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# Biology and Diseases of Rats

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## I. INTRODUCTION

### A. Major Taxonomic and Historical Considerations

The laboratory rat, *Rattus norvegicus*, is within the order Rodentia and family Muridae. The genus *Rattus* contains at least 56 species (retrieved January 28, 2014, from the Integrated Taxonomic Information System online database http://www.itis.gov); however, the Norway rat, *R. norvegicus*, and the black rat, *R. rattus*, are the two species most commonly associated with the genus. *Rattus rattus* preceded *R. norvegicus* in migration from Asia to Europe and the Americas by several hundred years. The former species reached Europe in the 12th century, and the Americas in the 16th century; whereas, *R. norvegicus* emerged in the 18th century in Europe and in the 19th century in the Western Hemisphere. Globally, the Norway rat has largely displaced the black rat, probably because of the Norway rat's larger size and aggressiveness. The domestication and introduction of the albino *R. norvegicus* is rooted by its use in Europe and America in the 1800s as prey for a sport (rat baiting) in which individuals would wager on which terrier dog would most swiftly kill the largest number of rats confined to a pit. Because of the large numbers of rats needed for this sport, wild

rats were purpose-bred, and albinos were selected out by some people as a hobby (Robinson, 1965; Mayhew, 1851).

Early experimental use of the rat was reviewed by Hedrich (Hedrich, 2000). Early experiments using rats in nutrition research date back to at least 1828, when rats were starved as part of fasting studies (McCay, 1973). Savory used them in 1863 in protein studies (Savory, 1863), and J.M. Philipeaux, reported on the effects of adrenalectomy in albino rats in 1856 (Philipeaux, 1856). Rats were used in experiments only sporadically in Europe and North America until about 1890. Pivotal to the development of the rat for use in research were Henry H. Donaldson, and Milton Greenman at the Wistar Institute in the early 20th century, who did much to produce and define early stocks of laboratory rats (Lindsey, 1979).

## B. Uses in Research

The rat is second only to the mouse as the most frequently used mammal in biomedical and behavioral research. Characteristics such as a short gestation and a relatively short life span, docile behavior, and ready availability of animals with well-defined health and genetic backgrounds are responsible for the importance of the rat as a laboratory animal. The rat is a standard species for toxicological, teratological, and carcinogenesis testing by the pharmaceutical industry and governmental regulatory agencies. Its early use in behavioral, neurological, nutritional, and endocrinology studies continues today. The size of the rat enables it to be used for surgical procedures, varying from organ transplantation to vascular techniques. Although the number of commonly used inbred strains is dwarfed by those of the mouse, inbred rat strains do represent an important repertoire of disease models (Table 4.1).

## C. Summary of Laboratory Management and Husbandry

### 1. Macroenvironment

Rooms in which rats are to be housed should meet the guidelines of the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011). Wall, ceiling, and floor surfaces should be made of materials that allow for effective sanitation and that resist damage from normal use and manipulation of equipment. The environment of the room should be well controlled, to ensure animal well-being and to help limit variables to those of the experimental design. Although rats, like most other species, can adapt to changes in temperature and humidity, room temperatures within a range of 70–76°F and with a relative humidity of 30–70% are typically accepted as being appropriate. Twenty-four-hour temperature/humidity recorders, either located in animal rooms or as a

**TABLE 4.1**  Commonly Used Rat Strains

| Inbred strains | Usefulness as models |
| --- | --- |
| ACI | Congenital genitourinary anomalies, prostatic adenocarcinomas |
| BB/Wor | Juvenile insulin-dependent diabetes mellitus |
| BN (Brown Norway) | Inducible, transplantable myeloid leukemia, hydronephrosis, bladder carcinoma |
| BUF (Buffalo) | Spontaneous autoimmune thyroiditis, host for transplantable Morris hepatoma |
| COP (Copenhagen) | Prostate adenocarcinoma |
| F-344 (Fischer 344) | Inbred rat model for National Toxicology Program's Carcinogen Bioassay Program and the National Institute on Aging |
| LEW (Lewis) | Multiple sclerosis, various experimentally induced autoimmune diseases |
| LOU/C | Myeloma, production of IgG autoantibody |
| SHR (spontaneous hypertensive rat) | Hypertension, cardiovascular research |
| WF (Wistar-Furth) | Mononuclear cell leukemia |
| Zucker | Obesity |
| **Mutant strains** | **Characteristics** |
| Brattleboro | Diabetes insipidus (autosomal recessive) |
| Gunn | Jaundice, kernicterus (autosomal recessive) |
| Nude | T cell deficient (autosomal recessive) |
| Obese SHR | Type 4 hyperlipoproteinemia (autosomal recessive) |

component of an electronic environmental management system, are useful in detecting changes in environmental conditions. Practice over many years has shown that, in general, ventilation rates of 10–15 air changes/hour of fresh air are sufficient to compensate for heat load and the generation of $NH_3$ and $CO_2$ from animals. A stable photoperiod is necessary to avoid changes in reproductive behavior, food intake, and weight gain. A cycle of 12–14h light and 10–12h dark is typically used for rats. Rats are particularly susceptible to phototoxic retinopathy. There is evidence to indicate that light intensity at cage level should be between 130 and 325 lux to prevent retinopathy (National Research Council, 2011). Management precautions such as not having exposed cages on the top shelves of tall racks should be considered.

Prevention and control of infectious diseases are partially a function of the location, size, and environmental conditions of a rat housing room. Strategies for limiting the transfer of pathogens will vary according to the potential impact that infectious agents may have on a particular group of rats and the study in which they are being used. For instance, an appropriate-sized room or cubicle may reflect the space necessary to separate rats

by such criteria as pathogen status, immunological status, vendor, protocol, or investigator. Modifications such as the incorporation of Class 100 flexible wall enclosures may be useful to help ensure the specific pathogen status of rats over an extended period of time. Because of stress produced by noise, rat rooms should be located distant from mechanical rooms, cage-washing centers, and species that are apt to produce noise (National Research Council, 2011).

## 2. Primary Enclosures

The amount of cage space needed for rats, whether group or individually housed, is a function of animal weight, age and sex, as well as the specific physiological or protocol requirements of the animal(s) (National Research Council, 2011). Unless there is an experimental need, rats should be housed in solid-bottom rather than in wire-bottom cages. This will help prevent pododermatitis and injuries that are more frequently associated with wire floors, and bedding within solid-bottom cages provides some minimum environmental enrichment. However, enrichment enhances animal well-being by providing stimulation of natural species behavior. The resulting psychological benefits to the animal also result in a more robust biological model system and can improve the quality of scientific data derived form those animals (National Research Council, 2011). The most frequently used materials for solid-bottom cages are polycarbonate and polypropylene. The former plastic is often preferred because it may be repeatedly autoclaved without damage and because its translucency allows for observation of animals. Various contact bedding materials are appropriate for rats (e.g., hardwood chips, ground corncob, cellulose sheets).

## 3. Sanitation

Solid-bottom cages should typically be sanitized at a frequency of 1–2 times per week. A less frequent cycle may be appropriate if cage density is very low, if there are perinatal considerations, or if ventilated cages are used; and a more frequent cycle, if cage density is high or if pathophysiological considerations exist (e.g., diabetes) which increase the rate of soiling of the bedding. A detailed description of appropriate sanitation for rodent housing is given in *The Guide* (National Research Council, 2011).

## II. BIOLOGY

## A. Morphophysiology

This section provides a summary of some of the morphophysiological characteristics of the rat that may be useful to the reader. For more comprehensive descriptions, see the references cited in this section.

## 1. General Appearance

The Norway rat has small, thick ears and a tail that is about 85% of the length of the body (in contrast, *R. rattus* has larger ears and a tail that is distinctly longer than the body). The hair coat is composed of two classes – long and short hair shafts, with the former being more sparse. Hair growth in the young rat is cyclic, with the resting period and the growing period each being 17 days. In the female, there are usually 12 teats, with three pairs in the pectoral and three pairs in the abdominal region (Greene, 1963). Body weights and growth rates are dependent on the stock, strain, and source of rats. Of the two most commonly used outbred stocks, the Sprague-Dawley is larger than the Wistar, and the inbred Fischer 344 rat is smaller than either of the outbreds.

## 2. Sensory Organs

Rat eyes are exophthalmic, which increases the risk of injury from trauma and drying during anesthesia. The eyelids are well developed, and only the cornea is visible. The cornea is moistened by secretions from the lacrimal glands and the Harderian gland, which is located medially to the orbit. When a rat is stressed (e.g., because of malnutrition, dehydration, disease, or environmental factors) there can be an excessive secretion of Harderian gland products containing porphyrins, which is termed chromodacryorrhea. This results in a reddish secretion or crust located periorbitally and at the nares that may be a useful indicator of an illness or a husbandry problem (Moore, 1995). Interestingly, the porphyrins in these secretions will fluoresce, and it has been suggested that the Harderian gland may have a function as a modulator of light-mediated responses (Cui *et al.*, 2003). The orbital venous plexus has a slightly different anatomy than the obrbital sinus observed in the mouse, but it is a useful site for blood collection in the anesthetized animal (Timm, 1979; Sharma *et al.*, 2014). Rats do not have robust color vision and their ability to discriminate based on color is difficult to demonstrate. It does, however, appear that they do have dichromatic color vision with two types of cones, one of which is responsive to ultraviolet (UV) wavelengths with a response centered around 359 nm (Koolhass, 1999; Jacobs *et al.*, 2001). This shift to the UV as compared to human vision is accompanied by a relative insensitivity of rat vision to longer wavelengths, since both the long-wavelength cone pigment and rod pigment have a peak sensitivity around 505–509 nm. This can be exploited by the use of dim red lighting (peak wavelengths greater than ~625 nm) generated using red bulbs or transparent red films/filters to view rats during their dark cycle, but it must be understood that intense red light (e.g., a bulb positioned close to the cage) can be perceived by the animals, and also that even dim red light can affect their photoperiodic physiology (McCormack and Sontag, 1980). Olfactory signals are strong determinants

for behavior in the rat. Male rats recognize the social status of other males, females in estrus, and kinship by olfactory cues. Rats also detect alarm pheromones from other rats (Koolhass, 1999). The vomeronasal organ is critical for many pheromone responses (Liman, 1996).

The hearing range of rats extends from 250 Hz to nearly 80 kHz (Sales and Milligan, 1992), with the most sensitive hearing in the range of 8–32 kHz. Except for the rat's high-frequency sensitivity, its hearing capability corresponds closely to that of other mammals (Kelly and Masterton, 1977). The ultrasonic range of hearing and vocalization in the 22- to 80-kHz range is used for a variety of positive and negative communications, such as those emitted by pups left alone by their dam, or by adults during sexual and aggressive behavior (Koolhass, 1999; Portfors, 2007). Because frequencies that are disruptive to rats may not even be audible to humans, it is important to minimize ultrasonic noise in animal facilities. Monitoring equipment capable of detecting ultrasound should be utilized when screening rodent housing or use areas for noise contamination, and care must be taken when selecting equipment to avoid those known to emit ultrasound including some models of occupancy sensors (Weigler et al., 2007) and some types of energy-efficient high-frequency electronic ballasts that are used to drive linear fluorescent lamps (personal experience; author G.O.).

An extremely important sensory organ for touch in the rat is the array of sensitive vibrissae that are used to sample the environment in a variety of ways (Hartmann, 2011).

### 3. Skeleton

The skull is composed of the following bones: paired nasal, premaxillary, maxillary, zygoma, palatine, lacrimal, frontal, parietal, squamosal, periotic capsule, tympanic bulla, and mandible; six auditory ossicles; four turbinates; and single vomer, ethmoid, basisphenoid, presphenoid, occipital, interparietal, and hyoid bones.

The vertebral column consists of seven cervical, 13 thoracic, six lumbar, four sacral, and 27–30 caudal vertebrae. The ribs consist of ventral calcified and dorsal ossified segments without true costal cartilages. The humerus, ulna, and radius are similar to those of other mammalian species. The carpus consists of nine bones. The pelvis is formed by two ossa coxae, which articulate with the first two sacral vertebrae. The bones of the hindlimb are the femur, the tibia, and the fibula, which articulates proximally with the tibia but is fused distally. The tarsus is composed of eight bones (Greene, 1963).

### 4. Digestive System

The dental formula of the rat is 2(I 1/1, C 0/0, PM 0/0, M 3/3). Incisors grow continuously. If the incisors are not worn evenly or are misaligned due to gingivitis or congenital defects, the resulting malocclusion may lead to nonfunctional, spiral elongation of the incisors, injury to the palate, and reduced food intake. The salivary glands are paired and consist of the parotid, the submandibular, and the smaller sublingual glands. The parotid gland is serous and consists of three or four lobes located ventrolaterally from the caudal border of the mandible to the clavicle. The submandibular glands are mixed glands situated ventrally between the caudal border of the mandibles and the thoracic inlet. The sublingual glands are mucous and located at the rostral pole of the submandibular glands. Multilocular adipose tissue, referred to as brown fat or the hibernating gland, is located in the ventral and lateral portions of the neck and can be confused with salivary glands. Figure 4.1 depicts the location of the salivary glands, cervical lymph nodes, and associated muscles and blood vessels (Constantinescu, 2011).

The stomach is divided into two parts: the forestomach, or cardiac portion, which is nonglandular; and the corpus, or pyloric portion, which is glandular. A structure referred to as the limiting ridge (margo plicatus) separates the two portions, with the esophagus entering at the lesser curvature of the stomach through a fold of the ridge. This anatomical configuration is sometimes mentioned as a reason that rats cannot vomit, but this is somewhat simplistic, since rats, along with other rodents, do not possess many of the anatomical and neurological components that are required for a functional vomiting reflex (Horn et al., 2013).

The small intestine of the adult rat consists of the duodenum (8 cm), jejunum (80 cm), and ileum (3 cm). The comma-shaped cecum is thin-walled, with a prominent mass of lymphoid tissue in its apical portion. The colon consists of the ascending colon, with prominent oblique mucosal ridges, and the transverse and descending portions, with longitudinal mucosa folds.

The liver has four lobes: the median, which has a deep fissure for the hepatic ligament; the right lateral, which is partially divided; the left, which is large; and the caudate, which is small and surrounds the esophagus. The rat does not have a gallbladder, and bile ducts from each lobe form the common bile duct, which enters the duodenum about 25 mm from the pyloric sphincter.

The pancreas is a very diffuse and lobulated organ that can be differentiated from adjacent adipose tissue by its darker color and firm consistency. Numerous excretory ducts fuse into two to eight large ducts, which empty into the common bile duct (Bivin et al., 1979). Figure 4.2 depicts the abdominal and thoracic viscera in situ (Greene, 1963).

### 5. Respiratory System

The rat has a maxillary recess (sinus) located between the maxillary bone and the lateral lamina of the ethmoid bone. The recess contains the lateral nasal gland (Steno's gland), which has morphologic similarities to a serous
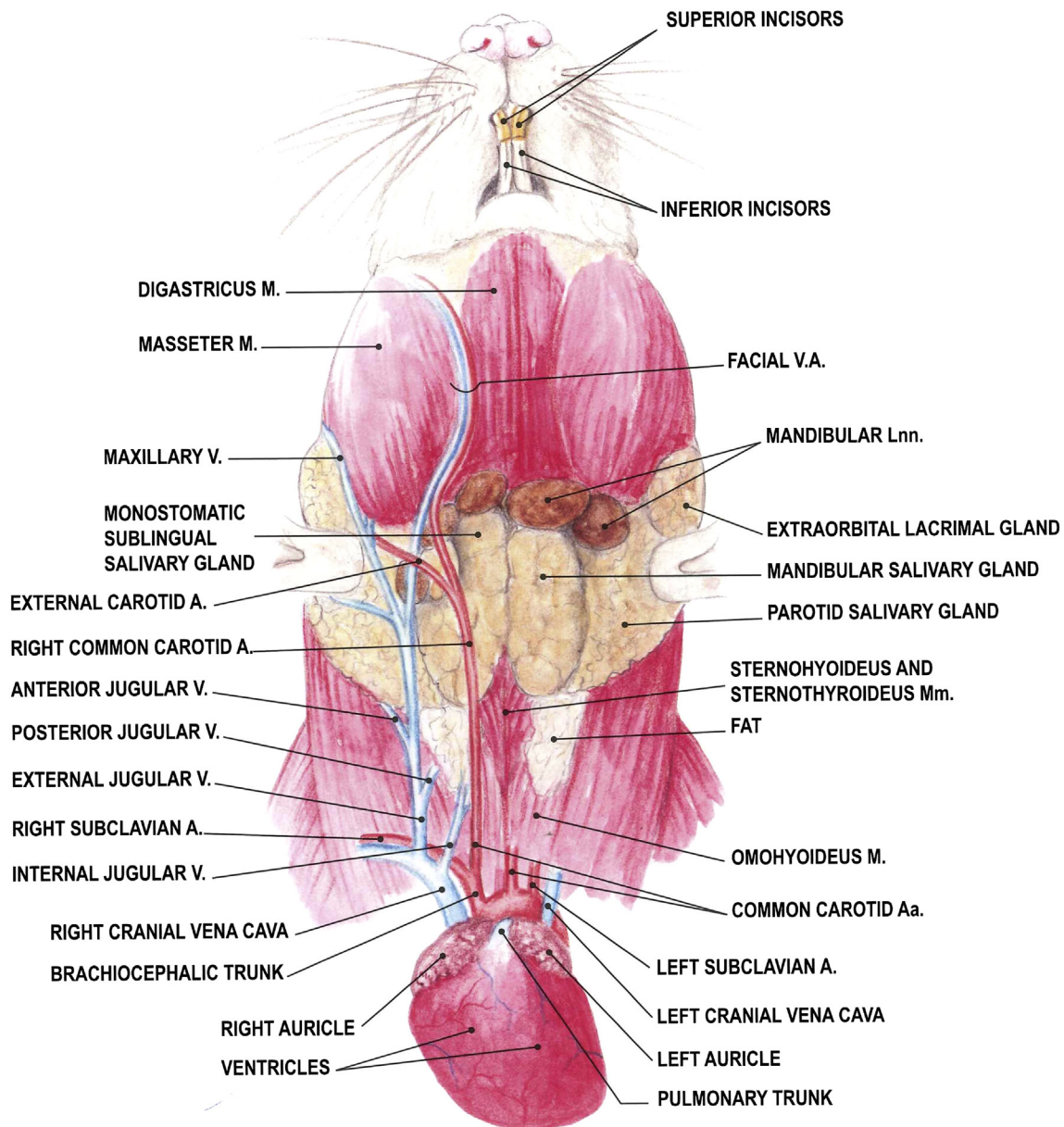
**FIGURE 4.1**  Anatomy of the superficial ventral cervical area including muscles, salivary glands, major vessels, and lymph nodes. *From Constantinescu (2011).*

salivary gland and secretes a watery product discharged at the rostral end of the nasal turbinate. It has been postulated that this secretion may act to regulate the viscosity of the mucous layer overlying the nasal epithelium.

The lungs consist of the left lung, which is single-lobed, and the right lung, which is divided into the cranial, middle, accessory, and caudal lobes. The pulmonary vein has cardiac striated muscle fibers within its wall that are contiguous with those in the heart. The rat does not have an adrenergic nerve supply to the bronchial musculature, and bronchoconstriction is controlled by vagal tone (Bivin *et al.*, 1979).

### 6. Genitourinary System

The male rat has a number of highly developed accessory sex glands (Fig. 4.3). The paired bulbourethral glands (Cowper's glands) at the base of the penis open into the dorsal surface of the urethral flexure. Within the abdominal cavity and surrounding the bladder are the large vesicular glands (seminal vesicles) and the prostate gland, which is composed of the dorsocranial (coagulation gland), ventral, and dorsolateral lobes. The female rat has a bicornate uterus, and although the uterine horns appear fused distally, there are two distinct ossa uteri and cervices.
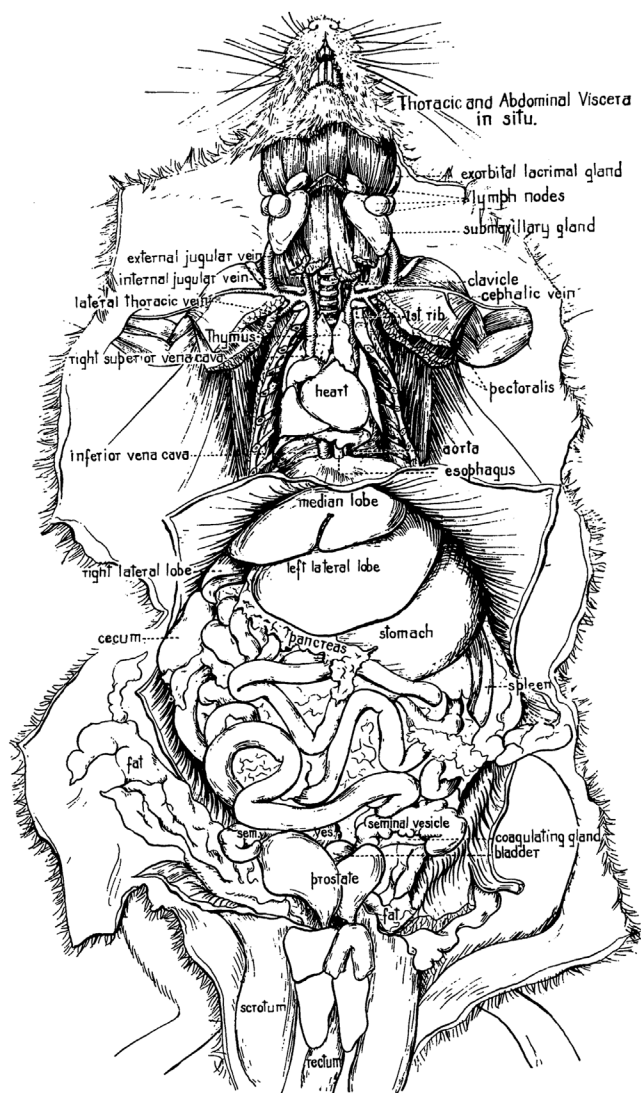
**FIGURE 4.2**  Abdominal and thoracic viscera *in situ*. From *Greene (1963)*.

The right kidney is more craniad than the left and has its cranial edge at the L1 vertebra and its caudal edge at the level of L3. Like the kidneys of other rodents, the rat kidney is unipapillate, making the rat useful for studies in which cannulization of the kidney is done. The rat is also widely used as a model for investigating nephron transport in an *in vivo* micropuncture system, because of the presence of superficial nephrons in the renal cortex (Vallon, 2009).

### 7. Central Nervous System

The brain is characterized by large olfactory bulbs, a lissencephalic (smooth) cerebrum, and the two parafloccular lobes of the cerebellum, which lie in deep sockets of the periotic capsule of the skull. The hypophysis (pituitary gland) lies behind the optic chiasma and is attached to the base of the brain by a thin hollow stalk, the infundibulum. The ventricular system is similar to that of other animals, but the rat lacks a foramen of Magendie. The spinal cord ends at the fourth lumbar vertebra, with the filum terminale ending at the level of the tail beyond the third caudal nerves (Greene, 1949).

### 8. Cardiovascular System

The heart is located on a midline in the thorax, with its apex near the diaphragm and its lateral aspects bounded mainly by the lungs. The heart is exposed to the left thoracic wall between the third and fifth ribs, making it a useful site for cardiac blood collection that for humane reasons is generally perfumed as a sedated, nonsurvival procedure. The blood supply to the atria of the rat, unlike that of higher mammals, is largely extra-coronary from branches of the internal mammary and subclavian arteries.

## B.  Normal Physiological Values

Many of the normal values determined for a specific group of rats may be accurate for only that source and stock/strain. Other factors such as age, pathogen status, sample collection methods, and husbandry conditions of the colony are also important variables (Suber and Kodell, 1985; Dameron *et al.*, 1992; Perez *et al.*, 1997). Selected physiological, clinical chemistry, and hematological values are listed in Tables 4.2–4.5. *Note*: the values provided in these tables are meant to be representative examples useful for educational purposes. Laboratory-specific normal ranges and/or strain, sex and age-matched control data should be used for research and diagnostic purposes.

## C.  Nutrition

Rats are categorized as omnivores, and nutritionally adequate diets are readily available from commercial sources. However, the refinement of ingredients within diet formulations may vary according to classifications of commercially available products. The three classifications of diets are (1) natural-ingredient, (2) purified, and (3) chemically defined. The most commonly used type for most research applications is the natural-ingredient diet, composed of agricultural products and by-products. This class of diet can be either an open-formula diet, in which the information on the amount of each ingredient is available, or a closed-formula diet, in which such information is held confidential by the producer. The nutrient composition of ingredients in natural-ingredient diets varies from batch to batch because of various factors (e.g., relative costs of grains, weather conditions, harvesting and storage conditions, and concentrations of contaminants). Certified, natural-ingredient diets
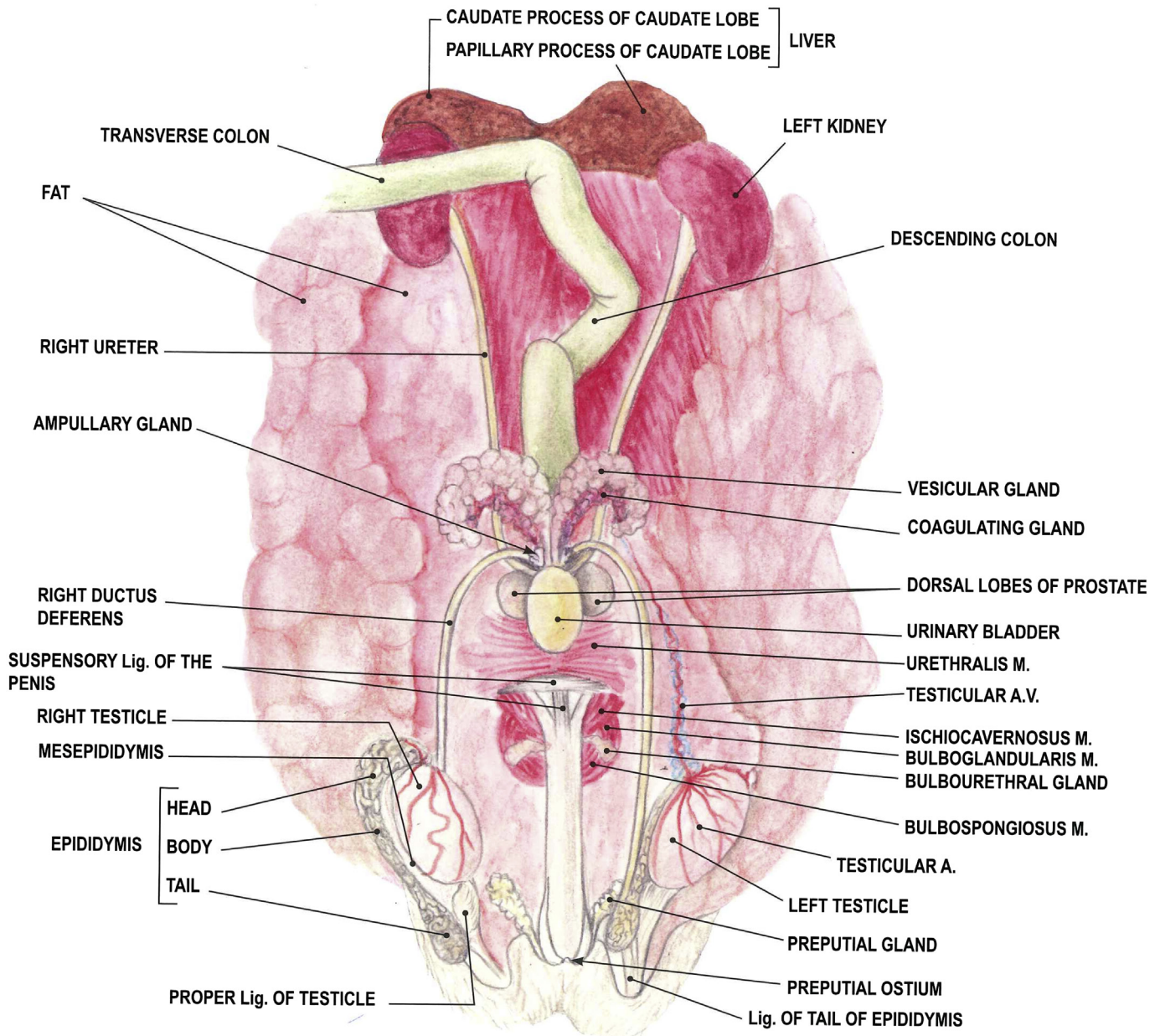
**FIGURE 4.3** Male urogenital system. *From Constantinescu (2011).*

are used for toxicological and other Good Laboratory Practice (GLP) studies because each lot is assayed and certified not to exceed established maximum concentrations of a set list of contaminants (e.g., pesticides, heavy metals, mycotoxins, and estrogens) that could influence study results.

