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Characterization of the Virus of Sialodacryoadenitis of Rats: A Member of the Coronavirus Group

Pravin N. Bhatt, Dean H. Percy, and Albert M. Jonas

From the Division of Health Science Resources, Section of Laboratory Animal Sciences, Yale University School of Medicine, New Haven, Connecticut

The virus that causes sialodacryoadenitis in rats has been isolated in mice and in primary cultures of rat-kidney cells and has been characterized as a heat-labile RNA virus that is sensitive to lipid solvents and is relatively stable at *p*H 3.0. This virus is antigenically related to the virus of hepatitis in mice and to coronavirus of rats. The range of hosts of this agent appears to be narrow. On the basis of available biologic characteristics, it has been placed in the coronavirus group.

Experimental transmission of sialodacryoadenitis (SDA) to germfree rats by intranasal inoculation of a suspension of infected salivary glands has been reported by Jonas et al. [1]. They demonstrated viruslike particles by electron-microscopic examination of thin sections of the salivary glands of infected animals and isolated an agent pathogenic for infant mice. With use of the mouse-brain-adapted agent, SDA was reproduced in rats. In a preliminary communication we reported the antigenic relationship of this agent to the virus of hepatitis in mice (MHV), which is a member of the coronavirus group [2, 3].

This communication describes the further characterization of the SDA virus after its adaptation to infant mice and to tissue culture.

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Please address requests for reprints to Dr. Pravin N. Bhatt, Division of Health Science Resources, Yale University School of Medicine, 375 Congress Avenue, New Haven, Connecticut 06510.

Materials and Methods

Viruses. A 10% suspension of salivary glands harvested from germfree rats that had been inoculated with the agent of rat sialodacryoadenitis was used for the initial work; later the mouse-brain-adapted virus was used. The original strain was designated no. 681. Stocks of rat coronavirus (RCV) [4] were prepared in cultures of kidney cells from germfree rats.

Mice. Mice of the CD(R)-1 HaM/ICR strain were obtained from a colony maintained for the Yale Arbovirus Research Unit. These mice had hemagglutination-inhibiting (HAI) antibodies to pneumonia virus of mice (PVM), sendai, and minute virus of mice (MVM, tests done by Microbiological Associates, Bethesda, Md.) but not to antigens of other murine viruses.

Infant and weanling mice were inoculated intracerebrally (ic) or intraperitoneally (ip) with 0.015 ml or 0.03 ml of infected-tissue suspension and observed daily for 21 days. Brains of sick mice were harvested and passaged in mice, rats, and tissue culture. In selected mice, brains were semisectioned along the midline; one half was frozen and the remainder was fixed in 10% buffered formalin. Other brains were frozen after coronal sectioning or were fixed whole in 10% buffered formalin and eventually processed for fluorescent-antibody procedures [5] or histology.

Rats. Germfree, cesarean-derived (CD) rats (Charles River Breeding Laboratories, Wilmington, Mass.), weighing approximately 200 g, or Wistar rats of similar weight (Manor Farms, Puerto Rico) were used. Previous studies indi-

cated that neither source had detectable serum antibodies to SDA virus and that both were susceptible to infection with this agent.

Rats were inoculated intranasally with 0.1 ml of virus-infected salivary-gland suspension, observed daily for evidence of overt illness, and sacrificed at various intervals. All rats were maintained in rigid plastic isolators with high-efficiency air filters.

Tissue culture. Three cell lines, baby-hamster kidney (BHK-21), VERO, and Hep–2, and primary monolayer cultures of rat embryo, rabbit kidney, rhesus-monkey kidney, guinea-pig-embryo skin, muscle, and kidney were used as previously described [1]. Monolayers obtained from explant cultures of submaxillary, parotid, Harderian, and exorbital glands of germfree rats, monolayers of trypsin-dispersed brain cells of infant mice, and a line of polyoma-transformed mouse cells (Py-AL/N) [6] were also tested. At a later stage in the study, primary rat-kidney (PRK) cultures prepared from kidneys of weanling Charles River CD germfree or conventional inbred dark Agouti (DA) rats were used.

