Characterization of Virus-Specific RNAs from Subacute Sclerosing Panencephalitis Virus-Infected CV-1 Cells

JEN YEH¹

Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, Pennsylvania 19104

Received for publication 5 June 1973

CV-1 cells infected with subacute sclerosing panencephalitis (SSPE) virus incorporated uridine-³H into at least four virus-specific RNA components in the presence of actinomycin D. The component sedimenting fastest had a sedimentation coefficient of 50s corresponding to a molecular weight of 6×10^6 . The other three RNA components have sedimentation constants of 35s, 22s, and 18s corresponding to molecular weights of 2.5×10^6 , 1.0×10^6 , and 0.75×10^6 , respectively. The base composition of the 50s RNA is distinct from that of cellular RNA and comparable with base compositions of viral RNAs of other paramyxoviruses. The base composition of the 18s RNA shows approximate complementarity with the 50s RNA. RNA-RNA annealing experiments using unlabeled 50s SSPE RNA with labeled 18s RNA from cells infected with SSPE virus or measles virus show 100% annealing with 18s SSPE RNA but only 60% annealing with 18s measles RNA. These experiments suggest some differences between the 18s RNAs of SSPE virus-infected cells and measles virus-infected cells.

Subacute sclerosing panencephalitis (SSPE) is a progressive degenerative disease of the human brain. Although the clinical and pathological changes that accompany this disease have been amply described and reviewed by ter Meulen et al. (15) and the possible involvement of paramyxoviruses has been suggested as early as 1965 (2), very few studies have attempted to elucidate the biochemical nature of the SSPE virus. Because of the high measles virus antibody titers found in blood serum and cerebral spinal fluid (CSF) of SSPE patients (4, 9, 17) the SSPE agent has been considered to be measles virus. Immunochemical (11) and morphological differences (11, 16) suggest that the virus is related to the measles family of paramyxovirus, but a different or a mutant form of measles virus.

After developing a tissue culture system for assaying and propagating sufficient quantities of the SSPE virus in vitro, we have reported the isolation and characterization of the SSPE virus nucleocapsids (22). The morphology, length, density, and chemical composition of the SSPE virus nucleocapsids were found to be similar to

those reported for other paramyxoviruses (5, 14), including measles (20).

We now report the characterization of the various virus-specific RNAs from SSPE-infected CV-1 cells and make some initial comparisons between SSPE RNA and measles virus RNA by the technique of cross-annealing.

MATERIALS AND METHODS

Virus and cell cultures. The LEC strain of SSPE virus, the CV-1 cells, and the culture conditions have been previously described (22). Woodfolk measles virus (16) had been passaged continuously in CV-1 cells.

Isotopes and chemicals. Uridine-5,6-³H (New England Nuclear Corp., Boston, Mass.; specific activity 42.4 Ci/mmol) was used at a concentration of 10 μ Ci/ml. Carrier-free ³²P-inorganic phosphate (New England Nuclear Corp.) was used at a concentration of 100 μ Ci/ml. All solutions, unless otherwise specified, were prepared in NT buffer (0.13 M NaCl, 0.05 M Tris-hydrochloride, pH 7.4). E buffer, used for electrophoresis, contained 0.04 M Tris, 0.02 M Na acetate, 0.002 M Na EDTA, and acetic acid sufficient to adjust the pH of the buffer to 7.8. The gels contained final concentrations of acrylamide, 2.2%, bis-acrylamide, 0.25%, TEMED, 0.05%, ammonium persulfate, 0.07%, and SDS, 0.1%. All of the items except SDS were obtained from Bio-Rad, Richmond,

¹ Present address: John L. Smith Memorial for Cancer Research, Pfizer Co., Maywood, N. J. 07607.

Calif. SDS was obtained from Sigma Chemical Co., St. Louis, Mo.

Infection of cell culture. Monolayers of CV-1 cells grown in 60-mm plastic culture dishes were infected with 0.2 ml of virus inoculum per dish at a multiplicity of infection (MOI) of 0.05 to 0.1. Plates were refed with fresh media after 1 h of adsorption at 37 C. Actinomycin D, 0.2 μ g/ml, was added 3 h postinfection and uridine- 3 H, 10 μ Ci/ml, was added 4 to 6 h postinfection. The plates were harvested at 36 h postinfection. For each experiment 10 to 20 plates were usually combined. When 32 P-inorganic phosphate was used as the radioactive label, plates were refed postinfection with phosphate-free media instead of complete media.

