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CHAPTER 7

Common Diseases

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Introduction

Many pathogens have been reported to cause disease in the laboratory rat. In today's modern laboratories many of the pathogens that were commonplace in the past are only occasionally observed today. Much of this progress is due to improvements in husbandry, breeding or **rederivation methods**, and health surveillance tests used by the supplier and the users. However, the breeder and the users must be forever vigilant and cognizant of some of the more common pathogens and the disease they can cause, because infections can and will continue to occur.

This chapter will concentrate on the pathology of the more common pathogens of the laboratory rat. Although other ancillary tests (e.g. serological, bacteriological, etc.) will be mentioned briefly, the reader is encouraged to consult the listed references for details.

Viral Diseases

Parvoviruses

Based on serologic surveys, parvoviruses are some of the most common viral pathogens in wild and laboratory rats (Kilham and Margolis, 1966; Robey *et al.*, 1968; Gannon and Carthew, 1980; Lussier and Descoteaux, 1986; Gilioli *et al.*, 1996; Ueno *et al.*, 1996).

Recently, a classification scheme was proposed for rat parvoviruses (Jacoby *et al.*, 1996). In general, there are three main serogroups including *Rat virus* (RV), *H-1 virus* and *Rat parvovirus* (RPV). In the past, RPV was referred to as rat **orphan** parvovirus (Ueno *et al.*, 1996, 1997). Each of these different parvoviruses were fortuitous discoveries. RV and H-1 were discovered during experiments with rat tumors (Kilham and Oliver, 1959) and transplantable human

Figure 7.1 These rat pups' hair coats have an oily appearance.

tumors (Toolan *et al.*, 1960), respectively. RPV was discovered during a pathogenesis experiment involving RV (Jacoby *et al.*, 1987; Ball-Goodrich *et al.*, 1998).

The rat is the natural host for RV; however, hamsters, mice and kittens have been experimentally infected with it (Kilham, 1961; Kilham and Margolis, 1965; ElDadah *et al.*, 1967; Margolis and Kilham, 1972).

The natural host for H-1 is not clear since the virus was originally recovered from transplantable human tumors, tissues from cancer patients, human embryos and a spontaneous tumor in a rat (Toolan, 1960, 1961; Toolan *et al.*, 1960, 1962). Hamsters and nonhuman primates have been experimentally infected with H-1 (Toolan, 1960, 1961, 1966; Kilham and Margolis, 1969). H-1 and RV share many of the same tissue tropisms and lesions (Margolis *et al.*, 1968; Kilham and Margolis, 1969; Margolis and Kilham, 1970).

Like RV, the natural host for RPV is the rat. To the authors' knowledge there has only been one strain of RPV discovered and it is has been referred to as rat parvovirus type 1a (RPV-1a) (Ball-Goodrich *et al.*, 1998). It appears that mice and hamsters are not susceptible (Ueno *et al.*, 1997). Both RPV and RV are **tropic** for many of the same tissues and they both may result in a persistent infection. However, RPV is antigenically and genetically distinct from RV, and it apparently does not cause clinical signs or lesions in infant rats. It also seems that RPV may be more tropic for the small intestine and this site maybe the primary portal of entrance for RPV. The lung is believed to be the portal of entry and the site of initial replication for RV (Gaertner *et al.*, 1993).

Since RV has probably been studied more extensively than any of the other parvoviruses of rats,

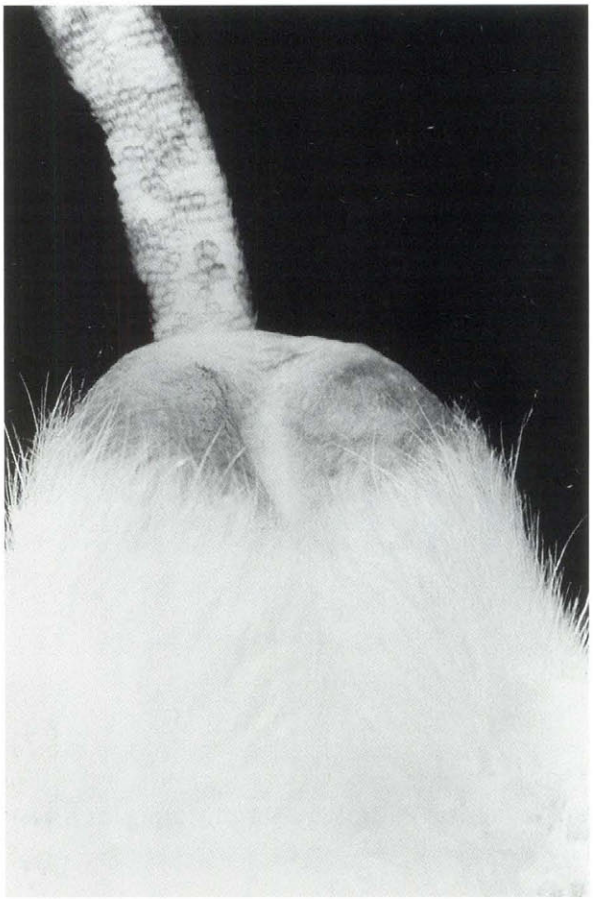

Figure 7.2 Rat virus infection. The testicles appear cyanotic.

much of the information involving clinical signs, pathology, etc. refers to this virus.

Clinical signs and the disease outcome depend on viral factors and host factors (Kilham and Ferm, 1964; Kilham and Margolis, 1966; ElDadah *et al.*, 1967; Robey *et al.*, 1968; Cole *et al.*, 1970; Margolis and Kilham, 1970; Nathanson *et al.*, 1970; Jacoby *et al.*, 1987; Gaertner *et al.*, 1989, 1996). Viral factors include the viral strain, its virulence and tissue tropism. Host factors include the age and the immune status of the rat at the time of infection. For example, neonates may exhibit tremors, **ataxia**, jaundice, stunted growth, oily hair coats (Figure 7.1), diarrhea and sudden death, whereas rats infected as juveniles (4 weeks old) or adults usually have latent asymptomatic infections. However, if such latently infected rats become immunocompromised or stressed they may exhibit paralysis resulting from hemorrhage and necrosis in the brain and spinal cord. In addition, scrotal cyanosis and hemorrhage (Figure 7.2) may be observed in juvenile and adult male rats. There may also be a transient drop in breeding efficiency. Similar clinical signs may also be observed in naïve

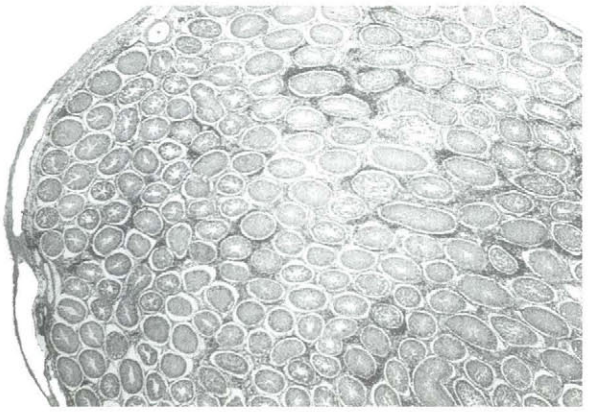

Figure 7.3 Rat virus infection. Multifocal acute hemorrhage in a testicle. Hematoxylin and eosin. × 15.

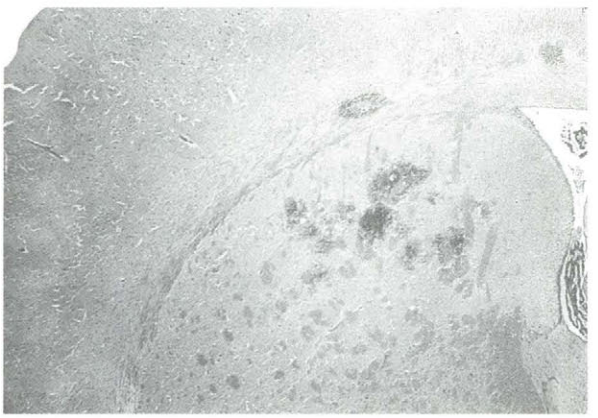

Figure 7.4 Rat virus infection. Multifocal hemorrhagic encephalopathy. Hematoxylin and eosin. × 15.

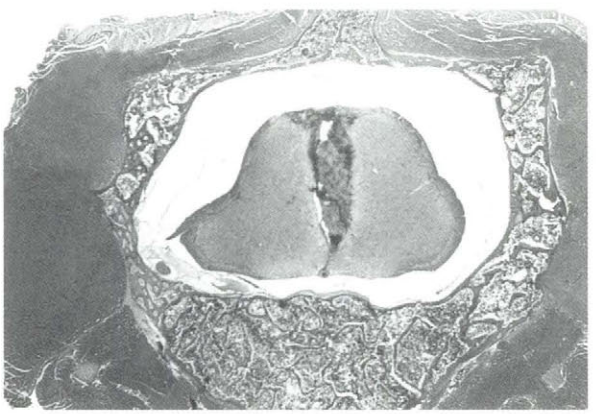

Figure 7.5 Rat virus infection. Hemorrhagic infarct in the spinal cord. Hematoxylin and eosin. × 15.

young adult rats which are introduced into an **enzootically infected** population of rats (Coleman *et al.*, 1983).

Persistent infections with parvoviruses are common, and the length of time in which virus can be detected in infected rats seems to depend on the rat's age at the time of infection and possibly its immune status (Jacoby *et al.*, 1987, 1991; Gaertner *et al.*, 1991, 1996). In addition, it seems that viral factors (e.g. the strain of RV) may also influence persistent infections.

RV can be shed in feces, milk and urine (Kilham and Ferm, 1964; Kilham and Margolis, 1966, 1974; Novotny and Hetrick, 1970; Lipton *et al.*, 1973; Jacoby *et al.*, 1987, 1991; Ball-Goodrich *et al.*, 1998). Similarly, RPV has also been detected in the feces, urine and saliva (Ueno *et al.*, 1997). Horizontal transmission of RV and RPV is thought to be primarily through direct animal-to-animal contact and fomites (Jacoby *et al.*, 1988; Yang *et al.*, 1995; Ueno *et al.*, 1996). Vertical or *in utero* transmission appears to be dependent on the virus strain, the route of inoculation and the inoculation dose (Jacoby *et al.*, 1988; Gaertner *et al.*, 1996). The duration of transmission after infection depends more on the age of the rat at the time of initial exposure than on the development of humoral immunity (Jacoby *et al.*, 1988). Experimentally infected neonates transmitted virus for 10 weeks after inoculation, whereas juvenile rats (4 weeks old) only transmitted RV for 3 weeks. In addition, Jacoby *et al.* also reported that the rats which had been experimentally infected as neonates continued to transmit virus for at least 7 weeks after **seroconversion**.

All parvoviruses are tropic for rapidly dividing cells and the clinical signs and lesions are the direct result of this tropism (Kilham and Margolis, 1966; Margolis *et al.*, 1968; Cole *et al.*, 1970; Margolis and Kilham, 1970, 1972; Baringer and Nathanson, 1972; Coleman *et al.*, 1983; Jacoby *et al.*, 1987; Gaertner *et al.*, 1993). RV attacks and destroys the cells in the external germinal cell layer of the cerebellar cortex of neonates, which results in granuloprival cerebellar hypoplasia and ataxia. Hepatocytes of the neonate and in some situations the adult are also targets for RV. Hepatic necrosis, hepatitis, fibrosis, nodular hyperplasia and jaundice result when the hepatocytes are targeted. The vascular endothelium and megakaryocytes are also attacked by RV and this along with the possible activation of the complement cascade may explain the hemorrhage observed in the testes (Figure 7.3), epididymis, central nervous system (Figures 7.4 and 7.5) and other tissues (Figure 7.6). The fetus is particularly vulnerable since a variety of tissues are mitotically active. In some *in utero* infections there are fetal deaths which result in decreased litter sizes and increased numbers of intrauterine resorption sites (Kilham and Margolis, 1966, 1969; Jacoby *et al.*, 1979).

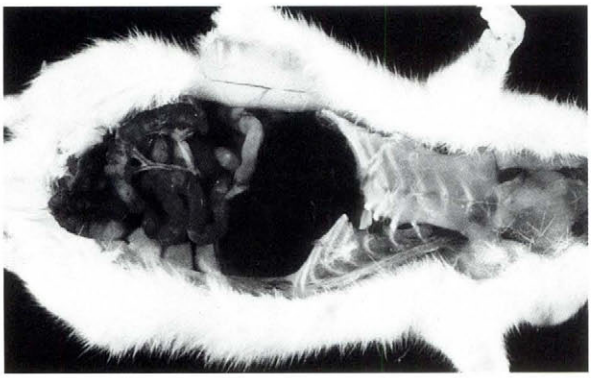

Figure 7.6 Rat virus infection. Note hemorrhage in the intestines and the cervical lymph nodes.

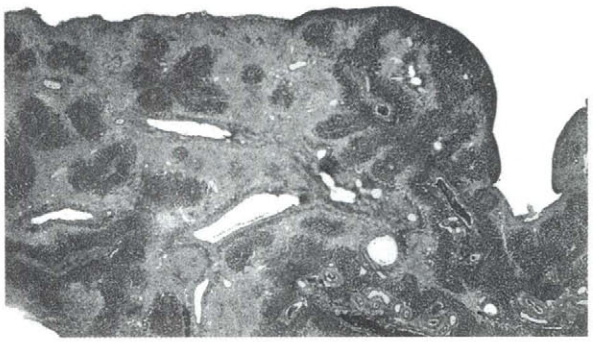

Figure 7.7 Chronic rat virus infection. There is extensive chronic inflammation in the hepatic parenchyma. Hematoxylin and eosin. × 15.

Parvoviruses of the rat are also tropic for many other tissues including lymphoid tissue (spleen, lymph nodes, Peyer's patches and thymus), smooth muscle (blood vessels, intestines and urogenital tract), heart (endocardium and myocardium), subependymal cells in the brain, erythropoietic cells, renal tubular epithelium, epididymal epithelium, uterine epithelium and bronchial epithelium (Margolis and Kilham, 1970; Jacoby *et al.*, 1987; Gaertner *et al.*, 1993, 1996; Ball-Goodrich *et al.*, 1998). This may result in foci of necrosis at any of those sites. Hypocellularity in the spleen and lymph nodes may be observed during the chronic stages of infection.

Additional lesions which may be observed in the liver during the subacute to chronic phases of infection include postnecrotic parenchymal collapse, hepatic fibrosis (Figure 7.7), nodular regenerative hyperplasia, **hepatocytomegaly**, **karyomegaly**, multinucleated hepatocytes, increased mitotic figures, bile duct hyperplasia and peliosis hepatis (Margolis *et al.*, 1968; Margolis and Kilham, 1970).

The diagnosis can be made with a variety of laboratory tests (Singh and Lang, 1984; ACLAD, 1991; Jacoby *et al.*, 1996; Ball-Goodrich *et al.*, 1998). The most commonly used serologic tests are the enzyme-linked immunosorbent assay **(ELISA)**, indirect immunofluorescence assay **(IFA)** and hemagglutination inhibition assay **(HAI)**. The ELISA and IFA can be used to identify antibodies to parvoviruses, and then the positive sera can be tested with the HAI assay to specifically identify whether or not the sera is reactive to either RV or H-1. There is currently no HAI test available to specifically identify RPV; however, negative results using the HAI test for both RV and H-1 is putative evidence of previous exposure to RPV (Ueno *et al.*, 1996).

Immunohistochemical staining of suspected tissues is also very useful if it is available. Additional tests which are being investigated and which seem to have great potential are the **polymerase chain reaction (PCR)** and *in situ* hybridization techniques (Gaertner *et al.*, 1993; Taylor and Copley, 1994a; Besselsen *et al.*, 1995; Yagami *et al.*, 1995; Ueno *et al.*, 1996; Ball-Goodrich *et al.*, 1998). Clinical signs, history and pathology may also be helpful in making the diagnosis. Histopathology is especially useful if intranuclear inclusion bodies are associated with lesions in the target tissues (e.g. cerebellar external germinal cells, hepatocytes, Kupffer cells, vascular endothelium, bile duct epithelium, etc.) (Kilham and Margolis, 1966; Ruffolo *et al.*, 1966; Margolis *et al.*, 1968; Cole *et al.*, 1970; Margolis and Kilham, 1970; Baringer and Nathanson, 1972; Coleman *et al.*, 1983).

Parvoviruses of rats have the potential to interfere with a variety of different types of research. Research utilizing cell cultures and transplantable tumors is especially vulnerable, as evidenced by the fact that some of the parvoviruses of rats were initially discovered as contaminants (Kilham and Oliver, 1959; Toolan *et al.*, 1960). Toxicological studies and surgical experiments involving the liver may also be compromised by the activation of a latent parvovirus infection during hepatocyte regeneration or proliferation (Ruffolo *et al.*, 1966; Margolis *et al.*, 1968). There is also the possibility that a latent parvovirus infection could be activated when rats are immunocompromised by cytotoxic or immunosuppressive chemicals (Nathanson *et al.*, 1970). In addition, it would not be too hard to imagine the problems which might be encountered by the pathologist and the toxicologist attempting to distinguish parvoviral lesions from toxic lesions. Similar problems also may be encountered with teratology studies. Parvoviruses may also interfere with carcinogenesis, oncology, cancer therapeutic and pathogenesis studies (Jacoby *et al.*, 1987; Ball-Goodrich *et al.*, 1998).

