The Laboratory MOUSE

Mark A. Suckow
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A Volume in The Laboratory Animal Pocket Reference Series

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preface

The use of laboratory animals, including mice, continues to be an important part of biomedical research. Individuals performing such research are often charged with broad responsibilities, including animal facility management, animal husbandry, regulatory compliance, and performance of technical procedures directly related to research projects. This handbook was written to provide a quick reference for investigators, technicians, and animal caretakers charged with the care or use of mice 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 mouse research programs 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). The final chapter, "Resources," provides the user with lists of possible sources and suppliers of additional information, animals, feed, sanitation supplies, cages, and research and veterinary supplies. The lists are not exhaustive and do not imply endorsement of listed vendors 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 mice are improved by initial and continuing education of personnel and will facilitate the overall success of programs using mice in research, teaching, or testing.

The authors wish to specifically acknowledge the contributions of several individuals. Dr. Muriel T. Davisson and Dorcas J. Corrow provided valuable guidance in their review of several chapters.

Colleen M. Vallee performed the manipulations illustrated in many of the photographs. For the skillfully drawn illustrations, the authors wish to thank Valerie A. Schroeder. This work has benefitted greatly from their efforts.

the authors

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Dr. Suckow earned the degree of Doctor of Veterinary Medicine from the University of Wisconsin in 1987, and completed a postdoctoral residency program in laboratory animal medicine at the University of Michigan in 1990. He is a diplomate of the American College of Laboratory Animal Medicine.

Dr. Suckow has published over 50 scientific papers and chapters in books. He was honored as the 1996 Young Investigator of the Year by the American Association for Laboratory Animal Science, and in 1998 with the Excellence in Laboratory Animal Research Award by the American Society of Laboratory Animal Practitioners and the American Veterinary Medical Association.

Peggy J. Danneman, M.S., V.M.D., received her Doctorate in Veterinary Medicine (VMD) from the University of Pennsylvania in 1982, then worked for two years in a small animal veterinary practice in Wernersville, PA. In 1984, she entered a residency program in laboratory animal medicine at the Pennsylvania State University's Hershey Medical Center. Upon receiving her Master of Science from this program, she joined the faculty at Pennsylvania State University and subsequently was certified as a diplomate by the American College of Laboratory Animal Medicine. At the present time, she is Chief of Veterinary Services at the Jackson Laboratory in Bar Harbor, ME. Dr. Danneman has maintained an active research program related to pain, anesthesia, analgesia, and humane euthanasia. She has lectured and published extensively on these topics, as well as other aspects of laboratory animal science and bioethics.

Cory Brayton, D.V.M., received her Doctorate in Veterinary Medicine from Cornell University in 1985, and is a diplomate of both the

American College of Laboratory Animal Medicine and the American College of Veterinary Pathologists. She did postdoctoral training at the Animal Medical Center, Cornell University, and the Rockefeller University in New York City. She served as Facility Director at the Hospital for Special Surgery in New York City from 1992 to 1998, and became Head of the Comparative Pathology Laboratory at Baylor College of Medicine in Houston, TX in 1998. Dr. Brayton wishes to dedicate her contributions to this volume to the mice and their investigators, to the husbandry and veterinary staffs that support humane and relevant research, and to the family and friends that tolerate her interest in great lesions and excellent research.

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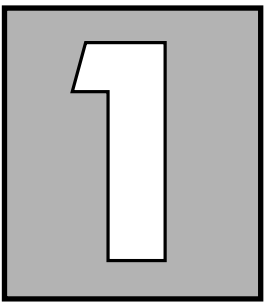


important biological features

introduction

Mice have been domesticated for centuries, even millennia, and have been used in scientific research since the 1600s. However, development of the laboratory mouse as a research model really began with genetic experiments in the early 1900s. Today, the laboratory mouse is recognized as the preeminent model for modern genetic research. Mice are also used in a variety of other types of research, including cancer, immunology, toxicology, metabolism, developmental biology, diabetes, obesity, aging, and cardiovascular research. They are prized for many qualities, including their small size, short generation time, and ease of breeding within the laboratory. The fact that they are genetically the best characterized of all mammals increases their value for all fields of study.

Mice belong to the order *Rodentia,* and most of the mice used in research belong to the genus *Mus.* The Old World house mouse, *Mus musculus*—including the substrains *M. musculus castaneus* and M. *musculus molossinus*—is most commonly used. Other mice used in research include *Mus spretus, Mus caroli, Mus pahari, Mus domesticus,* and *Peromyscus* spp.

stocks and strains

Both genetically diverse and genetically defined mice are used in research. NIH Swiss, Swiss Webster, ICR, and CD-1 are among the most commonly used genetically diverse stocks. There are over 3000 genetically defined strains, including:

- **Inbred mice**—Mice of a particular inbred strain result from a minimum of 20 consecutive generations of brother–sister matings and are virtually identical to all other mice of the same strain; C57BL/6, BALB/c, C3H, FVB, 129, DBA, and CBA are among the most commonly used inbred strains.
- **Hybrid mice**—These mice are first-generation (F1) crosses between 2 different inbred strains.
- **Recombinant inbred mice**—When F1 hybrids resulting from the same cross are mated together, the result is second-generation (F2) mice. Recombinant inbred strains result from 20 consecutive generations of brother–sister matings starting with the F2 mice.
- **Inbred mice that carry spontaneous mutations**—These are inbred mice that are perpetuated from a single mouse that was born with a significant genetic change. An example is the obese mouse (C57BL/6J-*Lepob*), which becomes massively obese because of a mutation that occurs in the gene that codes for the hormone *leptin.*
- **Coisogenic strains**—These are strains of mice that differ from each other only at one gene, the difference being due to a spontaneous mutation that occurred in that gene. After the appearance of the mutation, the animals with the mutation are maintained as a separate strain from the original inbred strain. (Note: coisogenic strains can also be created using the technology for producing "knockout" mice; see below.)
- **Mice that carry induced mutations**—Mutations may be induced by chemicals (e.g., ethylnitrosourea), irradiation, or retroviruses. This category also includes:
  - *Transgenic mice*—these mice carry foreign DNA that was intentionally inserted into their own DNA. Examples include several transgenic mouse models of amyotrophic lateral sclerosis (Lou Gehrig's disease), all of which carry inserted copies of a human gene that codes for an abnormal enzyme (e.g., B6SJL-TgN[SOD1-G93A]1Gur).

- *Knockout mice*—In these mice a normal mouse gene has been rendered nonfunctional by a complex process called **homologous recombination.** Examples include knockout mice that are severely immunodeficient because they lack a gene that is necessary for the development of B and T lymphocytes (e.g., C57BL/6J-*Rag1tm1Mom).*
- *Congenic mice*—Congenic mice are similar to coisogenic mice except that the genetic dissimilarity in otherwise identical strains is created through breeding rather than as a result of a mutation. This is accomplished by breeding animals with the desired gene(s) to animals of a selected inbred host strain, then breeding their offspring back to the same host strain, and so on for at least 10 generations.

nomenclature

Nomenclature is regulated by the International Committee on Standardized Genetic Nomenclature for Mice. Use of proper nomenclature for identifying mice is important, as the name of the mouse contains information that is essential to understanding its genetic makeup and differentiating it from the thousands of other stocks and strains of mice used in research. Mouse nomenclature is complex, and understanding and using it presents a challenge. The following guidelines represent a simplified overview of this topic. For a complete description of the rules and guidelines for nomenclature, refer to "Gene & Strain Nomenclature," under "JAX Mice" on the Jackson Laboratory website (www.jax.org). This information is also published every 4 years in the journal *Cancer Research* ("Standardized Nomenclature for Inbred Strains of Mice").

Outbred mice are referred to by a laboratory (supplier) code followed by a colon and stock designation. The stock designation consists of 2–4 capitalized letters. For example, Crl:ICR is an outbred ICR mouse from Charles River Laboratories.

Inbred mice are referred to by a combination of letters (generally capitalized) and numbers, e.g., FVB, C3H, or 129. **Substrains** are colonies of the same inbred strain that have been separated for at least 18 generations. Substrains are identified by a slash followed by a number, letters, or both, designating the institution/laboratory maintaining the colony. For example, DBA/1J and DBA/2J are two different substrains of DBA; both are maintained at the Jackson

Laboratory (*J*). **Hybrid** mice are designated using the standard strain abbreviations (e.g., D2 for DBA/1 and B6 for C57BL/6) for the two parental strains. The female parent is always indicated first. B6D2F1/J is a hybrid resulting from a mating between a female C57BL/6 mouse and a male DBA/2 mouse. *F1* indicates that the mouse is the first-generation offspring resulting from this mating. **Recombinant inbred** mice are designated by one- or two-letter strain abbreviations (e.g., D for DBA/2 and B for C57BL/6) for the progenitor strains separated by an *X*. For example, BXD is a recombinant inbred derived from C57BL/6 and DBA/2.

Inbred mice with **spontaneous mutations** are designated using the standard nomenclature for the inbred strain followed by the gene symbol for the mutated gene. C57BL/6J-*Lep*^{ob} is a C57BL/6J mouse with the *obese* (*ob*) mutation in the *leptin* gene (*Lep*).

Transgenic mice are denoted using the standard nomenclature for the inbred strain (or hybrid) followed by the three-part designation for the inserted gene, e.g., B6SJL-TgN(SOD1-G93A)1Gur.

1. The first part of this designation (*TgN*) refers to the **mode of insertion** of the foreign gene (in this case, insertion by microinjection of the foreign DNA into one of the pronuclei of a fertilized egg).
2. The second part of the designation (*SOD1-G93A*) refers to the **foreign gene** that was inserted (in this case, a mutant form of the human *SOD1* gene).
3. The third part of the designation (*1Gur*) refers to the **founder line number** (in this case, *1*) and the **registration code** assigned to the laboratory that produced the transgenic line (in this case, *Gur* for Dr. Mark Gurney).

Knockout mice are designated in the same manner as mice with spontaneous mutations, along with an indication of the method by which the mouse gene of interest was rendered nonfunctional and a designation of the laboratory that produced the knockout. C57BL/6J-*Rag1tm1Mom* is a C57BL/6J mouse with a disruption of the *Rag1* gene. This disruption was the first founder line (*1*) produced by a targeted mutation (*tm*) in the laboratory of Drs. P. Mombaerts and S. Tonegawa (*Mom*).

Congenic mice are identified by the strain name of the background or host strain (often abbreviated) followed by a period, the name of the strain (often abbreviated) that donated the desired trait, a hyphen, and the gene symbol of the transferred gene. For example,

B6.AKR-*H2k* is a C57BL/6 mouse that differs from other C57BL/6 mice in that it has the *H2k* major histocompatibility gene from an AKR mouse.

behavior

In general, the domestic mouse is not terribly aggressive and will attempt to evade rather than confront. The handler is most at risk when attempting to grasp or restrain the animal, as this may cause the animal to attempt biting. Occasionally, a mouse will bite a handler who is reaching toward the animal.

Although a social species, mice will fight with one another and may inflict serious injuries. This is particularly true with males and with some strains, such as the Balb/c. Fighting may be related to establishment of hierarchy and defense of territory, although a more common observation is focal loss of hair. **Barbering** is a focal loss of hair or whiskers with no wounds and a very sharp margin between the areas of hair loss and normal hair. It is inflicted upon subordinates by a dominant mouse whose hair coat and whiskers usually remain normal. In spite of these behaviors, it should be understood that mice are social animals and are best maintained in compatible groups.

Mice are most active in the evening and are therefore classified as **nocturnal.** In the laboratory environment, substantial activity is also noted during daytime as well. Mice commonly demonstrate burrowing and nesting behavior, and therefore abundant bedding and other material that encourages such behavior should be supplied. As an animal with a small body mass to surface area ratio, these behaviors also serve to help the mouse maintain body temperature.

anatomic and physiologic features

Important and unique anatomic and physiologic features of the mouse include:

- **Dentition**
  - The dental formula of the mouse is 2 (1/1 incisors, 0/0 canines, 0/0 premolars, and 3/3 molars).
  - The incisors are continuously erupting and will overgrow if malocclusion occurs.

- **Skeleton**
  - The normal vertebral formula is C7 T13 L6 S4 C28.
  - The mouse normally has 13 pairs of ribs. The cranial 7 pairs are "true" ribs and articulate with the sternum. In addition, there are 6 pairs of "false" ribs, the three most cranial of which connect to the caudal-most "true" rib, and the last three of which are "free" or "floating" with no attachment to other osseous structures.
- **External features**
  - Front and rear feet both have five digits each.
  - The female mouse normally has five pairs of nipples over the ventral thorax (three pairs) and abdomen (two pairs).
- **Gastrointestinal system**
  - The alimentary canal is similar to other mammals (except ruminants) and consists of the esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, and rectum.
  - The stomach is divided into cardiac (nonglandular) and pyloric (glandular) sections. The nonglandular portion is lined by squamous epithelium.
  - The mouse does not have an appendix.
- **Urogenital system**
  - The right kidney is normally anterior to the left kidney.
  - In males the inguinal canal remains open, and the testes may be retracted into the abdominal cavity. Males typically have an **os penis,** a small bone over the urethra near the tip of the penis. **Preputial glands** are paired structures that lie subcutaneously near the tip of the prepuce. Occasionally, an abscess will form in these glands and present as a small mass alongside the prepuce.
  - In females the reproductive tract includes two uterine horns that combine to form the **median corpus.** The **clitoral glands** lie subcutaneously just lateral to the opening of the urethra. As with the preputial glands, the clitoral glands will occasionally abscess.
  - Males are distinguished from females by the scrotal sac containing the testes and by a longer anogenital distance (Figure 1.1).
  - The placenta of the mouse is hemochorial.
  - The urine is normally clear, yellow, and quite concentrated (up to 4.3 osmol/kg).[1] Large amounts of protein are normally

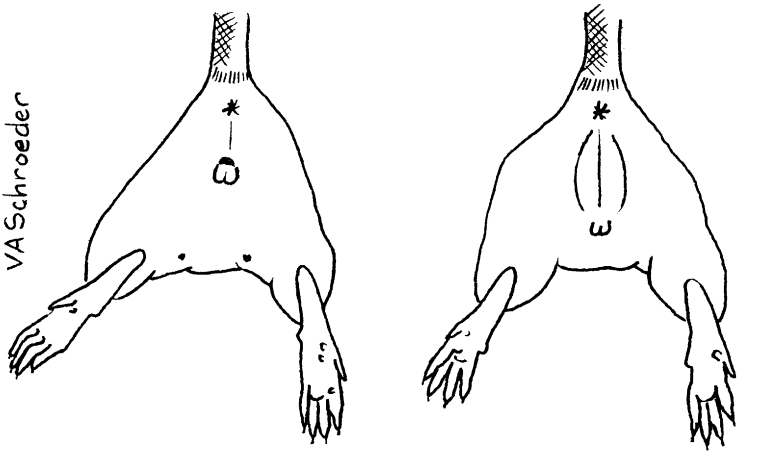


Fig. 1.1 Difference in anogenital distance between female (left) and male (right) mice. The distance between the anus and the external genitalia is shorter in females than in males.

excreted in the urine of mice, including uromucoid, alpha and beta globulins, and major urinary protein.[2] The pH of normal mouse urine is approximately 5.0.

- Maternal immunoglobulin is transferred to the pups across the placenta and across the intestinal epithelium from colostrum for 16 days after parturition.

- **Respiratory system**
  - The mouse has one lung lobe on the left side and four lobes (superior, middle, postcaval, and inferior) on the right.
- **Glands associated with the eye**
  - The **harderian** gland is horseshoe shaped and located within the orbit. It produces a secretion that lubricates the eyelids.
  - The **extraorbital** gland is located subcutaneously just ventral and anterior to the ear. It produces a secretion that lubricates the globe.
  - The **intraorbital** gland is found near the lateral canthus of the eye and produces a secretion that lubricates the globe.
- **Spleen**
  - Dark pigmentation of the spleen has been described as a non-pathogenic condition in the C57Bl mouse. Often, the pigmentation is focal. The specific pigment involved has been identified as melanin,[3,4] lipofuscin,[5] or hemosiderin.[6]

normative values

Basic Biological Parameters

Typical values for miscellaneous biological parameters (Table 1.1), clinical chemistry (Table 1.2), urine (Table 1.3), and hematology (Table 1.4) are presented below. (Note: Significant variation of values may occur between individual mice, strains and stocks, laboratories, and methods of sampling. It is imperative that individual laboratories establish normal values for their specific facility.)

Clinical Chemistry

Approximate values for clinical chemistry parameters are shown in Table 1.2. The values represent ranges in mean values reported for mice between 1 and 12 months of age. Table 1.2 represents data from mice of various strains, sexes, and laboratory and housing conditions.

Urinalysis

Evaluation of mouse urine is complicated by the small volumes that are usually available. For studies that require multiple or quantitative urinalyses, 24-h urine collections are usually obtained by using metabolic cages as described in Chapter 5 (note that in Table 1.3, some values are provided per 24 h). Increased drinking (polydipsia) and increased urination (polyuria) are typical of diabetes and some types or stages of renal disease. Sick mice, like other sick animals, may drink less than normal, resulting in decreased urine output. In addition, treatment of drinking water with chlorine, acid, or antibiotic can affect palatability of water, and may reduce water intake

TABLE 1.1: MISCELLANEOUS BIOLOGICAL PARAMETERS OF THE MOUSE

Parameter	Typical Value
Diploid chromosome number	40
Life span	2–3 years
Adult body weight	20–40 g
Body temperature	36.5–38.0°C (97.5–100.4°F)
Metabolic rate	180–505 kcal/kg/day
Food intake	12–18 g/100 g body weight/day
Water intake	15 ml/100 g body weight/day
Respiratory rate	80–230 breaths/min
Heart rate	500–600 beats/min

TABLE 1.2: CLINICAL CHEMISTRY VALUES OF THE MOUSE[a]

Analyte Evaluated	Reported Mean Values	Justification and/or Organ System
Glucose	106–278 mg/dl	Pancreas (diabetes)
Urea nitrogen (BUN)	19–34 mg/dl	Kidney
Creatinine	0.5–0.8 mg/dl[b]	Kidney
Sodium	147–167 meq/L	Electrolyte/water balance
Potassium	5–9 meq/L	Electrolyte/water balance
Chloride	104–120 meq/L	Electrolyte/water balance
Calcium	9–12 mg/dl	Thyroid/parathyroid, intestine, pancreas, kidney, bone metastasis
Phosphorus	6–13 mg/dl	Kidney
Iron	210–474 mg/dl	Iron transport and storage
Alanine aminotransferase (AST or SGPT)	26–120 IU/l	Liver
Aspartate aminotransferase (AST or SGOT)	69–191 IU/l	Liver, heart, skeletal muscle
Alkaline phosphatase (ALP)	44–118 IU/l	Liver, GI tract, kidney, bone
Lactic dehydrogenase (LDH)	26.8–34.4 mu/ml	Liver, heart, skeletal muscle, LDH-elevating virus infection
Sorbitol dehydrogenase (SDH)	27–37 IU/l	Liver
Creatinine kinase	2.5–3.7 IU/l	Heart and skeletal muscle, muscular dystrophies
Total protein	43–64 g/l	Liver function, immunoglobulin status
Albumin	20–47 g/l	Liver function
Cholesterol	63–174 mg/dl	Liver
Triglycerides	71–164 mg/dl	Cardiovascular disease
Total bilirubin	0.3–0.8 mg/dl	Heme catabolism, cholestasis

[a]Values from references 2, 7, and 8
[b]Creatinine levels > 0.7 mg/dl are generally in mice older than one year.

and urine output. Some mice chew and grind food, resulting in the disappearance of more food than is ingested. Much of this ground food, along with feces, can contaminate urine samples, resulting in

TABLE 1.3: NORMAL PARAMETERS OF URINE IN THE MOUSE[a]

Parameter	Approximate Normal Value
Color	Clear or slightly yellow
Volume	0.5–2.5 ml/24 hr
Specific gravity	1.030
pH	5.0
Glucose	0.5–3.0 mg/24 hr
Protein	0.6–2.6 mg/24 hr

[a]Values are from references 2 and 9.

abnormal urine sediment and bacterial growth that can alter protein and glucose values. Finally, small volumes of liquid are susceptible to evaporation because of their relatively large surface areas, so most 24-h collections of mouse urine are prone to evaporation artifact.

The common laboratory evaluations involved in urinalysis are tests for color, specific gravity, protein, and glucose, and evaluation of sediment. Typical values are shown in Table 1.3.

Urine sediment is the nonliquid material that remains after urine is centrifuged. This material is evaluated microscopically for the presence of cells, casts, crystals, and bacteria. With 24-h urine specimens, it is not uncommon to find contaminating plant material (from ground feed), bacteria, and pinworm eggs (in infested colonies, from fecal contamination). *Klossiella muris* is a protozoal parasite that can infect mouse kidneys and may be discerned in urine specimens, but it is very rare in modern, well-maintained mouse colonies. Gastrointestinal protozoa or their cysts and pinworm eggs may be discerned in fecal-contaminated urine specimens.

hematology

Hematology is the study of blood and usually refers to the study of its cellular components, including erythrocytes or red blood cells (RBCs), leukocytes or white blood cells (WBCs), and platelets. Blood can be analyzed with automated equipment (automated complete blood count) and by microscopic examination of stained blood smears. The blood smear is made by placing a spot (smaller than a drop) of blood near one end of the glass slide, usually near the label. It is important that the label side faces up so that the smear and the label are on the same side, thus avoiding the possibility of inadver-

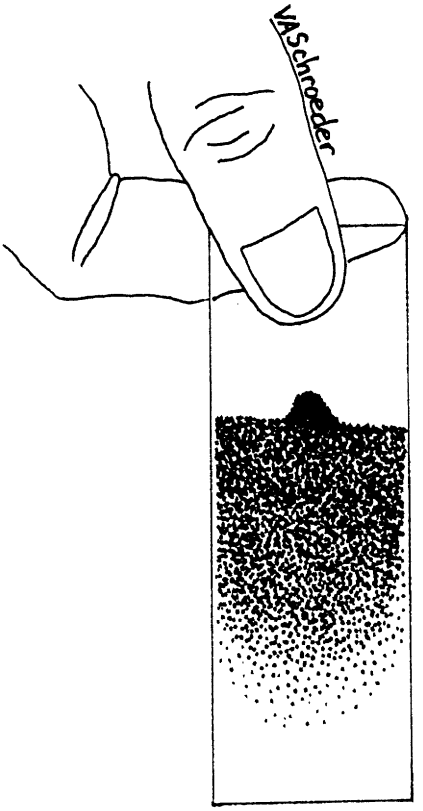


Fig. 1.2 Blood smear on a microscope slide, showing the thin "feathered" edge on the end away from the hand.

tently wiping the smear away during washing. Immediately, a second glass slide is rested upon the first slide, at approximately a 45° angle, and touched to the spot of blood. The spot of blood should be allowed to spread along the edge of the second slide, which is then slid down the first slide to spread the drop into a smear. The resultant smear should have an elliptical distal edge, known as the "feathered edge" (Figure 1.2), where the cells are distributed adequately for examination. Anticoagulant is not necessary if blood is placed on the slide directly from the animal and spread immediately. EDTA is the preferred anticoagulant for evaluating the morphology of blood cells, whereas heparin may interfere with staining or assessment of morphology.

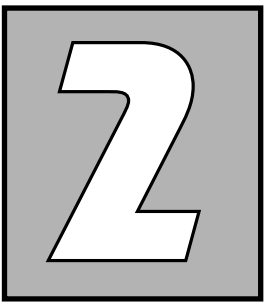
Hematology values can vary with mouse strain/stock, age, sex, blood sampling method, environmental conditions, pathogen status, and laboratory. The reference values provided below are based on

TABLE 1.4: TYPICAL HEMATOLOGIC VALUES OF THE MOUSE[a]

Parameter	Reported Mean Values	Units
Packed cell volume	38.5–45.1	%
Red blood cell number	5.0–9.5	10^6 cells/mm^3
Red blood cell diameter	5.5–6.0	μm
Hemoglobin concentration	10.9–16.3	g/dl
MCV	48.0–56.0	fl
MCH	11.9–19.0	pg
MCHC	25.9–35.1	g/dl
Platelets	1084–1992	10^3 platelets/μl
White blood cells	3.0–14.2	10^3 cells/μl
Neutrophils	0.46–2.20	10^3 cells/μl
Eosinophils	0.00–0.38	10^3 cells/μl
Basophils	0.00–0.09	10^3 cells/μl
Lymphocytes	3.22–11.20	10^3 cells/μl
Monocytes	0.40–1.43	10^3 cells/μl

[a]Values are for "adult" mice, or 1–8 month old mice (per referenced sources); sources referenced include 7, 8, 10, and 11; the table represents data obtained from male and female mice of various strains and laboratory and housing conditions. Reported ranges and standard deviations for some parameters are very large.

values from various strains, ages, sex, and laboratory conditions. They may provide a useful starting point, but individual laboratories should attempt to establish specific normal values based on these variables.



husbandry

Good husbandry is imperative for the optimal performance of mice in research, education, or testing. It involves attention to all aspects of the animals' environment, including both the **macroenvironment** (the room, cubicle, etc. in which the cage is kept) and the **microenvironment** (the cage itself). It also involves attention to sanitation, nutrition, and transportation of animals into and out of the facility. For many facilities knowledge of breeding systems is essential, as is a program of genetic quality control. Accurate identification of mice and carefully maintained records are vital for all facilities.

housing

Caging

Cages for laboratory mice should be made of a nonporous, nonopaque (for ready viewing of the mice), easily sanitizable material that is comparatively resistant to impact and can withstand frequent exposure to hot water and detergent in the cage washer. If caging will be autoclaved—recommended for high-risk mice such as severely immunodeficient animals—the material must be able to withstand autoclaving. Metal cages were commonly used to house mice in the past, but most mouse cages today are plastic, as plastics can meet all of the above criteria. Table 2.1 lists the plastics most commonly used for mouse cages, along with some of their advantages and disadvantages. For ease of cleaning, it is preferable to have cages with rounded corners that minimize packing of soiled bedding.

TABLE 2.1: PLASTICS COMMONLY USED FOR MOUSE CAGES

Material	Clarity	Impact Resistance	Chemical Resistance	Heat Resistance
Polypropylene	Variable	High	High	Medium
Polystyrene[a]	Transparent	Low	Low	Low
Polycarbonate	Transparent	Very high	Generally high[b]	Medium
High-temperature polycarbonate	Amber tint, transparent	High	Generally high[b]	High
Polyethylene	Opaque	Medium	Very high	Low
Polyetherimide	Dark amber tint, transparent	Low	High	Very high
Polysulfone[c]	Amber tint, transparent	High	High	High

[a]Inexpensive choice for disposable caging
[b]Susceptible to damage by strong alkaline agents
[c]Very expensive

Cage bottoms may be either solid or wire grid. Wire bottom cages are not provided with bedding, and they allow urine and feces to fall through onto a cage pan located below the cage. While they are advantageous for some species and may be necessary for some studies with mice (e.g., toxicity testing), they are generally not recommended for mice,[12] as they can interfere with thermoregulation and breeding performance and may cause foot problems.

There are many different types of cage tops. Typically, mouse cages are covered with a wire lid that contains a holder for feed and that may or may not have a place to put a water bottle. It is important that the bars or strands of wire be placed close enough together, or woven in a tight enough mesh, that mice cannot escape through—or become trapped in—the openings. This is a particular problem

TABLE 2.2: MOUSE CAGE SIZE RECOMMENDATIONS

Mouse weight (g)	Cage floor area (in.^{2})	Cage height (in.)
< 10	6[a]	5
15	8	5
25	12	5
> 25	> 15	5

[a]The *Guide* does not make any specific recommendations regarding the amount of space required by a nursing mother with pups, but preweanling pups are typically allotted considerably less than the 6 in.^{2} specified for mice < 10 g.

Fig. 2.1 An example of a commercial microisolator cage for housing mice.

with preweanling/weanling mice and with wild-derived mice of all ages. It is astonishing how small an opening a determined mouse can squeeze through. The wire lid may then be covered with some type of a filter top to minimize the spread of microorganisms between cages. There are many different types of filter tops, a typical example being the microisolator top (Figure 2.1). Filter tops should be sufficiently separated from the wire cover so that the mice cannot chew them. All filter tops will interfere to some extent with ventilation of the cage, thereby influencing the microenvironment within the cage.[13] For example, use of filter tops will generally lead to increases in the intracage temperature, ammonia concentration, and humidity.

Mouse cages come in many sizes, and no one size is preferable over any other. However, the cage must be large enough to comfortably accommodate the number of mice that will be housed within it. As seen in Table 2.2, The *Guide for the Care and Use of Laboratory Animals*[12] offers specific recommendations regarding the amount of space required by mice of different sizes.

Housing Systems

The following types of housing systems can be used to maintain mice:

1. **Open (no filter top) cages on static (nonventilated) racks**—This type of housing is typically used in conventional mouse colonies (see "Barriers and Containment"). It offers no protection against the cage-to-cage spread of undesirable microorganisms. This is the least labor-intensive system.
2. **Filter top cages on static racks**—This system offers protection against the spread of undesirable microorganisms between cages, but is typically associated with the poorest ventilation and offers the greatest potential for a poor microenvironment.[13–15] Optimum protection against the spread of microorganisms with this method is achieved using ventilated changing stations and appropriate microisolator technique.[16]
3. **Cages (generally filter top) on a laminar flow (or mass air displacement) rack**—This system offers protection against the spread of undesirable microorganisms, with better intracage ventilation than static microisolators.[17] Optimum protection against the spread of microorganisms is achieved using ventilated changing stations and appropriate "microisolator technique." A variation of the laminar flow rack is the mass air displacement room, which is ventilated by filtered air that flows unidirectionally in a vertical manner.
4. **Filter top cages on a rack designed to ventilate each cage individually**—This system offers good protection against the spread of undesirable microorganisms, along with the best intracage ventilation of any system (Figure 2.2).[13,18] Optimum protection against the spread of microorganisms is achieved using ventilated changing stations and appropriate microisolator technique.
5. **Cages (generally open) in an isolator**—This system offers the best protection against contamination with undesirable environmental microorganisms, with ventilation at least comparable to that achieved using open cages on static racks. All manipulations, including cage changing, should be performed within the isolator. This is the most labor-intensive system.

Bedding

Bedding may be used within solid-bottom mouse cages ("contact" bedding) or in catch pans beneath wire-bottom cages ("noncontact"

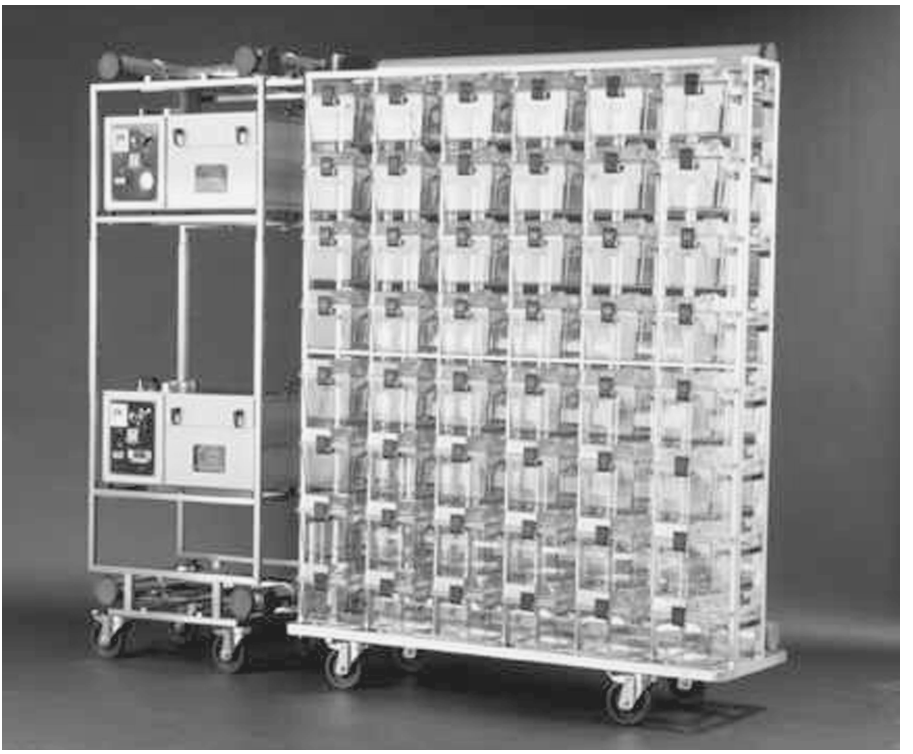


Fig. 2.2 A commercially available rack (Tecniplast USA) designed for housing mice in pressurized, individually ventilated cages.

bedding). Unless specifically contraindicated, bedded solid-bottom caging is preferred for housing mice. The bedding provides thermal insulation for the animals, absorbs moisture, enhances reproductive performance, provides environmental enrichment for the mice, and can significantly reduce odors and microenvironmental contaminants (e.g., ammonia) within cages.

Despite its advantages, bedding can also cause problems if not properly handled. Bedding should be free of splinters and excessive dust. It should also be free of microbial contaminants. Many commercially available beddings are decontaminated prior to shipping, but recontamination may occur during shipping or storage. Once received at the facility, bedding should be stored off the ground in a vermin-proof room. If possible, it is desirable to resterilize it in the facility prior to use. Once opened, it should be stored in a vermin-proof container with a tight-fitting lid. Bedding can also have inorganic contaminants that may adversely affect animal health, e.g., heavy metals.[19] Specifications regarding microbial and inorganic contaminants should be obtained from the manufacturer.