Isolation of nucleocapsid RNA. Virus nucleocapsids were harvested from the infected cells by the methods previously described (22). After centrifugation through 30% sucrose onto the 65% sucrose cushion, the interface material was collected, diluted with NT buffer, and extracted by phenol in the presence of SDS (7).

Isolation of RNAs from cells. Cultures were harvested by scraping the monolayers from the petri dishes with a rubber policeman. The scraped cells were allowed to swell for 10 min in 10% NT buffer at 4 C before homogenizing in a tight-fitting Dounce homogenizer. The homogenized cell extracts were centrifuged at 2,000 rpm for 10 min to remove cell debris and nuclei. RNA was extracted by the SDS-phenol method.

Polyacrylamide gel analysis. Polyacrylamide gels were made and prerun in E buffer before the samples were applied. Samples were applied in 10% sucrose and electrophoresed at 15 mA per gel for a predetermined length of time. After electrophoresis each gel was sliced into approximately 100 equal slices by a microtome. Slices were dissolved in 0.5 ml of 30% peroxide overnight at 65 C and counted in a scintillation spectrometer after adding scintillation fluid to each vial.

Sucrose gradient analysis. Nucleic acids redissolved from ethanol precipitates were applied onto a preformed 5 to 20% sucrose gradient containing 0.1% SDS. The samples were centrifuged in a SW 41 rotor at 200,000 $\times$ g at 20 C for 2 h 45 min. The gradient was fractionated into equal volume fractions from the bottom of the tube.

Base composition analysis. The sucrose gradient fractions containing the desired RNA species were combined and RNA reprecipitated by ethanol at -20 C. The samples were redissolved in a minimal amount of 0.3 N NaOH, sealed, and hydrolyzed overnight at 37 C. Yeast RNA (Sigma) hydrolyzed in the same manner, was used as carrier and also as independent marker. After neutralizing with perchloric acid, the hydrolysate was spotted on a pre-washed polyethylenimine (PEI)-cellulose thin-layer plate (Baker Chem. Corp., Phillipsburg, N.J.) and run in a one-dimension acetic acid-lithium chloride system (18). After the plates were dried the positions of the individual bases were marked under an ultraviolet light. Each spot was cut out and counted in a scintillation spectrometer.

RNA annealing. The RNA-RNA annealing technique used was that described by Bratt and Robinson (3) with some modifications. Predetermined quantities of the various RNAs in 0.01 M Tris buffer were placed in 12 ml conical centrifuge tubes. The volume of each tube was adjusted to 0.15 ml with buffer. The tubes were placed in boiling water for 5 min and quickly chilled in ice. NaCl and Tris were added to give the final concentrations of 0.2 M NaCl and 0.1 M Tris. The tubes were sealed and allowed to anneal overnight at 65 C. Base-paired RNA was detected by its resistance to RNase digestion. Pancreatic RNase (Sigma; DNase free) from a stock solution of 1 mg/ml was added to each tube at a final concentration of 75 μ g/ml and incubated at 37 C for 10 min. All nonresistant RNA were deemed digested as prolonged incubation did not change the amount of precipitable radioactivity. Cold 5% trichloroacetic acid was added to stop the reaction. Precipitable material collected on Millipore filters was counted for radioactivity.

RESULTS

Nucleocapsid RNA. Since the quantity of extracellular virus from the LEC strain SSPE infected CV-1 cells was insufficient for measurement of viral RNA, the nucleocapsid RNA from infected cells was used before it was degraded during the purification procedure (22). As a compromise to the lability of the nucleocapsids and the purity of the preparation, the extraction of the nucleocapsid RNA was carried out after the initial sucrose purification step. The material at the interface of the 30 to 65% sucrose step gradient was collected and the RNA was extracted by phenol in the presence of SDS. The ethanol-precipitated nucleic acid material was analyzed on the polyacrylamide gels. Figure 1

FIG. 1. Distribution of radioactivity of nucleocapsid preparation (●) and ribosomal RNA marker (○) after electrophoresis in a 2.2% polyacrylamide gel. SSPE virus-infected cells were harvested 36 h postinfection, homogenized, clarified, and passed through 30% sucrose onto a 65% sucrose cushion. The material at the 30 to 65% sucrose interface was extracted by SDS-phenol, precipitated by ethanol, redissolved in NT buffer and applied onto the gel.

