JOURNAL OF VIROLOGY, Jan. 1973, p. 61-68

Vol. 11, No. 1
Printed in U.S.A.

Characterization of Nucleic Acid of Pichinde Virus

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Received for publication 22 September 1972

The nucleic acid of Pichinde virus was found to be single-stranded ribonucleic acid (RNA) as determined by sensitivity to ribonuclease, by alkaline degradation, by buoyant density in cesium sulfate, and by analysis of the base composition. The RNA of the virion could be separated into five components which had sedimentation coefficients corresponding to 31*S*, 28*S*, 22*S*, 18*S* and 4 to 6*S*. The 28*S*, 18*S*, and possibly the 4 to 6*S* RNAs appear to be derived from host cell components incorporated into the virion, whereas the 31*S* and 22*S* components appear to represent the genome of the virus.

The arenaviruses are morphologically unique in that they contain multiple electron-dense granules instead of a discernible core (6, 16, 17, 22). The granules are similar in size and shape to cell ribosomes, and the possibility exists that host cell ribosomes may be incorporated into the virion (16, 17, 19). Pichinde virus, a member of the arenavirus group, is relatively stable, and the virus replicates well in tissue culture (15). The nucleic acids of Pichinde virus were, therefore, characterized to determine whether or not host cell ribosomal material was incorporated into the mature virions of members of the arenavirus group.

MATERIALS AND METHODS

Chemicals. Polyethylene glycol 6000 (PEG) was purchased from Union Carbide Corp., New York. Phenol, *m*-cresol and 8-hydroxyquinoline were obtained from Matheson, Coleman and Bell. Diethyl oxydiformate (DEP) and triisopropylnaphthalene sulfonic acid sodium salt (TINS) were purchased from Eastman Organic Chemical Co., Rochester, N.Y. Electrophoretically purified bovine pancreatic deoxyribonuclease and ribonuclease A were purchased from Worthington Biochemical Co., Freehold, N.J. Actinomycin D in 200-μg quantities (lot 025007) was obtained from Calbiochem. Acrylamide, *N,N'*-methylenebisacrylamide, *N,N,N',N'* tetramethylenediamine (TEMED), and ammonium persulfate were purchased from Canal Industrial Co., Rockville, Md. Agarose for electrophoresis were obtained from Sigma Chemical Co.

Uridine-5-^3H (25 Ci/mmole), uridine-2-^{14}C (50 mCi/mmole), and Aquasol liquid scintillation fluid were purchased from New England Nuclear Corp., Boston. L-Methionine-*methyl*-^3H (3.3Ci/mmole) was purchased from Schwarz/Mann, Orangeburg, N.Y. Carrier-free orthophosphate ^{32}P was obtained from Bionuclear, Inc., Friendswood, Texas.

Buffers and solutions. TNE buffer consisted of 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, *p*H 7.4, 0.1 M NaCl, and 0.001 M ethylenediaminetetraacetic acid (EDTA). TNM buffer contained 0.01 M Tris, *p*H 7.4, 0.1 M NaCl, and 0.001 M $MgCl_2$. PMQN was utilized as the basic deproteinizing agent and consisted of the following: 500 g of freshly distilled phenol, 70 ml of distilled *m*-cresol, 0.5 g of 8-hydroxyquinoline and 55 ml of 0.15 M NaCl. E buffer used for electrophoresis, described previously (2), consisted of 0.4 M Tris, *p*H 7.2, 0.02 M sodium acetate, and 0.001 M EDTA. The 3E buffer was three times the concentration of E buffer. Sucrose solutions utilized for density gradients were made in TNE buffer.

Media. Growth medium consisted of Eagle minimal medium supplemented with 10% fetal bovine serum (FBS), 0.75 g of sodium bicarbonate ($NaHCO_3$)/liter, and antibiotics (100 units of penicillin/ml and 100 μg of streptomycin/ml). Maintenance medium was composed of Eagle minimal medium supplemented with 2% FBS, 1.50 g of $NaHCO_3$/liter, and antibiotics.

Virus. Pichinde virus strain AN3739 has been described (15, 28). The virus was passaged once in BHK-21 cells, and the extracellular fluid from infected cultures was utilized as the source of virus in all experiments. The titer of the virus stock, assayed by the plaque counting method (15), was approximately 2×10^8 plaque-forming units (PFU)/ml.

Cell cultures. BHK-21 cells (26) in growth medium were seeded into 16-oz (ca. 0.47 liter) prescription bottles. Three days after seeding the cells were confluent monolayers with approximately 2×10^7 cells per bottle. HeLa cells were propagated in growth medium as previously described (12).