There are many types of contact bedding available for use in mouse cages, including:

1. **Wood shavings, chips, or pellets**—These are the least expensive bedding materials but are dusty and offer comparatively poor absorbency and ammonia control. Some softwoods (e.g., cedar) are aromatic, and volatilized hydrocarbons from the bedding may interfere with experimental results by affecting the activity of liver enzymes[20]—this is not a problem with hardwood beddings.
2. **Corn cob**—These products produce little dust, but offer only moderate absorbency and ammonia control. Mold is more of a problem with corn cob than with other bedding materials. Corn cob is moderately expensive compared with other bedding materials.
3. **Recycled paper, shredded or pelleted**—These products offer superior absorbency and ammonia control, but are dusty and more expensive than other types of bedding materials. The shredded products, in particular, tend to be well utilized by mice for nesting.
4. **Pads made of cotton fibers or cotton-based cellulose**—Many, but not all, mice will shred these pads to form a fluffy, highly absorbent bedding that offers excellent ammonia control. The effectiveness of the pads as a bedding material is poor if the mice do not shred them. Bedding pads are generally expensive.

There are also supplemental materials that can be used to increase absorbency (e.g., ALPHA-dri® and Omega-dri®) or improve reproductive performance by promoting nest building (e.g., Nestlets®). The ultra-high-absorbency products have been advocated as a primary bedding, but must be used with caution as they can be excessively dehydrating, especially for young pups.

barriers and containment

The environment within which a mouse is maintained may be designed to protect it from contact with undesirable microorganisms **(barrier)**, to prevent the dissemination of undesirable organisms that it may harbor **(biocontainment)**, or to contain the mouse without specific control with respect to the spread of microorganisms **(con-**

ventional). These terms imply nothing per se about the health status of the mouse, only the conditions in place to prevent the spread of contaminants. Both barriers and biocontainment facilities combine design features, environmental controls, and operating procedures necessary to achieve this goal. These elements are essentially the same for both types of facilities, but often function in reverse for barrier vs. biocontainment (e.g., shower *into* a barrier, shower *out* of biocontainment).

A barrier facility may consist of a single room, a suite of rooms, or an entire building. Barriers may also exist at the level of an isolator holding several cages. Individually ventilated racking systems and single *microisolator* cages can also function as barriers if animals are handled in ventilated changing stations or hoods using appropriate techniques. Barriers can be classified into four different levels, depending on methods used to introduce mice, humans, and supplies into the environment.[21] The highest level is the **maximum barrier** level, for which humans must shower and change into sterilized clothing prior to entry, and all supplies—including caging, food, and water—must be sterilized. Mice entering a maximum barrier facility are generally either redefined into it or are of a verified defined-flora health status. Most handling of animals is done using disinfected forceps (Figures 2.3 and 2.4). The lowest, **minimum barrier** level does not require humans to shower, and supplies may be either sterilized or sanitized prior to entry. The health status of mice entering minimum barrier facilities is often lower than for higher level barriers, but must still be both defined and verified. The mice may be handled by gloved hands. The security of any barrier is improved by opening cages and handling mice only within ventilated workstations, and by requiring personnel to wear disposable gloves that are changed after handling cages of mice. Equipment should be treated with a chemical disinfectant after use on cages of mice. Additional apparel, such as laboratory coats that remain in the animal room, disposable shoe covers, face masks, and hair bonnets all serve to further increase the security of the barrier. Finally, individuals should be restricted from entering the barrier if they have had contact with other, potentially contaminated rodents earlier in the day.

environment

The environment in the room (**macroenvironment**) typically differs from the environment in the cage (**microenvironment**), which will vary

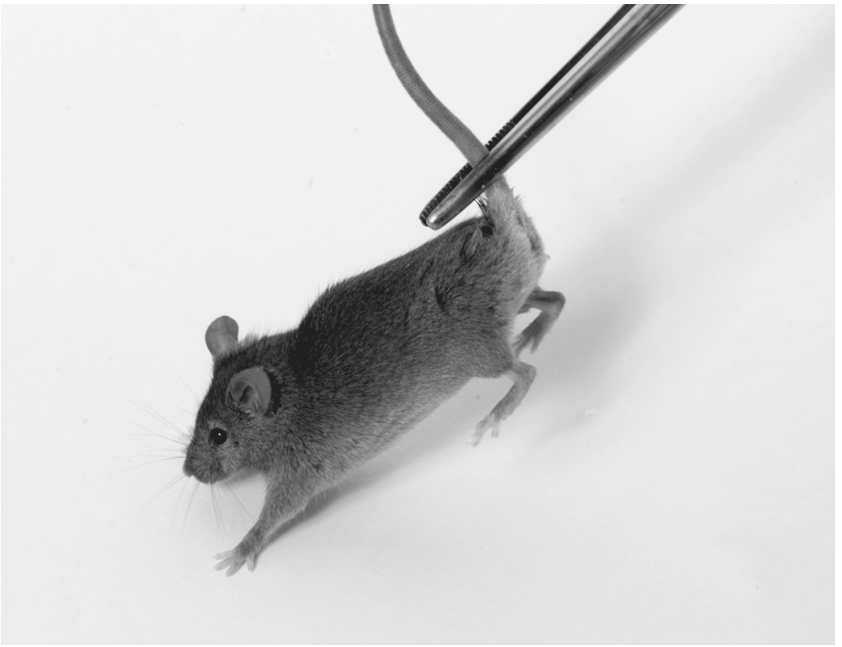


Fig. 2.3 Picking up a mouse by the tail using forceps.

Fig. 2.4 Technique for picking up a newborn mouse using forceps.

depending on the type of cage, caging system, position on the rack, number of mice, type of bedding, and frequency of cage changing, as well as macroenvironmental conditions. Although it is possible to measure the microenvironment, this is seldom done except for experimental purposes. When we talk about controlling and measuring environmental parameters, we are referring to the macroenvironment.

Temperature and Humidity

Environmental temperature and humidity work together in affecting the ability of a mouse to maintain a normal body temperature.

There is no specific environmental temperature or range of temperatures that can be stated unequivocally to promote optimal comfort, health, and performance in laboratory mice. However, considerable experience indicates that mice remain healthy and perform well if maintained within a range of 64 to 79°F.[12] It is important to minimize variability in temperature.

It is generally recommended that rooms housing mice be kept between 30% and 70% relative humidity.[12] There is anecdotal evidence that mice perform best when maintained in the range of 45 to 60% relative humidity; this appears to be particularly true of wild and wild-derived mice. If the humidity is maintained at this high level, the ideal environmental temperature is 68 to 69°F.

Ventilation

Ventilation in the room should be adequate to maintain a reasonable concentration of oxygen, minimize levels of gaseous contaminants such as carbon dioxide and ammonia, and dissipate heat generated by mice and equipment. Ventilation rates of 10 to 15 air changes per hour are recommended for rooms housing mice.[12] The air should be fresh, filtered, or both to remove contaminants. Individually ventilated caging can be used to improve ventilation in the mouse's immediate environment.[18]

Illumination

Light intensity in the room during working hours must be sufficient for humans to operate safely and inspect the mice, but not too intense, as higher levels may cause retinal damage in albino animals.[22] Light levels not exceeding 325 lux (30 foot-candles) measured

at 1 m above the floor have been recommended for rooms housing albino animals.[12]

In many species (e.g., hamster), photoperiod (the intervals of light and dark during a 24-h period) is more important than light intensity and has a critical effect on reproduction. Reproduction in domestic mice is not so dependent on photoperiod, although the light–dark cycle can affect other physiologic functions.[23–25] The two most commonly recommended photoperiods for laboratory mice are 12:12 (light to dark) and 14:10. The latter is typically chosen for breeding colonies. Shifts in photoperiod and interruptions in the light–dark cycle (e.g., turning on the lights briefly during the dark cycle) can also have disruptive effects on mice,[26] so these should be avoided.

Noise

Animal rooms are typically noisy and, if excessive, the noise can adversely affect both humans and mice. Noise levels greater than 85 dB are considered potentially damaging to both humans and animals.[12,15] To the greatest extent possible, noise levels within the mouse room should be kept well below this limit. This can be achieved with the use of soundproofing materials in room construction and by separation of mouse rooms from rooms housing noisy species (e.g., dogs or pigs) or noisy activities (e.g., cage wash). It is also important to minimize—preferably eliminate—sudden, loud noises from devices such as fire alarms and intercom systems. This is a particular problem with some mouse strains, such as DBA, which are prone to sound-induced seizures. Some facilities advocate the use of "white noise" (e.g., music played at low volume) to limit the disturbing impact on the mice of other sounds associated with work in the animal room.

sanitation and pest control

Sanitation

There are three components of an effective sanitation program: cage cleaning, room cleaning, and quality control.

Cage cleaning

The frequency of cage cleaning will depend on many factors, including the number of mice in the cage, the reproductive status of the

mice, the size of the cage, the type of bedding, and cage ventilation (e.g., microisolator cage vs. individually ventilated, air exchange rate). It may also vary with the strain of mouse (e.g., diabetic mice produce excessive amounts of urine and feces and must be changed more frequently) and the microbiological status of the mice (e.g., mice that harbor *Proteus* spp. will need to be changed more frequently than those that do not, as *Proteus* will increase the ammonia concentration in the cage).

As a general guideline, bedding should be changed with sufficient frequency to keep the mice clean and dry and to prevent excessive buildup of ammonia (>25 ppm) in the cage environment. However, this is not a case where more is better. If bedding is changed too frequently, it can interfere with breeding. Also, some mice will kill their newborn pups if the cage is disturbed too soon after parturition. Mouse cages are often changed concurrently with bedding changes, i.e., the mice are moved to a clean cage with fresh bedding. If this is not done, it is recommended that cages be changed at least once a week.[12] Under certain circumstances, including individually ventilated cages, this period may be extended to 2 weeks or more.[12,18]

The most effective sanitization of mouse cages and accessories is achieved using an automatic cage washer, although hand cleaning can be effective if done properly. Detergents, chemical disinfectants, hot water, or all of these can be used in this process. Procedures should be adequate to kill vegetative forms of common bacterial contaminants.[12] If chemicals are used, they must be completely removed during the rinsing process. Autoclaving is recommended for caging used for immunodeficient mice.

It is important not to neglect the cage rack. Portable racks should be sent through the cage washer on a regular schedule. Fixed racks that cannot be removed from the animal room should be cleaned and disinfected in place. Manifolds for automatic watering systems also need attention. In most cases they are flushed at the time of cage cleaning with either large quantities of water or a mild disinfectant followed by water.

Room cleaning

The mouse room should be cleaned daily to remove gross debris. Floors, walls, counters, etc. should also be cleaned daily with a mild disinfectant solution. Ceilings, light covers, ventilation covers, etc. can be cleaned on a less frequent, weekly or monthly schedule. Cleaning utensils such as brooms, mops, and dustpans should be

kept in designated areas and not moved from one room to another, as this can be a means of spreading contaminants. More thorough cleaning with a high-level disinfectant or sterilant should be done whenever all animals have been, or can be, removed from the room. To prevent spread of microbial contaminants, thorough cleaning should be done before a new group of mice is moved into a previously occupied room. Chemical sterilants and high-level disinfectants can be harmful, so precautions must be taken to prevent exposure of humans or other animals.

Quality control

The effectiveness of the sanitation program should be monitored on a regular basis. This can be done in part by visual and olfactory inspections for grossly visible dirt and odors. However, these inspections should be supplemented by more objective assessments. For example, the temperatures achieved within the cage washer can be monitored using a heat-sensitive tape that changes color when exposed to the high temperatures needed for disinfection. Another approach is to culture surfaces for bacteria following disinfection. Professional advice should be sought on methods of obtaining cultures, appropriate media and conditions for incubation, and interpretation of results.

Pest Control

Elimination of insects, feral rodents, and other pests from the vivarium is critical to a quality animal care program. These intruders often harbor disease and may spread contaminants throughout a facility. Poisons designed to eliminate pests pose a potential threat to laboratory mice and should be considered only as a last resort. Humane traps placed in protected locations near entryways and along walls—including feed and bedding storage areas—are useful for controlling wild mice. Baiting with peanut butter or sunflower seeds may improve their effectiveness. For both humane and sanitary reasons, traps should be checked at least once daily. Sticky traps are a less humane alternative for feral rodents, but are a desirable method for controlling crawling insects. If these approaches prove inadequate to control a vermin problem, a pest control expert should be consulted. The facility veterinarian should be involved in making any decisions regarding the use of toxic chemicals.

nutrition

Feed

Good nutrition is essential to health and optimal performance in laboratory mice, whether it be growth, reproduction, or simply maintenance within the vivarium. Nutrition is also quite complex, as nutrient requirements vary with age, strain, health status, reproduction, and use in research. It is advisable to purchase a palatable, high-quality mouse food from a reputable supplier of laboratory animal diets. For a review of dietary requirements of laboratory mice, refer to the 1995 publication of the National Research Council's Committee on Animal Nutrition.[27] For specialized needs consult a veterinarian or nutritionist.

Mice are typically fed *ad libitum* from food hoppers unless food restriction is required as part of the research protocol. The food is generally offered in the form of hard pellets. The hardness of the pellets is important. If the diet is too soft, it will tend to crumble easily and much will be wasted as the crumbs fall to the bottom of the cage. Soft food will also contribute to an increased incidence of malocclusion. If the food is too hard, the mice may not be able to chew it. Sick mice and mice of some fragile strains may find any pelleted feed too difficult to chew. These mice can be offered ground or moistened feed, which should be changed daily.

The feed should be as fresh as possible and never more than 6 months past the milling date (stamped on the bag). It must also be stored properly. If possible, the feed should be stored in a cool, dry, climate-controlled area. Warmth and humidity will hasten deterioration and spoilage. It should be kept off the ground and at least a few inches away from walls. These precautions will facilitate vermin control, which is essential if the feed is to remain uncontaminated. Once opened, any feed not used immediately should be stored in a vermin-proof container with a tight-fitting lid.

Even feed from a reliable supplier may be contaminated with undesirable microorganisms. For this reason, high-risk mice (e.g., immunodeficient) and other mice maintained in barrier facilities should be given autoclaved or irradiated feed. Autoclaving destroys some nutrients, so autoclavable feeds are specially fortified so that they will remain nutritionally complete after treatment. Irradiated feed can be purchased directly from some suppliers.

water

Mice can be provided with water from water bottles or automatic watering systems. Automatic watering systems offer the advantages of convenience and reduced labor. However, they can cause serious flooding—a particular problem for mice housed in solid-bottom cages—and they can serve as sources of microbial contamination if the water lines are not routinely sanitized. Water bottles are labor intensive but are preferable for situations where bacterial contamination is unacceptable (e.g., severely immunodeficient animals). Water for high-risk or otherwise protected colonies should be treated to minimize or eliminate contamination. This can be accomplished by a variety of techniques, including autoclaving, ultraviolet irradiation, reverse osmosis, or acidification (pH 2.5 to 3.0).

breeding

Breeding laboratory mice is seldom so simple as putting male and female together and waiting for the babies (called **pups**) to appear. The genetic background of the mice selected for breeding is almost always important, and for many purposes it is critical. For genetic experiments, the criteria for selection of breeders are quite specific and may vary from one generation to the next. Even for more general purposes, however, failure to choose breeders of the appropriate genetic background will eventually result in unwanted—albeit often subtle—changes in the characteristics of the mice. The following discussion covers some of the more basic aspects of breeding laboratory mice. More complete examinations of the topic can be found in other sources.[28–31]

Any discussion of mouse breeding requires an understanding of a few basic genetic terms:

- **Gene**—a single unit of DNA that produces a specific trait.
- **Allele**—One form of a specific gene. For most genes an individual animal carries one allele inherited from its mother and one allele inherited from its father. These alleles may be the same or they may be different. *Example:* Gene 1 may have alleles *a, b, c, d,* or *e.*
- **Homozygous**—Having the same allele from both parents for the same gene. *Example:* A specific mouse has two copies of the *b* allele for gene 1 (one from its father and one from its mother).

- **Heterozygous**—Having a different allele from each parent for the same gene. *Example:* A specific mouse has one copy of the *b* allele for gene 1 (inherited from its father) and one copy of the *c* allele (inherited from its mother).
- **Inbred**—All mice from a particular inbred strain are homozygous for virtually every gene. If mated together, two inbred mice would produce offspring that are genetically identical to each other and to their parents. *Example:* All mice from inbred strain Q carry two copies of the *a* allele for gene 1, two copies of the *e* allele for gene 2, two copies of the *b* allele for gene 3, etc.
- **Hybrid**—Hybrid mice have a mother of one inbred strain and a father of a different inbred strain. Although they are heterozygous for many different genes, the heterozygosity is limited in that there are only two alleles for each gene (one for the maternal inbred strain and one from the paternal inbred strain) within the entire population of hybrid animals. Hybrid animals are therefore genetically identical to each other, although, if mated together, two hybrids would produce offspring that differed genetically from each other and from their parents. *Example:* All hybrid mice resulting from a mating between strain Q and strain Z carry the *a* and *b* alleles for gene 1, the *e* and *d* alleles for gene 2, the *b* and *c* alleles for gene 3, etc.
- **Outbred**—Mice from a particular outbred stock are heterozygous for many genes. Outbreds differ from hybrids in that, within the entire stock, each gene has many different alleles that combine in different ways within individual animals. If mated together, two outbred mice would produce offspring that differed genetically from each other and from their parents. *Example:* One mouse from outbred stock T may carry the *e* and *b* alleles for gene 1, the *a* and *c* alleles for gene 2, the *b* and *c* alleles for gene 3, etc; another mouse from outbred stock T may carry the *c* and *d* alleles for gene 1, the *b* and *f* alleles for gene 2, the *a* and *e* alleles for gene 3, etc.

Genetic experiments aside, breeding schemes for laboratory mice are generally designed to either

1. develop or preserve a particular characteristic or group of characteristics; or
2. maintain maximum genetic variability.

Maintaining maximum genetic variability is almost always desirable when breeding outbred stocks of mice (e.g., ICR). This is

accomplished by random breeding of unrelated individuals. Even with random breeding, however, there will be a loss of genetic variability within a closed colony; this will happen sooner in a small colony versus later in a large colony. For this reason it is important to introduce outside animals of the same stock into the breeding colony on a regular basis.

With inbred mice, including inbred mice carrying spontaneous mutations, the goal is to preserve genetic uniformity in the colony. With most strains, this involves nothing more than mating males and females of the same strain. The only precaution is to avoid creation of a **substrain,** which occurs when a closed colony of inbred mice is isolated for many generations. A substrain may differ genetically from the original inbred strain and may yield different results when used in research. To avoid this, it is important to introduce outside animals from the same inbred strain on a regular basis. Some mutant mice do not make good parents because they are infertile or have poor maternal instinct. More complex breeding schemes are necessary to maintain these mice.[30]

Transgenic and knockout mice may be more difficult to breed than inbred mice. Among the many problems that may be encountered with these animals is a failure of homozygotes to survive to breeding age. If homozygotes are needed for a study, it may be necessary to use an alternative scheme, such as breeding heterozygotes.[30] Such matings will result in some homozygotes (statistically, about 25% of the pups), some heterozygotes (about 50% of the pups), and some pups that do not carry the mutation at all. As all of the pups may look the same, genetic testing is often required to distinguish those that carry the mutation from those that do not.[32–36]

Hybrid mice are desirable for some experiments. Breeding hybrids requires mating a male mouse of one inbred strain with a female mouse of another inbred strain. The resulting pups are first-generation, or F1, hybrids. In some cases second-generation (F2) hybrids are desired. These are obtained by mating an F1 female with an F1 male. Except for genetic experiments, it is rarely desirable to breed hybrids past the second generation. So, to keep the hybrid colony going, it is necessary to maintain colonies of the inbred parental lines to serve as breeders in the hybrid colony.

The maximum number of pups per female mouse is obtained by leaving the female with an adult male throughout her breeding life. Usually, mice become sexually mature by 7 to 8 weeks of age. Mice are continuously polyestrous and have an estrous cycle that typically lasts 4 to 5 days. The breeding productivity of the male can be

increased using a **harem system** (two or more females per male). Although mice can reproduce well beyond one year of age, their reproductive performance will typically diminish after 8 to 10 months of age. Other breeding systems can be used to achieve different goals. For example, if the goal is to maximize the number of pups weaned per litter, this is best achieved by moving the female to a separate cage prior to parturition (e.g., when she is visibly pregnant). If the goal is to maximize the genetic contribution to the colony from a single male, this is best achieved by mating him with many females using a **rotating system,** in which the male is moved between cages of females. Both of these systems will reduce female productivity, however, as no male will necessarily be present to breed with the female during her postpartum estrus, which occurs within hours after birth of the litter. The next opportunity for her to become pregnant will be after the litter is weaned.

Neonatal mortality can be a significant issue in some breeding colonies. Although mortality of pups can be related to the phenotype of genetic mutants, it can also involve a number of other factors. In this regard, maternal care of the pups should be evaluated. Close observation of dams and litters may help to determine if the dam has no milk or is not feeding the pups. For example, one should evaluate whether or not the pups move normally and attempt to nurse. Poor mothering may be influenced by strain, environment, and the pup itself. If litters are very large, weaker pups may not be able to nurse sufficiently. Removal of some pups or cross-fostering to other dams with similarly aged litters may promote survival of weaker pups. Busy, loud environments are generally not well tolerated, and some strains and dams seem to be especially sensitive to such conditions. Mice being bred may benefit from transfer to a quiet environment, removal of the male and other cagemates, isolation of the dam immediately prior to parturition so that the cage need not be cleaned or moved until at least a week after birth, and provision of nesting material.

Reproduction in mice is greatly influenced by pheromones, secreted chemical substances that elicit specific responses in other mice. For example, the **Whitten effect** occurs when synchronization of estrus occurs approximately 72 h after a group of female mice is introduced to a male mouse or to his odor. Group-housed female mice often become anestrous, but 40 to 50% of the females may enter estrus when exposed to a male or his odor. In contrast, the **Bruce effect** occurs when a pregnant female aborts after being exposed to a male or his odor within four days of breeding.

TABLE 2.3: TYPICAL REPRODUCTIVE PARAMETERS OF THE MOUSE

Parameter	Typical Value
Age of sexual maturity	7 to 8 weeks
Estrous cycle length	4 to 5 days
Gestation length	19 to 21 days
Litter size	10 to 12 pups
Birth weight	1 g
Age at weaning	21 to 28 days
Pseudopregnancy	10 to 13 days

Under some conditions female mice become **pseudopregnant.** The induction of pseudopregnancy in group-housed female mice is known as the **Lee-Boot effect.** Pseudopregnancy can also result from coitus or from stimulation of the vagina and cervix by swabbing for vaginal cytology. Pseudopregnant mice develop abdominal distension and exhibit behavior similar to a pregnant mouse; however, these changes begin to diminish after 10 to 13 days with pseudopregnancy.

Although reproductive parameters will vary with the strain/stock and age of the mouse and environmental conditions, typical values are presented in Table 2.3.

Timed Pregnancy

For some experimental procedures, it is necessary to know the date of conception of a litter (e.g., when prenatal mice at a certain day of gestation are needed). This is done by placing the female with a male, then checking her early the following morning for the presence of a **vaginal plug.** This is a whitish mass composed of coagulated secretions from the coagulating and vesicular glands of the male mouse, and it typically fills the vagina of the female for 8 to 24 h following breeding (which generally occurs at night). To visualize the plug, one should lift the female by the base of her tail and, if necessary, spread the lips of the vulva slightly with a cotton-tipped swab. If there is no plug, the female is left with the male and checked each morning until a plug is found. While the presence of a plug is no guarantee of pregnancy, it indicates that sexual activity occurred and pregnancy is likely. The first day of gestation is considered to be the day *after* the plug is found.

The Whitten effect can be exploited to synchronize estrus when larger numbers of timed pregnant mice are needed. When the females are placed with males (typically 1 to 3 females per male), the

estrous cycle will restart, and most will enter estrus within three days. Daily examination of the females for vaginal plugs will identify those that have mated and are likely to be pregnant.

Genetic Monitoring

Genetically defined mice are valued in research precisely because they are genetically defined. When a researcher selects a particular strain for an experiment, he/she counts on the fact that mice of this strain will reliably exhibit certain characteristics and that they will respond to experimental manipulations in the same manner as other mice of the same strain used in other laboratories. It is therefore essential that the investigator knows with certainty that his/her C57BL/6 mice are indeed C57BL/6 and not some other strain of black mice. Furthermore, one must be confident that a black mouse of another strain has not escaped and mated with one of his/her C57BL/6 mice.

In many cases it is easy to detect an "impostor." For example, no one will mistake a BALB/c mouse for a C57BL/6 mouse, because the BALB/c is white and the C57BL/6 is black. Similarly, it will be easy to tell if a BALB/c has gotten out and mated with a C57BL/6. The offspring will be **agouti** (where the shaft of the hair is one color—usually yellow or red—and the tip is black, giving the animal a golden color), not black. However, a black C57BL/10 mouse cannot be distinguished visually from a C57BL/6 mouse, and an unplanned mating between these two strains will produce pups that are indistinguishable from either parent. For this reason it is best, whenever possible, to keep mice of the same coat color in separate rooms, cubicles, etc. It is also essential to identify individual mice and keep accurate records, including properly labeled cage cards with **correct strain nomenclature.** If a mouse escapes, it should not be returned to the colony unless its identity can be determined with **absolute certainty.**

In addition to taking appropriate precautions to guard against inadvertent mix-ups between mice of similar-appearing strains, it may be necessary to test mice for genetic purity. If there is any suspicion that a genetic contamination may have occurred (e.g., that a C57BL/10 mouse may have gotten into the C57BL/6 colony), genetic testing should be performed. Periodic genetic testing is also advisable whenever breeding colonies of mice of the same coat color are maintained in the same room, particularly if the colonies are handled by different research or animal care personnel. Similarly, since mutations do occur and can lead to significant alterations in the genetic

makeup of a colony, genetic testing should be performed whenever closed breeding colonies are maintained over a long period of time.

Methods of genetic testing include electrophoretic testing for biochemical markers, serologic testing for immunological markers, skin grafting, mixed lymphocyte reaction testing, karyotype analyses, osteometric trait analysis, DNA typing, and screening for coat color alleles.[37–39] In addition, it is wise to remove from a breeding colony any mouse that is born with an obvious mutation (e.g., white spots on a mouse of a solid black strain). Technicians should be trained to recognize the normal appearance and behavior of strains with which they work.

identification and record keeping

Identification

Proper identification of mice is imperative for effective management of breeding colonies as well as for most research studies. All mice should be identified by a cage card and, if appropriate, group-housed mice should also be individually identified.

Cage cards are typically placed on individual cages of mice, although, under certain circumstances, it may be acceptable to have a single card for an entire section or room of mice. Cage cards of different colors can be used to facilitate distinction between mice of different strains, mice belonging to different investigators, or mice being used with different research protocols. The following information should be noted on cage cards:

1. **Complete and correct nomenclature.** Use of abbreviated nomenclature can be tempting but will almost inevitably lead to confusion and potentially serious mix-ups.
2. **Source of animals and date of arrival in the facility.**
3. **Sex.** If the cage contains more than one animal, note the number of animals of each sex.
4. **Name of the responsible investigator** and, if appropriate, the **protocol number** of the research project for which they will be used.
5. In breeding colonies, the cage card may indicate **pedigree number and reproductive performance history** (e.g., date mated, birth and weaning dates of litters, or the number of pups born and weaned).

Methods for permanent identification of individual mice include:

1. **Ear tags.** Tags designed for use on mice are commercially available. A disadvantage is that ear tags can be lost, especially in a cage where mice are fighting.
2. **Ear punch.** This technique is effective only if a numbering system is employed and personnel are trained to use it.
3. **Tattoo.** Devices for tattooing mice, usually on the tail, are commercially available.
4. **Subcutaneous transponder.** Anesthesia of the mouse prior to insertion of the transponder is recommended. A device designed to detect the transponder must be used as part of this system.
5. **Toe clip.** This method can be used to identify very young (, 7 days) pups when no other method is suitable.

Records

Records are kept within an animal facility for a variety of purposes, including documentation of health monitoring and veterinary care, tracking and census, and colony management.

Individual **health records** are generally not maintained for mice. However, it is important to keep "herd" records documenting health problems within a colony, and records delineating treatments of individual mice under veterinary care. Accurate **census records** are essential for determining per diem charges and can prove invaluable for managing and assigning space within a facility. Census records for mice are typically determined by counting cages rather than individual mice. **Colony management records** are necessary for breeding colonies but may not be needed for research colonies in which there is no breeding. The following types of records are recommended for breeding colonies:

1. **Pedigree charts**—A pedigree chart is essentially a register delineating the ancestors, progeny, and other relatives of a particular mouse. It may be kept as a hand-written ledger—traditionally in a bound book—or by computer. Today, there are computer software packages available for this specific purpose. Pedigree charts are necessary to maintain maximum genetic diversity within outbred stocks and to facilitate elimination of undesirable traits (i.e., spontaneous mutations) within inbred strains. Within large breeding colonies, a small foundation colony may be maintained as the source for a larger research or production colony.

A **foundation colony** is a small, isolated colony that is used to produce breeding stock for the larger colony, whereas a **production colony** is one that is maintained for the sole purpose of producing as many mice as possible within a given space. In such cases, pedigree records are maintained for the small foundation colony, but may not be maintained for the larger research/production colony.

2. **Breeding records**—A record should be kept of the reproductive performance of all breeding animals. This is often accomplished using a cage card designed for the purpose. The reproductive record should indicate the date of birth of each litter, the number of pups of each sex born, and the number of pups of each sex weaned. Combined with a good pedigree system, this information can be used to select the most productive lines and eliminate poorly productive lines, thereby increasing the overall productivity of the colony.

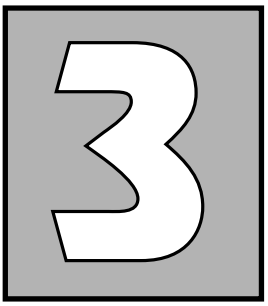
transportation

With the increasing popularity of transgenic mice and the increasing number of institutions that are creating transgenics, it is becoming more common for animal facilities to send mice to other institutions. Transportation is inevitably stressful to the animals, but stress can be minimized and the safety of the animals improved by attention to the following issues:

1. **The shipping container**—The shipping container must be durable enough to protect the mice during transit and to prevent them from chewing their way out. It must be free of sharp edges or other features that may trap or injure the animals. It should be resistant to moisture and it must be adequately ventilated. Ventilation openings must be covered with a chew-resistant screen and should be covered with a filter material to protect the mice from pathogenic organisms present in the environment. The box must be of sufficient size to accommodate all mice in the shipment without undue crowding. Keep in mind, however, that huddling with other mice can greatly reduce the risk of hypothermia during shipment in cold weather. Containers for mice that will be shipped by air should conform to the regulations of the International Air Transport Association (www.iata.org). Shipping con-

tainers suitable for shipment of mice can be purchased from many commercial mouse vendors.

2. **Feed, water, and bedding**—The shipping container should contain sufficient bedding to absorb urine and to provide insulation from the cold. A source of moisture should be provided, and food should be provided if the mice will be in transit for more than a few hours. Commercially available gel packs can be used as a water source. Alternatives such as moistened chow "meatballs" or sliced potatoes can provide both moisture and calories.
3. **Health records**—Most facilities receiving mice will want a record of the health status of the animals, and such a record is required for most international shipments. Many countries outside the U.S. also require an official U.S. Department of Agriculture (USDA) health certificate that may or may not have to be countersigned by a veterinarian employed by the USDA. These records should be attached to the outside of the box in such a manner that they are easily accessible during shipment.
4. **Requirements during shipment**—To the greatest extent possible, mice should be kept in a dry, well-ventilated, climate-controlled environment during shipping. Prolonged exposure to extremes of temperature must be avoided. Carrier(s) should be selected and shipping times arranged with these requirements in mind. Coordination between the shipping and receiving institutions will also help to ensure that any problems that might occur during shipping are quickly detected and rectified.



management

regulatory agencies and compliance

Specific regulatory agencies and requirements may vary with locale. At the time of publication, it is possible for research or teaching using mice to have no regulatory oversight. If the institution receives no funds from the Public Health Service (PHS) for research, the use and care of any mice used would be exempt from the *Public Health Service Policy on Humane Care and Use of Laboratory Animals*[40] and the standards of care described in the *Guide for the Care and Use of Laboratory Animals* (the *Guide*).[12] If the research or testing being conducted will not be used to support the approval process for drugs or medical devices intended for human or animal use, use of the mice would be exempt from the policies described in the "Good Laboratory Practices for Nonclinical Studies." Likewise, if the facility is not accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC International), it would be exempt from the standards described in the *Guide.*

Currently, mice are exempt from the standards for animal care and use proscribed by the USDA as described in the *Regulations of the Animal Welfare Act.*[41] Readers are cautioned, however, that these regulations are not static and may likely change to include mice in the future. Oversight responsibility is described in the **Animal Welfare Act** (P.L. 91–579, 94–279, 99–198). Registration with USDA and adherence to USDA regulations are required by all institutions, except elementary and secondary schools, using regulated species in teaching, testing, or research in the U.S.