shows the RNA profile of the preparation. A major peak of radioactivity is centered at slice 15. Using ^{32}P -labeled ribosomal RNA as molecular weight markers, this peak has a calculated molecular weight of approximately 6×10^6 . Uridine- ^3H -labeled viral RNA isolated from measles virus (19) and considered to have a molecular weight of about 6×10^6 also electrophoresed to that position in parallel polyacrylamide gel runs. Two smaller peaks of radioactivity were also present in the gel, centered at slices 37 and 57. Data presented later will suggest that these are RNAs complementary to viral RNA. This interpretation would seem reasonable considering that our initial nucleocapsid preparation was obtained from infected CV-1 cells and not from purified virus particles.

If the nucleocapsid RNA was extracted after the cesium chloride centrifugation step in our

nucleocapsid purification scheme, very little RNA of 6×10^6 daltons was recovered. Instead, one found a smear of radioactivity, corresponding to degraded RNA, spread through the lower regions of the gel.

Virus-specific RNAs. Virus-specific RNAs, extracted from virus-infected cell preparations 36 h postinfection were characterized by centrifugation in sucrose gradients and electrophoresis on polyacrylamide gels. Figure 2 is a 5 to 20% sucrose gradient profile of the extracted SSPE RNAs. Cellular ribosomal RNAs, measured by absorption at 260 nm, serve as position markers. Two major peaks of radioactivity and a shoulder are evident in the virus-infected culture as compared to the noninfected cell control. Compared to the 18s and 28s ribosomal RNAs, the major peaks have sedimentation rates of 50s and 18s. The shoulder has a sedimentation rate of 22s.

FIG. 2. Distribution of radioactivity of virus infected (●— —), uninfected (○), and A_{260} (●—) after 5 to 20% sucrose rate-zonal centrifugation of nucleic acid from SSPE virus-infected and uninfected cultures of CV-1 cells. The cultures were treated with actinomycin D, labeled with uridine- ^3H , and harvested 36 h postinfection. RNA was extracted by SDS-phenol, precipitated with ethanol, redissolved in NT buffer, and applied onto the gradient.

The polyacrylamide gel electrophoresis pattern of the same material is presented in Fig. 3. Uridine- ^{14}C -labeled cell ribosomal RNA was used as molecular weight markers. Four peaks of radioactivity in the virus-infected cell preparation was detectable over the uninfected cell controls. The first peak at slice 12 is calculated to have an approximate molecular weight of 6×10^6 and corresponds to the 50s viral RNA or nucleocapsid RNA. The next small peak at slice 35 has an approximate molecular weight of 2.5×10^6 and probably corresponds to the 32 to 35s RNA reported by others (1, 3) as one species of virus-specific RNAs found in paramyxovirus-infected cells. The broad peak centered at slice 55 represents RNA of about 10^6 daltons and is assumed to be the 22s RNA shoulder of the sucrose gradient. The last peak at slice 65 was the most abundant of the virus-specific RNA species. It corresponds to the 18s RNA of the sucrose gradient and calculates to an approximate molecular weight of 0.75×10^6 . RNA smaller than 18s were examined by polyacryl-

amide gel analysis. A peak of radioactivity corresponding to 4s RNA was detected, but the quantities present in virus-infected cell cultures did not exceed those present in control cells to a significant extent.

Studies to determine the time course of virus-specific RNA synthesis by changing labeling times and harvest times have been unsuccessful so far. The only RNA consistently found at 24 h postinfection has been the 18s RNA. Additional studies are underway.

Base composition analysis. The two more abundant moieties of virus-specific RNA, the 50s and the 18s RNA, were reprecipitated from the sucrose gradient and hydrolyzed for base composition studies and compared to 28s ribosomal RNA (Table 1). Both species of viral RNAs showed high adenosine and uridine contents, whereas ribosomal RNAs showed high guanosine and cytosine contents. More specifically, the 50s viral strand was particularly rich in uridine (31%), whereas the complementary strand was highest in adenosine (29%). Closer

FIG. 3. Distribution of radioactivity of SSPE virus-infected cells (●), uninfected cells (○), and ribosomal RNA marker (▲) after electrophoresis on a 2.2% polyacrylamide gel. The cells were infected, treated with actinomycin D, labeled with uridine- ^3H , and harvested 36 h postinfection. RNA was extracted by SDS-phenol, precipitated by ethanol, redissolved in NT buffer and applied onto the gels.