The nutrient concentrations in purified diets are less variable because defined ingredients, each composed of a single nutrient or nutrient class (e.g., casein, sugar, starch, vegetable oil, cellulose), are used in their formulation. A frequently used purified diet for rats is AIN-76. The downside of this class of diets is that they are more expensive and often less palatable. Chemically defined diets are formulated with very basically defined ingredients (e.g., specific amino acids, sugars, triglycerides, and essential fatty acids). These diets are costly and tend to lack palatability (National Research Council, 1996). A comprehensive description of the nutrient requirements of rats is available (National Research Council, 1995). Phytoestrogens are being increasingly recognized as a dietary variable that can have significant effects in both female and male rats (Boettger-Tong *et al.*, 1998; Weber *et al.*, 2001).

In most instances, rats are fed *ad libitum*. However, there are numerous reports that demonstrate that unlimited

TABLE 4.2    Selected Normative Data for the Rat[a]

### ADULT

| Weight | |
|---|---|
| Male | 300–500 g |
| Female | 250–300 g |
| Life span | 2.5–3 years |
| Body temperature | 37.5°C |
| Basal metabolism rate (400-g rat) | 35 kcal/24 h |
| Chromosome number (diploid) | 42 |
| Puberty | $50 \pm 10$ days |
| Gestation | 21–23 days |
| Litter size | 8–14 |
| Birth weight | 5–6 g |
| Eyes open | 10–12 days |
| Weaning | 21 days |
| Food consumption/24 h | 5 g/100 g body weight |
| Water consumption/24 h | 8–11 ml/100 g body weight |

### CARDIOVASCULAR

| Arterial blood pressure | |
|---|---|
| Mean systolic | 116 mmHg |
| Mean diastolic | 90 mmHg |
| Heart rate | 300–500 beats/min |
| Cardiac output | 50 ml/min |
| Blood volume | 6 ml/100 g body weight |

### RESPIRATORY

| Respirations/min | 85 |
|---|---|
| Tidal volume | 1.5 ml |
| Alveolar surface area (400 g rat) | 7.5 m$^2$ |

### RENAL

| Urine volume/24 h | 5.5 ml/100 g body weight |
|---|---|
| Na$^+$ excretion/24 h | 1.63 mEq/100 g body weight |
| K$^+$ excretion/24 h | 0.83 mEq/100 g body weight |
| Urine osmolarity | 1659 mOsm/kg of H$_2$O |
| Urine pH | 7.3–8.5 |
| Urine specific gravity | 1.04–1.07 |

[a]Data from Baker (1979) and Bivin et al. (1979).

feeding of rats on long-term carcinogenesis and toxicological studies reduces longevity and increases the incidence of neoplasia relative to rats fed at 70–80% of the *ad libitum* food amount. These effects have been found in Sprague-Dawley, Wistar, and F-344 rats and have caused increased variability among 2-year carcinogenicity and safety assessment studies, compromising the usefulness of bioassays in risk assessment (Keenan *et al.*, 1996). For instance, Wistar rats fed 80% of *ad libitum* beginning at 16 weeks of age had very significant reductions in the incidence of lung, mammary, pancreatic islet cell, and pituitary tumors relative to controls fed *ad libitum.* The overall incidence of malignant tumors was 16% in the feed-limited group and 37% in the *ad libitum* group, even though the feed-limited group had a greater longevity. There was also a reduction in chronic inflammation and fibrosis of the heart, acute inflammation of the prostate, radiculoneuropathy, and acinal hyperplasia of the mammary gland in feed-limited animals (Roe *et al.*, 1995). Four pathways have been implicated as potentially mediating the caloric restriction effect. These are the insulin-like growth factor (IGF-1)/insulin signaling pathway, the sirtuin pathway, the adenosine monophosphate (AMP)-activated protein kinase (AMPK) pathway, and the target of rapamycin (TOR) pathway (Speakman and Mitchell, 2011).

## D. Reproduction

### 1. Reproductive Physiology

In the rat, the vagina is closed at birth by compact epithelium, referred to as the vaginal plate (Del Vecchio, 1992). This begins to degenerate and cornify at 20–35 days of age and is completely open between 40 and 80 days of age. Persistence of the vaginal plate can result in a fully imperforate vagina or in the presence of a vaginal septum, anomalies that may be associated with infertility or metritis (Lezmi *et al.*, 2011). Vaginal opening can be used as an external indicator of impending puberty in female rats, and a similar phenomenon, preputial separation, occurs in male rats (Korenbrot *et al.*, 1977). A study in Wistar rats documented vaginal opening in control females at approximately 36 days of age and balano-preputial separation in control males at approximately 46 days of age (Engelbregt *et al.*, 2000). Puberty is defined as the onset of sexual maturity indicated by the ability to bear viable young, and it occurs before full body size and weight are attained. As in most species, puberty occurs in females earlier than in males and also varies with stock or strain. Puberty most often occurs at 2–3 months of age in the rat (Fox and Laird, 1970), although considerable variation exists in reported values. Kohn and Barthold (1984) report 40–60 days, Bennett and Vickery (1970) report 50–72 days, and Ayala *et al.* (1998) report 45–47 days. Estrus, which should be distinguished from puberty, begins before full reproductive competency is reached and has been reported to occur at 36 days in the Wistar rat (Eckstein *et al.*, 1973). However, some authors report successfully breeding Wistar BH rats at 35 days of age (Rosen *et al.*, 1987).

TABLE 4.3  Clinical Chemistry Reference Ranges for Adult Rats[a]

| Analyte | Units | Sprague-Dawley[b] | | Fisher 344[d] | |
| | | M | F | M | F |
|---|---|---|---|---|---|
| *SERUM* | | | | | |
| Glucose | mg/dl | 115 ± 16.9 | 111 ± 17.2 | 115 ± 12.5[c] | |
| Urea nitrogen | mg/dl | 19 ± 2.2 | 21 ± 3.4 | 15 ± 2.5[c] | |
| Creatinine | mg/dl | 0.70 ± 0.11 | 0.70 ± 0.13 | | |
| Sodium | mEq/l | 150 ± 3.4 | 148 ± 3.5 | 149 ± 3.0[c] | |
| Potassium | mEq/l | 7.00 ± 0.65 | 6.1 ± 0.67 | 4.80 ± 0.35[c] | |
| Chloride | mEq/l | 103.0 ± 1.90 | 104.0 ± 2.4 | 106 ± 3.0[c] | |
| Calcium | mg/dl | 12.0 ± 0.94 | 12.1 ± 0.71 | 10.5 ± 0.50[c] | |
| Phosphorus | mg/dl | 7.30 ± 1.5 | 5.80 ± 1.10 | | |
| Magnesium | | 3.12 ± 0.41[e] | 2.60 ± 0.21[e] | | |
| Iron | µg/dl | 152 ± 70 | 220 ± 130 | | |
| | | | (19–21 weeks) | | |
| Total iron-binding capacity | µg/dl | 368[c] | | | |
| Alanine aminotransferase | IU/l | 49 ± 24.1 | 69 ± 44.9 | 78 ± 11 | 49 ± 8 |
| Aspartate aminotransferase | IU/l | 95 ± 31.7 | 99 ± 54.5 | | |
| Alkaline phosphatase | IU/l | 130 ± 43.7 | 117 ± 41.7 | 49.5 ± 9.25[c] | |
| Lactate dehydrogenase | IU/l | 275 ± 112 | | 650 ± 75 | 650 ± 75 |
| | | | | | (20 weeks) |
| Sorbitol dehydrogenase | IU/l | 20 ± 5[c] | | 20.0 ± 7.5[c] | |
| | | (32 weeks) | | | |
| γ-Glutamyl transpeptidase | IU/l | 2.5 ± 1.25[c] | | | |
| Creatinine kinase | IU/l | 275 ± 112.5[c] | | 400 ± 50[c] | |
| Protein, total | g/l | 70 ± 5.0 | 75 ± 5 | 70.5 ± 4.75[c] | |
| Albumin | g/l | 34 ± 2.0 | 40 ± 2.5 | 42.5 ± 3.75[c] | |
| Cholesterol | mg/dl | 119 ± 51.3 | 119 ± 29.0 | 96.5 ± 14.25 | 130 ± 10.0 |
| Triglycerides | mg/dl | 266 ± 121.4 | 249 ± 159.7 | 122 ± 21.25 | 62.5 ± 11.25 |
| Bilirubin | mg/dl | 0.3 ± 0.16 | 0.4 ± 0.27 | 0.3 ± 0.1[c] | |
| Bile acids | µmol/l | 40 ± 10[c] | | 30 ± 10[c] | |
| | | (20 weeks) | | | |
| Uric acid | mg/dl | 1.52 ± 0.30 | 1.25 ± 0.36 | | |
| *URINE* | | | | | |
| Volume | ml/16h | | | 9.5 ± 4.0 | 9.3 ± 5.6 |
| | ml/22h | 15.7 ± 6.7 | 11.0 ± 5.0 | | |
| Specific gravity | | | | 1.022 ± 0.007 | 1.017 ± 0.007 |
| Osmolality | mOsm/kg | 943 ± 327 | 995 ± 367 | | |
| pH | | 7.8 ± 0.5 | 7.7 ± 0.5 | 6.0–6.5[c] | |
| Chloride | mmol/l | 148 ± 36 | 151 ± 51 | | |

(*Continued*)

**TABLE 4.3**   (Continued)

| Analyte | Units | Sprague-Dawley[b] | | Fisher 344[d] | |
|---|---|---|---|---|---|
| | | M | F | M | F |
| Sodium | mmol/l | 31 ± 11 | 50 ± 22 | | |
| Potassium | mmol/l | 121 ± 31 | 110 ± 45 | | |
| Phosphorus | mg/dl | 142 ± 34 | 156 ± 62 | | |
| Creatinine | mg/dl | 136 ± 40 | 116 ± 41 | 80 ± 28 | 54 ± 25 |
| Glucose | mg/dl | | | 9.9 ± 3.9 | 5.5 ± 2.3 |
| Protein | mg/dl | | | 98.8 ± 54.4 | 11.2 ± 5.5 |
| Alkaline phosphatase | IU/l | | | 152 ± 61 | 73 ± 37 |
| Lactate dehydrogenase | IU/l | | | 28 ± 15 | 16 ± 8 |
| $N$-Acetyl-$\beta$-glucosaminidase | IU/l | | | 12.2 ± 7.8 | 5.9 ± 2.7 |
| Aspartate aminotransferase | | | | 14.4 ± 6.5 | 3.6 ± 2.5 |
| $\gamma$-Glutamyl transpeptidase | IU/l | | | 4964 ± 780 | 1873 ± 215 |
| Insulin clearance | μl/min/100 g | 857 ± 178 | | | |
| Glomerular filtration rate | μl/min | 275 ± 33[c] | | | |
| Urine flow | μl/min/100 g | 5.2 ± 2.0 | | | |

[a]Values are for 12-month-old animals, unless noted otherwise, as summarized from Loeb and Quimby (1999).
[b]6–18 months old.
[c]Gender not specified.
[d]58–l 12 weeks old.
[e]Wistar strain.

**TABLE 4.4**

| Hormone[b] | Units | Male | Female |
|---|---|---|---|
| Luteinizing hormone | ng/ml | 0.16–0.64 | 0.32–0.64 (basal) |
| | | | 24.6–32.8 (late proestrus) |
| Follicle stimulating hormone | ng/ml | 5.56–11.1 (light period) | 2.22–4.44 (basal) |
| | | 11.1–20.0 (dark period) | 8.85–13.3 (preovulatory, estrus) |
| Prolactin | ng/ml | 28.6 (dark period) | 5.4–10.7 (basal) |
| | | 1.8–10.7 (light period) | 71.4–107 (late proestrus) |
| | | 71.4 (after coitus) | |
| Growth hormone | ng/ml | 0.4–80[a] | |
| Thyroid stimulating hormone | ng/ml | 2.27–3.4 | 0.57 (basal) |
| | | | 1.14 (early light period) |
| Adrenocorticotropic hormone | pg/ml | 30–100 | |
| Vasopressin | pg/ml | 1–8[a] | |
| Oxytocin | pg/ml | 4–10.5[a] | |
| Thyroxine ($T_4$) | μg/dl | 5.1 ± 0.4 | 4.9 ± 0.1 (Long-Evans) |
| Triiodothyronine ($T_3$) | ng/dl | 66 ± 3.5 | 83 ± 3 (Long-Evans) |
| Free $T_4$ | ng/dl | 2.212 ± 0.055[a] | |
| Free $T_3$ | pg/dl | 208.49 ± 8.55[a] | |

*(Continued)*

**TABLE 4.4** (Continued)

| Hormone[b] | Units | Male | Female |
|---|---|---|---|
| Calcitonin | pg/ml | 200–1000[a] (F-344/9 months) | |
| Parathyroid hormone | pg/ml | 140–180 | <50–400 (Sprague-Dawley) |
| 1,25-Dihydroxy vitamin D | pg/ml | 120 ± 24 | 96 ± 17 (Wistar) |
| Corticosterone | µg/dl | 15–23 (late light period) | 70 (late light period) |
| | | 1–6 (late dark period) | 17 (late dark period) |
| Aldosterone | ng/dl | 12–35 (late light period) | 25–35 (9–10 a.m.) |
| | | 4–11 (middle light period) | |
| Epinephrine | pg/dl | 253 ± 30[a] (awake, undisturbed; Sprague-Dawley) | |
| | | 460 ± 60[a] (handled) | |
| | | 180 ± 24[a] (asleep) | |
| Norepinephrine | pg/dl | 710 ± 110[a] (awake, undisturbed; Sprague-Dawley) | |
| | | 830 ± 130[a] (handled) | |
| | | 460 ± 80[a] (asleep) | |
| Progesterone | ng/ml | | 1–5 (early proestrus) |
| | | | 40–50 (estrus) |
| | | | 20–30 (first diestrus day) |
| Estradiol | pg/ml | | <10 (basal) |
| | | | 20–30 (2nd diestrus day) |
| | | | 40–50 (proestrus) |
| Testosterone | ng/ml | 3 (1330–1600 h) | |
| | | <1 (2130 h) | |
| | pg/ml | | 500–600 (proestrus) |
| | | | 100 (estrus) |

[a]Gender not specified.
[b]Strain not specified.

The estrous cycle of rats is most often 4–5 days in length and occurs throughout the year, as well as postpartum. Seasonal variation is not observed in laboratory colonies. For a 4-day estrous cycle, approximately 1 day is spent in each of the four stages: estrus, metestrus, diestrus, and proestrus. However, cycles of up to 6 days are not uncommon, with the additional time in diestrus or proestrus (Peluso, 1992). In proestrus, the uterus can appear 'ballooned' with fluid, especially in a peripubertal rat; this condition should not be mistaken for hydrometra.

Ovulation occurs approximately 8–11 h after the onset of estrus, usually between midnight and 2 a.m. (Peluso, 1992), although this would obviously depend on timing of the light cycle. Ova remain viable for approximately 10–12 h (Fox and Laird, 1970).

Testes descend from the abdomen into the scrotum at approximately 15 days of age (Russell, 1992). Sperm are first produced at about 45–46 days of age, but fertility (puberty) does not occur until approximately 62–65 days of age, and sperm production is not maximal until 75 days of age (Russell, 1992). Interestingly, on histologic examination of young rat testes, more degenerate germ cells are noted prior to 75 days of age than afterward, indicative of the poor efficiency of spermatogenesis at early ages in the rat (Russell et al., 1987). Male sexual behavior is, in part, dependent on age and experience. Prepubertal males have no preference for females in estrus, and female-oriented sexual behavior is reported to decrease after 150 days of age (Matuszczyk et al., 1994; Smith et al., 1992). Decreased serum testosterone may be partially responsible for age-related decreases in mating

TABLE 4.5  Hematological Parameters in the Rat[a]

| Parameter (units) | Crl:CD(SD) | | Crl:LE | | F344/DuCRL | |
|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female |
| WBC (K/μl) | 11 (4.0–18) | 9.5 (2.4–18) | 9.8 (5.6–14) | 8.7 (4.9–13) | 6.7 (2.1–9.6) | 7.4 (1.9–12) |
| Neutrophils (K/μl) | 3.4 (1.0–6.7) | 2.6 (0.7–5.9) | 4.0 (2.2–6.5) | 3.2 (1.7–5.4) | 2.5 (0.7–3.9) | 2.4 (0.5–4.4) |
| Lymphocytes(K/μl) | 6.5 (2.2–12) | 6.1 (1.5–11.5) | 5.0 (2.5–7.9) | 4.6 (2.6–7.9) | 3.6 (1.0–5.7) | 4.4 (1.2–7.5) |
| Monocytes (K/μl) | 0.7 (0.2–1.4) | 0.6 (0.1–1.2) | 0.7 (0.3–1.2) | 0.7 (0.3–1.3) | 0.5 (0.1–1.0) | 0.5 (0.1–1.0) |
| Eosinophils (K/μl) | 0.1 (0–0.5) | 0.1 (0–0.5) | 0.1 (0–0.5) | 0.2 (0–0.8) | 0.1 (0–0.3) | 0.1 (0–0.5) |
| Basophils (K/μl) | 0.03 (0–0.2) | 0.03 (0–0.2) | 0.03 (0–0.1) | 0.04 (0–0.3) | 0.02 (0–0.1) | 0.03 (0–0.1) |
| Neutrophils (%) | 32 (16–54) | 28 (14–55) | 41 (30–51) | 38 (25–51) | 37 (25–53) | 32 (19–47) |
| Lymphocytes (%) | 61 (38–79) | 63 (37–81) | 51 (41–63) | 53 (38–65) | 54 (40–67) | 59 (42–74) |
| Monocytes (%) | 6.3 (3–11) | 6.5 (3–11) | 6.6 (3–10) | 7.4 (4–12) | 7.6 (7.7–12) | 6.8 (2.9–12) |
| Eosinophils (%) | 1.2 (0.1–4.3) | 1.4 (0.2–4.8) | 1.2 (0.1–4.1) | 1.6 (0.1–7.3) | 0.9 (0–3.7) | 1.6 (0.1–5.5) |
| Basophils (%) | 0.3 (0–1.7) | 0.3 (0–1.6) | 0.3 (0–1.6) | 0.4 (0–2.4) | 0.4 (0–1.1) | 0.4 (0–1.3) |
| RBC (M/μl) | 7.7 (5.8–10) | 7.4 (5.5–9.8) | 7.6 (6.0–9.4) | 7.6 (6.7–8.6) | 8.3 (7.1–9.7) | 8.1 (6.6–9.4) |
| Hemoglobin (g/dl) | 17 (13–23) | 16 (13–22) | 16 (13–20) | 16 (14–18) | 16 (14–19) | 16 (13–18) |
| Hematocrit (%) | 51 (39–69) | 48 (37–64) | 52 (43–62) | 51 (43–60) | 54 (47–62) | 54 (44–62) |
| MCV (fl) | 67 (58–77) | 66 (58–75) | 69 (63–76) | 67 (61–75) | 65 (60–69) | 67 (63–70) |
| MCH (pg) | 22 (17–25) | 22 (18–25) | 21 (19–24) | 21 (20–23) | 20 (17–22) | 20 (17–22) |
| MCHC (g/dl) | 33 (27–38) | 33 (27–38) | 31 (29–33) | 31 (29–43) | 31 (27–34) | 30 (26–32) |
| RDW (%) | 16 (14–18) | 15 (13–17) | 16 (13–17) | 14 (12–15) | 15 (14–17) | 13 (12–15) |
| Platelets (M/μl) | 1.6 (0.8–2.6) | 1.6 (0.8–2.4) | 1.4 (0.7–1.9) | 1.3 (0.6–1.7) | 1.1 (0.8–1.6) | 1.1 (0.7–1.5) |
| MPV (fl) | 7.4 (5.9–10) | 7.3 (5.8–9.6) | 8.0 (6.7–9.2) | 8.1 (5.8–9.5) | 6.9 (5.8–8.2) | 6.8 (5.7–8.1) |

[a]Derived from vendor data (Charles River Research Models) on animals 8–10 weeks of age; mean (95% confidence interval).

behavior, but this does not appear to completely explain the phenomenon.

Coitus occurs more frequently during dark periods than light periods, and more frequently during the latter portion of the dark cycle than during the early portion (Mercier et al., 1987). Multiple intromissions (5–15), each lasting 0.3–0.6 s and with two to nine pelvic thrusts (Bennett and Vickery, 1970), precede the first ejaculation, which lasts about 1 s. This first series of intromissions is called the ejaculatory latency and lasts about 10 min, followed by a refractory period (Dewsbury, 1970). A single ejaculation with fewer intromissions is less likely to impregnate the female. Multiple series of intromissions and ejaculations occur, usually about seven, with increasing refractory periods between successive episodes. Coitus can be confirmed in rats by detection of spermatozoa on a vaginal smear, by observation of a vaginal plug (the pale coagulum formed by seminiferous fluid visible in the vagina), or by direct observation of sexual behavior.

Implantation of the blastocyst into the endometrium occurs between 5 and 7 days after fertilization and actually represents a process that requires 12–24 h for completion (Peluso, 1992; Enders, 1970; Garside et al., 1996). Parturition occurs 21–23 days after the time of coitus, although it can occur as early as 19 days (Bennett and Vickery, 1970; Baker, 1979). Pregnant rats whose time of coitus is known are therefore of known gestational stage and are referred to as timed pregnant.

### 2. Detection of Estrus and Pregnancy

Several methods can be used to determine if a rat is in estrus, which is useful in production of timed pregnant rats. Rats in estrus often exhibit specific behavior, including ear quivering when the back or head are stroked, and lordosis ('sway-back' posture) when the pelvic area is stimulated (Blandau et al., 1941). Additionally, the vulva becomes swollen, and the vaginal wall appears dry in estrus, instead of the moist pink appearance during metestrus or diestrus. This is due to cornification of the

vaginal epithelium during estrus (Baker, 1979). These changes in the vaginal epithelium can be assessed by cytologic examination of vaginal smears (Montes and Luque, 1988). In estrus, 25–100% of the epithelial cells are cornified (Bennett and Vickery, 1970). Changes in vaginal fluids and cytology also lead to changes in the electrical impedance in the vagina during estrus. This has been widely exploited as an alternative method of estrus detection (Koto et al., 1987a, b) using a device referred to as an impedance meter in which an electrical probe is inserted into the vagina. However, when compared to cytology, impedance measurement provides less information regarding the specific phase of estrus, and the physical stimulation of the probe may induce pseudopregnancy (Singletary et al., 2005).

Pregnancy is difficult to detect early in gestation, but conception rates of 85% or more are often observed for outbred rat stocks, somewhat less in inbred strains. After approximately 10 days, careful palpation can detect the developing fetuses; this is especially accurate after 12 days of gestation. Transabdominal real-time ultrasonography can begin to detect pregnancy at 9–10 days and is quite accurate thereafter, with fetal heartbeat detectable by Doppler by day 12 (Ypsilantis et al., 2009). By 14 days of gestation, mammary gland and nipple development are evident (Bennett and Vickery, 1970).

### 3. Husbandry Needs

Inbred rats are generally bred monogamously or in trios, with one male and two females in each cage. Outbred rats may also be bred monogamously but are more often bred polygamously by commercial breeders for reasons of economy. Pregnant females are removed and placed into separate cages a few days before parturition to minimize cannibalism or abandonment of litters.

A number of variables have been identified that may influence the husbandry requirements of a reproducing population of laboratory rats. Despite the lack of seasonal variation in estrous cycles in the rat, both ovarian function and the estrous cycle are influenced by light cycles. Continuous light has been reported to cause persistent estrus and cystic follicles in the ovaries, without formation of corpora lutea (Fox and Laird, 1970). Chronic exposure to even low-intensity light during the dark cycle has been reported to result in earlier vaginal opening and ovarian atrophy (Beys et al., 1995; Fox and Laird, 1970). Caloric restriction, a 15–30% decrease from ad libitum caloric intake, may cause cessation of estrous cycles and delayed sexual maturation (Fox and Laird, 1970).

High ambient temperatures can result in male infertility (Pucak et al., 1977) by causing irreversible degeneration of the seminiferous epithelium. Significantly, the damage may occur in rats as young as 4 days and in rats with prolonged exposure to temperatures as low as 26.6°C.

### 4. Parturition

Female rats increase nest-building activity approximately 5 days prepartum and continue through lactation (Bennett and Vickery, 1970). Nesting behavior adequate for successful reproduction can be expressed using typical hardwood chip bedding in solid-bottomed cages. Supplemental materials may be used by breeding females, but this behavior may be more learned than innate in laboratory rats (Van Loo and Baumans, 2004). Approximately 1.5–4 h before the first pup is born, clear mucoid fluid discharges from the vagina. In early labor, the female walks about the cage and stretches. This behavior becomes more exaggerated as events progress; then the female will lie on her abdomen with rear legs extended off the cage floor. As pups are born, the female pulls the placenta from the birth canal and eats it. Parturition averages 1–3.5 h, varying with litter size. Nursing usually begins only after all pups are born.