Many institutions use mice in activities that do fall under the purview of one or more of the oversight authorities described above. In this regard, the following summary is pertinent:

- **National Institutes of Health, PHS**—Oversight responsibility is described in the **Health Research Extension Act of 1985** (P.L. 99–158). The policy is described in the **Public Health Service Policy on Humane Care and Use of Laboratory Animals.** Adherence to the PHS Policy is required of those institutions conducting research using funds from PHS. Principles for implementation of PHS policy are those described in the *Guide for the Care and Use of Laboratory Animals.*

- **U.S. Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA)**—Policies are described in the **Good Laboratory Practices for Nonclinical Laboratory Studies** (CFR 21 [Food and Drugs], Part 58, Subparts A through K; CFR Title 40 [Protection of Environment], Part 792, Subparts A through L). In general, standard operating procedures must be outlined and rigorously followed and supported with detailed records. Adherence is required when using animals in studies used to request research or marketing permits as part of the approval process for drugs, medical devices, or pesticides.

- **AAALAC International**—A nonprofit organization designed to provide peer review–based accreditation to animal research programs. The basis for accreditation is adherence to principles described in the *Guide for the Care and Use of Laboratory Animals.* Accreditation is voluntary.

In addition to the above regulatory bodies, state and local regulations may exist.

institutional animal care and use committee

The basic unit of an effective animal care and use program is the Institutional Animal Care and Use Committee (IACUC). The USDA, PHS, and AAALAC International require an IACUC at all research institutions which fall under their purview. Important points regarding the composition of the IACUC include:

1. **Number of members.** PHS policy requires a minimum of five members. In contrast, USDA regulations require a minimum of three members.
2. **Qualifications of members.** The IACUC should include the following:
   - 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 fulfilled by clergypersons, lawyers, or local humane society or animal shelter officials.
   - A practicing scientist with experience in animal research (per the PHS policy)
   - One member whose primary concerns are in a nonscientific area (per the PHS policy). This individual may be an employee of the institution served by the IACUC.
   - It is acceptable for a single individual to fulfill more than one of the above categories.

Responsibilities of the IACUC

The written regulations should be consulted for an in-depth description of IACUC responsibilities. In general, the IACUC is charged with the following:

- To review proposed protocols for activities involving the use of animals in research, teaching, and testing. Protocols must be approved by the IACUC before animal use may begin.
- To inspect and assure that the animal research facilities and equipment meet an acceptable standard.
- To assure that personnel are adequately trained and qualified to conduct research using animals.
- To assure that animals are properly handled and cared for.
- To assure that the investigator has considered alternatives to potentially painful or distressful procedures and has determined that the research is nonduplicative.

- To assure that sedatives, analgesics, and anesthetics are used when appropriate.
- To assure that proper surgical preparation and technique are utilized.
- To assure that animals are euthanized appropriately.

occupational health and zoonotic diseases

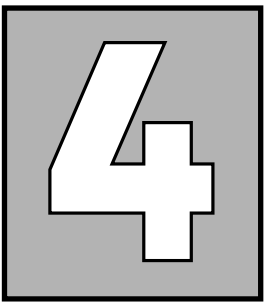
Domestic mice purchased from reputable vendors pose virtually no risk of infectious zoonotic disease, unless experimentally infected with zoonotic pathogens. The need for, and aspects of, a comprehensive occupational health and safety program for individuals working with laboratory animals, including mice, have been described.[42] In general, personnel should wear a clean lab coat or coveralls and gloves when working with research animals. Occupational health programs for personnel handling mice should be developed with consideration for the following potential health issues:

1. **Puncture and bite wounds**—Mice may attempt to bite when they feel threatened or during restraint. Puncture wounds resulting from bites carry risk of bacterial infection and should be thoroughly cleansed with an antiseptic. Puncture wounds may also result from handling equipment with sharp edges or points. For this reason personnel should be current with respect to tetanus immunization.
2. **Allergy**—Allergies to mice are not uncommon in personnel exposed to laboratory animals, including mice.[42–47] Prealbumin from the urine and albumin derived from the skin appear to be the major allergens.[48–51] Personnel may experience respiratory symptoms such as sneezing and rhinitis or skin symptoms such as redness, swelling, and pruritis following exposure. As with many allergies, extreme allergic sensitivity to mice can result in anaphylaxis and thus represents a serious occupational hazard for some individuals. It is advisable for personnel to wear a face mask or fitted respirator, gloves, and a clean, launderable lab coat or coveralls. One study found that dust respirators may vary from 65 to 98% in efficiency related to exclusion of mouse allergens.[52,53] Alternatively, ventilation and building designs, such as

down-ventilated benches and ventilated cages, can be used to decrease exposure of personnel to animal allergens.[54] The use of filter caps on cages and corncob bedding also appear to reduce the level of mouse allergens.[55] Ideally, sensitive personnel should be reassigned to job tasks that eliminate the possibility of exposure to allergens. The advice of an occupational health specialist should be sought and followed if reassignment away from mouse areas is not possible.[56] In addition, it is advisable for such individuals to undergo periodic respiratory function testing.

3. **Experimental biohazards**—Some studies may involve purposeful infection of mice with known human pathogens. In such cases it is recommended that standard operating procedures for safe handling of biohazardous materials and infected animals be established and followed. Guidelines for use of biohazardous agents are presented in detail elsewhere.[57]
4. **Hantavirus**—The genus *Hantavirus* is a member of the family *Bunyaviridae.* Infection with *Hantavirus* is associated with severe hemorrhagic disease with renal involvement in humans. Hantavirus does not appear to cause any apparent illness in rodents.[58] The virus is shed in the saliva, urine, and feces of infected mice. Infected humans may experience disease typically characterized by fever, myalgia, and respiratory distress. Most commonly, *Hantavirus* is isolated from wild rodents.[59] Mice from reputable commercial sources should be free from *Hantavirus;* however, the possibility of infection exists, albeit small, and is one reason why those working with mice should at least wear dust masks and gloves when handling mice or their waste products.
5. **Lymphocytic choriomeningitis**—An *Arenavirus,* lymphocytic choriomeningitis virus (LCMV) generally produces an asymptomatic infection in mice.[60] It should be noted that hamsters may also have LCMV as an inapparent infection.[61] Commercial colonies are generally free of LCMV; however, experimental transplantation of tumors into mice may serve as a route for passage of LCMV. LCMV may be transmitted to man by direct contact with feces and urine, inhalation of dried excreta, or by the bite of an infected mouse. In humans, severity of disease is variable but is usually described as having symptoms typical of a mild case of influenza. In severe cases, the central nervous system may be affected.

6. **Dwarf tapeworm**—The cestode *Hymenolepis nana* infrequently infects laboratory-bred research mice. Most infestations in mice are subclinical; however, the parasite is zoonotic and may result in disease in humans.



veterinary care

basic veterinary supplies

The following basic supplies are useful for the clinical care and experimental manipulation of mice:

1. Disposable syringes, 1 ml and 3 ml
2. Disposable hypodermic needles, particularly 23 to 26 gauge (diameter) and 5/8 in. (length)
3. Gauze sponges
4. Infrared ear thermometer
5. Disinfectant, such as povidone-iodine solution
6. Topical antibiotics, such as triple antibiotic ointment or ophthalmic gentamicin ointment
7. Sterile fluids, such as lactated Ringer's solution or 0.9% sodium chloride
8. Bacterial culture swabs in transport media
9. Several 22-gauge ball-tipped, stainless steel feeding needles for orogastric gavage

Additional supplies should supplement those listed above, depending upon the needs of the facility.

physical examination of the mouse

Physical examination should be performed on mice upon arrival at the facility and on mice exhibiting any abnormalities. With large groups of mice, a few representative individuals or any obviously abnormal animals should be examined. Findings should be recorded in a colony record or other appropriate health record. Physical examination of the mouse can be performed in the following manner:

1. General assessment of behavior of the animal within the cage and during removal from the cage. Findings such as lethargy or aggressiveness or hunched appearance should be noted.
2. The coat should be examined for hair loss, open or closed skin lesions, or abnormal masses. In addition, the overall care of the hair coat should be assessed, since an unkempt appearance is often evidence of underlying illness.
3. Overall body condition should be evaluated. Mice that are thin or abnormally small compared to littermates may have underlying illness.
4. Attention should be paid to any "chattering" noises as evidence of possible respiratory disease. Chattering is a stertorous breathing noise presumably due to mucopurulent material in the airways, nose or both.
5. The eyes, nose, ears, and perineal region should be examined for discharges.
6. The incisors should be examined for overgrowth.
7. Palpation for abnormal masses within the abdominal cavity can be performed by restraining the mouse by the scruff of the neck (Figure 4.1); firmly pressing the thumb and index finger of the free hand into the cranial part of the abdomen; and slowly drawing the fingers back caudally, being sure to palpate both ventral and caudal aspects of the abdomen.
8. The body temperature may be measured by several methods in mice, including:

    - The **infrared ear thermometer,** which has been used to measure body temperature in mice.[62] This method is quick, simple, and noninvasive.

Fig. 4.1 Abdominal palpation of the mouse (ventral side shown).

- **Thermcouple probes,** which can be used to accurately measure the rectal temperatures of mice.[63–65] This method requires a special temperature meter and is therefore relatively expensive. Repeated rectal insertion of thermocouple probes has been associated with mortality in mice.[66]
- **Telemetric monitoring,** which involves implantation of a small telemetric transmitter.[67,68] Transmitters may be implanted either subcutaneously or intraperitoneally.
- **Touch** may be used to roughly assess the body temperature, although only serious perturbations in body temperature can be detected by this method. The normal body temperature for mice may vary slightly depending upon the strain and the method of measurement; however, temperatures in the range of 36.5–38°C (97.5–100.4°F) are typical.[69]

common diseases

Although problems related to obvious masses or open wounds are generally apparent, mice often do not exhibit clinical evidence of disease. Depending upon the disease, the astute clinician may observe wasting, lethargy, alopecia, or an audible "chattering" (with respiratory disease). For many diseases, the animal assumes the appearance of a "sick mouse"–hunched, lethargic, and cool to the touch (Figure 4.2). Still, many infectious diseases are clinically inapparent and may be discovered only after the animal has been found dead.

It is important to understand that infection and disease are not synonymous. **Infection** refers to the invasion and multiplication of microorganisms in body tissues. Infection may occur without apparent disease. **Disease** refers to interruption or deviation from normal structure and function of any tissue, organ, or system. Many of the infections that cause concern may not cause discernible disease in many strains of mice; however, they may cause inapparent or subclinical changes that can interfere with research.

Fig. 4.2 This mouse was dyspneic and standing upright, but with a hunched posture. Note the pallor of the eyes due to anemia. Necropsy showed that the mouse had thymic lymphoma.

Viral Agents and Diseases

A variety of methods are commonly used to diagnose viral and other types of infections. Each has strengths and weaknesses, and frequently a combination of methods is the most effective means of diagnosis.

Serologic methods are the primary means to evaluate for the presence of viruses in a mouse or colony. Most serologic methods detect antibodies produced by the host against the infectious agent. They do not actually test for the presence of the agent. For example, an animal may have been infected, mounted an effective antibody response, and cleared the virus, but still remain seropositive for weeks or months (or longer even) though the virus is no longer present. Such is the case with mouse hepatitis virus (MHV). **Enzyme-linked immunosorbent assay** (ELISA), **hemagglutination inhibition assay** (HIA or HI), **complement fixation** (CF) and **immunofluorescent antibody** (IFA) tests are the most commonly used tests to detect antibodies to infectious agents in laboratory animal colonies. Ideally, such tests would be 100% sensitive (i.e., detect 100% of seropositive animals, with no false negative results) and 100% specific (i.e., detect 100% of the animals seropositive for a specific agent, with no false positive results). No test is 100% sensitive and 100% specific. Therefore, it is common practice to utilize the test with the higher sensitivity for screening (usually ELISA), followed by confirmation of positive results with tests of higher specificity. While it would be ideal to detect for the presence of the virus itself in an animal or colony, most current methods remain costly, time consuming, of low sensitivity or specificity, or all of these. **Virus isolation** remains the definitive method to determine the presence of a virus. A positive result means that the virus was definitely present. A negative result indicates only that the virus was not isolated, although it could conceivably have still been present.

Polymerase chain reaction (PCR) techniques detect the presence of nucleic acids specific to the infectious agent and can be very specific and sensitive, depending upon the probes used. Selection of the appropriate tissue specimen is critical. Although it is relatively rapid, it can be quite costly.

Electron microscopy can be used to visually identify a virus. Again, this method requires careful selection of tissue samples, can be quite time consuming and costly, and can have low specificity due to similar morphology of different viruses.

Histopathology (microscopic examination of tissues for abnormalities) is used to detect the damage done by infectious agents to the

host tissues. Some viruses, such as adenoviruses, herpesviruses, and papovaviruses may leave distinct "footprints" such as intranuclear inclusion bodies. Cytomegaloviruses can cause bizarrely enlarged "cytomegalic" cells. MHV infection can result in multinucleated cells called **syncytia.** Often, the viruses do not leave distinctive "footprints," and the pathologic changes or lesions are only suggestive of a virus or other agent. To further complicate matters, mice of different strains, ages, or sex may respond differently to pathogens, and different strains of a pathogen such as a virus can cause different lesions. For example, in adult B6 mice MHV may cause no lesions, but in young B6 mice it can cause diarrhea and death, and in athymic nude or otherwise immunocompromised mice it can result in massive hepatic necrosis. Some strains of MHV (enterotropic strains) tend to cause gastrointestinal and liver lesions in susceptible animals, while neurotropic strains may cause respiratory and neurologic lesions.

In general, commercial vendors of research mice survey serologically for antibodies to the viral agents shown in Table 4.1, on varying schedules. Health monitoring reports and specific information regarding their schedules and methods are usually available on the vendor's website and by request.

There are also several viruses for which serologic evaluations are not routinely performed. These include lactate-dehydrogenase-elevating virus (LDEV), murine retrovirus, murine leukemia virus, and mouse mammary tumor virus.

The following are summarized descriptions of some of the more important viral agents of mice:

Ectromelia virus

A double-stranded DNA virus, poxviruses have the distinction of being the largest of the viruses (approximately 300 x 240 x 100 nm). They have a characteristic brick or dumbbell morphology when examined by electron microscopy.

Transmission is by direct contact with infected mice or materials (fomites). DBA/2J, A/J, and BALB/cByJ mice are considered to be susceptible, while C57BL/6J and AKR/J mice are considered to be resistant to disease. Once considered to have been eradicated from research colonies in North America, an outbreak occurred in 1995 and resulted in the loss of thousands of mice. The source of the virus is believed to be commercial mouse serum.[70] Subsequent incidents have occurred.[71] These cases provide an excellent example of why

TABLE 4.1: IMPORTANT VIRUSES OF LABORATORY MICE

Acronym	Name of Virus	Family, Genus	Disease
DNA Viruses			
ECTRO	Ectromelia virus	*Poxviridae, Orthopoxvirus*	Mousepox, ectromelia
MCMV	Murine cytomegalovirus	*Herpesviridae, Betaherpesvirinae*	Usually subclinical
MTV	Mouse thymic virus	*Herpesviridae*	Thymic necrosis (only with experimental infections)
MAD	Mouse adenovirus	*Adenoviridae, Mastadenovirus*	Subclinical; wasting (nude mice)
K (MPV)	Polyoma K virus (mouse pneumonitis virus)	*Papovaviridae, Polyomavirus*	Subclinical; pneumonitis (experimental infections)
POLY	Mouse polyomavirus	*Papovaviridae, Polyomavirus*	Subclinical; wasting & paralysis (nude mice)
MVM	Minute virus of mice	*Parvoviridae, Parvovirus*	Subclinical
MPV	Mouse parvovirus (orphan parvovirus)	*Parvoviridae, Parvovirus*	Subclinical
RNA Viruses			
SEN	Sendai virus	*Paramyxovirus, Paramyxoviridae*	Subclinical or pneumonia
PVM	Pneumonia virus of mice	*Paramyxoviridae, Pneumovirus*	Subclinical; wasting (nude mice)
LCMV	Lymphocytic choriomeningitis virus	*Arenaviridae, Arenavirus*	Subclinical; runting, wasting—ZOONOTIC
MHV	Mouse hepatitis virus, lethal intestinal virus of infant mice (LIVIM)	*Coronaviridae, Coronavirus*	Subclinical; infant diarrhea; wasting (nude mice)
EDIM (ROTA)	EDIM virus	*Reoviridae, Rotavirus*	Epizootic diarrhea of infant mice
REO3	Reovirus 3, respiratory enteric orphan virus	*Reoviridae, Rotavirus*	Usually subclinical
MMTV	Murine mammary tumor virus	B type *Retroviridae*	Subclinical; mammary tumors
MuLV	Murine leukemia virus	C type *Retroviridae*	Subclinical; hematopoietic neoplasms
HAN (KHF)	Hantaan virus (Korean hemorrhagic fever)	*Bunyaviridae, Hantavirus*	Subclinical; ZOONOTIC
TMEV (GDVII)	Theiler's mouse encephalitis virus(es)	*Picornaviridae, Cardiovirus*	Usually subclinical; rarely, neurologic

testing should be performed on all biological products to be inoculated into mice or other rodents.

Infection may result in acute death with no premonitory signs in susceptible strains. For animals that survive longer, conjunctivitis and erosive and erythematous skin lesions (thus, the term *mousepox*) occur. **Ectromelia** (meaning "short limbs") is attributed to dry gangrene that occurs in survivors who have suffered severe skin lesions of the extremities.[70,72,73] Splenic necrosis, involving up to 50% of the spleen, may be considered to be the "classic" lesion, and splenic fibrosis is considered to be a pathognomonic lesion of survivors.

Histologic findings include necrosis of the liver, bone marrow, lymph nodes, skin, intestine, and other organs. Cytoplasmic inclusion bodies can be found in infected cells. In this regard, basophilic (Cowdry Type B) inclusion bodies are more likely to occur in hepatocytes, while eosinophilic (Cowdry Type A) inclusion bodies may occur in lymphoid tissues, mucosal epithelium, and skin.[70,72,73]

Differential diagnoses include MHV, helicobacteriosis, salmonellosis, and Tyzzer's disease, because of similar hepatic lesions, although infections with these agents do not result in inclusion bodies. Similar skin lesions, also without pox inclusions, may occur due to trauma. Serum antibodies can be detected by ELISA and IFA. Death with characteristic lesions may occur in disease-susceptible strains before seroconversion.

Culling of infected animals or colonies is the most appropriate means to eliminate the disease in most situations. Seronegative, apparently uninfected offspring have been produced by breeding immunocompetent seropositive survivors, a technique that has been used to preserve valuable colonies. It should be noted, however, that some immunocompromised strains cannot clear the virus. Intrauterine infection can occur, thus cesarean rederivation may not eliminate the virus.[74]

Herpesviruses of mice

There are two significant herpesviruses of mice:

1. Mouse cytomegalovirus (MCMV)

Cytomegaloviruses tend to be very species specific (able to infect only their own host species). MCMV results in persistent subclinical infection of mice with no apparent disease.[72,75] Infection with MCMV can profoundly alter immune responses that are being evaluated experimentally.[72,73,75]

Cytomegaly (enlarged cells) with large eosinophilic intranuclear and intracytoplasmic inclusion bodies, especially in the salivary glands, is pathognomonic for MCMV infection. Submaxillary salivary glands are affected more severely than sublingual glands, and parotid glands are affected the least. MCMV infection must be distinguished from polyomavirus infection, which can cause inflammation of the salivary glands with inclusion bodies.

Diagnosis is based upon histologic findings and supported by ELISA screening with IFA confirmation for antibodies to MCMV. Because prevalence of MCMV is believed to be low in the absence of contamination by wild mice, it is not included in many surveillance testing protocols.

Depopulation of infected colonies probably is the most appropriate means to eliminate MCMV in many situations. Cesarean rederivation and barrier maintenance should be effective.

2. *Mouse thymic virus (MTV)*

MTV infection is generally subclinical, although it causes transient immunosuppression related to thymic necrosis and destruction of T lymphocytes in suckling mice.[72,73,76,77] The lesion is difficult to discern grossly unless there are "normal" control mice for comparison. Histologically, massive necrosis of the thymus with intranuclear inclusion bodies can be seen. During recovery there is granulomatous inflammation of affected areas.[72,73,76,77]

IFA testing is commercially available to test for antibodies to MTV; however, screening for MTV is not widespread in diagnostic health surveillance programs.

The prevalence of MTV is uncertain, since screening for the agent is not widespread. Procurement of animals known to be negative for MTV is an appropriate strategy to prevent infection. Depopulation of infected colonies probably is the most appropriate means to eliminate MTV in many situations. Wild mice (*Mus musculus*) are considered to be the reservoir for MTV and must be excluded from rodent research facilities.

Mouse adenovirus

Adenoviruses are nonenveloped, double-stranded DNA viruses that replicate in nuclei and commonly cause characteristic large intranuclear inclusion bodies. There are two strains of adenovirus recognized in mice, MAD1 (Friend Leukemia Agent or FL) and MAD2 (K87). Seroprevalence in mouse colonies in the United States has been reported to be low.[78]

Infection with adenovirus could alter normal immune responses of mice and thereby skew experimental data. For example, mouse adenovirus is reported to accelerate experimental scrapie infection in mice.[79]

Adenoviral infection is generally subclinical in immunocompetent mice, although wasting may result in nude mice. Experimental infection in suckling mice can result in runting and death, with Type A intranuclear inclusions found in renal tubules. C57BL/6J mice are relatively susceptible to this condition, while BALB/c mice are relatively resistant, and outbred Swiss mice (CD1) vary in susceptibility.[80,81]

Serologic evaluation by ELISA and confirmation with IFA are in widespread use for detection of mouse adenovirus infections. Because mouse adenoviruses do not hemagglutinate, HIA is not useful. PCR techniques are also used by some laboratories to test for contamination of biological materials. When present, large intranuclear inclusions in intestinal epithelium may be considered virtually diagnostic for mouse adenovirus, but confirmation by serologic and PCR methods should be pursued. It should be remembered, however, that histology is not a reliable screening method for mouse adenovirus.

Culling of seropositive animals or colonies is probably the most appropriate means to eliminate the virus from the facility. Cesarean rederivation is also an effective means of elimination. Maintenance of mice under barrier conditions and exclusion of wild mice are useful for preventing contamination of uninfected colonies.

K virus (mouse pneumonitis virus)

K virus is a double-stranded DNA polyomavirus that is related to, but antigenically distinct from, mouse polyoma virus. It is named for Kilham, who originally identified the virus, but it should not be confused with Kilham rat virus, which is a parvovirus.[82,83]

K virus was originally discovered as a contaminant of transplantable murine tumors, emphasizing the importance of testing biological materials for mouse pathogens. As with many other viral infections, the normal immune response can be altered with K virus, thereby interfering with experimental data.

Natural polyomavirus infections are not generally associated with disease. Instead, infection tends to be subclinical and persistent. K virus may be enzootic in some wild mouse populations. The virus establishes persistent infection in the kidney and is shed in urine.[84] Experimentally infected neonates die with edema and hemorrhage in the lungs. In older mice viremia is blocked by neutralizing antibody,

and there are no clinical signs or gross lesions. Athymic nude mice develop disease similar to that seen in neonates.

ELISA screening and HIA confirmation are widely used for detection of antibodies to K virus in diagnostic and health surveillance programs. PCR techniques are also available to test for virus-specific nucleotide sequences in biological materials.

Culling infected animals or colonies is probably the most appropriate means to eliminate the agent in most situations. Cesarean or embryo rederivation are effective and could be used to preserve valuable lines or strains.

Polyomavirus

Polyomavirus is a double-stranded DNA virus that is antigenically distinct from K virus.

Natural infections with the virus are not generally associated with disease in immunocompetent mice. Athymic nude mice may develop tumors, wasting disease, and paralysis. Experimental infections of neonates can lead to development of tumors in multiple tissues, while immunocompetent adults mount an effective immune response and do not develop tumors.[72]

No histologic abnormalities are found in subclinically infected mice. Nude mice may develop multifocal necrosis and inflammation with intranuclear inclusion bodies. Paralysis in nude mice has been attributed to vertebral tumors and demyelinating central nervous system disease. The characteristic tumor resulting from experimental infections of neonates is a pleomorphic salivary gland tumor.

Differential diagnoses for intranuclear inclusion bodies in mouse tissues should include K virus, adenovirus and MCMV. Differential diagnoses for paralysis and demyelinating central nervous system disease in nude mice should include TMEV.

ELISA screening and IFA or HIA confirmation are in widespread use for detection of antibodies to polyoma virus in diagnostic health and surveillance programs. PCR techniques are also available to test for virus-specific nucleotide sequences in biological materials.

Prevalence of polyomavirus is generally very low. Most large vendors of mice screen for polyomavirus routinely and have long histories of negative serologic evaluations of barrier-maintained animals. Culling infected animals or colonies is the most appropriate means to eliminate the agent, although immunocompetent adults should confer protection on their offspring, and the infection should therefore "burn out" after the source is eliminated. Cesarean or embryo rederivation is effective and could be used to preserve valuable lines or strains.

Minute virus of mice (MVM)

MVM is a highly contagious parvovirus that results in no clinical disease or lesions in natural infections. Experimental infections of neonates cause abnormalities such as runting, cerebellar hypoplasia, renal infarcts, and anemia. MVM is a common contaminant of hybridomas and other transplantable tumors and cell lines,[85,86] emphasizing the need for testing of such materials to be administered to mice. Along with mouse parvovirus, MVM has the distinction of being one of the most frequently reported viral agents in domestic mouse colonies in the United States.[78] In addition, MVM is commonly found in wild mice. MVM is shed in urine and feces. Like other parvoviruses, MVM is hardy in the environment. Diagnosis is usually by ELISA, with IFA or HIA confirmation to distinguish cross-reactivity with MPV.

Elimination of MVM from mouse colonies is achieved by culling seropositive animals or entire colonies containing seropositive animals from the facility. Because parvoviruses are generally resistant in the environment, all contaminated rooms and equipment should be treated with a parvocidal disinfectant. Due to the highly infectious nature of rodent parvoviruses, strict barrier procedures and exclusion of wild mice and contaminated biological materials are extremely useful to prevent introduction of parvoviruses into the facility. MVM can be eliminated from mice through cesarean rederivation or by embryo transplantation.

Mouse parvovirus (orphan parvovirus)

Evidence for the existence of undescribed parvoviruses in both mice and rats first emerged in the 1980s, when several diagnostic testing laboratories began to report atypical serologic results. The mouse-origin species has been designated as mouse parvovirus or MPV;[87] the rat-origin species has been designated as rat parvovirus or RPV.[86] At least three strains or subtypes of MPV are recognized.[86] MPV has been shown to modulate *in vitro* and *in vivo* immune responses.[87,88]

MPV is distinct from other rodent parvoviruses such as MVM based on its genomic sequence, pathogenesis, and potential to interfere with research. Although natural infections with either MVM or MPV are asymptomatic, experimental infection studies suggest that MPV is less pathogenic than MVM since even neonatal and immunocompromised mice experimentally infected with MPV show no

clinical signs, whereas MVM infections of neonatal mice may be lethal.[89] Another difference between MPV and MVM is the apparent persistence of MPV. Infections with MPV in adult mice persist for at least 9 weeks after inoculation,[86] whereas infections with MVM are short-lived.

Diagnosis of MPV infections in mice relies on serology or PCR assays, since no clinical or histologic disease is evident even with experimental infections of neonatal and immunocompromised mice. Serology can be performed by ELISA or IFA using MPV, Kilham rat virus, or MVM as the antigen. Alternatively, an ELISA based on recombinant structural protein (NS1) can be used. Because of the cross-reactivity among rodent parvovirus species, ELISA and IFA results may not delineate the species of parvovirus; thus, an MPV-specific HIA is used as a secondary test. Positive HAI results indicate that MPV is the parvovirus species involved in the infection. PCR assays have also been developed to aid in diagnosis of rodent parvovirus infections. A universal rodent parvovirus assay detects any of the parvovirus species but does not delineate the specific virus species involved. Species-specific assays are also available for MVM and MPV, which identify individual rodent parvovirus species.

Elimination of MPV from mouse colonies is the same as for MVM.

Pneumonia virus of mice (PVM)

PVM is a paramyxovirus that is labile under typical room conditions. Worldwide, PVM is prevalent in mice, rats, and hamsters.

Infections are subclinical in immunocompetent mice; however, nude mice may develop chronic wasting disease with progressive interstitial pneumonia and wasting.[90,91] The target tissue is the bronchial epithelium and type 2 pneumocytes.

Typically, diagnosis is by ELISA, with confirmation by IFA or HIA. Biological materials to be administered to rodents should be tested for PVM contamination. PCR techniques are also available to test for virus-specific nucleotide sequences in biological materials.

The infection is controlled within a colony by either culling exposed animals or by cesarian rederivation. Alternatively, one may separate out several serologically negative pairs for breeding. Another approach is to take advantage of the fact that the infection persists for only about 9 days in individuals. With this in mind, one can separate and breed serologically positive mice nine days or more after they have tested positive. At that point, such mice should no longer be infected and can produce PVM-free offspring.

Sendai virus

Sendai virus may be the most significant viral pathogen of mice. Some strains of mice, such as the 129 and DBA, are particularly susceptible, while others, such as the C57 and SJL, are resistant.[72,92] This genetic susceptibility appears to be related to impaired mucociliary clearance and the kinetics of the immune response. These changes predispose infected mice to bacterial respiratory and ear infections. In general, Sendai virus is extremely contagious between mice.

Enzootic infections in breeding colonies are common, with mice becoming infected around 4 to 8 weeks of age, after maternal immunity wanes. Infection is relatively short-lived, with no clinical disease. The infection is maintained in the colony due to the continual supply of young, susceptible weanlings.

Epizootic infections may also occur, with the infection spreading rapidly among a mouse population over a short time period. An audible "chattering" can often be detected and is suggestive of respiratory disease. In addition, severely affected mice may be in apparent respiratory distress. Another indication of Sendai virus infection may include the poor growth of young mice.

Infections in nude mice and severe combined immunodeficient (SCID) mice are characterized by more severe clinical signs. Chronic wasting disease can also occur, and death is not uncommon.

The virus targets respiratory tissues, particularly respiratory epithelium and type 2 pneumocytes. Key histologic findings include necrotizing rhinitis, tracheitis, and bronchiolitis with interstitial pneumonia.[93] During recovery, cuboidal metaplasia in the alveoli and squamous metaplasia in the bronchioles, sometimes with fibrosis, are present. In nude and SCID mice, one may observe proliferative bronchiolitis and interstitial pneumonia with only minimal necrosis.

Diagnosis is made by serologic methods, usually the ELISA with confirmation by IFA or HIA. Histopathology of clinically affected animals is also a useful diagnostic tool. Biological materials to be administered to mice, rats, and hamsters should be tested for Sendai virus. PCR techniques are available to test for virus-specific nucleotide sequences in biological materials. Although Sendai virus is considered to be highly contagious via aerosol, studies have shown that sentinel mice exposed to dirty bedding from infected animals do not reliably seroconvert. In addition, some outbred stocks of mice do not seroconvert consistently.[94,95]

Sendai virus is difficult to control once it has entered a facility. The sacrifice of positive animals and any exposed animals should be

considered. Rigorous attention must be paid to minimizing contact with uninfected animals, and the flow of equipment and personnel should be such that the risk of spread by fomites is minimized. Alternatively, cessation of breeding and no introduction of any new animals into the colony for a period of 6 to 8 weeks will allow the infection to run its course. Seropositive animals, which would have cleared the infection by then, can then be bred, and the offspring tested to confirm their seronegative status.[72]

Lymphocytic choriomeningitis virus (LCMV)

A single-stranded RNA virus, LCMV can infect a number of species, including mice, rats and hamsters. In man, LCMV can cause lymphocytic choriomeningitis, a severe disease affecting the central nervous system, and is therefore considered to be a **zoonotic** pathogen.

Clinical signs vary with virus strain and with age, strain, and immunologic status of mice. *In utero* and early perinatal infection usually results in **persistent tolerant infection,** with lifelong viremia and shedding of virus. Disease in young mice usually is subclinical, although runting may occur.

Persistently infected mice may develop immune complex glomerulonephritis, known as **late onset disease,** 7–10 months postinfection and resulting in emaciation, ascites, and death. Gross lesions can include enlarged lymph nodes and spleen. Kidneys affected with glomerulonephritis may be enlarged and have a granular surface texture, although they may be shrunken in later stages of the disease process.

Natural infection in adult mice can be subclinical or can cause severe acute disease with high mortality, termed **nontolerant (acute) infection.** Gross lesions may not be apparent or there may be necrosis in the liver and lymphoid organs. Adult immunocompetent mice can be protected from disease (but not infection) by various immunosuppressive therapies. Athymic nude mice do not develop disease, but are persistently viremic and shed virus.[72,73,96]

The classic histologic picture of lymphocytic choriomeningitis is of dense perivascular accumulations of lymphocytes and plasma cells in the choroid and meninges due to immune complex (virus with specific IgG and complement) deposition in vessel walls. However, although this feature is seen after experimental intracerebral inoculations, it is probably not a feature of natural disease. In persistent tolerant infections, there is generalized lymphoid hyperplasia and immune complex deposition in glomeruli and vessel walls, resulting

in glomerulonephritis and perivascular cuffs of plasma cells and lymphocytes in all viscera. In nontolerant (acute) infection, there may be multifocal hepatic necrosis and lymphoid necrosis.[72,73,96,97]

Overall, the clinical signs and gross pathologic changes are nonspecific. Differential diagnoses for lymphadenopathy may include lymphoma. Differential diagnoses for glomerulonephritis may include TMEV or other persistent viral infections. Differential diagnoses for hepatic necrosis should include MHV, ectromelia, Tyzzer's disease and *Helicobacter hepaticus* infection.

