JOURNAL OF VIROLOGY, Jan. 1986, p. 183–190
0022-538X/86/010183-08$02.00/0

Vol. 57, No. 1

In Vitro Transcription of Human Pararotavirus

MATILDE JASHÉS,[1] ANA MARÍA SANDINO,[1] GUSTAVO FAÚNDEZ,[1] LUIS F. AVENDAÑO,[2] AND EUGENIO SPENCER[1]*

Laboratorio de Virología, Instituto de Nutrición y Tecnología de los Alimentos,[1] and Laboratorio de Virología, Departamento de Microbiología, División Ciencias Médicas Norte,[2] Universidad de Chile, Casilla 15138, Santiago 11, Chile

Received 13 February 1985/Accepted 16 August 1985

Purified human pararotavirus obtained from stool samples from a 6-month-old infant was characterized. Electron microscopy of the viral particles subjected to different treatments suggested that the protein shells differed from those described for rotavirus. Treatment with both EDTA or ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid in the presence or absence of Mg^{2+} seemed to convert the virions into core particles by removal of both the outer and inner shells, and no particles equivalent to single-shelled rotavirus were observed. Different procedures were used to activate the human pararotavirus-associated RNA-dependent RNA polymerase. The enzyme was not activated by chelating agents or by thermal shock as in rotavirus. Activation by thermal shock occurred only in the presence of the four ribonucleoside triphosphates and Mg^{2+}. However, the polymerase of pararotavirus was found to be similar to those described for rotaviruses. When in vitro transcripts were analyzed, 11 RNA species having a migration pattern similar to that of the original genomic RNA were detected.

Pararotavirus occasionally has been found associated with enteritis in several animal species, including humans (2, 3, 7, 11–13). This class of virus has been isolated with a low frequency, 1 in 200 cases of rotavirus, from stool samples obtained from infants with acute gastroenteritis (7). This class of virus differs from rotavirus in antigenic properties (12) but shares the same characteristics with respect to the structure of the genome; in both cases, the genome consists of 11 segments of double-stranded RNA (7). However, the electrophoretic mobilities of the individual RNA segments allow a clear differentiation between rotavirus and pararotavirus. Owing to the low isolation frequency, there is no information about the variability in electropherotypes of the viral genome of human pararotavirus as there is for rotavirus (7, 10, 16).

The morphological aspects of pararotavirus particles have been described previously (7). The virus structure seems to be similar to that of rotavirus when analyzed by negative-stain electron microscopy (EM). A protein organization similar to the double-shelled structure observed in rotavirus has been proposed. It has also been suggested that pararotavirus particles may lose the outer protein shell by treatment with EDTA, leaving single-shelled virions (3). A partial characterization of human pararotavirus structural proteins suggests that they differ from those of rotavirus (7).

In the present communication, we studied the effects of several agents and procedures on the structure of the virus particle and its significance in in vitro viral transcription. The properties of and substrate requirements for in vitro viral transcription in heat-activated virions were also analyzed.

MATERIALS AND METHODS

Virus. The virus was purified from stool samples obtained from a 6-month-old infant hospitalized with a diagnosis of acute diarrhea; no other pathogen was associated with the diarrhea event.

Virus purification and electrophoresis of the viral genome. The purification procedure was as follows. Stool samples (50 g) were diluted with 50 ml of distilled water. To the fecal suspension, 1 volume of Freon was added; after being mixed, the suspension was centrifuged in a Beckman L2-65B ultracentrifuge at 5,000 rpm at 4°C for 30 min. The pellet was discarded, and the supernatant was ultracentrifuged at 20,000 rpm for 30 min in a type 60 rotor (Beckman Instruments, Inc). The supernatant was discarded, and the pellet was suspended in 30 ml of 50 mM Tris hydrochloride buffer (pH 8.0) (buffer A) and centrifuged in a type 60 rotor at 30,000 rpm for 45 min through a 25% sucrose cushion. The pellet was resuspended in 15 ml of buffer A and then subjected to centrifugation through a continuous 25% to 35% sucrose gradient in an SW25 rotor at 12,000 rpm for 1 h. After centrifugation, 1-ml fractions were collected and analyzed for the presence of the virus by detection of the viral genome by polyacrylamide gel electrophoresis as previously described (6) and by using a silver nitrate stain. The virions were detected in the lower portion of the gradient. The fractions that contained virus were pooled, diluted three times with buffer A, and ultracentrifuged at 40,000 rpm for 45 min. The pellet was then diluted in buffer A and stored at −20°C. This isolate was characterized as strain Nociram. The characterization of the pararotavirus genome in polyacrylamide gels is shown in Fig. 1, in which human pararotavirus is compared by coelectrophoresis with "long" and "short" human rotaviruses. This isolate did not react in an enzyme-linked immunosorbent assay (ELISA) for rotavirus (Rotazyme; Abbott Laboratories), even when an amount of viral protein 10 times higher than that required to obtain a strong positive reaction with rotavirus was used.

Treatment of virus particles with chelating agents, trypsin, and $CaCl_2$. The partially purified virions were subjected to incubation with different concentrations of EDTA or ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) in the presence or absence of 20 mM $MgCl_2$. The incubation was carried out at 37°C for 30 min, and then the virions were diluted two times in buffer A and centrifuged in a Microfuge. The supernatant was discarded, and the pellet was suspended in buffer A. This suspension was used for EM analysis and also for in vitro transcription.

* Corresponding author.

