INFECTION AND IMMUNITY, Jan. 1974, p. 159-166
Vol. 9, No. 1
Printed in U.S.A.
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Characterization and Serotyping of Three Feline Reovirus Isolates

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Received for publication 20 August 1973

Three feline virus isolates were shown to be members of the reovirus group by their growth characteristics in cell cultures, physicochemical properties, and appearance under an electron microscope. Their close serological relationship to, or identity with, human reovirus type III was revealed by hemagglutination, hemagglutination inhibition, serum neutralization, and gel diffusion tests. One feline isolate (636) was pathogenic for suckling mice.

Reoviruses have been isolated from apparently healthy and diseased humans, subhuman primates, and lower animals (18). The first feline reovirus isolation was made by Scott et al. (20) from the intestine of a cat with a fatal illness suspected to be feline panleukopenia.

This investigation reports characterization and serotyping of three isolates with cytopathic changes characteristic of reovirus made in primary cat kidney cell cultures from tissue suspensions of the liver of a newborn kitten (isolate no. 577) and the large intestines of a 4-week-old (no. 23) and a 10-week-old (no. 636) ataxic cat. The isolates were made by the author and were unexpected findings while conducting research on cerebellar disease of cats. The isolates produced paranuclear intracytoplasmic inclusions which stained blue with May-Grünwald Giemsa and green with acridine orange and were Feulgen-negative (C. K. Csiza, Ph.D. thesis, Cornell University, Ithaca, N.Y., 1970).

MATERIALS AND METHODS

Viruses. Feline virus isolates 23, 577, and 636 were compared with human reovirus types I (strain 623, Lang), II (strain 624, Jones), and III (strain 625, Dearing). These type-specific reoviruses were obtained from Rudolf Deibel of this Division.

Feline stock virus was produced in LLCMK2 (rhesus monkey kidney continuous cell line) cells for physicochemical characterization and serum neutralization (SN) tests and in BHK-21 (baby hamster kidney continuous cell line) cells for hemagglutination inhibition (HI) and gel diffusion tests. The same cell system was used for production of human reovirus types. Virus grown in LLCMK2 cells was frozen at 7 to 9 days postinoculation (DPI), thawed once, and centrifuged at 1,120 × *g* for 15 min at 4 C. The supernatant was distributed in 1-dram screw-capped vials and stored at −70 C until used. Control and inoculated BHK-21 cell cultures were frozen at 4 DPI, thawed, sonically treated, and centrifuged as above. The preparations were concentrated 10 times in polyvinylpyrrolidone (A. H. Thomas Co., Philadelphia, Pa.) and dialyzed against phosphate-buffered saline (PBS) at pH 7.2. The preparation was distributed in 1-dram vials and held at −70 C until used.

Cell cultures. In addition to primary cat kidney cells, the feline isolates were passaged in primary cell cultures of rhesus and green monkey kidney; guinea pig lung and brain; rabbit lung, kidney, and testis; and in continuous cell lines of L2 (mouse fibroblast), LLCMK2, and BHK-21. Growth medium consisted of Melnick monkey medium A with 2% fetal bovine serum (FBS) for monkey kidney cell cultures; Eagle (Hanks base) medium with 12% rabbit sera and 10% tryptose phosphate broth (TPB) for rabbit cell cultures; and Eagle medium with 10% FBS and 10% TPB for guinea pig cells and continuous cell lines. Confluent cell cultures were changed to maintenance medium (Eagle with 2% FBS for all cultures). Growth and maintenance media containing penicillin (500 U/ml) and dihydrostreptomycin (0.1 mg/ml) were both adjusted to pH 7.2 with tris(hydroxymethyl)-aminomethane (Tris) (0.005 M/ml) buffer.

Antisera. Antisera were prepared in two separate groups: against the feline isolates, and against the three human reovirus types. To prevent possible cross-contamination, animals inoculated with different virus preparations were housed in separate buildings.