Inoculated tubes were kept in a roller drum at 37 C and observed for cytopathic effect (CPE) at intervals of two to four days for at least 21 days. The fluid medium was changed when necessary. In the absence of CPE, a blind passage was made between the eighth and 16th day after inoculation. Cultures for passage were observed for one to two weeks for development of CPE, and, in the absence of CPE, VERO, BHK-21, and PMK cultures were challenged on the 12th day after inoculation with Chandipura [8] virus, an arbovirus of the vesicular stomatitis viral group, for determination of interference. In addition, the fluid from each of the second-passage cultures was inoculated ic into infant mice, and the mice were observed for 21 days. In some instances tissue-culture fluid from inoculated tubes was passaged into infant mice without further passages in tissue culture. Monolayer cultures of PRK, infant-mouse brain, and Py-AL/N cells were also examined by indirect immunofluorescence for the presence of viral antigen [5].

Characterization of the virus. For determination of the effect of 5-bromodeoxyuridine (5-BUDR), lipid solvent, low *p*H, and various temperatures, methods described by Bhatt et al. [9] were used. The hemagglutination method will be described under Results. Staining with acridine orange was done according to the method of Hsiung [10].

Preparation of immune sera. Hyperimmune sera were prepared in rats and mice by repeated inoculation of a suspension of salivary glands from infected rats and of brains from infected mice, respectively.

Complement-fixation test. Complement-fixing antigen was prepared by sucrose-acetone extraction from infected brains of two- to four-day-old mice. Polyvalent mouse-hepatitis CF antigen prepared in tissue culture was obtained from Microbiological Associates. The CF test was performed by the micromethod [11] using two units of complement and four to eight units of antigen. Serum of mice immune to SDA strain 681 was tested against 118 viral antigens by the CF test.

Neutralization test. The neutralization (N) test with sera immune to murine viruses was performed in infant mice, and these animals were observed for 14 days after inoculation. PRK cultures were used for cross-N tests, using SDA strain 681 and Parker's RCV. Sera were inactivated at 56 C for 30 min. Cultures were examined on the third and fifth days after inoculation. The titers of antibody and virus were calculated by the method of Reed and Muench [12].

Fluorescent-antibody method. Pieces of mouse brain 2–4 mm thick were quick-frozen in a dry ice-alcohol bath and stored at —83 C. Sections 6–8 μm thick were cut in a cryostat, two sections were mounted per slide, and then the slides were fixed in acetone at 25 C for 15–20 min and dried at 37 C for 15 min. Sections were stained immediately or stored at —25 C for 1–30 days before use. Tissue-culture cover slips were similarly prepared but at times were kept in chilled acetone at —25 C for 18 hr. The section and cover-slip preparations were reacted with sera immune to virus for 20 min at 25 C and then exposed to mouse or rat antiglobulin conjugate for 20 min. Phosphate-buffered saline (PBS) was used for washing. Preparations were examined with a Carl Zeiss microscope fitted with an HBO 200 W/4 supermercury lamp, a UG-5 exciter filter, and a 47/65 barrier filter.

Histopathology. Histologic examination of tissue from inoculated rats included Harderian, exorbital, parotid, and submaxillary glands. In suckling mice, coronal sections of brain and serial

transverse sections of the thoracic and abdominal regions were examined.

Attempts to induce disease in weanling mice. Sixty female mice, three- to four-weeks old, were given 2.5 mg of cortisone im twice a week beginning a week before inoculation and continuing until the end of the experiment. Twenty mice each were inoculated ic and ip with 0.03 ml and 0.1 ml of viral suspension containing $2 \times 10^{3.9}$ and $6.3 \times 10^{3.9}$ infant mouse LD_{50} ($IMLD_{50}$), respectively. Twenty control mice were inoculated with diluent, 10 by the ic route and 10 by the ip route. Another group of controls was neither inoculated nor given cortisone. Six mice from each group were killed for histologic studies on the seventh day after inoculation and two were killed on the 14th day after inoculation. Remaining mice were observed until the 21st day after inoculation, when the experiment was terminated. A complete necropsy was done on each mouse.

