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Chapter 11

Viral Diseases

Robert O. Jacoby, Pravin N. Bhatt, and Albert M. Jonas

THE LABORATORY RAT, VOL. I

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I. INTRODUCTION

The number of viruses known to be naturally infectious for laboratory rats is small, and most cause inapparent infections which usually are detected by serological monitoring (Table I).

Table I

Viruses of Laboratory Rats

I. DNA viruses
  A. Viruses which cause naturally occurring infections in rats
    1. Rat parvoviruses (RV, H-1 virus, and related viruses)
      Natural host: Rat
      Signs: Usually asymptomatic; may have decreased litter size or decreased numbers of litters; neonatal deaths; occasional runting, jaundice or ataxis in sucklings or weanlings; neurological disease or sudden death in adults
      Lesions: Usually none; may be hemorrhages and necrosis in central nervous system, testes or elsewhere; cerebellar hypoplasia and/or hepatic necrosis and fibrosis in sucklings or weanlings; resorption sites in uterus; intranuclear inclusion bodies in brain, liver, endothelium, or elsewhere
      Epizootiology: Persistent and latent; highly infectious, spreads rapidly; transmission usually horizontal via oral or respiratory route but some strains can be transmitted vertically; virus may be excreted in feces, urine, and milk
      Diagnosis: Clinical signs and/or lesions, if present; detect antibody by HAI, CF, NT or FA tests;[a] isolate virus in primary rat embryo cultures; inoculate parvovirus-free sucklings with suspect tissues and test serum for antibody 2 to 4 weeks later
      Differentiate from: Sendai virus infection (production losses); chemical intoxication, tumors, trauma, genetic abnormalities
      Treatment: None
      Control: Destroy colony and repopulate from virus-free stock, disinfect facilities and equipment; institute routine serological surveillance to detect infection
    2. Cytomegalovirus
      Natural host: Rat (primarily wild rats)
      Signs: None
      Lesions: Usually none; intranuclear inclusion bodies in enlarged cells of salivary or lacrimal glands; mild nonsuppurative adenitis
      Epizootiology: Not well studied
      Diagnosis: Lesions with inclusion bodies; test serum for NT antiantibody have been detected in rats; virus has not been isolated from rats
      Treatment: None
      Control: Not studied
  B. Viruses which may infect rats[b]
    1. Minute virus of mice
      Natural host: Mouse
      Significance: Latent parvovirus of mice, but low titers of HAI antibody have been detected in rats; virus has not been isolated from rats
    2. Mouse adenovirus
      Natural host: Mouse
      Significance: CF antibodies have been detected in rats, but virus has not been isolated from rats
II. RNA Viruses
  A. Viruses which cause naturally occurring infections in rats
    1. Sialodacryoadenitis virus (SDAV)
      Natural host: Rat
      Signs: Photophobia; keratoconjunctivitis; red-brown (porphyrin-containing) tears staining skin around eyes and nares; sneezing; enlarged salivary glands; cervical edema
      Lesions: Acute rhinitis; necrosis and inflammation of submaxillary and parotid salivary glands and lacrimal glands; squamous metaplasia of glands during repair; cervical edema; cervical lymph node hyperplasia; transient thymic atrophy; keratoconjunctivitis with occasional megaloglobus; interstitial pneumonia not reported, but cannot be ruled out
      Epizootiology: Acute, self-limiting infection; nonimmune rats of all ages susceptible; high morbidity; low mortality; transmitted by aerosol; virus excreted for about 1 week then CF and NT antibody detectable in serum; virus antigenically related to rat coronavirus and mouse hepatitis virus; reinfection not reported, but cannot be ruled out
      Diagnosis: Clinical signs and/or lesions; test for serum antibody by CF or NT tests; isolate virus in primary rat kidney cell cultures (must differentiate from RCV)
      Differentiate from: Rat coronavirus infection; murine respiratory mycoplasmosis; Sendai virus infection; cytomegalovirus infection; eye irritation from chemical fumes (e.g. ammonia); bacterial infections
      Treatment: Usually none; eye lesions may be treated symptomatically
      Control: Quarantine rats for 6 to 8 weeks during outbreak, stop breeding, quarantine rats from infected colonies for 30 days before placing them among susceptible rats; maintain rats in barrier facility; institute routine serological surveillance to detect infection
    2. Rat coronavirus (RCV)
      Natural host: Rat
      Signs: Usually asymptomatic; may be occasional salivary gland enlargement and cervical edema
      Lesions: Acute rhinitis; mild focal interstitial pneumonia; occasional necrotizing sialoadenitis
      Epizootiology: Same as SDAV
      Diagnosis: Clinical signs if present; lesions; detect serum antibody by CF and NT tests; isolate virus in primary rat kidney cell cultures (must differentiate from SDAV)
      Differentiate from: SDAV infection; murine respiratory mycoplasmosis; Sendai virus infection
      Treatment: None
      Control: As described for SDAV
    3. Sendai virus (parainfluenza 1)
      Natural host: Rat, mouse, hamster, guinea pig
      Signs: Usually asymptomatic; may be rough haircoat, dyspnea, production losses in breeding colonies
      Lesions: Bronchopneumonia and interstitial pneumonia with necrosis of bronchial and/or bronchiolar epithelium; upper respiratory tract usually not involved; peribronchial lymphocytic infiltrates may persist for months
      Epizootiology: Acute, self-limiting; nonimmune rats of all ages susceptible; high morbidity, low mortality; transmitted by aerosol; antibody detectable in serum by 7 to 14 days postinfection; chronology of virus excretion and stability of immunity not established for rats
      Diagnosis: Clinical signs, if present; lesions; detect serum antibody by CF test; HAI and NT tests also available; isolate virus in BHK-21 cells or embryonated eggs
      Differentiate from: Murine respiratory mycoplasmosis; coronavirus infection; RV infection (production losses; intrauterine resorption sites)

(*continued*)

Table I (*Continued*)

Treatment: None
Control: Quarantine rats for 4 to 8 weeks during outbreak including cessation of breeding; quarantine rats from infected colonies for 30 days before placing them among susceptible rats; institute routine surveillance of vendor rats for infection

B. Viruses which may infect rats
1. Reovirus 3
Natural host: Many mammals
Significance: HAI antibodies have been detected in rats, but virus has not been isolated
2. Pneumonia virus of mice (PVM)
Natural host: Mouse
Significance: HAI and NT antibodies have been detected in rats, but virus has not been isolated
3. Mouse encephalitis virus
Natural host: Mouse
Significance: HAI and NT antibodies have been detected in rats but virus has not been isolated

III. Unclassified viruses or viruslike agents
A. MHG Virus
Natural host: Rat; other species?
Significance: Enterovirus-like neurotropic agent; can cause neurological signs and necrotizing inflammation of brain in experimentally infected rats; may be antigenically related to mouse encephalomyelitis virus; epizootiology unknown
B. Rat submaxillary gland (RSMG) virus
Natural host: Rat.
Significance: Latent, vertically-transmissible agent isolated from submaxillary gland; no signs or lesions; induces HAI antibody; unrelated antigenically to rat coronaviruses or cytomegalovirus; must differentiate isolates from coronaviruses, cytomegaloviruses
C. Novy virus
Natural host: Rat
Significance: Extremely stable filterable agent recovered from rat blood. Agent and its pathogenicity not well studied
D. Viruslike pneumotropic agents (enzootic bronchiectasis agent; gray lung virus; wild rat pneumonia agent)
Natural host: Rat; mouse?
Significance: Incompletely characterized agents associated with pneumonias of laboratory and/or wild rats.

[a]HAI, hemagglutination inhibition; CF, complement fixation; NT, neutralization; FA, fluorescent antibody.

[b]Mousepox virus may be mildly infectious for experimentally inoculated rats, but natural infections have not been reported and evidence of natural immunoreactivity to the virus has not been detected.

Agents from three families of viruses are especially well disseminated among rat colonies: rat virus (RV) and related strains (Parvoviridae), sialodacryoadenitis virus (SDAV) and rat coronavirus (RCV) (Coronaviridae), and Sendai virus (parainfluenza 1) (Paramyxoviridae). Rat parvoviruses normally induce latent infections, but, under conditions to be discussed, they may be lethal or cause vascular, neurological, and hepatic lesions. Sialodacryoadenitis virus causes nonlethal, self-limiting disease characterized clinically by oculonasal accumulations of lacrimal porphyrin pigment, photophobia, and cervical swelling from enlarged salivary glands and morphologically by necrotizing inflammation in the upper respiratory tract, salivary glands, and lacrimal glands. There is recent evidence that keratoconjunctivitis, sometimes resulting in permanent eye damage, can also accompany SDAV infection. Rat coronavirus may cause sialoadenitis, but appears to be more pneumotropic than SDAV. It can induce mild interstitial pneumonia in adult rats and can cause fatal pneumonias in sucklings. Sendai virus has received increased recognition as an important virus of rats, and current evidence indicates that natural infection causes pneumonia.

The impact of other viruses naturally infectious for rats has not been thoroughly studied. Humoral antibodies to minute virus of mice (MVM), pneumonia virus of mice (PVM), reovirus 3, mouse encephalomyelitis virus, and murine adenovirus have been detected in rats. The corresponding viruses, however, are currently considered nonpathogenic for rats. Similarly, cytomegalovirus inclusions have been found in rat tissues, but are not associated with clinical disease or significant lesions. Several other viruses [MGH virus, rat submaxillary gland virus (RSMG), and Novy virus] and viruslike agents (enzootic bronchiectasis, gray lung, wild rat pneumonia) have been reported in rats, but their significance is unclear. Therefore, this chapter concentrates primarily on rat parvoviruses, rat coronaviruses, and Sendai virus. The viruses are reviewed individually, but the reader is encouraged to peruse Section VII for broad approaches to the detection, diagnosis, and control of virus infections in rat colonies. Oncogenic viruses of rats, potential or confirmed, are not discussed.

II. DNA VIRUSES

A. Parvoviruses (Rat Virus, H-1 Virus, and Minute Virus of Mice)

1. Rat Virus (RV) and H-1 Virus

a. General. Parvoviruses are extremely small (18–26 nm) icosahedral viruses that are remarkably stable to heat, acid, lipid solvents, and prolonged exposure to room temperature. Some are defective and require adenovirus "helpers" to replicate, but rat parvoviruses are not defective. Parvoviruses have been isolated from rodents, birds, dogs, cats, pigs, cattle, nonhuman primates, and humans. They appear to have a predilection for tissues containing rapidly dividing cells and may induce latent or subclinical infections. Recent reviews offer additional background information (134,142).

Rat virus (RV) is the type species for the genus *Parvovirus* and was the first virus isolated of the family Parvoviridae. The literature on rat parvoviruses is extensive, but much of it pertains to experimentally induced infections in hamsters and several other species, as well as in rats. Since this report centers on natural parvovirus infections of rats, only experimentally

induced infections which contribute to understanding of natural infections will be included.

b. History. Rat virus was isolated from several tumor-bearing rats by Kilham and Olivier (78) during studies to determine if rats harbored a polyoma-like virus. Rat virus seemed nonpathogenic for newborn and weanling rats, suckling and weanling mice, suckling hamsters, and adult rabbits and guinea pigs. They also found natural RV neutralizing (NT) and hemagglutination inhibiting (HAI) antibodies in serum of conventional rats and germfree rats. This suggested that RV was not only naturally infectious for rats but also could be transmitted vertically. Toolan (148,149,155) isolated a second parvovirus from a human tumor cell line (HEp-1) that had been passaged in rats and named it H-1 virus. Dalldorf (37) found a third parvovirus-like agent in rat-passaged human tumor HEp-3 and named it H-3 virus (also known as OLV). Moore (110) showed that H-1 virus was antigenically distinct from RV, whereas Dalldorf's virus was antigenically related to RV, but it did not appear to share antigens with H-1. Nevertheless, RV, H-1, and H-3 were similar biochemically and physically. Furthermore, each could, under suitable conditions, induce severe developmental abnormalities, particularly in suckling hamsters, despite the fact that they usually caused latent persistent infections in weaned rats.

Additional strains of RV-related and H-1-related rat parvoviruses have been characterized since the pioneering work of Kilham, Toolan, and Dalldorf. Most are antigenically related to RV (Table II). There is some controversy about whether H-1 virus is of human or rat origin. It is clear, however, that natural infections with RV and H-1 viruses are widespread in laboratory rats and in wild rats.

c. Properties. i. Physiochemical. Rat virus and H-1 virus are single-stranded DNA viruses (62,102,139). They range in size from 18 to 30 nm depending on conditions of purification and measurement and appear to have 32 capsomeres (69,142,158). Rat virus has a bouyant density in CsCl of about 1.40 gm/ml, and its molecular weight is approximately 6.6×10^6 daltons (138). Rat virus and H-1 virus retain infectivity and hemagglutinating activity after exposure to ether, chloroform, or alcohol and are stable at pH's from 2 to 11 at 4°, 25°, and 37°C (47,78). They also are remarkably temperature resistant. Rat virus can remain infectious after exposure to 80°C for up to 2 h, for more than 6 months at −40°C, and for up to 60 days at 40°C (162). The stability of RV and H-1 virus to variations of temperature and pH may, however, depend on virus strain and supporting medium (18,86). These viruses are inactivated by ultraviolet light at room temperature (25,155). They are, however, resistant to ultrasonication, RNase, DNase, papain, trypsin, and chymotrypsin—properties which facilitate preparation of purified virus from infected cell cultures (20,46,97,154,158).

Table II

Antigenic Type of Some Rat Parvovirus Isolates

Antigenic type	Virus	Source of original isolate	Reference
RV	RV	Rat tumor	78
	H-3	HEp-3 cells	37
	X-14	Rat tumor	124
	L-5	Rat tumor	86
	HB	Human placenta	151
	SpRV	Rat tissue	73
	HER	Rat tissue	40
	HHP	Rat tissue	94
	Kirk	Detroit-6 cells human serum	16
H-1	H-1	HEp-1 cells	155
		Rat tissue	74
	H-T	Human placenta	151

ii. Hemagglutination and hemadsorption. Hemagglutination is a common property of rat parvoviruses and is the simplest method to detect them. Guinea pig erythrocytes are preferred to detect RV, H-1 virus, and H-3 virus. The test can be run at 4°, 25°, or 37°C (78,110). Rat parvoviruses agglutinate erythrocytes of other species to variable degrees, and it has been suggested that differential agglutination be used to identify virus strains (153,154). Since the hemagglutinin is cell associated, cell debris should be pretreated with deoxycholate, receptor-destroying enzyme, or, preferably, alkaline buffer (48). Portella (126) showed that RV, H-1, and H-3 caused adsorption of guinea pig erythrocytes to virus-infected cells, but this technique is not generally used as a diagnostic test.

iii. Antigens. Neutralizing (NT), hemagglutination inhibition (HAI), and complement fixation (CF) tests have shown that rat parvoviruses consist of two major antigenic groups (36,86,110,126,151,154). One group includes RV, H-3, X-14, and HER viruses, and the other includes H-1 and HT viruses (Table II). Some cross-reactivity has been detected by immunofluorescence (FA) among RV, H-1 virus, and MVM (36,54).

d. In Vitro Cultivation. Rat virus and H-1 replicate well in primary monolayer cultures of rat embryo cells (78). Hamster embryo cultures support viral growth less well, and cells of mouse, chicken, calf, or human origin are unsuitable for RV, but the H viruses can grow in human, monkey, rat, and hamster cells [reviewed by Siegl (142)]. Primary cultures should be prepared from rats free of RV and H-1 virus infection, and all cell lines should be checked for latent contamination with parvoviruses.

Rat parvovirus-infected cultures usually develop cytopathic

changes characterized by intranuclear inclusions and necrosis (78,110,156). The inclusions can be detected with standard histological stains. The course and severity of cytopathic effects (CPE) depend on several factors in addition to the target cells used. For example, high doses of virus may cause CPE in several days, whereas cultures for endpoint titrations should be held for up to 3 weeks (86,110,156). The sensitivity of methods used to detect CPE vary. Cole and Nathanson (29) detected antigen to the HER strain of RV using immunofluorescence by 5 to 6 h and infectious virus by 20 h after infection with a large dose (100 $TCID_{50}$/cell) of RV. Viral antigen was first detected in the cytoplasm and then in the nucleus before cell necrosis appeared. Bernhard *et al.* (8) showed that margination of chromatin occurred in some cells infected with RV by 36 to 48 h. Mayor and Ito (98) detected X-14 viral antigen with immunofluorescence by 12 h postinfection and hemagglutination (HA) activity and infectious virus by 48 h. Margolis and Kilham (89) reported that RV multiplication was enhanced in dividing cultured cells. Similarly, studies by Cole and Nathanson (29) suggested that dividing BHK-21 cells supported RV replication better than nondividing cells. Analogous results were reported for H-1 virus grown in WI-26 human diploid cells (81). The morphology of viral replication and inclusion body formation have been described in detail (8,38,99,126).

e. Host Range. Laboratory and wild rats appear to be the only natural hosts for rat parvoviruses. There is still debate as to whether the H viruses also may be naturally infectious for humans (154). Rat parvoviruses are, however, infectious for several species by experimental inoculation. Rat virus and H-1 virus can infect neonatal, suckling, and adult rats and hamsters (70,73,78,111,112,149,155). Experimental RV infection also has been reported in neonatal mice (40,96), *Praomys* (*Mastomys*) *natalensis* (129), and newborn kittens (72). H-1 virus infection has been induced in newborn and adult rhesus monkeys (*Macaca mulatta*) and in human volunteers (152). Attempts to induce RV infection in newborn rhesus monkeys were unsuccessful (154).

f. Clinical Disease. Kilham and Margolis (73) were first to report natural RV disease. They had purchased 13-day pregnant rats from a commercial breeder, and the embryos were harvested to prepare primary tissue cultures. Embryos cultured from one rat with three uterine resorption sites contained RV. Rat virus also was isolated from three undersized pups in a second litter of eleven, and one other pup from the litter developed ataxia from cerebellar hypoplasia and was euthanatized at 45 days. A third litter consisted of two small pups. They developed severe jaundice shortly after birth and were killed at 4 days; the dam had nine intrauterine resorption sites. A fourth pregnant rat was killed at gestation day 16. One of 10 fetuses was abnormal with a mottled placenta, and RV was isolated from the fetus.

The strain of RV isolated from these rats was designated SpRV. It proved highly infectious for pregnant and nonpregnant rats and could be isolated from tissues, milk, and feces after experimental inoculation. Furthermore, it crossed the placenta effectively and caused fetal death or teratogenesis when inoculated early in gestation, and postnatal cerebellar destruction and hepatitis when inoculated late in gestation. Simultaneous intraperitoneal (ip) and intracerebral (ic) inoculation of newborns with SpRV also caused cerebellar and liver lesions and pups usually died by 8 days. One control suckling developed cerebellar disease from contact infection.

Nevertheless, natural RV disease among newborn and suckling rats is sporadic (73), despite the high prevalence of RV infection among commercial and institutional colonies. In contrast, reduced litter size, runted litters, or decreased production with increased numbers of resorption sites in uteri of breeding females should be considered potential signs of parvovirus infection. RV infection of nonimmune pregnant dams in a production colony can be expressed initially by decreased litters or litter size followed by a progressive return to normal production as immunity to RV builds.

