Extraction of Infectious Ribonucleic Acid from a Feline Picornavirus

(Brief Report)

By

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Several serological types within a group of small, ether-resistant viruses have been isolated from the respiratory tract of domestic cats, and were tentatively classified as feline picornaviruses (1, 2). Recently, it was claimed that they possess morphological features which not only differ significantly from picornaviruses of other species but resemble those of reoviruses (3). Evidence for their nucleic acid type has been obtained by using indirect determination procedures (1, 2, 4). This report describes the extraction of an infectious, single-stranded ribonucleic acid (RNA) from a feline respiratory virus [FRV (5) and/or FR (6)], a serologically distinct, but typical member of the described picornaviruses of cats (1, 2). This agent presently is under investigation for further characterization (7).

Stock virus suspensions were prepared in primary feline kidney (FK) cell cultures grown in tissue culture medium 199 supplemented with 0.25% lactalbumin hydrolysate and 5% fetal calf serum (8). The virus was partially purified and concentrated by treatment with Freon 113 and precipitation with methanol (9) of clarified infectious tissue culture fluids. Infectivity of virus and nucleic acid preparations were assayed by cytopathic effect in FK cells grown in short Leighton tubes. Endpoints, calculated by the method of Kärber, are expressed as tissue culture infective doses (TCID_{50}). Coverslip preparations of infected, and non-infected control cultures for acridine-orange staining (10) were also prepared in Leighton tubes. Virus concentrates were extracted twice with water-saturated phenol at 1°C (11). Nucleic acid preparations diluted

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tenfold in PBS with and without the addition of DEAE-Dextran2 were
inoculated in 0.1 ml amounts on monolayers that had been washed twice
with PBS. After an adsorption period of 30 minutes at 37°C, medium 199
with 1% lamb serum was added. Exposure of phenol extracts to nuclease
was done as previously described (12).

The phenol extracts obtained showed nucleic acid (NA) characteristics
with some remaining protein contamination in their UV absorption spectra.
With maxima at 260 mμ and minima at 235 mμ, maximum, minimum
absorbance ratios ranged from 1.50 to 1.88 in different preparations.
Titration of the extracts showed infectivity up to \(10^{3.2}\) TCID_{50}/ml as
compared to that of the starting virus of \(10^{9.5}\) TCID_{50}/ml. This ratio could
not be improved by the addition of DEAE-Dextran (range: 30–250 μg/ml)
to the NA dilutions. Moreover, FK cells showed non-specific degeneration
when exposed to concentrations of DEAE-Dextran greater than 200 μg/ml
during the adsorption period. The infectivity of the extracts was completely
destroyed when incubated with 1 μg RNase. However, no loss in infectivity
was observed after exposure to 20 μg/ml of chromatographically
purified DNase. The starting virus was not affected by 25 μg/ml RNase.
When heated in PBS for 10 minutes, infectivity of the complete virus was
lost at 60°C, that of the NA at 65°C.

Acridine-orange staining produced flame-red fluorescent foci in the
cytoplasm of infected FK cells, while uninoculated control cells did not
show specific cytoplasmic fluorescence. These findings are in agreement
with earlier results on FRV (13). Progeny virus from cultures inoculated
with nucleic acid was neutralized by the same serum that neutralized
the intact starting virus. Although kittens inoculated intranasally with
progeny virus did not produce overt illness, they developed neutralizing
antibodies specific both for the starting virus and the progeny. Virus was
reisolated from throat swabs of these animals and identified as FRV by
means of neutralization tests with the type-specific rabbit antiserum.

The results show that an infectious RNA can be extracted from a
feline picorna virus using the cold phenol method. From the high sensitivity
to RNase, the heat inactivation data, and acridine-orange staining, the
RNA appears to be single-stranded. In contrast to findings with RNA
from some other viruses (14, 15), the plating efficiency of FRV-RNA was
not increased in the presence of DEAE-Dextran.

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