Immunological Relationship of Selected Feline Viruses by Complement Fixation. (26713)

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A number of viruses pathogenic for the domestic cat have been reported(1,2,3). In addition, a number of viruses of unknown clinical importance have been isolated from the nasopharyngeal region of domestic cats (4). The immunological relationship of a number of these agents has been established on the basis of serum neutralization tests in tissue culture(4,5). The purpose of this paper is to report the immunological relationship of certain viruses from both groups by means of the complement fixation test.

Materials and methods. The viruses selected for this study include feline viral rhinotracheitis (FVR)(2), kidney cell degenerating (KCD)(6), Bolin's panleukopenia (FPL)(7), California feline isolate (CFI)(3), and the feline viral isolates reported by Crandell as FRI 12, FRI 29, FRI 278(4).

Virus antigens for the complement fixation (CF) tests were prepared as follows: Viruses were grown in primary monolayer feline renal cell cultures prepared and maintained according to the method described by Madin(8). Viruses were harvested from 32-oz. culture bottles between 24-48 hours after inoculation. Harvesting consisted of storing the cells overnight at –20°C, thawing at room temperature, followed by centrifugation at 10,000 rpm for 10 minutes in a Servall SS-2 centrifuge to remove cellular debris. The supernatant was then centrifuged in a Spinco Model L at 30,000 rpm for one hour in a No. 30 rotor. The supernatant was discarded. The sediment was washed by resuspension in 0.02 M phosphate buffer pH 7.0 and recentrifuged in a like manner. The resultant pellet was resuspended to 1/10 the original volume, using 0.02 M phosphate buffer pH 7.0. This material is referred to in the text as 10X antigen and was used in the CF tests. Such material remains stable for periods up to 60 days at 4°C. Known positive sera were included in all tests to insure antigenic potency.

The antisera employed were obtained from 2 sources. The sera used to procure the data shown in Tables I and II were obtained from cats from which a specific virus had been isolated or from cats which had been infected with a single virus. The source is indicated in each table. Cats were inoculated under pentothal anesthesia by the intranasal route, using 0.25 to 0.50 ml of 30th tissue culture passage virus. Serum was obtained 30 days after inoculation.

The CF test employed in this study was a modified Kolmer technic using 0.1 ml amounts of antigen and antisera. Primary incubation was carried out at 4-8°C overnight. The usual controls were included in each test as well as a complement titration control series. Two units of complement (contained in 0.2 ml) as determined by titration in the presence of antigen were used. Block titrations were performed on all antigens, using at least 3 dilutions of antigen

TABLE I. Specificity of Immune Sera by Serum Neutralization Tests.

	CFI antisera*		FRI-278 antisera*		FRI-12 antiserum†
Virus	161	162	103	107	59–12
FVR	<20‡	<20	<20	<20	<20
CFI	*320*	*640*	<20	<20	<20
FRI-278	<20	<20	*160*	*320*	<20
FRI- 29	<20	<20	<20	<20	<20
FRI- 12	<20	<20	<20	<20	*640*
FPL	<20	<20	<20	<20	<20
KCD	<20	<20	<20	<20	<20

* Obtained from cats inoculated with the virus indicated.

† Obtained from a cat from which FRI-12 virus was isolated.

‡ Reciprocal of highest serum dilution (final) that inhibited cytopathic change in 2 out of 3 tubes inoculated with serum virus mixtures.

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TABLE II. Immunological Relationship of Feline Viruses by the Complement Fixation Test.

Virus	CFI antisera*		FRI-278 antisera*		FRI-12 antiserum†
	161	162	103	107	59-12
FVR	<4	<4	<4	<4	<4
CFI	256‡	256	256	64	128
FRI-278	256	256	256	64	128
FRI- 29	512	128	128	32	128
FRI- 12	128	128	64	32	64
FPL	512	512	512	128	256
KCD	<8	<8	<8	<8	<8

* Obtained from cats inoculated with the virus indicated.

† Obtained from a cat from which FRI-12 virus was isolated.

‡ Reciprocal of highest serum dilution showing positive fixation of complement.

and varying dilutions of known positive serum to determine the optimal antigen dilution. This optimal dilution of antigen was then used in all subsequent tests. Sera were inactivated at 56°C for 30 minutes. All titers are reported as the reciprocal of the highest dilution of serum showing positive fixation.

Neutralization tests were carried out by using primary feline renal cell tissue cultures. Between 100 and 200 $TCID_{50}$ of each virus, determined by replicate titration and calculated by the method of Reed and Muench(9), was employed. Equal volumes of serial serum dilutions were mixed with the test virus and allowed to stand for one hour at 24°C; 0.1 ml of each mixture was then inoculated into each of 3 tubes of tissue culture. Neutralization of the virus was indicated by the absence of a cytopathic effect (CPE). A virus control series was always included.

Results. The results of the serum neutralization test are shown in Table I and demonstrate the specificity of each of the antisera tested for its homologous virus. No cross neutralization was observed in any of the serum dilutions employed. These sera were then tested for CF antibody using 10X antigen described previously. The results are shown in Table II and indicate a serological cross reaction with 5 of the viruses tested. Two of the viruses failed to show any relationship.

Discussion. From the data shown in Tables I and II it would appear that each of the viruses is distinct when tested by serum neutralization and that 5 of the viruses appeared to be immunologically related according to the CF test. All of the viruses tested can arbitrarily be divided into 2 groups on the basis of inclusion body formation in feline renal cell cultures and time interval at which CPE occurs. Of these viruses, only FVR produces inclusion bodies in feline tissue culture and rate of CPE is slow when compared to the other feline isolates which do not produce inclusion bodies. The present study would tend to follow this line of separation immunologically since, with the exception of KCD, all of the other viruses inducing rapid CPE appear to be related, on the basis of the CF test. KCD virus was isolated by Fastier (6) in New Zealand and so might be expected to be serologically distinct from the other viruses.

Results similar to these have been obtained with 4 addiitonal viruses and their homologous antisera but are not included in the present report since these viruses have not yet been reported in the literature.

Summary. The relationship of 7 feline viruses was determined by means of the CF test. The results presented indicate that 5 of these viruses are antigenically related though immunologically distinct by the serum neutralization test in tissue culture.

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Received May 19, 1961. P.S.E.B.M., 1961, v107.