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Comparison of Human Rotaviruses Isolated in Mexico City and in Santiago, Chile, by Electrophoretic Migration of Their Double-Stranded Ribonucleic Acid Genome Segments

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During the period October to December 1979, rotaviruses were obtained from infants and young children hospitalized with acute gastroenteritis in Mexico City and were compared by analysis of the migration of their double-stranded ribonucleic acid (RNA) genome segments in gel electrophoresis. Comparison of the results of this analysis and of those of similar studies carried out in 1977 and 1978 showed that the two rotavirus electropherotypes designated 2s and 21 have been continuously present and that the proportion in which these two types have been found in hospitalized patients has varied greatly year to year. The RNAs from rotaviruses 2s and 21 differed in the electrophoretic migration of at least eight genome segments. However, RNAs from virus assigned to the same electropherotypes were not necessarily identical: on the basis of small but significant differences in the migration of segment 7, 8, or 9, isolates of types 2s and 21 could be assigned to two and three different subtypes, respectively. Human rotaviruses obtained in a distant geographical region, Santiago, Chile, in July 1979 had RNA electrophoretic patterns similar to that of electropherotype 21 but different from it in the migration of one or two of the larger RNA segments.

The genome of rotavirus, one of the most important etiological agents of infantile gastroenteritis, consists of 11 segments of double-stranded ribonucleic acid (dsRNA), which can be resolved by polyacrylamide gel electrophoresis (17). The existence of different types of rotavirus infecting humans has been shown by immunological studies (7, 16, 22) and by comparison of the viral RNA migration patterns in polyacrylamide gel electrophoresis (3, 9). In both 1977 and 1978, the two electropherotypes designated 21 and 2s were found in Mexico City by this last technique (4, 6). Electropherotypes 21 and 2s also differ in the polypeptide composition of the virions (5); however, their possible antigenic differences are not known. The relative importance of each rotavirus electropherotype as a cause of hospitalization of infants and young children was very different in the years 1977 and 1978: viruses of type 2s were found in only 11% (6 of 52) of the hospitalized children in 1977 (4), whereas in 1978, 89% (8 of 9) of the patients had this type (6). At that time it was predicted either that type 21 would completely disappear or that a cycle of prevalence exists between the two electropherotypes (6). In this paper we report that in 1979 rotavirus type 21 was prevalent, which suggests that the prevalence of each electropherotype is cyclic.

It was also previously reported that RNAs from rotavirus electropherotypes 2s and 21 differ in the electrophoretic migration of at least seven segments and that both types are not homogeneous since, among members of either type, small differences could be observed in the migration of segment 7, 8, or 9 (6). In this paper we report a previously unnoticed difference in migration of segment 1 of RNAs from types 2s and 21, and we also attempt to group viruses of the same type according to the electrophoretic migration of their dsRNA segments 7, 8, and 9.

Three distinct patterns differing in the electrophoretic migration of one, two, or three segments have been distinguished among RNAs from eight human rotaviruses obtained during four successive epidemics in the Washington, D.C., area (9). Verly and Cohen reported similar observations among calf rotaviruses obtained in France (20) and suggested that some variations appeared to be related to the geographical origin of the samples. Extensive diversity was later observed among bovine rotavirus isolates in Australia (15). Altogether, these observations suggest that rotaviruses are very heterogeneous, as

are reoviruses (12) and orbiviruses of the same serological groups (10), as demonstrated in each case by their RNA electrophoretic patterns, and that the extent of variation in these patterns may depend on geographical origin and time of collection, as previously observed for human reovirus (12).

In this paper we also report that isolates obtained from sick children in Santiago, Chile, in 1979 showed at least two different patterns of migration of RNA segments in gel electrophoresis and that both patterns, although similar to that of electropherotype 21, were different from those of isolates obtained in Mexico City during the period 1977 to 1979.