Litter size varies with stock, strain, source, and maternal age. The following are examples of the effect of maternal age. Wistar BH rats had an average of 1.69 more pups (11.80 vs. 10.11) when first mated at 105 days than when first mated at 35 days of age (Rosen et al., 1987). The second litter is usually the largest (Bennett and Vickery, 1970). After 9 months of age, litter size is further decreased, and the pregnancy rate declines after 12 months of age (Niggeschulze and Kast, 1994). Loss of fetuses, termed pregnancy wastage, occurs as a function of age (Mattheij and Swarts, 1991), with less than 5% wastage in 4-month-old rats, 30% in 9-month-old rats, and 65% at 11 months of age. Wastage is primarily due to preimplantation and early postimplantation mortality. In contrast to the decremental effects of aging on litter size, maternal behavior in virgin rats is enhanced at 19–20 months, when compared with those at 3–4 months of age (Gonzalez and Deis, 1990). In addition, at least some maternal stressors can lead to fetal wastage. An increase in fetal wastage was reported to be due to an earthquake that occurred when the dams were at 7, 8, or 9 days of gestation, although no difference was noted in the number of live births, fetal weight, or incidence of runts (Fujinaga et al., 1992). This raises the possibility of similar fetal loss when rats are shipped at this stage of gestation, although such has not been documented in the peer-reviewed literature. Strenuous maternal exercise, i.e., running on a treadmill, has also been reported to result in decreased litter size and decreased fetal weight (Mottola et al., 1992). Dystocia is rare in rats. Cannibalism is not frequently encountered and is an indicator of maternal stress.

### 5. Early Development of the Newborn

Rat pups are altricial and nidicolous; they are hairless and blind, with poorly developed limbs, short tails, and closed ear canals (Baker, 1979). There is an inverse

relationship between fetal or birth weight and litter size (Romero *et al.*, 1992). This phenomenon is significant for the reproductive toxicologist because the tendency of a test substance to cause decreased fetal weight may be masked if it also causes fetal loss. Other factors also influence birth weight and weaning weight, including the age of the dams. Pups of dams mated at 105 days of age weighed more at weaning than pups of dams mated at 35 or 70 days (Rosen *et al.*, 1987). The external acoustic meati open between 2.5 and 3.5 days of age. Internally, the cochlea and organ of Corti are immature at birth but develop rapidly to approximately adult morphology by the time of weaning. Rats appear to first be able to hear at about 9 days of age, although they are able to vocalize from the time of birth (Feldman, 1992). Incisors erupt at 6–8 days of age, although molars do not erupt until 16 (molar 1), 18 (molar 2), and 32–34 days of age (molar 3) (Brown and Leininger, 1992). The retina is poorly developed at birth, equivalent to a human fetus of 4–5 months. The eyelids open at about 14–17 days of age, although the retina does not fully mature until 30–40 days of age, and the final components in the angle of the anterior chamber are not fully formed until 60 days of age (Weisse, 1992). Some hairs may be present on the trunk at birth, usually associated with touch domes, indicating that they are guard hairs (English and Munger, 1992). Pups are considered fully haired at about 7–10 days of age. Maternal antibody is transferred passively across the yolk sac *in utero* (Laliberte *et al.*, 1984). Antibody can also be transferred across the intestinal mucosa from maternal colostrum and milk in the suckling rat. This transfer occurs at low rates shortly after birth, reaches maximal rates at day 14, and ceases by the 21 days, when gut closure is said to be complete (Martin *et al.*, 1997).

### 6. Sexing

Sex is readily determined in mature rats by direct observation of the perineal region. Males have a distinct scrotum located between the anus and the preputial opening. The penis is often visible and is larger than the urethral papilla of the female. In addition the distance between the anus and the genital opening, called the anogenital distance, is greater in the male than in the female.

Sex discrimination is more difficult in prepubertal rats but is possible even in neonates. Comparative evaluation will reveal that neonatal males have a greater anogenital distance than their female littermates, although the distinction is more subtle than in adults. A technique for sex determination of preblastocyst embryos has also been described (Utsumi *et al.*, 1991). Male embryos ceased development in the presence of antibody to the HY antigen, and resumed development only after the antibody was washed off. In contrast, 80% of the embryos that developed into blastocysts in the presence of the HY antibody produced female pups after the blastocysts were implanted. Polymerase

chain reaction (PCR) primers constructed from sequences found in the male sex determining region Y (SRY) have been used to determine the sex of embryonic tissues during postmortem analysis (Miyajima *et al.*, 2009).

### 7. Weaning

Rats are weaned at 20–21 days of age, although they may be weaned successfully as early as 17 days. The micturition reflex does not fully mature in rat pups until after day 15, prior to which the animals may not be fully capable of urination without maternal stimulation (Maggi *et al.*, 1986). As is the case with some other species, orphaned pups or those subjected to very early weaning for experimental purposes may require perianal stimulation to avoid urinary distension and possible urinary tract disease.

### 8. Synchronization of Estrus

Synchronization of estrus for timed pregnancy matings, or to prepare recipients in embryo transfer, can be performed by administering 40 µg of luteinizing hormone releasing hormone agonist (LHRH) to mature female rats (Borjeson *et al.*, 2014). Treated females can be bred four days later. Synchronization of estrus in the rat has also been accomplished by administration of 40 mg methoxyprogesterone in the drinking water for 6 days (in 200 ml ethanol/liter water, prepared fresh daily), followed by intramuscular injection with 1 IU of pregnant mare's serum (Baker, 1979). Although synchronization of estrus may be useful in the production of large numbers of timed pregnant rats, estrus staging via the use of vaginal cytology or an impedance meter, as described above, may be more practical in most circumstances.

### 9. Sperm Collection, Artificial Insemination, and Embryo Transfer

Electroejacuation of rats is possible, but the use of sperm collected in this fashion for artificial insemination (AI) in rats is complicated by the rapid coagulation of semen, especially when the semen is obtained by electroejaculation, due to the contributions of the coagulating glands and seminal vesicles (Bennett and Vickery, 1970). For this reason, electroejaculation may have more practicality as a method for evaluation of reproductive soundness in male rats (McCoy *et al.*, 2013). For artificial insemination or sperm cryopreservation, epididymal sperm is most commonly collected during a terminal dissection. Sperm harvested from the proximal portion (head) of the cauda epididymis are reported to have greater fertility than sperm for the middle (body) or caudal (tail) portions (Moore and Akhondi, 1996). Once collected, sperm may be surgically introduced directly into the uterus of estrous females (Nakatsukasa *et al.*, 2003). An essential step in ensuring the success of AI is the induction of pseudopregnancy in the recipient

female by prior mating with a vasectomized male, by mechanical stimulation of the vagina, or by electrical stimulation of the cervix (Bennett and Vickery, 1970; Rouleau et al., 1993).

Embryo transfer in rats is used as an alternative to cesarean rederivation in order to eliminate pathogens from breeding lines. Embryo transfer can also be used as a research technique to investigate whether specific characteristics are due to, or modified by, the uterine environment, in contrast to being solely determined by genetic factors (Kubisch and Gomez-Sanchez, 1999; Rouleau et al., 1993). Cryopreservation techniques allow harvested embryos to be stored for long periods prior to subsequent implantation. Hormonal superovulation of rats is commonly performed using pregnant mare serum gonadotropin (PMSG) followed by human chorionic gonadotropin (HCG) in a fashion similar to that used in mice, although other methods involving follicle stimulating hormone (FSH) with or without luteinizing hormone (LH) have also been utilized (Corbin and McCabe, 2002). For embryo manipulation, depending on the development stage required, embryos are collected 1–5 days after the females are bred. When embryo transfer is being used as a rederivation method, embryos are then suspended in PBS with BSA and fetal calf serum and surgically transferred into the uterus or oviduct of the pseudopregnant recipient (Kubisch and Gomez-Sanchez, 1999; Rouleau et al., 1993). Nonsurgical implantation of embryos through the cervix, using an otoscope, has also been reported (Bennett and Vickery, 1970) but has not found wide use. *In vitro* fertilization (IVF) is performed in the rat but is used primarily as a research tool for events in fertilization and early development rather than as a colony management tool (Gaddum-Rosse et al., 1984; Vanderhyden et al., 1986). Microinsemination of individual oocytes via intracytoplasmic sperm injection (ICSI) is a specific IVF technique that has the potential for being used to rescue or maintain strains that do not produce motile spermatozoa, as it has been used in mice (Tanemura et al., 1997; Songsasen and Leibo, 1998). By adding exogenous DNA to the process, it can also be exploited as a method for producing transgenic rats (Hirabayashi et al., 2005).

### 10. Cryopreservation

Cryopreservation has not been performed in rats as often as it has in mice, but the technique is becoming more widespread, for the same reasons that it is used widely in mice (Tada et al., 1995). Cryopreservation can be an efficient method of maintaining the potential of raising live mice of the thousands of genetically modified genotypes currently available (Songsasen and Leibo, 1998). It can serve as a fail-safe measure, should a strain become genetically contaminated. In addition to being used for murine reproductive purposes, frozen embryos are also used to test culture reagents and environments for human IVF (Meyer et al., 1997). Although embryos, two-cell through morula, are most frequently cryopreserved, the techniques for cryopreservation of mouse sperm have been developed (Songsasen and Leibo, 1998; Tanemura et al., 1997). Sperm cryopreservation has been successful in rats. Offspring can be obtained with thawed sperm using direct intrauterine insemination (Nakatsukasa et al., 2003) or via IVF using cryopreserved sperm (Seita et al., 2009).

## E. Behavior

Aspects of rat behavior relevant to experimental design and disease status may be considered in two broad and overlapping categories: normal behavior, and stressors and stress responses. Only a few examples will be cited here.

Laboratory rats of all stocks and strains have been selected for many years for a variety of traits, among which is docility. Nonetheless, strain differences exist. Rats from a Sprague-Dawley background (such as the CD and SD stocks) and Lewis rats are generally more docile than Brown Norway or F-344 rats. Frequent gentle handling will increase docility, which not only reduces the likelihood of occupational injury for animal workers but also avoids stress for the rats. Infrequent or rough handling will evoke fear responses and is used as a stressor in research projects. The presence or absence of acclimation to handling of both pre- and postweaning animals can modulate subsequent handling-induced stress and lead to altered responses in behavioral studies in animals (Hirsjarvi and Valiaho, 1995; Shalev et al., 1998) and 'playful' handling can be a refinement to reduce stresss when performing procedures such as injections (Cloutier et al., 2014). Handling also leads to vocalizations, often in the range of 22 kHz, typical for rat alarm calls (Brudzynski, 2009). Stress-induced vocalization from rats in one cage can make handling more difficult or have an effect on the behavior of other rats within hearing range (Brudzynski and Chiu, 1995). An additional interesting fact regarding rat vocalization, illustrative of its importance in rat behavior, is that rat pups vocalize in the ultrasonic range, probably to signal their mothers, even before their ears are sufficiently developed for them to be capable of hearing (Feldman, 1992).

Rats are most active at night but will also move and feed some during the day; they are also more active in the mornings than in afternoons (Saibaba et al., 1996). This circadian rhythm is relevant to a broad range of behavioral measurements. For example, pain threshold is often determined in a tail flick test. Female rats have shorter tail flick response times in the middle of the dark period, as well as during estrus and metestrus (Martinez-Gomez et al., 1994).

Rats, like other rodents, are coprophagic and vary considerably between individuals in the percentage of feces consumed. This may be of significance when fasting rats for gastric procedures, quantifying fecal output volume or measuring intestinal absorption of some agents.

Rats may be housed singly or in groups. In general, male rats are less likely to fight when housed together than are male mice, but they also do well when housed singly, as is the norm in many toxicology and safety assessment studies. Although historical experience has shown that laboratory rats appear to adapt well to single housing, there are demonstrable physiological and behavioral differences between rodents maintained in a social housing as compared to cage isolation (Gonder and Laber, 2007). The 2011 revision of the Guide for the Care and Use of Laboratory Animals strongly recommends that members of social species such as rats be housed in compatible groups whenever possible (National Research Council, 2011). However, as the emphasis on group housing begins to change the way institutions care for their animals, it needs to be recognized that a shift from single to group housing can function as a significant variable in a broad range of experimental studies. Such changes should be discussed with all groups involved so that the potential effects on the research study can be explored proactively by consulting the relevant literature, and also so that unanticipated effects can be recognized and evaluated retrospectively.

When afforded the choice, rats have shown preferences for solid flooring, bedding consisting of large particles of aspen wood chips, and nest boxes (Manser et al., 1995a, b, 1998a, b; Blom et al., 1995), although the consequences of being deprived of the preferred housing factors have not been reported. When provided with objects as part of an environmental enrichment program, rats will chew on inanimate objects such as wooden blocks and nylon bones and balls (Watson, 1993; Chmiel and Noonan, 1996). The provision of environmental enrichment is an important part of providing high-quality research animal care, but as is the case with social housing, it can significantly change the baseline behavior and experimental responses (Simpson and Kelly, 2011). As a result, when changes are made, it must be carefully considered as a research variable with the potential for unintended effects.

## III.  DISEASES

## A.  Infectious Diseases

### 1.  Bacterial, Mycoplasmal, and Rickettsial Infections

Although substantial evidence supports a salubrious role for a complex microflora in laboratory rodents, including contributions to gastrointestinal and immunologic development and function, endocrine and nervous systems, as well as metabolism, this section will be limited to detrimental, or potentially so, host–bacterial interactions, i.e., infection (Treuting et al., 2012; Rohde et al., 2007). Discussion of bacterial disease in laboratory rats is also compounded by changes in pathogen prevalence across North America, Europe, and Japan, such that many of the 'classical' pathogens of rats such as Corynebacterium kutscheri and Mycoplasma pulmonis are very rare (Pritchett-Corning et al., 2009; Livingston and Riley, 2003). However, they should not be forgotten, as they not only persist in wild populations and pet rodents, but there are many other regions of the bioresearch world where pathogen prevalence profiles cannot be assumed. Thus, we will briefly mention some bacteria which are primarily of historical interest in many areas of the world, as well as others likely to be of health or research significance to laboratory rats only in special situations or to individual rats, not to entire groups.

#### a.  Streptococcal Pulmonary Disease

*Etiology*　Pneumonia caused by *S. pneumoniae* has historically been referred to as streptococcosis. However, because the term *streptococcosis* could be used to describe any streptococcal infection, it is inherently nonspecific and should be avoided. Several species of *Streptococcus* are opportunistic pathogens in rats (i.e., they can cause clinical disease under at least some circumstances). *Streptococcus pneumoniae*, which is α-hemolytic, is the *Streptococcus* species of most historic concern in the rat.

*Epizootiology and Transmission*　*Streptococcus pneumoniae* is rare in commercially obtained rats and for more than 20 years has been considered to be a pathogen of low significance in laboratory animals (National Research Council, 1991). Numerous serotypes of *S. pneumoniae* exist; disease is predominantly associated with infection by more pathogenic serotypes, especially 2, 3, 8, 16, and 19 (Fallon et al., 1988). Humans are the natural host of *S. pneumoniae*, with both adults and children frequently colonized. *Streptococcus pneumoniae* is transmitted primarily via aerosol, although fomites may play a minor role.

*Clinical Signs*　Disease due to *S. pneumoniae* has been infrequently reported in rats, but infection is usually asymptomatic and results in colonization of the nasopharynx. No reports could be found of disease due to *S. pneumoniae* in laboratory rats in the last 20 years, and infection also appears to be rare (Pritchett-Corning et al., 2009). It is possible that older reports may have been confounded by concomitant infection with other respiratory pathogens such as *Pneumocystis carinii*, which was not recognized at that time as a cause of disease in immunocompetent rats. The rat, however, is used as an experimental model of human *S. pneumoniae* infection, although immunosuppression, neutropenia, or other

special techniques are usually necessary to induce disease (Chiavolini *et al.*, 2008).

*Pathology* Reports of disease associated with *S. pneumonia* in rats resembles that in both human and nonhuman primates, characterized by suppurative inflammation in the upper respiratory tract, which spreads to the lung to cause bronchopneumonia (Kohn and Barthold, 1984) and sometimes fibrinosuppurative pleuritis. Affected rats may become bacteremic and may develop fibrinopurulent inflammation of other serous surfaces (e.g., peritoneum, synovium) and other tissues.

*Diagnosis and Differential Diagnosis* Monitoring for *S. pneumoniae* infection is often conducted by nasopharyngeal culture onto blood agar. Differentiation of *S. pneumoniae* from other α-hemolytic streptococci is most often performed by the optochin inhibition test. Optochin inhibition is greater for most *S. pneumoniae* strains than for other α-hemolytic streptococci. PCR is also commercially available to detect *S. pneumoniae* in samples from the nasopharynx or lung. However, because of the occurrence of nonpathogenic isolates (Fallon *et al.*, 1988), detection of *S. pneumoniae* in rats, even if a respiratory problem is present in the colony, does not necessarily provide a diagnosis without corroborating histopathology, nor does isolation of *S. pneumoniae* from asymptomatic rats necessarily indicate a colony health threat.

*Prevention and Control* Contamination with this agent is rare in contemporary colonies, and since there may be nonpathogenic strains, action to eliminate *S. pneumoniae* is only indicated in the presence of characteristic lesions or detection of known pathogenic serotypes. Depopulation can be performed, followed by restocking with animals from a clean source that are maintained to remain SPF via the use of standard exclusion techniques.

*Research Complications* Animals affected by pneumonia or septicemia would be poor subjects for research, but physiologic variability associated with asymptomatic carriage have not been reported.

### b. Other Streptococcal Species

β-Hemolytic streptococci are also detected in laboratory rats but rarely cause disease. β-Hemolytic streptococci are divided into groups based on Lancefield antigens, with Lancefield groups B and G most commonly isolated from rats. Some of these organisms have been used in experimental infections in rats, but only once have they been associated with naturally occurring disease. In this single report, Group B streptococci were linked to myocarditis and abscesses found in a few 21- to 24-day old pups of a Munich Wistar–Frömter line transgenic for human diphtheria toxin (Shuster *et al.*, 2013). Exclusion of β-hemolytic streptococci from most rat colonies is neither necessary nor practical, for humans are often carriers. *Enterococcus* spp., which are not truly streptococci, are often considered together with

*Streptococcus* spp. So-called streptococcal enteropathy is actually due to nonhemolytic (γ-hemolytic) Lancefield group D enterococci, including *Enterococcus hirae*, *E. faecium-durans* 2, and *E. faecalis* 2 (Barthold, 1997). Streptococcal enteropathy is a disease that affects only suckling rats, not postweaning animals. Affected litters develop diarrhea or soft stools, with bright yellow pasty feces. Mortality can be high. Microscopically, the villi of the small intestine are carpeted with gram-positive cocci. Disease is clearly associated with some strains of enterococci and not with others, but the factors determining the pathogenic potential have not been elucidated. They may, however, involve the ability of pathogenic isolates to adhere to the surface of the microvilli. Control of *Streptococcus* spp. and *Enterococcus* spp. is problematic, because the organisms are widespread, including being cultivable in a high percentage of the human population (Del Vecchio, 1992). Some *Enterococcus* spp. have even been considered autochthonous flora of the rat (Savage, 1971). Streptococci can be excluded by aseptic microisolator technique or by use of isolators (Pleasants, 1974), yet the low incidence of disease may not warrant the additional time, expense, or other resources that such housing techniques would require.

### c. Pseudotuberculosis

*Etiology* *Corynebacterium kutscheri* is a gram-positive coryneform (club-shaped) bacterium which can be found in soil, sewage, and marine environments, and has been explored for possible utility of bioremediation of oil spills (Oyetibo *et al.*, 2013). In rats, mice, guinea pigs, and hamsters, *C. kutscheri* is the cause of pseudotuberculosis, although in the last two species, there is only bacteriological evidence, i.e., no disease has been reported.

*Epizootiology and Transmission* Transmission is probably through direct contact or oronasal exposure.

*Clinical Sign* Infections with *C. kutscheri* are rare (Pritchett-Corning *et al.*, 2009; Livingston and Riley, 2003) and usually clinically silent (Suzuki *et al.*, 1988; Amao *et al.*, 1995). Nonspecific clinical signs may be observed in advanced disease, such as ruffled fur, hunched posture, dyspnea and rales, porphyria, mucopurulent ocular and nasal discharges, lethargy, and lameness. These are usually followed by death in 1–7 days.

*Pathology* Gross lesions of *C. kutscheri* infection consist of solitary or multiple randomly distributed abscesses in the lung, liver, kidney, skin, and joints. The lesions are due to septic emboli becoming trapped in organs and tissues that contain an extensive capillary network. In the rat, the lung is the organ most frequently involved (Fig. 4.4). Suppurative inflammation may also be found in the preputial gland and tympanic bullae. Histopathologically, the lesions are generally as expected from the gross findings. Interstitial inflammation in the

**FIGURE 4.4**    Multiple abscesses in a rat lung caused by *C. kutscheri*. *Courtesy of Joanne Bella Hodges.*

lung is due to the hematogenous seeding of the lung with bacteria, although bronchi and bronchioles may also contain suppurative exudate. Caseous necrosis is often prominent, and epithelioid macrophages and multinucleated giant cells may be present in areas of abscessation. Large areas of caseous necrosis may also be present in the liver. Septic embolic glomerulitis may be present in the kidneys, as may abscesses with or without pyelonephritis. Abscesses and caseous necrosis may also be observed in virtually any tissue.

*Diagnosis*    In infected colonies, *C. kutscheri* will typically cause latent infections and may be detected by PCR or cultured from submaxillary (cervical) lymph nodes, oropharynx and nasopharynx, middle ears, and preputial glands of feces (Amao *et al.*, 1995, 2002, 2008). Latent infections may be triggered to become clinical by a variety of stressors that can cause immunosuppression in the host. These include poor husbandry, overcrowding, shipping, malnutrition, intercurrent infections, irradiation, and treatment with immunosuppressive drugs (Barthold and Brownstein, 1988). As with other persistent infections, such as mycoplasmosis, disease is more frequent in older animals. Definitive diagnosis is accomplished by bacteriologic culture (Fox *et al.*, 1987) or PCR (Jeong *et al.*, 2013). The best site, other than lesions, to culture is probably the submandibular (cervical) lymph nodes. The oral cavity, cecum, colon, and rectum may also harbor the organism. As feces have been identified in mice as a suitable culture site, they may also be a suitable sample for PCR, although this has not been confirmed in rats. Microscopic evaluation of lesions may reveal the characteristic irregularly branching arrays of

gram-positive rods in tissue sections (Brown and Brenn stain) or impression smears (Gram's stain). However, if possible, histopathology should always be confirmed by bacteriology or PCR. Serology has also been widely employed for detection of *C. kutscheri* infection in immunocompetent rats (Fox *et al.*, 1987; Boot *et al.*, 1995). As with other serologic assays, especially serologic assays for agents more antigenically complex than viruses, false positives and false negatives occasionally occur, so positive results should always be confirmed by culture or PCR.

*Differential Diagnosis*    Differential diagnosis for the presence of multiple abscesses in rats should include streptococcosis, streptobacillosis, mycoplasmosis (pulmonary abscesses), Cilia-associated respiratory (CAR) bacillus infection (pulmonary abscesses), or other miscellaneous bacteria. Of these, only mycoplasmosis and CAR bacillus infection would be found predominantly in older animals. PCR may be helpful in these situations as it can be performed on fresh, frozen, or formalin-fixed paraffin-embedded tissue, although the first two are preferable to the last.

*Prevention and Control*    Typical rederivation and bioexclusion techniques can be used to maintain colonies free of the agent.

*Research    Complications*    Morbidity/mortality from clinical disease can disrupt ongoing studies and is most likely to occur should the bacteria contaminate immunocompromised rats.

#### d. Tyzzer's Disease

*Etiology*    Tyzzer's disease, first discovered by Tyzzer in Japanese Waltzing mice (Tyzzer, 1917), is caused by *Clostridium piliforme* (Duncan *et al.*, 1993), formerly known as *Bacillus piliformis*. The host range is protean among mammals, including numerous rodent species, rabbits, carnivores, horses, and both nonhuman and human primates (DeLong and Manning, 1994; Skelton *et al.*, 1995).

*Epizootiology and Transmission*    *Clostridium piliforme* is transmitted horizontally in rats by spores through fecal–oral contamination. The spores are highly resistant to desiccation and some disinfectants (Ganaway, 1980). The delicate vegetative form, however, survives only inside of cells. After being ingested, *C. piliforme* spores produce a vegetative form, which is actively phagocytosed by mucosal epithelial cells covering the gut-associated lymphoid tissue, or Peyer's patches (Tyzzer, 1917; Duncan *et al.*, 1993).

*Clinical Signs*    *Clostridium piliforme* infection is usually clinically silent (Motzel and Riley, 1992; Hansen *et al.*, 1992b). Overt disease in rats, as in other species, is most likely to be observed in young, recently weaned animals. In these, the clinical signs are nonspecific (anorexia, lethargy, emaciation, ruffled fur) and may include

acute death without clinical signs. Diarrhea may be noted and may contain mucus and blood. Particularly in the rat, a distended abdomen has been observed in weanlings with Tyzzer's disease, albeit at a very low incidence (Hansen *et al.*, 1992a).

*Pathology* Grossly, in the minority of cases which produce disease, multiple, pale foci, pinpoint or larger, of necrosis are often visible on the surface of and within the liver. Megaloileitis – a greatly dilated, flaccid, and hyperemic ileum – may be present. Hyperemia, edema, hemorrhage, and ulceration may affect any part of the intestine, especially the terminal ileum, cecum, and colon. Secondary to intestinal involvement, mesenteric lymph nodes may be enlarged, hyperemic, and edematous. In the heart, pale circumscribed areas may be visible on the epicardium. Myocardial necrosis due to Tyzzer's disease may also appear as pale linear streaks or areas in the heart, especially near the apex. Histopathologically, characteristic lesions may be observed in the liver, ileum, cecum, and colon, and, less frequently, the heart. In the intestinal tract, there may be necrotizing enteritis, typhlitis, and colitis. Coagulative necrosis in the liver is the hallmark lesion and is often accompanied by a moderate leukocytic infiltrate, usually neutrophils and mononuclear cells, at the periphery of the lesions. Acute lesions may be hemorrhagic, and mineralization may occur with time. In the heart, myocardial degeneration and necrosis occurs in a minority of cases, often with a mixed leukocytic infiltrate and dystrophic calcification.

*Diagnosis* Diagnosis of clinical disease depends on demonstration of the organism in tissue. Histopathologic evaluation is diagnostic if the characteristic bacilli are observed (Tyzzer, 1917; Duncan *et al.*, 1993). The vegetative form of the organism is a filamentous bacillus, 8–20 μm long and 0.3–0.5 μm wide. Bacilli are intracellular, are often numerous, and may appear as either a jumbled array (pickup stick) or parallel arrangement, as dictated by the shape of the cell. The vegetative form may rarely be visible in hepatocytes in tissue sections stained with hematoxylin and eosin, but usually special stains are necessary, including Warthin–Starry silver (best), Giemsa, and methylene blue stains. Although gram-negative, *C. piliforme* stains very poorly with gram stains. Tissue smears may facilitate rapid diagnosis; Giemsa-stained smears of suspicious liver lesions are especially useful (Percy and Barthold, 2007). In the liver, the organisms are most often observed in surviving hepatocytes at the periphery or within lesions. In the intestine, normal gut flora within mucosal crypts and superimposed upon the mucosal epithelial cells may complicate evaluation. Organisms may also occasionally be observed in cardiac myocytes or myocytes of the tunica muscularis of the intestine. Colony screening for latent infection is problematic. Serologic screening is rapid and technically simple (Motzel and Riley, 1992)

but is subject to false positives, yielding results that can be difficult to put into context, as has been recently reported in rabbits (Pritt *et al.*, 2010). Disease provocation tests, or stress tests, to exacerbate latent infections are sometimes used as a follow-up test when serologic positive results are obtained. However, there is some doubt as to efficacy of stress tests that rely on chemical immunosuppression, usually with cyclophosphamide (Boivin *et al.*, 1990), followed by histopathologic evaluation. The doubt arises because test animals may have already cleared the *C. piliforme* infection and therefore may no longer be susceptible to activation of 'latent' infection. Alternatively, sentinel animals can be placed on soiled bedding, but this may require sentinels to be of the same species (to avoid species specificity causing false negatives), for not even gerbils are susceptible to all strains of *C. piliforme* (Motzel and Riley, 1992; Franklin *et al.*, 1994). PCR is also useful, as it is a sensitive and specific method to detect DNA from the organism. Thus, it can be used on fecal samples (Furukawa *et al.*, 2002), but will only detect animals which have not cleared infection. It can be used on ileum, cecum or colon, but only during the period when organisms are present. PCR is useful for screening grossly observed liver lesions for *C. piliforme*, and can additionally be used on paraffin-embedded tissues as a confirmatory test following histopathologic evaluation.

