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Bovine Coronavirus Genome

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The tissue culture-adapted strain (Mebus) of the bovine coronavirus was grown to titers of $>10^7$ 50% tissue culture infective doses per ml in secondary bovine embryo kidney cells, and the RNA was isotopically labeled with [^{3}H]uridine. The RNA was extracted from purified virus and was found to have the following properties. (i) It consisted primarily of a homogeneous large-molecular-weight species which comigrated electrophoretically with vesicular stomatitis viral RNA and therefore had an apparent molecular weight of 3.8×10^6. (ii) It remained as a 3.8×10^6-molecular-weight molecule after heat denaturation when rapidly harvested virus was examined. (iii) It was 80% susceptible to pancreatic RNase A digestion in high (0.3 M) NaCl, and the 20% resistant fraction was 4S to 7S in size. (iv) It was polyadenylated to the extent that 40 and 60% of the native RNA bound to polyuridylic acid-Sepharose and oligodeoxythymidylic acid-cellulose, respectively, under conditions of high (0.5 M) NaCl.

The coronaviruses are grouped together as a family primarily on the basis of their characteristically long (12- to 24-nm), widely spaced, bulbous surface projections, but also on the basis of a lipid-containing envelope and an RNA genome (21). To the extent that individual members have been studied, they possess single-stranded RNA (21).

Further characterization of the RNA genome structure and the mechanism by which the viral RNA replicates is needed to determine the taxonomic relatedness among members of this group (11, 21). Recently, an apparent diversity has been recognized within the coronavirus family with respect to the segmented structure of the viral RNA. The avian infectious bronchitis virus (9, 10, 16) (heretofore the prototype coronavirus) and the mouse hepatitis virus (7) have been shown to possess a nonsegmented molecule of single-stranded, polyadenylated, infectious RNA as their genome and hence appear to be "positive-stranded" viruses (2). The porcine coronaviruses, transmissible gastroenteritis virus, and hemagglutinating encephalomyelitis virus (5) and the human coronavirus OC-43 (20), on the other hand, appear to possess a segmented, single-stranded RNA genome. Polyadenylation has been reported for the human coronavirus (OC-43) genome (20). To what extent the RNA from the two porcine coronaviruses is polyadenylated and to what extent the RNA from these viruses and the human coronavirus is infectious remain to be determined.

The calf diarrheal coronavirus has recently been placed in the coronavirus family on the basis of its ultrastructure (12, 19). It apparently possesses an RNA genome based on studies with inhibitors of RNA and DNA replication (17). We describe studies aimed at characterizing the RNA genome structure of the bovine coronavirus (BCV, the calf diarrheal coronavirus).

MATERIALS AND METHODS

Cells. Secondary embryonic bovine kidney cells (BEK) between passages 4 and 18 were grown as monolayers in a growth medium consisting of Dulbecco-modified Eagle medium, 0.035% $NaHCO_3$, 20 mM HEPES (*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid) buffer, 50 μg of gentamicin per ml, and 10% donor calf serum (Flow).

Viruses. The Mebus strain of BCV, which has undergone approximately 40 tissue culture passages, was obtained from J. Black, Kord Animal Disease Laboratory, Nashville, Tenn. The Indiana strain of vesicular stomatitis virus (VSV) was obtained from A. Brown, University of Tennessee, Knoxville.

Infectivity assay. The infectivity assay (50% tissue culture infective dose [$TCID_{50}$]) was done on BEK cells grown to confluency in multiwell tissue culture plates (Falcon). Virus dilutions were made in serumless medium, and 0.1-ml amounts of each dilution were applied to cells that had been rinsed three times with Earle balanced salt solution. After 2 h of incubation, inoculum was replaced with 1 ml of serumless medium, and cells were incubated at 37°C for 5 days in a humidified incubator. Cells were then examined for hemadsorption after rinsing them once with phosphate-buffered saline (PBS), incubating them for 1 h at 4°C with a 0.2% suspension of hamster erythrocytes in PBS, and rinsing them twice again with PBS. Titers were calculated by the method of Reed and Muench (14).

Hemagglutination assay. The hemagglutination assay was carried out by the microtiter system of Hierholzer et al. (6). Virus was serially diluted with PBS, and then a 0.4% suspension of washed hamster erythrocytes was added to each dilution. Titers were reported as the reciprocal of the highest virus dilution causing complete hemagglutination.

Virus propagation for structural analyses. Confluent monolayers of BEK cells grown in 700-cm^2 glass roller bottles were drained and rinsed three times with Earle balanced salt solution. Cells were then infected with virus at a multiplicity of infection of approximately 1 $TCID_{50}$/cell. After 2 h of adsorption at 37°C, the inoculum was decanted, and 25 ml of serumless medium per bottle was added. Incubation was continued for 20 or 48 h. Viral titers of $>10^7$ $TCID_{50}$/ml were obtained at the end of the 48-h incubation period.

