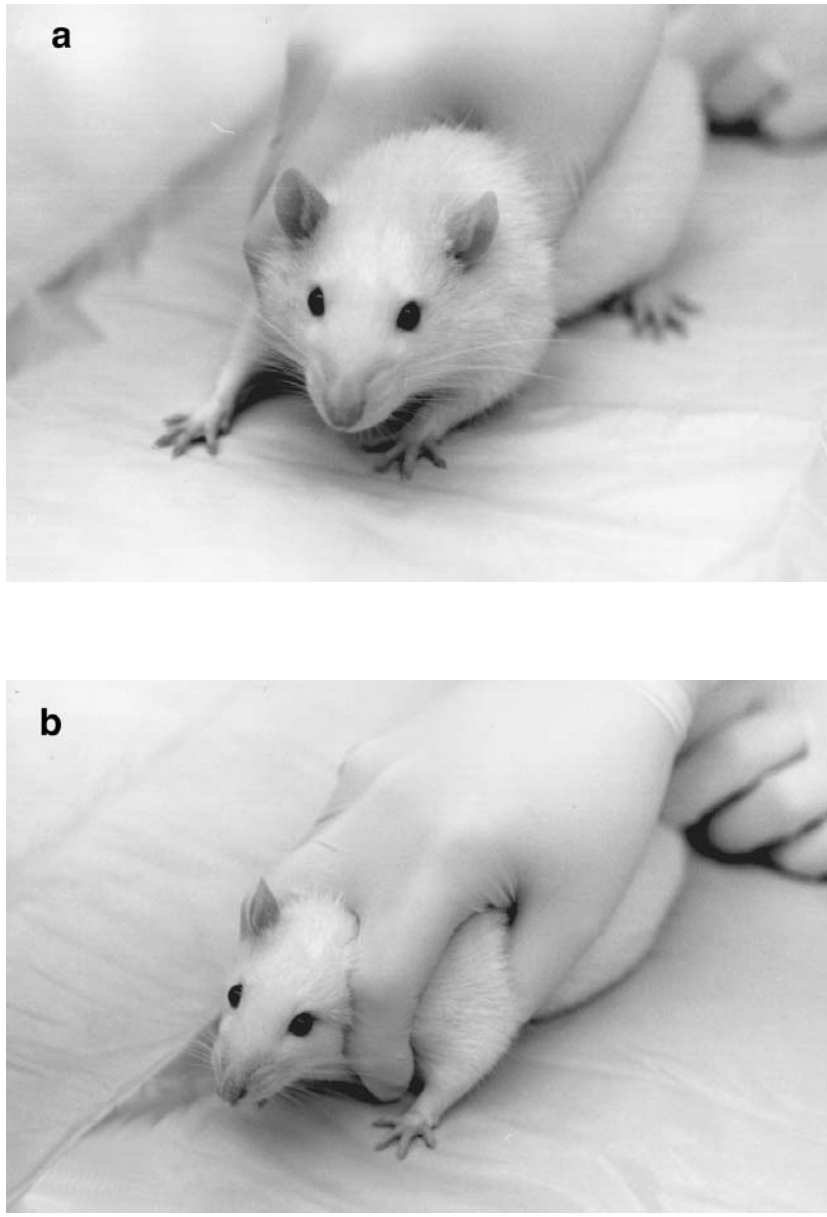


Fig. 23. Manual restraint techniques for the rat: (a) grasping the rat around the thorax with the index finger under the mandible; (b) palming the rat over the back while keeping the rat's head between the middle and index fingers.

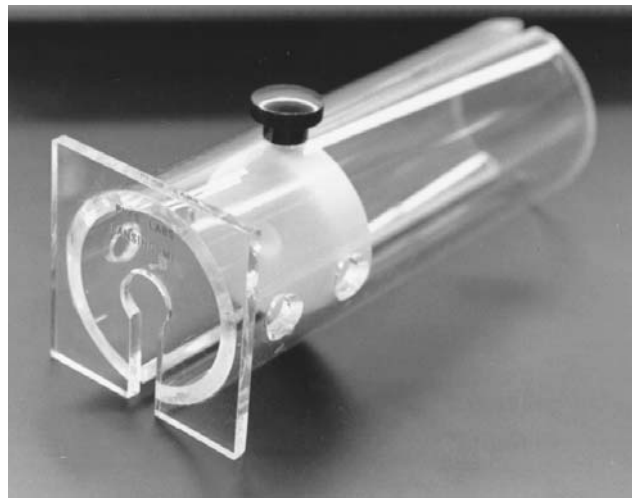


Fig. 24. Example of a rigid plastic restrainer. These adjustable devices come in various sizes to accommodate animals of different ages and sizes.

Mechanical Restraint

Several mechanical restraint devices are available commercially. They are designed for short-term handling and include various rigid plastic adjustable restrainers (Fig. 24); decapitation cones (Fig. 25); or towels (rat wrap, Fig. 26)). These devices provide good general restraint with the rigid restrainer and decapitation cone providing good visualization and access for injections.

Chemical Restraint

Chemical restraint is available and discussion is found in the anesthesia section. This may prove useful for procedures which are more than momentarily painful or needing strict immobilization.

sampling techniques

Blood Collection

Blood may be collected from many different sites in the rat. Survival blood collection sites include the tail (Fig. 27a), jugular

Fig. 25. Use of the decapicone to administer an IP injection.

(Fig. 27b), saphenous, and dorsal metatarsal veins; vena cava, and orbital plexus. Specific nonsurvival techniques would include open thoracic and abdominal puncture of vasculature, the heart, and decapitation. Serial blood samples may be collected through the above survival techniques or through the use of indwelling jugular or femoral catheters.

As a guide, the volume of blood taken during a single survival collection should be limited to 1.25 ml/100 g of body weight. If this maximum volume is taken, then a two-week interval between collections should be safe. If more frequent collections are needed, one should monitor the animal's hematocrit and serum protein levels. A recent study (Scipioni, et. al) evaluating multiple, frequent blood sampling, suggests the removal of up to 40% of the total blood volume of a rat over a 24-hour period has no untoward effects, even when repeated two weeks later.

tail vein

Drawing blood from the lateral tail vein of the rat requires no anesthesia, but will need a heat source which will dilate the vasculature. Following dilation of the vasculature, the rat

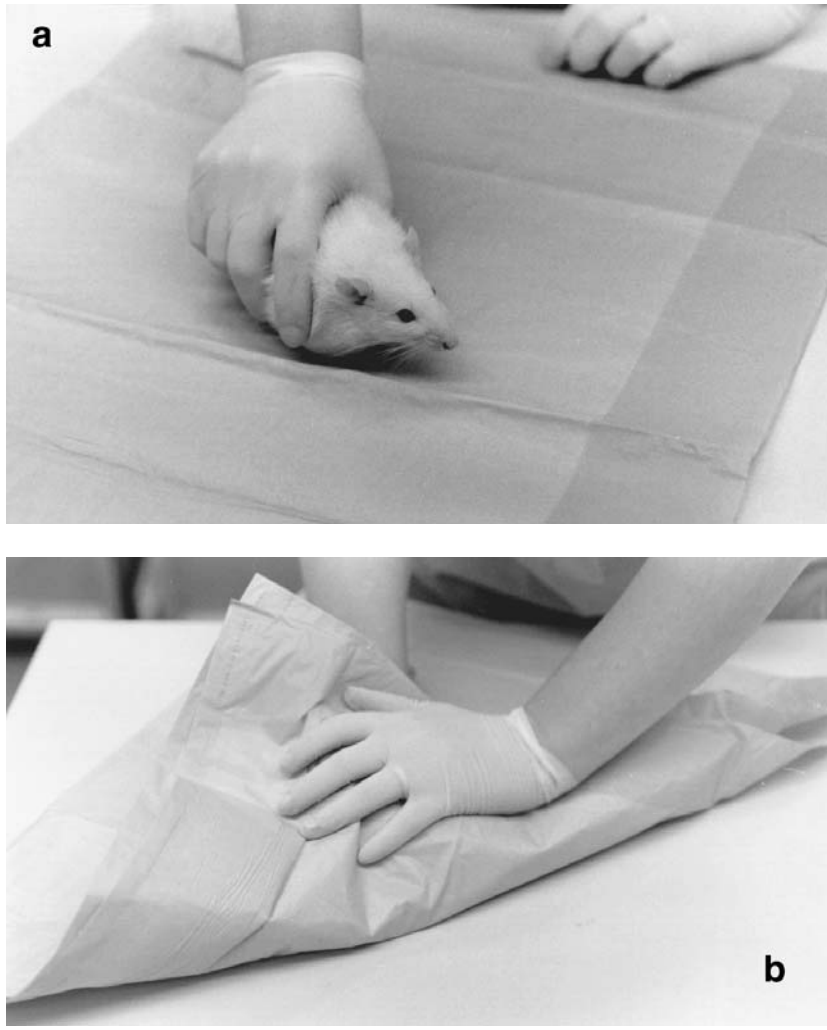
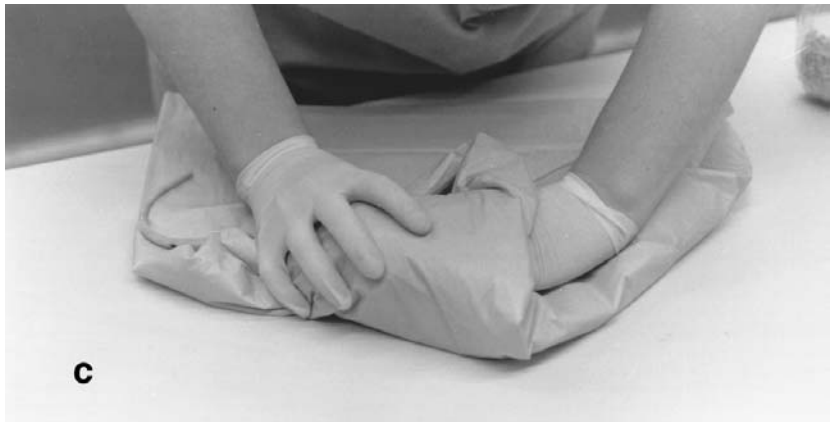


