JOURNAL OF VIROLOGY, May 1980, p. 410-415
0022-538X/80/05-0410/06$02.00/0

Vol. 34, No. 2

Genome of Porcine Transmissible Gastroenteritis Virus

DAVID A. BRIAN,* DOUGLAS E. DENNIS, AND JAMES S. GUY

Department of Microbiology, The University of Tennessee, Knoxville, Tennessee 37916

The Purdue strain of transmissible gastroenteritis virus, a porcine coronavirus, was grown to titers of $>10^8$ PFU/ml in a swine testicle cell line, 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 electrophoretically migrated with an apparent molecular weight of 6.8×10^6 under denaturing conditions. (ii) It migrated electrophoretically at the same rate on nondenaturing gels before and after heat denaturation, suggesting that it does not consist of subunits. (iii) It was susceptible to pancreatic RNase A digestion in high (0.3 M) NaCl. (iv) It was polyadenylated to the extent that >60% of the native RNA bound to oligodeoxythymidilic acid-cellulose under conditions of high (0.5 M) NaCl. RNA extracted from virions was infectious. This coronavirus can therefore be characterized as a positive-strand RNA virus.

Two species of the coronavirus family, namely the avian infectious bronchitis virus (9, 10, 16) and the mouse hepatitis virus (6, 20, 21), have been shown to possess an RNA genome that is single stranded, nonsegmented, polyadenylated, and infectious. These coronaviruses can therefore be categorized as positive-strand viruses (1). In addition, the human coronavirus strain 229E (12) and the bovine coronavirus (4) have been shown to possess a single-strand, nonsegmented, polyadenylated RNA as their genome. Should these molecules prove to be infectious, then the human coronavirus strain 229E and the bovine coronavirus can be classed as positive-strand viruses as well.

Three other members of the coronavirus family, namely the human coronavirus OC43 (18), transmissible gastroenteritis virus (TGEV) of swine (3), and hemagglutinating encephalomyelitis virus of swine (3), reportedly possess segmented, single-strand RNA. Polyadenylation has been reported for the human coronavirus OC43 genome (18). Further study is required to determine whether the apparent RNA segmentation in these viruses is the result of RNA degradation or whether it reflects a fundamentally different genetic scheme within the coronavirus family.

We describe studies aimed at characterizing the genome structure of the Purdue strain of TGEV of swine.

MATERIALS AND METHODS

Cells. The epithelioid swine testicle cell line (ST) developed by McClurkin (13) was obtained from R. Woods, National Animal Disease Center, Ames, Iowa. Cells were grown as monolayers in growth medium consisting of Dulbecco-modified Eagle medium, 4500 mg of glucose per liter, 0.35% $NaHCO_3$, 50 μg of gentamicin per ml, and 10% fetal calf serum (Sterile Systems, Inc.). Maden-Darby bovine kidney (MDKB) cells were obtained from N. Famulari, Memorial Sloan-Kettering Cancer Center, New York, N.Y., and were cultured in the same medium.

Viruses. Clone 116 of the Purdue strain of swine TGEV was obtained from E. Bohl, Ohio Agricultural Research and Development Center, Wooster, Ohio. The virus was cloned twice again by us. Cloned virus was passaged twice at a multiplicity of <0.1 PFU/cell, and then viral stocks were prepared from passages 3 through 6 by infecting cells at a multiplicity of approximately 10 PFU/cell. Viral titers ranging from 10^8 to 10^9 PFU/ml were obtained in stock virus preparations. The Indiana strain of vesicular stomatitis virus was obtained from A. Brown, University of Tennessee, Knoxville.

Virus titration. Virus was titered on cells in 24-well tissue culture plates (Falcon). Serial 10-fold dilutions of virus were made in serumless medium, and 0.1-ml amounts of each dilution were applied to cells that had been rinsed with Earle balanced salt solution. After 1 h of incubation, inoculum was replaced with medium containing 1% immunoglobulin G-free fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.) and 0.4% methylcellulose, and cells were incubated at 37°C for 4 days in a humidified incubator. Overlay medium was then removed, and cells were stained with a 1% solution of crystal violet. Alternatively, 0.1% agarose replaced methylcellulose in the overlay medium, and plaques were observed with the inverted microscope.