TABLE 1. *Base composition of ³²P-labeled RNA^a*

Source of RNA	Mol % ^b			
	G	U	A	C
50s SSPE RNA from CV-1 cells	21.4 ± 0.6	31.1 ± 0.8	24.2 ± 0.8	23.3 ± 1.0
18s SSPE RNA from CV-1 cells	21.2 ± 0.5	25.3 ± 0.6	29.3 ± 0.5	24.2 ± 0.9
28s rRNA from CV-1 cells	34.9 ± 0.2	17.6 ± 0.4	16.8 ± 0.3	30.6 ± 0.3
18s rRNA from CV-1 cells	29.7 ± 0.3	22.4 ± 0.3	22.5 ± 0.4	25.4 ± 0.4

^a Base compositions of ³²P-labeled RNA were determined by alkaline hydrolysis and PEI-cellulose thin-layer chromatography after initial separation of the RNA on a 5 to 20% sucrose gradient. Each point is an average of at least 10 different experiments.

^b Standard deviation, ±.

examination of the base compositions of the two viral RNAs shows that there is some degree of complementarity between the 50s and the 18s RNAs, especially between the adenosine and uridine moieties of the bases. The difference between guanosine and cytosine cannot be explained by this data.

Degree of complementarity of 18s and 50s SSPE RNAs. To further examine the relationship between the 50s and 18s viral RNAs, annealing experiments were performed to test sequence homology (Table 2). Unlabeled 50s SSPE nucleocapsid RNA was used as one of the RNA moieties and labeled 18s RNA as the other. A constant amount of uridine-³H-labeled 18s SSPE RNA was placed in a set of tubes and increasing amounts of unlabeled 50s SSPE RNA were added to the 18s SSPE RNA. Parallel sets, using labeled 18s ribosomal RNA were used as control. At saturation levels of 50s SSPE RNA, 100% of the 18s SSPE RNA was protected against RNase digestion. Control experiments using 18s ribosomal RNA showed only about 25% protection of the labeled 18s RNA at 50s viral RNA concentrations where 18s SSPE RNA was completely protected. Self-annealing of the 18s SSPE RNA was less than 6% at the concentrations of the 18s RNA used.

Cross-annealing with measles RNA. A valid comparison of the degree of similarity of two RNAs may be obtained by cross-annealing the two RNAs to a third RNA, known to be complementary to one of the two test RNAs. Since there appears to be a high degree of morphological and immunological similarity between measles virus and SSPE virus, the virus-specific RNAs induced by these viruses were compared on the basis of their relative efficiency of annealing with SSPE virus-nucleocapsid RNA (Fig. 4). The 18s RNAs used included: (i) those isolated from CV-1 cells infected by Woodfolk measles virus, (ii) those isolated from SSPE virus-infected cells, and (iii)

TABLE 2. *Annealing of 50s viral nucleocapsid RNA with 18s RNA from infected and uninfected CV-1 cells^a*

RNA species	RNase resistant radioactive material (% of control)
10 ⁴ counts/min of 18s RNA- ³ H from infected cells + 0 units of unlabeled 50s viral RNA treated with RNase before annealing overnight	< 1
10 ⁴ counts/min of 18s RNA- ³ H from infected cells + 0 units of unlabeled 50s viral RNA treated with RNase after annealing overnight	7
10 ⁴ counts/min of 18s RNA- ³ H from infected cells + 10 units of unlabeled 50s viral RNA	25
10 ⁴ counts/min of 18s RNA- ³ H from infected cells + 100 units of unlabeled 50s viral RNA	104
10 ⁴ counts/min of 18s RNA- ³ H from uninfected cells + 10 units of unlabeled 50s viral RNA	12
10 ⁴ counts/min of 18s RNA- ³ H from uninfected cells + 100 units of unlabeled 50s viral RNA	23

^a Samples of 18s RNA from infected cells were mixed with RNA from SSPE nucleocapsid preparations, boiled for 5 min, and quick chilled before annealing overnight at 65 C. After RNase treatment the RNase resistant material was precipitated with cold trichloroacetic acid and compared with controls not treated with RNase.