Fig. 26. The 'Rat Wrap' method of restraint. The rat is placed in the center of a towel (a). The four corners of the towel are folded on top of the rat (b,c,d) in such a manner that the back 1/4 to 1/2 of the rat is available for injections (e).

is held in a manual restraining device. An assistant can hold the tail steady during venipuncture and apply pressure to the base of the tail to further encourage dilation of the vessels. The vessel can be entered with a 21-gauge or smaller needle. If serial blood collections are desired, the first sample should be taken as distal as possible (close to the tip of the tail) and



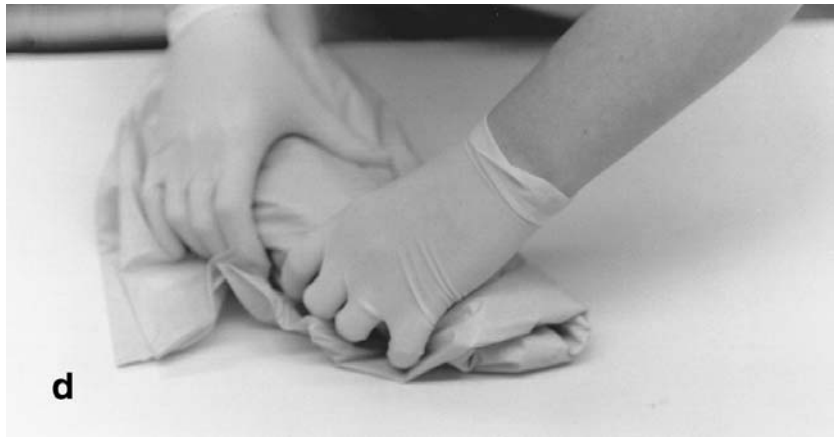


Fig. 26 (continued)

subsequent samples taken proximally. One must exercise caution to ensure the animals do not experience excessive heat exposure.

jugular vein

Blood collection from the jugular vein can be done with or without anesthesia using a 21-gauge needle. The anesthetized animal is placed in dorsal recumbancy, with the area over the jugular clipped and prepared. An incision is made over the jugular vein just as it passes the pectoral muscle. Following removal of the sample, the site is closed with suture, wound clips, or tissue adhesive. When performed without anesthesia the hair over the

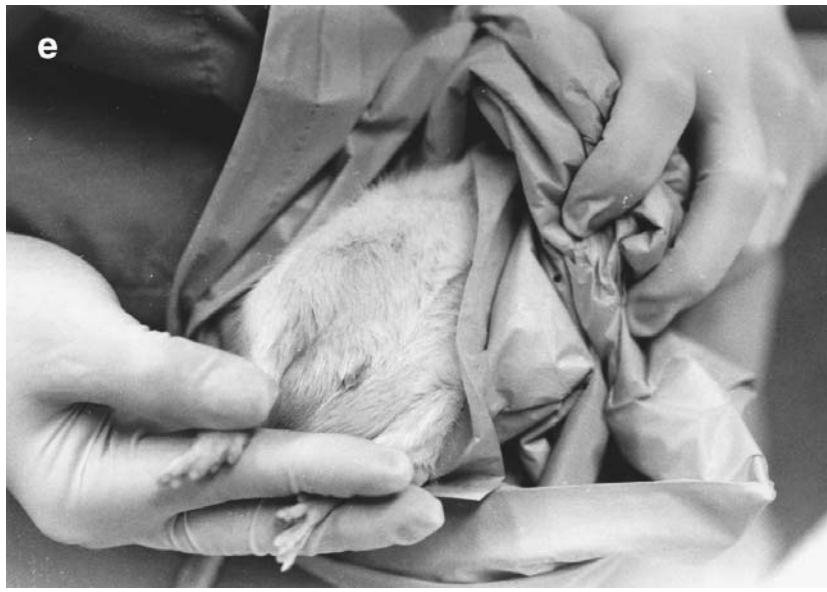


Fig. 26 (continued)

area is clipped (usually the right side), the neck is extended, and the skin is entered at an acute angle with a 21-gauge needle while gently aspirating. This technique may require practice to become proficient, initially the individual collecting the samples may choose to anesthetize the first few animals.

orbital plexus

Blood collection from the orbital plexus is quickly mastered. Properly performed orbital blood collection results in minimal trauma to the animal and its eyes. The animal should be anesthetized with the collector's non-dominant thumb and index finger used to hold the rat's head steady. With gentle caudal traction the eye will protrude and the thumb occludes the jugular vein. A capillary tube or pipette is positioned at the medial canthus (inner corner) of the eye and the ocular conjunctiva and the underlying orbital plexus entered caudomedially. Should blood fail to flow, the collector may be either not into the plexus or applying too much pressure which prevents blood from collecting in the tube or pipette. When the volume is collected, remove the tube or pipette and remove the thumb's pressure on the jugular vein. It is also sound to apply a small

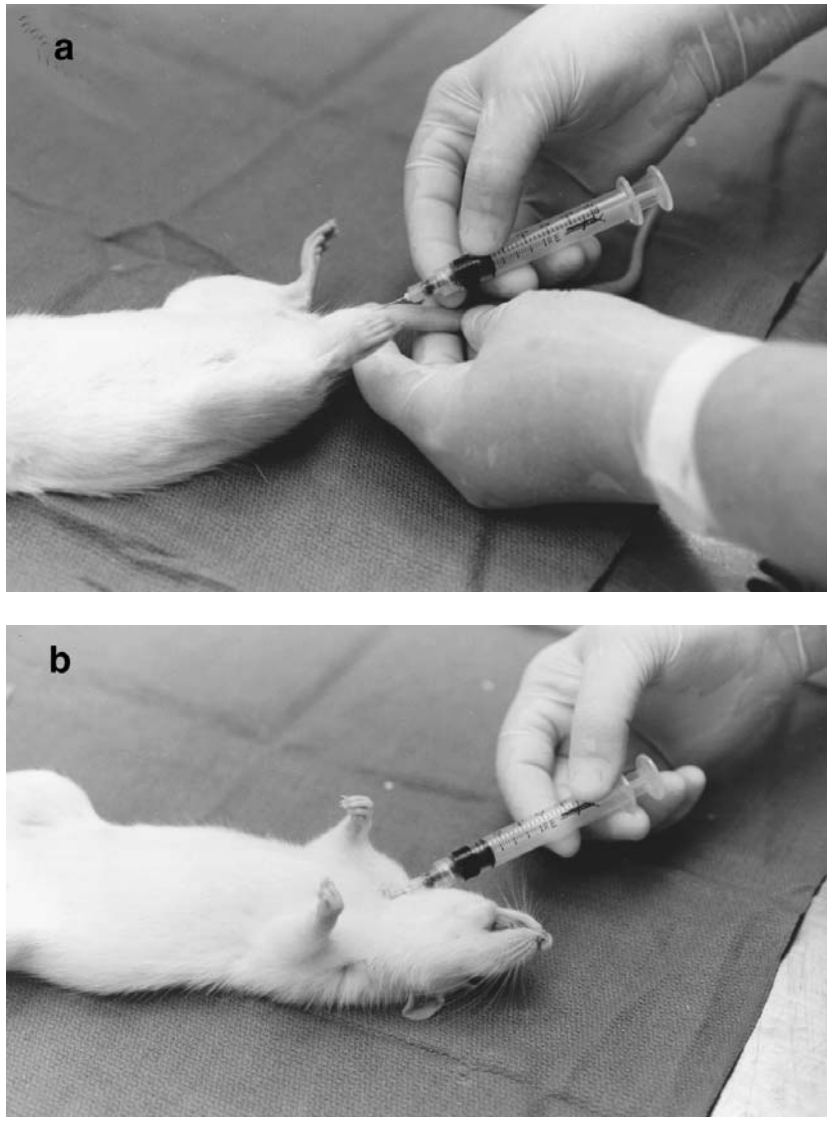


Fig. 27. Blood collection from the tail vein (a) and jugular vein (b).

quantity of ophthalmic ointment on the eye, to aid hemostasis and reduce the incidence of infection. Lesions from a single retroorbital bleeding appear to heal without appreciable scar tissue within four weeks. Rats with two retroorbital bleeds within two weeks had hemorrhagic remnants and an increase in connective tissue fibers.

Urine collection

Urine is typically collected through manual expression of the bladder or vesicular catheterization. One may easily perform vesicular catheterization in females; however, it is impossible to perform in males, due to the curves of the urethra.

Manually restraining a rat will yield a small urine sample. If this quantity is insufficient, one can apply pressure to the lower abdomen over the urinary bladder with increasing, but not excessive, force to manually express the bladder. Vesicular catheterization is possible in females with a 22-gauge, 5-cm, over-the needle catheter. The procedure should be done in a sterile manner to avoid the introduction of pathogens into the urinary bladder. The anesthetized rat is held in the non-dominant hand with the head facing the investigator, and the tail looped around the of the non-dominant index finger. The non-dominant thumb applies gentle cranial tension on the abdomen which makes the urethral opening apparent. The catheter is advanced caudally initially as the urethra rises over the pelvis, then cranially into the bladder.

Cerebrospinal Fluid (CSF)

CSF may be collected from lumbar or cisternal punctures, as a survival or nonsurvival procedure, and even through chronic lateral cerebral ventricle cannulation. Some procedures described require the use of a specially designed holding apparatus for the rat. The papers by Strake, Nakamura, Sanvitto, and DePasquale; and the text by Petty serve as good resources for the various collection techniques.

Bone Marrow

Bone marrow collection should occur under general anesthesia with a surgical preparation at the site of collection. One may use a low speed dental drill to create a small hole in either the tibia or femur large enough to insert a polyethylene tube or spatula tip. Further discussion on bone marrow collection may be found in a paper by Tavassoli.