Antisera against the human reovirus types II and III were prepared in rabbits. At a later date, antiserum was prepared against the feline isolates in a second group of rabbits. Immunization procedure was the same for human and feline viruses. Each of three adult rabbits was inoculated with LLCMK2-grown virus treated with Freon 113 (3). Four intravenous inoculations (0.5, 1.0, 1.5, and 2.0 ml, respectively) were given a week apart. With the first and third inoculations, 1 ml of virus adjuvant (Freund complete adjuvant, Difco Laboratories, Detroit, Mich.) mixture was also administered intramuscularly. One week

after the fourth inoculation, immune serum was collected.

Hamster-immune ascitic fluids were prepared against all three human reovirus types. The first inoculum (virus-adjuvant) was given intramuscularly. Three inoculations of virus adjuvant mixture were then given at weekly intervals by the intraperitoneal (ip) route. Ascitic fluid samples were collected from hamsters 5 to 15 days after the last injection.

Physicochemical characterization of feline isolates: heat stability (10, 24) and cationic stabilization (23, 24). Stock virus was diluted 1:10 with Eagle medium adjusted to pH 7.2 with Tris (0.005 M/ml) buffer. Half of the dilution was mixed with an equal volume of 2 M $MgCl_2$, and the other half was mixed with deionized water. The mixtures were incubated 1 h in a water bath at 50 C. For control, the isolates were diluted 1:20 with Eagle medium (pH 7.2) and kept at room temperature (RT: 20 to 25 C). The mixtures were titrated with 10-fold virus dilutions in LLCMK2 cells. Unstained cell cultures were examined for cytopathic effect (CPE) 9 DPI. The titers were calculated by the Spearman-Karber method (6).

pH stability, ether sensitivity, and chloroform sensitivity. These determinations were carried out by standard methods (10, 24). Virus titrations were done in LLCMK2 cells as above.

Effect of antimetabolites. The antimetabolites used in this experiment were 100 μg of 5-bromo-2'-deoxyuridine (BUdR; 22), 1 μg of dactinomycin (15), and 1 μg of mitomycin C per ml (14). Confluent LLCMK2 cell cultures were changed with BUdR in maintenance medium the day before inoculation. The treatment period was about 2 h with dactinomycin and mitomycin C. Before virus inoculation, the medium was decanted. Virus isolates 23, 577, and 636, titrating 4.0, 3.5, and 6.0 $\log_{10}$ per 0.1 ml. respectively, were adsorbed on six cell culture tubes at 37 C for 1.5 h. Unadsorbed virus was removed by washing cell cultures with PBS at pH 7.2. Maintenance medium with drugs was added, and the cultures were incubated at 37 C. Virus controls were treated as above except with drug-free maintenance medium. After 4 days of incubation (complete CPE), cell cultures were frozen and thawed once, pooled, and centrifuged 1,120 $\times g$ for 15 min. Supernatants from treated and control cultures were titrated in LLCMK2 cell cultures for infectivity.

To demonstrate that dactinomycin does enter the LLCMK2 cells and inhibits ribonucleic acid (RNA) synthesis, uninfected confluent cell cultures in Eagle maintenance medium were pulsed with 4.0 μCi of [^{3}H]uridine per ml (8 Ci/nmol, Schwartz Laboratories, Mt. Vernon, N.Y.) for 1, 2, and 3 h in the presence of 0, 1, 2, 5, and 10 μg of actinomycin. RNA was extracted three times with hot phenol, precipitated with alcohol, resuspended in 0.15 M NaCl and 10^{-3} M $MgCl_2$, treated with 10 μg of ribonuclease-free deoxyribonuclease (obtained from T. Plummer of this Division) for 1 h at 37 C, and passed through a Sephadex G-50 column. The RNA in the excluded volume was reprecipitated with 95% ethanol and resuspended in 0.15 M NaCl. For measurements of radioactivity, the RNA was precipitated onto membrane filters (Millipore Corp.) with 5% trichloroacetic acid. Measurements of RNA concentration were determined from the optical density at 260 nm.