LCMV is a zoonotic pathogen and can cause mild to serious or fatal disease in humans.[98–100] Historically, it has been a common and significant contaminant of biologic materials, such as transplantable tumors of mice, hamsters, guinea pigs, tissue culture cell lines, and virus stocks.[72,85,91] Its presence in such materials can be detected by MAP or PCR testing. Human cases of the disease have been associated with subclinically infected nude mice, hamsters bearing transplantable tumors, wild rodents, and pet hamsters. Persistently infected mice and hamsters can transmit virus and shed it in urine, saliva, and milk. Congenital infection in humans may result in hydrocephalus or fetal or neonatal death.

Serologic surveys indicate endemic human infection in Europe, Asia, and the Americas, and association with seropositive wild *Mus musculus* (house mouse) has been made.[101,102] Wild *Mus musculus* are the natural host for LCMV, and the presence of feral mice in a research animal facility should prompt serologic evaluation of the feral mice and of exposed laboratory mice for LCMV.

ELISA and IFA are in widespread use for detection of antibodies to LCMV in diagnostic and health surveillance programs. PCR techniques also are available to test for LCMV-specific nucleotide sequences in biological materials.

LCMV-infected animals or contaminated biologic materials should be destroyed. Cages and equipment exposed to infected animals should be autoclaved. Animal rooms should be fumigated with formalin or vaporized paraformaldehyde, and vacated for 7 to 10 days. Transovarian or transuterine infection has been documented, so cesarean or embryo rederivation is of no value and may be hazardous to personnel performing such procedures.

Mouse hepatitis virus (MHV)

MHV is a single-stranded RNA virus. It has a high prevalence worldwide and is very contagious between mice. The virus is shed in the feces and respiratory secretions, and transmission occurs through

contact with infected mice, fomites, and airborne particles. Transplacental infection has been documented experimentally.

Two major disease patterns exist and depend upon the tropism of the particular virus strain. **Respiratory** strains (MHV 1, 2, 3, and JHM) infect nasal and respiratory epithelium. In susceptible hosts, the virus then disseminates to other tissues, particularly the liver, through the bloodstream. Edothelial syncytia are sometimes seen with this syndrome. Typically, disease is minimal, with nonspecific hepatic lesions such as necrotizing hepatitis. However, neonates may develop necrotizing meningoencephalitis with syncytia, resulting in posterior paresis.

Enteric strains of the virus (MHVS, LIVIM, possibly A59) infect the mucosal epithelium of the gastrointestinal tract in all ages of mice. To some extent, virus will also infect the nasal epithelium and the liver. Multinucleated syncytial cells are a typical histologic finding. The ascending colon is the most frequent site of infection and of syncytia cells, both in the villi and the crypts. **Lethal intestinal virus of infant mice (LIVIM)** is an enteric strain of MHV and causes diarrhea and dehydration of neonates. Typically, affected neonates cease to nurse.

Enzootic infections of mouse colonies with MHV are very common. Although previously infected adults are immune, the continued introduction of susceptible mice into the colony, either through breeding or other sources, perpetuates the infection. Typically, clinical disease is limited with enzootic infections. When a previously nonexposed, susceptible mouse colony is exposed to MHV, an **epizootic** infection may occur. Usually, such infections spread rapidly. Many mice may become clinically ill, and deaths are common. Infection of athymic nude mice generally leads to severe **wasting disease** and death. Enterotropic strains may also cause diarrhea.

Diagnosis of MHV infection is usually made by use of the ELISA. IFA testing for MHV is also quite useful. Histopathology of tissues can aid the diagnosis, particularly if syncytial cells are observed in affected tissues. Blunting of intestinal villi is another typical histologic change.

If possible, infected and exposed mice should be sacrificed and the facilities thoroughly disinfected. MHV-infected mice should be isolated from other, susceptible mice to prevent the spread of infection. Strict attention should be paid to patterns of traffic flow within the facility and to sanitation of equipment to further aid this effort. The infection runs its course in 2 to 3 weeks in immunocompetent mice which subsequently become immune to reinfection. This means that

a **burn out** approach can be used, in which breeding and the introduction of new animals into the colony/facility is halted for a period of four weeks. This method is particularly valuable in instances where unique genetic strains of mice are infected that cannot be culled.

Epizootic disease of infant mice virus (EDIMV)

A double-stranded RNA virus, EDIMV is classified antigenically as a Group A rotavirus, similar to rotaviruses of other species that cause diarrhea in young animals.[72,103] In general, transmission of rotaviruses is through ingestion of material contaminated with the feces from infected animals. Rotaviruses tend to be susceptible to extremes of heat and cold, detergents and disinfectants, and elimination of infective virus from the environment can be accomplished by sanitation procedures that are standard in most well-managed rodent-housing facilities.

EDIMV infection of mouse pups younger than 12 days of age results in diarrhea and runting, although most pups continue to nurse and will survive. Infection is usually subclinical in older mice. Rotaviruses infect epithelial cells in the intestinal villi. These cells are replaced by less differentiated cells that initially lack a full complement of digestive enzymes, resulting in diarrhea due to maldigestion and malabsorption. Undigested milk in the small intestine promotes bacterial growth and exerts an osmotic effect, exacerbating damage to the villi.[72,73,95,104]

Histologic changes may be subtle even in animals with significant diarrhea. Hydropic change of villous absorptive (apical) epithelium is the hallmark finding in acute disease. Survivors may have short, atrophic villi.

In today's research mouse colonies, EDIMV and MHV (LIVIM) are the two most likely causes of diarrhea in mouse pups, although adenovirus, reovirus, salmonellosis, and Tyzzer's disease should be considered also. Pups with epizootic diarrhea of infant mice (EDIM) continue to suckle, and the white milk in their stomachs can be discerned through their translucent skin. Their dams are asymptomatic. Most pups survive, although they may be runted. In contrast, pups with diarrhea due to MHV usually stop suckling, do not have milk in their stomachs, and often die.

ELISA and IFA are in widespread use for detection of antibodies to EDIMV in diagnostic and health surveillance programs. PCR techniques also are available to test for rotavirus-specific nucleotide

sequences. Electron microscopy of feces of diarrheic pups should reveal 60–80-nm, wheel-shaped rotavirus particles. **Rotazyme** is a commercially available ELISA for fecal antigen, but some feeds are reported to cause false-positive results.

Culling of infected animals or colonies is the most appropriate means to eliminate the infection in most situations. Cesarean or embryo rederivation has been effective and could be used to preserve valuable lines or strains. In immunocompetent mice in which infection is cleared effectively, a breeding suspension strategy combined with excellent sanitation, filter tops, and conscientious serologic testing of offspring may be effective.[72] Elimination of infective virus from the environment can be accomplished by sanitation procedures that are standard in most modern rodent-housing facilities.

Reovirus 3 (REO3)

A double-stranded RNA virus, reovirus-3 can infect mice, rats, hamsters, and guinea pigs. Mammalian reoviruses are divided into three serotypes (1, 2, and 3). REO3 is the only serotype associated with disease, although natural infections in rats and mice are usually subclinical.

Although clinical disease is extremely rare with natural infections, stunted growth, diarrhea, jaundice, and oily hair coat have been associated with infection. Lesions can be found in the liver, gall bladder, and kidneys.

Experimental infections result in a number of lesions in young mice, including encephalitis, pneumonia, cholangitis, pancreatitis, adrenalitis, myocarditis, and others. In general, immunocompetent adult mice are relatively resistant to disease, even following experimental inoculation.

Serologic screening with ELISA and confirmation by IFA or HIA are commonly used to detect antibodies to REO3. In some serologic tests there is cross-reactivity with reovirus type 1 and reovirus type 2, and a weak or equivocal result may be due to presence of antibodies to these other serotypes. PCR techniques also are available to test for reovirus-specific nucleotide sequences. Transplantable tumors and cell lines can harbor the virus and should therefore be screened for contamination prior to administration to animals.

Culling of infected animals or colonies and cesarean rederivation are both effective means of eliminating the infection. Because humans may be subclinically infected with the virus, facility personnel are a potential source of contamination.[72,105]

Murine retroviruses

These viruses can exist as either endogenous or exogenous viruses. Endogenous viruses, or proviruses, consist of viral DNA integrated into the mouse genome and are inherited in a Mendelian pattern as are genes. Under certain conditions these proviruses can be induced to synthesize complementary RNA sequences and package themselves into virions, enter the bloodstream, and be shed in milk, saliva, semen, urine, or feces. Exogenous viruses occur outside the genome and behave like conventional transmissible viruses. Murine retroviruses include:

1. Murine mammary tumor viruses (MMTV)

MMTV exist as endogenous and exogenous viruses. All strains of mice have endogenous MMTV. Many of the endogenous MMTV have become named genes with the gene symbol *Mtv* followed by a locus number (e.g., Mtv1, Mtv2, Mtv46) on various chromosomes.[107] Exogenous MMTV or "Bittner" agent is transmitted vertically to nursing pups via milk, and is also shed and can be transmitted by saliva and semen. Bittner agent has been eliminated from most strains by cross-fostering and rederivation.

Infection with MMTV can result in mammary tumors, which can occur almost anywhere on the mouse, although they are usually found on the ventrum.[73,107] 100% of female C3H mice that received Bittner agent from their dam's milk develop mammary tumors by 9 months of age. C57BL/6 mice are considered to be resistant to development of mammary tumors.[107] Mammary tumors in mice are usually adenocarcinomas and frequently metastasize to the lungs.

Mammary neoplasia must be diagnostically distinguished from abscesses or other tumors. Diagnosis is confirmed by histopathology of the mass. Differential diagnoses for masses in the lung should include pulmonary adenoma. Masses found on the head may be mammary tumors or abscesses, frequently from *Staphylococcus* infection subsequent to fight wounds. In the inguinal region, abscesses or neoplasia of the preputial or clitoral glands should be considered.

Since MMTV occurs in all laboratory mouse strains, diagnostic testing is not considered to be necessary in most instances. Exogenous MMTV can be eliminated by foster nursing or cesarean rederivation.

2. Murine leukemia viruses (MuLV)

MuLV exist as endogenous and exogenous viruses. All strains of laboratory mice have endogenous MuLV,[72] and many of these have been

identified on various chromosomes and are named for genes with gene symbols such as Emv1-x (Ecotropic MuLV genes), Pmv1-x (Polytropic MuLV genes), and Xmv1-x (Xenotropic MuLV genes). Endogenous viruses are considered to be **ecotropic, polytropic,** or **xenotropic** depending on whether their virions are infectious *in vitro* to mouse cells only (ecotropic), to mouse and nonmouse cells (polytropic), or nonmouse cells only (xenotropic). Many endogenous MuLV are incomplete or "**defective,**" requiring the presence of another virus or viral parts, or require inducer genes in order to be expressed and produce complete virions.

Hematopoietic neoplasms are associated with endogenous MuLVs in thymus or other hematopoietic tissues. AKR and C58 mouse strains are especially predisposed to early onset of thymic lymphomas, while BALB/c and A/J mice often develop late onset lymphoma.[73] Although commonly called "leukemias," most of these spontaneous hematopoietic neoplasms in mice are malignant solid lymphoid tumors that are more correctly referred to as **lymphoma.** True myelogenous (granulocytic) or erythroid leukemias have been attributed to MuLV and can be induced experimentally.[73,107,108,109] **Leukemia** refers to the presence of neoplastic hematopoietic cells in blood and bone marrow. It may occur late in the progession of **lymphosarcoma,** in which there is invasion of marrow and release of neoplastic cells into the bloodstream, but the term *leukemia* is primarily used to refer to hematopoietic neoplasia that originates in bone marrow and manifests as neoplastic circulating blood cells.

With advanced stages of most hematopoietic neoplasms, a primary sign is lethargy and cachexia. Respiratory distress is a common clinical manifestation of advanced thymic lymphoma. The massively enlarged thymus may occupy a significant portion of the thoracic cavity and restrict breathing movement (Figure 4.3). Abdominal distension may occur when there is massive enlargement of lymph nodes, spleen, or liver by lymphosarcoma. Enlargement of peripheral lymph nodes may be seen with lymphosarcoma.

Histology of lymphoma in any tissue is characterized by sheets of similar lymphoid cells that efface the normal architecture. Depending on the type of lymphoma, cells may be large or small, with minimal or moderate cytoplasm, minimal or marked cellular atypia, and few or abundant mitotic figures.

Pneumonia due to infectious agents is the most important differential diagnosis for dyspnea in most situations. While an enlarged thymus is identified easily during necropsy, when presented with a dyspneic mouse the prosector should be prepared to withdraw blood

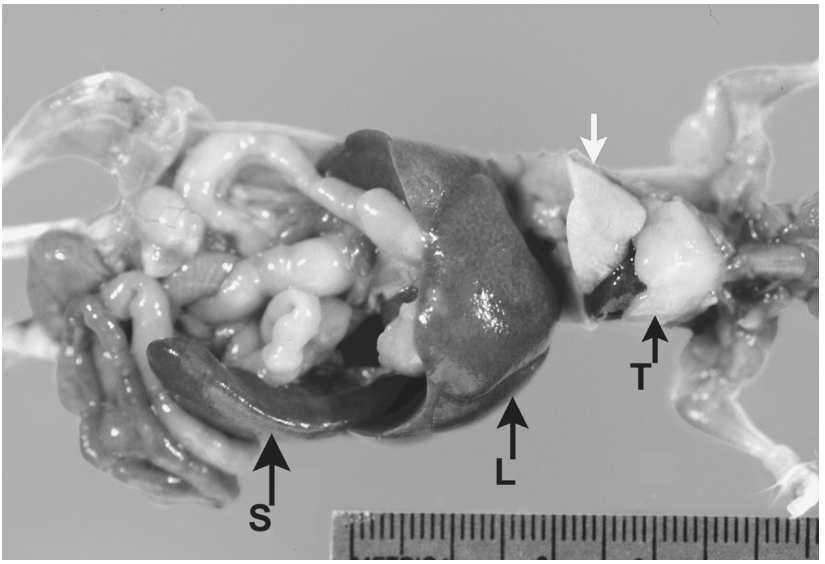


Fig. 4.3 Lymphoma in a mouse. The thymus (T) is massively enlarged, almost as large as the lungs (white arrow). There is hepatomegaly (L), and splenomegaly (S), also due to lymphoma.

for serologic evaluation and to procure suitable specimens for microbiological evaluation. Thymoma is a neoplasm of the epithelial (nonlymphoid) components of the thymus and is much less common than thymic lymphoma. Lymphadenopathy, splenomegaly, and hepatomegaly are strongly suggestive of lymphosarcoma in most strains of mice.

Since MuLV occurs in all laboratory mouse strains, diagnostic testing is not routinely performed. The role of MuLVs in neoplasms or other conditions is difficult to determine because MuLVs are ubiquitous, and viral nucleic acids or even intact virions can be detected routinely in various tissues.

Strain-specific maps of the distributions of endogenous MuLV have been proposed as tools for genetic monitoring of inbred strains.[110] Exogenous MuLVs are transmitted inefficiently and can be eliminated from laboratory mouse populations by rederivation.

Theiler's mouse encephalitis virus(es) (TMEV; includes GDVII)

TMEV is really a group of several picornaviruses, all of which are single-stranded RNA viruses. GDVII is one of several strains of TMEV

that infect mice and can cause disease. Transmission of TMEV is by ingestion of material contaminated with the feces of infected mice, although transmission via fomites and aerosols is also possible.

Most descriptions of the disease are based on experimental infections. The severity and nature of disease depends on virus strain, route of inoculation, host age and genotype.[72,73,111] Natural infection is usually subclinical, but may rarely cause posterior flaccid paralysis. Differential diagnoses for neurologic disease in mice should include trauma, neoplasia, middle and inner ear infections, MHV, and polyomavirus infection (in nude mice). CBA and C3H/He are considered to be relatively susceptible mouse strains, while C57BL/6, A, C57BL/10, and DBA/1 are considered to be resistant strains.[72,111–113]

TMEV infects neurons and glial cells, and histologic changes in the central nervous system include nonsuppurative meningitis, perivasculitis and poliomyelitis with neuronolysis, neuronophagia, and microgliosis in the brainstem and ventral horns of the spinal cord. TMEV is enterotropic, but histologic lesions in the gastrointestinal tract are not discernible.

ELISA and IFA are commonly used for detection of antibodies to GDVII virus in diagnostic and health surveillance programs. PCR techniques are also available to test for virus-specific nucleotide sequences. Tissues and cell lines to be administered to mice should be first tested for contamination with TMEV, as well as other murine pathogens.

Culling of infected animals or colonies is the most appropriate means to eliminate the infection in most situations. Although transplacental transmission has been experimentally demonstrated, foster nursing and cesarean rederivation have been reported to be effective in generating virus-free offspring.[114]

Lactate-dehydrogenase–elevating virus (LDEV)

LDEV is a relatively rare togavirus that causes persistent elevation in blood lactate dehydrogenase (LDH) levels. There is generally no clinical disease associated with natural infections with LDEV, although flaccid paralysis has been noted in immunosuppressed C58 and AKR mice 5 months or older.[115,116] In addition to elevations in LDH, aspartate transaminase (AST) and alanine transaminase (ALT) levels may be increased up to three-fold of normal levels. Most LDEV infections are attributable to inoculation of contaminated materials, such as cell lines that have been passaged in mice or products containing mouse serum. Horizontal (mouse-to-mouse) transmission is inefficient, but

is more likely to occur between fighting mice. Vertical (dam-to-pup) transmission also is unlikely but may occur if the dam is infected during pregnancy.

Diagnosis is based upon elevated (up to 20-fold) plasma LDH levels. Release of LDH by platelets during clotting may increase LDH in serum samples, so plasma is recommended over serum as a diagnostic specimen. Only nonhemolyzed samples should be used, since LDH levels will increase as a result of hemolysis. LDEV may contaminate tumor and cell lines; thus samples to be administered to mice should be tested for contamination. Testing these samples is done by injecting a small amount of the material into LDEV-free mice and testing serum samples from those mice after 72–96 h. Routine testing for LDEV is not part of most health surveillance programs because a simple, sensitive, specific serologic test is not available, and because horizontal transmission between animals is inefficient; thus reliable infection of sentinel animals cannot be anticipated.

Although the virus persists in the blood and viremia is lifelong, the virus can be eliminated from infected colonies by breeding animals with normal LDH levels or by cesarean rederivation. LDEV can be eliminated from transplantable tissues and tumors by passage through rats.

Hantaviruses (Han)

Hantaviruses are transmitted by urine and saliva (bites) of infected rodents, and aerosols containing infectious virus from rodent urine, feces, and saliva are considered to be extremely important means of transmission.[100] Many of the hantaviruses cause serious disease in humans and are considered **zoonotic.** Infected laboratory rats and wild-caught rodents have been identified as sources of infection for laboratory personnel in Europe and Asia.[72,117]

Hantaviruses cause persistent infection but no discernible disease in their reservoir hosts, which are wild rodents and insectivores of various species.[72] *Peromyscus* (deer mouse) species have been implicated as a primary reservoir in the U.S.

ELISA screening and IFA or HIA confirmation are available for detection of antibodies to hantaviruses. PCR techniques also are available to test for virus-specific nucleotide sequences in biologic materials.

Animals or biologic materials found to be positive for hantavirus should be destroyed with appropriate precautions for aerosolized material. Whenever possible, hantavirus-positive stock should be

excluded from laboratory facilities and steps taken to prevent entry of wild rodents into the facility.

Bacterial Agents and Diseases

A number of bacterial agents have import with respect to laboratory mice. The agents discussed here are considered to be of concern in contemporary mouse colonies. A summary of common agents is shown in Table 4.2.

Microbiological cultivation on artificial media is the primary means to evaluate for the presence of bacteria in mouse tissues, fluids, or excreta. The isolation of an agent from a site does not necessarily

TABLE 4.2: IMPORTANT BACTERIA OF LABORATORY MICE

Agent	Gram Type	Disease
Clostridium piliforme	Gram-negative rod	Tyzzer's disease; ileotyphylitis, necrotizing hepatitis, myocarditis
Pseudomonas aeruginosa	Gram-negative rod	Mostly in immunocompromised animals; abscess, sepsis.
Salmonella enteritidis typhimurium	Gram-negative rod	Salmonellosis; opportunist, usually subclinical; sepsis, hepatitis, ileotyphlitis; ZOONOTIC
Pasteurella pneumotropica	Gram-negative rod	Opportunist; abscess
Klebsiella pneumoniae	Gram-negative rod	Mostly in immunocompromised animals; cervical lymphadenopathy, sepsis
Helicobacter spp.	Gram-negative spiral; stains with modified Steiner stain.	Usually subclinical; hepatitis in some instances
Citrobacter rodentium	Gram-negative rod	Transmissible murine colonic hyperplasia
Streptobacillus moniliformis	Gram-negative rod	Abscesses, sepsis, lymphadenopathy; ZOONOTIC (rat bite fever)
Mycoplasma pulmonis	Gram-negative coccobacilli and other forms.	Respiratory disease, pneumonia
Corynebacterium bovis	Gram-positive pleomorphic rod	Hyperkeratotic dermatitis
Staphylococcus aureus	Gram-positive coccus	Dermatitis, abscesses
Streptococcus pneumoniae	Gram-positive coccus	Sepsis, otitis

mean that the agent is the cause of the lesion, since a number of bacteria are opportunistic and will secondarily infect lesions. **Opportunistic** agents do not ordinarily cause disease in a normal host, but under opportune circumstances such as immune compromise, debilitation due to other agents or stressors, or an open wound in an unclean environment, such agents can contribute to disease. The isolation of bacteria by culture can be complicated by numerous factors, such as procurement and handling of the proper specimen; overgrowth by other, more easily cultured bacteria present within the lesion; or inability to culture some agents (e.g., *Clostridium piliforme*) on artificial media. Other means to detect some of these agents include serologic evaluation (e.g., ELISA, IFA, HIA) and PCR.[118]

Clostridium piliforme

An obligate intracellular parasite, *C. piliforme* cannot be cultured on artificial media. The spores are infectious and can persist in the environment. Spore-contaminated food or bedding is believed to be a significant source of infection.[72,76]

Tyzzer's disease is the name of the clinical entity resulting from *C. piliforme* infection. Young animals, particularly sucklings or weanlings, are most often and most severely affected. Most infections are subclinical, with disease severity being affected by host strain, bacterial isolate or subtype, and environmental stressors.[76,119] DBA/2 mice are considered to be relatively susceptible and C57BL/6 mice are relatively resistant to disease.

Clinical signs include watery diarrhea, lethargy, ruffled hair coat, and sudden death. Important differential diagnoses for diarrhea in young mice include EDIMV and MHV infection.

The most consistent gross finding in mice is multiple pale foci in the liver (multifocal necrosis). The ileum and cecum may be thickened and red (inflammation), and the heart may have pale foci (myocardial necrosis).[72,76] Warthin Starry silver stain should demonstrate bundles of slender, filamentous bacteria within cytoplasm of cells at the edge of necrotic lesions, especially in the liver. PAS and Giemsa stains are also useful in this regard.

The recommended diagnostic test for *C. piliforme* is PCR. A "stress test" to expose the agent through immunosuppressive treatment with corticosteroids or cyclophosphamide and that encourages clinical disease and characteristic lesions can be useful in some circumstances. Various serologic tests have been developed but are plagued by false-positive results, possibly due to nonpathogenic commensal *Clostridium* species.[118]

Depopulation of infected colonies is the most appropriate means to eliminate *C. piliforme* in most situations. Cesarean or embryo rederivation and barrier maintenance, with provisions (e.g., autoclaving) to destroy spores in feed and bedding, should be effective and could be used to preserve valuable strains or lines.[72]

Pseudomonas aeruginosa

P. aeruginosa normally inhabits the nasal cavity, throat, and lower digestive tract of mice, rats, humans, and other vertebrates. Unless acidification, hyperchlorination, or other treatments are applied, animal facility water systems may be an important source of this agent.[72,76,120]

Clinical signs do not generally occur in immunocompetent mice; however, in immunocompromised mice (e.g., irradiated or chemically immunocompromised), septicemia commonly manifests as moribund and dead animals, while others may demonstrate hunched posture, rough hair coat, emaciation, and torticollis (head tilt and circling). Gross lesions may be absent or may include abscesses that sometimes contain pus that is green tinged due to the pigments **pyocyanin** or **pyoverdin.** Inflammatory exudate can sometimes be found in the inner or middle ear of animals exhibiting torticollis.

P. aeruginosa from lesions is easily cultivated on artificial media. In routine health monitoring of apparently normal mice, it may be isolated from the intestine or the nasopharynx. A distinctive odor is familiar to microbiologists, and colonies of some strains may be green tinged. Septicemia can be confirmed by culture of the organism from blood.

For studies that do not involve immunosuppression, control or elimination of *P. aeruginosa* is not necessary. Cesarean rederivation with maintenance under gnotobiotic conditions (including provision of defined gut flora) will eliminate the infection from the colony. Proper maintenance of watering systems and sanitation measures such as water acidification or hyperchlorination, frequent flushing of automatic watering systems, autoclaving sipper tubes, bottles, cages, feed, and bedding can minimize exposure of mice to the organism, but will not eliminate established infections.[72,76,120]

Salmonella enteritidis (serotype *typhimurium*)

Of the many (approximately 1500) serotypes of *S. enteritidis* identified, serotype *typhimurium* is the most common in laboratory rodents, although infection is rare overall.

Salmonellosis refers to disease due to infection with *Salmonella* organisms. Asymptomatic carriers that contaminate food can be an important source of infection. Development and severity of disease is influenced by a number of factors, including host age, strain or genotype, nutritional status, immune status, environmental stressors, and bacterial serotype. Clinical signs generally do not occur in immunocompetent mice, but when present they are nonspecific and include hunched posture, weight loss, ruffled coat, and reduced litter sizes and birth weights. Diarrhea, which is typical of salmonellosis in other species, is not a common finding in mice. Septicemia and death may occur.

There may be no gross lesions, although enlarged spleen and enlarged mesenteric lymph nodes are common findings, along with pale foci of necrosis in the liver. Histologic changes include necrosis and pyogranulomatous inflammation in mesenteric lymph nodes, spleen, liver, and ileum and cecum (ileotyphlitis). In the liver, multiple granulomas are highly suggestive of salmonellosis. Lesions consistent with septicemia include multifocal thrombosis.[72]

For diagnosis, *S. enteritidis typhimurium* is easily cultured on artificial media. Blood or organs, such as liver and spleen, should be cultured if septicemia is suspected. In surveillance or monitoring of apparently normal animals, cecal contents are commonly used specimens.

Once infection is detected, depopulation is the usual method of control. Cesarean rederivation and barrier maintenance are also effective. Rigorous sanitation and disinfection of facilities and equipment is critical. Personnel should be advised of the zoonotic risk.

Pasteurella pneumotropica

An opportunistic pathogen than can be isolated from the oropharynx, intestinal tract, and reproductive tract of clinically normal mice, *P. pneumotropica* has been implicated in various clinical syndromes, including conjunctivitis, infections of the reproductive tract, otitis, and subcutaneous abscess formation.[72,76]

Immunocompromised animals are more susceptible to disease, which may be manifested by infertility and abortions but is more often characterized by subcutaneous swellings due to abscesses or inflammation, often around the eyes or in the inguinal region. Specific gross lesions include periorbital inflammation or abscesses and inflammation or abscess of the preputial glands or other subcutaneous sites.[72,76] *Staphylococcus aureus* is a primary differential diagnostic consideration for subcutaneous abscesses in mice.

Isolation of *P. pneumotropica* on artificial media is the primary method of diagnosis.

Treatment with the antibiotic enrofloxacin is an effective alternative to cesarean rederivation or embryo transfer for the elimination of *P. pneumotropica* in mice.[76,121]

Klebsiella pneumoniae

An opportunistic agent that can be isolated from the intestinal tracts of clinically normal mice, *K. pneumoniae* rarely causes disease in immunocompetent mice. In immunocompromised mice, clinical signs may be severe and include cervical lymphadenopathy, lethargy, and death due to sepsis.

Gross necropsy of affected animals may demonstrate pneumonia, empyema (pus in the thoracic cavity), and abscesses in any organ.[72,76]

K. pneumoniae is cultivated readily on artificial media. For monitoring of clinically normal animals, cecal contents are frequently cultured.

Control of *K. pneumoniae* can be effected by strict sanitation and disinfection of the environment and equipment. Depopulation of infected colonies followed by repopulation with animals free of *K. pneumoniae* is often required to eliminate the infection. In addition, cesarean derivation and barrier maintenance is an effective option.[72,76]

Helicobacter *species*

Species naturally infecting mice include *H. hepaticus, H. bilis, H. muridarum, H. rodentium,* and *H. typhlonicus.*[122–424] *H. hepaticus* is considered to be the most important mouse pathogen of the genus. The prevalence of *Helicobacter* spp. is unknown because of erratic surveillance for the organisms.

Helicobacter spp. in mice have been identified as causes of hepatitis in various strains of mice and of proliferative colitis or proliferative typhlocolitis in immunocompromised mice.[76] In immunocompetent mice, clinical signs associated with these changes are usually not present. *H. hepaticus* infection has been associated with hepatic carcinoma in A/JCr mice.[76,121] In immunocompromised mice, rectal prolapse secondary to a thickened, proliferative colon is sometimes observed with *H. hepaticus, H. typhlonicus, H. rodentium,* and *H. bilis* infections.

H. hepaticus selectively and persistently colonizes the bile canaliculi and cecal and colonic mucosae. Histologic changes may be

absent or subtle depending on the mouse strain, age, sex, and other factors.[125] Chronic or necrotizing hepatitis due to *H. hepaticus* occurs more frequently and more severely in male mice, and lesions are more severe in older mice. *H. bilis* has been associated with chronic, multifocal hepatitis and can be isolated from the liver, bile, and lower intestine of infected mice. Modified Steiner's silver stain may demonstrate argyrophilic spiral organisms in less than 10% of infected mice, usually in bile ducts, but does not distinguish the species of *Helicobacter.* The gastrointestinal (GI) tract of mice contains many argyrophilic organisms, so the stain is not helpful to identify *Helicobacter* in the GI tract.

Primary diagnostic considerations for necrotizing hepatitis in mice should include MHV and *Clostridium piliforme.* Differential diagnostic considerations for rectal prolapse should include *Citrobacter rodentium* and pinworms.

Diagnosis of *Helicobacter* by PCR of fecal specimens is recommended. Serologic tests to detect antibodies to *Helicobacter* spp. have been developed, but are less sensitive than PCR methods. Culture of gastrointestinal or fecal samples for *Helicobacter* is possible; however, *Helicobacter* spp. are slow growing, microaerophilic, and easily overgrown by other GI tract flora, making diagnosis by culture difficult.

Especially for studies involving the liver and GI tract, and studies involving immunocompromised mice, mice from colonies documented by PCR to be free from *Helicobacter* should be used. Cesarean derivation and barrier maintenance are effective methods to eliminate infection when used in tandem. Several treatment protocols for active infections are reported, but their long-term efficacy remains to be demonstrated.[121]

Citrobacter rodentium

Formerly *C. freundii* biotype 4280, *C. rodentium* is the etiologic agent of **transmissible murine colonic hyperplasia.** *C. rodentium* is generally considered to be an opportunistic pathogen. In some cases, antibiotic therapy may eliminate normal gut flora and permit overgrowth of *C. rodentium* in the mouse intestine.[72,76,126]

Clinical signs, when present, are nonspecific and may include ruffled coat, weight loss, lethargy, runting, perianal fecal staining, and rectal prolapse. Suckling mice are most susceptible. Strain differences in susceptibility exist, with C3H/HeJ mice more susceptible than DBA/2J, NIHS (Swiss), or C57BL/6J mice. Especially in cases

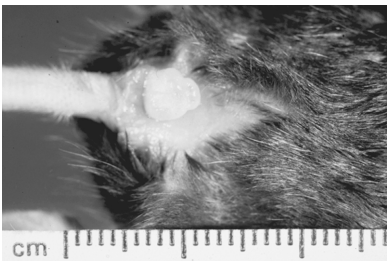


Fig. 4.4 Rectal prolapse in a mouse. This condition has been associated with infection by *Helicobacter* spp., *Citrobacter rodentium,* EDIMV, pinworms, or other conditions that cause diarrhea or straining.

with rectal prolapse, the colon may be grossly and remarkably thickened (Figure 4.4).[72,76,126]

The hallmark histologic lesion of *C. rodentium* infection is colonic hyperplasia. Generally, the descending colon is most affected; however, the entire colon and cecum may be involved, with crypt elongation and up to three-fold increase in villus height.