*Differential Diagnosis* Differential diagnoses for necrotizing hepatitis in the rat should include other bacterial septicemias, such as *Corynebacterium kutscheri*, as well as infection with rat virus.

*Prevention and Control* As is the case with the other bacterial diseases described in this section, control in contemporary colonies is through the use of exclusion techniques. However, the fact the organism produces spores suggests that (1) cross-contamination from conventional or wild-caught colonies could be more likely, and (2) very thorough decontamination would be indicated after an outbreak.

*Research Complications* Interference of *Clostridium piliforme* with research has primarily been attributed to the morbidity and mortality, although effects on coagulation and leukokines have also been reported in mice with subclinical *C. piliforme* infection (Van Andel *et al.*, 2000).

### e. Pasteurellosis

*Etiology* *Pasteurella pneumotropica* is a gram-negative coccobacillus. It grows aerobically on sheep blood agar without producing hemolysis, producing smooth, gray or occasionally yellow translucent colonies (Carter, 1984; Nicklas, 2007). It has been isolated from numerous mammalian species, including humans, and has long been considered to be of low significance in immunocompetent rats (National Research Council, 1991).

*Epizootiology and Transmission*   *Pasteurella pneumotropica* has a high prevalence in infected colonies and is most often isolated from the nasopharynx, cecum, vagina, uterus, and conjunctiva during routine monitoring (National Research Council, 1991).

*Clinical Signs and Pathology*   The vast majority of animals are asymptomatic, with only rare instances of conjunctivitis, metritis, and mastitis (Percy and Barthold, 2007). Histologically, any lesions that occur are characterized by necrosuppurative inflammation.

*Diagnosis and Differential DiagnosiS*   Screening for *P. pneumotropica* may be conducted by either culture or PCR. Culture is best conducted on the sites mentioned above, although feces, oral swabs, or even dust that has accumulated in animal air spaces, such as within the plenum of some individually ventilated caging systems, are all suitable for PCR (Henderson *et al.*, 2013).

*Prevention and Control*   Control of the agent may not be necessary in immunocompetent rats, because of the rarity of *P. pneumotropica*-induced disease. Treatment with enrofloxacin has been described for the mouse (Goelz *et al.*, 1997). Rederivation by either cesarean section or embryo transfer will also eliminate the agent (Nicklas, 2007). Offspring should also be held in strict isolation – i.e., not mixed in with a breeding colony – until confirmed negative for *P. pneumotropica*. *Pasteurella pneumotropica* is not transmitted to a significant degree by fomites, does not persist or multiply in the environment, and only very rarely colonizes humans. Therefore, once a colony is free of the agent, there is relatively little risk of reinfection except through introduction or incursion of infected animals.

*Research Complications*   Studies that involve tissues and organs affected by the associated inflammation would be affected.

### f.  Salmonellosis

*Etiology*   Salmonellosis is the disease caused by bacteria of the genus *Salmonella*. The taxonomic classification and subdivision of the genus remain somewhat controversial and, like much taxonomy, subject to change. All salmonellas that one is likely to encounter in rats belong to *S. enterica* which comprises more than 2500 serovars (Tindall *et al.*, 2005; Timme *et al.*, 2013). These vary greatly in pathogenicity and geographic distribution, which makes serovar classification useful in epizootiologic investigations. For simplicity sake, however, this discussion will treat *S. enterica* as a single entity.

*Epizootiology and Transmission*   Salmonellosis may be virtually nonexistent in laboratory rats in the United States, but because infection is thought to be prevalent among many other species of vertebrates, including wild rodents, the potential for introduction remains. *Salmonella enterica* is transmitted by ingestion of contaminated materials, including feed, bedding, or water. Incursion of wild or feral rodents into a laboratory facility poses a further risk.

*Clinical Signs*   As in most species, clinical signs of infection with S. enterica in rats are rare but may include a hunched posture, ruffled fur, lethargy, weight loss, and conjunctivitis. Soft stools and diarrhea may also be observed, usually in less than 20% of animals.

*Pathology*   In rats with subclinical infections, gross and microscopic lesions will usually be absent. Rats with clinical disease may have evidence of gastrointestinal involvement and septicemia, including mural thickening and mucosal ulcers in the cecum and ileum, as well as splenomegaly. Microscopically, enteric lesions are characterized by edema of the lamina propria, leukocyte infiltration in areas of ulceration, and reactive hyperplasia of crypt epithelial cells. Lymphoid hyperplasia, with focal necrosis and neutrophil infiltration, may be observed in Peyer's patches, as well as in the spleen and mesenteric lymph nodes. Septicemic rats will have necrosis in the spleen and liver, with emboli composed of fibrin, bacteria, and debris present in liver, spleen, and lymph nodes (Percy and Barthold, 2007).

*Diagnosis*   Colony screening is most often conducted on fecal samples by microbiologic culture or by PCR. Suspected cases of salmonellosis may also be tested by culture or PCR of feces, mesenteric lymph nodes, liver, spleen, or blood. Material is placed in enrichment broth and then inoculated onto selective growth medium. Although symptomatic animals should be culture-positive, an infected colony may have only a low incidence of asymptomatic carriers, perhaps less than 5%. Detection of *S. enterica* in these colonies may require repeated testing of significant numbers of samples. It is unknown at this time if PCR would find a higher rate of carriage than detected by culture.

*Differential Diagnosis*   Differential diagnoses for diarrheal disease in rats include Tyzzer's disease, rotavirus infection, enterococcal enteropathy, cryptosporidiosis, and problems with feed and/or water.

*Prevention and Control*   Salmonellosis is prevented by rigorous pest control and by ensuring that food and bedding are not contaminated. Good personal hygiene of employees will prevent them from serving as a source of *Salmonella* or other enteric pathogens to the colony. Once *S. enterica* is detected in a colony, all animals are usually destroyed, and all surfaces and materials either sterilized or safely discarded. Strict quarantine of a small group of animals may be practical in some situations, prior to rederivation by embryo transfer or cesarian section. This may be most feasible in a flexible film or semirigid isolator. Treatment is not recommended, because a chronic carrier state may result and there is the potential for zoonotic disease.

*Research Complications*   Rats infected with *S. enterica* should not be used in research, because of the zoonotic potential and the risk they animals pose to other animals.

### g. Pseudomoniasis

*Etiology*  Pseudomoniasis refers to clinical disease caused by *Pseudomonas aeruginosa*, a gram-negative bacillus of the order Eubacteriales, family Pseudomonadaceae.

*Epizootiology and Transmission*  *Pseudomonas aeruginosa* is motile, aerobic, oxidase-positive, and widely distributed in water, soil, sewage, and the skin and gastrointestinal tract of many animals. It is considered as part of the common commensal flora of humans, domestic animals, and laboratory rodents and isolation frequency increases in animals and humans receiving antibiotics (Kiska and Gilligan, 1999).

*Clinical Signs and Pathology*  Despite its near ubiquity, *P. aeruginosa* is rarely implicated in disease except in mammals with specific and severe host defense deficits, particularly hosts or tissues deficient in functional phagocytes (i.e., macrophages and neutrophils, and their serum opsonins). Thus, athymic nude mice are not subject to a high incidence of pseudomoniasis unless irradiated or treated with myelosuppressive agents. In general, pseudomoniasis is considered to be of low significance in rats (National Research Council, 1991) but should be suspected when rats that are irradiated or treated with radiomimetic agents die earlier than expected (Percy and Barthold, 2007). In particular, pseudomoniasis has been reported as a consequence of infection of indwelling jugular catheters (Wyand and Jonas, 1967). Signs were those of septicemia. Necropsy findings included vegetative valvular endocarditis and multifocal hemorrhagic pneumonia. Histologically, fibrin emboli, leukocytes, and gram-negative bacteria were observed in the heart, lung, and occasionally other organs.

*Diagnosis*  Pseudomoniasis is diagnosed by either cultural identification of the organism or by PCR. However, caution should be exercised in attributing observed morbidity and mortality to an organism as nearly ubiquitous as *P. aeruginosa*. Although *Pseudomonas* will grow on blood agar, isolation is enhanced by the use of selective media, such as *Pseudomonas* isolation agar, or *Pseudomonas* agar P. The use of selective media is particularly recommended when screening clinically healthy animals, because only low numbers of organisms may be harbored in the common sites for isolation, the cecum, and nasopharynx.

*Prevention and Control*  Exclusion of *P. aeruginosa* is rarely justified in a research setting as it requires gnotobiotic methods and sterilization of all water reaching the animals, as well as sterilization of cages, feed, and bedding. Animals must be maintained in isolators or microisolators and must be routinely monitored. All possible sources of contamination from human skin or any wet surface must be strictly prohibited. Control of *P. aeruginosa* infection often begins with the watering system. *Pseudomonas aeruginosa* is one of many bacteria that form biofilms, layers of bacteria, usually with reduced metabolic activity, embedded in a dense glycocalyx. Bacteria in a biofilm are extraordinarily resistant to chlorine (150- to 3000-times more resistant than free-floating bacteria) and monochloramine (2- to 100-fold; LeChevallier *et al.*, 1988) and may be inaccessible to antibiotics. Nonetheless, chlorination (10–13 ppm) or acidification (pH 2.5–3.0) can significantly reduce the colonization of mice with *P. aeruginosa* but will not eliminate infection. Rederivation by cesarian section or embryo transfer is required to eliminate *P. aeruginosa* from an infected colony. Treatment with gentamycin in the animal drinking water, at 1 g/l, has been reported to eliminate the infection in mice but is probably not practical for large groups of rats (Urano *et al.*, 1977).

*Research Complications*  As mentioned above, infection of indwelling catheters has been reported, and disease caused by the organism could interfere with radiation studies or those using strains with specific immune deficits.

### h. Streptobacillosis

One cause of rat-bite fever, *Streptobacillus moniliformis* is primarily of historic interest in laboratory rats. This zoonotic agent is virtually nonexistent in modern laboratory animals but nonetheless bears brief mention because of the potentially serious consequences of infection (Anderson *et al.*, 1983; Wullenweber, 1995). The agent is a gram-negative pleomorphic bacillus, which will grow nonhemolytically on sheep blood agar, although trypticase soy agar enriched with 20% horse serum is preferred (Weisbroth, 1982; Savage, 1984).

*Streptobacillus moniliformis* is commensal in wild rats, inhabiting the nasopharynx, middle ear, and respiratory tract. It is present in blood and urine of infected rats and is transmitted to humans by bite wounds, aerosols, and fomites (Will, 1994). The organism is nonpathogenic in rats. Clinical signs in humans follow a 3- to 10-day incubation period and include fever, vomiting, arthralgia, and rash. Disease is treated with antibiotics, and mortality is low.

Colonies of laboratory rats are monitored by PCR or by culture of blood and nasopharyngeal swabs for *Streptobacillus moniliformis*, and any colony in which the organism is confirmed should immediately be terminated. Because wild rats are the reservoir for *S. moniliformis*, detection of this delicate bacterium in a laboratory rat colony would indicate close-range exposure to infected wild rats.

### i. Helicobacteriosis

*Etiology*  A number of enterohepatic *Helicobacter* spp. have been found as natural infections of rats. *Helicobacter muridarum*, one of the first helicobacters identified in rodents, was initially reported in 1992 (Lee *et al.*, 1992). *H. trogontum* has now been identified as

the most prevalent of the naturally occurring intestinal helicobacters (Mendes *et al.*, 1996) in rats, and *H. bilis* has been reported from the large bowel of immunodeficient rats (Haines *et al.*, 1998). More recently, *H. pullorum* was isolated from a breeding colony of inbred Brown Norway rats housed in the same room as an infected mouse colony. Persistent infection of Brown Norway rats was also established by oral inoculation (Cacioppo *et al.*, 2012b). A similar inoculum resulted in persistent infection of four of 10 Sprague-Dawley rats; none of eight other Sprague-Dawley rats receiving dirty bedding from infected Brown Norway rats became infected (Cacioppo *et al.*, 2012a). No lesions were attributed to *Helicobacter* in any of the rats. All currently identified helicobacters of laboratory rats are microaerophilic, gram-negative flagellated bacteria that may be spiral, slightly curved, or straight. Coccoid forms have also been described for *H. bilis* and *H. pylori* (Fox *et al.*, 1995).

***Epizootiology and Transmission***    The host range of rat *Helicobacter* species is not fully elucidated. Clearly, *H. bilis* has been found in rats and mice, and there is also an additional report of it in a dog (Eaton *et al.*, 1996). *Helicobacter muridarum* has been reported in rats and mice. *H. pullorum* has been reported in mice, rats, chickens, and humans. No host range has been reported for *H. trogontum.* Many other *Helicobacter* species, however, are able to colonize a phylogenetically wide range of mammalian hosts. With the exception of an attempt to transmit *H. pullorum* by soiled bedding, no studies have been published on the transmission of naturally occurring *Helicobacter* infections in rats. In mice, horizontal transmission by soiled bedding, probably fecal–oral transmission, has been demonstrated (Livingston *et al.*, 1998), although it has recently been shown that soiled bedding sentinels will only detect a subset of infected mice (Henderson *et al.*, 2013). Fecal–oral transmission is also presumed in rats.

***Clinical Signs***    Once established, infection by any *Helicobacter* species is typically lifelong. Infection, or colonization, should be distinguished from disease. *Helicobacter muridarum* and *H. trogontum* may be nonpathogenic in rats, although *H. muridarum* has been reported to cause lymphocytic gastritis in aged mice, possibly associated with a loss of parietal cell mass leading to increased gastric pH.

***Pathology***    Key pathogenic factors for *H. pylori* include urease, a vacuolating cytotoxin (*vacA*), and the presence of a pathogenicity island. All of the enterohepatic *Helicobacter* spp. currently identified in rats are urease-positive (Fox and Lee, 1997). Other virulence factors have not been reported. Lesions reported in athymic nude rats infected with *H. bilis* are similar to those reported in immunodeficient mice inoculated with *H. bilis* or *H. hepaticus* and include proliferative and ulcerative typhlitis, colitis, and proctitis (Haines *et al.*, 1998),

although no causal role was confirmed. No lesions due to any naturally occurring *Helicobacter* species have been reported in immunocompetent laboratory rats. The only report of lesions in rats due to natural *Helicobacter* infection involved a small group of 11 male athymic nude rats, 5–8 months of age, infected with *H. bilis* (Haines *et al.*, 1998). In these rats, gross lesions consisted of focal or diffuse thickening of the cecal wall, with normal-appearing colon and rectum. Cystic mesenteric lymph nodes were also noted in eight of the 11 rats. Histologically, all 11 rats had proliferative typhlitis, with eight of the animals also having similar lesions in the colon and rectum. Crypt epithelium was hyperplastic, with cytoplasmic basophilia, increased mitoses, and fewer goblet cells than normally observed. The lamina propria was infiltrated by lymphocytes, plasma cells, and a few eosinophils. Mucosal erosion and ulceration were observed in the cecum of the most severely affected rats. The authors experimentally reproduced many aspects of the disease by intraperitoneal injection of approximately $5 \times 10^8$ *H. bilis* bacteria in phosphate-buffered saline. *Helicobacter* infection should, therefore, be a prime differential diagnosis when proliferative lesions of the large bowel are observed in rats. Spontaneous chronic ulcerative colitis has also been reported in athymic nude rats apparently free of *Helicobacter* infection (Thomas and Pass, 1997). The authors were unable to culture *Helicobacter* spp. from affected animals, although no molecular techniques were employed.

***Diagnosis***    Diagnosis of *Helicobacter* infection in laboratory rats is best accomplished by PCR (Riley *et al.*, 1996; Fox and Lee, 1997; Henderson *et al.*, 2013). Samples are most often fecal pellets or fecal dust, although cecal mucosal scrapings or tissue may also be used. *Helicobacter* spp may also be cultured from a variety of sources. Culture from contents of the large intestine is greatly complicated, however, by the rich flora of the site. Fox and Lee (1997) recommend passage of cecal contents through a 0.65-µm filter, then culture on *Brucella* agar with antibiotics (trimethoprim, vancomycin, polymyxin) to suppress growth of unwanted organisms that are not removed by the filter. Culture is generally considered to have less sensitivity for detecting helicobacters, so negative culture results must be interpreted cautiously.

***Prevention and Control***    In general, it is relatively simple to exclude from animal colonies those rodent-specific organisms that do not multiply or survive for long in the environment. However, given the uncertainty as to the full host range of rat helicobacters, the possibility of transmission by humans is difficult to exclude. The usual source of infection for *Helicobacter* infection in rats is nonetheless expected to be contaminated rodents or other laboratory animals. No indication of transmission by feed, bedding, water, or aerosols has been reported, although *Helicobacter* DNA can be detected on surfaces

contaminated by dust, presumably fecal in origin, from animal cages.

Once a colony is infected, treatment of small groups of animals may be possible. Several antibiotic regimens have been reported to be successful for mice and might similarly be attempted in rats. These have primarily involved oral dosing with antibiotics by gavage several times each day or incorporation of antibiotics into the diet. Elimination of infection from large groups of rats would be less likely to be 100% effective, even if practical obstacles of dosing could be overcome, because even a single rat retaining any viable *Helicobacter* could lead to reinfection of the entire colony. Cross-fostering pups from infected dams onto nursing uninfected dams has been reported as successful for mice (Singletary *et al.*, 2003), although it has not been reported in rats. Rederivation of infected stocks by caesarian section or embryo transfer is also successful.

*Research Complications* Research complications due to infection by *Helicobacter* spp. in rats have not been reported.

### j. Cilia-Associated Respiratory Bacillus

*Etiology* Usually referred to as CAR bacillus, the CAR bacillus is not taxonomically classified in the genus *Bacillus.* Rather, it has been tentatively placed in a group of bacteria known as 'gliding bacteria,' based on the fact that they are motile but without visible means for such motility, and may be related to *Flavobacterium* or *Flexispira*, based on 16S rRNA sequencing (Cundiff *et al.*, 1995a; Kawano *et al.*, 2000). Final identification, however, is still pending. CAR bacillus has been identified in rats, mice, and rabbits among common laboratory animals (van Zwieten *et al.*, 1980; MacKenzie *et al.*, 1981; Waggie *et al.*, 1987; Griffith *et al.*, 1988).

*Epizootiology and Transmission* Transmission is primarily via direct contact with infected animals. Fomites probably do not play a significant role in natural transmission of CAR bacillus, and bedding does not transmit the infection well (Matsushita *et al.*, 1989; Cundiff *et al.*, 1995b). Airborne exposure is not an important means of transmission (Itoh *et al.*, 1987).

*Clinical Signs* In rats, infection is usually asymptomatic, although nonspecific clinical signs such as weight loss and dyspnea may occur.

*Pathology* CAR bacillus infection may not always present gross lesions, although translucent gray cystic lesions, representing dilated, mucus-filled airways may be visible on the pleural surface (Itoh *et al.*, 1987). Coinfection with *Mycoplasma pulmonis* or other pathogens may occur, resulting in suppurative bronchopneumonia. Histopathologically, hyperplastic peribronchial and peribronchiolar mononuclear cell cuffs are observed in the lungs (Itoh *et al.*, 1987; Matsushita and Joshima, 1989). A thin basophilic layer may be observed on the surface of the airway epithelium in hematoxylin–eosin-stained sections, giving the impression that the cilia are more basophilic than normal, but this is not specific and should not be used as a definitive diagnostic feature. With Warthin–Starry or methenamine silver stain, filamentous bacilli are readily observed among cilia of respiratory epithelium from the nasal cavity to the bronchioles (Fig. 4.5). The upper respiratory tract is involved earlier in the course of infection than the lower tract and should be included in histologic examinations for CAR bacillus infection.

*Diagnosis* Colonies are easily screened for CAR bacillus infection by serologic techniques (Matsushita *et al.*, 1987; Shoji *et al.*, 1988; Lukas *et al.*, 1987). Because
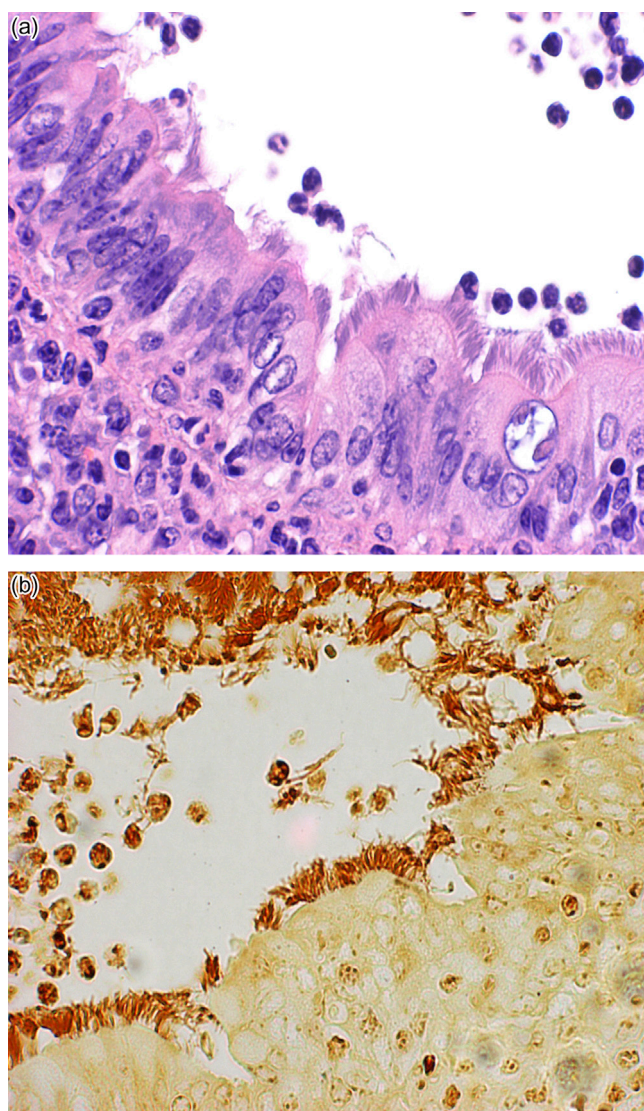


**FIGURE 4.5** Rat bronchus stained via H & E (a) and Warthin–Starry (b). Innumerable filamentous bacteria are densely clustered at the ciliated surface of the columnar epithelium. Note the polymorphonuclear cell exudate in the bronchial lumen. Magnification: × 400.

false-positive reactions can occur (Hook *et al.*, 1998), any positive results should be confirmed by a Steiner stain of tracheal mucosal scraping or histopathology with use of special stains, or by PCR. Interestingly, because infection is not readily transmitted by soiled bedding (Cundiff *et al.*, 1995b), many sentinel programs may fail to detect CAR bacillus infection. Infection is lifelong, and the organisms are readily retrievable by tracheal lavage or scraping (Medina *et al.*, 1998). Therefore, CAR bacillus may be readily detected by PCR (Cundiff *et al.*, 1994), which may serve as an important confirmatory test to follow positive serologic results. PCR may be positive prior to serologic conversion, and samples for PCR may be collected as nasal swabs as a nonterminal procedure (Franklin *et al.*, 1999).

*Differential Diagnosis* CAR bacillus infection should be distinguished from murine respiratory mycoplasmosis, pneumonia due to other bacteria (i.e., *Streptococcus pneumoniae*, *Corynebacterium kutscheri*, etc.), and viruses. Detection of CAR bacillus infection should also raise the suspicion of coinfection with other pathogens, especially *Mycoplasma pulmonis* (van Zwieten *et al.*, 1980; MacKenzie *et al.*, 1981).

*Prevention and Control* CAR bacillus infection is prevented by exclusion of infected animals. No effective treatment has been described. As an alternative to elimination and rederivation of entire infected colonies, the requirement for direct contact for transmission may possibly be exploited to advantage. If individual animals or cages are monitored by serology, and then negative individuals are monitored by PCR, all rats that are positive (or all cages that have a positive rat) by either test may be eliminated or quarantined. Because the infection is not transmitted well by aerosol or fomites, it may be possible to control the spread of infection. However, the expense, the labor, and the consequences of possible failure would have to be weighed against the value of saving some of the rats.

*Research Complications* The interference of CAR bacillus with research is unknown. Interference with ciliary function has been suspected but not measured. Effects of CAR bacillus on other respiratory functions and on the immune response have been not been reported in rats, although in mice it incited an antibody response and increased some serum and pulmonary cytokines (Kendall *et al.*, 2000, 2001).

### k. Mycoplasmosis

*Etiology* Murine respiratory mycoplasmosis (MRM), also known as chronic respiratory disease, is caused by *Mycoplasma pulmonis* (Kohn and Kirk, 1969; Lindsey *et al.*, 1971).

*Epizootiology and Transmission* The infection is rare in North America (Pritchett-Corning *et al.*, 2009). *Mycoplasma pulmonis* is transmitted horizontally by direct contact and aerosol and vertically by *in utero* transmission (Lindsey *et al.*, 1982). Venereal transmission may also be possible.

*Clinical Signs* Although infection can begin in young rats, clinical signs are usually observed only in older animals; *M. pulmonis* infection is clinically silent in young animals. Clinical signs are nonspecific, referable to the respiratory and auditory involvement, and include rales and dyspnea, snuffling and chattering, and ocular and nasal discharges, as well as chromodacryorrhea, rubbing of eyes, and head tilt. Rats with severe middle ear involvement may spin when held up by the tail. Decreased reproductive efficiency has also been reported in rats (Leader *et al.*, 1970).

*Pathology* The disease outcome depends on a complex interaction of factors relating to host, pathogen, and environment (Lindsey *et al.*, 1985). Host factors include age, strain (Davis and Cassell, 1982), immune status and lymphoreticular function, and the presence of intercurrent infections such as Sendai virus (Schoeb *et al.*, 1985); host nutritional deficiencies such as vitamin A and E deficiencies may exacerbate disease (Tvedten *et al.*, 1973). *Mycoplasma* isolates may also vary in virulence (Davidson *et al.*, 1988). Environmental factors may include intracage ammonia, temperature, humidity, etc. (Schoeb *et al.*, 1982). *Mycoplasma pulmonis* possibly damages host cells by causing dysfunction and/or loss of cilia (Kohn, 1971), which is a likely cause of the accumulation of exudate, opportunistic bacterial infections, and impaired transport of ova (infertility). *Mycoplasma pulmonis* competes for the host cell nutrients and metabolites (Cassell *et al.*, 1986) and may also produce toxic metabolites, such as peroxides and nonspecific mitogens (Naot *et al.*, 1979a, b). The latter may cause proliferation of autoreactive clones of lymphocytes, leading to the host becoming a victim of its own immune system. *Mycoplasma pulmonis* successfully evades the host's immune defenses, so infection and some lesions (especially those in the upper respiratory tract) are persistent and often progressive. The exact mechanism by which *M. pulmonis* evades the host immune system, however, is unknown.