Isotopic labeling of RNA and virus purification. For labeling TGEV RNA, confluent monolayers of ST cells grown in 700-cm^2 glass roller bottles were drained and rinsed with Earle balanced salt solution. Cells were then infected at a multiplicity of infection of approximately 10 PFU/cell. After 1 h of adsorption at 37°C, the inoculum was removed and 20 ml of

medium containing 1% immunoglobulin G-free fetal calf serum and 500 μCi of [^{3}H]uridine (8 Ci/mmol, Schwarz/Mann) was added per bottle. Incubation was continued for 15 to 17 h, at which time virus was harvested. Supernatant fluids were clarified by centrifugation at 10,000 × *g* for 10 min and layered onto a 24-ml continuous 20 to 60% (wt/wt) sucrose gradient. Virus was isopycnically sedimented by centrifugation at 90,000 × *g* for 4 h at 4°C in a Sorvall AH-627 rotor. Virus was located by radioactivity and infectivity, diluted with an equal volume of TNE (0.01 M Tris-hydrochloride [pH 7.5]-0.1 M NaCl-1.0 mM EDTA) and pelleted at 150,000 × *g* for 2 h in a Sorvall AH-650 rotor. The pellet was either used immediately or stored at −80°C. Gradients were harvested from the top with an Isco gradient fractionator. Sucrose solutions were made in TNE.

Vesicular stomatitis virus RNA was labeled by infecting a roller bottle of MDBK cells at a multiplicity of infection of 0.1 and incubating cells for 20 h in the presence of [^{3}H]uridine as described for TGEV. Vesicular stomatitis virus was purified as described for TGEV.

Cellular RNA was labeled by incubating MDBK cells at 90% confluency for 24 h with [^{3}H]uridine (20 μCi/ml) in a 150-cm^2 flask.

Extraction of RNA. Viral RNA from both TGEV and vesicular stomatitis virus was extracted by dissolving viral pellets in 0.5 ml of TNE-2% sodium dodecyl sulfate, immediately adding a small crystal (0.05 mg) of proteinase K (E. M. Laboratories), incubating at 20°C for 0.5 h, and extracting twice with an equal volume of TNE (pH 9.0)-saturated phenol. RNA was precipitated at −20°C for at least 15 h with 2 volumes of 95% ethanol.

Cellular RNA was extracted with phenol as described by Erikson et al (2).

Agarose gel electrophoresis and fluorography. The method of Lerach et al. (8) was used to analyze RNA in agarose gels containing 2.2 M formaldehyde. RNA samples were heated in 2.2 M formaldehyde-50% formamide-0.018 M Na_2HPO_4-0.002 M NaH_2PO_4, for 5 min at 60°C. The electrophoresis buffer was 2.2 M formaldehyde-0.018 M Na_2HPO_4-0.002 M NaH_2PO_4. Slab gels of 0.75 or 1.0% agarose were used with either a vertical apparatus or a horizontal apparatus of 13- by 10-cm dimensions. Gels were 1.5 mm thick. Electrophoresis was carried out using 5 to 15 mA of constant current (1.5 to 4.5 V/cm). The method of Laskey and Mills (7) was used to analyze the slab gels by fluorography. At times Enhance (New England Nuclear) was used to prepare gels for fluorography.

Assay for infectious viral RNA. Approximately 10^9 ST cells infected at a multiplicity of infection of 10 were incubated for 20 h and then frozen at −80°C. After thawing, the medium was clarified by centrifugation at 1,500 × *g* for 10 min, and virus was pelleted by centrifugation at 90,000 × *g* for 2 h at 4°C. RNA was extracted from the viral pellet as described above and ethanol precipitated. The RNA precipitate was washed three times with ethanol and dissolved in serumless medium containing 25 μg of DEAE-dextran per ml (Pharmacia). Cells to be infected had reached confluency within 12 h before the time of infection. Before infection, cells were rinsed three times with Earle balanced salt solution and incubated for 0.5 h with serumless medium containing 25 μg of DEAE-dextran per ml. RNA in serumless medium containing 25 μg of DEAE-dextran per ml was applied to the cells for 2 h at 37°C, and then the inoculum was replaced with Dulbecco-modified Eagle medium containing 1% immunoglobulin G-free fetal calf serum and 0.1% agarose. Progeny virus was confirmed to be TGEV by indirect immunofluorescence with hyperimmune porcine anti-TGEV serum from a gnotobiotic pig obtained from L. Kemeny, National Animal Disease Center, Ames Iowa, and rabbit anti-porcine immunoglobulin G obtained from Miles Laboratories, Inc., and by electron microscopy.