18s ribosomal RNA of CV-1 cells. At concentrations of unlabeled 50s SSPE RNA sufficient to completely protect the 18s SSPE RNA from ribonuclease digestion, only 60% of the labeled 18s measles RNA was protected and only 25% of the ribosomal RNA was protected, suggesting that there are differences in nucleotide sequences between the 18s RNAs of measles virus and SSPE virus.

FIG. 4. Annealing of uridine- ^3H -labeled 18S RNA from SSPE virus-infected cells (●), measles-infected cells (■), and uninfected cells (○) with increasing amounts of unlabeled 50S SSPE virus nucleocapsid RNA. The appropriate RNAs were mixed, boiled for 5 min, and quick chilled before annealing at 65°C overnight. After RNase treatment the RNase resistant material was precipitated with cold trichloroacetic acid and counted.

DISCUSSION

We have demonstrated that SSPE viral RNA, isolated both from partially purified nucleocapsid preparations and from infected CV-1 cell monolayers have a sedimentation rate of 50S and a molecular weight of 6×10^6 . These properties of the SSPE viral RNA are similar to those of the viral RNA from both the purified virus of, and from cells infected by, a number of paramyxoviruses including measles virus (6, 7, 19). We have also demonstrated that at least three other species of viral-specific RNAs are present in SSPE virus-infected CV-1 cultures. These RNAs have sedimentation rates of 35S, 22S, and 18S and molecular weights of 2.5×10^6 , 1.0×10^6 , and 0.75×10^6 , respectively. Similar sizes of viral-specific RNAs induced by Sendai (1) and NDV (3) viruses have also been reported. These characteristics of the SSPE and

nucleocapsid RNA unquestionably identify the SSPE virus as a member of the paramyxovirus family.

The base compositions of the SSPE virus RNAs also show striking similarities to the base compositions of the other paramyxoviruses. The base composition of SV5 (6), Sendai virus (1, 10), NDV (7, 12), and measles viruses (D. J. Waters, Ph.D. thesis, Univ. of Kansas, Lawrence, Kansas, 1973) are all similar. All these 50S virus RNAs have higher contents of uridine and adenosine than of guanosine and cytosine. All have larger amounts of uridine (around 30%) than of the other three nucleotides. The fact that the 18S SSPE RNA had a base composition somewhat complementary to the 50S RNA suggests that it is RNA copied from the 50S virion RNA. The fact that the 18S SSPE RNA was able to anneal completely with the 50S RNA further substantiates this supposition.

Since so many of the morphological and physiochemical properties of the paramyxoviruses are similar, any clearcut method of differentiating the viruses would be useful. Many of the viruses have definite antigenic differences, but others, such as the measles, distemper, and rinderpest group of viruses, have varying degrees of antigenic cross-reactions (21), and need other means of differentiation. Because of the anti-measles antibody titer in the blood serum and CSF of SSPE patients, SSPE has long been considered a measles virus. While this does suggest a close relationship, identity cannot be assumed.

The technique of cross-annealing of RNA provides a quantitative method of determining the relationship of two viruses. As Kingsbury (13) has shown in cross-annealing studies of NDV, different strains of NDV all cross-annealed so that 95% of the labeled RNA was RNase resistant. Cross-annealing of NDV with Sendai showed less than 25% RNase resistance. Our cross-annealing data of measles and SSPE RNAs resulting in 60% RNase resistance suggests that though measles virus and SSPE virus share some common RNA base sequences, there are also RNA sequences that are different from each other. If the NDV studies can be used as an example, then this difference is more than just strain variation.

ACKNOWLEDGMENTS

I thank Phyllis Dennis for excellent technical assistance. This investigation was supported by Public Health Service research grants NS-06859 and RR-05540 and funds from the John A. Hartford Foundation.