C. rodentium can be isolated from colon contents or feces on Maconkey agar. The agent may be found in the presence of profound clinically apparent lesions, and isolation is likely to be successful early in the disease. Identification of the organism in fecal specimens by PCR is also diagnostic.[118]

If *C. rodentium* is identified, cesarean rederivation and barrier maintenance should be effective in eliminating the infection and preserving clean stock. Neomycin and tetracyline in drinking water may reduce losses during epizootics but may not eliminate infection.[76,127]

Streptobacillus moniliformis

An agent that causes the zoonotic disease, **rat bite fever,** *S. moniliformis* is a normal inhabitant of the upper respiratory tract of wild and laboratory rats. Most infections of laboratory mice occur when there is exposure to wild rats.[128–130] The disease in humans is usually transmitted via rat bites and is characterized by fever, myalgia, vomiting, headache, and rash.

Epizootics may occur in mice with high mortality due to septicemia. Survivors often develop arthritis, which may result in amputation of affected limbs (like ectromelia), with the hindlimbs more commonly affected.

Gross lesions can include multifocal hemorrhage on serosal surfaces, multifocal necrosis in spleen, liver, and other tissues; or

enlarged spleen and lymph nodes. Histologic changes of septicemia may be subtle or there may be thrombosis and multifocal necrosis in liver, spleen, and lymph nodes.

S. moniliformis grows well on blood agar and various other artificial media. It can be isolated from blood of septicemic mice or joint fluid of arthritic mice.[131]

Depopulation of infected animals, and identification and elimination of the rat source of the infection are the safest and most appropriate means to control this zoonotic agent.

Mycoplasma pulmonis

One of the most important pathogens of rats and mice, *M. pulmonis* is primarily a pathogen of the respiratory tract. Mycoplasmas are the smallest and simplest of the bacteria. They lack a rigid cell wall and are gram negative, although they stain poorly.

Murine respiratory mycoplasmosis (MRM) is the name of the disease resulting from infection in mice and rats. Susceptibility to disease varies with mouse strain, with resistant strains including C57BL/6J and susceptible strains including C3H. Infected mice are often asymptomatic; however, clinical signs in susceptible, chronically infected animals include "chattering," dyspnea, weight loss, hunched posture, and lethargy. Immunodeficient mice are particularly susceptible to pneumonia and death, and may develop severe arthritis following infection. Infection may be widely disseminated and infections of the reproductive tract may reduce breeding performance.[72,132]

Grossly affected lungs have multifocal consolidation, and airways can be dilated (bronchiectasis) and filled with thick exudate. Histologic changes may include suppurative rhinitis, otitis media, tracheitis, bronchiectasis, and pulmonary abscesses. Perivascular and peribronchiolar lymphoid infiltrates occur in chronic disease but are not as intense as in rats with MRM. Syncytial giant cells in the respiratory epithelium are a feature of the disease in mice.

The recommended diagnostic method is PCR, and acceptable serologic tests are available as well. *M. pulmonis* can be cultured, although it requires special media, grows slowly, and is easily overgrown by other bacteria.[117]

Optimally, mice should be documented free of *M. pulmonis* before entering the facility. Depopulation of infected animals probably is the most appropriate strategy to eliminate the infection in most situations. Cesarean rederivation and barrier maintenance can be effective in preserving valuable lines of mice.[117]

Corynebacterium bovis

C. bovis is a small, pleomorphic Gram-positive rod with terminal club-shaped swellings. Colonies of the bacteria are said to resemble Chinese characters when seen microscopically.[76] The clinical disease is referred to as **hyperkeratotic dermatitis.**

Infection in immunocompetent mice is asymptomatic. Clinical signs in nude mice include flaking of the skin, which can be very severe, primarily along the dorsum, and pruritis in some animals.[76,133] SCID mice may develop mild clinical signs that include mild alopecia and flakey skin.[134,135]

The primary differential diagnostic consideration for flakey skin in nude mice is low humidity. Dermatophytosis should also be considered. *C. bovis* can be isolated on blood agar from affected skin and identified by biochemical tests.[134]

Depopulation of infected colonies and repopulation with mice documented to be free of *C. bovis* is the most appropriate strategy to eliminate the organism.

Staphylococcus aureus

S. aureus is a common normal inhabitant of the skin of many animals, including mice. **Botromycosis** refers to lesions in which grapelike colonies of *Staphylococcus* (*aureus* and other species) occur in abscesses or granulomas. **Furunculosis** in nude mice refers to abscesses or granulomas of the muzzle from which *S. aureus* usually is isolated. Disease may be precipitated by stress or immunosuppression. Entry of the organism into the body is via breaks in normal barriers (e.g., wounds).[72,76]

Clinical signs of *S. aureus* in mice include ulcerative dermatitis, swellings due to facial abscesses, or preputial and clitoral gland abscesses. Pus from the abscess is usually white or creamy.

S. aureus infection can be diagnosed by blood agar culture of samples from lesions. Alternatively, histologic examination of lesions will show suppurative inflammation with abscessation, most commonly in the skin and subcutis, although any organ can be affected. Tissue gram stains should reveal large Gram-positive cocci.

Improved and rigorous sanitation procedures and methods to eliminate traumatic lesions, such as reducing the number of mice housed per cage and elimination of mites, should help to control disease in immunocompetent animals. In immunocompromised mice, cesarean rederivation and barrier maintenance with stringent sanitation measures may be necessary to eliminate infection.[72]

Streptococcus pneumoniae

A commensal organism under most conditions, *S. pneumoniae* can often be identified in pairs or chains of Gram-positive cocci. In asymptomatic carrier mice, *S. pneumoniae* colonizes nasal passages and middle ears.[72,76]

Clinical signs associated with infection are uncommon in immunocompetent mice. When present, signs are nonspecific and may include dyspnea, weight loss, hunched posture, and sniffling. A head tilt or tremor may be present due to otitis media or interna, or meningitis. Gross lesions are usually not discernible.

Infection spreads from the lung to the pleura and pericardium, and is disseminated throughout the body via the blood. Histologic lesions of disseminated infections can include suppurative or fibrinosuppurative lesions throughout the respiratory tract and serosal surfaces (i.e., pleura, peritoneum, epicardium, and meninges). Bacterial emboli may be present in various tissues, especially the glomeruli.

Isolation of *S. pneumoniae* from suppurative lesions, blood, or spleen of bacteremic mice is diagnostic of the disease. Isolation of *S. pneumoniae* from the respiratory tract is diagnostic of infection, but because the carrier state is common, it is not diagnostic of the disease, and other causes of respiratory disease should be considered.

Cesarean rederivation and barrier maintenance are effective in eliminating infection from colonies of mice. The high incidence of asymptomatic human carriers suggests that personnel may be an important source of infection for laboratory rodents, thus steps (e.g., wearing of gloves, face masks, and lab coats) should be taken to prevent contact of mice with *S. pneumoniae* from humans.[72]

Arthropod Parasites of Mice

Of the potential arthropod parasites of mice, mites, especially *Myobia musculi,* represent the greatest concern. Mites were detected in more than 15% of SPF colonies and almost 40% of non-SPF colonies in the U.S. in 1996.[78] Mites can cause overt disease in some strains of mice, alter immune responses, and interfere with reproduction and colony maintenance because of wounds and secondary infections. That said, elimination of parasites from valuable colonies can be very frustrating.

Mites

This group includes *Myobia musculi* (Figure 4.5) and *Myocoptes musculinus* (Figure 4.6). *M. musculi* is considered to be more pathogenic because it feeds on skin secretions and interstitial fluid (but not on

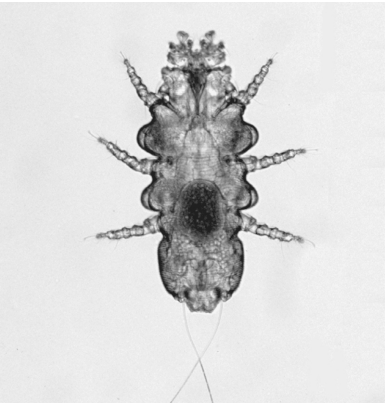


Fig. 4.5 *Myobia musculi*

blood), while *M. musculinus* feeds more superficially. Both species are nonburrowing, and mixed infestations are common. The life cycles of these parasites are direct, with all stages (egg, nymph, and adult) attached to hairs on the host. Consequently, hairless mice are not susceptible.[72,76,136] The term **acariasis** refers to mite infestation in any species.

Clinical signs vary in severity, depending upon host factors and mite species. C57BL and related strains of mice are most susceptible to severe disease, due to exuberant type 1 hypersensitivity reactions. Infestations may be asymptomatic or there may be intense pruritis, extensive alopecia, rough hair coat, accumulation of fine bran-like material (keratin debris) over affected areas, and self-trauma to the

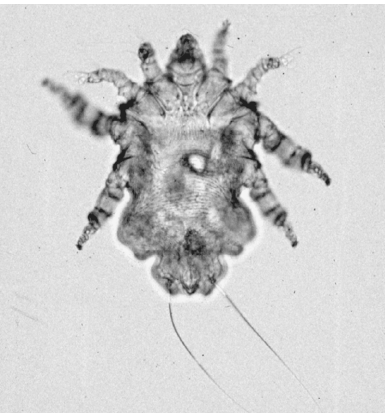


Fig. 4.6 *Myocoptes musculinus*

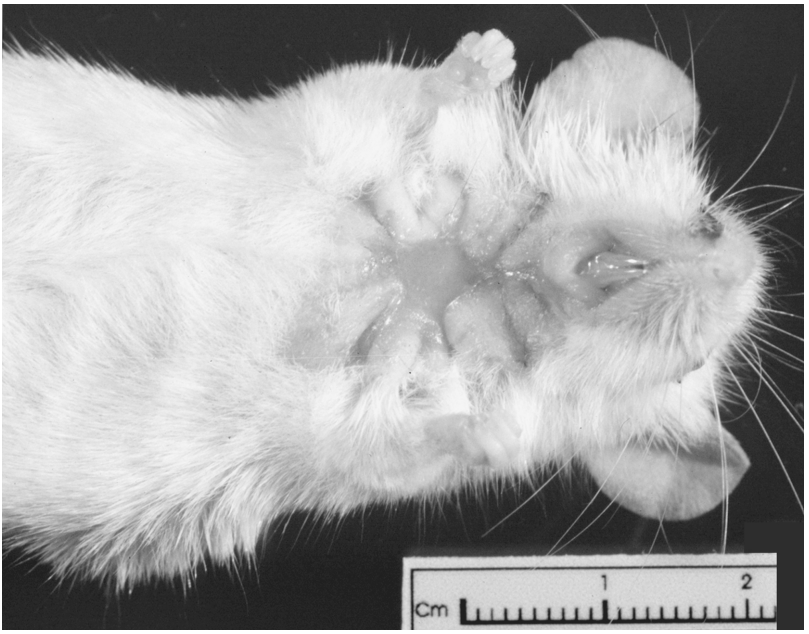


Fig. 4.7 Although it is more common in black mice of C57BL/6 origin, ulcerative dermatitis can occur in other strains of mice. In this white mouse, the lesion involves the ventral neck. Frequently, the back of the head and neck are affected. This mouse had severe cervical and axillary lymphadenopathy. It was negative for mites.

point of excoriative ulcerative dermatitis with secondary bacterial infections and auto-amputation of the ears (Figure 4.7). Mites and lesions are most common on the dorsum, primarily on the back of the neck and between the shoulder blades where the fur is dense. Regional, usually cervical, lymphadenopathy and splenomegaly may be profound (Figure 4.8).[72,76] Differential diagnoses should include barbering and dermatophytosis (ringworm). In addition, ulcerative dermatitis can occur without acariasis in C57BL and related strains, and diagnosis and treatment of the condition can be very frustrating.[72,76]

Histologic changes may be mild in resistant mouse strains, consisting of subtle epithelial hyperplasia and mild dermal infiltrates of eosinophils and mast cells. In susceptible mice, lesions can be severe and include marked hyperkeratosis, dense eosinophil and mast cell infiltration, ulcerative dermatitis, profound plasmalymphocytic hyperplasia in spleen and lymph nodes, and eventual secondary amyloidosis.[72,76]

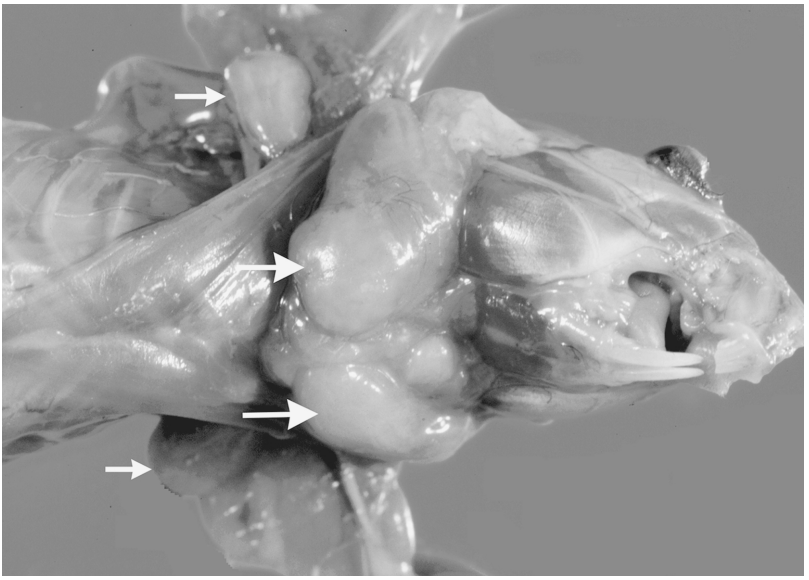


Fig. 4.8 Cervical and axillary lymphadenopathy. View of ventral neck and chest with skin removed. Large arrows indicate submandibular lymph nodes. Small arrows indicate axillary lymph node. This mouse had severe ulcerative dermatitis.

For diagnosis, direct visualization of the skin and pelage, especially of the hair bases at low magnification (10×) with a dissecting microscope, is recommended. Alternatively, one can permit a fresh cadaver to cool on black paper and then lift off motile (white) forms with cellophane tape for identification under a dissecting microscope. Evaluation of skin scrapings is not recommended.[136] *M. musculi* has an elongate body with bulges between the legs and has a single empodial claw on the second pair of legs. *M. musculinus* is oval with thick third and fourth legs, and a sucker on the tarsi.

Once a facility is infested, eradication of mites is achievable but is labor intensive. Cesarean rederivation along with barrier maintenance is the most effective method to eradicate mites. Ivermectin can be delivered in the drinking water, by orogastric gavage, or applied topically as an effective treatment.[72,137,138]

Lice

Polyplax serrata is the louse of mice. The parasite sucks blood from the host and in this way can impact the health of the animals. It is common in wild mice, but rare in well-managed research mouse colonies.[136] Infestation with lice is referred to as **pediculosis.**

Bites from lice are pruritic and can result in intense scratching and dermatitis. In severe infestations there may be anemia and debilitation.[136] Mite infestation is a major differential diagnosis with these clinical signs.

Direct visualization of the skin and pelage at low magnification (10×) with a dissecting microscope is recommended for diagnosis. Alternatively, one can allow a fresh cadaver to cool on black paper and then lift off the motile (white) forms with cellophane tape for identification under a dissecting microscope. The eggs ("nits") can be seen attached to the bases of hair shafts. Evaluation of skin scrapings is not valuable as a diagnostic tool for pediculosis.[118,136]

Cesarean rederivation along with barrier maintenance is the most effective method to eradicate lice from mouse colonies. As with acariasis, ivermectin can be used as an effective treatment.[72,137,138]

Enteric Helminth Parasites

The important enteric helminth (wormlike) parasites of mice include several nematodes and one cestode. The nematodes, both pinworms, are very common in colonies of laboratory mice, and were detected in more than 30% of SPF colonies and almost 70% of non-SPF colonies in the U.S. in 1996.[78]

Nematodes

The important nematodes of mice are pinworms. *Syphacia obvelata* is a parasite of mice, but can also infest rats, hamsters, gerbils, and wild rodents. *Syphacia muris* is a parasite primarily of rats, but can also infest mice, hamsters, gerbils, and wild rodents. For practical purposes of detection and elimination, there is no real need to distinguish these agents. They have similar life cycles and both deposit asymmetric eggs around the anus of the host mouse. *Aspiculuris tetraptera* is a parasite primarily of mice, but it can also infest wild rodents and rarely rats.[72,76,137,140]

Syphacia has a direct life cycle (i.e., does not require an intermediate host), which is completed in only 11–15 days. Adults are found primarily in the cecum and in the rectum, where gravid females deposit their eggs in the perianal region. Eggs are infectious within six hours of deposition. *S. obvelata* eggs are 36 μm wide × 134 μm long and are distinctly asymmetrical (banana shaped). *S. muris* eggs are smaller and plumper, only 20 μm wide × 75 μm long, and are only mildly asymmetrical, being only slightly flattened on one side (Figure 4.9).[72,139] *Aspiculuris tetraptera* has a direct life cycle that requires 23–25 days. Adults are found primarily in the colon where females

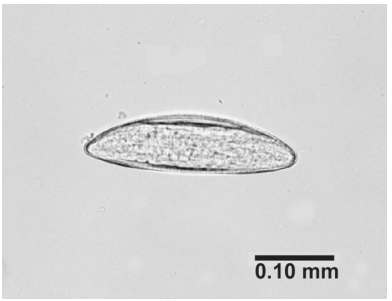


Fig. 4.9 A *Syphacia* egg found during examination of a fecal flotation sample. Note the flattening to one side. These eggs may also be found by applying transparent tape to the perianal area of the mouse, where they are deposited. Magnification 66×.

lay their eggs. Eggs leave the host on fecal pellets. The eggs become infectious after 6 to 7 days at room temperature. *A. tetraptera* eggs are ellipsoid and symmetrical, only 41 μm wide × 90 μm long, and are slightly flattened on one side (Figure 4.10).[72,139]

Oxyuriasis refers to infestation or disease by oxyurid nematodes (pinworms). Ingestion of the environmentally-resistant eggs is the primary route of infestation.

Oxyuriasis is frequently subclinical, although heavy infestations can contribute to poor condition, rough hair coat, runting, and rectal prolapse. The prevalence of pinworms in an infested colony depends on the age, sex, and host immune status. In enzootically infested colonies, weanlings develop the greatest parasite loads. *Syphacia* numbers tend to diminish with increasing age of the host, and males are often more heavily parasitized than females. Athymic nude (nu/nu) mice are extremely susceptible and may have a remarkable parasite burden, which may result in a wasting condition.[72,76,140]

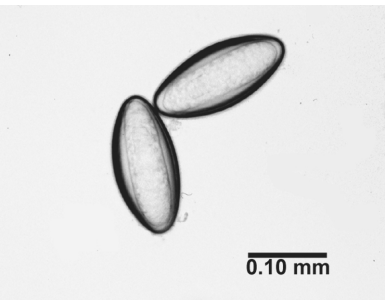


Fig. 4.10 Two *Aspiculuris* eggs from a fecal flotation. Note the bilateral symmetry. Magnification 66×.

At necropsy, adult parasites may distend the intestinal lumen in young mice or athymic nude mice. Histologic changes are usually characterized by mild infiltrates of eosinophils, mast cells, and mononuclear inflammatory cells in the lamina propria of the large intestine.

Diagnosis is by direct visualization of the contents of the cecum and colon at low magnification (10×) with a dissecting microscope. Fecal flotation in hypertonic saline solution to detect pinworm eggs is also useful. Detection of pinworm eggs by microscopic examination of cellophane tape pressed against the perianal region of the mouse and then placed on a glass microscope slide is a quick but less sensitive means of diagnosis.

Pinworm eggs are very light and will easily aerosolize, resulting in widespread environmental contamination. The eggs can survive for extended periods of time at room temperature. Once a facility is infested, eradication is achievable but is labor intensive. Cesarean rederivation along with barrier maintenance is the most effective method to eradicate these parasites.[72,76] Various treatment regimens involving ivermectin in combination with other anthelmintics, such as piperazine or fenbendazole, have been used with variable success. Elimination of resistant eggs from the environment and equipment is the major obstacle to overcome for such methods to succeed. Ivermectin can be delivered in the drinking water or via gavage, or it can be administered topically.[141–143] Consideration should be given to the possible effects anthelmintics may have on experimental parameters.[138]

Cestodes

The dwarf tapeworm, *Hymenolepis nana,* is generally uncommon in contemporary research rodent colonies. Mice, rats, hamsters, other rodents, humans, and nonhuman primates are all potential hosts; thus this agent constitutes a zoonotic concern. *H. nana* has a direct life cycle, requiring no intermediate host, although it can utilize an arthropod intermediate host (flea or beetle).[72,76,136]

Cestodiasis refers to infestation with tapeworm parasites. Infestation with *H. nana* is usually subclinical, but heavy infestations may cause weight loss and growth retardation.

Diagnosis is made by detection of cestode eggs through fecal flotation. The embryonated eggs are oval, 30 to 60 μm in diameter, and have three distinctive hooks.[144] Threadlike adult tapeworms, usually less than 1 mm in diameter and up to 40 mm in length, can be found in the small intestine during necropsy. Histologically, the presence of

cysticerci (larval tapeworms) within the lamina propria of intestinal villi is diagnostic of *H. nana,* but these are usually very sparse.[72,140]

Prevention of cestodiasis by procurement of parasite-negative mice is strongly recommended. Depopulation of infested colonies is the most appropriate strategy to eliminate *H. nana,* particularly because of zoonotic concerns. Cesarean rederivation along with barrier maintenance is also an effective means of elimination. Ivermectin treatment is ineffective.

Protozoal Parasites

Cryptosporidium muris

A coccidian parasite of the mouse stomach, *C. muris* is considered to be commensal or only mildly pathogenic in mice.[145] Normal, immunocompetent mice clear the infection within several weeks, and there are generally no clinical signs associated with infection. Immunocompromised mice may not readily clear the infection and may continue to shed infectious oocysts within the feces.[146]

The organisms are approximately 5 μm in diameter and found in the gastric brush border or within the gastric glands. Inflammation is usually absent or mild.

Cesarean rederivation and barrier maintenance can be used to eliminate infections from colonies of mice.

Cryptosporidium parvum

The term **cryptosporidiosis** usually refers to infection with this pathogen (versus *C. muris*), which is also a cause of diarrhea in neonatal calves and immunocompromised humans.

Infection of immunocompetent mice is subclinical, and the infection is readily cleared. In immunocompromised mice, the infection may persist and result in wasting, icterus, and death.[147,148]

The organisms are approximately 5 μm in diameter and can be found in and on the mucosal surfaces of the intestine, particularly the ileum, with little associated inflammation. Cholangitis, periportal hepatitis, and cholecystitis are associated with icterus and death.[148,149]

Acid fast staining of intestinal contents or feces and histologic sections can facilitate identification of the organism.

Depopulation of infected groups of animals and disinfection of premises is a reasonable strategy to eliminate *C. parvum* in most situations. Cesarean rederivation along with barrier maintenance is another effective approach.

Giardia muris

A flagellated intestinal protozoan, *G. muris* infections are occasionally detected in laboratory rodents. **Giardiasis** is the term used to refer to infection with this parasite. Most infections in immunocompetent mice are asymptomatic, and the infection is readily cleared. When apparent, clinical signs are nonspecific and include weight loss, lethargy, poor growth, rough coat, and abdominal distension. In athymic nude mice, symptoms may be more severe and include diarrhea and death. Shedding of infectious cysts may be prolonged.

Grossly, the small intestine may be distended with pale fluid. Histologic findings include blunting of intestinal villi and presence of flattened, pear-shaped trophozoite forms of the parasite, approximately 5 μm in diameter, on the epithelial surfaces at the villous base or free in the lumen.[72]

Diagnosis can be made by examination of wet mounts of small intestinal scrapings for the pear-shaped trophozoites. The parasite can sometimes be seen moving with a characteristic rolling motion. Histologic examination of the small intestine is another option for diagnosis.

Depopulation of infected colonies along with rigorous disinfection of rooms and equipment is an appropriate strategy to eliminate *G. muris* in most situations, although cesarean rederivation with barrier maintenance is another option.

Spironucelus muris (Hexamita muris)

Infections by *S. muris,* a flagellated protozoan, are generally asymptomatic in immunocompetent mice and rats. Weanling and immunocompromised mice may develop nonspecific clinical signs, including diarrhea, weight loss, dehydration, rough coat, lethargy, abdominal distension, hunched posture, and death.

Histologically, dilated hyperplastic crypts filled with granular eosinophilic material composed of tiny, elongate *S. muris* trophozoites are characteristic, primarily in the duodenum and gastric pylorus (Figure 4.11). The organism stains poorly with hematoxylin and eosin, and PAS and silver stains may assist in diagnosis of mild infections.[72]

Diagnosis can be made by microscopic examination of wet mounts of pyloric or duodenal scrapings for the typical elongate, torpedo-shaped trophozoites that measure approximately 4 μm diameter × 12 μm long. Cysts can also be found in the large intestine and feces and are 4–7 μm in diameter and egg-shaped. Identification of the organism by histologic examination of the pylorus and duodenum is another approach.

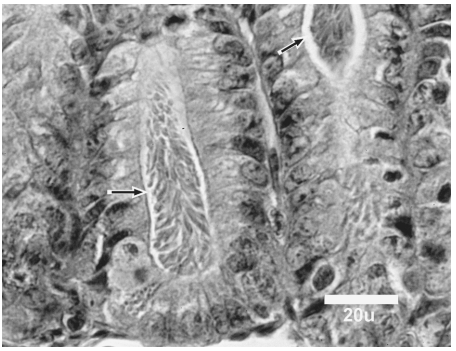


Fig. 4.11 H & E skin of small intestine demonstrating numerous elongate *Spironucleus muris* trophozoites (arrows) between villi and within a crypt. Magnification is 132×, bar 5 20 μm.

Treatment with dimetridazole in the drinking water may control, but not eliminate, infection. Depopulation of infected groups of animals along with disinfection of the premises and equipment is an appropriate strategy for elimination, as is cesarean rederivation with barrier maintenance.

Trichomonas muris

A flagellated protozoan that occurs in the large intestine of rodents, *T. muris* is commensal and not associated with any disease. Trophozoites measure 12–20 μm in diameter and can be found in the feces, cecum, and colon. Control measures are not usually undertaken due to the incidental nature of the organism.

Entamoeba muris

Like *T. muris*, *E. muris* is a commensal organism found in the large intestine of rodents (Figures 4.12 and 4.13). *E. muris* is an amoeba that is not associated with any disease in mice. Cysts can be found in the feces and measure 9–20 μm in diameter. Control measures are not usually undertaken due to the incidental nature of the organism.

Eimeria *species*

Several *Eimeria* species have been reported in mice, but infections are rare in laboratory mice and mostly associated with wild mice. **Coccidiosis** is the term used to describe infection with *Eimeria.* In wild mice, coccidiosis has been reported to cause colitis and runting in juvenile animals.

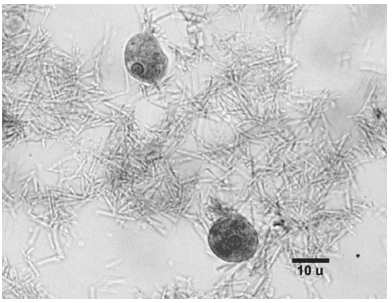


Fig. 4.12 Photomicrograph of *Entamoeba muris* in the colon of a mouse. Numerous bacilli surrounding the entamoebae are usual in colon contents. Magnification 132×.

Encephalitozoon cuniculi

E. cuniculi is a microsporidian parasite that can infect a wide range of hosts, including laboratory mice and rats. Its prevalence is considered to be low in laboratory mice.

Infection is usually asymptomatic in immunocompetent rodents. In contrast, athymic nude mice can experience high mortality with infection.

When present, pitting of the renal surface is the only gross lesion. Histologic lesions may be seen in both the kidney and the brain. In the kidneys, lesions consist of intracellular birefringent spores (, 3 μm in diameter) in renal tubule epithelium and inflammatory changes, with eventual focal destruction of tubules and replacement by fibrous connective tissue, resulting in pitting of the renal surface. The spores are Gram positive. When infected tubule epithelial cells rupture, spores enter the tubules and are shed in the urine. Meningoencephalitis is sometimes also present.

Klossiella muris is a coccidian parasite that once was common in laboratory mice, but has largely been eliminated from most well-managed colonies. All developmental stages of *K. muris* develop within cells of the renal tubules, and oocysts are shed in the urine. Because of the similarity, *E. cuniculi* must be distinguished from *K. muris.* Most of the developmental stages of *K. muris* are much larger than *E. cuniculi* spores and can be distinguished on this basis.

Serologic tests or histology are the recommended techniques for diagnosis of *E. cuniculi.*

Depopulation is an appropriate strategy for elimination of *E. cuniculi* from mice. Cesarean rederivation along with barrier maintenance is likely to be effective as well.

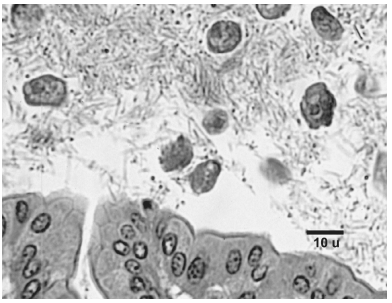


Fig. 4.13 Photomicrograph of *Entamoeba muris* in the lumen of the colon. Magnification 132×.

Fungal Agents

Two fungal agents are commonly considered as agents with importance to the health of mice:

Pneumocystis carinii

P. carinii is classified as a fungus, although it has some protozoan characteristics. It inhabits the respiratory tracts of many species, including laboratory mice and rats. It is a pathogen only under conditions of induced or inherent immunodeficiency. Transmission is via inhalation of infective cysts. Differences in host specificity and susceptibility have been shown, suggesting that organisms infecting mice originate from other mice and not from other species (including animal caretakers).[72,76,150]

Clinical signs are absent in immunocompetent animals; however, clinical signs in immunosuppressed (e.g., nude, scid mice) include wasting, rough hair coat, dyspnea, cyanosis, and death. Differential diagnoses for dyspnea and pneumonia in immunocompromised mice include infections with *M. pulmonis,* Sendai virus, or PVM.

Histologic changes include thickening of alveolar septa, interstitial pneumonia, and distension of alveoli with foamy, eosinophilic material composed of *P. carinii* organisms and cell debris. Gomori's Methenamine Silver (GMS) stain will reveal numerous *P. carinii* cysts that measure 3–5 μm in diameter.

Disease in infected immunocompromised animals can be controlled but not eradicated by long-term, intermittent administration of trimethoprim sulfamethoxizole in the drinking water. Placental transmission does not occur, so cesarean rederivation is effective in eliminating the agent.[72,76]

Dermatophytes

Trichophyton mentagrophytes is the most common agent in both subclinical and overt **dermatophytosis** ("ringworm") in mice. Other *Trichophyton* spp. and *Microsporum* spp. have been implicated less frequently.[72]

Many animals, including laboratory mice, carry dermatophytes without any signs of disease. On occasion, however, some will exhibit dermatophytosis characterized by hair loss with crusty material usually around the head, although lesions may occur anywhere on the body. In some cases lesions are pruritic, and mice will scratch vigorously. Differential diagnoses for hair loss include barbering and acariasis, although the presence of crusts is suggestive of dermatophytosis. In nude and scid mice, hyperkeratotic dermatitis due to infection with *Corynebacterium bovis* should be considered.

Diagnosis is made by culturing hair shaft samples from the edge of the lesion in special fungal media for dermatophytes (Sabouraud's or dermatophyte test media). Histologic examination of lesions will demonstrate dermatitis with hyperkeratosis. Fungal elements can be demonstrated with periodic acid Schiff (PAS) stain or silver stains.[72]

Depopulation of infected colonies along with disinfection of premises and equipment is the most appropriate means to eliminate dermatophytes in most situations. Cesarean rederivation with barrier maintenance would also be a possible approach.

Miscellaneous Conditions

***Barbering*—**As described in Chapter 1, barbering is characterized by focal loss of hair or whiskers with no wounds and a very sharp margin between the areas of hair loss and normal hair. The condition is due to depilation of the hair of a submissive mouse by a dominant mouse as part of the hierarchical structure within the cage. Barbering results in no debilitation, and the hair coat will grow back if the submissive mouse is moved to a separate cage. This approach should be used only after consideration has been given to the social needs of the animals.

***Dental malocclusion*—**Because they are continuously erupting, the incisors of mice will overgrow if they are not normally aligned and do not properly occlude. The condition may have an inherited component. Often, mice lose weight as they are unable to properly chew and masticate food. It is possible to clip the overgrown teeth with a nail clipper, although the tooth may fracture. Preferably, the overgrown teeth are reduced with a dental bur.

***Bite wounds*—**Mice placed in groups often fight as the social hierarchy of the cage is established. In general, male mice are more aggressive than females. Bite wounds often occur on the neck, back, and feet. Aggressive mice should be separated from others. Bite wounds can be treated with topical antibiotics or systemic antibiotics, as described in the section "General Treatment of Open Skin Lesions" later in this chapter. Severely wounded mice should be euthanized.

***Neoplastic disease*—**Mice develop a variety of neoplastic diseases, many of which are significantly more common in some strains of mice than others.[151] As with other species, cancer in mice is more common in older individuals. In addition, some cancers of mice are associated with viral infections or exposure to chemical carcinogens. In addition to neoplasias caused by MuLV and MMTV as described earlier, another relatively common neoplasm is **pulmonary adenoma.** Pulmonary adenomas usually arise from either type 2 pneumocytes or from Clara cells of the terminal bronchioles. The tumor is more common in some strains of mice (e.g., A strain) than others (e.g., C57BL). As with many neoplasms, pulmonary adenomas occur with increasing prevalence as the age of the mouse increases. Often, the tumors appear as distinct, whitish nodules on the pleural surface of the lung. The tumor usually is found incidentally at necropsy with no associated clinical signs.