VSV was propagated similarly on BEK cells except that a multiplicity of infection of 0.1 was used and virus was harvested after 24 h of incubation.

Isotopic labeling of RNA. BCV RNA was labeled by adding 250 to 500 μCi per roller bottle of [^{3}H]-uridine (8 Ci/mmol, Schwarz/Mann) to the serumless medium immediately after viral adsorption. VSV RNA was labeled by adding 3 mCi of ^{32}P as orthophosphate (144 Ci/mg from Amersham) or 40 μCi of [^{14}C]uridine (56 mCi/mmol, Amersham) to a 150-cm^2 flask of cells in 15-ml serumless medium immediately after viral adsorption. Cellular marker RNA species were prepared by incubating BEK cells at 90% confluency for 24 h with [^{32}P]orthophosphate, 1 mCi/25 ml in a 150-cm^2 flask, and extracting with phenol as described by Erikson et al. (4).

Virus purification. BCV was harvested from infected cells and cell supernatant fluids after a series of three freeze-thaw cycles to rupture the cells. All subsequent steps were carried out at 4°C. The suspension was clarified by centrifugation at 15,000 × *g* for 20 min, and the pellet was resuspended in TNE (0.01 M Tris-hydrochloride [pH 7.5]-0.1 M NaCl-1.0 mM EDTA) to one-tenth the original volume, sonically disrupted for 0.5 min at 50 W with a Branson sonic oscillator with a miniprobe, and clarified at 15,000 × *g* for 20 min. The supernatant fluids were pooled, and polyethylene glycol was added to make a final concentration of 10%. After 12 h of incubation, the precipitate was collected by centrifugation at 15,000 × *g* for 20 min and then resuspended in TNE to one-tenth the original volume. This was layered onto a 10/60% (wt/wt) sucrose step gradient and centrifuged at 90,000 × *g* for 4.0 h in a Sorval AH-627 rotor. The interphase was collected and diluted with an equal volume of TNE, then layered onto a 24-ml continuous 20 to 40% (wt/wt) sucrose gradient for velocity sedimentation, and centrifuged at 70,000 × *g* for 1.5 h in a Sorval AH-627 rotor. The gradient was fractionated, and the virus was located by radioactivity and hemagglutination. The fractions showing peak radioactivity and hemagglutinating activity were diluted with an equal volume of TNE, layered onto a 24-ml continuous 20 to 60% (wt/wt) sucrose gradient for isopycnic sedimentation and centrifuged at 90,000 × *g* for 4.0 h or overnight in a Sorvall AH-627 rotor. The virus was again located by radioactivity and hemagglutinating activity, diluted with an equal volume of TNE, and pelleted at 150,000 × *g* for 2.0 h in a Sorvall AH-650 rotor. The pellet was resuspended in approximately 0.025 ml of TNE and either used immediately or stored at −80°C. All gradients were harvested from the top with an Isco gradient fractionator. All sucrose solutions were made in TNE.

VSV was partially purified by sedimentation from clarified supernatant fluids directly on a 10/60% (wt/wt) sucrose step gradient as described above. The interphase containing VSV was diluted in TNE, and the virus was pelleted as described for BCV.

Polyacrylamide-agarose gel electrophoresis. The method of Peacock and Dingman (13) as described by Brian et al. (3) for making combination 2.0% polyacrylamide-0.5% agarose gels in cylinders and for the electrophoresis of RNA was used. Electrophoresis buffer was composed of 0.09 M Tris-hydrochloride (pH 8.3)-0.09 M boric acid-2.0 mM EDTA. Gels were subjected to pre-electrophoresis for 1 h at 150 V. Electrophoresis was carried out at 150-V constant voltage at 20°C until the bromophenol blue tracking dye migrated 8.0 cm. Gels were fractionated into 1- or 2-mm fractions with a Gilson gel slicer.

Velocity sedimentation in Me_2SO-sucrose gradients. RNA was layered in 0.3-ml quantities onto a 4.4-ml continuous gradient of 5 to 25% (wt/wt) sucrose (RNase-free from Bio-Rad) made up in 99% dimethyl sulfoxide (Me_2SO)-1 mM EDTA-10 mM LiCl-1% water and centrifuged for 7.0 h at 23,000 rpm and 26°C in a Sorvall 650 rotor.

Radioactivity assays. Aliquots from sucrose gradient fractions or gel slices were counted directly in 3 ml of Scintiverse (Fisher) with 5-ml plastic minivials.