Gross lesions of MRM (Percy and Barthold, 2007) include suppurative rhinitis, otitis media, laryngitis, and tracheitis in the upper respiratory tract. In the lung, suppurative bronchopneumonia with or without atelectasis, bronchiectasis, and abscesses may occur; widespread bronchiectatic abscesses lead to the appearance referred to as 'cobblestone' lung, primarily seen in adults with endstage disease (Fig. 4.6). This classic lesion of MRM is rare in recent years. Arthritis may rarely be observed. No genital tract lesions are usually observed, but occasionally partially resorbed fetuses and suppurative salpingitis may be found. Histopathologically (Kohn and Kirk, 1969; Percy and Barthold, 2007), airway lesions in
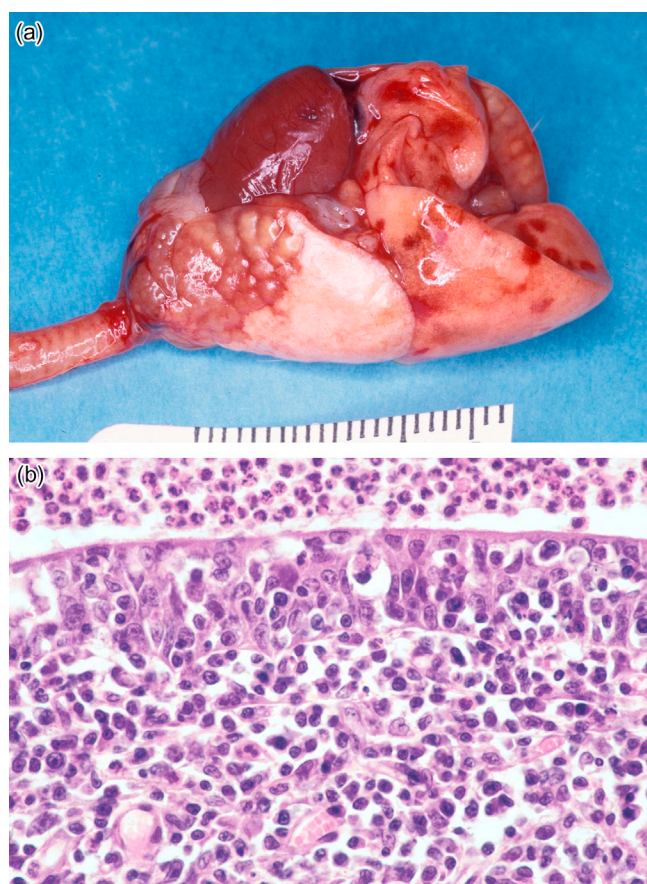
**FIGURE 4.6** *M. pulmonis* infection in a rat. Note the 'cobblestone' appearance of the lungs in the gross photograph (a). Histologically, a polymorphonuclear exudate is present in the lumen of the airway while a chronic inflammatory infiltrate is present below the respiratory epithelium. *Courtesy of Dr. T. R. Schoeb.*

the respiratory tract are usually characterized by suppurative exudate, hyperplasia (squamous metaplasia) of mucosal epithelium, and often striking hyperplasia of the bronchial-associated lymphoid tissue (BALT) that could even potentially be confused with lymphoma. Other respiratory tract lesions include pseudoglandular hyperplasia of the nasal epithelium in chronic cases, and hyperplasia of peribronchial alveolar type II pneumocytes. CAR bacillus and/or other secondary bacterial pneumonias also frequently accompany MRM. Lesions in the female genital tract of rats with mycoplasmosis may include suppurative oophoritis and salpingitis, or hydrosalpingitis, and chronic suppurative endometritis or pyometra.

*Diagnosis* Diagnosis of mycoplasmosis in an individual rat is usually based on cultural isolation (especially exudate in the upper respiratory tract and middle ears) or PCR. Surveillance of infections in colonies, however, is most effectively accomplished by serology (Cassell *et al.*, 1981; Lussier, 1991) or by PCR on

respiratory samples. Note that due to the sensitivity of the organism to desiccation, it is doubtful whether soiled bedding would be effective in transmitting it to sentinels. Pathology, including gross examination, and histopathology should not be considered diagnostic by themselves but may provide guidance in selecting more definitive tests such as PCR on deparaffinized tissue.

*Differential Diagnosis* Differential diagnoses for MRM include other bacterial pneumonias, such as *Corynebacterium kutscheri* infection, streptococcosis, CAR bacillus infection, and (rarely) mycotic pneumonia. All of these infections are considered rare (Pritchett-Corning *et al.*, 2009). Iatrogenic lesions due to gavage errors should also be considered a possibility. Viral infections are less likely to be mistaken for MRM, but intercurrent infections are common, including Sendai virus, pneumonia virus of mice, and others. All of these are also rare in contemporary laboratory rats (Pritchett-Corning *et al.*, 2009; Livingston and Riley, 2003).

*Prevention and Control* Use of high-quality vendors, careful sanitation practices, segregation of clean colonies from conventional or wild rodents and isolation and testing of rats imported from suspect sources are steps commonly taken to protect research colonies. Antibiotics such as tetracycline have been used historically to minimize symptoms and prolong survival of affected animals in order to salvage long-tern studies, but antimicrobial eradication is unlikely. Depopulation and restocking (or rederivation in the case of unique strains) are indicated to eliminate colony contamination.

*Research Complications* Mycoplasma pulmonis interferes with research by its effects on the immune system, the respiratory system, and the reproductive system and by being a primary cause of early mortality in infected colonies (Cassell *et al.*, 1986; Swing *et al.*, 1995; Lindsey *et al.*, 1971, 1982). It has also confounded the diagnosis of pulmonary lymphoproliferative disease in toxicology safety testing (Schoeb *et al.*, 2009a, b).

### l. Mycoplasma Haemomuris (Hemobartonellosis)

*Etiology* Mycoplasma haemomuris, formerly *Haemobartonella muris*, is a gram-negative bacterium parasitizes erythrocytes of rats (Neimark *et al.*, 2001, 2002). It is an obligate parasite and cannot be grown *in vitro*.

*Epizootiology and Transmission* Because *M. haemomuris* is transmitted by the spiny rat louse, *Polyplax spinulosa*, which is very rare in modern laboratory animal facilities, erythrocytic mycoplasmosis is also correspondingly rare (National Research Council, 1991). However, the potential exists for infection of biological materials, which would provide a route of introduction into rat colonies. In addition, both the agent and the vector are still extant in North America and presumably elsewhere, indicating a continuing, albeit low-level, threat.

*Clinical Signs*    Clinical signs are typically observed only if the normally latent infection is activated by immunosuppression or splenectomy (National Research Council, 1991). Signs are due to erythrocyte destruction and may include weight loss, hemoglobinuria, pallor, and dyspnea. Clinical pathology demonstrates anemia, reticulocytosis, increased coagulation times, decreased plasma proteins, and increased serum immunoglobulins (IgG and IgM).

*Pathology*    Necropsy of rats with *M. haemomuris* infection is unrewarding except in the case of active infections, when anemia, hemoglobinuria, and splenomegaly may be observed. Blood films are likely to show parasitemia only in active infections.

*Diagnosis*    *M. haemomuris* infection should be suspected whenever lice are found in a rat colony or whenever anemia and hemoglobinuria are observed. Diagnosis should be based on detection of the organisms on erythrocytes, where they appear as round (coccoid), elongate (rod), or dumbbell-shaped densities on the erythrocyte surface.

*Prevention and Control*    *M. haemomuris* infection is readily prevented by excluding *Polyplax spinulosa* and controlling biologic materials being introduced into a colony. Once the disease is confirmed in a colony, rederivation by embryo transfer or cesarian section is warranted, although treatment with antirickettsial compounds such as tetracyclines or arsenicals may be appropriate for small groups of rats (Ristic and Kreier, 1984).

*Research Complications*    *M. haemomuris* exerts its effects on research by virtue of its parasitism of erythrocytes. It reduces the half-life of erythrocytes, can alter function of the mononuclear phagocyte system, and can increase rejection of transplantable tumors, as well as interfering with research in other blood-borne parasitic diseases such as malaria and trypanosomiasis.

## 2. Viral Infections

As with bacteria, many of the 'classical' viral pathogens of rats such as Sendai virus are very rare (Pritchett-Corning *et al.*, 2009; Livingston and Riley, 2003). However, they should not be forgotten as they not only persist in wild populations and pet rodents, but there are many other regions of the bioresearch world where they may possibly persist. Thus, we will briefly mention a few viruses that are primarily of historical interest in many areas of the world.

### a. Sendai Virus Infection

*Etiology*    *Sendai virus* is an RNA virus of the family *Paramyxoviridae*, genus and species *Respirovirus*. The species contains strains that are antigenically homologous (Jacoby and Gaertner, 2006).

*Epizootiology and Transmission*    Although Sendai virus was once very prevalent in commercial sources of mice and rats, it is rarely seen in today's colonies (Pritchett-Corning *et al.*, 2009; Liang *et al.*, 2009; Schoondermark-van de Ven *et al.*, 2006; Livingston and Riley, 2003; Mahler and Kohl, 2009, McInnes *et al.*, 2011). Sendai virus is highly contagious, with transmission occurring through the respiratory tract either by aerosol or direct contact.

*Clinical Signs*    Unlike Sendai virus-induced disease in mice, an asymptomatic and self-limiting disease is usually induced by Sendai virus in rats. Clinical signs associated with the virus may include reduced production and litter sizes, as well as retarded growth of young within breeding colonies. Infrequently, clinical respiratory signs occur (Makino *et al.*, 1973). It has been shown in Lewis rats, inoculated intranasally with Sendai virus, that draining lymph nodes of the upper respiratory tract are the initial and major site of antibody production. Development of serum immunoglobulin G (IgG) antibodies coincides with clearance of respiratory tract infection and recovery from viral infection (Liang *et al.*, 1999). Coinfection with other respiratory pathogens such as *Mycoplasma pulmonis*, CAR bacillus, *Pasteurella pneumotropica*, and pneumonia virus of mice (PVM) increases the severity of clinical disease and pulmonary lesions (Besch-Williford *et al.*, 1987; Carthew and Aldred, 1988).

*Pathology*    After exposure, the initial tropism in the upper respiratory tract induces a rhinitis characterized by focal to diffuse necrosis of the epithelial cells, and a leukocytic infiltrate composed of neutrophils, lymphocytes, and plasma cells. Within the lungs there is a hyperplastic to suppurative bronchitis and focal alveolitis. Alveolar septa are hypercellular, with infiltrates of alveolar macrophages, neutrophils, and lymphocytes. Viral replication occurs in bronchial epithelial cells, type I and type II pneumocytes, and alveolar macrophages. Later, there is pronounced perivascular and peribronchial cuffing with a lymphocytic and plasmacytic infiltrate that may remain 7 months after the acute phase of the infection (Burek *et al.*, 1977; Percy and Barthold, 2007). Based upon experimental infection, lesion severity has been shown to be more severe in Brown Norway and LEW rats than in F-344 rats (Liang *et al.*, 1995; Sorden and Castleman, 1991).

*Diagnosis*    Due to the low prevalence of clinical signs, diagnosis is best achieved by detection of antibodies to the virus and demonstration of typical lesions in the respiratory tract. The multiplex fluorescent immunoassay (MFI or MFIA; Hsu *et al.*, 2007; Wunderlich *et al.*, 2011) has replaced ELISA as the test of choice for diagnosis of Sendai virus infections in rats due to improved sensitivity and specificity and the requirement of much lower amounts of serum. In situations where confirmation is desired, IFAs or western blots can be used to confirm or refute MFI results. Moreover, PCR assays are now available for the diagnosis of active infections; trachea and lungs are preferred sites of sample collection.

*Prevention and Control* Prevention of Sendai virus introduction into an existing colony requires knowledge of the pathogen status of the source and, in some cases, quarantine with serological testing of incoming rats and mice. Regular and periodic serologic testing within colonies of rats and mice should be done to help prevent and control infection within rodent housing facilities. While the prevalence of infection is likely low in wild rats, the latter remain a potential source of infection if not appropriately controlled (Easterbrook *et al.*, 2008). Moreover, the use of Sendai virus as a research tool continues and these studies also serve as a source of potential infection. PCR testing should be done on all transplantable tumors, cell lines, and other biological materials to prevent transmission of Sendai virus from infected materials to recipient animals (Bauer *et al.*, 2004). Of note, Sendai virus is not readily detected in indirect sentinel monitoring of programs of mice (Artwohl *et al.*, 1994; Compton *et al.*, 2004). Thus sentinel programs may fail to detect this infection, but the limited transmission may also prevent inadvertent infection from wild rodents or experimental studies. If Sendai virus is introduced into a rat colony of immunocompetent rats, neutralizing antibody in infected rats renders the infection self-limiting. Accordingly, if antibody-naive rats are not introduced and if pregnant and preweanling rats are killed and breeding is halted, the virus will be eliminated from the colony within 4–8 weeks (Jacoby and Gaertner, 2006). This presumes that either all rats in the colony have been exposed, as might occur where rats are housed in open-top cages, or that all further interindividual transmission is prevented, so that no new infections occur during the clearing, or 'burnout' phase, which could perpetuate the infection.

*Research Complications* In addition to research complications associated with the respiratory tract tropisms of the virus, it may modulate some immunological responses, e.g., reducing the severity of adjuvant arthritis (Garlinghouse and Van Hoosier, 1978) and depressing T cell and thymocytotoxic autoantibody (Takeichi *et al.*, 1988).

### b. Rat Coronavirus Infection

*Etiology* In the family *Coronaviridae*, the type species of the genus *Betacoronavirus* is *Murine coronavirus*. This species contains several distinct serotypes, including murine hepatitis virus and rat coronaviruses. The two prototype coronaviruses in rats are Parker's rat coronavirus (RCV-P) and sialodacryoadenitis virus (RCV-SDA). In addition to these two coronavirus strains, there are others that have been isolated and found to differ antigenically from either RCV-P or RCV-SDA. Historically, RCV-P and RCV-SDA were considered to induce two rather distinct sets of clinical signs and types of lesions in rats (Jacoby and Gaertner, 2006). More recently, however, the clinical signs, pathogenicity, and histological lesions are considered to be variable but similar for both RCV-P and RCV-SDA, and defining the neutralization group of a new RCV isolate is not useful in predicting its pathogenic potential (Compton *et al.*, 1999). Moreover, because these are highly mutable coronaviruses, field isolates may exhibit additional genetic and antigenic differences, but are likely capable of inducing a similar spectrum of disease. The antigenic differences between RCV-P and RCV-SDA are significant enough to allow cross-infection with either virus. Probably the most important point to be made from a clinical perspective is that neutralizing antibodies to one virus prototype will not offer significant cross protection from the other virus strain, thus allowing viral shedding and recurrence of clinical signs and lesions, albeit diminished (Percy and Barthold, 2007; Jacoby, 1986; Bihun and Percy, 1994; Kojima and Okaniwa, 1991; Weir *et al.*, 1990). However, with the low prevalence of these viruses in contemporary laboratory rat colonies, this scenario is unlikely to occur.

*Epizootiology* Rat coronaviruses are very contagious, with transfer to susceptible rats by direct contact with infected rats, and indirectly by aerosol and fomites (La Regina *et al.*, 1992). During outbreaks, morbidity is high, but mortality is very low (Percy and Barthold, 2007). Virus is present in target tissues for about 1 week after exposure, at which time heightened antibody levels render the infection self-limiting. However, immunity is not lifelong. Under experimental conditions, it has been shown that rats are susceptible to reinfection with a homologous strain as early as 6 months after initial infection and that such rats are able to transfer infection to naive rats by cage contact. However, the severity of lesions in reinfected rats is minimal compared with those associated with primary infections (Percy *et al.*, 1990; Weir *et al.*, 1990). Differences in pathogenicity have been reported among a few rat strains (Jacoby and Gaertner, 2006; Carthew and Slinger, 1981).

*Clinical Signs* Rat coronaviruses may induce either asymptomatic infections or transient clinical infections (sialodacryoadenitis) associated with tissue tropisms for the salivary glands, lacrimal glands, Harderian glands, and respiratory epithelium. There are two distinctive types of clinical disease associated with the virus. The first is associated with breeding colonies in which the virus is endemic with mature rats immune to infection, and in which clinical disease is primarily associated with preweanling, nonimmune animals that display ocular signs associated with conjunctivitis. These signs are transient, lasting for a week or less. The second type of clinical picture is associated with a sudden onset of clinical signs in naive postweanling-to-adult rats that have been exposed to infected rats. Signs include cervical swelling due to inflammation and edema of submaxillary salivary glands (Fig. 4.7), nasal and ocular discharges
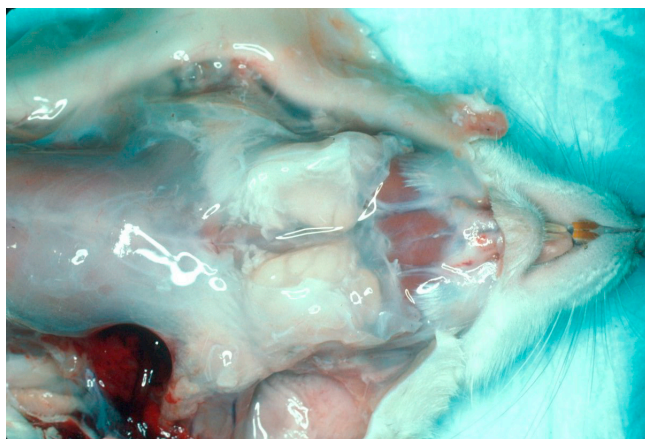
**FIGURE 4.7**   Edematous, swollen, and pale submaxillary salivary glands caused by coronaviral infection. *Courtesy of Dr. T. R. Schoeb.*

that are usually porphyrin stained, photophobia, corneal opacities, and corneal ulcers. In most animals, the signs last for less than 2 weeks. However, in some animals a chronic keratitis and megaloglobus may persist (Jacoby and Gaertner, 2006).

*Pathology*   The microscopic changes associated with rhinitis, tracheitis, and focal bronchitis during the acute stage of the disease include a mononuclear and polymorphonuclear cell infiltration, hyperplastic respiratory epithelia with loss of ciliated surfaces, and focal alveolitis. The lesions within the lower respiratory tract abate in about 7–10 days, and those in the nasopharynx remain somewhat longer (Percy and Barthold, 2007). Histological changes associated with sialodacryoadenitis (SDA) in affected salivary and lacrimal glands include coagulation necrosis of ductal and acinar epithelial cells during the acute stages of the disease, followed by squamous metaplasia during the reparative period that begins 7–10 days after infection. There is a mixed leukocyte infiltrate. Regeneration of the epithelial cells occurs in about 4 weeks postinfection. However, focal lesions may persist an additional several weeks in the Harderian glands.

*Diagnosis*   Once antibodies are produced, about 7 days after infection, diagnosis of rat coronavirus infection is best achieved by serology using the MFI method with confirmatory IFA or western blot testing when desired. During outbreaks, especially prior to seroconversion, histological examination of the Harderian glands and the submaxillary and parotid salivary glands may aid in diagnosis (Percy and Barthold, 2007) and PCR of the infected glands is useful in confirmation of active infections (Besselsen *et al.*, 2002; Compton and Riley, 2001). However, because the disease is often subclinical, typical signs associated with salivary gland and Harderian gland tropisms may not be useful.

*Differential Diagnosis*   Differential diagnoses include *Mycoplasma* and Sendai virus infections, and stress-associated factors that induce chromodacryorrhea (Percy and Barthold, 2007). Due to its contagiousness, rat coronavirus is readily detectable by indirect sentinel programs.

*Prevention and Control*   Preventing transfer of this highly contagious coronavirus to naive colonies is predicated upon preventing entry of infected rats into a facility through knowledge of the pathogen status of vendor colonies and an effective quarantine program. Like most viral agents of rats, the prevalence of RCV in laboratory rats is declining markedly (Liang *et al.*, 2009; Livingston and Riley, 2003; Mahler and Kohl, 2009; McInnes *et al.*, 2011; Pritchett-Corning *et al.*, 2009; Schoondermark-van de Ven *et al.*, 2006). However, in one report, this agent was prevalent in wild rats, so effective control of the latter is of paramount importance (Easterbrook *et al.*, 2008). Control of infection within a colony or facility is based upon the fact that rats shed the virus for only about 1 week, after which they are immune and not latently infected. The virus is not transmitted vertically. Eliminating rat coronavirus from a colony is achieved by allowing the virus to spread quickly to all animals, preventing entry of susceptible rats to the room, and suspension of breeding and removal of preweanlings. The rapidity in which all animals will seroconvert and no longer shed the virus will determine the period of time needed before susceptible animals can be safely introduced or breeding resumed. In most instances, a 6- to 8-week period should be allowed (Jacoby and Gaertner, 2006). Alternatively, if suspension of breeding cannot be done, a method to continue breeding and eliminate SDA is to define a subset of the breeding colony that is seropositive and to relocate these breeding animals to a separate room, allow litters to be born in the original colony until the relocated breeders are in late gestation, and then kill all animals in the original colony (Brammer *et al.*, 1993).

*Research Complications*   Research complications associated with SDA reflect tropisms for the lacrimal and salivary glands, vomeronasal organ, and respiratory epithelium (Percy and Barthold, 2007). Exposure keratitis may also result from lack of tear production or exophthalmus associated with edema of Harderian glands. Except for long-term ocular lesions, research complications would be expected to be linked to the period of active infection and the 2- to 3-week reparative period. During this period, food intake frequently decreases if cervical swelling occurs. Rat coronavirus infection may also have unanticipated effects on ongoing studies as evidenced by impairment of nerve regeneration that occurred in during a recent outbreak (Yu *et al.*, 2011).

### c. Rat Parvovirus Infection

*Etiology* Parvoviruses are single-stranded DNA viruses that have a predilection for mitotically active host cells. Parvoviruses that infect rats include (Kilham's) rat virus (RV), (Toolan's) H-1 virus, rat parvovirus (RPV), and rat minute virus (RMV). In the nomenclature proposed by the International Committee on Taxonomy of Viruses, all of these viruses, plus minute virus of mice and mouse parvovirus are merged into one species, *Rodent protoparvovirus 1.* The first two viruses were initially isolated from a transplantable tumor (RV) and a tumor cell line passed in rats (H-1). In the 1980s, testing of rat sera indicated the presence a parvovirus that was neither RV nor H-1. This virus, which was initially referred to as rat orphan parvovirus (OPV), is now designated rat parvovirus (RPV; Jacoby *et al.*, 1996). In 2002, three variants of another novel parvovirus, rat minute virus (RMV), were isolated from naturally infected rats (Wan *et al.*, 2002).

*Epizootiology and Transmission* Rat virus is excreted in urine and milk and is transmitted by aerosol through direct contact or fomites (Jacoby *et al.*, 1996). RV-contaminated bedding, stored at room temperature for up to 5 weeks, is capable of inducing seroconversion of rats for up to 5 weeks (Yang *et al.*, 1995). Rats may harbor and transmit RV long after seroconversion occurs, with the frequency of persistent infection during natural outbreaks (Gaertner *et al.*, 1996) and experimental infections (Ball-Goodrich *et al.*, 2001) being RV strain-dependent. After experimental inoculation of RV into neonatal rats, the virus persists in tissues for up to 14 weeks, and the duration of infectivity to cage contacts up to 10 weeks. Infection of pregnant females also results in persistent infection of progeny; however pups of persistently infected dams are protected, presumably by maternal antibody (Jacoby *et al.*, 2001). If weanling rats are inoculated, the duration of viral recovery and infectivity is decreased to 7 and 3 weeks, respectively (Paturzo *et al.*, 1987; Jacoby *et al.*, 1988). In persistent infections, DNA and antigenic evidence of RV is most likely to be observed in lymphoid tissues, endothelium, vascular muscle tunics, and renal tubular epithelium (Gaertner *et al.*, 1996; Jacoby *et al.*, 1991).

*Clinical Signs* Clinical signs associated with RV infection occur very sporadically in colonies showing serological evidence of infection and are usually seen only in preweanling animals. In such colonies, reduced litter size, runted litters, and fetal and neonatal death may be observed. Although subclinical infections in postweanling rats are the rule, an outbreak characterized by hemorrhage and necrosis of the brain, testes, and epididymides has been reported in young adult rats (Coleman *et al.*, 1983). The ability of RV to cross the placenta appears to depend on the virus strain, dose, and time of gestation. Resistance to lethal infection develops during the first postpartum week (Gaertner *et al.*, 1996; Jacoby *et al.*, 1988). Serological surveys of rat colonies have indicated that rat parvoviruses, especially RPV (Liang *et al.*, 2009; Livingston and Riley, 2003; Mahler and Kohl, 2009; McInnes *et al.*, 2011; Pritchett-Corning *et al.*, 2009; Schoondermark-van de Ven *et al.*, 2006; Clifford and Watson, 2008) are among the most prevalent viruses of contemporary research rat colonies; however, clinical disease is rarely reported. Natural infectious by H-1, RPV, and RMV have received very little study. To date, none have been associated with naturally occurring clinical disease, suggesting that they are primarily subclinical infections (Ball-Goodrich *et al.*, 1998; Wan *et al.*, 2002; Weisbroth *et al.*, 1998).

*Pathology* The correlation of age and RV pathogenicity is thought to be due to the decreased complement of target cells in the S-phase of division needed for productive infection. The immune status of the host is also significant to the outcome of RV infection. Immunocompetent adult rats mount a classic Th1 immune response that results in viral clearance (Ball-Goodrich *et al.*, 2002), whereas rat virus in athymic rats induces a more severe and persistent infection than in euthymic rats (Gaertner *et al.*, 1995, 1989).

*Diagnosis* Diagnosis of RV, RPV, RMV, and H-1 virus can be accomplished by serology. Serologic tests usually include recombinant capsid viral protein (VP2) antigens of each parvovirus along with a recombinant nonstructural protein (NS1) antigen. The latter is shared among parvoviruses so that animals infected with any parvovirus will seroconvert to this antigen. NS1-based tests thus represent a non-specific assay that provides confirmatory testing for the specific VP2-based assays (Besselsen *et al.*, 2008; Kunita *et al.*, 2006; Livingston *et al.*, 2002). PCR assays for RV, H-1, RPV and RMV have also been developed that provide a rapid, specific and sensitive means for detecting viral DNA in tissue (Besselsen *et al.*, 1995a, b), feces or the environment.

