STUDIES ON CYTOPATHOGENIC VIRUSES FROM CATS WITH RESPIRATORY INFECTIONS

II. CHARACTERIZATION OF FELINE PICORNAVIRUSES

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INTRODUCTION

The report on cytopathogenic viruses isolated from domestic kittens with respiratory diseases in Japan was previously published¹⁴⁾. These viruses produced cytopathic effects in feline kidney cell cultures. The characteristic feature of their cytopathic effects was the rapid cell rounding without formation of inclusion bodies. These viruses were resistant to ether and had no hemagglutination. They were divided antigenically into at least two types by the neutralization test, while they had common complement fixing antigens. However, these viruses remained unclassified.

This paper deals with the investigations to determine some of their biophysical and biochemical properties, and the attempts to classify them.

Additionally, the serologic difference of 27 strains of the viruses isolated in our laboratory was investigated and the serologic relationship between foreign isolates and our isolates was also examined.

MATERIALS AND METHODS

Cell cultures

Primary feline kidney cell (FKC) cultures were prepared as described previously¹⁴⁾.

Vero cell cultures: a cell line of African green monkey kidney established by YASUMURA et al.²¹⁾: They were grown in cell culture tubes and were inoculated with 0.2 ml of 10⁻¹ dilutions of the feline viruses.

Viruses

The 27 strains of the feline viruses isolated in our laboratory¹⁴⁾ were used. The 15 foreign isolates used for serologic examination were as follows.

American isolates^{2,4,7,8,9,11)}: FC, FS, CFI, FRI, FJ, FRV, FPL, KCD, FVR.

Swiss isolates⁵⁾ : 135/62, 227/62, 337/61, 344/61, 377/61.

Italian isolate¹⁹⁾ : VGP.

American isolates were supplied by Dr. J. H. GILLESPIE, Swiss isolates by Dr. F. BÜRKI and Italian isolate by Dr. V. TORLONE.

Nucleic acid determination

The type of the nucleic acid of the viruses was indirectly determined by use of 5-Bromo-2'-Deoxyuridine (BUDR). After the growth medium was changed for the medium containing 10⁻⁵ M BUDR, the FKC cultures were sensitized with BUDR at 37°C

for 2 hours. The viruses were then diluted to contain about 100 TCID₅₀ per 0.2 ml and inoculated into the FKC cultures. After incubation with maintenance medium containing 10⁻⁵ BUDR at 37°C for 3 days, the cell culture fluid was harvested and titrated for virus content by inoculating 0.2 ml of tenfold dilution into each of three cell culture tubes. The cytopathic effect was recorded during 7 days postinoculation and the titers were calculated by REED and MUENCH method¹⁸⁾.

Acid lability

Acid lability was tested according to the modified method of KETLER et al.¹⁶⁾. Each virus was diluted 1:10 in Veronal buffer (Sodium veronal-Sodium acetate-HCl) adjusted to pH 3.0, 5.0 and 7.2. After 3 hours at room temperature, infective titer was determined in the FKC cultures.

Size determination

Undiluted viruses were passed through membrane filters with an average pore diameter of 50 m μ (Millipore VM). These filtrates were titrated in FKC cultures.

Heat stability

Each of the viruses was diluted 1:10 in Hanks' balanced salt solution (HBSS) and incubated in a water bath at 50°C for 30 minutes in stoppered tubes. As controls, each virus was kept at room temperature. After incubation, the virus infectivity titer was determined in the FKC cultures.

Cationic stabilization

The viruses were diluted 1:10 in lactalbumin medium (HBSS containing 5% lactalbumin hydrolysate) containing 1 M MgCl₂. Each of the virus solutions was incubated at 50°C for 1 hour according to the method of WALLIS and MELNICK²⁰⁾ and titrated in FKC cultures.

Antisera

Antisera of the four viruses (F14:KN5-B, F17:K6253-B, F18:K6254-O and F22:K6321-Lg) were prepared in rabbits as described previously¹⁴⁾. Guinea pigs were also immunized by a similar inoculation schedule.