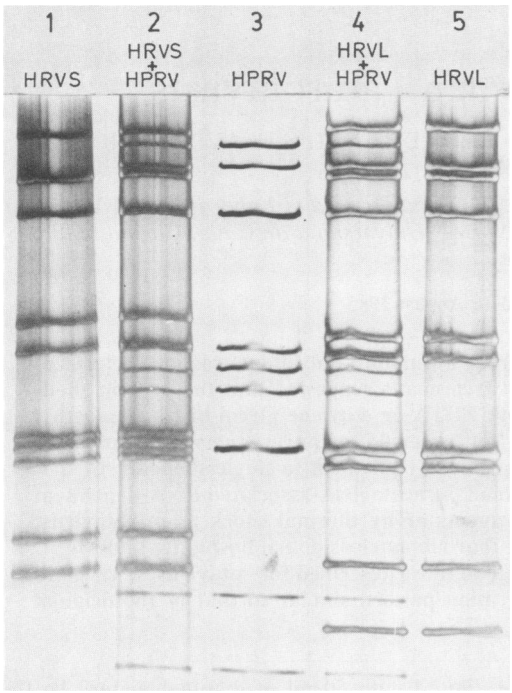

FIG. 1. Comparison of the genome profiles of pararotavirus and rotaviruses of different electropherotypes. RNAs from long (HRVL) and short (HRVS) electropherotypes of human rotavirus were coelectrophoresed with the RNA of the human pararotavirus isolate (HPRV). Analysis was performed on an 8% (wt/vol) polyacrylamide gel at 20 to 25 mA overnight at room temperature.

When virions were treated with trypsin, the reaction was carried out with 0.8 μg of enzyme per ml as described by Espejo et al. (6), and the particles were reisolated and observed by EM. Treatment with $CaCl_2$ was carried out as described by Bican et al. (1).

EM of virus particles. Purified virions and virus particles treated by the procedures described above were negatively stained with 3% ammonium molybdate (pH 7) on carbon-coated Formvar grids and viewed in a Zeiss model ME-109 EM.

RNA polymerase assay. Assays were performed with a reaction mixture containing, in 120 mM Tris hydrochloride buffer (pH 8.5), a 5 mM concentration each of ATP, CTP, and GTP; 20 mM $MgCl_2$; 0.5 mM *S*-adenosylmethionine (Adomet); 240 μM [^{3}H]UTP (specific activity, 40 cpm/pmol); and 0.2 μg of purified virus. This mixture was subjected to a 60-s heat shock treatment at 55°C and incubated at 45°C for different periods of time, depending on the experiment. The reaction was halted by the addition of 0.4 ml of a solution containing 200 mM sodium PP_i, 1 mg of denatured calf thymus DNA per ml, and 4 ml of ice-cold 10% trichloroacetic acid. The acid-insoluble material was collected on glass fiber filters, which were then extensively washed with 10% trichloroacetic acid–cold ethanol. The filters were dried, and the radioactivities were measured with a liquid scintillation counter. The radioactivity was expressed as picomoles of [^{3}H]UMP incorporated into acid-insoluble material.

Polyacrylamide gel electrophoresis of the in vitro transcripts. A reaction mixture similar to the one described above was used to prepare in vitro RNA transcripts, except that the total volume was doubled and [^{3}H]UTP was replaced by [α-^{32}P]UTP (specific activity, 400 to 272 cpm/pmol). After incubation, the reaction mixture was centrifuged for 15 min in an Eppendorf centrifuge, and the supernatant was mixed with 100 mM Tris hydrochloride buffer (pH 7.5) containing 99% formamide, 1 mM EDTA, and 0.01% xylene cyanol. The mixture was then loaded in a 5% acrylamide–0.13% bisacrylamide gel in 0.089 M Tris–0.089 M boric acid–0.008 M EDTA buffer (pH 8.4) and 8 M urea. The gel was electrophoresed at 25 mA for 24 h. After the run, the gel was washed with a solution containing 6% acetic acid for 2 h, dried, and exposed to X-ray film with an intensifying screen.

RESULTS

Virus isolate. The pararotavirus isolate was from stools from an infant suffering from acute diarrhea with vomiting and an increase in body temperature up to 38°C. The duration of the illness was 6 days.

EM of purified virions. The majority of the virus particles appeared to be very homogeneous in size, with a mean diameter of 70 nm, and had a structure similar to the double-shelled arrangement characteristic of rotavirus (Fig. 2A and B). However, a relatively low proportion (less than 15%) may correspond to empty particles because they were penetrated by the stain (Fig. 2A, EC). Furthermore, no virus particles lacking the outer protein shell and having a structure resembling single-shelled rotavirus were observed. A very low proportion of the virus particles seemed to have lost both inner and outer protein shells, leaving structures that may correspond to viral cores (Fig. 2A, inset) and that were clearly different from single-shelled rotavirus. Even fragments of the protein shell present in the purified viral fractions seemed to contain both inner and outer layers (Fig. 2A, bps).