RESULTS

Growth curve. The growth curve for BCV (Fig. 1) depicts a maximal number of infectious virus particles being produced at around 48 h postinfection. Thereafter, the total number of infectious particles declines, suggesting a gradual loss in some aspect of viral integrity. Because of the precedent that retrovirus genome degradation correlates with loss of viral infectivity (1), we presumed that the 48-h harvest would offer a high probability of yielding biologically active genomes for structural analysis; hence, we chose this time of harvest for our initial studies. The continual rise in hemagglutinating activity to 72 h may reflect a continual production of virion particles (not necessarily infectious) or alternatively the production of hemagglutin-containing cellular membrane components.

Virus purification. The virus was located by measuring tritium radioactivity and hemagglutinating activity in the sucrose gradients after velocity sedimentation and isopycnic sedimentation. As shown in Fig. 2 and 3, the peaks of radioactivity and hemagglutinating activity coincided during each of these procedures. After isopycnic sedimentation, the buoyant density of

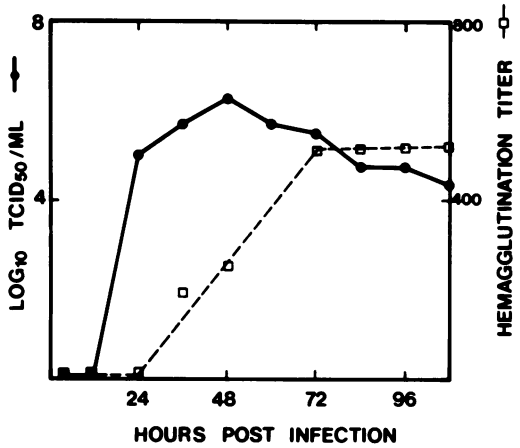

FIG. 1. *Growth curve of BCV. BEK cells were grown in tissue culture tubes (16 by 125 mm) by using a roller tube apparatus. Cells were washed twice with maintenance medium and infected with BCV at a multiplicity of infection of approximately 1 $TCID_{50}$/cell. Virus was adsorbed for 3.0 h at 37°C, and then the cells were rinsed once with serumless medium and incubated at 37°C in maintenance medium. At 12-h intervals, tubes were removed and stored at −80°C until completion of the experiment. Viral titers were determined for the supernatant (clarified after a 3× freeze-thaw cycle) by infectivity and hemagglutination as described in the text.*

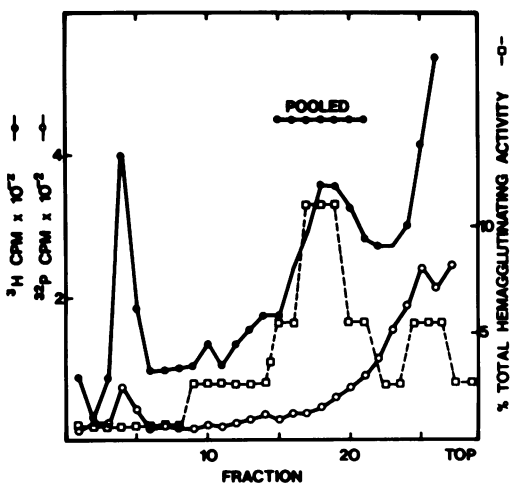

FIG. 2. *Velocity sedimentation of BCV. Radioactively labeled virus from one roller bottle was purified through the stage of velocity sedimentation as described in the text. Sedimentation is from right to left. Radioactivity in 10-μl aliquots was measured. The peak fractions were pooled and isopycnically sedimented as described in the legend to Fig. 3.*

the activities was found to be 1.19 g/cm^3 which is within the density range (1.15 to 1.21 g/cm^3) reported for BCV by Sharpee et al. (17). In the

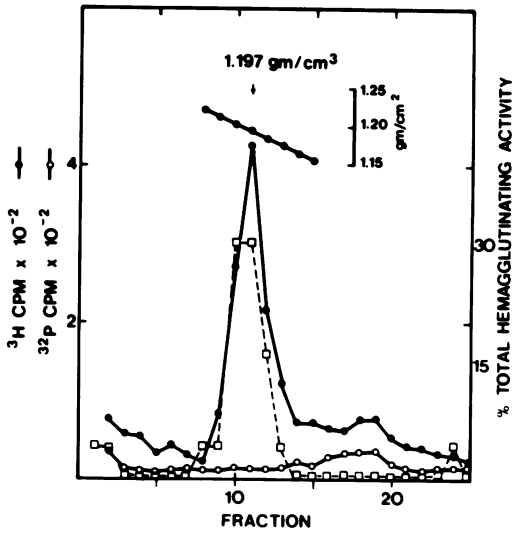

FIG. 3. *Isopycnic sedimentation of BCV. Virus from peak fractions of radioactivity and hemagglutinating activity on the velocity sedimentation gradient (Fig. 2) was isopycnically sedimented as described in the text. Radioactivity in 10-μl aliquots was measured. The density of each gradient fraction was determined by reading the refractive index of each fraction.*