MATERIALS AND METHODS

Patients were infants and children less than 3 years old admitted with gastroenteritis and diarrhea with duration of 5 days or less to the Hospital de Pediatria, I.M.S.S., México, D.F, during the period October to December 1979 or to the Hospital Roberto del Rio, Santiago, Chile, in July 1979. Amounts of 1 to 3 g of stool samples were collected and screened for rotavirus by analysis of the electrophoretic patterns of the dsRNA segments, upon disruption of the viral particles which had been obtained by precipitation with polyethylene glycol from a Freon-treated suspension of stool (3). RNA was extracted either from purified virus or, if the amount of virus seemed small, from a polyethylene glycol precipitate of a stool suspension treated with trifluorotrichloroethane (6). For electrophoresis of RNA, slab polyacrylamide-bisacrylamide (5% and 0.125%, wt/vol) gels prepared as described (6) or 5 to 10% polyacrylamide linear gradient gels were used. Gradient gels were prepared in a gradient former using equal volumes of two acrylamide solutions made in Loening E buffer (14) containing 0.1% (wt/vol) sodium dodecyl sulfate. The first contained 5% (wt/vol) acrylamide, 0.125% (wt/vol) bisacrylamide, 0.1% (vol/vol) *N,N,N',N'*-tetramethylethylenediamine, and 0.1% (wt/vol) ammonium persulfate; and the other contained 10% (wt/vol) acrylamide, 0.25% (wt/vol) bisacrylamide, 0.05% (vol/vol) *N,N,N',N'*-tetramethylethylenediamine, 0.05% (wt/vol) acrylamide, 0.05% (wt/vol) ammonium persulfate, and 20% (vol/vol) glycerol. After electrophoresis, gels were stained with an aqueous solution of ethidium bromide (1 μg/ml) and photographed (4). Growth and purification of SAll and extraction of its RNA were as previously described (5).

RESULTS

Electrophoresis of the RNAs extracted from rotavirus obtained from October to December 1979 from nine infants or young children hospitalized with diarrhea showed that all these patients were infected with rotavirus electropherotype 21. During the same period in 1978, only one of nine patients (11%) infected with rotavirus excreted virus of type 21; the remainder excreted type 2s (6). In 1977 during this 3-month time span, all of the 30 rotavirus-infected patients excreted rotavirus of type 21 (4). Therefore, during these 3 months, the proportion of type 21 found in 1979 (100%) was the same as that found in 1977, but was significantly different ($P < 0.001$) from that found in 1978 (11%). The probability (P) of obtaining such a difference, had the proportion of the electropherotypes been the same, was calculated with the use of $\chi^2 = 18.4$, which was obtained with a 2×2 contingency table.

Difference in migration of dsRNA genome segments 2, 3, 5, 10, and 11 from rotavirus 2s and 21 has been previously illustrated (6). However, when the electrophoresis was run for longer periods to optimize resolution of the larger dsRNA segments, differences appeared in the migration of segment 1 from these two rotavirus electropherotypes (Fig. 1). Since this difference was not noticed previously, four isolates of each electropherotype, taken in different years, were reexamined as described in Fig. 1 to search for a possible intratype heterogeneity in dsRNA segment 1. The results (not shown) did not show intratype heterogeneity but always showed the same difference in the electrophoretic migration of segment 1 between rotaviruses of electropherotypes 21 and 2s.

Rotavirus isolates assigned to either electropherotype are not necessarily completely alike: small but significant differences could be detected in the electrophoretic migration of one or two of the segments 7, 8, and 9 between some isolates of the same electropherotype. This intratype heterogeneity was reexamined by electrophoresis on a 5 to 10% polyacrylamide gradient gel, which gave better resolution of the smaller segments. Figure 2 shows the results after electrophoresis of five isolates of electropherotype 2s, one of which (track 2) can be distinguished by the increased electrophoretic migration of segment 7. Figure 3 shows the three different electrophoretic patterns of segments 7, 8, and 9 that have been distinguished among rotaviruses of electropherotype 21.

Polyacrylamide gradient gels were also employed to examine the differences in segments 7, 8, and 9 between electropherotypes 21 and 2s. Since these segments are resolved into five bands after coelectrophoresis of the RNAs from both electropherotypes (Fig. 4), at least two of the dsRNA segments in the cluster made by segments 7, 8, and 9 are different between these isolates of types 21 and 2s.

