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RNA Polymerase Associated with Human Rotaviruses in Diarrhea Stools

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RNA polymerase activity was detected in six stools which were partially purified by high-speed centrifugation from infants with rotavirus gastroenteritis, but was not detected in five stools which were negative for rotavirus by counterimmunoelectrophoresis and radioimmunoassay. The polymerase activity was associated with the 1.38-g/ml rotavirus band after purification in a CsCl gradient.

The human rotavirus (HRV) is a major cause of winter diarrhea in infants and young children (3). Similar viruses have been found associated with acute gastroenteritis in calves, mice, piglets, foals, lambs, and rabbits (16). Although they can be distinguished from the reoviruses and orbiviruses morphologically (4) and antigenically (5, 6) the rotaviruses are classified in the *Reoviridae* family because they contain segmented, double-stranded RNA. Inclusion of these viruses in the *Reoviridae* family led us to examine two rotaviruses, Nebraska calf diarrhea virus (NCDV) and HRV, for the presence of a virion-associated RNA polymerase which has been described for several species within the family (7, 14, 15). Our group and Cohen (2) independently found an RNA polymerase to be associated with the purified virions of NCDV grown in tissue culture in a CV-1 cell line (CCL-70, Flow Laboratories). The NCDV RNA polymerase resembled the previously described polymerases. It required four nucleoside triphosphates, $MgCl_2$ (optimum concentration, 0.006 to 0.012 M), a pH optimum between 7.5 and 8.0, and a salt concentration of less than 0.1 M. Cohen (2) showed that the complete double-shelled virus (density = 1.36 g/ml) had to be treated with EDTA to "activate" the RNA polymerase. We had routinely included EDTA in our TNE buffer (Tris, 0.05 M [pH 7.5]; NaCl, 0.15 M; EDTA, 0.01 M), and our purified virus had a density of 1.38, which is the reported density for rotavirus single-shelled cores (13) or "uncoated" virus particles. Our 1.38-g/ml-density particle exhibited RNA polymerase activity without any need of activation. The RNA polymerase product appeared to be single stranded because it was susceptible to RNase digestion. In preformed CsCl gradient, the RNA polymerase product remained near the top and distinct from the intact, unlabeled cores which sedimented again at a density of 1.38 g/ml.

Next, we examined stools of infants with rotavirus infection diagnosed by counterimmunoelectrophoresis (9), radioimmunoassay (8), and electron microscopy for the presence of an RNA polymerase. We found that direct assay was not possible due to the presence of nucleases in the stools which digested the product or any exogenously added [^{3}H]uridine-labeled HEp-2 RNA. The nucleases were present in stools from both normal patients and patients with diarrhea. Subsequently, we partially purified stools to eliminate the nucleases, as follows. Crude 5% stool solutions in IRMA buffer (barbital, 0.02 M [pH 7.5]; NaCl, 0.5 M; sodium azide, 0.02%; bovine serum albumin, 0.1%; and fetal calf serum, 0.1%) were clarified in a Brinkman tabletop centrifuge for 2 min. Next, 50 μl of supernatant was overlaid onto a 5 to 20% sucrose gradient in TNE and pelleted to the bottom by centrifugation in an SW40 rotor for 2.5 h at 38,000 rpm and 4°C. The pellet was dissolved in TNE and used for radioimmunoassay and the RNA polymerase reaction (0.15 ml of mixture containing ATP, CTP, and GTP, 8×10^{-4} M; $MgCl_2$, 0.012 M; Tris-hydrochloride [pH 8.0], 0.1 M; and 5 μCi of [^{3}H]UTP); samples were incubated for 2 h at 37°C, and 100 μl of the mixture was spotted on Whatman no. 3 filter paper. The filters were batch washed through a series of 5% trichloroacetic acid baths (11), dried, and counted in toluene scintillation fluid (Ominfluor). The results (Table 1) demonstrated that all the samples positive for rotavirus by counterimmunoelectrophoresis and radioimmunoassay also incorporated [^{3}H]uridine 3 to 72 times more than the control and well-patient samples.