Milk Collection

Milk collection may be necessary for studying compounds which may pass in the milk. The paper by Rodgers provides a comprehensive and concise discussion on study design and collection technique.

TABLE 21. VOLUME ADMINISTRATION GUIDELINES FOR THE RAT

Route	Guideline	Notes
SQ	5 mL/kg	Excludes Freund's adjuvant Administer volume in 2–3 sites
IM	0.1–0.2 mL/site	
IP	10 mL/kg	
IV	5 mL	Warm fluids to body temperature; administer over 2–3 min; single injection volume should not exceed 10% of the circulating volume; continuous 24 h IV infusion should be ð4 mL/kg/h
ID	0.05 mL/site	
Tracheal	40 µl	For a 200 g rat
Intranasal	100 µl	
Gastric gavage	20 mL/kg	
Subplantar	0.1 mL/foot	Usually inject only 1 foot

compound administration

One may deliver compounds via different routes; PO (orally), IV (intravenous), IM (intramuscularly), or SQ (subcutaneously); and vehicles (normal or physiologic saline, sterile water, propylene glycol, etc.). The solubility characteristics of the compound will determine the vehicle which one will use. Water soluble compounds could use sterile water, 5% dextrose, or normal saline; whereas non-water soluble compounds may use propylene glycol or DMSO (dimethylsulfoxide) as a vehicle. One may deliver water miscible vehicles by any route, whereas those vehicles which are oils should not be administered IV. Certain vehicles, such as 5% dextrose, may need reconsideration when considering their use for chronic continuous IV administration due to the potential for opportunistic infections. One should also consider the pH and injection volume of the injected material. Table 21 provides guidelines for optimal compound administration volumes.

One should observe the following guidelines for pH:

- The pH should be 4.5–8.0.

- The greatest pH tolerance is seen with IV administration (due to blood's buffering capacity), followed by IM, and last SQ administration.
- Solutions less acidic than 0.1 N HCl are well tolerated by oral administration.
- Alkaline solutions are not well tolerated by the stomach.

When administering injectable compounds, one should use the smallest gauge needle possible and the most accurate and appropriate syringe for delivering the necessary volume. The needle gauge will depend on the thickness or viscosity of the sample. Syringes are available in various sizes, usually 1–60 mL, with smaller volumes being administered by a Hamilton syringe. This syringe permits accurate delivery of very small volumes (1–50 µl). One should keep in mind 100 µl = 0.1 mL, which makes the volume of the 1 mL syringe 1000 µl. Depending on the nature of the solution, the syringe may need to be of plastic or glass construction. For most applications plastic syringes are satisfactory. One may use a 21–25 gauge needle to administer injectable compounds depending on the solutions viscosity. Once the needle is inserted, one should aspirate prior to injection. Failure to aspirate undesirable material (urine or gastrointestinal contents) indicates the needle is in the proper location before injection.

Subcutaneous (SQ, SC)

While tenting the skin, one inserts a needle between the skin and underlying tissues. If nothing is aspirated into the syringe, the injection is then made under the skin over the back and sides of the rat (Fig. 28). Most individuals can incorporate this administration technique into their restraint.

Intraperitoneal (IP)

Injection into the peritoneum should be made into the animal's lower right quadrant to avoid vital organs. One may facilitate proper administration by orienting the animal in a head-downward position. The technique is most easily performed with the assistance of another individual. The gauge of the needle

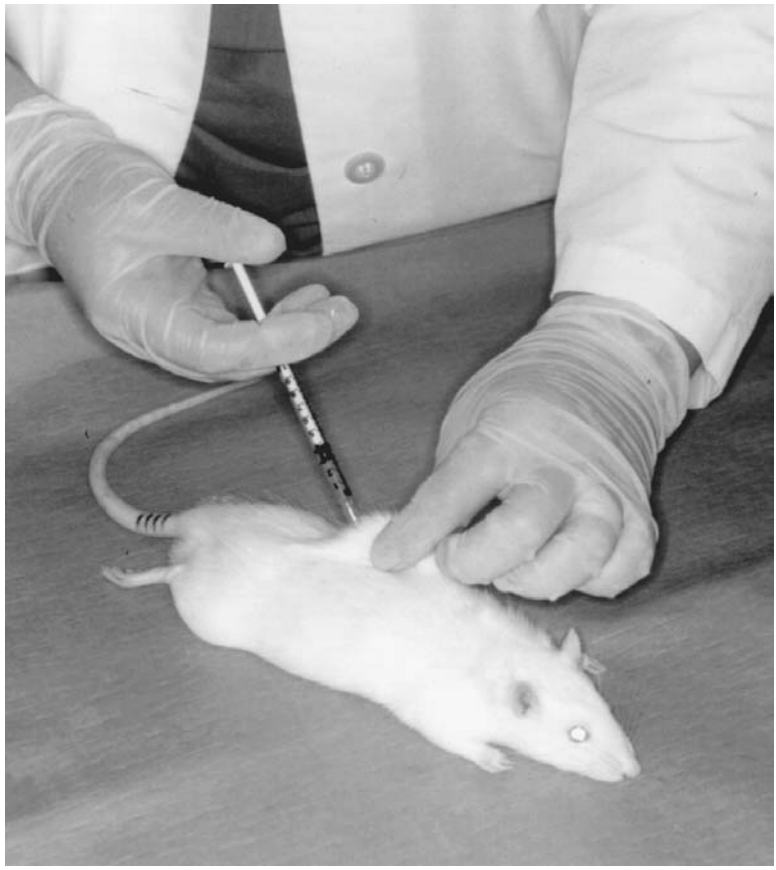


Fig. 28. Subcutaneous administration of a compound to a rat.

should be chosen with the viscosity of the compound in mind. The needle should enter the abdomen at a shallow angle, and once into peritoneum the investigator should gently aspirate. Should aspiration of intestinal contents occur following placement of the needle into the peritoneum, one should dispose of the needle, syringe, and solution due to contamination.

Administering compounds IP results in their absorption into the hepatic circulation prior to their distribution to other organs.

Intramuscular (IM)

The caudal hindlimb muscles are a common site for IM administration in the rat. Injection should not be made too deeply, as it is important to avoid the femoral nerve and hitting bone.

Intravenous (IV)

Various sites are available for IV administration of compounds. They include the lateral tail, dorsal metatarsal, jugular, femoral, sublingual, and penile veins. Injections into the lateral tail veins are the easiest to perform and the best tolerated by the rat. Typically a small quantity of blood will "flash" back into the hub of the needle if properly positioned in the vein. The formation of a bleb or bubble indicates the injection is extravascular. When performing any IV injection, one should start distally (farthest from the heart) and move proximally in case serial injections are given, or the animal flinches and the venipuncture is disrupted.

One may perform lateral tail-vein injections in the rat alone, with the assistance of a mechanical restraining device. To best visualize the vein, one may vasodilate it using warm water (38–40°C for 1–2 minutes) or with careful use of a heat lamp (1–2 minutes). One may use a 23 to 25-gauge needle or 24-gauge over-the-needle catheter to administer the solution. The catheter should then be securely fastened to the tail. Upon removal of the needle or catheter one should apply adequate pressure to minimize hematoma formation.

One may inject into the dorsal metatarsal vein of the (hind) foot with an assistant restraining the rat in one hand and firmly extending the leg with the other. Shaving the dorsal surface of the hind foot will permit better visualization, as will having the assistant use their thumb to distend the vessel at the level of the ankle and keep the skin taught. The vessel is entered using a 25-gauge needle.

Other veins may be used for intravenous injections. The jugular and femoral veins typically require cutdowns and a 25-gauge needle for the injections, whereas the sublingual and penile veins require anesthesia and a 25- to 30-gauge needle for the injection.

Intradermal (ID)

ID injections are given into shaved areas over the thorax and abdomen (Fig. 29). One should position the needle at an acute angle to the skin which is held taught so the bevel of the 23-gauge or smaller needle enters only the skin and not the subcutaneous tissue. One may ensure proper placement upon injection when a small bleb forms.

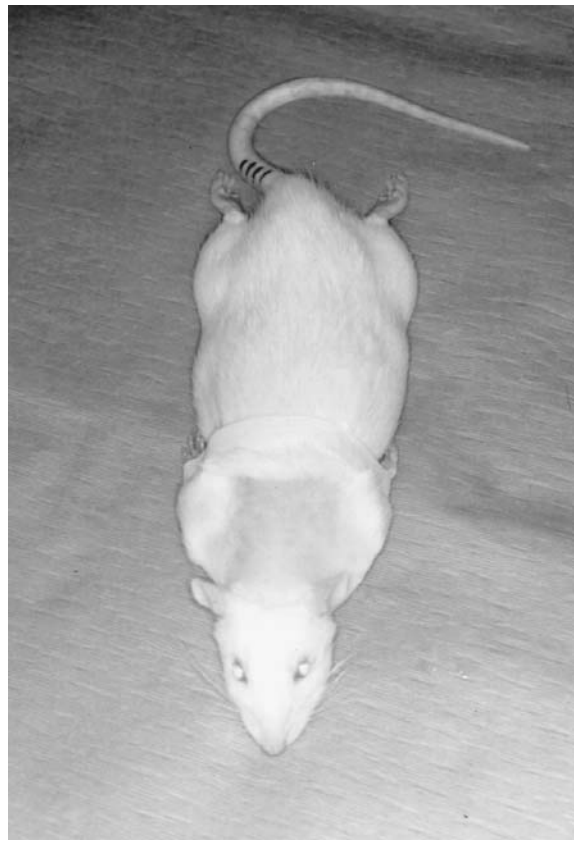


Fig. 29. Prepping the area by shaving prior to the administration of a compound intradermally.

Gastric Gavage/Per Os (PO)

Administering substances for gastric gavage involves the use of a bulb-tipped gastric gavage needle (Fig. 30). Prior to use, one should determine that the length of the needle is sufficient. The length of the needle should approximate the distance from the mouth to just beyond the last rib. Firmly restrain the animal and pass the needle through the side of the mouth. Now advance the needle towards the esophagus. The animal will swallow, yet one must ensure that the needle is in the stomach rather than the lungs. Administration of a small amount of the substance without vocalization or struggling indicates proper positioning.