Dactinomycin effectively inhibited [^{3}H]uridine incorporation into RNA. Treatment with 1 μg of actinomycin for 1 h inhibited incorporation by 69%; treatment with 10 μg for 3 h produced 97% inhibition. Intermediate concentrations of dactinomycin for differing pulse intervals produced levels of inhibition between these values. The specific activities of the isolated RNAs indicated similar levels of inhibition.

Electron microscope examination. Concentrated BHK-21 cell culture-grown viruses used for hemagglutination (HA) and agar-gel diffusion studies were examined with a Philips 300 electron microscope (M. Appel, Veterinary Virus Research Institute, Cornell University, Ithaca, N.Y.). The preparations were negatively stained with 2% phosphotungstate in cacodylate buffer.

Hemagglutination tests. HA tests were done in a microtiter system. Twofold serial dilutions of 0.025 ml of virus were prepared with 25-μliter diluters in wells of U-plates containing 0.025 ml of PBS. To each well 0.025 ml of 1.5% erythrocyte suspension was added with a 25-μliter pipette dropper. The microplate was shaken gently and then incubated. Titers were expressed as the reciprocal of the highest dilution of virus which gave complete agglutination of cells.

Human erythrocytes of types O, A, B, and AB and cow, sheep, horse, chicken, goose, rabbit, rat, hamster, guinea pig, and mouse erythrocytes were tested. PBS of pH 6 to 8, at 0.5 $\log_{10}$ increments, was used to test the effect of pH on HA. HA tests were done at three different temperatures: 4 C, RT, and 37 C. For routine testing, HA was performed at RT, with 1.5% human type O erythrocytes in PBS at pH 7.2.

Hemagglutination inhibition tests. HI tests were done at RT in a microtiter system, with 8 HA units of virus diluted in PBS at pH 7.2. Before use in the HI test, antisera were adsorbed at RT for 30 min each with human type O erythrocyte (0.1 ml of 50% erythrocytes to 1.0 ml of serum) and acid-washed kaolin (25 g of kaolin in 100 ml of saline, 0.1 ml per 1.0 ml of serum). Serially diluted sera (0.025 ml) were mixed with an equal volume of virus and incubated at 37 C for 1 h. After incubation, 0.05 ml of 1.5% human type O erythrocytes was added. Plates were shaken to suspend the cells, sealed with tape, and incubated. Antibody titers were expressed as the reciprocals of the highest serum dilutions that completely inhibited HA.

SN tests. SN tests were carried out on heat-inactivated serum samples (56 C for 30 min) by using constant samples of virus with varying serum dilutions. The stock virus was diluted to contain 100 mean tissue culture infective dose ($TCID_{50}$) units/0.1 ml. Equal volumes of virus and twofold serum dilutions were mixed and incubated at 37 C for 45 min. A 0.2-ml portion of each mixture was inoculated into each of two confluent LLCMK2 culture tubes. Tests were read at 9 DPI. Neutralizing antibody titers were calculated by the Spearman-Karber method (6).

Gel diffusion test (11, 13, 19). Double-diffusion was carried out on microscope slides with a template containing six peripheral wells, the center of each being 6 mm from that of the central well. This technique has the advantage of smaller quantities of antigen, greater ease of performance, and finer sensitivity than the macro-Ouchterlony method. The gel in the diffusion plates was 1% agar (in 0.85% saline) at a thickness of two layers of electrical tape. Antiserum was placed in the central well, and the peripheral wells were filled with concentrated reovirus antigen used in the HI test. The plates were kept in humidified chambers to prevent drying. The wells were refilled as required, and diffusion was allowed to proceed for 3 days at RT. The template was then removed, and the slide was soaked in 0.85% saline for about 12 h, rinsed in distilled water for 3 h, and stained with naphthol blue black (Buffalo Black NBR, Allied Chemical, Morristown, N.J.). Excess stain was washed off with a mixture of acetic acid (10%), methanol (70%), and water, examined for precipitin lines, and dried.