Parvovirus infection in adult rats is usually asymptomatic, but latent infection can be activated by immunosuppression (40). We have, however, followed a natural enzootic of severe RV infection in a colony of Sprague-Dawley rats (66). Two groups of nonimmune male weanling rats, numbering 100 and 50, respectively, were introduced to a colony known to be latently infected with RV. Two weeks later most of the newly introduced rats developed ruffled haircoats, dehydration, and cyanotic scrotums. Twenty-five rats from the first group and seven from the second group died. Rat virus was isolated from affected rats and hemorrhagic lesions compatible with RV infection were detected (see Section II, A, 1, g).

The spectrum of clinical signs associated with RV infection likely depends on the strain of virus involved in a given outbreak. Strains capable of infecting the gravid uterus of nonimmune dams may affect production. Strains restricted to horizontal transmission probably produce largely silent infections, but may occasionally provoke acute fatal disease in neonates or adult rats. The transmission of parvoviruses is discussed in more detail in Section II, A, 1, h.

g. Pathology. Parvoviruses can be distributed widely in tissues of infected rats, but they most commonly cause lesions in tissues containing mitotically active cells, such as the developing liver and cerebellum. Since resistance to these agents usually develops during the first week or two of life, much information about the pathogenesis of parvovirus lesions stems from study of infected pregnant dams, neonates, or young sucklings. Rat parvoviruses also have been studied extensively

in heterologous hosts, particularly suckling hamsters, in which they not only cause cerebellar lesions but also induce severe mongoloid developmental abnormalities and dental deformities. The reader is referred to several reviews for details about these lesions (37,89,154).

The cerebellar lesions in pups, either naturally or experimentally infected with RV, are characterized by development of intranuclear inclusions in rapidly dividing cells of the external germinal layer of cerebellum (Fig. 1) and then by necrosis (Fig. 2) (73). Since cells of the external granular layer migrate to form the internal granular layer, their depletion aborts cerebellar development and results in granuloprival cerebellar hypoplasia with ataxia (Fig. 3).

The liver lesions also begin with intranuclear inclusions in hepatocytes (Fig. 4), and less frequently in Kupffer cells, biliary epithelium, endothelial cells (Fig. 5), and connective tissue cells (73). Ensuing cytopathic changes are characterized by nuclear pyknosis, cytoplasmic eosinophilia, ballooning degeneration, dissociation of affected cells from adjacent cells and finally cell lysis. Other changes may include hepatocytic bile retention and pigmented casts (ostensibly bile) in renal tubules.

Margolis *et al.* (94) reported that fresh isolates of RV and H-1 virus were more virulent for experimentally infected newborn rats than tissue culture passaged strains. Furthermore, rats inoculated as sucklings (3 to 8 days) rather than as neonates

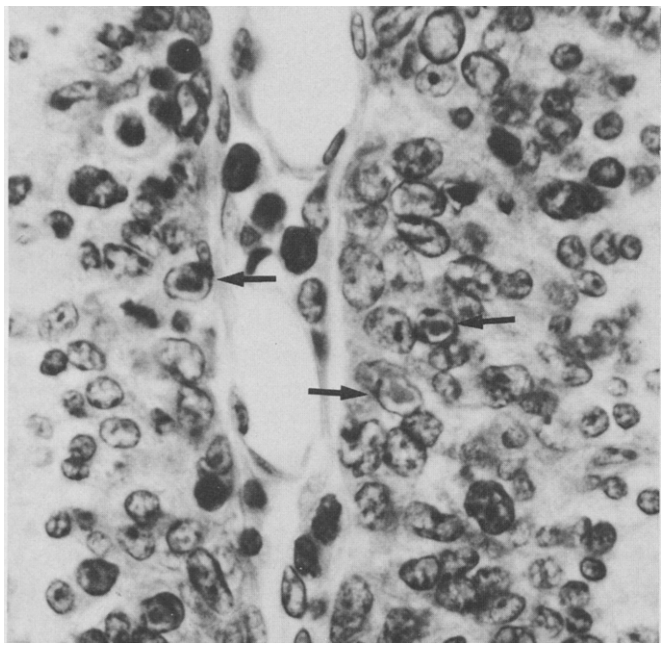

Fig. 1. Cerebellum from a 4-day-old rat naturally infected with RV. The inclusion body phase is shown. The central fissure is bordered by the external germinal layer, several cells of which contain intranuclear inclusion bodies (arrows). [Courtesy of Dr. Kilham and Dr. Margolis (73); and the *American Journal of Pathology.*]

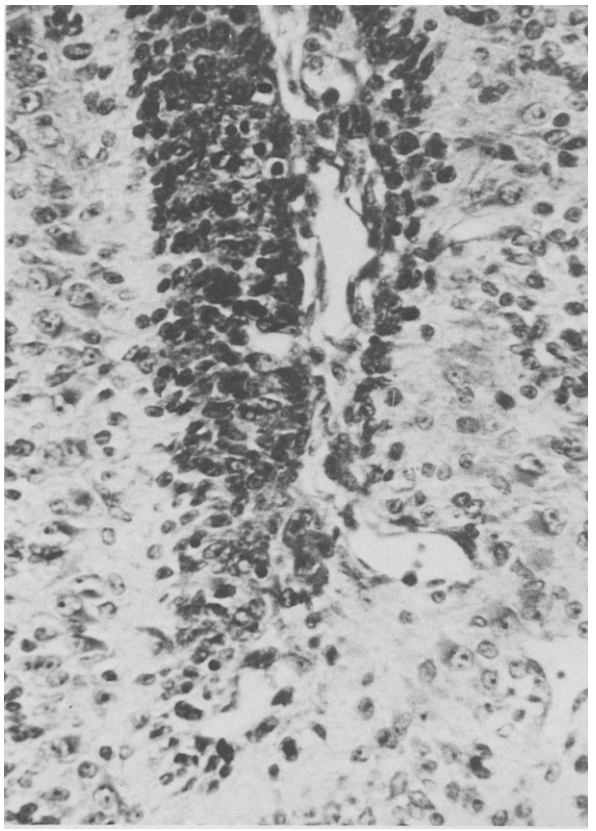

Fig. 2. Cerebellum from a 4-day-old rat naturally infected with RV. The external germinal layer has been partially destroyed. The leptomeninges are moderately infiltrated with mononuclear cells. [Courtesy of Dr. Kilham and Dr. Margolis (73); and the *American Journal of Pathology.*]

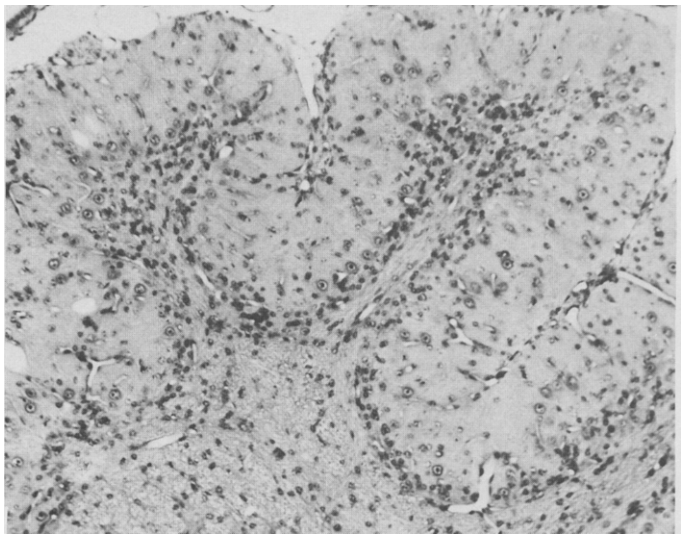

Fig. 3. Cerebellum from a 37-day-old rat inoculated intracerebrally at birth with SpRV. There is severe granuloprival hypoplasia and haphazard distribution of Purkinje cells. [Courtesy of Dr. Kilham and Dr. Margolis (73); and the *American Journal of Pathology.*]

had less severe infections, even with virulent strains. Other workers also have found that acute lethal disease, often accompanied by hemorrhage, was most easily induced in neonatally inoculated rats (111,116). Kilham and Margolis (74) found that typical cerebellar lesions developed only in rats inoculated before day 4. Hepatitis with jaundice but without fulminating generalized disease occurred in sucklings inoculated between days 6 and 12. Sucklings inoculated after day 12 developed only mild self-limiting hepatitis. The only sign of infection in adults was seroconversion.

Inclusions appeared in liver by 24 h after infection and persisted in some rats for up to 3 weeks. Persistence of virus in liver was attributed to increased mitotic activity postpartum which lasts up to 6 weeks. Ruffolo *et al.* (137) supported this view by showing that the susceptibility of adult rat liver to H-1 virus could be reestablished by partial hepatectomy or by carbon tetrachloride hepatotoxicity. Postnecrotic progression and repair of liver lesions occurred in several stages (94): (a) giant cell formation and polyploidy (Fig. 6); (b) biliary hyperplasia (Fig. 7), adenomatoid distortion of lobular architecture, and formation of blood-filled spaces (peliosis hepatitis) (Fig. 8); and (c) postnecrotic stromal collapse, fibrosis, and nodular hyperplasia (Fig. 9). Peliosis hepatitis has been produced in several strains of suckling rats and is presumably a sequel of RV-induced hepatic necrosis (7).

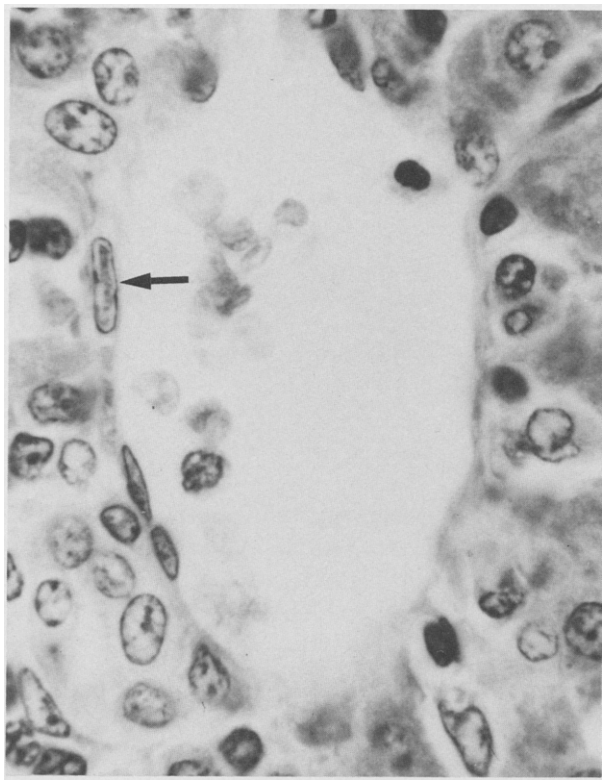

Fig. 5. An endothelial cell in an hepatic vein has an elongated intranuclear RV inclusion body (arrow). [Courtesy of Dr. Kilham and Dr. Margolis (73); and the *American Journal of Pathology*.]

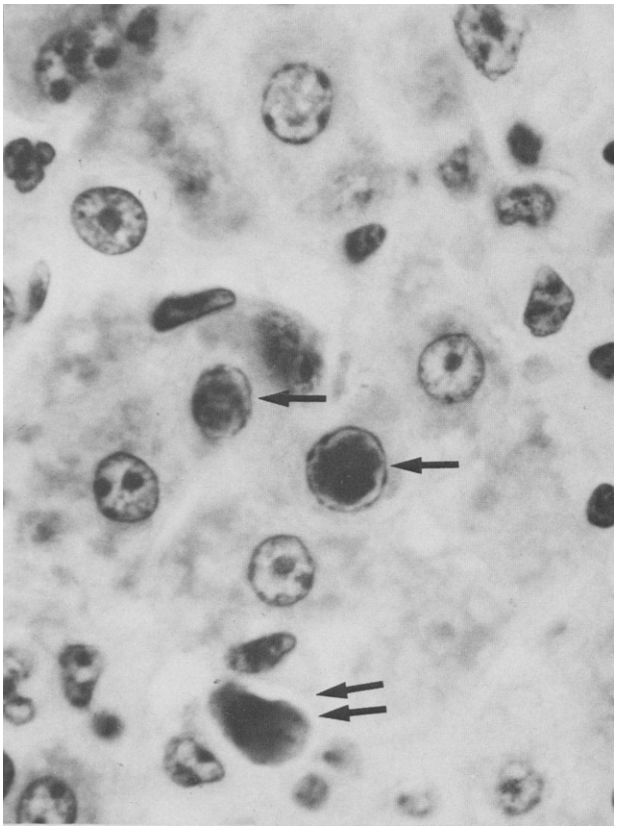

Fig. 4. Liver of a 4-day-old rat with natural RV infection. Several phases of inclusion body formation (arrows) are demonstrated in hepatocytes. A rounded eosinophilic necrotic hepatic cell (double arrows) is also shown. [Courtesy of Dr. Kilham and Dr. Margolis (73); and the *American Journal of Pathology*.]

Hemorrhagic encephalomyelopathy was first detected in about 2% of more than 1500 adult Lewis rats given a single dose of 100–225 mg/kg of cyclophosphamide (40,112). Lesions developed 10 to 25 days after treatment and consisted of multiple hemorrhages in medulla and spinal cord (Fig. 10). They were manifested clinically by paralysis. An agent, designated HER virus, was isolated from affected rats, and subsequently was shown to be a strain of RV. Hemorrhagic encephalomyelopathy was produced experimentally by intracerebral inoculation of suckling rats. It also occurred after intraperitoneal inoculation of virus into young adult rats given cyclophosphamide. Typical neurological disease and hemorrhagic lesions appeared in 1 to 4 weeks, but in only 20% of inoculated rats. Margolis and Kilham (91) showed that similar lesions could be induced by parenteral inoculation with several strains of RV and with H-1 virus (Figs. 11 and 12). Cole *et al.*

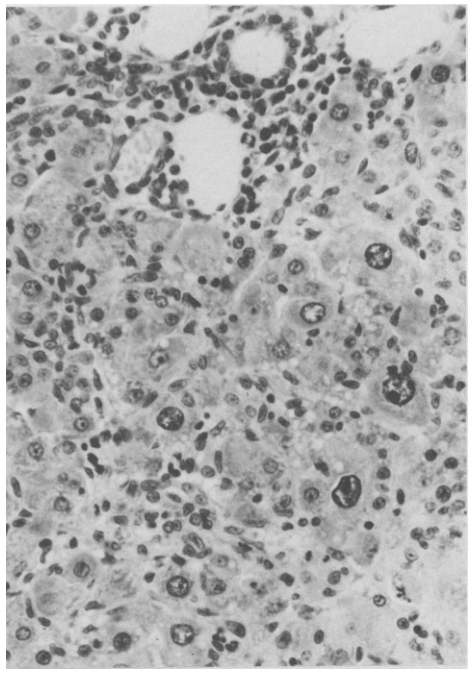

Fig. 6. Spectrum of cell and nuclear size in the liver of a rat inoculated with RV. There is also biliary hyperplasia and nonsuppurative portal hepatitis. Rats from this study were inoculated as neonates or sucklings. Lesions were detected in rats 16 to 43 days old. [Courtesy of Dr. Margolis, Dr. Kilham, and Dr. Ruffolo (94); and *Experimental and Molecular Pathology.*]

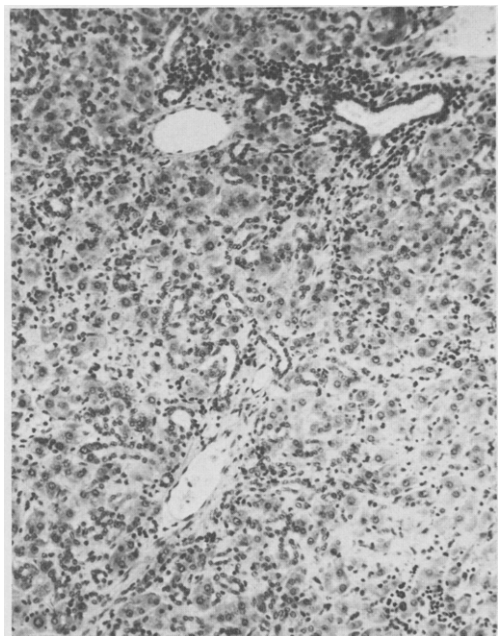

Fig. 7. Liver of a 19-day-old rat inoculated at 8 days of age with H-1 virus. Numerous, small, dilated, hyperplastic biliary ducts extend from portal areas deep into hepatic lobules. Hypocellular zones are remnants of necrotic foci. [Courtesy of Dr. Margolis, Dr. Kilham, and Dr. Ruffolo (94); and *Experimental and Molecular Pathology.*]

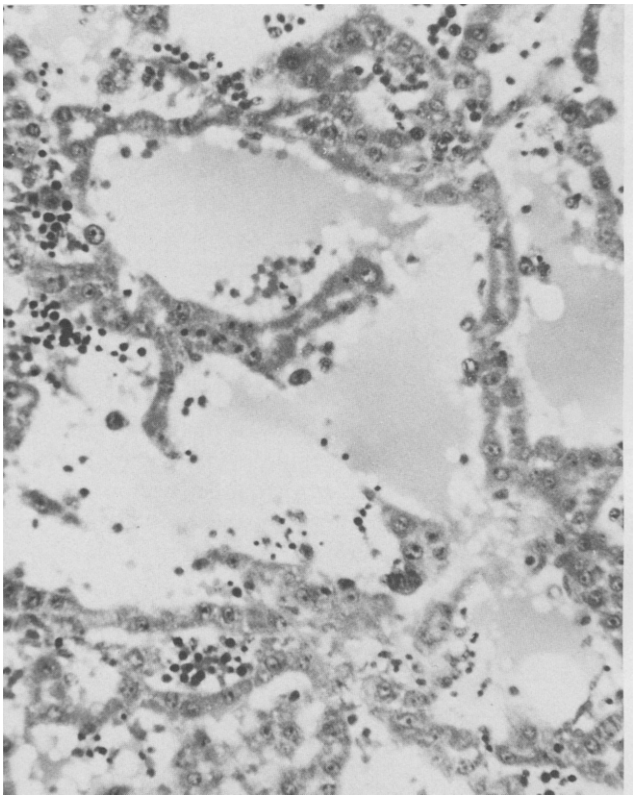

Fig. 8. Large vascular lakes (peliosis hepatitis) in the liver of a rat surviving RV hepatitis. Except for a rare endothelial cell or Kupffer cell only plates of hepatic cells line the cysts. [Courtesy of Dr. Margolis, Dr. Kilham, and Dr. Ruffolo (94); and *Experimental and Molecular Pathology.*]

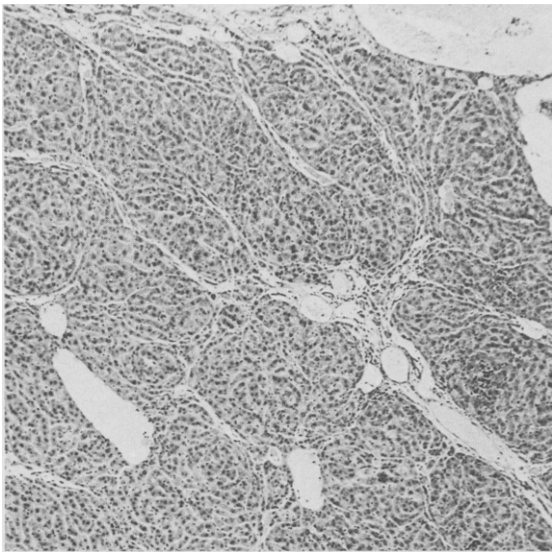

Fig. 9. Nodular hyperplasia and stromal collapse in the liver of a rat surviving RV hepatitis. Rat was inoculated at 8 days with HHP strain of RV and necropsied at 61 days. [Courtesy of Dr. Margolis, Dr. Kilham, and Dr. Ruffolo (94); and *Experimental and Molecular Pathology.*]

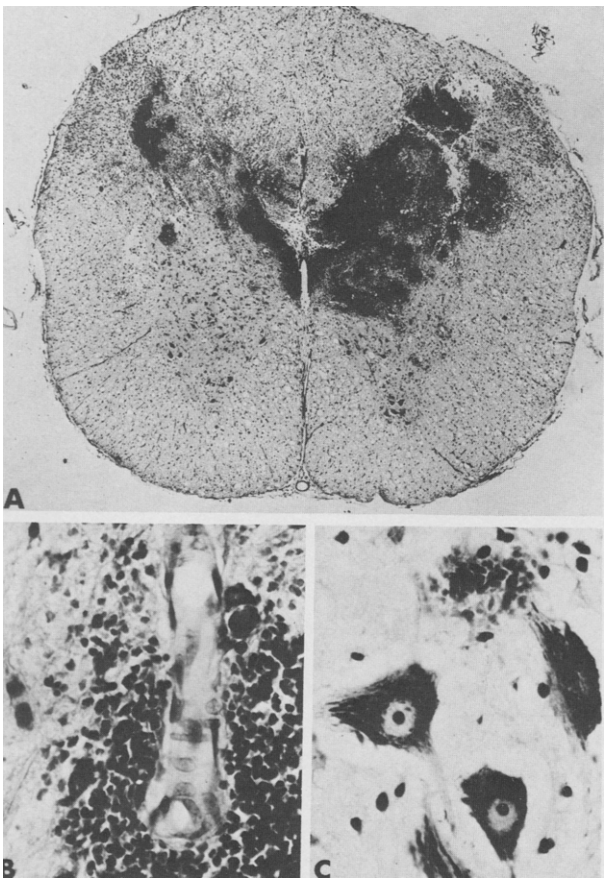

Fig. 10. Hemorrhagic myelopathy. Adult Lewis rat perfused at onset of paralysis 13 days after a single intraperitoneal dose of cyclophosphamide 100 mg/kg. Lumbar spinal cord. (A) Massive hemorrhage in both gray and white matter. (B) Area of typical bland hemorrhage in white matter, surrounding perfused vessel. (C). Small hemorrhage (top of frame) in anterior gray horn. [Courtesy Drs. N. Nathanson, G. A. Cole, G. W. Santos, R. A. Squire, and K. O. Smith (112); and the *American Journal of Epidemiology*.]