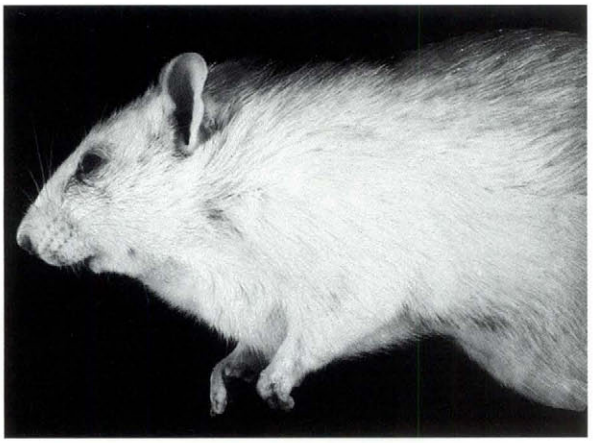

Figure 7.8 Experimental *Sialodacryoadenitis virus* (SDAV) infection. There is considerable ventral cervical swelling. (Charles River Laboratories, Technical Bulletin, 1983.)

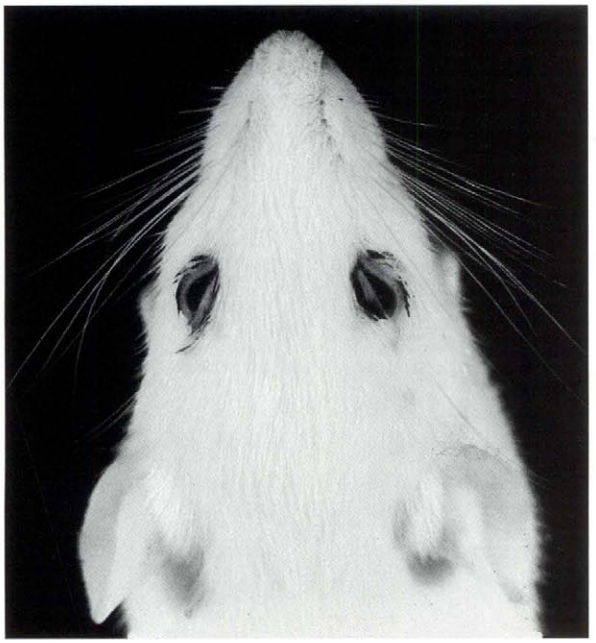

Figure 7.9 Experimental *Sialodacryoadenitis virus* (SDAV) infection. Note the red porphyrin staining around the eyes.

Coronaviruses

Infections with coronaviruses are very common in laboratory and wild rats (Parker *et al.*, 1970; Gannon and Carthew, 1980; Lussier and Descoteaux, 1986; Rao *et al.*, 1989; Gilioli *et al.*, 1996). Several different coronaviruses have been discovered in rats including *Sialodacryoadenitis virus* (SDAV), *Rat coronavirus* (RCV) (Parker *et al.*, 1970), and the *Causative agent of rat sialoadenitis virus* (CARS) (Maru and Sato, 1982). Since the diseases caused by each of these viruses are similar they will be considered together; however, significant differences will be noted. In fact, Percy and Williams (1990) suggested that the term 'rat coronavirus group' may be a more appropriate designation until we know more about these coronaviruses.

The rat is the natural host for SDAV, RCV and CARS; however, mice have been experimentally infected with SDAV and CARS (Parker *et al.*, 1970; Bhatt *et al.*, 1977; Maru and Sato, 1982; Barthold *et al.*, 1990; La Regina *et al.*, 1992).

SDAV is transmitted through aerosol exposure, **fomites**, handling and close contact, and this may also be true for RCV and CARS (Thigpen and Ross, 1983; Percy and Wojcinski, 1986; La Regina *et al.*, 1992). SDAV and CARS are highly tropic for the salivary glands; however, RCV's tropism for the salivary glands is relatively low (Bhatt and Jacoby, 1977; Maru and Sato, 1982; La Regina *et al.*, 1992). In addition, SDAV and RCV also have an affinity for lacrimal glands and the respiratory tract (Percy and Williams, 1990). The respiratory tract is the first site of replication for SDAV and RCV, and from there the virus attacks the salivary and lacrimal glands (Bhatt and Jacoby, 1977; Wojcinski and Percy, 1986). SDAV also appears to have an affinity for the transitional epithelium of the kidney and urinary bladder of athymic rats (Weir *et al.*, 1990).

The most common clinical sign associated with SDAV and CARS infections in rats is ventral cervical and/or intermandibular swelling (Figure 7.8) (Maru and Sato, 1982; Bhatt and Jacoby, 1985; Percy and Wojcinski, 1986; Bihun and Percy, 1995; Schunk *et al.*, 1995). Ventral cervical swelling also has been reported with RCV infections (Macy *et al.*, 1996). This particular clinical sign is very suggestive of a coronavirus infection; however, ventral cervical swelling can be caused by other things (e.g. tumors).

Additional nonspecific clinical signs which may be observed with coronaviruses of the rat include red tears**(chromodacryorrhea)** 7.9), red porphyrin staining of the front paws, respiratory disturbances (sneezing, rales, etc.), anorexia, decreased breeding efficiency and occasional ophthalmic signs (e.g. **blepharospasms;**, photophobia, keratoconjunctivitis, etc.) (Parker *et al.*, 1970; Lai *et al.*, 1976; Fox, 1977; Weisbroth and Peress, 1977; Maru and Sato, 1982; Percy *et al.*, 1984; Bhatt and Jacoby, 1985; Percy and Wojcinski, 1986; La Regina *et al.*, 1992; Schunk *et al.*, 1995; Macy *et al.*, 1996).

Morbidity is usually high and mortality is usually low in uncomplicated cases of SDAV; however, mortality rates can be quite high in some rat strains infected with RCV (Parker *et al.*, 1970; Weisbroth and

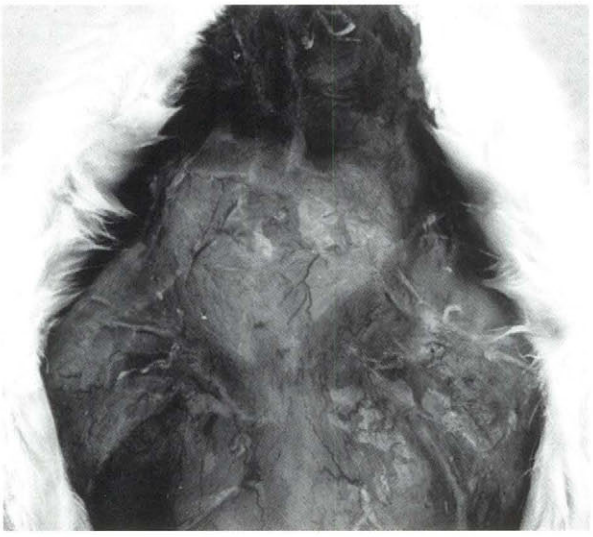

Figure 7.10 Experimental SDAV infection. There is an enormous amount of edema associated with the salivary glands. (Charles River Laboratories, Technical Bulletin, 1983.)

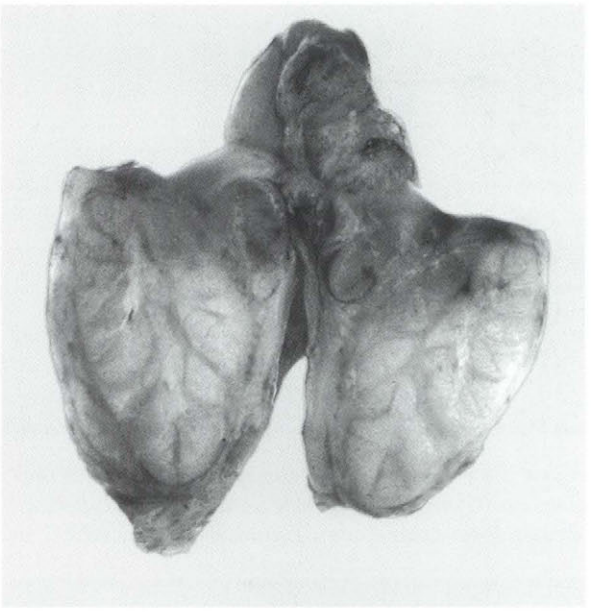

Figure 7.11 Experimental SDAV infection. The salivary glands in Figure 7.10 were removed and incised. Note the widening of the interlobular connective tissue with edema.

Peress, 1977; Percy and Wojcinski, 1986; Hajjar *et al.*, 1991; Macy *et al.*, 1996). Death may be observed in suckling rats infected as neonates with RCV (Parker *et al.*, 1970; Macy *et al.*, 1996). Otherwise the disease course is usually short and self-limiting with CARS, SDAV and older RCV-infected rats. However, SDAV and the disease it causes may persist for several months in athymic rats (Weir *et al.*, 1990; Hajjar *et al.*, 1991). It also seems that SDAV may have a synergistic relationship with *Mycoplasma pulmonis* and this may also influence the disease expression and outcome (Schoeb and Lindsey, 1987).

Occasional chronic ophthalmologic problems may persist, probably as a result of the destructive effects on the Harderian gland and other lacrimal glands (Percy *et al.*, 1989). It is believed that SDAV and RCV cause decreased lacrimation and this may leave the eye vulnerable to foreign bodies and opportunistic bacteria (e.g. *Pasteurella pneumotropica* and *Staphylococcus* spp). In spontaneous infections, secondary ophthalmic problems are most commonly seen in Lewis and **SHR** rats, and keratoconjunctivitis has resulted in experimentally infected athymic rats (Lai *et al.*, 1976; Weisbroth and Peress, 1977; Weir *et al.*, 1990).

Macroscopic lesions may include swollen and edematous salivary glands (Figures 7.10 and 7.11), with enlargement of the regional lymph nodes (Maru and Sato, 1982; Percy *et al.*, 1984; Percy and Wojcinski, 1986; Percy and Williams, 1990; La Regina *et al.*, 1992; Macy *et al.*, 1996). Hyperemia and exudation may be present at any location in the respiratory tract of rats infected with SDAV and RCV (Parker *et al.*, 1970; La Regina *et al.*, 1992). Occasional ophthalmic lesions include keratoconjunctivitis, corneal opacities and ulcers (Figure 7.12), megaloglobus, hypopyon (Figure 7.13), and **hyphema** (Lai *et al.*, 1976; Fox, 1977; Weisbroth and Peress, 1977).

Histopathologic lesions are related to the virus strain, virus tropism, clinical signs and gross lesions (Parker *et al.*, 1970; Bhatt and Jacoby, 1977; Maru and Sato, 1982; Percy *et al.*, 1984, 1989; Percy and

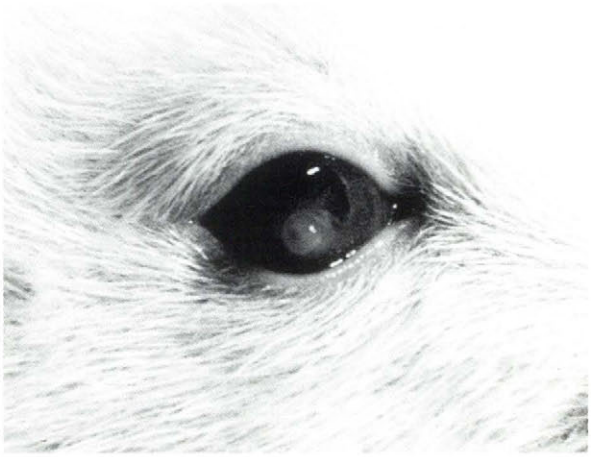

Figure 7.12 Chronic SDAV infection. This rat has ulcerative keratitis which was secondary to the inflammation and necrosis in the Harderian gland. (Charles River Laboratories, Technical Bulletin, 1983.)

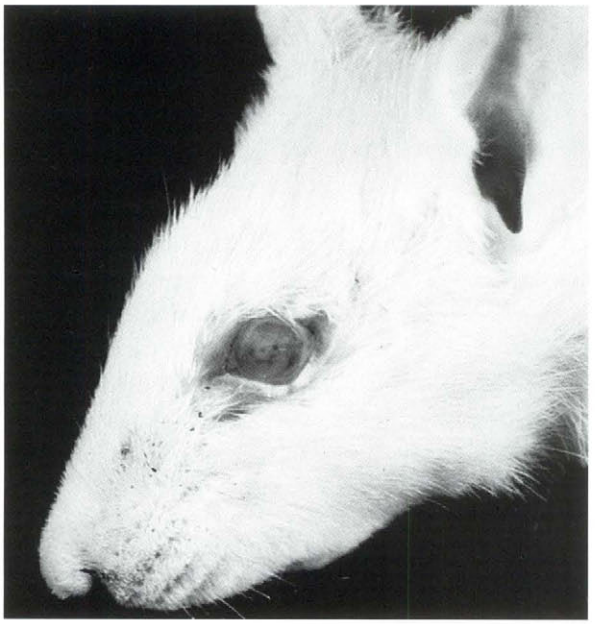

Figure 7.13 Chronic SDAV infection. This rat has both purulent exudate and blood in its eye.

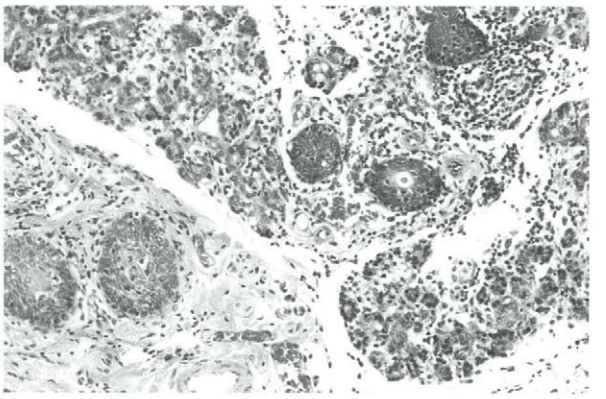

Figure 7.14 Spontaneous SDAV infection. The parotid salivary gland is mildly infiltrated with mononuclear cells and there is squamous metaplasia of intralobular and interlobular ducts. Hematoxylin and eosin. × 30.

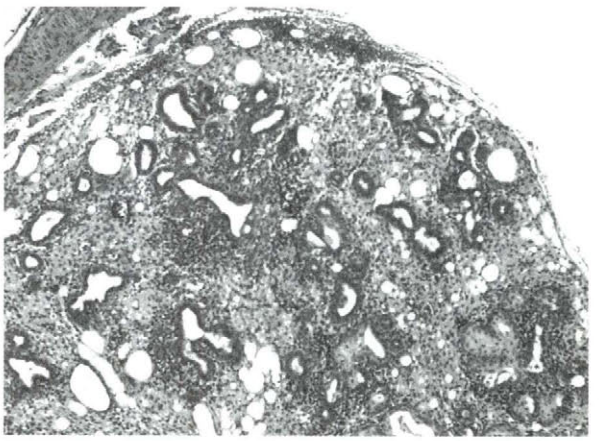

Figure 7.15 Chronic SDAV infection. This Harderian gland has chronic nonsuppurative inflammation. Hematoxylin and eosin. × 15

Wojcinski, 1986; Wojcinski and Percy, 1986; Schoeb and Lindsey, 1987; Percy and Williams, 1990; La Regina *et al.*, 1992; Bihun and Percy, 1995; Liang *et al.*, 1995; Macy *et al.*, 1996; Compton *et al.*, 1998). All three coronaviruses cause inflammation and necrosis of the submaxillary (submandibular) salivary glands. Depending on the stage of the infection there may also be necrosis and/or squamous metaplasia of the ducts in the submaxillary salivary gland. Similar findings may be observed in the parotid salivary gland (Figure 7.14), Harderian gland (Figure 7.15) and other lacrimal glands of rats infected with SDAV or RCV. Lesions may also be observed in the respiratory tract with SDAV and RCV including interstitial pneumonia as well as inflammation, necrosis, and hyperplasia of the airways throughout the upper and lower respiratory tract. **Bronchiectasis** and squamous metaplasia of the bronchial epithelium have also been reported in persistently infected athymic rats (Weir *et al.*, 1990). Pulmonary atelectasis with compensatory **emphysema**, nonsuppurative perivasculitis and hyperplasia of the bronchial associated lymphoid tissue (BALT) may also be observed with RCV. Inflammation in the upper respiratory tract has been reported with CARS; however, there was apparently no involvement of the lower respiratory tract, parotid salivary gland or lacrimal glands (Maru and Sato, 1982). Enlargement of the lymph nodes in the neck is due to reactive hyperplasia and/or inflammation. Ophthalmic lesions which may be seen include those observed at necropsy as well as lenticular degeneration, retinal degeneration and anterior **synechia** (Lai *et al.*, 1976; Weisbroth and Peress, 1977; Weir *et al.*, 1990).

Percy *et al.* (1986) observed a necrotizing encephalitis in rats which had been intranasally inoculated with SDAV during the first week of life. They speculated that similar lesions could possibly be seen in young suckling rats during natural epizootics; however, it appears that no cases have been reported in the literature. They also believed that the virus was probably carried to the brain via the blood, and not through the cribriform plate.

The clinical signs and lesions are not specific for coronaviruses of rats because similar findings can be observed with other viruses (Sendai virus, pneumonia virus of mice, papovavirus in athymic rats and cytomegalovirus), hypovitaminosis A, stress,

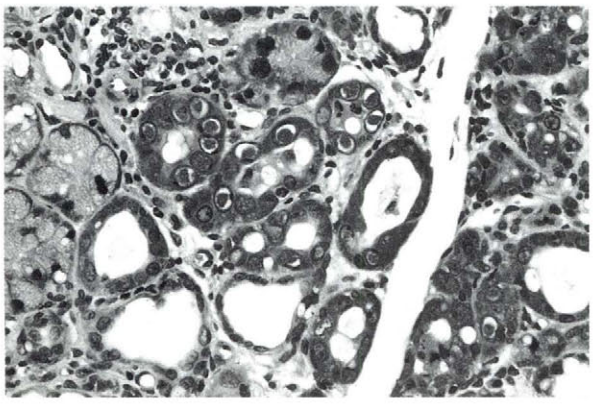

Figure 7.16 Papovavirus infection in an athymic nude rat. The parotid salivary gland is mildly infiltrated with inflammatory cells and there is a conspicuous reduction in the number of acini. Note the relatively large basophilic intranuclear inclusion bodies which are surrounded by halos in several epithelial cells lining ducts. Hematoxylin and eosin. × 60. (Courtesy of R. Rahija and J. Dennis, Duke University, Division of Laboratory Animal Resources.)