Infection of cells and preparation of radioactive virus. Confluent monolayers were infected with virus at a multiplicity of infection of approximately 2 PFU/cell. After adsorption of the virus for 90 min at 37 C, the monolayers were washed once with maintenance medium, and 25 ml of fresh medium was added to

each monolayer. Three different radioisotopes were used to label the viral nucleic acid. ^{3}H-uridine-labeled virions were grown in the presence of 5 μCi of the radioisotope/ml and 25 μg each of deoxycytidine and thymidine/ml. *Methyl ^3H*-labeled virions were grown in methionine-free maintenance medium containing methionine-*methyl*-^3H (15 μCi/ml). ^{32}P-labeled virions were grown in phosphate-free maintenance medium supplemented with 25 μCi of ^{32}P-orthophosphate/ml. Extracellular fluids were harvested 72 hr after infection. Extracellular fluids from uninfected cells served as controls.

In some experiments the radiolabeled virus was grown in the medium containing actinomycin D. Maintenance medium supplemented with radioisotope and actinomycin D (0.05 μg/ml) was added to the cultures after virus adsorption. The extracellular fluids containing the virus were harvested 48 hr after infection. Uninfected cultures were used as controls. In all experiments, however, label was added at the time of infection and maintained throughout the entire time of infection.

Concentration and purification of Pichinde virus. Extracellular fluids were harvested at 48 or 72 hr after infection and clarified by centrifugation at 1,100 $\times$ *g* for 20 min, and the virus was concentrated by precipitation with polyethylene glycol (13). PEG and NaCl were added to a final concentration of 6.0% and 0.4 M, respectively, and the mixture was held overnight at 4 C. The precipitate was pelleted by centrifugation at 11,000 $\times$ *g* for 45 min and resuspended in TNE (3 ml of buffer per 100 ml of original volume). After sonic treatment at 50 kc for 30 sec, the resuspended material was layered on a discontinuous 20 and 50% (w/w) sucrose gradient and centrifuged for 90 min at 30,000 rev/min and 4 C in a Spinco SW50 rotor. A distinct band on the 50% sucrose was observed, and it was collected by puncturing the side of the tube. The band was diluted to less than 20% sucrose with TNE, and 10% FBS was added to stabilize the virus. One milliliter of the diluted virus was carefully layered on a 20 to 50% (w/w) linear sucrose density gradient and centrifuged at 35,000 rev/min for 2 hr at 4 C in the SW50 rotor. After centrifugation, approximately 20 fractions (0.25 ml) were collected from the bottom by using a piercing unit (Buchler Instruments). The refractive index (*n*) of every fifth fraction was obtained using a Bausch and Lomb refractometer, and the refractive index was used to calculate the buoyant density. When the infectious virus titer was to be determined, an 0.05-ml sample of each fraction was diluted 100-fold in maintenance medium and stored at -70 C until assayed.

Isolation of RNA from virus. The fractions of the linear sucrose gradient which contained the virus (density = 1.14 to 1.18 g/cm^3) were pooled, and the nuclease inhibitors 2-mercaptoethanol and DEP were added to 0.1% and 0.2% in the final volume, respectively. Sodium lauryl sarcosinate (SLS) was added to 1% in the final volume, the mixture was diluted fourfold in TNE, and the nucleic acid was extracted as previously described (4) using the PMQN solution for deproteinization.

Isolation of RNA from cells. Confluent monolayers of HeLa cells in 16-oz bottles were labeled for 24 hr with ^{14}C-uridine (2 μCi/ml). The cells were collected and suspended in 0.01 M acetate buffer, *p*H 5.1, and the RNA was extracted by the hot phenol procedure described by Scherer and Darnell (24).

RNA was extracted from uninfected BHK-21 cells by using a method which was modified from that previously described by Biswal et al. (4). Cells from four 16-oz bottles were suspended in 10 ml of TNM, partially lysed by treatment with 0.05 ml of SDS (10%) for 5 min at room temp and treated with deoxyribonuclease (50 μg) for an additional 5 min to remove most of the cell DNA. DEP (0.2 ml) and 0.2 ml of 0.25 M EDTA were added, followed by the addition of 0.5 ml of SLS (10%) and 0.3 ml of SDS. After shaking at 37 C for 5 min, 0.25 g of TINS was added. An equal volume of PMQN was added and gently shaken at 0 to 4 C for 10 min. The aqueous phase was separated by centrifugation (6,000 $\times$ *g*, 15 min) and adjusted to 0.2 M NaCl, and the deproteinization with PMQN was repeated until the white precipitate at the interface was no longer present. After the final extraction, the aqueous phase was collected, precipitated in two volumes of 95% ethanol at -20 C for at least 1 hr, and then centrifuged at 18,000 $\times$ *g* for 20 min. The pellet was resuspended in 2 ml of TNM, deoxyribonuclease (50 μg) was again added, and the mixture was incubated at 37 C for 15 min. To remove the deoxyribonuclease, an equal volume of PMQN was added, and the mixture was gently shaken at 4 C for 10 min. The phases were separated by centrifugation, the ribonucleic acid (RNA) in the aqueous phase was precipitated, and the precipitate was dried in the cold and suspended in 1 ml of TNE.