study by Sharpee et al., virus was located by infectivity and hemagglutinating activity in a similar gradient system. Material from the peak fractions having a density of 1.18 to 1.20 g/cm^3 was pelleted, negatively stained, and shown to consist of coronavirus-like particles by electron microscopy (Fig. 4), although a fewer than normal number of peplomers were visible. This may be the result of peplomers becoming redistributed on the virion surface during the process of viral aggregation (L. S. Sturman and K. V. Holmes, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, S231, p. 251) or alternatively some peplomers may have been lost during virus purification. In one experiment, depicted in Fig. 2 and 3, uninfected BEK cells were labeled with [^{32}P]-orthophosphate and then mixed with [^{3}H]uridine-labeled infected BEK cells and cell fluids before viral purification for the purpose of determining what ^{32}P-labeled cellular components would purify with the virus. No ^{32}P-labeled material was found in the isopycnic gradient at the density of 1.18 to 1.20 g/cm^3, indicating that the ^{3}H-labeled material is probably all viral RNA.

Virion RNA characterization. Approximately 5 μg of BCV RNA was obtained from one 700-cm^2 roller bottle when the virus was harvested at 48 h (1 absorbance at 260 nm unit was taken to be equivalent to 42 μg of RNA). When 250 μCi of [^{3}H]uridine was used per roller bottle,

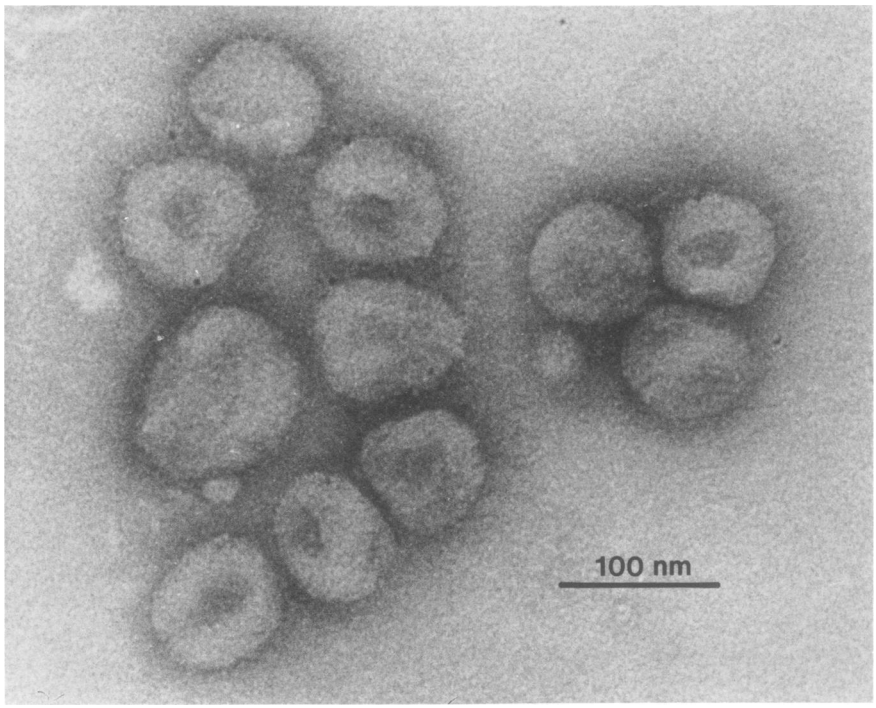

FIG. 4. *Electron micrograph of purified BCV. Virus was purified and pelleted as described in the text. The pellet was drained to near dryness and resuspended in 0.05 ml of TNE. A 5-μl quantity was applied to a carbon-coated, formvar-filmed copper grid, and then stained for 2 min with a 0.5% solution of uranyl acetate. Electron microscopy was done on a Zeiss model EM9 S-2. The bar represents 100 nm.*

a specific activity of approximately 20,000 cpm/μg of RNA was obtained for virus harvested at 48 h.

When native radiolabeled BCV RNA from viruses harvested at 48 h was electrophoretically analyzed as described in Fig. 5, it was found to migrate primarily (85%) as a molecule either coincident with 43S VSV RNA or at times up to one fraction faster than 43S VSV RNA. The remainder was heterogeneous in size, migrating between the 18S and 43S marker RNA species. Consistently, no small-molecular-weight species (4S to 7S) were seen. By plotting the $\log_{10}$ of the molecular weight versus the distance migrated, a straight line was obtained for the 18S to 43S marker RNA species (Fig. 6). Using 3.8×10^6 as the molecular weight for VSV marker RNA (15), we estimate the high-molecular-weight BCV RNA to be approximately 3.8×10^6, or by interpolation, to be no smaller than 3.6×10^6. Hereafter, we refer to the molecule as being 3.8×10^6 molecular weight in size.