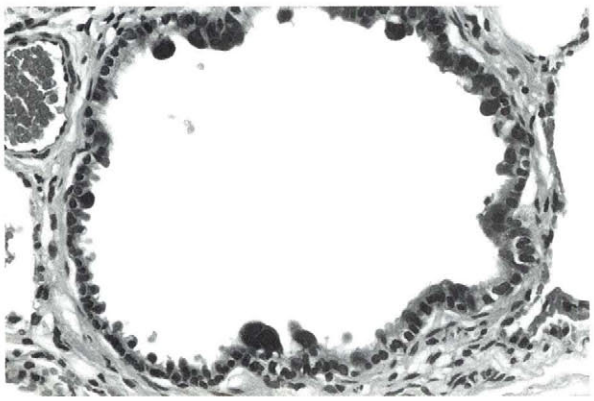

Figure 7.17 Papovavirus infection in an athymic nude rat. Note the large basophilic intranuclear inclusion bodies in several bronchiolar epithelial cells. × 60. (Courtesy of R. Rahija and J. Dennis, Duke University, Division of Laboratory Animal Resources.)

dehydration and exposure to noxious gases (e.g. ammonia) (Eaton and Van Herick, 1944; Broderson *et al.*, 1976; Fox, 1977; Rogers, 1979; Harkness and Ridgeway, 1980; Vogtsberger *et al.*, 1982; Ward *et al.*, 1984; Wojcinski and Percy, 1986; Percy and Barthold, 1993a; Liang *et al.*, 1995).

The history, clinical signs and pathology may be very helpful in making a diagnosis; however, ancillary tests are needed for confirmation (ACLAD, 1991). Serologic tests (e.g. ELISA and IFA) can be used to confirm the diagnosis after the 7th to 10th day of infection. In addition, virus isolation, *in situ* hybridization, polymerase chain reaction (PCR), **reverse transcriptase-PCR (RT-PCR)** and immunofluorescent/immunohistochemical staining of tissues can also be used to arrive at a definitive diagnosis (Percy *et al.*, 1984; Taylor and Copley, 1994b; Bihun and Percy, 1995; Macy *et al.*, 1996; Compton *et al.*, 1998). RT-PCR can also be used to detect rat coronaviruses using cage swabs (Compton and Vivas-Gonzalez, 1998).

Coronaviruses of rats have the potential to interfere with inhalation research, respiratory disease research, reproductive research, nutritional research, toxicologic research, physiology research, tumor transplantation research and ophthalmologic research (Lai *et al.*, 1976; Wojcinski and Percy, 1986; McDonald, 1988; Rao *et al.*, 1989; Hajjar *et al.*, 1991; Bihun and Percy, 1995; Macy *et al.*, 1996).

Papovavirus

In 1984, Ward *et al.* reported on a papovavirus which caused **parotid sialoadenitis** in athymic nude rats. There have been no other published reports of this virus causing disease in athymic rats; however, one of the authors of this chapter has seen the disease in three different cases, and the clinical disease and lesions were similar to those described by Ward and his colleagues (Gaillard, 1998, unpublished observation).

Ward *et al.* (1984) reported that approximately 10–15% of the athymic nude rats developed a wasting disease with respiratory **dyspnea**. The later clinical sign may have been due to opportunistic bacteria which had also been isolated from pneumonic lungs. A few of the rats died during that epizootic.

At necropsy the rats were emaciated and many of the rats' parotid salivary glands were atrophic and dark. In addition, some of the parotid salivary glands contained small white foci.

Microscopically, the parotid salivary glands had necrosis of acinar cells and ductular epithelium. The parotid salivary glands were also infiltrated with mononuclear cells (Figure 7.16). In some rats there was also epithelial hyperplasia in the ducts. Large basophilic intranuclear inclusion bodies, surrounded by a clear halo, were common in the affected cells in the parotid salivary gland (Figure 7.16), but were also occasionally observed in the laryngeal glands, bronchial/bronchiolar epithelium (Figure 7.17), and the Harderian gland. Intranuclear inclusion bodies also have been observed in alveolar epithelial cells (Figure 7.18) (Gaillard, 1998, unpublished observation). Ward and his colleagues also reported that tracheitis, bronchitis, **bronchiolitis** and secondary bacterial pneumonias were common findings.

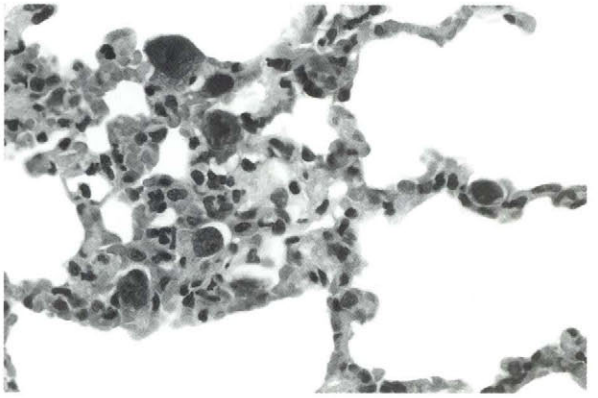

Figure 7.18 Papovavirus infection in an athymic nude rat. This is a photomicrograph of a different area of the same lung lobe as that in Figure 7.17. Several alveolar pneumocytes have large basophilic intranuclear inclusion bodies. A modicum of inflammatory cells are also present. Hematoxylin and eosin. × 95. (Courtesy of R. Rahija and J. Dennis, Duke University, Division of Laboratory Animal Resources.)

The prevalence of papovavirus infections in rats is not known, since there apparently are no serologic tests available at the present time. The clinical signs and lesions can be very suggestive and can be useful in making a presumptive diagnosis. If available, electron microscopy and immunoperoxidase tests can be used to make a definitive diagnosis (Ward *et al.*, 1984). Initial attempts at virus isolation were unsuccessful.

The list of differential diagnoses includes coronaviruses of rats, cytomegalovirus and Sendai virus. Coronaviruses may cause similar lesions in the parotid salivary glands and lung, but intranuclear inclusion bodies are not a characteristic since they are RNA viruses. In addition, coronaviruses of rat may also cause lesions in the submaxillary salivary gland, whereas that salivary gland does not appear to be a target of papovavirus. The rat cytomegalovirus can cause similar lesions and intranuclear inclusion bodies in the salivary and lacrimal glands; however, it also causes cytomegaly and intracytoplasmic inclusion bodies (Percy and Barthold, 1993a). Although Sendai virus can cause lesions in the respiratory tract, it does not cause a sialoadenitis.

Sendai Virus

Sendai virus (SV) is a paramyxovirus (parainfluenza virus type 1) and infections of rats and mice are relatively common (Parker *et al.*, 1966; Tennant *et al.*, 1966; Parker and Reynolds, 1968; Zurcher *et al.*, 1977; Ishida and Homma, 1978; Gannon and Carthew, 1980; Lussier and Descoteaux, 1986; Rao *et al.*, 1989; Gilioli *et al.*, 1996). The mouse is particularly susceptible and acute **epizootic** and **enzootic** infections have been reported. In addition, there is evidence that hamsters, guinea pigs, rabbits and marmosets are also susceptible to infection with SV; however, some apparently seropositive guinea pigs may in fact be seropositive to other parainfluenza viruses instead of *Sendai virus* (Parker *et al.*, 1966; Profeta *et al.*, 1969; Hawthorne *et al.*, 1982; Machii *et al.*, 1989; Percy and Palmer, 1997).

Natural infections in rats are usually asymptomatic (Burek *et al.*, 1977). However, nonspecific clinical signs referable to the respiratory tract have been reported in experimental infections (Carthew and Sparrow, 1980a; Castleman, 1983). Those clinical signs included moist nasal sounds, wheezing, snuffling and increased respiratory rates. Additional clinical signs which have been reported in experimental and in some natural infections are decreased weight gain/weight loss, decreased breeding efficiency, anorexia and lowered activity (Coid and Wardman, 1971, 1972; Castleman, 1983; Carthew and Aldred, 1988; Rao *et al.*, 1989). Morbidity may be quite high but there may be little or no increases in the mortality rates in rats (Burek *et al.*, 1977; Castleman, 1983).

The results from experiments conducted by Thigpen and Ross (1983) suggested that SV can be transmitted via the airborne route in rats. In mice, SV appears to be primarily transmitted by direct animal contact or by contact with contaminated **fomites** (Tennant *et al.*, 1966; Parker and Reynolds, 1968; Carthew and Aldred, 1988). It is likely that these same methods of transmission also apply to the rat.

SV is tropic for the epithelium lining the nasal cavity, bronchi/bronchioles, alveoli and alveolar macrophages (Carthew and Sparrow, 1980a; Castleman, 1984; Castleman *et al.*, 1987; Giddens *et al.*, 1987). The epithelium in the trachea is probably also a target since lesions are frequently present at that location (Castleman, 1983).

Similar to other viruses which infect rodents, host factors may influence the disease patterns observed in rats infected with SV. The host's genotype, immune status and age are important factors (Burek *et al.*, 1977; Carthew and Sparrow, 1980a; Castleman *et al.*, 1987; Liang *et al.*, 1995). For example, in one epizootic with SV the disease was more severe in Sprague-Dawley, BN/Bi and (WAG × BN) F1 rats, whereas WAG/Rij rats had a relatively milder disease (Burek *et al.*, 1977). In this same epizootic, rats younger than

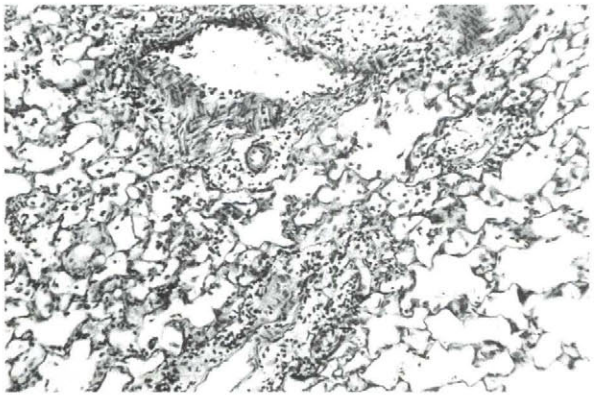

Figure 7.19 Experimental Sendai virus infection in a young Lewis rat. There is acute necrosis and inflammation of the bronchiole (top) and adjacent alveoli. Hematoxylin and eosin. (Courtesy of S. Botts.)

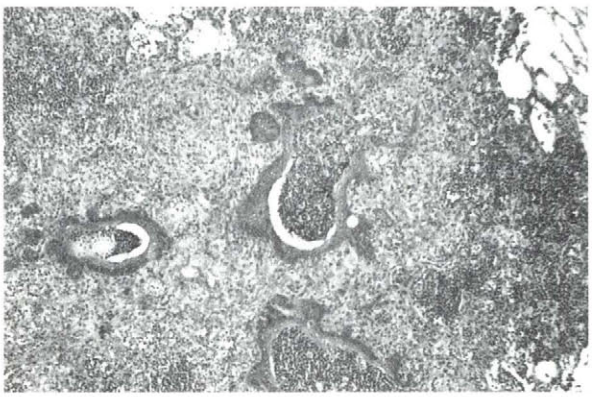

Figure 7.20 Intercurrent infection with Sendai virus and *Streptococcus pneumoniae*. Note the chronic active inflammation in the interstitium and the squamous metaplasia of the bronchiolar epithelium. Hematoxylin and eosin. × 15.

eight months had relatively more severe lung lesions than older rats. In addition, the infection is more severe and the virus persists for a longer time in **athymic nude rats**, compared with euthymic rats. The microbial status of the host is also important, since the disease outcome can be significantly affected by secondary opportunistic bacteria and mycoplasma (Burek *et al.*, 1977; Schoeb *et al.*, 1985).

Macroscopic lesions are frequently absent, but when present there may be small red to gray randomly distributed foci on the surface of the lungs or the lungs may be diffusely reddened (Castleman, 1983). In addition, the lungs may fail to collapse when the thoracic cavity is opened. Purulent exudate within the airways, ventral consolidation or abscesses in the lungs, as well as bronchiectasis would be suggestive of a possible infection with opportunistic bacteria and/or mycoplasma (Burek *et al.*, 1977; Schoeb *et al.*, 1985; Carthew and Aldred, 1988).

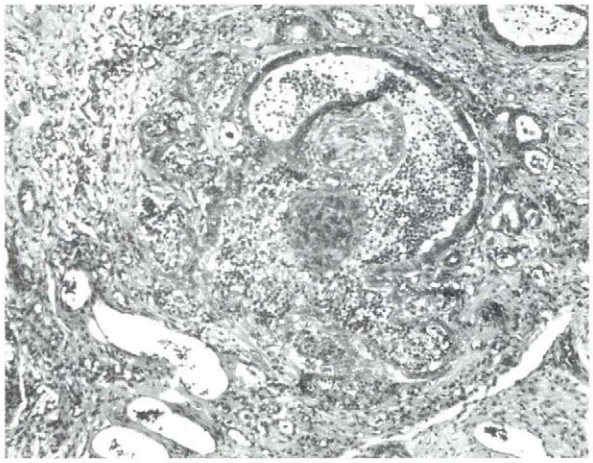

Figure 7.21 Intercurrent infection with *Sendai virus* and *Streptococcus pneumoniae*. Bronchiolitis obliterans which is characterized by the epithelial covered polypoid structure in the lumen of an inflamed bronchiole. Hematoxylin and eosin. × 20.

In pregnant females there may be a bloody vaginal discharge, retarded embryonic development and increased resorption sites in the uterus (Coid and Wardman, 1971; Carthew and Aldred, 1988).

Histopathologic lesions are variable and depend on the stage of the infection (Burek *et al.*, 1977; Carthew and Sparrow, 1980a; Castleman, 1983, 1984; Schoeb *et al.*, 1985; Castleman *et al.*, 1987, 1988; Giddens *et al.*, 1987; Liang *et al.*, 1995). During the acute stage the lesions include epithelial necrosis and inflammation in the nasal cavity, trachea, larynx, airways in the lung (Figure 7.19), and alveoli. The inflammatory cell infiltrates in these locations include variable numbers of mononuclear cells (lymphocytes and plasma cells) and neutrophils. Increased numbers of alveolar macrophages may also be present in the alveoli. In general, the lesions in the lung are centered around the airways, especially the terminal bronchioles, and the adjacent alveoli; therefore, these lesions can be collectively referred to as a necrotizing bronchointerstitial pneumonia. During the reparative stage there may be hyperplasia and squamous metaplasia of the epithelium in the nasal cavity, bronchi, bronchioles (Figure 7.20), and alveoli. Multinucleated syncytial epithelial cells may also be present in the bronchiolar epithelium and alveoli during the early and chronic stages of infection. In some lungs there may be collections of inflammatory cells and fibroblasts in the lumina of bronchioles, and presumably these structures may develop into epithelial covered polypoid structures (Figure 7.21). This finding is referred to as **bronchiolitis obliterans**. The lungs may completely return

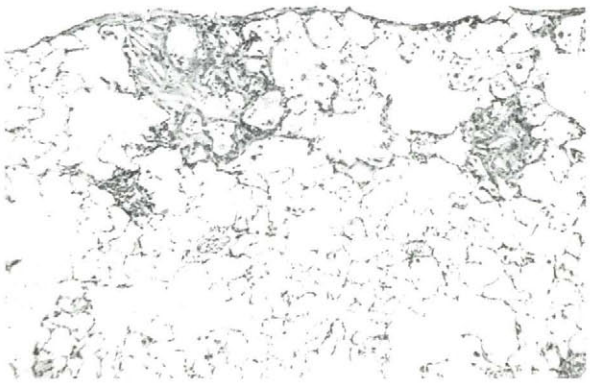

Figure 7.22 Focal scar from a previous infection with *Sendai virus*. Note the fibrosis, cholesterol clefts and alveolar macrophages. Hematoxylin and eosin. × 20.

to normal or there may be residual scars (Figure 7.22) including interstitial fibrosis, cholesterol clefts, and peribronchial and perivascular mononuclear cell cuffs. This later lesion may be the only lesion observed in aged rats during any stage of infection.

The lesions are suggestive of a possible SV infection but they are not **pathognomonic** since similar lesions may be caused by coronaviruses of rats and less likely *pneumonia virus of mice* (Eaton and Van Herick, 1944; Vogtsberger *et al.*, 1982; Liang *et al.*, 1995).

A definitive diagnosis can be made with serology (ELISA or IFA), virology and immunohistochemistry (Rottinghaus *et al.*, 1986; Castleman *et al.*, 1987; ACLAD, 1991). Immunohistochemistry and virology are useful in making a diagnosis during the acute stage of infection, but are less helpful after the second week of infection because the virus may have been eliminated from the tissues at that time (Castleman *et al.*, 1987; Giddens *et al.*, 1987; ACLAD, 1991). However, serology is useful for a much longer time, in that antibodies can be detected from about the first to second week of infection and remain detectable long after virus has been eliminated from the tissues. Taylor and Copley (1994b) have also evaluated a polymerase chain reaction (PCR) test as a diagnostic technique for SV infections and it seems to have great potential; however, it too will probably only be useful during the acute stage of infection when the virus is present.

Similar to other viruses of rats, SV has the potential to interfere with many different types of research including research in gerontology, toxicology, physiology, immunology, respiratory disease, reproduction, teratology, carcinogenesis and others (Coid and Wardman, 1971, 1972; Burek *et al.*, 1977; Kay, 1978; Castleman, 1983, 1984; Carthew and Aldred, 1988; Castleman *et al.*, 1988; McDonald, 1988; Lussier, 1988; Rao *et al.*, 1989; Piedimonte *et al.*, 1990; Baker, 1998).