Sensitivity to nucleases. Viral RNA suspended in TNM buffer was tested for sensitivity to nucleases. Ribonuclease (20 μg/ml) or deoxyribonuclease (50 μg/ml) was added to the samples of the viral RNA preparations, and, after 1 hr of incubation at 37 C, acid-precipitable counts were determined (4).

Velocity sedimentation of viral and cell RNA. An 0.2-ml sample of RNA was layered on top of 5.0 ml of a 5 to 20% (w/w) linear sucrose density gradient. A few drops of mineral oil was layered above the sample to insure uniform distribution of the sample on the sucrose. ^{14}C-labeled HeLa cell RNA was used as reference marker. After centrifugation, fractions (0.15 ml) were collected by bottom puncture of the tube, and the acid-precipitable radioactivity of each fraction was determined (4, 5).

Buoyant density of viral RNA in cesium sulfate. A solution of TNE (*p*H 4.5) containing 2% dimethylsulfoxide was added to the viral RNA preparation to a total volume of 4.0 ml. Crystalline cesium sulfate (3.2 g) was added, the refractive index of the solution was adjusted to 1.3780, and 4.6 ml was covered with 0.6 ml of mineral oil in polyallomer tubes. After centrifugation, the refractive index of selected fractions and the acid-precipitable radioactivity of each fraction (4) were determined.

Determination of base composition. The base composition of RNA species from the virion or from BHK-21 cells was determined by a slightly modified

method previously described by Biswal et al. (4). The ^{32}P-labeled viral or cellular RNA species were mixed with 500 μg of yeast "carrier" RNA and digested in 0.05 ml of 0.5 M KOH for 16 hr at 37 C. The hydrolysates were spotted on Whatman 3M paper for electrophoresis, and the nucleotides were electrophoresed for 3 hr at 1,500 v in 0.05 M ammonium formate, *p*H 3.5. The ultraviolet light-absorbing spots on the paper were cut out, and the radioactivity of each was determined.

Polyacrylamide gel electrophoresis. Composite gels (8 cm in length) of 2.4% polyacrylamide and 0.5% agarose were prepared using methods previously described (7, 27). After polymerization for approximately 4 hr, the gels were removed to E buffer solution containing 0.2% SDS. Electrophoresis buffer was the E buffer containing 0.2% SDS. The gels were preelectrophoresed at 20 C for 30 min at 5 mA per tube using a Buchler DC power supply. For electrophoresis, 0.05 ml of RNA sample was mixed with 0.005 ml of bromophenol blue in 30% sucrose, and the mixture was carefully layered on the gel. Electrophoresis was usually carried out at 20 C and 5 mA per gel for 2.5 hr. After electrophoresis, the gels were sliced into 2-mm fractions by using a Gilson gel fractionator, model B100/GMA/GCB (Middleton, Wis.). A 10% solution of Bio Solv (Beckman Instruments, Palo Alto, Calif., was used for elution of the isotopically labeled materials from minced gel fractions. Ten milliliters of scintillation fluid was added, and radioactivity of each gel fraction was measured.

RESULTS

Purification and buoyant density of Pichinde virus. When BHK-21 cells were infected at an input multiplicity of 1 to 2 PFU/cell, maximum virus titers of 10^7 PFU/ml were consistently observed at 48 and 72 hr after infection. Pichinde virus was concentrated and purified from extracellular fluids as described above. To determine the buoyant density, purified Pichinde virus which had been labeled with ^{3}H-uridine was centrifuged to equilibrium in a sucrose density gradient (Fig. 1). Fractions were analyzed for both infectivity and radioactivity. Maximum infectivity and radioactivity were recovered at a buoyant density of about 1.15 to 1.16 g/cm^3. In some of the experiments maximum infectivity banded at a slightly greater density than maximum radioactivity although the difference was never more than a single fraction which represented a density difference of 0.01 g/cm^3. In all experiments no less than 15% of the original quantity of infectious virus was recovered in the fractions containing maximum virus. When culture fluids from uninfected cells were processed under identical conditions, a negligible amount of the radioactivity at the density of 1.16 g/cm^3 in the linear sucrose gradient was observed (Fig. 1).