Should lung administration occur, euthanasia of the animal should be considered due to the potential for pneumonia to develop. One may wish to practice the gavage technique with normal saline, which will be absorbed if a small quantity enters the lungs.

Tracheal/Intranasal

Following endotracheal intubation one may administer substances into the trachea, using the method for endotracheal intubation described in this text. One should administer no more than 40 µl per 200 g rat under anesthesia intratracheally or up to 100 µl intranasally to rat.

Subplantar

One may administer up to 100 µl subcutaneously on the plantar aspect of a hind foot. In general, only one hind foot should be injected, since feeding requires the animals to use both of their hind feet to access the overhead feeders found in most cages.

Osmotic Pumps

Osmotic pumps continuously and reliably deliver compounds at controlled rates over a given interval. These implantable pumps can deliver compounds IP, SQ, IV, intracerebrally, or intraarterially. One may use the pumps to target delivery to specific tissues or organs. These pumps are available in a wide spectrum of delivery rates and intervals. The pumps function through an osmotic gradient between the pump and the tissue where the pump is implanted.

other techniques

Electroencephalogram (EEG)

Several techniques are available to measure EEGs in rats, permitting either free movement (telemetry) or tethering (cabled system) of rats. These techniques may assist behavior or brain electrophysiology studies in control and experimental animals. A more complete description of tethered EEG techniques is given by Gohd and Petty, and of telemetry techniques by Troncoso, Fodor, and Petty.

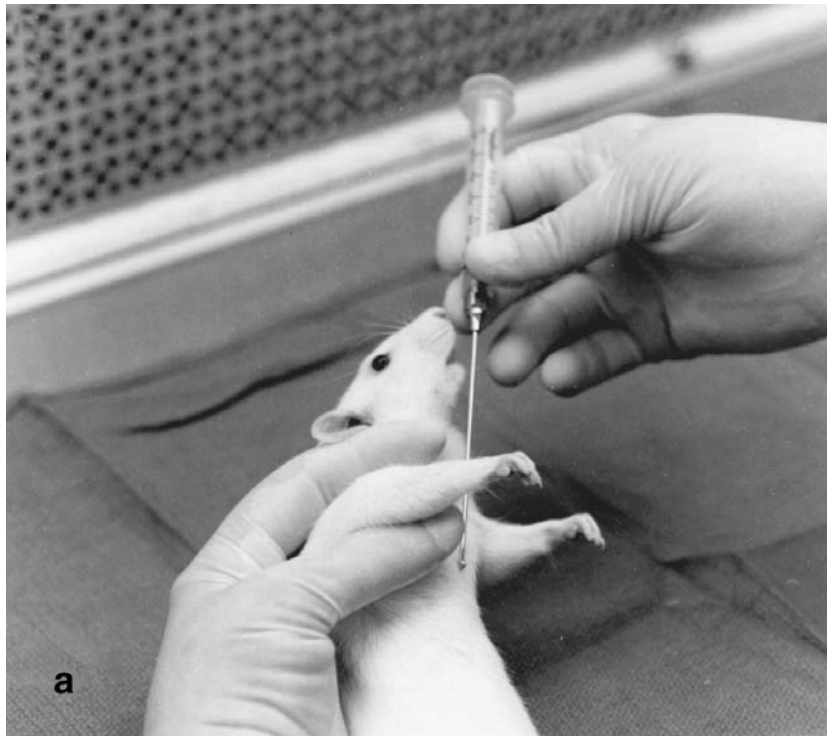


Fig. 30. Oral gavage. (a) Measure the bulb-tipped oral gavage needle prior to placement; (b) gently, but firmly advance the bulb-tipped oral gavage needle into the conscious rat from the side of the mouth. The rat will swallow the needle and once placement is in the stomach, the compound may be administered.

Electrocardiogram (ECG)

The use of ECG has been described in the rat and has value in cardiac toxicology and transplants, and other studies where information on the heart's electrical activity is vital. The use of three-dimensional ECG and cardiac gated magnetic resonance microscopy are other valuable research tools. The use of telemetry for ECG (Kuwahara, 1994; Troncoso, 1995; Fodor, 1978; Gottesmann, 1977) permits evaluation of conscious animals over a given interval. The authors direct the reader to techniques described by Lombard, Petty, and Sambhi for techniques on anesthetized animals.

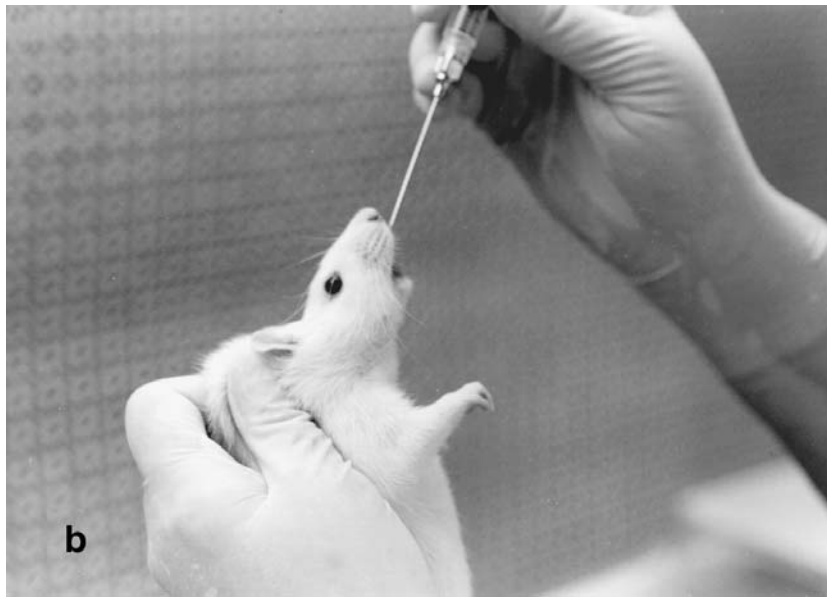


Fig. 30 (continued)

common research uses of rats

The laboratory rat has many common research uses including aging studies, oncology, toxicology, and teratology.

Aging Studies

The laboratory rat is useful for lifetime studies, whether the questions are related to toxicology, neoplasia, or aging, because of its short lifespan, moderate size, and low maintenance cost. Rodents are most appropriate for those aging studies which address the fundamental mechanisms of senescence. Specific aging changes such as lifespan, type and incidence of neoplasia, and other age-related conditions vary with the strain or stock of rat, sex, breeding status, nutrition, health status, and husbandry. All these factors must be considered in light of the experimental questions the researcher wishes to answer.

- Rodent populations used to study normal aging processes must be free of infectious disease, since the basic assumption is that infectious disease is not a component of normal aging. In addition, infectious disease affects survivability.

- Diets fed to rodents during their lifetime should be free of contaminants and nutritionally complete. It has been shown that food restriction (decreased calories) after six months of age, increases the lifespan of rats. Diet can also influence the onset of age-related pathology such as chronic glomerulonephropathy.
- Husbandry of the aging rodent colony should take into consideration prevention of infectious disease through a barrier system and reduction of environmental stresses such as noise and animal density.
- The Fischer 344 rat, the Brown Norway rat, and the F1 hybrid of these two are the three most commonly used inbred rat strains for aging studies in the United States.
- The Sprague Dawley, Wistar, and Long Evans outbred stocks are common in aging studies in the United States. Despite efforts to minimize inbreeding, suppliers often have stocks which differ genetically. Therefore, researchers using outbred stocks should be familiar with the colony history and breeding dynamics to assess the degree to which a sample genotype will be representative of the whole species.
- Whatever model is chosen, genetic quality control of the rodent population is essential. Genetic monitoring for individual loci include biochemical or immunological markers, DNA restriction length polymorphisms, and coat color markers. Skin grafting, polyvalent alloantisera, DNA fingerprinting, morphology, and breeding performance are methods which monitor several loci concurrently.

Oncology, Toxicology, and Teratology

These headings were combined because they share many of the same methods and principles. Along with mice, rats are the most common species used in studies to identify the toxic, carcinogenic, and teratologic potential of chemicals and drugs. In addition, they are frequently used to study the carcinogenic process, both tumor initiation and promotion. Rat models of various cancers also are important in the development of cancer therapy.

- As for aging studies, the health status, diet, genetics, and husbandry must be monitored to assure reliable data.
- Experimentally induced tumors are those which arise in various organs in the rat following administration of known cancer causing agents.
- Spontaneous tumors are those which arise during the natural course of a rat's lifetime. The incidence of spontaneous tumors varies with age, strain, and sex of the rat.
- The choice of model will depend on the nature of the oncology study. A model which produces a tumor in a target tissue with high relevance to human cancer may be most useful in a chemotherapy trial. On the other hand, a model with spontaneous tumors that are less common in humans may be the better choice for understanding the basic mechanisms of tumor initiation or promotion.
- The rat has long been used as a model for toxicity testing of various agents. It is widely used for evaluating the safety and efficacy of new drugs.
- The National Toxicology Program (NTP) has established detailed specifications for experimental bioassay protocols to evaluate the toxic and carcinogenic potential of chemical, biological, and physical agents.
- The rat is useful in teratologic studies because of its short reproductive cycle, large litter size, and relatively few spontaneous congenital anomalies.
- The route of administration of a potential teratogen as well as dose can influence its effect on the fetus. Potential teratogens are administered to the dam at a specific time during gestation. The feti are removed about 24 hours before birth, to prevent cannibalism by the dam. Embryos are methodically examined grossly and histologically for evidence of malformations.
- Common stocks and strains of rats used in toxicological, carcinogenic, and teratologic studies are Sprague Dawley, F344, Wistar, and Long Evans.

other rats used in research

Listed below are other rats that are sometimes used in research. These rats have characteristics which sometimes make them valuable research animals. These rodents are typically unavailable commercially, with institutional breeding colonies frequently serving as sources.