Pneumonia Virus of Mice

Pneumonia virus of mice (PVM) is another paramyxovirus, although not a parainfluenza virus, which is relatively common in mice and rats (Parker *et al.*, 1966; Tennant *et al.*, 1966; Gannon and Carthew, 1980; Lussier and Descoteaux, 1986; Richter, 1986; Rao *et al.*, 1989; Gilioli *et al.*, 1996). Antibodies to PVM have also been detected in hamsters, gerbils, guinea pigs, rabbits, mongooses and perhaps even monkeys and humans (Pearson and Eaton, 1940; Eaton and Van Herick, 1944; Horsfall and Curnen, 1946; Tennant *et al.*, 1966; Pringle and Eglin, 1986). In addition, guinea pigs have been experimentally infected with PVM, but neither clinical signs nor lesions could be attributed to PVM (Griffith *et al.*, 1997).

PVM is usually considered to be a relatively innocuous virus in rodents since it usually does not cause disease nor lesions in natural infections (Gannon and Carthew, 1980; Baker, 1998). However, natural disease and lesions do occur in some cases. In particular, athymic nude mice seem to be especially susceptible to PVM which can result in a wasting type syndrome, dyspnea, cyanosis, a necrotizing and hemorrhagic pneumonia and death (Richter *et al.*, 1988; Weir *et al.*, 1988). Similar lesions, along with desquamation and hyperplasia of the bronchial epithelium, have been reported in natural infections involving **euthymic mice**; however, clinical signs and deaths were not observed (Carthew and Sparrow, 1980b). Experimentally infected rats reportedly develop interstitial pneumonia, but clinical signs were not observed (Eaton and Van Herick, 1944; Vogtsberger *et al.*, 1982). **Vasculitis** may also be present in areas of pneumonia in experimentally infected mice and rats.

PVM is probably transmitted by direct animal-to-animal contact (Pearson and Eaton, 1940). The virus is tropic for the bronchial and alveolar epithelium of mice (Carthew and Sparrow, 1980b).

PVM reportedly does not cause clinical disease nor lesions in natural infections in rats; therefore, it is usually detected in rats with serology (HAI, ELISA and IFA) during routine monitoring (ACLAD, 1991). Although virus isolation can be used to detect PVM in tissues, it can be time consuming, whereas

immunohistochemistry (e.g. immunoperoxidase) can also be used and is faster (Carthew and Sparrow, 1980b; Weir *et al.*, 1988; ACLAD, 1991).

Reports of PVM interfering with research involving rats are rare. For example, Rao *et al.* (1989) reported that PVM and Sendai were associated with significant decreases in the body weights of rats in carcinogenicity studies. In contrast, there have been slightly more reports of PVM interfering with research involving mice (Horsfall and Curnen, 1946; Tennant *et al.*, 1966; Richter, 1986). PVM has the potential to have devastating effects on research using athymic nude mice since they are particularly prone to develop fatal disease (Richter *et al.*, 1988; Weir *et al.*, 1988). Although similar fatal infections have yet to be reported in athymic rats, PVM might have the same effect with research involving them.

Infectious Diarrhea of Infant Rats Virus

Vonderfecht and his colleagues (1984) were the first to identify a virus which caused diarrhea in suckling rats. The virus was morphologically identical to typical rotaviruses, but it was antigenically different from other rotaviruses. They gave this virus the name of *Infectious diarrhea of infant rats virus* (IDIR) because it apparently only caused diarrhea in infant rats. Subsequent to their discovery, the IDIR virus was assigned to the group B rotavirus group which contains similar atypical rotaviruses that infect and cause similar disease in humans, calves and pigs (Mebus *et al.*, 1978; Vonderfecht *et al.*, 1988; Chasey *et al.*, 1989; Eiden *et al.*, 1991). The prevalence of IDIR virus in rats is not known since rats are not routinely tested for this virus.

Diarrhea was the only clinical sign described in the original report of the natural outbreak with IDIR virus (Vonderfecht *et al.*, 1984; Huber *et al.*, 1989). However, in experimental infections not only was diarrhea described, but also erythema, cracking and bleeding of the perianal skin along with anorexia, dehydration and rapid weight loss (Vonderfecht *et al.*, 1984; Salim *et al.*, 1995). The animals may also be stunted and have dry flaky skin during the latter stages of the infection. The infection is self-limiting, with the rats rapidly returning to normal.

The mode of transmission is not known, but probably involves the fecal–oral route of infection. Rats older than 14 days of age are apparently resistant to infections, but the exact reason for this age-associated resistance is not known (Vonderfecht *et al.*, 1984). The virus is tropic for the small intestinal villous epithelium, particularly on the luminal one-third to one-fourth of the villi (Vonderfecht *et al.*, 1984; Huber *et al.*, 1989). IDIR virus seems to have more of an affinity for the middle and distal small intestines and less affinity for the proximal small intestines and even less for the colon. The virus causes loss of small intestinal villous enterocytes which are quickly replaced with immature cuboidal epithelium. These changes lead to a reduction in the absorption of water and sodium which results in diarrhea (Salim *et al.*, 1995). The diarrhea continues until the villi are again lined by mature columnar epithelium.

Gross lesions which may be observed in suckling rats include perianal fecal staining, hyperemia, cracking and hemorrhage. The intestinal contents are watery and gaseous and may also contain mucus and poorly formed fecal pellets (Vonderfecht *et al.*, 1984). The rats' stomachs contain curdled milk.

Microscopic lesions are restricted to the small intestine and are characterized by villous atrophy, villous epithelial necrosis, villous epithelial syncytial cell formation and mucosal flattening (Vonderfecht *et al.*, 1984; Huber *et al.*, 1989; Salim *et al.*, 1995). As expected, these lesions are most prominent in the distal small intestine. Small, eosinophilic intracytoplasmic inclusion bodies may be observed in the syncytial cells. Little if any inflammation is present in the intestine; however, there maybe compensatory hyperplasia of the small intestinal crypt epithelium.

Good timing and correct sampling are critical for establishing the diagnosis (Vonderfecht *et al.*, 1988; Huber *et al.*, 1989; Salim *et al.*, 1995). The chance of making a diagnosis is greatly enhanced if samples for histopathology or other tests (e.g. immunofluorescent staining of tissues) are collected from the distal small intestine during the first 24 hours of the infection. During this time the characteristic syncytial cells and sometimes eosinophilic intracytoplasmic inclusion bodies may be observed with histopathology. IDIR viral antigens are also more abundant during this time period and are rapidly eliminated after that time. Other laboratory tests are being investigated to detect group B rotaviruses in feces and intestinal specimens, but they may not be commercially available (Vonderfecht *et al.*, 1985, 1988; ACLAD, 1991). A combined reverse transcriptase reaction–PCR assay seems to have potential as a diagnostic tool

for the detection of IDIR virus in fecal samples (Eiden *et al.*, 1991).

This virus has the potential to interfere with any research utilizing suckling rats, as evidenced by the concerns of the researcher who submitted the original rats which were experiencing diarrhea (Huber *et al.*, 1989).

Bacterial and Mycoplasmal Diseases

Murine Respiratory Mycoplasmosis

Mycoplasma pulmonis is the etiology of murine respiratory mycoplasmosis (MRM). *M. pulmonis* causes natural disease in rats and mice; however, it also has been cultured from hamsters and guinea pigs (Tully, 1986).

The infection in young rats is usually clinically silent. In older rats, nonspecific clinical signs such as snuffling, sneezing, rattling, **moist rales**, **dyspnea**, **hyperpnea**, head shaking, chromodacryorrhea, face and ear rubbing, ruffled hair coat, twirling when suspended by the tail, head tilts, weight loss and decreased breeding efficiency may be observed (Lane-Petter *et al.*, 1970; Lindsey *et al.*, 1971; Jersey *et al.*, 1973; Broderson *et al.*, 1976; Fox, 1977; Cassell *et al.*, 1979, 1981a; Harkness and Ridgeway, 1980; Cassell, 1982; Schunk *et al.*, 1995).

M. pulmonis is transmitted both horizontally (airborne and possibly sexually) and vertically (Jersey *et al.*, 1973; Cassell *et al.*, 1979, 1981a; Cassell, 1982; Lindsey *et al.*, 1985; Cassell *et al.*, 1986). The infection progresses slowly and may eventually result in respiratory and/or genital disease.

The principal portal of entry is the respiratory tract, beginning in the nasal cavity with probable progressive colonization of the middle ears, larynx, trachea and inconsistently the lungs (Cassell *et al.*, 1973, 1979; Lindsey *et al.*, 1985). The exact means by which it reaches the genital tract is not clear. It has been speculated that the organism may reach the genital tract from the oropharynx during the rat's coprophagic activities or via the blood from the respiratory tract (Cassell, 1982).

The disease outcome depends on a complex interaction of host factors, environmental factors and *M. pulmonis* factors. Host factors include the rat strain, age and microbial status. For example, LEW and CFE rats appear to be more susceptible than F-344, CFHB and CFY rats to the disease-producing potential of *M. pulmonis*, and the lesions in the respiratory and genital tracts tend to be more severe in LEW rats (Lane-Petter *et al.*, 1970; Cassell, 1982; Davis and Cassell, 1982). Older rats tend to have more severe disease than suckling rats, probably because of the declining immune function, the slow progression of the disease, and lung involvement in older rats (Jersey *et al.*, 1973; Cassell *et al.*, 1981a; Lindsey *et al.*, 1985). Intercurrent infections with Sendai virus, rat coronaviruses, opportunistic bacteria and possibly cilia-associated respiratory (CAR) bacillus can also significantly influence the disease outcome (MacKenzie *et al.*, 1981; Lindsey *et al.*, 1985; Schoeb and Lindsey, 1985, 1987; Schoeb *et al.*, 1985; Schunk *et al.*, 1995). High environmental ammonia levels and other irritating gases can also exacerbate the disease (Lane-Petter *et al.*, 1970; Cassell *et al.*, 1973, 1981a; Broderson *et al.*, 1976; Schoeb *et al.*, 1982; Lindsey *et al.*, 1985; Pinson *et al.*, 1988). Such increases in environmental irritating gases may result from faulty ventilation systems, overcrowding and infrequent bedding changes. Similarly, exposure to some toxic chemicals (e.g. hexamethylphosphoramide) can also exacerbate the disease (Cassell *et al.*, 1973; Overcash *et al.*, 1976). Some nutritional deficiencies (e.g. vitamins A and E) may predispose rats to infection with *M. pulmonis* (Tvedten *et al.*, 1973). Apparently, most strains of *M. pulmonis* are capable of causing upper respiratory tract lesions; however, not all are capable of causing significant pulmonary lesions (Lindsey *et al.*, 1971; Cassell *et al.*, 1973). In addition, the inoculation dose of *M. pulmonis* also influences the disease outcome, especially in mice (Cassell *et al.*, 1973, 1981a).

As was alluded to above, the pathologic expression of the disease can vary considerably because of a number of factors; therefore, the gross and microscopic lesions can also vary or not be present at all. Rats of all ages may have evidence of acute (suppurative) to chronic rhinitis, otitis media, otitis interna and laryngotracheitis (Lane-Petter *et al.*, 1970; Lindsey *et al.*, 1971, 1985; Broderson *et al.*, 1976; Cassell *et al.*, 1981a; Davis and Cassell, 1982; Schoeb *et al.*, 1985; Schoeb and Lindsey, 1987). In addition, there may be pseudoglandular epithelial hyperplasia and/or squamous metaplasia in the nasal and tracheal

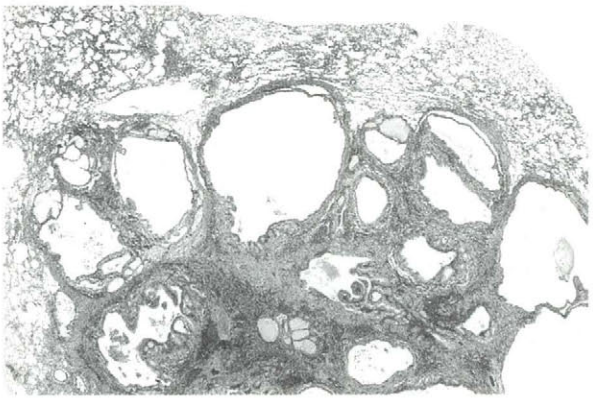

Figure 7.23 Murine respiratory mycoplasmosis. There is severe bronchiectasis and inflammation in the lung. Hematoxylin and eosin. × 2.4.

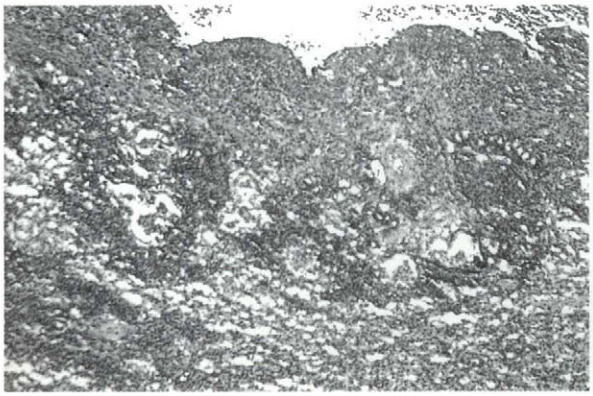

Figure 7.24 Murine respiratory mycoplasmosis. There is moderate hyperplasia and dysplasia of the bronchiolar epithelium along with moderate inflammation and luminal exudate. Hematoxylin and eosin. × 24.

Figure 7.25 Murine respiratory mycoplasmosis. This is a higher magnification of Figure 7.23 showing a few peribronchiolar pseudoglands. Hematoxylin and eosin. × 24.

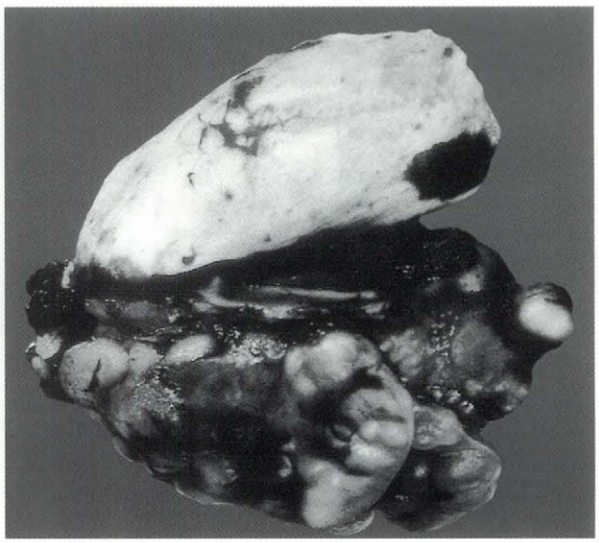

Figure 7.26 Murine respiratory mycoplasmosis. One lobe of the lung from an adult rat is dark and has multiple, raised, opaque blebs (bronchiectasis) on its surface.

mucosa, as well as a reduction in the number of cilia which normally lines different parts of the airways (Lindsey *et al.*, 1971, 1985; Davis and Cassell, 1982; Schoeb and Lindsey, 1987). In contrast, lesions in the lower respiratory tract are most common in rats beginning at 4 weeks old and may include one or more of the following lesions: hyperplasia of the bronchial-associated lymphoid tissue, bronchiectasis (Figure 7.23), bronchiolectasis, suppurative bronchopneumonia, suppurative bronchiolitis, epithelial hyperplasia with associated submucosal mononuclear cell infiltrates in the upper and lower air passages (Figure 7.24), peribronchial and perivascular mononuclear cell infiltrates, nonsuppurative to suppurative alveolitis, atelectasis and peribronchial pseudoglands (Figure 7.25) (Lindsey *et al.*, 1971, 1985; Cassell *et al.*, 1973; Jersey *et al.*, 1973; Broderson *et al.*, 1976; Davis and Cassell, 1982; Schoeb and Lindsey, 1985, 1987; Schoeb *et al.*, 1985). In addition, abscess-like structures, apparently arising from airways, and squamous metaplasia of the bronchial epithelium may be observed. Grossly, the lungs of adult rats may have a cobblestone appearance (Figure 7.26), due to the bronchiectasis and/or abscess-like structures, or there may only be dark red, purple, brown, or gray depressed foci with or without yellow areas (Lane-Petter *et al.*, 1970; Lindsey *et al.*, 1971; Davis and Cassell, 1982; Schoeb and Lindsey, 1985). Mucopurulent exudate may be observed in the upper respiratory tract and the tympanic bullae.

Lesions outside the respiratory tract are less common and are more likely to be observed microscopically. In the female genital tract there may be **salpingitis**, perioophoritis, endometritis, **pyometra**, partially resorbed fetuses, vaginitis and cervicitis (Lane-Petter *et al.*, 1970; Cassell *et al.*, 1979, 1981a,b;

Cassell, 1982; Lindsey *et al.*, 1985). In addition, there also may be epithelial hyperplasia in the oviducts with associated submucosal mononuclear cells. Chronic inflammation of the epididymis, vas deferens and urethra may be observed in male rats. Arthritis has been reported with some *M. pulmonis* infections in rats (Cassell *et al.*, 1986).

The diagnosis can be made with a variety of tests and a combination of methods is recommended, because no one test alone is entirely perfect (Lindsey *et al.*, 1971, 1985; Cassell *et al.*, 1979, 1986; Davidson *et al.*, 1981, 1982; Schoeb and Lindsey, 1985). Microbiologic cultures of the respiratory tract, particularly the upper respiratory tract (e.g. the nasopharyngeal duct), is effective. Serology (IFA and ELISA), IFA or immunohistochemical staining of tissues, and electron microscopy may also be helpful. It is not recommended that pathology alone be used to make a diagnosis because lesions may be either absent or minimal, and when present, similar lesions can be caused by other bacteria (e.g. CAR bacillus) and viruses (e.g. Sendai virus and coronaviruses) (Lindsey *et al.*, 1985; Matsushita and Joshima, 1989; Weir *et al.*, 1990).