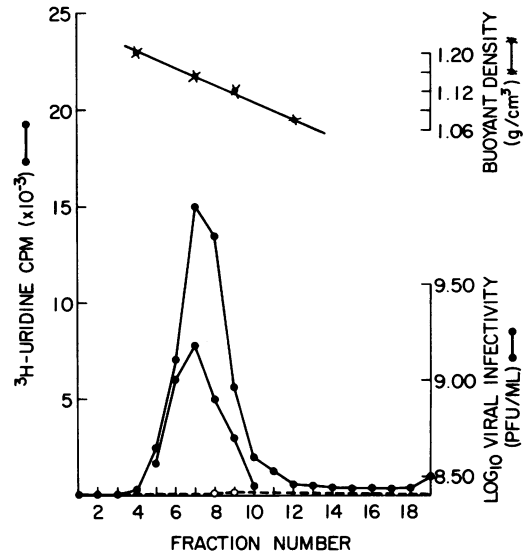

FIG. 1. *Equilibrium density gradient centrifugation of ^{3}H-uridine-labeled Pichinde virus in sucrose density gradient. Partially purified virus (1.0 ml) was layered on the top of a 4.0-ml 20 to 50% (w/w) linear sucrose gradient. The virus was centrifuged in a Spinco SW50 rotor at 35,000 rev/min for 2 hr at 4 C. Fractions were collected from the bottom of the tube, and the radioactivity (●) and infectivity (⊙) of each fraction were determined. Radioactivity of purified uninfected culture fluids (○) was also measured.*

Sedimentation characteristics of Pichinde virus RNA. The nucleic acid which was isolated from purified Pichinde virus was analyzed by velocity sedimentation in 5 to 20% sucrose density gradients (Fig. 2). The ^{3}H-uridine-labeled material was separated into four components, and the sedimentation coefficient of each was calculated using ^{14}C-uridine-labeled HeLa cell RNA as reference marker (14). The major component corresponding to 28*S* was consistently observed as were the 22*S* and 4 to 6*S* components. A minor 18*S* species was observed, but often this species appeared as a shoulder on the 22*S* peak. The sucrose sedimentation pattern observed in Fig. 2 represents RNA extracted from virus harvested at 72 hr after infection. Identical sedimentation patterns were observed using RNA from virus harvested at 24, 48, and 96 hr after infection.

The fractions collected from the sucrose density gradient were tested for sensitivity to enzyme digestion. Figure 2 shows that all fractions were sensitive to ribonuclease but resistant to deoxyribonuclease digestions. These results suggested that the Pichinde virus nucleic acid is single-stranded RNA.

Polyacrylamide gel electrophoresis of Pichinde virus RNA. When ^{3}H-uridine-labeled Pichinde virus RNA was electrophoresed in 2.4% polyacrylamide gels for 2.5 hr, four distinct viral RNA components were observed (see Fig. 6). With HeLa cell ribosomal RNA as reference, the sizes of these RNA components corresponded to 31*S*, 28*S*, 22*S*, and 18*S*, respectively (2). The low-molecular-weight component (4 to 6*S*) observed in sucrose density gradients (Fig. 2) migrated off the gel under these conditions (*unpublished data*). The major peak corresponded to the size of 28*S*, but the 31*S*, 22*S*, and 18*S* segments were also well resolved. These data, plus those obtained by sucrose velocity sedimentation, indicate that the Pichinde virus RNA consists of at least five components. By using the method of Spirin (25), the molecular weights of the components were calculated to be 2.1×10^6 (31*S*), 1.7×10^6 (28*S*), 1.1×10^6 (22*S*), 7×10^5 (18*S*), and 2.9×10^4 (4*S*). The 28*S*, 18*S*, and 4 to 6*S* components are similar in size to certain species of host cell RNA.