Electrophoretic analysis of RNA genome segments from seven rotavirus isolates obtained from different patients, among 13 hospitalized

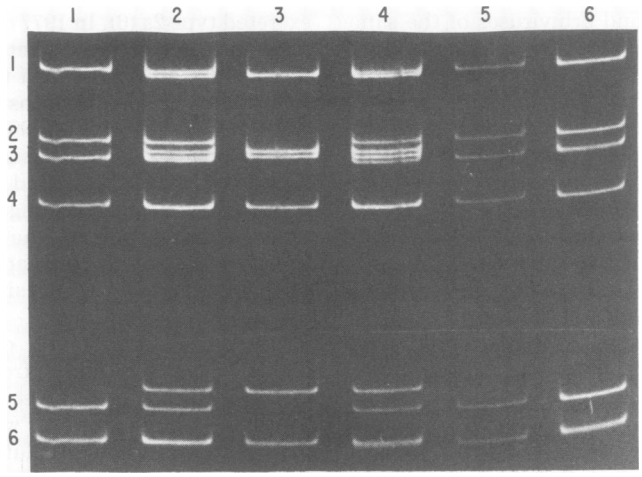

FIG. 1. *Electrophoresis of RNA from human strains of rotavirus (HRV) 2s and 21. Electrophoresis was performed in 5% polyacrylamide gels, 1.5 mm thick, for 20 h at 40 mA to obtain better resolution of the larger segments. Tracks: (1) HRV 4185 (21, August 1979), (2) HRV 4185 + HRV 186 (2s, December 1978), (3) HRV 186, (4) HRV 186 + HRV 770 (21, September 1977), (5) HRV 770, (6) HRV 770 + HRV 4185. Strain numbers are the numbers given to the hospitalized patients, 2s or 21 indicates virus type, and the dates are those of patient admission. Numbers on the left of the photograph are the assigned segment numbers.*

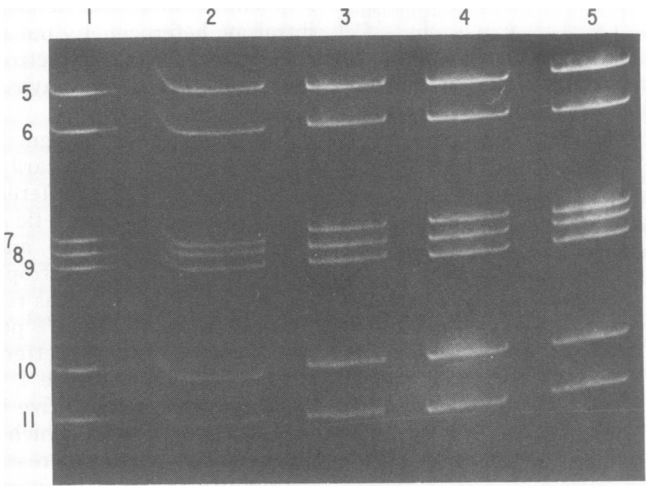

FIG. 2. *Electrophoresis of RNA from different isolates of human rotavirus (HRV) type 2s. Electrophoresis was performed in 5 to 10% polyacrylamide gradient gels, 0.75 mm thick, for 15 h at 27 mA to optimize resolution of the smaller segments. Tracks: (1) HRV 9923 (October 1978), (2) HRV 1383 (November 1978), (3) HRV 2017 (November 1978), (4) HRV 186 (December 1978), (5) HRV 5859 (October 1978). Meanings of numbers and dates are explained in Fig. 1.*

young children with acute gastroenteritis in Santiago, Chile, during July 1979, showed the presence of two electropherotypes of rotavirus, distinguishable by the relative electrophoretic migration of dsRNA segment 2. Figure 5 shows these two patterns in tracks 5 and 6; the pattern shown in track 6 was observed in isolates from two patients, and that in track 5, in isolates from five patients. Coelectrophoresis of the RNA from these different isolates showed only the aforementioned difference in segment 2 (data not shown). The patterns obtained by coelectrophoresis of each of the two distinct human rotavirus RNAs with simian rotavirus (SA11) RNA are shown in tracks 2 and 4. SA11 RNA (track 3) is a suitable standard for comparison of rotavirus RNAs because it is generally available, a property not shared by human rotaviruses as a consequence of their failure to grow efficiently in vitro. The difference between hu-