M. pulmonis can interfere with many different types of research, including that in the fields of immunology, respiratory disease, tissue culture, gerontology, toxicology, carcinogenesis, nutrition, behavior, pathophysiology and reproduction (Lindsey *et al.*, 1971; Tvedten *et al.*, 1973; Overcash *et al.*, 1976; Cassell *et al.*, 1979, 1981a; Naot *et al.*, 1979; Barile, 1981; Davis *et al.*, 1982; Naot, 1982; Schoeb *et al.*, 1985; Aguila *et al.*, 1988; McDonald, 1988).

Pseudotuberculosis

Corynebacterium kutscheri is the cause of pseudotuberculosis, a naturally occurring disease in mice and rats (Giddens *et al.*, 1968; Weisbroth and Scher, 1968; McEwen and Percy, 1985; Fox *et al.*, 1987). *C. kutscheri* has been cultured from hamsters which had neither clinical signs nor lesions (Amao *et al.*, 1991). Vallee *et al.* (1969), as cited by Weisbroth (1979), also isolated *C. kutscheri* from guinea pigs.

In the rat, transmission is supposedly via the fecal–oral route (Baker, 1998). Direct and indirect contact is reported to be the mode of exposure in mice (Shechmeister and Adler, 1953). Both modes of transmission are probably operative in both the rat and the mouse.

C. kutscheri apparently resides quietly in the upper and lower digestive tract, submaxillary lymph nodes, and the upper respiratory tract of rats and mice (Amao *et al.*, 1995a,b). These latent infections may be precipitated by a number of stressors including shipping, overcrowding, nutritional deficiencies (e.g. pantothenic acid), immunosuppressive drugs (e.g. corticosteroids), irradiation, experimental manipulations, and concurrent infections (Antopol, 1950; Wolff, 1950; LeMaistre and Tompsett, 1952; Shechmeister and Adler, 1953; Seronde, 1954; Zucker and Zucker, 1954; Seronde *et al.*, 1955, 1956; Giddens *et al.*, 1968; Fujiwara, 1980; McEwen and Percy, 1985). In addition, one of the authors observed an epizootic which was associated with high environmental temperatures and elevated ammonia levels in an animal room with a defective ventilation system (Gaillard, 1998, unpublished observation). High ammonia levels may have also been responsible for an epizootic reported by Giddens *et al.* (1968). Apparently some strains of mice do not require stress or experimental manipulations for the disease to occur (Weisbroth and Scher, 1968).

The bacteria are spread throughout the body via the blood and septic emboli become lodged in organs with extensive capillary networks (e.g. lung, kidney and liver) or those which filter blood (e.g. kidney, choroid plexus and synovial membrane) (Weisbroth and Scher, 1968). This accounts for the distribution of most lesions in the host. Although virtually any organ can develop lesions, the lung is the most frequently involved organ in the rat, whereas the kidney and liver are the most common organs in mice (Seronde, 1954; Giddens *et al.*, 1968; Weisbroth and Scher, 1968).

Clinical signs, when present, are nonspecific and may include porphyrin and mucopurulent ocular and nasal discharge, respiratory rales, dyspnea, hyperpnea, hunched posture, lethargy, emaciation, weight loss, subcutaneous nodules, swollen joints and lameness (Zucker and Zucker, 1954; Giddens *et al.*, 1968; Nelson, 1973; Fox *et al.*, 1987).

External gross lesions may include subcutaneous abscesses, preputial gland abscesses and suppurative arthritis (Giddens *et al.*, 1968). Internal gross lesions may include necrosis and/or abscesses in the lung (Figures 7.27 and 7.28), liver, kidney, brain and other tissues (Giddens *et al.*, 1968; McEwen and Percy, 1985; Fox *et al.*, 1987). In addition, there may also be hepatic necrosis, and fibrinous or fibrous pleuritis, and fibrinous pericarditis (LeMaistre and Tompsett, 1952; Zucker and Zucker, 1954; Seronde *et al.*, 1956; Giddens *et al.*, 1968; McEwen and Percy, 1985; Fox *et al.*, 1987).

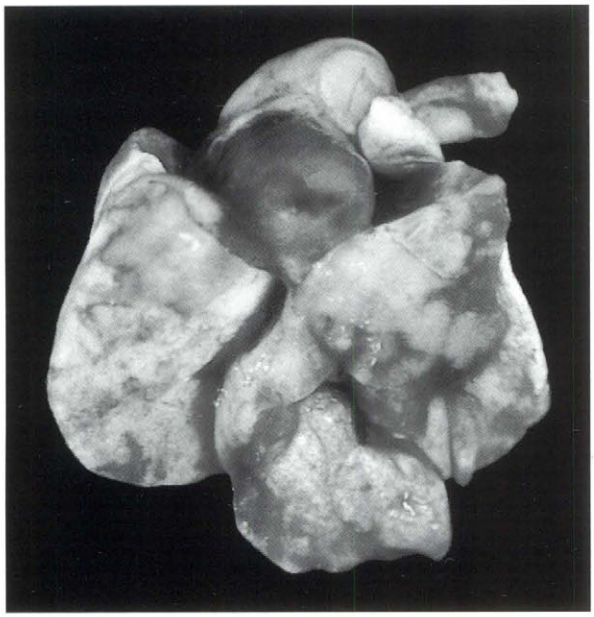

Figure 7.27 Pseudotuberculosis. Note the irregular pale areas of acute caseous necrosis in the lung of a rat.

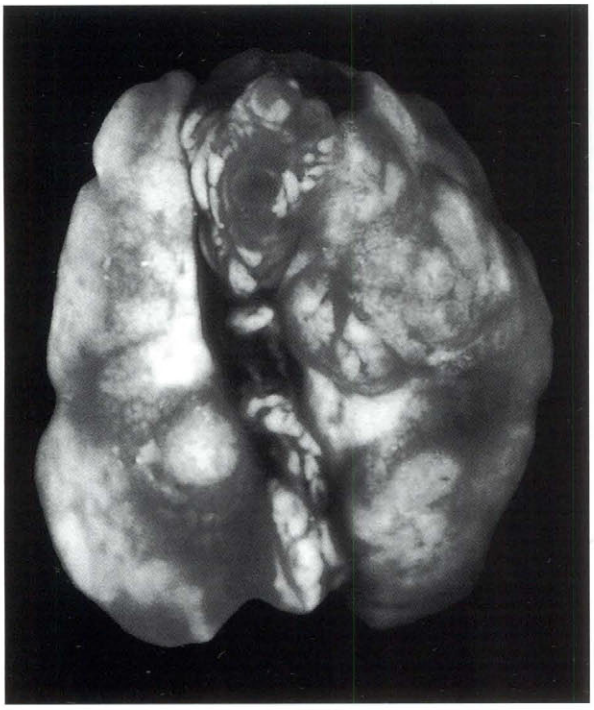

Figure 7.28 Pseudotuberculosis. There are multiple pale abscesses on the dorsal aspect of the lung.

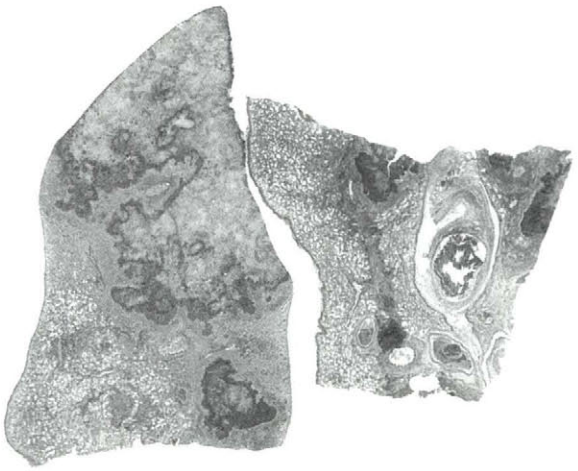

Figure 7.29 Pseudotuberculosis. Subgross photograph of the lung in Figure 7.27. Note the large, irregular areas of caseous necrosis. Hematoxylin and eosin.

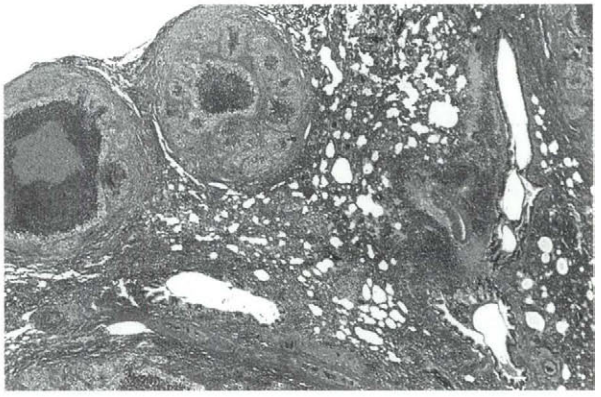

Figure 7.30 Pseudotuberculosis. Photomicrograph of the lung in Figure 7.28. There are multiple abscesses in the pulmonary parenchyma. Note that the airways near the center and the right of the photomicrograph are normal. Hematoxylin and eosin. × 4.

The microscopic lesions are related to the gross lesions (LeMaistre and Tompsett, 1952; Seronde, 1954; Giddens *et al.*, 1968; McEwen and Percy, 1985; Fox *et al.*, 1987). In the lungs there may be either irregular foci of caseous necrosis surrounded by mixed inflammatory cells (Figure 7.29) or there may be abscesses (Figure 7.30). The lesions in the lungs are primarily found in the interstitium; however, in some instances abscesses can erode through the airways and parietal pleura resulting in the seepage of septic exudate and ultimately pleuritis. In contrast to some reports in the literature, multinucleated giant cells may be present at the margins of the lesions (Figure 7.31). Necrosis and/or abscesses may be also present in the liver, kidney, subcutaneous tissues (Figure 7.32), brain (Figure 7.33), and other sites. Clusters of pleomorphic Gram-positive rods can be observed in the lesions. These bacterial colonies typically have either a Chinese letter (Figure 7.34) or bottle brush configuration.

Pathology, with the help of a Gram stain, is very helpful in making a diagnosis when lesions are present. However, the major problem is identifying animals which are latently infected with *C. kutscheri*.

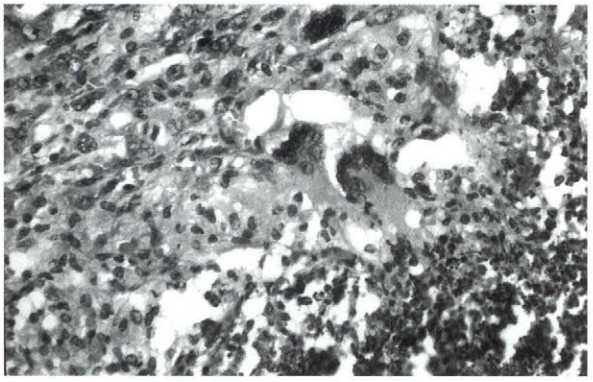

Figure 7.31 Pseudotuberculosis. Photomicrograph of the lung in Figures 7.27 and 7.29. Note the multinucleated giant cells at the margin of the area of caseous necrosis. Hematoxylin and eosin. × 60.

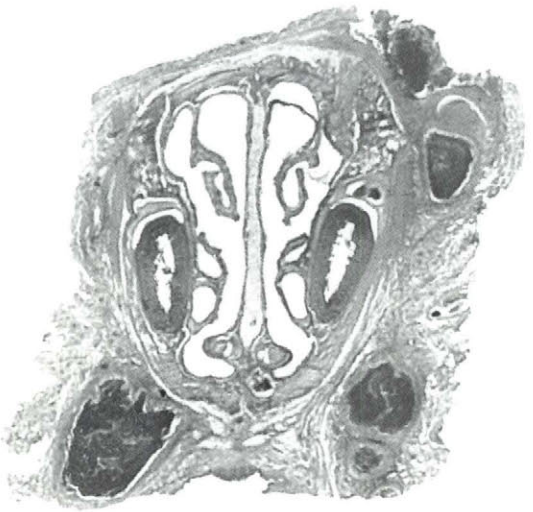

Figure 7.32 Pseudotuberculosis. Subgross photograph of the muzzle from the same rat in Figure 7.28. Note the abscesses in the subcutaneous tissues. Hematoxylin and eosin.

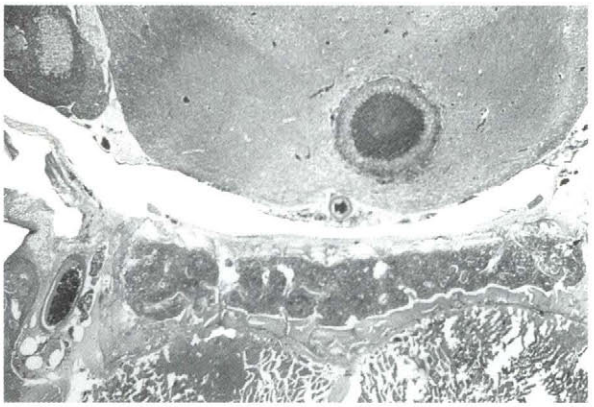

Figure 7.33 Pseudotuberculosis. Photomicrograph of an abscess in the brain of the same rat in Figure 7.32. Hematoxylin and eosin. × 15.

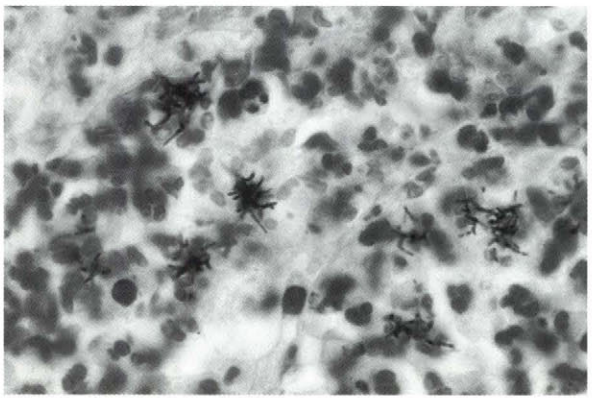

Figure 7.34 Pseudotuberculosis. Several clusters of Gram-positive bacilli arranged in a Chinese letter configuration. Gram stain. × 240

Amao *et al.* (1995b) recommended culturing the oral cavities of rats, using furazolidone–nalidixic acid–colimycin (FNC) agar to detect **latent infections** with *C. kutscheri*. Additional sites along the upper and lower digestive tract also had a very high isolation rate, but the oral cavity was the best site. Serological tests (e.g. ELISA) as well as DNA–DNA hybridization techniques on tissue touch blots may also be helpful if available (Saltzgaber-Muller and Stone, 1986; Fox *et al.*, 1987; Boot *et al.*, 1995). Subcutaneous injections with cortisone can also be used to detect latent infections by precipitating the disease (Fauve *et al.*, 1964).

C. kutscheri has the potential to interfere with virtually any type of research which causes stress, immunosuppression, nutritional deficiencies, etc. (Antopol 1950; Antopol *et al.*, 1951; LeMaistre and Tompsett, 1952; Seronde, 1954; Zucker and Zucker, 1954; Baker, 1998).

Streptococcal Infections

At least several bacteria of the genus *Streptococcus* can cause clinical disease in rats. All of the streptococci of concern in rats are Gram-positive cocci, and are catalase-negative, nonfermentative, and generally nonmotile (Ruoff, 1995). The name *Streptococcus* is derived from the tendency of many members of the genus to form chains, visible on wet mounts of cultured colonies or in histologic section. Streptococci are differentiated on their ability to hemolyse erythrocytes in blood agar. **Beta hemolysis** refers to a clear, colorless zone of red cell lysis surrounding the bacterial colonies. This contrasts with **alpha hemolysis**, an often greenish to brownish indistinct

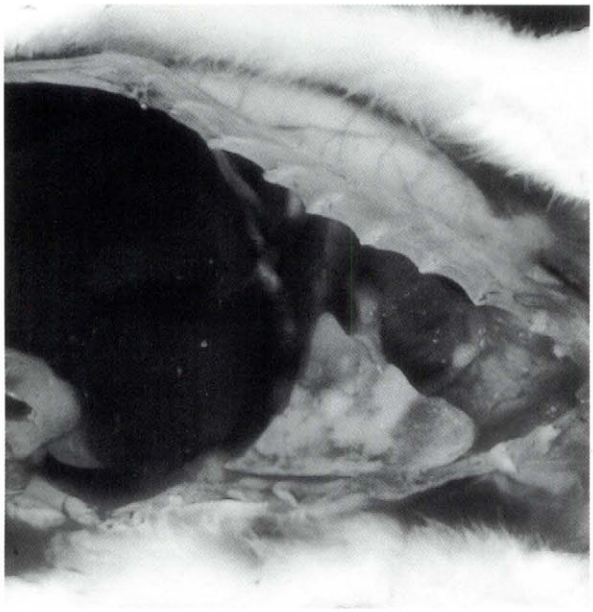

Figure 7.35 *Streptococcus pneumoniae* bronchopneumonia. Note the ventral consolidation in the lung lobe.