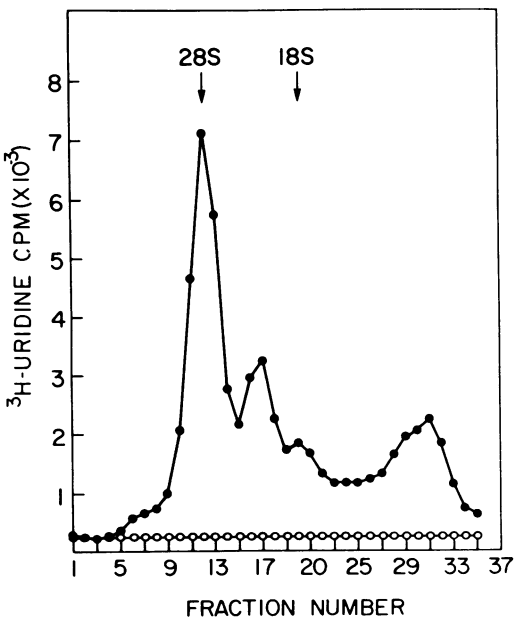

FIG. 2. *Velocity sedimentation of RNA from Pichinde virus labeled with ^{3}H-uridine. Viral RNA in TNE was layered on the top of a 5 to 20% (w/w) linear sucrose gradient, and centrifugation was carried out at 43,000 rev/min for 3.25 hr at 4 C in a Spinco SW50 rotor. Two 0.05-ml samples of each fraction were incubated for 1 hr at 37 C with either ribonuclease (20 μg/ml) or deoxyribonuclease (50 μg/ml) before determination of trichloroacetic acid-precipitable radioactivity. ^{14}C-uridine-labeled HeLa cell ribosomal RNA was centrifuged in a separate tube and used as a marker. Symbols: ●, ^{3}H-uridine-labeled Pichinde RNA after no enzyme treatment or after deoxyribonuclease treatment; ○, ^{3}H-uridine-labeled Pichinde RNA after treatment with ribonuclease.*

Buoyant density of Pichinde virus RNA. The buoyant density of ^{3}H-uridine-labeled Pichinde virus RNA in cesium sulfate was determined (Fig. 3). ^{14}C-uridine-labeled HeLa cell ribosomal RNA was used as a density marker. When the buoyant density of the HeLa cell ribosomal RNA was 1.685 g/cm^3, the density of the viral RNA was found to be 1.677 g/cm^3, a value which corresponds to the density of single-stranded RNA. The density of the Pichinde virus RNA varied from 1.685 to 1.669 g/cm^3 from experiment to experiment.

Identification of host cell ribosomal RNA in Pichinde virus. Since part of the RNA isolated from Pichinde virions consisted of 28*S* and 18*S* components, the possibility that these species were of host cell ribosomal RNA origin was examined by using actinomycin D to inhibit the 28*S* and 18*S* ribosomal RNAs produced in the infected cell. Low concentrations of actinomycin D have been found to inhibit both the synthesis of the large precursor ribosomal RNAs found in the cell nucleus and the appearance of cytoplasmic 28*S* and 18*S* ribosomal RNAs (9, 21). Pichinde virus was replicated in medium containing actinomycin D,

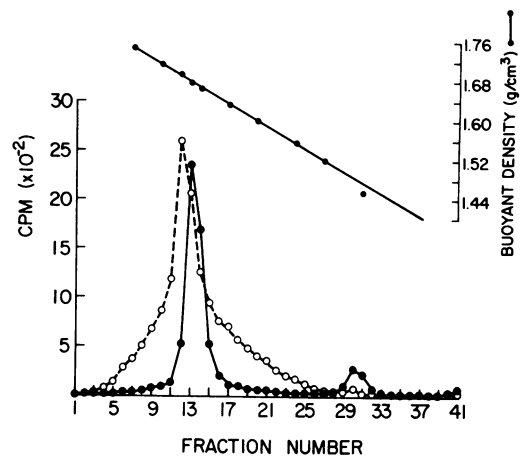

FIG. 3. *Equilibrium density centrifugation of Pichinde virus RNA in cesium sulfate. ^{3}H-uridine-labeled Pichinde virus RNA (●) was centrifuged for 72 hr at 30,000 rev/min in a Spinco SW50 rotor at 20 C. Fractions were collected by bottom puncture, and the trichloroacetic acid-precipitable radioactivity of each fraction was determined. ^{14}C-uridine-labeled HeLa cell ribosomal RNA was centrifuged in a separate tube and used as a density marker (○).*

and the RNA from resulting virions was analyzed. It was found that, when the culture medium was supplemented with 0.05 μg of actinomycin D/ml, the virus titers obtained 48 hr after infection were consistently two- to fivefold greater than in cultures maintained in medium lacking the drug (*unpublished observations*).

The effect of 0.05 μg of actinomycin D/ml on RNA synthesis in uninfected cells was measured by examining extracted RNA by sucrose velocity centrifugation. Figure 4 shows that the inhibitory effect of the drug on RNA synthesis was quite marked. In the absence of actinomycin D the 28*S*, 18*S*, and 4*S* species of host cell RNA were synthesized. Cells that were exposed to actinomycin D, however, failed to synthesize the 28*S* and 18*S* ribosomal RNAs.