*Research Complications* Research complications induced by RV are associated with its tropism for mitotically active cells of fetuses, neonates, cell cultures, and tumors. Rat virus has been shown to modulate immune function through its tropism for T-cell lymphocytes (McKisic *et al.*, 1995). Rat virus infection in the diabetes-resistant BioBreeding rat increases the expression of macrophage cytokines, leading to an autoimmune diabetes (Chung *et al.*, 1997). The effect of RV on the immune system has been shown to be rat strain dependent for natural killer cell activity. Natural killer cell-mediated cytotoxicity is increased in Brown Norway rats, whereas it is decreased in Wistar–Furth rats (Darrigrand *et al.*, 1984).

The effects, if any, that naturally occurring RPV, H-1 or RMV infections may have on research are unknown.

### d. Rat Theilovirus

There has long been suspicion that rats may harbor a host specific cardiovirus as evidenced by seroconversion to the closely related Theiler's murine encephalomyelitis virus (TMEV; genus *Cardiovirus* and species *Theilovirus*). Isolation of the MHG virus (McConnell *et al.*, 1964) and subsequently a virus designated NSG910 (Ohsawa *et al.*, 2003) from rats provided further evidence of distinct rat cardioviruses. In 2008, a novel rat theilovirus (RTV) was isolated from the feces of asymptomatic rats.

In immunocompetent rats, RTV replicates in enterocytes of the small intestine and following experimental inoculation, is shed for 4–8 weeks. No clinical signs have been associated with either experimental inoculation or natural infection, and the virus does not spread beyond the intestinal tract. While experimental intracranial inoculations with the MHG virus resulted in paralysis in suckling rats (McConnell *et al.*, 1964), this finding was not reproduced with RTV (Drake *et al.*, 2011). Differential strain susceptibility has been identified with experimentally infected SD rats from one vendor exhibiting prolonged fecal shedding and higher infectivity and seroconversion when compared to SD rats from another vendor. In contrast to immunocompetent rats, immunodeficient nude rats exhibit persistent fecal shedding and the virus can be found in extraintestinal sites. The latter findings suggest that adaptive immunity is important in elimination of this virus.

Infections with RTV are best diagnosed by serology. In addition, PCR tests are available for detection of active infections, with feces or intestine serving as optimal samples for testing (Drake *et al.*, 2011). Little is known about control of RTV infections, although measures employed for TMEV in mice are likely effective with RTV infections. In one report, a test and cull strategy was shown to be effective in eliminating the virus from a contaminated colony (Dyson, 2010). Serological surveys, including those that previously used TMEV as an antigen suggest that RTV is of low to moderate prevalence in contemporary research rat colonies (Liang *et al.*, 2009; Livingston and Riley, 2003; Mahler and Kohl, 2009; McInnes *et al.*, 2011; Pritchett-Corning *et al.*, 2009; Schoondermark-van de Ven *et al.*, 2006).

### e. Pneumonia Virus of Mice Infection

Pneumonia virus of mice (Genus *Pneumovirus* and species *Murine pneumonia virus* (PVM) is a pneumovirus in the family *Paramyxoviridae*. Contrary to the virus's common name, serological evidence indicates infectivity in mice, rats, hamsters, gerbils, guinea pigs, and rabbits. The prevalence of seropositive rat colonies was reported in 1982 to exceed 50%; however, today serological evidence of PVM infection is rare to non-existent in rats (Liang *et al.*, 2009; Livingston and Riley, 2003; Mahler and Kohl, 2009; McInnes *et al.*, 2011; Pritchett-Corning

*et al.*, 2009; Schoondermark-van de Ven *et al.*, 2006). Diagnosis is typically accomplished by serology. PCRs are also available for detection of active infections with trachea or lungs, the sites of infection, serving as optimal samples for testing. The virus does not cause clinical disease, but multifocal, nonsuppurative vasculitis and interstitial pneumonitis with necrosis are prominent lesions seen in the acute phase of the disease. These lesions persist for several weeks. Historically, the virus was considered to be a significant co-pathogen with other respiratory agents such as *Mycoplasma pulmonis*, and cross species transmission is a potential concern (Percy and Barthold, 2007).

### f. Group B Rotavirus Infection

Diarrhea in suckling rats has been associated with a virus in the species *Rotavirus B*, from the genus *Rotavirus* and family *Reoviridae* (Eiden *et al.*, 1985). The authors who first reported this disease named it 'infectious diarrhea of infant rats' (Vonderfecht *et al.*, 1984) so it is sometimes referred to by the acronym, IDIR. Affected infant rats excreted feces that varied from liquid to being poorly formed, and the animals displayed erythema and bleeding of the perianal skin. Pathology associated with infection included small intestinal villous atrophy, villous epithelial necrosis, and syncytial cell formation. This same agent was found to be associated with diarrhea in humans and has been shown by enzyme immunoassay inhibition assay to be prevalent in children and adults. Human isolates were shown to induce diarrhea in infant rats (Eiden *et al.*, 1985; Vonderfecht *et al.*, 1985). This suggests that under nonexperimental conditions there may be cross-infectivity between humans and rats. Although the virus is not frequently included in screening panels, commercial serology and PCR assays are available. The prevalence is unknown but generally thought to be low.

### g. Hantavirus Infection

Hantaviruses are enveloped RNA viruses of the genus *Hantavirus*, family *Bunyaviridae*. Rodents serve as the natural reservoirs for hantaviruses, with each virus in the genus being associated with a specific rodent species. Hantavirus infections in rodents are characterized by being chronic and subclinical, with virus being shed persistently in the feces and urine. Hantaviruses pose as significant zoonotic agents. *Rattus norvegicus* is the natural host for the Seoul Hantavirus, causing hemorrhagic fever with renal syndrome (HFRS) in humans. Hantavirus has been isolated from wild rats in Baltimore and several other cities in the United States. One report cites evidence of human infection with a rat-associated Hantavirus (Childs *et al.*, 1988). Cotton rats, *Sigmodon hispidus*, are reservoirs for a Hantavirus that has induced Hantavirus pulmonary syndrome in individuals living

in Florida (Hutchinson *et al.*, 1998). Transmission of Hantavirus from laboratory rats to laboratory personnel has been reported in Japan, Belgium, and the United Kingdom (Desmyter *et al.*, 1983; Lloyd *et al.*, 1984). In both reports, multiple cases occurred that resulted in hemorrhagic fever with renal syndrome.

### h. Rat Respiratory Virus

'Rat Respiratory Virus' was a working name given to a putative viral agent thought to be the cause of idiopathic histiocytic pneumonia often seen in rats (Elwell and Mahler, 1997; Gilbert *et al.*, 1997; Slaoui *et al.*, 1998; Riley *et al.*, 1997). It is now known that these lesions are due to *Pneumocystis carinii* infection (Livingston *et al.*, 2011; Henderson *et al.*, 2012; see Section II.A.4. in this chapter).

### i. Other Viral Infections

There are several rodent viruses for which there is serological evidence of infection in the rat, but for which there are negligible data demonstrating any clinical or pathological importance. These viruses include mouse adenovirus, reovirus 3, parainfluenza virus 3, and endogenous retroviruses (Kohn and Barthold, 1984; Percy and Barthold, 2007; National Research Council, 1991).

### 3. *Parasitic Infections*

#### a. Protozoa

Protozoa are of little consequence in laboratory rats in recent decades (National Research Council, 1991; Kohn and Barthold, 1984). There are several reasons for this. First, no spontaneous disease due to any naturally occurring enteric protozoa of laboratory rats has been reported. Second, parenteral infections are rare in laboratory rats because of absence of vectors. Third, there is almost universal use of high-quality diets, which are generally subjected to heat disinfection prior to use. The days of giving rats fresh produce have happily slipped into the past. Protozoa of potential significance in rodent facilities include *Encephalitozoon cuniculi* and two enteric flagellates, *Spironucleus muris* and *Giardia muris*, which may contribute to disease and alter immune responses in mice.

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii.* Toxoplasmosis in rats is usually subclinical. The definitive host is the domestic cat and other felids, which shed oocysts in the feces. Rats, like many other vertebrates, serve as intermediate hosts. Transmission to rats is via ingestion of cat feces. Ingestion of infected intermediate hosts might also horizontally transmit the infection, although it would not be expected as a mode of transmission in a well-managed rat colony. Infected rats can transmit *T. gondii* vertically, but only very poorly. Therefore, in order for a rat colony to remain infected with *T. gondii*, cat feces would need to be repeatedly introduced. As a result, *T. gondii* is an organism is of little current significance in research facilities, and routine monitoring for toxoplasmosis in rats is not commonplace.

Numerous enteric flagellates have been reported in laboratory rats over the years, but none are of known significance. The most commonly seen are in the order Trichmonadida (trichmonads) or of the genera *Chilomastix* or *Hexamastix* (Pritchett-Corning *et al.*, 2009). The life-cycle of all flagellates and another common commensal protozoan, *Entamoeba muris*, is direct (Baker, 2007), with fecal–oral transmission. Trophozoites, the feeding form, are present in the gastroinestinal tract. Reproduction is asexual and produces resistant cyst forms, which are shed in the feces (Kunstyr, 1977).

*Spironucleus muris* colonizes mice, rats, and hamsters, where it inhabits glandular crypts and the lumen of the small intestine (Gruber and Osborne, 1979; Baker, 2007). Age-infection relationships have not been reported for rats but are probably similar to that of mice, in which animals under 6 weeks of age are more susceptible to infection. Transmission of cloned *S. muris* between rats and mice has been attempted (Schagemann *et al.*, 1990). An isolate from rats was not infective to hamsters, immunocompetent mice, or athymic nude mice. Similarly, rats were not persistently colonized by isolates from mice or hamsters.

Cysts of *S. muris* are resistant to drying (room temperature for 14 days), freezing (−20°C for 6 months), pH 2.2 for 1 day, or 0.1% glutaraldehyde for 1 h (Kunstyr and Ammerpohl, 1978). *Spironucleus muris* infection is diagnosed by examination of wet mounts of duodenal scrapings of weanling rats. Phase-contrast microscopy is especially helpful in observing the trophozoites. Identification is usually based on the size, $3–4 × 10–15 \mu m$, and characteristic rolling motion of the flagellated trophozoites. Cysts may be observed in wet mounts or in fecal smears. These measure $4 × 7 \mu m$ and are reported to have a characteristic banded pattern (Kunstyr, 1977). Recently, a PCR for *S. muris* was developed that has superior sensitivity when compared to wet mounts or fecal smears (Jackson *et al.*, 2013).

*Giardia* spp. are ancient, with one of the most highly conserved genomes of all eukaryotes (Yu *et al.*, 1996, 1998; 1996). *Giardia* also has its own microbiota, including mycoplasma-like particles and bacteria (Feely *et al.*, 1988) and viruses (Tai *et al.*, 1991, 1996). *Giardia muris* colonizes a wide variety of mammalian hosts, including rats, mice, and hamsters (Baker, 2007). Trophozoites attach to the surface of intestinal epithelial cells via a surface membrane mannose-binding lectin and can occur via any point on the parasite surface (Inge *et al.*, 1988). Cysts stored in liquid feces have remained infective for at least 1 year (Craft, 1982).

No naturally occurring clinical disease has been reported in rats infected with *G. muris.* Experimental infection with *G. lamblia* and *G. duodenalis* has resulted

in secretion of specific immunoglobulin A into bile (Loftness *et al.*, 1984; Sharma and Mayrhofer, 1988a).

Giardiasis is diagnosed similarly to spironucleosis. Trophozoites, measuring $7–13 \times 5–10\,\mu m$, have a characteristic piriform or teardrop shape, with a broad, rounded anterior tapering to a pointed posterior end. The trophozoites have a slight curvature toward the ventral side, which causes the motion of their multiple flagella to impart a rolling motion to the organisms in wet mounts (Baker, 2007). In stained preparations, the darkly stained dual nuclei are prominent. Two small, dark median bodies are also visible, immediately posterior to the nuclei. Cysts may also be identified on fecal smears or with fecal flotation methods. PCR tests with high sensitivity are also available for the diagnosis of *G. muris*.

*Entamoeba muris* is a nonpathogenic commensal amoeba of rats, mice, and hamsters (Baker, 2007). Trophozoites, measuring $8–30\,\mu m$ in length, are found in wet-mount preparations of contents from the cecum and colon, where they feed on bacteria. Cysts $9–20\,\mu m$ in diameter have eight nuclei and can be observed in feces.

Control measures in rats for all intestinal flagellates and *Entamoeba muris* are similar. Rederivation, either by cesarean section or by embryo transfer, is effective. Contaminated animal rooms should be thoroughly cleaned, then disinfected with chlorine dioxide solutions or other suitable disinfectants (Weaver and Wickramanayake, 2001), prior to repopulation introduction. All materials brought into the room, which may have had prior exposure to rodents or rodent feces should be autoclaved. All animals should be monitored for infection prior to introduction. This should include examination of rats of appropriate age, i.e., 3–6 weeks.

Treatment of animals to eliminate infection with intestinal flagellates has met with limited success. Metronidazole (Flagyl) or dimetridazole can be added to the drinking water but is ineffective against cysts in the environment. Other authors have reported success in eliminating *Giardia* spp., using metronidazole in rats and mice (Sharma and Mayrhofer, 1988b). Significantly, however, metronidazole has been shown to be carcinogenic in rats and mice (Koch-Weser and Goldman, 1980).

### b. Nematodes

#### OXYURIASIS

*Etiology*    Three species of oxyurid nematodes (pinworms) – *Syphacia muris*, *S. obvelata*, and *Aspiculuris tetraptera* – occur in the laboratory rat. Their continued prevalence (Liang *et al.*, 2009; Livingston and Riley, 2003; McInnes *et al.*, 2011, Pritchett-Corning *et al.*, 2009, Schoondermark-van de Ven *et al.*, 2006, Clifford and Watson, 2008), despite the dramatic progress in eliminating viral and bacterial pathogens, is due both to the persistence of the eggs in the environment and to the low degree of attention paid to these parasites.
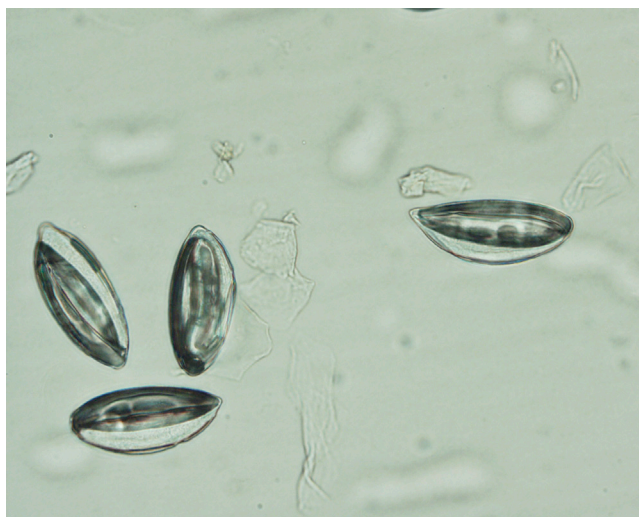


**FIGURE 4.8**    *Syphacia muris* ova.

*Syphacia muris* is the most common oxyurid of the rat (Liang *et al.*, 2009; Livingston and Riley, 2003; Pritchett-Corning *et al.*, 2009). *Syphacia obvelata* is more frequently found in mice, hamsters, and gerbils but is also occasionally found in the rat, especially when housed in the same room with infested mice. The morphology of adults of both *S. muris* and *S. obvelata* is similar, although *S. muris* is slightly smaller and the male has a longer tail, measured as a proportion of body width (Baker, 2007). Eggs vary more markedly between the species, with eggs of *S. muris* being $72–82 \times 25–36\,\mu m$ and those of *S. obvelata* being $118–153 \times 33–55\,\mu m$. In addition, the eggs of *S. obvelata* are almost completely flat along one side, whereas those of *S. muris* are only slightly flattened on one side (Fig. 4.8).

Adult *A. tetraptera* are readily recognized by the four alae present at the anterior end of the body (Fig. 4.9). Eggs of *A. tetraptera* are approximately the same size as *S. muris* eggs, measuring $89–93 \times 36–42\,\mu m$, and are bilaterally symmetrical.

*Epizootiology and Transmission*    *Syphacia* spp. have a direct life-cycle, requiring 11–15 days for completion (Baker, 2007). Transmission is horizontal via ingestion of eggs. Eggs, which remain viable at room conditions for weeks to months, are deposited around the anus and in the colon and become infective in approximately 6h. They are ingested during self-cleaning and hatch in the small intestine. The larvae then mature in the cecum in 10–11 days. *Aspiculuris tetraptera* is also transmitted horizontally by ingestion of eggs, which are extremely persistent in the environment (Baker, 2007). The direct life-cycle is longer than that of *Syphacia*, requiring 23–25 days. Also unlike in *Syphacia*, *Aspiculuris* eggs are passed in the feces and are not deposited around the anus.
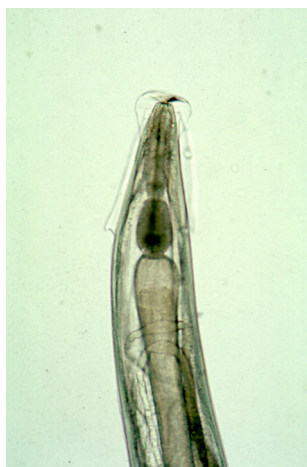
FIGURE 4.9 *Aspiculuris tetraptera* adult pinworm. Note that the prominent cervical alae extend all the way to the level of the pharyngeal bulb.

***Clinical Signs and Pathology*** Rats infected with pinworms are generally asymptomatic. Gross lesions of oxyuriasis are very rare (Baker, 2007), and histologic lesions of oxyuriasis have not been reported.

***Diagnosis*** Diagnosis of oxyuriasis is most practically accomplished by direct examination of macerated cecum and colon under low magnification with a stereomicroscope. This is almost as sensitive as complete direct examination of the large bowel and is significantly less time-consuming. Examination for eggs must be tailored to the infesting species suspected. The perianal tape test is effective only for *Syphacia* spp., and fecal flotation or fecal concentration and centrifugation is effective for *A. tetraptera* (Parkinson *et al.*, 2011). Screening for oxyurid eggs is significantly less sensitive than direct examination of the bowel for the adult helminths (Klement *et al.*, 1996; West *et al.*, 1992), although as a posttreatment diagnostic tool, perianal tape testing was found to be highly sensitive for *S. muris* infection (Hill *et al.*, 2009). For optimal diagnostics, use of more than one diagnostic method is recommended (Effler *et al.*, 2008). Recently, PCR assays have been developed, which add a highly sensitive methodology for detection of these agents (Feldman and Bowman, 2007; Parel *et al.*, 2008; Parkinson *et al.*, 2011). Because pinworm transmission to sentinel animals may be delayed, PCR testing may be particularly amenable to health monitoring programs as it allows for easy testing of colony animals.

***Prevention and Control*** Several anthelmintics, including fenbendazole (Barlow *et al.*, 2005; Coghlan *et al.*, 1993; Huerkamp *et al.*, 2000, 2004), ivermectin (Zenner, 1998; Sueta *et al.*, 2002) new generation avermectins (Oge *et al.*, 2000; Sevimli *et al.*, 2009) and levamisole (Ince *et al.*, 2010) have been shown to eliminate pinworms in mice or rats when administered through a variety of routes (reviewed in Pritchett and Johnston (2002)). However, some treatments such as topical selemectin and diet-delivered moxidectin have been ineffective (Gonenc *et al.*, 2006; Hill *et al.*, 2006; Oge *et al.*, 2000). Fenbendazole-medicated feed is perhaps the most common therapeutic because of its ovacidal, larvacidal, and adulticidal properties and the ease of use; ivermectin is not ovicidal (Pritchett and Johnston, 2002). However, when treating rats for pinworms, consideration should be given to potential effects of the anthelmintic. Ivermectin has known deleterious effects (reviewed in Pritchett and Johnston (2002)) whereas fenbendazole is considered either inconsequential or potentially detrimental to ongoing studies (Barron *et al.*, 2000; Cray *et al.*, 2008, 2013; Duan *et al.*, 2012; Gao *et al.*, 2008; Hunter *et al.*, 2007b; Landin *et al.*, 2009; Vento *et al.*, 2008; Villar *et al.*, 2007). Because ova can readily contaminate and persist in the environment (Lytvynets *et al.*, 2013), decontamination of the latter may also be warranted. Heat, ethylene oxide formaldehyde gas, and chlorine dioxide with or without didecyl di-methyl ammonium chloride have been shown to be effective, while potassium peroxysulphate, alcohol/chlorhexidine, and ultraviolet light were not (Dix *et al.*, 2004). Of note, treatment failures have occurred, but these likely involved lack of consideration of biology and risk factors associated with re-infection (Huerkamp *et al.*, 2004). Oxyuriasis can also be eliminated by rederivation and is readily excluded by proper adherence to modern practices of barrier room technology (Hasslinger and Wiethe, 1987).

***Research Complications*** Numerous research effects of oxyuriasis have been described. In rats, oxyuriasis has been reported to interfere with adjuvant arthritis (Pearson and Taylor, 1975), growth rate (Wagner, 1988), intestinal electrolyte transport (Lubcke *et al.*, 1992), cardiac reactivity to b-adrenergic stimulation (Silveira *et al.*, 2003) and the immune response to allergic sensitization (Demirturk *et al.*, 2007). In other studies, infections have had no deleterious effects (Carlberg and Lang, 2004).

### TRICHOSOMOIDES CRASSICAUDA

***Etiology*** This trichurid nematode is found only in the rat (Baker, 2007). Although geographically widespread, *Trichosomoides crassicauda* is virtually nonexistent in barrier-maintained rodents that have been rederived by cesarean section or embryo transfer. Adult females, approximately 10 mm long, live in the urinary bladder, either free in the lumen or embedded in the mucosa (Baker, 2007; Antonakopoulos *et al.*, 1991; Cornish *et al.*, 1988). The males are anatomically degenerate and exist symbiotically in the vagina or uterus of the females.

***Clinical Signs*** Infestation with *T. crassicauda*, although persistent, is usually clinically inapparent (Baker, 2007). Usually very few worms, perhaps averaging three in number (Barthold, 1996b), are present in the

bladder, where they cause mild uroepithelial hyperplasia (Antonakopoulos *et al.*, 1991; Zubaidy and Majeed, 1981). When found in the renal pelvis, they are associated with mild pyelitis and pyelonephritis.

*Epizootiology* Embryonated eggs are laid and pass in the urine. Transmission of *T. crassicauda* is via ingestion of these eggs and probably occurs from dam to pups prior to weaning. The eggs hatch in the stomach, where the larvae penetrate the wall and pass through the peritoneal cavity or bloodstream to reach the lungs and other tissues. Most larvae lodge in tissues other than the kidneys and may cause hemorrhages or granulomas. Only those that reach the kidney or bladder survive and develop to maturity. The entire life-cycle is 8–9 weeks, so eggs are not present in the urine until the rats are 8–12 weeks of age.

*Pathology* Early speculation concerning the possible etiologic role of *T. crassicauda* infestation in causing bladder tumors in a famous study in rats that were administered high doses of saccharin in the diet (Anonymous, 1977; Homburger, 1977) has not been supported by later investigators (Barthold, 1996b). However, proliferative changes in uroepithelium caused by *T. crassicauda* infestation are identical to those produced early in carcinogenesis by chemical compounds such as *N*-methylnitrosourea (MNU) (Pauli *et al.*, 1996).

*Diagnosis* *Trichosomoides crassicauda* infestation is diagnosed in live rats by filtration of urine and then examination of the filter medium for the eggs. Diagnosis in recently killed rats is by direct examination of the bladder wall, histopathology, scanning electron microscopy, or microscopic examination of cryostat sections stained with acridine orange (Barthold, 1996b; Cornish *et al.*, 1988). The last two methods are purported to be more reliable but are probably not practical for routine, large-scale screening.

*Prevention and Control* Treatment for *T. crassicauda* infestation has been reported, using a single dose of ivermectin (Summa *et al.*, 1992). Follow-up found that the infestation was not eliminated in one of 30 rats, perhaps because of reinfection. Once a colony is free of this parasite, however, there should be little chance of reintroduction if no infected rats enter the colony.

*Research Complications* No confirmed research effects of *T. crassicauda* infestation have been reported in the scientific literature, although proliferative changes in the urothelium would render these animals unsuitable for research involving the urinary system (Cohen *et al.*, 1998).

### c. Cestodes

*Etiology* There are only two adult cestodes that are likely to be encountered in laboratory rats: *Rodentolepis nana* (also often referred to as *Hymenolepis nana*) and *Hymenolepis diminuta.* The primary differences of

consequence between the two species are that *R. nana* is zoonotic and can have a direct life-cycle, whereas *H. diminuta* always has an indirect life-cycle, utilizing an intermediate host, and is not zoonotic. Although not uncommon in wild rats (Easterbrook *et al.*, 2008), both are rare in laboratory rats (Livingston and Riley, 2003).

*Rodentolepis nana* averages 20–40 mm long but can vary greatly. It is slender and less than 1 mm wide. The scolex has four suckers, and a rostellum armed with 20–27 hooks (Fig. 4.10). Mature proglottids are trapezoidal and contain as many as 200 eggs, which are thin-shelled, oval, and colorless and have six visible polar filaments. Within the eggs, the embryo, or oncosphere, has three pairs of hooklets within an inner envelope (Fig. 4.11). The eggs are approximately 30–56 × 44–62 μm and do not persist for long periods outside the host.

*Hymenolepis diminuta* is larger than *H. nana*, 20–60 mm long and 3–4 mm wide. The scolex of *H. diminuta* also has four suckers but the rostellum is unarmed – it has no hooks. Eggs of *H. diminuta* are 60–88 × 52–81 μm, and the oncosphere has three pairs of hooks, but no polar filaments.



**FIGURE 4.10** *Rodentolepis (Hymenolepis) nana* adult tapeworm. Note the rostellum armed with hooks.



**FIGURE 4.11** *Rodentolepis (Hymenolepis) nana* ova. Note the hooklets.

In addition to the adult cestodes, one may occasionally encounter larvae of *Taenia taeniaformis*, also called cysticercus fasciolaris. The cysts are found in the livers of rats, mice, and hamsters and are up to several centimeters in diameter. They are readily identified by the presence in the cyst of a scolex, strobila, and bladder (Hsu, 1979). Although considered nonpathogenic (Baker, 2007), the cyst may be associated with the development of hepatic sarcomas, probably in a mechanism similar to the induction of sarcomas in the rat by a variety of foreign bodies (Altman and Goodman, 1979; Elcock *et al.*, 2001). Because the definitive host is the cat, detection of the cysticercus is evidence that materials in the animals' immediate environment, usually feed, were contaminated with unsterilized feces from an infected cat. Control, therefore, is simple.

*Epizootiology*    *Rodentolepis nana* lives in the small intestine of rats, mice, hamsters, and primates, including humans. It is primarily the ability to parasitize humans that gives *R. nana* significance, for it causes little damage in rats or mice. Furthermore, it is not clear whether or not human infestations represent infestation with distinct strains, i.e., whether or not the strains of *R. nana* present in rats are infective for humans (Baker, 2007). In the direct life-cycle (Hsu, 1979), which requires 14–16 days, embryonated eggs are ingested and hatch in the small intestine. The oncospheres penetrate villi and develop into cysticercoid larvae in 4–5 days. These larvae reenter the lumen, the scolex evaginates, and they attach to the mucosa. An additional 10–12 days are required before mature proglottids are formed. Adults live only a few weeks. Infection normally results in some level of immunity, which prevents autoinfection. When autoinfection occurs, eggs hatch in the small intestine and develop, without being passed in the feces, and can result in very high worm burdens. In the indirect life-cycle, grain beetles (*Tenebrio molitor* and *T. obscurus*) and fleas (*Pulex irritans, Ctenocephalus canis, Xenopsylla cheopis*) serve as intermediate hosts. Rats and other definitive hosts are infected by ingesting the intermediate host.