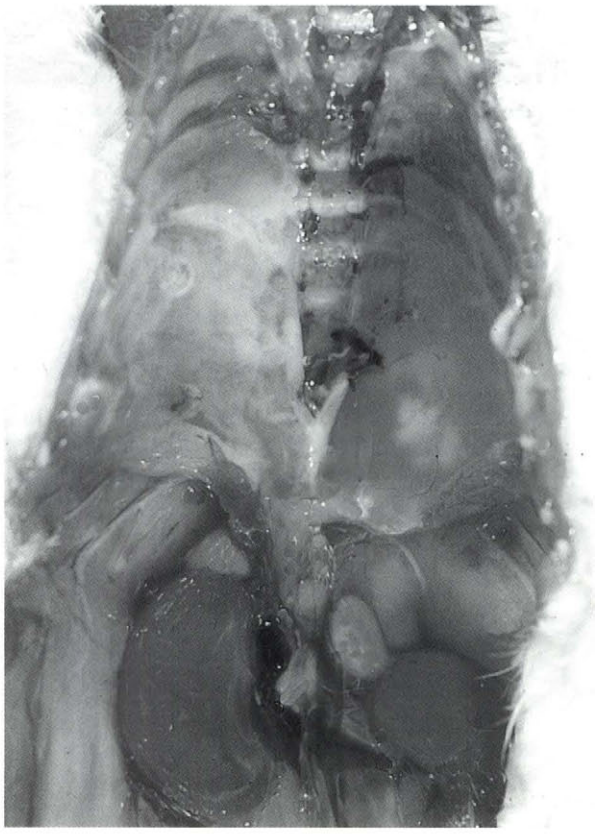

Figure 7.36 *Streptococcus pneumoniae* fibrinopurulent pleuritis. The lungs have been removed and there is exudate on the parietal pleura.

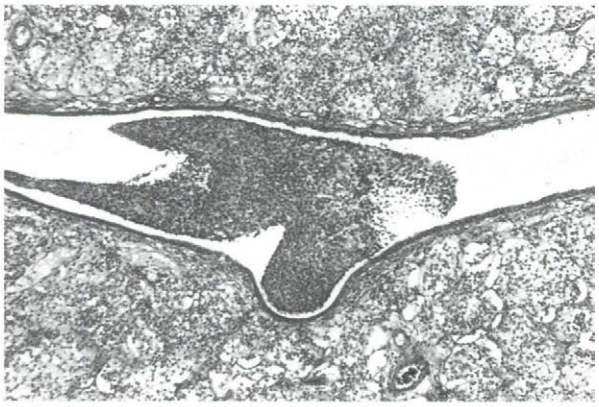

Figure 7.37 *Streptococcus pneumoniae* fibrinopurulent bronchopneumonia. Hematoxylin and eosin. × 15.

zone of partially lysed erythrocytes, as well as with **gamma hemolysis**, the term used when no hemolysis is observed. Of primary interest in the rat is *S. pneumoniae*, which is alpha-hemolytic. There is also lesser interest for various members of the beta-hemolytic group, and for *Enterococcus* spp., which are not truly streptococci.

Pneumonia caused by *S. pneumoniae* has previously been referred to as streptococcosis, but this term should be avoided, as it is inherently nonspecific. *S. pneumoniae* may be said to have more notoriety than true impact, and it has been recently considered to be of low significance in laboratory animals (National Research Council, 1991). Humans are the natural host for *S. pneumoniae* (Austrian, 1998), with both adults and children frequently colonized. Transmission is primarily via aerosol; fomites may play a less important role. Infection in rats is usually asymptomatic although disease has, albeit infrequently, been reported. Asymptomatic rats harbor the infection in the nasopharynx. 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).

Grossly, disease begins with suppurative inflammation in the upper respiratory tract, then spreads to the lung. In the lung, multifocal areas of bronchopneumonia (Figure 7.35) expand and may coalesce (Kohn and Barthold, 1984), and may be accompanied by fibrinopurulent pleuritis (Figure 7.36). Histologically, the bronchopneumonia is characterized by edema, fibrinopurulent inflammation (Figure 7.37) and necrosis of the pulmonary parenchyma, often with fibrinopurulent pleuritis. Affected rats may become **bacteremic**, and develop fibrinopurulent inflammation of other serous surfaces (peritoneum, synovium, etc.) and other tissues.

Colonies of laboratory rats are screened for *S. pneumoniae* infection by nasopharyngeal culture onto

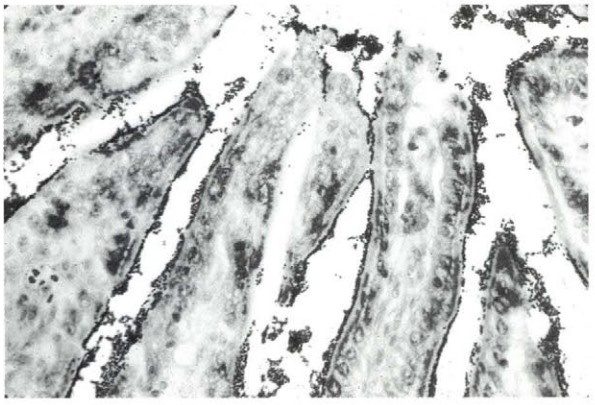

Figure 7.38 Enterococcal (streptococcal) enteropathy in a suckling rat. Gram-positive cocci form a thick blue layer on the surface of the small intestine's villi. Gram stain. × 180.

blood agar (Kohn and Barthold, 1984). However, *S. pneumoniae* must be differentiated from other alpha-hemolytic streptococci. This is most often performed by the **optochin inhibition test** which measures the sensitivity of the test isolate to optochin disks; most *S. pneumoniae* strains are inhibited to a greater degree than other alpha-hemolytic streptococci. Isolates displaying intermediate degrees of inhibition should be tested for bile solubility for confirmation; *S. pneumoniae* colonies are bile soluble (Ruoff, 1995). However, due to the occurrence of nonpathogenic isolates (Fallon *et al.*, 1988), isolation of *S. pneumoniae* from rats, even if a respiratory problem is present, does not necessarily provide a diagnosis, nor does isolation of *S. pneumoniae* from asymptomatic rats necessarily indicate a colony health threat.

Beta-hemolytic streptococci are also present in many rats, with disease only a rare occurrence. Beta-hemolytic streptococci are divided into groups based on Lancefield antigens, with Lancefield groups B and G most commonly isolated from rats. On rare occasions, they may be isolated from abscesses, but exclusion from most colonies is neither practical nor necessary.

So-called 'streptococcal enteropathy' is actually due to nonhemolytic Lancefield group D enterococci, including *Enterococcus hirae*, *E. faecium-durans-2*, and *E. fecalis-2* (Barthold, 1997). Enterococci are differentiated from streptococci by biochemical tests, as well as by 16S rRNA sequencing (Facklam and Sahm, 1995). Streptococcal enteropathy is a disease of suckling rats, and does not affect post-weaning animals. Affected litters develop diarrhea or soft stool, with bright yellow, pasty feces. Microscopically, the villi in the small intestine are covered with a carpet of Gram-positive cocci (Figure 7.38). Disease is clearly associated with some strains of enterococci and not with others. The factors determining the pathogenicity, however, have not been elucidated, but may involve the ability of the pathogenic isolates to adhere to the microvillous surface.

Control of *Streptococcus* spp. and *Enterococcus* spp. is problematic. The organisms are virtually ubiquitous, including being present in a high percentage of the human population (Weisbroth, 1982; Facklam and Sahm, 1995). Some *Enterococcus* spp. are even considered **autochthonous flora** of the rat (Savage, 1971). Clearly, the bacteria 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, money and other resources such housing techniques would require.

Cilia-associated Respiratory Bacillus

Often referred to as CAR bacillus, cilia-associated respiratory bacillus is not taxonomically classified in the genus *Bacillus*. Rather, it has recently 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 thus may be related to *Flavobacterium* or *Flexispira*, based on 16S ribosomal RNA sequencing (Cundiff *et al.*, 1995). At this point, however, final identification is still pending.

Amongst the common laboratory animals CAR bacillus has been identified in rats, mice, and rabbits (van Zwieten *et al.*, 1980; MacKenzie *et al.*, 1981; Waggie *et al.*, 1987; Griffith *et al.*, 1988). In rats, infection is usually asymptomatic although nonspecific clinical signs, such as weight loss and dyspnea, may be observed.

Transmission is probably primarily via direct contact with infected animals. Bedding does not transmit the infection well (Cundiff *et al.*, 1995), so the role of fomites in natural transmission of CAR bacillus is probably insignificant (Matsushita *et al.*, 1989). Airborne exposure is not an important means of transmission (Itoh *et al.*, 1987).

Gross lesions in uncomplicated CAR bacillus infections may not always be present, although translucent gray cystic lesions may be visible on the pleural surface (Figure 7.39) (Itoh *et al.*, 1987). If there is coinfection with *Mycoplasma pulmonis* or other

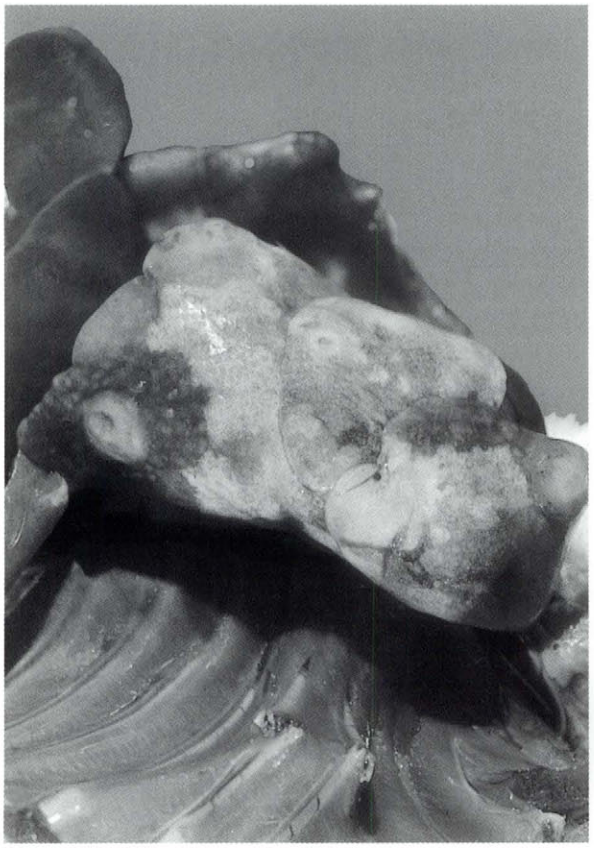

Figure 7.39 Cilia-associated respiratory (CAR) bacillus infection in a rat. The lung has multiple red areas of consolidation and a few gray, translucent, cystic areas on the pleural surface.

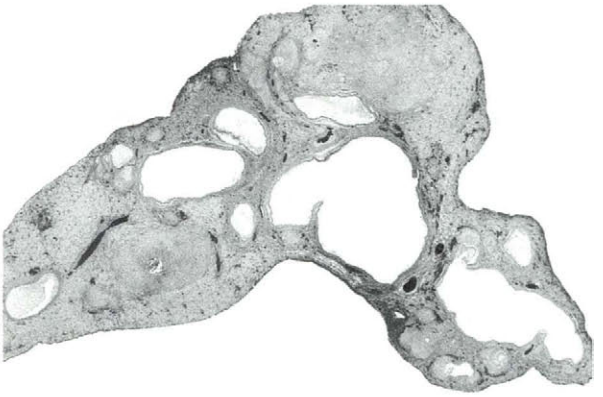

Figure 7.40 CAR bacillus infection in a rat. There is severe bronchiectasis and intense inflammation and mucus in the interstitium. Hematoxylin and eosin. × 1.5

Figure 7.41 CAR bacillus infection in a rat. This is a higher magnification of Figure 7.40. Note the pale basophilic structures on the luminal surface of the bronchial epithelial cells. Additionally, there are moderate numbers of neutrophils in the lumen. Hematoxylin and eosin. × 95.

pathogens, the resulting lesions resemble those of chronic respiratory disease (CRD) (MacKenzie *et al.*, 1981). Although *Mycoplasma pulmonis* is accepted as the cause of chronic respiratory disease (Jersey *et al.*, 1973), some think that CAR bacillus may contribute to the lesions of CRD.

Histopathologically, hyperplastic peribronchial lymphoid tissue and peribronchiolar mononuclear cell cuffs are observed in the lungs (Itoh *et al.*, 1987; Matsushita and Joshima, 1989). Additionally, there may be bronchiectasis and considerable amounts of mucopurulent exudate in the airways and alveolar spaces (Figure 7.40). A thin basophilic layer may be observed on the surface of the respiratory epithelium of airways in hematoxylin and eosin stained sections (Figure 7.41), 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 a Warthin-Starry or Grocott's methenamine silver stain, filamentous bacilli are readily observed among cilia of respiratory epithelium from the nasal cavity to the bronchioles (Figure 7.42). The upper respiratory tract is involved earlier than the lower tract during the course of the infection, and both sites should be included n histologic examinations of the disease. With secondary bacterial infections, there may also be a suppurative bronchopneumonia.

CAR bacillus infection should be distinguished from murine respiratory mycoplasmosis, other bacterial pneumonias (i.e. *Streptococcus pneumoniae*, *Corynebacterium kutscheri*, etc.) and viral pneumonias. Diagnosis of CAR bacillus infection should also raise the suspicion of coinfection with the other pathogens that often accompany CAR bacillus infections (van Zwieten *et al.*, 1980; MacKenzie *et al.*, 1981).

Colony screening is usually performed by serologic techniques, such as ELISA or IFA (Matsushita *et al.*, 1987; Lukas *et al.*, 1987; Shoji *et al.*, 1988). As false positive reactions can occur (Hook *et al.*, 1998), any

Figure 7.42 CAR bacillus infection in a rat. Darkly stained bacteria are readily visible in the normally pale layer of cilia on the surface of the bronchial epithelium. Bacilli are also present in the lumen. Warthin–Starry Silver. × 240

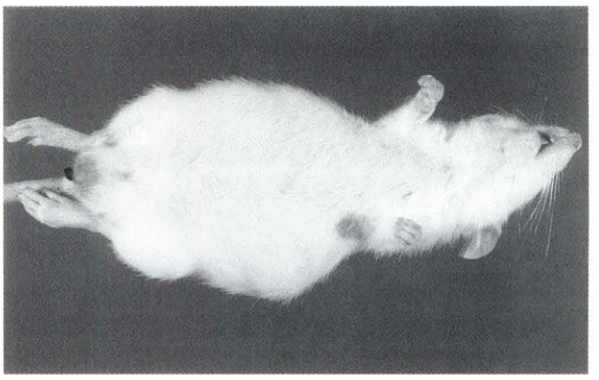

Figure 7.43 Tyzzer's disease. A 3–4-week-old Wistar rat has a distended abdomen due to megaloileitis associated with Tyzzer's disease.

positive results should be confirmed by a Steiner stain of tracheal mucosal scrapings or histopathology with use of special stains, as discussed previously. Interestingly, infection is not readily transmitted by soiled bedding (Cundiff *et al.*, 1995), so many sentinel programs may fail to detect this organism. Infection is lifelong, and the organisms are readily retrievable by tracheal lavage or scraping (Medina *et al.*, 1998). Therefore, CAR bacillus can also be detected by PCR (Cundiff *et al.*, 1994), which may serve as an important confirmatory test should serologic screening be positive.

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 also been postulated but not documented in the scientific literature.

Tyzzer's Disease

Tyzzer's disease, first discovered in Japanese waltzing mice (Tyzzer, 1917), is caused by *Clostridium piliforme* (Duncan *et al.*, 1993), formerly known as *Bacillus piliformis*. The host range is extremely broad (Kohn and Barthold, 1984), including numerous rodent species, rabbits, carnivores, horses, and both nonhuman and human (Skelton *et al.*, 1995) primates.

C. piliforme infections are most often clinically silent (Motzel and Riley, 1992; Hansen *et al.*, 1992a). 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 diarrhea with or without mucus and blood), but may also include acute death without clinical signs. In the rat, in particular, a distended abdomen (Figure 7.43) has been observed in weanlings with Tyzzer's disease (Hansen *et al.*, 1992b).

C. piliforme is transmitted horizontally in rats via the fecal–oral route by spores, which are highly resistant to desiccation and some disinfectants (Ganaway, 1980; Hansen *et al.*, 1992b). The vegetative form, however, survives only inside cells.

After a rat ingests *C. piliforme* spores, the spores produce the vegetative form, which is actively phagocytosed by mucosal epithelial cells covering the gut-associated lymphoid tissue or Peyer's patches in the jejunum, ileum and cecum (Franklin *et al.*, 1993; Riley and Franklin, 1997). The vegetative form escapes from the phagosome and multiplies in the epithelial cells and possibly the reticuloendothelial cells in Peyer's patches. The vegetative form reaches the liver, probably via the portal circulation. It then infects and multiplies in the hepatocytes, following which it may enter the bloodstream or lymphatics to colonize the myocardium. It may also possibly enter the biliary epithelium to multiply and be shed into the bile to reinfect the intestine and liver (autoinfection). Alternatively, the infection may be cleared (Motzel and Riley, 1992).

A number of factors influence the infection and disease outcome, especially age; recently weaned rats are more susceptible to overt disease. Immune function also is important (Livingston *et al.*, 1996), and immunosuppression from stress or immunosuppression by treatment with cyclophosphamide or corticosteroids may precipitate a latent infection (Boivin *et al.*, 1990).

Bacterial factors also play an important role in the pathogenesis of Tyzzer's disease. Pathogenicity has

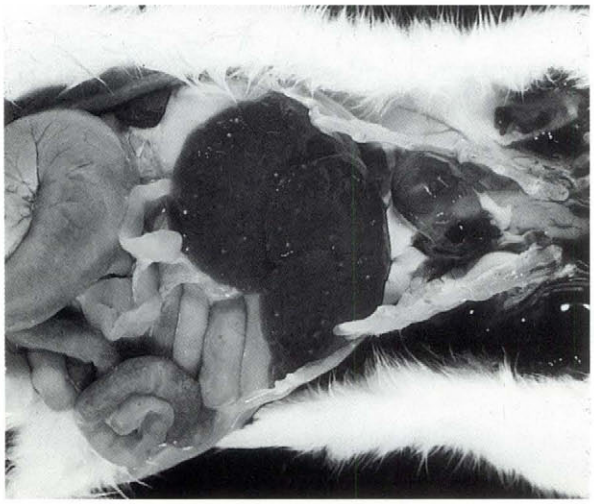

Figure 7.44 Tyzzer's disease. Numerous white foci of necrosis and inflammation are scattered in the liver of this 3–4-week-old Wistar rat.