Effect on chelating agents on pararotavirus. The effect of chelating agents on pararotavirus was explored to compare the properties of the outer protein shell with those of the outer protein shell of rotavirus. When EDTA-treated virions were observed after negative staining with phosphotungstic acid, the same smooth core particles described by Bican et al. (1) for rotavirus were observed (results not shown). However, 3% ammonium molybdate (pH 7) was used as the negative stain in our experiments because this stain revealed more details of the surfaces of whole and disrupted virions than were observed with phosphotungstic acid. The effect of treatment with two concentrations of EDTA (5 and 10 mM) is shown in Fig. 3A and B. The treated virus particles appeared to be disrupted and were penetrated by the stain to a higher degree than were the virions. The effect of both various EDTA concentrations and incubation times was a variety of disrupted virus particles having different outer protein shell contents but having a structure like that of a core rather than that of single-shelled rotavirus. Since EGTA has the same effect as EDTA (Fig. 3C), purified pararotavirus was treated with EGTA in the presence of $MgCl_2$ to explore the possible existence of single-shelled particles unstable in the absence of divalent ions (Fig. 3D and E). When Mg^{2+} was added, the loss of the protein cover was again observed, but the particles were penetrated by the stain to a lower degree (Fig. 3D); prolonged incubation with EGTA or higher EGTA concentrations (Fig. 3E), both in the presence of Mg^{2+}, yielded particles similar to those obtained with EDTA or with EGTA in the absence of Mg^{2+}. However, particles equivalent to single-shelled rotavirus were not observed under any conditions; the particles were modified from complete virions to partial cores or viral cores. An interesting feature of the treated pararotavirus (Fig. 3D; see

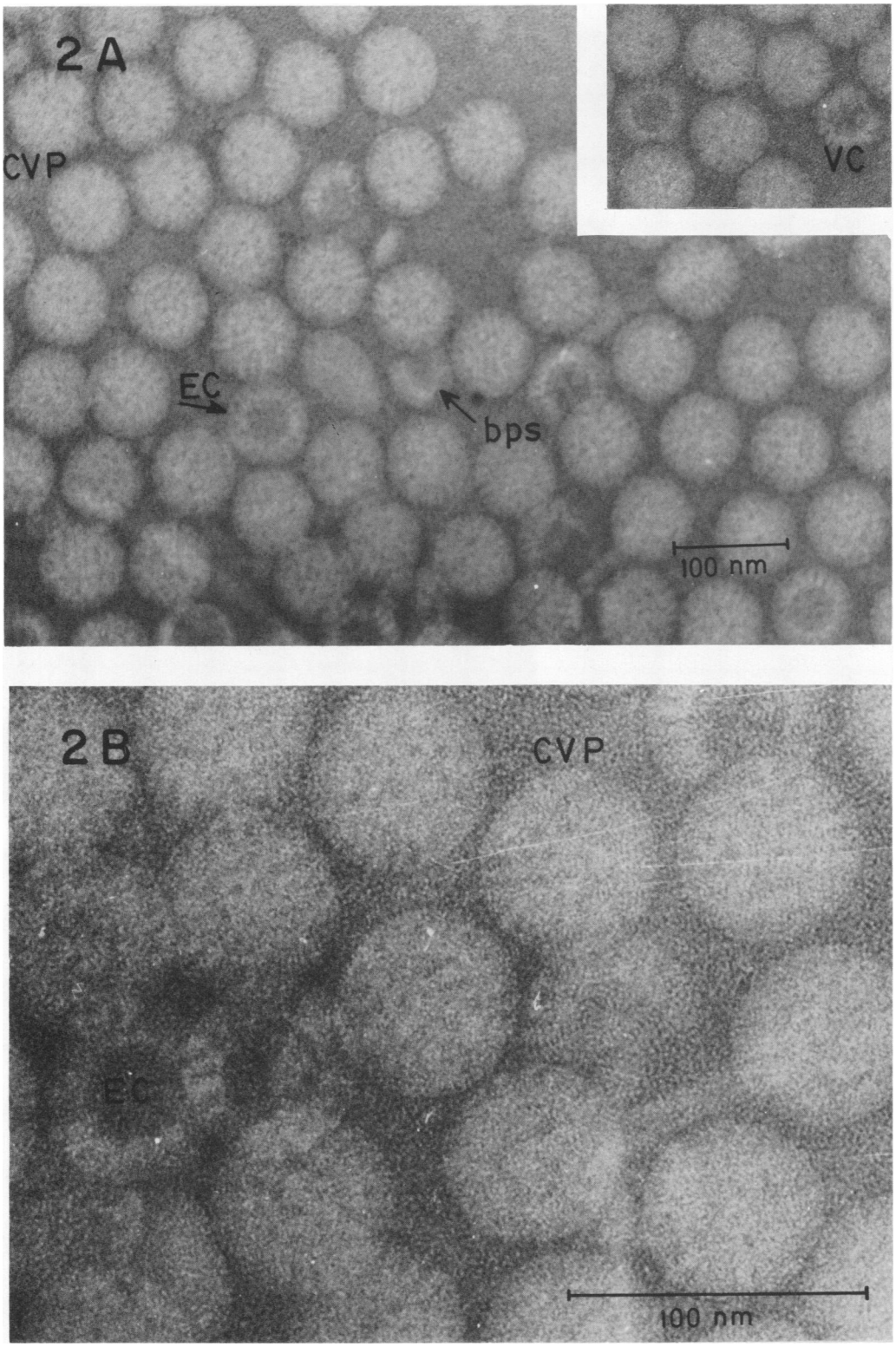

FIG. 2. Electron micrographs of negatively stained human pararotavirus. Purified virus particles were observed under different magnifications. CVP, Complete virus particles; EC, empty capsid; VC, viral core; bps, broken protein shell.