Dipodomys spp. (Kangaroo rat)

Characteristics include:

- approximately 20 species found in North America
- adapted to bipedal locomotion, with the tail used for balance
- seldom drink, but produce water from the breakdown of food
- kidneys have four times the concentrating capacity of human kidneys
- non-receptive females are very aggressive and reportedly will kill males
- kangaroo rats possess fragile tails which will break off if used to restrain the animal; for proper restraint one should firmly grasp the skin on the dorsum of the neck, and restrain the hindlimbs.

Research uses:

- renal physiology and water conservation
- whole body irradiation
- psychotropic drug effects

Millardia meltoda (Indian soft-furred rat)

Characteristics include:

- animals are slow-moving and easy to handle
- young are born with thin hair and well-grown incisors
- young attach to the mother's teats with their incisors
- X chromosome is large and metacentric

TABLE 22. REPRODUCTIVE VALUES FOR *DIPODOMYS* SPP.

Parameter	Range
Sexual maturity	2 mo
Breeding season	All year
Litters per year	3
Gestation	29–33 d
Litter size	3.5 pups
Wean	21–24 d
Cycle length	6 d

TABLE 23. NORMAL VALUES FOR *MILLARDIA MELTODA*

Parameter	Range
Adult weight (g)	84.5–100
Lifespan: usual	6.2 m
Lifespan: maximum	16 m
Chromosome number	2n = 50
Vagina opens	35–66 d
Gestation	20 d
Average litter size	4.09 pups
Eyes open	12 d
Mean blood pressure	95.1 ± 14.5 mm Hg

Research uses:

- parasitic infections
- androgen-dependent mammary tumors
- reproduction studies

Praomys coucha (multimammate rat)

Characteristics include:

- widely distributed throughout Africa
- Y, Z, and GRA-Giessen strains have been described
- gall bladder absent

TABLE 24. NORMAL VALUES FOR *PRAOMYS COUCHA*

Parameter	Range
Adult weight	40–80 g
Lifespan: usual	2–3 y
Lifespan: maximum	38 m
Food consumption	6 g/d
Chromosome number	2n = 36
Body temperature	M: 35.9, F*: 36.9–37.5
Puberty: male	55–75 d
Puberty: female	55–75 d
Breeding season	All year
Estrous cycle: range	6–8 d
Postpartum estrus	Yes
Litters per year	Several
Gestation	23 d
Litter size	5–20 pups
Birth weight	2.0–3.0 g
Eyes open	13–17 d
Wean	19–21 d

* Temperature of females depends on lactation, nonlactating females, 36.9°C; lactating females, 37.5°C

TABLE 25. HEMATOLOGIC VALUES FOR *PRAOMYS COUCHA*

Parameter	Range
WBC count (Total $\times 10^3/\mu l$)	2.8–13.0
Neutrophils (%)	8–48
Lymphocytes (%)	48–93
Monocytes (%)	0–1
Eosinophils (%)	0–9
Basophils (%)	0–1
Platelets ($\times 10^3/\mu l$)	208–754
PCV (%)	40
RBC ($\times 10^6/mm^3$)	7.5
Hemoglobin (g/dl)	13.0

- Females
  - 8–10 pair of mammary glands
  - clitoral gland is absent
  - have a well-developed prostate
  - have strong cannibalistic tendencies with the first litter

- Males
    - preputial glands absent

Husbandry:

- One should establish monogamous pairs
- Animals will bite without provocation

Research uses:

- high incidence of neoplastic and pre-neoplastic lesions
- thyroid disease
- gastric/duodenal ulcers
- degenerative joint disease
- Lassa virus studies — only nonhuman natural host

Mystromys albicaudatus (white-tailed rat, South African hamsters)

Features include:

- Found in Central and South Africa
- No cheek pouches
- Large ventral sebaceous gland
- No gall bladder
- Females
    - have a rudimentary prostate
    - have two pair of inguinal mammae
    - Newborns attach to the mammary glands for 2–3 weeks
- Males
    - have an os penis

Husbandry:

- one should establish breeding pairs at a young age and maintain as monogamous lifetime mates
- remove the male prior to parturition
- the tail is too fragile for lifting, so lift by the thorax

TABLE 26. NORMAL VALUES FOR *MYSTROMYS ALBICAUDATUS*

Parameter	Range
Adult weight: male	85 g
Adult weight: female	130 g
Lifespan: usual	2.4 y
Lifespan: maximum	6.2 y
Water consumption	5 mL/d
Chromosome number	2n = 32
Puberty: male	4–7 m
Puberty: female	4–5 m
Breeding season	all year
Estrous cycle: range	4–9 d
Postpartum estrus	Yes
Litters per year	Several
Gestation	36–39 d
Litter size	1–5 pups
Birth weight	5.0–7.8 g
Eyes open	16–20 d
Wean	25 d

TABLE 27. NORMAL VALUES FOR *OCTODON DEGU*

Parameter	Range
Adult weight	200–300 g
Litters per year	2
Gestation	90 d
Litter size	5–6 pups
WBC count (total $\times 10^3/\mu l$)	8.5 ± 0.39
Neutrophil:Lymphocyte ratio	40:60
PCV (%)	42.1 ± 0.59
RBC ($\times 10^6/mm^3$)	8.69 ± 0.19
Hemoglobin (g/dl)	12.0 ± 0.15
Total protein (g/dl)	5.7 ± 0.2

- high mortality associated with the application of polymyxin B sulfate, bacitracin, and neomycin sulfate (triple antibiotic ointment) for two weeks

Research uses:

- diabetes mellitus
- dental caries/periodontal disease

- infectious disease
- radiation research

Octodon degu (trumpet-tailed rat, degu)

Characteristics include:

- found in South America
- cheek teeth have a peculiar deep fold resembling the number 8
- cervical and thoracic thymus
- left and right anterior vena cava
- dental formula: 2(1/1 0/0 1/1 3/3) = 20
- diurnal and active throughout the year
- young are born fully furred with eyes open (precocious)
- enhanced metabolism of morphine and pentobarbital
- a stereotaxic atlas of the brain is available (Wright and Kern, 1992)
- Females
    - induced ovulators
    - 8 mammae
    - vaginal closure membrane

Husbandry:

- can climb trees, possible source of environmental enrichment
- do not handle by the tail; scoop up with the tail as the skin may slip from the tail

Research uses:

- diabetes mellitus
- reproduction
- thymus research
- ocular pathology

TABLE 28. NORMAL VALUES FOR *PSAMMOMYS OBESUS*

Parameter	Range
Adult weight	150–180 g
Lifespan: usual	3 y
Breeding season	all year
Gestation	23–25 d
Litter size	2–5 pups

Psammomys obesus (fat sand rat)

Features include:

- found in Northern Africa and the Middle East.
- prefers leaves and stems from plants of the family Chenopodiaceae as food items. These plants have a high concentration of salt and water.
- kidneys produce highly concentrated urine.
- communicate through foot thumps and high-pitched squeaks.
- feed: 5 g of commercial rodent ration with 50 g of beets and spinach per animal per day. Also provide vitamin D or a general vitamin/mineral supplement.
- water: should consist of a 1.5% (15 mg/ml) sodium chloride solution.
- best reproductive ability with 14 hours of light.
- cornified vaginal epithelium shed as a cast.
- nongrooved incisors.
- secretes more insulin than most other species.

Research uses:

- diabetes mellitus type II
- renal physiology
- reproduction

TABLE 29. NORMAL VALUES FOR *SIGMODON HISPIDUS*

Parameter	Range
Adult weight: male	70–200 g
Adult weight: female	70–200 g
Body temperature	36.5 ± 0.1°C 97.7 ± 0.2°F
Chromosome number	2n = 52
Lifespan: usual	23 m
Lifespan: maximum	3 y
Puberty: male	30–50 d
Puberty: female	30–50 d
Breeding season	All year
Estrous cycle: usual	9 d
Estrous cycle: range	4–20 d
Postpartum estrus	Yes
Litters per year	Several
Gestation	26–28 d
Litter size	2–10 pups
Birth weight	7.0 g
Eyes: open	1 d
Wean	21 d

Sigmodon hispidus (cotton rat)

Characteristics include:

- found in the southern U.S., Central and South America
- S-shaped molar cusps
- young are precocious
- permanent monogamous pairing by 6–7 weeks of age is most successful

Research uses:

- dental caries
- respiratory syncytial virus, parainfluenza type 3, and human adenovirus 5 studies