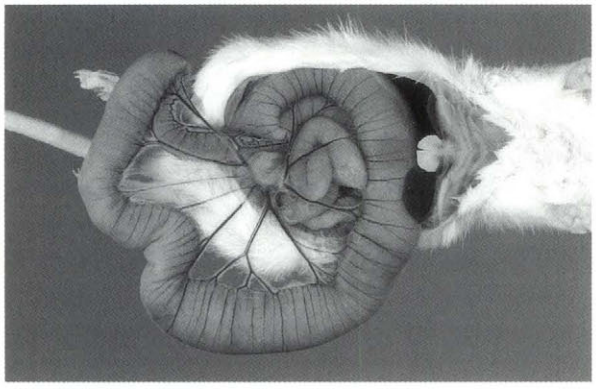

Figure 7.45 Tyzzer's disease. This is the same 3–4-week-old Wistar rat depicted in Figure 7.43. The megaloileitis is due to *Clostridium piliforme*.

Figure 7.46 Tyzzer's disease. A distinct white area of necrosis and inflammation is present in the heart of this 3–4-week-old Wistar rat.

been associated with production of a high-molecular weight, cytotoxic protein (Riley *et al.*, 1992). In addition, some degree of species-specificity has been demonstrated for *C. piliforme* isolates, such that a rat isolate may not readily infect other host species, and vice versa (Motzel and Riley, 1992; Franklin *et al.*, 1994).

Grossly, Tyzzer's disease may cause perianal fecal staining, but the most characteristic lesions are internal (Tyzzer, 1917; Duncan *et al.*, 1993). Multiple, pinpoint or larger, pale foci of necrosis are often visible on the surface of and within the liver (Figure 7.44). Megaloileitis (Figure 7.45), a greatly dilated, flaccid and hyperemic small intestine (ileum) may be present (Hansen *et al.*, 1992a). Hyperemia, edema, hemorrhage and possibly ulceration may affect any part of the intestine, especially the terminal ileum, cecum and colon. Probably as a consequence of intestinal involvement, mesenteric lymph nodes may be enlarged, hyperemic and edematous. In the heart, pale, circumscribed, sometimes raised foci may be present on the surface. Myocardial necrosis and inflammation due to Tyzzer's disease may also appear as pale linear streaks or circumscribed raised areas on the heart (Figure 7.46), especially near the apex.

Histopathologically, characteristic lesions (Duncan *et al.*, 1993) may be observed in the liver, ileum, cecum, colon, and less frequently the heart (Kohn and Barthold, 1984). In the intestinal tract there is often necrotizing enteritis, typhlitis and colitis, which may be accompanied by edema, blunted and fused villi, crypt epithelial hyperplasia, ulceration and hemorrhage, with cellular debris in crypts and lymphatics. In the liver, coagulative necrosis (Figure 7.47) is a frequent finding, and is often accompanied by a moderate leukocytic infiltrate (neutrophils, mononuclear cells, macrophages and, rarely, multinucleated giant cells) at the periphery of the lesions. Hemorrhage may accompany acute lesions, and mineralization may be visible with time as a consequence of the necrosis. In the heart, myocardial degeneration and necrosis occurs in a minority of cases, often associated with a mixed leukocytic infiltrate and dystrophic calcification.

Histopathology is diagnostic if the characteristic bacilli are observed (Tyzzer, 1917; Kohn and Barthold, 1984; Duncan *et al.*, 1993). The vegetative form of the organism is a filamentous bacillus, 8.0–20.0 × 0.3–0.5 μm. One or usually more bacilli are present in cells in either a jumbled array (pickup stick) or parallel arrangement, as dictated by the shape of the cell. Occasionally, the vegetative form

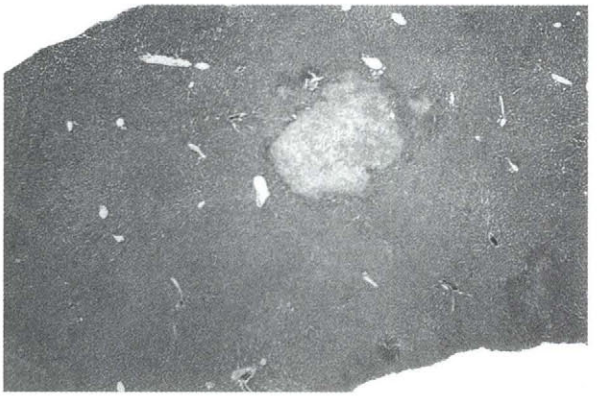

Figure 7.47 Tyzzer's disease. Foci of hepatic necrosis and inflammation. Hematoxylin and eosin. × 15

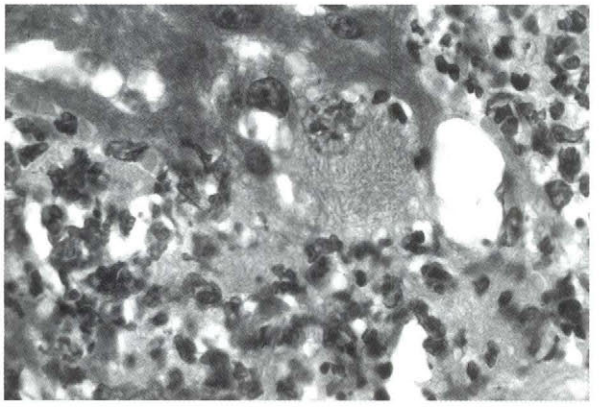

Figure 7.48 Tyzzer's disease. Numerous basophilic organisms lie in a jumbled array within the cytoplasm of a hepatocyte at the periphery of a focus of necrosis. Hematoxylin and eosin. × 295.

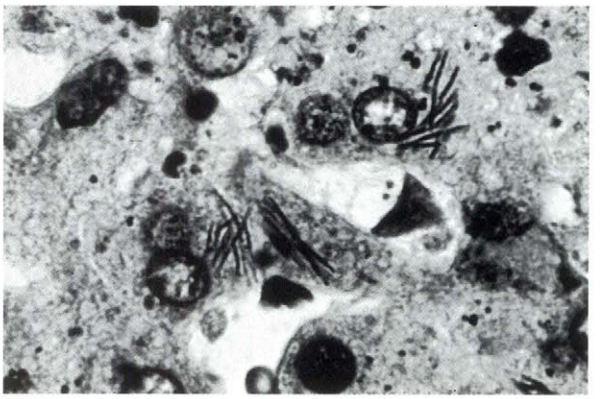

Figure 7.49 Tyzzer's disease. Numerous argyrophilic organisms lie in a jumbled array within the cytoplasm of hepatocytes. Warthin–Starry silver. × 295.

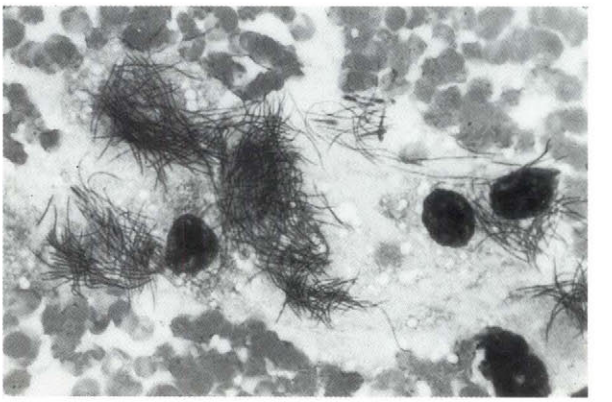

Figure 7.50 Tyzzer's disease. A Giemsa-stained impression smear from liver lesions demonstrates numerous intracellular and extracellular bacilli. × 740.

may be visible in hepatocytes in tissue sections stained with hematoxylin and eosin (Figure 7.48), but usually special stains are necessary, including Warthin–Starry silver (best), Giemsa and methylene blue stains. The organism is Gram-negative, but stains very poorly with Gram stains. In the liver, the organisms are most often observed in surviving hepatocytes at the periphery or within lesions (Figure 7.49), but may also be in hepatocytes not associated with a lesion. In the intestine, normal gut flora within mucosal crypts and superimposed upon the mucosal epithelial cells may complicate evaluation. Organisms may also be observed in myocytes of the heart when there is cardiac involvement. Less frequently, they may be seen in myocytes of the tunica muscularis of the intestine.

Differential diagnoses for necrotizing hepatitis in the rat should include other bacterial septicemias, such as *Corynebacterium kutscheri*, as well as infection with *Rat virus*. Diagnosis of clinical disease depends on demonstration of the organism in tissue. Tissue smears may facilitate rapid diagnosis (Figure 7.50); Giemsa-stained smears of suspicious liver lesions are especially useful (Percy and Barthold, 1993b).

Colony screening for latent infections is problematic. Serologic screening is rapid and technically simple (Motzel *et al.*, 1991), but subject to false positives, yielding results which can be difficult to put into context. Disease provocation tests to exacerbate latent infections are widely used, and are recommended as a follow-up test when serologic positive results are obtained. However, there is some doubt as to the efficacy of these tests which rely on immunosuppression, usually with cyclophosphamide (Boivin *et al.*, 1990) and less frequently with corticosteroids, followed by histopathologic evaluation. The doubt arises since animals may clear the *C. piliforme* infection and, therefore, would no longer be susceptible to activation of 'latent' infection (Motzel and Riley, 1992). Alternatively, sentinel animals can be placed on soiled bedding, but this may require sentinels to be of the same species (to

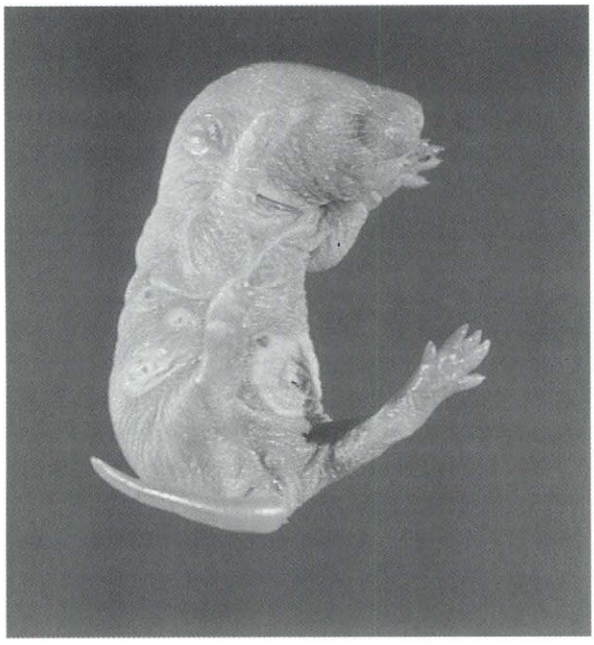

Figure 7.51 Staphylococcal dermatitis in a neonatal rat. Culture of vesicles on the skin yielded heavy growth of *S. aureus* in pure culture.

avoid species-specificity causing false negatives), as not even gerbils are susceptible to all strains of *C. piliforme* (Motzel and Riley, 1992).

Research effects of *C. piliforme* have primarily been ascribed to the morbidity and mortality, although recent effects on coagulation and leukokines (Van Andel *et al.*, 1996) have also been reported.

Staphylococcosis

Despite the attention given to more exotic organisms, one of the most widespread and mundane of bacteria, *Staphylococcus aureus*, may cause some of the most frequently observed lesions in laboratory rats (National Research Council, 1991). *S. aureus* is present as a commensal in laboratory rodents, as well as most other mammals, including humans. In fact, it is probably accurate to say that *S. aureus* is always present unless strictly excluded.

In any given rat population, most will be asymptomatic carriers. Occasionally, an ulcerative dermatitis (Figure 7.51) will be observed in one or more adults or, rarely, a vesicular or ulcerative condition in young animals (Clifford, 1998, unpublished observation).

S. aureus is a Gram-positive, coagulase-positive coccus (Schleifer, 1986). The name is derived from Greek, *staphyle*, meaning like a cluster of grapes. It is readily cultured from the nasopharynx, skin, bedding and surfaces onto blood agar, where it produces yellow-gold colonies.

Figure 7.52 Staphylococcal dermatitis. Multiple ulcerated skin lesions on the shoulders, back and base of the tail may have originated from bite wounds in these group-housed Brown-Norway rats.

Transmission is horizontal. Caretakers may serve as reservoirs for *S. aureus* and may spread it directly or by fomites (Blackmore and Francis, 1970). The organism can also multiply in dirty bedding or other materials.

S. aureus normally inhabits the skin and mucous membranes (National Research Council, 1991), and results in disease only when there is a break in the integrity of these structures from trauma or foreign bodies. It can also penetrate the oral mucosa at the gingival–tooth margin, resulting in periodontal abscesses. Some *S. aureus* strains produce an exfoliative toxin which may predispose the skin of neonatal rats to gentle rubbing, therefore, possibly providing a portal of entry for this opportunist. It is also one of the most common organisms isolated from preputial glands, although less frequently in rats than in mice. Involvement of deeper tissues or hematogenous spread is rare in immunocompetent rats.

Gross lesions generally are those of an ulcerative dermatitis, although subcutaneous abscess may also, rarely, be observed (National Research Council, 1991). Lesions are typically intensely pruritic, and are most frequent on the dorsolateral surface of the anterior thorax, head and neck (Figures 7.52 and 7.53).

Histopathologically (Carlton and Hunt, 1978), ulcerative dermatitis is a chronic suppurative condition, usually with numerous prominent dense colonies of large Gram-positive cocci embedded in serum exudate and occasionally in the dermis. Clusters of Gram-positive cocci in the dermis or other tissues may be surrounded by an eosinophilic to amphophilic dense ring or corona, referred to as Splendore–Hoeppli material.

Figure 7.53 Staphylococcal dermatitis. The ulcerated skin lesion on the ear of this Long-Evans rat may have originated as a self-inflicted wound. The rat was negative for mites.

The list of differential diagnoses to be considered should include other bacterial infections, especially *Corynebacterium kutscheri*, as well as higher bacteria (*Actinomyces* spp., *Actinobacillus* spp., *Nocardia* spp.) and fungi. Chemical injury, such as inadequate rinsing of sterilants from gloves or forceps can also result in ulcerative skin lesions. Trauma from handling, sharp edges in cages and from fighting should also be considered as primary causes of ulcerative skin lesions.

Diagnosis is made by bacterial culture or by identification of characteristic organisms in ulcerative and/or suppurative lesions.

Pasteurellosis

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

P. pneumotropica has a high prevalence in positive colonies, and is most often isolated from the nasopharynx, cecum, vagina, uterus and conjunctiva during routine monitoring (National Research Council, 1991). The vast majority of animals are asymptomatic, with only rare instances of conjunctivitis, **metritis** and mastitis (Percy and Barthold, 1993b). Histologically, lesions are characterized by necrotizing, suppurative inflammation.

Control of the agent may not be necessary in immunocompetent animals due to the rarity of *P. pneumotropica*-induced disease. However, treatment with enrofloxacin has been described (Goelz *et al.*, 1997). Rederivation by either cesarean section or embryo transfer will also eliminate the agent (National Research Council, 1991). Antibiotic treatment of infected dams prior to cesarean section is recommended by at least one major rodent vendor (Clifford, 1998, unpublished observation), since *P. pneumotropica* can be present in the uterus. The probability of successful elimination of *P. pneumotropica* by cesarean section can be further increased by culturing all uteri after the pups have been removed, and eliminating any offspring from a culture-positive uterus. Offspring should also be held in strict isolation (i.e. not mixed in with a breeding colony) until repeatedly cultured negative for *P. pneumotropica*. *P. pneumotropica* is not transmitted to a significant degree by fomites, does not persist or multiply in the environment, and only 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.

Streptobacillus moniliformis

A cause of 'rat-bite fever', *Streptobacillus moniliformis* is primarily of historic interest. Although this zoonotic agent is virtually nonexistent in modern laboratory animals, it nonetheless bears brief mention due to the potential consequences of infection (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).

S. moniliformis is commensal in wild rats, inhabiting the nasopharynx, middle ear and respiratory tract. It is also 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 3–10 day incubation period, and include fever, vomiting, **arthralgia** and rash. Disease is treated with antibiotics, and mortality is usually low.

Colonies of laboratory rats are monitored by culture of blood and nasopharyngeal swabs for *S. moniliformis*, and any colony in which the organism is

confirmed should immediately be terminated. As wild rats are the reservoir for *S. moniliformis*, its detection in a laboratory rat colony would indicate exposure to infected wild rats.

Parasitic Diseases

Oxyuriasis

Three species of oxyurid nematodes (pinworms), *Syphacia muris*, *S. obvelata*, and *Aspiculuris tetraptera*, occur in the laboratory rat. Their continued occurrence, despite the dramatic progress in the last decades in eliminating and excluding 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.

Syphacia muris is the most common oxyurid of the rat (National Research Council, 1991; Owen, 1992). *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 as mice with the parasite.

Syphacia spp. have a direct life cycle, requiring 11–15 days for completion (Flynn, 1973b). Transmission is horizontal via ingestion of eggs. Eggs, which remain viable at room conditions for weeks to months, are deposited by the female in the colon and around the anus, and become infective in approximately 6 hours. They are ingested during self-cleaning behavior, and hatch in the small intestine. The larvae then mature in the cecum in 10–11 days. The morphology of adults of both species is similar, although *S. muris* is slightly smaller and the male has a longer tail, measured as a proportion of body width (Flynn, 1973b). Eggs vary more markedly between the species, with *S. muris* (Figure 7.54) being 72–82 × 25–36 µm, compared to 118–153 × 33–55 µm for *S. obvelata*. In addition, the eggs of *S. obvelata* (Figure 7.55) are almost completely flat along one side, whereas those of *S. muris* are only slightly flattened on one side.