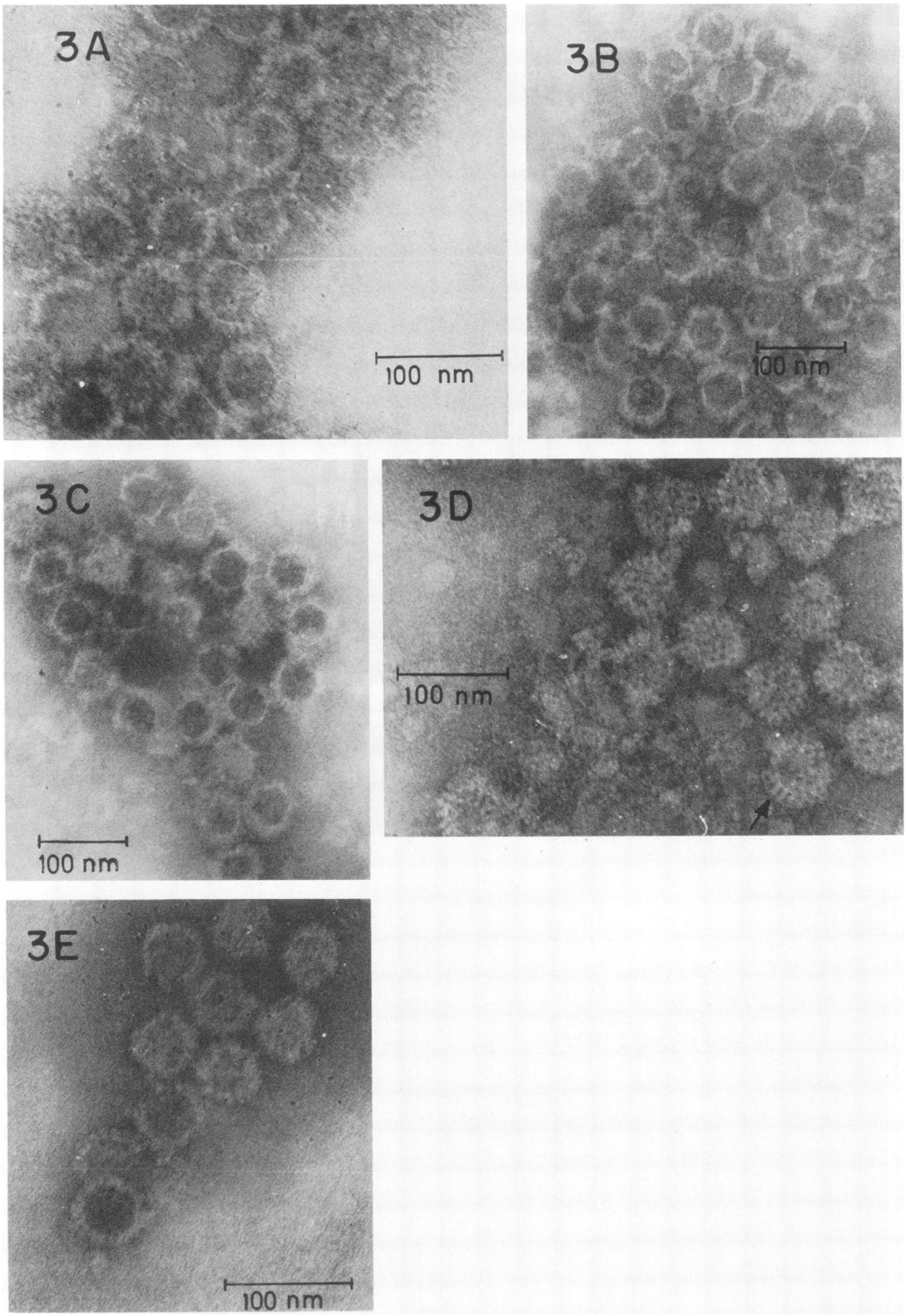

FIG. 3. Comparison of the morphologies of pararotavirus treated with chelating agents. Virus particles were treated with chelating agents (EDTA and EGTA) at different concentrations for 30 min at 37°C. The particles thus obtained were reisolated by centrifugation, suspended in 50 mM Tris hydrochloride buffer (pH 8.0), and spread on Formvar-covered grids. (A) 5 mM EDTA; (B) 10 mM EDTA; (C) 5 mM EGTA; (D) 5 mM EGTA–20 mM $MgCl_2$; (E) 10 mM EGTA–20 mM $MgCl_2$.