Complement fixation test

The complement fixation (CF) test was performed by a modified KOLMER's method.

Neutralization test

Serum neutralization tests were carried out in FKC culture tubes. All antisera were inactivated at 50°C for 30 minutes. An infected cell culture fluid containing approximately 100 to 1000 TCID₅₀ per 0.2 ml of the virus was mixed with equal volume of serial fourfold or eightfold dilution of rabbit antisera and incubated at room temperature for 1 hour prior to inoculation into cell cultures. The titer was the highest initial dilution of serum which causes 50% suppression of cytopathic effects.

RESULTS

Biophysical and biochemical properties

Results obtained on the nucleic acid type, acid lability and the size determination of five feline viruses are summarized in Table 1. Growth of all five viruses was not inhibited by BUDR. This fact indicated that these viruses were of RNA type indirectly. The viruses were shown to be less stable at pH 3.0 as indicated by loss in infectivity titer of 2.0 of a log₁₀ unit or more. All viruses passed easily through a Millipore filter with a pore diameter of 50 m μ .

The thermal stability and effect of 1 M MgCl₂ of the viruses are shown in Table 2. All viruses were shown to be less stable to heat and indicated minimum loss in infectivity titer of 3.0 in log₁₀ unit on heating at 50°C for 30 minutes. Each virus did not show

any noticeable stabilization in the presence of 1 M $MgCl_2$.

Serologic studies

Serum neutralization tests were carried out to determine the serologic relationship among 27 feline viruses isolated in our laboratory. The results are summarized in Table 3.

These feline viruses were divided into the following serotypes.

- 1) F 4 (KN2-O) type : F1, 2, 4, 5, 6 and 24.
- 2) F14 (KN5-B) and F18 (K6254-O) type: F7, 11, 12, 14 and 18.
- 3) F17 (K6253-B) type : F16, 17, 21 and 23.
- 4) F22 (K6321-Lg) type : F8, 9, 13, 15, 20, 22, 26 and 27.
- 5) Others : F3, 10, 19 and 25.

Serologic relationships between foreign isolates and our isolates are shown in Table 4. There was an antigenic similarity among them in the CF test except in the SVR strain which was identified as feline herpesvirus⁷⁾.

In neutralization tests, most of foreign strains had no cross reaction against our three antisera. However, two American strains, FPL and KCD, were clearly neutralized by F14 antiserum.

Table 1. Biophysical and Biochemical Properties of the Feline Viruses

Virus	Indirect evidence for nucleic acid type			Acid lability				Size		
	With BUDR	Without BUDR	Nucleic acid	pH 3.0	pH 5.0	pH 7.2	Titer reduction	Millipore filter VM 50 m μ	Control	Titer reduction
F 4 (KN 2-O)	6.5*	6.3	RNA	4.5	6.5	7.0	2.5	6.8	7.3	0.5
F 14 (KN 5-O)	5.8	6.3	RNA	5.5	6.5	7.5	2.0	7.3	7.3	0
F 17 (K 6253-B)	6.5	6.5	RNA	4.5	7.5	7.5	3.0	6.8	6.8	0
F 18 (K 6254-O)	6.5	6.5	RNA	4.0	5.8	7.5	3.5	7.3	7.5	0.2
F 22 (K 6321-Lg)	5.8	6.3	RNA	5.2	7.5	7.8	2.6	7.3	7.5	0.2

* Log TCID₅₀/0.2 ml

Table 2. Thermal Stability and Effect of 1 M $MgCl_2$ on Feline Viruses

Virus	Influence of temperature at 50° C for 30 min.			Effect of $MgCl_2$ at 50° C for 1 hour	
	Heated	Control	Titer reduction	With 1 M $MgCl_2$	Hanks' BSS
F 4 (KN 2-O)	1.0*	8.0	7.0	3.0	1.0
F 14 (KN 5-O)	3.5	6.5	3.0	4.0	1.5
F 17 (K 6253-B)	3.0	8.0	5.0	4.0	2.2
F 18 (K 6254-O)	2.5	6.8	4.3	3.0	2.2
F 22 (K 6321-Lg)	2.8	7.5	4.7	3.0	2.2