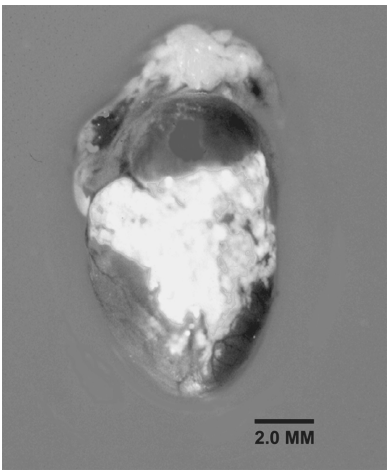


Fig. 4.14 Epicardial mineralization in an 11-month-old BALB/C mouse. The white (mineral) material covers the epicardial surface and infiltrates the underlying myocardium. In this strain, the right ventricular free wall typically is affected earliest and most severely. DBA and several other strains are also susceptible to this condition.

***Epicardial mineralization*—**Occasionally at necropsy, white, granular material is observed on the surface of the heart. Often, this finding represents epicardial mineralization, deposition of mineralized material in the superficial tissues surrounding the heart (Figure 4.14). Some strains of mice, such as DBA/2, are more predisposed to this condition than other strains. Clinical disease does not generally result from epicardial mineralization.

health surveillance and monitoring

Effective health management and care of large numbers of mice maintained at high population density is partially achieved through periodic colony health surveillance. Evaluation and documentation of the microbial status of the colony is a critical component of the overall animal care program. The degree to which such evaluation is carried out will vary with the character and goals of the institution.

A basic component of the health surveillance program should begin with monitoring of **vendor health reports.** Most reputable vendors supply information related to the routine monitoring for infectious pathogens within their colonies. Institutions should scrutinize such reports to assure that mice acquired from vendors are free of serious infectious pathogens. Similarly, mice received from other research institutions should be preceded by paperwork detailing the recent health monitoring history of the colony from which they have been obtained. It is typical to quarantine newly received mice and in some cases to rederive mice, especially from noncommercial sources, into the barrier.

Health surveillance programs typically include batteries or panels of tests to determine microbial or health status, performed at specific frequencies. Samples are generally obtained from either animals which are part of the colony or **sentinels,** animals that have been exposed to the colony for the specific purpose of health monitoring. Colony-raised animals are preferred, since there is always a chance that sentinels might introduce an unwanted agent into the colony. If the colony is being evaluated for a specific pathogen, it is sometimes advisable to use a mouse strain that is particularly susceptible to that agent as the sentinel. For example, if mites are a concern, B6 mice might be a good choice for a sentinel, since they tend to develop overt disease rapidly. If nematode parasites are a concern, athymic nude mice might be a good choice, because they rapidly develop heavy parasite burdens. It is worth noting that athymic nude mice do

not typically make good sentinel animals, since they do not mount good antibody responses for serologic testing.

Practices to enhance exposure of sentinels to infectious material include removing the filter tops from cages (if used) and administering a small amount of dirty bedding from cages of colony animals to the cages of the sentinels.[152] In some cases, contact sentinel animals are placed in the cage with the potentially infected colony animals. This strategy may be necessary to detect agents of low infectivity. It is advisable to allow approximately four weeks of exposure to the colony before evaluation is performed.

Testing usually involves evaluation of sentinels (or colony animals if available) for endo- and ectoparasites; necropsy with gross and histologic examination for signs of disease; cultures of appropriate samples for some bacterial pathogens; and evaluation of serum (serologic tests) or other samples (PCR) for infectious pathogens. Again, the degree to which sentinels are evaluated and the specific pathogens considered will depend upon the overall needs of the research facility and the investigator.

Frequency of testing varies with the situation. In a small, closed facility in which new animals are seldom introduced from outside, a single annual evaluation with evaluation of any animals that become ill might be sufficient. In production facilities, where documentation of "clean" or specific pathogen-free status is critical, short "core" panels of evaluation may be done every two weeks, with larger panels performed on a less frequent basis. Most research facilities fall somewhere in between these two extremes of testing frequency, and quarterly evaluations are fairly common.

The number of animals to be sampled varies according to population size, the presumed prevalence of the agent(s) that are being tested for, and practical or economic constraints. It is not uncommon to include a minimum of three or four mice to test each group of mice, although considerable variation exists in practice.

testing of biological materials

Biological materials such as cells, sera, transplantable tumors, and virus stocks have all been implicated as potential sources of infectious pathogens in mice. For this reason, it is critical that biological materials that are to be administered to mice be evaluated to assure freedom from infectious murine pathogens. The **mouse antibody production (MAP) test** is effective in testing for the presence of murine

viruses or other agents of concern. Mice that are defined as SPF for the agents to be tested are inoculated with the test material, and then their serum is evaluated two to three weeks later for antibodies to the pathogen(s) of concern. PCR testing is another means to evaluate biological materials for evidence of infectious pathogens. The test is rapid, utilizes fewer animals, and may be cost effective in many situations.

rederivation

For many types of infectious agents, rederivation is the most reasonable means to eliminate the infection from the colony. There are two common methods of rederivation:

Cesarean rederivation

This method involves transfer of pups from the infected female to an uninfected foster mother.[153,154] Variations on the basic theme of transfer of pups to a foster mother have been used successfully. To perform cesarean rederivation, the following basic points should be considered:

***Foster mother*—**A specific pathogen-free foster mother with a litter of pups nearly the same age as the unborn pups should be used. Usually, timed matings are planned such that the foster mother delivers her litter one to three days prior to the expected birth of the litter to be rederived. Lactating females of many strains of mice will readily accept and nurse pups that are not their own, and many investigators have successfully used ICR and BALB/C mice as fosters. Typically, the pups from the foster mother are removed and euthanized immediately prior to placement of the litter to be rederived.

***Procedures*—**After the litter of the foster mother has been removed and euthanized, the pregnant mouse from the group to be rederived is euthanized. This should be done as close as possible to the time of expected parturition. Euthanasia is best performed by physical means, such as cervical dislocation, since pharmacologic compounds may influence the viability of the pups, particularly those of fragile strains. The uterus is removed using aseptic procedures and transferred to a sterile petri dish. The pups are then delicately removed from the incised uterus, usually within a biological safety cabinet, a laminar flow hood, or an isolator. Excess mucus and secretions can be removed from the face of the pups with a sterile, moistened swab,

and respiration stimulated by gentle rubbing of the pups with soft, sterile gauze. The pups can then be placed with the foster mother. Abundant bedding and nesting material should be provided to assure warmth of the pups. In some cases, it is advantageous to direct a drop or two of urine from the foster mother onto the transferred pups, since this may lead her to identify them as her own.

Embryo Transfer

For many pathogens, it is feasible to perform rederivation through embryo transfer. The specific procedures are described in detail elsewhere, but can involve either collection of embryos from donor mice[155] or superovulation followed by *in vitro* fertilization.[156] In either case, embryos are transferred to specific pathogen-free surrogate mothers under aseptic conditions.

treatment of disease

Drug Dosages

Treatment of sick mice should be implemented under the direction of a qualified veterinarian, following appropriate diagnostic measures. A short list of drugs that have been used in mice is provided in Table 4.3. Dosages in Table 4.3 are expressed per unit of body weight as shown. Abbreviations for route of administration are PO (oral), IV (intravenous), IM (intramuscular), IP (intraperitoneal), and SC (subcutaneous). Abbreviations for frequency of administration are SID (once daily), and BID (twice daily).

General Treatment of Open Skin Lesions

Mice may develop open skin lesions for a variety of reasons, including fighting and self-trauma resulting from infestation with ectoparasites. In all cases, it is important to keep the lesion free of contaminating debris. In addition, it is advisable to clean all open lesions at least every other day with an antiseptic solution such as betadine. Whether or not the inciting cause of the lesion was a bacterial pathogen, any open wound is susceptible to secondary bacterial infection. For this reason, application of a topical antibacterial ointment should be considered. In addition, one may wish to perform bacterial culture on lesions that have drainage suggestive of infection. It is not always necessary to suture or otherwise close draining lesions. To the contrary, drainage should be permitted as part of the normal healing process.

TABLE 4.3: DRUGS COMMONLY USED IN MICE

Drug	Dosage Information	General Application
Amikacin	100 mg/kg, SC, BID	Bacterial infections
Atropine	0.05 mg/kg, IM or SC or IV	Parasympatholytic, which decreases respiratory secretions and relaxes smooth muscle
Chloramphenicol succinate	50 mg/mg, SC or IM, BID	Bacterial infections
Dexamethasone	0.6 mg/kg, IM	Anti-inflammatory
Doxapram	5–10 mg/kg, IV	Respiratory stimulant
Enrofloxacin	85 mg/kg, SC, BID,14 days; 5 mg/kg in drinking water (deionized), 14 days	Bacterial infections
Ivermectin	0.2 mg/kg, PO or SC, every 7 days for 3 weeks; 1% ivermectin diluted 1:10 in water, 1–2 ml applied as a mist into cage, every 7 days for 3 weeks; 8 mg/ml in drinking water for 4 days on, then 3 off, for 4 treatments	Ectoparasites, oxyurids
Metronidazole	2.5 mg/ml of drinking water, for 5 days	Endoparasites
Piperazine citrate	5–7 mg/ml of drinking water, for 14 days	Oxyurids
Prednisolone	0.5–2.2 mg/kg, SC or IM	Anti-inflammatory
Tetracycline	3–5 mg/ml of drinking water, 7 days; 20 mg/kg, PO, BID	Bacterial infections
Yohimbine	0.5–1.0 mg/kg, IV	Reversal of xylazine

disease prevention through sanitation

Practicing proper sanitation is an important adjunct to control of infectious disease in animals, including mice. Cages should be routinely cleaned and disinfected. Instruments and equipment used on more than a single animal should be cleaned and disinfected between mice to the greatest practical extent. The use of disposable gloves will also facilitate control of infectious disease. Personnel should wash their hands after handling animals suspected of harboring infectious agents. Optimally, mice infected with pathogens should be isolated from noninfected animals.

anesthesia and analgesia

Anesthesia is derived from the Greek *anaisthesis*, meaning "loss of sensation."[157] With respect to surgical anesthesia, the loss of sensation is reversible, and it may involve the entire body or only a specific part or region of the body. It may or may not be accompanied by loss of conscious awareness. The goal of anesthesia is to eliminate the perception of pain, to immobilize the animal to permit some manipulation, or both. These goals must be accomplished without posing a serious threat to the life of the animal. In addition, to be useful in a research setting, the anesthetic must have as little effect as possible on the parameter being studied. No single anesthetic or combination of drugs or techniques is the best choice for all situations. The key to choosing a method of anesthesia is to give careful thought beforehand to both what you want the anesthetic to do and what you *don't* want it to do. Consultation with a veterinarian can then help you decide what method is likely to work the best. It is important to remember that no anesthetic is both 100% effective and absolutely safe.

The following variables should be considered when selecting a method of anesthesia:

1. **The animal**—Mice vary significantly in their sensitivity to both the desired and undesired effects of anesthetics. Age, body composition, health status, temperament, and sex may all affect the response to an anesthetic, and remarkable variability may be seen in the responses of some mice of different stocks/strains.
2. **The procedure**—Anesthetics vary in their duration of effect and degree of analgesia provided. Thus, consideration should be given to the length of the procedure for which the animal will be anesthetized and the amount of pain that might result.
3. **The laboratory**—In this sense, the laboratory is the environment in which the animal will be anesthetized. What kind of equipment is available for anesthetic administration (e.g., precision anesthetic vaporizer vs. bell jar)? Will someone be able to closely monitor the condition of the anesthetized animal? Is there any equipment available for monitoring the condition of the animal? Will someone be available who knows what to do if the animal fails to respond satisfactorily to the anesthetic or experiences a severe adverse reaction? If the answer to many of these questions is "no," then it would be preferable to choose a drug or method that can be injected, has a wide margin of safety, or both.

4. **Postprocedural Use of the Animal**—Most drugs will have some kind of lingering effect on the animal that may or may not be desired. For example, some drugs (e.g., pentobarbital) will have a prolonged depressant effect on respiration, temperature regulation, or both. If the mouse cannot be monitored and kept warm after the procedure, the postprocedural mortality rate might be high with these drugs. If it will be difficult to monitor the animal closely after the procedure, it would be desirable to choose a drug that has a low potential for a long or difficult recovery from anesthesia. Alternatively, a drug could be chosen that can be reversed by administration of a specific antagonist. Anesthetics may also have other types of lingering effects. For example, pentobarbital can have a prolonged effect on the metabolism of other drugs.

Methods and Drugs

There are many drugs and methods available for mouse anesthesia and many ways in which various drugs and methods can be combined to improve the outcome. The reader is referred to an excellent review of this topic by Wixson and Smiler.[158] Some of the more commonly used compounds for anesthesia of mice are described below and summarized, with doses, in Table 4.4.

TABLE 4.4: COMMONLY USED COMPOUNDS FOR ANESTHESIA OF MICE

Anesthetic Compound	Dosage	Route of Administration
Inhalant anesthetics (halothane, isoflurane, methoxyflurane, ether)	1–4% (to effect)	Inhalation
Pentobarbital	50–90 mg/kg (diluted 1:9 in sterile saline)	IP
Thiopental	25–50 mg/kg	IP
ETMU	80 mg/kg	IP
Ketamine 1 xylazine	100 mg/kg (ketamine) 1 10mg/kg (xylazine	IP or IM
Ketamine 1 xylazine 1 acepromazine	30 mg/kg (ketamine) 1 5 mg/lg (xylazine) 1 1 mg/kg (acepromazine)	IP or IM
Ketamine 1 medetomidine	75 mg/kg (ketamine) 1 1 mg/kg (medetomidine)	IP
Tiletamine/zolazepam	80–100 mg/kg	IP
Tribromoethanol	180–250 mg/kg	IP

Inhalant anesthetics

Inhalant anesthetics are delivered to the patient as gasses or vapors. In general, these agents act by depressing transmission of nerve impulses in the brain, which results in a blocking of the transmission of messages from the brain to the rest of the body. This results in a loss of motor control, depression of the cerebral cortex, and loss of consciousness.

The most commonly used inhalant anesthetics for mice are **halothane** and **isoflurane.** Two other inhalant anesthetics—**methoxyflurane** and **ether**—are less commonly used, methoxyflurane because it is no longer commercially available and because of its many disadvantages. All of these drugs are delivered by inhalation, and two of them—halothane and isoflurane—can be safely administered only with the use of specialized anesthetic vaporizers that precisely regulate the concentration of anesthetic in the inhaled air or oxygen.[159]

Initial anesthesia of mice with gaseous anesthetics is generally accomplished by placing the animal in an induction chamber. This is a small chamber with a tight-fitting lid. Halothane or isoflurane in the appropriate concentration is piped into the chamber from the anesthetic vaporizer (Figure 4.15). Once the mouse is anesthetized, it is removed from the chamber, and anesthesia is maintained by delivery of the gas through a face mask, which should fit as snugly as possible around the nose of the mouse (Figure 4.16). It is also possible to deliver anesthetic to a mouse through a tube inserted into the trachea,[160] although this is seldom done for recovery procedures as the intubation procedure requires special miniaturized equipment and skill to perform safely. Whenever inhalant anesthetics are used, it is essential that appropriate measures be taken to prevent exposure of human personnel to anesthetic vapors. This may be accomplished by performing the procedure in a properly ventilated fume hood or by using any of a variety of scavenging systems.[159]

Ether and methoxyflurane are most often administered by the "open drop" technique, which involves placement of the mouse in a closed chamber (often a glass bell jar or syringe casing) with an anesthetic-soaked gauze sponge or cotton ball. The mouse must not be allowed to come into direct contact with the sponge, so the mouse is often placed on a wire grid just above the anesthetic-soaked material. Once the mouse is anesthetized, anesthesia is maintained using a nose cone (often a syringe casing) or face mask containing an anesthetic-soaked sponge. The "open drop" approach is risky and

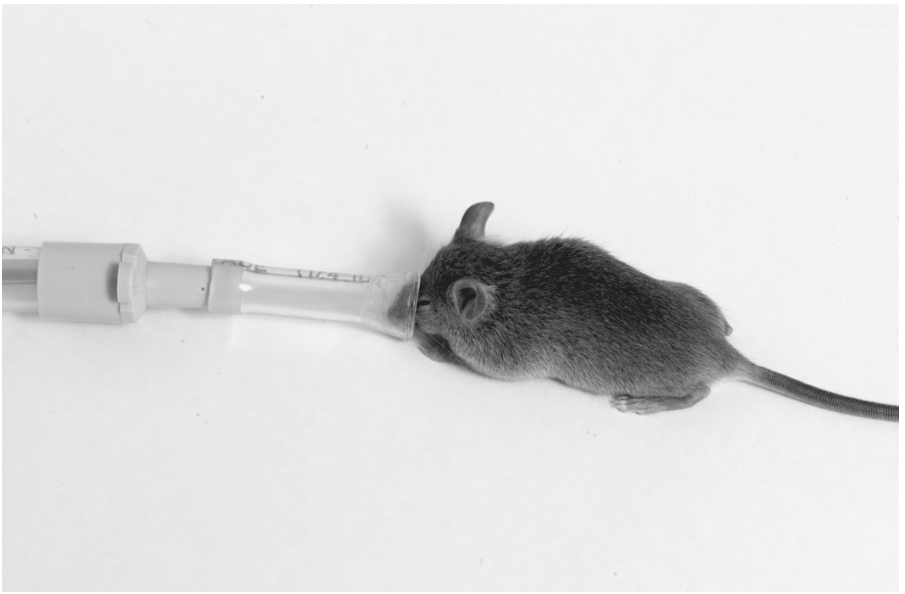


Fig. 4.16 Homemade mask for maintaining anesthesia following induction.

requires careful attention to the mouse to avoid inadvertent anesthetic overdose and death. Scavenging of environmental anesthetic vapors is essential to protect human health.

Compounds used for inhalational anesthesia include:

1. **Halothane** (Fluothane®)
   Advantages of halothane include:
   - Comparatively inexpensive and effective in mice
   - Suppresses salivary and bronchial secretions

   Disadvantages of halothane include:
   - Elevated cerebral blood flow and intracranial pressure
   - Hypotension and myocardial depression with bradycardia
   - Sensitization of the myocardium to the dysrhythmogenic effects of catecholamines
   - Dose-related respiratory depression with compensatory increase in respiratory rate
   - Subclinical hepatic necrosis is common, although clinically significant hepatic necrosis is rare.
   - Poor analgesia and muscle relaxation.

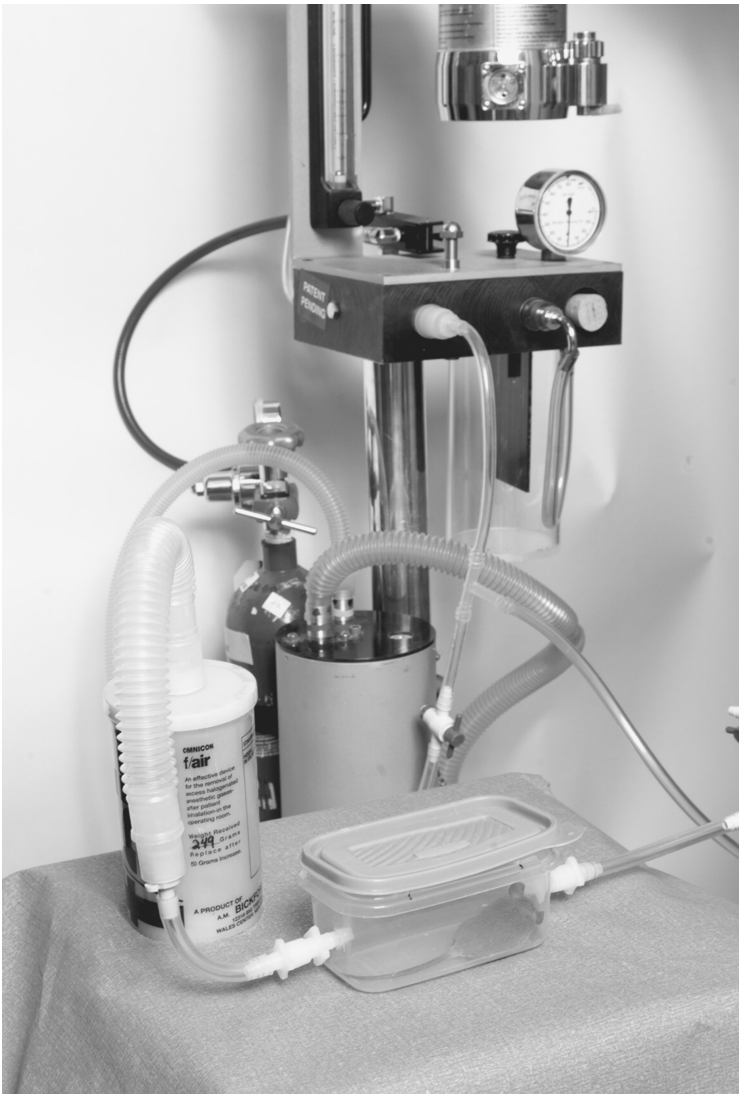


Fig. 4.15 A typical setup for inhalation anesthesia of mice with a commercially available precision vaporizer with homemade induction chamber for induction of anesthesia.

- Possible malignant hyperthermia.

Halothane used to be considered the inhalant anesthetic of choice for those using precision vaporizers (except for intracranial procedures). It is still a reasonable choice, although isoflurane is generally preferable.

2. **Isoflurane** (Forane®)
   Advantages of isoflurane include:
   - Rapid induction and recovery
   - Preferred to halothane for intracranial surgery (less effect on cerebral blood flow and cerebrospinal fluid pressure).
   - Reduced effects on cardiovascular parameters compared to halothane
   - Less induction of microsomal enzymes than halothane
   - Good muscle relaxation

   Disadvantages of isoflurane include:
   - Expensive
   - Pungent odor (can cause animals to hold breath during induction)
   - Increased airway secretions and reflexes
   - Requires vigilant monitoring, since the depth of anesthesia may change rapidly

   With the proper vaporizer and scavening equipment, isoflurane is probably the most desirable anesthetic for mice, especially for long procedures. It is important that the mouse be monitored carefully to avoid overdose, however.

3. **Methoxyflurane** (Metophane®, Penthrane®)
   Advantages of methoxyflurane include:
   - Can be safely used by "open drop" technique
   - Less induction of microsomal enzymes than halothane or ether (more than isoflurane)
   - Profound analgesia, good muscle relaxation
   - Less cardiovascular depression than ether, although hypotension may develop with prolonged anesthesia
   - Less respiratory depression than halothane

   Disadvantages of methoxyflurane include:
   - Long induction and recovery times
   - Potential for nephrotoxicity with prolonged or frequent use in older animals and in animals given other nephrotoxic drugs, such as aminoglycoside antibiotics

   Despite very slow induction and recovery compared to newer inhalant anesthetics, methoxyflurane offers many advantages.

Before it was taken off the market, it was considered an excellent choice to replace ether for those who had no access to precision vaporizers.

4. **Ether**

   Advantages of ether include:

   - Can be safely used by open drop method
   - Good analgesia and muscle relaxation
   - Stimulates respiration at all but deepest planes of anesthesia

   Disadvantages of ether include:

   - Flammable and explosive (even in animals recovering from ether anesthesia or carcasses of animals that were anesthetized with ether immediately prior to death). Because of this, explosion-proof fume hoods and carcass coolers/freezers must be used.
   - Prolonged induction and recovery times
   - Causes excitement during induction and recovery, which may be intense
   - Respiratory irritant
   - Increases bronchial secretions
   - Causes myocardial depression which may progress rapidly, leading to sudden death
   - Induction of hepatic microsomal enzymes (15% of the dose undergoes metabolism by liver)

   The disadvantages of ether outweigh the advantages. It is advisable to select another agent for inhalant anesthesia.

Injectable anesthetics

Barbiturates

The barbiturate anesthetics are injectable drugs that appear to interact with specific receptors in the central nervous system to inhibit the transmission of nerve impulses. They can be used to produce all levels of clinical depression, from hypnosis to coma. The following are general characteristics of barbiturates:

- They have a relatively narrow margin of safety, with wide variability in the effective and lethal doses. Mortality with light anesthetic doses is possible.

- All cause respiratory depression, which increases with the dose of the drug and may be severe in unborn pups (if the drug is administered to the pregnant dam). They also cause disturbances in respiratory rhythm. Complete cessation of breathing (apnea) occurs commonly, especially after intravenous administration.
- All cause cardiac depression, increases in cerebral blood flow and intracranial pressure, and possible cardiac arrhythmias.
- They may cause excitement during induction, especially if the drug is injected too slowly.
- They have no analgesic effect and may actually increase the perception of pain when used at subanesthetic doses.[161]
- Muscle relaxation is moderate.
- They interfere with the regulation of body temperature, which may lead to significant hypothermia.
- They must be administered IV or IP. Administration by the SC or IM routes will result in irritation or even necrosis at the site of injection.

Specific examples of barbiturates include:

- **Pentobarbital** is a short-acting barbiturate, with a dose-dependent duration of effect. High doses provide 20–45 min of light surgical anesthesia. There is considerable variation in response with respect to mouse strain, age, sex, bedding, environmental temperature, and nutritional status.[158]
- **Thiopental and Thiamylal** are ultrashort-acting thiobarbiturates. They may cause respiratory depression and transient apnea. These compounds are adequate for minor surgery, but are not commonly used in mice.
- **ETMU** (Inactin®) is a longer-acting barbiturate with a duration of anesthesia of up to several hours. It produces a variable depth of anesthesia that is often inadequate for surgery. This compound is more commonly used in rats than in mice.

The barbiturates, particularly pentobarbital, have many advantages that made them popular for mouse anesthesia in the past. They are readily available, comparatively inexpensive, and generally suitable for administration by the intraperitoneal route. There is also a large body of data related to their effects on various experimental parameters. However, they also have many disadvantages, and, for most situations, there are better choices.

Ketamine hydrochloride

Ketamine induces a state of **dissociative anesthesia,** in which the subject experiences an altered state of consciousness without complete loss of consciousness. In mice this state is characterized by:

- Immobility associated with increased muscle tone and sudden, jerky movements
- Variable analgesia, which is generally inadequate for surgery in mice
- General stability of respiratory function
- Stimulation of most cardiovascular parameters, increased cerebral blood flow, intracranial pressure, and intraocular pressure

Muscle relaxation can be greatly improved and the duration of anesthesia prolonged by combining ketamine with sedative drugs such as **diazepam** or **acepromazine.** The degree of analgesia with these combinations is inadequate for surgery in mice, but they provide good restraint for mildly painful, nonsurgical manipulations.

For moderate surgical anesthesia, ketamine can be combined with an alpha-2 agonist such as **xylazine** or **medetomidine.** In addition to better muscle relaxation and analgesia, these combinations cause moderate to severe hypothermia and respiratory and cardiovascular depression. These effects, along with the analgesia and muscle relaxation, can be reversed with **atipamezole** or **yohimbine.**

Ketamine alone is not too useful for anesthesia of mice. Combined with diazepam or acepromazine, it can be used to restrain mice for mildly painful, nonsurgical manipulations. Combined with an alpha-2 (preferably medetomidine), it can be used for moderately painful procedures, and the effects are reversed at the end of the procedure.

Tribromoethanol

Tribromoethanol is a white, crystalline powder that is typically diluted in vehicle (butanol, amylene hydrate, or amyl alcohol) to form a stock solution. The stock solution is diluted in distilled water or saline at 40–50°C to form a working solution. Characteristics of tribromoethanol include:

- Rapid induction with 15–20 min of moderate surgical anesthesia
- Good muscle relaxation, fair analgesia
- Moderate cardiovascular and respiratory depression at moderate anesthetic doses (severe at high doses)

The breakdown products of tribromoethanol are highly irritating to tissues. Following IP administration of the drug, these products can cause adhesions, peritonitis, intestinal disorders (including ileus), and death. These effects are most likely to occur:

- If the solution is old or was prepared or stored improperly. It should be stored in a cool, dark place and used within a few weeks of preparation.
- Following the second exposure to the drug, regardless of the dosing interval.[162]

If properly prepared and stored, tribromoethanol is a reliable anesthetic for mildly to moderately painful procedures of short duration. Supplemental administration of the drug to prolong anesthesia or repeated use of the drug within individual animals for recovery procedures should be avoided.

Tiletamine/zolazepam (Telazol®)

This is a combination of two drugs. Tiletamine is similar to ketamine but two to three times more potent. Characteristics of this combination include:

- There is a dose-dependent duration of effect, typically 30 to 60 min.
- High doses are associated with high mortality. The depth of anesthesia with lower doses is generally inadequate for surgery in mice.
- It can be combined with xylazine or medetomidine for surgical anesthesia, although cardiovascular and respiratory depression and prolonged hypothermia may result.

When used alone this combination can provide effective sedation and restraint, but provides inadequate analgesia for surgery. When combined with xylazine or medetomidine, it can be used for surgery, but necessitates prolonged monitoring and support of the animal.

Special techniques

Carbon dioxide

Carbon dioxide, alone or mixed with air, can be used to produce light to deep anesthesia of very short duration. Unconsciousness results from direct depression of the central nervous system. Further depression may result from hypoxia. In addition, carbon dioxide has

a direct analgesic effect that is apparent with very low concentrations (as low as 5%). Characteristics of carbon dioxide with respect to its use as an anesthetic agent include:

- The duration of anesthesia once returned to room air averages 30 seconds, with abrupt recovery.
- Mice exposed to concentrations of 80–100% become anesthetized within two to four minutes, but then experience rapid progression to severe hypercapnia, respiratory failure, and death if maintained at these high concentrations.
- Inhalation of higher concentrations is painful.[163]
- Lower concentrations are less painful and less rapidly fatal, but are associated with a correspondingly longer induction time (often exceeding 30 min with 50% carbon dioxide).
- Lower concentrations may cause adverse effects such as copious salivation, seizures, and/or capillary hemorrhage.

In summary, carbon dioxide can be used to anesthetize postweanling mice for very brief, painful procedures (e.g., cardiac puncture or periorbital blood sampling). One should be prepared for abrupt recovery of the mouse following return to inhalation of room air. For humane reasons, exposure to carbon dioxide is not recommended for anesthesia of neonates, as they are able to withstand exposure to significant concentrations of carbon dioxide with no resulting induction of anesthesia.

Neonates

Neonatal mice are difficult to anesthetize safely. The margin of safety with most anesthetics is much narrower for neonates than adults, and the mortality rate associated with dosages adequate to produce surgical anesthesia can be quite high. Nonetheless, neonatal mice appear to be at least as capable as adults of perceiving pain and must be anesthetized for painful procedures. The following techniques can be used to anesthetize young mouse pups:

1. **Hypothermia**—Anesthesia and analgesia result from cold-induced depression of neural conduction and synaptic transmission, which completely cease at 9°C. Neonatal mice cool rapidly and tolerate cooling down to 1°C. Profound hypothermia can be safely maintained for up to 30 min. Hypothermia is sufficient for surgery in mouse pups up to 6 to 7 days of age. To prevent pain

during cooling, pups should not be placed in direct contact with a freezing or frozen surface.

2. **Halothane or isoflurane**—both are safe and effective for anesthesia of neonatal mice. As with anesthesia of adults, these compounds should be used with proper delivery and scavenging equipment.

Several other agents, including **ketamine** and **pentobarbital** are associated with high mortality rates in neonatal altricial rodents[164] and are not recommended for use with young mouse pups.

Periprocedural Care

If a mouse is to be anesthetized for any reason, it is important that the animal be given special attention before, during, and after the procedure. All anesthetics have the potential to significantly alter the animal's physiologic status and, without proper care, the outcome could be fatal. The type and severity of physiological alterations will vary with the anesthetic chosen, but most will cause at least some degree of respiratory and cardiovascular depression, and virtually all will cause some degree of hypothermia. These changes will persist and may even worsen until the animal has recovered fully from anesthesia, so supportive care and monitoring may be necessary for many hours after the procedure has ended. As with adverse physiological effects, the time to complete recovery will vary with the anesthetic.

The ideal candidate for anesthesia is a normal, healthy mouse. The type of preoperative evaluation and blood work that is typically performed on larger animals are seldom done with mice. However, the mouse should look active and alert, with bright eyes and a well-groomed appearance. Realistically, this standard cannot always be met. Many mutant and genetically engineered mice will not appear normal, and many of these mice are less than ideal candidates for anesthesia. Successful anesthesia of such mice can be tricky, and it is particularly important that they be given careful attention during and after the procedure. The anesthetic chosen for these animals should be one that offers a wide margin of safety, one that can be reversed if the mouse experiences severe adverse effects, or both.

Intraprocedural monitoring involves two aspects: (1) assuring adequate depth of anesthesia for the type of procedure to be performed, and (2) evaluation of the physiological status of the anesthetized animal. For procedures that cause little or no pain, monitoring can be limited to assessments of physiologic function. However, for more

painful procedures, it is essential to assure that the mouse has been rendered insensitive to pain before the procedure begins. The most reliable indicator is the response to toe or tail pinch. If the mouse moves or increases its rate of breathing in response to the pinch, it is inadequately anesthetized for a painful procedure. Note that the corneal and palpebral reflexes, which are commonly used to assess the depth of anesthesia in larger animals, are unreliable in mice.