Mouse pathogenicity. Isolate 636 was titrated in 2-day-old NYLAR (New York State Department of Health, Albany) mice by the ip route. Eight young per family were inoculated with 0.05 ml of 10-fold virus dilution and observed daily. In addition, families of suckling mice were inoculated with 1.5×10^5 $TCID_{50}$ of virus. This group of animals was killed 1 to 44 DPI for virus isolation. Tissue suspensions (10%) were made from the lung, kidney, and small intestine (jejunum), and reisolation of the virus was attempted in tissue culture.

RESULTS

Susceptibility of cell cultures derived from different animal species to the three isolates. In addition to primary cat kidney cells, the isolates multiplied in primary cell cultures of rhesus and green monkey kidney and of guinea pig lung and brain, as well as in continuous cell lines of L2, LLCMK2, and BHK-21. The isolates failed to produce CPE or hemagglutinin in primary cell cultures from rabbit kidney, testis, and lung.

CPE was detectable 48 h after infection in L2 and BHK-21 cells, and after 4 to 5 days the destruction of the cell sheet was complete. CPE was observed 4 to 5 DPI in primary cat kidney cells, rhesus and green monkey kidney cells, and in the continuous cell line LLCMK2. Cell degeneration increased progressively until 8 to 10 DPI, when the entire cell sheet sloughed off. The isolates produced paranuclear cytoplasmic inclusions in a small percentage of cells in primary cell cultures from guinea pigs but failed to cause readily recognizable destruction of monolayers. The cytoplasmic inclusions, which usually did not completely surround the nucleus, were detectable upon staining.

Physicochemical characteristics of the isolates. The isolates were resistant (Table 1) to acid, heat, ether, and chloroform, with no loss of titer in the presence of 1 M $MgCl_2$.

The effect of antimetabolites is shown in Table 2. The addition of BUdR and mitomycin C to the tissue culture fluid resulted in 1.4 and 0.9 $\log_{10}$ increase in the titer of virus isolate 23. Presence of dactinomycin in the medium had an opposite effect on virus isolate 577, decreasing virus titer by 1.2 $\log_{10}$.

Electron microscopy. Viral particles (Fig. 1 and 2) are about 70 nm in diameter. The nucleocapsid has icosahedral symmetry, with 92 hollow capsomeres projecting from the surface. No envelope surrounds the capsid.

HA test. Hydrogen ion concentrations between pH 6 and 8 did not affect HA titers. Erythrocytes of human blood types O, A, B, and AB were agglutinated by the feline isolates as well as by the three human types of reovirus. The titers were slightly lower at 4 C than at RT and 37 C. The feline isolates and human type III agglutinated bovine cells at 4 C. Erythrocytes of some rabbits were agglutinated by the isolates

TABLE 1. *Sensitivity of virus isolates 23, 577, and 636 to physical and chemical agents*[a]

Treatment	Virus titer ($-\log_{10}$/0.1 ml 50% end points)		
	23	577	636
$MgCl_2$, 1 h	4.8	3.8	4.8
Deionized H_2O, 1 h	5.3	4.3	5.3
pH 3.0, 3 h	5.3	3.8	4.8
pH 7.2, 3 h	5.3	4.3	5.3
Ether, overnight	4.8	4.3	4.8
Chloroform, 10 min	4.8	4.3	4.8

[a] Isolates were propagated in LLCMK2 cells.

TABLE 2. *Effect of antimetabolites on virus isolates 23, 577, and 636*[a]

Treatment	Virus titer ($-\log_{10}$/0.1 ml 50% end points)[b]		
	23	577	636
Inoculum/0.1 ml	4.0	3.5	6.0
Control[c]	5.1	5.3	6.1
BUdR (100 μg/ml)	6.5	5.5	6.5
Dactinomycin (1 μg/ml)	5.1	4.1	6.1
Mitomycin C (1 μg/ml)	6.0	5.5	6.0

[a] Propagated in LLCMK2 cells.
[b] Mean of three titrations.
[c] One set of controls served for all three antimetabolite titrations (carried out simultaneously in the same cultures).