To determine whether the 3.8×10^6-molecular-weight molecule from virus harvested at 48 h was contiguous or whether it was composed of smaller subunits that would be revealed upon denaturation, BCV RNA was analyzed after being denatured by two methods. In the first, total viral RNA was heated at 90°C for 3 min, rapidly cooled, and analyzed immediately by gel electrophoresis (Fig. 7), by using the same markers as employed in Fig. 5. In this experiment, 12% of the RNA comigrated with 43S RNA, and the remainder was heterogeneous and migrated between the 18S and 43S marker species. In several experiments of this type, 10 to 20% of the RNA comigrated with 48S marker RNA. In addition, no consistent pattern was seen in the heterogeneous species; hence, no definite subunit structure(s) could be resolved. No 4S to 7S RNA was seen after heat denaturation. In the second method, total viral RNA was suspended in 90% Me_2SO and sedimented through a sucrose gradient made up in 99% Me_2SO with cell cytoplasmic markers (Fig. 8). In this experiment 30% of the RNA sedimented as a homogeneous species with 1.3 times the velocity of 28S rRNA, and 70% was heterogeneously dispersed between 18S rRNA and the high-molecular-weight species. Again, no 4S to 7S RNA was seen. Because of the precedent that avian infectious bronchitis virus RNA and

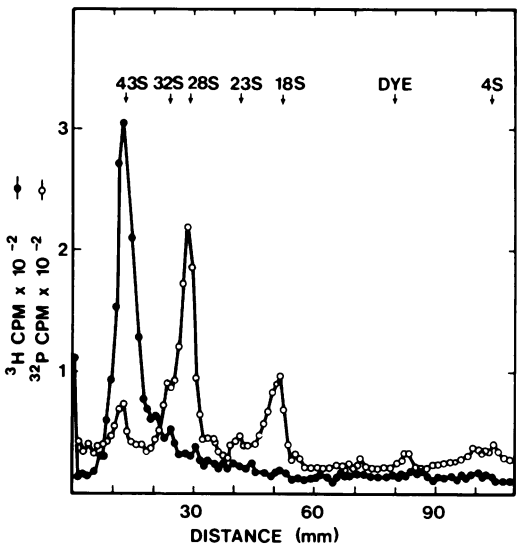

FIG. 5. *Electrophoresis of native BCV RNA. BCV was labeled with [^{3}H]uridine for 48 h and purified as described in the text. The viral pellet was then treated with 0.1 ml of 2.0% SDS in TNE at 20°C for 0.5 h, mixed with ^{32}P-labeled cellular (32S, 28S, 18S, and 4S) and VSV (43S and 23S) marker RNAs, made 10% sucrose and 0.005% bromophenol blue, and subjected to electrophoresis as described in the text.*

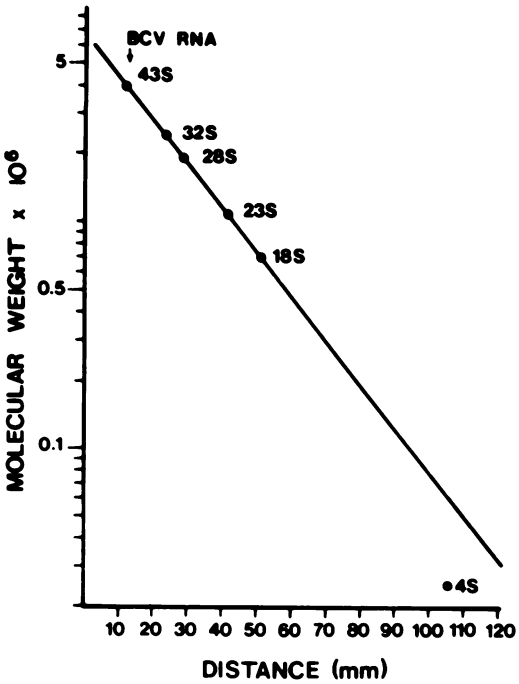

FIG. 6. *Plot of molecular weight versus distance migrated for 43S VSV RNA (3.8 × 10^6 molecular weight), 32S nuclear RNA (2.4 × 10^6 molecular weight), 28S rRNA (1.9 × 10^6 molecular weight), 18S rRNA (0.07 × 10^6 molecular weight), and 4S tRNA (2.5 × 10^4 molecular weight). Data are taken from the experiment depicted in Fig. 5.*

