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NOTES

Characteristics of the Genome of Human Infantile Enteritis Virus (Rotavirus)

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Thermal denaturation studies and polyacrylamide gel electrophoretic analysis have shown that the genome of human infantile enteritis virus (human rotavirus) consists of approximately 11 double-stranded segments. Differences in the molecular weights of some of the corresponding genome segments are apparent between different isolates of the virus.

Human rotavirus, which appears to be one of the most widespread causes of human infantile enteritis, has been identified only in recent years (1, 2). In morphology and morphogenesis the virus closely resembles the viruses of neonatal calf diarrhea (calf rotavirus), epizootic diarrhea of infant mice (EDIM), and simian virus SA 11 (4). Human rotavirus also has a number of features of morphology and morphogenesis in common with reoviruses and orbiviruses (4). Antigenic similarities between the human virus, calf rotavirus, and EDIM virus have been demonstrated, whereas no cross-reactions between rotaviruses and reo- or orbiviruses have been found (3, 5, 6). As a result, the placement of human rotavirus in the family *Reoviridae* in a genus together with calf rotavirus, EDIM and SA 11 viruses but separate from the reo- and orbiviruses has been proposed (4, 11).

Human rotavirus has recently been shown to contain RNA (8), and in this communication we characterize the human rotavirus genome further and compare it with those of typical representatives of the family *Reoviridae*. Calf rota-, reo-, and orbiviruses have all been shown to possess genomes consisting of double-stranded segments of RNA (11-13, 15).

Human rotavirus was extracted from stools collected from infants with acute enteritis. The clinical material was kindly supplied by I. Gust (Fairfield Infectious Diseases Hospital, Melbourne, Australia) and R. F. Bishop and G. P. Davidson (Royal Children's Hospital, Melbourne, Australia).

Reovirus type 3 (Abney strain) (research reference reagent) was obtained from the National Institutes of Health, Bethesda, Md.

The orbivirus D'Aguilar was obtained through the courtesy of R. L. Doherty (Queensland Institute of Medical Research, Brisbane, Australia).

S. M. Rodger (Department of Microbiology, University of Melbourne, Melbourne, Australia) kindly provided the polyacrylamide gel of the calf rotavirus genome.

The method of virus purification, using fluorocarbon extraction, sucrose gradient rate zonal centrifugation, and caesium chloride density gradient centrifugation, has been described previously (11). Deproteinization of the purified virus, construction of the thermal denaturation curves, and the conditions used for electrophoresis of deproteinized human rotavirus on 7.5% polyacrylamide gels were as described for calf rotavirus (11), with the exception that electrophoresis was carried out for 40 h. After staining with methylene blue, gels were scanned on a microdensitometer and photographed by the method of Oliver and Chalkley (10) as described previously (11). The molecular weights of the genome segments were calculated as described for calf rotavirus (11) after co-electrophoresis on the same gel of nucleic acid preparations of human rotavirus and reovirus type 3.

Figure 1 shows the melting curve obtained when nucleic acid of human rotavirus was heated to 96 C in 0.1× SSC (0.015 M NaCl plus 0.0015 M sodium citrate, pH 7.2). The sharp increase observed in the relative absorbance indicates that, like the nucleic acids of reoviruses, orbiviruses, and calf rotavirus (11), the nucleic acid of the human rotavirus is double stranded in nature. The measured T_m was 77 C, which is very similar to the T_m of 78 C obtained for calf rotavirus in the same buffer (11).

An essentially parallel curve was obtained

when the nucleic acid of the human rotavirus was heated in Loening electrophoresis buffer (7) but displaced so that the measured T_m was 58 C.