man rotavirus RNAs can be observed in these coelectrophoresis: segment 2 of the virus electropherotype (track 2) comigrated with segment 2 of SA11 RNA, whereas the respective segment of the other electropherotype (track 4) migrated slightly slower. Comparison of the patterns obtained by coelectrophoresis of SA11 RNA with RNA extracted from human rotaviruses obtained in Santiago, Chile (Fig. 5), and human rotaviruses obtained in Mexico City (Fig. 6), shows that the RNAs from human rotavirus obtained in Santiago, Chile, were very similar to that of the rotaviruses electropherotype 21 obtained in Mexico City (Fig. 6, track 4). Coelectrophoresis of the RNAs from rotavirus obtained in Santiago and from rotavirus type 21 obtained in Mexico City suggested only small differences in the migration of some of the larger segments. These differences were more clearly shown when the electrophoresis was performed for a longer time, to optimize resolution of the larger segments (Fig. 7). The patterns in Fig. 7 were obtained by coelectrophoresis of Mexican type 21 rotavirus (track 1) with either of the Chilean isolate electropherotypes (tracks 3 and 5) and show differences in the migration of either segment 4 (track 4) or segments 2 and 4 (track 2). The difference in dsRNA segment 2 between the isolates obtained in Santiago, Chile, is illustrated better in Fig. 7 (track 6). Small differences also seem to exist between the migration of segments 1 and 3 of the RNAs of rotaviruses from Santiago and that of the rotavirus type 21 from Mexico City because the bands corresponding to these segments were broader after coelectrophoresis and, in some cases, barely resolved into two (tracks 2 and 4).

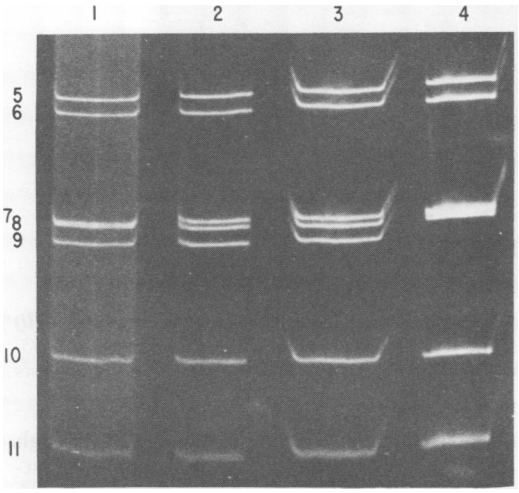

FIG. 3. *Electrophoresis of RNA from different isolates of human rotavirus (HRV) type 21. Tracks: (1) HRV 8451 (July 1977), (2) HRV 770 (September 1977), (3) HRV 4185 (August 1979), (4) HRV 3222 (November 1978). Meanings of numbers and dates are explained in Fig. 1. Conditions of electrophoresis were as in Fig. 2.*

DISCUSSION

Two electropherotypes of rotavirus designated 2s and 21 have been found in infected infants and young children in Mexico City during the period December 1976 to December 1979, and the proportion in which these two types have been found has varied greatly from year to year (4, 6, this paper). The persistence of these two types for at least the 3 years of this study suggests that the appearance of new rotavirus