*Hymenolepis diminuta* has a similar host range: mice, rats, hamsters, and primates, including humans (Hsu, 1979). The life-cycle of *H. diminuta* is always indirect and is similar to the indirect life-cycle of *R. nana.*

*Clinical    Signs*    Both    *Rodentolepis    nana*    and *Hymenolepis diminuta* are pathogenic in rats only in severe infections, where retarded growth, weight loss, impaction, and death have been reported in the older literature (Hsu, 1979), although no recent reports excluding the contributions of other potential pathogens have been published.

*Pathology*    Although presence of the parasites can be identified during gross examination or microscopically in tissue sections, there are no associated significant lesions.

*Diagnosis*    Infection is diagnosed by detection of the adult cestodes on direct examination of the small intestine, by observation of the eggs in feces (smear or fecal flotation), or by histopathologic detection of cysticercoids or adults in the small intestine (Hsu, 1979). Infection is most common in recently weaned rats and young adults, probably because of acquired immunity in older animals.

*Prevention and Control*    *Rodentolepis nana* and *H. diminuta* infection is prevented by purchase of clean stocks of rodents, by adequate disinfection of barrier room supplies, and thorough insect control and exclusion of wild rodents (Baker, 2007). Treatment of infected animals is not generally recommended, because of the zoonotic implications of this disease.

*Research    Complications*    Experimentally,    *H. diminuta* has been shown to elicit a host Th2 type immune response (McKay, 2010; Starke and Oaks, 2001; Webb *et al.*, 2007) that may modulate models of intestinal and extraintestinal disease (Graepel *et al.*, 2013; Hunter *et al.*, 2005, 2007a; Reardon *et al.*, 2001). However, inadvertent interference of research has been not been reported likely because of the rarity of hymenolepiasis in rat research colonies.

### d. Trematodes

Numerous trematodes have been reported in wild rats, including *Plagiorchis muris, P. philippinensis*, and *P. javensis.* Some are zoonotic (Hong *et al.*, 1996), but none are significant in laboratory rats and trematodes are not even addressed in some texts (Baker, 2007).

### e. Mites

#### FUR MITES

*Etiology*    *Radfordia ensifera* is the fur mite most likely to be encountered in laboratory rats, although other fur mites, such as *Radfordia affinis* or *Myobia musculi*, could possibly be harbored on the pelage (Fig. 4.12).
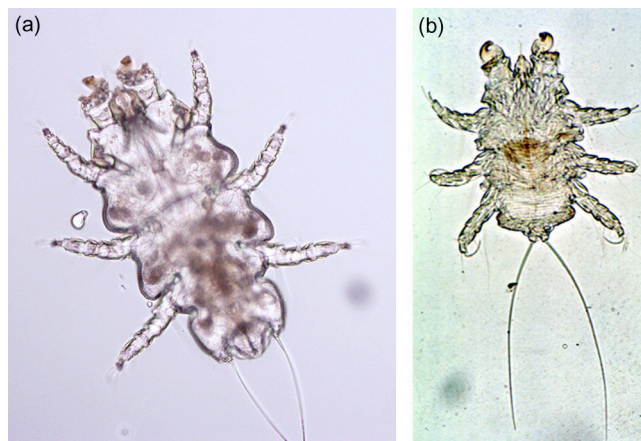


**FIGURE 4.12**    Adult fur mites. *Radfordia ensifera* (a) and *Myobia musculi* (b).

*Epizootiology*   Fur mites are transmitted by eggs, which can persist in the environment for long periods. The eggs hatch in 7–8 days, and females can begin to lay eggs after another 16 days.

*Clinical Signs*   Infestation can result in pruritus, self-excoriation, and secondary bacterial infection.

*Diagnosis*   A number of diagnostic methods for the detection of fur mites have been developed including fur plucks, tape tests, and skin scrapes which can be used on live animals and examination of cooled rats or pelts using a dissecting microscope; the latter may be aided by placing the animal or pelt on a black background. Because mites or eggs may be found on different parts of the body (Metcalf Pate *et al.*, 2011; Ricart Arbona *et al.*, 2010b), examination of several sites is recommended. Studies comparing sensitivity of various assays have been performed in mice (Metcalf Pate *et al.*, 2011) but are likely applicable to rats. Recently, PCR assays have been developed that show superior sensitivity to traditional techniques (Karlsson *et al.*, 2014; Parkinson *et al.*, 2011; Rice *et al.*, 2013; Weiss *et al.*, 2012; Jensen *et al.*, 2013). These assays are also useful in assessment of posttreatment efficacy although animal environmental samples may remain positive for several weeks (Rice *et al.*, 2013). Of note, detection of fur mites using indirect sentinel monitoring programs has met with mixed success in mice (Lindstrom *et al.*, 2011; Ricart Arbona *et al.*, 2010b).

*Prevention and Control*   Several fur mite treatments have been developed in mice that are likely applicable to rats. Compounds used include topical selemectin alone (Gonenc *et al.*, 2006) or in conjunction with amitraz- and fipronil-treated nestlets (Bornstein *et al.*, 2006), topical moxidectin (Mook and Benjamin, 2008; Pollicino *et al.*, 2008; Pullium *et al.*, 2005), and ivermectin (administered topically or in food or water) with or without cross fostering (Conole *et al.*, 2003; Huerkamp *et al.*, 2005; Ricart Arbona *et al.*, 2010c). As with pinworm treatment, consideration should be given to possible effects of the treatment. For example, although uncommon, ivermectin has been associated with neurologic clinical signs in mice being treated for mite infestations (Ricart Arbona *et al.*, 2010a). Of note, there are numerous anecdotal reports of infection recurrence, thus animals should be monitored regularly to ensure effective treatment. Infestation can also be eliminated by rederivation and is readily excluded by proper adherence to modern practices of barrier room technology.

*Research Complications*   In mice, fur mite infestation has been associated with increased mitotic activity in the skin, immunologic alterations, amyloidosis and a Th2 immune response that results in elevation of serum IgE (Pochanke *et al.*, 2006; Roble *et al.*, 2012).

*Other Mites*   Rats may also be infested with the mesostigmatid mites, *Ornithonyssus bacoti* (the tropical rat mite) and *Laelaps echidnina* (Watson, 2008). Unlike fur mites, these are blood-sucking mites that live in the environment and are only found on the rat during periods of feeding. Clinical signs may range from none to anemia and debility with severe infestations. These mites can also carry a number of rodent pathogens. Moreover, they will bite humans and can carry several zoonotic diseases. Suspicion of mesostigmatid mite infestation often arises when animal care personnel are bitten. Diagnosis may be achieved by direct observation of blood engorged mites on rats or in the environment. Mites may also be found in sticky insect traps, although the latter may lack sensitivity as a sole diagnostic tool (Watson, 2008). Elimination of mites must include treatment of both animals and environment. Treatments reported to be effective include permethrin-impregnated cotton balls for mice and pyrethrin permethrin or dichlorvos treatment of the environment (Cole *et al.*, 2005; Hill *et al.*, 2005; Watson, 2008; Chu and Couto, 2005).

### f. Lice

Pediculosis in the laboratory rat is currently rare and is attributed to only one species, *Polyplax spinulosa* (Baker, 2007). *Polyplax spinulosa* females are approximately 0.6–1.5 mm long; females are larger than males. Like all insects, they have six legs (Fig. 4.13). The female lays eggs, called nits, which are cemented to hairs. The eggs have a distinct operculum, with a row of pores near the operculated end. The eggs hatch by a pneumatic mechanism in 5–6 days; the larvae ingest air through the pores, pass it through the body, and then use that pressure to force open the operculum (Owen, 1992). The young nymphs are paler than the yellow–brown adults but are morphologically similar. After three ecdyses, or molts, they become adults. Depending on environmental conditions, the ecdyses require 1–3 weeks. The entire life-cycle is completed in 2–5 weeks. Adults live only 25–28 days. Transmission is by direct contact (Baker, 2007).



**FIGURE 4.13**   *Polyplax spinulosa* adult louse.

Pediculosis is usually inapparent, although heavily parasitized animals may appear unthrifty and pruritic. *Polyplax spinulosa* is also the vector of *Mycoplasma haemomuris* and other pathogens (Baker, 2007). Diagnosis is by direct examination of the pelt for adults, nymphs, and eggs (Baker, 2007). Any time that infestation with *P. spinulosa* is detected, blood smears should be screened for *Haemobartonella muris.*

Pediculosis is prevented by introducing only animals free of the condition. Insecticides approved for use in veterinary medicine may be used effectively to treat infestations, as may subcutaneous administration of ivermectin (Baker, 2007), but would probably be advisable only in especially valuable rats in the absence of significant intercurrent infections.

### 4. Fungal Infections

#### a. Pneumocystis Carinii

*Etiology* *Pneumocystis carinii* is classified as a fungus based upon DNA base sequences in genes encoding ribosomal RNAs (Stringer, 1993). Our understanding of pneumocystosis in rats has changed significantly in the last few years. *P. carinii* causes a host-specific infection (rats only) with two distinct diseases seen in immunodeficient vs. immunocompetent rats.

*Epizootiology and Transmission* *Pneumocystis carinii* is naturally acquired by rats through airborne transmission (Hughes, 1982) or possibly by fomites such as soiled bedding (Albers *et al.*, 2009).

*Disease Presentation in Immunodeficient Rats* In rats with impaired immunity, such as athymic nude rats, the organism proliferates uncontrolled within the lung, filling alveoli and resulting in dyspnea, wasting and death (Pohlmeyer *et al.*, 1993; Deerberg *et al.*, 1993). In addition to the foamy material comprising trophozoites, phospholipids, macrophages, and debris filling aveoli, the infection in nude rats was also noted to have interstitial and perivascular lymphocytic infiltration. The abundant organisms in these cases are usually easily identified by special stains such as methenamine silver that demonstrate the fungal cysts within the alveoli and bronchioles. Rats which are immunocompromised by various methods are also susceptible to this form of infection and have been used as a model of *P. jirovecii* pneumonitis that occurs in some human patients with uncontrolled HIV infections (Oz and Hughes, 1996).

*Disease Presentation in Immunocompetent Rats* *P. carinii* is also prevalent in immunocompetent rats, where it has been shown to cause the interstitial pneumonia that had been previously informally referred to as 'Rat Respiratory Virus, RRV' (Livingston *et al.*, 2011; Henderson *et al.*, 2012). In immunocompetent rats, lesions occur following infection, regardless of the age of the rats. *P. carinii* has a slow doubling time, estimated at 4.5 days (Aliouat *et al.*, 1999), and does not appear to incite a host response until a population density threshold is reached. At that point, lesions begin to be visible and specific antibodies are produced. Thereafter, the population of *P. carinii* diminishes and the infection is eventually cleared. It is noteworthy that even at peak population, the number of organisms present is magnitudes less than the population densities achieved in immunodeficient rats. The lower population density may explain the difficulty in finding *Pneumocystis* cysts using special stains in infected immunocompetent rats, although diligent searching of multiple sections may eventually be fruitful.

*Pathology* Gross lesions of *P. carinii* infection become visible in 50% or more of infected rats 4–5 weeks after infection, and have been observed in many strains, suggesting that all strains and stocks of rats are probably susceptible. At necropsy, red–brown or tan lesions are scattered throughout the lung in a pattern typical of interstitial pneumonia. These lesions may be resolved after another 8–12 weeks. Microscopically, the lesions are characteristic enough they were reported as diagnostic for this disease (Albers *et al.*, 2009) and this has been supported by subsequent molecular diagnosis (Henderson *et al.*, 2012). Microscopic lesions will occur in 50–100% of infected rats and are typically characterized as lymphohistiocytic interstitial pneumonia. In *Pneumocystis* interstitial pneumonia in immunocompetent rats, alveolar septa are thickened with macrophages and lymphocytes, and alveoli contain macrophages, lymphocytes, and debris. Multinucleated giant cells may occasionally be present, and slight interstitial hemorrhage is common. Eosinophils and/or neutrophils are occasionally present but not a useful diagnostic criterion. The other hallmark of the histopathologic appearance is perivascular lymphocyte cuffing (Fig. 4.14). Bands, or cuffs, of macrophages
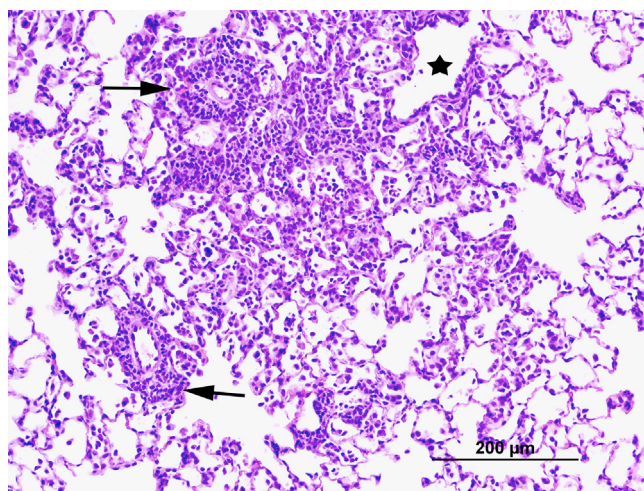


FIGURE 4.14 Photomicrograph of rat lung with interstitial pneumonia due to *Pneumocystis carinii*. Note the bands, or cuffs, of lymphocytes and macrophages encircling interstitial vessels (black arrows). Alveolar septa are thickened. Bronchioles (black star) are much less affected than the vessels.

and lymphocytes encircle interstitial arteries when the first lesions of interstitial pneumonia are observed. Over the next few weeks, lymphocytes come to predominate, and these lymphoid cuffs will persist after the infiltrates in the alveoli and septa have resolved. Increased BALT is not considered to be a major component of the lesions.

*Diagnosis*   The diagnosis in immunocompetent rats may be made provisionally based on the characteristic histologic lesions, although it is recommended to confirm it with PCR on lung tissue. Special stains such as methenamine silver may demonstrate the fungal cysts within the alveoli. Screening of groups of rats for *P. carinii* may be accomplished by serology or by PCR. Rats will produce antibodies by approximately 6–8 weeks after infection and will remain antibody positive for life. Lung tissue is the best sample for PCR, bronchial wash is somewhat less sensitive (perhaps because of the relatively low population density of the fungus), nasal samples may occasionally be positive, and oral swabs are ineffective (Henderson *et al.*, 2012).

Differential diagnosis. The characteristic lymphohistiocytic interstitial pneumonia must be differentiated from bronchial or bronchiolar inflammation as observed with *Mycoplasma pulmonis*, CAR bacillus, Sendai virus and coronavirus.

*Research Complications*   The impact of *P. carinii* infection on research using immunocompetent rats has not been well-explored, although it has been a confounding factor in inhalation studies (Gilbert *et al.*, 1997). In addition, because of its high prevalence and the fact that it results in observable pulmonary inflammation, it might be a differential diagnosis to consider in instances where unexpected pulmonary or cardiopulmonary results are observed.

### b. *Encephalitozoon cuniculi*

*Etiology*   *Encephalitozoon cuniculi*, is a microsporidian fungal parasite of a wide variety of mammalian hosts, including rodents, lagomorphs, carnivores, and primates, including humans. It has also been reported in birds (Poonacha *et al.*, 1985; Reetz, 1993). Resistance to infection and the outcome of infection are dependent on T-cell function, which is strain dependent (Liu *et al.*, 1989; Niederkorn *et al.*, 1981). Athymic nude mice, and presumably athymic nude rats, are more susceptible to lethal infection than are euthymic animals. *Encephalitozoon cuniculi* has also been recovered from transplantable ascites tumors in rats (Petri, 1969).

*Epizootiology and Transmission*   Encephalitozoonosis is occasionally found in conventional rabbit colonies, but is rare in rats. It is transmitted by ingestion, and possibly inhalation, of spores shed in urine (Wilson, 1979). Vertical transmission has been proposed in primates, foxes, mice, rabbits, and guinea pigs but not in rats (Boot *et al.*, 1988; Liu *et al.*, 1988).

*Clinical Signs*   Clinical signs and gross lesions of *E. cuniculi* infection are not reported in rats.

*Pathology*   On histopathologic examination (Majeed and Zubaidy, 1982), rats with *E. cuniculi* infection may have nonsuppurative or granulomatous meningoencephalitis in any or all parts of the brain and occasionally the spinal cord. Interstitial nephritis may also be observed. Less frequently, similar lesions may be observed in other tissues. Spores may be observed in, or more frequently adjacent to, any of the lesions. Spores stain poorly with hematoxylin and eosin but are strongly gram-positive.

*Diagnosis*   Diagnosis of *E. cuniculi* infection is usually based on serology (Pakes *et al.*, 1984). Screening of colonies by serology is probably the most efficient method, because infected colonies normally have a high prevalence (Gannon, 1980). As with all serologic assays, positive serologic results should be confirmed by a second method or by repeating the assay on groups of animals to establish a pattern of positive results. Histopathologic observation of the organism is definitive as is the detection of *E. cuniculi* DNA by PCR; kidney, urine and brain are preferred sampling sites for the latter.

*Differential Diagnosis*   The primary histopathologic differential diagnosis for *E. cuniculi* infection in rats is toxoplasmosis, which is very rare. *Encephalitozoon cuniculi* measures $1 \times 2\,\mu m$, stains well with Gram stain and poorly with hematoxylin and eosin. *Toxoplasma gondii* measures $2 \times 4\,\mu m$, stains well with hematoxylin and eosin, and poorly with Gram stain (Wilson, 1979).

*Prevention and Control*   Encephalitozoonosis is controlled by purchasing only animals that are free of *Encephalitozoon* and by maintaining them away from infected animals. There is currently no effective treatment.

*Research Complications*   Research complications of *E. cuniculi* infections have not been reported in rats, although it is potentially a confounding factor if histopathologic evaluation of the central nervous system and kidney is part of the study (Majeed and Zubaidy, 1982).

### c. Other Fungal Infections

Other fungal infections in the rat have been infrequently reported and are associated with predisposing factors that reduce immunocompetence. In one report, about one-fifth of Wistar rats on a 2-year carcinogenesis study had chronic rhinitis associated with *Aspergillus fumigatus* (Rehm *et al.*, 1988). The predisposing factor in these animals was thought to be Sendai virus infection; Sendai is currently very rare. Clinical signs included sniffing and nasal exudation. At necropsy, yellowish, friable material was present either unilaterally or bilaterally in the nasal cavities, and in the most severe cases, the nasal cavities were completely blocked. The *A. fumigatus*-induced rhinitis was, in most cases, limited

to the naso- and maxilloturbinates. A bronchial abscess containing hyphae and multiple fruiting heads occurred in one rat.

Tracheobronchial aspergillosis was reported in an aged F-344 rat with concomitant large granular-cell leukemia. Immunodeficiency due to the leukemia was thought to be involved with the multifocal, transmural necrotic lesions of the trachea and bronchi (Hubbs *et al.*, 1991).

Royals *et al.* (Royals *et al.*, 1999) reported two cases of fungal-induced rhinitis in rats that had no known immunosuppression. Corncob and hardwood bedding from two sources were tested to determine if the source of the *Aspergillus* infection was bedding material. A range of 700–5400 fungal spores per gram of nonautoclaved corncob bedding was found. Six genera of fungi (*Cladosporidium, Acremonium, Penicillium, Aspergillus, Fusarium*, and *Scolobasidium*) were isolated from the samples of corncob bedding, whereas only negligible counts were isolated from hardwood bedding samples. The authors suggested that either the use of autoclaved or γ-irradiated corncob bedding should be considered as a means to eliminate fungal contamination of bedding.

Dermatomycosis (ringworm) due to *Trichophyton mentagrophytes* has been reported in wild and laboratory rats. However, it has not been reported in laboratory rats for many years. In rats, dermatomycosis may be presented clinically by patchy hair loss and scurfy or erythematous papular-pustular lesions (Weisbroth *et al.*, 1998).

## B. Noninfectious Diseases

### 1. Genetic Predisposition to Disease

Traits that may be desirable to one investigator may be undesirable to another. Every stock and strain of laboratory rat has been carefully selected for specific genetic traits for decades. Common among these are albinism, behavioral characteristics, such as docility and willingness to breed in captivity, and certain tumor profiles.

Overt metabolic diseases such as obesity (Zucker rat), diabetes (BB rat), and hypertension (SHR and fawn-hooded and Dahl rats) make these strains valuable models in biomedical research, whereas spontaneous appearance of the same characteristics in outbred stocks may complicate other research studies. More subtle strain-related tendencies, such as immunologic responsiveness characteristics in Brown Norway and Lewis rats, are exploited by researchers in particular areas of research. In recent years, genetic manipulation has allowed further development of specifically tailored metabolic disease to model critical human defects. It is beyond the scope of this chapter to catalog the innumerable genetic traits or strain-related variations that occur in laboratory rats, and the reader is encouraged to consult the literature and genomic databases such as the NIH-supported Rat Genome Database for specific information on particular genes, strains, and conditions (Laulederkind *et al.*, 2013).

In addition to known and characterized, spontaneous or induced, genetic variation in rats, isolated colonies of breeding rats inevitably experience some degree of genetic drift. Although this may be monitored to some degree in inbred rats by molecular techniques such as restriction fragment length polymorphisms, it is more difficult to assess the degree to which it has occurred in outbred stocks, where expected interindividual variation may obscure intercolony differences. Nonetheless, any two colonies started from the same source will vary increasingly with time unless there is a sufficient and ongoing exchange of breeders between the colonies. Genetic drift within a population can also be reduced by careful adherence to specific outbreeding programs, such as line breeding with systematic exchange of breeders between multiple lines. 'Randomized' breeding is an important tool used to maintain genetic heterogeneity, but this is a carefully planned process that must not be confused with a haphazard approach that simply pairs 'random' animals.

The inevitability of some degree of genetic drift should not, however, blind researchers to the large role played by environmental, husbandry, dietary, and experimental variables in apparent differences between succeeding groups of animals. These extraneous factors can also have a major impact on the expression of underlying genetic traits. An example of modification of lesion prevalence is the impact of *ad libitum* overfeeding on increasing the incidence of progressive renal disease (Keenan *et al.*, 1996, 1998).

Brown Norway rats have a high incidence of eosinophilic granulomatous pulmonary inflammation, nearing 100% incidence in both males and females at 3–4 months of age. Brown Norway rats from colonies worldwide are affected, including those maintained in isolators. Affected colonies are seronegative for all known agents, and rats of other strains maintained with the Brown Norway rats do not develop lung lesions. The lung lesions are scattered throughout the parenchyma and are characterized by generally well-organized granulomas of Langhans' giant cells, macrophages, and eosinophils. No foreign material, fungi, or bacteria are routinely visible or can be demonstrated by polarized light or special stains. This inbred strain is used for studies of allergy and asthma due to the inherent pulmonary hyperresponsiveness, and it has been hypothesized that the eosinophilic inflammatory lesion seen in this syndrome may be an allergic or reactive response to an environmental insult (Noritake *et al.*, 2007).

Polyarteritis (panarteritis) nodosa is a vascular disease that has been identified in Sprague-Dawley and spontaneously hypertensive rats. Gross lesions are apparent in the large, muscular arteries of the mesentery

and visceral organs, which become enlarged and tortuous. Histological changes in the walls of affected arteries include fibrinoid necrosis and muscular hypertrophy. It is often identified as an incidental lesion, but in a study of the disease in rats on an inbred ACI background the rupture of affected arteries was identified as a potential cause of sporadic death (Cohen et al., 2007).

## 2. Nutritional Diseases

Frank dietary deficiencies are uncommon, probably for several reasons. First, high-quality commercial diets are in almost universal use. Second, rats store fat-soluble vitamins and vitamin $B_{12}$, manufacture vitamin C, and can fulfill many of their requirements for other B vitamins by coprophagy. However, heat and moisture, such as are associated with autoclaving, can reduce vitamin levels, particularly lysine, vitamin A, vitamin E, riboflavin, and thiamin. Prolonged storage can have similar effects. In addition, diets designed for maintenance of adult rodents may be too low in protein and fat for optimal growth of young animals or successful reproduction. Clinical evidence of dietary insufficiency may include decreased reproductive performance, litter loss, poor growth, and sparse hair coat. Signs of severe deficiencies of specific vitamins are rare. If they occur, they include squamous metaplasia of salivary ducts with hypovitaminosis A, disseminated hemorrhage with hypovitaminosis K, and embryonic death and testicular degeneration with hypovitaminosis E. Nutritional deficiencies can also alter disease susceptibility and severity.

In addition, feed qualities, aside from total levels of calories and specific nutrients, must be considered, including contaminating chemicals, microbes, and the size and hardness of pellets. For example, feeding a powdered diet will result in an increased incidence of malocclusion.

## 3. Management-Related Diseases

There are a wide variety of health problems that may be caused by suboptimal care and management, including those relating to experimental manipulations. Only a few of the most common will be mentioned. Sanitation of the animal's cage, bedding, water, and feed, as well as of experimental equipment, is critical. High moisture content in bedding leads to rapid growth of bacteria, which can increase the incidence of urinary tract infections and, possibly, mastitis and skin lesions. Bacterial urease production in urine-saturated zones of bedding can increase ammonia levels. High moisture content in food may increase environmental fungal spore contamination through the proliferation of mold. Some softwood bedding materials emit aromatic compounds that may increase hepatic microsomal levels and affect responses to test compounds, although these compounds are usually completely sublimated during the drying phase in bedding manufacture. Considerations of air quality might also include factors such as ammonia concentration, $CO_2$ levels, dusts, disinfectant residues, and pollutants.

Rats are sensitive to temperature, humidity, noise, vibration, light, room activity, and other perceptible changes in their environment. Relative humidity of less than 40% has been linked to the poorly characterized condition known as ringtail, although there can be other factors involved (Crippa et al., 2000). Ringtail is primarily a condition of young rats, usually sucklings, characterized by the formation of prominent annular constrictions of the tail and occasionally of the digits. Portions of affected extremities distal to the constrictions often become necrotic and are sloughed. This condition should not be confused with bite wounds or the normal, more subtle annulations of rat tails which develop with age.