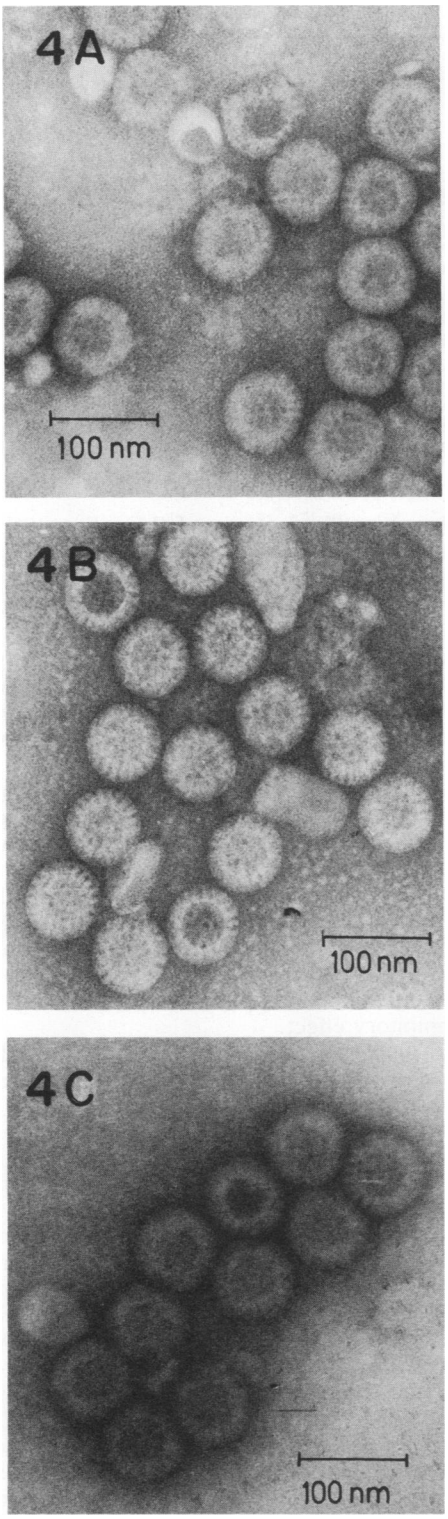

FIG. 4. Effects of $CaCl_2$, trypsin, and heat shock on the structure of pararotavirus. Virus particles were treated with 1.5 M $CaCl_2$ (A), 0.8 μg of trypsin per ml (B), or a 60-min heat shock treatment at 55°C (C) under the transcriptional conditions described in Materials and Methods.

TABLE 1. Ribonucleoside triphosphate and Mg^{2+} requirements for the in vitro activation of human pararotavirus-associated RNA polymerase[a]

Addition(s) before thermal shock	Addition(s) after thermal shock	[^{3}H]UMP incorporated into acid-insoluble material (pmol/30 min)
None	Virus + ATP + CTP + GTP + [^{3}H]UTP + MG^{2+}	4.1
Virus	ATP + CTP + GTP + [^{3}H]UTP + Mg^{2+}	7.0
Virus + Mg^{2+}	ATP + CTP + GTP + [^{3}H]UTP	2.0
Virus + Mg^{2+} + ATP	CTP + GTP + [^{3}H]UTP	5.8
Virus + Mg^{2+} + CTP	ATP + GTP + [^{3}H]UTP	4.8
Virus + Mg^{2+} + GTP	ATP + CTP + [^{3}H]UTP	5.6
Virus + Mg^{2+} + ATP + CTP	GTP + [^{3}H]UTP	21.4
Virus + Mg^{2+} + ATP + GTP	CTP + [^{3}H]UTP	33.2
Virus + Mg^{2+} + CTP + GTP	ATP + [^{3}H]UTP	25.9
Virus + Mg^{2+} + ATP + GTP + CTP	[^{3}H]UTP	53.8
Virus + Mg^{2+} + ATP + GTP + CTP + [^{3}H]UTP	None	75.0

[a] Standard reaction mixtures (25 μl) contained 0.2 μg of human pararotavirus and a final concentration of each ribonucleoside triphosphate of 5 mM.

arrow) was that pieces of the remaining shell seemed to contain both inner and outer layers.

Owing to the unexpected results obtained when this pararotavirus isolate was treated with chelating agents, we explored other procedures known to be able to modify the structure of viruses of the *Reoviridae* family. Pararotavirus treated with either 1.5 M $CaCl_2$ or trypsin or heat shocked are shown in Fig. 4. When double-shelled rotavirus particles were incubated with $CaCl_2$ at concentrations of 0.5 M to 1.5 M, viral cores were observed. However, $CaCl_2$ at those concentrations did not have the effect on pararotavirus that it had on rotavirus; the pararotavirus particles appeared to be apparently intact (Fig. 4A). Concentrations of $CaCl_2$ higher than 2 M led to the complete disruption of both rotavirus and pararotavirus. When human pararotavirus was treated to different concentrations of trypsin, no structural modifications were observed (Fig. 4B). The 60-s thermal shock at 55°C used to activate the polymerase did not have a detectable effect on the structure, as observed by EM (Fig. 4C).

Requirements of in vitro transcription. Direct incubation with appropriate substrates of the untreated virus or of the virus treated with either trypsin or EDTA yielded no detectable RNA synthesis, measured as [^{3}H]UMP incorporated into acid-insoluble material. An activation procedure previously described for rotavirus (15) and consisting of thermal shock for 60 s at 55°C was also used. These attempts were successful only when Mg^{2+} and ribonucleoside triphosphates were present during thermal shock. The results of such experiments are shown in Table 1, suggesting that to

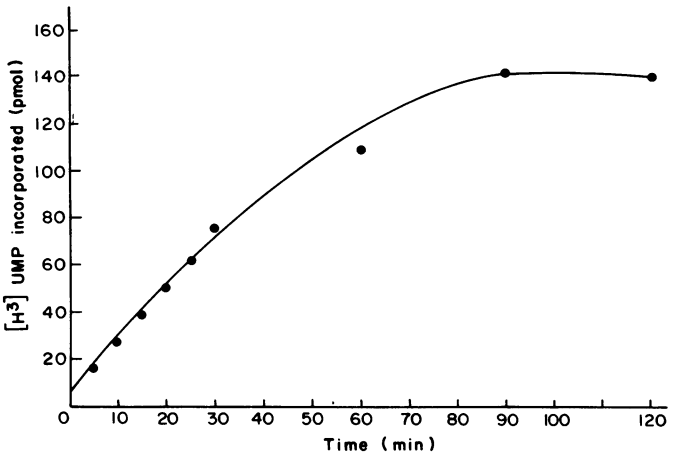

FIG. 5. Time course of RNA synthesis. Standard reaction mixtures containing 0.2 μg of heat-treated virus were incubated for the indicated times at 45°C.

obtain optimal RNA synthesis, all four ribonucleoside triphosphates and Mg^{2+} must be present during thermal shock.

The time course of the incorporation of ribonucleoside triphosphates into acid-insoluble material with activated virus exhibited linear kinetics for at least 30 min, reaching a plateau after 75 min of incubation (Fig. 5). Optimal temperatures for both the thermal shock and the subsequent incubation were, respectively, 55 and 45°C. Other combinations also explored resulted in lower enzymatic activity.