RESULTS

Growth characteristics. When confluent monolayers of ST cells were infected with TGEV at a multiplicity of 10 PFU/cell, there was total destruction of the monolayer by 20 to 24 h postinfection. Affected cells became rounded and detached from the glass. Peak titers under these conditions were obtained by 19 to 20 h postinfection (Fig. 1), a time preceding the development of maximal cytopathic effect. For RNA structural studies, supernatant fluids were harvested at 15 to 17 h postinfection, a time when cells were showing 50 to 75% of maximal cytopathic effect.

Virus purification. The results of isopycnic sedimentation of TGEV are shown in Fig. 2. From a 15 to 17-h postinfection harvest, a major peak of [^{3}H]uridine activity with a buoyant density of 1.20 g/cm^3 and a minor peak (or sometimes only a shoulder) with a buoyant density of 1.18 g/cm^3 were seen. The results of infectivity assays (Fig. 2) and electronmicroscopic exami-

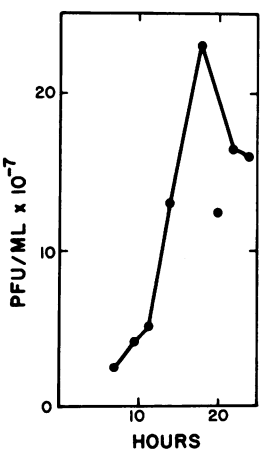

FIG. 1. *Growth curve of TGEV. A confluent roller bottle of ST cells was infected at a multiplicity of infection of 10. Samples of the medium were taken at 2-h intervals and immediately frozen at −80°C. Virus was titrated as described in the text.*

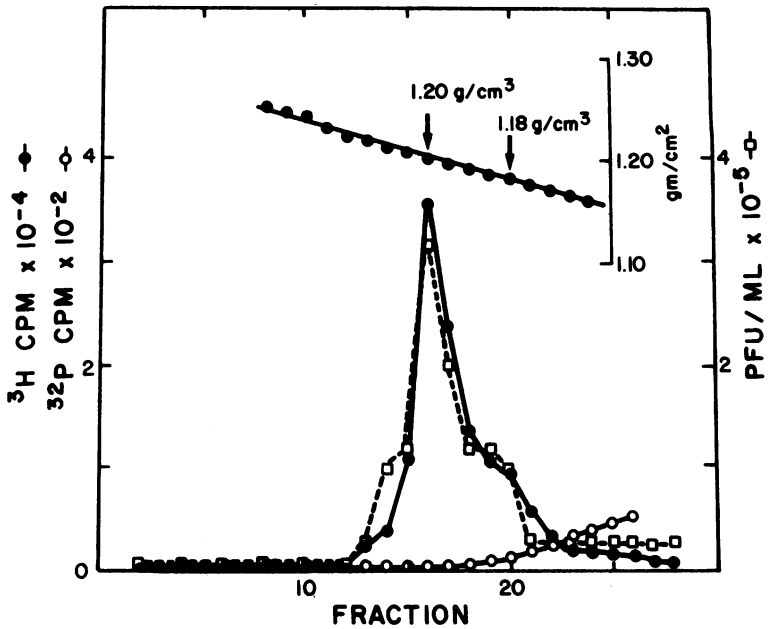

FIG. 2. *Isopycnic sedimentation of TGEV. Virus labeled with [^{3}H]uridine was purified as described in the text. Infectivity and trichloroacetic acid-precipitable radioactivity was measured in 10-μl portions. The buoyant density of each fraction was determined by reading the refractive index. ^{32}P-labeled material from uninfected cells was sedimented in parallel as described in the text.*

nation (data not shown) indicate that TGEV is found at both densities, but that more are found at the buoyant density of 1.20 g/cm^3. Our impression from preliminary studies is that particles with a buoyant density of 1.18 g/cm^3 have fewer surface projections and many appear to be ruptured. As described below, RNA extracted from particles of both densities was indistinguishable in our analyses.