mouse hepatitis virus RNA harvested early after infection (14 to 20 h) show minimal heterogeneity after denaturation (7, 10, 16), we prepared 20-h [^{3}H]uridine-labeled BCV by rapid purification (bypassing polyethylene glycol precipitation and isopycnic sedimentation), heat denatured it at 90°C for 3 min, and analyzed it by gel electrophoresis (Fig. 9). In this experiment, heterogeneity was restricted to a shoulder on the low-molecular-weight side of the high-molecular-weight species, and no definite subunit species could be resolved by this method. A total of 77% of the RNA migrated as a homogeneous peak, one fraction faster than the 43S VSV RNA. It therefore appears that the 3.8 × 10^6-molecular-weight BCV RNA is a contiguous molecule which is not broken under conditions that would destroy hydrogen bonding. Again, no 4S to 7S RNA was found.

To determine whether the genomic RNA is single-stranded, extracted RNA was incubated with pancreatic RNase under conditions known to protect double-stranded RNA (18) (Fig. 10) and electrophoretically analyzed. A total of 80% of the input RNA was sensitive, and the resistant RNA migrated in the 4S to 7S region of the gel.

Poly(A) content of BCV RNA. To determine whether the BCV RNA genome contains stretches of polyadenylic acid [poly(A)], [^{3}H]uridine-labeled BCV RNA was released by using sodium dodecyl sulfate (SDS) and proteinase K and chromatographed on polyuridylic acid [poly(U)]-Sepharose or oligodeoxythymidylic acid [oligo(dT)]-cellulose as described in Table 1. A total of 40 to 60% of the viral RNA bound under conditions which bind greater than 90% of commercially prepared [^{3}H]poly(A), and less than 7% of commercially prepared [^{3}H]polycytidylic acid[poly(C)].

DISCUSSION

At the time this study was initiated, coronaviruses were described as possessing noninfectious RNA and having a virion-associated RNA-dependent RNA polymerase (21). Recent reports, however, have described the RNA genome of the avian bronchitis virus (9, 10, 16) and the mouse hepatitis virus (7) as nonsegmented, infectious molecules which are polyadenylated. By the convention of Baltimore (2), these two coronaviruses are plus-stranded, and their genomes probably serve a messenger function by being translated immediately after infection. We

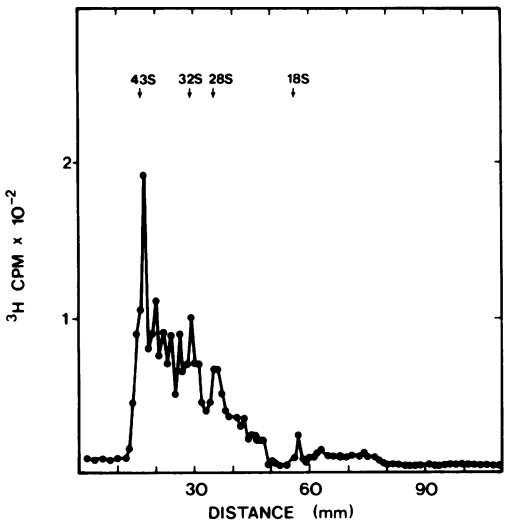

FIG. 7. *Electrophoresis of heated BCV RNA from virus harvested 48 h postinfection. BCV was labeled with [^{3}H]uridine for 48 h and purified as described in the text. The viral pellet was treated with 0.1 ml of 2.0% SDS in TNE at 20°C for 0.5 h, and then heated at 90°C for 3 min in a sealed ampoule and rapidly cooled. The sample was immediately mixed with ^{32}P-labeled cellular (32S, 28S, 18S, and 4S) and VSV (43S) marker RNAs, made 10% sucrose and 0.005% bromophenol blue, and subjected to electrophoresis as described in the text.*