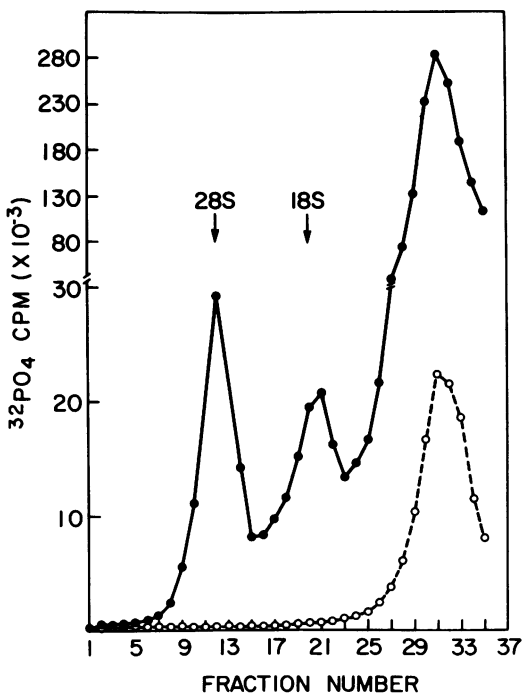

FIG. 4. *Velocity sedimentation of ^{32}P-labeled RNA from BHK-21 cells treated with actinomycin D. Confluent monolayers were maintained for 48 hr in either Eagle phosphate-free maintenance medium supplemented with ^{32}P or maintenance medium supplemented with ^{32}P and actinomycin D (0.05 μg/ml). The cellular RNA was extracted as described in Materials and Methods. A 0.2-ml sample of RNA from actinomycin D-treated (○) or untreated cells (●) was layered on top of a 5.0-ml, 5 to 20% linear sucrose gradient and centrifuged as described in Fig. 2. Fractions were collected by bottom puncture, and the trichloroacetic acid-precipitable radioactivity of each fraction was measured.*

These results confirmed the inhibitory effect of the actinomycin D for host cell ribosomal RNA (21).

RNA was extracted from virions which were replicated in the presence or absence of 0.05 μg of actinomycin D/ml. The RNAs were analyzed by sucrose velocity centrifugation and polyacrylamide gel electrophoresis. The sedimentation pattern of RNA from virions grown in the presence of the drug was considerably altered (Fig. 5). The 28*S* RNA species was significantly reduced, and a smaller peak corresponding to 30 to 31*S* was observed. The 18*S* species was also absent. However, the 22*S* and 4 to 6*S* peaks were essentially unchanged. The selective reduction in the 28*S* and 18*S* RNA species from virions grown in the presence of actinomycin D was also observed when the viral RNA was examined by gel electrophoresis (Fig. 6). Replication of the virus in the presence of actinomycin D resulted in virions whose RNA resolved into 31*S* and 22*S* components.

Incorporation of methyl-^{3}H group into Pichinde virus RNA. Cellular ribosomal and

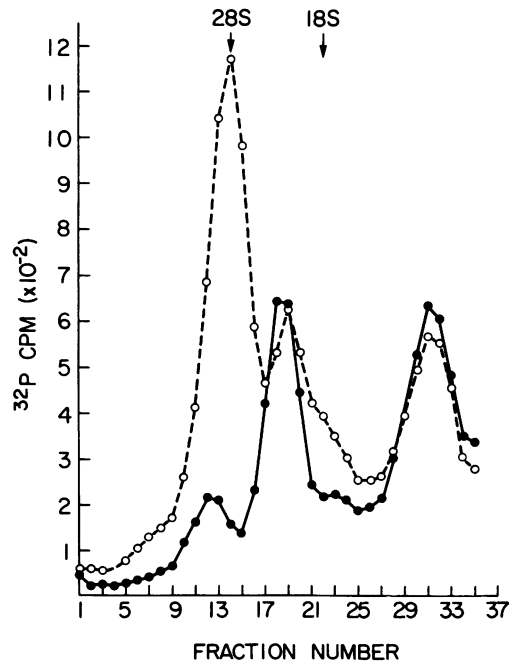

FIG. 5. *Velocity sedimentation of ^{32}P-labeled viral RNA synthesized in the presence of actinomycin D. The ^{32}P-labeled RNA from Pichinde virions grown in the presence (●) or absence (○) of actinomycin D (see Materials and Methods) was centrifuged as described in Fig. 2. Fractions were collected by bottom puncture of the tube, and the trichloroacetic acid-precipitable radioactivity of each fraction was measured.*

transfer RNAs have been found to contain methylated nucleotides (10, 29). In contrast, however, there is no evidence that the nucleotides which comprise the RNAs of animal viruses are methylated. To obtain ^{3}H-methylated RNA, Pichinde virus was grown in medium containing methionine-*methyl*-^{3}*H* (see above), and the virion RNA was analyzed by sucrose velocity centrifugation (Fig. 7). The 28*S*, 18*S*, and 4*S* components of the viral RNA contained methylated nucleotides. The 22*S* component observed when the RNA was labeled with ^{3}H-uridine (see Fig. 2) was not detected under these experimental conditions. The effect of actinomycin D on the incorporation of *methyl*-^{3}*H* group into viral RNA is also shown in Fig. 7. Labeled 28*S* and 18*S* RNA components were almost completely absent from virions grown in the presence of actinomycin D. The 4 to 6*S* RNA was labeled, however, and incorporated into the virions.