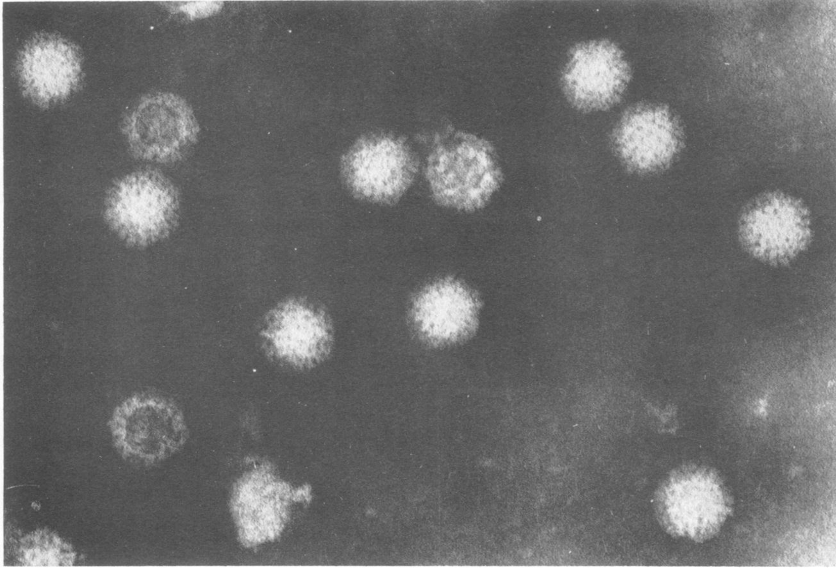

FIG. 1. *Virions of feline isolate 636, approximately 70 nm in diameter, showing icosahedral symmetry with capsomeres projecting from the surface. Intact and coreless particles are present. Negatively stained with 2% phosphotungstic acid, pH 7.2. ×137,500.*

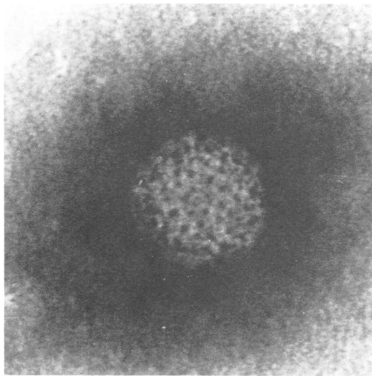

FIG. 2. *Higher magnification of Fig. 1. Hollow capsomeres can be seen negatively stained with 2% phosphotungstic acid, pH 7.2. ×272,000.*

as well as by the three human types. Although HA titers were much higher with these rabbits than with human or bovine erythrocytes (Table 3), they were not routinely employed because of their tendency to agglutinate spontaneously and because HA was influenced by the donor animal. Furthermore, these rabbit erythrocytes, previously shown to be satisfactory for HA tests, were also agglutinated by some hyperimmune serum used in the HI tests and therefore could not be used. None of the reoviruses used in this study agglutinated sheep, horse, chicken, goose, rat, hamster, mouse, or guinea pig erythrocytes.

The feline isolates and human type III reovirus produced hemagglutinins in low titers (1:8 to 1:64) when they multiplied in L2 or BHK-21 cells. They produced little or no demonstrable hemagglutinins when propagated in primary cat kidney, rhesus, and green monkey kidney, and in guinea pig lung and brain cells and continuous cell line LLCMK2 tissue culture cells.

Hemagglutination inhibition test. Antigenic comparison of reoviruses by the HI test is shown in Table 4. The feline isolates cross-reacted with one another and with human type III reoviruses. Rabbits hyperimmunized with types II and III developed high heterotypic antibody titers. Antibody produced in a second group of rabbits (against the three feline isolates) and in hamsters (against the three human types) was quite specific for the homologous virus.

Serum neutralization test. Cross-reaction among the reoviruses by the SN test is shown in Table 5. The feline isolates cross-reacted with one another and with the three human types, although the cross-reaction varied according to the serum and virus used. Heterologous serum titers were significantly higher with the feline isolates and human type III than with types I and II.