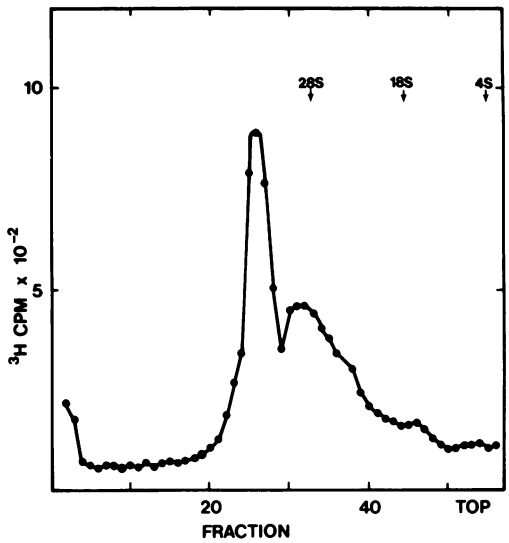

FIG. 8. *Me_2SO_4-sucrose gradient sedimentation of BCV RNA. BCV was labeled with [^{3}H]uridine for 48 h and purified as described in the text. The viral pellet was treated with 0.5 ml of 2.0% SDS in TNE at 20°C for 0.5 h, extracted twice with 2 volumes of TNE-saturated phenol (50%)-creosol (50%) mixture, precipitated at −20°C for 15 h with 2 volumes of 95% ethanol with 5 absorbance at 260 nm units of Torula grade B (Sigma) RNA as carrier, and dried in a stream of N_2. The precipitate was dissolved in 0.1 ml of 0.1% SDS in TNE, then 0.2 ml of 99% Me_2SO–0.01 M LiCl–1.0 mM EDTA was added, and the mixture was incubated for 0.5 h at 37°C before the addition of marker RNA. Sedimentation was as described in the text.*

have shown many structural features of the bovine coronavirus RNA to be similar to those described for avian bronchitis virus (9, 10, 16) and mouse hepatitis virus (7) RNA.

Our data give direct evidence that the BCV genome is RNA by the fact that it becomes labeled with isotopic uridine and is sensitive to RNase (Fig. 10). We have further demonstrated that the native bovine coronavirus RNA, whether it is released by SDS alone (Fig. 5), extracted by an SDS-phenol method (Figs. 9 and 10), or extracted by an SDS-proteinase K-phenol method (data not shown), consists primarily of a high-molecular-weight molecule that is approximately 3.8×10^6 based on its electrophoretic comigration or near comigration with VSV RNA under nondenaturing conditions. As has been reported for other coronaviruses (7, 10), intravirion degradation of the genomic RNA seems to have occurred in the aged (in this case 48 h) bovine coronavirion. An analysis of virus harvested a short time (20 h) postinfection is necessary to find undegraded RNA and to make meaningful conclusions concerning the subunit structure of the genome. The 3.8×10^6-molecular-weight molecule, if segmented, is apparently not held together by short hydrogen-bonded regions as is the genome of oncornaviruses. Denaturation of the RNA with heat and a subsequent analysis in an electrophoretic system that reveals the subunit structure for oncornavirus RNA (3) reveal no such subunit structure for BCV RNA (Fig. 9). We can further rule out the possibility of protein-nucleic acid linkages between segments, because analysis of SDS-proteinase K-extracted RNA yields results essentially identical to those depicted in Fig. 9 (data not shown). Although approximately 30% of the [^{3}H]BCV RNA sedimented as a high-molecular-weight species on Me_2SO-sucrose gradients at a velocity consistent with a 3.8×10^6-molecular-weight molecule, we cannot at this time rule out the possibility of aggregate formation which has been a problem during the analysis of avian coronaviral RNA in Me_2SO gradients (10).

Two features of bovine coronavirus RNA contrast with those of other coronaviruses. (i) A molecular weight of 3.8×10^6 is decidedly less than the molecular weights of 5.6×10^6 (16) or 8.1×10^6 (10) reported for the avian infectious

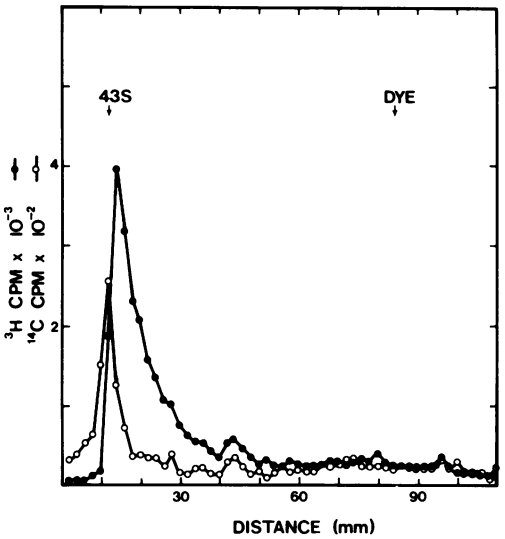

FIG. 9. *Electrophoresis of heated BCV RNA from virus harvested 20 h postinfection. BCV was labeled with [^{3}H]uridine for 20 h and purified as described in the text, except that the polyethylene glycol precipitation and isopycnic sedimentation steps were bypassed to shorten the time of purification, and pelleted. The viral pellet was treated with 0.5 ml of 2.0% SDS in TNE at 20°C for 0.5 h, extracted twice with an equal volume of TNE-saturated phenol, precipitated at −20°C for 15 h with 2 volumes of 95% ethanol using 5 absorbance at 260 nm units of Torula grade B (Sigma) RNA as carrier, and dried in a stream of N_2. The precipitate was dissolved in 0.05 ml of electrophoresis buffer, heated at 90°C for 3 min, and rapidly cooled. The sample was immediately mixed with ^{14}C-labeled VSV (43S) marker RNA, made 10% sucrose and 0.005% bromophenol blue, and subjected to electrophoresis as described in the text.*