Most of the experiments were done with 0.2 μg of viral protein per assay. However, there was a linear relationship between the amount of heat-treated virus and RNA synthesis (Fig. 6). Also, no RNA synthesis was detected when the virus was not previously activated (Fig. 6).

The incorporation of [^{3}H]UMP into acid-insoluble material showed an absolute requirement of Mg^{2+}; when purified virus and a 5 mM concentration of each ribonucleoside triphosphate were used , the optimal concentration of Mg^{2+} was 20 mM. There was no detectable reaction when Mg^{2+}

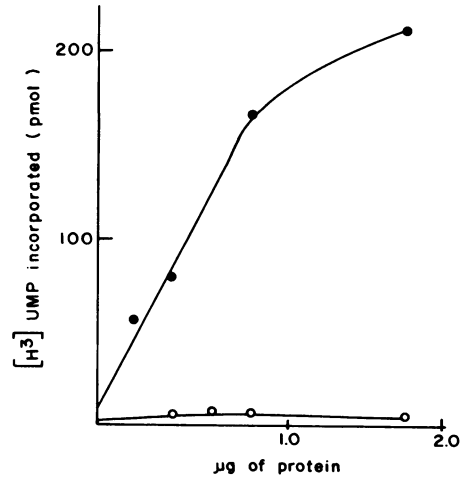

FIG. 6. Dependence of RNA synthesis on human pararotavirus concentrations. Standard reaction mixtures containing the indicated amounts of heat-treated (●) or nontreated (○) virions were incubated for 30 min at 45°C.

TABLE 2. Ribonucleoside triphosphate requirements for the in vitro reaction catalyzed by human pararotavirus-dependent RNA polymerase[a]

Condition(s)	[^{3}H]UMP incorporated into acid-insoluble material (pmol/30 min)
Complete	74.0
Lacking ATP or CTP or GTP	2.1
Lacking ATP, containing dATP	3.0
Lacking GTP, containing dGTP	0.7
Lacking CTP, containing dCTP	2.0
Lacking ATP, containing β-γ-methylene ATP	12.0
Lacking GTP, containing β-γ-methylene GTP	7.0
Lacking ATP, containing cordycepin triphosphate	1.0

[a] Standard reaction mixtures contained 0.2 μg of heat-treated human pararotavirus and a final concentration of each ribonucleoside triphosphate of 5mM, except for [^{3}H]UTP, which was present at a concentration of 240 μM.

was replaced by Mn^{2+} at any of the cation concentrations studied (results not shown).

As expected, viral RNA synthesis required all four ribonucleoside triphosphates. In the absence of a single ribonucleotide, the corresponding deoxyribonucleotide was unable to replace it. When ATP was replaced by cordycepin triphosphate, no RNA synthesis was detected. However, the replacement of ATP or GTP by the corresponding β-γ-methylene analog produced a decrease in incorporation of only eightfold instead of complete inhibition, as was observed with rotavirus (15) (Table 2). Results similar to those shown in Table 2 were obtained with different concentrations of the nucleotides and analogs. The ribonucleoside triphosphate requirement is shown in Fig. 7.

The effects of NaCl, $(NH_4)_2SO_4$, and P_i on the rate of RNA synthesis catalyzed by the virus-associated RNA polymerase were explored. The results showed that none of these salts produced a significant increase in the rate of RNA synthesis, but at concentrations of 40 mM or higher they all had an inhibitory effect.

Optimal incorporation of labeled ribonucleoside triphosphates was observed when thermal shock was performed at pH 8.5.

Actinomycin D at concentrations higher than 200 μg/ml had no effect on viral polymerase. α-Amanitin did not inhibit the reaction, even at a concentration of 200 μg/ml.

Effect of Adomet and Adohcy on transcription. In several eucaryotic and viral models it has been shown that Adomet has a stimulatory effect on transcription. In some viruses transcription may become dependent upon its addition, lowering the ribonucleoside triphosphate requirement (8, 18). Figure 8 shows the stimulatory effect of Adomet at different concentrations and shows that *S*-adenoylhomocysteine (Adohcy) at similar concentrations was unable to stimulate transcription; instead, it had a slightly inhibitory effect. Figure 7 shows the stimulatory effect of Adomet at different concentrations of ribonucleoside triphosphates. The addition of Adomet did not alter the ribonucleoside triphosphate concentration optimal for transcription of the viral genome. It seems worthwhile to remark that the stimulatory effect of Adomet was observed only at concentrations of ribonucleoside triphosphates able to support RNA synthesis in its absence.

Analysis of the RNA product. RNA synthesis in vitro by heat-treated human pararotavirus was analyzed under