(30) reported that large doses of HER inoculated intracerebrally into newborn rats produced cerebellar destruction with hemorrhages throughout brain and spinal cord, whereas small doses produced jaundice and runting. Sucklings infected after day 10 remained well.

We have recently observed hemorrhagic lesions with thrombosis, and necrosis in the testicles and epididymis of weanling rats with naturally occurring RV infection (Figs. 13 and 14) (see Section II, A, 1, f.).

The hemorrhagic lesions appear to result from viral-induced injury to vessel walls. Inclusion bodies have been demonstrated in the vascular endothelium of small blood vessels and in arteriolar smooth muscle before extravasation of blood begins (Fig. 15) and hemorrhage is accompanied by vascular thrombosis and liquifaction necrosis (infarction) in the central

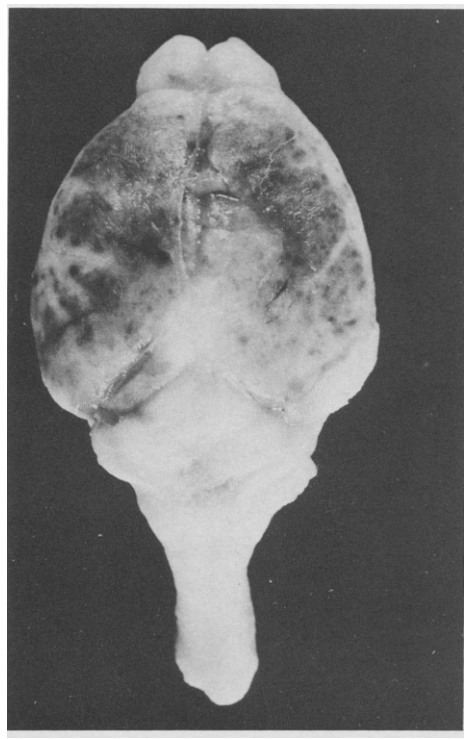

Fig. 11. Hemorrhagic encephalopathy in a suckling rat inoculated with RV. [Courtesy of Dr. Margolis and Dr. Kilham (91); and *Laboratory Investigation*.]

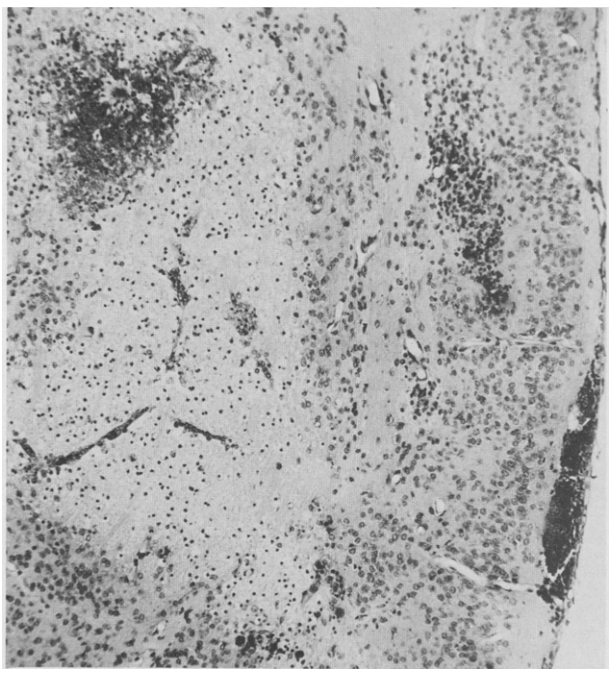

Fig. 12. Hemorrhagic encephalopathy in an 11-day-old rat infected 19 days previously with H-1 virus via intracerebral inoculation of the mother with H-1 virus. Hemorrhage, thrombosis, and malacia are evident. [Courtesy of Dr. Margolis and Dr. Kilham (91); and *Laboratory Investigation*.]

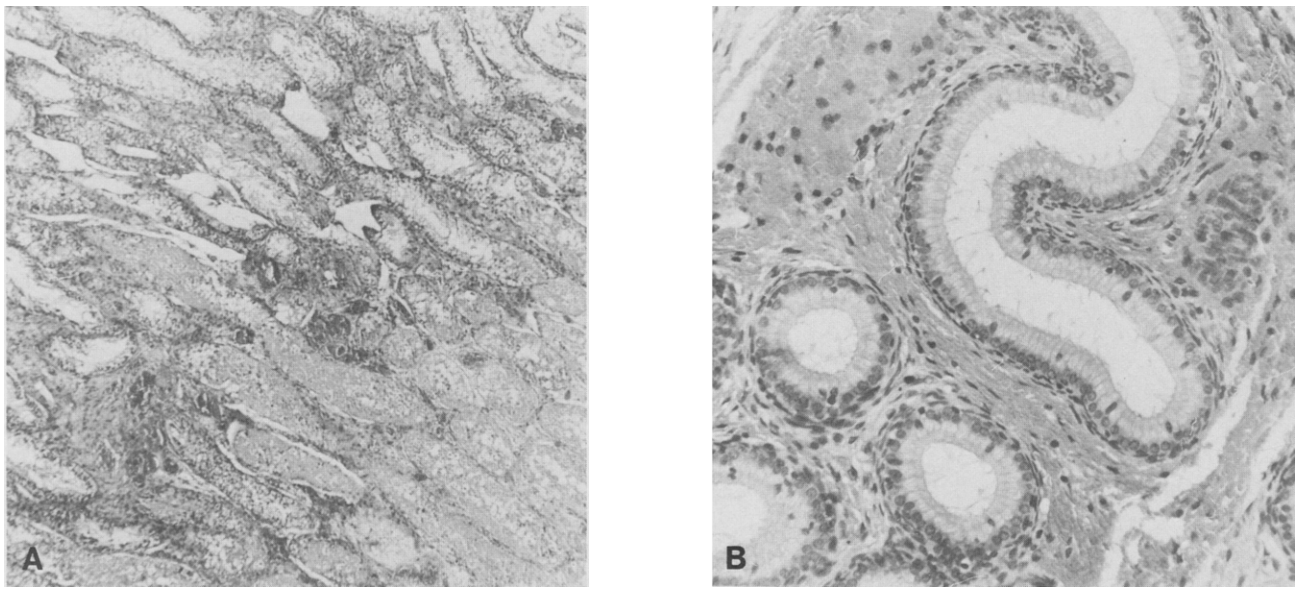

Fig. 13. Typical lesions in the testicle and epididymis from a natural outbreak of RV infection in young adult rats. (A) Testicle with coagulation necrosis of seminiferous tubules and interstitial hemorrhage. (B) Epididymis with interstitial hemorrhage.

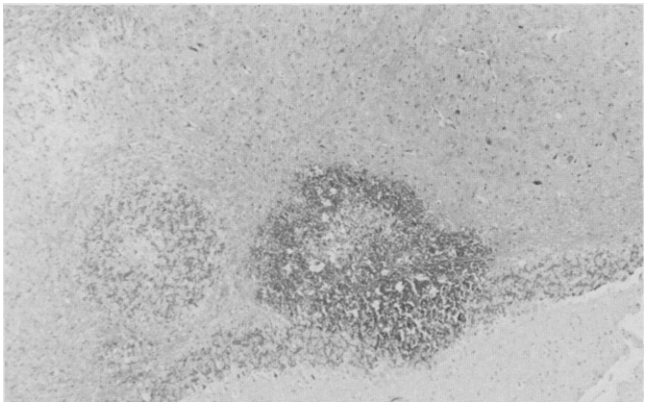

Fig. 14. Focal hemorrhage and malacia in the cerebellum of a young adult rat with naturally occurring RV infection.

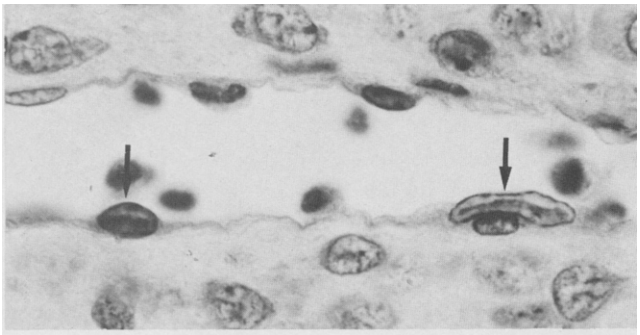

Fig. 15. Intranuclear inclusions in three endothelial cells (arrows). The rodlike form with surrounding halo is especially well shown in one cell cut in its long axis. [Courtesy of Dr. Margolis and Dr. Kilham (91); and *Laboratory Investigation.*]

nervous system (Fig. 16) (30,91). Baringer and Nathanson (5) found that platelet-fibrin aggregates attached perferentially to RV-infected cells and suggested that endothelial injury and hemorrhage followed activation of clotting near infected cells rather than from a direct cytolytic effect of RV. There is indirect evidence indicating RV may also interfere with blood coagulation. Margolis and Kilham (91) hypothesized that RV could cause coagulative disorders and promote hemorrhagic lesions through infection of hematopoietic tissues. They demonstrated, in a retrospective histological study (92), that RV inclusions can be found in megakaryocytes, and earlier reports by Portella (126) showed that RV had an affinity for erythrocytes. Corroborative coagulation studies on RV-infected rats have not been reported.

Since rat parvoviruses have a broad tissue tropism, clinical signs and lesions other than those described may occur. It seems likely that lesions of natural parvovirus infection will depend, as do their experimental counterparts, on the age and strain of the host and on the virulence and tropism of the strain of virus.

h. Epizootiology. Serological surveys show clearly that natural infections with RV and H-1 virus are common among laboratory and wild rats. Robey *et al.* (130) found that about 85% of more than 350 sera collected from Sprague-Dawley rats from three commercial breeders and from their own colony had HAI antibody to RV. Kilham (71) surveyed several populations of wild rats near Hanover, New Hampshire, and detected HAI antibody to both RV and H-1 virus. The proportion

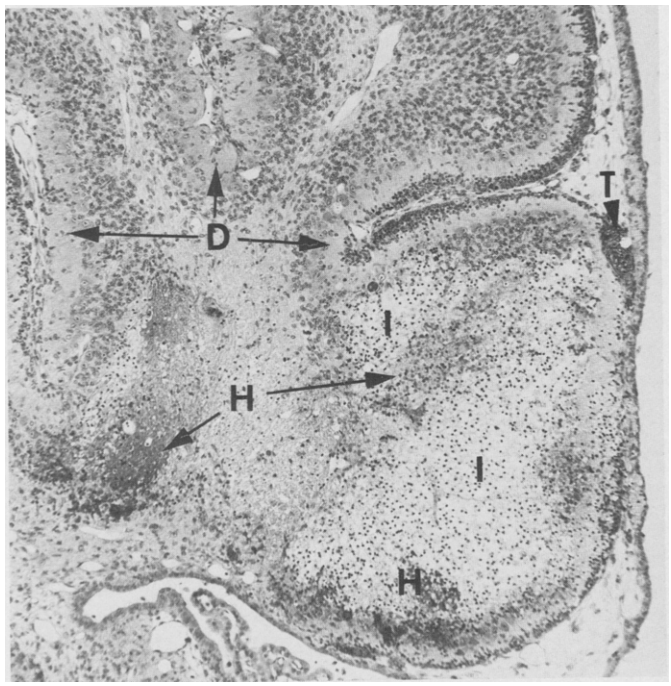

Fig. 16. Cerebellum of a 10-day-old rat infected with RV 7 days previously. There is incomplete destruction of the external germinal layer (D) a thrombosed superficial vessel (T), zones of hemorrhage (H), and infarction (I) of a folium. [Courtesy of Dr. Kilham and Dr. Margolis (91); and *Laboratory Investigation.*]

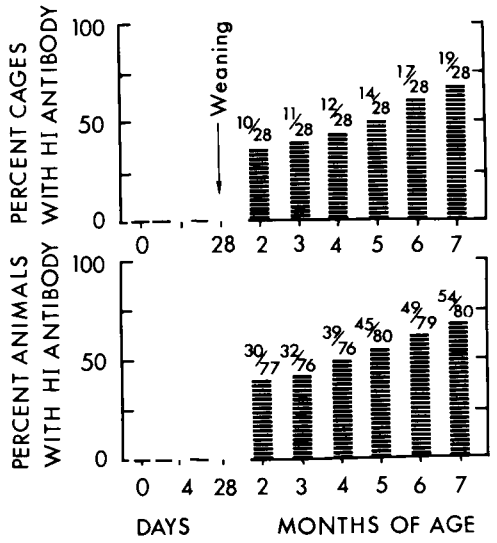

Fig. 17. Cohort study of RV infection in a closed colony. Frequency of hemagglutination inhibiting (HAI) antibody by age. After weaning, males and females were separated; cages refers to the littermates of one sex. Passively acquired maternal antibody is excluded. [Courtesy of Dr. N. Nathanson (131); and the *American Journal of Epidemiology.*]

of rats with antibody to each virus varied independently for each population. Robinson and co-workers (131) made an extensive seroepizootiological study of RV in a closed colony of 2000 McCollum rats with an annual production of about 3000 rats. Forty percent of rats without maternal antibody seroconverted to RV by 7 weeks of age, and 67% had anti-RV antibody by 7 months (Fig. 17). If several pups in a litter sustained infection, most cagemates developed antibody (Fig. 18). Passive antibody titers disappeared by 2 months. About 70% of 1000 juvenile rats were susceptible to infection at any given time, whereas 30% were actively immune (Fig. 19). Several attempts to isolate RV from immune rats were unsuccessful. Seroconversion to H-1 virus was not detected, but about half of the litters had transient maternal antibody to H-1 virus. These studies demonstrated that RV is readily transmitted among rats held in confined quarters and that infections are perpetuated by sustained introduction of susceptible hosts.

The epizootiological studies suggest that oral and perhaps respiratory infection are primarily responsible for natural horizontal transmission of RV. The studies of Kilham and Olivier (78) and Kilham and Margolis (73) indicated that natural vertical transmission can occur. Experimental studies from several laboratories support these impressions. Kilham and Margolis (74) inoculated pregnant rats orally with RV on the eleventh day of gestation and showed that transplacental and fetal infection with fetal death occurred although dams remained asymptomatic. They also found that RV replication was widespread in tissues of pregnant dams and that dams developed viremia which contributed to placental infection. These observations parallel those of natural RV infection where signs may be limited to fetal resorption and reduced litter size (see Section II, A, 1, f). In contrast, H-1 virus infected rat fetuses only after intraperitoneal inoculation but not after oral inoculation of pregnant mothers. These workers suggested that in rats H-1 virus is more likely restricted to horizontal transmission primarily among sucklings and that adults are susceptible to clinical disease only if they are debilitated by intercurrent infections or leukemia. Lipton *et al.* (84) reported that viremia and virus infection of intestine, lung, liver, spleen, brain, and kidney followed intragastric inoculation of adult rats with the HER strain of RV. Furthermore, virus was excreted in feces but not in urine for up to 12 days, and inoculated rats were infectious for contact cohorts for 15 to 20 days. They were also able to infect rats by intranasal inoculation. Novotny and Hetrick (116) did find RV in urine of suckling rats inoculated parenterally as newborns with high doses (10^6 LD_{50}) of RV. Dams and siblings of infected sucklings developed HAI antibodies to RV which indicated that horizontal infection had ensued. Vertical transmission of RV was also confirmed, since litters born 2 to 5 days after intraperitoneal or intravenous (iv) inoculations of mothers had 100% mortality, whereas rats inoculated at least 2 weeks before conception delivered normal litters. Furthermore, litters of infected dams developed disease

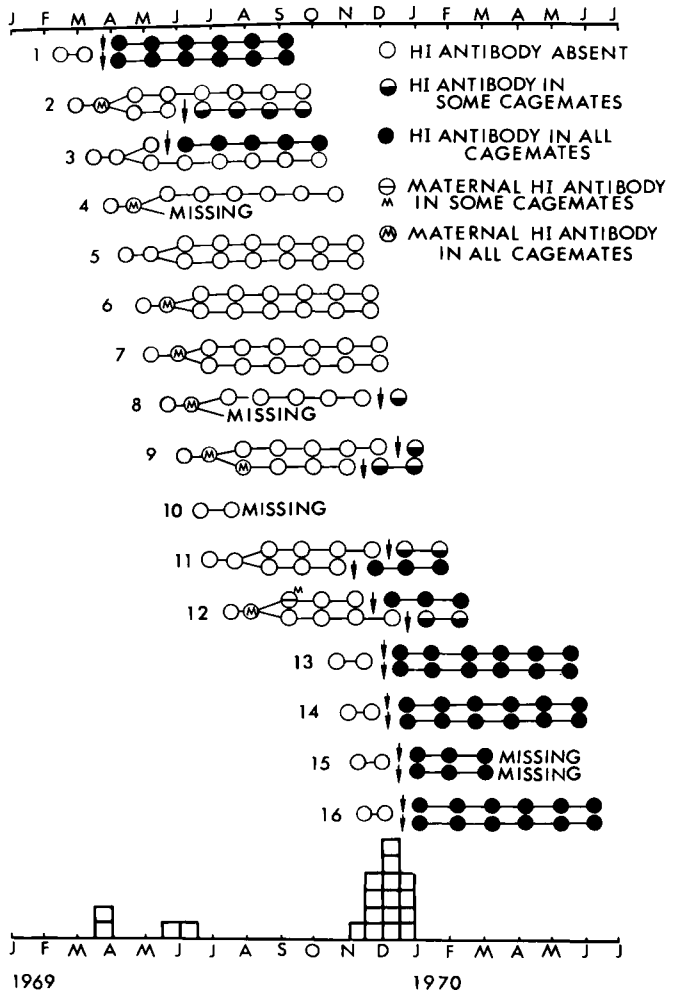

Fig. 18. Cohort study of RV infection in a closed colony. Litters of rats bled periodically from 1 day to 27 weeks of age. Each litter (identified by number) is represented by a single line to age 3 weeks (when sexes were segregated) and by a double line (males above) thereafter. Date of infection of each group of cagemates is represented by an arrow and by a square on the histogram. [Courtesy of Dr. N. Nathanson (131); and the *American Journal of Epidemiology*.]