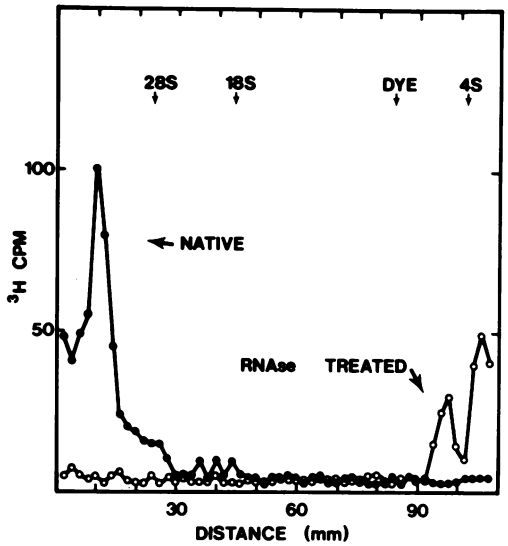

FIG. 10. *Effect of RNase on BCV RNA. The RNA was extracted as described in the legend to Fig. 8 and ethanol precipitated in two equal portions. The first portion was dissolved in electrophoresis buffer and subjected to electrophoresis after the addition of dye, sucrose, and cytoplasmic (28S, 18S, and 4S) marker RNAs. The second portion was treated for 1 h at 37°C in 0.3 M NaCl–0.01 M Tris-hydrochloride (pH 7.5)–10 μg of pancreatic A RNase (Sigma) per ml and subjected to electrophoresis on a parallel gel after the addition of dye and sucrose.*

bronchitis virus genome, or the molecular weight of 5.4 × 10^6 reported for the mouse hepatitis virus genome (7). Caution on this point is needed, however, because molecular weight estimates of RNA molecules made under nondenaturing conditions, or when denaturation is incomplete, can lead to erroneously small molecular weight estimates due to the regions of intramolecular hydrogen bonding (8). This appears to be born out by the disparate results for the same strain of avian infectious bronchitis virus. A molecular weight of 5 × 10^6 was obtained by electrophoresis under nondenaturing conditions (16), and 8.1 × 10^6 was obtained by electrophoresis under denaturing conditions and by oligonucleotide digest analysis (10). Because RNA was subjected to electrophoresis under nondenaturing conditions in our studies, our molecular weight measurement of 3.8 × 10^6 for BCV RNA must be regarded as a minimum molecular weight. (ii) No 4S to 7S RNA species are found

TABLE 1. *Presence of poly(A) in BCV RNA*

Experiment	RNA applied	Radioactivity bound (cpm)[a]		RNA bound (%)
		Poly(U)-Sepharose	Oligo(dT)-cellulose	
1[b]	[^{3}H]poly(A)[c]	23,150	—	91.1
	[^{3}H]poly(C)[c]	1,670	—	<7.0
	^{3}H-labeled native BCV[d]	22,200	—	40.7
2[e]	[^{3}H]poly(A)[c]	—	5,044	98.8
	[^{3}H]poly(C)[c]	—	29	<0.1
	^{3}H-labeled native BCV[d]	—	32,240	61.4

[a] Recovery ranged from 83 to 100%.

[b] RNA was bound to 1 ml of swollen poly(U)-Sepharose (Pharmacia) in 0.5 M NaCl–0.01 M Tris-hydrochloride (pH 7.4)–0.2% SDS (wt/vol)–1.0 mM EDTA and eluted with water.

[c] Purchased from Miles Laboratories.

[d] Released from purified BCV by using 0.2 ml of 1% SDS (wt/vol) in TNE and 0.2 mg of proteinase K (E. M. Laboratories) and then diluted to 1 ml in binding buffer before applying to column.

[e] RNA was bound to 1 ml of swollen oligo(dT)-cellulose (Collaborative Research) in 0.5 M NaCl–0.01 M Tris-hydrochloride (pH 7.8)–0.2% SDS (wt/vol) and eluted with water.

in the BCV RNA either before or after heat denaturation, whereas 4S to 7S RNA comprises 5 to 10% of heat denatured porcine (5) and human (20) coronavirus RNA. Presence of low-molecular-weight RNA in the avian (16) and mouse (7) coronavirus was illustrated but not discussed.

The bovine coronavirus genome is polyadenylated to the extent that 40 and 60% of it binds to poly(U)-Sepharose and oligo(dT)-cellulose, respectively. This, therefore, shows further similarity between the BCV genome and the genomes of the avian infectious bronchitis virus and the mouse hepatitis virus and leads us to expect that the BCV RNA genome will also be infectious.

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