In one experiment depicted in Fig. 2, uninfected ST cells were labeled with [^{32}P]orthophosphate and the supernatant fluids were centrifuged in parallel on a sucrose gradient for the purpose of determining the degree to which ^{32}P-labeled cellular components might contaminate the virus preparation. No ^{32}P-labeled material was found in the isopycnic gradient at the density region of 1.18 to 1.20 g/cm^3, indicating that the ^{3}H-labeled material is probably all viral RNA. In other experiments (data not shown) in which differentially labeled infected and uninfected cells are mixed, ruptured by freezing, and copurified, there was also no evidence of cellular RNA contamination of purified TGEV. These tests, however, do not rule out the possibility that RNA-containing vesicles are being produced by infected cells which could copurify with TGEV.

Molecular weight analysis of virion RNA. TGEV genomic RNA was labeled to a specific activity of approximately 20,000 cpm/μg when 500 μCi of [^{3}H]uridine/10^8 cells was used for a 20-h labeling period.

When radiolabeled TGEV RNA from viruses harvested at 15 to 17 h postinfection was electrophoretically analyzed on gels of 0.75% (Fig. 3) or 1.0% (data not shown) agarose concentration in the presence of 2.2 M formaldehyde, only one distinct RNA species could be seen which migrated as a band with a mobility of 0.6 relative to vesicular stomatitis virus RNA. At times heterogeneous material migrated ahead of the genomic RNA and at the sides of the lane. The amount of this material was variable, and it apparently represented fragments of genomic RNA. By plotting the square root of the molecular weight versus the log of the relative mobilities for the standard RNA species (8) (Fig. 4), a straight line relationship was observed on the 0.75 and 1.0% gels. The molecular weight of TGEV RNA was estimated by extrapolation. From six separate preparations, we obtained a mean molecular weight of 6.8 × 10^6. Our estimates ranged from 6.4 × 10^6 to 7.2 × 10^6.

Analysis of RNA from particles having buoyant densities ranging from 1.20 to 1.18 g/cm^3, i.e., from fractions 16 through 20 of the gradient described in Fig. 2, demonstrated no detectable

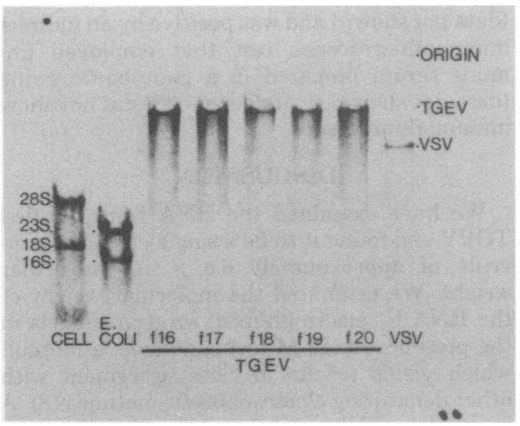

FIG. 3. *Molecular weight analysis of TGEV RNA on a formaldehyde-agarose gel. Samples were subjected to electrophoresis on a 0.75% agarose gel in the presence of 2.2 M formaldehyde (8). The molecular weight of TGEV RNA was estimated by extrapolation from a plot of the square root of the molecular weight verus the log of the relative distance migrated (8) for standards as shown in Fig. 4. Standards used were vesicular stomatis virus RNA (3.8 × 10^6 molecular weight [15]), 28S and 18S MDBK cell rRNA (1.75 × 10^6 and 0.68 × 10^6 molecular weight, respectively), and 23S and 16S Escherichia coli rRNA (1.1 × 10^6 and 0.56 × 10^6 molecular weight, respectively). The estimated molecular weight of TGEV RNA was 6.8 × 10^6.*

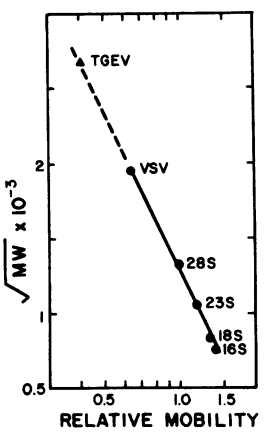

FIG. 4. *Plot of the square root of the molecular weight versus the log of the relative mobility for RNA species analyzed as described in Fig. 3.*

differences in the size or distribution of RNA species (Fig. 3). We conclude therefore that the differences seen in buoyant densities between the particles of 1.20 and 1.18 g/cm^3 must be due to a virion structural component involving something other than the RNA.