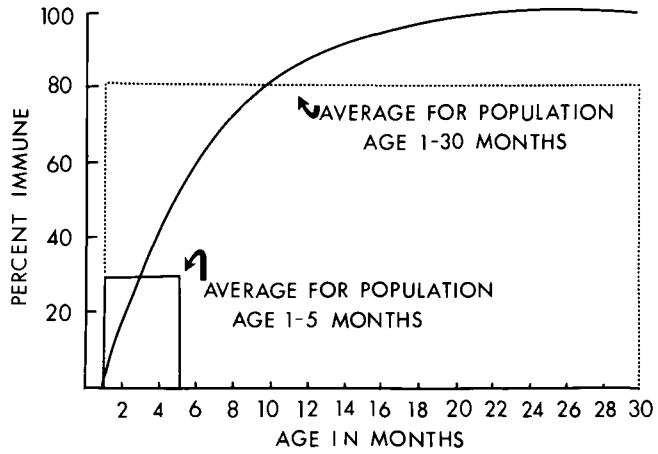

Fig. 19. Dynamics of RV in a population of juvenile rats (1 to 5 months old). The total population at any given time was about 1000 animals. Every month 250 rats entered the group, and an equal number were removed. Results are compared with those obtained from testing rats 1 to 30 months old. [Courtesy of Dr. N. Nathanson (131); and the *American Journal of Epidemiology*.]

when nursed by normal mothers, whereas normal litters nursed on infected mothers remained well. Kilham and Margolis (73) showed that RV may be excreted briefly in milk by rats inoculated with RV in late pregnancy. Dams infected on the first postpartum day had RV in milk by 24 h and could excrete virus in milk up to 12 days and up to 5 days after HAI antibody appeared in their serum.

There is additional evidence that RV can persist as a latent infection in the presence of high titers of HAI antibody. Robey *et al.* (130) isolated RV from five clinically normal laboratory rats, all of which had HAI antibody to RV, and the rat with the highest antibody titer had the most widespread infection. Latent RV infection also was activated in clinically normal rats by a single nonlethal dose (150 mg/kg) of cyclophosphamide (40). The repository of latent RV is unknown, but Margolis and Kilham (91) suggested that, since RV favors rapidly dividing cells, the gastrointestinal tract or hematopoietic system are likely sites. In addition, factors that contribute to RV latency and the role of latency in H-1 virus infection are not understood. Nevertheless, it must be assumed that seropositive rats are latently infected and can serve as a potential source of infection to all susceptible rats in a colony. Furthermore, since some strains of rat parvoviruses can be transmitted vertically, even caesarian-derived or germfree rats can not be assumed free of infection until they have been tested serologically.

i. Diagnosis. The diagnosis of parvovirus infection should be approached on four levels: (a) clinical, (b) morphological, (c) serological, and (d) virological.

Clinical signs in adult rats include neurological disturbances, sudden death, and hemorrhagic diatheses (e.g., scrotal hemorrhage and cyanosis). The incidence of clinical signs among infected adults is likely to be low, but may be exacerbated by immunological crippling. In breeding colonies, production may decrease, or neurological deficits and jaundice may occur among sucklings.

Morphological evidence of parvovirus infection is more difficult to detect since viruses are usually nonpathogenic for adults. An increase in fetal resorption sites in gravid uteri may occur. Spontaneous lesions rarely develop in young animals, but histological examination for persisting inclusion bodies occasionally may be rewarding. Other lesions in suckling and adult rats have been described in Section II, A, 1, g.

Inapparent infection is detected most easily by serological testing. Four serological tests are available (HAI, CF, NT, and

FA), but only the first three distinguish reliably between RV and H-1 infection (36,110,151,154). Hemagglutination inhibition tests are the simplest and least expensive to perform (131). The use of kaolin to remove nonspecific inhibitors from test serum has been recommended (49,110,131), but we and others have not found this necessary (31).

Serological evidence of infection should be confirmed, whenever possible, by viral isolation (see Section II, A, 1, d). Virus may be found in blood early in disease (viremia can persist for 2 to 10 days after experimental infection) (74). Fetuses, placenta, hematopoietic tissue, or gastrointestinal tract should, however, yield virus more consistently. Confirmation of RV infection in our laboratory is made by inoculation of test material into primary rat embryo cultures and into RV-free suckling rats. Cultures are checked for hemagglutinin production and CPE. The identity of isolates is confirmed by HAI assay with anti-RV and anti-H-1 virus immune serum. Sera are collected from inoculated rats after 2 to 4 weeks and are tested for anti-RV HAI antibody.

j. Differential Diagnosis. Rat virus infection may be subclinical or may cause lesions in the central nervous system, liver, vascular system, testes, and fetal death or resorption. Sporadic illness affecting these organs must be carefully evaluated for infection by other viruses, chemical toxicity (e.g. dicoumarin toxicity), neoplastic diseases including leukemia or pituitary adenomas, trauma, and genetic abnormalities such as hydrocephalus. Reduction in litters or litter size may be associated with Sendai virus and possibly with environmental factors. Since RV-infected colonies are common, specific antibodies per se only indicate subclinical infection has occurred. Associations between infection and clinical signs or lesions must be carefully made to confirm a cause and effect relationship. For example, dual viral infections may occur. Further, environmental or experimental factors may activate latent RV, while simultaneously causing lesions unrelated to RV infection.

k. Control. Since rat parvoviruses cause latent infections and may be transmitted vertically and/or horizontally, procedures to control infection must be planned carefully and adhered to stringently. Once a colony has been infected, infection can be eliminated effectively only by destroying the colony and repopulating from parvovirus-free stock. For experimental colonies, this usually requires purchase of rats from vendors who have adequate serological monitoring programs. For production colonies, it means rederiving breeding stock by caesarian section, nursing sucklings on parvovirus-free foster dams, and testing weanlings for antibody before they are used for breeding. Physical facilities for rederived stock should include pathogen-free barriers with personnel locks and proper air balancing to prevent reinfection. Periodic serological surveillance also is essential to detect possible reinfection as early as possible.

l. Interference with Research. The effects of rat parvoviruses on biological research are only partially understood and are potentially serious. For example, they can contaminate transplantable neoplasms (78,150) and established cell lines (49,163). Therefore, it is reasonable to assume, because of their predilection for rapidly dividing cells, that they could alter the growth characteristics of affected cells. Furthermore, parvoviruses are pathogenic for fetal and suckling rats and hamsters, so inadvertent inoculation, especially of young animals, with virus-contaminated cells could cause fatal infection. Immunosuppression also can activate latent RV infection in older rats (40), so rats subjected to immunosuppression or severe stresses, such as intercurrent infections or surgical trauma, may be at risk. Conversely, there is recent evidence that RV can suppress proliferative responses of infected lymphocytes to concanavalin A, phytohemagglutinin, or allogeneic lymphoid cells (25,26). If infected adult rats are used for experiments unaffected by latent viral infections, parvovirus infection can be tolerated, because it is unlikely that rats will develop clinical signs or lesions. Since all factors relevant to latent infection have not been identified, a decision to work with infected animals should be made cautiously. Finally, since these viruses are so hardy, instruments should be thoroughly disinfected after contacting infected animals to reduce the spread of infection.

2. Minute Virus of Mice (MVM)

This is a widely disseminated latent virus of mice. Experimentally inoculated neonatal rats developed viremia and mild necrosis of ependyma and choroid plexus, and infected cells contained intranuclear inclusion bodies. Virus also was detected on postinoculation day 6 in brain, liver, intestine, and urine (76). Low titers ($\leq$ 1 : 40) of HAI antibody to MVM have been found in wild rats (76) and in laboratory rats (119). Titers were reduced, however, by pretreating sera with receptor-destroying enzyme and attempts, to recover MVM from more than 100 seropositive rats proved unsuccessful. Therefore, the significance of anti-MVM antibody in rat serum is unresolved. Clinical signs or lesions due to natural MVM infection of rats have not been reported, and MVM has not been isolated from rats.

B. Herpesvirus (Cytomegalovirus)

Cytomegalovirus is the only known herpesvirus of rats. Similar viruses have been described for many species including humans, nonhuman primates, mice, guinea pigs, and rats

(133). They have physical and chemical characteristics typical of the Herpetoviridae, including a penchant for latency. As with other cytomegaloviruses, rat cytomegalovirus appears to have a predilection for salivary glands and has also been found in lacrimal glands (87). Typical lesions include cytomegaly, intranuclear inclusion body formation in acinar or ductal epithelium, and mild nonsuppurative interstitial inflammation (79,87,128). Kuttner and Wang (79) also showed that salivary glands of affected wild rats contained infectious virus by transmitting disease by intraglandular and ic inoculation of several "young" rats with emulsions of submaxillary gland.

The epizootiology of rat cytomegalovirus has not been well studied, but virus and/or antibody have been detected in wild rats in several widely separated areas of the world (79,128). The potential the virus has for interfering with research has not been evaluated. Infection is usually diagnosed by histological examination. Anti-viral antibody can be detected by NT test (128), but rapid serological tests are not available. Rat cytomegalovirus can be grown in primary rat fibroblasts, rat kidney cells, and hamster kidney cells (6,128).

C. DNA Virus Which May Infect Rats

Mouse Adenovirus

We have found that some rat sera contain CF antibodies to mouse adenovirus, but clinical disease or lesions attributable to infection of rats with this virus have not been detected, and virus has not been recovered from rats.

III. RNA VIRUSES

A. Coronaviruses (Sialodacryoadenitis Virus and Rat Coronavirus)

1. General

Coronaviruses are lipid solvent-labile, pleomorphic, 60 to 220 nm particles with characteristic clublike projections (corona) uniformly arranged on their surfaces. They multiply in the cytoplasm and mature by budding through endoplasmic reticulum. Coronaviridae are fairly species specific and have been identified as etiologic agents in diseases of humans, pigs, bovines, rats, mice, dogs, chickens, and turkeys. They generally infect the gastrointestinal tract and its associated glandular organs and/or the respiratory tract. Reviews of coronavirus biology are available (17,103). Two strains of coronavirus have been identified as important pathogens of laboratory rats; sialodacryoadenitis virus (SDAV) (15) and rat coronavirus (RCV) (120).

2. History

In 1961, Innes and Stanton reported two outbreaks of clinical disease in weanling rats characterized by cervical edema and "red tears" (59). They described the morphology of the disease in considerable detail and named it sialodacryoadenitis from the characteristic lesions: inflammation and edema of salivary and lacrimal glands. Hunt (58) described a similar disease of young rats, but inflammation was restricted to the intraorbital lacrimal glands and was accompanied by keratoconjunctivitis. Innes and Stanton suggested an infectious agent caused the disease, and Hunt detected acidophilic intranuclear inclusion bodies in affected Harderian glands and in conjunctival mucosa, but viral isolations were not attempted in either study. Ashe and co-workers (3,4) isolated a transmissible cytopathic viral agent from the submaxillary glands of gnotobiotic rats that hemagglutinated rabbit erythrocytes. Ashe's virus apparently was not associated with clinical signs or lesions in infected rats (see Section IV,B). Jonas *et al.* (67) induced sialodacryoadenitis in germfree rats by intranasal inoculation of an ultrafiltrate of diseased submaxillary salivary gland. Virus particles were detected in ducts of submaxillary glands from experimentally infected rats by electron microscopy, but attempts to isolate an agent *in vitro* were initially unsuccessful. However, when neonatal mice were inoculated ic with submaxillary gland homogenate they developed severe neurological deficits and died in 3 to 6 days. Brain homogenates from affected mice caused sialodacryoadenitis in intranasally inoculated rats. Bhatt and co-workers (15) subsequently isolated a virus from salivary glands of affected rats by inoculation of neonatal mice and primary rat kidney (PRK) monolayer cultures. The isolate had serological and physicochemical characteristics of a coronavirus. It was lethal for infant mice after ic inoculation but not for weanling mice. Mouse brain-passaged virus induced sialodacryoadenitis in susceptible rats.

In 1964 Hartley and associates found that some rat sera contained antibody to mouse hepatitis virus (MHV) (51). They suggested that an agent antigenically related to MHV could elicit anti-MHV antibody in rats. Parker *et al.* (120) offered support for Hartley's theory by isolating a coronavirus antigenically related to MHV from lungs of infected but clinically normal rats. Parker's virus (RCV) was subsequently shown to be antigenically related to both SDAV and MHV (15), and the current view is that SDAV and RCV may be different strains of one rat coronavirus. The biology of each virus is discussed separately in each of the following sections.

3. Viral Characteristics

a. SDAV. The diameter of SDAV particles, determined by ultrafiltration, is 100 to 220 nm. Jonas *et al.* (67) described a 60- to 70-nm particle in ductal epithelium of experimentally

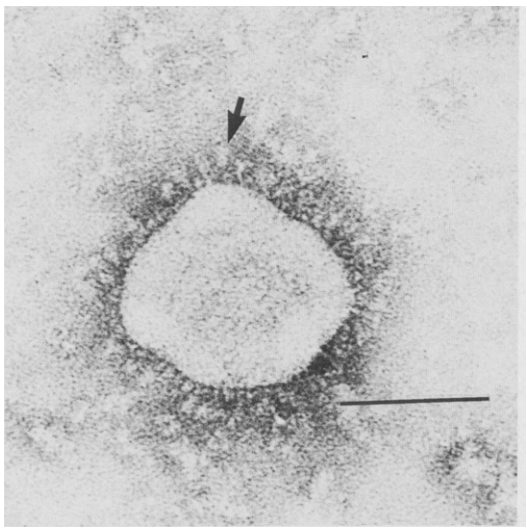

Fig. 20. Negative stained preparation of SDAV. Note projections typical of coronaviruses (arrow). Bar represents 100 nm. ×200,000. (Courtesy of Dr. M. Lipman.)

infected submaxillary glands by electron microscopy. Preliminary ultrastructural studies of SDAV propagated *in vitro* indicate it has the typical morphology of a coronavirus (Fig. 20) and is about 114 nm in diameter (83). The differences in size reported probably reflect differences in the methods of measurement and the source of virus as well as the pleomorphism characteristic of coronaviruses. Sialodacryoadenitis virus is sensitive to lipid solvents, but it is relatively stable at acid pH (3.0). It also is stable in 3% fetal bovine serum (in phosphate-buffered saline) at 4°C for up to 7 days, at 37°C for up to 3 h, and at 56°C for less than 5 min. It can be stored at −60°C for more than 7 years but loses infectivity rapidly if stored at −20°C. The virus replicates intracytoplasmically and replication is not affected by 5-bromodeoxyuridine (BUdR). Detailed morphological studies of viral maturation and release have not been reported. The virus does not hemagglutinate rabbit, guinea pig, or goose erythrocytes at 4°, 25° or 37°C (15).

Sialodacryoadenitis virus is closely related antigenically to RCV, to MHV (Tables III and IV) and a human coronavirus (OC38), but its antigenic relationship to other coronaviruses has not been tested. The virus is not antigenically related to the pox-, herpes-, areno-, paramyxo-, rhabdo-, reo- or togavirus groups (15).

Table III

Results of Cross-Complement-Fixation Tests with SDAV and MHV and Their Respective Immune Sera[a]

	Antisera		
Antigen	SDAV-A	SDAV-B	MHV
SDAV	128/≥256[b]	64/128	160/128
MHV	32/32	16/16	80/≥64

[a] After Bhatt *et al.* (15) with modification.

[b] Highest dilution of serum reacting with the lowest dilution of antigen divided by the highest dilution of antigen reacting with the lowest dilution of serum.

Table IV

Results of Cross-Neutralization Tests with SDAV and RCV and Their Respective Immune Sera[a]

		Antibody titer versus			
	Species	SDA		RCV	
Antigen	immunized	1	2	1	2
SDA	Mice	1:253	1:452	1:67	1:100
RCV	Rats	1:67	1:100	1:284	1:272

[a] After Bhatt *et al.* (15) with modification.

b. RCV. Rat coronavirus also is a typical coronavirus morphologically and, by negative staining, measures 50–118 nm in diameter including the corona. Like SDAV, it is destroyed by ether, chloroform, or heating to 56°C for 30 min. It does not hemagglutinate mouse, chicken, guinea pig, sheep, or human erythrocytes at 4°, 22°, or 37°C (120).

4. *In Vitro* Cultivation

a. SDAV. Sialodacryoadenitis virus causes a characteristic CPE in PRK monolayer cultures with formation of multinucleated giant cells (15). Cultures can be prepared from SDAV-free or infected rats, since SDAV does not infect kidney. Cultures are most sensitive if inoculated by 1 week after seeding. Older cultures appear to be less sensitive to viral replication, and CPE may not develop. Virus can be detected in the cytoplasm by immunofluorescence within 12 h after inoculation. Cytopathic effects appear by 24 h and extensive lysis occurs shortly after. Sialodacryoadenitis virus does not replicate in BHK-21, VERO, or HEp-2 cell lines; a line of polyoma-transformed mouse cells (Py-A1/N); or mouse cell line NCTC 1469 which supports growth of MHV (15). Sialodacryoadenitis virus can be plaqued in PRK monolayers only after serial *in vitro* passage.

b. RCV. Methods for and results of cultivation of RCV resemble those described for SDAV (120).

5. Host Range

a. SDAV. Early work showed that SDAV is pathogenic for in inoculated adult rats and for ic inoculated infant mice (15,61,67). Recent evidence indicates that SDAV also is infectious and pathogenic for weanling mice (12). Contact-exposed

mice developed NT antibody to SDAV, whereas in inoculated mice developed NT and CF antibody. The virus was recovered from the respiratory tract for up to 7 days postinfection, and mice developed interstitial pneumonia. Anti-SDAV and anti-MHV antibody also has been found among retired breeder mice from colonies thought to be free of MHV. Since SDAV and MHV are antigenically related, SDAV infection should be considered if unexpected or unexplained seroconversions to MHV occur in mouse colonies. Seroconversions to MHV from infection of mice or rats exposed to human coronaviruses (e.g., carried by animal technicians) also should be considered (Hartley *et al.*, 1964) but has not been studied.