Induction of sialodacryoadenitis in susceptible rats by mouse-brain-adapted virus. Eleven rats, weighing 250 g and from a colony known to be susceptible to SDA virus, were inoculated by the intranasal route with fourth passage, infected-mouse-brain material. The inoculum contained approximately $6.6 \times 10^{3.8}$ $IMLD_{50}$ of virus. Rats were sacrificed on the fifth, sixth, and eighth days after inoculation. Harderian and submaxillary glands were processed for isolation of virus and histologic examination, whereas parotid gland was collected for histologic examination only.

Results

Adaptation to mice and related observations. A 10% suspension of infected salivary glands was inoculated ic into one-day-old mice. One mouse was sick on the fifth day after inoculation, eight more were sick on the seventh day after inoculation, and six on the eighth day after inoculation. Some of these animals were killed, and tissues were harvested for passages and histologic study, but mice that were sick but not killed died on the 10th day after inoculation. One mouse was unaffected and survived until the 21st day, when it was discarded. The disease was characterized by ataxia and uncoordination, followed by paresis, paralysis, and death. The same pattern of illness was observed on further passages. By the fifth mouse-brain passage, the incubation period was shortened to two to three days. There was usually a random pattern of illness and death from two to eight days and occasionally up to 10 days after inoculation. The pattern has remained unchanged for 29 passages with this strain of virus. One other observation made during the first passage in mice and amply confirmed during subsequent work was emaciation of sick mice as compared to uninoculated control mice of the same age. These differences were more marked in mice that were two to four days old or older when inoculated.

Other significant observations can be summarized as follows:

(*1*) The agent of SDA does not cause detectable illness in weanling (three- to four-week-old) mice when inoculated ic or ip or in infant mice inoculated ip.

(*2*) A comparative titration was done in mice two days old, 13 days old, and 22 days old that were inoculated ic with viral stock passaged 12 times in mouse brain. Titers were $10^{4.00}$, $10^{4.25}$, and $<10^{2.0}$ $IMLD_{50}$/0.015 ml, respectively.

(*3*) Virus has undergone 29 serial ic passages in zero- to six-day-old mice, the cumulative dilution of which exceeds 10^{-100}.

(*4*) The titer of virus between the fifth and 29th passage in mouse brain has remained relatively stable at $10^{3.5}$–$10^{5.0}$ $IMLD_{50}$/0.015 ml (usually around $10^{3.7}$ $IMLD_{50}$/0.015 ml).

(*5*) The original salivary-gland suspension was titrated in one-day-old mice and had a titer of $10^{3.6}$ $IMLD_{50}$/0.015 ml.

(*6*) When inoculated intranasally into susceptible rats, mouse-brain-adapted virus produced sialodacryoadenitis.

(*7*) Brains from two uninoculated mice (two days old) were harvested as controls; seven serial ic passages of this material were made at intervals of six to seven days in mice three to four days old. No agent pathogenic for mice was isolated from these control animals.

Histopathologic and immunofluorescent observations in inoculated mice. In general, histologic changes observed in the central nervous system of inoculated mice were characterized by diffuse and focal neuronal degeneration with minimal inflammatory cell response. Regions of brain most frequently involved were the cortices of the occipital and parietal lobes. Other foci of neuronal destruction were scattered elsewhere in the central nervous system; there was relatively little destruction

in the cerebellum. Affected neurons were pyknotic and densely eosinophilic. In addition, there was a scattering of shrunken, densely staining astrocytes in these areas. Occasionally there was hypertrophy and hyperplasia of capillary endothelial cells and minimal perivascular cuffing with mononuclear cells. Sometimes a few polymorphonuclear leukocytes were scattered in areas of destruction. Spinal cord, salivary glands, lung, heart, liver, kidney, spleen, and intestine were histologically normal.

Immunofluorescence procedures detected viral antigen in regions where frank cellular necrosis was seen by standard histologic techniques. In addition, intense staining was observed in neuronal cytoplasm of scattered cells that were intact and not associated with frank necrosis. Serial coronal sections had immunofluorescence staining in dorsal cortical areas, the ventral portion of Ammon's horn, the hypothalamus, and the brain stem, but fluorescence was rarely found in cerebellar folia and white matter.

Attempts to adapt the agent to monolayer cell cultures. The original salivary-gland suspension inoculated onto various monolayer cell cultures produced no detectable CPE up to 21 days after inoculation. When blind passages were made and cultures were challenged with Chandipura virus [8], interference was not observed. Fluid from the second passage in tissue culture was inoculated ic into infant mice; the results were negative.