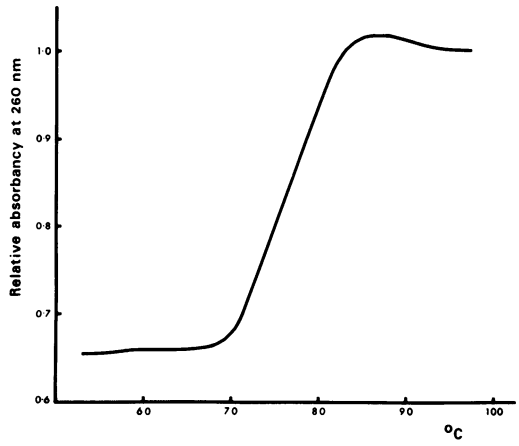

FIG. 1. *Thermal denaturation curve of human rotavirus nucleic acid.*

Polyacrylamide gel electrophoresis resolved the genome of human rotavirus into eight distinct bands (Fig. 2). So that a direct comparison can be made, polyacrylamide gels showing band patterns obtained by electrophoresis of the genomes of calf rotavirus, reovirus, and D'Aguilar virus have been included together with that of human rotavirus in Fig. 2. Conditions of electrophoresis for the other three viruses were identical to those for the human rotavirus. The molecular weights of the bands of human rotavirus are listed in Table 1.

On a densitometer tracing of the gel of the human rotavirus, eight peaks were obtained (Fig. 3); to determine whether any of the gel bands contained more than one nucleic acid component, molar ratios were calculated as previously described (11). These are listed in Table 1. The results suggest that the genome of human rotavirus is composed of 11 segments. In Table 1 and Fig. 2 the genome segments have been numbered in order of decreasing molecular weight by the system employed by Verwoerd et al. (14).

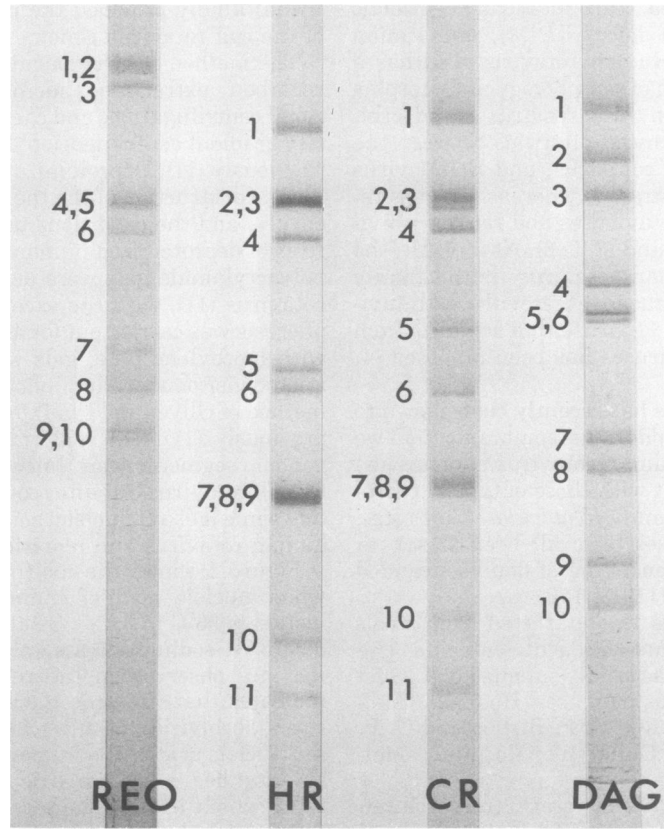

FIG. 2. *Electrophoresis of the genomes of reovirus type 3 (REO), human rotavirus (HR), calf rotavirus (CR), and the orbivirus D'Aguilar (DAG) on 7.5% polyacrylamide gels. Migration was from top to bottom.*

TABLE 1. *Molecular weights and molar ratios of the nucleic acid molecules obtained by electrophoretic fractionation of the genome of human rotavirus*

Peak no.[a]	Genome segment no.[b]	Mol wt ($\times 10^6$)	Molar ratio[c]
1	1	2.04	0.62
2	2,3	1.58	1.61
3	4	1.40	0.99
4	5	0.81	1.09
5	6	0.75	1.06
6	7,8,9	0.50	3.38
7	10	0.28	0.99
8	11	0.23	1.14

[a] From densitometer tracing (Fig. 3).
[b] Determined from molar ratio calculations.
[c] Calculated according to Rodger et al. (11).