Extensive host range studies of SDAV have not been done, but preliminary trials with several strains of rats and mice suggest that various strains of SDAV may vary in infectivity and antigenicity (14). For example, during spontaneous outbreaks, WAG/Rij rats developed severe clinical disease, whereas DA rats developed primarily subclinical disease. Furthermore, some strains of mice developed both CF and NT antibody following experimental SDAV infection, whereas others produced only NT antibody. Conversely, one strain of SDAV induced only NT antibody in a given mouse strain whereas a second strain of SDAV induced both CF and SN antibody. These variations are important for interpretation of diagnostic and epizootiological data.

b. RCV. Host range studies with RCV also have been limited. Rat coronavirus is infectious for rats and induces seroconversion to RCV, MHV, and SDAV (11,15,120). Its pathogenicity varies with strain and age but is greatest for suckling rats. For example, mortality among intranasally inoculated Fischer 344 rats less than 48 h old approached 100%, whereas comparable Wistar rats had only 10 to 25% mortality. Furthermore, deaths among Fischer 344 sucklings occurred 6 to 12 days after infection, whereas Wistar sucklings usually died after 12 days. Resistance to mortality, however, among even highly susceptible sucklings, increased rapidly so that rats inoculated after 7 days of age had nonfatal respiratory disease and weanlings were asymptomatic (120). The pathogenicity and infectivity of RCV for other species have not been reported.

6. Clinical Disease

a. SDAV. Susceptible rats can be infected at any age, but clinical disease usually occurs in one of two patterns: endemic infection of breeding colonies or explosive outbreaks among nonimmune rats exposed to virus as weanlings or adults. In the former setting, adults may have clinical signs, but more commonly they are immune. Therefore, clinical disease develops among susceptible sucklings and is characterized by so-called "winking and blinking" associated with acute inflammation of the eye and adnexae. Signs are transient (1 week or less) among individual sucklings, but affected animals will be prevalent among the suckling population as long as newly susceptible litters are available to become infected. In the latter situation, either new, SDAV-susceptible rats are placed in a room with infected rats or an infected rat(s) is placed in a room housing nonimmune weanlings or adults. Generally, within 1 week, the susceptible population will develop typical signs of SDAV infection. For individual rats they include cervical swelling (edema) with palpable enlargement of submaxillary salivary glands, sneezing or repeated wiping of the external nares with the forepaws, photophobia and nasal, and ocular discharges which are often red-tinged due to a high content of porphyrin pigment. Clinical signs last about 1 week. They may be mild or severe, and all signs do not occur in every infected rat. This last point is especially significant, since a single subclinically infected rat placed in a susceptible colony can initiate a full enzootic episode.

Keratoconjunctivitis has been associated with several natural outbreaks of SDAV (80,161) and may be the only clinically detectable evidence of SDAV infection. Signs and lesions commonly begin by the time of weaning, but also can occur in adults. They include photophobia, lacrimation, circumcorneal flush, diffuse corneal opacities, corneal ulcers, pannus, hypopyon, and hyphema. Lesions usually resolve completely in 1 to 2 weeks, but chronic active keratitis and megaloglobus may develop in some rats. The morbidity of eye lesions during an acute outbreak of SDAV infection varies from 0 to 100%, but is usually 10 to 30%. The prevalence of eye lesions seems greater among breeding colonies chronically infected with SDAV (65). The severity of lesions also may vary among strains of rats. In our experience, inbred Lewis and WAG/Rij rats are more susceptible to SDAV-associated eye disease than DA rats or outbred CD rats (65). Weisbroth and Peress (161) found that the spontaneously hypertensive strain TAC:SHR/N also was highly susceptible. The pathology of the eye lesions is discussed in greater detail in Section III, A, 7.

b. RCV. Rat coronavirus infection is subclinical in postweaned rats. Nonfatal respiratory disease can occur in intranasally inoculated sucklings, and intranasally infected susceptible neonates may die (11,120).

7. Pathology

a. SDAV. The lesions of SDAV infection have been described in detail by several groups of workers (59,61,67,80,161).

Gross Lesions of SDAV infection usually are restricted to mixed or serous salivary glands, lacrimal glands, cervical lymph nodes, thymus, and occasionally lung. Submaxillary and parotid salivary glands and cervical lymph nodes are un-

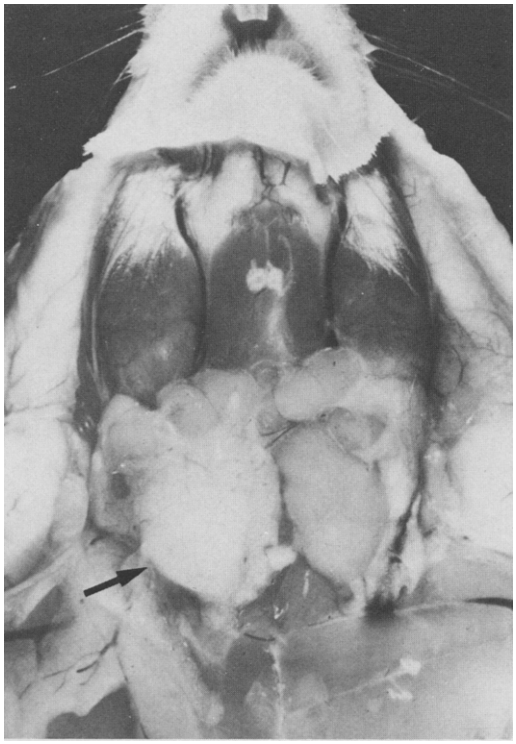

Fig. 21. Swollen pale submaxillary gland (arrow) in a rat inoculated intranasally with SDAV 5 days previously. The cervical lymph nodes are also moderately enlarged.

ilaterally or bilaterally enlarged, pale yellow to white, and edematous (Fig. 21), although they may have red spots or a red tinge if they are congested. Periglandular connective tissue usually is severely edematous. The exorbital and intraorbital lacrimal glands also may be swollen, and the Harderian gland may be flecked with yellow-gray foci. Brown-red mottling of the Harderian gland is due to its normal content of porphyrin pigment and should not be interpreted as a lesion. The thymus may be small and the lungs may be spotted with small grey foci.

Histological changes of SDAV infection are found in the respiratory tract, salivary glands, thymus, cervical lymph nodes, eye, exorbital and intraorbital lacrimal glands, Harderian gland and other ocular adnexae.

Nasopharyngeal lesions include multifocal necrosis of respiratory epithelium with inflammatory edema of the lamina propria (Figs. 22 and 23). Nasal meatuses may contain neutrophils, mucus, and necrotic cell debris. Focal necrosis of glands in the lamina propria also occurs. Mild, nonsuppurative tracheitis with focal necrosis of mucosal epithelium also may occur, and hyperplastic peribronchial lymphoid nodules develop. Pneumonia has not been reported in either naturally or experimentally infected adult rats, but interstitial pneumonia may occur in naturally infected sucklings (65).

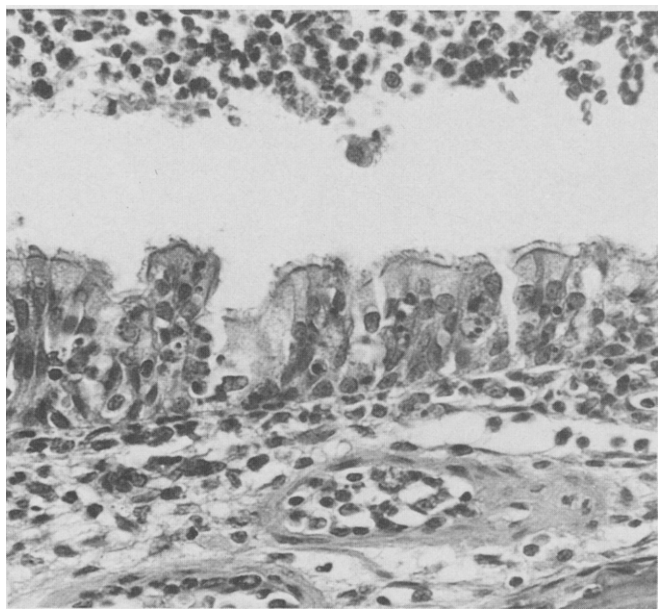

Fig. 22. Nasal mucosa from a rat inoculated intranasally with SDAV 2 days previously. There is focal necrosis of epithelium and the lamina propria is infiltrated by lymphoid cells and neutrophils. Inflammatory exudate is present in the meatus. [Courtesy of Dr. R. O. Jacoby, Dr. P. N. Bhatt, and Dr. A. M. Jonas; and *Veterinary Pathology*.]

Salivary gland lesions (submaxillary and parotid) begin as necrosis of ductular epithelium which rapidly progresses to diffuse acinar necrosis (Fig. 24). Ultrastructural and immunofluorescence studies of experimentally infected rats have confirmed that SDAV has a predilection for salivary ductal epithelium (Fig. 25) (61,67). Moderate to severe interstitial inflammatory edema develops and glandular architecture is rapidly effaced (Fig. 26). Inflammatory edema and occasional focal hemorrhage are prominent in periglandular connective

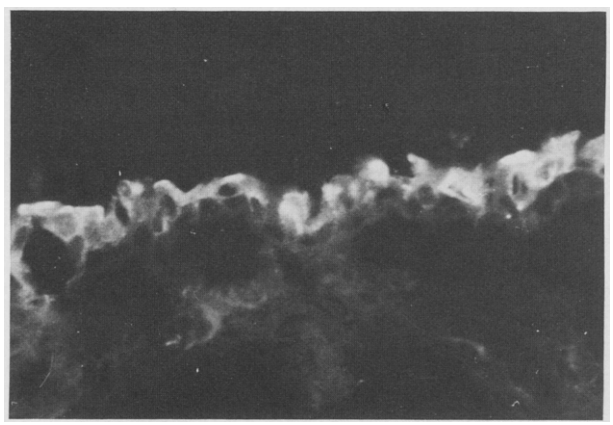

Fig. 23. Nasal mucosa with fluorescing SDAV antigen in the cytoplasm of epithelial cells. [Courtesy of Dr. R. O. Jacoby, Dr. P. N. Bhatt, and Dr. A. M. Jonas; and *Veterinary Pathology*.]

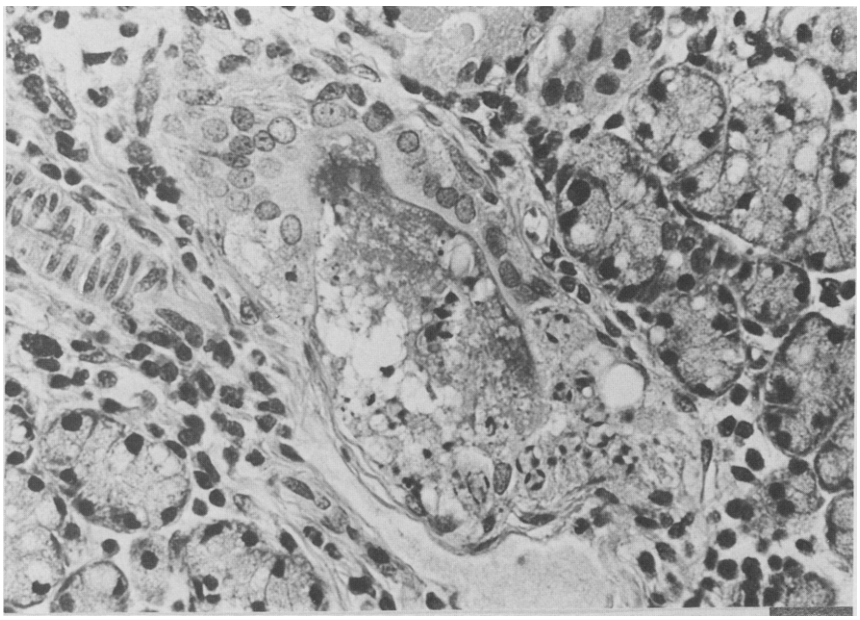

Fig. 24. Submaxillary gland 4 days after intranasal inoculation of SDAV. There is necrosis of a large salivary duct and periductular inflammation. Adjacent acini have early signs of degeneration characterized by formation of intracytoplasmic vacuoles. [Courtesy of Dr. R. O. Jacoby, Dr. P. N. Bhatt, and Dr. A. M. Jonas; and *Veterinary Pathology*.]

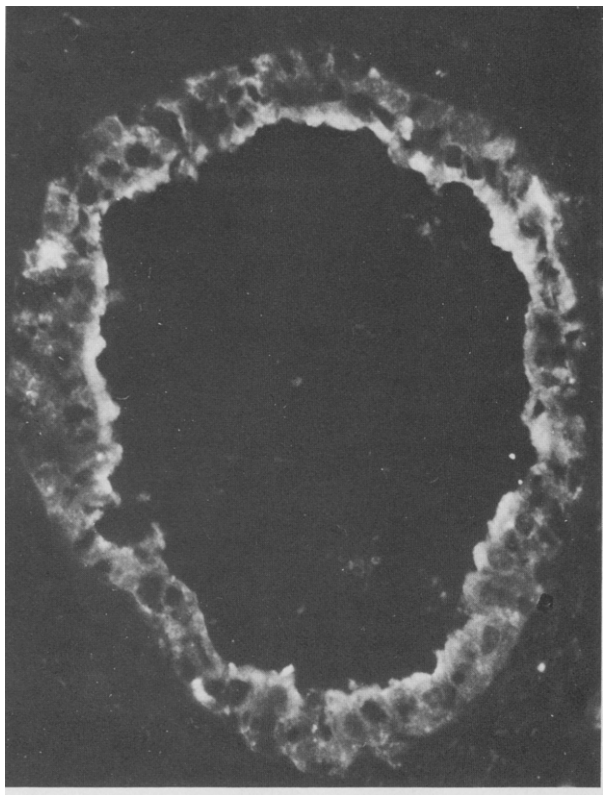

Fig. 25. A large salivary duct from a rat infected with SDAV. SDAV antigen in the cytoplasm of ductal epithelial cells demonstrated by immunofluorescence.

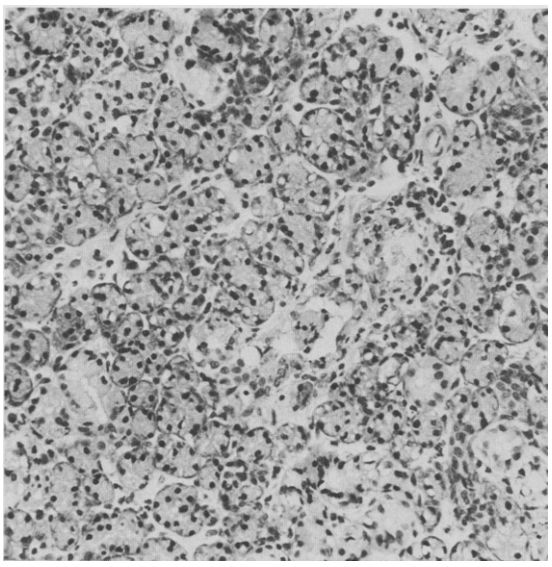

Fig. 26. Submaxillary gland from a rat infected intranasally with SDAV 5 days previously. There are advanced degenerative changes including acinar necrosis, interstitial edema, and inflammation. [Courtesy of Dr. R. O. Jacoby, Dr. P. N. Bhatt, and Dr. A. M. Jonas; and *Veterinary Pathology*.]

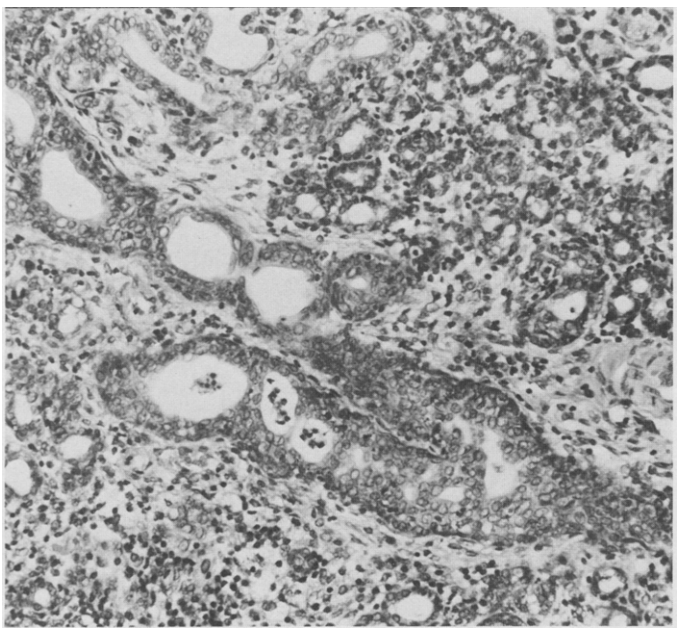

Fig. 27. Submaxillary gland 9 days after intranasal inoculation with SDAV. There is prominent squamous metaplasia of ducts. Note regenerating acini at upper right. [Courtesy of Dr. R. O. Jacoby, Dr. P. N. Bhatt, and Dr. A M. Jonas; and *Veterinary Pathology.*]

tissue. Repair begins 5 to 7 days after infection and is characterized by prominent squamous metaplasia of ductular epithelium and by proliferation of hyperchromatic regenerating acinar cells (Fig. 27). The sublingual salivary gland which is exclusively of the mucus-secreting type, is not affected.

Lacrimal gland lesions develop in essentially the same pattern as salivary gland lesions, i.e., necrosis and inflammation is followed by regeneration with squamous metaplasia of ducts or tuboalveolar units (harderian gland) and acinar regeneration (Figs. 28 and 29).

Squamous metaplasia in all glands subsides by 30 days postinfection, and the cytoarchitecture is restored to normal because basement membrane is not destroyed during necrotic and inflammatory phases of the disease.

Cervical lymph nodes may be hyperplastic with focal necrosis and perinodal edema, and focal necrosis of thymus with widening of interlobular septums is common.

After experimental intranasal inoculation of adult rats, viral replication and lesions occur first in the respiratory tract, then in the salivary glands and exorbital glands, and finally in the intraorbital lacrimal glands (Fig. 30) (61). It is common, however, in either spontaneous or experimental infection, to encounter a varied distribution of lesions. For example, a rat may have marked submaxillary sialoadenitis, whereas the parotid gland may remain normal or dacryoadenitis may occur without sialoadenitis. Furthermore, lesions may be unilateral or bilateral. The factors responsible for these variations have not been

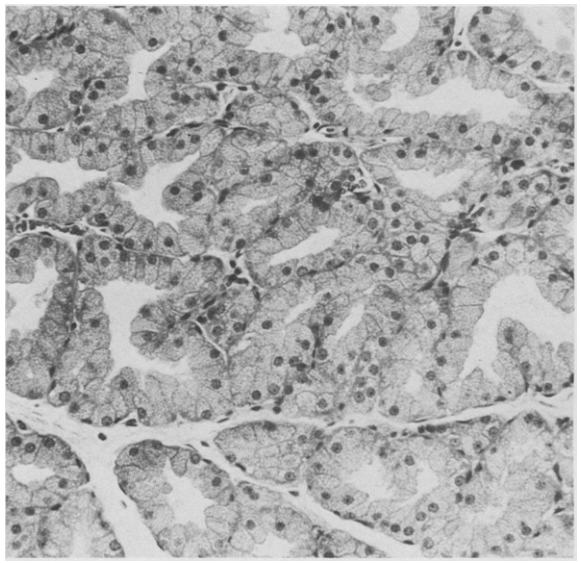

Fig. 28. Normal rat Harderian gland. [Courtesy of Dr. R. O. Jacoby, Dr. P. N. Bhatt, and Dr. A. M. Jonas; and *Veterinary Pathology.*]

identified. Also it is not clear how virus spreads from the respiratory tract to the salivary glands, since attempts to detect viremia or retrograde infection of salivary excretory ducts were not successful (61).