TABLE 30. HEMATOLOGIC VALUES FOR *SIGMODON HISPIDUS*

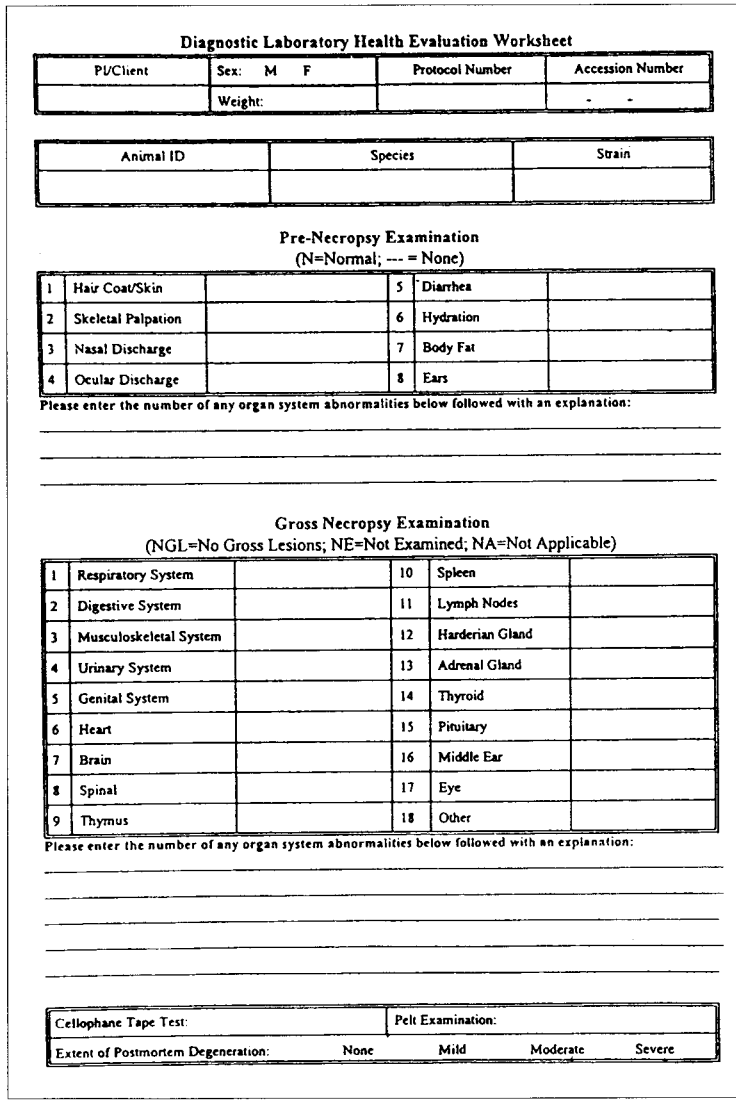
Parameter	Range
WBC count (total $\times 10^3/\mu l$)	4.3–11.9
Neutrophils ($\times 10^3/\mu l$)	2.8–9.2
Lymphocytes ($\times 10^3/\mu l$)	3.2–5.9
Monocytes ($\times 10^3/\mu l$)	0.1
Eosinophils ($\times 10^3/\mu l$)	0.3–0.5
Basophils ($\times 10^3/\mu l$)	0.1
Platelets ($\times 10^5/\mu l$)	5.4–8.0
PCV (%)	33.3–43.5
RBC ($\times 10^6/mm^3$)	3.9–6.2
Hemoglobin (g/dl)	10.5–17.1
MCV (mm^3)	62.0–71.3
MCH (pg)	19.5–27.8
MCHC (g/dl)	31.3–32.6

necropsy

In general, rats and other rodents should be necropsied soon after death. If an infectious disease is suspected, a preferred method is to humanely euthanize a rat with clinical signs. This allows the prosector to take blood for serologic assay, and assures that any bacterial pathogens are not overgrown by those bacteria associated with post-mortem degeneration (PMD). It is highly recommended to consult with the laboratory animal veterinarian before the necropsy, especially if murine pathogens are suspected. For toxicologic studies it is imperative to avoid any PMD which could compromise the interpretation of histologic results. Refrigeration of the carcass will slow PMD but will not prevent it. Freezing the carcass is generally unacceptable due to the resulting destruction of tissue architecture. Detailed desciptions of necropsy techniques are available in Feldman and Seeley's text, with the following serving as a brief outline of a rat necropsy.

➤ *Technique*

1. Proper necropsy reports or data sheets should be used to record results of the necropsy. An example of such a record is given in Fig. 31.
2. The rat is weighed and any identification is recorded.
3. A pre-necropsy exam is performed, consisting of a visual inspection of the pelt and all orifices and palpation of the limbs and torso.
4. The fur can be wetted with alcohol to make dissection easier. A quick method to view the organs *in-situ* is to take heavy scissors and cut through the skin and muscle of the lower abdomen. Extend the incision on both sides of the abdomen toward the thorax and through the ribs. This will expose all major thoracic and abdominal viscera.
5. The viscera are examined for abnormalities in size, shape, texture, and color. Special attention should be given to the teeth, salivary glands, Harderian glands, lungs, intestinal tract, and genitourinary tract as these are the most common tissues involved in spontaneous disease in the rat.
6. Samples for routine histopathologic interpretation can be immersion-fixed in 10% neutral buffered formalin at a ratio of one part tissue to 10 parts formalin. Organs such as the lungs and intestinal tract should be inflated or the lumens infiltrated with formalin to adequately fix these tissues. Choice of fixative will vary depending on the purpose of the necropsy. For example, a better choice of fixative for evaluation of the testes may be Bouin's solution. Regardless of fixative, samples should be small, less than 1 cm thick, to allow for adequate penetration of the fixative. The prosector should check with the pathologist or researcher evaluating the tissues as to the best method of preservation. Studies of the brain may require perfusion of the whole animal with formalin or other fixative.



Diagnostic Laboratory Health Evaluation Worksheet

PI/Client	Sex: M F	Protocol Number	Accession Number
	Weight:		- -

Animal ID	Species	Strain

Pre-Necropsy Examination
(N=Normal; --- = None)

1	Hair Coat/Skin		5	Diarrhea	
2	Skeletal Palpation		6	Hydration	
3	Nasal Discharge		7	Body Fat	
4	Ocular Discharge		8	Ears	

Please enter the number of any organ system abnormalities below followed with an explanation:

Gross Necropsy Examination
(NGL=No Gross Lesions; NE=Not Examined; NA=Not Applicable)

1	Respiratory System		10	Spleen	
2	Digestive System		11	Lymph Nodes	
3	Musculoskeletal System		12	Harderian Gland	
4	Urinary System		13	Adrenal Gland	
5	Genital System		14	Thyroid	
6	Heart		15	Pituitary	
7	Brain		16	Middle Ear	
8	Spinal		17	Eye	
9	Thymus		18	Other	

Please enter the number of any organ system abnormalities below followed with an explanation:

Cellophane Tape Test:				Pelt Examination:
Extent of Postmortem Degeneration:	None	Mild	Moderate	Severe

Fig. 31. This gross necropsy record helps organize the pathologic findings and indicates to the prosector which organ systems should be examined.

Tissues Taken for Histopathology

Lung		Brain		Testes		Ovaries	
Heart		Liver		Adrenal		Uterus	
Stomach		Kidney		Thyroid		G. Bladder	
Duodenum		Spleen		Lymph Nodes		Ureters	
Jejunum		Pancreas		Spinal Cord		Middle Ear	
Ileum		U. Bladder		Trachea		Nasal Passages	
Cecum		Pituitary		Salivary Gland		Harderian Gland	
Colon							

Histopathology Evaluation:

_____

_____

_____

_____

_____

Additional Comments:

_____

_____

_____

_____

_____

_____
Signature of Prosector

Fig. 31. (continued)

6

resources

organizations

A number of professional organizations exist which can serve as initial contacts for obtaining information regarding specific professional issues related to the care and use of laboratory rats. One should consider membership in these organizations, since it allows the laboratory animal professional to stay abreast of regulatory issues, improved procedures for the use of animals, management issues, and animal health issues. These organizations include:

AALAS (American Association of Laboratory Animal Science), 70 Timber Creek Drive, Cordova, TN 38018. Telephone: (901) 754-8620. AALAS serves a diverse professional group, ranging from principal investigators to animal care technicians to veterinarians. The journals, *Laboratory Animal Science* and *Contemporary Topics in Laboratory Animal Science* are both published by AALAS and serve to communicate relevant information. AALAS sponsors a program for certification of laboratory animal science professionals at three levels: assistant laboratory animal technician (ALAT), laboratory animal technician (LAT), and laboratory animal technologist (LATG). The AALAS-affiliated Institute for Laboratory Animal Management (ILAM) is a program

designed to improve state of the art training in laboratory animal facility management. In addition, the association sponsors an annual meeting. Local groups have also organized into smaller branches.

AAALAC, International (Association for the Assessment and Accreditation of Laboratory Animal Care, International), 11300 Rockville Pike, Suite 1211, Rockville, MD 20852-3035. Telephone: (301) 231-5353. AAALAC is a nonprofit organization which provides a mechanism for peer evaluation of laboratory animal care programs. AAALAC accreditation is widely accepted as strong evidence of a quality research animal care and use program.

ACLAM (American College of Laboratory Animal Medicine), can be contacted through the Executive Director, who at the time of publication is Dr. Charles McPherson, 200 Summerwinds Drive, Cary, NC 27511. ACLAM is an association of laboratory animal veterinarians founded to encourage education, training, and research in laboratory animal medicine. ACLAM is recognized as a specialty of veterinary medicine by the American Veterinary Medical Association (AVMA) and board certifies veterinarians as Diplomates in laboratory animal medicine by means of examination, experience requirements, and publication requirements. The group sponsors the annual ACLAM forum and sessions at the annual AALAS meeting.

ANZCCART (Australia and New Zealand Council for the Care of Animals in Research and Teaching), ANZCCART Australia, contact: The Executive Officer, P.O. Box 19, Glen Osmond, South Australia 5064. Telephone: +61-8-303-7393; ANZCCART New Zealand, contact: The Executive Officer, C/- The Royal Society of New Zealand, P.O. Box 598, Wellington, New Zealand. Telephone: +64-4-472-7421. ANZCCART is an independent body which provides a national focus for the use of animals in research teaching and testing. This organization acts to promote a level of understanding between the scientific and outside communities. ANZCCART acts to minimize animal discomfort, ensure worthwhile scientific outcomes, and cultivate discussion and debate over the scientific uses of animals.

ASLAP (American Society of Laboratory Animal Practitioners) is an association of veterinarians engaged in some aspect of laboratory animal medicine. The society publishes a newsletter to foster communication between members. In addition, the group sponsors annual meetings, generally in conjunction with annual meetings of AALAS and the AVMA. The contact for ASLAP changes annually with the elected president. Current contact information may be obtained from AALAS.

CALAS/ACTAL (Canadian Association for Laboratory Animal Science/L'association Canadienne pour la Technologie des Animeaux Laboratoire) is a multidisciplinary association concerning itself with animal use in research, teaching, and testing. The goals of the association are to advance the knowledge, skills, and status of those who care for and use laboratory animals; improve animal care and research standards; and provide a forum for the exchange and dissemination of knowledge regarding animal care and research. The association maintains a laboratory animal technician registry, publishes a bimonthly newsletter, and hosts a national meeting. Contact may be made through the executive secretary, who at the time of publication is Dr. Donald McKay, CW401 Biological Science Building, Bioscience Animal Service, University of Alberta, Edmonton, Alberta, Canada T6G 2E9. Telephone: (403) 492-5173.