Attempts were made to propagate mouse-brain-adapted virus to cell-culture systems, such as monolayers obtained from explant cultures of parotid, Harderian, exorbital, and submaxillary glands of germfree rats and monolayers of trypsin-dispersed infant-mouse-brain cultures. There was no detectable CPE. Similar results were obtained with the Py-AL/N cell line. Infectious virus or viral antigen was not detected when tissue-culture fluids from infected-mouse-brain and Py-AL/N cultures were inoculated ic into infant mice or when monolayers were examined by indirect immunofluorescence. However, PRK cultures showed CPE characterized by formation of multinucleated giant cells, which were seen as highly reflective masses. These cells fell off the glass wall a few hours later and were seen floating in medium. Tissue-culture fluids of these cultures contained virus as detected in infant mice, and cultures were positive for viral antigen by indirect immunofluorescence.

Some important observations are summarized as follows.

(*1*) Cultures were most sensitive when used within a week after seeding; then sensitivity decreased. The CPE was delayed and less extensive in older cultures.

(*2*) Development of virus in PRK cells was monitored by CPE, detection of viral antigen by indirect immunofluorescence, and quantitation of infectious virus in PRK tubes. Results are presented in table 1.

Significantly, detectable viral antigen developed by 12 hr and was followed by release of infectious virus into the medium. CPE was detected at 24 hr. Beyond 24 hr, quantitation of viral antigen was difficult due to lysis of cell sheets, and after 36 hr, titer of infectious virus decreased.

(*3*) The sensitivity of inoculation of mice ic with strain 681 virus was compared with that of inoculation of PRK cultures. Titers obtained with a mouse-brain-adapted virus were 2.5×10^4 $IMLD_{50}$ in mice and $10 \times 10^{4.3}$ $TCID_{50}$ in PRK cultures. Similar differences were also noted in other experiments.

Characterization of the agent. The effect of 5-BUDR on viral multiplication was determined by the method of Bhatt et al. [9]. Chandipura virus was used as RNA control (P. N. Bhatt, unpub-

Table 1. Development of viral antigen (VA), CPE, and infectious virus after infection of primary cultures of rat-kidney cells with sialodacryoadenitis virus.

Hour	VA*	CPE†	Viral titer ($log_{10}TCID_{50}$)
0	0	0	0.5
6	±	0	Trace
12	6/20‡	0	1.2
18	10/20	0	1.5
24	47/20	+	1.8
30	L§	++	2.5
36	L	+++	2.7
48	L	+++	1.7
72	L	+++	0.7
96	L	+++	Trace
120	L	+++	Trace
168	L	+++	Trace

* Determined by indirect fluorescent antibody method.

† 0 = no detectable CPE; + = approximately 1%–25% of cells showed CPE; ++ = approximately 26%–50% of cells showed CPE; +++ = approximately 51%–75% of cells showed CPE.

‡ Number of fluorescent foci/number of fields examined.

§ = Lysis of cell sheet; fluorescence detected but quantitation not possible.

Table 2. Effect of 5-bromodeoxyuridine (5-BUDR) on multiplication of the sialodacryoadenitis virus in primary cultures of rat-kidney cells.

Virus	Inoculum (log_{10} $TCID_{50}$)	Viral yield (log_{10} $TCID_{50}$/0.1 ml) With 10^{-5} M BUDR	Viral yield (log_{10} $TCID_{50}$/0.1 ml) Without BUDR
Chandipura	3.5	5.7	5.3
SDV	2.7	3.7	3.3
Vaccinia	$\geq$3.7	2.8	5.0

lished observation) and vaccinia as DNA control. 5-BUDR did not affect the multiplication of the SDA virus and of Chandipura virus, but multiplication of vaccinia virus was inhibited. Results are presented in table 2.

Sensitivity of SDA virus to a lipid solvent was also tested. The titer of virus was $10^{3.8}$ and $<10^{2.0}$ $IMLD_{50}$/0.015 ml for controls and chloroform-treated samples, respectively. The test was repeated with similar results, and it was concluded that the agent is sensitive to lipid solvents.