LITERATURE CITED

1. Blair, C. D., and W. S. Robinson. 1968. Replication of

- Sendai virus. I. Comparison of the viral RNA and virus-specific RNA synthesis with Newcastle disease virus. *Virology* **35**:537-549.
2. Bouteille, M., C. Fontaine, D. Vedrenne, and J. Delarue. 1965. Sur un cas d'encephalite subaiguë à inclusions. Étude anatomo-clinique et ultrastructurale. *Rev. Neurol.* **113**:454-458.
3. Bratt, M. A., and W. S. Robinson. 1967. Ribonucleic acid synthesis in cells infected with Newcastle disease virus. *J. Mol. Biol.* **23**:1-21.
4. Chen, T. T., I. Watanabe, W. Zeman, and J. Mealey, Jr. 1969. Subacute sclerosing panencephalitis: propagation of measles virus from brain biopsy in tissue culture. *Science* **163**:1193-1194.
5. Compans, R. W., and P. W. Choppin. 1967. Isolation and properties of the helical nucleocapsid of the parainfluenza virus SV5. *Proc. Nat. Acad. Sci. U.S.A.* **57**:949-956.
6. Compans, R. W., and P. W. Choppin. 1968. The nucleic acid of the parainfluenza virus SV5. *Virology* **35**:289-296.
7. Duesberg, P. H., and W. S. Robinson. 1965. Isolation of the nucleic acid of Newcastle disease virus (NDV). *Proc. Nat. Acad. Sci. U.S.A.* **54**:794-800.
8. Horta-Barbosa, L., D. A. Fuccillo, R. Hamilton, R. Traub, A. Ley, and J. L. Sever. 1970. Some characteristics of SSPE measles virus. *Proc. Soc. Exp. Biol. Med.* **134**:17-21.
9. Horta-Barbosa, L., D. A. Fuccillo, J. Sever, and W. Zeman. 1969. Subacute sclerosing panencephalitis: isolation of measles virus from a brain biopsy. *Nature (London)* **221**:974.
10. Iwai, Y., M. Iwai, M. Okumoto, Y. Hosokawa, and T. Asai. 1966. Properties of the nucleic acid isolated from HVJ. *Biken's J.* **9**:241-248.
11. Katz, M., V. ter Meulen, L. Leonard, and H. Koprowski. 1970. Subacute sclerosing panencephalitis: a new serological test in the diagnosis of the disease. *Pediat. Res.* **4**:482.
12. Kingsbury, D. W. 1966. Newcastle disease virus RNA. I. Isolation and preliminary characterization of RNA from virus particles. *J. Mol. Biol.* **18**:195-203.
13. Kingsbury, D. W. 1966. Newcastle disease virus RNA. II. Preferential synthesis of RNA complementary to parental viral RNA by chick embryo cells. *J. Mol. Biol.* **18**:204-214.
14. Kingsbury, D. W., and R. W. Darlington. 1968. Isolation and properties of Newcastle disease virus nucleocapsids. *J. Virol.* **2**:248-255.
15. Meulen, V. ter, M. Katz, and D. Müller. 1972. Subacute sclerosing panencephalitis: a review. *Curr. Top. Microbiol. Immunol.* **57**:1-38.
16. Oyanagi, S., V. ter Meulen, M. Katz, and H. Koprowski. 1971. Comparison of subacute sclerosing panencephalitis and measles virus: an electron microscope study. *J. Virol.* **7**:176-187.
17. Payne, F. E., J. V. Baublis, and H. H. Itabashi. 1969. Isolation of measles virus from cell cultures of brain from a patient with subacute sclerosing panencephalitis. *N. Engl. J. Med.* **281**:585-589.
18. Randerath, E., and K. Randerath. 1965. Ion-exchange thin-layer chromatography. XII. Quantitative elution and micro-determination of nucleoside monophosphates, ATP, and other nucleotide coenzymes. *Anal. Biochem.* **12**:83-93.
19. Schluderberg, A. 1971. Measles virus RNA. *Biochem. Biophys. Res. Commun.* **42**:1012-1015.
20. Waters, D. J., R. T. Hersch, and R. H. Bussell. 1972. Isolation and characterization of measles nucleocapsid from infected cells. *Virology* **48**:278-281.
21. Yamanouchi, K., F. Kobune, A. Fukuda, M. Hayami, and A. Shishido. 1970. Comparative immunofluorescent studies on measles, canine distemper, and rinderpest viruses. *Arch. Gesamte Virusforsch.* **29**:90-100.
22. Yeh, J., and Y. Iwasaki. 1972. Isolation and characterization of subacute sclerosing panencephalitis virus nucleocapsids. *J. Virol.* **10**:1220-1227.