ICLAS (International Council for Laboratory Animal Science) was organized to promote and coordinate the development of laboratory animal science throughout the world. ICLAS sponsors international meetings every fourth year, with regional meetings being held on a more frequent basis. The organization is composed of national, scientific, and union members. At the time of publication, the contact for ICLAS is Professor Osmo Hanninen, Secretary General, Department of Physiology, University of Kuopio, P.O. Box 1627, SF-70211, Kuopio, Finland.

ILAR (Institute of Laboratory Animal Resources) functions under the auspices of the National Research Council (NRC) to develop and make available scientific and technical information on laboratory animals and other biologic resources. A number of useful publications are available from ILAR, including the *Guide for the Care and Use of Laboratory Animals* and the *ILAR*

Journal. Contact ILAR at 2101 Constitution Avenue, NW, Washington, D.C. 20418. Telephone: (202) 334-2590.

JALAS (Japanese Association for Laboratory Animal Science) serves to enhance the development of laboratory animal science through publication, knowledge exchange, and supplying information on laboratory animals. The organization holds an annual meeting and publishes the journal *Experimental Animals* quarterly. Contact JALAS through The Director, T. Nomura, Central Institute for Experimental Animals, 1430 Nogowa, Miyamae, Kawasaki 216, Japan.

LAMA (Laboratory Animal Management Association) serves as a mechanism for information exchange between individuals charged with management responsibilities for laboratory animal facilities. In this regard, the association publishes the *LAMA Review* and sponsors periodic meetings. The contact for LAMA changes annually with the elected president. The current contact for LAMA may be obtained from AALAS.

publications

A number of publications are valuable as reference materials. These publications include periodicals and texts.

Periodicals

The following periodicals are excellent sources of current relevant information:

Contemporary Topics in Laboratory Animal Medicine, published by AALAS, see above.

ILAR Journal, published by ILAR, see above.

Lab Animal, published by Nature Publishing Co., 345 Park Avenue South, NY, NY 10010-1707.

Laboratory Animals, published by the Royal Society of Medicine Press, 1 Wimpole Street, London W1M 8AE, UK.

Laboratory Animal Science, published by AALAS, see above.

Textbooks

The following textbooks may be worthwhile sources of additional information:

***The Laboratory Rat,* Volumes 1 and 2**, edited by H.J. Baker, J.R. Lindsey, and S.H. Weisbroth; 1979. Academic Press, New York.

***Necropsy Guide:* Rodents and the Rabbit**, edited by D.B. Feldman and J.C. Seeley; 1988. CRC Press, Boca Raton, FL.

Laboratory Animal Medicine, J.G. Fox, B.J. Cohen, and F.M. Lowe; 1984. Academic Press, Orlando, FL.

Laboratory Animal Anaesthesia, P.A. Flecknell, 1996. Academic Press, London.

The Biology and Medicine of Rabbits and Rodents, J.E. Harkness and J.E. Wagner, 1995. Williams & Wilkins, Baltimore, MD.

Formulary for Laboratory Animals, C.T. Hawk and S.L. Leary, 1995. Iowa State University Press, Ames, Iowa.

Exotic Companion Medicine Handbook for Veterinarians, C. Johnson-Delaney, 1996. Wingers Publishing, Lake Worth, FL.

Infectious Diseases of Mice and Rats, National Research Council, 1991. National Academy Press, Washington, D.C.

The Rat Nervous System, 2nd edition, G. Paxinos, 1994. Academic Press, London.

Pathology of Laboratory Rodents and Rabbits, D.H. Percy and S.W. Barthold, 1993. Iowa State University Press, Ames, Iowa.

Research Techniques in the Rat, C. Petty, 1982. Charles C Thomas Publisher, Springfield, IL, USA.

The UFAW Handbook on the Care and Management of Laboratory Animals, 6th Edition, T.B. Poole, 1987. Churchill Livingstone, Inc., New York.

Handbook of Laboratory Animal Science, Volume 1, P. Svendsen and J. Hau, 1994. CRC Press, Inc., Boca Raton, FL.

Experimental and Surgical Technique in the Rat, H.B. Waynforth and P.A. Flecknell, 1992. Academic Press, London.

Principles of Laboratory Animal Science: A Contribution to the Humane Use and Care of Animals and to the Quality of Experimental Results, L.F.M. Zutphen, V. Baumans and A.C. Beynen, 1993. Elsevier Science Publishers, Amsterdam.

electronic resources

Many online sources of information relevant to the care and use of laboratory animals, including rats are available. These sources include websites, listserv mailing lists, and biomedical databases. The websites are divided into key websites and additional websites.

Websites

key websites

American Association for Accreditation of Laboratory Animal Care, International (AALAC)
www.aaalac.org

American Association for Laboratory Animal Science (AALAS)
www.aalas.org

American Committee on Laboratory Animal Diseases (ACLAD)
www4.ncsu.edu/unity/users/b/bweigler/Web/ACLAD/Index.html

American College of Laboratory Animal Medicine
www.aclam.org

American College of Veterinary Anesthesiologists (ACVA)
a.cvm.okstate.edu/~ACVA/

American Society of Laboratory Animal Practitioners (ASLAP)
netvet.wustl.edu/aslap.htm

Animal and Plant Health Inspection Service
www.aphis.usda.gov
www.aphis.usda.gov/vs/vshome.html
www.aphis.usda.gov/forms/index.html

Animal Welfare Information Center (AWIC)
www.nalusda.gov/awic/awic.html

Armed Forces Institute of Pathology Department of Veterinary Pathology
vetpath1.afip.mil/

Canadian Association for Laboratory Animal Science (CALAS)
www.utoronto.ca/calas/

Center for Alternatives to Animal Testing (CAAT)
infonet.welch.jhu.edu/~caat/

Gnotobiote Laboratory Investigator's Manual
www.biostat.wisc.edu/gnotolab.html

Help Searching in the Code of Federal Regulations
law.house.gov/cfrhelp.html

Institute of Laboratory Animal Resources (ILAR)
www2.nas.edu/ilarhome

International Council for Laboratory Animal Science (ICLAS)
www.uib.no/vivariet/iclaswww/iclashomepage

Lab Animal Magazine
www.labanimals.com/

National Association for Biomedical Research (NABR) (US)
www.nabr.org/

National Institutes of Health Genetic Resource
www.ncrr.nih.gov/access.nihagrex.html

NetVet/Electronic Zoo
netvet.wustl.edu
www.avma.org

Office for Protection from Research Risks
www.nih.gov:80/grants/oprr/oprr.html

United Federation for Animal Welfare (UFAW)
www.users.dircon.co.uk/~ufaw3/

USDA Animal and Plant Health Inspection Service
www.aphis.usda.gov/vs/vshome.html

additional websites

Alza Scientific Products
www.alza.com

American Veterinary Medical Association
www.avma.org/

Americans for Medical Progress (AMP)
www.ampef.org/

Association of Veterinary Anesthetists (AVA)
www.mandm.ncl.ac.uk/ava.html

Australian and New Zealand Council for the Care of Animals in Research and Teaching
www.adelaide.edu.au/ANZCCART

The Ultimate Biomedical Internet Directory
www.yahoo.com/Health/tree.html

British Laboratory Animal Veterinary Association (BLAVA)
www.mandm.ncl.ac.uk/BLAVA.html

Center for Animal Alternatives
www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm

Centers for Disease Control and Prevention (CDC)
www.cdc.gov/

Charles Lewis Davis Foundation for the Advancement of Veterinary Pathology
vetpath1.afip.mil/CLDavis/CLDavis.html

Charles River Laboratories, Inc.
www.criver.com

Chemical Industry Institute of Toxicology (CIIT)
www.ciit.org/

Cold Spring Harbor Laboratory
www.cshl.org/

Communicable Disease Surveillance Centre (UK)
www.open.gov.uk/cdsc/cdschome.htm

Covance (HRP, Inc.)
www.hrpinc.com

DNX Transgenics
www.dnxtrans.com

Environmental Protection Agency Good Automated Laboratory Practices
www.epa.gov/docs/irm_galp

European Centre for the Validation of Alternative Methods
www.ei.jrc.it/report/ecvam.html

European College of Veterinary Anaesthesia (ECVA)
www.mandm.ncl.ac.uk/MANDMWEB/AVA/ECVAhome.html

Federal Biotechnology Transfer Directory
www.bioingo.com/biotech/

Federal Register
www.clay.net/fedreg.html

Federation of American Societies for Experimental Biology (FASEB)
www.faseb.org/

Food and Drug Administration Center for Veterinary Medicine (FDA CVM)
www.cvm.fda.gov/

Food and Drug Administration (FDA) Homepage
www.fda.gov/fdahomepage.html

Finnish Laboratory Animal Scientists (FinLAS)
www.uku.fi/~kostet/FinLAS/index.html

Foundation for Biomedical Research (FBR) (US)
www.fbresearch.org/

Gesellschaft für Versuchstierkunde-Society of Laboratory Animal Science (GV-SOLAS)
www.unizh.ch/labtier/gvsolas

Harlan
www.harlan.com/

Information Technology Standards and Organizations Page
www.nlc-bnc.ca/ifla/ll/standard.htm

Institute of Laboratory Animal Science
www.unizh.ch:80/labtier/

International Committee on Taxonomy of Viruses (ICTV)
life.anu.edu.au/viruses/Ictv/index.htm
life.anu.edu.au/viruses/at-ictv.htm

International Registry of Reproductive Pathology
www.cvm.uiuc.edu/homepages/gfoley/Foley.html

The Jackson Laboratory
www.jax.org

Jackson Laboratory Bioinformatics
www.informatics.jax.org/

Japanese Association for Laboratory Animal Medicine (JALAM)
hayoto.med.osaka-u.ac.jp/index/societies-j/jalam-j.html