* Log TCID₅₀/0.2 ml

Table 3. Neutralization Tests of 27 Feline Viruses

Virus	Antiserum			
	F 4 (KN 2-O)	F 14 (KN 5-O)	F 17 (K 6253-B)	F 22 (K 6321-Lg)
F 1 (KI-O)	45*	<8	<8	<8
F 2 (KI-N)	45	<8	<8	<8
F 3 (KI-T)	6	16	<8	<8
F 4 (KN 2-O)	23	<8	<8	<8
F 5 (KN 2-T)	64	<8	<8	<8
F 6 (KN 2-T)	23	<8	<8	<8
F 7 (KN 3-O)	<4	64	<8	12
F 8 (KN 3-N)	<4	<8	<8	128
F 9 (KN 3-B)	<4	<8	<8	320
F 10 (KN 3-R)	<4	<8	<8	12
F 11 (KN 4-O)	<4	80	<8	12
F 12 (KN 4-B)	<4	80	<8	12
F 13 (KN 5-O)	<4	24	<8	80
F 14 (KN 5-B)	<4	96	<8	12
F 15 (K 6252-B)	<4	12	<8	64
F 16 (K 6253-O)	<4	16	320	12
F 17 (K 6253-B)	<4	16	320	12
F 18 (K 6254-O)	<4	64	<8	<8
F 19 (K 6254-B)	<4	<8	<8	<8
F 20 (K 6254-R)	<4	16	<8	128
F 21 (K 6321-B)	<4	<8	320	<8
F 22 (K 6321-Lg)	<4	<8	<8	80
F 23 (K 6322-B)	<4	12	320	12
F 24 (K 6323-O)	<16	<8	<8	<8
F 25 (K 6323-B)	<4	16	<8	<8
F 26 (K 6323-R)	<4	16	<8	80
F 27 (K 6324-B)	<4	<8	<8	128

* Reciprocal of the serum dilution that inhibited cytopathic change in 50%.

Cytopathic effects in Vero cell

Four of 27 strains (F2:KI-N, F4:KN2-O, F14:KN5-B and F25:K6323-B) grew and showed cytopathic effects in Vero cell. The cytopathic effects in Vero cell appeared as cellular rounding within 2 or 3 days after inoculation.

DISCUSSION

The biophysical and biochemical characteristics of the five feline viruses were investigated. All five strains of feline viruses were shown to be RNA type, ether resistant and small in size. These characteristics indicated that they belonged to the picornaviruses of animals.

Further investigations of their properties were performed in order to compare them to the recognized criteria for the classification of the enteroviruses¹⁷⁾, rhinoviruses¹²⁾, and reoviruses¹²⁾. They were shown to be less stable at pH 3.0 than enteroviruses, but not as labile as the rhinoviruses. They appeared less stable at 50°C for 30 minutes on heating. The stabilization in the presence of 1 M MgCl₂ did not noticeably appear. They differed from reoviruses by the lack of hemagglutination, heat lability and being small in size. Therefore, these feline picornaviruses should be placed between the

Table 4. Serologic Relationship between Feline Viruses Isolated in Japan and Foreign Countries

Virus	Complement fixation	Neutralization test		
	Antiserum F 18 (K 6254-B)	F 14 (KN 5-O)	Antiserum F 17 (K 6253-B)	F 22 (K 6254-O)
FC	64*	<8**	<8	<8
FS	64	<8	<8	<8
CFI	64	<8	<8	<8
FRI	32	<8	<8	<8
FJ	128	<8	<8	<8
FRV	64	<8	<8	<8
FPL	64	64<	<8	<8
KCD	32	64	<8	<8
FVR	<4	NT	NT	NT
GP	64	<8	<8	<8
135/62	128	<8	<8	<8
227/62	128	<8	<8	<8
337/61	128	<8	<8	<8
344/61	64	<8	<8	<8
377/61	128	<8	<8	<8
F 14 (KN 5-O)	64	96	<8	12
F 17 (K 6253-B)	256	16	320	<8
F 22 (K 6254-O)	64	<8	<8	80

* Reciprocal of the highest serum dilution appeared positive fixation of complement (50% inhibition of hemolysis)

** Reciprocal of the serum dilution that inhibited cytopathic change in 50%

enteroviruses and rhinoviruses.