Base composition of the RNA. BHK cell RNA from uninfected cells and viral RNA from virions replicated in the presence or absence of actinomycin D were labeled with ^{32}P. Different components of BHK-21 cellular RNA or viral RNA were separated by sucrose density gradient centrifugation, and the base composition of the various RNA components was determined (Table 1). The base composition of the 28*S* component from viruses replicated in the absence of actinomycin D was similar but not identical to host cell 28*S* RNA. Both RNAs had high guanosine and cytosine (GC) content. The base composition of the virion 22*S* RNA was distinct from either host cell 28*S* or 18*S* RNAs. When the virus was grown in the presence of actinomycin D, the virion 31*S* and 22*S* RNAs showed a decreased GC content.

DISCUSSION

While these studies were in progress, Pedersen reported that the RNA of lymphocytic choriomeningitis virus could be separated into four components corresponding to 31*S*, 28*S*, 22*S*, and 18*S*. He concluded, on the basis of selective inhibition of the 28*S* and 18*S* RNAs with actinomycin D, that the 31*S* and 22*S* RNAs were virus specified and the 28*S* and 18*S* RNAs were derived from host cell ribosomes (19, 20). Our findings with Pichinde virus, another member of the arenavirus group, in essence confirm these observations, and we agree with the conclusion that host cell ribosomal RNA is incorporated into the virion.

The nucleic acid of Pichinde virus clearly consists of single-stranded RNA. The sensitiv-

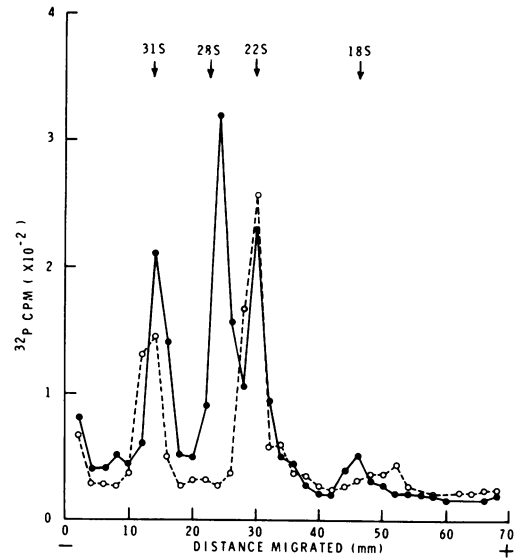

FIG. 6. *Polyacrylamide gel electrophoresis of Pichinde virus RNA synthesized in the presence of actinomycin D. The ^{32}P-labeled viral RNA synthesized in the presence* (○) *or absence* (●) *of actinomycin D were electrophoresed as described in Materials and Methods. ^{14}C-uridine-labeled HeLa cell ribosomal RNA was used as reference.*

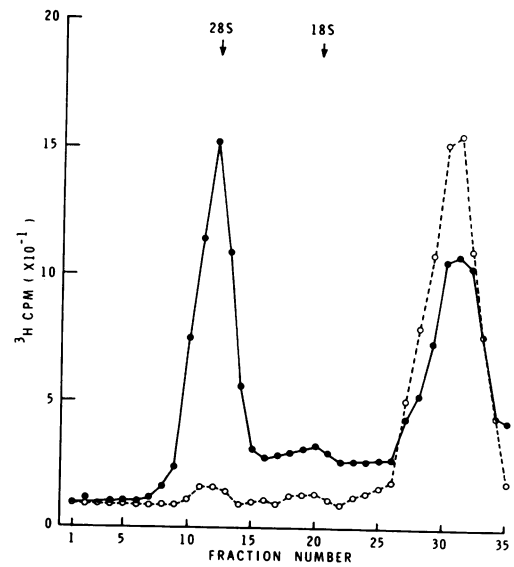

FIG. 7. *Velocity sedimentation of methyl-^{3}H-labeled Pichinde virus RNA synthesized in the presence of actinomycin D. Labeled RNA from Pichinde virus grown in the presence* (○) *or absence* (●) *of actinomycin D was centrifuged in 5 to 20% sucrose density gradients as described in Fig. 2. Fractions were collected by bottom puncture of the tube, and the trichloroacetic acid-precipitable radioactivity of each fraction was measured.*

TABLE 1. *Nucleotide composition of Pichinde viral RNA and BHK cell RNA*[a]