Kalilus Peter's Castle
www.elim.net/~kalilus

Kansas Animal Welfare Information Collection
www.vet.ksu.edu/library/kawic/habond.htm

Laboratory Animal Science Association
www.mandm.ncl.ac.uk/lasa.html

Laboratory Animal Welfare Training Exchange (LAWTE)
netvet.wustl.edu/org/lawte/homepg.htm

Laboratory Animals
netvet.wustl.edu/lahome.htm

Massachusetts Society for Medical Research (MSMR)
www.msmr.org/

MedFinder
www.netmedicine.com

Medical, Clinical, and Occupational Toxicology Resource Home Page
www.pitt.edu/~martint/

Medical Education Information Center
medic.med.uth.tmc.edu/

Medical Matrix-Guide to Internet Clinical Medicine Resources
www.kume.edu:80/mmatrix/

Medical Research Council (UK)
www.mrc.ac.uk/MRC

Medicine (Biosciences)
golgi.harvard.edu/biopages/medicine.html

Medscape
www.medscape.com/

Metris
www.xs4all.nl/~metris

Michigan Society for Medical Research (MISMR)
www.med.umich.edu/~mismr/mismr.html

Morbidity and Mortality Weekly Report
www.cdc.gov/epo/mmwr/mmwr.html
gopher://cwis.usc.edu/11/The_Health_Sciences_Campus/Periodicals/mmwr

Multimedia Medical Reference Library
www.tiac.net/users/jtward/

National Academy of Science
www.nas.edu/

National Agricultural Library (US)
www.nalusda.gov/

National Cancer Institute
www.nci.nih.gov

National Center for Infectious Diseases
www.cdc.gov/ncidod/ncid.htm

National Library of Medicine (US)
www.nlm.nih.gov/

NCRR
www.ncrr.nih.gov/

Norweigan Reference Center for Laboratory Animal Science and Alternatives
oslovet.veths.no/

NORINA Database of Alternatives in Education
oslovet.veths.no/NORINA

National Center for Infectious Diseases (US)
www.cdc.gov/ncidod/ncid.htm

National Health Information Center (US)
nhic-nt.health.org

National Institutes of Health (US) Animal Genetic Resource
www.ncrr.nih.gov/access/nihagrex.htm

National Institutes of Health (US) Library
libwww.ncrr.nih.gov/ADDSITE.HTM

National Institutes of Health (NIH) Homepage
www.nih.gov

Outbreak
ichiban.objarts.com/outbreak-unreg/index.html

Pan American Health Organization
www.paho.org

Ralston-Purina, Inc.
www.ralston.com

Regulatory Enforcement of Animal Care
www.aphis.usda.gov/reac

Research Defence Society
www.ucl.ac.uk:80/research/rds/

Scandinavian Federation for Laboratory Animal Science
www.uib.no/vivariet/SCANDLAS.html

ScienceNet
www.campus.bt.com/CampusWorld/pub/ScienceNet/first.html

Society of Study for the Science and Technology of Laboratory Animals
www.elim.nit/~kalilus/ssstla.htm

Swiss Society of Laboratory Animal Science
www.unizh.ch/labtier/sgv

Taconic
www.taconic.com/

The Ultimate Biomedical Internet Directory
www.yahoo.com/Health/tree.html

US Government Printing Office
www.gpo.gov/

Veterinary Informatics and Epidemiology (Glasgow)
www.dis.strath.ac.uk/research/vie/

Wistar Institute
www.wistar.upenn.edu/

World Congress on Alternative Home Page
www.pdk.dgk.ruu.nl/wca.ht

World Health Organization (WHO)
www.who.ch/

World Health Organization World Wide Web Server
www.who.ch/

Listserv Mailing Lists

Animal Health/Emerging Animal Diseases
majordomo@usa.healthnet.org

Biz-Biotech
grunwald@netcom.com

CompMed
ken@wudcm.wustl.edu

Embryo Mail
Embryo-L@ggpl.arsusda.gov

Occupational and Environmental Medicine
green011@mc.duke.edu

RAT-TALK (Reseach Animal Topics)
decock@rullf2.leidenuniv.nl

Toxinet
mailserv@levels.unisa.edu.au

Transgenic-list
majordomo@ic.ac.uk

Biomedical Databases

PREX Biomedical Database
Dr. Hans Kuiper
Utrecht University
PO Box 80166
NL 3508 TD Utrecht
The Netherlands
Telephone: 31302533158
Fax: 31302536747
e.mail: prex@pdk.dgk.ruu.nl
dgkp.pdk.dgk.ruu.nl/

National Library of Medicine
www.nlm.nih.gov

BioMedNet
BioMedNet.com/db/medline

vendors

The following is a partial list of suppliers of rats, feed, bedding, caging, and equipment. It is not an endorsement of any supplier, but merely an initial starting place for seeking additional information. For a more comprehensive listing, the reader is referred to the yearly buying guides published by *Lab Animal* and *Laboratory Animals*.

Rats

1. Ace Animals, P.O. Box 122, Boyertown, PA 19512, (Tel: 610-367-6047).
2. B and K Universal, 3403 Yale Way, Fremont, CA 94538, (Tel: 800-USA-MICE).
3. Buckshire, P.O. Box 155, 2025 Ridge Road, Perkasie, PA 18944, (Tel: 800-229-2825).
4. CAMM Research Laboratory Animals, 414 Black Oak Ridge Road, Wayne, NJ, (Tel: 201-694-0703).
5. Charles River Laboratories, 251 Ballardville St., Wilmington, MA 01887, (Tel: 800-LAB-RATS).
6. Genetic Models, Inc., P.O. Box 68737, Indianapolis, IN 46268-0737, (Tel: 317-824-7070).
7. Harlan Sprague Dawley, Inc., P.O. Box 29176, Indianapolis, IN 46229-0176, (Tel: 317-894-7521).

8. Hilltop Laboratory Animals, P.O. Box 183, Scottdale, PA 15683, (Tel: 800-245-6921).
9. Simonsen Laboratories, 1180-C Day Road, Gilroy, CA 95020-9308, (Tel: 408-847-4176).
10. Taconic, 273 Hover Avenue, Germantown, NY 12526, (Tel: 518-537-6208).

Feed

1. Bio-Serv, P.O. Box 450, 8th and Harrison St., Frenchtown, NJ 08825, (Tel: 800-473-2155).
2. Harlan Teklad, P.O. Box 44200, Madison, WI 53744-4220, (Tel: 800-473-2155).
3. PMI/Purina Mills, 505 North 4th St, P.O. Box 548, Richmond, IN 47375, (Tel: 800-227-8941).

Caging and Equipment

1. Allentown Caging Equipment, P.O. Box 698, Allentown, PA 08501-0698, (Tel: 800-762-2243).
2. Ancare Corp., 2475 Charles Court, P.O. Box 661, North Belmore, NY 11710, (Tel: 800-645-637?).
3. Britz-Heidbrink, P.O. Box 1179, Wheatland, WY 82201-1179, (Tel: 307-322-4040).
4. Caster Technology, 11552 Markon Dr., Garden Grove, CA 92641, (Tel: 800-627-2008).
5. Edstrom Industries, 819 Bakke Ave., Waterford, WI 53185-4299, (Tel: 800-558-5913).
6. Fenco Cage Products, 1188 Dorchester Ave., Dorchester, MA 02125-1503, (Tel: 800-233-2243).
7. Lab Products, 255 West Spring Valley Ave., P.O. Box 808, Maywood, NJ 07607, (Tel: 800-526-0469).
8. Lenderking Caging Products, Inc., 1000 South Linwood Ave., Baltimore, MD 21224, (Tel: 410-276-2237).
9. Lock Solutions, P.O. Box 1099, Laurence Harbor, NJ 08879, (Tel: 800-947-0304).

Bedding

1. Anacare Corp, 2475 Charles Court, P.O. Box 661, North Belmore, NY 11710, (Tel: 800-645-637?).
2. Andersons/Bed O' Cobs, P.O. Box 119, Maumee, OH 43537, (Tel: 800-537-3370).
3. Green Products, P.O. Box 756, Conrod, IA 50621, (Tel: 800-247-7807).

Research Animal Diagnostic Laboratories

1. Anmed/Biosafe, Inc., 7642 Standish Plaza, Rockville, MD 20855, (Tel: 301-762-0366).
2. Charles River Laboratories, 251 Ballardvale Street, Wilmington, MA 01887, (Tel: 800-LAB-RATS).
3. Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, MD 20850, (Tel: 301-738-1000).

Sanitation Supplies

1. BioSentry, Inc., 1481 Rock Mountain Blvd., Stone Mountain, GA 30083-9986, (Tel: 800-788-4246).
2. Convatec/Calgon Vestal Contamination Control, P.O. Box 147, St. Louis, MO 63166-0147, (Tel: 800-325-0966).
3. Rochester Midland, Inc., 333 Hollenbeck St., P.O. Box 1515, Rochester, NY 14603-1515, (Tel: 800-836-1627).

Veterinary and Surgical Supplies

1. Butler Co. Inc., 500 Bradenton Ave., Dublin, OH 43017, (Tel: 800-241-2888).
2. Harvard Apparatus, 22 Pleasant St., South Natick, MA 01760, (Tel: 800-272-2775).
3. IDE Interstate, Inc., 1500 New Horizons Blvd., Amityville, NY 11701, (Tel: 800-666-8100).
4. Otto Environmental, 6914 North 124th St., Milwaukee, WI 53224, (Tel: 800-484-5363 Ext. 6886).

5. Vetamac, Inc., P.O. Box 178, Rossville, IN 46065, (Tel: 800-334-1583).
6. Viking Products, Inc., P.O. Box 2142, Medford Lakes, NJ 08055, (Tel: 609-953-0138).
7. J.A. Webster, Inc., 86 Leominster Road, Sterling, MA 01564, (Tel: 800-225-7911).

bibliography

Chapter 1

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