To examine whether high-molecular-weight TGEV RNA is composed of subunits that can be revealed upon heat denaturation, as reported earlier for this virus (3), native TGEV RNA, that is, RNA that had not seen conditions of hydrogen bond destruction, was analyzed before and after heating (Fig. 5). No change in the migration rate of the large-molecular-weight molecule was detectable. Minor amounts of broadly heterodisperse breakdown material was observed in the heated RNA, however. An estimate of the molecular weight from a plot of log molecular weight versus distance migrated for the virion RNA and for RNA standards (not shown) gave an approximate value of 6 × 10^6. It is apparent, then, that the high-molecular-weight RNA seen on nondenaturing gels is not segmented, and it is unlikely that the 6.8 × 10^6 molecule isolated on denaturing gels is a subunit of a still larger molecule.

Analysis of RNAse-treated virion RNA. TGEV genomic RNA is single stranded since it is totally degraded after RNase digestion in 0.3 M NaCl (Fig. 6).

Polyadenylic acid content of TGEV RNA. To determine whether the TGEV RNA genome contains polyadenylic acid, [^{3}H]uridine-labeled TGEV RNA was released by treatment with sodium dodecyl sulfate and proteinase K and was chromatographed on oligodeoxythymidylic acid-cellulose as described in Table 1. A total of 60 to 70% of the viral RNA bound under conditions which bind greater than 90% of commercially obtained [^{3}H]polyadenylic acid and less

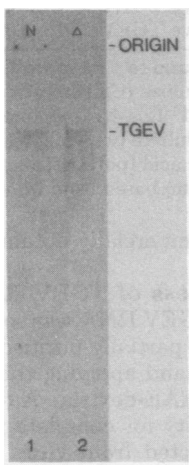

FIG. 5. *Analysis of heat-denatured TGEV RNA on nondenaturing agarose gels. Samples were analyzed on a 0.75% agarose gel by using the system of Lerach et al. (8), except that formaldehyde was omitted. Native TGEV RNA was dissolved in TNE-1.0% sodium dodecyl sulfate and subjected to electrophoresis without further treatment (lane 1), or after heating at 90°C for 3 min followed by quick cooling (lane 2).*

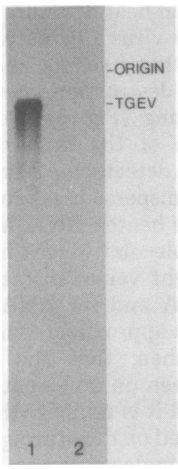

FIG. 6. *Analysis of RNase-treated TGEV RNA on a formaldehyde-agarose gel. Virion RNA (lane 1) and RNase-treated virion RNA (lane 2) were analyzed on a 0.75% agarose gel in the presence of 2.2 M formaldehyde (8). RNase treatment was for 1 h at 37°C in 0.3 M NaCl–0.01 M Tris-hydrochloride (pH 7.5)–10 of μg pancreatic A RNase (Sigma) per ml. (17).*

TABLE 1. *Presence of polyadenylic acid in TGEV RNA*

Expt[a]	^{3}H-labeled RNA applied	Radioactivity bound (cpm)	RNA bound (%)
	Poly(A)[b]	5,044	>98
	Poly(C)[b]	29	<0.1
1	Native TGEV	418	66
2	Native TGEV	1,391	72

[a] RNA was bound to 1 ml of swollen oligodeoxythymidylic acid-cellulose (Collaborative Research) in 0.5 M NaCl–0.01 M Tris-hydrochloride (pH 7.8)–0.2% sodium dodecyl sulfate (wt/vol) and eluted with water.

[b] Polyadenylic acid [poly(A)] and polycytidylic acid [poly(C)] were purchased from Miles Laboratories.

than 1% of commercially obtained [^{3}H]polycytidylic acid.