Gel diffusion test. Precipitin lines formed in 3 days between virus concentrates and their homologous antisera and between human type

III reovirus and the feline isolates. When antigens of type III virus and feline isolates were placed in contiguous peripheral wells, they diffused toward centrally placed antibody prepared against either type III virus or one of the feline isolates. A line of precipitate formed, which fused without spur formation at the angular junctions. Occasionally, the number of lines formed with the same antigen-antiserum combination varied from one to three on repeated tests. Regardless of the number of precipitin lines formed, they always fused perfectly at the angular junction. No cross-reaction was demonstrated between the feline isolates and types I and II.

Mouse pathogenicity. Two-day-old NYLAR mice were inoculated ip with isolate 636, and after 5 to 10 days they showed some signs of illness. Runting, weakness, and unhealthy appearing coats were the most common signs. Circling and a sideways tilting of the head were observed from 13 to 16 DPI in 6 of 16 mice that were inoculated with 1.5×10^5 $TCID_{50}$ of virus and survived 13 days or longer. The mean lethal dose of mouse-adapted virus preparation was 3×10^3/0.05 ml as compared with 1.5×10^5 $TCID_{50}$/0.05 ml.

The virus was reisolated 24 h postinoculation from lung and jejunum but not from kidney in LLCMK2 cell cultures. Recovery of virus from

TABLE 3. *Hemagglutinin titers using erythrocytes of different species, and tissue culture infectivity of concentrated reovirus preparation*[a]

Erythrocyte types	Titer of reovirus preparation[b]					
	Human type			Feline isolate		
	I	II	III	23	577	636
Human O	1,024	8,192	256	256	128	512
Bovine	0	2	512	256	256	2,048
Rabbit[c]	4,096	4,096	4,096	2,048	2,048	4,096
Tissue culture infectivity[d]	5.5	7.0	8.5	9.0	9.0	7.5

[a] BHK-21-propagated virus concentrated 10 times.

[b] For HA tests, viruses were incubated at RT with human type O erythrocytes and at 4 C with bovine and rabbit erythrocytes. Titers are expressed as reciprocals of virus dilutions.

[c] HA was inconsistent (influenced by the donor animal).

[d] BHK-21 cells, $TCID_{50}$, $-\log_{10}$/0.1 ml.

TABLE 4. *Cross-reaction among human reovirus types I, II, and III and feline isolates 23, 577, and 636 as shown by the hemagglutination inhibition test*

Antibody type	No. tested	Reovirus antigens (8 HA units)					
		Human type			Feline isolate		
		I	II	III	23	577	636
Hamster							
I	3	215[a]	4	4	3	3	3
II	5	3	120	2	6	5	6
III	6	4	4	64	42	42	128
Rabbit (group 1)							
preinoculation	6	4	3	0	5	3	3
II	5	17	112	2	61	24	18
III	4	20	40	64	168	28	176
Rabbit (group 2)							
preinoculation	9	0	0	2	0	3	2
23	6	0	0	101	93	37	59
577	6	5	0	149	91	45	59
636	6	1	0	160	36	32	64

[a] Each titer, expressed as a reciprocal, is a mean of the highest serum dilutions that completely inhibited HA. Fractions were rounded off to the nearest whole number. Homologous titers are underlined.

TABLE 5. *Cross-reaction among human reovirus types I, II, and III and feline isolates 23, 577, and 636 as shown by serum neutralization test*