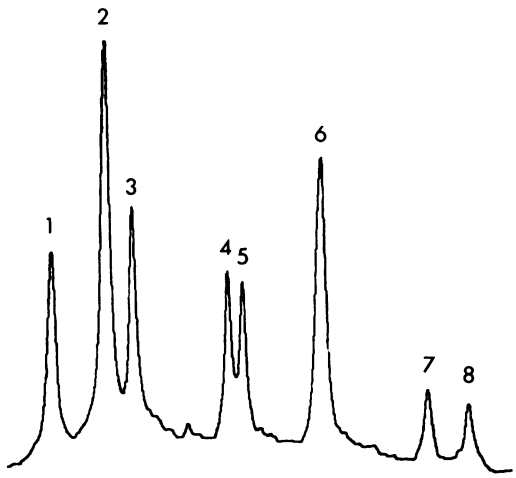

FIG. 3. *Densitometer tracing of the fractionated genome of human rotavirus. The tracing starts with the top of the gel on the left.*

When purified nucleic acid preparations from virus isolates obtained from two different children were subjected to co-electrophoresis on the same gel, more than eight bands were observed. Two distinct bands were apparent in each of the positions occupied by the bands 1, 2 and 5 shown in Fig. 2. The difference in the molecular weights of the two components of each of the double bands was quite small—of the order of 2%. The molecular weights of some of the corresponding genome segments of these isolates apparently differed from those of the isolate that was subjected to co-electrophoresis with reovirus and provided the values listed in Table 1. Lack of material made further comparisons by co-electrophoresis impossible. The splitting of some but not all bands in a gel containing a pool of two isolates at least establishes that detectable variations in the molecular weight of particular genome segments can occur in different isolates of human rotavirus and that further work will be required to determine the extent of these variations among different isolates and their resultant effects on the virus polypeptides.

The genome of the human rotavirus has proved to be very similar to that of the calf rotavirus. Not only do both viruses possess genomes with similar melting temperatures and probably consisting of 11 double-stranded segments, but the molecular weights of corresponding segments also appear to be generally very similar. The molecular weights of the calf rotavirus genome segments used in the comparison are those obtained by Rodger et al. (11).

The genomes of the human and calf rotaviruses are clearly basically very similar to those of the reoviruses and orbiviruses, although the band patterns are characteristic for each virus group (Fig. 2). A significant difference is that rotaviruses possess 11 genome segments, whereas the genomes of reoviruses and orbiviruses are known to consist of only 10 double-stranded segments (12, 13, 15).

Molecular weight differences between the human and calf rotavirus genomes appear to be most marked in segments 1, 4, and 5. As evidenced by a comparison of the band patterns on polyacrylamide gels (Fig. 2), these differences seem to allow the genomes of the human and calf viruses to be readily distinguished from one another. Further work needs to be done on distinct isolates of both human and calf rotaviruses to determine the range of variation in molecular weights of particular genome segments. Until this is established we cannot be certain whether it will always be possible to distinguish human rotavirus isolates from those of calf origin by polyacrylamide gel electrophoresis of the genomes. It will of course also be of great interest to compare human and calf rotavirus genomes with those of other animal rotaviruses.

Newman et al. (9) have suggested that the genome of calf rotavirus could consist of 11 or 12 segments, as they were unsure whether their peak 6 from radioactively labeled virus nucleic acid fractionated on polyacrylamide gels represented three or four species. We would agree that the corresponding peak 6 in our present study of the human virus could conceivably represent four rather than three species, but our data support the lower estimate.

Overall, we feel that the results presented here showing the double-stranded-segment nature of the human rotavirus genome have further underlined the similarity of this virus not only to the calf rotavirus but also to the reoviruses and orbiviruses. This lends further sup-

port to the proposal to classify the human and calf rotaviruses together in a genus within the family *Reoviridae*.

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