Several groups have reported keratoconjunctivitis in rats with naturally occurring SDAV infection, but eye lesions have

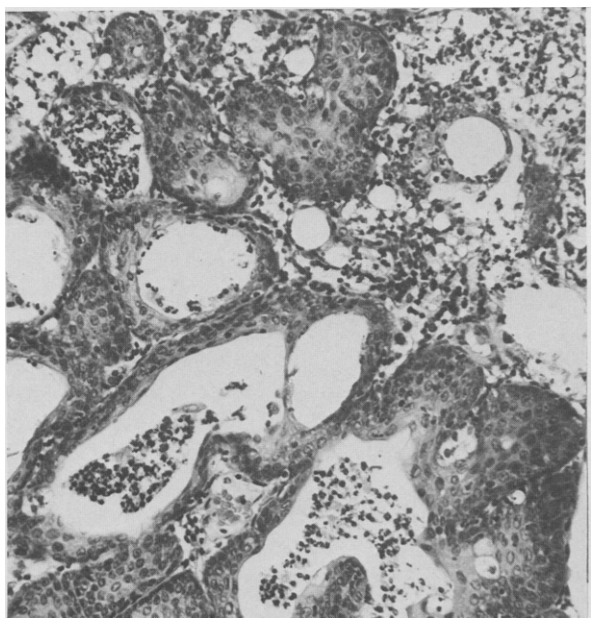

Fig. 29. Harderian gland from a rat inoculated with SDAV 10 days previously. There is prominent squamous metaplasia indicating repair, but some necrosis and inflammation remains. [Courtesy of Dr. R. O. Jacoby, Dr. P. N. Bhatt, and Dr. A. M. Jonas; and *Veterinary Pathology.*]

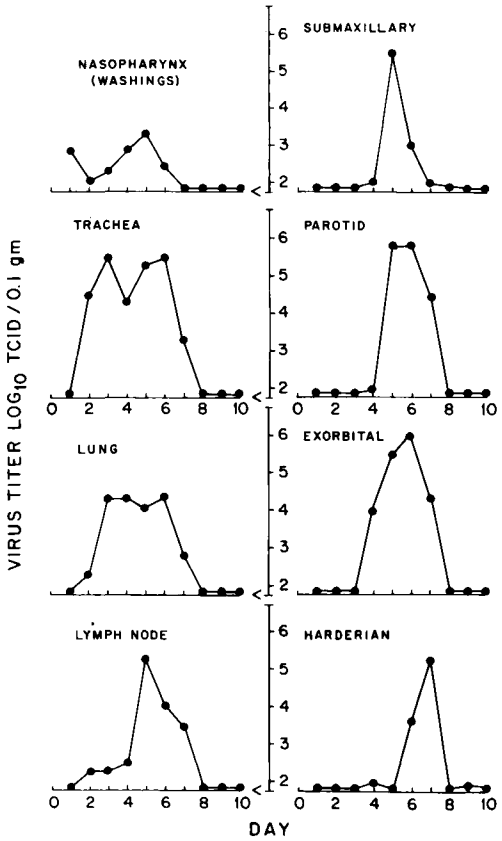

Fig. 30. Titers of virus in tissues of germfree rats 10 days after intranasal inoculation of $10^{4.0}$ $TCID_{50}$ of SDAV. [Courtesy of Dr. R. O. Jacoby, Dr. P. N. Bhatt, and Dr. A. M. Jonas; and *Veterinary Pathology.*]

produced experimentally (52,80,161). Lai *et al.* (80) recently described an outbreak of keratoconjunctivitis in a closed colony of inbred Lewis rats. Lesions were most prominent in weanlings and included focal or diffuse interstitial keratitis, corneal ulceration, synechia, hypopyon, hyphema, and conjunctivitis (Figs. 31-34). Lesions resolved in most rats by 6 weeks of age, but about 6% of affected rats developed chronic keratitis with megaloglobus and lenticular and retinal degeneration. Similar lesions in adult rats, excluding chronic sequellae, were reported by Weisbroth and Peress (161). Affected rats from both outbreaks had a high incidence of dacryoadenitis and serum antibody to SDAV. In the Yale study (80) SDAV was recovered from the respiratory tract of some rats, but virus was not detected in the eyes either by tissue culture isolation techniques or by immunofluorescence. It has been suggested that inflammation and necrosis of lacrimal glands during SDAV infection results in impedence to flow of lacrimal fluids, proptosis, and keratitis sicca. Ostensibly, normal conjunctival bacteria could proliferate under these conditions and increase the severity of lesions (80,161).

Fig. 31. Weanling rat with SDAV-associated keratitis. The cornea is opaque and ulcerated. There is keratinaceous debris on the surface of the cornea and hypopyon.

b. RCV. Parker and colleagues (120) showed that RCV induced lethal interstitial pneumonia in intranasally inoculated newborn rats. Lesions developed by 4 days postinoculation and

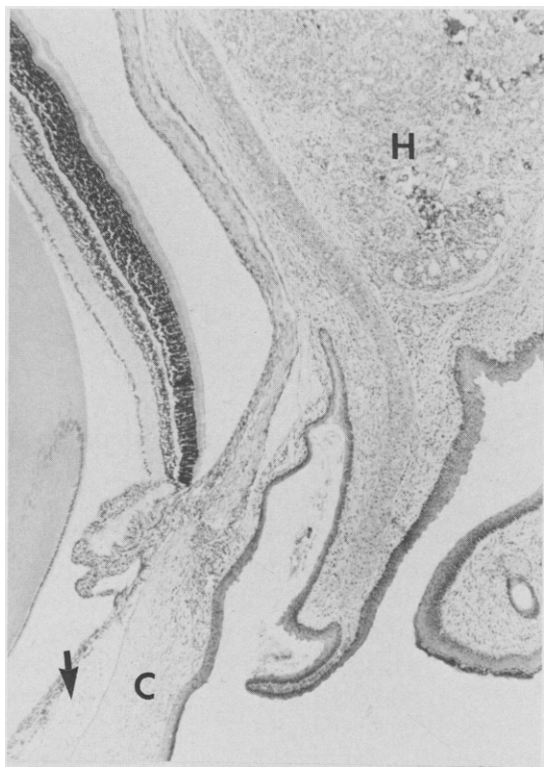

Fig. 32. SDAV-associated keratoconjunctivitis. The Harderian gland (H) is effaced. The cornea (C) and conjunctival mucosa are diffusely infiltrated with inflammatory cells. There are also inflammatory cells in the anterior chamber (arrow). The retina is artifactually detached.

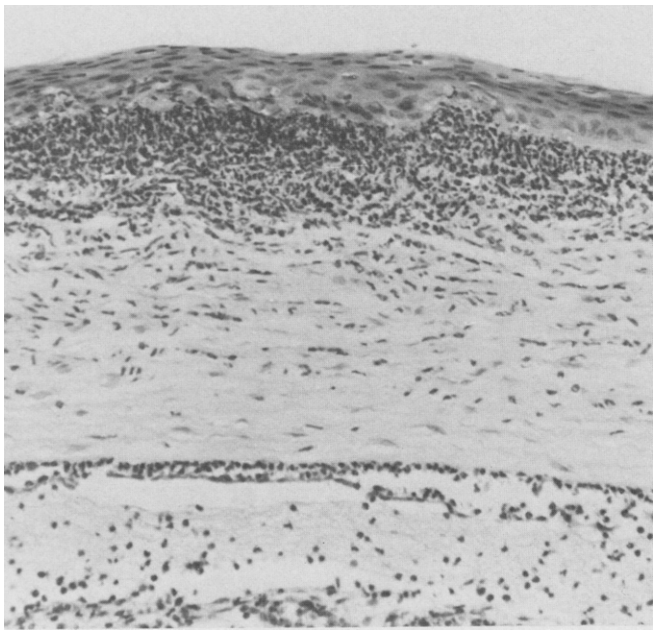

Fig. 33. Cornea from a weanling rat with SDAV-associated keratoconjunctivitis. The stroma is mildly edematous and diffusely infiltrated with neutrophils, but the heaviest accumulations are in the superficial layers. The anterior chamber contains inflammatory exudate.

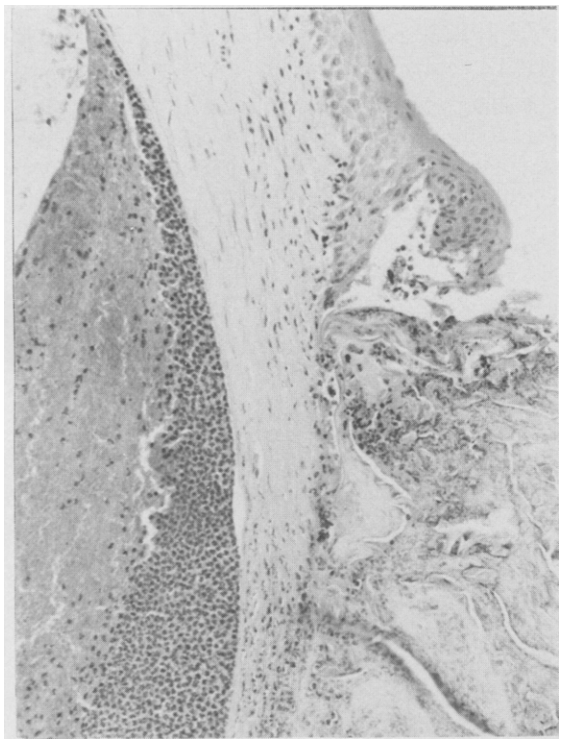

Fig. 34. Corneal ulceration in SDAV-associated keratoconjunctivitis. Masses of keratinized, necrotic cell debris have accumulated over the ulcer. There is also severe hyphema.

included hyperemia, mononuclear cell infiltrates, focal atelectasis, and emphysema. Sialodacryoadenitis was not observed.

Bhatt and Jacoby (11) have described rhinotracheitis and focal interstitial pneumonia in adult germfree rats inoculated intranasally with RCV. Virus was recovered from the respiratory tract for 7 days (Fig. 35), and viral antigen was detected in mucosal epithelium of the nasopharynx and in alveolar septae of some rats. Upper respiratory lesions were nearly identical to those described for experimental SDAV infection. Gross pulmonary lesions were limited to scattered red-brown to gray foci. Histologically, peribronchial lymphoid cell hyperplasia was detected by day 5. Alveolar septa contained mononuclear cells and neutrophils, and inflammatory cells occupied some adjacent alveolar spaces. Pneumocytes, macrophages, and edema fluid also filled alveolar spaces in some lungs. Lesions were mild and focal and subsided by day 7 (Fig. 36). Salivary gland lesions were rare, but, when present, were identical to those caused by SDAV. Dacryoadenitis was not detected.

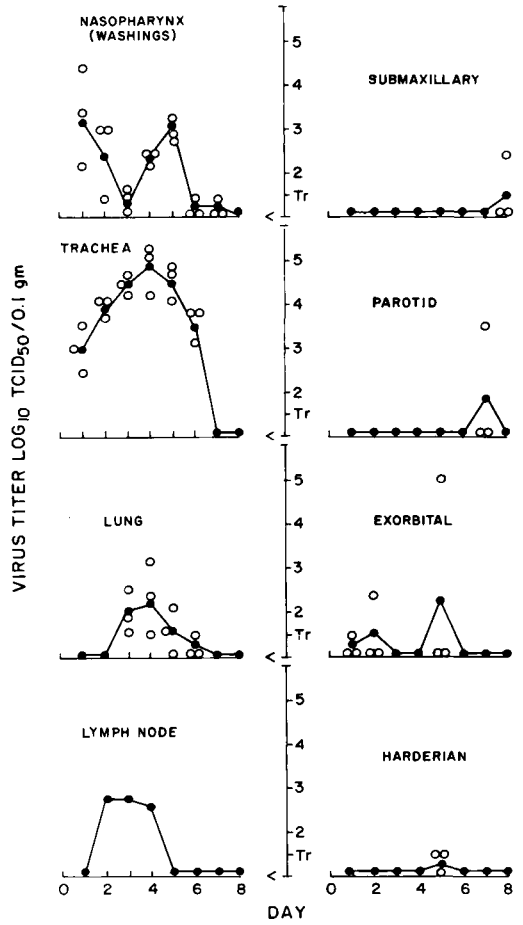

Fig. 35. Titers of virus in tissues of germfree rats inoculated intranasally with $10^{4.6}$ $TCID_{50}$ of RCV. [Courtesy of Dr. P. N. Bhatt and Dr. R. O. Jacoby; and *Archives of Virology*.]

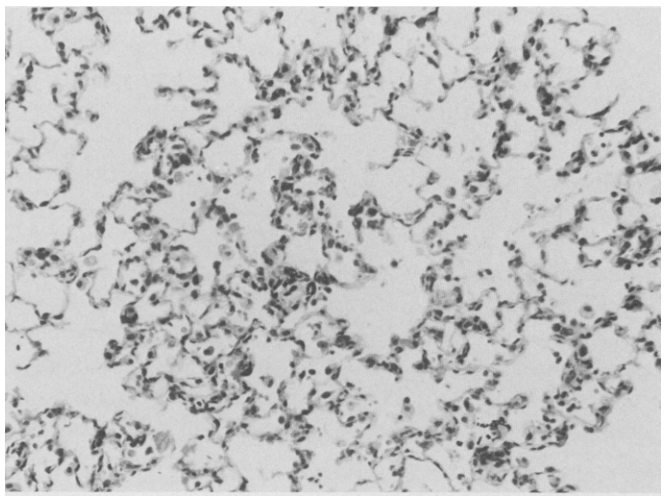

Fig. 36. Lung from a rat 5 days after intranasal inoculation with RCV. There is interstitial pneumonia characterized by infiltration of alveolar septae with mononuclear cells. [Courtesy of Dr. P. N. Bhatt and Dr. R. O. Jacoby; and *Archives of Virology*.]

Table V

Serum Antibody Titers of Germfree Rats Inoculated Intranasally with SDAV[a]

Test	Titer	No. of rats with titer days after inoculation									
		1	2	3	4	5	6	7	8	9	10
Complement fixation	<1:10	3	3	3	3	3	3	2	2		
	1:10							1			
	1:20								1	2	1
	1:40									1	1
	>1:40										1
Neutralization											
	<1:10	3	3	3	3	3	3				
	1:10							3	3	1	1
	1:20									2	
	1:40										2
	>1:40										

[a] After Jacoby *et al.* (61) with modification. Three rats sampled each day.

8. Epizootiology

a. SDAV. Sialodacryoadenitis virus infection is nonfatal, but is highly contagious and spreads easily and rapidly among susceptible rats in a colony room by contact, aerosol, or fomite. Morbidity is normally highest among late suckling or weanling rats, but susceptible rats of any age can be infected. Infected rats excrete virus from the respiratory tract for about 7 days, at which time anti-SDAV antibody is first detectable in serum by either NT or CF tests (Fig. 30 and Table V) (61). There is no evidence for a carrier state, so recovered rats should be considered free of virus and immune. Therefore, propagation of virus in a colony depends on continuous introduction of susceptible rats. The possibility remains, however, that as with some human coronavirus infections (109) rats immune to a strain of SDAV could be reinfected with the same strain or with an antigenically different strain. There also is no evidence that SDAV can be vertically transmitted, but this point has not been adequately tested.

The incidence of SDAV infection cannot be determined reliably by clinical signs, since infection may remain subclinical. Therefore, serological methods are preferred. Serum titers of CF and NT antibody should be determined simultaneously, since recent studies of natural and experimental SDA indicate that CF antibody titers rise during outbreaks then decline relatively rapidly, whereas NT titers increase during infection, but decline more slowly (Fig. 37) (14). Therefore, CF antibody titers appear be a better marker for current or recent infection, whereas NT antibody titers are a more reliable indicator of previous infections.

Sialodacryoadenitis virus infection is probably widespread among rat colonies. We recently tested retired breeders from six vendors, and all had anti-SDAV antibody (10). Nevertheless, confirmation that antibody detected during a given outbreak is due exclusively to SDAV infection may be difficult to obtain, since RCV and SDAV are closely related antigenically, and seroconversions for both viruses and to MHV follow infection by either RCV or SDAV. Bhatt *et al.* (15) have shown, however, that antibody titers to the homologous virus are usually higher than those to the heterologous, antigenically related viruses (Tables III and IV). This finding, coupled with documentation of clinical signs and lesions can improve the accuracy of epizootiological studies.

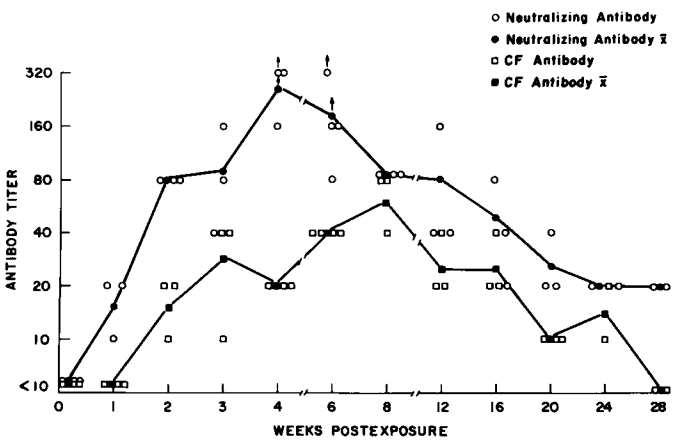

Fig. 37. Serum antibody titers to SDAV in specific pathogen-free rats after a single intranasal inoculation of virus. Rats were kept in germfree isolators for this study. Arrows = titer equal to or greater than indicated value; $\bar{X}$ = average titer.

b. RCV. The epizootiology of RCV has not been reported in detail aside from Parker *et al.* (120) who demonstrated that RCV-infected rats developed anti-MHV antibody. Rat coronavirus is cleared from tissues of experimentally inoculated rats by postinoculation day 7, and there is no evidence for a carrier state. It may be assumed, for the present, that RCV is highly infectious and that it follows epizootiological patterns similar to those of SDAV.

9. Diagnosis of SDAV and RCV Infection

Infections of SDAV or RCV can be diagnosed on the basis of clinical signs, lesions, and serological profiles of NT and CF antibody and confirmed by isolation of the causative virus. Active SDAV infection should not be diagnosed solely on the basis of serological data, since anti-SDAV antibody can persist in previously infected rats for many weeks (see Section III, A, 8). The duration of anti-RCV antibody in rat serum after infection has not been reported. The viruses can be demonstrated in tissues by immunofluorescence for about 7 days after exposure and can be isolated in PRK cultures or by ic inoculation of neonatal mice (15).

It is difficult to differentiate RCV from SDAV infection virologically or serologically since the viruses are similar antigenically, physicochemically, and in their *in vitro* growth characteristics. As noted previously, however, if CF and NT antibody titers to SDAV and RCV are compared, titers to the homologous virus will likely be slightly higher than those to the heterologous virus (Table III) (15). Comparison of experimental infections with SDAV and RCV have revealed additional differences (Table VI). First, clinical signs of rhinitis and sialodacryoadenitis are common during SDAV infection and keratitis may occur, whereas RCV infection is asymptomatic. Second, RCV causes mild interstitial pneumonia in adults, whereas SDAV does not. Third, RCV replicates poorly in salivary and lacrimal glands and only rarely produces lesions, whereas SDAV is highly pathogenic for these tissues. Nevertheless, suitable caution should be maintained in differentiating these infections since the experimental data described were obtained solely from CD rats. Mitigating factors such as strain of virus, strain of rat, and age remain to be examined.