Infectiousness of TGEV RNA. The infectiousness of TGEV RNA was sought by extracting RNA from partially purified virus (Table 2, experiment 1) and applying this to cells in the presence of DEAE-dextran. A total of 0.00024% of the infectivity for complete virus was found in RNA extracted from virus. Infectivity was completely destroyed by incubation with RNase. This, along with evidence that virion infectivity is not destroyed by ribonuclease (Table 2, experiment 2), indicates that RNA, and not residual virus, was the infectious moiety. Clones resulting from infectious RNA were selected and grown. Progeny virus was shown to have typical TGEV morphology by electron microscopy (data not shown) and was positive by an indirect immunofluorescence test that employed immune serum prepared in a gnotobiotic swine (data not shown). Uninfected cells did not show immunofluorescence.

DISCUSSION

We have examined the RNA from purified TGEV and found it to be a single-stranded molecule of approximately 6.8×10^6 molecular weight. We estimated the molecular weight of the RNA by electrophoresis on agarose gels in the presence of 2.2 M formaldehyde, a method which yields results in close agreement with other denaturing electrophoretic methods (8). A molecular weight of 6.8×10^6 approaches the size reported for the avian infectious bronchitis virus genome which was measured by electrophoresis on methylmercury gels and by oligo T_1 nuclease digest analysis to be $8.0 \pm 0.1 \times 10^6$ (10). It is somewhat larger than a molecular weight of 5.4×10^6 to 6.5×10^6 measured for the mouse hepatitis virus RNA (6, 20) or 5.8×10^6 measured for the human coronavirus 229E (12). Earlier (4) we reported a molecular weight of 3.8×10^6 for the bovine coronavirus genome which was determined by electrophoresis under nondenaturing conditions. We now know this to be an erroneously small molecular weight estimate since bovine coronavirus RNA coelectrophoreses with TGEV RNA in formaldehyde-agarose gels and therefore has an apparent molecular weight of 6.8×10^6 as well (data not shown). These five coronaviruses, then, infectious bronchitis virus, mouse hepatitis virus, human coronavirus 229E, TGEV, and bovine coronavirus have the largest single-strand RNA genomes of RNA viruses reported to date. Native and heat-denatured TGEV RNAs were analyzed on nondenaturing gels to determine whether the genome might be segmented as reported earlier (3). We found no difference in electrophoretic migration after heat denatura-

TABLE 2. *Infectiousness of TGEV RNA*

Expt	Inoculum	Treatment	Titer (PFU/ml)	% of original viral infectivity
1	TGEV	None	1×10^7	100
	Viral RNA[a]	None	630	0.00024
	Viral RNA[a]	RNase[b]	0	0
2	TGEV	None	3×10^7	100
	TGEV	RNase[b]	2.3×10^7	76

[a] Prepared from 38 ml of virus-containing supernatant fluids as described in the text and dissolved in 1.5 ml of serumless medium.

[b] RNA was treated at 10 μg of pancreatic RNase per ml (Sigma) for 15 min at 20°C in serumless medium.

tion and conclude that the 6.8×10^6 RNA is a contiguous molecule. Although our studies and the studies of Garwes and Pocock employed different strains of TGEV, it is unlikely that any fundamental difference exists between the two strains since all TGEV isolates made so far are antigenically monotypic (14, 19). We attribute our success in obtaining unbroken RNA molecules to the use of early harvest virus and to the use of proteinase K in the RNA extraction procedure.

We demonstrated that TGEV RNA is infectious, and, on the basis of preliminary data which shows single-hit kinetics of infectivity by the RNA, we conclude that the 6.8×10^6 molecule acts alone in initiating the infection. Inclusion of TGEV with infectious bronchitis virus and mouse hepatitis virus as viruses with infectious genomes brings to three the number of coronaviruses with demonstrated plus-strand polarity. The coronavirus family, therefore, if it can be characterized by infectious bronchitis virus, mouse hepatitis virus TGEV, bovine coronavirus, and human coronavirus 229E, is a truely unique RNA virus family with positive strandedness. Unlike the picornaviruses and togaviruses, coronaviruses are large (80 to 120 nm in diameter) with an apparent helical nucleocapsid symmetry (5, 11). The genome is roughly three times the size of the picornavirus genome and twice the size of the togavirus genome. Whether or not coronavirus RNA transcriptional and translational processes follow patterns utilized by the picornaviruses and togaviruses is being investigated. Studies are in progress to determine the length of the coronaviral polyadenylic acid stretch and to determine the nature of the 5′ end of the TGEV genomic molecule.