Mononucleotides[b]	Viral RNA			Cell RNA			Viral RNA (+ actinomycin D)	
	28S	22S	4S	28S	18S	4S	31S	22S
CMP	28.5 ± 0.9[c]	25.8 ± 1.2	26.0 ± 1.5	29.0 ± 0.8	27.4 ± 0.8	26.6 ± 1.5	22.4 ± 0.5	21.8 ± 1.0
AMP	19.7 ± 0.8	23.0 ± 0.2	21.3 ± 0.7	18.7 ± 0.3	21.6 ± 0.9	20.3 ± 0.4	27.5 ± 0.1	25.5 ± 1.2
GMP	31.8 ± 1.2	27.6 ± 1.2	31.0 ± 1.8	34.7 ± 1.0	30.4 ± 0.4	31.4 ± 0.3	19.3 ± 0.2	24.7 ± 1.3
UMP	19.9 ± 0.7	23.6 ± 0.9	20.9 ± 2.6	17.5 ± 1.0	20.6 ± 1.0	21.7 ± 0.7	30.8 ± 0.4	27.8 ± 0.8
GMP + CMP	60.3 ± 2.1	53.4 ± 2.4	57.9 ± 3.3	63.7 ± 1.8	57.8 ± 1.2	58.0 ± 1.8	41.7 ± 0.7	46.5 ± 2.3

[a] Expressed as percent mononucleotides.
[b] Abbreviations: CMP, cytidine monophosphate; AMP, adenosine monophosphate; GMP, guanosine monophosphate; UMP, uridine monophosphate.
[c] Average of at least three determinations.

ity to ribonuclease and alkali, buoyant density in cesium sulfate, and base composition are all characteristics of single-stranded RNA. We found the RNA of Pichinde virus to be composed of five discernible species when analyzed by sucrose gradient centrifugation and polyacrylamide gel electrophoresis. Three of the species, 28*S*, 18*S* and 4 to 6*S*, appear, at least in part, to be of host cell origin. Virions grown in the presence of concentrations of actinomycin D, which inhibited ribosomal RNA synthesis in host cells, yielded RNA devoid of radiolabel in the 28*S* and 18*S* components. In addition, methylated nucleotides were found associated with the 28*S*, 18*S*, and 4 to 6*S* segments of virions replicated in the presence of methionine-*methyl*-^3H; methylation of nucleotides is characteristic of cell ribosomal and transfer RNAs (21).

The data obtained upon analysis of the base composition of the RNA components isolated from virions replicated in the presence and absence of actinomycin D also suggested a ribosomal origin of the 28*S* and 18*S* RNAs. The base composition of the viral 28*S* to 31*S* component observed in virus grown in the presence of the drug was completely different than that observed in virus grown in the absence of the drug; the percent GC was markedly less. This is compatible with a significant portion of the 28*S* RNA component being ribosomal RNA which is rich in guanosine and cytosine.

The 22*S* viral RNA component was also found to be altered when the RNA was extracted from virions replicated in the presence of actinomycin D. The differences observed probably represent technical difficulties in efficiently separating virion 22*S* and 18*S* RNAs by sucrose centrifugation. The values of base compositions obtained from viruses grown in the presence of actinomycin D would appear to more accurately portray the composition of the viral 31*S* and 22*S* RNAs. These RNAs appear to be viral-coded RNAs and correspond to molecular weights of 2.1×10^6 and 1.1×10^6, respectively. The RNA responsible for viral-coded products, can, thus, be estimated to be about 3.2×10^6 daltons.

The origin of the 4 to 6*S* virion RNA is difficult to determine. A similarity of viral and host cell 4 to 6*S* RNA was found by base composition analysis, and this RNA was methylated (observations indicating a host cell origin). Comparisons of the base composition of the viral RNA species also suggest that the virion 4 to 6*S* RNA is not the breakdown product of one of the larger RNA components. A 4*S* RNA can also be extracted from the oncornaviruses

(3, 8) and visna virus (11). This low-molecular-weight RNA in the oncornavirus is apparently a mixture of host cell and viral-specified RNA, whereas the 4*S* RNA of visna virus is thought to be virus specified (11). At present the origin of the 4 to 6*S* RNA of Pichinde virus cannot be stated with certainty.

The nature of the arrangement of the RNAs within the virion is unknown. Ribosome-like particles have been observed in electron micrographs of sections of Pichinde virus (17). In addition, the replication of arenaviruses is intimately associated with ribosome-like inclusions in infected cells (1, 17), and aggregates of ribosome-like particles have been observed at the site of virus maturation for several arenaviruses (6, 16, 17). These observations strongly suggest host cell ribosomes are incorporated into mature virions and serve as source of the 28*S* and 18*S* RNA. The 31*S* and 22*S* components then correspond to the major viral-specific RNAs. It is not known if these two components are present as distinct segments, as described for orthomyxoviruses (20) and diplornaviruses (24), or if they are breakdown products from a larger single-stranded molecule with specific weak points. These points will require further study.

ACKNOWLEDGMENTS

This work was supported by Public Health Service research grant AI 10,125 and training grant 5 T1AI74 from the National Institute of Allergy and Infectious Diseases and research grant HE 05425 from the National Heart and Lung Institute.

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