Reovirus antiserum	No. tested	Reovirus antigens (100 $TCID_{50}$ units/0.1 ml)					
		Human type			Feline isolate		
		I	II	III	23	577	636
Hamster ascitic fluid							
I	3	2.76 ± 0.23[a]	0.43 ± 0.75	1.38 ± 0.35	0.78 ± 0.83	0.38 ± 0.66	0.00
II	5	0.00	1.66 ± 0.29	0.11 ± 0.25	ND[c]	ND	0.00
III	6	0.50 ± 0.56	0.40 ± 0.65	1.82 ± 0.34	ND	ND	1.70 ± 0.28
Rabbit serum[b]							
II	5	0.95 ± 0.59	1.69 ± 0.54	1.04 ± 0.71	ND	ND	0.65 ± 0.37
III	4	1.52 ± 0.09	1.52 ± 0.19	2.16 ± 0.28	ND	ND	2.12 ± 0.19
23	3	1.75 ± 0.10	1.30 ± 0.26	2.75 ± 0.17	2.63 ± 0.33	2.50 ± 0.26	2.30 ± 0.23
577	3	1.60 ± 0.15	1.55 ± 0.17	2.90 ± 0.46	2.62 ± 0.47	3.15 ± 0.23	2.40 ± 0.23
636	3	1.70 ± 0.15	1.30 ± 0.15	2.95 ± 0.30	2.20 ± 0.23	2.65 ± 0.26	2.65 ± 0.26

[a] Mean SN titer ($-\log_{10}$/0.1 ml, 50% end points) ± standard deviation. Homologous titers are underlined.
[b] Preinoculation serum samples from one rabbit (immunized with type III renovirus) neutralized type II and isolate 636 at a dilution of 1:2. No SN antibody was demonstrated in the other preinoculation serum samples.
[c] ND, not done.

kidney was successful 2 DPI. Viral isolations were obtained up to 15 DPI from lung and up to 20 DPI from kidney and jejunum. Virus isolation from the cerebellum and cerebrum of one circling mouse at 13 DPI was successful.

DISCUSSION

Feline virus isolates 23, 577, and 636 were shown to be members of the reovirus group. They are resistant to acid, heat, and lipid solvents. Electron microscopy of negatively stained preparations revealed viral particles, about 70 nm in diameter, morphologically similar to other reovirus particles (20). Primary cell cultures from cat kidney, rhesus, and green monkey kidney, and from guinea pig lung and brain and continuous cell lines L2, BHK-21, and LLCMK2 supported viral replication with formation of paranuclear intracytoplasmic inclusions. The inclusions had previously been shown to contain double-stranded RNA (C. K. Csiza, Ph.D. thesis, Cornell University, 1970), and reoviruses are known to contain double-stranded RNA (24).

Reoviruses multiply in cytoplasm in association with acromatic mitotic apparatus (21). The relationship of the multiplying virus to the host cell's apparatus can be examined by incorporating appropriate chemical inhibitors into tissue culture fluids. For example, BUdR (22) and mitomycin C (14) can interfere with nucleic acid synthesis; dactinomycin can interfere with deoxyribonucleic acid (DNA)-dependent RNA synthesis (15); and puromycin can interfere with protein synthesis (26). Infection of cells with reoviruses results in the inhibition of DNA synthesis without detectable inhibition of cellular protein synthesis (5, 8). Host protein synthesis in mouse L cells in the presence of dactinomycin (0.5 and 2.0 μg/ml) decreased in parallel in cells infected with reovirus type III and in uninfected cells, indicating that reovirus did not switch off protein synthesis (27).

Wong et al. (25) observed that dactinomycin does not enter Vero cells very efficiently. Since search of the literature revealed no data about the permeability of LLCMK2 cells to dactinomycin, an experiment was conducted to determine this. The results indicated that dactinomycin enters the cells relatively freely and inhibits the synthesis of cellular RNA.

Gomatos et al. (7) have shown that dactinomycin and mitomycin C inhibit replication of reovirus type III. In the present study, cell cultures of LLCMK2 were infected with isolates 23, 577, and 636 in the absence of chemical inhibitors and in the presence of BUdR (100 μg/ml), mitomycin C (1 μg/ml), and dactinomycin (1 μg/ml). Presence of BUdR and mitomycin C in culture fluids enhanced slightly the development of CPE and resulted in an increased virus titer of 0.5 to 1.0 $\log_{10}$ for virus isolate 23. This is an indication that formation of virions does not depend upon synthesis and function of host cell DNA. Presence of dactinomycin in culture fluids also enhanced slightly the development of CPE; but, in contrast to BUdR and mitomycin C, the synthesis

of new virus particles decreased by 0.5 to 1.0 $\log_{10}$. One log increase in titer of culture fluids (isolate 23) with BUdR and one log titer decrease in culture fluids (90% loss, isolate 577) in the presence of dactinomycin are probably significant. The mechanism by which these antimetabolites affect the replication of two of the three feline reovirus isolates is not clear.