The requirement for physiological monitoring and support will depend on the procedure itself and on the anesthetic used. Brief anesthesia with an inhalant anesthetic or CO_2 will require no such care, whereas both monitoring and support should be provided during any procedure that requires prolonged anesthesia. At the very least, the mouse should be checked frequently to assure that it is still breathing regularly. More sophisticated monitoring techniques are also available for use in mice, including techniques for monitoring blood oxygen saturation, blood pressure, and electrical activity of the heart. The mouse should also be kept warm. This can be done with a heating pad or a heat lamp. The best type of heating pad is a recirculating warm-water blanket. Care must be taken with other types of heating pads and lamps to guard against thermal injury to the mouse. Finally, for all but very brief periods of anesthesia, use of a lubricant for the eyes is recommended. Ophthalmic ointments for this purpose are commercially available.

Monitoring and physiologic support should continue after the procedure has ended. Until the mouse is up and moving about, it should be checked frequently to assure that it is breathing normally. It should also be kept warm. For procedures that involve prolonged anesthesia or a slow return to normal function, it may be desirable to increase the ambient temperature in the room for up to several days. The administration of warm, sterile fluids subcutaneously can help to promote a quicker, smoother recovery. Typically, 0.25 ml of supplemental fluid is adequate. It is important that one not administer too great of a volume of fluid. If recovery is prolonged, reapplication of ophthalmic ointment will protect against dessication of the eyes. Once the mouse is able to move around the cage, it should be checked frequently to make sure that it is eating and drinking. The provision of soft, moistened food will encourage eating if the mouse seems to be ignoring its normal hard food. Mice that are not feeling well will also be more inclined to drink from a water bottle than from an automatic waterer.

Analgesia

Administration of an analgesic should be considered for procedures that may involve significant postoperative pain. Signs of pain in mice include:

- Partial/complete closing of the eyelids; sunken eyes
- Changes in respiration, which may include increased or decreased, shallow or labored respiratory patterns
- Rough haircoat from lack of grooming; incontinence with soiled haircoat
- Increased or decreased vibrissal movements
- Severe pain or distress indicated by decreased responsiveness to handling, or withdrawal from other mice in the group
- Writhing, scratching, biting, or self-mutilation
- Hunched posture
- Sudden, sharp movement, such as running
- Vocalization when being handled or palpated
- Dehydration or weight loss, with wasting of the muscles on the back and a sunken or distended abdomen
- Ataxia or circling
- Hypothermia

Many drugs are available that provide effective analgesia in mice. Unfortunately, most of those that have been proven effective must be administered by injection or oral gavage. This poses a dilemma for the investigator, since the distress caused by handling and injecting the mouse may outweigh the benefits of the drug, particularly since many analgesics must be administered every one to five hours to maintain effective analgesia.

For mild to moderate pain, the most effective course of action often is to reduce pain indirectly by minimizing stressors in the animal's environment. The mouse should be kept clean and dry, protected from extremes of temperature, and provided with sufficient quantities of clean water and palatable, nutritious food. Soft, moistened food may be more appealing than regular rodent chow, and water may be more readily taken from water bottles than from automatic watering devices. Although mice that are accustomed to group housing may be stressed by isolation, efforts must be made to protect vulnerable animals (e.g., those recovering from surgery) from aggression by cagemates. Changes in the general environment may be quite

TABLE 4.5: USEFUL ANALGESICS FOR MICE

Analgesic	Dosage	Route	Duration of Effect
Morphine	2–10 mg/kg	IP, SC	2–4 h
Oxymorphone	0.1–0.3 mg/kg	SC	3–4 h
Buprenorphine	1.5–2.5 mg/kg	SC	3–5 h
Butorphanol	3–5 mg/kg	SC	1–2 h
Flunixin meglumine	2.5 mg/kg	SC	12–24 h
Aspirin	100–300 mg/kg	PO	Not determined
Acetominophen	100–300 mg/kg; 1–2 mg/ml in the drinking water	PO	Not determined
Ibuprofen	7.5 mg/kg	PO	Not determined

stressful. It is important to minimize changes in food (other than softening or moistening the food), water, lighting, social grouping, and position within the room. A possible exception to this is room temperature. Mice that are experiencing a difficult or painful recovery from surgery will often do better in a warmer than normal (but **not excessively hot**) environment.

For pain that cannot be controlled by the methods described above, it will be necessary to administer analgesic drugs. Some of the drugs that have proven effective for reducing pain in mice are listed in Table 4.5.

euthanasia

Introduction

The term *euthanasia* is derived from the Greek, meaning "easy or good death." In keeping with this definition, euthanasia must, above all, be humane. This means a rapid loss of consciousness without pain or fear, followed by a reliable progression to death without regaining consciousness. Also, to minimize fear and distress in other animals, they should not be subjected to the sight, sound, or smell of animals being euthanized. To achieve these goals, consideration must be given to the method to be used, the time and place where the euthanasia will be performed, and appropriate training of the person who will perform the euthanasia. Thought should also be given to the possible effects of the procedure on human participants, possible effects on the scientific goals of the study, and practical constraints that would render one method more desirable than another.

Laws, Regulations, and Guidelines for Euthanasia

Euthanasia of mice in conjunction with biomedical research, testing, or education in U.S. institutions receiving NIH funding must be performed in accordance with PHS Policy on Humane Care and Use of Laboratory Animals.[40] PHS policy requires adherence to the provision of the Animal Welfare Act,[41] the *Guide for Care and Use of Laboratory Animals*,[12] and the most recent report of the AVMA Panel on Euthanasia.[165] All of these documents emphasize the importance of: (1) performing euthanasia in a humane manner, and (2) assuring that people charged with performing euthanasia have the necessary training and skills to perform it correctly. The report of the AVMA Panel on Euthanasia contains considerably more detail on the implications of euthanasia and advantages and disadvantages of specific techniques that can be used. Some states have specific guidelines regarding methods of euthanasia. It is important to know the regulations in your state. The institutional veterinarian can be consulted regarding the details of federal and state laws and guidelines pertaining to euthanasia.

Management Considerations for Euthanasia

Euthanasia may affect people or other aspects related to facility management in a number of ways. The necessity of assuring that personnel who will perform euthanasia are fully trained in the method(s) to be used has already been mentioned. Other points to consider include the following:

- The psychologic implications of euthanasia. The act of taking life, by any method, can be difficult for humans performing the euthanasia, especially if it must be performed often or if an emotional attachment to the animal has developed.
- The aesthetic implications of different methods. Some methods (e.g., decapitation) can be particularly unpleasant for human participants and observers.
- The health and safety of humans and other animals. Some methods of euthanasia are inherently more dangerous than others (e.g., decapitation or carbon monoxide).
- The potential for human abuse. Some methods require the use of substances that may be abused by humans (e.g., pentobarbital). Appropriate facilities and procedures must exist to control access to these substances. In the U.S., controlled substances such as pentobarbital can be purchased only by individuals registered with

the Drug Enforcement Administration (DEA). Detailed records must be kept of the use and disposal of controlled substances.

- The expense and availability of drugs and equipment needed for some methods of euthanasia. In addition the difficulty and expense of maintaining equipment in proper working order can be a consideration.

Scientific Considerations for Euthanasia

It is important to recognize that the method of euthanasia may have confounding effects on postmortem evaluations or other scientific objectives of the research. Possible effects on scientific goals should be taken into account when choosing among the various methods that can be used to euthanize mice.

Methods

The following methods are acceptable for the euthanasia of mice:

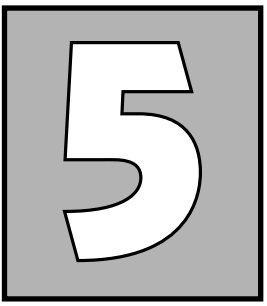
1. **Inhalant anesthetic overdose**—Effective scavenging of waste gasses is essential to protect humans and other animals. This method is not appropriate for neonates, except with prolonged exposure.
2. **Carbon monoxide**—Appropriate precautions (good ventilation and monitors) must be taken to prevent human exposure. One should use compressed CO only. The concentration of CO in the euthanasia chamber should be at least 6%.
3. **Carbon dioxide**—To minimize pain, conscious animals should not be exposed to concentrations . 70%. This method is not appropriate for neonates unless exposure times are prolonged.
4. **Barbiturate overdose**—Pentobarbital (100 mg/kg, IP) is the most commonly used barbiturate for euthanasia of mice.
5. **Microwave irradiation**—Microwave ovens specifically designed for euthanasia are sometimes used for mice. It is important never to use an oven designed for food preparation.

The following methods are acceptable for euthanasia of mice only if certain conditions are met:

1. **Decapitation**—Scientific justification and IACUC approval are required, and appropriate training and demonstrated skill are absolutely essential. Performance of this technique requires either a small guillotine or heavy, sharp scissors. Decapitation is

potentially dangerous to humans (guillotine), is aesthetically displeasing to many individuals, and may cause significant pain to the mouse if performed incorrectly. The potential for pain to the mouse is reduced or eliminated if the mouse is first anesthetized. Many believe that, if done properly, this is the most humane method of euthanasia for neonatal mice.

2. **Cervical dislocation**—Scientific justification and IACUC approval are required, and appropriate training and demonstrated skill are absolutely essential. Cervical dislocation does not require specialized equipment, but mechanical cervical dislocators are commercially available and can be quite useful. Like decapitation, cervical dislocation is aesthetically displeasing, and may cause significant pain for the mouse if performed incorrectly. The potential for pain to the mouse is reduced or eliminated if the mouse is first anesthetized. Many believe that, if done correctly, cervical dislocation is a humane form of euthanasia for mice. Anyone wishing to use this technique should be thoroughly trained by someone who is proficient in its performance.



experimental methodology

restraint

As with any animal, safe handling requires gentleness, firmness, and respect for the animal. People who must handle mice should be thoroughly trained and confident in their ability before they are given the responsibility to operate independently. In particular fearfulness on the part of the human handler is antithetical to safe handling and must be overcome by practice.

Laboratory mice vary significantly in temperament. Some strains, such as A, are typically placid in response to handling, while others, such as SJL, tend to be aggressive. Handling wild mice requires focused attention and a gentle, knowledgeable approach, as they are particularly nervous and amazingly adept at escape. Exuberant young mice of two to four weeks of age must also be approached with extra care, as they may jump out of the cage as soon as the lid is lifted.

Cage Transfer

To transfer a mouse from one cage to another, the mouse may be picked up by the base of the tail (not the tip!) using a gloved hand. Docile or very young (< two weeks old) mice can also be grasped by the scruff of the neck or lifted in a cupped hand. Mice in protected environments (e.g., high level barriers) should be lifted by blunt or rubber-tipped forceps. Older mice can be grasped by the scruff of the neck or the base of the tail. Neonates should be grasped *gently*

around the shoulders; alternatively they can be transferred in a cupped, gloved hand. Forceps, if used, should be disinfected between cages. In all cases, mice should be gently placed in the new case, *never* dropped.

Restraint for Manipulation

If the mouse is to be manipulated in any manner, greater control is needed. To manually restrain a mouse for further manipulation, lift it by the base of the tail and place it on the cage lid or other rough surface. Pull gently back on the tail, which will induce the mouse to dig in with all four feet and pull in the opposite direction. Then quickly and firmly, reach down and grasp the mouse by the scruff near the base of the head (Figure 5.1). With the tail in one hand and the scruff in the other, lift the mouse and tuck the base of the tail between the palm and third or fourth finger of the hand holding the scruff. While effective control necessitates a firm hold on the scruff, make sure that the skin around the neck is not stretched so tightly that it interferes with the animal's ability to breathe.

Restraint Devices

Various devices can be obtained commercially or made in the laboratory to restrain mice for further manipulations. For quick procedures, these devices seldom offer any advantage over manual restraint, but they can be quite useful for procedures that require more prolonged restraint or repeated manipulations at short intervals. Continuous restraint of a mouse for more than two hours should be scientifically justified by the investigator and approved by the IACUC.

sampling methods

Blood

The blood volume of the average adult mouse is only 2–2.75 ml, and the peripheral veins are small. This means that blood samples must be comparatively small and may be challenging to obtain without harming the mouse. The following are some guidelines regarding sample size:

1. **Approximately 10%** of the total blood volume, or 0.75% of the body weight, can be removed safely at one time without fluid replacement (0.18–0.2 ml from a 25-g mouse; 0.24–0.28 ml from a 35-g mouse).

Fig. 5.1 Technique for manual restraint of a mouse. A. The mouse is picked up by the scruff of the neck. B. The tail is pinned between the palm and third or fourth finger.

2. **Up to 15%** of the blood volume, or approximately 1.5% of the body weight, can be removed if the blood is withdrawn slowly and

replacement fluids are given (0.35–0.4 ml from a 25-g mouse; 0.5–0.56 ml from a 35-g mouse).

3. **For repeated sampling, 7.5%** of the total blood volume can be taken per week, or 10% of the total blood volume can be taken every two weeks (0.15 ml weekly or 0.2 ml every two weeks from a 25-g mouse; 0.26 ml weekly or 0.35 ml every two weeks from a 35-g mouse). The hematocrit content, hemoglobin content, or both should be monitored in all animals that will have large volumes (i.e., approaching the recommended maximum) drawn repeatedly (more than three times).

The following are some of the more common methods that are used to obtain blood samples from mice:

1. **Retro-orbital sinus puncture**—The mouse is manually restrained, and a microhematocrit tube or small-bore pipette is placed at the medial or lateral canthus of the eye. The tube is rotated and directed caudally at a 30° angle. As the sinus is ruptured, blood will flow back into and through the tube (Figure 5.2). After the tube is withdrawn, mild pressure should be applied with cotton or a gauze sponge to stop the bleeding. If personnel are properly trained and skilled in this technique, it can be accomplished with little trauma and will be followed by rapid healing. If there is any question about the training or skill of the person performing the procedure, the mouse should be anesthetized. The need for anesthesia with skilled operators is controversial. Retro-orbital blood sampling can be performed more than once at the same site, although the risk of permanent damage increases each time the procedure is performed. It is preferable to allow at least two weeks between repeated sampling from the same sinus.
2. **Tail laceration**—The mouse is restrained on a flat surface or placed in a restraint device with the tail protruding. A cut may be made on the ventral surface of the tail to lacerate the artery, or the tip of the tail may be amputated. Warming the animal or its tail beforehand will increase the flow of blood, which will generally not exceed a few drops.
3. **Cardiac puncture**—This should be done only as a terminal procedure in an anesthetized mouse. The animal is placed on its back on a flat surface. A 24-gauge needle is either (1) inserted through the diaphragm lateral to the xiphoid cartilage and directed for-

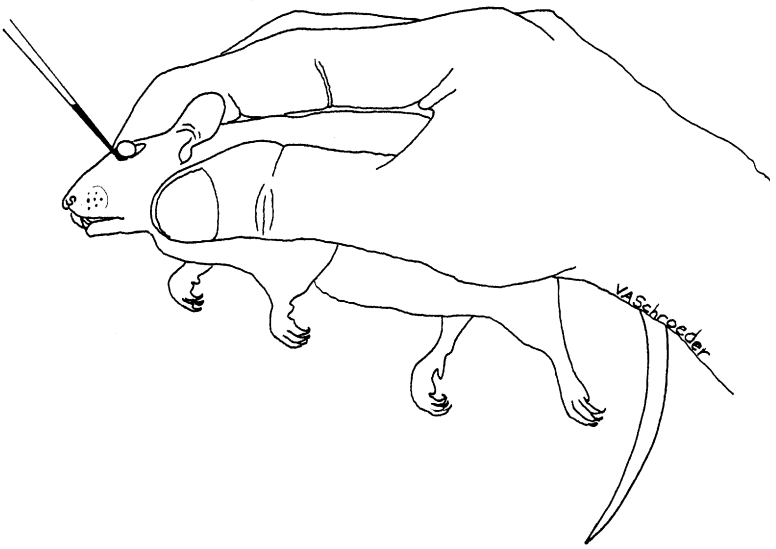


Fig. 5.2 Blood collection from the retro-orbital sinus. A glass capillary tube is inserted at the medial canthus and gently rotated until the sinus is penetrated and blood flows into the tube.

ward and medially toward the heart; or (2) inserted between the fifth and sixth ribs on the left side and directed forward toward the heart (Figure 5.3). This method requires training and skill, but can be used to obtain comparatively large volumes of blood.

4. **Decapitation**—This technique can be used to obtain comparatively large volumes of blood, provided that contamination of the blood with hair and body fluids is not contraindicated. Personnel must be well trained to perform this method safely, and it should be used only when scientifically justified by the investigator.[165] Decapitation may cause significant pain for the mouse if performed incorrectly (especially using scissors). The potential for pain is reduced or eliminated if the mouse is anesthetized.

Urine

Mice will often urinate upon handling and, if a test tube is readily at hand, a drop or two of urine can be caught from the genital papilla after the mouse has been lifted from the cage. If necessary, the mouse can be restrained manually and the caudal abdomen (over the bladder) gently massaged to stimulate urination. The catch tube

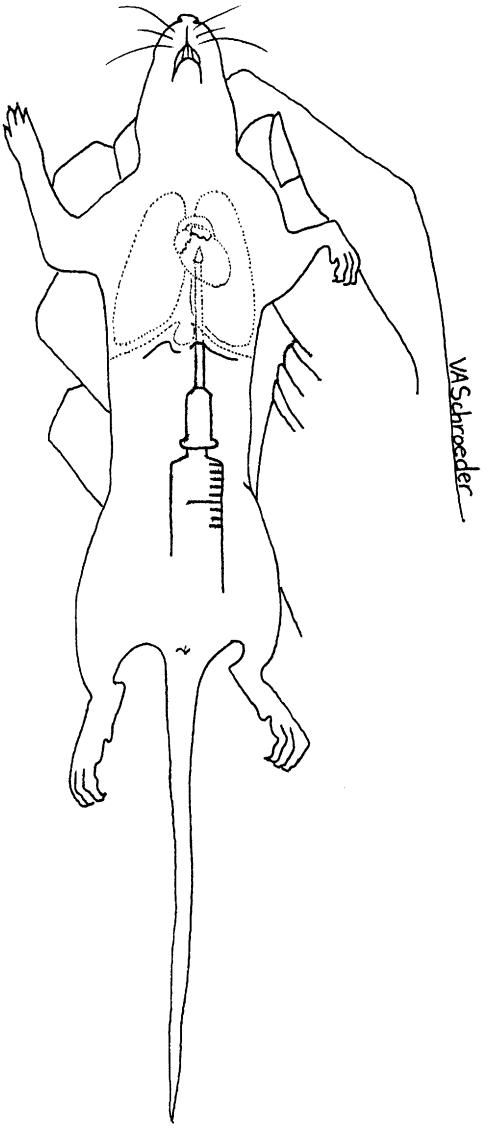


Fig. 5.3 Intracardiac blood collection. The needle is inserted just lateral to the manubrium and directed through the diaphragm and into the cardiac ventricle. Diaphragm, lungs, and heart are all represented by dotted lines.

should be pre-positioned beneath the genital papilla to catch the urine.

If more than a drop or two of urine is needed, it will be necessary to either make multiple collections from the mouse or place the

mouse in a **metabolism cage.** This is a special cage with a wire-bottom floor on top of a funnel-shaped base. Urine (and feces) drop through the wire floor and are funneled into a collection vessel placed underneath the cage. Samples obtained in this manner are not useful for microbiological analysis.

Feces

Dry, and sometimes fresh, feces can be obtained from the cage bottom or, in the case of wire-bottom cages, from the catch pan beneath the cage. To obtain a fresh sample from the rectum, lift the mouse from its cage by the base of the tail. Place a test tube beneath the anus and gently press it up and back to "milk" a fecal pellet out of the rectum and into the tube. With some mice, it may be possible to obtain two or three pellets within a short period of time by placing the mouse back in the cage after collecting a pellet, then coming back and repeating the process a few minutes later. Feces can also be collected using a metabolism cage.

Samples for DNA Analysis

Genetic testing is a common requirement in modern mouse colonies (e.g., to determine which mice carry a transgene of interest). This is typically done by analyzing a tissue sample from the mouse by Southern blotting or the polymerase chain reaction (PCR).[166,167] A variety of tissues have been used for this purpose, including blood,[168] saliva,[169] and rectal epithelial cells.[170] The most commonly used tissue, however, is the tip of the tail. When performed on young mice before or shortly after weaning, the procedure is typically performed without anesthesia. The mouse is restrained and a section of up to 5 mm is removed from the end of the tail using a single stroke with a scalpel blade. Pressure over the wound may be needed to stop bleeding. With older mice, or if more tissue will be removed, the mouse should be anesthetized for the procedure. Bleeding may also be a problem with older mice, and the wound will generally take longer to heal.

Vaginal Swabs

Vaginal swabs can be used to identify the stage of the estrous cycle in a female mouse. Although of limited practical utility for breeding of mice, this technique is sometimes employed for experimental purposes. To obtain a swab, the mouse is restrained manually and a moistened cotton-tipped swab inserted into her vagina (note that

most commercially available swabs are too large for this purpose; an appropriately sized swab can be made using a bit of sterile cotton wool wrapped around a blunt toothpick). The swab is gently but firmly rotated, then removed and wiped onto a clean glass microscope slide. Once the slide has air-dried, it is stained with a 0.1% aqueous solution of methylene blue. After the stain has dried, the slide is examined under a microscope and interpreted as follows:

- **Diestrus**—Cells consist primary of polymorphonuclear lymphocytes (PMNs), with some epithelial cells.
- **Proestrus**—Nucleated and cornified epithelial cells, with some PMNs in the early stage.
- **Estrus**—Cornified epithelial cells predominate, with a few nucleated cells seen in the early stage.
- **Metestrus**—Cornified epithelial cells and PMNs predominate, with some nucleated epithelial cells.

compound administration

Drugs and other substances can be administered to laboratory mice by a variety of routes. People using these techniques should be thoroughly trained and skilled before working with live, unanesthetized animals. Common routes of compound administration include:

Oral (PO)

Some compounds can be administered in the food or water; however, under the best of circumstances, it is difficult to give a precise amount or dose in this manner, and mice may be reluctant to consume the treated food or water at all. For this reason, direct administration by oral **gavage** is often a preferred approach. For this procedure, the mouse is manually restrained—firm restraint with complete immobilization of the mouse's head is essential for this procedure—and a gavage needle is passed through the animal's mouth and into the stomach (Figure 5.4). The compound is administered slowly using a syringe attached to the gavage needle. The primary complication that may occur using this technique is passage of the needle into the lungs rather than the stomach. This may be indicated

if obstruction to passage of the needle is encountered, if the mouse coughs, chokes, or begins to struggle vigorously after compound administration begins, or if fluid is seen coming out through the nose. Any of these signs would necessitate immediate withdrawal of the needle. In addition, traumatic rupture of the pharynx or esophagus may occur if excessive force is used in passing the gavage needle. Upon injection of the sample, hydrothorax will result if these tissues have been ruptured. If it appears that fluid has gotten into the lungs, the mouse should be euthanized.

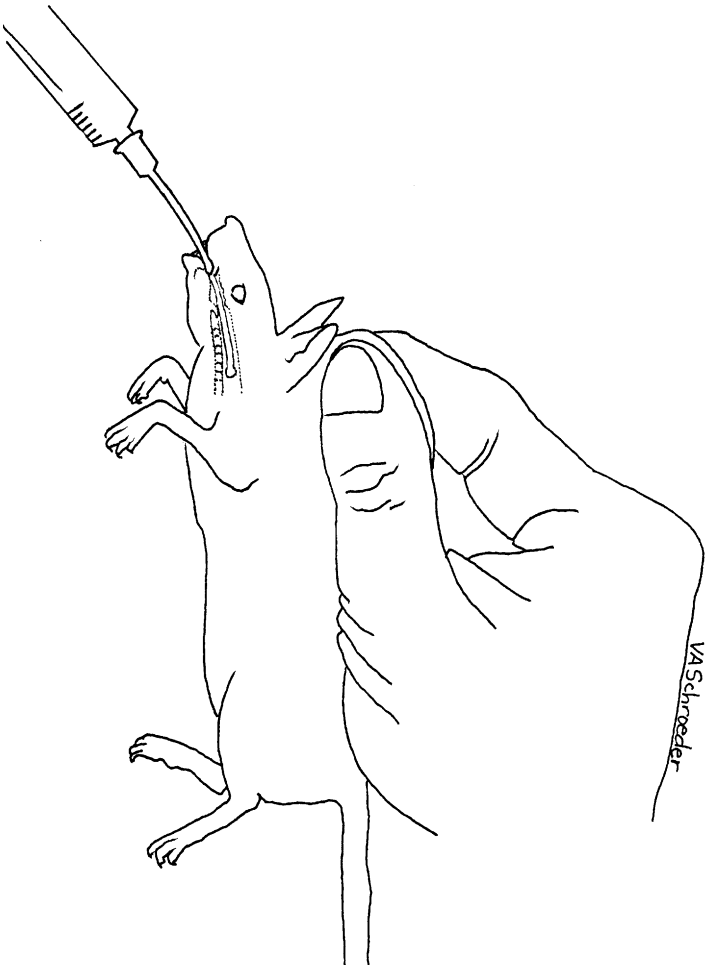


Fig. 5.4 Orogastric gavage. The tip of the feeding needle is directed to the back of the pharynx, through the esophagus and into the stomach. Care must be taken not to enter the trachea.

Intramuscular (IM)

Intramuscular injections are generally made into the muscles at the back or top of the thigh. The mouse is restrained manually and the tip of a 25-gauge needle (attached to a syringe) is inserted firmly through the skin and into the muscle. Prior to administration of the compound, it is advisable to pull back lightly on the plunger of the syringe. If blood is pulled back into the syringe, the needle is probably in a blood vessel and should be repositioned.

Intraperitoneal (IP)

Intraperitoneal injections are made into the caudal left (to avoid the cecum on the right) abdominal quadrant. The mouse is restrained manually and held with the head and body tilted downward. With a quick, firm motion, the tip of the needle is inserted through the skin and just past the abdominal wall (Figure 5.5). Slow or hesitant insertion of the needle may result in failure to penetrate the abdominal musculature. Prior to administration of the compound, it is advisable to pull back lightly on the plunger of the syringe. If any kind of fluid is pulled back into the syringe, the needle is probably in one of the abdominal organs and should be repositioned. With training and practice, it is possible to achieve a high rate of success with IP injections. However, there is always a risk of injection into an abdominal organ. This may have no serious consequences for the mouse, but may be associated with slow and erratic absorption of the compound. This is a particular problem in the case of anesthetics, as the mouse will not respond as expected to the drug. Redosing under these circumstances is tricky, as the initial dose will eventually be absorbed and, combined with the second dose, may prove fatal.

Subcutaneous (SC)

Depending on how the mouse is restrained, SC injections may be made through the loose skin over the upper back and neck (Figure 5.6) or through the considerably tighter skin over the ventral abdomen. The tip of the needle is inserted firmly through the skin, and the needle is advanced several millimeters further. No resistance should be encountered as the plunger of the syringe is depressed. Resistance suggests that the needle has not completely penetrated the skin.

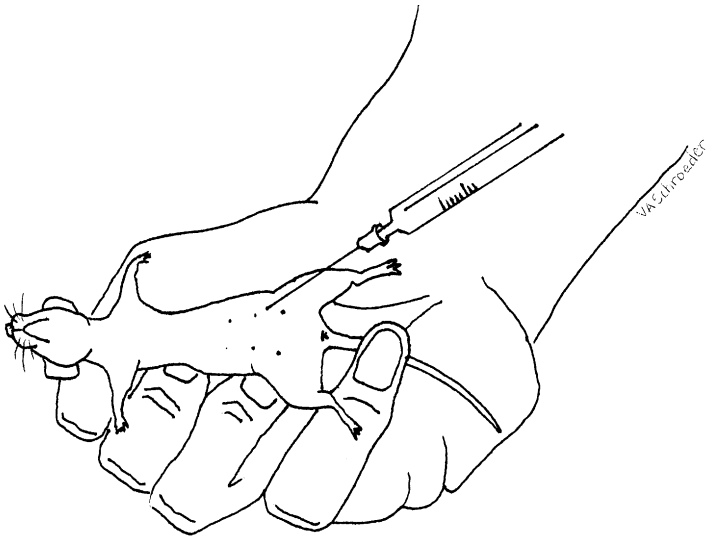


Fig. 5.5 Intraperitoneal injection. The needle is inserted into the caudal left rear abdominal quadrant, and the syringe aspirated to assure that neither vasculature nor viscera have been penetrated.

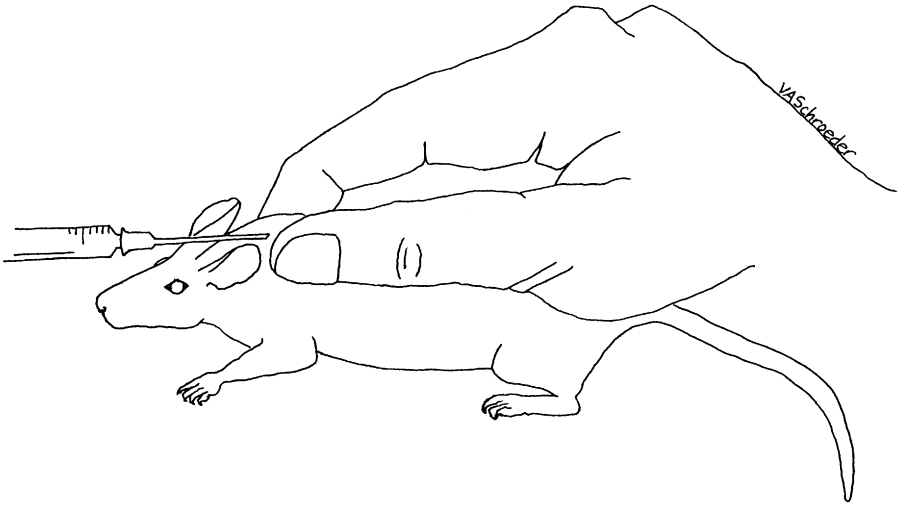


Fig. 5.6 Subcutaneous injection. Injections into the subcutaneous space overlying the upper back and shoulders can be easily made.

Intradermal (ID)

Intradermal injections are made into the skin over the back/neck, ventral abdomen, or hind footpad. The technique is much the same as for SC injections except that the tip of the needle is placed

between the layers of skin rather than through the skin. In contrast to SC injections, resistance should be felt both as the needle is advanced and as the compound is injected. A hard bleb will be seen upon successful ID injection of even a small quantity of fluid. This is a tricky procedure in any species, but is particularly difficult in the mouse because the skin is so thin. Removal of the hair overlying the injection site will facilitate visualization and improve the likelihood of success.

Intravenous (IV)

The most common sites for IV injections in the mouse are the lateral tail veins. These vessels are readily visualized, but are quite small in diameter and injection into them requires considerable practice and skill. The mouse is typically placed in a restraint device for this procedure. Warming the mouse, or just its tail, will dilate the veins and make the procedure easier. Excellent lighting will also prove helpful. A small-gauge (27-gauge or smaller) needle is inserted just through the skin and into the vein; the tip is then advanced a couple of millimeters (Figures 5.7 and 5.8). If placement has been successful, there will be no resistance felt as the compound is administered, and the vein will appear to "bleach out" as the administered fluid temporarily replaces the blood within it.

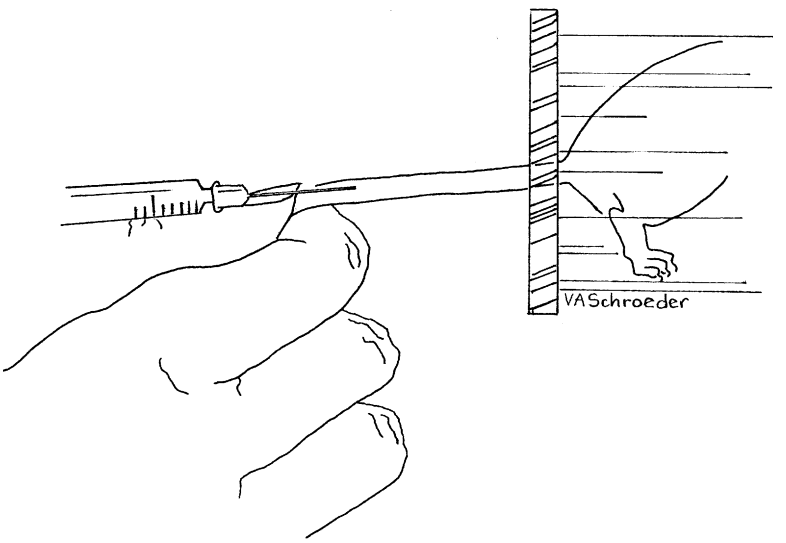


Fig. 5.7 Intravenous injection. Injections into one of the lateral tail veins can be made with the mouse placed into a restrainer.