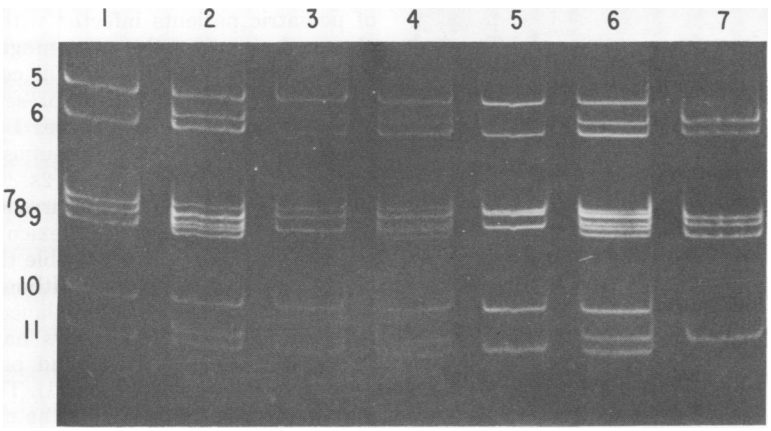

FIG. 4. *Electrophoresis of RNA from different isolates of human rotavirus (HRV) types 2s and 21. Tracks: (1) HRV 2017 (2s, November 1978), (2) HRV 2017 + HRV 4185 (21, August 1979), (3) HRV 5859 (2s, October 1978), (4) HRV 5859 + HRV 4185, (5) HRV 1383 (2s, November 1978), (6) HRV 1383 + HRV 4185, (7) HRV 4185. Meanings of numbers and dates are explained in Fig. 1. Conditions of electrophoresis were as in Fig. 2.*

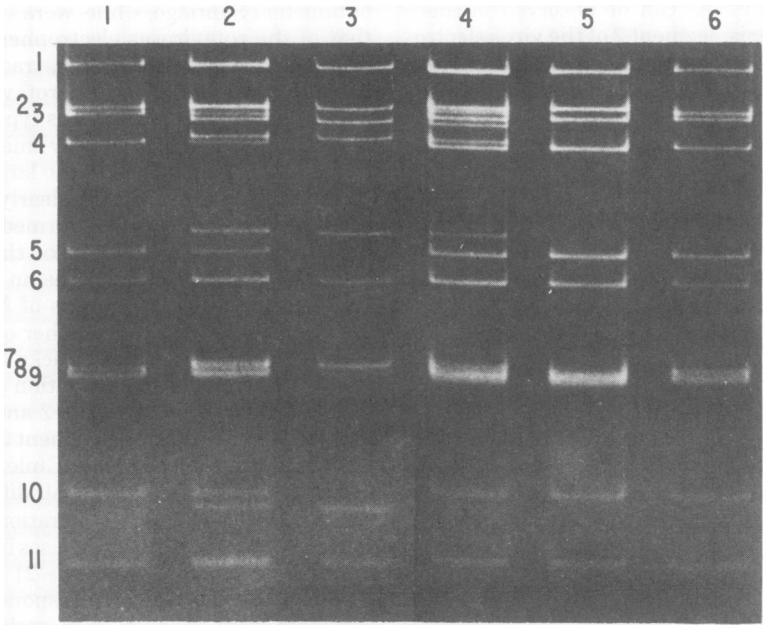

FIG. 5. *Electrophoresis of human rotavirus (HRV) RNA from Santiago, Chile, with simian rotavirus (SAll) RNA used as standard. Tracks: (1) HRV 23 (July 1979), (2) HRV 23 + SA11, (3) SAll, (4) HRV 28 + SAll, (5) HRV 28 (July 1979) and (6) HRV 23. Meanings of numbers and dates are explained in Fig. 1. Electrophoresis was run at 20 mA for 20 h.*

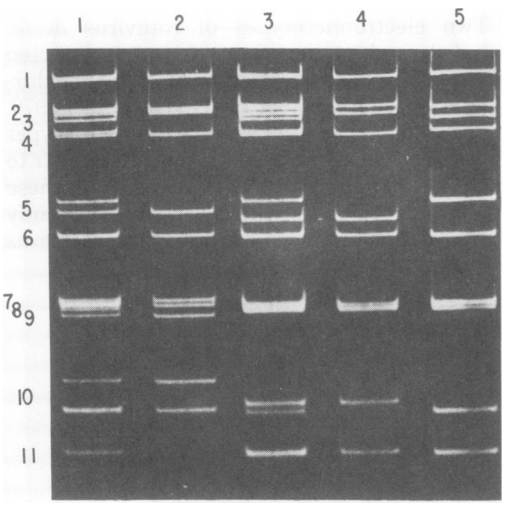

FIG. 6. *Electrophoresis of RNA from human rotavirus (HRV) isolated in Mexico City, types 2s and 21, using SAll RNA as standard. Tracks: (1) HRV 0454 (2s, November 1978) + SAll, (2) HRV 0454, (3) HRV 3222 (21, November 1978) + SAll, (4) HRV 3222, (5) SAll. Electrophoresis was run at 20 mA for 20 h. Meanings of dates and numbers are explained in Fig. 1.*