Effect of low pH on infectivity. The test was performed as described by Leibhaber [13]. Ten-fold serial dilutions of infected-mouse-brain suspension kept at different *p*H values were made in Eagle's minimal essential medium in Earle's base with 3% fetal bovine serum (FBS). The *p*H of each dilution was adjusted to approximately 7.0 by addition of Tris, and this solution was inoculated into mice. End points of infectivity were calculated by the method of Reed and Muench [12].

The titers of infectious virus detected in PBS after incubation for 3.0 hr at 25 C was $10^{3.6}$ $IMLD_{50}$/0.015 ml, whereas at *p*H 7.0 and *p*H 3.0 it was $10^{3.9}$ $IMLD_{50}$/0.015 ml and $10^{2.8}$ $IMLD_{50}$/0.015 ml, respectively. Thus infectious virus was relatively stable at low *p*H.

Effect of temperature on infectivity. The effect of a temperature of 37 C on infectious virus was determined as outlined by Bhatt et al. [9]. To determine the effect of a temperature of 56 C, infectivity was determined at intervals of 0, 5, and 10 min. An aliquot of viral stock was kept at 4 C, and infectivity was determined on days 0, 7, and 28.

Infectivity of viral strain 681 was stable in PBS plus 3% FBS at 37 C for 3 hr; the titer then decreased by 1.1 log_{10} by 5 hr. At 56 C the infectivity decreased from $10^{3.8}$ $IMLD_{50}$ at zero time to trace levels by 5 min and was undetectable by 10 min. Titers of viral stock kept at 4 C were $10^{3.8}$, $>10^{3.5}$, and $<10^{1}$ $IMLD_{50}$/0.015 ml on days 0, 7, and 28, respectively.

The size of infectious viral particles. The approximate size of infectious viral particles was determined by the method of Atoynatan and Hsiung [14] and Casals [15] as modified by Bhatt et al. [9]. A fresh, 10% suspension of mouse brain was made in PBS plus FBS, clarified by centrifugation at 1,000 *g* for 20 min, and filtered through Millipore filters (Millipore Corp., Bedford, Mass.) of various pore sizes.

Viral titers obtained were $10^{3.5}$, $10^{4.2}$, $10^{3.8}$, $10^{3.0}$, and $<10^{2}$ $IMLD_{50}$/0.015 ml for unfiltered virus and after filtration through pore sizes 1,200 μm, 450 μm, 220 μm, and 100 μm, respectively. Results indicate that the size of the virus is less than 220 μm but greater than 100 μm. The particles without membranes measured by electron microscopy were previously reported to be 60–70 μm [1].

Detection of hemagglutination. Two sources of antigen were used. (*1*) A 10% suspension of infected salivary gland was tested for its capacity to hemagglutinate red blood cells (RBCs) of rabbits, guinea pigs, and geese by the method of Ashe [16]. (*2*) Either a 10% suspension of infected mouse brain in PBS or infected mouse brain extracted with sucrose and acetone was tested for hemagglutinability with RBCs of rats, mice, guinea pigs, and geese. The microtiter method [11] was used for all tests, and incubation was at 4 C, 25 C, and 37 C. RBCs were suspended (0.5%) in PBS.

Hemagglutination was not detected at 4 C, 25 C, or 37 C using a suspension of infected salivary gland and rabbit, guinea pig, or goose cells. A 10% suspension of infected brain in PBS or sucrose-acetone-extracted antigen from mouse brain gave unsatisfactory results with RBCs of rats, guinea pigs, and mice at 25 C and 37 C, and there was no HA activity after incubation overnight at 4 C. Neither was HA detected for goose RBCs at 4 C, 25 C, or 37 C.

Acridine-orange staining. The cytoplasm of infected cells stained orange-red with acridine orange, indicating that virus replicates in the cytoplasm.

Antigenic relationship to other viruses. Results of these experiments are summarized as follows.

(*1*) Serum-neutralization tests were conducted with strain 681 SDA virus with immune sera to

13 murine viruses. Immune serum to MHV had a titer of neutralizing antibody of 1:80 for strain 681, but sera immune to Reo virus 3, K virus, Theiler encephalomyelitis virus (strain GD VII), Sendai, MVM, and mouse adenovirus were negative. Immune sera to Toolan H-1 virus, Kilham rat virus, and simian myxovirus SV_5 (at dilutions of 1:5) did not neutralize SDA virus.