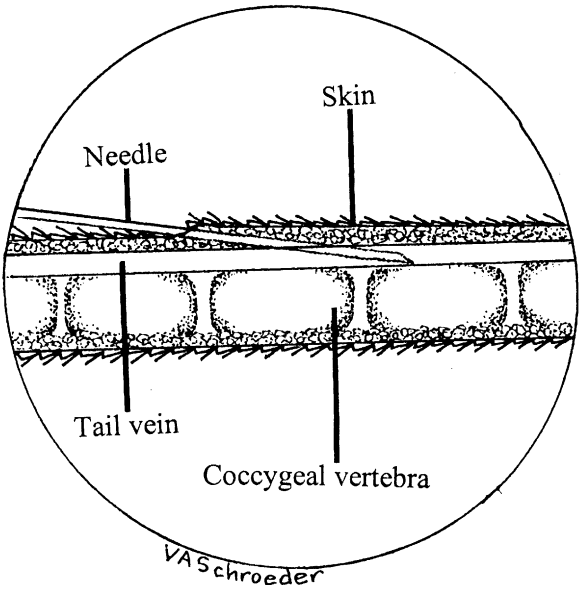


Fig. 5.8 Close-up internal appearance of the tail during intravenous injection. Note the penetration of the needle through the skin and into the tail vein.

Implantable cannulas and pumps

Osmotic pumps can be placed subcutaneously or into the abdominal cavity to deliver compounds at a slow, steady rate over a period of days or weeks. Implantation is a surgical procedure and should be performed using aseptic technique. Implantable cannulas permit continuous access to the venous or arterial system for either IV compound administration or blood withdrawal. This technology is used more often in larger animals, but miniaturized devices for mice are commercially available. Using strict aseptic technique, the cannula is inserted into a vein or artery (the femoral vessels, jugular vein, and carotid artery are common sites), and secured in place. The other end of the cannula is attached to a small port that is secured in a subcutaneous location, most often over the shoulders. When the surgical incisions are closed, the entire system, port and cannula, is internal. To access the port for injections or blood withdrawal, the skin over it must be shaved and disinfected. Special **Huber point** needles may be used to prolong the life of the port. More information on implantable cannulas can be found elsewhere.[171]

monoclonal antibody (Mab) production

Monoclonal antibodies are used extensively in biomedical research, in the diagnosis of disease, and in the treatment of conditions such as cancer. Until recently, the standard method of producing Mab was the **mouse ascites method.** With this method, **hybridoma** tumor cells—created by fusing selected splenic immune cells with cancer cells—are injected into the abdominal cavities of mice. As the tumors grow, they produce fluid (**ascites**) that accumulates in the mouse's abdomen. The fluid is rich in the particular Mab of interest. The fluid is withdrawn from the abdomen using a needle, a procedure referred to as an **abdominal tap,** and the Mab extracted and purified in the laboratory.

The mouse ascites method has fallen into disfavor with many because of possible adverse consequences for the mouse.

Pain and distress can occur as a result of:

1. Aggressive or invasive tumor growth (the aggressiveness and invasiveness of the tumor varies with different hybridomas)
2. Overdistention of the abdomen with ascites
3. Repeated abdominal taps combined with slow growth of even comparatively nonaggressive hybridomas

In an effort to limit the use of live mice for the production of Mab, alternative methods have been developed for producing Mab *in vitro.* Even *in vitro* techniques require some use of animals as sources of cells for the hybridomas, but they avoid the potentially serious humane consequences of *in vivo* ascites production. *In vitro* methods should be used whenever possible; however, it is not always possible to produce Mab in the desired quantity and concentration using *in vitro* techniques. The following procedure represents a humane method for producing Mab by the mouse ascites method. This method involves a single abdominal tap to remove ascites. The tap is performed after the mouse has been euthanized.

1. Each mouse is primed with pristane (0.5 ml, IP) at least ten days prior to administration of hybridoma cells. This step is critical to ensure the development of ascites-producing tumors rather than solid-tumor development. Sterile disposable syringes with sterile tissue culture grade reagents should be used.

2. Each mouse is injected (IP) with 10^6 to 10^7 hybridoma cells.
3. The animals are palpated daily to assess tumor development and ascites production. At the same time, the mice are evaluated for any signs of distress, including reduced activity, respiratory distress, and reduced food and water intake.
4. The mice are euthanized after abdominal girth has doubled (this occurs prior to maximum ascites production). If any animal shows signs of impaired mobility, respiratory distress, or decreased activity, it is euthanized earlier.
5. The ascites is removed with a syringe and needle by tapping the abdomen after euthanasia.

For a more in-depth discussion of the advantages and disadvantages of both *in vivo* and *in vitro* methods of Mab production, the reader is referred to a recent report from the National Research Council.[172]

necropsy

Many types of studies require the postmortem examination of organs and tissues (**necropsy**). In addition, necropsy is frequently performed to diagnose disease problems. Proper necropsy technique should permit and facilitate evaluation of all organs and lesions and be efficient and simple. In this regard, prior to the necropsy a plan should be developed that outlines all procedures that will be performed (e.g., photography, radiography, and gross necropsy), all tissues or organs that will be weighed, and in what order they will be done. A check list may facilitate this process.

Equipment and Materials

Useful equipment and materials needed to conduct a necropsy on a mouse include the following:

1. Ventilated workstation or other means to protect the prosector (person performing the necropsy) from formalin fumes
2. Glasses or goggles—While infectious or zoonotic disease is not a common issue when working with specific pathogen-free mice, glasses or goggles will protect the eyes from splashes with formalin. Magnifying reading glasses may facilitate dissection and examination of small specimens.

3. Gloves—When multiple animals are to be examined, hand lotion and double gloving may protect the hands better than frequent glove changes. When the top gloves are damaged or soiled, they can be replaced with minimal exposure of the hands to drying or contaminating materials. Latex gloves are commonly used, although vinyl gloves are useful for those individuals who are allergic to latex.
4. Lab coat or other protective uniform should be worn to protect the skin and clothes from contaminating materials and fixatives.
5. Cutting board—An inexpensive, plastic cutting board is adequate for most purposes. It should be relatively easy to clean and able to withstand frequent use.
6. Paper towels—Some tissues (e.g., skin and reproductive tract) can be laid out on a paper towel and will adhere to it to facilitate examination and ensure uniform fixation.
7. Small metric ruler—Such a ruler should be included in any photograph of specimens. When a lesion is described in a report as being small or large, it should be measured and, if possible, weighed. Masses and organs with three dimensions should have measurements recorded for all three. For example, a spot may be 2$_3$ 2 mm, but a mass may be 2$_3$ 2$_3$ 2 mm.
8. Forceps—Blunt-ended, serrated, or toothed forceps seem to cause the least damage to delicate mouse tissues. Fine-pointed forceps can create artifactual holes or tears in tissues. Smooth forceps require considerable compression to grip slippery tissue.
9. Scissors—Fine, blunt-ended scissors seem to cause the least damage to delicate tissues. Sharp-tipped corneal scissors are used commonly but tend to create holes or tears, especially in inexperienced hands.
10. Scalpel blades—These should be used sparingly in dissection of the whole animal, but sharp, fresh blades are critical to trimming tissues appropriately for histologic processing. Single-edge blades are suitable for most purposes.
11. Syringe and needle—A 3-ml syringe with a 21-gauge needle works well for infusing the lungs and gastrointestinal tract with formalin.
12. Fixative—To preserve and prepare tissues for further processing, tissues are normally placed in a fixative solution. Neutral buffered formalin (10%) is suitable for soft tissues in most situations.

13. Decalcifying solution—Bones must be decalcified for histologic processing and evaluation. A 26% formic acid solution provides fixation and decalcification in a single step and is suitable for mouse tissues in many situations. Typically, 20 to 48 hours are required for this process. Some immunohistochemical techniques will work on tissues decalcified in this manner.
14. Specimen containers with labels

Necropsy Procedure

Ideally, the animal should be necropsied immediately after death. Alternatively, carcasses may be stored for a short time (several hours) under refrigeration to delay tissue decomposition. Carcasses thus stored should be kept in refrigerators not used for storage of food for animals or personnel. Freezing of carcasses can significantly interfere with meaningful necropsy. General procedures for necropsy of a mouse are as follows:

External examination

The animal should be weighed, and the color of the eyes and hair coat and identification should be recorded. External lesions (e.g., domed head, anophthalmia, masses, or open wounds) should be described and measured. A simple assessment of body condition should be recorded (e.g., thin, adequate or good body condition, or obese).

Palpation

Gentle abdominal palpation may reveal pups or other abdominal masses or suggest the presence of fluid. When the abdomen is distended with fluid (e.g., ascites), a sterile sample can be obtained with a needle and syringe for cytologic or microbiologic evaluation. The consistency of any palpated masses should be described as soft or fluctuant, firm or hard. Hard should be reserved for boney or mineralized masses.

Dissection

It is helpful to always orient animals in the same direction (e.g., head up or head right) so that the side of the lesion can be recalled accurately. It is useful to weigh organs and to record descriptions of any lesions, including coloration, size, presence of abnormal masses or

fluids, and any other abnormal findings. Specific steps in dissection are as follows:

1. Removing the pelt facilitates assessment of subcutaneous fat (minimal, adequate, or abundant), and reveals subcutaneous lesions and abdominal organs *in situ.* Removal of the pelt is performed by incising the abdominal skin and exerting gentle pressure cranially and caudally until the pelt has been removed.
2. After examining the animal with the skin removed, remove the "chain" of parotid glands, submandibular salivary glands, and lymph nodes that extend from ear to ear under the chin.
3. Open the abdomen, xiphoid to pubis, and examine the contents *in situ.*
4. Lift the sternum by the xiphoid process and remove it to expose the thoracic cavity. Examine the contents, noting any fluid or masses, and absence or enlargement of the thymus.
5. Expose the trachea by blunt dissection and use the 3-ml syringe/21-gauge needle to infuse the lung with fixative. The lungs should expand fully, and excess fixative will reflux up the trachea. It is not necessary to clamp or tie the trachea. After infusion, it is important not to compress the lungs during subsequent dissection.
6. Split the mandibular symphysis with a scissors, grasp the tongue with the forceps, and gently retract to remove the tongue, larnyx, trachea, and esophagus from the head and neck; continue retracting to remove the heart, thymus, and lungs from the thorax. Use blunt dissection with the scissors to free these tissues.
7. The thyroid glands are immediately caudal to the larynx on either side of the trachea. They may be difficult to see without magnification, but if a 2-mm section of trachea immediately caudal to the larynx is removed, the thyroid glands are usually included and can be observed microscopically on cross-section.
8. Split the pelvis at the pubic symphysis to facilitate removal of abdominal contents. Grasp the diaphragm with the forceps, cut at its deepest extent, and retract gently to lift out all of the abdominal contents together. The adrenal glands and kidneys tend to remain, deep in the retroperitoneal space; blunt dissection is usually required for their removal. Abdominal contents can be examined individually, organs weighed, and any abnormalities recorded.

9. The liver should be separated from the rest of the gastrointestinal tract. The small caudate lobes may be folded into the lesser curvature of the stomach, and care should be taken to ensure that they are removed so that the entire liver is included in weighing that organ. When manipulating the liver, lift it gently, or grasp parts that will not be submitted for histology (e.g., diaphragm or smaller liver lobes). The median and left lateral lobes are the largest and are usually selected for histology, unless lesions are present in other lobes, or if experimental protocol dictates otherwise.
10. The gastrointestinal tract should be isolated from all other organs. Different methods to prepare the tract for histologic evaluation are preferred by different pathologists; however, the following is provided as a simple method to evaluate representative sections from the different segments. First, the stomach is held in one hand and the rectum in the other, as the tissues are gently stretched apart to break the mesenteric attachments. Next, the mesentery, lymph nodes, and pancreas are removed as completely as possible. These pale, soft tissues may be difficult to distinguish grossly. With the 3-ml syringe/21-gauge needle, 0.5–1.0 ml of formalin is infused into the stomach, duodenum, ileum, cecum, and colon.
11. Bone must be decalcified prior to further processing for histology. Skin and other soft tissues should be removed from the bone as completely as possible because they may interfere with decalcification. The sternum is preferred by some pathologists for histologic evaluation of bone marrow, especially if the marrow from femurs was removed for cytologic or other evaluations. Soft tissues and bones can be trimmed as early as 20 hours after dissection. Although 26% formic acid is gentle compared to other decalcifying solutions, tissues should not be exposed to it for greater than 72 hours or the tissues will become overdecalcified and uniformly pink with diminished cellular detail. As with formalin, the ratio of tissue to solution should be approximately 1:10 and the tissue covered completely by the solution.

Trimming of tissues

During dissection, several tissues can be placed directly in cassettes and submitted for histologic processing as is, including lung, thyroid, trachea, salivary glands, lymph nodes, and pancreas. Other tissues

must be "trimmed"; that is, smaller sections of the tissue must be prepared and placed into cassettes for further processing.

Trimming of tissue should be performed in a well-ventilated area or hood. Used formalin should be discarded as a hazardous waste. After trimming, labeled cassettes should be submitted in clean formalin. In addition, tissues to be saved should be stored in clean formalin.

During trimming, tissues should be cut with a single clean swipe, not squished or sawed. Inexpensive single-edge blades are suitable for trimming most tissues, and they should be replaced as soon as they become dull so that tissue is not damaged. Trimmed specimens should not be more than 3mm deep to fit into the cassette without generating grid marks and "squish artifact." For decalcified skulls, trimming may be facilitated by use of Weck blades, which have a longer cutting edge and seem to be sharper than the single-edge blades. Decalcified tissue should be soft and easily cut. Crunchy tissue requires additional decalcification. Decalcified specimens should be rinsed in water (preferably distilled), and cassettes should be kept in water until histologic processing. Remaining rinsed, decalcified tissue can be saved with formalin-fixed tissue.

Numbering of cassettes should be systematic to facilitate retrieval of specific tissues from archived material. Cassettes are best labeled with a graphite pencil, since many inks are soluble in formalin and markings will become illegible. The numbering system in Table 5.1 is provided as an example, but other systems may be better suited to specific situations or preferences. Additional cassettes can be prepared to include tissues with lesions that were observed during the dissection.

Some additional tips for trimming of tissues include the following:

1. The fixed heart can be hemisected (cut in half, longitudinally) to expose all the chambers and valves. The right ventricle has a thinner wall than the left ventricle and may wrinkle slightly to facilitate identification. For some purposes, multiple cross-sections may be preferred.
2. The right kidney can be transected (cross-section) and the left kidney cut longitudinally for identification after processing. Both kidney sections should include cortex and medulla.
3. The following gastrointestinal tract segments can be included in a single cassette:

TABLE 5.1: SUGGESTED CASSETTE NUMBERING SYSTEM

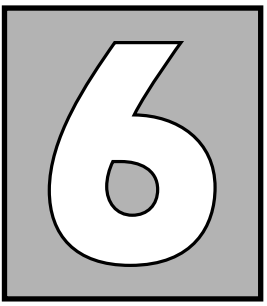
Cassette Number	Tissues Included in Cassette
1	Muscle: diaphragm, tongue, soleus; also thymus
2	Lung: all lobes, formalin-infused, along with thyroid and trachea
3	Kidneys, adrenals
4	Submandibular and parotid salivary glands and lymph nodes
5	Gastrointestinal tract (formalin-infused)
6	Lymph nodes (mesenteric and any enlarged nodes)
7	Liver, spleen
8	Urogenital tract
9	Skin: dorsal neck, inguinal, any skin with lesions
10	Heart
11	Decalcified hind limb, sternum
12	Decalcified head, with ears, eyes, and brain

- Stomach, including squamous (white) and glandular (pink) portions
- Duodenum (cross section) with pancreas
- Mid-jejunum (cross section)
- Ileum (cross section) from last 2cm before cecum, including a pale lymphoid nodule (Peyer's patch) if it can be identified grossly
- Cecum (V-like section), including a pale lymphoid nodule
- Proximal colon (one or two cross sections), from first 2 cm after cecum
- Include distal colon/rectum (cross section) from between fecal pellets. Fecal pellets should not be included, since they may contain indigestible material that can damage the microtome blade.

4. The liver's median lobe is cut to include the gall bladder between the left and right parts of the lobe. A section or wedge of the left lateral lobe is cut from hilus to the edge.
5. The female reproductive tract should be trimmed to provide representative sections of ovaries and uterus, and one or two

cross sections through rectum, vagina, and urinary bladder to demonstrate their anatomic relationships. The male reproductive tract should be trimmed to include sections of testes, epididymis, seminal vesicle, and coagulating gland, and a cross-section through the rectum and the neck of the urinary bladder to include the prostate gland.

6. The decalcified hind limb should be trimmed (i.e., muscle removed by a clean cut) so that the bone can be seen on the flat (cut) surface of the submitted specimen, and minimal sectioning by the microtome will be enough to enter the bone for a good section. It is often useful to include the femur and stifle joint or the tibia and tarsus in single sections. The sternum is often placed in the same cassette as the hind limb specimen.
7. For trimming the decalcified head, the external ear canal openings and the eyes can be used as landmarks. Using clean, single strokes, cuts should be made on either side (rostral and caudal) of the ear canal for a section that includes the middle ear, the internal ear, or both; the pituitary, and the hippocampus. A cut should be made on either side (rostral and caudal) of the eyes for a section that includes the eyes, Harderian glands, and molars. Usually, the cerebrum section (section from between ear and eye), the cerebellum section (section from behind the ear), and a section from the nose will fit into the same cassette.
8. Many pathologists or investigators have very specific methods for evaluation of the spinal cord and vertebrae. The following method is suggested if a simple screening is desired. Cervicothoracic and lumbosacral specimens can usually be accommodated in a total of two cassettes. Cross-sections are cut at each end of the specimens, and then each segment is hemisected (cut longitudinally). The cross-sections and hemisections from each segment are then placed together in a single cassette. Biopsy ink or a distinctive nick in the section can be used to identify left from right, if desired.



resources

To provide the user of this handbook with information regarding sources of mice, equipment and materials, and additional information, examples of relevant vendors and organizations are included in this chapter. The lists are not exhaustive, nor do they imply endorsement of one vendor over others. Rather, these lists provide a starting point for development of one's own list of resources. Sources for equipment are provided with contact information provided at the end of the chapter.

organizations

A number of professional organizations exist that can serve as initial contacts for obtaining information regarding specific professional issues related to the care and use of laboratory mice. Membership in these organizations should be considered, since it allows the laboratory animal science professional to stay abreast of regulatory issues, improved procedures for the use of animals, management issues, and animal health issues. Relevant organizations include:

- **American Association for Laboratory Animal Science (AALAS),** 9190 Crestwyn Hills Drive, Memphis, TN 38125 (Tel: 901-754-8620; E-mail: info@aalas.org; WWW: http://www.aalas.org), AALAS serves a diverse professional group, ranging from principal investigators to animal care technicians to veterinarians. *Comparative Medicine* 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 Institute for Laboratory Animal Management (ILAM) is a program designed to provide state-of-the-art training in laboratory animal facility management. Local groups have also organized into smaller branches.

- The **Laboratory Animal Management Association (LAMA)** 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. Information regarding the current contact for LAMA may be obtained through AALAS.
- The **American Society of Laboratory Animal Practitioners (ASLAP)** 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 periodic meetings, generally in conjunction with annual meetings of AALAS and the American Veterinary Medical Association (AVMA). Information regarding ASLAP can be obtained by visiting the Web site at www.aslap.org.
- The **American College of Laboratory Animal Medicine (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 AVMA, and the 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 as well as sessions at the annual AALAS meeting. Information regarding ACLAM can be obtained by visiting the Web site at www.aclam.org.
- The **International Council for Laboratory Animal Science (ICLAS)** 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. Current contact information can be obtained by visiting the Web site at www.iclas.org.
- The **Institute of Laboratory Animal Resources (ILAR)** functions under the auspices of the National Research Council to develop and make

available scientific and technical information on laboratory animals and other biological resources. A number of useful publications are available from ILAR, including the *Guide for the Care and Use of Laboratory Animals*. In addition, ILAR maintains the International Registry of Laboratory Registration Codes on behalf of the International Committee on Standardized Genetic Nomenclature for Mice. The Registry is a means of identifying substrains of mice, rats, and rabbits held in different institutions by different investigators. Information on ILAR can be obtained by visiting the Web site at www4.nas.edu/cls/ilarhome.nsf.

- The **Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International** 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. Additional information can be obtained by visiting the Web site at www.aaalac.org.

publications

A number of published materials are valuable as additional reference materials, including both books and periodicals.

Books

The following books may be worthwhile sources of additional information:

1. *The Mouse in Biomedical Research* (Volumes I–IV), edited by H. L. Foster, J. D. Small, and J. G. Fox, 1981 (Vol. I), 1982 (Vol. II), 1983 (Vol. III), 1984 (Vol. IV). Academic Press, Inc., San Diego, CA 92101.
2. *The Biology and Medicine of Rabbits and Rodents,* by J. E. Harkness and J. E. Wagner, 1995. Williams and Wilkins, Baltimore, MD 21298–9724.
3. *Clinical Laboratory Animal Medicine,* by K. Hrapkiewicz, L. Medina, and D. D. Holmes, 1998. Iowa State University Press, Ames, IA 50014.
4. *Laboratory Animal Anesthesia: A Practical Introduction for Research Workers and Technicians,* by P. A. Flecknell, 1996. Academic Press, Inc., San Diego, CA 92101.

5. *Formulary for Laboratory Animals*, by C. T. Hawk and S. L. Leary, 1995. Iowa State University Press, Ames, IA 50014.
6. *Infectious Diseases of Mice and Rats*, by the Committee on Infectious Diseases of Mice and Rats, ILAR, 1991. National Academy Press, Washington, D.C. 20418.
7. *Systematic Approach to Mouse Mutations*, by J. P. Sundberg and D. Boggess, 1999. CRC Press, Inc., Boca Raton, FL 33431.
8. *Necropsy Guide: Rodents and the Rabbit*, by D. B. Feldman and J. C. Seely, 1988. CRC Press, Inc., Boca Raton, FL 33431.
9. *Pathology of the Mouse*, edited by R. R. Maronpot, G. A. Boorman, and B. W. Gaul, 1999. Cache River Press, Vienna, IL 62995.

Periodicals

The following periodicals are excellent sources of current relevant information:

1. *Comparative Medicine.* Published by the American Association for Laboratory Animal Science. For contact information see above listing for AALAS.
2. *Contemporary Topics in Laboratory Animal Science.* Published by the American Association for Laboratory Animal Science. For contact information see above listing for AALAS.
3. *Laboratory Animals.* Published by the Royal Society of Medicine Press, 1 Wimpole Street, London W1M 8AE, UK.
4. *Lab Animal.* Published by Nature Publishing Co., 345 Park Avenue South, New York, 10010–1707.

electronic resources

Many online sources of information relevant to the care and use of laboratory animals, including mice, are available. Only a representative few are listed below:

1. *Comparative Medicine Discussion List (COMPMED).* An electronic mailing list available through the Internet, COMPMED is a valuable means to quickly tap into the expertise of laboratory animal science professionals around the world. Subscription information can be obtained by contacting AALAS, the host of this list.

2. *The Jackson Laboratory.* A nonprofit breeder of mice, including many unique mutant strains, the Jackson Laboratory website also has extensive information related to mouse genomics and nomenclature. Information can be accessed at www.jax.org/resources/documents/.
3. The *MRC Mammalian Genetics Unit, Harwell* contains extensive information related to mouse genetics. This site can be accessed at http://imsr.har.mrc.ac.uk/.

mice

Mice may be obtained from vendors of varying size and quality. Most commercial vendors to research facilities supply SPF mice, which have come from colonies tested for the presence of certain infectious agents. The purchase and use of such mice is advisable. Prior to purchase, vendors should be asked to supply information regarding the health status of the colony from which the mice are to come. It is impractical to list all vendors here; however, the following are examples of vendors which supply mice:

1. Ace Animals, Inc., P.O. Box 122, Boyertown PA 19512 (Tel.: 610-367-6047; WWW: www.netjunction.com/aceanimals)
2. B & K Universal, Inc. 3403 Yale Way, Fremont, CA 94538 (Tel: 1-800-USA-MICE; WWW: www.bku.com)
3. Charles River Laboratories, 251 Ballardville St., Wilmington, MA 01887 (Tel: 1-800-LAB RATS; WWW: www.criver.com)
4. Covance Research Products, Inc., P.O. Box 7200, Denver, PA (Tel: 1-800-345-4114)
5. Harlan Sprague Dawley, Inc., P.O. Box 29176, Indianapolis, IN 46229-0176 (Tel: 317-894-7521; WWW: www.harlan.com/hsd.htm).
6. Hilltop Lab Animals, Inc., P.O. Box 183, Hilltop Dr., Scottdale, PA 15683 (Tel: 1-800-245-6921; e-mail: edmied@aol.com).
7. The Jackson Laboratory, 600 Main St., Bar Harbor, ME 04609 (Tel: 1-800-422-6423; WWW: www.jax.org)
8. Simonsen Laboratories, Inc., 1180-C Day Rd., Gilroy, CA 95020 (Tel: 408-847-2002; WWW: www.simlab.com)

9. Taconic Farms, Inc., 273 Hover Ave., Germantown, NY 12526 (Tel: 888-TAC-ONIC; WWW: www.taconic.com)

feed

Feed for laboratory mice is typically obtained from large vendors with rigorous quality assurance programs. Examples of such vendors include the following:

1. Dyets, Inc., 2508 Easton Ave., Bethlehem, PA 18017 (1-800-275-3938; WWW: www.dyets.com)
2. Harlan-Teklad, Inc., P.O. Box 44220, Madison, WI 53744-4220 (Tel: 608-277-2070; WWW: www.harlan.com)
3. P.J. Noyes, Inc., P.O. Box 381, Lancaster, NH 03584 (Tel: 1-800-522-2469; WWW: www.pjnoyes.com)
4. Purina Mills, Inc., P.O. Box 66812, St. Louis, MO 63166-6812 (Tel: 314-768-4593; WWW: www.labdiet.com)
5. Zeigler Bros., Inc., P.O. Box 95, Gardners, PA 17324 (Tel: 1-800-841-6800; WWW: www.zeiglerfeed.com)

equipment

Sanitation

Several sources of disinfectants and other sanitation supplies are listed below:

1. BioSentry, Inc., 1481 Rock Mountain Blvd., Stone Mountain, GA 30083 (Tel: 1-800-788-4246; WWW: www.sentrychem.com/biosentry/default.com)
2. Pharmacal Research Labs, Inc., P.O. Box 369, Naugatuck, CT 06770-0369 (Tel: 1-800-243-5350; WWW: www.pharmacal.com)
3. Quip Laboratories, Inc., 1500 Eastlawn Ave., Wilmington, DE 19802 (Tel: 302-761-2600; WWW: www.quiplabs.com)
4. Rochester Midland Corp., P.O. Box 1515, Rochester, NY 14603-1515 (Tel: 716-336-2260; WWW: www.rochestermidland.com)
5. Steris Corp., 5960 Heisley Rd., Mentor, OH (Tel: 1-800-548-4873; WWW: www.steris.com/steris/)

Cages and Research and Veterinary Supplies

Examples of sources for pharmaceuticals, general veterinary supplies, surgical equipment, cages, and other related equipment are provided below. Items are listed and vendors indicated numerically, with contact information for vendors provided following this section. Cages should meet the size requirements as specified by any applicable regulatory or oversight agencies.

Possible Sources of Cages and Research and Veterinary Supplies

Item	Source
Bedding	1, 2, 5, 10, 12, 22, 23, 25
Cages and related equipment	3, 4, 7, 9, 16, 17, 20, 24, 28, 30
Diagnostic laboratories	6, 11, 19, 29, 32
Veterinary and surgical supplies	1, 8, 13, 14, 15, 18, 26, 34
Inhalant anesthesia equipment	1, 8, 14, 15, 27, 33, 34
Restrainers	3, 4, 8, 14, 16, 15, 24
Shipping containers	11, 21, 31
Necropsy tools	1, 8, 13, 14, 15, 26

Contact Information for Cages and Research and Veterinary Supplies

1. A.J. Buck & Son, Inc., 11407 Cronhill Drive, Owing Mills, MD 21117 (Tel: 1-800-638-8672; WWW: www.ajbuck.com)
2. Absorption corp., 1051 Hilton Ave., Bellingham, WA 98225 (Tel: 1-800-242-2287, ext. 3007; e-mail: absorbs@absorption-corp.com)
3. Allentown Caging Equipment Co., Inc., P.O. Box 698, Allentown, NJ (Tel: 1-800-762-2243; WWW: www.acecaging.com)
4. Ancare, Inc., 2647 Grand Ave., P.O. Box 814, Bellmore, NY 11710-0814 (Tel: 1-800-645-6379; WWW: www.ancare.com)
5. Anderson's/Bed-O-Cobs, Inc. P.O. Box 119, Maumee, OH 43537 (Tel: 1-800-537-3370; e-mail: dr_cobs@andersonsinc.com)
6. Anmed/Biosafe, Inc., 7642 Standish Place, Rockville, MD 20855 (Tel: 301-762-0366; e-mail: anmedbio@erols.com)
7. Biozone, Inc., 377 Carowinds Blvd., Suite 209, Fort Mill, SC 29708 (Tel: 1-800-992-6267; WWW: www.biozoneglobal.com).
8. Braintree Scientific, Inc., P.O. Box 850929, Braintree, MA 02185-0929 (Tel: 718-843-2202; WWW: www.braintreesci.com)

9. Britz-Heidbrink, Inc., P.O. Box 1179, Wheatland, WY 82201 (Tel: 307-322-4040; WWW: www.cages-bh.com)
10. Canbrands International, Ltd., 6299 Airport Rd., Suite 303, Mississauga, Ontario L4V 1N3 Canada (Tel: 1-800-267-5287 ext. 3667; WWW: www.paperchip.canbrands.com).
11. Charles River Laboratories, 251 Ballardville St., Wilmington, MA 01887 (Tel: 1-800-522-7287; WWW: www.criver.com)
12. Converdis US, Inc., 210 Walnut St., Lockport, NY 14094 (Tel: 716-433-7430)
13. Fine Science Tools, Inc., 373-G Vintage Park Dr., Foster City, CA 94404 (Tel: 1-800-521-2109; WWW: www.finescience.com)
14. Harvard Apparatus, Inc., 84 October Hill Rd., Holliston, MA 01746 (Tel: 1-800-272-2775; WWW: www.harvardapparatus.com)
15. Kent Scientific Corp., 457 Bantam Rd., Litchfield, CT 06759 (Tel: 860-567-5496; WWW: www.kentscientific.com)
16. Lab Products, Inc., P.O. Box 639, Seaford, DE 19973 (Tel: 1-800-526-0469; WWW: www.labproductsinc.dom)
17. Lenderking Caging Products, 8370 Jumping Hole Rd., Millersville, MD 21108 (Tel: 410-544-8795 Ext. 11; WWW: www.lenderking.com/caging.htm)
18. Lomir Biomedical, Inc., 95 Huot, Notre-Dame Perrot, PQ J7V M4 Canada (Tel: 514-425-3604; WWW: www.lomir.com)
19. MA BioServices, Inc., 9900 Blackwell Rd., Rockville, MD 20850 (Tel: 1-800-756-5658 ext. 3234)
20. Nalge, Inc., 75 Panorama Creek Drive, P.O. Box 20365, Rochester, NY 14602-0365 (Tel: 716-586-8800)
21. Negus Container and Packaging, Inc., 114 N. Bedford St., Madison, WI 53703 (Tel: 608-251-2533)
22. Northeastern Products, Inc., Old Route 9, Box 98, Warrensburg, NY 12885 (Tel: 1-800-873-8233)
23. P.J. Murphy Forest Products Corp., P.O. Box 300, 150 River Rd., Bldg. G2-A, Montville, NJ 07045-0300 (Tel: 1-800-631-1936; WWW: www.pjmurphy.net).
24. Plas-Labs, Inc., 917 E. Chilson St., Lansing, MI 48906 (Tel: 1-800-866-7527; WWW: www.plas-labs.com)
25. Shepherd Specialty Papers, Inc., P.O. Box 2887, 7950 Moorsbridge Rd., Kalamazoo, MI 49003-2887 (Tel: 1-800-253-3286)

26. Roboz Surgical Instrument Co., 9210 Corporate Blvd., Suite 220, Rockville, MD 20850 (Tel: 1-800-424-2984; WWW: www.roboz.com)
27. SurgiVet/Anesco, Inc., N7 W22025 Johnson Road, Suite A, Waukesha, WI 53186 (Tel: 1-888-745-6562; WWW: www.surgivet.com)
28. Tecniplast, USA, P.O. Box 181, Birchrunville, PA 19421 (Tel: 610-827-5006; e-mail: tecniplast@worldnet.att.net)
29. Therion Corp., 185 Jordan Rd., Troy, NY 12180-7617 (Tel: 518-286-0016; WWW: www.theriondna.com)
30. Thoren Caging Systems, Inc., P.O. Box 586, Hazleton, PA 18201 (Tel: 570-455-5041; e-mail: sathomas@thoren.com)
31. Transport Container, Inc., P.O. Box 163183, Columbus, OH 43216 (Tel: 614-459-8140)
32. University of Missouri-Columbia Animal Diagnostic and Investigative Laboratory, 1660 East Rollins, Columbia, MO 65211 (Tel: 1-800-669-0825; WWW: www.hsc.missouri.edu/~radil)
33. VetEquip, Inc., P.O. Box 10785, Pleasonton, CA 94588 (Tel: 925-463-1828; WWW: www.VetEquip.com)
34. Viking Medical, Inc., P.O. Box 2142, Medford Lakes, NJ 08055 (Tel: 1-800-920-1033; WWW: www.vikingmedical.com)

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