The three feline isolates have hemagglutinating properties similar to those reported for reoviruses, particularly of human type III (2, 4, 9, 17). Hemagglutinin titers were low. The viruses agglutinated all groups of human erythrocytes at 4 C, RT, and 37 C and bovine erythrocytes at 4 C, with no effect of pH in the range of 6 to 8 on the HA titers.

The isolates, as well as the three distinct human serotypes, agglutinated erythrocytes of certain rabbits. Although such HA titers were considerably higher than those with human or bovine cells, their routine use was avoided because this species of cell had a tendency to agglutinate spontaneously.

Correlation between the infectivity and hemagglutinin titers of the isolates was not routinely tested, since HA titers of tissue-culture fluids and cells were either undetectable or very low after infection (high infectivity titers without detectable hemagglutination).

Because of the ubiquitous distribution of reoviruses and their antibodies in various animal species (18), it is difficult to produce monospecific antisera. Type-specific antibodies have been produced mostly in selected avian species such as roosters (17) and white domestic geese (1). In this study, reovirus antisera were prepared in rabbits and hamsters. The first group of rabbits had low preinoculation HI antibody titers to feline isolates and human types I and II. Immunization of these animals with types II and III resulted in production of antisera, with extensive cross-reaction between the serotypes in HI and SN tests. Antibodies prepared against the feline isolates in a second group of rabbits, and against the three human types in hamster ascitic fluid, were more type-specific. The isolates cross-reacted with one another and with type III in both HI and SN tests. Crossing was insignificant with types I and II in HI and only partial in SN tests.

A common antigenic factor was demonstrated between the feline reovirus isolates and type III reovirus by immunodiffusion. The number of lines developed varied from one to three on repeated tests. Development of the multiple precipitin lines was considered a result of refilling of wells, temperature fluctuation, etc., during the lengthy diffusion period.

Cats are susceptible to various respiratory pathogens. *Myagawanella felis*, herpesvirus, and picornavirus (calicivirus) (numerous serotypes), the most common agents that produce upper respiratory infection, spread rapidly from cat to cat, and often persist and shed from asymptomatic carriers for several months (16). Human reovirus type III also produces a mild respiratory illness in cats (20) and spreads readily from animal to animal. In this study the cats were not reinoculated with isolates because of the difficulty of assembling enough susceptible cats free of reovirus antibody and complicating respiratory agents. Since reovirus type III has been shown to produce spontaneous disease in laboratory mouse colonies (18), it was simpler to test the effect of feline isolates in mice known to be free of reovirus antibodies. Isolate 636 was lethal to 2-day-old NYLAR mice when inoculated ip. Mouse LD_{50} of tissue culture-grown virus was 2 $\log_{10}$ lower than its $TCID_{50}$.

Isolate 636 also produced central nervous system disease in a number of mice that survived the infection. It has been observed that the prototype strain of human type III replicates in close relationship to the neurotubules and has a greater neurovirulence than type I (12). A close serological relationship has been shown between isolate 636 (as well as the two other isolates) and human reovirus type III by HI, SN, and gel diffusion tests.

ACKNOWLEDGMENTS

This investigation was supported in part by Public Health Service General Research Support Grant 5SO1-RR05649-05 awarded by the Department of Health, Education and Welfare.

I thank Max Appel (Veterinary Virus Research Institute, New York State Veterinary College at Cornell University, Ithaca, N.Y.) for the electron microscopy. The technical assistance of Bonnie Hall and Robert Vinson is gratefully acknowledged.

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