(*2*) Sera of mice immune to strain 681 were tested by CF at dilutions of 1:4, 1:8, and 1:16 for reactivity to 118 viral antigens. These agents are listed in table 3. There was no reaction, indicating absence of antigenic relationship between our agent and these 118 agents under the conditions of the CF test.

(*3*) A checkerboard cross-CF test was performed, using two different sera from mice immune to SDA agent and commercial MHV antigen and corresponding immune serum. On the basis of this test, neither agent was distinguishable from the other. The results of both tests are given in table 4.

Table 3. Viral antigens tested by complement fixation with hyperimmune serum to sialodacryoadenitis virus.

Viral group	Virus used
Pox	Vaccinia
Herpes	Herpes simplex
Areno	Lymphocytic choriomeningitis, Tacaribe, Tamiami, Junin
Paramyxo	Newcastle disease virus
Rabies	Rabies
Reo	Reo type 3
Arboviruses*	
Bunyamwera super group	28 viruses
Anophelese A	3 viruses
Mossuril group	2 viruses
Kemerovo group	3 viruses
Vesicular stomatitis group	3 viruses
Qalyub	2 viruses
Quaranfil	2 viruses
Phlebotomus fever group	8 viruses
Uukuniemi	3 viruses
Ungrouped	46 viruses
Turlock	Turlock
Bakau	Bakau
Congo	Congo
Boracea	Boracea
Changuinola	Changuinola
Palyam	Palyam
Kaisodi	Silver water
Epidemic hemorrhagic fever of deer	IHD New Jersey
Flanders	Flanders

* Names of the individual arboviruses will be furnished upon request.

Table 4. Results of cross-complement-fixation tests with antigens of the viruses of sialodacryoadenitis and mouse hepatitis and their respective immune sera.

	Antisera		
Antigen	681 A	681 B	MHV*
681	128/≥256†	64/128	160/128
MHV	32/32	16/16	80/≥64

* Mouse-hepatitis virus.

† The highest dilution of serum reacting with the lowest dilution of antigen/the highest dilution of antigen reacting with the lowest dilution of serum.

(*4*) Cross-neutralization tests were performed with Parker's rat coronavirus and strain 681, using both homologous and heterologous immune sera. As shown in table 5, there is cross-neutralization, but there are also antigenic differences between these viruses.

Induction of disease in weanling mice. Neither overt illness nor any gross or histologic evidence of lesions was associated with this agent. More specifically, there were no lesions compatible with infection by MHV either in the group treated with cortisone or in the untreated group.

Production of SDA in susceptible rats by mouse-brain-adapted strain 681. There was no overt illness in any rat, and at necropsy all organs, including the lacrimal and salivary glands, were grossly normal.

Histopathologic examination of the Harderian, exorbital, parotid, and submaxillary salivary glands showed evidence of SDA. There was considerable variation in the severity of the reaction of affected glands. In general, lesions were most numerous and most severe in the parotid and exorbital glands. Two important features of this study were the relative severity of lesions in the parotid salivary glands and the failure to isolate an agent pathogenic for mice from these animals.

Table 5. Results of cross-neutralization tests with sialodacryoadenitis virus (SDA) and rat coronavirus (RCV) and respective immune sera.

Immunizing agents	Animals immunized	Antibody titer vs. 681		Antibody titer vs. RCV	
		1	2	1	2
SDA	Mice	1:253	1:452	1:67	1:100
RCV	Rats	1:67	1:100	1:284	1:272

These findings suggest that the mouse-brain-adapted virus caused mild but definite lesions compatible with SDA.

Discussion

It has been approximately 10 years since the first recognized outbreak of SDA in rats. On the basis of the information presented in this report, it is concluded that a viral agent causing this disease in rats has been isolated and adapted to grow in brains of infant mice and in primary rat-kidney cultures.