electropherotypes is not very frequent. Kalica et al. (8) found three different electrophoretic patterns of RNA from eight human rotaviruses, obtained from each of four successive annual rotavirus epidemics occurring from 1974 through 1977 in the Washington, D.C., area. Interestingly, the RNA patterns of both 1974 and both 1977 strains were identical. However, epidemiological studies of the two serotypes of rotavirus (1 and 2), which are distinguishable by enzyme-linked immunosorbent assay, did not suggest a cyclic variation in the type of rotavirus found in the Washington, D.C., area (21). In that study, from 1974 to 1978, an ever-increasing percentage of pediatric patients infected with type 1 was observed, whereas the percentage of patients infected with rotavirus type 2 constantly decreased. We do not know, at present, if there is any relation between serotypes 1 and 2 determined by enzyme-linked immunosorbent assay and electropherotypes 21 and 2s.

Abrupt changes in the occurrence of type 21 or 2s observed each year in Mexico City have no simple explanation. It is possible that the prevalence of each type is cyclic although confirmation of a cycle requires surveillance for the next few years. Cyclical epidemics have been observed with other viruses and probably have been best defined in measles (1). The important parameters used to describe the cycles are the number of persons susceptible and the number of persons infected and capable of spreading the disease. According to Bartlett's equation (2), the average interval between epidemics will become shorter with increasing community size. It is

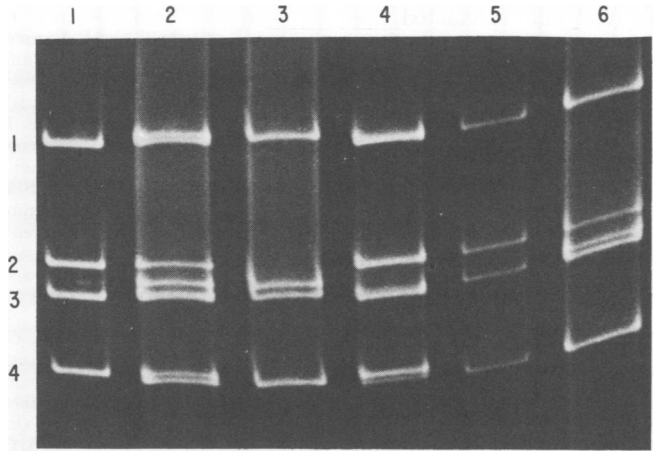

FIG. 7. *Electrophoresis of human rotavirus (HRV) RNA from both Santiago, Chile, and Mexico City to resolve the larger RNA segments. Tracks: (1) HRV 3222 (Mexico City November 1978) (2) HRV 3222 + HRV 23 (Santiago July 1979), (3) HRV 23, (4) HRV 3222 + HRV 28 (Santiago July 1979), (5) HRV 28, (6) HRV 23 + HRV 28. Electrophoresis was run for 20 h at 40 mA. Meanings of dates and numbers are explained in Fig. 1.*

possible that these parameters and perhaps others, such as hygiene, may determine a cycle, in Mexico City, between these two rotavirus electropherotypes, should there be no cross-protection between electropherotypes 21 and 2s.

The remarkable difference in the RNA of rotavirus types 2s and 21 in polyacrylamide gel electrophoresis is shown in the migration of their genome segments 1, 2, 3, 5, 10, and 11, and usually in two segments of the cluster made by segments 7, 8, and 9. Although these two electropherotypes apparently infect the same population, we have not detected the appearance of new types that might arise from "segment reassortment." "Segment reassortment" has been shown to occur at high frequency in mixed infections in vitro with different serotypes of reovirus (18) or orbivirus (11), which also contain a segmented dsRNA genome. Segment exchange has been postulated to occur in nature for influenza virus (13). Exchange of the dsRNA segment 2, 5, 10, or 11 between rotavirus 2s and 21 would have been readily detected in polyacrylamide gel electrophoresis.