Recently in America, CRANDELL¹⁰⁾ reported that eight feline respiratory viruses were classified as picornaviruses. Since then similar studies have been reported^{1,15)}. In Europe, BÜRKI⁵⁾ reported the isolation of five serotypes of feline viruses belonging to the picornaviruses of lower animals and being more stable to low pH.

CRANDELL¹⁰⁾ reported that the feline viruses, unlike rhinoviruses showed multiplication only in tissue of feline origin, but high titers were easily achieved in stationary cultures at 35~37°C. On the other hand, KARPAS et al.¹⁵⁾ reported the isolation of feline rhinoviruses from the oral cavity of a cat suffering from a mild respiratory disease and also exhibiting symptoms of ulcerative glossitis and that the virus multiplied in a line of green monkey kidney cell monolayers (GMK). We found that four strains of 27 feline picornaviruses grew and showed cytopathic effects in Vero cell. This evidence assumed that those four strains might be rather similar to feline rhinoviruses.

The anti-feline picornavirus rabbit sera had antibodies in the CF test as well as neutralization test, but the guinea pig antisera had them only in the CF test. It has not been confirmed yet why guinea pig antisera had no neutralizing antibody to feline picornaviruses.

The results of serologic examination obtained here indicated that these feline picornaviruses including foreign strains had various antigenicity. Our 27 strains were subdivided into more than five serotypes by neutralization tests. It is interesting that two American strains were neutralized by our F14 antiserum, though several workers have reported that each of American feline viruses have no antigenic similarity in neutralization tests^{3,6,8,12)}.

Recently, in various fields a great many cats have been reared as experimental animals. And in these fields the infectious diseases, especially respiratory virus infections of cats are becoming serious problem.

It will be the subject for future study to develop some useful polyvalent vaccines or antisera against these diseases.

SUMMARY

The biophysical and biochemical properties of the feline cytopathogenic viruses which cause respiratory diseases in cats were investigated. These viruses were of RNA type, ether resistant, acid labile, heat labile and smaller than 50 m μ in size. Therefore, these viruses were picornaviruses of animals. However, it was not yet confirmed whether they should be placed in enterovirus or rhinovirus group. Some of 27 strains might be similar to feline rhinoviruses owing to their growth in Vero cell.

Serologic investigations were carried out in order to make clear serologic relationship among 27 feline viruses isolated in our laboratory, and between our isolates and 15 foreign isolates. Twenty seven viruses were subdivided into more than five serotypes by neutralization test. Only two strains, FPL and KCD isolated in America were neutralized by our F14 antiserum.

Therefore, these feline picornaviruses including foreign isolates were similar antigenically in CF test, but subdivided into more than five serotypes by neutralization test.