ACKNOWLEDGMENTS

This work was supported by Public Health Service Biomedical Research Support Grant RR-07088 from the Bureau of Health Professions, Education and Manpower Training and by Public Health Service grant AI-4367 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Baltimore, D.** 1971. Expression of animal virus genomes. Bacteriol. Rev. **35**:235–241.
2. **Erikson, E., R. L. Erikson, B. Henry, and N. R. Pace.** 1973. Comparison of oligonucleotides produced by RNase T1 digestion of 7S RNA from avian and murine oncornaviruses and from uninfected cells. Virology **53**: 40–46.
3. **Garwes, D. J., D. H. Pocock, and T. M. Wijaszka.** 1975. Identification of heat-dissociable RNA complexes in two porcine coronaviruses Nature (London) **257**:508–510.
4. **Guy, J. S., and D. A. Brian.** 1979. Bovine coronavirus genome. J. Virol. **29**:293–300.
5. **Kennedy, D. A., and C. M. Johnson-Lussenburg.** 1975/76. Isolation and morphology of the internal component of human coronavirus, strain 229 E. Intervirology **6**:197–206.
6. **Lai, M. M. C., and S. A. Stohlman.** 1978. RNA of mouse hepatitis virus. J. Virol. **26**:236–242.
7. **Laskey, R. A., and A. D. Mills.** 1975. Quantitative film detection of ^3H and ^{14}C in polyacrylamide gels by fluorography. Eur. J. Biochem. **56**:335–341.
8. **Lehrach, H., D. Diamond, J. Wozney, and H. Boedtker.** 1977. RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical review. Biochemistry **16**:4743–4751.
9. **Lomniczi, B.** 1977. Biological properties of avian coronavirus RNA. J. Gen. Virol. **36**:531–533.
10. **Lomniczi, B., and I. Kennedy.** 1977. Genome of infectious bronchitis virus. J. Virol. **24**:99–107.
11. **Macnaughton, M. R., H. A. Davies, and M. V. Nermut.** 1978. Ribonucleoprotein-like structures from coronavirus particles. J. Gen. Virol. **39**:545–549.
12. **Macnaughton, M. R., and M. Hilary Madge.** 1978. The genome of human coronavirus strain 229E. J. Gen. Virol. **39**:497–504.
13. **McClurkin, A. W., and J. O. Norman.** 1966. Studies on transmissible gastroenteritis of swine II. Selected characteristics of a cytopathogenic virus common to five isolates from transmissible gastroenteritis. Can. J. Comp. Med. Vet. Sci. **30**:190–198.
14. **McIntosh, K.** 1974. Coronaviruses: a comparative review. Curr. Top. Microbiol. Immunol. **63**:85–129.
15. **Repik, P., and D. H. L. Bishop.** 1973. Determination of the molecular weight of animal RNA viral genomes by nuclease digestions. I. Vesicular stomatitis virus and its defective T particle. J. Virol. **12**:969–983.
16. **Schochetman, G., R. H. Stevens, and R. W. Simpson.** 1977. Presence of infectious polyadenylated RNA in the coronavirus avian bronchitis virus. Virology **77**:772–782.
17. **Simmons, D. T., and J. H. Strauss.** 1972. Replication of Sindbis virus. II. Multiple forms of double-stranded RNA isolated from infected cells. J. Mol. Biol. **71**:615–631.
18. **Tannock, G. A., and J. C. Hierholzer.** 1977. The RNA of human coronavirus OC-43. Virology **78**:500–510.
19. **Tyrrell, D. A. S., J. D. Almeida, C. H. Cunningham, W. R. Dowdle, M. S. Hofstad, K. McIntosh, M. Tajima, L. Ya. Zakstelskaya, B. C. Easterday, A. Kapikian, and R. W. Bingham.** 1975. Coronaviridae. Intervirology **5**:76–82.
20. **Wege, H., A. Muller, and V. Ter Meulen.** 1978. Genomic RNA of the murine coronavirus JHM. J. Gen. Virol. **41**:217–227.
21. **Yogo, Y., N. Hirano, S. Hino, H. Shibuta, and M. Matumoto.** 1977. Polyadenylate in the virion RNA of mouse hepatitis virus. J. Biochem. **82**:1103–1108.