Differences observed in one or two of the segments of the cluster made by 7, 8, and 9 may have significance in the antigenic properties of rotavirus, especially if they code for some of the outer layer polypeptides. On the basis of the electrophoretic migration of these segments, rotaviruses of electropherotypes 21 and 2s were assigned to two or three subtypes, respectively (Table 1). Table 1 demonstrates that viruses of the same subtype have been present in different years.

The RNA patterns of the rotavirus isolates obtained in 1979, in Santiago, Chile, could be assigned to two types on the basis of the migration of segment 2. Even though both patterns were very similar to that of Mexican rotavirus 21, one of these electropherotypes differed in the migration of both segments 2 and 4, whereas the other differed only in that of segment 4. They might also differ from rotavirus 21 in the migration of dsRNA segments 1 and 3; however, further attempts to establish more clearly these

TABLE 1. *Rotavirus assigned to indicated type and group*[a]

Type	Group	
2s	a	1383 (November 1978)
	b	9923 (October 1978), 2017 (November 1978), 186 (December 1978), 5859 (October 1978), 454[b] (November 1978), 154047[b] (March 1977)
21	a	3222 (November 1978)
	b	4185 (August 1979), 2332[b] (August 1979), 770 (September 1977), 480[b] (November 1979), 1514[b] (November 1979), 1751[b] (November 1979), 4435 (November 1979)
	c	1116 (November 1977), 8451 (July, 1977), 166872 (December 1977), 5614 (November 1979)

[a] Group assignments were made on the basis of the electrophoretic migration of dsRNA segments 7, 8, and 9. The patterns of each subgroup are shown in the figures: 2sa in track 2, Fig. 2; 2sb in tracks 1, 3, 4, and 5, Fig. 2; 21a in track 4, Fig. 3; 21b in tracks 2 and 3, Fig. 3; and 21c in track 1, Fig. 3.

[b] Subtypes reexamined (electrophoretic data not shown).

possible differences failed and exhausted the sample RNAs from the rotaviruses obtained in Chile.

These results are in agreement with a previous observation with reovirus (12) that samples isolated from widely different geographical origins showed different patterns, whereas samples from the same area over a period of time showed more limited variations. Although the consequence of dsRNA genome segment variation is not known, these observations should be considered in designing a strategy to develop a possible vaccine that would be effective worldwide. Extensive variability in the RNA patterns has been found among all three serotypes of reovirus (12) and in some serological groups of orbivirus (10). The observations to date suggest a similar situation among rotaviruses (6, 8, 15, 20). Variation among human rotaviruses, however, may be less extensive than that observed with reovirus, since the rotavirus isolates subjected to analysis have been only those infecting infants and capable of causing a diarrhea serious enough to justify hospitalization.

The significance of the different electrophoretic migration of RNA segments has yet to be established, but it seems likely that the variation observed in the RNA genome is translated into the viral polypeptides. Extensive variation has been observed in the electrophoretic patterns of viral polypeptides from cells infected with rotavirus found in different species (19), and it has been observed that human rotaviruses with different RNA electrophoretic patterns are also distinguishable by the electrophoretic patterns of their polypeptides (5). The study of the relationship between RNA pattern and antigenic properties seems difficult at present as a consequence of the inability to apply serological techniques such as neutralization and hemagglutination inhibition to human rotavirus.