The agent probably has RNA as its nucleic acid and is sensitive to lipid solvents. It is antigenically related to the rat coronavirus of Parker and to mouse-hepatitis virus. It apparently multiplies in the cytoplasm and forms multinucleated giant cells in tissue culture. Some coronaviruses are acid labile, but the SDA agent is relatively stable at *p*H 3.0. The virus of transmissible gastroenteritis is also stable at *p*H 3.0, yet is considered a coronavirus [17]. Thus *p*H stability may be a variable feature of this group. On the basis of this information, we conclude that this virus belongs to the corona group, even though information on the morphology of the negatively stained viral particle and its mode of replication in cells (as determined by electron microscopy) has yet to be obtained. In the affected tissues, the particles are frequently found in cytoplasmic vesicles that eventually contain lysosomal activity (A. M. Jonas, unpublished observation).

The titer of infectious virus in mouse brain was not increased by serial passage. This may be a reflection of the facts that only selected cells are involved in viral multiplication and that there may be a low yield of infectious virus per infected cell. (The former supposition has been confirmed by detailed histopathologic study supplemented by fluorescent-antibody tracing of infected cells.)

When working with mouse-adapted viruses of rats, it is essential to prove that one has not picked up agents from mice. Evidence against this possibility includes the following. (*1*) Sickness in mice is produced only when the animals are inoculated with a suspension of infected salivary gland and not when normal salivary gland is used. (*2*) Agents pathogenic for mice were not recovered when brains from normal mice were passaged consecutively several times by the intracerebral route in mice. (*3*) Dr. R. E. Shope tested 118 viral antigens prepared from infected mouse brains with antiserum to SDA virus. These mice were from the same colony used for our work. He did not find any reaction in CF tests with immune serum to SDA virus. (*4*) Immune sera prepared in rats with virus passaged in salivary glands reacted with antigen from murine brain, while sera taken from the same rats before immunization did not. (*5*) SDA was reproduced in rats by inoculation of mouse-brain-adapted virus.

Failure to adapt SDA virus to tissue-culture systems other than primary rat-kidney cultures confirms the observation that coronaviruses are fastidious in their cultural requirements [18]. Absence of viral multiplication in monolayers of rat-salivary-gland and mouse-brain tissue may be due to absence in culture of cells that normally support growth of virus in the intact host.

Complement-fixing and neutralizing antibodies to MHV were detected in the sera of men and rats by Hartley et al. [19] and were interpreted as indication of infection by antigenically-related, species-specific viruses. This hypothesis has been confirmed in part by isolation of coronaviruses from man [18]. SDA agent and Parker's rat corona agent may be responsible for antibodies to MHV found in rats. Biologically, SDA virus behaves differently from known strains of MHV, since the former does not multiply in the Py-AL/N cell line, which is highly susceptible to MHV [6]. In addition, SDA virus does not induce lesions compatible with those due to MHV if inoculated ic into infant mice or if inoculated ic or ip into cortisone-treated weanling mice. Therefore, our failure to detect antigenic differences between these agents by the complement-fixation test should be interpreted with caution. More information may be obtained for serologic differentiation by use of immune sera prepared by different schedules of immunization and then tested by neutralization, complement-fixation, fluorescent-antibody, and gel-diffusion methods.

Since there is more than one serotype of human and mouse coronavirus [18, 20], it is not surprising that, although there is much cross-neutralization, there seem to be antigenic differences between our virus and the one isolated by Parker et al. [4]. Several serotypes of rat coronaviruses may therefore exist. It is necessary to delineate further the serotypes, the disease poten-

tials, and the epidemiology of both agents. For example, our agent is known to cause SDA, while Parker's rat coronavirus causes pulmonary lesions.

The ability of mouse-brain-adapted virus to produce the disease in rats indicates that we are dealing with the same agent. An evaluation of the glands involved and the extent of involvement may indicate a shift in tissue tropism and also an apparent reduction in virulence of virus.

SDA virus was not recovered from submaxillary salivary glands when mouse-adapted-virus was inoculated into rats, but a change in tissue tropism may have accounted for this difficulty. Attempts were not made to isolate virus from parotid and exorbital glands, but histopathologically they demonstrated classical lesions. These tissues were only minimally involved in previous studies with the strain passaged in rats. The apparent shift in tissue tropism needs confirmation in both germfree and conventional, susceptible rats of the same strain, particularly since the transmission experiments were performed in susceptible, specific-pathogen-free Wistar rats.

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