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REFERENCES

- 1) BARTHOLOMEW, P.T. and GILLESPIE, J.H. (1968): *Cornell Vet.*, **58**, 248~265.
- 2) BITTLE, J. L., YORK, C.J., NEWBERNE, J.W. and MARTIN, M. (1960): *Amer. J. vet. Res.*, **21**, 547~550.
- 3) BITTLE, J.L., EMERY, J.B., YORK, C.J. and McMILLEN, J.K. (1961): *Amer. J. vet. Res.*, **22**, 374~378.
- 4) BOLIN, V.S. (1957): *Virology*, **4**, 389~390.
- 5) BÜRKI, F. (1965): *Arch. ges. Virusforsch.*, **15**, 690~696.
- 6) COHEN, D., YOHAN, D.S., PANIA, R.A. and HAMMON, W.M. (1961): *Amer. J. vet. Res.*, **22**, 637~643.
- 7) CRANDELL, R.A. and MAURER, F.D. (1958): *Proc. Soc. exp. Biol. (N.Y.)*, **97**, 487~490.
- 8) CRANDELL, R.A. and MADIN, S.H. (1960): *Amer. J. vet. Res.*, **21**, 551~556.
- 9) CRANDELL, R.A., NIEMANN, V.H., GANAWAY, J.R. and MAURER, F.D. (1960): *Virology*, **10**, 283~285.
- 10) CRANDELL, R.A. (1967): *Proc. Soc. exp. Biol. (N.Y.)*, **126**, 240~245.
- 11) FASTIER, L.B. (1957): *Amer. J. vet. Res.*, **18**, 382~389.
- 12) HAMPARIAN, V.V., HILLEMANN, M.R. and KETLER, A. (1963): *Proc. Soc. exp. Biol. (N.Y.)*, **112**, 1040~1050.
- 13) HERSEY, D.F. and MAURER, F.D. (1961): *Proc. Soc. exp. Biol. (N.Y.)*, **110**, 821~831.
- 14) KAMIZONO, M., KONISHI, S., OGATA, M. and KOBORI, S. (1968): *Jap. J. vet. Sci.*, **30**, 197~206.

- 15) KARPAS, A. and ROUTLEGE, J.K. (1969): *Zbl. Vet.-Med.*, **16**, 91~93.
- 16) KETLER, A., HAMPARIAN, V.V. and HILLEMANN, M.R. (1962): *Proc. Soc. exp. Biol.* (N. Y.), **110**, 821~831.
- 17) MELNICK, J.L. DALLDORF, G., ENDERS, J.F., GELFAND, H.M., HAMMON, W.M., HUEBNER, R.J. and ROSEN, L. (1962): *Virology*, **16**, 501~504.
- 18) REED, L.J. and MUENCH, H. (1938): *Amer. J. Hyg.*, **27**, 493~497.
- 19) TORLONE, V. (1960): *Vet. Itali.*, **11**, 915~928.
- 20) WALLIS, C. and MELNICK, J.L. (1962): *Virology*, **16**, 504~506.
- 21) YASUMURA, Y. and KAWAKITA, Y. (1963): *Jap. J. clinic. Med.*, **21**, 1201~1215.

ネコの呼吸器疾患より分離した細胞変性 ウイルスに関する研究

II. ネコのピコルナウイルスの性状と分類

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著者らは、すでにネコの呼吸器疾患より分離した細胞変性ウイルスが、エーテル耐性で赤血球凝集性がなく、CF 抗原性は共通であるが、中和試験で 2 型以上に分けられることなどを報告した (1968 年)。

今回の報告では、それらのウイルスの物理化学的性状を明らかにし、ウイルスの分類を検討するとともに、米国、スイス、イタリアから分与された外国分離株との血清学的関連性を検討した。

これらのウイルスは、RNA 型であり、pH 3.0 にやや不安定、50°C 30 分の加熱にやや不安定で、さらに 1 M MgCl₂ 存在下での熱抵抗性の増進が若干みられた。また孔径 50 mμ のフィル

ターを容易に通過した。これらのことから、このウイルスは動物のピコルナウイルスに属し、エンテロウイルスとライノウイルスの中間に位置するものと考えられた。また 27 株中 4 株が、Vero 細胞中で増殖を示し、CPE を起こすことが認められ、とくにこの 4 株は、ライノウイルスにより近縁であることも想像される。

血清学的試験の結果、教室分離株 27 株は中和試験により、少なくとも 5 型以上の血清型に分けられた。外国分離株と比較すると、CF 抗原性は共通であるが、中和試験では米国外分離株の 2 株のみが教室分離株 F 14 の抗血清により中和された。