Aspiculuris tetraptera is also transmitted horizontally by ingestion of eggs which are extremely persistent in the environment (Flynn, 1973b). The direct life cycle is longer than *Syphacia*, requiring 23–25 days. Also unlike *Syphacia*, *Aspiculuris* eggs are passed in the feces, and are not deposited around the anus. They become infective in 6–7 days. After ingestion they hatch in the colon where the larvae also mature. Adult *A. tetraptera* are readily recognized by the four alae present at the anterior end of the body (Figure 7.56). The bilaterally symmetrical eggs (Figure 7.57) are smaller than those of *Syphacia obvelata*; however, they are approximately the same size as *S. muris*, measuring 89–93 × 36–42 µm.

Gross lesions of **oxyuriasis** are very rare (Flynn, 1973b). Rectal prolapse, constipation and intussusception have been reported in mice infested with *S. obvelata*, but these findings were not experimentally reproduced. The authors also did not exclude other potential causes of rectal prolapse in mice which have since been discovered (Percy and Barthold, 1993a; Ward *et al.*, 1996), such as *Citrobacter rodentium* or *Helicobacter hepaticus*. Histologic lesions of oxyuriasis have not been reported.

Diagnosis of oxyuriasis is most practically accomplished by direct examination of macerated

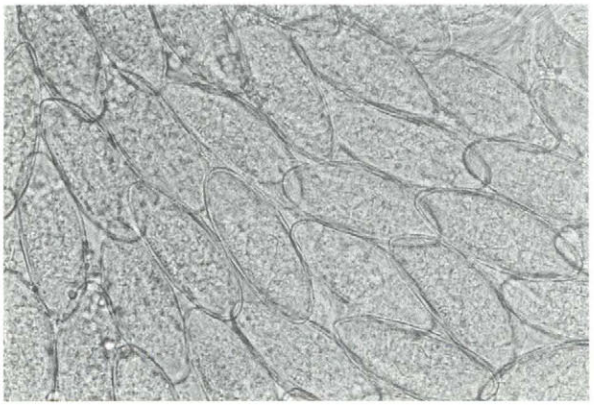

Figure 7.54 *Syphacia muris* eggs inside the uterus of an adult parasite. × 295

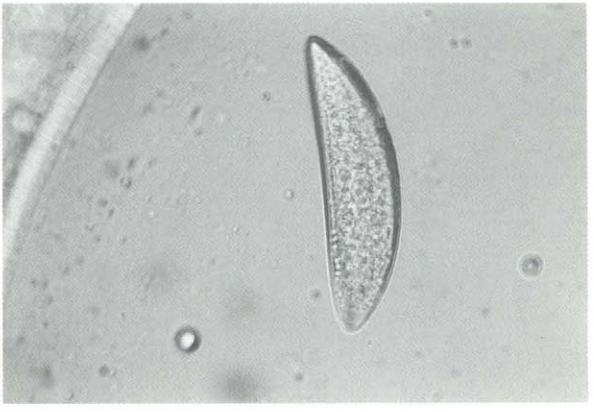

Figure 7.55 *Syphacia obvelata* egg. Compared to the eggs in Figure 7.54, this egg is larger and more markedly flattened along one side. × 295.

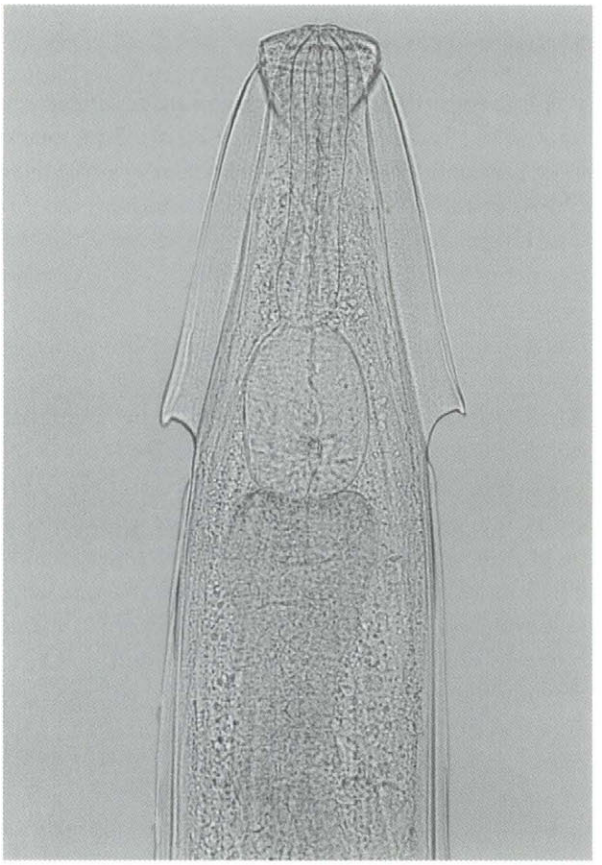

Figure 7.56 Adult *Aspiculuris tetraptera*. Lateral alae are visible along the sides of the parasite. × 295.

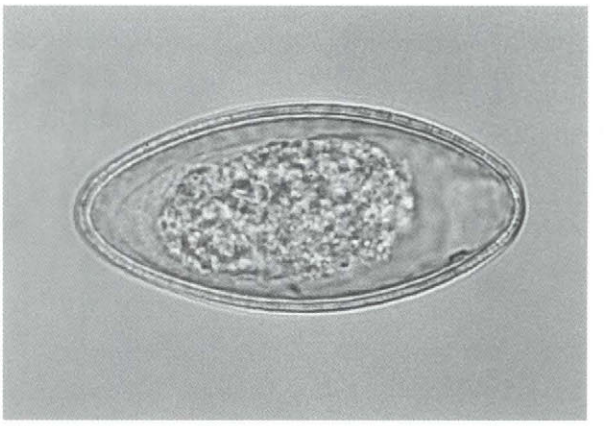

Figure 7.57 *Aspiculuris tetraptera* egg. The egg is symmetrical, and approximately the same size as the egg of *Syphacia muris*.

cecum and colon under low magnification with a stereomicroscope. Although not quite as sensitive as complete direct examination of the large bowel, it is significantly less time consuming. Examination for eggs must be tailored to the suspected infesting species of nematode. As dictated by the method of egg transmission, the perianal tape test is effective only for *Syphacia* spp., and fecal flotation is only effective for *A. tetraptera*. Screening for oxyurid eggs is significantly less sensitive than direct examination of the bowel for the adult helminths (West *et al.*, 1992; Klement *et al.*, 1996).

Oxyuriasis can be eliminated in individual rats with Ivermectin (Hasslinger and Wiethe, 1987; Huerkamp, 1993; Klement *et al.*, 1996). However, the source of the original infestation should be identified, and the premises should be thoroughly disinfected so as to prevent reinfestation. Ivermectin is not effective against eggs, which can persist for long periods in the environment. Oxyuriasis can also be eliminated by rederivation. It is readily excluded by proper adherence to modern practices of barrier room technology (Hasslinger and Wiethe, 1987).

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), and intestinal electrolyte transport (Lubcke *et al.*, 1992). In mice, oxyuriasis has been reported to alter exploratory behavior (McNair and Timmons, 1977) and to increase antibody production to other antigens (Sato *et al.*, 1995).

Acariasis

Radfordia ensifera is the only ectoparasite of rats that one is likely to encounter in a laboratory animal environment, although it should be distinguished from other acarids, such as *Myobia musculi*, which could possibly be harbored briefly on the rat's pelage. *R. ensifera*, like *R. affinis*, which is more commonly found in mice, has two empodial claws on the second pair of legs (Figures 7.58 and 7.59), whereas *Myobia* only has one (Flynn, 1973a). In *R. ensifera* the empodial claws are unequal in length, whereas in *R. affinis* they are equal. In the author's experience, this difference in length is subtle.

The infestation is 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. Infestation can result in pruritus, self-excoriation and secondary bacterial infections. In mice, **acariasis** has also been associated with increased mitotic activity in the skin, immunologic alterations and amyloidosis (Weisbroth, 1982).

Acariasis is most practically diagnosed by direct examination of the animals with a dissecting scope

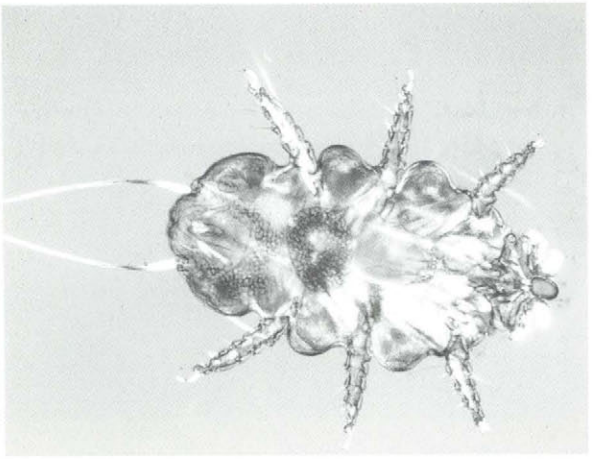

Figure 7.58 Female *Radfordia* sp. Use of polarized light helps in assessment of the two claws on the second pair of legs, since the more sclerotized body parts are anisotropic, as is the internal striated musculature. The first pair of legs, located near the mouth parts, is short and compressed for grasping hairs. × 74.

(Flynn, 1973a). As an alternative, dead rats or their pelts can also be placed in a sealed clear glass or plastic container and refrigerated overnight, then examined against a black background.

Control of acariasis is similar to that for other parasitic metazoa. As with oxyuriasis, acariasis can be eliminated in individual rats with Ivermectin (West *et al.*, 1992). However, the source of the original infestation should be identified, and the premises should be thoroughly disinfected so as to prevent reinfestation. Ivermectin is not effective against eggs, which can persist for long periods in the environment. Infestation can also be eliminated by rederivation, and is readily excluded by proper adherence to modern practices of barrier room technology (Weisbroth, 1982).

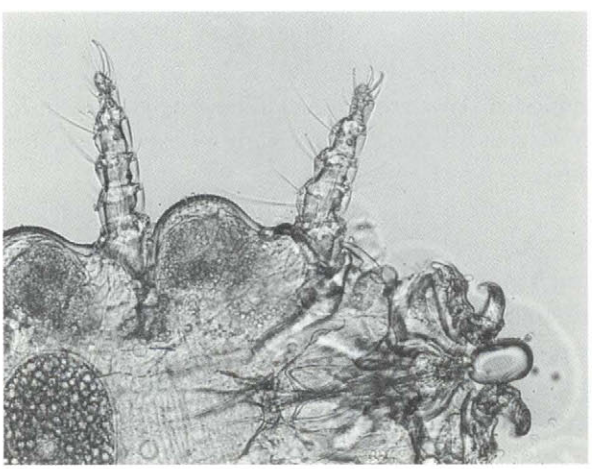

Figure 7.59 Female *Radfordia* sp. The second pair of legs has two claws. × 295.

Pneumocystosis

Pneumocystosis, caused by *Pneumocystis carinii*, has long been recognized as a disease of immunodeficiency in a multitude of mammalian host species (Franklin and Riley, 1993). Molecular biology techniques have determined that *P. carinii* is a fungus (Stringer, 1993; Keely *et al.*, 1994), and have also demonstrated considerable genetic variation between the *P. carinii* strains infecting different host species (Cushion *et al.*, 1993). For example, *P. carinii* strains infecting humans and rats are so widely divergent that they are not considered to be cross-infective.

In rats, clinical signs are observed only with congenital immunodeficiency (Furuta *et al.*, 1993) or prolonged impairment of host defenses, including administration of immunosuppressive agents (Sukura *et al.*, 1991; Armstrong *et al.*, 1991; Oz and Hughes, 1996), such as cyclophosphamide and corticosteroids. Infection is persistent and progressive; therefore, clinical signs are more likely to be observed in animals older than six months of age. In immunodeficient animals, emaciation, dyspnea and cyanosis may be observed (Furuta *et al.*, 1993).

P. carinii spores are widespread in the environment, and infection is transmitted via inhalation of these spores (Chandler *et al.*, 1979). Trophozoites attach primarily to type I alveolar epithelial cells, and to a lesser extent type II alveolar epithelial cells (Yoneda and Walzer, 1980). Infection results in pulmonary insufficiency through damage to type I alveolar epithelial cells, damage to alveolar capillaries and filling of alveoli with a frothy material. This material is composed of disintegrating parasites, antigen–antibody complexes, fibrin and other serum proteins. Infection interferes with research utilizing immunodeficient and immunosuppressed animals primarily through the morbidity it causes.

Gross lesions are absent in **occult infections**. In clinical infections, lungs often fail to collapse when the thorax is opened, and may have gray-brown foci suggestive of interstitial pneumonia (Figure 7.60). The affected animals are often emaciated and occasionally cyanotic.

Histopathologic examination of hematoxylin and eosin stained sections from affected rats reveals a frothy to honeycombed, eosinophilic material with small, faint, basophilic granules (trophozoite nuclei) in alveolar spaces and the lumina of the airways (Figures 7.61 and 7.62) (Chandler *et al.*, 1979; Lanken *et al.*, 1980). In some occult infections, only meager amounts of the honeycombed material

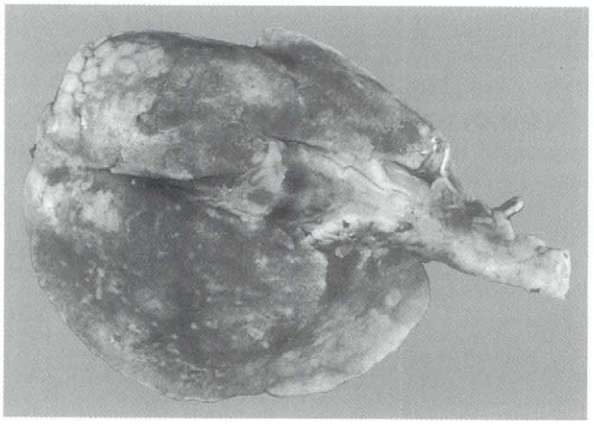

Figure 7.60 *Pneumocystis carinii* pneumonia in an athymic nude rat. The posterior portion of both lung lobes is pale and consolidated.

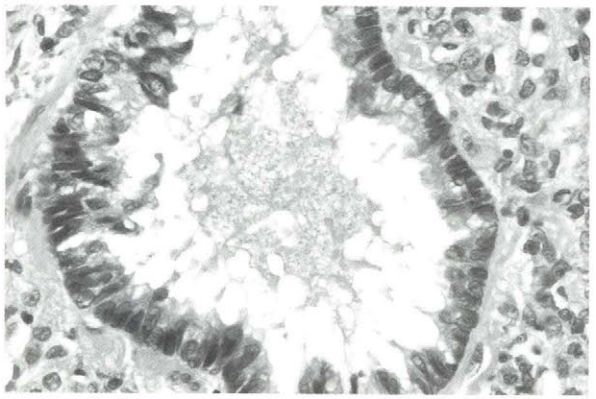

Figure 7.61 *Pneumocystis carinii* pneumonia in an athymic nude rat. There is a modest amount of a frothy eosinophilic material in the lumen of a bronchiole. Pale basophilic coccoid bodies (trophozoite nuclei) are barely visible in the frothy material. Hematoxylin and eosin. × 95.

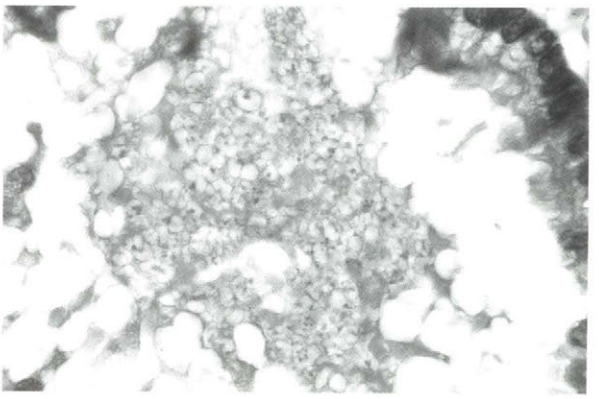

Figure 7.62 *Pneumocystis carinii* pneumonia in an athymic nude rat. This is a higher magnification of Figure 7.61. At this magnification the trophozoite nuclei (pale basophilic coccoid bodies) can be visualized. Hematoxylin and eosin. × 180.

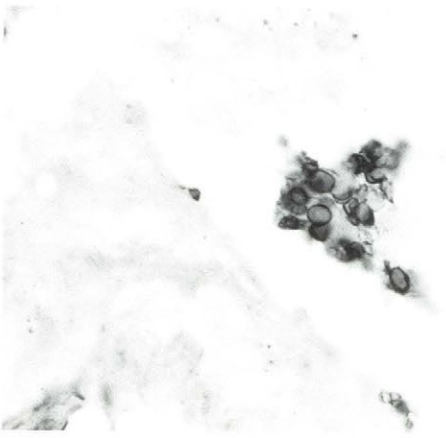

Figure 7.63 *Pneumocystis carinii* pneumonia in an athymic nude rat. There are numerous dark staining mature *P. carinii* cysts. Grocott's methenamine silver. × 180.

may be observed attached to alveolar septa. An interstitial pneumonia may or may not be observed in association with the infection. Methenamine silver stains (Kusnitz *et al.*, 1994) reveal round, oval and folded cysts (Figure 7.63), although these may be few in number, and should not be confused with artifactual staining of host cells. Immunohistochemical stains are sometimes employed as an adjunct for definitive identification.

Diagnosis of pneumocystosis is most often based on histopathologic examination of appropriately stained sections. Molecular biologic techniques employing the polymerase chain reaction have been developed in recent years, and are useful in both immunocompetent and immunodeficient animals (Kitada *et al.*, 1991; Lu *et al.*, 1995). Immunosuppression, followed by histologic techniques, is sometimes used to screen immunocompetent rats for latent infection (Sukura *et al.*, 1991; Oz and Hughes, 1996). Serologic screening of immunocompetent rat populations is also possible, but is not widely employed.

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