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LITERATURE CITED

1. **Bartlet, M. S.** 1956. Deterministic and stochastic models for recurrent epidemics. Third Berkeley Symposium on Mathematics and Statistical Problems, vol. 4, p. 81-109.
2. **Bartlet, M. S.** 1957. Measles periodicity and community size. J. R. Stat. Soc. Ser. A **120**:40-70.
3. **Espejo, R. T., E. Calderon, and N. Gonzalez.** 1977. Distinct reovirus-like agents associated with acute infantile gastroenteritis. J. Clin. Microbiol. **6**:502-506.
4. **Espejo, R. T., E. Calderon, N. Gonzalez, A. Salomon, A. Martuscelli, and P. Romero.** 1979. Presence of two distinct types of rotavirus in infants and young children hospitalized with acute gastroenteritis in Mexico City, 1977. J. Infect. Dis. **139**:474-477.
5. **Espejo, R. T., E. Martinez, S. Lopez, and O. Muñoz.** 1980. Different polypeptide composition of two human rotavirus types. Infect. Immun. **28**:230-237.
6. **Espejo, R. T., O. Muñoz, F. Serafin, and P. Romero.** 1980. Shift in the prevalent rotavirus detected by ribonucleic acid segments differences. Infect. Immun. **27**: 351-354.
7. **Flewett, T. H., M. E. Thouless, J. N. Pilfold, and A. S. Bryden.** 1978. More serotypes of human rotavirus. Lancet **2**:632.
8. **Kalica, A. R., C. F. Garon, R. G. Wyatt, C. A. Mebus, D. H. Van Kirk, R. M. Chanock, and A. Z. Kapikian.** 1976. Differentiation of human and calf reovirus-like agents associated with diarrhea using polyacrylamide gel electrophoresis of RNA. Virology **74**:86-92.
9. **Kalica, A. R., M. M. Sereno, R. G. Wyatt, C. A. Mebus, R. M. Chanock, and A. Z. Kapikian.** 1978. Comparison of human and animal rotavirus strains by gel electrophoresis of viral RNA. Virology **87**:247-255.
10. **Gorman, B. M. and J. Taylor.** 1978. The RNA genome of Tilliperry virus. Aust. J. Exp. Biol. Med. Sci. **56**:369-371.
11. **Gorman, B. M., J. Taylor, P. J. Walker, and P. P. Young.** 1978. The isolation of recombinants between related orbiviruses. J. Gen. Virol. **41**:333-342.
12. **Hardy, D. B., L. Rosen, and B. N. Fields.** 1979. Polymorphism of the migration of double-stranded RNA genome segments of reovirus isolates from humans, cattle, and mice. J. Virol. **31**:104-111.
13. **Laver, W. G., and R. G. Webster.** 1973. Studies on the origin of pandemic influenza. III. Evidence implicating duck and equine influenza viruses as possible progenitors of the Hong Kong strain of human influenza. Virology **51**:383-391.
14. **Loening, U. E.** 1969. The determination of the molecular weight of ribonucleic acid by polyacrylamide gel electrophoresis. Biochem. J. **113**:131-138.
15. **Rodger, S. M., and I. H. Holmes.** 1979. Comparison of the genomes of simian, bovine, and human rotaviruses by gel electrophoresis and detection of genomic variation among bovine isolates. J. Virol. **30**:839-846.
16. **Rodriguez, W. J., H. W. Kim, C. D. Brandt, R. H. Yolken, J. O. Arrobio, A. Z. Kapikian, R. M. Chanock, and R. H. Parrot.** 1978. Sequential enteric illnesses associated with different rotavirus serotypes. Lancet **2**:37.
17. **Schnagl, R. D., and I. H. Holmes.** 1976. Characteristics of the genome of human infantile enteritis virus (rotavirus). J. Virol. **19**:267-270.
18. **Sharpe, A. H., R. F. Ramig, R. F. Mustoe, T. A. Fields, and B. N. Fields.** 1978. A genetic map of reovirus. I. Correlation of genome RNAs between serotypes 1, 2 and 3. Virology **84**:63-74.
19. **Thouless, M. E.** 1979. Rotavirus polypeptides. J. Gen. Virol. **44**:187-197.
20. **Verly, E., and J. Cohen.** 1977. Demonstration of size variation of RNA segments between different isolates of calf rotavirus. J. Gen. Virol. **35**:583-586.
21. **Yolken, R. H., R. G. Wyatt, G. Zissis, C. D. Brandt, W. J. Rodriguez, H. W. Kim, R. H. Parrott, J. J. Urrutia, L. Mata, H. B. Greenberg, A. Z. Kapikian, and R. M. Chanock.** 1978. Epidemiology of human rotavirus types 1 and 2 as studied by enzyme-linked immunosorbent assay. N. Engl. J. Med. **299**:1156-1161.
22. **Zissis, G., and J. P. Lambert.** 1978. Different serotypes of human rotavirus. Lancet **1**:38-39.