Rats, especially albino rats that have no protective iris pigmentation, can be susceptible to retinal degeneration when exposed to ambient light levels above a certain threshold, which can vary depending on the intensity of the light exposure, the duration of the light cycle, and the illumination levels the animals have been exposed to previously (Semple-Rowland and Dawson, 1987). For rats raised under very dim conditions (6 lux), functional and histological damage can occur with as little as 270 lux of irradiation as measured at the cage level with a 12:12 light:dark cycle. Beyond simple retinal injury, exposure to very high light levels of 1600 lux for 12 h each day for 8 days resulted in necrosis in Harderian glands, likely as a result of the photoreactive properties of the porphyrin secretions (Kurisu et al., 1996). Historically recommended room light levels are in the range of 325–400 lux as measured 1 m above the floor (National Research Council, 2011). Note that these recommended levels as measured 1 m above the floor do not necessarily reflect actual light levels to which rats are exposed in individual cages at various levels in racks at varying distances from light fixtures. Light energy exposure from a generating source is a distance-squared function, so the exposure of animals in cages at the top levels of the rack can be greatly increased over those in the lower levels. This should be recognized as a potential study variable, and if retinal function or structure is to be evaluated as part of a study, the rack location of animals in various experimental groups should be randomized at the initiation of the study or uniformly rotated throughout the study. In addition to intensity-related adverse effects, disruption of the light:dark cycle and resulting exposure to constant light may cause anestrus and other breeding problems. Light exposure (contamination) during the dark phase of the light cycle, with light levels as low as 0.21 lux, has been reported to interfere with rat tumor and metabolism studies, and there are design and management strategies that can be utilized to minimize the physiologic disruptions of light leaks during the dark cycle (Dauchy et al., 2011).

## 4. Traumatic and Iatrogenic Diseases

Traumatic lesions are uncommon in the rat. Rats housed in wire-bottom cages may develop pododermatitis or lesions on their hocks if housed long-term in such caging. Occasionally, wire-grid floors will allow a rat's foot to become entrapped in the wire grid causing severe edema and injury to the foot and leg, a complication which may occur when rats are allowed to recover from anesthesia in a wire-bottom cage. Group housing of rats is much less likely to result in traumatic injuries due to fighting than is seen in mice. Ulcerative dermatitis, associated with *Staphylococcus aureus* and self-induced trauma from scratching, has been reported (Fox et al., 1977; Wagner et al., 1977). In one report (Fox et al., 1977), the skin lesions were observed only in rats originating from two breeding colonies of one commercial vendor, leading to the hypothesis that the lesions may have been associated with specific *Staphylococcus* phage types or host susceptibility factors.

Adynamic ileus, sometimes leading to death, may occur subsequent to intraperitoneal administration of chloral hydrate. This lesion is noteworthy as it may be mistaken for pathology due to Tyzzer's disease in young rats. Clinical signs occur several days after anesthesia and include lethargy, anorexia, and abdominal distension. The most prominent dilatation occurs in the jejunum, ileum, and cecum. The usual anesthetic dose of chloral hydrate is 400 mg/kg; however, the concentration of the drug, not the dosage, appears to be correlated with the induction of ileus (Fleischman et al., 1977). Use of chloral hydrate in contemporary laboratories is rare except for certain neuroscience models, and comparative studies with more commonly used anesthetic agents are ongoing in order to identify acceptable alternatives (Maud et al., 2014).

## C. Neoplastic Diseases

The prevalence of neoplastic disease in the rat is well defined because this species has been routinely used for decades in large-scale carcinogenic, aging, and toxicological studies. Stock- and strain-specific differences in the prevalence of some types of tumors are well documented (Boorman and Everitt, 2006). However, the overall prevalence of neoplasia and that of specific tumor types may vary considerably within stocks or strains because of genetic variation, environmental influences, and differences in laboratory methodologies and diagnostic criteria. The age at which rats are surveyed is also important, because most tumors, other than mammary gland fibroadenomas in many stocks and testicular tumors in F-344 rats, occur in animals greater than 18 months old (Kohn and Barthold, 1984). Table 4.6 compares the incidence of the most frequently occurring tumors in Sprague-Dawley and F-344 rats.

TABLE 4.6  Incidence of Most Prevalent Tumors in Two-Year-Old Rats[a]

| Organ/tissue | Incidence (%) | | | |
| --- | --- | --- | --- | --- |
| | Sprague-Dawley (Crl:CD(SD)) | | Wistar Han (Crl:WI(Han)) | |
| | Male | Female | Male | Female |
| **TESTES** | | | | |
| Interstitial cell tumor | 4.0 | — | 1.2 | — |
| **UTERUS** | | | | |
| Endometrial stromal polyp | — | 1.2 | — | 6.8 |
| **OVARY** | | | | |
| Granulosa cell/theca cell tumor | — | 0.8 | — | 1.1 |
| **MAMMARY GLAND** | | | | |
| Fibroadenoma | 0.8 | 32.7 | 0.2 | 12.6 |
| Carcinoma | 0.2 | 9.2 | 0 | 3.0 |
| **LIVER** | | | | |
| Hepatocellular adenoma/carcinoma | 2.9 | 2.2 | 5.3 | 2.2 |
| **LYMPHORETICULAR** | | | | |
| Lymphocytic leukemia | 0.6 | 0.3 | 1.2 | 0.8 |
| Histiocytic sarcoma | 1.0 | 0.6 | 0.7 | 0.3 |
| **PITUITARY** | | | | |
| Adenoma/carcinoma, pars distalis | 60.9 | 66.1 | 24.7 | 45.9 |
| **ADRENAL GLAND** | | | | |
| Cortical adenoma | 1.8 | 4.7 | 1.7 | 1.2 |
| Pheochromocytoma, benign | 12.0 | 2.5 | 1.3 | 0.4 |
| Pheochromocytoma, malignant | 1.9 | 0.1 | 0.3 | 0 |
| **PANCREAS** | | | | |
| Islet cell adenoma | 7.8 | 3.3 | 2.1 | 0.4 |
| Islet cell carcinoma | 0.8 | 0 | 0.8 | 0.2 |
| **THYROID** | | | | |
| C-cell adenoma | 7.8 | 7.5 | 6.9 | 7.0 |
| C-cell carcinoma | 0.3 | 0.6 | 1.4 | 1.6 |
| Follicular cell adenoma | 0.8 | 1.1 | 4.4 | 2.7 |
| Follicular cell carcinoma | 0.2 | 0 | 1.0 | 1.2 |

[a]*Adapted from: Giknis, M.L.A. and Clifford, C.B. 2011 Neoplastic and Non-Neoplastic Lesions in the Charles River Wistar Hannover [Crl:WI(Han)] Rat. [online] Charles River Laboratories. Available at: <http://www.criver.com/files/pdfs/rms/wistarhan/rm_rm_r_wistar_han_tox_data_2011.aspx>[Accessed 27 March 2014]; and Giknis, M.L.A. and Clifford, C.B. 2013. Compilation of Spontaneous Neoplastic Lesions and Survival in Crl:CD®(SD) Rats From Control Groups. [online] Charles River Laboratories. Available at: <http://www.criver.com/files/pdfs/rms/cd/rm_rm_r_cd_rat_tox_data_2013.aspx> [Accessed 27 March 2014].*

Among the environmental influences, diet has long been known to be an important factor in modulating tumor prevalence (Boorman and Everitt, 2006). Dietary factors may include manipulations of dietary composition (Rogers et al., 1993) such as high fat (Eustis and Boorman, 1985), specific amino acid deficiencies (Nakae, 1999), or even high protein levels. High protein may alter chronic progressive nephropathy (CPN) which can, in turn, alter the development of renal neoplasia (Seely et al., 2002). Overall caloric intake is also a key factor in the age of onset, rate of tumor growth, and types of tumors observed. Rats in two-years studies fed ad libitum, will have lower survival and more tumors, especially those tumors such as pancreatic, mammary, and pituitary where endocrine influences play a role (Keenan et al., 1995).

Another environmental influence on the prevalence of tumors is the pathogen or disease status of the rats in a particular report. Data on tumor risk can be significantly influenced by the effect that some infectious diseases may have on longevity, pre-neoplastic or neoplastic changes, and masking of small tumors (Boorman and Everitt, 2006).

### 1. Mammary Gland

Mammary gland tumors are the most frequently occurring tumors in most stocks and strains of rats. Sprague-Dawley stocks often have an incidence of 50% in aged female animals, whereas F-344 have a lower incidence of about 25–30%, and Wistar Han have an incidence of 30% or less (Boorman et al., 1990b; Giknis and Clifford, 2011, 2013). Most mammary tumors are benign fibroadenomas, with carcinomas occurring less frequently. Both types can occur in aged males; however, the incidence is usually less than 1%. The tumors may arise in mammary tissue at any point from the neck to the inguinal area, and they tend to attain a large size and become ulcerated unless surgically excised. On gross examination, fibroadenomas are freely movable in subcutaneous tissues, circumscribed, firm, and lobulated. Histologically, they are characterized by well-differentiated acinar epithelial components surrounded by inter- and intralobular connective tissue components (Boorman et al., 1990b; Percy and Barthold, 2007).

### 2. Testicle

Interstitial cell tumors occur in about 80% of F-344 rats by the age of 15 months (Boorman et al., 1990a), whereas they occur at a much lower (Giknis and Clifford, 2011, 2013) frequency in Wistar Han and Sprague-Dawley rats. They are discrete, soft, and yellow to brown, with areas of hemorrhage, and may occur in multiple sites unilaterally or bilaterally. Histologically, their Leydig's cell origin is apparent. Tumors have two cell types that are arranged in solid sheets or in an organoid pattern.

The cell types are (1) polyhedral to elongated cells with granular to vacuolated cytoplasm, and (2) smaller cells with hyperchromatic nuclei and scanty cytoplasm (Boorman and Everitt, 2006; Percy and Barthold, 2007).

### 3. Pituitary Gland

Pituitary tumors occur frequently in aged rats of many stocks and strains, including Sprague-Dawley, F-344 and Wistar Han rats (Giknis and Clifford, 2011, 2013; Percy and Barthold, 2007). Most pituitary tumors are classified as chromophobe adenomas, originating from the pars distalis. In some reports, the prevalence of pituitary tumors is greater in female F-344 and Sprague-Dawley rats. Carcinomas of the pars distalis are reported with much less frequency; however, their reported prevalence may vary considerably because of differences in classification protocols by pathologists. As was previously noted, caloric restriction significantly reduces the incidence of pituitary tumors in rats.

Chromophobe adenomas vary in size, often reaching 0.5 cm in diameter. Grossly, the tumors are soft and dark red due to prominent hemorrhagic areas. They are well circumscribed and, because of their size, often compress adjacent brain tissue and induce hydrocephalus. Microscopically, they consist of large polygonal cells with prominent vesicular nuclei and eosinophilic cytoplasm. The architecture of the tumors consists of cells arranged in nests, cords, or sheets separated by vascular sinusoids (Boorman and Everitt, 2006).

### 4. Adrenal Gland

The incidence of cortical adenoma in Sprague-Dawley rats has been recently reported as 2% in males and 5% in females. Pheochromocytoma (approximately 85% benign) was reported to have an incidence of 14% in males and 2% in females of this stock, although as with other tumors, there was tremendous variation between studies. For example, the range of benign pheochromocytoma, reported in all 20 of 20 studies included in the survey, was from 1% to 28%. F-344 rats have a relatively low incidence of adrenal cortical neoplasia; pheochromocytomas occur at a higher rate, and are observed in approximately 32% of males and 5% of females (Table 4.6).

### 5. Pancreas

Endocrine tumors of the pancreatic islet cells are relatively common in some stocks of rats. A mean control group incidence of 8% has recently been reported in males, and 3% in female Sprague-Dawley rats (Giknis and Clifford, 2013). For Wistar Han, islet tumors were observed in 36 of 1217 males (2%) in control groups in 16 studies, of which 26 were adenomas and 10 were classified as carcinomas (Giknis and Clifford, 2011). Among the 1217 Wistar Han females in control groups

in the same set of studies, there were only seven islet cell tumors, five adenomas and two carcinomas. In the F-344 rat, the incidence is similar, 4% in males and 1.5% in females (Boorman and Everitt, 2006). Grossly, islet cell tumors may be either single or multiple and are circumscribed and reddish brown. Islet cell carcinomas are distinguished from adenomas by capsular invasion and metastases. Tumors of the exocrine pancreas are less common (Boorman and Everitt, 2006).

### 6. Lymphoreticular System

Large granular lymphocytic leukemia is a major cause of death in F-344 rats (Percy and Barthold, 2007), with a reported incidences of up to 50%, with average incidences around 34% in males and 20% in females (Boorman and Everitt, 2006). The initial site of malignancy is thought to be the spleen (Fig. 4.15). The neoplastic cells are transplantable to rats of the same strain. Unlike leukemia in mice, this leukemia in rats is not associated with a retrovirus. Diagnosis is based upon clinical signs of anemia, jaundice, weight loss, and laboratory findings of splenomegaly, elevated leukocyte counts of up to 400,000/ml, and diffuse infiltration of malignant lymphocytes in various organs (Percy and Barthold, 2007). In the Sprague-Dawley rat and most other stocks, the incidence is quite low.

Other lymphoreticular tumors include lymphocytic lymphoma and histiocytic sarcoma, each of having an incidence of approximately 1% in both Sprague-Dawley and Wistar Han rats (Giknis and Clifford, 2011, 2013).

## D. Miscellaneous Conditions

### 1. Congenital/Hereditary Anomalies

It is beyond the scope of this chapter to catalog congenital defects in rats. The incidence of such defects is obviously influenced by administration of mutagenic and teratogenic substances, but it also varies with strain, age of mother, disease status, coincidences of statistics, and human terminology. Rats are susceptible to a wide variety of genetic diseases, some of which make them valuable models and others of which are confounding variables. Only a few spontaneous defects, involving the urinary tract, heart, and central nervous system, will be mentioned here. The growing range of genetically engineered diseases, and their unintentional side effects, will not be addressed. Researchers and supporting animal resource professionals are strongly urged to investigate, with due scientific scrutiny, background information concerning specific stocks and strains, prior to embarking on courses of research involving any laboratory animal. Databases of defects observed in reproductive studies may be available from animal vendors and some contract research organizations.

Hydronephrosis is one of the more commonly reported congenital defects of rats, characterized by unilateral or bilateral dilation of the renal pelvis. Although it may be inherited as a single dominant gene in the Gunn rat, it appears to be polygenic in the Brown Norway and Sprague-Dawley rats (Van Winkle et al., 1988). The right kidney is affected more often than the left. Severity of hydronephrosis can vary from a slight dilation of the renal pelvis to such severe dilation that the kidney appears as a transparent cystic structure. The ureter may also be affected to varying degrees. The normal renal pelvis of young animals may appear to be dilated, however, so some caution is required in identifying hydronephrosis (Maronpot, 1996). Hydronephrosis may also be mistaken for pyelonephritis, in which the material in the dilated pelvis is typically cloudy; for polycystic kidneys; and for renal papillary necrosis. Culture and histopathology of the affected site will distinguish among these conditions.

Congenital lesions of the cardiovascular system are less frequently reported but include ventricular and atrial
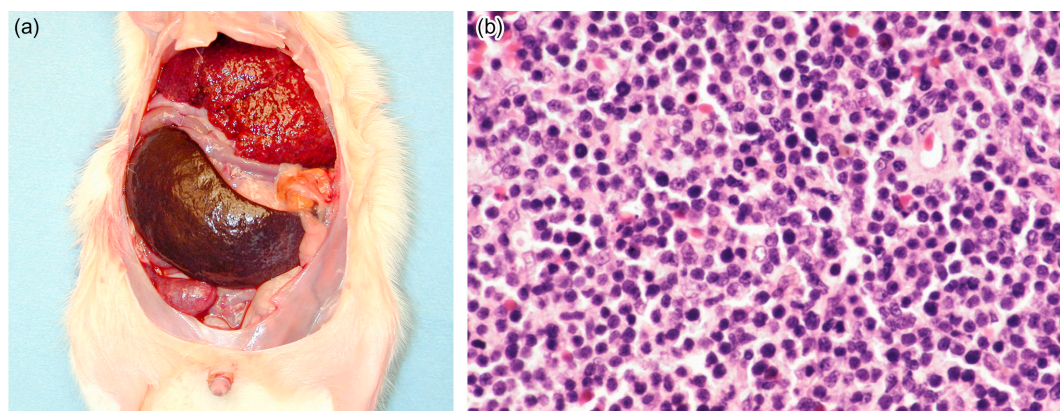


**FIGURE 4.15** Splenomegaly and hepatomegaly associated with large granular lymphocytic leukemia in an F344 rat (a). Light micrograph (b) demonstrates the pleomorphic neoplastic cells in an affected spleen. *Courtesy of Dr. T. R. Schoeb.*

septal defects, dextrocardia, and defects of the valves and endocardial cushion, as well as various anomalies of the great vessels. Overall incidence of cardiac defects has been estimated in one colony of Sprague-Dawley rats at 2.3% (Johnson et al., 1993). Interestingly, high rates of cardiac septal defects, resulting in right ventricular hypertrophy, are observed in the Wistar-Kyoto inbred rats used as control for the outbred spontaneous hypertensive rat (SHR) stock (Slama et al., 2002).

Hydrocephalus is an example of a relatively uncommon congenital defect that may arise as a more prevalent anomaly in small, isolated breeding populations. When such situations are recognized, it may be possible to intentionally further increase the prevalence through inbreeding in order to establish a strain that can serve as an animal model for the disease. Selective inbreeding from such a colony resulted in the establishment of breeding lines that had a prevalence of hydrocephalus well over 25% (Kohn et al., 1981). Seizures have also been reported in a variety of stocks and strains of rat but have been reported most frequently in various Wistar stocks (Nunn and Macpherson, 1995). Wistar rats are especially used in investigation of audiogenic seizures (Garcia-Cairasco et al., 1998).

Congenital and genetically determined ocular defects are very common in some strains of rats. Retinal degeneration is an age-related lesion that can be accelerated by exposure to light (Lai et al., 1978). In albino rats, the lack of a pigmented tapetum increases light exposure to the retina and predisposes for the development of retinal atrophy. Fischer rats (F-344) have an incidence of corneal mineralization that varies from 10 to 100%, depending on subline (Bruner et al., 1992; Yoshitomi and Boorman, 1990). This is characterized by deposition of calcium salts, often visible in routinely stained sections as basophilic granules, along the interface of the corneal epithelium and the stroma. Other ocular abnormalities reported in laboratory rats include retinal degeneration, cataracts, osseous and cartilaginous metaplasia of the sclera, and colobomas.

Several abnormalities of the reproductive tract have been reported in laboratory rats, including transverse vaginal septum in female Wistar and Sprague-Dawley (Barbolt and Brown, 1989; De Schaepdrijver et al., 1995; Lezmi et al., 2011). Affected animals are functionally sterile if the septum is complete, and subfertile if the septum only partially prevents spermatozoa from entering the uterus. Pseudohermaphroditism is occasionally observed in rats, most often male pseudohermaphroditism, also known as testicular feminization; i.e., testes are present internally, but the external genitalia are approximately female. Affected rats are karyotypically XY but express the default feminine phenotype. Although mutant strains have been selected for this characteristic (Allison et al., 1965), it is also occasionally observed in other strains

as well. In the testicular-feminized rat (*tfm*), the defect is a lack of androgen receptors due to a point mutation (Yarbrough et al., 1990), although defects in other genes could potentially result in similar syndromes.

## 2. Age-Related Diseases

Laboratory rats are subject to a wide range of neoplastic and nonneoplastic age-related diseases, as are most aging mammals. Because of the use of rats in 2-year carcinogenicity studies, and as models of gerontology for humans, diseases of the geriatric rat have particular significance to the laboratory animal professional. The type, incidence, and severity of these lesions vary greatly with stock or strain of rat, infectious disease status, experimental manipulation, and husbandry practices, including dietary restriction. Only a few of the most common nonneoplastic conditions will be discussed here, and readers are encouraged to consult the scientific literature, perhaps starting with these excellent reviews for additional information concerning the particular stock or strain with which they are concerned (Boorman et al., 1990; Mohr et al., 1992). In addition, the National Toxicology Program Nonneoplastic Lesion Atlas available on the Internet at <http://ntp.niehs.nih.gov/nnl/> is an excellent resource (Cesta et al., 2014). Neoplastic conditions are afforded a separate section in this chapter.

CPN is an important age-related disease of rat kidneys and is among the most common causes of death in rats in lifetime studies. Synonyms abound, including 'chronic progressive nephrosis' and 'old rat nephropathy'. The condition is more common in males than in females and is progressive, as correctly indicated by its appellation (Hard and Khan, 2004). Gross lesions of CPN are first observed in rats more than 6 months of age and are characterized by pitting of the cortical surface. Because of cortical interstitial fibrosis, removal of the renal capsule may tear the cortical parenchyma. As it becomes more severe in rats more than 1 year of age, the cortical surface becomes increasingly irregular and may develop areas of pallor. Microscopically, glomerular changes are characterized by thickened basement membranes, thickening of the capillary tufts, adhesions to the parietal layer of Bowman's membrane, and segmental glomerulosclerosis (Short and Goldstein, 1992). As the disease advances, numerous tubules in both the cortex and medulla are often dilated and filled with eosinophilic proteinaceous casts. Secondary hyperparathyroidism may occur subsequent to renal functional compromise in advanced cases, resulting in widespread dystrophic mineralization. The etiopathogenesis of CPN is poorly understood and is probably multifactorial. However, several of the major contributing factors have been described (Barthold, 1996a; Percy and Barthold, 2007). First, the reported incidence varies with strain. This indicates probably at

least some genetic predisposition for the development of CPN. Sprague-Dawley and F-344 rats have high incidences, whereas Wistar and Long-Evans stocks have a lower incidence. Reported incidences, however, are difficult to interpret, because of geographic variation in use of different stocks and strains (Wistar rats have been used more predominantly in Europe, and Sprague-Dawley rats in the United States), which could lead to other factors, such as housing and diet, actually causing what otherwise appears to be a strain-related change. For example, when many of the reports of the incidence of CPN in European rats were published, rats were housed five per cage, which is known to result in decreased feed consumption and decreased weight gain, relative to single housing. Second, gender is a determining factor in the development of CPN. Male rats have an earlier onset, higher incidence at any given age, and greater severity of lesions than do females. Third, diet is a critical factor and is also the factor that may be the most amenable to management solutions. It is now clear that moderate dietary restriction will greatly reduce the incidence and severity of CPN at any given age, relative to *ad libitum* overfeeding. The mechanism is hypothesized to be that overfeeding results in prolonged increases in renal blood flow and glomerular filtration rate (Gumprecht *et al.*, 1993). This hyperfiltration causes glomerular hypertrophy, leading to macromolecule filtration deficits, mesangial damage, glomerulosclerosis, and protein leakage. Whatever the mechanism, however, 25–30% reduction in caloric intake, relative to *ad libitum*, results in decreased incidence and severity of CPN in female rats, and decreased severity of CPN in male rats, as well as increased survival in both sexes (Keenan *et al.*, 1995).

Nephrocalcinosis is defined as the deposition of calcium phosphate in renal tissue, although a variety of additional terms are sometimes employed to reflect the localization of the mineral in the cortex, medulla, and so on. In contrast to CPN, which is more common in males, nephrocalcinosis is more common in female rats. In addition to gender, the incidence varies with age and strain and may occur in F-344 rats as young as 7 weeks old. The incidence in F-344 rats may reach 50%, whereas the lower incidence of 0–7% is reported in stocks of Sprague-Dawley and Wistar rats (Montgomery and Seely, 1990). An especially high incidence is observed in BDIX rats. The incidence and severity of nephrocalcinosis may be increased by several dietary manipulations, including high levels of calcium, high phosphorus, low calcium/phosphorus ratios, or low magnesium (Percy and Barthold, 2007). However, it is not clear if dietary levels of these minerals are key determining factors in the background incidence of nephrocalcinosis. Histologically (Short and Goldstein, 1992), mineral deposition is observed most frequently at the corticomedullary junction, in cells of the pars recta and thin loops of Henle, as well as in the lumen of these tubules.

Urolithiasis is the formation of mineral deposits within the urine, occasionally observed in the renal pelvis and/or the urinary bladder, more frequently the latter. The incidence of urolithiasis is generally low, but it bears brief discussion for a couple reasons. First, copulatory plugs (Percy and Barthold, 2007), very firm proteinaceous bodies formed by seminal fluids, can often be found in the bladders and urethras of male rats and are occasionally mistaken for uroliths. Copulatory plugs, however, are not considered to be lesions. Second, although uroliths are sporadically observed in aging rats of both sexes, their detection in rats less than six months of age usually indicates bacterial infection, with cystic uroliths most often associated with an ascending *E. coli* infection; renal uroliths, rare in younger rats, are more often associated with an embolic spread of infection. Rats predisposed to urinary tract infection such as the Zucker Diabetic Fatty rat, may have an elevated incidence, as do rats with hydronephrosis. Uroliths are usually calcium phosphate and struvite, although determination of the composition is rarely helpful.

Chronic myocardial disease is a major cause of death in aged male rats of multiple strains, including Sprague-Dawley, when fed *ad libitum* (Keenan *et al.*, 1995). The condition is often known as cardiomyopathy, or chronic progressive cardiomyopathy, and may be observed as early as 3 months of age. Grossly, the heart is enlarged, occasionally with pale streaks visible. Increased weight of the heart correlates well with the degree of damage observed on histologic examination. Microscopically (Lewis, 1992), there is necrosis of myocardial fibers and an interstitial infiltration of mononuclear cells. Later in the course of the disease, fibrosis may be more prominent. Large reactive nuclei are also observed in myofibers. The most commonly affected myocardial sites are the papillary muscles and interventricular septum. As with CPN, the incidence of chronic progressive cardiomyopathy can be dramatically reduced at any age by moderate dietary restriction, i.e., reduction of 25–30% of total caloric intake relative to *ad libitum* overfed rats (Keenan *et al.*, 1995).

Changes in skin and pelage in geriatric laboratory rats are often observed but rarely reported, which may cause concern to the inexperienced observer. The most common change is thinning or loss of hair, especially over the back (Elwell *et al.*, 1990, 1992). This may be observed in any stock or strain but is especially common in the Brown Norway rat. Old albino rats also have a more yellow appearance at times, because of the accumulation of sebum in the skin. The rings of scales covering the tail increase in number with age to 190 at 1 year (English and Munger, 1992). They continue to become more prominent and more yellowed with time after that. The yellowish material which accumulates on the tail and adjacent to the ear also may become black with

time, probably from oxidation and/or bacterial action. In addition, male rats accumulate brown-pigmented foci on the skin, termed scales (Tayama and Shisa, 1994). These scales can be detached and can overlay skin of 'normal' color. They are found on the dorsum, with some on the tail and perineum. Scale formation is abrogated by gonadectomy. The nature of the pigment is unclear, but it may be oxidized lipid or amino acids.

Alveolar histiocytosis is a very common incidental finding in the lung of aging rats of many stocks and strains (Dungworth et al., 1992). Grossly, alveolar histiocytosis is visible as white to pale tan foci, usually about 1mm in diameter, visible on the pleural surface. The foci may extend slightly above the pleural surface in uninflated lung. Microscopically (Boorman and Eustis, 1990), clusters of alveoli, often in a subpleural locations or adjacent to a terminal bronchiole, contain increased numbers of large, pale, foamy-appearing macrophages. Occasionally, cholesterol clefts may be visible in the more dense aggregates of macrophages, and a slight infiltration of lymphocytes may be present around adjacent vessels, probably as a response to proinflammatory mediators released by the macrophages. Alveolar histiocytosis should not be mistaken for any of the viral pneumonias of rats, because affected animals are seronegative, and any lymphoid infiltrate is slight and localized to the areas of macrophage aggregation. The cause of alveolar histiocytosis is not known, but it is not considered to be infectious.

## Acknowledgment

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