10. Differential Diagnosis

Clinically, cervical swelling from inflammatory edema and salivary gland enlargement is virtually pathognomic for rat coronavirus infection and is more characteristic of SDAV infection than of RCV infection. Porphyrin-tinged nasoocular exudates can accumulate during SDAV infection, but they also occur in murine respiratory mycoplasmosis. Ammonia fumes, especially from urine-soaked bedding, can cause acute inflammation of the eye and nose resembling SDAV infection.

Table VI

Comparison of the Major Features of Experimental Infection with SDAV and RCV in Adult Germfree CD Rats[a,b]

Feature	SDAV	RCV
Clinical signs		
Photophobia	Yes	No
Sneezing	Yes	No
Cervical swelling	Yes	No
Viral replication		
Respiratory system	Yes	Yes
Salivary glands	Yes	Trace
Lacrimal glands	Yes	Trace
Lesions		
Acute rhinotracheitis	Yes	Yes
Focal interstitial pneumonia	No	Yes
Sialoadenitis	Yes	Trace
Dacryoadenitis	Yes	No
Antibody response		
Complement fixing	Yes	No[c]
Neutralizing	Yes	Yes

[a] After Bhatt and Jacoby (11) with modification.
[b] See text for additional discussion.
[c] Up to 8 days postinoculation. Complement fixing antibody can occur in rats tested at later times.

Morphologically, sialoadenitis and dacryoadenitis are characteristic of rat coronavirus infections. Salivary and lacrimal gland lesions may, however, be mild and transient or may not develop, especially during RCV infection, and detectable lesions may involve only the respiratory tract. They must then be differentiated from infections caused by *Mycoplasma pulmonis,* Sendai virus, or pathogenic bacteria. Lesions associated with these agents are generally more severe than those caused by coronaviruses, and have been described elsewhere* (21,82). Dual infections, for example, with SDAV and Sendai virus, may also complicate interpretation of lesions unless adequate serological and virological tests are performed.

Infection with SDAV also must be differentiated from rat cytomegalovirus infection. The latter is clinically silent, lesions are usually mild and are characterized by enlarged salivary ductal epithelial cells with intranuclear inclusions (79). Hunt (58) detected intranuclear inclusions in the Harderian glands of rats with dacryoadenitis which may have been virus related, but inclusions have not been found in confirmed natural or experimental SDAV infection. Bacterial keratocon-

*"A Guide to Infectious Diseases of Mice and Rats," A Report of the Committee on Laboratory and Animal Diseases, Institute of Laboratory Animal Resources. Natl. Research Council, National Academy of Sciences, Washington, D.C., 1971.

junctivitis can also occur in rats, but SDAV must be eliminated as an underlying cause (53,164).

11. Control of SDAV and RCV Infection

Rat colonies can be maintained free of coronaviruses if they are kept under rigid barrier conditions and are handled by personnel familiar with proper disinfection procedures for specific pathogen-free facilities. The key to effective control in infected colonies stems from recognition that infected rats shed virus for about 7 days and then are immune and that latent infections do not occur. Since SDAV spreads rapidly through a susceptible colony, all rats in a room should be infected and immune in 3 to 5 weeks. Thus, infected holding or experimental colonies should be quarantined for at least 4 weeks and preferably 6 to 8 weeks after infection is detected. In production colonies, breeding should cease for 6 weeks and weanlings should be removed from the room, since sucklings and weanlings of infected dams are particularly susceptible to infection. The quarantine period may be reduced by increasing contact exposure among susceptible rats. If susceptible rats are eliminated (e.g., weanlings and rats introduced from other colonies) the infection will disappear (14). However, once immune rats are replaced by susceptible rats, the opportunity for infection again increases. In addition, the possibility of reinfection with the same strain or with a different strain of coronavirus has not been ruled out. The immune status of the colony and of any rats to be introduced can be established by serological monitoring. There are no published reports of effective vaccination protocols for SDAV or RCV infection.

Rat coronaviruses are quite labile, so routine disinfection of facilities and equipment will destroy environmental sources of infection. There is no evidence that RCV or SDAV are communicable to humans. It is not known whether human coronaviruses can infect rats.

12. Interference with Research

Rat coronavirus infections may hamper, if only transiently, studies on the respiratory system or salivary glands of rats. Furthermore, infected rats may be a greater risk for inhalation anesthesia, since excess mucus produced during acute coronavirus rhinitis can obstruct major airways. Eye research may be hampered by SDAV-associated keratoconjunctivitis, and chronically affected rats are obviously unsuitable for study. The eye lesion may be particularly troublesome for long-term studies such as those required for toxicological programs (80). Finally, since both SDAV and RCV are primary pathogens for the respiratory tract, it has been suggested they may act as initiators or as copathogens in murine respiratory mycoplasmosis (61,120).

B. Paramyxoviruses [Sendai Virus (Parainfluenza 1)]

1. General

Sendai virus was isolated originally from mice in Japan (44), but it can infect hamsters and guinea pigs (127). In mice, it causes silent respiratory infections, particularly in adults, but severe or even fatal bronchopneumonia and interstitial pneumonia can occur in sucklings and in genetically susceptible adults (13,118,121,122). The infectivity and pathogenicity of Sendai virus for rats is not well characterized, and there are few reports of natural outbreaks. Serological studies from our laboratory indicate, however, that Sendai virus is widely disseminated in rats from commercial breeders. (Rats from six of nine colonies had anti-Sendai virus antibody.) Therefore, we feel that Sendai virus infection is common among rats.

2. Properties

Sendai virus is a pleomorphic, filamentous virus with a lipid coat derived from host cell plasma membrane. Therefore, it is readily inactivated by organic solvents such as ether. It can also be inactivated by ultraviolet light and is unstable at temperatures above 37°C.

Sendai virus has hemolytic activity and two major surface antigens: a hemagglutinin and a neuramidase. It also has a nonhemagglutinating internal CF antigen. Sendai virus is antigenically related to parainfluenza types 2 and 3, but can be differentiated from them serologically by CF, HAI, or NT tests. The virus grows well in the amnion and allantois of embryonated eggs and in a variety of cell cultures, including human and monkey kidney and in perfused rat lung organ cultures (19). We prefer to propogate Sendai virus in BHK-21 cells to minimize the danger of contamination with latent passenger viruses which are particularly troublesome in primary monkey kidney cultures (13). Sendai virus induces syncytial giant cells in culture as is typical for other parainfluenza viruses. The biochemical, physical, antigenic, and cultural characteristics of Sendai virus has been described at length by others (27,33,44,60).

3. Clinical Disease

Spontaneous Sendai virus infection of rats appears to follow patterns described for mice (121). In our experience and in the experience of others, infection is usually asymptomatic (21), but it may be associated with signs of pneumonia (88). Makino and colleagues (88) also observed a decrease in average litter size and retarded growth of young rats during a cyclic epizootic of Sendai virus infection. Jonas (65) has also noticed transient decreases in production during active Sendai infection in a large breeding colony. Intranasally inoculated rats developed

extensive pulmonary lesions with respiratory difficulty, anorexia, and starry haircoats within 1 week after exposure. Tyrrell and Coid (157) found that clinical disease in experimentally infected weanlings was self-limiting and that mortality was negligible. Contact exposed rats seroconverted, but remained asymptomatic.

Coid and Wardman (28) examined the effects of Sendai virus on pregnant rats. Nine of 12 rats exposed to aerosols of virus at 4 to 5 days of pregnancy resorbed all embryos, but virus was recovered from the conceptus of only 1 of 14 additional rats. Each dam developed respiratory distress, inappetence, and a rough haircoat within 1 week postinfection, and virus was recovered from lungs of all rats. The authors concluded that resorption was probably related to systemic distress from respiratory disease in the dams, rather than to direct virus infection of embryos.

4. Pathology

The pathogenesis and lesions of natural and experimental Sendai virus infection have been well described for mice (2,132,160), but only limited information on rats is available. Burek and co-workers (21) found that naturally infected rats seroconverted to Sendai virus and developed multifocal interstitial pneumonia. Unfortunately, limited attempts to isolate Sendai virus from affected rats were unsuccessful. Lesions included perivascular and peribronchial lymphocytic and plasmacytic infiltrates; focal necrosis and hyperplasia of bronchiolar epithelium with infiltration by lymphocytes and neutrophils; and accumulation of mucus, inflammatory cells, and necrotic cell debris in some airways. In some rats, interstitial inflammation was accompanied by syncytial giant cell formation. Rats younger than 8 months seemed predisposed to severe interstitial lesions compared to older rats in which peribronchial and perivascular inflammation were prominent and interstitial lesions were less severe. The frequency and severity of interstitial and perivascular lesions subsided over a 7-month period, and antibody titers to Sendai virus also decreased. Peribronchial lesions persisted in many rats, however, for at least 7 months postinfection.

They also reported that rats seroconverted to pneumonia virus of mice (PVM) as well as to Sendai virus. Since PVM can cause interstitial pneumonia in mice, they could not eliminate the possibility that PVM contributed to the Sendai-associated lesions they described in rats.

Sendai virus lesions have been produced in rats experimentally, but macroscopic descriptions were sketchy and histological findings were not reported (28,157). An example of an early lesion in a germfree rat inoculated intranasally with Sendai virus is shown in Fig. 38.

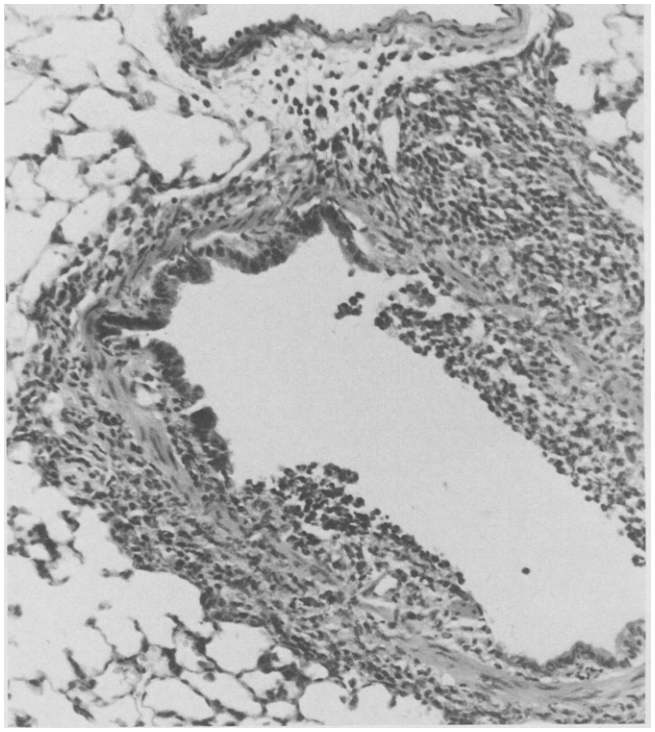

Fig. 38. Necrotizing bronchitis in a germfree rat inoculated intranasally with Sendai virus.

5. Epizootiology

Epizootiological studies of Sendai virus infection must be expanded before its natural history in rats is well defined, but based on the few reports available and our own experience, it seems likely that infection of rats will follow patterns reported previously for mice (13,121).

Sendai virus is highly infectious and can be expected to disseminate widely and rapidly through a colony. A typical outbreak was reported by Makino and co-workers (88) in a breeding colony of 500 rats. Infection spread rapidly as determined by development of HAI antibody in exposed rats, but clinical signs of respiratory disease and retarded growth lasted for several weeks during each episode. Furthermore, Sendai virus was isolated from lungs of affected rats. Outbreaks recurred at 8- to 10-month intervals among rats born after each preceding outbreak had subsided. Susceptible rats remained seronegative until the colony was reinfected. Hemagglutination inhibition antibody titers to Sendai virus increased during each outbreak and were detectable for at least 1 year postinfection. Burek *et al.* (21) reported an epizootic of Sendai virus infection in breeding and aging colonies of WAG/Rij rats. Rats seroconverted and had interstitial pneumonia, but they remained asymptomatic. Attempts to isolate virus from six seropositive rats were unsuccessful.

6. Diagnosis and Differential Diagnosis

Sendai virus infection may provoke clinical signs, such as

ruffled haircoats, inappetence, respiratory distress, growth retardation, or decreased litter size. Sendai virus infection should also be considered if deaths occur during routine anesthesia or if unexplained shifts in immunological responses occur (45). More commonly, however, infection remains subclinical, so laboratory diagnostic tests are required for diagnosis. Detection of anti-Sendai antibody in serum provides strong evidence for infection. There are HAI and NT tests available, but the CF test appears to be most sensitive (118,141). Since a single antibody survey may indicate past or current infection, diagnostic procedures should include histopathological examination for pneumonia characterized by epithelial necrosis in bronchi and bronchioles and, when possible, virus isolation from lung during acute stages of infection. Virus can be isolated in embryonated eggs, primary monkey kidney cells (121), or BHK-21 cells, but in our laboratory the last is preferred. If CPE does not appear by 1 week, it has been recommended that cultures be checked for Sendai antigen by hemadsorption with guinea pig erythrocytes (27). In our experience, however, BHK-21 cultures that are CPE negative are never hemadsorption positive.

Sendai virus infection of rats must be differentiated from respiratory infection of rats due to coronaviruses, *Mycoplasma,* and bacteria. It is helpful to remember that uncomplicated Sendai virus infection involves the lungs, but not the upper respiratory tract, whereas coronavirus and *Mycoplasma* infections regularly induce rhinitis. Furthermore, in contrast to Sendai virus infection, coronavirus infection is not associated with necrotizing bronchitis and bronchiolitis, and *Mycoplasma* infection produces chronic inflammation of the lung culminating in bronchiectasis and bronchiolectasis (11,61,82). It has been suggested that PVM may cause pneumonia in rats, but this has not been confirmed (21). Since Sendai virus infection also has been associated with embryonic resorption and retarded growth, RV infection must also be ruled out.

7. Control

Based on studies in mice (13,121), it is likely that Sendai virus infection in rats is self-limiting. Persistence of infection in a colony probably depends on continued introduction of susceptible animals, especially sucklings and weanlings. Thus, infection should be controlled by halting introduction (including breeding) of susceptible rats into a colony for 4 to 8 weeks. This will allow infection to run its course in exposed rats.

Rats can be kept Sendai virus-free in suitable barrier facilities serviced by technical personnel schooled in the control of infectious diseases. Filter lids for animal boxes have, in our experience, neither prevented nor contained Sendai virus outbreaks. Furthermore, since Sendai virus infection is prevalent among mice and hamsters, susceptible rats should not be housed with infected mice and hamsters if they must remain Sendai virus-negative. If valuable animals from an infected colony must be placed in a Sendai virus-free colony, seropositive animals should be selected and quarantined for 30 days in a laminar air flow unit or other appropriate isolation unit. Production colonies, experimental colonies, and, if possible, animal vendors should be monitored serologically for Sendai virus infection at regular intervals (e.g., semiannually). Additional discussion on control of virus infections in rat colonies is found in Section VII.

8. Interference with Research

Sendai virus can cause pneumonia in rats, but infection is frequently subclinical. Therefore, any experimental procedure involving the respiratory system, from anesthesia to inhalation toxicology, could be at risk during active infection. In addition, histological interpretation of experimentally induced pulmonary lesions may be complicated by residual lesions of Sendai viral pneumonia. Tissue harvests from inapparently infected rats also present a danger of tissue culture contamination or inadvertent infection of recipient rats subsequently inoculated with virus-infected material. There is recent evidence that selected immunological responses of rats may be suppressed during Sendai virus infection (45) and that the virus can induce numerous long-lasting immunological changes in mice (69a). Sendai virus also should be considered a potential copathogen or aggrevating factor in other respiratory diseases of rats. Finally, infected rats may disseminate infection to other susceptible rodents.

C. RNA Viruses Which May Infect Rats [Reovirus 3, Pneumonia Virus of Mice (PVM), and Mouse Encephalomyelitis Virus]

1. General

These viruses are not known to be naturally pathogenic for rats. Subclinical infections may occur, however, since antibodies to them have been detected in rat sera.

2. Reovirus 3

Mammalian reoviruses consist of three serotypes: 1, 2, and 3. They cross-react by CF and immunofluorescence assays, but can be separated antigenically by NT and HAI tests (135). Reovirus 3 is a natural pathogen of mice and produces a syndrome in sucklings characterized clinically by runting, oily skin, jaundice, conjunctivitis, hair loss, neurological disturbances, and sporadic deaths and pathologically by focal nec-

rosis of liver, pancreas, heart, and brain with nonsuppurative encephalitis (32,143–145). Spontaneous reovirus infection has not been described for rats, but we and others (10,31) have detected HAI antibody to reovirus 3 in rat serum. Experimental infections of rats with reoviruses 1 and 3 have been reported as described in Section VI.

3. Pneumonia Virus of Mice (PVM)

This virus is closely related to respiratory syncytial virus of humans. It produces silent infections in mice. However, virulent, mouse lung-passaged strains can cause severe interstitial pneumonia after intranasal inoculation in mice (56,57). It can be cultivated *in vitro* in BHK-21 cells and produces CPE in about 1 week (50). Neutralizing and HAI antibodies have been detected in rats (55), but clinical signs or lesions have not been reported. Burek *et al.* (21) detected seroconversions to PVM among rats sustaining Sendai virus-associated interstitial pneumonia and suggested that PVM may have contributed to the development of pneumonia.

4. Mouse Encephalomyelitis Virus

Mouse encephalomyelitis virus is a picornavirus that causes persistent latent infection of mice, but which can occasionally cause a paralytic syndrome similar clinically and morphologically to human poliomyelitis (24,147). There are no published reports documenting infection of rats either by isolation of virus or by detection of serum antibody. We and others (10; J. C. Parker, personal communication) have, however, detected occasional low titers of HAI or NT antibody to the GD VII strain of mouse encephalomyelitis virus in rats. The biological significance of these antibodies is unknown.

IV. UNCLASSIFIED VIRUSES OR VIRUSLIKE AGENTS

A. MHG Virus

McConnell and co-workers (101) isolated a neurotropic agent from adult Sprague-Dawley rats that was biologically and physically similar to an enterovirus. A clinical disease characterized by circling, incoordination, tremors, torticollis, and high mortality was produced in suckling rats and mice by ic inoculation. Virus was isolated from brain, lung, and intestine. Lesions included hydrocephalus, brain edema, neuronal destruction, and gliosis in the gray matter of spinal cord and brainstem and mild encephalitis. The agent was nonhemagglutinating with erythrocytes from a variety of species, but it reacted at low titer (up to 1 : 16) with antibody to mouse encephalomyelitis virus (GD VII strain) by CF assay.

Interestingly, these workers also found CF antibody to their agent in human sera. Additional characterization of the agent has, to our knowledge, not been reported.