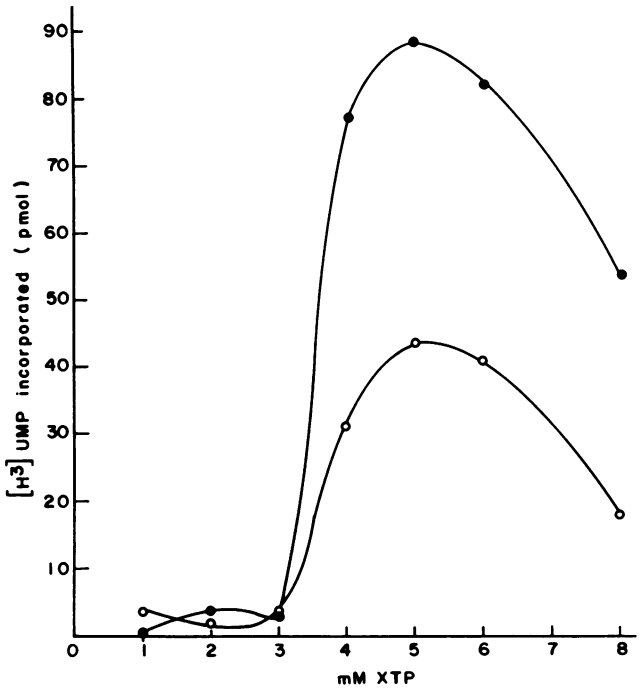

FIG. 7. Dependence of the reaction catalyzed by the virus-associated RNA polymerase on the concentrations of ribonucleoside triphosphates. Standard reaction mixtures containing 0.2 μg of heat-treated human pararotavirus were incubated for 30 min at 45°C in the absence (○) or presence (●) of 0.5 mM Adomet. The concentrations of the ribonucleoside triphosphates varied as indicated. The concentration of [^{3}H]UTP was 0.24 mM (specific activity, 40 cpm/pmol).

denaturating conditions by urea-formamide polyacrylamide gel electrophoresis (Fig. 9). The distribution of radioactive bands in the radioautograph indicates the presence of at least 11 different RNA species, with a migration pattern resembling that of genomic RNA.

DISCUSSION

In the present communication, we report the structural characterization of a human pararotavirus isolate and the results obtained from studies of the in vitro transcription catalyzed by the virus-associated RNA polymerase.

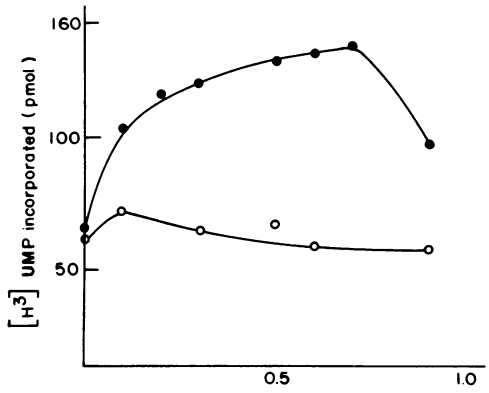

FIG. 8. Effect of Adomet and Adohcy on RNA polymerase activity. Standard reaction mixtures containing 0.2 μg of human pararotavirus were supplemented with Adomet (●) or Adohcy (○) (millimolar concentrations indicated on the abscissa) and incubated for 30 min at 45°C after heat shock.

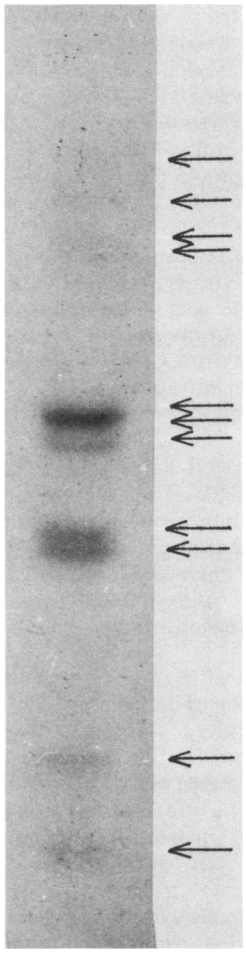

FIG. 9. Electrophoretic analysis of RNA products synthesized in vitro. ^{32}P-labeled RNA was subjected to 5% acrylamide–8 M urea gel electrophoresis, dried, and autoradiographed. The arrows indicate the RNA products.

A peculiarity of pararotavirus seems to be that, in contrast to rotavirus, all of the particles had an apparently intact outer layer and single-shelled particles were not observed. Previous reports showed that EDTA removes the outer protein shell of rotavirus (4). However, when pararotavirus was subjected to treatment with EDTA, even at lower concentrations and shorter times than those used for rotavirus, the inner protein shell that covers the viral core was partially removed, together with the outer protein shell, suggesting a quite different stability of both shells in rotavirus and pararotavirus. The virus particles thus obtained did not correspond in size or structure to single-shelled virions (5). The results of EGTA treatments also showed that even in the presence of Mg^{2+}, the inner protein shell was removed together with the outer protein shell. The above results suggest that in pararotavirus, Ca^{2+} may play an important function in maintaining the association of the virol core with both inner and outer protein shells. EDTA-treated pararotavirus seemed to lack the capacity to transcribe in vitro. This observation agrees with the loss of the inner layer after EDTA treatment observed by EM, indicating that these proteins may be required for in vitro transcrip-

tion of pararotavirus, as described for rotavirus (1). Several procedures were used to activate the virion. As with rotavirus, thermal shock was able to activate the human pararotavirus-associated RNA polymerase (15). The requirements for thermal shock were similar to those with rotavirus in terms of both optimal temperature (55°C) and optimal incubation condition (45°C). However, the major difference from rotavirus was the strict requirement for the presence of the ribonucleoside triphosphates and Mg^{2+} during thermal shock to obtain virus activation. This different requirement for activation of the transcriptase, together with the different modification of the virion with chelating agents, argues in favor of a pararotavirus structure different from that observed for rotavirus. The resistance of pararotavirus virions to high concentrations of $CaCl_2$ also argues in favor of a structural difference between rotavirus and pararotavirus.

The virus-associated RNA polymerase had well-defined substrate requirements. The reaction also required the presence of all four ribonucleotides. The optimal ribonucleoside triphosphate concentration, when $MgCl_2$ was added at 20 mM, was 5 mM for each ribonucleoside triphosphate, except for [^{3}H]UTP. If the ratio of Mg^{2+} to nucleotides was either increased or decreased, there was a decrease in RNA synthesis.