Finally, we proved that the polymerase activity was due to rotavirus by purifying a diarrhea

TABLE 1. *Presence of RNA polymerase associated with human rotavirus in purified stools of patients with infantile gastroenteritis*[a]

Stool	Clinical status	CIE	RIA (cpm of ^{125}I)	RNA polymerase (cpm of ^{3}H)
(A) 1	Diarrhea	+	5,212	20,200
2[b]	Diarrhea	+	4,292	5,460
6[b]	Diarrhea	+	5,062	12,074
7[b]	Diarrhea	+	5,812	43,440
8[b]	Diarrhea	+	4,539	17,587
9	Diarrhea	+	4,415	1,854
(B) 3	Diarrhea	−	3,351	436
(C) 21	Well	−	2,632	617
22	Well	−	2,366	645
23	Well	−	2,370	804
24	Well	−	2,617	556
TE buffer[c] control			3,115	528
			3,151	440
NCDV control			28,612	17,864

[a] CIE, Counterimmunoelectrophoresis; RIA, radioimmunoassay; cpm, counts per minute.

[b] Rotavirus particles observed by electron microscopy by L. Spence.

[c] TE buffer is 0.10 M Tris (pH 7.5)–0.01 M EDTA.

stool via CsCl density gradient centrifugation and correlating the observed RNA polymerase activity with the viral band. The results of this experiment are shown in Fig. 1. Two predominant antigenic peaks are seen at 1.38 and 1.29 g/ml, which correspond to the "full" cores and "empty" particles (1, 10, 12) of NCDV when it is similarly treated. In addition, only the 1.38-g/ml-density peak of NCDV was labeled with the isotope when it was grown in the presence of [^{3}H]uridine, thus supporting the hypothesis that this peak in HRV is also due to "full" cores. As in the case of NCDV, the RNA polymerase activity of the HRV was found to be associated only with the "full" core antigen band. (Direct assay of the CsCl gradient could not be done because the high salt concentration interfered with the RNA polymerase, and therefore pelleting or dialysis had to be performed first).

Our demonstration of an RNA-polymerase activity associated with HRV and NCDV gives further support for the inclusion of these two viruses in the family *Reoviridae* and also provides a potentially useful tool for the study of the molecular biology and laboratory diagnosis of this interesting group of viruses.

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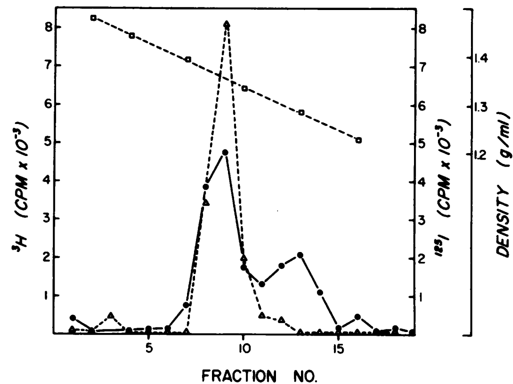

FIG. 1. *CsCl gradient centrifugation of HRV-like antigen: association of RNA polymerase with the virion peak. A diarrhea stool, no. 174, known to be positive for HRV by counterimmunoelectrophoresis and radioimmunoassay, was diluted 1:2 with IRMA buffer and clarified for 2 min in a Brinkman tabletop centrifuge. A 200-μl sample of the stool supernatant was then overlaid onto a preformed CsCl gradient and centrifuged in an SW65 rotor at 11°C, 45,000 rpm, for 2.5 h. Fractions were collected dropwise from the bottom of the gradient and measured for density* (□) *and NCDV antigen by radioimmunoassay* (●). *Subsequently each fraction was diluted with TE (0.10 M Tris [pH 7.5]-0.01 M EDTA), repelleted through a 25% sucrose pad, and assayed for RNA polymerase activity* (△).

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