B. Rat Submaxillary Gland (RSMG) Virus

Ashe and co-workers (4) isolated a cytopathic agent from submaxillary glands of 74 of 97 clinically normal conventional, monoinfected, or germfree rats and designated it RSMG virus. It measured approximately 50 to 300 nm by filtration techniques and was not sensitive to lipid solvents (in contrast to cytomegalovirus). It was, inactivated, however, at pHs below 5, after exposure to bactericidal light or by heating to 60°C for 30 min. The RSMG virus was originally isolated by inoculating salivary gland extracts into primary monolayer cultures of rabbit kidney and was passed repeatedly in similar cultures. Cytopathic effect developed in 2 to 5 days, and cultures were rapidly destroyed. Efforts to grow RSMG virus in cultured cells from monkeys, rats, hamsters, mice, and chickens were unsuccessful. Virus could be adapted to HeLa cells and to human skin cells. Clinical signs did not develop in experimentally inoculated suckling, weanling, or adult rats, mice, or hamsters.

Infected salivary glands were histologically normal, but contained a hemagglutinin which was first detected at 8 weeks, was apparently specific for rabbit erythrocytes, and was ostensibly virus associated. Neutralizing antibody to RSMG virus and HAI antibody to the hemagglutinin were detected in sera of affected rats, the latter antibody being detected in rats as young as 3 weeks. The hemagglutinin was not inhibited by antisera to RV, H-1 virus, MVM, or reovirus 3. Additional studies of RSMG virus have not been reported, but Ashe's work indicates that it is a persistent latent virus that can probably be transmitted vertically and that is not related to other sialotropic rat viruses such as RCV, SDAV, or rat cytomegalovirus (3).

C. Novy Virus

Jordan *et al.* (68) recovered a filterable agent from virus-infected rat blood stored in glycerin at about 9°C. Remarkably, the blood had been collected by Novy 35 years prior to Jordan's reisolation (117). It was subsequently shown to cause fatal infection of ic- or ip-inoculated weanling rats and mice, but lesions were not described. Further work with this agent has not been reported.

D. Viruslike Pneumotropic Agents (Enzootic Bronchiectasis Agent, Gray Lung Virus, and Wild Rat Pneumonia Agent)

1. Enzootic Bronchiectasis

Nelson *et al.* [reviewed by Nelson (114)] found that suspensions of pneumonic lung from *Mycoplasma*-free rats caused chronic pneumonia in intranasally inoculated mice and rats. The putative etiological agent was filterable, but was not isolated. Nelson indicated that it may be a copathogen with *M. pulmonis* in chronic respiratory disease of rats, but this view has not been confirmed.

2. Gray Lung Virus

Andrews and Glover (1) found a pneumotropic agent in mice inoculated with bovine and human material. It caused red-gray consolidation of lungs due to interstitial pneumonia and pulmonary edema. Vrolijk *et al.* (159) described similar naturally occurring lesions in laboratory and wild rats. A viruslike agent infectious for mice was isolated from affected animals, but it has not been characterized in laboratory rats.

3. Wild Rat Pneumonia Agent

Nelson (113) also recovered an agent from lungs of several wild rats which produced interstitial pneumonia in mice. He was not able to isolate the etiological agent, but suggested it was related to the agent of gray lung pneumonia (114).

The association of these agents with rat pneumonias needs further definition. None has been adequately characterized *in vitro* or *in vivo,* and serological tests to detect them have not been developed.

V. EXPERIMENTAL VIRAL INFECTIONS

A. General

Excluding studies of natural infections, rats have been used sparingly as experimental animals for *in vivo* virological investigations compared to mice and hamsters. Examples of experimental infections of rats with heterologous viruses are listed in Table VII. Three viruses have been selected for additional comment either because they induce interesting lesions in inoculated rats or because they are natural pathogens for other rodents.

Table VII

Examples of Experimental Infections of Rats with Heterologous Viruses

Virus	Experimental disease	Reference
Borna	Slow virus infection	100
		115
Herpes simplex	Encephalitis, hepatitis, skin lesions	95
		125
		146
Lymphocytic choriomeningitis	Cerebellar hypoplasia, retinopathy	104–108
		85
Measles	Encephalitis	23
		140
Reovirus 1	Hydrocephalus	75
		90
Rubella	Congenital defects of heart, eye, other organs	34
		35
SV40	Retinopathy	42
Toga	Encephalitis	39

B. Lymphocytic Choriomeningitis (LCM) Virus

Wild mice are the natural hosts for LCM virus, an arenavirus which may occur as a latent infection of mice and hamsters and as an acute infection of guinea pigs. The pathobiology of LCM infection has been studied extensively, since in mice it is a prototype for cell-mediated immunological injury to the nervous system of animals infected as adults and a prototype for immune complex-mediated glomerular disease in adult mice infected as fetuses or neonates [reviewed by Lehmann-Grube (81a)]. LCM infection has not been detected in laboratory rats, but infection can be induced experimentally. Monjan and colleagues (104,105,107,108) have induced cerebellar "hypoplasia" and retinopathy in ic inoculated suckling rats. Cerebellar hypoplasia was secondary to necrosis of the cortex which was most severe in rats inoculated 4 days postpartum. Interestingly, cerebellar lesions were prevented by immunosuppressing rats with anti-lymphocytic serum (106) and were elicited by adoptive immunization of infected rats with LCM virus-immune splenic cells (105). Therefore, the pathogenesis is similar to that observed for LCM disease in mice in that cell-mediated immunity to LCM virus is required to cause acute lesions. The retinal lesions were characterized by progressive destruction of all retinal layers and modest inflammation, and the presence of LCM virus in all layers of retina. The retinal lesions were also inhibited by immunosuppression (108).

Löhler and associates (85) showed that the WE strain of LCM virus caused widespread infection of the brain after ic inoculation of adult Sprague-Dawley rats. Lesions were characterized by lymphocytic infiltration of meninges, choroid plexus, and paraventricular areas. They closely resembled

changes seen in brains of inoculated adult mice. Recently, Zinkernagel *et al.* (165) have shown that rats sensitized with live LCM virus develop potent cell-mediated immune responses to the virus.

C. Reoviruses 1 and 3

The infectivity and pathogenicity of reoviruses for naturally infected rats are believed to be low; however, suckling rats inoculated ic and ip with reovirus 1 at 3 days of age develop encephalitis. Some rats also develop hydrocephalus secondary to viral infection of ependymal cells inoculated with reovirus 3. Pregnant rats (75,90) developed viremia in the presence of maternal serum antibodies, and transient nonfatal infection of fetuses occurred. When virus was inoculated directly into fetuses, however, there was a high rate of death and resorption (77,93).

D. Mousepox Virus (Ectromelia)

We are not aware of adequately documented studies showing that mousepox virus produces natural infections of rats. Burnet and Lush (22) found that rats inoculated intranasally with large doses of virus developed inapparent infection of olfactory mucosa. Neutralizing antibody was detected in serum of infected rats. Rats inoculated intradermally developed either a minute papule at the injection site or no lesions, but HAI antibodies appeared in serum (41). Intraperitoneally inoculated rats also developed antiviral antibody.

VI. COLLECTION OF SAMPLES FOR SEROLOGICAL TESTS AND VIRUS ISOLATION

A. Serum for Antibody Titrations

The demonstration of anti-viral antibody in serum is good evidence for infection with the homologous virus or with an antigenically related virus. Serological testing, in fact, often provides the first evidence of asymptomatic or latent viral infections and, in laboratories with limited capabilities in tissue culture or pathology, may be the only method to detect infection. Sampling protocols must reflect the population under study and the goals of the surveillance program. Consideration must be given to the number, age, and sex of rats to be tested for each sampling period, to the geographical distribution of rats selected from a colony or a vendor shipment, and to the frequency of sampling. These variables are discussed further in Section VII.

Blood can be collected at necropsy by cardiac, intraaortic, or intravenacaval puncture and should be allowed to clot at room temperature. If an animal must be saved, smaller samples can be obtained under ether anesthesia by aseptic cardiac puncture or by retroorbital bleeding with a heparinized capillary tube. Use of nonheparinized tubes frequently results in premature clotting. Heparinized samples (plasma) should be held in ice, separated shortly after collection, and assayed immediately or frozen as described below. Clotted blood samples can be held overnight at 4°C and centrifuged and 0.5- to 1.0-ml aliquots of serum should be stored at −20°C or lower until tested. We recommend that samples not be pooled. Frozen or refrigerated samples may be packed for shipment as described at the end of Section VI, B. If serum samples are collected with reasonable care (aseptically) and rapid shipment is assured, they can be shipped unrefrigerated. It is best, however, to discuss shipping details with the respective testing laboratory.

B. Tissues for Virus Isolation

Clinical signs, serological profiles, lesions, and immunofluorescence staining can incriminate a particular virus and thus narrow the selection of tissues for virus isolation. Conversely, there is considerable overlap in the spectrum of tissues susceptible to the common rat viruses, so it is better to "overcollect" than to "undercollect." Timing is also critical for successful isolations during acute, self-limiting infection such as those caused by SDAV or Sendai virus, since rats may harbor virus before lesions develop or only during early or florid stages of disease. Attempts to isolate infectious virus once seroconversion has occurred may therefore be unsuccessful. On the other hand, isolation of latent viruses such as RV may be difficult without proper culturing techniques, regardless of when tissues are harvested. As a rule fetal, suckling, or weanling rats with low levels of antibody should be selected (Table VIII).

Regardless of the tissues to be harvested, aseptic techniques should be followed. The pelt should be wet with an antiseptic solution (e.g., 70% ethyl alcohol), but care must be taken not to contaminate the tissues so that virus is not inactivated. Instruments should be autoclaved or flame-sterilized (after immersion in 95% or absolute alcohol) and allowed to cool at room temperature. Small pieces of tissue 0.5-1.0 cm^2 should be placed in labeled sterile vials (2 dram screwcap glass vials are adequate) and held on ice until they are frozen. Clotted or anticoagulant-treated blood also can be stored in vials.

Tissues should be stored at −60°C or below. If deep cold storage must be delayed, tissues may be held overnight in ice. Storage of tissues at −20°C should be avoided, since virus titers may drop quickly. If tissues are triturated before freezing, antibody-free protein (e.g., fetal bovine serum) should be added to the suspension to a final concentration of 50% to

Table VIII

Tissues Recommended for Isolation of Viruses from Naturally Infected Rats[a]

	Virus	Tissues
I.	Common viruses	
	Parvoviruses[b]	Lesions, spleen, liver, intestine
	Rat coronavirus[c]	Nasal wash, lung
	Sendai virus	Nasal wash, lung
	Sialodacryoadenitis virus[d]	Nasal wash, submaxillary salivary gland, Harderian gland
II.	Other viruses	
	Adenovirus[e]	Intestines, mesenteric lymph nodes
	Cytomegalovirus	Submaxillary salivary gland, saliva
	Mouse encephalomyelitis virus[e]	Intestines, central nervous system
	Pneumonia virus of mice[e]	Nasal wash, lung
	Rat submaxillary gland virus	Submaxillary gland
	Reovirus 3[e]	Intestines

[a] See footnote, p. 301.
[b] Select young rats with low titers of HAI antibody.
[c] See Fig. 35.
[d] See Fig. 30.
[e] Has not been isolated from rats, but antibody has been detected in rat serum. See text for details.

prevent inactivation of virus. Tissues stored in dry ice should be placed in heat-sealed glass ampules, since CO_2 can infiltrate tightened screwcap vials and may inactivate virus.

If facilities for serological testing or virus isolation are not available locally, samples can be shipped in sealed ampules encased in rigid cardboard carriers placed in a styrofoam or other suitable insulated shipping carton filled with dry ice. Alternatively, serum can be shipped in containers containing ice cubes or crushed ice. Containers should be clearly marked according to federal regulations,* and the receiving laboratory should be notified of the time and mode of shipping in advance.

VII. GENERAL COMMENTS ON THE DETECTION, DIAGNOSIS, AND CONTROL OF VIRAL INFECTIONS

A. Detection

A healthy laboratory rat has been defined as one free of all currently detectable known viruses, with a defined microbial flora and maintained in a protective barrier.† In practice, however, investigators usually use rats with a varied history of exposure to viruses, so documentation of exposure of infection should be integrated into quality assurance programs for breeding and experimental colonies.

Recognition of viral infection may be relatively simple if mortality or characteristic clinical signs occur, but detection is more difficult if infection is asymptomatic or latent. Detection procedures must, therefore, be designed to encompass all possibilities so that preventive measures can be instituted to reduce the negative impact of infection on animal-related research.

Infectious disease can be detected by intramural routine surveillance programs. "Routine surveillance" is, however, a general term which, depending on the needs and judgment of the professional staff, may imply minimal effort or a thorough multidimensional program. Minimal surveillance procedures may be limited to clinical spot checks of production or research colonies or newly received vendor rats with follow-up clinical checks. This may detect active infections such as SDAV, but will usually miss latent infections such as those caused by rat parvoviruses. Effective surveillance must, therefore, include adequate diagnostic laboratory support in virology, serology, and pathology as well as thorough clinical evaluations (63,64).

Signs of disease may be reported to the veterinary staff by veterinary assistants or animal health technicians, animal care technicians, research technicians, or the investigator. The role of investigators in the early recognition of disease can be extremely helpful, since they often have unique opportunities to observe effects of potential viral infection through unexplained changes of responses in experimental procedures. Increased mortality, decreased survival times, increased or unexplained anesthesia-associated deaths, altered metabolic or immunological responses, or variations in tumor growth *in vivo* or *in vitro* all may be potentially associated with viral infection.

The detection of subclinical or latent infections depends on well conceived, statistically valid sampling procedures, since it is impossible to test every animal. Sampling protocols must consider the age, sex, and number of rats to be tested for each sampling period, the distribution of rats selected from a colony room or vendor, and the frequency of sampling. As a rule, we recommend testing equal numbers of males and females at 90 days of age and also retired breeders at 9 to 12 months of age. The number of rats tested may vary according to the goals of the program, but we suggest that a minimum of 10 rats per age group from a given room be screened twice yearly for a total of 40 rats annually. For intramural production colonies or long-term holding colonies, no more than 1 rat per cage should be included in a single sample population, but blood or tissues from all rats selected should be collected on the same day. In addition, rats should be chosen from as many different racks or shelves in a room as possible. A useful technique, especially for intramural sampling, is to place sentinel weanling rats in separate cages among other rats in a room and test them when

*"Transportation of Hazardous Materials," Vol. 4, No. 1. National Institutes of Health Guide for Grants and Contracts, United States Department of Health, Education and Welfare, Washington, D.C., 1975.

†"Long-Term Holding of Laboratory Rodents," A Report of the Committee on Long-Term Holding of Laboratory Rodents, Institute of Laboratory Animal Resources. *ILAR News* **19,** No. 4 (1976).

Table IX

Sample Size Required to Detect at Least One Infected Animal in a Population for a Given Expected Incidence of Infection[a-c]

No. of animals in sample	Incidence of infection in population (%)
29	10
14	20
9	30
6	40
5	50
4	60
3	70
2	80
2	90

[a]Confidence limit = 95%.

[b]See text for additional explanation.

[c]From formulas contributed by Dr. C. White, Yale University School of Medicine and described in "Long-term Holding of Laboratory Rodents." A report of the Committee on Long-Term Holding of Laboratory Rodents, Institute of Laboratory Animal Resources. *ILAR News*, **19**, No. 4 (1976).

they reach the appropriate age.

Vendor sampling is more difficult to control, but if the source of the rats (specific room or area) is requested, reliable data can be obtained. We recommend testing only 90-day-old vendor-derived rats according to the protocol outlined above, unless screening of retired breeders can be justified.

The recommendations for sample size were derived from a statistical formula provided by White and extrapolated to Table IX. For example, from the table one can infer that if a population of rats with a 30% incidence of viral infection is properly sampled, there is a 95% probability that at least one infected rat will be detected in a sample of nine rats from that population. The sampling protocol must include a thorough serological and pathological evaluation. The reader is referred to Table IX for additional information.

B. Diagnosis

Accurate diagnosis is critical for control of infection and for evaluating the impact of infection on research, not only with respect to requirements of individual investigators but also with respect to hazards infection may present to other animals. Seroconversion or increasing titers of antibody to a particular viral antigen over several weeks is commonly accepted as good presumptive evidence of active or recent viral disease. Antibody alone, however, indicates only that exposure to a viral agent or to an antigenically related agent may have occurred. For example, it was known that rats developed humoral antibody to MHV (51); however, signs or lesions of MHV infection were never seen in rats. Parker *et al.* (120) and Bhatt *et al.* (15) showed subsequently that rat antibodies to MHV were elicited by rat coronaviruses (SDA and RCV), viruses which are antigenically related to MHV. Thus, antibody induced by one virus reacted with all three viruses. Similarly, isolation of a virus per se during a suspected outbreak may be misleading, since passenger viruses, multiple viral infections, or latent viruses with no influence on the problem under investigation may occur. Therefore the significance of serological and virological findings must be assessed after evaluating clinical signs, lesions, and epizootiological data. Furthermore, before a final diagnosis is confirmed, the veterinarian must critically evaluate information about an outbreak and compare it to his or her knowledge about the suspected infection derived from personal experience or from the scientific literature. Diagnosis of viral infection must be combined with a search for its source. For example, rats lose colostrum-derived antibody progressively and, around the time of weaning, can become susceptible to viruses to which they were passively immune. Therefore, although infection may be present in a vendor colony, it is possible that weanling rats can be infected by an "in-house" source after arrival when passive immunity has decayed. It may be helpful in such situations to test whether rats received from a vendor become seronegative and remain free of infection when held in isolation for several weeks.*

C. Control

Effective control of viral infection requires that the veterinary clinician have thorough knowledge of the epizootiology of the etiological agent. For example, the clinician must be aware of the influence viruses may have on research so that control procedures are neither overzealous nor inadequate. The clinician must also consider the implications of spread from a colony where the impact of the virus may be negligible to a colony where its impact may be major and engender significant losses in time and money.

Generally, infections such as those caused by SDAV or Sendai virus can be eliminated, as discussed in previous sections, by appropriate quarantining procedures. On the other hand, persistent latent infections, such as those due to RV and which can occasionally be transmitted vertically, may require that all stock be killed, that rooms and equipment be thoroughly decontaminated, and that colonies be restocked with virus-free rats.

*The mouse antibody production (MAP) test has been used to detect viruses in mouse tissues and has been useful in detecting viral contamination of tumors (136). The test is based on the principle that inoculation of test tissues into a nonimmune recipient will elicit antibody to viruses in the inoculum. This test can be adapted for rats and is particularly valuable when dealing with latent infections such as those caused by RV.

Spread of air-borne virus from infected to susceptible animals can be reduced by methods ranging from (a) improved air handling procedures, such as maintaining infected rooms under constant negative pressure, to (b) reducing animal populations, separating cages, and covering cage tops with filter lids, to (c) use of more sophisticated air moving equipment, such as mass air flow rooms, laminar air flow racks, or Horsfall-type cabinets. Technicians should be instructed about proper animal handling procedures and more frequent cage changes to reduce ammonia levels.

Adequate surveillance programs to prevent entry of infected animals is a major priority. Rats received from other institutions should be quarantined for 30 days and tested, at least serologically, before introducing them to holding rooms. Portable isolators, like those used for gnotobiotic work are satisfactory for this purpose. Tissues for animal inoculation, such as tumors, should also be tested for passenger viruses prior to their use in animal rooms.

In summary, evaluation of a viral disease and recommendations for its control depend on many factors, including (a) detection of a problem, (b) accurate diagnosis of the disease, (c) complete knowledge of its epizootiology, (d) knowledge of the research using the rats being evaluated, (e) thorough assessment of the risk infection implies for other colonies or research projects, and (e) the economic and logistical feasibility of implementing adequate control measures successfully.

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