The replacement of ribonucleotides by the β-γ-methylene ATP or GTP analog reduced the yield of the reaction by approximately eightfold, although some activity could still be detected, suggesting that in the virion there is no ATP hydrolysis requirement, as described for rotavirus RNA polymerase (15). The effect of Adomet on in vitro synthesis seemed to be different from that for some members of the *Reoviridae* family but similar to that for rotavirus and reovirus in the sense that in vitro transcription was not dependent on the methylating agent but was stimulated at least twofold (9). Also, Adomet did not diminish the requirement for ribonucleotides, as in vesicular stomatitis virus or polyhedrosis virus (8, 18). The stimulatory effect of Adomet may be associated with the synthesis of a 5′ cap in the mRNA, an event that normally takes place early during transcription and allows for a more effective elongation of the RNA molecule (14, 17). Therefore, it is possible that the virus particle contains an associated guanylyl-methyltransferase system capable of synthesizing the cap in vitro, as in reovirus and rotavirus (9, 17).

The above characteristics of in vitro RNA synthesis catalyzed by human pararotavirus-associated RNA polymerase suggest that this reaction is different from that described for rotavirus not only in the activation procedure but also in the effect of monovalent salts and the ATP requirement (4, 16).

Analysis of the RNA products by gel electrophoresis revealed that each genome segment was transcribed into a single product, rendering a pattern that was similar to that obtained with the original RNA genome. However, the number of transcripts associated with each genome segment seemed to be different. At present, no information exists for in vitro or in vivo procedures for pararotavirus culturing; therefore, the mechanisms of virus replication and in vivo transcription remain unknown.

ACKNOWLEDGMENTS

We thank Romilio Espejo for his cooperation and patience in editing and discussing the manuscript.

This investigation was supported by grant B-15078213 from Dirección de Investigación y Biblioteca de la Universidad de Chile.

ADDENDUM

The results reported in the present communication regarding the virus structure and the effect of the chelating agents were also obtained recently with other pararotavirus isolates of either the same or different electropherotypes.

LITERATURE CITED

1. **Bican, P., J. Cohen, A. Charpilienne, and R. Scherrer.** 1982. Purification and characterization of bovine rotavirus cores. J. Virol. **43**:1113–1117.
2. **Bohl, E. H., L. J. Saif, K. W. Theil, A. G. Agnes, and R. F. Cross.** 1982. Porcine pararotavirus: detection, differentiation from rotavirus, and pathogenesis in gnotobiotic pigs. J. Clin. Microbiol. **15**:312–319.
3. **Bridger, J. C., I. N. Clarke, and M. A. McCrae.** 1982. Characterization of an antigenically distinct porcine rotavirus. Infect. Immun. **35**:1058–1062.
4. **Cohen, J., J. Laporte, A. Charpilienne, and R. Scherrer.** 1979. Ribonucleic acid polymerase activity associated with purified calf rotavirus. J. Gen. Virol. **36**:395–402.
5. **Esparza, J., and F. Gil.** 1978. A study on the ultrastructure of human rotavirus. Virology **91**:141–150.
6. **Espejo, R. T., S. López, and C. Arias.** 1981. Structural polypeptides of simian rotavirus SA11 and the effect of trypsin. J. Virol. **37**:156–160.
7. **Espejo, R. T., F. Puerto, C. Soler, and N. González.** 1984. Characterization of a human pararotavirus. Infect. Immun. **44**:112–116.
8. **Furuichi, Y.** 1981. Allosteric stimulatory effect of S-adenosyl methionine on the RNA polymerase in cytoplasmic polyhedrosis virus: a model for the positive control of eukaryotic transcription. J. Biol. Chem. **256**:483–493.
9. **Joklik, W.** 1981. Structure and function of the reovirus genome. Microbiol. Rev. **45**:483–501.
10. **Lourenco, M. H., J. C. Nicolas, J. Cohen, R. Scherrer, and F. Bricout.** 1981. Study of human rotavirus genome by electrophoresis: attempt of classification among strains isolated in France. Ann. Virol. (Paris) **132**:161–173.
11. **McNulty, M., G. Allan, J. Tood, J. McFerran, and R. McCracken.** 1981. Isolation from chickens of a rotavirus lacking the rotavirus group antigen. J. Gen. Virol. **55**:405–413.
12. **Nicolas, J. C., J. Cohen, B. Fortier, M. Lourenco, and F. Bricout.** 1983. Isolation of a human pararotavirus. Virology **124**:181–184.
13. **Rodger, S. M., R. F. Bishop, and I. H. Holmes.** 1982. Detection of a rotavirus-like agent associated with diarrhea in an infant. J. Clin. Microbiol. **16**:724–726.
14. **Shatkin, A.** 1980. Capping of eucaryotic mRNA. Cell **9**:654–667.
15. **Spencer, E., and M. L. Arias.** 1981. In vitro transcription catalyzed by heat-treated human rotavirus. J. Virol. **40**:1–10.
16. **Spencer, E., F. Avendaño, and M. Araya.** 1983. Characteristics and analysis of electrophoretypes of human rotavirus isolated in Chile. J. Infect. Dis. **148**:41–48.
17. **Spencer, E., and B. I. García.** 1984. Effect of *S*-adenosylmethionine on human rotavirus RNA synthesis. J. Virol. **52**:188–197.
18. **Testa, D., and A. Barnargee.** 1979. Initiation of RNA synthesis in vitro by vesicular stomatitis virus: role of ATP. J. Biol. Chem. **254**:2058–2068.